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

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Abstract:

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 \textit{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.

**Introduction**

The megamouth shark, \textit{Megachasma pelagios}, was accidentally captured in 1976 off
the coast of Kāne‘ohe, Hawai‘i, and was examined and described as a new species in 1983 (Taylor et al., 1983). More than forty years since its discovery, only about 100 specimens have as yet been caught or documented. There are only few official records including a review by Nakaya (2010), which documented 40 records of these sharks being either caught or released from 1976 to 2007. The Ichthyology section of the Florida Museum of Natural History has documented 65 sighting records from 1976 to 2016 (https://www.floridamuseum.ufl.edu/fish/discover/sharks/megamouths/reported-sightings). In addition, with recently added records from Taiwan (34 individuals) and Puerto Rico (1 individual) (Hsu et al., 2015; Rodriguez-Ferrer et al., 2017), only 99 individuals have been officially recorded (a global sighting record list based on scientific literature is given as supplementary information in Table S1). To date, relatively few studies have focused on this species compared to other, better known sharks. It is suggested to be a widely distributed species across the world’s oceans, including the Indian, Pacific, and Atlantic. Males become mature at about 4 m in total length and females at about 5 m, and mating occurs all year round based on the record.
of 40 specimens sampled from 1976-2008 (Nakaya, 2010). Their daily movements were recorded by acoustic tags and showed a very clear vertical movement. This vertical movement indicated they swim at depths around 200 m during daytime, move toward the surface at dusk, remain around 20 m during nighttime, and move back down to a deeper layer at dawn (Nelson et al., 1997). This shark feeds exclusively on euphausiids (Taylor et al., 1983; Yano et al., 1998; Sawamato & Matsumoto, 2012) and employs engulfment feeding analogous to humpback whales (Nakaya et al., 2008). Their pectoral fins are very flexible and mobile, which enhance dynamic lift control and thus give stability while swimming at slow speed (Tomita et al., 2014). In addition, due to the scarcity and vulnerability of these sharks, satellite tagging has not yet been feasible. Therefore, information about their horizontal movement and migration is still unknown. Among the sharks recorded, only few specimens have been used for genetic studies (i.e. phylogenetic relationships, mitochondrial genome) (Martin & Naylor, 1997; Chang et al. 2014), and most of them were discarded or consumed. Due to its rarity, population studies such as demographics, population structure, and genetic diversity among different geographic regions are difficult to
The region along the Kuroshio Current path, including the Philippines, Taiwan, and Japan, are the countries where the megamouth shark is frequently found (74 out of 99). The number of documented records from Taiwan (45 out of 99) was the highest in the world.

Taiwan initiated its National Plan of Action concerning sharks in May 2006 (Taiwan Fisheries Agency, 2006) and implemented a ban on shark finning in 2012. Additionally, to monitor the catch of several threatened shark species, the Taiwan Fisheries Agency implemented a mandatory catch and report measurement scheme in 2013 that included the megamouth shark (M. plagios), basking shark (Cetorhinus maximus), and great white shark (Carcharodon carcharias) in addition to the whale shark (Rhincodon typus). When these species are caught, fishers must immediately inform the local Fishery Agency, Taiwan Fisheries Agency, and shark experts (National Taiwan Ocean University) before further processing. Due to this management measure, our team was able to obtain fishery biology information such as total length, body weight, sex, and the relationships between measurements and
tissue samples before the sharks were processed and sold (Hsu et al., 2015). Sharks are facing global decline, and the effects (i.e. lost of genetic diversity) of population decline are of major concern in marine conservation (Pinsky & Plumbi, 2014). Loss of genetic diversity has several potential consequences on reducing evolutionary potential and adaptive ability (i.e. decreasing fitness and resistance) (Frankham 2005; Allendorf et al., 2008). The objectives of this study were to reveal the genetic diversity and connectivity of the megamouth shark with 2 tissue samples collected from the Baja California, Mexico, 27 tissue samples from Hualien, eastern Taiwan, and published sequences from GenBank.

