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Characterization and Conservation Concerns of Green Turtles (*Chelonia mydas*) Nesting in Hong Kong, China¹

Connie Ka-yan Ng,^{2,5} Peter H. Dutton,³ Simon Kin-fung Chan,² Ka-sbing Cheung,² Jian-wen Qiu,⁴ and Ya-nan Sun⁴

Abstract: Hong Kong has one of the last remaining nesting populations of endangered green turtles (*Chelonia mydas*) in southern China. Because nesting individuals are vital to sustain populations, this study characterizes and reports essential baseline information about nesting pattern, postnesting movement, and genetic composition of green turtles nesting in Hong Kong to provide a basis for effective scientific-based management of this migratory species. The number of nesters observed in Hong Kong was relatively low compared with other rookeries in southern China, but the nesting pattern in terms of clutch size and internesting interval was comparable with that of other nearby rookeries. These nesters are likely a remnant of a small population previously depleted as a result of historical harvesting of eggs in Hong Kong. Based on available DNA sequences and literature, we identified two mtDNA haplotypes, CmP18 (which is also common in the rookery in Taiwan) and a novel endemic haplotype (CmP116). We found significant differentiation based on haplotype frequencies between populations in Hong Kong and Lanyu, Taiwan, indicating that these nesting populations are demographically isolated. Loss of these populations would therefore result in loss of genetic diversity for this species in the region. Satellite tracking of the local nesters revealed postnesting movement to foraging habitats in Vietnam and Hainan Island. International cooperation and consistent dedicated research are of paramount importance to conservation and recovery of green turtle assemblages in the region.

SEA TURTLES ARE globally endangered species (International Union for the Conservation of Nature and Natural Resources 2011) and face anthropogenic threats of all kinds, including incidental capture in coastal arti-

sanal and high seas industrial-scale fisheries (Cheng and Chen 1997, Alfaro-Shigueto et al. 2007, 2011, Wallace et al. 2010), pollution and marine debris (Lam et al. 2006, Wabnitz and Nichols 2010), and habitat degradation (Mazaris et al. 2009). Due to their endangered status, sea turtles are listed in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and protected by law in China and Hong Kong.

Hong Kong is located in southern China, where five species of sea turtles, including the green turtle (*Chelonia mydas*), leatherback (*Dermochelys coriacea*), olive ridley (*Lepidochelys olivacea*), loggerhead (*Caretta caretta*), and hawksbill (*Eretmochelys imbricata*), can be found (Frazier et al. 1988, Wang 1993, Chan et al. 2007). The green turtle is the most common of the five species recorded in Hong Kong, and the only species that nests there, representing one of the last of the dwindling

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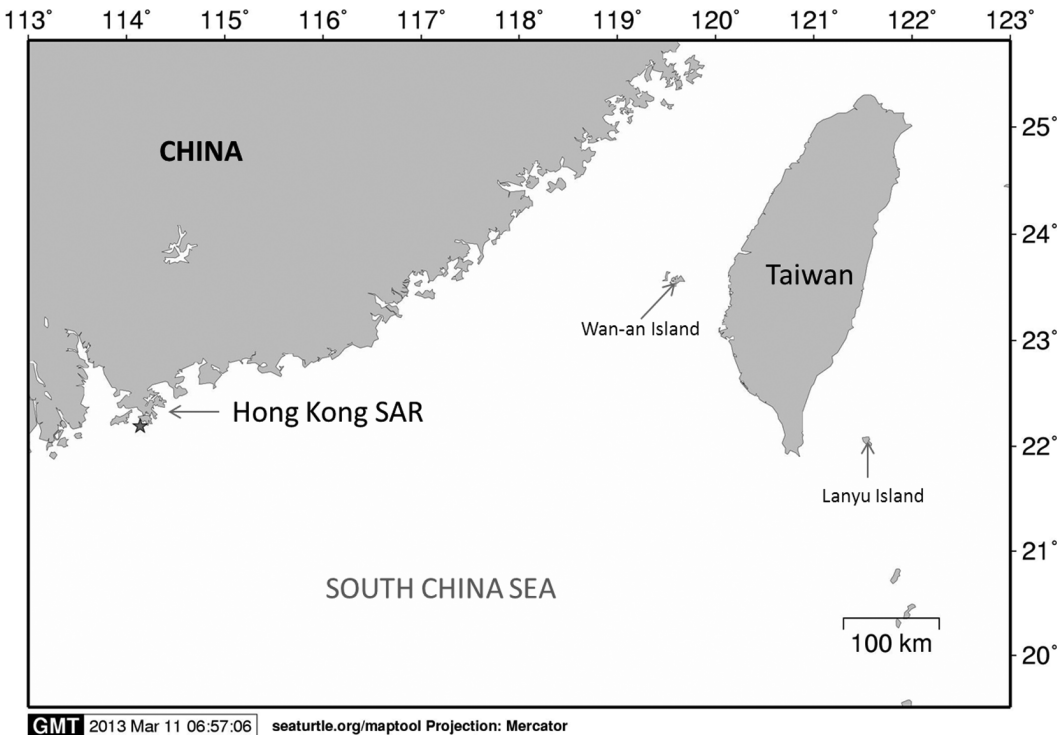


