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New management unit for conservation of the Endangered green turtle *Chelonia mydas* at the Xisha (Paracel) Islands, South China Sea

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ABSTRACT: The Qilianyu cluster of the Xisha (Paracel) Islands has one of the few remaining green turtle *Chelonia mydas* rookeries in the China region. Genetic samples were obtained from dead green turtle embryos and hatchlings salvaged from post-hatched nests at Middle Island (n = 3), North Island (n = 9) and South Sand (n = 1) of the Qilianyu cluster in 2017–2019. The ~800 bp mitochondrial DNA control region was sequenced from the samples, and 5 haplotypes were identified belonging to 2 documented clades (clades III and VIII), including 2 new haplotypes (CmP243.1 and CmP244.1) and 3 previously reported haplotypes (CmP18.1, CmP19.1, CmP20.1). These results were combined with previously published mtDNA data for the Qilianyu cluster and nearby (~93 km) Yongle Islands indicating a lack of differentiation based on truncated 384 bp control region sequences (exact test, p = 0.0997; F_{ST} = 0.015, p = 0.2760), to represent a single Xisha Islands rookery. The rookery at the Xisha Islands was significantly differentiated (p < 0.01) from all 19 management units (MUs) documented in the Indo-Pacific and Japan regions, supporting recognition of the Xisha Islands rookery as a new independent MU. The results will help inform national and international conservation action plans by China and the countries around the South China Sea to protect green turtles in the West Pacific Ocean.

KEY WORDS: *Chelonia mydas* · Mitochondrial DNA · Haplotypes · Indo-Pacific · Nesting grounds

1. INTRODUCTION

The green turtle *Chelonia mydas* (Linnaeus, 1758) has a global distribution in tropical and subtropical coastal waters, across at least 140 countries, of which more than 80 contain nesting grounds (Groombridge & Luxmoore 1989, Hirth 1997). Green turtles undertake complex movements and migrations through geographically disparate habitats for foraging and nesting purposes (Lutz & Musick 1997). The decline of green turtle populations has been a longstanding worldwide issue (Schwartz et al. 2007). The green turtle was placed on Appendix I by CITES in 1981 and listed as Endangered on the IUCN Red List in 2004 due to continued population declines resulting from overexploitation of eggs and adult females at nesting beaches, and of juveniles and adults in foraging areas (Seminoff 2004, https://cites.org/eng/gallery/species/reptile/green_turtle.html). It is challenging to design consistent conservation action plans

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for such a globally distributed marine species, particularly since some green turtle populations with longer histories of conservation appear to have been recovering in recent years (Bjorndal & Bolten 2008), despite continued declines of others (Seminoff et al. 2015).

Global green turtle population assessments have identified subpopulations in order to conduct status and risk assessment at the appropriate geographic scale useful for conserving genetic diversity (Seminoff 2004, Komoroske et al. 2017). These regional subpopulations include regional management units (RMUs) (Wallace et al. 2010) and distinct population segments (DPSs) (Seminoff et al. 2015). Each of the green turtle regional populations, regardless of whether listed as an IUCN subpopulation, an RMU or a DPS, consists of several demographically independent nesting populations, or management units (MUs), made up of one or more rookeries (Jensen et al. 2019). Typically, for green turtles, the nesting range of an MU is no more than 500 km (Dethmers et al. 2006).

For green turtles, recognizing MUs is generally based on the significant divergence of allele (haplotype) frequencies of the mitochondrial DNA (mtDNA), irrespective of the phylogenetic differentiation of the haplotypes (Moritz 1994). The 860 bp mtDNA control region contains sufficient variation for detecting fine-scale stock or population structure (Taylor & Dizon 1999, Formia et al. 2006). However, shorter control region sequences (≥384 bp) from older studies are also informative (Norman et al. 1994). To date, 25 haplotypes, 5 clades and 17 MUs have been recognized in Australian waters by using the 384 bp control region segment from 27 rookeries of green turtles (Dethmers et al. 2006). Furthermore, based on the 386 bp control region segments, 11 clades (clades I–XI) were identified from 127 rookeries of green turtles globally, and 58 MUs were proposed in 12 geographical regions considered to be evolutionarily distinct (Jensen et al. 2019). Among the 12 regions, the Indo-Pacific region consists of 15 MUs covering a large area from southern China to Southeast Asian countries and northern Australia, and includes 5 clades (III, IV, V, VII and VIII), and the Japan region consists of 4 MUs, all located in archipelagos (e.g. Ogasawara, Ryukus and Yeayama) south of mainland Japan, and includes 4 clades (III, IV, VII and VIII).

