Genetic diversity and connectivity of the megamouth shark (*Megachasma pelagios*)

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The megamouth shark (*Megachasma pelagios*) was described as a new species in 1983. Since then, only *ca*. 100 individuals have been observed or caught. Its horizontal migration, dispersal, and connectivity patterns are still unknown due to its rarity. Two genetic markers were used in this study to reveal its genetic diversity and connectivity pattern. This approach provides a proxy to indirectly measure gene flow between populations. Tissues from 27 megamouth sharks caught by drift nets off the Hualien coast (eastern Taiwan) were collected from 2013 to 2015. With two additional tissue samples from megamouths caught in Baja California, Mexico, and sequences obtained from GenBank, we were able to perform the first population genetic analyses of the megamouth shark. The mtDNA *cox1* gene and a microsatellite (Loc 6) were sequenced and analyzed. Our results showed that there is no genetic structure in the megamouth shark, suggesting a possible panmictic population. Based on occurrence data, we also suggest that the Kuroshio region, including the Philippines, Taiwan, and Japan, may act as a passageway for megamouth sharks to reach their feeding grounds from April to August. Our results provide insights into the dispersal and connectivity of megamouth sharks. Future studies should focus on collecting more samples and conducting satellite tagging to better understand the global migration and connectivity pattern of the megamouth shark.

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18   **Abstract:**

19   The megamouth shark (*Megachasma pelagios*) was described as a new species in 1983. Since

20   then, only *ca*. 100 individuals have been observed or caught. Its horizontal migration, dispersal,

21   and connectivity patterns are still unknown due to its rarity. Two genetic markers were used in

22   this study to reveal its genetic diversity and connectivity pattern. This approach provides a proxy

23   to indirectly measure gene flow between populations. Tissues from 27 megamouth sharks caught

24   by drift nets off the Hualien coast (eastern Taiwan) were collected from 2013 to 2015. With two

25   additional tissue samples from megamouths caught in Baja California, Mexico, and sequences

26   obtained from GenBank, we were able to perform the first population genetic analyses of the

27   megamouth shark. The mtDNA *cox1* gene and a microsatellite (Loc 6) were sequenced and

28   analyzed. Our results showed that there is no genetic structure in the megamouth shark,

29   suggesting a possible panmictic population. Based on occurrence data, we also suggest that the

30   Kuroshio region, including the Philippines, Taiwan, and Japan, may act as a passageway for

31   megamouth sharks to reach their feeding grounds from April to August. Our results provide

32   insights into the dispersal and connectivity of megamouth sharks. Future studies should focus on

33   collecting more samples and conducting satellite tagging to better understand the global

34   migration and connectivity pattern of the megamouth shark.

35   **Introduction**

36   The megamouth shark, *Megachasma pelagios*, was accidentally captured in 1976 off the coast of

37   Kāneʻohe, Hawaiʻi, and was examined and described as a new species in 1983 (*Taylor et al.,*

38   *1983*). More than forty years since its discovery, only about 100 specimens have as yet been

39   caught or documented. There are only few official tracking records including a review by Nakaya

40   (2010), which documented 40 records of these sharks being either caught or released from 1976

41   to 2007. The Ichthyology section of the Florida Museum of Natural History has documented 65

42   sighting records from 1976 to 2016

43   (https://www.floridamuseum.ufl.edu/fish/discover/sharks/megamouths/reported-sightings). In

44   addition, Hsu et al. (2015) published the catch and fishery information of 34 megamouth sharks

45   collected off Hualian, Taiwan, between 2013 and 2015. With these documents and an additional

46   specimen recently caught off the coast of Puerto Rico in 2016 (*Rodriguez-Ferrer et al., 2017*),

47   only 99 individuals have been officially recorded. To date, relatively few studies have focused on

48   this species compared to other, better known sharks. It is suggested to be a widely distributed

49   species across the world's oceans, including the Indian, Pacific, and Atlantic. Males become

50   mature at about 4 m in total length and females at about 5 m, and mating occurs all year round

