

# Multi-Genetic Marker Approach and Spatio-Temporal Analysis Suggest There Is a Single Panmictic Population of Swordfish *Xiphias gladius* in the Indian Ocean

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## Abstract

Genetic population structure of swordfish *Xiphias gladius* was examined based on 2231 individual samples, collected mainly between 2009 and 2010, among three major sampling areas within the Indian Ocean (IO; twelve distinct sites), Atlantic (two sites) and Pacific (one site) Oceans using analysis of nineteen microsatellite loci ( $n = 2146$ ) and mitochondrial ND2 sequences ( $n = 2001$ ) data. Sample collection was stratified in time and space in order to investigate the stability of the genetic structure observed with a special focus on the South West Indian Ocean. Significant AMOVA variance was observed for both markers indicating genetic population subdivision was present between oceans. Overall value of F-statistics for ND2 sequences confirmed that Atlantic and Indian Oceans swordfish represent two distinct genetic stocks. Indo-Pacific differentiation was also significant but lower than that observed between Atlantic and Indian Oceans. However, microsatellite F-statistics failed to reveal structure even at the inter-oceanic scale, indicating that resolving power of our microsatellite loci was insufficient for detecting population subdivision. At the scale of the Indian Ocean, results obtained from both markers are consistent with swordfish belonging to a single unique panmictic population. Analyses partitioned by sampling area, season, or sex also failed to identify any clear structure within this ocean. Such large spatial and temporal homogeneity of genetic structure, observed for such a large highly mobile pelagic species, suggests as satisfactory to consider swordfish as a single panmictic population in the Indian Ocean.

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## Introduction

Large pelagic species have commonly been thought to lack genetic spatial structure due to their cosmopolitan distribution, large population size, high fecundity, production of numerous pelagic larvae and ability to easily migrate inter-ocean distances [1]. However, examples of single panmictic worldwide distributed populations appear to be rare such as in the case of the wahoo, *Acanthocybium solandri* [2]. Long-distance migrants are, by definition, highly mobile but factors maintaining observed genetic population structure are poorly understood yet these are common (examples such as Atlantic Bluefin tuna [3], bigeye tuna [4]). Structure has often been observed for large pelagic species with cosmopolitan distribution demonstrating genetic population structure partitioned among or even within ocean basins. Examples of genetic sub-structure in cosmopolitan species include albacore tuna *Thunnus alalunga* [5] and at the intra-oceanic level, the blue marlin *Makaira nigricans* [6] or the white marlin *Tetrapturus albidus* [7]. Geographic partition was also shown in the most widely distributed species of pelagic fishes, the broadbill swordfish *Xiphias gladius* with two subdivisions in the Pacific Ocean (North-west versus South-East; [8]), as well as in the Atlantic Ocean (North-west versus South [9]), with a Mediterranean population clearly isolated from those of the Atlantic Ocean [10]. Furthermore, evidence of

northwest and south Atlantic stocks of swordfish was supported by both parasite [11] and genetic data [9] strongly demonstrating that this species has the potential to evolve intra-ocean population subdivision.

Genetic divergence in migratory species may exist if individuals are philopatric and consistently return to the same breeding grounds. Conventional and electronic tagging of swordfish shows that individuals are capable of extensive movements of thousands of kilometres [12] across the North Atlantic - 2500 km [13,14] and even larger migration in the Indian Ocean with one swordfish recaptured 6670 km south-eastward from the point of release (975 releases -29 recaptures over the last 20 years; [15]). However, migratory behavior of swordfish seems to be constrained and delineated by equatorial boundaries, at least in the Atlantic Ocean [12], explaining why genetic pattern was more similar between southern regions of two adjacent oceans than from the southern to the northern parts of a same ocean [16,17]. Moreover, a great number of tagged swordfish were recaptured near the release site [13,18,19]. For instance, in the North Atlantic, Neilson et al. [14] showed evidence of precise homing from nesting to feeding areas on 4 swordfishes over 25 tracked individuals. This homing behaviour thus may explain discrete mtDNA boundaries observed between samples from North-west Atlantic and South Atlantic known to be distinct breeding grounds [20].

Swordfish population structure has previously been explored within the Indian Ocean with most studies identifying some genetic differentiation in the Indian Ocean [17,21,22,23], although there appears to be inconsistency among the various conclusions. However, these studies were in fact conducted on different geographic scales, with too few individuals analyzed, the reproduction behavior was not taken in consideration, there were heterogeneous sampling periods, and often only one genetic marker was used. This could explain incongruity of results among studies in terms of stock structure. A hypothesis of strong philopatry would suggest that genetic differentiation could be observed in the Indian Ocean between different spawning grounds and/or between Northern and Southern hemispheres like in the other oceans. However, reproduction data are scarce in the Indian Ocean (see review in: [24]) and reproductive season and spawning ground remain unclear. Three spawning grounds were however described: the Gulf of Bengal and off the Somalia coast - where spawning is supposed to occur after April for both areas - and around Reunion island - where spawning is supposed to take place from October to April. Evidences from past studies suggest that spawning may occur in the West Equatorial area [25] the Gulf of Bengal [26] and the East Madagascar Tropical area from October to April [24]. To date no genetic study has investigated population structuring of Indian Ocean swordfish with a sufficient sampling strategy to address population structure within the Indian Ocean.