Materials and Methods

A total of 27 tissue samples of *M. plagios* were collected between 2013 and 2015 off Hualien county, eastern Taiwan (Figure 1). These sharks were caught at night before dawn between April and August and were the bycatch of drift-gillnet fishery. This fishery operated year round, mainly targeting sun fishes during spring and summer and bill fish during fall and winter. Basic information recorded included catch date, sex, body weight, and total length. Additionally, maturity stages were determined by examining the developmental status of sexual organs. Males having fully calcified
claspers that twisted easily and fully developed testes and epididymides were determined to be adults. Females with mature ova in their ovaries (both ovaries in the megamouth shark) and having swollen oviducts and uteri were determined to be adults. If only one or portions of these organs were developed, individuals were determined to be subadults, and those whose sexual organs were in undeveloped stages were determined to be juveniles. Meanwhile, tissue samples were collected at the harbor before further commercial processing, preserved in 95% alcohol, and stored at 4 °C. In addition to samples collected from Taiwan, we obtained two tissue samples deposited in the Scripps Institute of Oceanography, University of California, San Diego, that were collected from the coastal area of Baja California, Mexico (SIO-07-53, Bahia Tortugas; SIO11-299, Bahia Sebastian Vizcaino). One cox1 sequence downloaded from GenBank was derived from a specimen collected from Mojacasabe Beach, Cabo Rojo, Puerto Rico (17.980570 N,−67.210663 W), and one from Indonesia (Figure 1).

Genomic DNA was extracted from tissue fragments using commercial DNA extraction kits (Geneaid Tissue Genomic DNA mini Kit, Geneaid Biotech, Taiwan).
DNA extracts were diluted in TE buffer and stored at -20 °C until amplification by polymerase chain reaction (PCR).

Amplification of genetic markers

The partial mitochondrial DNA gene *cox1* was amplified with the primer pair F1/R1 described by Ward et al., (2005). An additional microsatellite locus (Loc6) that has been successfully cross-amplified in lamniform sharks was also amplified, since it showed a high variation in not only repeat number but also flanking regions (Martin et al., 2002). PCRs were run in 30 µL reactions containing 10–40 ng template DNA, 3 µL 10X buffer, 0.2 mM dNTPs, 1.5 mM MgCl₂, 10 mM of each primer, and 0.2 units of Taq polymerase (MDbio, Taipei). The thermocycling profile consisted of initial denaturation at 94 °C for 2 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 40 s, and a final extension at 72 °C for 2 min. This program was used to amplify the *cox1* gene and Loc6. The nucleotide sequences of PCR products of both loci were determined using an ABI 377 automated sequencer (Carlsbad CA, U.S.A.). Nucleotide sequences were assembled
and edited using Geneious 9.1.2 (Biomatters, New Zealand).

Genetic analyses

Two cox1 gene sequences of individuals from Indonesia (EU3938905) and Puerto Rico (KY392958.1) were downloaded from GenBank. In addition, a Loc6 sequence derived from a Japanese specimen was downloaded (AF423063) (Figure 1). Arlequin 3.5 (Excoffier & Lischer, 2010) was used to analyze genetic diversity indexes, including haplotype diversity ($h$) and nucleotide diversity ($\pi$). Sequences were aligned and exported to MEGA 7 (Tamura et al., 2013) to visually inspect all alignments.

Phylogenetic analyses were used to reveal potential genetic divergences among specimens from different geographic locations, with maximum likelihood (ML) and Bayesian inference assessments being performed on the CIPRES Science Gateway (Miller et al., 2015) and MrBayes (MB) version 3.2.2 (Ronquist et al., 2012), respectively. The latter implemented two parallel runs of four simultaneous Markov chains for 10 million generations, sampling every 1000 generations and using default parameters. The first million generations (10%) were discarded as burn-in, based on the stationarity of log-likelihood tree scores. ML analyses were conducted in RAxML version 8.1.24 (Stamatakis, 2014) using the HKY substitution model chosen by MEGA 7. Supporting values on the branch were evaluated by non-parametric bootstrapping with 1000 replicates performed with RAxML (ML) and by posterior probabilities (MB). Moreover, median-joining haplotype networks were generated based on cox1 and Loc6 sequence datasets by using Popart 1.7 (Leigh & Bryant, 2015).