FIGURE 1. Hong Kong Special Administrative Region is located in southern China. Star icon denotes location of green turtle nesting site: Sham Wan in the southeastern part of Hong Kong. Also shown are the two nearest primary nesting sites with genetics information available in the South China Sea. (SEATURTLE.ORG Maptool, 2002, SEATURTLE.ORG, Inc. <http://www.seaturtle.org/maptool/> October 2012.)

nesting population of green turtles in the southern China region (Chan et al. 2007, Ng et al. 2011). Some decades ago, green turtle nesting was observed at many remote beaches and islands in Hong Kong (Chan 2004), but now the only known remaining nesting site is a sandy beach of 0.5 ha called Sham Wan on Lamma Island (22.191° N, 114.139° E) in southeastern Hong Kong (Figure 1). Observed nesting was reported to have been 20–50 nests each season several decades ago, but nesting has substantially decreased since then (McGilvray and Geermans 1997). Sporadic nesting of green turtles has also been recorded at other beaches in the territory in the last two decades (Figure 2). In Hong Kong, an array of management measures has been implemented for the conservation of sea turtles, including protection and management of the nesting beach, enforcement, and scien-

tific research (Chan 2004, Agriculture, Fisheries and Conservation Department [AFCD] 2011, Ng and Wan 2011).

Green turtles are migratory species of circumtropical distribution with adults traveling hundreds to thousands of kilometers between nesting beaches and foraging grounds (Hirth 1997). Characterization of regional stock structure and connectivity of breeding and foraging populations is thus important for spatial management and conservation (Wallace et al. 2011). Genetic analysis of mitochondrial (mt) DNA sequences (FitzSimmons et al. 1999, Cheng et al. 2008, Dutton et al. 2008, Jensen et al. 2013) and satellite telemetry (Song et al. 2002, Chan et al. 2003) have been useful research tools in defining stock structure and connectivity among populations to lay out effective conservation measures.

