

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

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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. According 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

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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 Mascarene 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

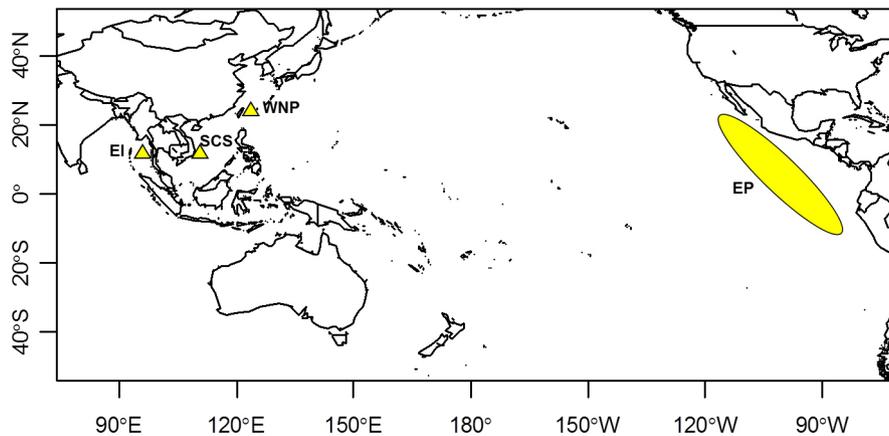
We used a small piece (~2 mm<sup>3</sup>) of muscle and fin clip for DNA extraction, following the protocol of the Genomic DNA Mini Kit (Tissue) (Genomic, New Taipei City, Taiwan). The extracted DNA samples were stored at 4°C until we conducted the polymerase chain reaction. Two pairs of primers, one 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 <i>cyt b</i>	Source
Outgroup								
<i>Istiophorus platypterus</i>						AP006035		
<i>Tetrapturus angustirostris</i>						AB470303		
Ingroup								
<i>Makaira nigricans</i>	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
	Eastern Atlantic (EA)		18	CAS_N8	CAS_H8	EF607802	-	McDowell et al. 2007
				CAS_N9	CAS_H9	EF607803	-	
				CAS_N10	CAS_H10	EF607804	-	
				CAS_N11	CAS_H11	EF607805	-	
				EA_N1	EA_H1	EF607806	-	
				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	-	
	<b>Atlantic Ocean</b> eastern Pacific (EP)	2004-2006	56 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 <i>b</i>	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	
	<b>Pacific Ocean</b>		<b>150</b>					
	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	KP184949	
				EI_N31	EI_H31	KP219227	KP184950	
				EI_N32	EI_H32	KP219228	KP184951	
				EI_N33	EI_H33	KP219229	KP184952	
	<b>Indian Ocean</b>		<b>33</b>					
	<b>Total</b>		<b>239</b>					

We also used Bayesian analysis (BA), as implemented in MrBAYES 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 model) 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 ( $N_e$ ) 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 a spatial 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

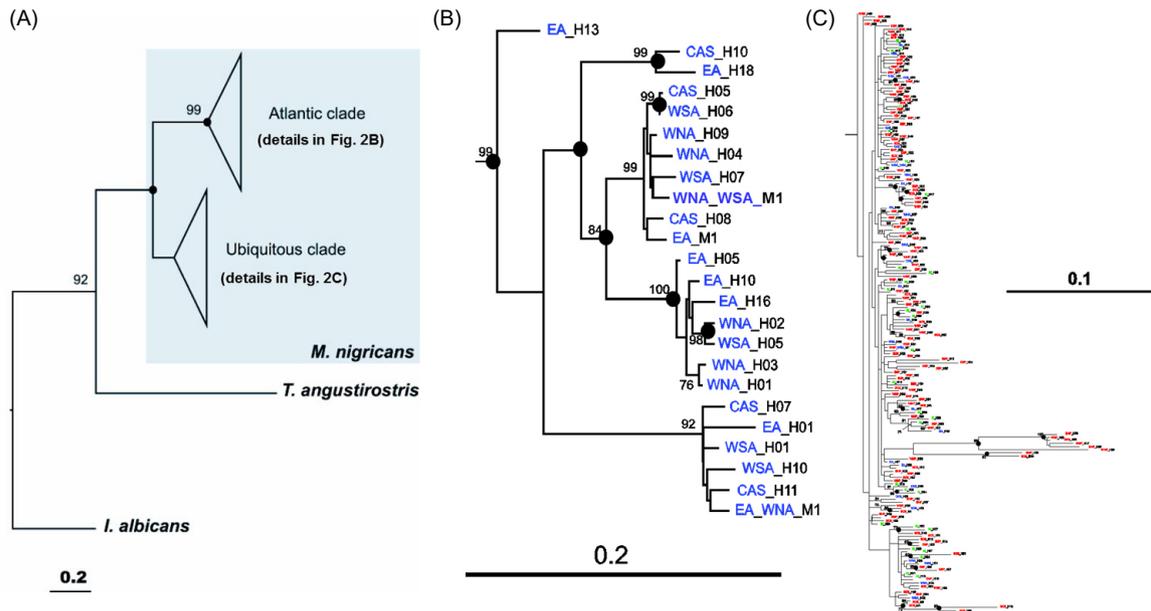
For the mtDNA CR, a total of 220 haplotypes were verified in 239 individual blue marlin. There were 52 haplotypes found in 56 Atlantic specimens, 137 haplotypes in 150 Pacific samples, and 32 haplotypes in 33 eastern Indian Ocean samples. In the eastern Indian and Pacific samples, 158 haplotypes were 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