Results

Catch information
Basic catch information showed that megamouth sharks were mainly caught between April and August, with total weights ranging 210-1147 kg and total lengths ranging 341-710 cm. The sex ratio (female : male) was 16:11, which was not significantly different from 1:1. Five of the 27 individuals were determined to be adults and the others were sub-adults (Table 1).

**Genetic information**

The cox1 gene (623 bp) and Loc6 microsatellite sequence (592 bp) were amplified and analyzed for 29 individuals obtained from Taiwan and Mexico. Three individuals failed to amplify on both loci, including MP3, MP16, and MP21, due to low DNA quality. There were two parsimony informative sites, and the nucleotide diversity (p) and haplotype diversity (h) of the cox1 gene was 0.000616 ± 0.000695 (mean ± SD) and 0.3305 ± 0.1083, respectively. Twenty-seven cox1 sequences were composed of three unique haplotypes, and the sequences from Taiwan, Mexico, Indonesia, and Puerto Rico shared a dominant haplotype (Figure 2A haplotype network). The phylogenetic analyses showed that the sequences we used in the present study formed a monophyletic clade and that there were two nodes with substantial support, including...
one composed of MP2, MP7, and MP26, and the other composed of MP11 and MP18 (Figure 2A). On the other hand, MP7 and MP24 failed to amplify for Loc6 from a sequence downloaded from GenBank derived from a Japanese specimen; therefore, a total of 25 sequences were obtained for further genetic analyses. Our results showed that the 23 sequences from Taiwan and 2 from the Mexico were identical. The haplotype derived from the Japanese coast specimen had one singleton and formed a unique haplotype separate from the dominant one. No parsimony informative sites were found, and in addition, phylogenetic analyses showed that those sequences were clustered as a single clade in the topology of the cox1 gene tree.

Discussion

Kuroshio as the passage to feeding grounds

More than 74% (74/99) of sighting records were from countries along the Kuroshio Current, including the Philippines, Taiwan, and Japan. Therefore, this region is likely a hotspot for the occurrence of the megamouth shark. Along the east coast of Taiwan particularly, different sizes of megamouth sharks were caught mainly from April to August off the Hualien coast (Table 1). The stomach contents of a megamouth shark
caught off Ibaraki Prefecture (Japan) suggested that it fed almost exclusively on

_Euphausia pacifica_ (Sawamoto & Matsumoto, 2012). _Euphausia pacifica_ is the
dominant species of euphasiid in the North Pacific (Boden et al., 1955; Brinton,
1975) and dominates the zooplankton community in the East Sea (Sea of Japan)
(Mauchline, 1980) and Yellow Sea (Yoon et al., 2000). Endo (1981) reported that the
eggs and larvae of this species occur throughout the year in Sanriku waters, but are
most abundant in April–June. In the Yellow Sea, _E. pacifica_ was the most dominant
euphasiid species in both summer and winter (Yoon et al., 2000). Therefore, we
propose that the Kuroshio Current may be the lower latitude passage for the
megamouth shark to reach its feeding grounds in higher latitudes such as the Yellow
Sea and Sanriku waters where _E. pacifica_ is abundant. Seasonal movements between
productive high-latitude feeding grounds and low-latitude breeding grounds have
been commonly used to explain the migration of baleen whales (e.g., Norris, 1967),
and we suggest this may also explain the seasonal migration of the megamouth shark.
However, a future satellite tagging study is needed to track the movement and habitat
use of the megamouth shark to verify this hypothesis.
Although the megamouth shark appears to be very rarely encountered throughout its range, IUCN assessed its population status as Least Concern based on its wide distribution (Simpfendorfer & Compagno, 2015). This rarity may lead to intrinsic sensitivity to overexploitation since the effects of genetic drift are stronger in smaller populations, which ultimately leads to a substantial loss of genetic variation (Allendorf et al. 2008) and consequently increases the probability of the fixation of deleterious alleles and reduces the resilience of overfished species (Hare et al. 2011).

