Pakistan J. Zool., vol. 51(4), pp 1281-1288, 2019. DOI: http://dx.doi.org/10.17582/journal.pjz/2019.51.4.1281.1288

Genetic Homogeneity among Bull Sharks *Carcharhinus leucas* in the South China Sea

Zhaochao Deng[1], Jingchen Chen[1], Na Song[2], Yongzhen Li[3] and Zhiqiang Han[1,*]

[1]*Fishery College, Zhejiang Ocean University, Zhoushan, Zhejiang 316022, China*
[2]*Fishery College, Ocean University of China, Qingdao 266003, China*
[3]*South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou 510300, China*

ABSTRACT

Carcharhinus leucas has a cosmopolitan distribution and marked genetic structure. However, analysis of this structure has not included the South China Sea population. In the present study, we collected a total of 23 individuals from Xisha, and Nansha Islands in the South China Sea and a 503 bp segment of mitochondrial DNA control region was sequenced. 17 polymorphic sites were obtained defining 7 haplotypes in the South China Sea population. Contrary to heterogeneous expectation, AMOVA and pairwise F_{ST} values showed that the genetic differences in this region were all non-significant. The pattern of population demography showed a stable model in this species in the South China Sea population. To reveal its population structure within the large-scale geography distribution, we added 169 sequences of the mitochondrial control region already genotyped from northern Australia. The South China Sea and northern Australia groups were successfully distinguished by the NJ tree with significant genealogical branches of haplotypes. These results indicated that there might have only one fishery management units of *C. leucas* in the South China Sea and genetic heterogeneity among *C. leucas* in the center of Indo-Pacific regions.

Article Information
Received 03 May 2018
Revised 30 June 2018
Accepted 10 July 2018
Available online 01 May 2019

Authors' Contribution
ZH conceived and designed the work. JC performed the experiments. YL collected the specimens. ZD analyzed the data and wrote the manuscript. NS and ZH revised the manuscript.

Key words
***Carcharhinus leucas*, Genetic structure, South China Sea, Mitochondrial DNA, Control region.**

INTRODUCTION

The bull shark *Carcharhinus leucas*, belonging to family Carcharhinidae, is a coral-reef or coastal shark species, widely distributes in tropical and subtropical waters (Gadig *et al*., 2006; Brunnschweiler and Earle, 2006; Chen *et al*., 2015). This species is famous for its ability to penetrate freshwater and is known to travel long distances (>1500 km) (Thomerson *et al*., 1977; Montoya and Thorson, 1982; Carlson *et al*., 2010). It matures at approximately 210–220 cm in males and >225 cm in females (Branstetter and Stiles, 1987). The large size and abundance of *C. leucas* makes it to be a substantial part of developing commercial fishery (Compagno, 1984; Branstetter and Stiles, 1987), and the high fishing pressure makes this species vulnerable to local extirpation and enhances the global potential for extinction (Martin, 2005). For the past few years, a great many studies have been carried out on the fishery biology and life history of *C. leucas*, such as food habits (Snelson *et al*., 1984), age and growth (Neer *et al*., 2005), distribution and movement (Heupel and Simpfendorfer, 2008), and so on. However, few studies have investigated the genetic structure and genetic diversity of *C. leucas*.

Understanding fish genetic structure will contribute to a successful and sustainable long-term management. On the contrary, the failure to detect population structure can result in long-term declines or localized extirpation (Hueter *et al*., 2005). To some extent, it is more important for *C. leucas*, since it is a 'near threatened' species (Camhi *et al*., 2009). Meanwhile, due to the ability of bull sharks in penetrating freshwater and travelling long distances, coupled with the long life span and late age of maturity, one might predict global genetic exchange among bull shark populations (Karl *et al*., 2011). Nowadays, molecular approaches have been very useful in identifying and delineating fish stock structure (Chen and Gao, 2017), and have been used successfully to understand the structure of sharks (Feldheim *et al*., 2001; Keeney *et al*., 2005; Castro *et al*., 2007). Such approaches could also facilitate the study of this species.

The South China Sea locates in the center of Indo-Pacific regions and represents an area of globally significant marine shallow-water, tropical biodiversity. The Xisha and Nansha Islands are two important islands in the South China Sea and *C. leucas* is commonly found in this region. The Xisha and Nansha Islands become the ideal study locations to understand the genetic structure in this species, because ofong distances between two islands. A previous study including samples from 13 river systems across northern Australia, demonstrated that significant genetic structure existed among different nurseries and the females did not disperse randomly but were philopatric,

* Corresponding author: d6339124@163.com
0030-9923/2019/0004-1281 $ 9.00/0

returning to reproduce in the nurseries where they were born (Tillett *et al*., 2012). However, little is known about its population structure in the South China Sea or in a large-scale geography distribution including the South China Sea and northern Australia.

