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Regional Conservation Implications of Green Turtle (*Chelonia mydas*) Genetic Stock Composition in China

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ABSTRACT. – Sea turtles are globally endangered and face anthropogenic threats, such as direct harvest, bycatch, and habitat degradation. Genetic studies help identify connectivity between nesting and foraging grounds for conservation and management. However, information on genetic stock composition of green turtles (*Chelonia mydas*) in the South China Region (including Hong Kong, Guangdong Province, and Taiwan) is severely limited. In this study, mixed stock analysis based on the 760-bp mitochondrial DNA (mtDNA) control region of green turtles ($n = 110$) revealed that the primary source rookeries in the Pacific contributing to foraging green turtle aggregations in the South China Region were Peninsular Malaysia, Yap in the Federated States of Micronesia, Aru of Indonesia, Sulu Sea, northeastern Borneo, Republic of Marshall Islands, Wan-an of Taiwan, and the central Ryukyu and Yaeyama of Japan. This study is the first to investigate and report the source nesting populations of a relatively large number of foraging green turtles in the region. The genetic results also indicate possible use of coastal Guangdong, the Taiwan Strait, and the East China Sea as habitat by pelagic-phase green turtles hatched from nesting beaches in Taiwan and mainland China. As a precautionary approach for effective sea turtle conservation, conservation and management of each distinct green turtle source rookery as well as foraging aggregations sourced from multiple natal origins in the South China Region is needed to preserve genetic diversity for the species. Anthropogenic threats to nesting and foraging habitats and migratory pathways, such as direct take for trade and fishery impacts, should be thoroughly assessed and effectively mitigated by regional collaboration to sustain these populations.

CHINESE ABSTRACT. – 海龜是全球瀕危的物種，牠們每天面對各種的人為威脅，包括捕獵、混捕及生境破壞等。綠海龜的基因結構可找出產卵地與覓食地的連繫，這有助確定綠海龜群種相互的關聯，從而為地域層面上保育海龜的合作定下科學性的基礎。可是，有關資料在南中國地區極其缺乏。本研究分析南中國地區覓食綠海龜($n = 110$)中 760 bp mtDNA control region 的單倍體頻率(haplotype frequency)，透過 Mixed Stock Analysis 與其他地區群種的比較及統計，顯示南中國地區的覓食綠海龜與馬來西亞、密克羅尼西亞聯邦的雅浦島、印尼的阿魯群島、蘇祿海、婆羅洲東北部、馬紹爾群島共和國、台灣望安島，及日本中央琉球和八重山地區的繁殖種群有連繫。本研究是南中國地區首次用相對大量覓食綠海龜來調查其源頭繁殖種群。本研究亦指出，於中國內地及台灣出生的綠海龜在其外洋性階段可能會棲息於廣東沿海、台灣海峽及東海。為保存基因多樣性，我們須保護各綠海龜繁殖種群及南中國地區覓食綠海龜群落。未來的保育及研究亦要集中評估及緩和對綠海龜產卵地、覓食地及遷移路線的人為威脅，包括捕獵及混捕。正因為綠海龜廣大的活動範圍及生境聯繫，我們須聯合各地域各方利益相關者的力量及合作以更有效地保護南中國地區綠海龜及其生境。

KEY WORDS. – genetics; mixed stock analysis; connectivity; conservation; green turtle; China

Of the 7 sea turtles species in the world, 5 species are found in the South China Sea: green turtle (*Chelonia mydas*), leatherback (*Dermochelys coriacea*), olive ridley (*Lepidochelys olivacea*), loggerhead (*Caretta caretta*), and hawksbill (*Eretmochelys imbricata*) (Wang 1993; Chan et al. 2007). Among the 5 sea turtle species recorded in South China, the green turtle is the most common and is the only species that nests in the area (Wang 1993; Chan et al. 2007; Wang and Li 2008; Ng and Wan 2011). Green turtles are listed as globally endangered (International Union for Conservation of Nature [IUCN] 2012) and face various anthropogenic threats such as direct harvest and by-catch (Cheng and Chen 1997; Wang and Li 2008), trade pressure (Pilcher et al. 2009; Lam et al. 2011), habitat degradation (Wang and Li 2008), and marine pollution and debris (Lam et al. 2006; Nelms et al. 2015). Nesting populations of green turtles in South China have been dwindling over the past several decades (Chan et al. 2007; Wang and Li 2008; Ng et al. 2014a).

Green turtles are migratory species of circumtropical distribution with adults travelling hundreds to thousands of kilometers between nesting beaches and foraging grounds (Hirth 1997). Green turtle foraging grounds have been described as indicators of the quality of tropical coastal marine ecosystems (Scott et al. 2012). Protection of the areas frequently used by green turtles could also synergistically promote ecosystem management in areas where other marine species of conservation importance are also present, such as dugongs (Gredzens et al. 2014). Green turtles show site fidelity to both breeding (FitzSimmons et al. 1997; Broderick et al. 2007; Rees et al. 2013; Stiebens et al. 2013) and foraging (Makowski et al. 2006; Schofield et al. 2010; Senko et al. 2010) areas. Knowledge of regional stock structure and connectivity of breeding and foraging populations is important for spatial manage-