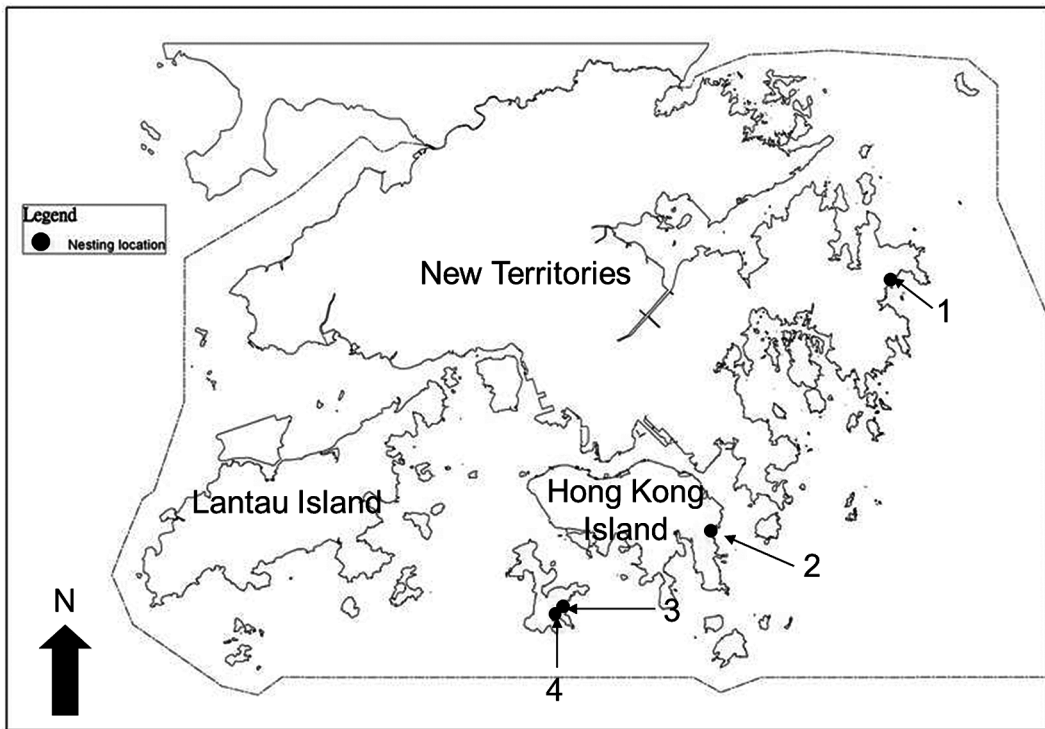


FIGURE 2. Overview of nesting locations of green turtles recorded in Hong Kong: 1, Tai Wan in Sai Kung in the New Territories; 2, Tai Long Wan in Shek O on Hong Kong Island; 3, Tung O on Lamma Island; 4, Sham Wan on Lamma Island.

Chan *et al.* (2007) and the action plan formulated at the Sea Turtle Conservation and Integrated Marine Management International Workshop (Conservation International 2011) stressed the need for comprehensive scientific research to develop effective actions for conserving sea turtles in China. Current research efforts in southern China focus on captive-rearing and husbandry techniques (Hu 1996, Zhu 2002, 2005, Wang and Shao 2005, Zhang and Gu 2005, Chen *et al.* 2006, Gu *et al.* 2006, Chen *et al.* 2007, Gu *et al.* 2010), as well as hematology and blood chemistry (Li *et al.* 2008, Zhang *et al.* 2009, Gu *et al.* 2011, Zhang *et al.* 2011). There is, however, a paucity of information on free-ranging sea turtles in the area, apart from some telemetry information on postnesting green turtles (Song *et al.* 2002, Chan *et al.* 2003). Because adult nesters are vital to sustain populations, the primary objective of this study is to characterize and

report essential baseline components of the biology and ecology of nesting green turtles, including nesting patterns, postnesting movement, and genetic composition in Hong Kong, which ultimately helps in devising scientific-based conservation actions for effective management of the species in the wild.

MATERIALS AND METHODS

Nesting Patterns

Since 1998, the Agriculture, Fisheries and Conservation Department (AFCD) has been actively monitoring the status of nesting green turtles in Hong Kong. Both daytime and overnight beach patrols were conducted at Sham Wan to monitor and count any nesting green turtles and their nests. Nesting green turtles were constrained in a cushioned wooden pen after nesting and measured for

curved carapace length (CCL). The number of nesters and nests and clutch sizes were counted after nesting using methods described in Schroeder and Murphy (1999). Local villagers were also interviewed to collect historical information on the nesting activity of green turtles at Sham Wan.

Postnesting Movement

After a general field assessment to ensure that each nesting green turtle was physically fit, tags (Inconel) were placed on its fore- and/or hind flippers for identification before release (Balazs 1999). A satellite-linked transmitter (Telonics Inc., model ST-14, A-2400, TAM-2619, TAM-2639, A-2010B, or TGM-4510) was attached to the carapace of the nester with fiberglass resin protocols described by Balazs et al. (1996). The weight of the transmitter package was less than 5% of the body weight of the nester to minimize potential impact to the turtles (Watson and Granger 1998). The transmitter was given a duty cycle of 24 hr on and 12 hr off for the nester in 2002 (Chan et al. 2003) and set to transmit continuously for the same nester in 2003 and 2008. Dual transmitters programmed at 8 hr on and 24 hr off and 8 hr on and 14 hr off, respectively, were deployed on the nester in 2012, which also nested in 2003 and 2008. Each turtle was released with the attached transmitter immediately after deployment at the nesting site. Tracks were plotted using Maptool (SEATURTLE.ORG, Inc. <http://www.seaturtle.org/maptool/>) with mostly positional data derived from LC1 to 3 signals, but large spatial gaps were filled using data points of

LC 0, A and B, where appropriate following visual filtering for obvious inaccurate points (Chan et al. 2003).