In the present study, we sequenced the 5'end of the mtDNA control region of *C. leucas* collected from the Xisha and Nansha islands in the South China Sea to reveal the population structure and genetic connectivity in this region. Furthermore, the data was also used to obtain more exact information on the population structure within the large-scale geography distribution of this species. The study will provide theoretical basis for fishery management and be helpful for the protection of *C. leucas*.

MATERIALS AND METHODS

Sample collection and DNA extraction

Through a scientific fishery resources survey, twenty three individuals were collected from Xisha Islands (16°77'N, 112°26'E; 16°67'N, 112°75'E), and Nansha Islands (9°43`N,115°52`E; 9°95`N,114°67`E; 10°19`N,114°23`E) in the South China Sea during May 2004 to July 2004 (Fig. 1; Table I). Muscle samples were preserved in 95% ethanol before DNA extraction. Genomic DNA was extracted from muscle tissue by proteinase K digestion followed by a standard phenol-chloroform method (Sambrook *et al*., 1982).

MtDNA control region amplification and sequencing

The first hypervariable fragment of mtDNA control region was amplified using forward primer DL-S: 5′-CCCACCACTAACTCCCAAAGC-3′ and reverse primer DL-R: 5′-CTGGAAAGAACGCCCGGCATG-3′ (Lee *et al*., 1995). Each polymerase chain reaction (PCR) was performed in a volume of 50 µL containing 20-50 ng template DNA, 5 µL of 10×reaction buffer, 5 µL of $MgCl_2$ (25 mM), 1 µL of dNTPs (10 mM), 10 pM of each primer and 2.5 units of *Taq* DNA polymerase (Promega, Madison, WI, USA) in an Eppendorf Mastercycler 5333 (Eppendorf, Hamburg, Germany). Sterile distilled H_2O was added to reach a total volume of 50 µL. Initial denaturation was for 3 min at 94°C, followed by 40 cycles of 45 s at 94°C for denaturation, 45 s at 50°C for annealing, 45 s at 72°C for extension and a final extension at 72°C for 10 min. Negative controls were conducted with all reagents included, except template DNA. PCR product was separated on a 1.5% agarose gel. Then clear PCR products were selected to purify with the Gel Extraction Mini Kit (Watson BioTechnologies Inc., Shanghai) and both strands were sequenced at last. Control region sequences have been deposited in the GenBank database under Accession Nos. MH488888 to MH488894.

Data analyses

Sequences were edited and aligned using DNASTAR software (DNASTAR, Inc., Madison, USA). Molecular diversity indices such as number of haplotypes, polymorphic sites, indels, transitions and transversions were obtained using the program ARLEQUIN (Ver.3.5) (Excoffier *et al*., 2010). Based on Tamura 3-parameter (Tamura, 1992) model, haplotype diversity (h), nucleotide diversity (π) and their corresponding variances were calculated in ARLEQUIN.

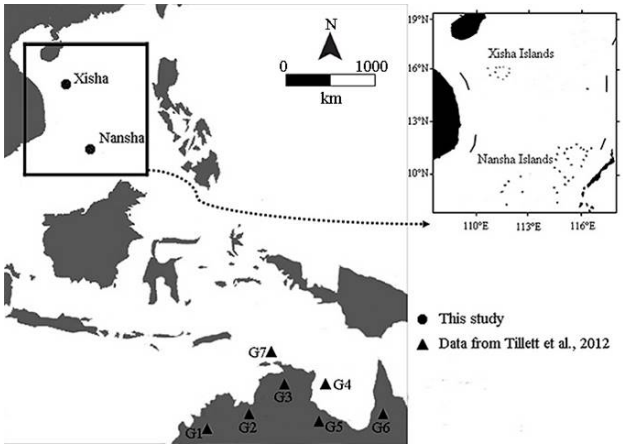

Fig. 1. Sampling localities for this study. Triangles represent populations from Tillett *et al*. (2012). G1, Mitchell River, Robison River and Fitzroy River; G2, Daly River and Ord River; G3, East Alliagator River; G4, Blue Mud Bay; G5, Roper River, Towns River, Limmen River and Robinson River; G6, Wenlock River, Mission River and Mitchell River; G7, Tiwi Islands. Circles represent locations sampled in this study (Xisha and Nansha Islands).