ment and conservation (Hays 2008; Wallace et al. 2011). In addition to satellite telemetry and mark-and-recapture with conventional flipper tagging, analysis of genetic variation in terms of mitochondrial DNA (mtDNA) sequences (FitzSimmons et al. 1999; Dethmers et al. 2006, 2010; Cheng et al. 2008; Dutton et al. 2008; Shamblin et al. 2015; Jensen et al. 2016), microsatellites (Chassin-Noria et al. 2004; Nishizawa et al. 2011; Roden et al. 2013), and single nucleotide polymorphisms (SNP) (Roden et al. 2013) has been a useful tool to define population connectivity of green turtles (see Komoroske et al. 2017). The hypervariable mtDNA control region has provided the most informative marker for detecting population differentiation in sea turtles because of its relative variability compared with other regions of the mitogenome (Jensen et al. 2013). Most early studies used a 384-base pair (bp) sequence of this control region, but more recently primers designed to amplify a 760-bp region encompassing the entire mtDNA control region have uncovered additional variation that has been able to further differentiate common, widespread haplotypes (Dutton et al. 2014b, Jensen et al. 2016). Advances in whole mitogenome sequencing have provided new markers to detect fine-scale population structure in green turtles in the Caribbean (Shamblin et al. 2015), and recent studies have expanded the baseline data set with longer sequences for the Pacific Ocean, although gaps still remain (Jensen et al. 2016, submitted for publication).

Management Units (MUs), which form part of broader regional populations such as Regional Management Units (RMU) (Wallace et al. 2010) or Distinct Population Segments (DPS) (Seminoff et al. 2015), are identified according to the distinctiveness of the genetic composition of breeding populations of sea turtles (Komoroske et al. 2017). MUs serve to designate functionally independent

populations in which any loss of individuals in one population is unlikely to be replenished by individuals from another population within management time frames (Moritz 1994). Designation of MUs, which may encompass several adjacent nesting sites or rookeries, informs and facilitates regional collaboration to mitigate anthropogenic threats and to spatially highlight priority areas for international conservation of species (Hays 2008; Wallace et al. 2011). The frequencies of mtDNA haplotypes detected in nesting populations of green turtles are compared with those of other rookeries to assess genetic variation with the aim of determining population connectivity. A total of 5 MUs have been identified to date for green turtles in the Northwest Pacific, including rookeries in Japan and Taiwan (Cheng et al. 2008; Nishizawa et al. 2011, 2013; Hamabata et al. 2014), 5 MUs for the Central and Eastern Pacific (Dutton et al. 2014a, 2014b), and 17 MUs for the Southwest Pacific (Dethmers et al. 2006; Read et al. 2015; Jensen et al. 2016, submitted for publication).

The existing evidence for genetic connectivity between foraging and nesting populations of sea turtles underscores the importance of collaboration on conservation of both nesting sites and foraging grounds, often across national borders. Recruitment and composition of sea turtles at foraging aggregations is affected by a combination of factors, e.g., natal rookery size, geographic distance between nesting and foraging grounds, oceanic currents, and ontogenetic shifts of development habitats among life stages (see Jensen et al. 2013). Mixed stock analysis (MSA) was originally used in fisheries management such as to identify the stock origin and estimate the population structure of salmon and other marine fishes (Utter and Ryman 1993). MSA was later used in sea turtle conservation to estimate the relative contribution of various rookeries, or MUs (the source), to foraging aggregations (the mix) based on patterns of haplotype frequencies (Bolker et al. 2007; Jensen et al. 2013). Sea turtles at a foraging aggregation are usually sourced from various genetically distinct breeding populations, which are often large distances apart, e.g., several thousand kilometers. Amorochio et al. (2012) identified multiple distant origins for green turtles foraging around Gorgona Island in the Colombian Eastern Pacific from rookeries in the Central and Eastern Pacific including Mexico, the Galapagos Island, and Australasia and suggested that dispersal of juvenile green turtles along the eastern Pacific coast and eastward from the distant western and central Pacific was facilitated by oceanic currents. Saied et al. (2012) used MSA to identify dispersal patterns of loggerhead turtles with sea surface currents from a nesting site in Libya to oceanic and neritic foraging grounds that spanned the Atlantic Ocean. Nishizawa et al. (2013) reported that current flow influenced the composition of feeding aggregations along the Japanese archipelago. Most hatchlings from the western Pacific rookeries settled in upstream feeding grounds from the southern to northern

Japanese archipelago as the Kuroshio Current flows from southwest to northeast. Recently, Naro-Maciel et al. (2014) found that green turtles foraging at Palmyra Atoll in the Central Pacific were assigned to nesting populations in the West Central and South Central Pacific, with some turtles also from the East Pacific, and demonstrated that oceanic currents influence the dispersal of juvenile turtles to this foraging area.

On the other hand, some studies have revealed dispersal of sea turtles over relatively shorter distances of several hundred kilometers and within national boundaries. Dutton et al. (2008) found the same unique genetic composition in terms of the mtDNA control region in multiple foraging populations of green turtles in the Hawaiian Islands and, hence, these aggregations are considered to be part of the same distinct breeding population of green turtles for the purposes of management and risk assessment in Hawaii (Dutton et al. 2008; Wallace et al. 2011). Based on MSA in terms of the mtDNA control region, foraging green turtles aggregated in the western Japanese main islands were mainly found to originate from rookeries in the Ogasawara Island Group (Nishizawa et al. 2013; Hamabata et al. 2015). Hamabata et al. (2016) also found that pelagic-stage juvenile green turtles collected in Japanese coastal waters had hatched from nesting sites on the Ogasawara Islands and islands in the Ryukyu Archipelago of Japan. Hamabata et al. (2016) stressed the need to prioritize protection of Ogasawara nesting populations on the grounds that the rookeries contribute to various foraging aggregations in Japan. By knowing the geographical extent of dispersal of sea turtles between rookeries and foraging grounds, targeted measures can be developed and taken to mitigate threats in a more-spatially appropriate manner and prioritize the conservation needs of a specific area for more-effective protection of sea turtle populations in both habitats.