51   based on the record of 40 specimens sampled from 1976-2008 (*Nakaya, 2010*). Their daily

52   movements were recorded by acoustic tags and showed a very clear vertical movement. This

53   vertical movement indicated they swim at depths around 200 m during daytime, move toward the

54   surface at dusk, remain around 20 m during nighttime, and move back down to a deeper layer at

55   dawn (*Nelson et al., 1997*). This shark feeds exclusively on euphausiids (*Taylor et al., 1983*; *Yano*

56   *et al.*, *1998*; *Sawamato & Matsumoto, 2012*) and employs engulfment feeding analogous to

57   humpback whales (*Nakaya et al., 2008*). Their pectoral fins are very flexible and mobile, which

58   enhance dynamic lift control and thus give stability while swimming at slow speed (*Tomita et al.,*

59   *2014*). In addition, due to the scarcity and vulnerability of these sharks, satellite tagging has not

60   yet been feasible. Therefore, information about their horizontal movement and migration is still

61   unknown. Among the sharks recorded, only few specimens have been used for genetic studies

62   (*i.e.* phylogenetic relationships, mitochondrial genome) (*Martin & Naylor*, *1997*; *Chang et al.*

63   *2014*), and most of them were discarded or consumed. Due to its rarity, population studies such as

64   demographics, population structure, and genetic diversity among different geographic regions are

65   difficult to conduct.

66   The region along the Kuroshio Current path, including the Philippines, Taiwan, and Japan, are the

67   countries where the megamouth shark is frequently found (74 out of 99). The number of

68   documented records from Taiwan (45 out of 99) was the highest in the world.

69   Taiwan initiated its National Plan of Action concerning sharks in May 2006 (*Taiwan Fisheries*

70   *Agency*, *2006*) and implemented a ban on shark finning in 2012. Additionally, to monitor the

71   catch of several threatened shark species, the Taiwan Fisheries Agency implemented a mandatory

72 catch and report measurement scheme in 2013 that included the megamouth shark (*M. plagios*),

73 basking shark (*Cetorhinus maximus*), and great white shark (*Carcharodon carcharias*) in addition

74 to the whale shark (*Rhincodon typus*). When these species are caught, fishers must immediately

75 inform the local Fishery Agency, Taiwan Fisheries Agency, and shark experts (National Taiwan

76 Ocean University) before further processing. Due to this management measure, our team was

77 able to obtain fishery biology information such as total length, body weight, sex, and the

78 relationships between measurements and tissue samples before the sharks were processed and

79 sold (*Hsu et al., 2015*).

80 Sharks are facing global decline, and the effects (*i.e.* lost of genetic diversity) of population

81 decline are of major concern in marine conservation (*Pinsky & Plumbi, 2014*). Loss of genetic

82 diversity has several potential consequences on the reduction in evolutionary potential and

83 adaptive ability (*i.e.* decreasing fitness and resistance) (*Frankham 2005*; *Allendorf et al., 2008*).

84 The objectives of this study were to reveal the genetic diversity and connectivity of the

85 megamouth shark with 2 tissue samples collected from the Baja California, Mexico, 27 tissue

86 samples from Hualien, eastern Taiwan, and published sequences from GenBank.

87 **Materials and Methods**

88 A total of 27 tissue samples of *M. plagios* were collected between 2013 and 2015 off Hualien

89   county, eastern Taiwan (Figure 1). These sharks were caught at night before dawn between April

90   and August and were the bycatch of drift-gillnet fishery. This fishery operated year round, mainly

91   targeting sun fishes during spring and summer and bill fish during fall and winter. Basic

92   information recorded included catch date, sex, body weight, and total length. Additionally,

93   maturity stages were determined by examining the developmental status of sexual organs. Males

94   having fully calcified claspers that twisted easily and fully developed testes and epididymides

95   were determined to be adults. Females with mature ova in their ovaries (both ovaries in the

96   megamouth shark) and having swollen oviducts and uteri were determined to be adults. If only

97   one or portions of these organs were developed, individuals were determined to be sub-adults,

98   and those whose sexual organs were in undeveloped stages were determined to be juveniles.