Table I.- Sampling information of *C. leucas* including sample size, date of collection, and molecular diversity indices.

Sample	Date of collection	Sample size	Number of haplotypes	Haplotype diversity	Nucleotide diversity	Mean pairwise difference
Xisha	2004.06	13	5	0.6923±0.1187	0.0059±0.0037	2.9519±1.6510
Nansha	2004.05	10	5	0.6667±0.1633	0.0140± 0.0081	7.0177±3.6025
Total		23	7	0.6640±0.0992	0.0097±0.0055	4.8686±2.4635

Table II.- Nucleotide sequences of control region used in the present study.

Region	Species	Sample locations	Sample size	GenBank accession numbers	References
Northern Australia	*C. leucas*	Mitchell River, Robison River, Fitzroy River	15	HQ324914, HQ324919, HQ324920	Hueter *et al*. (2005)
	C. leucas	Daly River, Ord River	44	HQ324914-HQ324916, HQ324920-HQ324922, HQ324925, HQ324926	
	C. leucas	East Alliagator River	22	HQ324914, HQ324915, HQ324919, HQ324920	
	C. leucas	Blue Mud Bay	18	HQ324914, HQ324919	
	C. leucas	Roper River, Towns River, Limmen River, Robinson River	27	HQ324914-HQ324919, HQ324926	
	C. leucas	Wenlock River, Mission River, Mitchell River	17	HQ324914, HQ324919, HQ324924	
	C. leucas	Tiwi Islands	26	HQ324914, HQ324919, HQ324920, HQ324923, HQ324924	
Western Atlantic	*C. limbatus*	Brazil		JX025760, JX025761	Sodre *et al*. (2012)

Table III.- Distribution of haplotypes in nine regions of *C. leucas*.

Haplotype	Xisha	Nansha	G1	G2	G3	G4	G5	G6	G7	Total
H1	7	6								13
H2		1								1
H3	1	1								2
H4	3	1								4
H5	1									1
H6		1								1
H7	1									1
H8*			14	31	16	18	24	16	23	142
H9*				3	1		1			5
H10*							1			1
H11*							1			1
H12*			1	6	5				1	13
H13*				3						3
H14*									1	1
H15*								1	1	2
H16*				1						1

The marked haplotypes (*) represented the sequences from northern Australia. The details of the places (G1- G7) have been marked in Figure 1.

Genetic relationships among haplotypes were reconstructed using the neighbor-joining method (NJ) (Saitou *et al*., 1987) implemented in MEGA (6.0) (Tamura *et al*., 2013). The homologous sequences of the mtDNA control region from genus *Carcharhinus* were downloaded from GenBank, which were used to construct NJ tree. *C. limbatus* was treated as out-group. The sources and numbers of the sequence were given in Table II. These sequences varied from 503 to 1068 bp and the consensus sequence was 503 bp.

Genetic differentiation between sample sites were tested by the fixation index F_{ST}, which was performed in ARLEQUIN. The significance of the F_{ST} was tested by 1000 permutations. In addition, analysis of molecular variation (AMOVA), performed in ARLEQUIN, was used to examine the population genetic structure. For these, the data provided by Tillett *et al*. (2012) (Tables II, III) were added in this study. We conducted AMOVA analysis with two groups representing the South China Sea and northern Australia. Two sample sites within the South China Sea and seven sample sites within northern Australia (Fig. 1) were measured to verify the significance of genetic variance within group, respectively. Their significance of the covariance components was tested using 1000 permutations.

The historical demographic pattern of *C. leucas* was investigated by using neutrality test (Fu, 1997) and mismatch distribution analysis (Rogers and Harpending, 1992). Both of them were applied in ARLEQUIN.

RESULTS

Sequence variation and genetic diversity

A 503 bp segment of the 5' end of the control region was obtained from 23 individuals (13 from Xisha Islands, 10 from Nansha Islands). Sequence comparison of this segment revealed 17 polymorphic sites, eleven of which were transitions, with six transversions and no deletions/insertions. These polymorphic sites defined seven haplotypes and three of them were shared in both islands

(Table III). Among all the haplotypes, H1 was the most common one (13 individuals; 56.52%). The C, T, A and G composition of the sequence were 21.02%, 37.50%, 32.17% and 9.31%, respectively, and the composition of A+T was richer than G+C. The overall nucleotide diversity (π) was 0.0097±0.0055 and haplotype diversity (h) was 0.6640±0.0992. The haplotype diversity was almost alike in Xisha and Nansha islands, but the nucleotide diversity of Nansha population was much higher than that of Xisha' (Table I).

