*Zoological Studies* **55:** 33 (2016)
doi:10.6620/ZS.2016.55-33

Zoological Studies

# Population Structure of Blue Marlin, *Makaira nigricans*, in the Pacific and Eastern Indian Oceans

Hui Chen[1], Chia-Hao Chang[2], Chi-Lu Sun[1,3,*], Kwang-Tsao Shao[2], Su-Zan Yeh[1], and Gerard DiNardo[4]

[1]*Institute of Oceanography, National Taiwan University, Taipei 10617, Taiwan*
[2]*Biodiversity Research Center, Academia Sinica, Taipei 11529, Taiwan*
[3]*Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung, 20224, Taiwan*
[4]*NOAA Fisheries, National Marine Fisheries Service, Southwest Fisheries Science Center, La Jolla, CA 92037-1508, USA*

*(Received 20 March 2015; Accepted 20 April 2016)*

**Hui Chen, Chia-Hao Chang, Chi-Lu Sun, Kwang-Tsao Shao, Su-Zan Yeh, and Gerard DiNardo (2016)** Blue marlin *Makaira nigricans* is economically important for fisheries worldwide. However, overfishing has substantially reduced the stock size. Better knowledge of blue marlin population genetics will help improve management and conservation. Previous genetic studies concluded that the Pacific blue marlin should be considered a single stock. This study investigated the population genetic structure of blue marlin inhabiting the Pacific and eastern Indian oceans based on mtDNA cytochrome *b* (cyt *b*) and control region (CR) sequence variation. We collected tissue samples ($n$ = 183) from three Pacific and one Indian Ocean, and determined the sequences of 1140 bp of cyt *b* and 905 bp of CR. Phylogenetic analysis revealed that blue marlin contain two clades, the Atlantic clade and the ubiquitous clade, and that all the eastern Indian and Pacific individuals collected for this study belonged to the ubiquitous clade. All eastern Indian and Pacific blue marlin possess extremely high haplotype diversity ($h$) and low nucleotide diversity ($\pi$). The results of pairwise $\Phi_{ST}$, hierarchical analysis of molecular variance (AMOVA) and spatial analysis of molecular variance (SAMOVA) all support that there is no population differentiation among eastern Indian and Pacific blue marlin. Neutrality tests and pairwise mismatch distribution analysis both indicated that eastern Indian and Pacific blue marlin have undergone a rapid population expansion on the order of 0.30 to 0.65 million years ago (mya). This study demonstrates that blue marlin in the Pacific and eastern Indian oceans constitute a single stock. International cooperation will be required to preserve blue marlin as a resource; moreover, the high genetic variation of blue marlin in this region suggests that unique haplotypes in the population are sensitive to high harvesting levels and could disappear.

**Key words:** Blue marlin, Cytochrome *b*, Control region, Population genetics.

## BACKGROUND

Blue marlin *Makaira nigricans* (Lacépède, 1802), famously featured in Ernest Hemingway's novel *The Old Man and the Sea*, is one of the most popular and valuable game fish; it has circumtropical distribution and is high migratory. It dwells in pelagic waters where the surface temperature exceeds 22°C (Nakamura 1985).

Blue marlin represents an important commercial and recreational resource. It is caught primarily in pelagic longline fisheries, although small catches are also taken by gill-nets and purse seine as well as by surface trolls, handlines and harpoons. Accorrding to a 2013 report, the global production of blue marlin was 38,722 mt, with approximately 71% harvested from the Pacific, 25% from the Indian Ocean, and 4% from the Atlantic (FAO Fishery statistical collections global capture production, 2015). Blue marlin is threatened by over-exploitation (Collette et al. 2011); blue marlin catches in the Pacific decreased

*Correspondence: E-mail: chilu@ntu.edu.tw

from 39,684 metric tons (mt) to 27,599 mt and those in the Western central Pacific fell from 38,284 mt to 8,765 mt from 1963 to 2013. Despite this critical situation, a conservation program to protect against overexploitation is lacking (Collette et al. 2011).

Blue marlin larva have been extensively collected in the western and central Pacific, south of the Maldive Islands, around the Mascalene Islands, and off the southern coasts of Java and Sumatra in the Indian Ocean (Nakamura 1985). Although reproducing year-round in equatorial waters to 10°N/S, blue marlin in the Pacific and Indian oceans seasonally spawn in summer periods in both hemispheres to 30°N/S (Kailola et al. 1993). Tagging data also indicated about 85% of blue marlin was recaptured in the same general area where they had been released after a space of three years or less (Ortiz et al. 2003). Both the reported seasonal reproductive behavior of blue marlin and their tendency towards annual fidelity may be expected to restrict gene flow, conceivably suppose the significant intra-oceanic genetic differentiation. The genetic differentiation within Pacific fish populations has been observed in other billfishes, such as sailfish *Istiophorus platypterus* (McDowell 2002; Lu et al. 2015), striped marlin *Kajikia audax* (McDowell and Graves 2008), and swordfish *Xiphias gladius* (Lu 2014).

Several studies have focused on the genetic population structure of blue marlin using different geographic scales. McDowell et al. (2007) demonstrated that blue marlin in the Atlantic should be viewed as a single stock by analyzing the mitochondrial control region (CR) sequences of 57 individuals gathered from four localities. Finnerty and Block (1992), Graves and McDowell (1995), Buonaccorsi et al. (1999, 2001) revealed significant genetic differentiation in samples of blue marlin from the Atlantic and Pacific. Graves and McDowell (1995) and Buonaccorsi et al. (1999, 2001) asserted that all the Pacific blue marlin have a single genetic stock structure; nevertheless, the blue marlin they sampled was mainly from Hawaii and Mexico, not pan-pacific. Moreover, information on blue marlin from the Indian Ocean has been scarce (Graves and McDowell 2015).

Since blue marlin is threatened by over-exploitation and the current catch comes mainly from the Pacific and Indian oceans, we urgently need to investigate the genetic structure of blue marlin in this region as a basis for a more comprehensive resource conservation program. To truly understand whether the blue marlin population structure in the Pacific and Indian oceans should be considered a single stock, it is necessary to increase the sampling locales in the Pacific and to include Indian Ocean samples for analysis. The objective of this study is to investigate the genetic structure of blue marlin in the Pacific and eastern Indian oceans and provide a scientific basis for effective fishery management.

## MATERIALS AND METHODS

### Sample collection and storage

A total of 183 blue marlin was collected from three Pacific localities, eastern Pacific (EP), western North Pacific (WNP), and South China Sea (SCS), and one Indian Ocean locality (EI) between September 2004 and June 2011. For each individual collected, we cut a piece of muscle tissue or fin clip and preserved it in 95% ethanol at 4°C. Details of sampling and the map of sampling localities are shown in table 1 and figure 1.

### DNA extraction and data collection

We used a small piece (~2 $mm^3$) of muscle and fin clip for DNA extraction, following the protocol of the Genomic DNA Mini Kit (Tissue) (Genomic, New Taipei City, Taiwan). The extracted DNA samples were stored at 4°C until we conducted the polymerase chain reaction. Two pairs of primers, one for amplifying the mitochondrial cytochrome *b* (cyt *b*) (1140 bp) and the other for control region (CR) (905 bp), were employed. The newly cyt *b* primers, Cytb-F (5'- GCC AGG ACT CTA ACC ACC ACT A -3') and Cytb-R (5'- ACC TCC GGC ATC CGG YTT ACA A -3'), were designed on the basis of the mitochondrial genomes of *Istiompax indica* (AB470305) and *M. nigricans* (AB470304), and the CR were amplified with two primers, designed by Bernatchez et al. (1992) and Palumbi et al. (1991). We performed PCR amplifications of these two segments in a mixture with a final volume of 100 μL, containing 10 ng template DNA, 5 μmol of each specific primer, 50 μL of Fast-Run™ Advanced *Taq* Master Mix (ProTech, Taipei, Taiwan) and distilled water. Thermal cycling began with one cycle at 94°C for 4 min; followed by 35 denaturation cycles at 94°C for 30 seconds, annealing at 54°C for 30 seconds in cyt *b* and 52°C for 50 seconds in the control region, and 72°C for 1 min; and finally, a single extension step at 72°C

for 10 min. PCR products were purified using a PCR DNA Fragment Extraction Kit (Geneaid, Taipei, Taiwan). Approximately 50 ng of the purified PCR products was employed as the template for sequencing, which we performed following the protocol of the ABI PRISM BigDye Sequencing Kit (PE Applied Biosystems, Foster City, CA, USA) with each of the two PCR primers. The cyt *b* gene sequences (1,140 bp) were directly aligned and the CR sequences were aligned by using Clustal W (Thompson et al. 1994) and then checked by eye.

### Phylogenetic analysis

In order to incorporate the molecular information from 56 Atlantic blue marlin samples (the details of sampling of the Atlantic marlin are shown in Table 1), only the CR data set was used to construct the phylogenetic tree. Following Santini and Sorenson (2013), we downloaded the CR sequences of *Istiophorus platypterus* (AP006035) and *Tetrapturus angustirostris* (AB470303) from GenBank to serve as outgroups for the phylogenetic analysis. A maximum-likelihood (ML) tree was constructed utilizing RAxML 7.0.4 (Stamatakis 2006). In setting the parameters of RAxML 7.0.4, data were analyzed under the GTR + G + I model as suggested by jModeltest 0.1.1 (Posada 2008). We obtained the ML tree by performing 100 different runs using the default algorithm of the program, and chose the best ML tree by the likelihood scores among suboptimal trees created during each run. Nodal support was certified by bootstrap analysis with 1,000 nonparametric bootstrap iterations.