Genetic Analysis

Skin biopsy and tissue samples were collected from green turtle nesters and salvaged from dead hatchlings from two sites, Sham Wan and Tai Wan, from 1998 to 2008 and preserved in 90% ethanol (Table 1) (Dutton and Balazs 1995, Dutton 1996). Genomic DNA was extracted from these six tissue samples from different nesters/hatchlings using the Qiagen DNeasy Blood and Tissue Extraction Kit. An approximately 850 base pair (bp) fragment of the mtDNA control region was amplified using the primers LCM15382 (5'-GCTTAACCCTAAAGCATTGG-3') and H950 (5'-GTCTCGGATTTAGGGGTTTG-3') (Abreu-Grobois et al. 2006), which encompass the 384 bp segment at site 251–635 amplified using TCR5 and TCR6GC primers (Norman et al. 1994, Dethmers et al. 2006). Polymerase chain reaction (PCR) conditions were 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; 72°C for 7 min. Samples with sufficient PCR product (CM-HK-3 and CM-HK-4) were sent to Beijing Genomics Institute (BGI) for sequencing. Samples with insufficient DNA product (CM-HK-1, CM-HK-2, CM-HK-5, and CM-HK-6) were cloned directly into the pMD 18-T Vector (Takara, Dalian, China) to target segments before sequencing. Sequencing reactions were performed using an AB SOLiDTM 4.0 automatic sequencer. At least two clones of these samples were

TABLE 1
Specimens of Green Turtles (*Chelonia mydas*) Collected for Genetic Analysis

ID	Collection Date	Life Stage	Collection Location
CM-HK-1	1998	Hatchling	Sham Wan, Lamma Island
CM-HK-2	1998	Hatchling	Sham Wan, Lamma Island
CM-HK-3	1998	Hatchling	Sham Wan, Lamma Island
CM-HK-4	5 September 2008	Adult nesting female	Sham Wan, Lamma Island
CM-HK-5	4 October 2006	Hatchling	Tai Wan, Sai Kung
CM-HK-6	4 October 2006	Hatchling	Tai Wan, Sai Kung

sequenced to verify the results. For each sample, both strands of the PCR product were sequenced.

To determine the relationship between haplotypes found in Hong Kong with those of green turtle rookeries in other nearby regions (e.g., Taiwan, Australasia, and the Indo-Pacific), the 384 bp mtDNA segments were compared with those in GenBank using Basic Local Alignment Search Tool (BLAST) and against a reference data set of standardized mtDNA haplotype nomenclature maintained on the National Oceanic and Atmospheric Administration's Southwest Fisheries Science Center website (Southwest Fisheries Science Center 2011) using ClustalX (Thompson et al. 1997). Phylogenetic analyses were performed with Neighbor-joining (NJ) procedures as follows, using a published loggerhead turtle (*Caretta caretta*) CR sequence as an outgroup. NJ analysis was performed with the HKY85 model (Hasegawa-Kishino-Yano, 85). Support for NJ nodes was assessed by bootstrapping using 1,000 replicates.

We identified green turtle nesting populations in the published literature containing haplotypes found in Hong Kong and tested for differentiation using pairwise F_{ST} comparisons, and pairwise exact tests of population differentiation with Arlequin v 3.5.1.2 (Excoffier and Lischer 2010). Exact tests of popula-

tion differentiation were conducted with 100,000 permutations and 10,000 dememorization steps (Raymond and Rousset 1995).