The identification of genetic structure in the Indian swordfish should be of importance in term of fish management as one of the challenging issues commonly recognized is to match the artificial spatial scale of stock assessment with the natural spatial structure of the species [27]. Swordfish has the largest commercial value among billfish fisheries and is currently heavily exploited by commercial fisheries in the Indian Ocean. On the basis of the last swordfish stock assessment [28], levels of catches in the whole Indian Ocean for 2006–2010 (average of 24 008 tons) were considered below the estimated *maximum sustainable yield* (MSY; 29 900–34 200 tons). Nevertheless, when population structure was considered and when the assessment focused on the southwest Indian Ocean as an independent stock – a case considered by the India Ocean Tuna Commission (IOTC) on the basis of the fishery data [29], most of the evidence indicated that the resource has been overfished in the past decade, with the current level of catches indicating a stock fully exploited (8 112 tons in 2010 with an estimated MSY: 7 100–9 400; [26]). Therefore, deeper investigation on the swordfish stock structure was recommended as one of the top priorities by the IOTC Scientific Committee to reduce the uncertainty in assessment [30].

The present study aims to determine the swordfish genetic population structure in the Indian Ocean. For this purpose, an intensive sampling was conducted over the whole Indian Ocean, at several periods during two consecutive years (2009–2010). We examined genetic variation of more than two thousands swordfish using newly developed genetic markers, supposedly more discriminating than older ones: 19 microsatellite loci [31,32] and mitochondrial sequences of the Nicotinamide Dehydrogenase subunit 2 (ND2) [22].

## Materials and Methods

### Sample Collection

Sampling for the present study focused on the Indian Ocean (IO) – as defined by international conventions as the waters delineated from the Atlantic Ocean by the 20° east meridian, from the Pacific by the meridian of 146°55' east and a southern limit at 60°S [33]. Ethical approval was not required for this study, as all

fish were collected as part of routine fishing procedures. Swordfish samples were collected from different zones within the Indian and adjacent Oceans (Figure 1, Table 1 and Appendix F1 in File S1) by onboard observers on commercial fishing vessels or at landing (with due care collecting the related fishing information). Swordfish were killed by the fishermen by cutting their head off. For each sample, muscle tissue biopsies were so taken on already dead animals; they were then stored in 90% ethanol then frozen until DNA was isolated. Information on sample location (exact latitude and longitude or 5° square) was systematically noted. Whenever it was possible fish sex information were collected.

Initial sampling strategy was to sample one hundred fish per zone at two targeted seasons (April–June that we considered as the non-spawning season for this species and October–December, the season where swordfish should be in spawning condition) over two consecutive years (2009 and 2010). Initial sampling seasons were defined based on known information of swordfish reproductive condition in IO (see Introduction and [24,25,26]). Due to field realities, not all samples were collected during those periods but were in fact collected over 46 months from February 2008 to October 2011 (see Appendix F2 in File S1) and then classified in five seasons numbered from 1 to 5 (see Table 1).

### Genetic Analysis

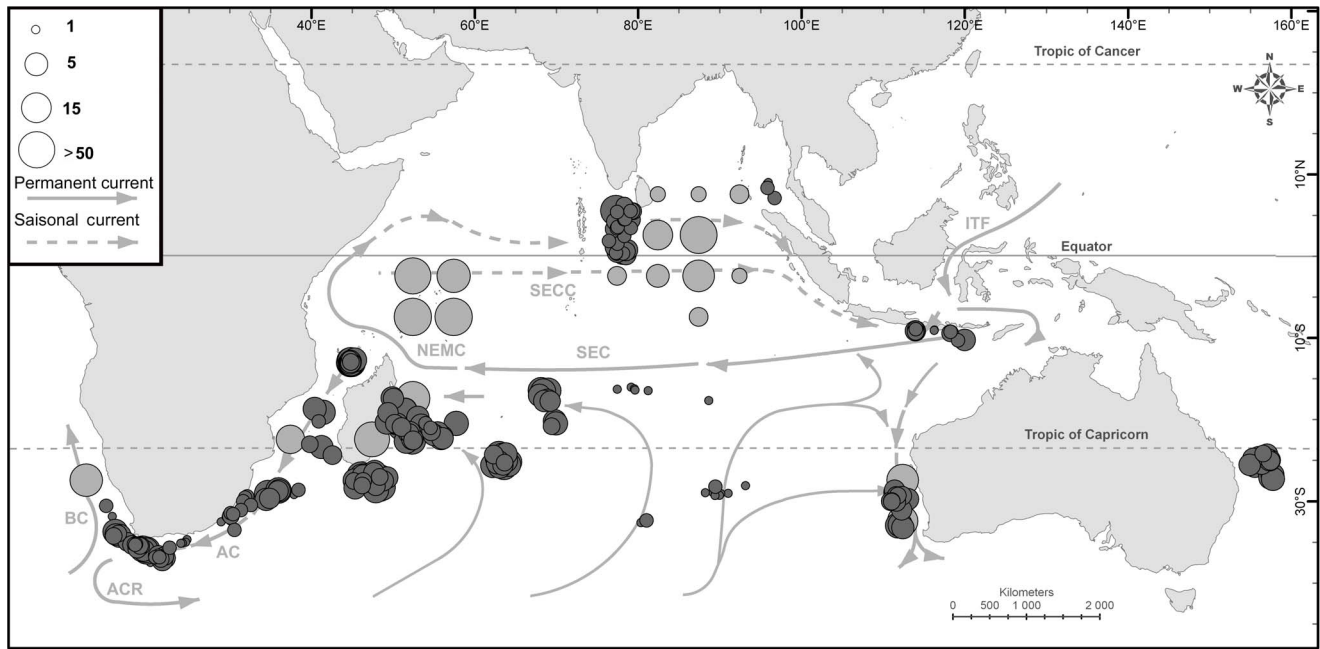
Total genomic DNA was extracted using DNAeasy Tissue Kit (Qiagen) following the manufacturer instructions. A 1007 bp fragment of the mitochondrial ND2 gene was amplified by PCR using the primers and recommended conditions defined in Bradman et al. [22]. PCR products were purified and sequenced in forward and reverse directions on an ABI 3100 sequencer (Macrogen Inc.). Sequences were edited using Chromas version 1.6 [34] and aligned using CLUSTALW [35] in BIOEDIT Sequence Alignment Editor [36]. Sequences were submitted to GenBank (Accession numbers JQ353203 to JQ353484).