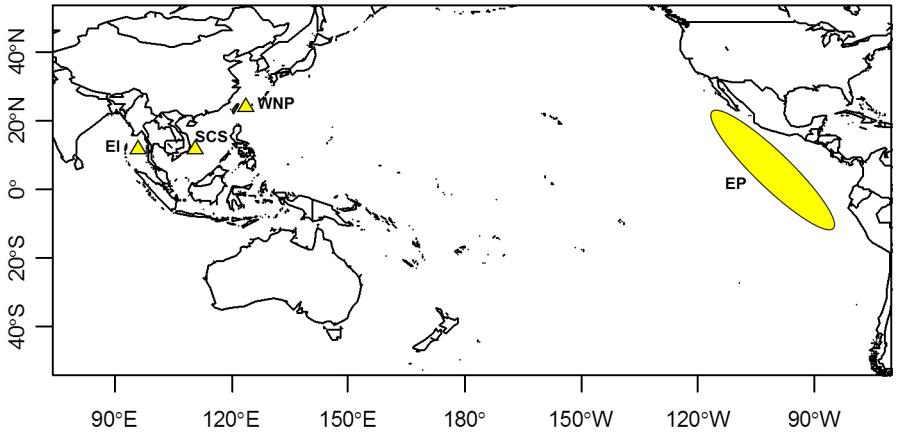


**Fig. 1.** This map illustrates the areas where blue marlin samples were taken. The triangle and ellipse symbols indicate sampling sites. EP, eastern Pacific; WNP, western North Pacific; SCS, South China Sea; EI, eastern Indian Ocean.

**Table 1.** Haplotypes of cyt *b* and CR genes. List of blue marlin specimens and the outgroup taxa sequenced for cytochrome *b* (cyt *b*) and control region (CR) with sampling location, as shown in figure 1; sampling date; individual number and GenBank accession numbers

| Scientific name | Collection locality (Code) | Date | Number of specimens | Specimen code | Haplotype code of CR | Accession number of CR | Accession number of cyt *b* | Source |
|---|---|---|---|---|---|---|---|---|
| Outgroup | | | | | | | | |
| *Istiophorus platypterus* | | | | | | AP006035 | | |
| *Tetrapturus angustirostris* | | | | | | AB470303 | | |
| Ingroup | | | | | | | | |
| *Makaira nigricans* | Caribbean Sea (CAS) | | 11 | CAS_N1 | CAS_H1 | EF607795 | - | McDowell et al. 2007 |
| | | | | CAS_N2 | CAS_H2 | EF607796 | - | |
| | | | | CAS_N3 | CAS_H3 | EF607797 | - | |
| | | | | CAS_N4 | CAS_H4 | EF607798 | - | |
| | | | | CAS_N5 | CAS_H5 | EF607799 | - | |
| | | | | CAS_N6 | CAS_H6 | EF607800 | - | |
| | | | | CAS_N7 | CAS_H7 | EF607801 | - | |

**Table 1.** (continued)

| Scientific name | Collection locality (Code) | Date | Number of specimens | Specimen code | Haplotype code of CR | Accession number of CR | Accession number of cyt *b* | Source |
|---|---|---|---|---|---|---|---|---|
| | | | | CAS_N8 | CAS_H8 | EF607802 | - | |
| | | | | CAS_N9 | CAS_H9 | EF607803 | - | |
| | | | | CAS_N10 | CAS_H10 | EF607804 | - | |
| | | | | CAS_N11 | CAS_H11 | EF607805 | - | |
| | Eastern Atlantic (EA) | | 18 | EA_N1 | EA_H1 | EF607806 | | McDowell et al. 2007 |
| | | | | EA_N2 | EA_M1 | EF607807 | - | |
| | | | | EA_N3 | EA_M1 | EF607808 | - | |
| | | | | EA_N4 | EA_WNA_M1 | EF607809 | - | |
| | | | | EA_N5 | EA_H5 | EF607810 | - | |
| | | | | EA_N6 | EA_H6 | EF607811 | - | |
| | | | | EA_N7 | EA_H7 | EF607812 | - | |
| | | | | EA_N8 | EA_H8 | EF607813 | - | |
| | | | | EA_N9 | EA_H9 | EF607814 | - | |
| | | | | EA_N10 | EA_H10 | EF607815 | - | |
| | | | | EA_N11 | EA_H11 | EF607816 | - | |
| | | | | EA_N12 | EA_H12 | EF607817 | - | |
| | | | | EA_N13 | EA_H13 | EF607818 | - | |
| | | | | EA_N14 | EA_H14 | EF607819 | - | |
| | | | | EA_N15 | EA_H15 | EF607820 | - | |
| | | | | EA_N16 | EA_H16 | EF607821 | - | |
| | | | | EA_N17 | EA_H17 | EF607822 | - | |
| | | | | EA_N18 | EA_H18 | EF607823 | - | |
| | western North Atlantic (WNA) | | 15 | WNA_N1 | WNA_H1 | EF607836 | - | McDowell et al. 2007 |
| | | | | WNA_N2 | WNA_H2 | EF607837 | - | |
| | | | | WNA_N3 | WNA_H3 | EF607838 | - | |
| | | | | WNA_N4 | WNA_H4 | EF607839 | - | |
| | | | | WNA_N5 | WNA_H5 | EF607840 | - | |
| | | | | WNA_N6 | WNA_H6 | EF607841 | - | |
| | | | | WNA_N7 | WNA_H7 | EF607842 | - | |
| | | | | WNA_N8 | WNA_H8 | EF607843 | - | |
| | | | | WNA_N9 | WNA_H9 | EF607844 | - | |
| | | | | WNA_N10 | WNA_WSA_M1 | EF607845 | - | |
| | | | | WNA_N11 | WNA_WSA_M2 | EF607846 | - | |
| | | | | WNA_N12 | WNA_H12 | EF607847 | - | |
| | | | | WNA_N13 | EA_WNA_M1 | EF607848 | - | |
| | | | | WNA_N14 | WNA_H14 | EF607849 | - | |
| | | | | WNA_N15 | WNA_H15 | EF607850 | - | |
| | western South Atlantic (WSA) | | 12 | WSA_N1 | WSA_H1 | EF607824 | - | McDowell et al. 2007 |
| | | | | WSA_N2 | WNP_WSA_M1 | EF607825 | - | |
| | | | | WSA_N3 | WSA_H3 | EF607826 | - | |
| | | | | WSA_N4 | WSA_H4 | EF607827 | - | |
| | | | | WSA_N5 | WSA_H5 | EF607828 | - | |
| | | | | WSA_N6 | WSA_H6 | EF607829 | - | |
| | | | | WSA_N7 | WSA_H7 | EF607830 | - | |
| | | | | WSA_N8 | WNA_WSA_M2 | EF607831 | - | |
| | | | | WSA_N9 | WSA_H9 | EF607832 | - | |
| | | | | WSA_N10 | WSA_H10 | EF607833 | - | |
| | | | | WSA_N11 | WSA_H11 | EF607834 | - | |
| | | | | WSA_N12 | WNA_WSA_M1 | EF607835 | - | |
| | **Atlantic Ocean** | | **56** | | | | | |
| | eastern Pacific (EP) | 2004-2006 | 54 | EP_N1 | EP_H1 | KP219230 | KP184953 | this study |
| | | | | EP_N2 | EP_H2 | KP219231 | KP184954 | |
| | | | | EP_N3 | EP_H3 | KP219232 | KP184955 | |
| | | | | EP_N4 | EP_H4 | KP219233 | KP184956 | |
| | | | | EP_N5 | EP_H5 | KP219234 | KP184957 | |
| | | | | EP_N6 | EP_H6 | KP219235 | KP184958 | |
| | | | | EP_N7 | EP_H7 | KP219236 | KP184959 | |
| | | | | EP_N8 | EP_H8 | KP219237 | KP184960 | |
| | | | | EP_N9 | EP_H9 | KP219238 | KP184961 | |
| | | | | EP_N10 | EP_H10 | KP219239 | KP184962 | |