Genetic diversity is also one of the important indexes to be considered in shark management and conservation policies because the long-term survival of a species is strongly dependent on the levels of genetic diversity within and between populations (Domingues et al. 2017). In the present study, the increasing number of captures in the Kuroshio region (Table S1), particularly Taiwan, may indicate increasing fishing pressure on megamouth sharks. Comparing its cox1 genetic diversity with other sharks (Alopias pelagicus, Scyliorhinus canicula, Squalus blainville, and R. typus; Table 2), the megamouth shark has the lowest nucleotide diversity (0.000616), and
relatively lower haplotype diversity (0.3305). Among these sharks, the pelagic thresher shark (*A. pelagicus*) is one of the most abundant open ocean sharks and one of the most over-exploited shark species in the Pacific (*Tsai et al. 2010; Caballera et al. 2011*). Even under great fishing pressure, its nucleotide diversity was higher than that of the megamouth shark. With its rarity, increasing capture in the Kuroshio region and potentially low genetic diversity found in the present study, establishing species-specific regulations or management schemes for the megamouth shark is urgently needed.

On the other hand, information regarding population connectivity is an important consideration when establishing conservation strategies to manage threatened species. In sharks, habitat usage could be one of the major factors influencing the connectivity pattern. For example, pelagic sharks (e.g., the basking shark *Cetorhinus maximus*, whale shark *R. typus*, and blue shark *Prionace glauca*) that undergo long oceanic movements showed less genetic structure either within-ocean or between-ocean scales compared to coastal sharks, except that the whale shark showed a genetic break between the Pacific and Atlantic Oceans (Table 3). In the present study, neither the
mitochondrial cox1 gene nor Loc6 sequence revealed any genetic structure. While a
cox1 gene sequence from a specimen caught in the Caribbean was included in the
analysis, it was identical to the dominant cox1 gene haplotype found in the Pacific.
This suggests that the megamouth shark might travel across the world’s oceans, which
corresponds to its pelagic-oceanic life. Therefore, the careful tracking of fisheries
captures and the implementation of a long-term global monitoring program are
needed to reassess its population status and ensure that this species does not become
threatened in the near future.

Conclusions

In conclusion, the Kuroshio Current region may act as a passageway for the
megamouth shark to reach its feeding grounds during April to August. No genetic
structure and low genetic diversity were found in the megamouth shark, suggesting a
small population and the ability to travel across oceans. However, due to the small
sample size and lower variability of the loci used in the present study, connectivity
between sites could be overestimated. Nonetheless, to better understand the
movement and migration of the megamouth shark, we recommend that in future

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studies the sample size be increased, hyper variable loci (microsatellite loci or SNPs)
be used, and the pop-up satellite tag method be applied.

**Acknowledgements**

We deeply thank the staffs of George Chen Shark Research Center, National Taiwan Ocean University who helped to carry out the biological measurements in the field.

**References**

Allendorf FW, England PR, Luikart G, Ritchie PA, Ryman N. 2008. Genetic effects of harvest on wild animal populations. *Trends in Ecology and Evolution* 23: 327-337

Ahonen H, Harcourt RG, Stow AJ. 2009. Nuclear and mitochondrial DNA reveals isolation of imperilled grey nurse shark populations (*Carcharias taurus*). *Molecular Ecology* 18: 4409–4421.

Benavides MT, Horn RL, Feldheim KA, Shivji MS, Clarke SC, Wintner S, Natanson L, Braccini M, Boomer JJ, Gulak SJB, Chapman DD. 2011. Global phylogeography of the dusky shark *Carcharhinus obscurus*: implications for fisheries management and monitoring the shark fin trade. *Endangered Species Research* 14: 13–22.

Boden BP, Johnson MW, Brinton E. 1955. The Euphausiacea (Crustacea) of the North Pacific. *Bulletin of the Scripps Institution of Oceanography* 6: 287–400.

Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C, Xie D, Suchard MA, Bowen BW. 2012. Global phylogeography with mixed-marker analysis reveals male-mediated dispersal in the endangered scalloped hammerhead shark (*Sphyrna lewini*). *PLoS ONE* 7: e29986

Brinton E. 1975. Euphausiids of Southeast Asian waters. *Naga Report* 4: 287.