In the South China Region, studies on the genetic composition of sea turtles are limited to nesting populations and their connectivity with other rookeries nearby. Cheng et al. (2008) conducted analysis of mtDNA sequences of 53 nesting green turtles from 2 rookeries in Wan-an Island and Lanyu Island, Taiwan, and reported that both rookeries were genetically distinct. Two haplotypes found in nesting green turtles in Hong Kong (CmP18 and CmP116, identified based on a 384-bp sequence of the mtDNA control region) are closely related to each other along with other Taiwan haplotypes (CmP49 and CmP19) (Ng et al. 2014a). Significant differentiation in haplotype frequencies between rookeries in Hong Kong and Lanyu Island in Taiwan was detected but not between Hong Kong and Wan-an Island. Information on genetic stock composition of foraging aggregations of green turtles in the South China Region is therefore scarce. The limited published information available is the complete cytochrome *b* gene and D-loop sequence of mtDNA of 4 green turtle museum specimens collected from Hainan Island and Leizhou Peninsula in mainland China and their

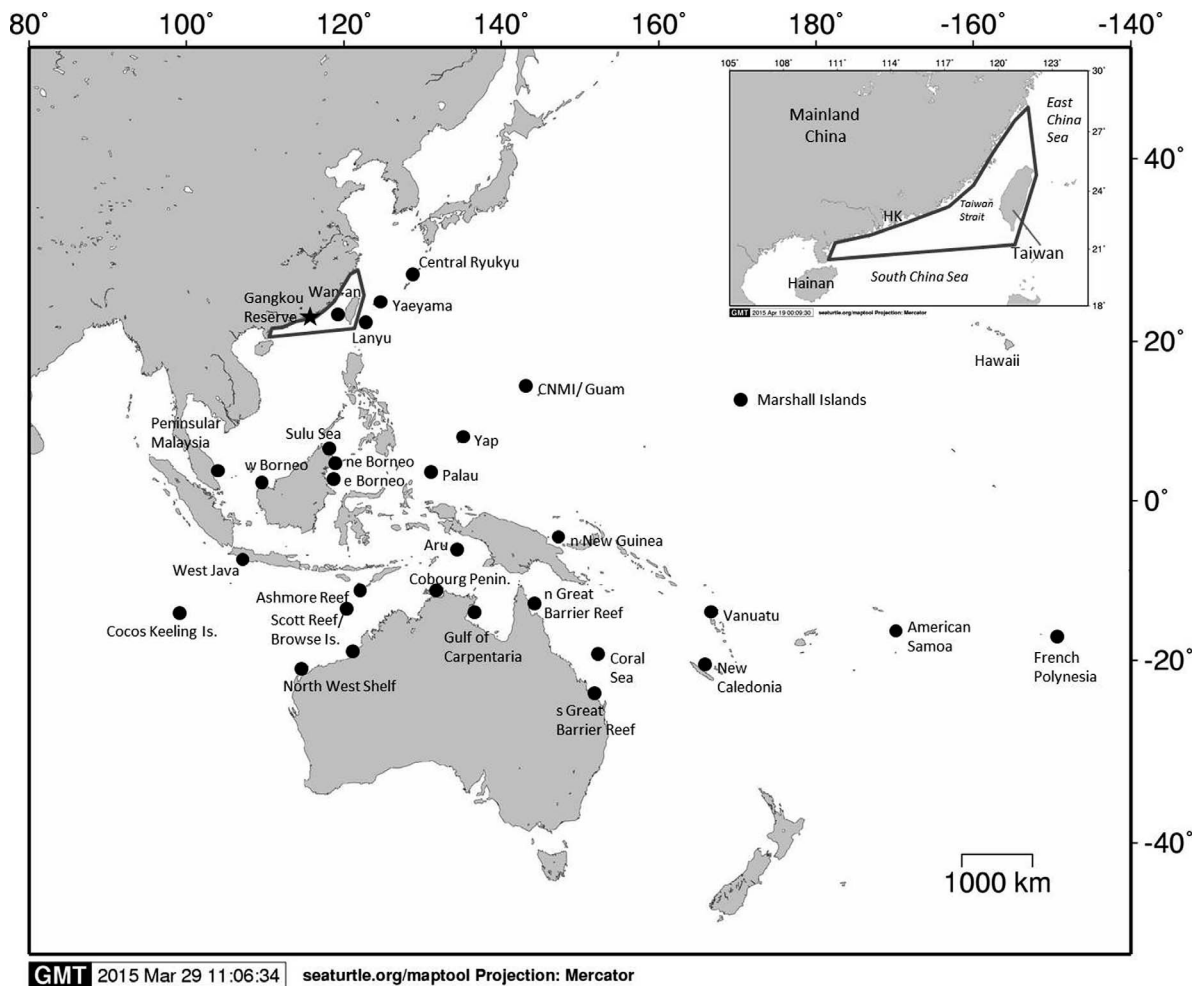


Figure 1. Locations of green turtle foraging aggregations sampled in this study (polygon in insert) and potential source rookeries (filled circles) in the Pacific (SEATURTLE.ORG Maptool 2002). Location of the rookery at Gangkou Sea Turtle National Nature Reserve is marked by a star.

inferred connectivity with rookeries in the Central Eastern Pacific, the Southwestern Pacific, and the Indian Ocean (Guo et al. 2009). Based on the analysis of the mtDNA control region of 88 small-sized juveniles sampled around Hainan Island, Yang et al. (2015) identified 8 haplotypes and found that green turtles of the South China Sea shared a close genetic relationship with green turtle populations in the Northwest Pacific (including Japan), the Southwest Pacific (including Malaysia), and the Indian Ocean. Yang et al. (2015) suggested that the South China Sea represented a transition area for green turtles migrating between the Pacific and Indian oceans. This kind of information is fundamental to define connectivity between foraging grounds in South China and potential source rookeries in the Pacific for more-effective, spatially based management of the species.