**Table 1.** (continued)

| Scientific name | Collection locality (Code) | Date | Number of specimens | Specimen code | Haplotype code of CR | Accession number of CR | Accession number of cyt *b* | Source |
|---|---|---|---|---|---|---|---|---|
| | | | | EP_N11 | EP_H11 | KP219240 | KP184963 | |
| | | | | EP_N12 | EP_H12 | KP219241 | KP184964 | |
| | | | | EP_N13 | NP_H13 | KP219242 | KP184965 | |
| | | | | EP_N14 | EP_H14 | KP219243 | KP184966 | |
| | | | | EP_N15 | EP_H15 | KP219244 | KP184967 | |
| | | | | EP_N16 | EP_H16 | KP219245 | KP184968 | |
| | | | | EP_N17 | EP_H17 | KP219246 | KP184969 | |
| | | | | EP_N18 | EP_H18 | KP219247 | KP184970 | |
| | | | | EP_N19 | EP_H19 | KP219248 | KP184971 | |
| | | | | EP_N20 | EP_H20 | KP219249 | KP184972 | |
| | | | | EP_N21 | EP_H21 | KP219250 | KP184973 | |
| | | | | EP_N22 | EP_H22 | KP219251 | KP184974 | |
| | | | | EP_N23 | EP_H23 | KP219252 | KP184975 | |
| | | | | EP_N24 | EP_H24 | KP219253 | KP184976 | |
| | | | | EP_N25 | EP_H25 | KP219254 | KP184977 | |
| | | | | EP_N26 | EP_H26 | KP219255 | KP184978 | |
| | | | | EP_N27 | EP_H27 | KP219256 | KP184979 | |
| | | | | EP_N28 | EP_H28 | KP219257 | KP184980 | |
| | | | | EP_N29 | EP_H29 | KP219258 | KP184981 | |
| | | | | EP_N30 | EP_H30 | KP219259 | KP184982 | |
| | | | | EP_N31 | EP_H31 | KP219260 | KP184983 | |
| | | | | EP_N32 | EP_H32 | KP219261 | KP184984 | |
| | | | | EP_N33 | EP_H33 | KP219262 | KP184985 | |
| | | | | EP_N34 | EP_H34 | KP219263 | KP184986 | |
| | | | | EP_N35 | EP_H35 | KP219264 | KP184987 | |
| | | | | EP_N36 | EP_H36 | KP219265 | KP184988 | |
| | | | | EP_N37 | EP_H37 | KP219266 | KP184989 | |
| | | | | EP_N38 | EP_H38 | KP219267 | KP184990 | |
| | | | | EP_N39 | EP_H39 | KP219268 | KP184991 | |
| | | | | EP_N40 | EP_H40 | KP219269 | KP184992 | |
| | | | | EP_N41 | EP_H41 | KP219270 | KP184993 | |
| | | | | EP_N42 | EP_H42 | KP219271 | KP184994 | |
| | | | | ESP_N43 | EP_H43 | KP219272 | KP184995 | |
| | | | | ESP_N44 | EP_H44 | KP219273 | KP184996 | |
| | | | | ESP_N45 | EP_H45 | KP219274 | KP184997 | |
| | | | | ESP_N46 | EP_H46 | KP219275 | KP184998 | |
| | | | | ESP_N47 | EP_H47 | KP219276 | KP184999 | |
| | | | | ESP_N48 | EP_H48 | KP219277 | KP185000 | |
| | | | | ESP_N49 | EP_H49 | KP219278 | KP185001 | |
| | | | | ESP_N50 | EP_H50 | KP219279 | KP185002 | |
| | | | | ESP_N51 | EP_H51 | KP219280 | KP185003 | |
| | | | | ESP_N52 | EP_H52 | KP219281 | KP185004 | |
| | | | | ESP_N53 | EP_H53 | KP219282 | KP185005 | |
| | | | | ESP_N54 | EP_H54 | KP219283 | KP185006 | |
| | western North Pacific (WNP) | 2008-2014 | 56 | WNP_N1 | WNP_H1 | KP219324 | KP185047 | this study |
| | | | | WNP_N2 | WNP_H2 | KP219325 | KP185048 | |
| | | | | WNP_N3 | WNP_H3 | KP219326 | KP185049 | |
| | | | | WNP_N4 | WNP_H4 | KP219327 | KP185050 | |
| | | | | WNP_N5 | WNP_H5 | KP219328 | KP185051 | |
| | | | | WNP_N6 | WNP_H6 | KP219329 | KP185052 | |
| | | | | WNP_N7 | WNP_H7 | KP219330 | KP185053 | |
| | | | | WNP_N8 | WNP_H8 | KP219331 | KP185054 | |
| | | | | WNP_N9 | WNP_H9 | KP219332 | KP185055 | |
| | | | | WNP_N10 | WNP_H10 | KP219333 | KP185056 | |
| | | | | WNP_N11 | WNP_H11 | KP219334 | KP185057 | |
| | | | | WNP_N12 | WNP_H12 | KP219335 | KP185058 | |
| | | | | WNP_N13 | WNP_H13 | KP219336 | KP185059 | |
| | | | | WNP_N14 | WNP_H14 | KP219337 | KP185060 | |
| | | | | WNP_N15 | WNP_H15 | KP219338 | KP185061 | |
| | | | | WNP_N16 | WNP_H16 | KP219339 | KP185062 | |

**Table 1.** (continued)

| Scientific name | Collection locality (Code) | Date | Number of specimens | Specimen code | Haplotype code of CR | Accession number of CR | Accession number of cyt *b* | Source |
|---|---|---|---|---|---|---|---|---|
| | | | | WNP_N17 | WNP_H17 | KP219340 | KP185063 | |
| | | | | WNP_N18 | WNP_H18 | KP219341 | KP185064 | |
| | | | | WNP_N19 | WNP_H19 | KP219342 | KP185065 | |
| | | | | WNP_N20 | WNP_H20 | KP219343 | KP185066 | |
| | | | | WNP_N21 | WNP_H21 | KP219344 | KP185067 | |
| | | | | WNP_N22 | WNP_H22 | KP219345 | KP185068 | |
| | | | | WNP_N23 | WNP_H23 | KP219346 | KP185069 | |
| | | | | WNP_N24 | WNP_WSA_M1 | KP219347 | KP185070 | |
| | | | | WNP_N25 | WNP_H25 | KP219348 | KP185071 | |
| | | | | WNP_N26 | WNP_H26 | KP219349 | KP185072 | |
| | | | | WNP_N27 | WNP_H27 | KP219350 | KP185073 | |
| | | | | WNP_N28 | WNP_H28 | KP219351 | KP185074 | |
| | | | | WNP_N29 | WNP_H29 | KP219352 | KP185075 | |
| | | | | WNP_N30 | WNP_H30 | KP219353 | KP185076 | |
| | | | | WNP_N31 | WNP_H31 | KP219354 | KP185077 | |
| | | | | WNP_N32 | WNP_H32 | KP219355 | KP185078 | |
| | | | | WNP_N35 | WNP_M1 | KP219356 | KP185079 | |
| | | | | WNP_N36 | WNP_H36 | KP219357 | KP185080 | |
| | | | | WNP_N37 | WNP_H37 | KP219358 | KP185081 | |
| | | | | WNP_N38 | WNP_H38 | KP219359 | KP185082 | |
| | | | | WNP_N39 | WNP_H39 | KP219360 | KP185083 | |
| | | | | WNP_N40 | WNP_H40 | KP219361 | KP185084 | |
| | | | | WNP_N41 | WNP_H41 | KP219362 | KP185085 | |
| | | | | WNP_N42 | WNP_H42 | KP219363 | KP185086 | |
| | | | | WNP_N43 | WNP_H43 | KP219364 | KP185087 | |
| | | | | WNP_N45 | WNP_H45 | KP219365 | KP185088 | |
| | | | | WNP_N46 | WNP_M1 | KP219366 | KP185089 | |
| | | | | WNP_N47 | WNP_H47 | KP219367 | KP185090 | |
| | | | | WNP_N48 | WNP_H48 | KP219368 | KP185091 | |
| | | | | WNP_N49 | WNP_H49 | KP219369 | KP185092 | |
| | | | | WNP_N50 | WNP_M3 | KP219370 | KP185093 | |
| | | | | WNP_N51 | WNP_M3 | KP219371 | KP185094 | |
| | | | | WNP_N52 | WNP_M2 | KP219372 | KP185095 | |
| | | | | WNP_N53 | WNP_M2 | KP219373 | KP185096 | |
| | | | | WNP_N54 | WNP_H54 | KP219374 | KP185097 | |
| | | | | WNP_N55 | WNP_H55 | KP219375 | KP185098 | |
| | | | | WNP_N56 | WNP_H56 | KP219376 | KP185099 | |
| | | | | WNP_N57 | WNP_H57 | KP219377 | KP185100 | |
| | | | | WNP_N58 | WNP_H58 | KP219378 | KP185101 | |
| | | | | WNP_N59 | WNP_M2 | KP219379 | KP185102 | |
| | South China Sea (SCS) | 2010 | 40 | SCS_N1 | SCS_H1 | KP219284 | KP185007 | this study |
| | | | | SCS_N2 | SCS_M1 | KP219285 | KP185008 | |
| | | | | SCS_N3 | SCS_M1 | KP219286 | KP185009 | |
| | | | | SCS_N4 | SCS_H4 | KP219287 | KP185010 | |
| | | | | SCS_N5 | SCS_H5 | KP219288 | KP185011 | |
| | | | | SCS_N6 | SCS_H6 | KP219289 | KP185012 | |
| | | | | SCS_N7 | SCS_H7 | KP219290 | KP185013 | |
| | | | | SCS_N8 | SCS_H8 | KP219291 | KP185014 | |
| | | | | SCS_N9 | SCS_H9 | KP219292 | KP185015 | |
| | | | | SCS_N10 | SCS_H10 | KP219293 | KP185016 | |
| | | | | SCS_N11 | SCS_H11 | KP219294 | KP185017 | |
| | | | | SCS_N12 | SCS_H12 | KP219295 | KP185018 | |
| | | | | SCS_N13 | SCS_M2 | KP219296 | KP185019 | |
| | | | | SCS_N14 | SCS_H14 | KP219297 | KP185020 | |
| | | | | SCS_N15 | SCS_H15 | KP219298 | KP185021 | |
| | | | | SCS_N16 | SCS_H16 | KP219299 | KP185022 | |
| | | | | SCS_N17 | SCS_M2 | KP219300 | KP185023 | |
| | | | | SCS_N18 | SCS_H18 | KP219301 | KP185024 | |
| | | | | SCS_N19 | SCS_H19 | KP219302 | KP185025 | |
| | | | | SCS_N20 | SCS_H20 | KP219303 | KP185026 | |