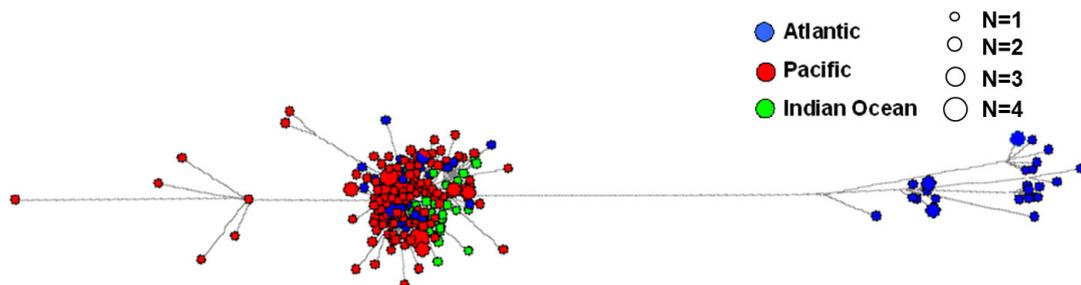
Pacific localities included 179 haplotypes from 183 individuals, and among these 179 haplotypes, 175 haplotypes were only detected one time; the other four haplotypes were found in more than one individual in a locality. The haplotype diversity values ( $h$ ) of these localities were very high, ranging 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  $\geq 0.95$ .



**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.

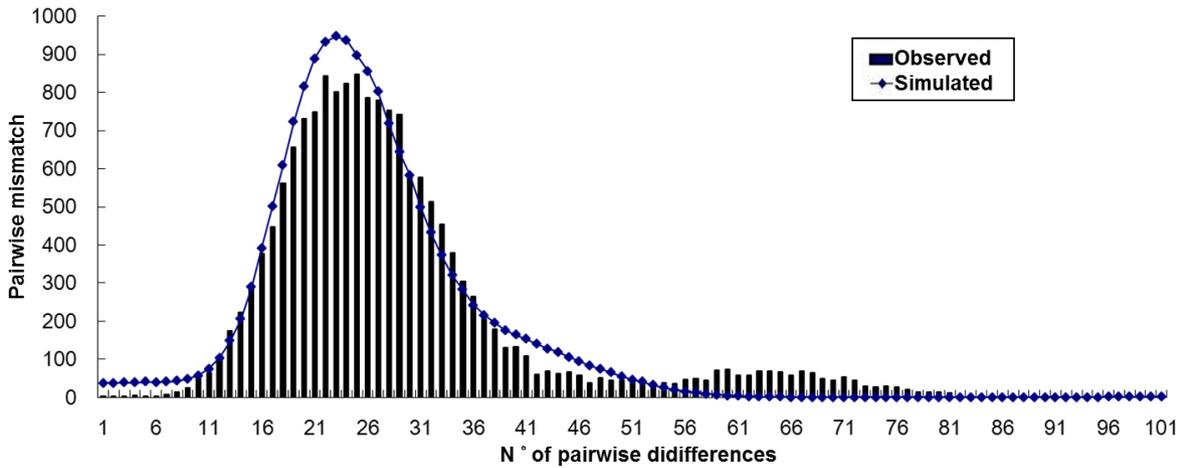


Fig. 4. Pairwise mismatch distribution of blue marlin in the Pacific and eastern Indian oceans.

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

	<i>h</i>	$\pi$ %	Tajima's <i>D</i>	Fu's <i>F<sub>s</sub></i>	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 ( <i>F<sub>ST</sub></i> )	<i>d.f.</i>	Sum of squares	Variance components	Percentage of variation	Fixation indices	<i>p</i> value
Among locations	3	144.446	0.766	5.33	-	-
Within locations	179	2434.346	13.600	94.67	0.0534	<i>p</i> < 0.0001

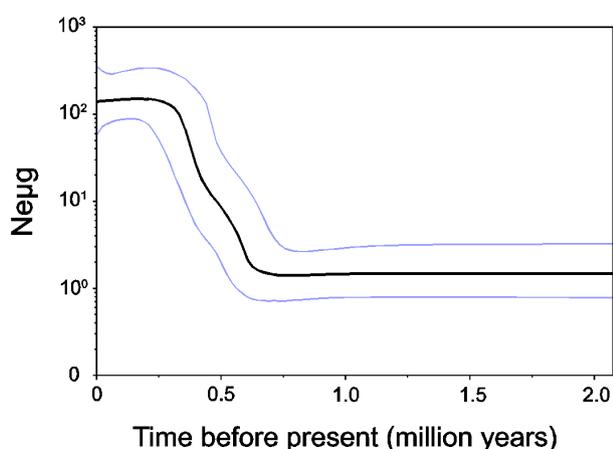
## DISCUSSION

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 occurred 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 differentiation 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

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 Ushiyama 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



**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.

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.

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