RESULTS

Nesting Patterns

Green turtle nesting in Hong Kong was observed and reported to occur from June to October. In the 1970s, 30–40 green turtle nests were observed each season at Sham Wan by the local villagers, implying that four to 13 green turtles nested at Sham Wan if each green turtle deposited three to seven clutches in a season. Between 1998 and 2012, up to five green turtle nesters, ranging from 86 cm to 109 cm CCL, were observed each nesting season at Sham Wan (Table 2). An average number of 93 to 152 eggs were laid per clutch at a mean internesting interval of 11 to 13 days in a nesting season, producing over 2,700 eggs since 1998 (Table 2). One of the females was observed nesting in 2003, 2008, and 2012, indicating a remigration interval of 4 and 5 yr for this nesting turtle.

Postnesting Movement

The female green turtle tagged in 2002 was tracked from Hong Kong for about 20 days to

TABLE 2
Nesting Pattern of Green Turtles in Sham Wan, Lamma Island, Hong Kong

Year	No. of Nesters Observed	No. of Clutches Observed ^a	No. of Clutches Uncovered ^b	Total No. of Eggs ^b	Clutch Size		Interesting Interval (days)	
					Mean ^c	SD	Mean	SD
1998	5	12	5	561	112	25	—	—
2000	1	3	1	129	129	0	—	—
2001	1	5	1	152	152	0	—	—
2002	1	5	4	373	93	22	11.0	3.6
2003	2	9	4	401	100	13	11.0	0.0
2008	1	7	4	544	136	29	13.0	1.8
2012	1	5	4	548	137	29	12.0	1.8

Note: No nesting was observed in the years 1999, 2004–2007, and 2009–2011.

^a Number of clutches observed may be underestimated due to bad weather or failure to locate the nest.

^b Number of clutches uncovered indicates that the eggs of these clutches were counted and recorded.

^c Mean number of eggs per clutch was determined by dividing the total number of eggs counted in all clutches by the number of clutches uncovered.

its foraging ground in coastal waters off Wanning City of Hainan Island of China, which is about 500 km from Hong Kong (Chan et al. 2003). Another female green turtle migrated to its foraging ground in Dao Bach Long Vi, a marine protected area in Vietnam, approximately 700 km from Hong Kong, within a month after nesting at Sham Wan in both 2003 and 2008 (Figure 3a). The green turtle that nested in both 2003 and 2008 was tracked again after nesting in Hong Kong in 2012. The turtle, on its migratory pathway back to the foraging ground in Dao Bach Long Vi in Vietnam, was reported entangled in a fishing net and found dead when fishermen recovered the net in early October 2012. The turtle was subsequently buried with Buddhist prayers in a sandy beach at Xuwen of Guangdong Province, China (Figure 3b).

Genetic Analysis

Two haplotypes were identified from the 384 bp portions of the consensus sequences compiled for the six individual turtles sampled. The sequence of CM-HK-5 (i.e., a green turtle hatchling from Tai Wan of Sai Kung in 2006) was novel, differing from that of CmP18 by two substitutions, and was assigned the haplotype name CmP116. Sequences of the other five specimens that corresponded to haplotype CmP18 (Cheng et al. 2008) included hatchlings and a nesting green turtle from Sham Wan of Lamma Island in the years 1998, 2000, and 2008, and a green turtle hatchling from Tai Wan of Sai Kung in Hong Kong East in 2006. Comparisons with published sequences revealed that CmP18, the common Hong Kong haplotype, has been observed in Taiwan only at the Wan-An nesting site (Cheng et al. 2008) (Figure 4). The two haplotypes found in Hong Kong (CmP18 and CmP116) are closely related to each other along with the other Taiwan haplotypes (CmP49 and CmP19 [Figure 4]) and cluster together within a broader regional clade (Figure 4). Pairwise F_{ST} and exact tests of haplotype frequencies between Hong Kong and each of the Taiwan rookeries showed significant differentiation between Hong Kong and

Lanyu ($F_{ST} = 0.90$, $P < .001$) but not between Hong Kong and Wan-An ($P > .05$).