In view of the information gap on population connectivity of this globally endangered species in the South China Region, the present study aims to characterize the genetic composition of green turtles encountered as stranded individuals and as fisheries by-catch around Hong Kong, Guangdong Province of mainland China, and

Taiwan (Fig. 1) in terms of the mtDNA control region variation and to compare haplotype frequencies with those of rookeries to identify their source breeding populations.

METHODS

Sample Collection. — A total of 110 by-catch or stranded green turtles (curved carapace length [CCL] ranging from 13 to 105 cm, mean 60.2 ± 21.9 cm SD) found in Hong Kong, Guangdong Province, and Taiwan from 2001 to 2014 were sampled including 3 green turtles with CCL < 35 cm (i.e., when the Pacific pelagic juveniles switch to a neritic life-style according to Balazs [1980]). Blood, skin biopsy, or muscle tissue samples were collected from by-catch or stranded green turtles in Hong Kong, Guangdong, and Taiwan (Fig. 1). Skin biopsy and muscle tissue samples were preserved in 90% alcohol at 4°C (Dutton and Balazs 1995; Dutton 1996). Blood was also collected from 4 green turtle yearlings from a single nester in 2010 and 5 yearlings from another nester in 2011 at Gangkou Sea Turtle National Nature Reserve of Guangdong. These specimens were analysed for the 760-

bp mtDNA control region. Blood was preserved in Corning plastic vials (in EDTA or saline solution). Foraging grounds of green turtles in the South China Region are mainly distributed along the coasts of Hainan Island and Guangdong of mainland China as well as coasts of Taiwan and the Philippines, the East China Sea, and outlying islands in the South China Sea (Ng 2015).

Laboratory Analysis. — Genomic DNA was extracted from tissue samples using the Qiagen DNeasy Blood & Tissue Extraction Kit. An approximately 760-bp fragment of the mtDNA control region was amplified using the primers LCM15382 (5'-GCTTAACCCTAAAGCATTGG-3') and H950 (5'-GTCTCGGATTTAGGGTTG-3') (Abreu-Grobois et al. 2006), which encompass the 384-bp segment at site 251–635 previously amplified using TCR5 and TCR6GC primers (Norman et al. 1994, Dethmers et al. 2006). PCR conditions were set as follows: 95°C for 5 min, 34 cycles of 95°C for 30 sec, 56°C for 30 sec, 72°C for 1 min, and 72°C for 7 min.

Data Analysis. — Connectivity of foraging populations of green turtles in the South China Region with nesting populations in the Pacific was determined by estimating stock composition using Bayesian MSA using the program BAYES from Pella and Masuda (2000). BAYES was run using 2 models: 1) flat priors, where the rookeries are treated with equal weightings and the results are driven by the genetic information alone for all parameters; and 2) weighted priors, which take into account the relative size of each rookery on the assumption that when genetic information is similar for rookeries, the larger population is more likely to contribute to the foraging aggregation than would the smaller one. The use of weighted priors can be helpful when genetic diversity is weak, rookeries share common haplotypes that are widely distributed, and the relative size of each rookery differs greatly (Bolker et al. 2007; LaCasella et al. 2013).

The haplotype composition and frequency of all foraging green turtles sampled (as the stock mix) in the South China Region was compared with those documented for 30 MUs (as potential sources) across the Pacific (e.g., Taiwan, Australasia, and the Indo-Pacific region) (Fig. 1; Supplemental Table 1, available online at <http://dx.doi.org/10.2744/CCB-1253.1.s1>). The 760-bp mtDNA sequences from the specimens collected in this study were aligned and compared using BLAST and Clustal X (Thompson et al. 1997) with published rookery haplotypes (Dethmers et al. 2006; Cheng et al. 2008; Guo et al. 2009; Hamabata et al. 2009, 2014; Nishizawa et al. 2013; Dutton et al. 2014b; Read et al. 2015; Jensen et al. 2016), those available on GenBank, and unpublished data (Jensen et al., submitted for publication; P.H.D., unpubl. data, 2017). Because 760-bp haplotype data were not available for the Taiwan rookeries, we assumed that the shorter 384-bp published haplotypes for Wan-an and Lanyu reported in Cheng et al. (2008) represented unique equivalent 760-bp variants (e.g., CmP18 represents CmP18.1, CmP19 is CmP19.1, CmP20 is CmP20.1; see Supplemental Table 1)

in order to include the local rookeries in the MSA. As the rookery sizes of Hong Kong and Gangkou in Guangdong Province are small (annual estimates of nesting individuals range from 1 to 5 in Hong Kong and from 2 to 20 in Gangkou according to Chan et al. 2007 and Ng et al. 2014a, respectively), these 2 rookeries were not included as potential sources in the MSA. Orphan haplotypes (i.e., those observed only in foraging grounds and not in any of the nesting rookeries) were removed from the analyses. A total of 200,000 Markov Chain Monte Carlo steps were run for 4 independent chains, each with different starting points. A burn-in of 50,000 runs was used to calculate the posterior distribution. The Gelman and Rubin shrink factor diagnostic was calculated as part of the BAYES analysis to test for convergence of all chains (Pella and Masuda 2000; Jensen et al. 2016). Nomenclature of the haplotypes was named after the data set of CmP# maintained at the National Oceanic and Atmospheric Administration's (NOAA) Southwest Fisheries Science Center Web site (<https://swfsc.noaa.gov/textblock.aspx?Division=PRD&ParentMenuId=212&id=1331>).