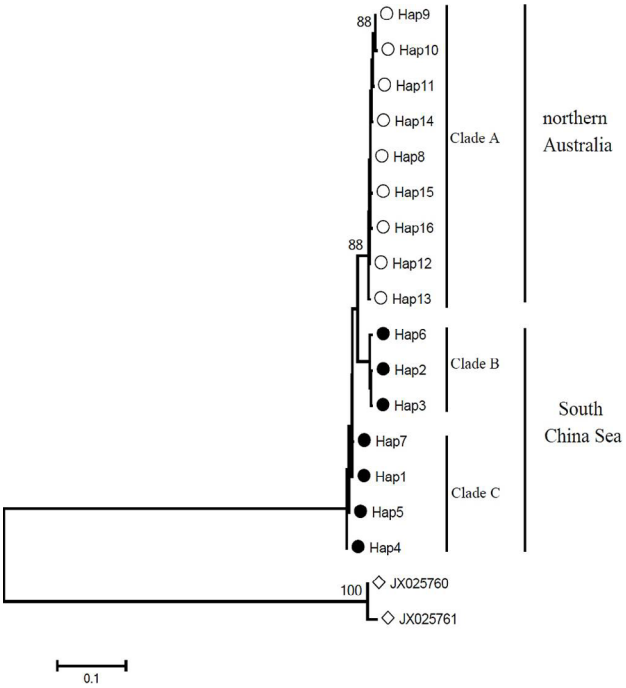

Fig. 2. Neighbor-joining tree constructed using Tamura 3-parameter model for control region haplotypes of *C. leucas*. The congener *C. limbatus* was chosen as out-group. The black solid and hollow circles represented the South China Sea and the northern Australia population, respectively. Triangles represented the haplotype of *C. limbatus*. Bootstrap supports of > 85% in 1000 replicates are shown.

Genetic structure

The NJ tree of *C. leucas* showed significant genealogical branches of haplotypes distinguishing them from the South China Sea or northern Australia group (Fig. 2). One branch (clade A) represented the haplotypes from northern Australia group and two branches (clade B and clade C) were from the South China Sea. Clade B from the South China Sea was firstly clustered with clade A from northern Australia and then the other clade from the South China Sea (clade C) was clustered with them. It indicated the close relationship between clade C and the ancestral lineage of clade A and clade B.

With regard to genetic differences, the F_{ST} values (data not shown) between the northern Australia and the South China Sea samples were high (> 0.80) and significant, but it was low (0.067) and non-significant within the South China Sea population. There was significant genetic structure between northern Australia and the South China Sea group revealed by AMOVA, with 92.29% of genetic variation was found among groups (P = 0.036) (Table IV). A small (0.34%) and no significant (P=0.111) of genetic variation was found among sampling sites within groups, while the remaining 7.37% (F_{ST} = 0.926, P < 0.05) resulted from variation within populations. To obtain more detail information, we conducted AMOVA analysis on the South China Sea and northern Australia group separately. The results revealed no significant genetic differences in the South China Sea group (P=0.181), but significant genetic differences in northern Australia group (P=0.027).

Table IV.- Results of AMOVA analysis of *C. leucas* populations.

Source of variation	Variance components	Percentage of variance	F/φ-statistics	P
All populations				
Among groups	6.415	92.29	0.923	0.036
Among sites within groups	0.024	0.34	0.044	0.111
Within populations	0.512	7.37	0.926	0.000
Northern Australia group				
Among sites	0.009	3.27	0.033	0.027
Within populations	0.274	96.73		
South China Sea group				
Among sites	0.170	6.73	0.067	0.181
Within populations	2.347	93.27		

Historical demographics

A plot of the mismatch distribution that included all of the samples of northern Australia and the South China Sea resulted in a multimodal curve (Fig. 3A) that could be accounted for by structuring of the different groups of haplotypes. When only the northern Australia samples were included, the curve was unimodal (Fig. 3B), which corresponded to the result obtained by Tillett *et al*. (2012). The bimodal curve was found in the South China Sea samples (Fig. 3C), which may be related to the existence of two groups of haplotypes or the less individuals. All

of them did not significantly deviate the demographics expansion model (P>0.05), which indicated they could be used to analyze historical demographics.