Nineteen microsatellite loci were also used, three from [32] (Xg-66, Xg-144, Xg-166) and sixteen from Bradman et al. [31]: A3, A4, A7, A8, A10, A113, A115, B108, B112, B6, C10, C4, C7, C8, D11, D2B. Reactions were performed in 20 µl containing 1X PCR buffer, 2.5 mM MgCl<sub>2</sub>, 2 µM of each dNTPs, 0.3 µM of each primer, 0.5 U of SilverStar Polymerase Taq (Eurogentec), 25 ng of genomic DNA. Cycling parameters were 93°C for 3 min, followed by 35 cycles of 93°C for 30 s, 50–62°C for 50 s, and 72°C for 50 s and a final elongation at 72°C for 30 min. PCR were amplified separately and electrophoresed in three multiplex panels. Amplified fragments were separated on an ABI Prism 3100 genetic analyser. Alleles were scored using a co-migrating size standard (Genescan500, Applied Biosystems, Inc.) and identified using GENE MAPPER v4 (Applied Biosystems Inc.).

### Data Analyses

Data analysis was first conducted on the whole dataset to identify global level of structure. We also defined spatially and temporarily stratified sampling sets (*i.e.* swordfish sampled in a given area at a given time; each sample set was named as XXX\_00\_### (XXX for the area name, 00 for the sampling year, ## for the period within the respective 00 year - see details in Table 1) for more meaningful comparisons (*e.g.* comparison of fish from a same area at different times or from different areas at a same time).

For ND2 sequences, haplotype (*h*) and nucleotide ( $\pi$ ) diversities and Fu's [37] F-statistic were estimated per sampling sets with DNAsp 5.0 [38]. Fu's F-statistic tests for departure from equilibrium between the addition of variation by mutation and the removal of variation by genetic drift; theoretically, mutation-



**Figure 1. Geographic location of Swordfish tissue samples analysed in this study.** The size of circles is proportional to number of swordfish sampled; the colour indicates the accuracy of the localisation data collected (dark grey for exact coordinates and light grey for 5° square position). This map also shows the main Indian Ocean currents (from Schott et al. 2009); SEC: South Equatorial Current; SECC: South Equatorial Counter Current; NEMC: Northeast Madagascar Current; AC: Agulhas Current; ACR: Agulhas Current Retroflexion; BC = Benguela Current; ITF: Indonesian Trough Flow. doi:10.1371/journal.pone.0063558.g001

drift equilibrium should be reached if the effective population size has remained stable in the past. Phylogenetic relation between all available ND2 sequences were represented by a neighbour-joining tree constructed using MEGA 5 [39]. Correlations between haplotype frequencies and longitude were tested using Pearson coefficient. For microsatellites, allele frequencies, mean number of alleles ( $N_{all}$ ), and the observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosities [40] were calculated per samples sets with ARLEQUIN 3.5 [41]. To account for differences in sample size, allelic diversity was adjusted by estimating the allelic richness ( $R_s$ ) using the rarefaction process of the standArich package (available at <http://www.cmar.ualg.pt/maree/software.php?soft=sarich>) for R [42]. Deviations from Hardy-Weinberg equilibrium were examined for each sampling set, at each locus, by calculating Wright's [43] fixation index  $F_{is}$  as estimated by Weir and Cockerham [44] and tested using exact tests performed with Arlequin 3.5 [41]. MICRO-CHECKER 2.2.3 [45] was used to detect possible null alleles. Microsatellite dataset was analysed using the software STRUCTURE 2.3.2 [46] to determine if the genotypes could be partitioned in one or more genetic pools. For this analysis, an admixture model assuming independent allele frequencies was used and ten replicates were run (each with  $1.10^5$  burn-in samples/generations and  $5.10^5$  iterations) for  $K$  values from 1 to 5.