In the China region, nearly all historically recorded green turtle rookeries along the coastline including Guangdong Province, Hainan Province and the Hong Kong Special Administration Region have disappeared or are severely depleted (Chan et al. 2007). Among the 17 RMUs globally, Taiwan rookeries belong to the Northwest Pacific RMU, and the South China Sea rookeries belong to the West Pacific/Southeast Asia RMU (Wallace et al. 2010). Furthermore, among the 12 DPSs identified globally, the China region falls within the East Indian–West Pacific DPS (Seminoff et al. 2015). Only 2 MUs are recognized in the China region: one from western Taiwan and the other from eastern Taiwan (Cheng et al. 2008, Jensen et al. 2019). However, in general, little current information is available on nesting green turtles in the China region, particularly in the South China Sea, and this represents a pressing data gap for the West Pacific region.

The South China Sea contains 4 main archipelagos where green turtle nesting has been observed. These include Dongsha Island (Pratas Archipelago) to the north (Chan et al. 2007), Taiping Island (Itu Aba Island) of Nansha (Spratly) Islands to the south (Cheng 1996) and Xisha (Paracel) Islands in the central region (Chan et al. 2007). Recently, the largest extant green turtle rookery in the China region has been identified at the Qilianyu cluster of the Xisha Islands, with >100 nests annually recorded since 2016 (Jia et al. 2019). The Qilianyu cluster includes 8 islands, found in the same coral barrier reef system with the farthest 2 approximately 16 km apart; 7 of these islands have green turtle nests, which are not documented on Zhaoshu Island (Fig. 1a). Furthermore, nesting sites of green turtles have also been reported at the Yongle Islands of the Xisha Islands, including Jinqing Island and Ganquan Island; however, the nesting scale is still unclear (Jia et al. 2019) (Fig. 1a).

Genetic population structure of nesting green turtles in the South China Sea merits further investigation. In a recent mtDNA study assessing the origin of illegally traded green turtles in Hainan Province, Gaillard et al. (2021) included data for 16 samples obtained from nests at the Xisha Islands, and suggested that nesting green turtles in this regional archipelago are unique and may represent a distinct population. In addition, Ng et al. (2014, 2017) reported data for a few samples that they were able to sequence from Guangdong Province and Hong Kong (a linear distance of approximately 90 km from each other); however, the population connectivity between these rookeries and the Xisha Islands is unclear due to the small sample sizes.

In this study, genetic samples were collected in 2017–2019 from post-hatched green turtle nests in the Qilianyu cluster in order to characterize the mtDNA diversity and to conduct comparative analyses with published data to determine population