RESULTS

Genetic Analysis

Nesting Green Turtles in Guangdong, China. — Four green turtle yearlings hatched from eggs laid by a single nesting green turtle in 2010 at the Gangkou Sea Turtle National Nature Reserve shared the same haplotype CmP19.1. All 5 yearlings from another nester in 2011 had the same haplotype CmP49.1.

By-catch or Stranded Green Turtles in Hong Kong, Guangdong, and Taiwan. — A total of 27 haplotypes in terms of 760 bp of the mtDNA control region were identified among the 110 samples sequenced (Supplemental Table 1). The most common haplotype found in this study was CmP20.1 (17% of the 27 haplotypes identified) followed by CmP19.1 (13%) and CmP50.1 (10%). One orphan haplotype (CmP154.1) was previously identified in juvenile green turtles at foraging areas in Malaysia (Jensen et al. 2016). Two new orphan haplotypes (i.e., CmP132.1 and CmP219.1) have not been described or found anywhere to date (GenBank Accession no. KU378111 and KU378112, respectively). CmP132.1 corresponded to a juvenile green turtle stranded in Hong Kong and CmP219.1 to a subadult green turtle accidentally caught in Guangdong. Excluding the 3 orphan haplotypes, 23 of the 27 haplotypes found in this study were detected within rookeries in Wan-an and Lanyu islands of Taiwan; central Ryukyu and Yaeyama of Japan; Peninsular Malaysia; Sulu Sea; western Java; Aru; Republic of Marshall Islands; Yap in the Federated States of Micronesia; Palau; Commonwealth of the Northern Mariana Islands/Guam; northern New Guinea; Ashmore Reef; Scott Reef/Browse Island (Scott/Browse); Gulf of Carpentaria (GoC); Vanuatu; Coral Sea/Chesterfields; western New Caledonia; eastern, northeastern, and western Borneo; North West Shelf;

Table 1. Mean estimated stock contribution (%) of each rookery: (a) flat priors and (b) weighted priors, with credible intervals (CI).

Source rookeries	Population size	(a) Flat priors			(b) Weighted priors		
		Mean	CI (quantile)		Mean	CI (quantile)	
Location			2.5%	97.5%		2.5%	97.5%
Peninsular Malaysia	350	23.2	2.8	39.4	37.0	26.4	47.7
Central Ryukyu, Japan	50	18.4	10.2	27.5	20.6	12.6	29.6
Yap, Federated States of Micronesia	750	16.2	4.8	29.4	18.4	0.0	33.3
Aru, Indonesia	1000	2.0	0.0	9.8	0.2	0.0	3.6
Sulu Sea	13,900	11.0	5.3	18.0	11.6	6.0	18.6
Northeast Borneo	300	0.3	0.0	3.2	0.0	0.0	0.0
Republic of Marshall Islands	350	9.2	0.0	23.9	9.5	0.0	28.1
Wan-an, Taiwan	20	5.6	0.0	26.2	0.1	0.0	0.0
South Yaeyama, Japan	50	5.4	0.0	26.8	0.8	0.0	10.0
North Yaeyama, Japan	50	3.2	0.0	12.8	0.1	0.0	0.0
American Samoa	70	1.2	0.0	16.1	0.0	0.0	0.0
West Borneo	300	1.0	0.0	6.7	0.0	0.0	0.0
East Borneo	7100	1.0	0.0	10.3	0.0	0.0	0.0
Vanuatu	200	0.6	0.0	6.9	0.0	0.0	0.0
Northern Great Barrier Reef	24,300	0.4	0.0	2.7	0.9	0.0	3.5
Commonwealth of the Northern Mariana Islands/Guam	20	0.3	0.0	4.0	0.0	0.0	0.0
Long Island, Papua New Guinea	800	0.3	0.0	3.3	0.0	0.0	0.0
Palau, Micronesia	300	0.1	0.0	1.7	0.0	0.0	0.0
West Java, Indonesia	300	0.1	0.0	1.6	0.0	0.0	0.0
Ashmore Reef, Australia	600	0.1	0.0	1.2	0.0	0.0	0.0
Lanyu, Taiwan	20	0.1	0.0	1.0	0.0	0.0	0.0
Cocos Keeling, Indian Ocean	300	0.1	0.0	1.0	0.0	0.0	0.0
Scott Reef, Australia	300	0.1	0.0	0.6	0.0	0.0	0.0
Gulf of Carpentaria	6600	0.1	0.0	0.5	0.1	0.0	0.5
Cobourg Peninsula, Australia	200	0.0	0.0	0.5	0.0	0.0	0.0
North West Cape, Australia	125,300	0.0	0.0	0.4	0.6	0.0	2.9
Coral Sea/Chesterfields,	2800	0.0	0.0	0.4	0.0	0.0	0.1
Southern Great Barrier Reef	6600	0.0	0.0	0.4	0.0	0.0	0.4
New Caledonia	2000	0.0	0.0	0.4	0.0	0.0	0.0
French Polynesia	350	0.0	0.0	0.4	0.0	0.0	0.0

Cocos “Keeling” Island; and Cobourg Peninsula (Fig. 1; Supplemental Table 1).