**Table 1.** (continued)

| Scientific name | Collection locality (Code) | Date | Number of specimens | Specimen code | Haplotype code of CR | Accession number of CR | Accession number of cyt *b* | Source |
|---|---|---|---|---|---|---|---|---|
| | | | | SCS_N21 | SCS_H21 | KP219304 | KP185027 | |
| | | | | SCS_N22 | SCS_H22 | KP219305 | KP185028 | |
| | | | | SCS_N23 | SCS_M3 | KP219306 | KP185029 | |
| | | | | SCS_N24 | SCS_M3 | KP219307 | KP185030 | |
| | | | | SCS_N25 | SCS_H25 | KP219308 | KP185031 | |
| | | | | SCS_N26 | SCS_M3 | KP219309 | KP185032 | |
| | | | | SCS_N27 | SCS_H27 | KP219310 | KP185033 | |
| | | | | SCS_N28 | SCS_M3 | KP219311 | KP185034 | |
| | | | | SCS_N29 | SCS_M4 | KP219312 | KP185035 | |
| | | | | SCS_N30 | SCS_M5 | KP219313 | KP185036 | |
| | | | | SCS_N31 | SCS_H31 | KP219314 | KP185037 | |
| | | | | SCS_N32 | SCS_M4 | KP219315 | KP185038 | |
| | | | | SCS_N33 | SCS_M5 | KP219316 | KP185039 | |
| | | | | SCS_N34 | SCS_M6 | KP219317 | KP185040 | |
| | | | | SCS_N35 | SCS_M6 | KP219318 | KP185041 | |
| | | | | SCS_N36 | SCS_H36 | KP219319 | KP185042 | |
| | | | | SCS_N37 | SCS_M6 | KP219320 | KP185043 | |
| | | | | SCS_N38 | SCS_M6 | KP219321 | KP185044 | |
| | | | | SCS_N39 | SCS_H39 | KP219322 | KP185045 | |
| | | | | SCS_N40 | SCS_H40 | KP219323 | KP185046 | |
| | **Pacific Ocean** | | **150** | | | | | |
| | Eastern Indian Ocean (EI) | 2010 | 33 | EI_N1 | EI_H1 | KP219197 | KP184920 | this study |
| | | | | EI_N2 | EI_H2 | KP219198 | KP184921 | |
| | | | | EI_N3 | EI_M1 | KP219199 | KP184922 | |
| | | | | EI_N4 | EI_H4 | KP219200 | KP184923 | |
| | | | | EI_N5 | EI_H5 | KP219201 | KP184924 | |
| | | | | EI_N6 | EI_H6 | KP219202 | KP184925 | |
| | | | | EI_N7 | EI_H7 | KP219203 | KP184926 | |
| | | | | EI_N8 | EI_H8 | KP219204 | KP184927 | |
| | | | | EI_N9 | EI_H9 | KP219205 | KP184928 | |
| | | | | EI_N10 | EI_H10 | KP219206 | KP184929 | |
| | | | | EI_N11 | EI_H11 | KP219207 | KP184930 | |
| | | | | EI_N12 | EI_H12 | KP219208 | KP184931 | |
| | | | | EI_N13 | EI_H13 | KP219209 | KP184932 | |
| | | | | EI_N14 | EI_H14 | KP219210 | KP184933 | |
| | | | | EI_N15 | EI_H15 | KP219211 | KP184934 | |
| | | | | EI_N16 | EI_H16 | KP219212 | KP184935 | |
| | | | | EI_N17 | EI_H17 | KP219213 | KP184936 | |
| | | | | EI_N18 | EI_H18 | KP219214 | KP184937 | |
| | | | | EI_N19 | EI_H19 | KP219215 | KP184938 | |
| | | | | EI_N20 | EI_H20 | KP219216 | KP184939 | |
| | | | | EI_N21 | EI_H21 | KP219217 | KP184940 | |
| | | | | EI_N22 | EI_H22 | KP219218 | KP184941 | |
| | | | | EI_N23 | EI_H23 | KP219219 | KP184942 | |
| | | | | EI_N24 | EI_H24 | KP219220 | KP184943 | |
| | | | | EI_N25 | EI_M1 | KP219221 | KP184944 | |
| | | | | EI_N26 | EI_H26 | KP219222 | KP184945 | |
| | | | | EI_N27 | EI_H27 | KP219223 | KP184946 | |
| | | | | EI_N28 | EI_H28 | KP219224 | KP184847 | |
| | | | | EI_N29 | EI_H29 | KP219225 | KP184948 | |
| | | | | EI_N30 | EI_H30 | KP219226 | KP84949 | |
| | | | | EI_N31 | EI_H31 | KP219227 | KP184950 | |
| | | | | EI_N32 | EI_H32 | KP219228 | KP184951 | |
| | | | | EI_N33 | EI_H33 | KP219229 | KP184952 | |
| | **Indian Ocean** | | **33** | | | | | |
| | **Total** | | **239** | | | | | |

We also used Bayesian analysis (BA), as implemented in $M_RB_{AYES}$ 3.1.1 (Huelsenbeck and Ronquist 2001), for the CR data set. Parameters for performing partitioned BA were as follows: "lset nst = 6" (for GTR modle) and "rates = invgamma" (G + I). Two independent MCMC chains were performed with 50,000,000 replicates, sampling one tree per 100 replications for each run. We examined the distribution of log likelihood scores to determine both stationarity for each search and the need for additional runs to reach convergence in log likelihoods. We discarded the initial trees with non-stationary log likelihood (as burn-in), and combined the remaining trees that resulted in convergent log likelihood scores from both independent searches. We used these trees to construct a 50 % majority rule consensus tree. The values represented are *a posteriori* probabilities (PP) for BA. Nodal support for the BA tree was based on PP.

A median joining haplotype network was also constructed using Network version 4.6.1.3 (Copyright Fluxus Technology Ltd 1999-2015) to reveal the relationship between haplotypes and each locality. Network calculations did not include gaps in the sequence alignment.

**Population genetic variation**

We conducted the population genetic analyses for 183 individuals from the four Indian and Pacific Ocean using the combined data set of cyt *b* (GenBank accession numbers KP184920 - 185102) and CR (Table 1). The DNASP 5.0 (Librado and Rozas 2009) was utilized to calculate haplotype diversity (*h*) and nucleotide diversity ($\pi$) of each Indian and Pacific locality, and also to obtain the fixation indexes ($\Phi_{ST}$) between each pair of sampled localities. Moreover, we used the Arlequin version 3.5 (Excoffier and Lischer 2010) to analyze Tajima's *D* (Tajima 1989) and Fu's $F_S$ (Fu 1997) test, to obtain the mismatch distributions of pairwise differences, and to calculate the sum of the squared deviation (SSD) and Harpending's raggedness index (RI) (Harpending 1994) of all individuals in the Pacific and eastern Indian oceans. Furthermore, the Arlequin version 3.5 is also employed to demonstrate the statistical significance of $\Phi_{ST}$ by Exact test. Bayesian skyline analysis, which is calculated by using BEAST ver. 1.8.0 (Drummond et al. 2012), is employed to infer the vicissitude of the effective population size (*Ne*) with time. In this analysis, the substitution models of cyt *b* and CR are both GTR + G, which is recommended by jModelTest 2.0 (Darriba et al. 2012), and no partition into codon position. Based on Bermingham et al. (1997), the evolutionary rate of cyt *b* is set to 2.0% per million years and CR is set to 3.6%. The analysis is conducted with 50 million steps in a Markov chain Monte Carlo (MCMC) simulation with a relaxed molecular clock model. The result is generated by Tracer ver. 1.6 (Rambaut et al. 2014).

We then figured out whether blue marlin in the Pacific and eastern Indian oceans is one stock or not by performing a nested analysis of molecular variance (AMOVA) to estimate population differentiation from the genetic variation of different hierarchical levels. We used apatial analysis of molecular variance (SAMOVA) to identify the grouping based on the maximized and significant $F_{CT}$ values, setting the number of groups of populations to identify (k) = 2 and 3 (Dupanloup et al. 2002).

## RESULTS

For the mtDNA CR, a total of 220 haplotypes were verified in 239 individual blue marlin. There were 52 haplotypes found in 56 Atlantic specimens, 137 haplotypes in 150 Pacific samples, and 32 haplotypes in 33 eastern Indian Ocean samples. In the eastern Indian and Pacific samples, 158 haplotypes were only detected one time (singleton), and the other 11 haplotypes were found in more than one individual in a locality. Globally, there was only one haplotype, WNP_WSA_M1, could be detected transoceanically in both the Pacific and Atlantic. Both ML and BA phylogenetic analyses revealed there were two main clades of blue marlin; the Atlantic clade and the ubiquitous clade (Fig. 2). The Atlantic clade was constructed by the haplotypes restricted to the Atlantic, but the ubiquitous clade contains the haplotypes from the Indian, Pacific and Atlantic oceans. The result of median joining haplotype networks also revealed two clades, similar to the results of the ML and BA phylogenetic analyses (Fig. 3). These analyses indicate that all the eastern Indian and Pacific blue marlin belong to the ubiquitous clade which also contributed 53.6% to the sampled Atlantic blue marlin. The 183 individual blue marlin gathered from the Pacific and eastern Indian oceans did not display any association between similar haplotypes and sampling locations (Fig. 3).

