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Genetic structure and diversity of green sea turtle (*Chelonia mydas*) from South China Sea inferred by mtDNA control region sequence

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ABSTRACT

We analyzed 88 control region sequences of green sea turtle (*Chelonia mydas*) from around Hainan Island in the South China Sea. These sequences had a length of 489 bp and revealed 8 mtDNA haplotypes of which four haplotypes (CMC1, CMC4, CMC7, and CMC8) had not been discovered before. Haplotype diversity (h) and nucleotide diversity (π) were 0.45 ± 0.054 and 0.0035 ± 0.0014, respectively. Neighbor-Joining tree based on control region sequences revealed that genetic relationship between green sea turtles from the South China Sea and from Japan Sea were very close. Clustering relationship based on control region sequences indicated that the South China Sea is an important breeding site and feeding habitat for green sea turtles, which connects with the Middle East Pacific, the Southwest Pacific and the Indian Ocean.

1. Introduction

Green sea turtles (*Chelonia mydas*) are herbivorous and migratory marine animals distributed in tropical and subtropical waters. These animals present a highly migratory behavior. Female green sea turtles possess a precise positioning capability about its migration routes, foraging areas and nest sites (Bagda et al., 2012). There are seven species of sea turtles in the world of which five are located in China: namely the green sea turtle (*C. mydas*), hawksbill (*Eretmochelys imbricata*), loggerhead turtle (*Carette carette*), leatherback turtle (*Dermochelys coriacea*) and Pacific ridley turtles (*Lepidochelys olivacea*). In China, over 80% of sea turtle resources are green sea turtles. Most of the green sea turtles live in the South China Sea (SCS), including the Paracel Islands (latitude 15°40′–17°10′, longitude 111°–113°), Spratly Islands (latitude 3°40′ to 11°55′, longitude 109°33′ to 117°50′) and Hainan Island (longitude 108°37′–111°05′, latitude 18°–20°10′). Green sea turtles are classified by the World Conservation Unit as endangered (IUCN, 2007). The population of green sea turtles in China reduced sharply over the past 50 years, mainly because of fishing activities and marine pollution (Jian-feng et al., 2013).

Chinese government has been taking efforts to protect green sea turtles. Effective conservation strategies would benefit from an accurate and comprehensive understanding of the genetic diversity of the species. Phylogeographic studies, which are beneficial to understand the temporal and spatial distribution of a species, can help to reform the protective measures. Populations of marine turtles tend to be structured along female lineages. In light of the matrilineal inheritance of

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mitochondrial DNA, analyses of this genetic marker can therefore be useful in sea turtle phylogeographic studies (Formia et al., 2007). Researchers have been studied cytochrome b (Cyt b) (Bowen et al., 1993), control region, ND4 (Dutton et al., 1996) and 12S and 16S (Naro-Maciel et al., 2008) in terms of mitochondrial phylogenetics (Sebastian et al., 2012) . Studies of control region sequence have been increasingly applied to marine turtles, whereby the development of genetic tags for these animals has contributed to the acquisition of valuable data on their evolution, population structure, reproductive behavior and migration ecology, besides providing a foundation for conservation and management strategies (Proietti et al., 2009). Dutton et al. (2008) analyzed control region sequences of green sea turtles on the Hawaiian island and found the Hawaiian feeding ground populations comprise one genetic stock derived from the nesting population at French Frigate Shoals. Prosdocimi et al. (2012) in the analysis of green sea turtles control region sequences in Argentina confirmed that green sea turtles at Argentinean feeding grounds originate mainly in the Ascension Island rookery, with less contribution on from rookeries in Suriname, Aves Island and Trindade Island. Compared to other countries, genetics research of green sea turtle in China is still in its infancy. Guo et al. (2009) studied control region sequences of only four green sea turtles in SCS and inferred that the SCS was not only the breeding site, but also an important feeding habitat for green sea turtles from the central eastern Pacific, the southwestern Pacific, and the Indian Ocean.

The main purposes of this paper are: (1) to further our knowledge about the genetic diversity of Chinese green sea turtle; (2) explore the phylogeographic distribution of Chinese green sea turtles in the world; (3) to provide scientific information that can inform the strategy for the protection of SCS turtles resources.

2. Material and methods

2.1. Sample collection

From June to October in 2011 and 2012 we collected skin samples from 175 sea turtles around Hainan Island (19°12′N, 109°42′E) in China. Most of them are juvenile turtles, while adult turtles account for about ten percent. The skin samples are stored in 95% ethanol at −20 °C. The mean curved carapace length (CCL) was 19.6 ± 8.15 cm (range: 13.0–54.4 cm).