Bernard AM, Feldheim KA, Heithaus MR, Wintner SP, Wetherbee BM, Shivji MS. 2016. Global population genetic dynamics of a highly migratory, apex predator shark. *Molecular Ecology* 25: 5312–5329.

Caballero S, Cardena D, Soler G, Hyde J. 2011. Application of multiplex PCR approaches for shark molecular identification: feasibility and applications for fisheries.
management and conservation in the Eastern Tropical Pacific. *Molecular Ecology Resources* 12: 233–237.

Cardeñosa D, Hyde J, Caballero S. 2014. Genetic Diversity and Population Structure of the Pelagic Thresher Shark (*Alopias pelagicus*) in the Pacific Ocean: Evidence for Two Evolutionarily Significant Units. *PLOS One* 9: e110193.

Chabot CL, Allen LG. 2009. Global population structure of the tope (*Galeorhinus galeus*) inferred by mitochondrial control region sequence data. *Molecular Ecology* 18: 545–552.

Chang CH, Shao KT, Lin YS, Chiang WC, Jang-Liaw NH. 2013. Complete mitochondrial genome of the megamouth shark *Megachasma pelagios* (Chondrichthyes, Megachasmidae). *Mitochondrial DNA* 25:185-187.

Chapman DD, Babcock EA, Gruber SH, Dibattista JD, Franks BR, Kessel SA, Guttridge T, Pikitch EK, Feldheim KA. 2009. Long-term natal site-fidelity by immature lemon sharks (*Negaprion brevirostris*) at a subtropical island. *Molecular Ecology* 18: 3500–3507.

Daly-Engel TS, Seraphin KD, Holland KN, Coffey JP, Nance HA, Toonen RJ, Barnett A, Abrantes KG, Stevens JD, Semmens JM. 2012. Site fidelity and sex-specific migration in a mobile apex predator: implications for conservation and ecosystem dynamics. *Animal Behaviour* 81:1039-1048

Domingues RR, Hilsdorf AWS, Gadig OBF. 2018. The importance of considering genetic diversity in shark and ray conservation policies. *Conservation Genetics* (Online first)

Duncan KM, Martin AP, Bowen BW, de Couet G. 2006. Global phylogeography of the scalloped hammerhead shark (*Sphyrna lewini*). *Molecular Ecology*: 15: 2238–2251.

Excoffier L, Lischer H. 2010. ARLEQUIN suite ver 3.5: a new series of pro- grams to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10: 564–567.

Endo Y. 1981. Ecological studies on the Euphausiids occurring in the Sanriku waters with special reference to their life history and aggregated distribution, PhD. thesis. Sendai: Tohoku University (in Japanese with English abstract).

Frankham R. 2005. Genetics and extinction. *Biological Conservation* 126: 131–140.

Frankham R. 2006. Relationship of genetic variation to population size in wildlife. *Conservation Biology* 10: 1500–1508.

Feldheim KA, Gruber SH, De Marignac JRC, Ashley MV. 2002. Genetic tracking
to determine passive integrated transponder tag loss in lemon sharks. *Journal of Fish Biology* **61**: 1309–1313.

Hare MT, Nunney L, Schwartz MK, Ruzzante DE, Burford M, Waples RS, Palstra F. 2011. Understanding and estimating effective population size for practical application in marine species management. *Conservation Biology* **3**: 438–449.

Herbert TD, Peterson LC, Lawrence KT, Liu Z. 2010. Tropical ocean temperatures over the past 3.5 million years. *Science* **328**: 1530–1534.

Hoelzel AR, Shivji MS, Magnusen J, Francis MP. 2006. Low worldwide genetic diversity in the basking shark (*Cetorhinus maximus*). *Biology Letters* **2**: 639–642.

Grant WS. 2015. Problems and cautions with sequence mismatch analysis and Bayesian skyline plots to infer historical demography. *Journal of Heredity* **106**: 333–346.