For both ND2 sequence and microsatellite data, the analysis package ARLEQUIN 3.5 [41] was used to estimate pairwise values of genetic differentiation. A total of 10 000 permutations were used with the fixation index  $\Phi_{st}$  for sequence data and Wright's  $F_{ST}$  statistic for microsatellite data. In both cases, critical significance levels for multiple testing were corrected in agreement with Narum [47] using a sequential Benjamini-Yekutieli procedure [48]. Pairwise values of genetic differentiation between sample sets were used as input data in order to construct neighbour-joining trees with the program MEGA 5 [39]. Jost's [49] unbiased estimator of divergence ( $D$ ), based on the effective

number of alleles rather than on the expected levels of heterozygosity) was also calculated per pair of localities using the software SPADE (available at <http://chao.stat.nthu.edu.tw/softwareCE.html>) for ND2 sequences and SMOGD [50] for microsatellite data. To test for patterns of isolation-by-distance, marine distances between localities (estimated on the <http://www.geodistance.com> website) were plotted against genetic distance (using  $\Phi_{st}/(1-\Phi_{st})$  for mitochondrial data or  $F_{ST}/(1-F_{ST})$  for microsatellite data following the recommendations of Rousset [51]). The significance of this relationship was tested with a Mantel test, performed in R [42] using the *ncf* package (available at <http://onb.ent.psu.edu/onb1/R>).

ARLEQUIN 3.5 [41] was also used to perform analysis of molecular variance (AMOVA) with *a priori* grouping based on geographical or temporal proximity, within or between oceans. The software SAMOVA 1.2 [52] was finally used to perform spatial analysis of molecular variance (SAMOVA) on localities that were sampled within the same period. This approach could detect genetic barriers in a sampling region without *a priori* group definition and identify geographic partitions that maximize genetic differences between groups and geographic homogeneity within groups; it was tested for  $K$  group values ranging from 1 to 4, with 100 annealing replicates each time.

Finally, the same analytical approaches (pairwise values of genetic differentiation and AMOVA) were processed to assess whether or not sex of individual had an effect on the genetic structure.

## Results

A total of 2 231 swordfish were sampled from the three major study areas during this study (1920 from the IO; 186 from Atlantic Ocean; and 125 from the Pacific Ocean). Sampling details are provided in Table 1. A total of 2 146 were genotyped with

**Table 1.** Swordfish sampling and genetic characteristics from the Indian and adjacent Oceans sampling areas.

Ocean	Sample set name	Geographic area	Dates (begin - end)	Corres- ponding season	Sex ratio	ND2 sequences						Microsatellite					
						N	Nhap	h	π	Main Hap. #11	Private hap. %	Fu's F	N	Rs	Fis19	Fis16	
																	N
INDIAN OCEAN	AUS_08_1	Australia (West)	22/02/08 02/03/08	-	NA	36	16	0.866	2.202	0.33	0	-9.5	38	6.4	0.088	0.036	
	AUS_08_2	Australia (West)	25/11/08 30/11/08	-	NA	27	16	0.866	1.573	0.37	0	-15.5	28	6.3	0.108	0.048	
	AUS_09_1	Australia (West)	12/12/09 15/12/09	2	NA	27	14	0.803	1.954	0.44	0	-9.3	29	6.0	0.110	0.045	
	AUS_11_1	Australia (West)	28/05/11 31/05/11	-	NA	17	9	0.860	1.544	0.35	0	-5.1	19	5.8	0.107	0.060	
	CA1_10_1	Mozambic chanel (North)	06/04/10 17/04/10	4	0.55	42	26	0.914	2.213	0.28	0	-26.7	47	6.1	0.092	0.030	
	CA2_10_1	Mozambic chanel (South)	03/01/10 04/02/10	3	NA	53	29	0.912	2.735	0.28	0.05	-26.1	53	6.4	0.108	0.067	
	GB1_09_1b	Gulf of Bengal (West)	27/09/09 09/12/09	2	NA	40	23	0.890	2.097	0.32	0.02	-22.9	48	5.9	0.107	0.046	
	GB1_10_1	Gulf of Bengal (West)	27/06/10 30/07/10	4	NA	77	43	0.927	2.231	0.25	0	-27.1	83	6.1	0.100	0.030	
	GB2_10_1	Gulf of Bengal (central)	21/01/10 21/01/10	3	NA	31	19	0.944	2.361	0.19	0.03	-15.8	38	5.8	0.064	0.010	
	GB2_10_2	Gulf of Bengal (central)	12/12/10 22/01/11	5	NA	19	11	0.888	1.731	0.31	0	-7.2	21	6.0	0.061	0.052	
ATLANTIC OCEAN	IND_09_1	Indonesia	02/05/09 12/06/09	1	NA	41	22	0.891	1.995	0.31	0.07	-21.1	40	6.2	0.128	0.055	
	MAD_09_1	Madagascar (South-east)	28/04/09 10/05/09	1	0.74	42	21	0.890	2.918	0.30	0.07	-13.4	91	6.2	0.080	0.020	
	MAD_09_2	Madagascar (South-east)	07/11/09 18/11/09	2	0.74	93	45	0.838	1.945	0.39	0.05	-27.4	97	6.1	0.116	0.053	
	MAD_10_1	Madagascar (South-east)	21/04/10 01/05/10	4	0.70	93	40	0.908	3.456	0.27	0.11	-26.1	94	5.8	0.060	0.022	
	MAY_09_1	Mayotte	26/10/09 11/12/09	2	NA	85	39	0.881	2.210	0.32	0.10	-27.1	95	6.2	0.103	0.053	
	MAY_10_1	Mayotte	12/10/10 18/11/10	5	NA	76	30	0.892	2.149	0.30	0.03	-27.0	68	5.8	0.064	0.037	
	RON_09_1	Rodrigues (North)	26/09/09 06/10/09	2	0.66	70	39	0.906	2.730	0.30	0.08	-26.6	81	6.0	0.143	0.088	
	ROS_09_1	Rodrigues (South)	09/10/09 18/10/09	2	NA	11	8	0.927	2.109	0.27	0	-4.2	15	5.9	0.116	0.085	
	ROS_10_1	Rodrigues (South)	19/05/10 24/05/10	4	0.70	34	20	0.921	2.046	0.26	0.05	-18.6	35	6.2	0.051	-0.008	
	ROS_10_2	Rodrigues (South)	20/07/10 26/07/10	4	0.71	44	23	0.887	2.001	0.31	0.13	-22.3	45	6.1	0.049	0.001	
ATLANTIC OCEAN	ROS_10_3	Rodrigues (South)	12/10/10 18/10/10	5	0.67	84	35	0.801	2.177	0.44	0.08	-27.1	78	6.2	0.033	-0.007	
	RUN_09_1	Reunion island	29/05/09 03/06/09	1	0.5	63	26	0.795	1.746	0.44	0.07	-26.4	63	6.0	0.123	0.051	
	RUN_09_2	Reunion island	15/10/09 29/11/09	2	0.67	59	27	0.916	2.891	0.23	0.05	-20.3	73	5.9	0.108	0.031	
	RUN_10_1	Reunion island	16/06/10 31/07/10	4	0.56	78	39	0.921	2.695	0.25	0.08	-26.6	93	6.1	0.046	0.015	
	RUN_10_2	Reunion island	21/10/10 22/11/10	5	0.47	92	41	0.903	1.999	0.28	0.08	-27.4	85	6.1	0.069	0.028	
	SEY_09_1	Seychelles	22/11/09 17/12/09	2	0.93	85	34	0.826	2.017	0.40	0	-27.3	91	6.1	0.092	0.027	
	SEY_10_1	Seychelles	02/07/10 08/07/10	4	NA	67	28	0.874	1.888	0.32	0	-27.2	68	6.2	0.109	0.048	
	SEY_10_2	Seychelles	04/11/10 17/11/10	5	NA	21	11	0.814	1.581	0.42	0	-7.2	21	6.2	0.087	0.046	
	SEY_11_1	Seychelles	21/01/11 21/01/11	5	NA	24	15	0.837	2.228	0.41	0	-11.0	30	6.3	0.092	0.029	
	AFS_09_1b	South Africa	29/07/09 01/11/09	2	NA	64	32	0.929	3.064	0.23	0.09	-26.1	53	6.4	0.075	0.036	
AFS_10_1	South Africa	24/01/10 04/02/10	3	NA	15	11	0.904	2.857	0.33	0	-6.0	15	6.2	0.021	0.008		