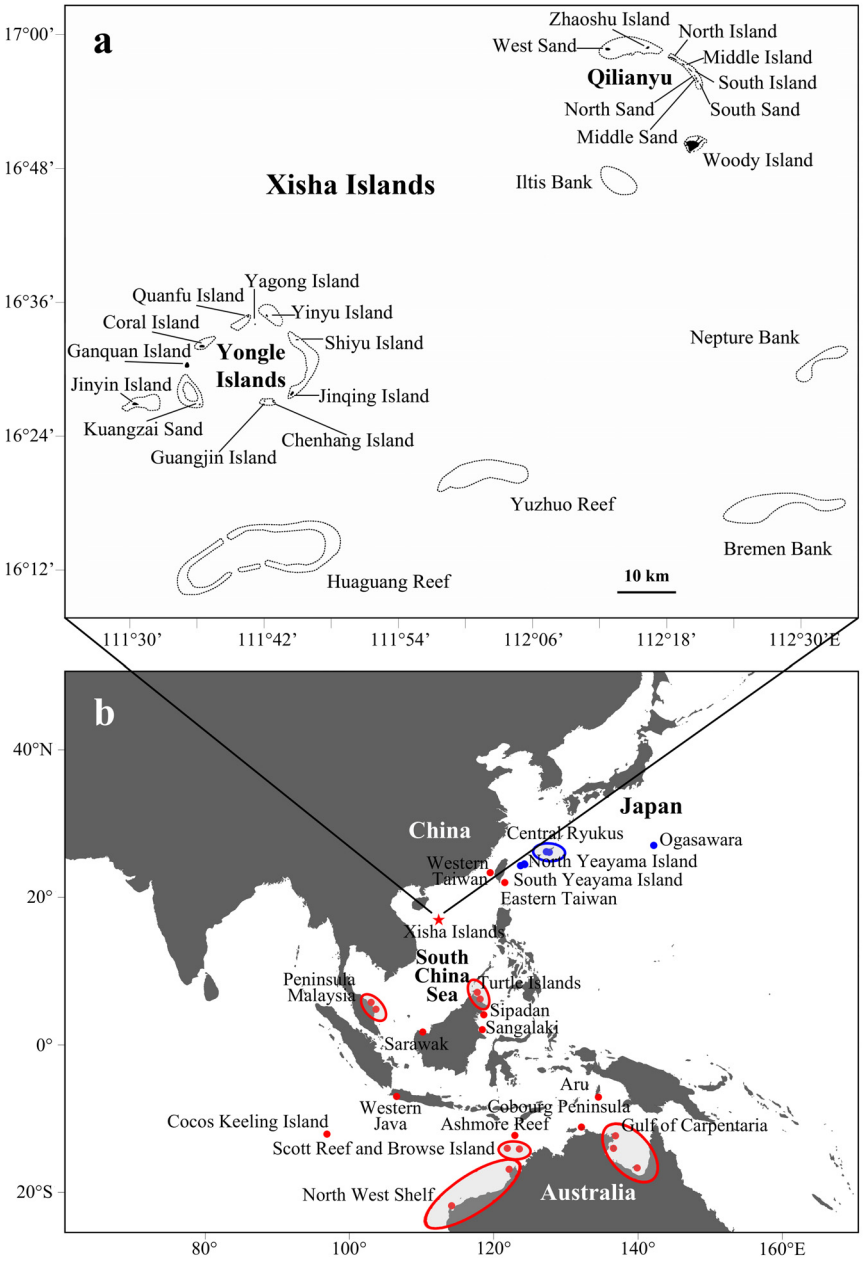

Fig. 1. (a) Sampling sites used in this study and Gaillard et al. (2021) at the Xisha Islands, including Middle Island, North Island and South Sand in the Qilianyu cluster, and Ganquan Island and Jinqing Island in the Yongle Islands. (b) Twenty management units (MUs) of green turtles *Chelonia mydas* proposed in the Indo-Pacific (red dots) and Japan regions (blue dots) (adapted from Jensen et al. 2019, this study). Red and blue circles indicate MUs having >1 rookery

structure and phylogeographic relationships within the Xisha Islands and the broader West Pacific region. The results of this study provide a basis for drawing up informed national and international conservation action plans to protect green turtle rookeries in the South China Sea.

2. MATERIALS AND METHODS

2.1. Sampling

Dead embryos and hatchlings from 13 post-hatched nests were collected in 2017–2019 from 3 islands at the Qilianyu cluster, Middle Island (n = 3), North Island (n = 9) and South Sand (n = 1) (Table 1, Fig. 1a). Based on the nesting sites and dates, these nests were very likely from different female turtles. The linear distances between Middle Island, North Island and South Sand are all less than 6 km without geological barriers, and all belong to the same coral reef system. Tissue (~2 g) was dissected from each sample and preserved in 95% ethanol for further molecular analysis. Tissue was obtained from multiple embryos and hatchlings from each nest, if available, for quality control and in case of tissue degradation.

2.2. Analysis of control region gene sequences

DNA was extracted using Chelex® 100 Resin (Bio-Rad Laboratories). Both the forward and reverse stands of an ~800 bp mtDNA control region segment were PCR-amplified using the primer pair LCM15382 (5′-GCT TAA CCC TAA AGC ATT GG-3′) and H950 (5′-GTC TCG GAT TTA GGG GTT TG-3′) (Abreu-Grobois et al. 2006). Briefly, 1–2 µl of template (20–50 ng μl^{-1}) was added into a 25 µl PCR reaction with 1.0 µl forward primer (at 10 µM), 1.0 µl reverse primer (at 10 µM), 1.0 µl dNTP (at 10 mM), 2.5 µl 10× *Taq* buffer (with 15 mM $MgCl_2$) and 0.2 µl *Taq* polymerase (at 5 U μl^{-1}) using standardized conditions of denaturing at 94°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 60 s for 38 cycles. Amplicons of the expected size were purified and sequenced (Sangon Biotech), and sequences were assembled by DNASTAR Lasergene SeqMan Pro 7.1.0.

Sequences were aligned using BioEdit software against available reference sequences obtained with

Table 1. Number of nests, nesting dates, nucleotide diversity (π) and haplotype diversity (*h*) of the 5 haplotypes (~800 bp control region) found from green turtles *Chelonia mydas* at Middle Island, North Island and South Sand of the Qilianyu cluster, Xisha Islands. The clades used here follow Jensen et al. (2019)

Sampling site	Nests (n)	Nesting date	Haplotype	Clade	π (mean ± SD)	*h* (mean ± SD)
Middle Island	1	5 July 2017	CmP20.1	III	0.039 ± 0.030	0.667 ± 0.314
	2	23 June 2017, 5 July 2017	CmP243.1	VIII		
North Island	1	August 2019	CmP18.1	VIII	0.014 ± 0.009	0.417 ± 0.191
	7	August 2018, 16 July 2019, 27 July 2019, 1 August 2019, 3 August 2019, August 2019, October 2019	CmP19.1	VIII		
	1	August 2019	CmP244.1	III		
South Sand	1	23 July 2019	CmP19.1	VIII	0.000 ± 0.000	1.000 ± 0.000
Total	13		5		0.018 ± 0.010	0.628 ± 0.143