DISCUSSION

Our results confirm that the Hong Kong nesting population of green turtles is small, and our estimates of four to 13 females per season in the 1970s are consistent with those estimated by McGilvray and Geermans (1997) of 20–50 nests (i.e., three to 17 nesters) each season in the past several decades. This number of nesting green turtles observed in Hong Kong (i.e., zero to five nesters per nesting season) was only slightly lower than numbers in other regions in the South China Sea (Table 3: e.g., one to 20 in Huidong Gangkou National Sea Turtle Nature Reserve; two to 19 on Wan-an Island of Penghu Archipelago; three to 11 on Lanyu Island), but the orders of magnitude were much lower when compared with the high number of nestings observed in the Turtle Islands in the Philippines (International Union for the Conservation of Nature and Natural Resources 2012 [e.g., a record high of 14,220 in 2011]). The clutch sizes and internesting interval of nesters in Hong Kong were comparable with those in other areas in the South China Sea (Table 3). There is no information available on the abundance of nesting green turtles in Hong Kong before the 1970s, but if they were once abundant, our surveys show that the nesting population was depleted by the 1990s or was never very large. Historical extensive harvesting of sea turtle eggs over the last several decades was partly the cause of this decline, and recently in-water impacts such as bycatch (Alfaro-Shigueto et al. 2011) pose considerable threats to the recovery and long-term viability of the species.

Regarding connectivity between rookery and foraging grounds, results of our satellite tracking show that Hong Kong nesters migrated to foraging areas in coastal waters of Wanning City on Hainan Island in China and Dao Bach Long Vi Island in Vietnam, identifying these areas and the associated migratory corridors as important habitat for survival and of conservation concern, which was supported by the reported death of the nesting green

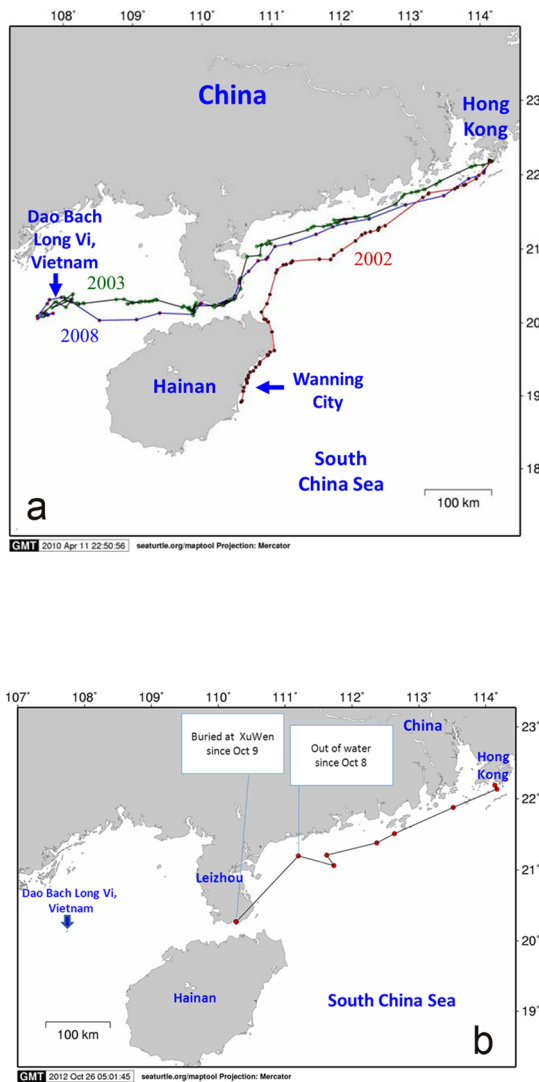


FIGURE 3. *a*, Three postnesting tracks of two green turtles from Sham Wan in Hong Kong in 2002, 2003, and 2008; *b*, postnesting track in 2012 of the green turtle that also nested in 2003 and 2008. (SEATURTLE.ORG Maptool, 2002, SEATURTLE.ORG, Inc. <http://www.seaturtle.org/maptool/> October 2012.)

turtle in 2012 due to interaction with fishing gear.