According to the 2045 bp of the combined data set of cyt *b* and CR, the four Indian and

Pacific localities included 179 haplotypes from 183 individuals, and among these 179 haplotypes, 175 haplotypes were only detected one time; the other four haplotypes were found in more than one individual in a locality. The haplotype diversity values ($h$) of these localities were very high, ranging from 0.998 to 1.000; however, nucleotide diversity ($\pi$) was quite low, ranging from 1.0% to 1.3% (Table 2). The pairwise $\Phi_{ST}$ values of the four Indian Ocean and Pacific localities were shown in table 3. All the pairwise $\Phi_{ST}$ values were significant, but lower than 0.15; the Exact test showed no population differentiation between all pairwise localities. The results of AMOVA analysis also demonstrated that the most variation (94.67%) was found within the eastern Indian and Pacific blue marlin locality (Table 4). SAMOVA analysis indicated that the $F_{CT}$ values of two or three groups were not significant (k = 2, $F_{CT}$ = 0.0383, $p$ = 0.2483; k = 3, $F_{CT}$ = 0.0195, $p$ = 0.1760).

The Tajima's $D$, Fu's $F_S$, SSD, and RI values of the entire Indian and Pacific blue marlin set are presented in table 2. Both of the Tajima's $D$ and Fu's $F_S$ values were significantly negative; neither the SSD nor RI values were significant. Mismatch distribution analysis of the entire Indian and Pacific blue marlin revealed a unimodal curve (Fig. 4). The Bayesian skyline plot of the eastern Indian and Pacific blue marlin displays a demographic expansion during 0.30 to 0.65 million years ago (mya) (Fig. 5).

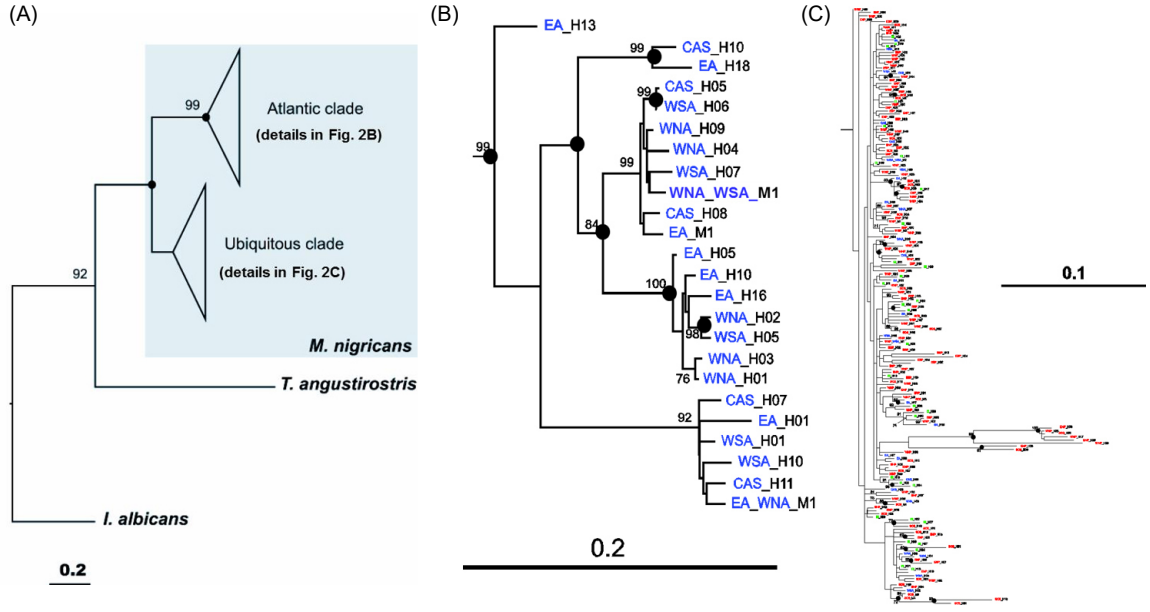


**Fig. 2.** Phylogenetic tree of 239 blue marlins based on CR sequences. Rooted phylogeny of 239 blue marlin CR sequences from maximum likelihood (ML) analysis and Bayesian (BA) analysis. Topologies of ML and BA analyses are similar; differences exist only in those relationships with weak statistical support. Numbers on branches are ML bootstrap values (Those below 70% are not shown) and solid circles on branch nodes indicate statistically robust nodes with posteriori probabilities from partitioned Bayesian analysis ≥ 0.95.

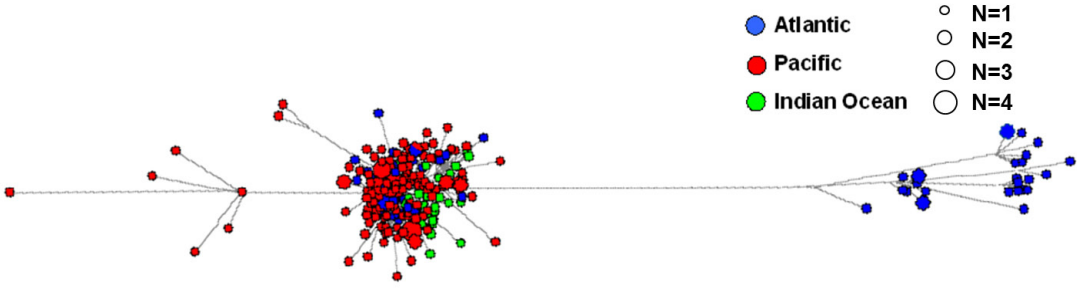


**Fig. 3.** Median joining network of 239 of blue marlin CR haplotypes. Each circle means a unique haplotype, and diameter is proportional to the individual number shading that haplotype.

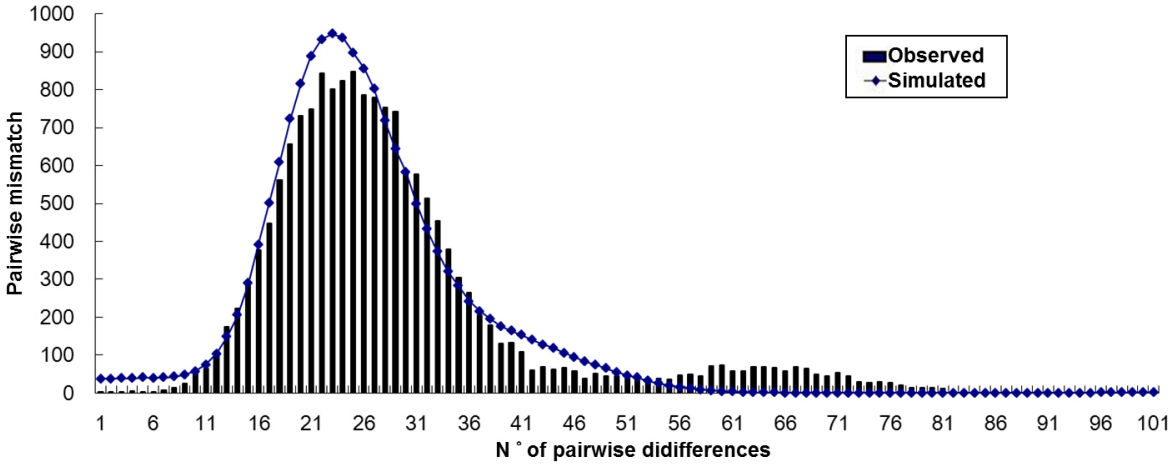


**Fig. 4.** Pairwise mismatch distribution of blue marlin in the Pacific and eastern Indian oceans.

**Table 2.** Summary of genetic diversity indexes. The measures of cyt *b* and CR diversity, *h*, haplotypes; π, nucleotide diversity were for four sampling localities of blue marlin. The *h*; π; Tajima's *D*; Fu's $F_S$; SSD, the sum of squared deviation; RI, Harpending's raggedness index were for all individuals in four sampling localities

| | *h* | π % | Tajima's *D* | Fu's $F_S$ | SSD | RI |
|---|---|---|---|---|---|---|
| EP | 1.0000 | 1.1 | | | | |
| WNP | 0.9994 | 1.1 | | | | |
| SCS | 1.0000 | 1.3 | | | | |
| EI | 0.9981 | 1.0 | | | | |
| Total (Pacific and eastern Indian oceans) | 0.9999 | 1.1 | -2.272* | -23.714* | 0.00048 | 0.00047 |

*$p < 0.05$.