BLAST from NCBI GenBank to identify haplotypes from our new sequences collected from the Qilianyu cluster (Sayers et al. 2019). Because shorter control region segments were commonly used to align and analyze population structures of green turtles, especially 384 bp in the Indo-Pacific region, the ~800 bp sequences obtained were subsequently trimmed to a length of 384 bp for further comparison with the available data using the standardized CmP nomenclature (Jensen et al. 2019, Gaillard et al. 2021, P. H. Dutton unpubl. data). Briefly, the haplotypes represent 384 bp sequences, while those with decimal suffixes (e.g. CmPx.1) represent equivalent variants of these 384 bp haplotypes based on variation identified by the additional longer sequences. One sample sequence was selected from each nest for further analysis because the random samples from the same nest for quality control were 100% identical.

2.3. Statistical analysis

In a recent publication, 16 dead green turtle hatchlings from different nests were collected in 2012–2018 at the Qilianyu cluster and nearby (~93 km) Yongle Islands in the Xisha Islands group (Gaillard et al. 2021, L. Lin pers. comm.) (Fig. 1a). To test for homogeneity between the 2 datasets, genetic distance-based (Φ_{ST}) analysis of molecular variance (AMOVA) was conducted. Additional tests for differentiation were conducted using a pairwise exact test (500 000 steps in a Markov chain with a 10 000-step dememorization) and pairwise F_{ST} analysis (Nishizawa et al. 2013). In the absence of significant differentiation, the data from the 2 studies were combined for further analyses (see Section 3).

The green turtle rookery of the Xisha Islands and all 19 documented green turtle MUs from the Indo-Pacific and Japan regions (Jensen et al. 2019) were compared. Pairwise tests for population structure were conducted using exact tests and F_{ST} analyses based on haplotype frequencies, and using Φ_{ST} that incorporated information on genetic distance (computing distance matrix with a Kimura 2-parameter model). The alpha significance level was set at 0.05 to determine whether the Xisha Islands can be considered as a new MU (sensu Moritz 1994). All MUs in each of the 2 regional sub-groups representing the Indo-Pacific and Japan regions (Jensen et al. 2019) were further compared with AMOVA. The analyses above, along with determination of nucleotide diversity (π) and haplotype diversity (*h*), were conducted using Arlequin 3.5.2.2 (Excoffier & Lischer 2010).

Phylogeographic relationships of all haplotypes documented in the Indo-Pacific and Japan regions (Ng et al. 2014, 2017, Jensen et al. 2019, Gaillard et al. 2021) and this study were inferred by constructing a neighbor-joining (NJ) tree, including the flatback turtle *Natator depressus* as an outgroup, using MEGA 6.06 with 10 000 bootstrap pseudo-replications (Tamura et al. 2013).

3. RESULTS

3.1. Haplotypes and clades

Five longer haplotypes (~800 bp) were identified, including 3 previously documented (CmP18.1, CmP19.1, CmP20.1) and 2 new haplotypes (CmP243.1 and CmP244.1, GenBank accession numbers MW631941 and MW631940, respectively). CmP19.1

Table 2. Clades, haplotypes (384 bp control region) and number of green turtle nests in the Xisha Islands rookery, South China Sea. The clades used here follow Jensen et al. (2019)

Clade	Haplotype	No. of nests	Reference
III	CmP20	1	This study
	CmP54	1	Gaillard et al. (2021)
	CmP244	1	This study
VIII	CmP18	2	Gaillard et al. (2021), this study
	CmP19	18	Gaillard et al. (2021), this study
	CmP49[a]	4	Gaillard et al. (2021)
	CmP243	2	This study
	Total	29	

[a]This haplotype was mistakenly listed as CmP83 in Table 1 of Gaillard et al. (2021). According to the Appendix provided by the authors and personal communications with a co-author (L. Lin), CmP49 is correct

was the most common haplotype, found in 8 nests (61.5%), i.e. 7 from North Island and 1 from South Sand (Table 1).

The 5 truncated 384 bp haplotypes contained 24 polymorphic sites, all transitions. CmP243 (MW 013461) is identified by 1 substitution site, and Cm P244 (MW013460) is distinguished by 2 substitution sites. Another 4 truncated 384 bp haplotypes (CmP18, CmP19, CmP49, CmP54) were reported at the Xisha Islands (Gaillard et al. 2021), giving a total of 7 haplotypes observed at the Xisha Islands (Table 2). CmP18, CmP19, CmP49 and CmP243 fall into clade VIII, and CmP20, CmP54 and CmP244 fall into clade III as identified by Jensen et al. (2019) (Fig. 2).