Despite the small sample size, our genetic results indicate that Hong Kong green turtle nesters are demographically distinct based on the presence of an endemic mtDNA haplotype and significant differentiation with Lanyu on Taiwan, and reveal that this small population contributes to the overall genetic

diversity of this species in the region. Because small populations are prone to lineage assortment resulting from genetic drift (Avice 1994), the detection of unique haplotypes in the depleted nesting populations of Lanyu in Taiwan and Hong Kong is not surprising (see Cheng et al. 2008). The close relationship between the new haplotype (CmP116) that we found in Hong Kong and CmP18, which is

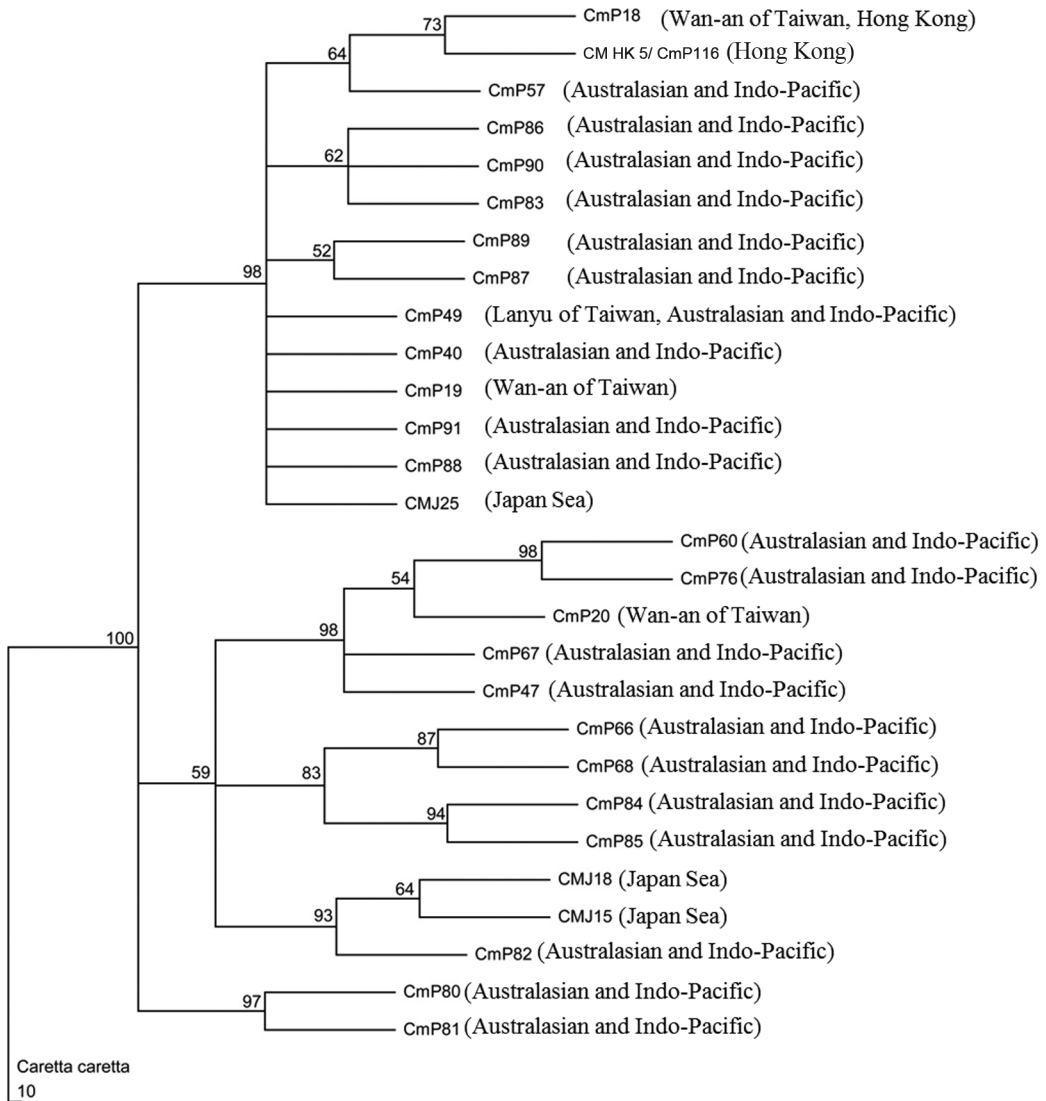


FIGURE 4. Neighbor-joining tree of HKY85 distance of 28 haplotypes based on 384 bp mtDNA sequences. Numbers above branches represent bootstrap values based on 1,000 replicates. Bootstrap values <50 are not shown. Scale below the tree indicates sequence divergence estimates. The sequences were observed in rookeries of Hong Kong, Wan-an Island and Lanyu Island in Taiwan, Sea of Japan, Australasia, and the Indo-Pacific (Norman et al. 1994, Dethmers et al. 2006, Cheng et al. 2008, Hamabata et al. 2009). Australasian and Indo-Pacific refer to the Southwest Pacific Ocean, Northwest Pacific Ocean, South China Sea, Sulu Sea, Celebes Sea, Timor Sea, and East Indian Ocean.