99   Meanwhile, tissue samples were collected at the harbor before further commercial processing,

100   preserved in 95% alcohol, and stored at 4 °C. In addition to samples collected from Taiwan, we

101   obtained two tissue samples deposited in the Scripps Institute of Oceanography, University of

102   California, San Diego, that were collected from the coastal area of Baja California, Mexico (SIO-

103   07-53, Bahia Tortugas; SIO11-299, Bahia Sebastian Vizcaino). One *cox1* sequence downloaded

104   from GenBank was derived from a specimen collected from Mojacasabe Beach, Cabo Rojo,

105   Puerto Rico (17.980570 N,−67.210663 W), and one from Indonesia (Figure 1).

106   Genomic DNA was extracted from tissue fragments using commercial DNA extraction kits

107   (Geneaid Tissue Genomic DNA mini Kit, Geneaid Biotech, Taiwan). DNA extracts were diluted

108   in TE buffer and stored at -20 °C until amplification by polymerase chain reaction (PCR).

109   **Amplification of genetic markers**

110   The partial mitochondrial DNA gene *cox1* was amplified with the primer pair F1/R1 described by

111   *Ward et al*., (2005). An additional microsatellite locus (Loc6) that has been successfully cross-

112   amplified in lamniform sharks was also amplified, since it showed a high variation in not only

113   repeat number but also flanking regions (*Martin et al*., *2002*). PCRs were run in 30 μ*L* reactions

114   containing 10-40 ng template DNA, 3 μL 10X buffer, 0.2 m*M* dNTPs, 1.5 m*M* MgCl$_2$, 10 m*M* of

115   each primer, and 0.2 units of Taq polymerase (MDbio, Taipei). The thermocycling profile

116   consisted of initial denaturation at 94 °C for 2 min followed by 35 cycles of denaturation at 94 °C

117   for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 40 s, and a final extension at 72 °C

118   for 2 min. This program was used to amplify the *cox1* gene and Loc6. The nucleotide sequences

119   of PCR products of both loci were determined using an ABI 377 automated sequencer (Carlsbad

120   CA, U.S.A.). Nucleotide sequences were assembled and edited using Geneious 9.1.2 (Biomatters,

121   New Zealand).

122   **Genetic analyses**

123   Two *cox1* gene sequences of individuals from Indonesia (EU3938905) and Puerto Rico

124   (KY392958.1) were downloaded from GenBank. In addition, a Loc6 sequence derived from a

125   Japanese specimen was downloaded (AF423063) (Figure 1). Arlequin 3.5 (*Excoffier & Lischer,*

126   *2010*) was used to analyze genetic diversity indexes, including haplotype diversity (*h*) and

127   nucleotide diversity (π). Sequences were aligned and exported to MEGA 7 (*Tamura et al., 2013*)

128   to visually inspect all alignments. Phylogenetic analyses were used to reveal potential genetic

129   divergences among specimens from different geographic locations, with maximum likelihood

130   (ML) and Bayesian inference assessments being performed on the CIPRES Science Gateway

131   (*Miller et al., 2015*) and MrBayes (MB) version 3.2.2 (*Ronquist et al., 2012*), respectively. The

132   latter implemented two parallel runs of four simultaneous Markov chains for 10 million

133   generations, sampling every 1000 generations and using default parameters. The first million

134   generations (10%) were discarded as burn-in, based on the stationarity of log- likelihood tree

135   scores. ML analyses were conducted in RAxML version 8.1.24 (*Stamatakis, 2014*) using the

136   HKY substitution model chosen by MEGA 7. Supporting values on the branch were evaluated by

137   non-parametric bootstrapping with 1000 replicates performed with RAxML (ML) and by

138   posterior probabilities (MB). Moreover, median-joining haplotype networks were generated

139   based on *cox1* and Loc6 sequence datasets by using Popart 1.7 (*Leigh & Bryant, 2015*).