2.2. DNA extraction and laboratory procedures

The DNA was extracted by kit “DNeasy Blood & Tissue Kit” (QIAGEN, Germany) and stored in TE buffer at −20 °C. About 490 bp sequence in the 5′ end of mtDNA control region was amplified using the primers the LTCM1 and HDCM1 (Allard et al., 1994). Polymerase chain reactions (PCR) consisted of 12 μl Premix Ex Taq (TAKARA), 1 μl template DNA, 1 μl each primer, 1 μl BSA, and 9 μl distilled ddH_2O to a total volume of 25 μl. PCR cycling parameters were as follows: 94 °C for 3 min; 35 cycles of 94 °C for 45 s, 55 °C for 30 s, 72 °C for 90 s; and a final extension of 72 °C for 5 min. The PCR products were purified and sequenced by Shanghai Sangon Biological Engineering Technology and Services Co. Ltd.

2.3. Data analysis

Sequences were edited and aligned by the Bioedit 7.0. Haplotype (h) and nucleotide diversities (π) were calculated with DnaSP 5.10 and DAMBE. Phylogenetic trees were reconstructed through the neighbor-joining method (MEGA package, version 3) by 1000 bootstrap resampling used the Kimura two-parameter model with MEGA5.1 software. MEGA5.1 software was also used to calculate the base composition, variable sites and Kimura two-parameter genetic distance.

3. Results

A fragment of 485–490 bp at the 5' end of the mtDNA control region was successfully amplified from 88 green sea turtles from SCS. There are 460 conserved sites, 29 variable sites and 23 parsimony informative sites. There are seven base transitions and no transversion. The base composition was T (31.7%), C (19.9%), A (35.2%), G (13.3%). C + G% (33.2%) is lower than the A + T % (66.9%), which is in line with the fact that G and C are in shortage in vertebrate mitochondrial DNA. Overall mean distance was 0.004. Eight haplotypes of CMC1~CMC8 were detected from 88 samples (accession numbers in Genbank, KJ598121–KJ598128), while haplotype CMC1, CMC4, CMC7, CMC8 were newly discovered. The haplotype CMC2 (71%) was most common one and CMC1 (21%) was the second most common one. Haplotype diversities (h) and nucleotide diversities (π) were 0.45 ± 0.054 and 0.0035 ± 0.0014, respectively.

A Neighbor-Joining tree (Fig. 1) was constructed based on the eight haplotypes in combination with other control region sequences which were tested as the most similar sequences by BLAST from Genbank. We used hawksbill (*E. imbricata*) (AB485806, FR798951, JF510457, JF926555, and JX441892) as the outside group.

In the analysis of control region sequence, we found that green sea turtles of the SCS shared a close genetic relationship with ones in the northwest Pacific, the southwest Pacific and the Indian Ocean. By comparison, CMC2 and AB485793 from Japan have a consistent sequence; CMC3 and AB472329 from Japan, AF529028 from Comoros of Indian Ocean have a consistent sequence. The following sequences are the same: CMC5 and AB472330 of Japan, AF529029 of Comoros; CMC6 and AB472307 of Japan, JX454976 of Malaysian, JX454985 of Micronesia.