3.2. A new management unit recognized

The results of the AMOVA, exact test (p = 0.0997) and F_{ST} (F_{ST} = 0.015, p = 0.2760) indicated homogeneity and lack of differentiation between datasets

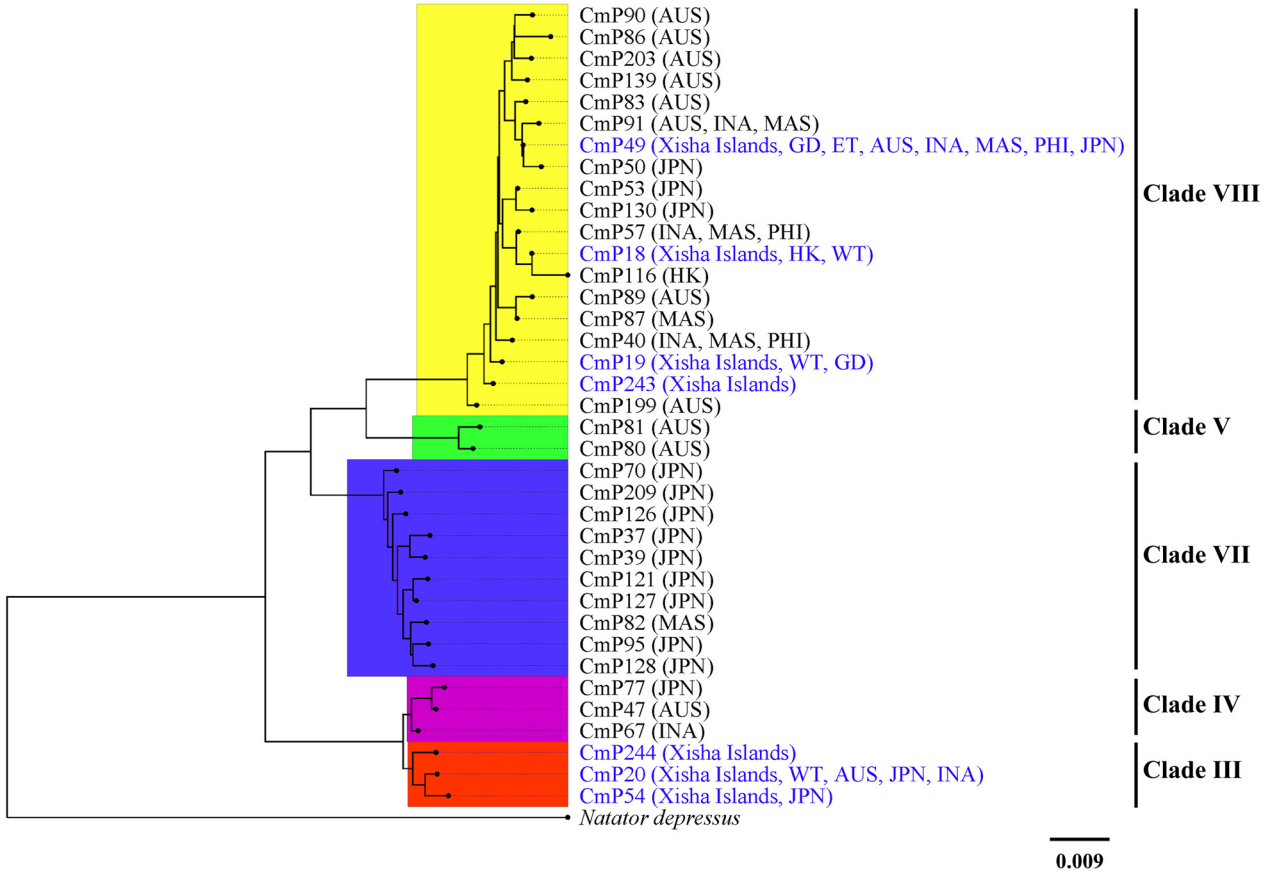

Fig. 2. Neighbor-joining tree of all 37 haplotypes (384 bp control region) from rookeries of green turtles *Chelonia mydas* in the Indo-Pacific and Japan regions, including 7 haplotypes from the Xisha Islands (blue text) (Ng et al. 2014, 2017, Jensen et al. 2019, Gaillard et al. 2021, the present study). The clades used here follow Jensen et al. (2019). AUS: Australia; ET: Eastern Taiwan; GD: Guangdong; HK: Hong Kong; INA: Indonesia; JPN: Japan; MAS: Malaysia; PHI: Philippines; WT: Western Taiwan

from the 2 independent sampling efforts at the Xisha Islands. Therefore, data from Gaillard et al. (2021) and this study were combined to represent a single Xisha Islands rookery for further analysis (Table 2).