Hsu HH, Ebert DA, Joung SJ, Liu KM, Yu CJ, Lin CY. 2015. Catch and preliminary fishery biological information of megamouth sharks *Megachasma pelagios* in eastern waters off Taiwan. AES Annual Meeting, 9-13 July, Rino, Nevada, USA.

Keeney DB, Heist EJ. 2006. Worldwide phylogeography of the blacktip shark (*Carcharhinus limbatus*) inferred from mitochondrial DNA reveals isolation of western Atlantic populations coupled with recent Pacific dispersal. *Molecular Ecology* **15**: 3669–3679.

Kousteni V, Kasapidis P, Kotoulas G, Mealofonou P. 2015. Strong population genetic structure and contrasting demographic histories for the small-spotted catshark (*Scyliorhinus canicula*) in the Mediterranean Sea. *Heredity* **114**: 333–343.

Kousteni V, Kasapidis P, Kotoulas G and Megalofonou P. 2016. Evidence of high genetic connectivity for the longnose spurdog *Squalus blainville* in the Mediterranean Sea. *Mediterranean Marine Science* **17**: 371–383.

Lavenberg RJ. 1997. An acoustic tracking of a megamouth shark, *Megachasma pelagios*; a crepuscular vertical migrator. *Environmental Biology of Fish* **49**: 389–399.

Leigh, JW, Bryant D. 2015. PopART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution* **6**: 1110–1116.

Leighton RT, Compagno LJV, Struhsaker PJ. 1983. Megamouth – a new species, genus, and family of lamnoid shark (*Megachasma pelagios*, family Megachasmidae) from the Hawaiian Islands. *Proceedings of the California Academy of Sciences* **43**: ...
Martin AP., Naylor GJP. 1997. Independent origins of filter-feeding in megamouth
and basking sharks (order Lamniformes) inferred from phylogenetic analysis of
cytochrome b gene sequences, in: K. Yano, J.F. Morrissey, Y. Yabumoto & K. Nakaya
(ed.), Biology of Megamouth Shark, Tokai University Press, Tokyo. pp. 39–50.
Martin AP, Pardini AT, Noble LR, Jones CS. 2002. Conservation of a dinucleotide
simple sequence repeat locus in sharks. *Molecular Phylogenetics and Evolution* 23:
205–213.
Mauchline J. 1980. The biology of euphausiids. *Advances in Marine Biology* 18:
370–637.
Miller MA, Schwartz T, Pickett BE, He S, Klem EB, Scheuermann RH,
Passarotti M, Kaufman S, O’Leary MA. 2015. A RESTful API for Access to
Phylogenetic Tools via the CIPRES Science Gateway. *Evolutionary Bioinformatics* 11:
43-48.
Pinsky ML, Palumbi SR. 2014. Meta-analysis reveals lower genetic diversity in
overfished populations. *Molecular Ecology* 23: 29–39.
Nakaya K. 2010. Biology of the megamouth shark, *Megachasma pelagios*
(Lamniformes: Megachasmidae). In: UchidaS, editor. Proceedings of an International
Symposium—Into the Unknown, Researching Mysterious Deep-Sea Animals.
Okinawa: Okinawa Churaumi Aquarium. pp 69–83.
Nakaya K, Matsumoto R, Suda K. 2008. Feeding strategy of the megamouth shark
*Megachasma pelagios* (Lamniformes: Megachasmidae). *Journal of Fish Biology* 73:
17–34.
Nelson SR, McKibben JN, Strong WR, Lowe CG, Sisneros JA, Schroeder DM,
Lavenberg RJ. 1997. An acoustic tracking of a megamouth shark, *Megachasma
pelagios*: a crepuscular vertical migrator. *Environmental Biology of Fishes* 49:
389-399.
Norris KS. 1967. Some observations on the migration and orientation of marine
mammals. In Animal orientation and migration (ed. R. M. Storm), pp. 101–125.
Corvallis, OR: Oregon State University Press.
Planes S. 2014. Genetic structure of populations of whale sharks among ocean basins
and evidence for their historic rise and recent decline. *Molecular Ecology* 23: 2590–
2601.
Portnoy DS, McDowell JR, Heist EJ, Musick JA, Graves JE. 2010. World phylogeography and male-mediated gene flow in the sandbar shark, *Carcharhinus plumbeus*. *Molecular Ecology* 19:1994–2010.