**Table 3.** The pairwise $\Phi_{ST}$ values for four localities of blue marlin. $\Phi_{ST}$ values are below the diagonal and corresponding *p* values are above the diagonal

| | EP | WNP | SCS | EI |
|---|---|---|---|---|
| EP | - | < 0.0001 | < 0.0001 | < 0.0001 |
| WNP | 0.0318 | - | < 0.0001 | < 0.0001 |
| SCS | 0.0454 | 0.0375 | - | < 0.0001 |
| EI | 0.0626 | 0.0784 | 0.0918 | - |

**Table 4.** Results of hierarchical analysis of molecular variance (AMOVA) of blue marlin in different locations. All individuals in four sampling localities were assigned to one group

| Source of variation ($F_{ST}$) | *d.f.* | Sum of squares | Variance components | Percentage of variation | Fixation indices | *p* value |
|---|---|---|---|---|---|---|
| Among locations | 3 | 144.446 | 0.766 | 5.33 | - | - |
| Within locations | 179 | 2434.346 | 13.600 | 94.67 | 0.0534 | $p < 0.0001$ |

## DISCUSSION

Previous phylogenetic analyses based on mtDNA of blue marlin from the Pacific and Atlantic has demonstrated that the phylogeny of blue marlin consists of two distinct clades (Finnerty and Block 1992; Graves and McDowell 1995; McDowell et al. 2007). Compared with former phylogenetic studies, our study is the first to sample the blue marlin from the Indian Ocean and includes a larger number of Pacific blue marlin; it also confirms the two clade pattern of blue marlin phylogeny: one is widespread in the Indian, Pacific and Atlantic oceans and the other is endemic to the Atlantic (Fig. 2).

The presence of distinct two clades in the blue marlin phylogeny indicates they were isolated for a considerable period of time; Finnerty and Block (1992) speculated this divergence occured 1.5-3.0 mya, but Graves and McDowell (1995) estimated 0.6 mya. However, both agreed that the two distinct clades most likely occurred during the Pleistocene. The divergence of blue marlin could be driven by two factors: first, Graves and McDowell (1995) suggested that the diffentiation of blue marlin occurred allopatrically in the Pacific (ubiquitous clade) and in the Atlantic (Atlantic clade), a result of the formation of the Isthmus of Panama constraining the gene flow between the Pacific and Atlantic populations of blue marlin during the Pleistocene. Population

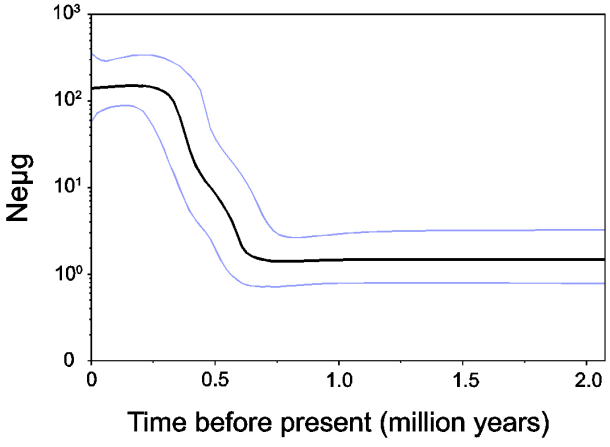


**Fig. 5.** Bayesian skyline plots of mitochondrial cytochrome *b* (cyt *b*) and control region (CR) haplotypes for Pacific and Indian Ocean blue marlin. The Bayesian skyline plot is based on cyt *b* and CR sequence data, in which the x-axis is time and the y-axis is the production ($Ne\mu g$) of effective population size (*Ne*), mutation rate ($\mu$), and generation length (*g*). The median estimate (black line) and 95% highest posterior density (blue lines) limits are displayed.

differentiation caused by the formation of the Isthmus of Panama can be observed in other pelagic fishes (Bermingham et al. 1997; Knowlton and Weigt 1998). Second, Buonaccorsi et al. (2001) proposed that the cooler water mass in the south of the Cape of Good Hope during the Pleistocene could act as a barrier limiting gene flow between the Atlantic and Indian-Pacific blue marlin. Global marine fishes forming clades through allopatry have been observed in other pelagic species, such as swordfish *Xiphias gladius* (Alvarado Bremer et al. 1995, 1996; Rosel and Block 1996), sailfish *Istiophorus platypterus* (Graves and McDowell 1995, 2003), albacore *Thunnus alalunga* (Chow and Ushiama 1995; Vinas et al. 2004), and bigeye tuna *Thunnus obesus* (Alvarado Bremer et al. 1998; Chow et al. 2000; Martínez et al. 2006).

This study detected the haplotype, WNP_WSA_M1, both in the Pacific and the Atlantic and the tagging data (Scott et al. 1990; Anon. 1994; Ortiz et al. 2003) likewise demonstrated that blue marlin nowadays is capable of inter-oceanic movement. Consequently, there are three possible reasons why the haplotypes of the ubiquitous clade are distributed globally and the ones of the Atlantic clade are restricted to the Atlantic: first, Graves and McDowell (1995, 2003) asserted that the migration of blue marlin between the Atlantic and Pacific Ocean was uni-directional. The strong westward flow of the tropical Agulhas current and the eastward passage hindered by Benguela water assisted the movement of blue marlin from the Indian Ocean to the Atlantic but inhibited migration in the reverse direction (Talbot and Penrith 1962; Penrith and Cram 1974; Buonaccorsi et al. 2001); second, Consuegra et al. (2015) demonstrated that the mitochondrial genome acclimatizes to the local environment and Chien et al. (2015) also discovered the correlation between special mitochondrial haplotypes and physiological traits; therefore, it is possible that the blue marlin bearing the mitochondrial haplotypes of the Atlantic clade are restricted to the Atlantic Ocean by natural selection; in contrast, there is speculation that the ubiquitous-clade blue marlin are more adaptive and capable of inter-oceanic migration, as indicated by the blue marlin tagging data (Ortiz et al. 2003); finally, if we only take mitochondrial markers into consideration, sex-biased migration may bring about a wrong conclusion as the mitochondrial markers are maternal. In the future, use of nuclear DNA markers, such as microsatellites and single nucleotide polymorphisms (SNP), in genetic population studies of blue marlin could shed light

on the fine structure of the global blue marlin and the gene flow between distinct populations.

For the eastern Indian and Pacific blue marlin, neither the phylogenetic analyses nor the median joining haplotype network reveals an association between similar haplotypes and sampling locations. The pairwise $\Phi_{ST}$ values for four localities were very low (Table 3) and the Exact test showed no population differentiation between any two localities; furthermore, AMOVA analysis reveals that most variation (94.67 %) comes from within the locality rather than between localities. SAMOVA analysis also does not obtain significant $F_{CT}$ values. These results support Graves and McDowell (2003) assertion that Pacific blue marlin populations have no significant differentiation. Nevertheless, previous research has suggested that marine biogeographic barriers could be defined using faunal breaks in composition and diversity patterns and levels of endemism (Rocha et al. 2007). The eastern Pacific barrier, an expanse of ~5000 km of water, separates the eastern and central Pacific Ocean and is the world's largest marine biogeographic barrier (Lessios and Robertson 2006). Three large pelagic fishes, sailfish *Istiophorus platypterus* (McDowell 2002; Lu et al. 2015), striped marlin *Kajikia audax* (McDowell and Graves 2008), and yellowfin tuna *Thunnus albacares* (Sharp, 1978; Ward et al. 1994; Ely et al. 2005), all developed genetic differentiation based on this biogeographic barrier. Evidence for discrete spawning cycles of blue marlin has been reported (Hopper 1990; Serafy et al. 2003), and the larval distribution in the Pacific and eastern Indian oceans are apparently separated by the Indo-Australian Archipelago (Howard and Ueyanagi 1965; Matsumoto and Kazama 1974; Nishikawa et al. 1985). However, as information derived from cyt *b* and CR show, these barriers cannot impede the gene flow within blue marlin in the Indian and Pacific oceans. In this study, the phylogenetic and population genetic analyses based on the mitochondrial DNA fail to identify the locality of individual blue marlin, rendering it useless in the investigation of illegal trade of Atlantic blue marlin in the United States. Fortunately, Sorenson et al. (2013) successfully advanced discrimination of the population structure of Atlantic and Pacific blue marlin using 13 microsatellite markers. A greater collection of blue marlin samples from the Indian and Pacific Oceans and more sensitive nuclear markers will be required in future studies to unveil the detailed population structure of blue marlin in this region. Therefore, the application of hypervariable genetic markers to blue marlin studies in the future could better depict the population structure of blue marlin across the oceans and assist in constructing a more comprehensive fishery management.