The exact tests showed significant differentiation between the Xisha Islands rookery and all 19 MUs in the Indo-Pacific and Japan regions ($p < 0.0001$) (Table 3). F_{ST} values ranged from 0.283 to 0.659 for all pairwise comparisons, and all were significant ($p < 0.0001$). For Φ_{ST}, significant differences were also found with 18 of the 19 MUs (16 MUs with $p < 0.01$, 2 MUs with $0.01 < p < 0.05$), with the Cocos

Table 3. Comparison of exact test, F_{ST} and Φ_{ST} of all 19 management units for green turtles *Chelonia mydas* in the Indo-Pacific and Japan regions (defined by Jensen et al. 2019) and 2 rookeries in Hong Kong (Ng et al. 2014) and Guangdong (Ng et al. 2017) with the Xisha Islands rookery (sample size = 29, Gaillard et al. 2021 and the present study). *$p < 0.05$; **$p < 0.01$

Region	Management unit	Country	Sample size	Exact test (p-value)	F_{ST}	Φ_{ST}	Source
Indo-Pacific	Guangdong (rookery)	China	2	0.6341	−0.161 (p = 0.9999)	−0.257 (p = 0.9999)	Ng et al. (2017)
Indo-Pacific	Hong Kong (rookery)	China	6	0.0003	0.448 (p < 0.0001**)	0.322 (p = 0.0041**)	Ng et al. (2014)
Indo-Pacific	Western Taiwan	China	40	<0.0001	0.420 (p < 0.0001**)	0.150 (p = 0.0050**)	Cheng et al. (2008)
Indo-Pacific	Eastern Taiwan	China	14	<0.0001	0.571 (p < 0.0001**)	0.151 (p = 0.0313*)	Cheng et al. (2008)
Indo-Pacific	Western Java	Indonesia	23	<0.0001	0.433 (p < 0.0001**)	0.187 (p < 0.0001**)	Dethmers et al. (2006)
Indo-Pacific	Aru	Indonesia	28	<0.0001	0.659 (p < 0.0001**)	0.278 (p < 0.0001**)	Dethmers et al. (2006)
Indo-Pacific	Sangalaki	Indonesia	29	<0.0001	0.283 (p < 0.0001**)	0.126 (p < 0.0001**)	Dethmers et al. (2006)
Indo-Pacific	Sipadan	Malaysia	30	<0.0001	0.357 (p < 0.0001**)	0.199 (p < 0.0001**)	Dethmers et al. (2006)
Indo-Pacific	Turtle Islands	Malaysia and Philippines	67	<0.0001	0.545 (p < 0.0001**)	0.409 (p < 0.0001**)	Dethmers et al. (2006)
Indo-Pacific	Sarawak	Malaysia	22	<0.0001	0.517 (p < 0.0001**)	0.197 (p = 0.0001**)	Dethmers et al. (2006)
Indo-Pacific	Peninsula Malaysia	Malaysia	27	<0.0001	0.469 p < 0.0001**)	0.080 (p = 0.0242*)	Dethmers et al. (2006)
Indo-Pacific	Cocos Keeling Island	Australia	19	<0.0001	0.514 (p < 0.0001**)	0.045 (p = 0.1409)	Jensen et al. (2016)
Indo-Pacific	Ashmore Reef	Australia	20	<0.0001	0.315 (p < 0.0001**)	0.280 (p = 0.0009**)	Dethmers et al. (2006)
Indo-Pacific	Scott Reef and Browse Island	Australia	65	<0.0001	0.395 (p < 0.0001**)	0.181 (p = 0.0001**)	Jensen et al. (2016)
Indo-Pacific	North West Shelf	Australia	45	<0.0001	0.529 (p < 0.0001**)	0.296 (p < 0.0001**)	Dethmers et al. (2006)
Indo-Pacific	Cobourg Peninsula	Australia	37	<0.0001	0.412 (p < 0.0001**)	0.321 (p < 0.0001**)	Jensen et al. (2016)
Indo-Pacific	Gulf of Carpentaria	Australia	132	<0.0001	0.354 (p < 0.0001**)	0.324 (p < 0.0001**)	Dethmers et al. (2006)
Japan	South Yeayama Island	Japan	26	<0.0001	0.395 (p < 0.0001**)	0.377 (p < 0.0001**)	Nishizawa et al. (2011)
Japan	North Yeayama Island	Japan	41	<0.0001	0.365 (p < 0.0001**)	0.659 (p < 0.0001**)	Nishizawa et al. (2011)
Japan	Central Ryukus	Japan	70	<0.0001	0.343 (p < 0.0001**)	0.165 (p = 0.0009**)	Hamabata et al. (2014)
Japan	Ogasawara	Japan	103	<0.0001	0.331 (p < 0.0001**)	0.548 (p < 0.0001**)	Nishizawa et al. (2013)

Keeling Island MU being the exception ($p > 0.1$). Based on the significant differences between the Xisha Islands rookery and other rookeries in the Indo-Pacific region, a new MU is proposed for the Xisha Islands, noting the absence of any other rookeries within a distance of 500 km (Fig. 1b).