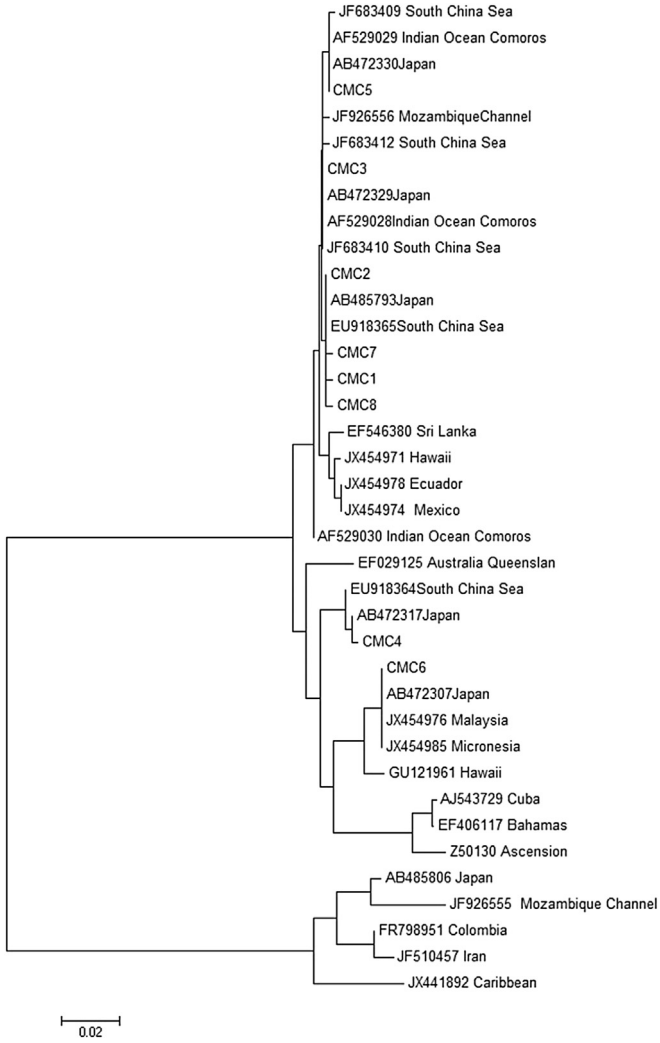

Fig. 1. Neighbor-Joining tree based on mtDNA control region sequences of green sea turtles in SCS.

Clustering relationship based on control region sequences also inferred that there was gene exchange between green sea turtles of SCS and ones of Middle East Pacific such as Hawaii, Mexico, Ecuador and Australia.

4. Discussion

Genetic information obtained from this study pointed out that the status of SCS green sea turtles is quite worrisome and not optimistic. For example Hamabata et al. (2009) studied control region sequences of green sea turtles in Japan and found h was 0.63–0.87 and π was 0.0190–0.0336. By contrast h (0.45 ± 0.054) and π (0.0035 ± 0.0014) of green sea turtles in SCS were much lower than the normal range. Nucleotide diversity in Japan was 4 times higher than in SCS. This result may be explained by the fact that haplotypes in Japan (32 haplotypes) were more diverse with respect to SCS.

By comparison SCS and Japan Sea have the most frequent gene exchange of green sea turtles. Most samples (including the most common haplotype CMC2) can find consistent sequence in Japan Sea. We assumed that Japanese Kuroshio Current played an important role. By satellite tracking, Song et al. (2002) found a migratory route of green sea turtles from SCS to Japan Sea. After released at Gangkou Sea Turtle National Nature Reserve, a green sea turtle swam across Taiwan Strait and took a final move north to Japan. This is consistent with our findings. We can determine a significant migratory route from the SCS to Japan Sea. Around this route are the East China Sea and Yellow Sea. The economic development and population explosion occurred in these coastal areas urged fishing activities and ocean pollution appeared offshore, which is against green sea turtles during migration.

The green sea turtles in SCS and the Indian Ocean and other areas of the Southwest Pacific were genetically closely related. The present geological boundary, which is the cluster of islands around SCS, seems not to effectively prevent the gene flow

from SCS to the Southwest Pacific and the Indian Ocean. From Fig. 1 we can infer that part of the SCS green sea turtles migrated southward towards the equator. The possible route is as following: They moved southward and passed Malaysia, then some migrated eastward towards the Pacific Ocean and some migrated westward towards the Indian Ocean.

A strong feature of the mtDNA data for SCS green turtles is the distinction between the Pacific vs. Indian Ocean and Southeast Asian populations, which is consistent with Dethmers' research about green sea turtles in Australia (Dethmers et al., 2006). Gene flow between green sea turtles in SCS and in Hawaii was at a relatively low level, although The sampling location (19°12′N, 109°42′E) and Hawaii (18°55′N–29°N, 154°40′W–162°W) are almost at the same latitude and three famous currents are between them: North Equatorial Current, South Equatorial Current and Equatorial Counter Current.

In conclusion the migratory routes of SCS green sea turtles are variegated. SCS, as an integral section of green sea turtles migrating between Pacific and Indian Ocean, needs more attention. There is primary evidence for joint management between China and neighboring nations like Indonesia and Malaysia, given that these migration routes extend over a large geographical area.

The experiment samples were almost collected near Hainan Island. We can not survey the entire SCS because of sovereign issues, which seriously obstructed our research and caused the data shortage of sea turtles in this area (Chan et al., 2007). Existing results came mostly from field survey and satellite tracking and can not fully display the present situation of green sea turtle in SCS. Not to mention the dense and intricate distribution of islands around SCS go against to determine the precise migratory routes. Therefore, we need to further expand the scope of sampling and surveys and utilize more satellite tracking techniques. Migration routes, spawning grounds and feeding grounds of green sea turtles in SCS need to be explored in depth.

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