Table 1. Cont.

Sampling	ND2 sequences										Microsatellite					
	Sample set name	Geographic area	Dates (begin - end)	Corresponding season	Sex ratio	N	Nhap	h	$\pi$ %	Main Hap. #11	Private hap. %	Fu's F	N	Rs	Fis19	Fis16
	AFS_10_2	South Africa	21/04/10 26/04/10	4	NA	49	30	0.916	2.493	0.28	0	-26.7	49	6.2	0.086	0.027
	AFS_10_3	South Africa	25/10/10 30/10/10	5	0.70	10	9	0.977	2.911	0	0	-5.6	12	5.8	0.117	0.080
	NAM_11_1	Namibia	10/11	-	NA	24	13	0.873	1.996	0.16	0	-8.2	23	6.5	0.023	-0.026
PACIFIC OCEAN	COR_09_1b	Coral Sea	10/04/09 08/07/09	1	NA	50	20	0.884	2.076	0.30	0.10	-14.4	53	6.5	0.061	0.002
	COR_09_3	Coral Sea	26/10/09 08/11/09	2	NA	63	33	0.916	2.229	0.26	0.15	-27.0	72	6.2	0.047	0.009
	Out Class		04/07/08 27/02/11	-	0.92	185	65						202			
	TOTAL				0.67	2001	282									2146

- Sampling characteristics: GPS coordinates, sampling dates and corresponding season, sex ratio (estimated as the number of females on the whole number of swordfish), Sampling sets (i.e. swordfish sampled in a given area at a given time) were named as XXX\_00\_# (XXX for the area name, 00 for the sampling year, ## for the period within the respective 00 year). 'Out Class' samples correspond to swordfish collected out of a sampling season and/or out of a sampling site.

- ND2 characteristics: Nhap the number of haplotypes per population, h the nucleotide diversity,  $\pi$  the percentage of main (#11) and private haplotypes, Fu's Fs value.

- Microsatellite characteristics: Rs the allelic richness (estimated on 10 individuals), Fis19 and Fis16 the fixation index respectively estimated with 19 and 16 loci. Significance at  $p < 0.001$  is noted by bold characters. doi:10.1371/journal.pone.0063558.t001

microsatellites and 2 001 were sequenced at the mitochondrial ND2 gene.