Additionally, CmP19 and CmP49 were found in the Guangdong rookery from 2 samples (Ng et al. 2017), and CmP18 and CmP116 were found in the Hong Kong rookery from 6 samples (Ng et al. 2014) (Table 3). In the Xisha Islands rookery, approximately 700 km away from Guangdong and Hong rookeries, CmP19 was the most common haplotype, and CmP116 was not found (Table 2). CmP18, the most common haplotype in the Hong Kong rookery (83.3% of samples), only made up a small proportion (6.9%) in the Xisha Islands rookery (Table 2). Additionally, the Xisha Islands rookery showed no significant difference with the Guangdong rookery based on the exact tests, F_{ST} and Φ_{ST} analyses, but was significantly differentiated from the nearly extinct Hong Kong rookery in all 3 tests (Table 3).

The AMOVA showed that the variation of the MUs between the Indo-Pacific region and the Japan region (45.73%) was higher than those among MUs within regions (18.22%) and within MUs (36.05%).

4. DISCUSSION

4.1. Characteristics of the new Xisha Islands MU

Based on this study and that of Gaillard et al. (2021), 7 haplotypes, including 2 new ones, were identified from the Xisha Islands rookery from a total of 29 green turtle nest samples (Table 2). The results indicated a high haplotype diversity, despite the small population size, in this rookery, relative to MUs in the Philippines (3 haplotypes), Malaysia (6 haplotypes) and Indonesia (6 haplotypes) in the West Pacific (Fig. 2).

In the Indo-Pacific and Japan regions, haplotype CmP19 (or CmP19.1) is not common and has only been found in western Taiwan and Guangdong rookeries (Cheng et al. 2008, Ng et al. 2017) (Fig. 2), and in the foraging grounds of northeastern Australia (Jensen et al. 2016) and Japan (CMJ35, a 517 bp sequence equivalent to a truncated portion of CmP19.1) (Nishizawa et al. 2010). However, CmP19 (or CmP19.1) was the most common at the Xisha Islands, contributing to approximately 62% of the samples (Gaillard et al. 2021, this study) (Table 2). Haplotype CMC2 (489 bp sequence equivalent to a truncated portion of CmP19.1) was found in juvenile green turtles (71%) captured in Hainan waters, less than 300 km northwest of the Xisha Islands (Yang et al. 2015). CmP19 was also found in nearly 46% of green turtles caught by fishermen in Hainan waters (Gaillard et al. 2021). Considering the relatively short distance between Hainan and the Xisha Islands, the Xisha Islands rookery is probably an important source of the green turtles foraging in Hainan waters. The proportions of haplotypes CmP18.1 and CmP20.1 at the Xisha Islands were low (10.3%). CmP18 is the dominant haplotype found in the nearly extinct rookery in Hong Kong and in the western Taiwan rookery, and is also found in foraging grounds of the Japan region (Cheng et al. 2008, Nishizawa et al. 2010, Ng et al. 2014, Hamabata et al. 2015). CmP20 is the second dominant haplotype in the western Taiwan rookery (32.5%) and widely distributed in the nesting grounds and foraging areas of the Japan, Indo-Pacific and Central West Pacific regions (Dethmers et al. 2006, Hamabata et al. 2014, Read et al. 2015, Jensen et al. 2019). Haplotype CmP20.1 is dominant in the Central West Pacific region (Dutton et al. 2014, Read et al. 2015, Boissin et al. 2019), but is less frequent in northern Australia (Dethmers et al. 2006, Cheng et al. 2008, Jensen et al. 2016). The aforementioned changes in the proportions of different haplotypes in the Xisha Islands are significant, and the connectivity among the Xisha Islands and other rookeries merits further investigation.

The NJ tree revealed that the Xisha Islands haplotypes were nested within clades III and VIII, accounting for 10.3 and 89.7% of nests sampled, respectively (Table 2). Clade VIII is dominant in the Indo-Pacific region (Jensen et al. 2019). Eastern Taiwan only consists of clade VIII (Cheng et al. 2008). Our study confirmed that clade VIII is also dominant in the Xisha Islands rookery, consistent with phylogeographic placement of this MU within the Indo-Pacific region.