140   **Results**

141   **Catch information**

142   Basic catch information showed that megamouth sharks were mainly caught between April and

143   August, with total weights ranging 210-1147 kg and total lengths ranging 341-710 cm. The sex

144   ratio (female : male) was 16:11, which was not significantly different from 1:1. Five of the 27

145   individuals were determined to be adults and the others were sub-adults (Table 1). A global

146   sighting record list based on the scientific literature comprising 99 specimens is given as

147   supplementary information (Table S1).

148   **Genetic information**

149   The *cox1* gene (623 bp) and Loc6 microsatellite sequence (592 bp) were amplified and analyzed

150   for 29 individuals obtained from Taiwan and Mexico. Three individuals failed to amplify on both

151   loci, including MP3, MP16, and MP21, due to low DNA quality. There were two parsimony

152   informative sites, and the nucleotide diversity (p) and haplotype diversity (h) of the *cox1* gene

153   was 0.000616 ± 0.000695 (mean ± SD) and 0.3305 ± 0.1083, respectively. Twenty-seven *cox1*

154   sequences were composed of three unique haplotypes, and the sequences from Taiwan, Mexico,

155   Indonesia, and Puerto Rico shared a dominant haplotype (Figure 2A haplotype network). The

156   phylogenetic analyses showed that the sequences we used in the present study formed a

157   monophyletic clade and that there were two nodes with substantial support, including one

158   composed of MP2, MP7, and MP26, and the other composed of MP11 and MP18 (Figure 2A).

159   On the other hand, MP7 and MP24 failed to amplify for Loc6 from a sequence downloaded from

160   GenBank derived from a Japanese specimen; therefore, a total of 25 sequences were obtained for

161   further genetic analyses. Our results showed that the 23 sequences from Taiwan and 2 from the

162   Mexico were identical. The haplotype derived from the Japanese coast specimen had one

163   singleton and formed a unique haplotype separate from the dominant one. No parsimony

164   informative sites were found, and in addition, phylogenetic analyses showed that those sequences

165   were clustered as a single clade in the topology of the *cox1* gene tree.

166   **Discussion**

167   **Kuroshio as the passage to feeding grounds**

168   More than 74% (74/99) of sighting records were from countries along the Kuroshio Current,

169   including the Philippines, Taiwan, and Japan. Therefore, this region is likely a hotspot for the

170   occurrence of the megamouth shark. Along the east coast of Taiwan particularly, different sizes of

171   megamouth sharks were caught mainly from April to August off the Hualien coast (Table1). The

172   stomach contents of a megamouth shark caught off Ibaraki Prefecture (Japan) suggested that it

173   fed almost exclusively on *Euphausia pacifica* (*Sawamoto & Matsumoto, 2012*). *Euphausia*

174   *pacifica* is the dominant species of euphausiid in the North Pacific (*Boden et al., 1955; Brinton,*

175   *1975*) and dominates the zooplankton community in the East Sea (Sea of Japan) (*Mauchline,*

176   *1980*) and Yellow Sea (*Yoon et al., 2000*). Endo (1981) reported that the eggs and larvae of this

177   species occur throughout the year in Sanriku waters, but are most abundant in April–June. In the

178   Yellow Sea, *E. pacifica* was the most dominant euphausiid species in both summer and winter

179   (*Yoon et al., 2000*). Therefore, we propose that the Kuroshio Current may be the lower latitude

180   passage for the megamouth shark to reach its feeding grounds in higher latitudes such as the

181   Yellow Sea and Sanriku waters where *E. pacifica* is abundant. Seasonal movements between

182   productive high-latitude feeding grounds and low-latitude breeding grounds have been commonly

183   used to explain the migration of baleen whales (*e.g.*, Norris, 1967), and we suggest this may also

184   explain the seasonal migration of the megamouth shark. However, a future satellite tagging study

185   is needed to track the movement and habitat use of the megamouth shark to verify this

186   hypothesis.