One green turtle with CCL of 24 cm (i.e., the Pacific pelagic juveniles according to Balazs [1980]) found stranded in Wanshan Archipelago of mainland China and 2 other individuals (CCL, 32 and 33 cm) in Hong Kong eastern waters shared the CmP19.1 haplotype. Of the 2 turtles found in Hong Kong, 1 individual with a metal plate engraved with Chinese characters attached to its carapace was suspected to have been released from Shanwei in mainland China for religious reasons. All 3 of these individuals were found alive and collected from May to October.

Statistical Analysis

The MSA using both flat priors and weighted priors revealed that rookeries in Peninsular Malaysia (Paka and Pulau Redang in Terengganu) contributed the most to the foraging aggregations in South China Region, with estimated means of 23.2% (flat) and 37.0% (weighted) (Table 1). Central Ryukyu of Japan accounted for the second-most contribution to the foraging aggregations with estimated means of 18.4% (flat) and 20.6% (weighted). Yap in the Federated States of Micronesia, Aru of Indonesia, Sulu Sea, northeastern Borneo, Republic of Marshall Islands, Wan-an of Taiwan, and Yaeyama of

Japan also contributed to the foraging aggregations, with mean estimates ranging from 3.2% to 16.2% (flat) and from 0.1% to 18.4% (weighted) (Table 1). Large credible intervals (CI) (i.e., the lower bound of the CI was close to 0.0%) were observed around the means of contributions from rookeries in Wan-an Island of Taiwan, Yaeyama of Japan, Aru, northeastern Borneo, and Republic of Marshall Islands (Table 1).

DISCUSSION

Genetic Stock Composition of Green Turtle Rookeries in South China. — The haplotypes found in rookeries in Hong Kong and Gangkou in Guangdong of mainland China are widely distributed in the Pacific region. In this study, the 2 haplotypes (CmP 19.1 and 49.1) detected in the yearlings from 2 nests sampled at Gangkou were also reported in rookeries in Wan-an Island (CmP 19), Lanyu Island in Taiwan, Australasia, and the Indo-Pacific (CmP 49) (Norman et al. 1994; Dethmers et al. 2006; Cheng et al. 2008). The 27 green turtle rookeries tested by Dethmers et al. (2006) in “Australasia and Indo-Pacific” refer to those in the Southwest Pacific Ocean, Northwest Pacific Ocean, South China Sea, Sulu Sea, Celebes Sea, Timor Sea, and East Indian Ocean. Ng et al. (2014a) reported that the 2 haplotypes found in nesting green turtles in Hong Kong (CmP18 [$n = 5$] and CmP116 [$n = 1$]) are closely

related to each other along with the other Taiwan haplotypes (CmP49 and CmP19). CmP116 is a novel haplotype which differs from CmP18 by 2 substitutions.

In general, the genetic composition of rookeries in terms of haplotype identified from mtDNA control region at Hong Kong and Gangkou is similar to that of other nesting populations in the Pacific Region except that significant differentiation in haplotype frequency of rookeries between Hong Kong and Lanyu Island was detected (Ng et al. 2014a). Moreover, the presence of the novel CmP116 haplotype implies that there is regional uniqueness in genetic diversity in the Hong Kong rookery (Ng et al. 2014a). It should be noted, however, that this nesting population in Hong Kong is extremely small (< 10 turtles), and the genetic study was based on a total of 6 samples (Ng et al. 2014a), whereas only 2 individuals are represented in the genetic analysis from Gangkou. These 2 nesting areas should perhaps be characterized as having sporadic nesting as opposed to significant nesting populations and are unlikely to provide a detectable source for the foraging turtles sampled in this study.

Connectivity between Rookeries in the Pacific and Foraging Aggregations in South China. — The results of the MSA using both flat and weighted priors in this study revealed a similar pattern. The major rookeries that contributed to foraging aggregations in the South China Region were Peninsular Malaysia, Yap in the Federated States of Micronesia, Aru of Indonesia, Sulu Sea, northeastern Borneo, Republic of Marshall Islands, Wan-an of Taiwan, and central Ryukyu and Yaeyama of Japan (Table 1; Fig. 1). The most-common haplotypes found in by-catch or stranded green turtles in South China Region in this study, CmP20.1, CmP19.1, and CmP50.1, were also observed in the rookeries in the Pacific. CmP20.1 was predominant and widespread in rookeries in Micronesia (Dutton et al. 2014b), and CmP20 was also reported in rookeries at Wan-an Island (Cheng et al. 2008) and the Yaeyama Group (Nishizawa et al. 2011). CmP19 was observed in 1 nesting green turtle from Wan-an Island (Cheng et al. 2008). The 860-bp haplotype CmP50.1 was the most-dominant haplotype in the green turtle rookeries in central Ryukyu (Hamabata et al. 2014) and was also common in rookeries in the Yaeyama and Ogasawara Groups (Nishizawa et al. 2011, 2013) but was not detected in Taiwan (Cheng et al. 2008). The MSA results indicate that the contributions of Yap (Micronesia) are greater than those of the Sulu Sea population, whereas the size of the Sulu Sea population is about 10 times as large as that of Micronesia. These results suggest that rookery size is not always the most important factor determining contributions to foraging aggregations in the South China Region. Moreover, although nesting populations along the Australian coast and in the East Indian Ocean are included as potential sources in the MSA, some of them, such as the Ashmore Reef of Australia and Cocos Keeling of Indian Ocean, show little contribution to foraging grounds in the South China Region (Table 1). As revealed in other studies