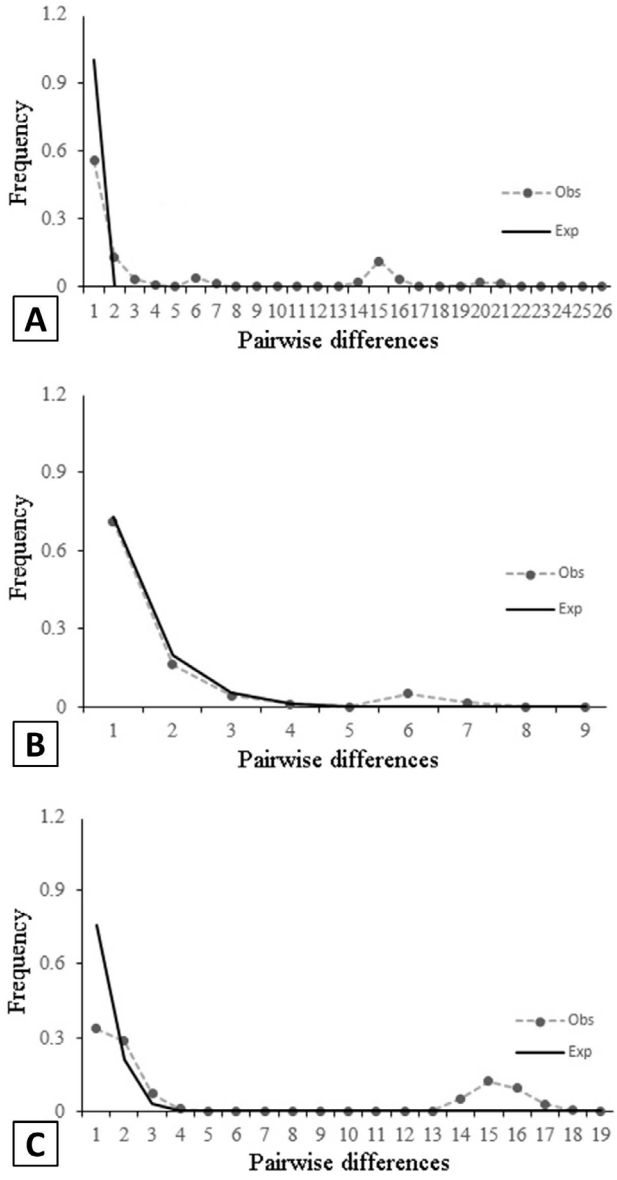

Fig. 3. Mismatch distribution of all populations (A), northern Australia populations (B) and South China Sea populations (C). The solid line represents the curve expected (Exp.) based on the expansion model. Obs., observed.

To obtain more precise estimates, the neutrality test was performed for each population. The result of neutrality test for northern Australia population was negative (Fu's F_S= -4.37, P = 0.046), but the result of the South China Sea was different (Fu's F_S=1.90, P=0.796). Both of mismatch distribution and neutrality test revealed that the northern Australia population had experienced demographic expansions, while the South China Sea population was relatively stable. The result of neutrality test for northern Australia population was in line with it obtained by Tillett *et al*. (2012).

DISCUSSION

The overall haplotype and nucleotide diversities observed for the mtDNA control region of the bull sharks were low for such a large and geographically widespread shark species. These values were lower than the species which showed similar ability to make large-scale movements, such as blue sharks (*Prionace glauca*) (h= 0.9973 ± 0.0014, π= 0.0151 ± 0.0009) (Zheng *et al*., 2014), but were higher than oceanic whitetip shark, (*Carcharhinus longimanus*) (h= 0.5953, π= 0.0013). One of the main explanations might be that the changes in population size would affect levels of standing genetic variation (Nei *et al*., 1975). The close ecological association of the bull sharks with heavily impacted coastal estuarine environments might make them highly susceptible to human-induced population size reductions (Martin, 2005). The comparison with *C.longimanus* seemed to be an evidence for it, as *C. longimanus* became "vulnerable" throughout its range and "critically endangered" in the western north Atlantic (Baum *et al*., 2006; Camhi *et al*., 2009). Of course, any recent human impact on population size was not sufficient to have substantially changed levels of genetic variability in these species. Moreover, we cannot rule out the possibility that selection can reduce standing genetic variation dramatically.