## Genetic Diversity

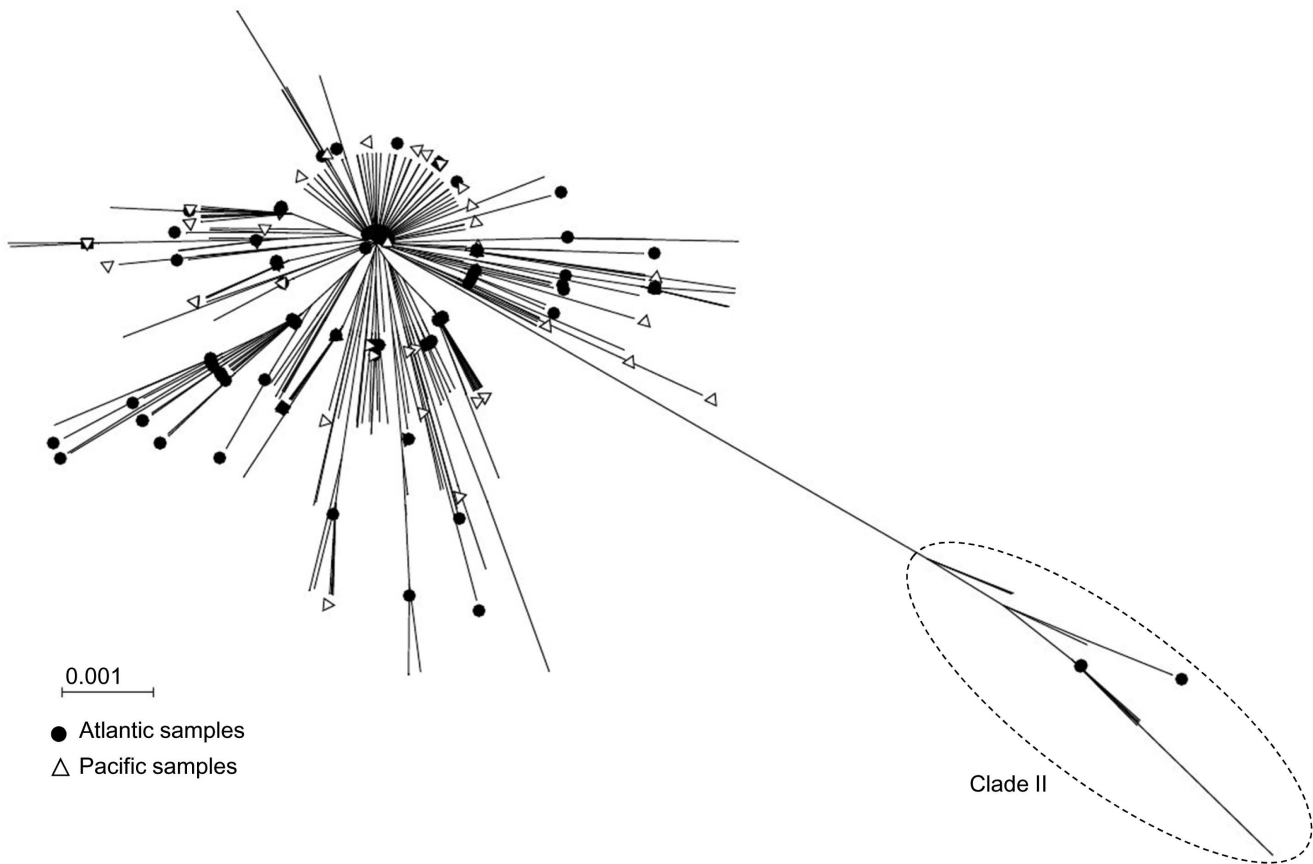
**ND2 sequences.** A total of 195 variable sites, constituting 282 haplotypes, were detected among the ND2 sequences (1007 bp) of 2 001 swordfish. Approximately 48% of these 282 haplotypes were represented more than once. Mean haplotype diversity ( $H_d$ ) and mean nucleotide diversity ( $\pi$ ) were high, respectively 0.886 ( $\pm 0.04$ ) and 0.0022 ( $\pm 0.0004$ ), and similarly high within each sample set (Table 1). The most common haplotype (#11) was well represented in all localities, except AFS\_10\_3, where it was absent. The mean frequency of this haplotype per sampling locality was 31% ( $\pm 7\%$ ), varying from 16% in NAM\_11\_1 to 44% in AUS\_09\_1, ROS\_10\_3 and RUN\_09\_1 (Table 1). The private haplotypes constituted a small proportion of the individuals, with a mean frequency of 4% per sample set and the highest frequency of 13% in ROS\_10\_2 (Table 1). Fu's F values were highly negative and significant ( $F = -682.3$ ,  $p < 0.001$ ; Table 1) and the mismatch distribution for overall dataset presented one peak, implying that the sudden population expansion model could not be rejected (Appendix F3 in File S1).

The relationship between ND2 sequences as represented by a neighbor-joining tree is shown in Figure 2. Sequence analysis revealed two divergent clades, a dominant one, which contained 98% of the samples, and a second clade separated by seven fixed mutations from the main clade. This clade structure observed in this study was similar to one previously described from examination of mitochondrial cytochrome b sequence data [53]. By analogy to the aforementioned study, the clades herein were called clade I and clade II respectively for the common and the rare clades. The clade I is equally represented in all sample sets while the clade II is absent from the Pacific Ocean sample sets (COR\_09\_1 and COR\_09\_3). The proportion of the clade II within each sample was thus significantly and negatively correlated to decreasing longitude ( $r = 0.14$ ;  $p < 0.02$ ).