in the Pacific (Amorocho et al. 2012; Nishizawa et al. 2013; Naro-Maciel et al. 2014), the dispersal of green turtle hatchlings from Malaysia and the Philippines to the South China Region is also likely owing to the seasonal oceanic current that flows from the southern to northern South China Sea (Hu et al. 2000). Green turtle hatchlings from Yap and the far southern Japanese archipelagos may be transported to the South China Region by the North Pacific Gyre that flows cyclically from the Central Pacific to West Pacific (Bowen et al. 1995). Sea turtle nesting and foraging grounds are protected in the Turtle Islands Heritage Protected Area managed by Malaysia and the Philippines and in several National Marine Parks in the Ryukyu and Ogasawara Groups in Japan. The green turtle nesting beaches on Wan-an Island and at the Gangkou Reserve are also protected (IUCN and UNEP-WCMC 2010). Nesting populations in some of the source rookeries appear to be stable or increasing in recent years; a record high number of 14,220 green turtle nests was reported in the Turtle Islands (Sulu Sea) in 2011 according to IUCN (2012). Numbers of nesting green turtles were consistent on Okinawa of the Ryukyu Islands (Abe et al. 2003) and appeared to be increasing in the Yaeyama group (Tanaka 2009). Stable rookery stocks were also reported for Terengganu, Malaysia (Chaloupka et al. 2007). Nevertheless, any decline in foraging aggregations in the South China Region could potentially cause adverse and irreversible impacts to source rookeries in Malaysia, Micronesia, Japan, and Taiwan.

The genetic connectivity between source rookeries and foraging grounds identified in this study is further supported by various satellite telemetry and conventional flipper tagging studies. Nesting green turtles from Terengganu of Peninsular Malaysia travelled in the South China Sea to their foraging grounds in Vietnam (Abdullah 2007; van de Merwe et al. 2009). A nesting green turtle from Sabah, Malaysia, with metal tags was taken as incidental catch offshore of Nanao Island, Shantou, Guangdong Province (Ng 2015). Nesting green turtles from Gielop Island of Yap in the Federated States of Micronesia found their foraging grounds in the Pacific, including the Marshall Islands and Papua New Guinea, and in Southeast Asia including the Philippines (Kolinski 1995). Some also moved through the South China Sea to forage in the Philippines, Malaysia, and through Taiwan coastal waters to the Ryukyu Archipelago of Japan (Kolinski et al. 2014). Nesting green turtles from Wan-an Island migrated to their foraging pastures in coastal waters off Taiwan, Qinpeng-Dao of Shantou, and along the Guangdong coastline to Dangan Liedo and Hainan Island (Cheng 2000). Tagged nesting green turtles in Ogasawara, Japan, travelled to the main island of Japan and Ryukyu Archipelago as well as to Taiwan (Tachikawa and Sasaki 1990). Interestingly, previous tracking studies on nesting green turtles also revealed their postnesting movement from the rookery in the Gangkou Reserve (Song et al. 2002) and in Liuciou Island of Taiwan (Ng