It had been proven that *C. leucas* exhibited a greater degree of reproductive philopatry in female (Karl *et al*., 2011; Tillett *et al*., 2012). This behavior can lead to marked genetic heterogeneity among nurseries (Keeney *et al*., 2003). The North Pacfic humpback whales, Australian white sharks and blacktip sharks that practiced reproductive philopatry were also found with restricted maternal gene flow among different nurseries, despite the high vagility of them (Baker *et al*., 2013; Blower *et al*., 2012; Keeney *et al*., 2003). Contrary to this phenomenon, the result of AMOVA analysis in the South China Sea samples revealed the absence of genetic heterogeneity in mitochondrial control region. Additionally, the F_{ST} value between Xisha and Nansha was also low and non-significant. These results indicated that Xisha and Nansha' *C. leucas* might have a mutual nursery. This assumption was supported by the conclusion of Tillett *et al*. (2012) that significant mtDNA population genetic structure existed between individual nurseries, but not within. Nevertheless,

we couldn't find the nursery of the *C. leucas* in the South China Sea. After all, the ability of travelling long distances (>1500 km) (Carlson *et al*., 2010) in this species made it easy for them to reach every corner of the South China Sea.

The analysis of a wider spread of samples showed that there was significant genealogical branches of haplotypes corresponding to sampling locality and no mutual haplotype between the South China Sea and northern Australia population. The explanation for the close relationship between clade C and the ancestral lineage of clade A and clade B might be that the ancestral lineage of clade A and clade B existed in both the South China Sea and northern Australia, but after a long period of isolation, it diverged. The F_{ST} values between the northern Australia and the South China Sea samples were high (> 0.80) and significant, but F_{ST} value was low (0.067) and non- significant within the South China Sea population. The results indicated that this species had strong ability of locomotion, but it cannot span so large-scale region (>3000 km). Geographical isolation generated genetic heterogeneity among bull sharks in the center of Indo-Pacific regions.The result of neutrality test for the South China Sea population indicated relatively stable historical demographics of this species in this region, but it was not the case in northern Australia. The main explanation for it might be that the habitat of this species did not change significantly in the South China Sea, but changed significantly in northern Australia. According to the report of maps of Pleistocene sea levels in Southeast Asia (Voris, 2000), with the decline of sea level during the Pleistocene ice age, the marginal area of the South China Sea changed little, but the area of Timor Sea and Arafura Sea experienced a "cliff-like drop". Hence, this species from northern Australia experienced a process of rapid habitat expansion, which caused demographic expansions in this population. It was the different surroundings that resulted different historical demographics in the same species.

CONCLUSIONS

In the present study, we concluded that there might have only one fishery management unit of *C. leucas* in the South China Sea. Meanwhile, we also investigated the structure within the large-scale geography distribution of this species, which revealed genetic heterogeneity among *C. leucas* in the center of Indo-Pacific regions and this result could be helpful for biological conservation and fishery management for *C. leucas*. The genetic diversity of *C. leucas* was low in our study, which indicated that the quality of its population was poor. As a result, we suggested the conservation priority for *C. leucas*. Without doubt, the lack of individuals and single molecular marker will bring incomprehensive analyses. Consequently, we will collect more specimens in the South China Sea and the surrounding sea of Malaysia and Republic of Indonesia, using various molecular markers, and attempt to reveal the population genetic structure and genetic diversity comprehensively in our future studies.

ACKNOWLEDGEMENTS

We sincerely thank the reviewer for the constructive criticisms and valuable comments. The research was funded by the National Natural Science Foundation of China (31472281) and the National Key Research and Development Program of China (2017YFA0604904).

Statement of conflict of interest

The authors declare no conflicts of interest.

REFERENCES

Baker, C.S., Steel, D., Calambokidis, J., Falcone, E.A., González-Peral, U., Barlow, J., Burdin, A.M., Clapham, P.J., Ford, J.K.B., Gabriele, C.M., Mattila, D., Rojas-Bracho, L., Straley, J.M., Taylor, B.L., Urbán, J., Wade, P.R., Weller, D., Witteveen, B.H. and Yamaguchi, M., 2013. Strong maternal fidelity and natal philopatry shape genetic structure in North Pacfic humpback whales. *Mar. Ecol. Progr. Ser*., **494**: 291-306. https://doi.org/10.3354/meps10508

Baum, J., Medina, E., Musick, J.A. and Smale, M., 2006. *Carcharhinus longimanus*. In: *IUCN red list of threatened species*. International Union for Conservation of Nature, Gland, Switzerland.