**Microsatellites.** Allelic richness was of the same order between the different sample sets, with a mean at 6.16 ( $\pm 0.19$ ) varying from 5.8 for MAD\_10\_1 to 6.50 for COR\_09\_1 (Table 1). Fixation indices Fis were highly significant in most sample sets with values ranging from 0.02 to 0.14 (Table 1), mostly because of significant heterozygote deficiencies at the three loci A3, B6 and B108. These loci were indeed characterised by the presence of null alleles ( $p < 0.05$ ); consequently, these three loci were excluded from following analyses which were therefore run using 16 loci. Fis values became non-significant for more than half of the sample sets when removing these 3 loci (Table 1). No loci were in disequilibrium ( $p < 0.001$ ) over the whole dataset, supporting the independent assortment of alleles at different loci.

## Inter-ocean Structure

**ND2 sequences.** Overall  $\Phi_{ST}$  was 0.006 ( $p < 0.001$ ) when considering all the samples; it decreased to 0.001 and was non-significant ( $p > 0.05$ ) when considering only the swordfish sampled within the IO (i.e. excluding all the sample sets from NAM, AFS and COR areas). Pairwise genetic distance estimates ( $\Phi_{ST}$ ) between sample sets are summarized by a neighbor-joining (NJ) tree in Appendix F4 in File S1. This analysis clearly segregated NAM\_11\_1 and AFS\_09\_1 from all the others sets but showed no clear structure among the Indo-Pacific sample sets. Of the 630 pairwise comparisons used in this NJ tree (see complete table in Appendix S1), 80 were significant ( $p < 0.05$ ) from which 64 concerned interoceanic comparisons (i.e. including at least one



**Figure 2. Unrooted neighbor-joining tree showing the relationship between the ND2 sequences (n = 2001).** White triangles are samples from the Pacific Ocean (5.5% of the samples), black circles from Atlantic Ocean (8% of the samples) and branches without symbol are from Indian Ocean (86.5% of the samples). Clade I and clade II names refer to the same nomenclature proposed in Alvarado-Bremer *et al.*, (2005a). doi:10.1371/journal.pone.0063558.g002

sample set from NAM, AFS or COR areas in the pairwise comparisons). The highest  $\Phi_{ST}$  values were observed for the NAM sample sets (mean  $\Phi_{ST}=0.098$ ), followed by COR and AFS comparisons (0.012 and 0.010 respectively). Consistent with these

significant differentiation results, values of Jost's D were very high when comparisons included NAM samples ( $D > 0.25$ ), even if the highest values of Jost's D regarded AFS\_10\_3 (mean  $D = 0.74$ ). An AMOVA analysis undertaken with grouping made per ocean also

**Table 2. Results of AMOVAs made for both markers according to different grouping (bold underline significant values).**