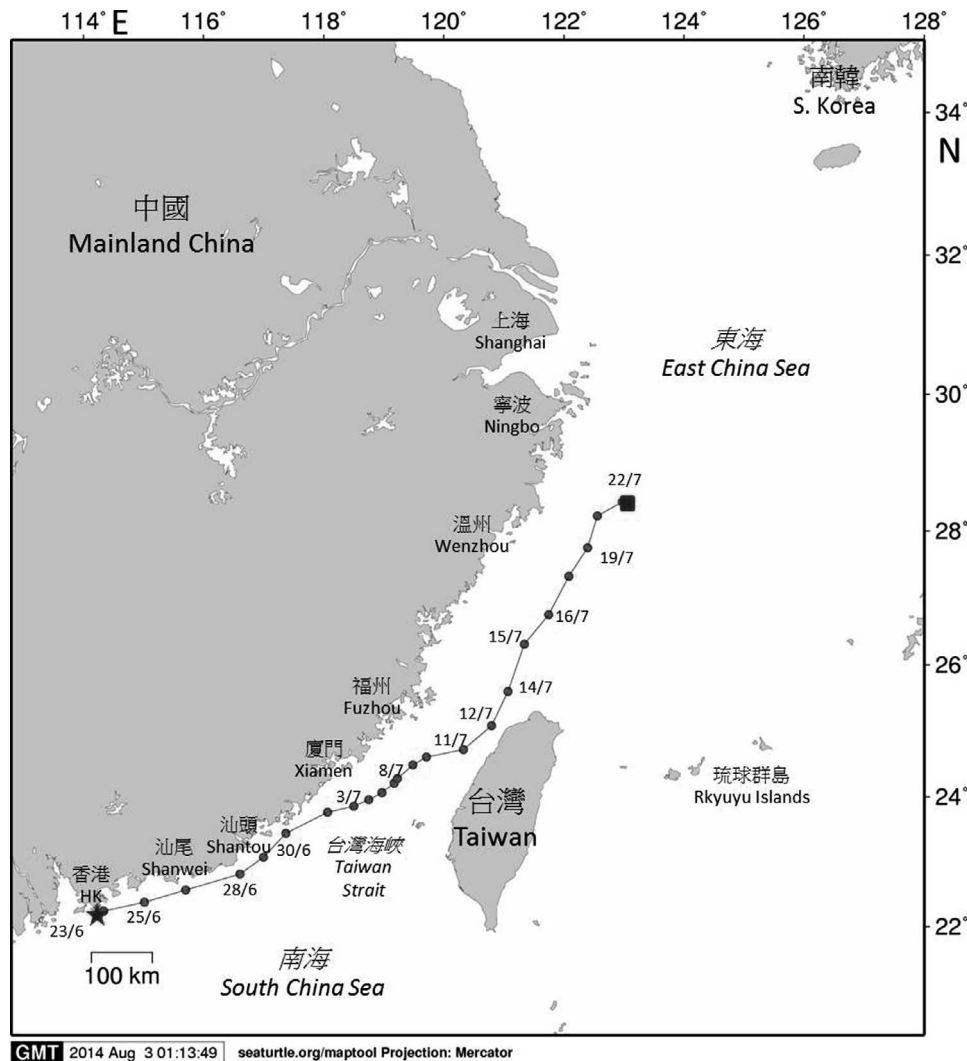


Figure 2. Movement of a green turtle (CCL = 35 cm) released from Hong Kong by satellite telemetry in 2014 (star icon denotes starting point, square denotes end point of the track) extracted from Ng (2015). This pelagic-phase green turtle had the haplotype CmP19.1, which was only observed in the rookeries at Wan-an Island (Cheng et al. 2008) and Gangkou Reserve in this study.

2015) to foraging grounds in Okinawa in the Ryukyu Archipelago in Japan.

The large credible intervals around the mean values for the Wan-an Island of Taiwan, Yaeyama of Japan, Aru, northeastern Borneo, and Republic of Marshall Islands imply relatively uncertain MSA results regarding these rookeries. This is typical when there are widespread, shared, common haplotypes that reduce analytical power, the presence of many potential source rookeries (LaCasella et al. 2013), relatively small sample sizes of both nesting and foraging populations, the presence of novel or orphan haplotypes (Dethmers et al. 2010), and incomplete sampling of potential nesting populations (Anderson et al. 2013; Shamblin et al. 2017). It should also be noted that the shorter sequence haplotypes for Taiwan incorporated into the MSA may also contribute to this uncertainty. However, the previous tracking and tagging studies as discussed above revealed that nesting green turtles from Japan and Taiwan moved to their foraging grounds in the South China Region.

Occurrence and Natal Origin of Pelagic-Phase Green Turtles in South China. — One of the turtles sampled in this study, a pelagic-phase green turtle (fig. 2 from another study conducted by Ng 2015) was satellite-tracked from the Wanshan Archipelago through Hong Kong eastern waters and the Taiwan Strait to Wenzhou in the East China Sea. Together with the other 2 pelagic-phase green turtles, these 3 individuals shared the same haplotype CmP19.1, which was only observed in the rookeries at Wan-an Island (Cheng et al. 2008) and Gangkou Reserve in this study. The occurrence of pelagic-phase live green turtles, coupled with the genetic composition and satellite tracking information, suggests the potential use of waters along Guangdong, the Taiwan Strait, and the East China Sea as habitat by pelagic-phase green turtles hatched from Taiwan and mainland China. Ng et al. (2014b) also reported the potential habitat use by pelagic-phase green turtles of waters associated with *Sargassum* spp. drifting mats off Keelung of northern Taiwan. The East China Sea, which covers coastal and pelagic waters next to Taiwan,

mainland China, and Japan, has also been identified as a hotspot for pelagic-feeding loggerhead turtles based on satellite tracking and oceanography features (Kobayashi et al. 2011). Based on the collective findings of these previous studies and the present study, the East China Sea is likely to be important developmental habitat for pelagic-phase green turtles as well as for other sea turtle species.

Conclusions

This study is the first of its kind to investigate and report the source nesting populations of a relatively large number of foraging green turtles in the South China Region. The results of this study broadly show that major source nesting populations contributing to the foraging aggregations in South China Region were from Peninsular Malaysia, Yap in the Federated States of Micronesia, Aru of Indonesia, Sulu Sea, northeastern Borneo, Republic of Marshall Islands, Wan-an of Taiwan, and central Ryukyu and Yaeyama of Japan. More targeted research should be conducted to identify and genetically sample additional rookeries such as the Pratas (Dongsha) Islands (Cheng 1995) and Paracel (Xisha) Island (Anonymous 1975; Wang and Li 2008) and to assess their genetic composition at higher resolution using additional markers such as microsatellites and SNPs that may detect fine-scale population differences to refine genetic stock structure (Shamblin et al. 2012; Dutton et al. 2013; Roden et al. 2013). Nesting populations in the northwestern Pacific, comprising Taiwan, the Ogasawara and Yaeyama Groups, and central Ryukyu in Japan, are characterized by high genetic diversity and phylogenetic endemism (Cheng et al. 2008; Nishizawa et al. 2011, 2013; Hamabata et al. 2014). Green turtle populations in some parts of the South China Region appear to be stable or increasing in recent years. Taking a precautionary approach, effective conservation and management of each distinct green turtle source rookery, as well as foraging aggregations sourced from multiple natal origins in the South China Region, are needed to preserve this genetic diversity. Anthropogenic threats at both nesting and foraging habitats and migratory pathways, such as direct take for trade and fisheries impacts, should be thoroughly assessed and properly mitigated as needed via regional collaboration to sustain these populations.

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