Blower, D.C., Pandolfi, J.M., Bruce, B.D., Gomezcabrera, M.D.C. and Ovenden, J.R., 2012. Population genetics of Australian white sharks reveals fine-scale spatial structure, transoceanic dispersal events and low effective population sizes. *Mar. Ecol. Progr. Ser*., **455**: 229-244. https://doi.org/10.3354/meps09659

Branstetter, S. and Stiles, R., 1987. Age and growth estimates of the bull shark, *Carcharhinus leucas*, from the northern gulf of Mexico. *Environ. Biol. Fishes*, **20**: 169-181. https://doi.org/10.1007/BF00004952

Brunnschweiler, J.M. and Earle, J.L., 2006. A contribution to marine life conservation efforts in the South Pacific: The Shark Reef Marine Reserve, Fiji. *Cybium*, **30**: 133-139.

Camhi, M.D., Valenti, S.V., Fordham, S.V., Fowler, S.L.

and Gibson, C., 2009. *The conservation status of pelagic sharks and rays: Report of the IUCN shark specialist group pelagic shark red list workshop*. IUCN Species Survival Commission Shark Specialist Group, Newbury, UK, pp. 8-9.

Castro, A.L.F., Stewart, B.S., Wilson, S.G., Hueter, R.E., Meekan, M.G., and Motta, P.J., Bowen, B.W. and Karl, S.A., 2007. Population genetic structure of earth's largest fish, the whale shark (*Rhincodon typus*). *Mol. Ecol*., **16**: 5183-5192. https://doi.org/10.1111/j.1365-294X.2007.03597.x

Compagno, L.J.V., 1984. *FAO species catalogue. Sharks of the world. An annotated and illustrated catalogue of shark species known to date. II. Carcharhiniformes*. FAO Fisheries Synopsis, Rome, Italy.

Carlson, J.K., Ribera, M.M., Conrath, C.L., Heupel, M.R. and Burgess, G.H., 2010. Habitat use and movement patterns of bull sharks, *Carcharhinus leucas* determined using pop-up satellite archival tags. *J. Fish Biol*., **77**: 661-675. https://doi.org/10.1111/j.1095-8649.2010.02707.x

Chen, D. and Zhang, M., 2015. *Marine fishes of China*. China Ocean University Press, Qingdao, China, pp. 73-74.

Chen, Z. and Gao, T., 2017. Morphological re-description and DNA barcoding of *Platycephalus indicus* collected from Beihai, China. *Pakistan J. Zool.,* **49**: 1647-1655. http://dx.doi.org/10.17582/journal.pjz/2017.49.5.1647.1655

Excoffier, L. and Lischer, H.E.L., 2010. Arlequin suite, ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour*., **10**: 564-567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Feldheim, K.A., Gruber, S.H. and Ashley, M.V., 2001. Population genetic structure of the lemon shark (*Negaprion brevirostris*) in the western Atlantic: DNA microsatellite variation. *Mol. Ecol*., **10**: 295-303. https://doi.org/10.1046/j.1365-294x.2001.01182.x

Fu, Y.X., 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**: 915-925.

Gadig, O.B.F., Juliano, M.F. and Barreiros, J.P., 2006. Further notes on the capture of a *Carcharhinus leucas* in a northeastern Atlantic oceanic insular shelf, the Azores Archipelago, Portugal. *Cybium*, **30**: 31-33.

Heupel, M.R. and Simpfendorfer, C.A., 2008. Movement and distribution of young bull sharks *Carcharhinus leucas* in a variable estuarine environment. *Aquat. Biol*., **1**: 277-289. https://doi.org/10.3354/ab00030

Hueter, R.E., Heupel, M.R., Heist, E.J. and Keeney, D.B., 2005. Evidence of philopatry in sharks and implications for the management of shark fisheries. *J. N. Atlant. Fish. Sci*., **37**: 239-247.