Haplotype and nucleotide diversity values provide information on the population history of blue marlin. High $h$ and low $\pi$ values were found in all four localities in the Pacific and eastern Indian oceans, a finding which concurs with those of McDowell et al. (2007). Avise et al. (1984) and Rogers and Harpending (1992) suggesting this pattern of genetic diversity could result from expansion of a population from a low effective population size, because rapid population growth stimulates new mutations. Such demographic scenarios have been proposed for western Atlantic Spanish sardine *Sardina pilchardus* (Tringali and Wilson 1993), Atlantic bluefin tuna *Thunnus thynnus* (Carlsson et al 2004.), and yellowfin tuna *Thunnus albacares* (Ely et al. 2005). The median joining haplotype network of the eastern Indian and Pacific blue marlin also revealed a star-like configuration, consistent with the population expansion model. The Tajima's $D$ and Fu's $F_S$ values are both significantly negative (Table 2), which indicates an expanding population. The mismatch distribution analysis of eastern Indian and Pacific blue marlin is uni-modal, indicating that the accumulation of a new mutation in a population is greater than the loss of variation through genetic drift, and that this population has undergone rapid expansion (possibly after a bottleneck) (Rogers and Harpending 1992). Furthermore, both of the SSD and RI values were not significant ($P > 0.05$) (Table 2) and the steep curve of the Bayesian skyline plot (Fig. 5) confirms the population expansion of eastern Indian and Pacific blue marlin. Santini and Sorenson (2013) suggest that the divergent time of Atlantic and Indo-Pacific blue marlin is approximately one to nine mya and the eastern Indian and Pacific blue marlin may have existed for 0.35 to 8.35 mya before population expansion. The historical event that promoted the demographic expansion of the eastern Indian and Pacific blue marlin during 0.30 to 0.65 mya is worth investigating in the future, though this expansion may simply be the result of the blue marlin's prey species in the Pleistocene (Liu et al. 2006, López et al. 2010, Chou et al. 2015, Sukumaran et al. 2015).

## CONCLUSIONS

Effective management and conservation of blue marlin as a resource is required to fully understand the population structure. This study shows that the blue marlin in the Pacific and eastern Indian oceans could be viewed as a single genetic stock, so international cooperation is needed to effectively manage the resource of blue marlin. In addition, it is clear that over-exploitation causes damage to the genetic diversity of fish (Allendorf et al. 2014), so the genetic variation of Indian and Pacific blue marlin may be very sensitive to pressure from intensive fishing, as seen in the Atlantic (McDowell et al. 2007). Since it is established that the severe loss of genetic diversity of a fish as a result of overfishing leads to irreversible trait shift, such as body size and maturation (Pinsky and Palumbi 2014). Unlike the Atlantic blue marlin, which is supervised by the International Commission for the Conservation of Atlantic Tunas (ICCAT), the Indian and Pacific blue marlin are not yet well monitored or managed; we therefore appeal to the countries for a sustainable blue marlin fishing program.

**Acknowledgments:** We are grateful to Michael Hinton in IATTC for providing the samples from the eastern Pacific Ocean; Mr. Yu Paol Chu and Mrs. Kanya Yu for providing samples from the eastern Indian Ocean; Vu Le and Wei-Chuan Chiang for assistance in field sampling. We especially thank three anonymous reviewers and Dr. John E. Graves for constructive and valuable comments which greatly improved this manuscript. This study was financially supported by research grants of Fish Ecology and Evolution Laboratory (Academia Sinica of Taiwan) and partially by the research grant NSC98-2611-M-002-002 from National Science Council (the current Ministry of Science and Technology) to Dr. Chi-Lu Sun.

## REFERENCES

Allendrof FW, Berry O, Ryman N. 2014. So long to genetic diversity, and thanks for all the fish. Mol Ecol **23(1):**23-25. doi:10.1111/mec.12574.

Alvarado Bremer JR, Mejuto J, Baker AJ. 1995. Mitochondrial DNA control region sequences indicate extensive mixing of swordfish (*Xiphias gladius*) populations in the Atlantic Ocean. Can J Fish Aquat Sci **52(8):**1720-1732. doi:10.1139/f95-764.

Alvarado Bremer JR, Mejuto J, Thomas WG, Ely B. 1996. Global population structure of the swordfish (*Xiphias gladius*) as revealed by the analysis of the mitochondrial control region. J Exp Mar Biol Ecol **44(3):**206-216. doi:10.1016/0022-0981(95)00164-6.

Alvarado Bremer JR, Stequert B, Robertson NW, Ely B. 1998. Genetic evidence for inter-oceanic subdivision of bigeye tuna (*Thunnus obesus*) populations. Mar Biol **132:**547-557. doi:10.1007/s002270050420.

Anonymous. 1994. Cooperative game fish tagging program annual newsletter: 1992. Southeast Fisheries Science Center, Miami, FL.

Avise JC, Neigel JE, Arnold J. 1984. Demographic influences on mitochondrial DNA lineage survivorship in animal populations. J Mol Evol **20:**99-105. doi:10.1007/BF02257369.

Bermingham E, McCafferty SS, Martin AP. 1997. Fish biogeography and molecular clocks: perspectives from the Panamanian Isthmus. *In*: Kocher TD and Stepien CA (eds) Molecular systematics of fishes. Academic Press, San Diego, 113-128.

Bernatchez L, Guyomard R, Bonhomme F. 1992. DNA sequence variation of the mitochondrial control region among geographically and morphologically remote European brown trout, *Salmo trutta*, populations. Mol Ecol **1:**161-73. doi:10.1111/j.1365-294X.1992.tb00172.x.

Buonaccorsi VP, McDowell JR, Graves JE. 2001. Reconciling patterns of inter-ocean molecular variance from four classes of molecular markers in blue marlin (*Makaira nigricans*). Mol Ecol **10(5):**1179-1196. doi:10.1046/j.1365-294X.2001.01270.x.

Buonaccorsi VP, Reece KS, Morgan LW, Graves JE. 1999. Geographic distribution of molecular variance within the blue marlin (*Makaira nigricans*): A hierarchical analysis of allozyme, single-copy nuclear DNA, and mitochondrial DNA markers. Evolution **53(2):**568-579. doi:10.2307/2640793.

Carlsson J, McDowell JR, Diaz-Jaimes P, Carlsson JE, Boles SB, Gold JR, Graves JE. 2004. Microsatellite and mitochondrial DNA analyses of Atlantic bluefin tuna (*Thunnus thynnus*) population structure in the Mediterranean Sea. Mol Ecol **13:**3345-3356. doi:10.1111/j.1365-294X.2004.02336.x.

Chien A, Kirby R, Sheen SS. 2015. The relevance of mitochondrial lineages of Taiwanese cultured grey mullet, *Mugil cephalus*, to commercial products of Roe. Aquac Res pp. 1-6. doi:10.1111/are.12693.

Chou CE, Liao TY, Chang HW, Chang SK. 2015. Population structure of *Hirundichthys oxycephalus* in the northwestern Pacific inferred from mitochondrial cytochrome oxidase I gene. Zool Stud **54:**19. doi:10.1186/s40555-014-0085-4.

Chow S, Okamoto H, Miyabe N, Hiramatsu K, Barut N. 2000. Genetic divergence between Atlantic and Indo-Pacific stocks of bigeye tuna (*Thunnus obesus*) and admixture around South Africa. Mol Ecol **9:**221-227. doi:10.1046/j.1365-294x.2000.00851.x.

Chow S, Ushiama H. 1995. Global population structure of albacore (*Thunnus alalunga*) inferred by RFLP analysis of the mitochondrial ATPase gene. Mar Biol **123:**39-45. doi:10.1007/BF00350321.

Collette BB, Carpenter KE, Polidoro BA, Juan-Jorda MJ, Boustany A, Die DJ, Elfes C, Fox W, Graves J, Harrison L, McManus R, Minte-Vera C, Nelson R, Restrepo V, Schratwieser J, Sun CL, Brick Peres M, Canales C, Cardenas G, Chang SK, Chiang WC, de Oliveira Leite N, Harwell H, Lessa R, Fredou FL, Oxenford HA, Serra

R, Shao KT, Sumalia R, Wang SP, Watson R, Yanez E. 2011. High value and long life - Double Jeopardy for tuna and billfishes. Science **333:**291-292. doi:10.1126/science.1208730.

Consuegra S, Elgan J, Eric V, de Leaniz CG. 2015. Patterns of natural selection acting on the mitochondrial genome of a locally adapted fish species. Genet Sel Evol **47:**58. doi:10.1186/s12711-015-0138-0.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat Methods **9:**772. doi:10.1038/nmeth.2109.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol Biol Evo **29:**1969-1973. doi:10.1093/molbev/mss075.

Dupanloup I, Schneider S, Excoffier L. 2002. A simulated annealing approach to define the genetic structure of populations. Mol Ecol **11:**2571-2581. doi:10.1046/j.1365-294X.2002.01650.x.

Ely B, Viñas J, Alvarado-Bremer JR, Black D, Lucas L, Covello K, Labrie A, Thelen VE. 2005. Consequences of the historical demography on the global population structure of two highly migratory cosmopolitan marine fishes: the yellowfin tuna (*Thunnus albacares*) and the skipjack tuna (*Katsuwonus pelamis*). BMC Evol Biol **5:**19. doi:10.1186/1471-2148-5-19.

Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Res **10(3):**564-567. dio:10.1111/j.1755-0998.2010.02847.x.

Finnerty JR, Block BA. 1992. Direct sequencing of mitochondrial DNA detects highly divergent haplotypes in blue marlin (*Makaira nigricans*). Mol Mar Biol Biotechnol **1(3):**206-214.

Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics **147(2):**915-925.

Graves JE, McDowell JR. 1995. Inter-ocean genetic-divergence of Istiophorid billfishes. Mar Biol **122(2):**193-203. doi:10.1007/BF00348932.

Graves JE, McDowell JR. 2003. Stock structure of the world's Istiophorid billfishes: a genetic perspective. Mar Freshwater Res **54(4):**287-298. doi:10.1071/MF01290.