also present in Taiwan (Figure 4), suggests that CmP116 evolved recently (in evolutionary timescales) relative to other widespread, and presumably ancestral, haplotypes (such as CmP49) in the same clade (Figure 4) that likely represent an older regional colonization

and dispersal within the broader Australasian and Indo-Pacific region. More extensive research is required to uncover the regional and global phylogeography of green turtles. On a finer scale, the presence of the shared common haplotype (CmP18) in Wan-an Island

TABLE 3
Nesting Pattern of Green Turtles in the South China Sea from the late 1990s to 2000s

Nesting Site	No. of Nesting Green Turtles per Season	Clutch Size	No. of Clutches per Season	Hatching Rate (%)	Interesting Interval (days)	References
Sham Wan, Lamma Island, Hong Kong	0–5	93–152	1–5	40–90	Mean=11–13	Chan <i>et al.</i> (2007), This study
Huidong Gangkou National Sea Turtle Nature Reserve	1–20	Mean=113	Mean=5	34–96	N/A	Chan <i>et al.</i> (2007), Wang and Li (2008)
Wan-an Island, Penghu Archipelago	2–19	70–154	1–9	50–87	N/A	Chan <i>et al.</i> (2007)
Lanyu Island	3–11	73–110	1–4	53–94	Mean=9.7–12	Cheng <i>et al.</i> (2009)

and in Hong Kong suggests that these rookeries have an ancestral connection. The lack of significant differentiation between haplotypes of Wan-an in Taiwan and Hong Kong does not necessarily indicate ongoing interchange of nesting females but could also be due to recent shared common ancestry (Avice 1994). However, given the small sample sizes and lack of diversity, the statistical power to detect structure would be low (see Dutton *et al.* 2013), and acceptance of the null hypothesis could represent a false negative (Type II error) and therefore warrants caution (Taylor and Dizon 1999). Yet the results of long-term tagging and monitoring of these beaches do not indicate that any of the nesting females nest on different beaches within the same nesting season or in subsequent seasons, further suggesting that the genetic connectivity apparent in mtDNA lineages in Wan-an in Taiwan and in Hong Kong may not be the result of contemporary movement by adult nesting females themselves but could possibly result from other reasons, such as “migration” at maturity by females (because of imperfect natal homing) that originate as hatchlings from different nesting sites (see Dutton *et al.* 2008). Alternatively this apparent connectivity could be a statistical artifact related to the presence of common and shared haplotypes.

The characterization and linkage of the nesting green turtle population in Hong Kong with foraging habitat in Vietnam,

Hainan Island, and the rookery in Taiwan collectively supported by our satellite telemetry and genetic findings highlight the need for collaborative management and recovery of the population of Hong Kong nesting green turtles among concerned parties in the West Pacific region.

CONCLUSION AND RECOMMENDATIONS

The genetic distinction of the remnant breeding green turtle population in Hong Kong, as indicated by the newly reported haplotype, infers uniqueness to the genetic diversity of the population. Despite the small size of the breeding population, conservation is of high regional ecological importance in terms of genetic diversity, given the continuing threats and the generally depleted status of green turtle nesting populations in the South China Sea relative to historical records and to current abundance in other regions (Zhang 1996, Chan *et al.* 2007, Wang and Li 2008). Loss of the few nesters remaining in Hong Kong would result in an important loss of genetic diversity in the region, which escalates the level of conservation concern for the species. It is critical to strengthen and enforce legal protection, and to continue and expand monitoring and research of nesting populations at potential nesting sites in the region. Reinforcement of regional cooperation will facilitate concerted conservation efforts to protect

this shared migratory species. Future research studies in Hong Kong and southern China should focus on habitat use and genetic composition of foraging or migrating sea turtles to identify and mitigate threats to the wild population for the recovery of the green turtle assemblage in the area.

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