187   **Genetic diversity and connectivity in the megamouth shark**

188   Although the megamouth shark appears to be very rarely encountered throughout its range, IUCN

189   assessed its population status as Least Concern based on its wide distribution. This rarity may

190   lead to intrinsic sensitivity to overexploitation since the effects of genetic drift are stronger in

191   smaller populations, which ultimately leads to a substantial loss of genetic variation (*Allendorf et*

192 *al. 2008*) and consequently increases the probability of the fixation of deleterious alleles and

193 reduces the resilience of overfished species (*Hare et al. 2011*). Genetic diversity is also one of the

194 important indexes to be considered in shark management and conservation polices because the

195 long-term survival of a species is strongly dependent on the levels of genetic diversity within and

196 between populations (*Domingues et al. 2017*). In the present study, the increasing number of

197 captures in the Kuroshio region (Table S1), particularly Taiwan, may indicate increasing fishing

198 pressure on megamouth sharks. Comparing its *cox1* genetic diversity that of with other sharks

199 (*Alopias pelagicus*, *Scyliorhinus canicula*, *Squalus blainville*, and *R. typus*; Table 2), the

200 megamouth shark has the lowest nucleotide diversity (0.000616), and relatively lower haplotype

201 diversity (0.3305). Among these sharks, the pelagic thresher shark (*A. pelagicus*) is one of the

202 most abundant open ocean sharks and one of the most over-exploited shark species in the Pacific

203 (*Tsai et al. 2010*; *Caballera et al. 2011*). Even under great fishing pressure, its nucleotide

204 diversity was more than 20-fold higher than that of the megamouth shark. This may imply that

205 the megamouth shark has a smaller population according to the positive correlation between

206 population size and genetic variation found in a previous study (*Frankham et al. 1996*), whether

207 being overexploited or a combination of both.

208   On the other hand, information regarding population connectivity is an important consideration

209   when establishing conservation strategies to manage threatened species. In sharks, habitat usage

210   could be one of the major factors influencing the connectivity pattern. For example, pelagic

211   sharks (*e.g.*, the basking shark *Cetorhinus maximus*, whale shark *R. typus*, and blue shark

212   *Prionace glauca*) undergoing long oceanic movements showed less genetic structure either

213   within-ocean or between-ocean scales compared to coastal sharks, except that the whale shark

214   showed a genetic break between the Pacific and Atlantic Oceans (Table 3). In the present study,

215   neither the mitochondrial *cox1* gene nor Loc6 sequence revealed any genetic structure. While a

216   *cox1* gene sequence from a specimen caught in the Caribbean was included in the analysis, it was

217   identical to the dominant *cox1* gene haplotype found in the Pacific. This indicates the megamouth

218   shark might travel across the world's oceans, which corresponds to its pelagic-oceanic life.

219   Therefore, the careful tracking of fisheries captures and the implementation of a long-term global

220   monitoring program are needed to reassess its population status and ensure that this species does

221   not become threatened in the near future.

222   **Conclusions**

223   In conclusion, the Kuroshio Current region may act as a passageway for the megamouth shark to

224 reach its feeding grounds during April to August. No genetic structure and low genetic diversity

225 were found in the megamouth shark, suggesting a small population and the ability to travel across

226 oceans. However, due to the small sample size and lower variability of the loci used in the

227 present study, connectivity between sites could be overestimated. Even though most recorded

228 megamouth sharks have been discarded, sold, or merely observed in the wild and thus provided

229 only 29 tissue samples, our data set is still the best dataset available. Nonetheless, to better

230 understand the movement and migration of the megamouth shark, we recommend that in future

231 studies the sample size be increased, hyper variable loci (microsatellite loci or SNPs) be used,

232 and the pop-up satellite tag method be applied.