Previous phylogenetic studies have determined that sea turtles show evidence of connectivity resulting from episodic single colonization or long-distance dispersal events followed by secondary or multiple contact over evolutionary timescales (Leroux et al. 2012). Jensen et al. (2019) noted that the Indo-West Pacific was a source for green turtles, or center of origin for the present-day global mtDNA lineage diversity. The presence of haplotypes from the deeply divergent evolutionary lineages (clades III and VIII, 2–3.5 million years ago, Jensen et al. 2019) within the remnant green turtle population in this study (the Xisha Islands), including new unique haplotypes, suggests that this newly recognized MU is important for preserving the diversity of this species. Our results

are consistent with the evolutionary connectivity with mtDNA lineages in the Indo-Pacific region to the south and the Japan region to the north bordering on the Xisha Islands (see Jensen et al. 2019), spanning a linear distance of >1000 km (Fig. 1b).

While the results of this study support recognition of the Xisha Islands as a separate MU within the South China Sea, further work is needed to resolve 2 caveats. First, the Xisha Islands rookery could not be differentiated from the Guangdong rookery with a linear distance of approximately 680 km, almost certainly due to the sample size (n = 2) from the Guangdong rookery (Ng et al. 2017). Second, no differentiation ($p > 0.1$) was found between the Xisha Islands MU and the Cocos Keeling Island MU with a distance of approximately 3500 km, based on Φ_{ST} analysis that incorporates the genetic distance and the influence of shared haplotypes (Table 3). CmP19 is the predominant haplotype (62.1%) at the Xisha Islands, while CmP49 is the main haplotype (89.5%) at the Cocos Keeling Island. Of note, CmP19 and CmP49 are only distinguished by a 2 bp sequence variation. This apparent lack of differentiation may reflect deeper evolutionary connectivity rather than contemporary gene flow, and warrants further study.

4.2. Conservation implications

The accurate identification of MUs is a necessary first step toward developing appropriate assessment, monitoring and management plans that are tailored to address threats. This study establishes a new MU in the data-poor South China Sea region that will help policy makers to draft MU-focused national and international conservation action plans and management strategies that can be modeled on others in the West Pacific region. For instance, where an MU spans the jurisdiction of more than one country, international coordination is needed, as illustrated by the protected areas of the Turtle Islands MU that is shared by the Philippines and Malaysia (Palma 1997, Dethmers et al. 2006) (Fig. 1b). For the countries with more than one MU identified, different management strategies can be tailored to address MU-specific management priorities. In Australia, for instance, 9 green turtle MUs have been identified that span a large geographic area and are subject to different population trends and threats (Dethmers et al. 2006, Jensen et al. 2016, Commonwealth of Australia 2017). The 10 yr Australian National Sea Turtle Recovery Plan was based on a multi-stakeholder process that incorporated scientific, socio-economic and environmental factors to develop conservation actions addressing MU-specific threats (Commonwealth of Australia 2017). Extensive oil and gas industry activities, for example, primarily impact the nesting beaches of the North West Shelf MU (Fig. 1b), so the control of threats from artificial lights and chemical spills are management priorities. In contrast, mortality and injury from incidental capture by trawlers, ghost nets and marine debris at foraging areas are primary threats to the Gulf of Carpentaria MU and are therefore a mitigation priority for that MU.

In the past, green turtle nesting was widespread along the coastline of southern China, but this has now almost disappeared. The nesting that has been documented at offshore islands in Taiwan, and in Dongsha Island, Taiping Island and the Xisha Islands in the South China Sea, may be the last remaining in the China region (Frazier et al. 1988, Cheng 1996, Chan et al. 2007, Jia et al. 2019). As a result, the green turtle was upgraded to the highest (Class I protection) national protected species category by China in January 2021 (www.forestry.gov.cn/html/main/main_5461/20210205122239482485322/file/20210205122347636743107.pdf, accessed 18 February 2021), indicating a commitment to conservation and providing an opportunity for updating the action plan for sea turtle conservation in China (People's Republic of China 2018). The identification of a new MU for the Xisha Islands rookery, together with the previous 2 Taiwan MUs, bring the total number of MUs to 3 in the China region (Fig. 1b). The new Xisha Islands MU warrants attention due to its relatively high haplotype diversity that can potentially provide a basis for conserving as much diversity as possible for green turtles in the West Pacific region. With the growing research interest focusing on the Xisha Islands (Yang et al. 2015, Jia et al. 2019, Gaillard et al. 2021), basic information is accumulating, and our results provide an improved rookery baseline to enable more accurate genetic assignment of stock origin of foraging, stranded and illegally harvested turtles in the region. In a recent study, 85 green turtle juveniles and adults confiscated in Hainan Province revealed that most turtles were from the Xisha Islands and the Sulu Sea (including the Philippines and Malaysia) (Gaillard et al. 2021). The results confirmed the existence of illegal trans-national trade in the West Pacific region. Satellite tracking post-nesting movements by females from the Xisha Islands rookery would help identify hotspots and foraging grounds connected to this MU, which combined with expanded genetic sampling will be necessary for expanding understanding of habitat

connectivity and RMU delineation to inform green turtle conservation in the China region, as well as the broader Indo-Pacific region.

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