Karl, S.A., Castro, A.L.F., Lopez, J.A., Charvet, P. and Burgess, G.H., 2011. Phylogeography and conservation of the bull shark (*Carcharhinus leucas*) inferred from mitochondrial and microsatellite DNA. *Conserv. Genet*., **12**: 371-382. https://doi.org/10.1007/s10592-010-0145-1

Keeney, D.B., Heupel, M.R., Hueter, R.E. and Heist, E.J., 2003. Genetic heterogeneity among blacktip shark, *Carcharhinus limbatus*, continental nurseries along the US Atlantic and Gulf of Mexico. *Mar. Biol*., **143**: 1039-1046. https://doi.org/10.1007/s00227-003-1166-9

Keeney, D.B., Heupel, M.R., Hueter, R.E. and Heist, E.J., 2005. Microsatellite and mitochondrial DNA analyses of the genetic structure of blacktip shark (*Carcharhinus limbatus*) nurseries in the northwestern Atlantic, Gulf of Mexico, and Caribbean Sea. *Mol. Ecol*., **14**: 1911-1923. https://doi.org/10.1111/j.1365-294X.2005.02549.x

Kitamura, T., Takemura, A., Watabe, S., Taniuchi, T. and Shimizu, M., 1996. Mitochondrial DNA analysis for the cytochrome b gene and D-loop region from the bull shark *Carcharhinus leucas*. *Fish. Sci*., **62**: 21-27. https://doi.org/10.2331/fishsci.62.21

Lee, W.J, Conroy, J., Howell, W.H. and Kocher, T.D., 1995. Structure and evolution of teleost mitochondrial control regions. *J. mol. Evolut*., **41**: 54-66.

Martin, R.A., 2005. Conservation of freshwater and euryhaline elasmobranchs: A review. *J. Mar. biol. Assoc. U.K*., **85**: 1025-1047. https://doi.org/10.1017/S0025315405012105

Montoya, R.V. and Thorson, T.B., 1982. The bull shark (*Carcharhinus leucas*) and largetooth sawfish (*Pristis perotteti*) in Lake Bayano, a tropical man-made impoundment in Panama. *Environ. Biol. Fishes*, **7**: 341-347. https://doi.org/10.1007/BF00005568

Neer, J., Thompson, B. and Carlson, J., 2010. Age and growth of *Carcharhinus leucas* in the northern Gulf of Mexico: Incorporating variability in size at birth. *J. Fish Biol.,* **67**: 370-383. https://doi.org/10.1111/j.0022-1112.2005.00743.x

Nei, M., Maruyama, T. and Chakraborty, R., 1975. The bottleneck effect and genetic variability in populations. *Evolution*, **29**: 1-10. https://doi.org/10.1111/j.1558-5646.1975.tb00807.x

Rogers, A.R. and Harpending, H., 1992. Population growth makes waves in the distribution of pairwise genetic differences. *Mol. Biol. Evolut.,* **9**: 552-569.

Saitou, N. and Nei, M., 1987. The neighbour-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evolut.,* **4**: 406-425.

Sambrook, J., Fritsch, E.F. and Maniatis, T., 1982. *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory, pp. 895-909.

Snelson, F.F., Timothy, J., Mulligan, J. and Williams, S.H., 1984. Food habits, occurrence, and population structure of the bull shark, *Carcharhinus leucas*, in Florida coastal lagoons. *Bull. Mar. Sci*., **34**: 71-80.

Tamura, K., 1992. The rate and pattern of nucleotide substitution in drosophila mitochondrial DNA. *Mol. Biol. Evolut.,* **9**: 814-825.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S., 2013. Mega6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evolut.,* **30**: 2725-2729. https://doi.org/10.1093/molbev/mst197

Thomerson, J.E., Thorson, T.B. and Hempel, R.L., 1977. The bull shark, *Carcharhinus leucas*, from the upper Mississippi River near Alton, Illinois. *Copeia*, **1977**: 166-168. https://doi.org/10.2307/1443522

Tillett, B.J., Meekan, M.G., Field, I.C., Thorburn, D.C. and Ovenden, J.R., 2012. Evidence for reproductive philopatry in the bull shark *Carcharhinus leucas*. *J. Fish Biol*., **80**: 2140-2158. https://doi.org/10.1111/j.1095-8649.2012.03228.x

Voris, H.K., 2000. Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. *J. Biogeogr*., **27**: 1153-1167. https://doi.org/10.1046/j.1365-2699.2000.00489.x

Wang, P., Tian, J., Cheng, X., Liu, C. and Xu, J., 2004. Major Pleistocene stages in a carbon perspective: The South China Sea record and its global comparison. *Paleoceanography*, **19**: PA4005. https://doi.org/10.1029/2003PA000991

Zheng, Z.Z., Xu, Q.H., Dai, X.J. and Zhu, J.F., 2014. Population genetic structure of blue shark *Prionace glauca* based on partial control region sequence in mitochondrial DNA. *J. Dalian Ocean Univ*., **5**: 463-468.