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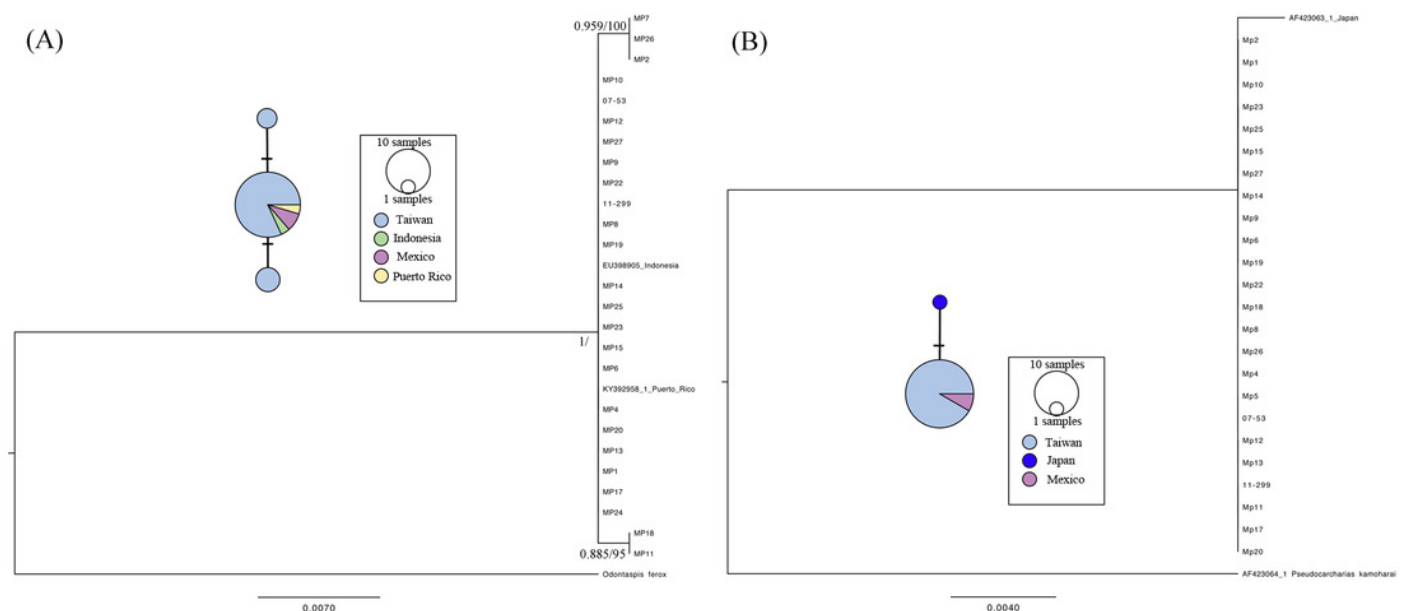
Figure 1

Sampling sites of the megamouth shark (*M. plagios*).

* indicates tissue sample sites and + indicates sequences downloaded from GenBank.

Figure 2

Maximum-likelihood phylogenetic trees and the median joining haplotype network based on the *cox 1* gene (A) and Loc6 (B) sequence data.

Nodes are presented only for those with bootstrap scores >85% majority rule for maximum likelihood and >95% majority probabilities for Bayesian probability values (BI/ML). Different colors indicate different sampling localities (*e.g.*, light blue = Taiwan, green = Indonesia, purple = Mexico, yellow = Puerto Rico, and deep blue = Japan).

Biological information and GenBank accession number
Table 1. Biological information and GenBank accession number of megamouth shark samples used in the present study.