Rambaut A, Drummond AJ. 2014. BEAST 2: A software platform for bayesian evolutionary analysis. *PLoS Computational Biology* 10: e1003537.

Rodriguez-Ferrer G, Wetherbee BM, Schärer M, Liñestrom C, Zegarra JP, Shivji M. 2017. First record of the megamouth shark, *Megachasma pelagios*, (family Megachasmidae) in the tropical western North Atlantic Ocean. *Marine Biodiversity Records* 10:20.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–42.

Sawamoto S, Matsumoto R. 2012. Stomach contents of a megamouth shark *Megachasma pelagios* from the Kuroshio extension: evidence for feeding on a euphausiid swarm. *Plankton and Benthos Research* 7: 203–206.

Schultz JK, Feldheim KA, Gruber SH, Ashley MV, McGovern TM, Bowen BW. 2008. Global phylogeography and seascape genetics of the lemon sharks (genus *Negaprion*). *Molecular Ecology* 17: 5336–5348.

Simpfendorfer C, Compagno LJV. 2015. *Megachasma pelagios*, The IUCN Red List of Threatened Species 2015: e.T39338A2900476.

Stamatakis A. 2014. RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–3.

Taiwan Fisheries Agency 2006. Taiwan’s National Plan of Action for the Conservation and Management of Sharks. Fisheries Agency, Taipei, Taiwan. www.fa.gov.tw/eng/guide/npoasharke.php.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30: 2725–2729.

Taylor LR, Compagno LJV, Struhsaker PJ. 1983. Megamouth-a new species, genus, and family of lamnoid shark (*Megachasma pelagios*, family Megachasmidae) from the Hawaiian Islands. *Proceedings of the California Academy of Natural Sciences* 43: 87-110.
Tomita T, Tanaka S, Sato K, Nakaya K. 2014. Pectoral fin of the megamouth shark: Skeletal and muscular systems, skin histology, and functional morphology. *PLoS ONE* 9: e86205.

Toha AH, Widodo N, Subhan B, Himawan MR, Tania Casandra, Noor BA, Stewart BS, Madduppa HH. 2016. Close genetic relatedness of whale sharks, *Rhincodon typus* in the Indo-Pacific region. *AACL Bioflux* 9: 458–465.

Tsai WP, Liu KM, Joung SJ. 2010. Demographic analysis of the pelagic thresher shark, *Alopias pelagicus*, in the north-western Pacific using a stochastic stage-based model. *Marine and Freshwater Research* 61: 1056.

Verissimo A, McDowell JR, Graves JE. 2010. Global population structure of the spiny dogfish *Squalus acanthias*, a temperate shark with an antitropical distribution. *Molecular Ecology* 19: 1651–1662.

Verissimo A, Sampaio Í, Medowell J R, Alexandrino P, Mucientes G, Queiroz N, da Silva C, Jones C S, Noble I R. 2017. World without borders—genetic population structure of a highly migratory marine predator, the blue shark (*Prionace glauca*). *Ecology and Evolution* 7: 4768–4781.

Vignaud TM, Maynard JA, Leblois R, Meekan MG, Vázquez-Juárez R, Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN. 2005. DNA barcoding Australia’s fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences* 360: 1847–1851.

Yagishita N. 2014. Genetic population structure of the Pacific bluefin tuna *Thunnus orientalis* and the yellowfin tuna *Thunnus albacares* in the North Pacific Ocean. *Fisheries science* 80: 1193–1204.

Yano K, Tsukada O, Furuta M. 1998. Capture of megamouth shark No. 12 from Atawa, Mie, Japan. *Ichthyological Research* 45: 424–426.

Yoon WD, Cho SH, Lim D, Choi YK, Lee Y. 2000. Spatial distribution of Euphausia pacifica (*Euphausiacea: Crustacea*) in the Yellow Sea. *Journal of Plankton Research* 22: 939–949.