Graves JE, McDowell JR. 2015. Population structure of istiophorid billfishes. Fish Res **166:**21-28. doi:10.1016/j.fishres.2014.08.016.

Harpending HC. 1994. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. Hum Biol **66:**591-600.

Hopper CN. 1990. Patterns of Pacific blue marlin reproduction in Hawaiian waters. *In*: Stroud RH (ed) Planning the future of billfishes: research and management in the 90s and Beyond. Part II. Contributed papers. National Coalition for Marine Conservation Inc., Savannah, GA, pp. 29-39.

Howard JK, Ueyanagi S. 1965. Distribution and relative abundance of billfishes (Istiophoridae) of the Pacific Ocean. Univ. Miami Inst. Mar. Sci., Stud. Trop. Oceanography **2:**1-134.

Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics **17:**754-755.

Kailola PJ, Williams MJ, Stewart PC, Reichelt RE, McNee A, Grieve C. 1993. Australian fisheries resources. Bureau of Resource Sciences, Canberra, Australia.

Knowlton N, Weigt LA. 1998. New dates and new rates for divergence across the Isthmus of Panama. Proc R Soc Lond B **265:**2257-2263. doi:10.1098/rspb.1998.0568.

Lessios HA, Robertson DR. 2006. Crossing the impassable: genetic connections in 20 reef fishes across the eastern Pacific barrier. Proc Roy Soc **273:**2201-2208. doi:10.1098/rspb.2006.3543.

Librado P, Rozas J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics **25(11):**1451-1452. doi:10.1093/bioinformatics/btp187.

Liu JX, Gao TX, Zhuang ZM, Jin XS, Yokogawa K, Zhang YP. 2006. Late Pleistocene divergence and subsequent population expansion of two closely related fish species, Japanese anchovy (*Engraulis japonicus*) and Australian anchovy (*Engraulis australis*). Mol Phylognet Evol **40:**712-723. doi:10.1016/j.ympev.2006.04.019.

López MD, Alcocer MU, Jaimes PD. 2010. Phylogeography and historical demography of the Pacific Sierra mackerel (*Scomberomorus sierra*) in the Eastern Pacific. BMC Genetics **11:**34. doi:10.1186/1471-2156-11-34.

Lu CP. 2014. Bayesian Analyses of Genetic Variation and Population Differentiation in Pacific Swordfish (*Xiphias gladius* L.) and the Development of High Resolution Melting Assays for Species Identification and Potential Sex-Linked Marker Survey in Istiophorid Billfish. PhD dissertation, Texas A&M University, College Station, TX, USA.

Lu CP, Alvarado Bremer JR, Mckenzie JL, Chiang WC. 2015. Analysis of sailfish (*Istiophorus platypetrus*) population structure in the North Pacific Ocean. Fish Res **166:**33-38. doi:10.1016/j.fishres.2014.09.018.

Martínez P, Gonzalez EG, Castilho R, Zardoya R. 2006. Genetic diversity and historical demography of Atlantic bigeye tuna (*Thunnus obesus*). Mol Phyl Evol **39:**404-416. doi:10.1016/j.ympev.2005.07.022.

Matsumoto WM, Kazama TK. 1974. Occurrence of young billfishes in the central Pacific Ocean. *In*: Shomura RS, Williams F (eds) Proceedings of the International Billfish Symposium, Kailua-Kona, Hawaii, 9-12 August 1972, Pt. 2. Review and Contributed Papers. NOAA Tech. Rep. NMFS SSRF-**675:**239-251.

McDowell JR. 2002. Genetic stock structure of the sailfish, *Istiophorus platypterus*, based on nuclear and mitochondrial DNA. PhD dissertation, College of William and Mary: Virginia Institute of Marine Science, USA.

McDowell JR, Carlsson JEL, Graves JE. 2007. Genetic analysis of blue marlin (*Makaira nigricans*) stock structure in the Atlantic Ocean. Gulf Caribb Res **19(2):**75-82. doi:10.18785/gcr.1902.09.

McDowell JR, Graves JE. 2008. Population structure of striped marlin (*Kajikia audax*) in the Pacific Ocean based on analysis of microsatellite and mitochondrial DNA. Can J Fish Aquat Sci **65:**1307-1320. doi:10.1139/F08-054.

Nakamura I. 1985. FAO species catalog vol 5. billfishes of the world an annotated and illustrated catalog of marlins sailfishes spearfishes and swordfishes known to date. FAO (Food and Agriculture Organization of the United Nations) Fisheries Synopsis FAO/UNDP, Rome.

Nishikawa Y, Honma M, Ueyanagi S, Kikawa S. 1985. Average distribution of larvae of oceanic species of Scombroid fishes, 1956-1981. Far Seas Fish Res Lab S Ser **12:**1-99.

Ortiz M, Prince E, Serafy J, Holts D, Davy K, Pepperell J, Lowery M, Holdsworth J. 2003. Global overview of the major constituent-based billfish tagging programs and their results since 1954. Mar Freshwater Res **54(4):**489-

507. doi:10.1071/MF02028.
Palumbi S, Martin A, Romano S, McMillan WO, Stice L, Grabowski G. 1991. The Simple Fool's Guide to PCR, version 2, Department of Zoology, University of Hawaii, Hawaii.
Penrith MJ, Cram DL. 1974. The Cape of Good Hope: A hidden barrier to billfishes. *In*: Shomura RS, Williams F (eds) Proceedings of the International Billfish Symposium, Kailua-Kona, Hawaii, 9-12 August 1972. Part 2 Reviewed and Contributed Papers. NOAA Technical Report, NMFS SSRF-675, pp. 175-187 (NOAA: Seattle, WA).
Pinsky ML, Palumbi SR. 2014. Meta-analysis reveals lower genetic diversity in overfished population. Mol Ecol **23(1):**29-39. doi:10.1111/mec.12509.
Posada D. 2008. jModelTest: Phylogenetic model averaging. Mol Biol Evol **25:**1253-1256. doi:10.1093/molbev/msn083.
Rambaut A, Suchard MA, Xie D, Drummond AJ. 2014. Tracer v1.6, Available from http://beast.bio.ed.ac.uk/Tracer.
Rocha LA, Craig MT, Bowen BW. 2007. Phylogeography and the conservation of coral reef fishes. Coral Reefs **26(3):**501-512. doi:10.1007/s00338-007-0261-7.
Rogers AR, Harpending H. 1992. Population-growth makes waves in the distribution of pairwise genetic-differences. Mol Biol Evol **9(3):**552-569.
Rosel PE, Block BA. 1996. Mitochondrial control region variability and global population structure in the sword-fish, *Xiphias gladius*. Mar Biol **125:**11-22. doi:10.1007/BF00350756.
Santini F, Sorenson L. 2013. First molecular timetree of billfishes (Istiophoriformes: Acanthomorpha) shows a Late Miocene radiation of marlins and allies. Ital J Zool **80(4):**481-489. doi:10.1080/11250003.2013.848945.
Scott EL, Prince ED, Goodyear CD. 1990. History of the cooperative game fish tagging program in the Atlantic Ocean, Gulf of Mexico, and Caribbean Sea 1954-1987. Am Fish Soc Symp **7:**841-853.
Serafy JE, Cowen RK, Paris CB, Capo TR, Luthy SA. 2003. Evidence of blue marlin, *Makaira nigricans*, spawning in the vicinity of Exuma Sound, Bahamas. Mar Freshwater Res **54:**299-306. doi:10.1071/MF01273.
Sharp GD. 1978. Behavioral and physiological properties of tunas and their effects on vulnerability to fishing gear. *In*: Sharp GD, Dizon AE (eds) The Physiological Ecology of Tunas. Academic Press, New York, pp. 397-449.
Sorenson L, McDowell JR, Knott T, Graves JE. 2013. Assignment test method using hypervariable markers for blue marlin (*Makaira nigricans*) stock identification. Conserv Genet Resour **5:**293-297. doi:10.1007/s12686-012-9747-x.
Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics **22:**2688-2690. doi:10.1093/bioinformatics/btl446.
Sukumaran S, Sebastian W, Gopalakrishnan A. 2015. Population genetic structure of Indian oil sardine, *Sardinella longiceps* along Indian coast. Gene **576(1):**372-378. doi:10.1016/j.gene.2015.10.043.
Tajima F. 1989. The effect of change in population size on DNA polymorphisms. Genetics **123(3):**597-601.
Talbot FH, Penrith MJ. 1962. Tunnies and marlins of South Africa. Nature **193:**558-559. doi:10.1038/193558a0.
Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucl Acids Res **22(22):**4673-4680. doi:10.1093/nar/22.22.4673.
Tringali MD, Wilson RR. 1993. Differences in haplotype frequencies of mtDNA of the Spanish sardine *Sardinella aurita* between specimens from the eastern Gulf of Mexico and southern Brazil. Fish Bull **91:**362-370.
Vinas J, Alvarado Bremer JR, Pla C. 2004. Inter-oceanic genetic differentiation among albacore (*Thunnus alalunga*) populations. Mar Biol **145:**225-232. doi:10.1007/s00227-004-1319-5.
Ward RD, Elliott NG, Grewe PM, Smolenski AJ. 1994. Allozyme and mitochondrial DNA variation in yellowfin tuna (*Thunnus albacares*) from the Pacific Ocean. Mar Biol **118:**531-539. doi:10.1007/BF00347499.