Name	Date of collecting	Sex	Weight (kg)	TL (cm)	Life stage	cox1	Loc 6
MP1	2013/4/18	F	366	387	sub-adult	HQ010081	MG461954
MP2	2013/4/30	F	383	373	sub-adult	MG461955	MG461954
MP3	2013/5/6	F	1090	476	adult	-	-
MP4	2013/5/6	M	413	368	sub-adult	HQ010081	MG461954
MP5	2013/5/7	M	328	385	sub-adult	-	MG461954
MP6	2013/5/8	F	408	413	sub-adult	HQ010081	MG461954
MP7	2013/5/18	F	516	524	sub-adult	MG461955	-
MP8	2013/5/18	F	452	552	sub-adult	HQ010081	MG461954
MP9	2013/5/19	M	320	395	sub-adult	HQ010081	MG461954
MP10	2013/5/21	M	320	363	sub-adult	HQ010081	MG461954
MP11	2013/5/30	F	516	426	sub-adult	MG461956	MG461954
MP12	2013/6/13	M	348	380	sub-adult	HQ010081	MG461954
MP13	2013/7/10	F	549	463	sub-adult	HQ010081	MG461954
MP14	2013/7/10	F	348	398	sub-adult	HQ010081	MG461954
MP15	2013/7/10	M	653	484	adult	HQ010081	MG461954
MP16	2013/7/17	F	1147	710	adult	-	-
MP17	2014/5/5	F	916	341	sub-adult	HQ010081	MG461954
MP18	2014/5/22	F	210	352	sub-adult	MG461956	MG461954
MP19	2014/5/30	F	752	660	adult	HQ010081	MG461954
MP20	2014/5/31	M	532	478	sub-adult	HQ010081	MG461954
MP21	2014/5/31	M	277	377	sub-adult	-	-
MP22	2014/5/31	F	734	517	adult	HQ010081	MG461954
MP23	2014/6/1	M	355	370	sub-adult	HQ010081	MG461954
MP24	2014/6/4	M	490	390	sub-adult	HQ010081	-
MP25	2014/6/8	M	296	370	sub-adult	HQ010081	MG461954
MP26	2014/8/3	F	330	366	sub-adult	MG461955	MG461954
MP27	2015/5/15	F	307	345	sub-adult	HQ010081	MG461954
sio07-53	2006/11/16	F	-	215	juvenile	HQ010081	MG461954
sio11-299	-	-	-	-	-	HQ010081	MG461954

Examples of shark genetic diversity based on mitochondrial *cox1* gene.

2 Table 2. Examples of shark genetic diversity based on mitochondrial *cox1* gene.

Species	MtDNA nucleotide diversity	MtDNA haplotype diversity	Reference
Alopias pelagicus (Pelagic thresher shark)	0.013	0.3066	Cardenosa et al. 2014 (Taiwan)
Scyliorhinus canicula (Small-spotted catshark)	0.0032	0.808	Kousteni et al. 2015
Squalus blainville (Longnose spurdog)	0.0029	0.763	Kousteni et al. 2016
Rhincodon typus (Whale shark)	0.00244	0.1871	Toha et al. 2016
Megachasma pelagios (Mega-mouth shark)	0.000616	0.3305	present study

Examples of shark population genetic structure studied at the global scale.

Table 3. Examples of shark population genetic structure studied at the global scale.

Species	Structure within ocean	Structure between Pacifc and Atlantic	Genetic marker	Habitat	Reference
Carcharhinus obscurus (dusky shark)	North and South Atlantic	Yes	Control region	reef-associated	Benavides et al. 2011
Carcharhinus limbatus (blacktip shark)	East and West Atlantic	Yes	Control region	reef-associated	Keeney and Heist 2006
Carcharhinus plumbeus (sandbar shark)	Pacific	Yes	Control region; Microsatellite	benthopelagic	Portnoy et al. 2010
Carcharias taurus (grey nurse shark)	Atlantic, Pacific, Indian	Yes	Control region; Microsatellite	reef-associated	Ahonen et al. 2009
Galeorhinus galeus (school shark)	North and South East Pacific, Atlantic	Yes	Control region	benthopelagic	Chabot and Allen 2009
Galeocerdo cuvier (tiger shark)	North and South Atlantic, Hawaii	Yes	Control region; Microsatellite	benthopelagic	Bernard et al. 2016
Sphyrna lewini (scalloped hammerhead shark)	Pacific and Atlantic	Yes	Microsatellite	pelagic-oceanic	Daly-Engel et al. 2012
Rhincodon typus (whale shark)	No	Yes	Control region; Microsatellite	pelagic-oceanic	Vignaud et al. 2014
Cetorhinus maximus (basking shark)	No	No	Control region	pelagic-oceanic	Hoelzel et al. 2006
Prionace glauca (blue shark)	No	No	Control region; Microsatellite	pelagic-oceanic	Veríssimo et al. 2017
Megachasma pelagios (mega-mouth shark)	No	No	Cox 1; Microsatellite sequence	pelagic-oceanic	present study