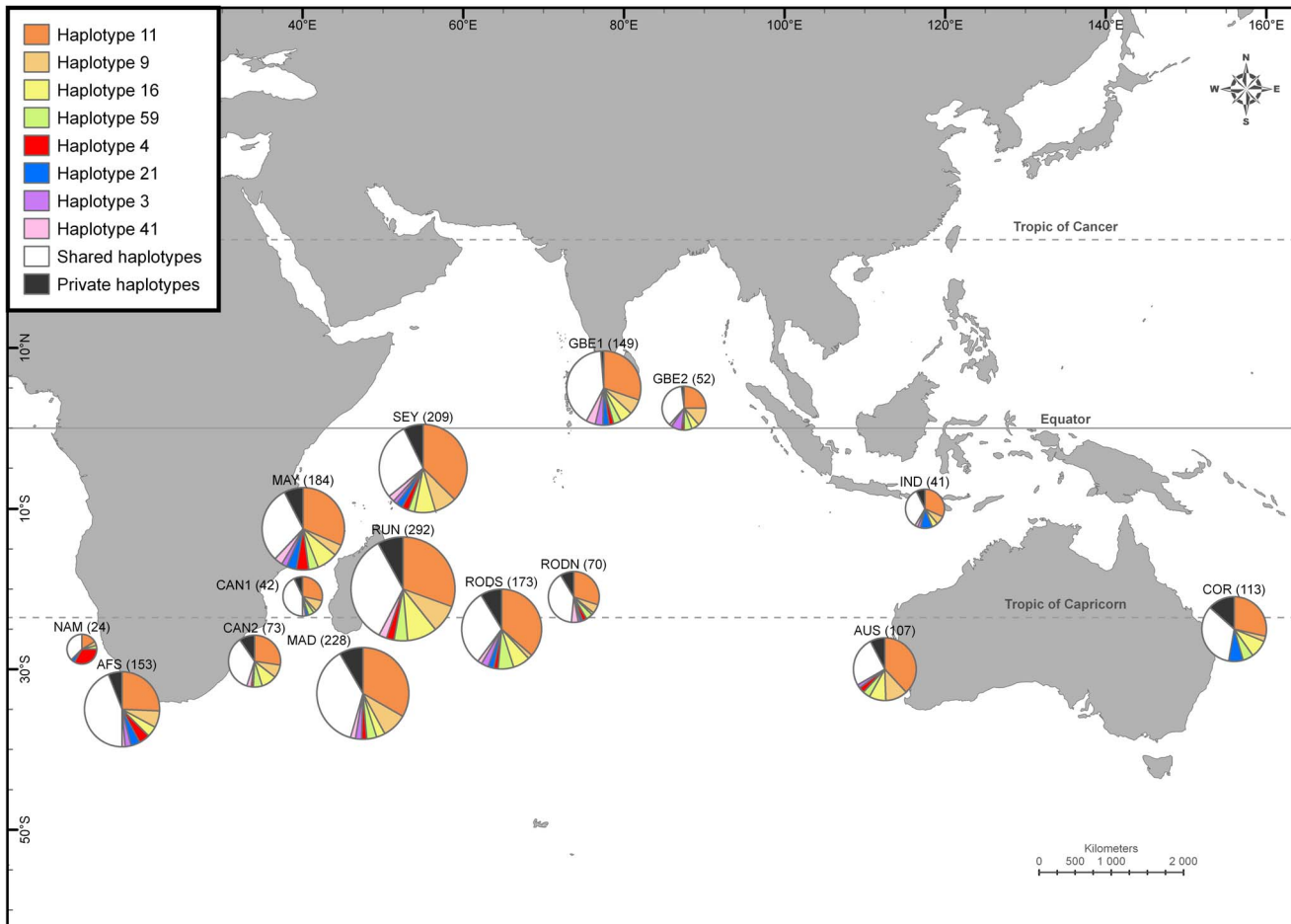
Source of variation	ND2				Microsatellite			
	d.f.	% variation	Fixation index	p	d.f.	% variation	Fixation index	p
<b>(1)</b> Among oceans ( $\Phi_{CT}$ )	2	1.18	0.0118	<b>&lt;0.001</b>	2	0.09	0.0008	<b>0.01</b>
Among areas within oceans ( $\Phi_{SC}$ )	33	0.30	0.0030	0.02	33	0.26	0.0026	<b>&lt;0.001</b>
Among individuals within areas ( $\Phi_{ST}$ )	1770	98.52	0.0118	<b>&lt;0.001</b>	3852	99.65	0.0035	<b>&lt;0.001</b>
<b>(2)</b> Among 3 groups within IO only ( $\Phi_{CT}$ )	2	0.08	0.0008	0.21	2	0.03	0.0003	0.09
Among areas ( $\Phi_{SC}$ )	26	0.12	0.0012	0.12	26	0.25	0.0025	<b>&lt;0.001</b>
Among individuals within areas ( $\Phi_{ST}$ )	1502	99.80	0.0020	0.09	3305	99.72	0.0028	<b>&lt;0.001</b>
<b>(3)</b> Among 5 seasons within IO ( $\Phi_{CT}$ )	3	-0.14	-0.0010	0.98	4	0.02	0.0002	0.14
Among areas within seasons ( $\Phi_{SC}$ )	22	0.34	0.0019	<0.05	21	0.25	0.0025	<b>&lt;0.001</b>
Among individuals within pop. ( $\Phi_{ST}$ )	1435	99.8	0.0033	<0.06	3138	99.73	0.0027	<b>&lt;0.001</b>

(1) between the 3 oceans.

(2) among 3 geographical groups within IO, among SEIO (Australia and Indonesia), Gulf of Bengal and SWIO (all the others).

(3) among the 5 seasons within the IO (see Table 1 for details).

doi:10.1371/journal.pone.0063558.t002



**Figure 3. Map of plotted frequencies of the main ND2 haplotypes, shared haplotype and private haplotypes per identified areas.** Number of samples is shown in brackets.  
doi:10.1371/journal.pone.0063558.g003

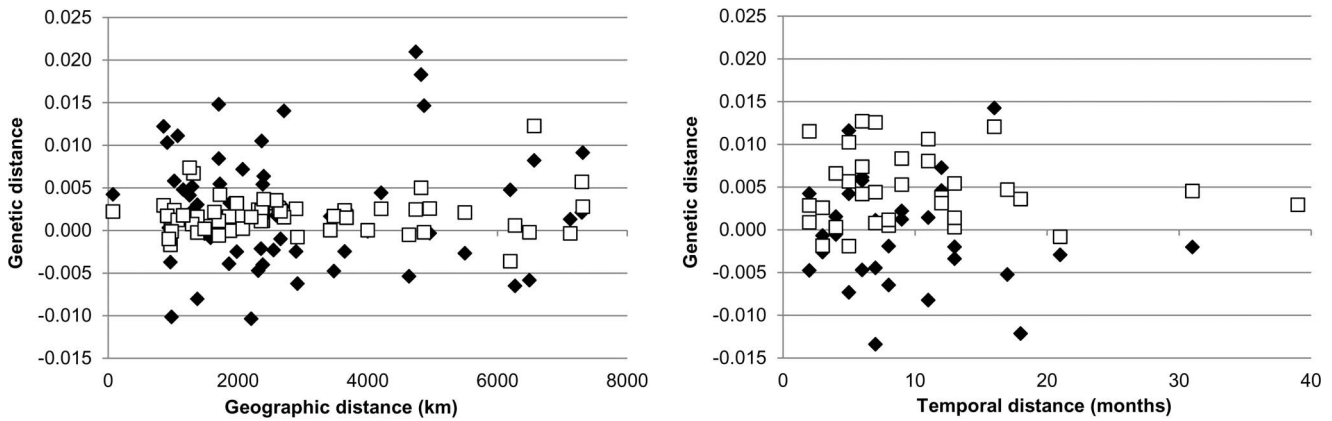
demonstrated a small but significant level of structure between oceans ( $\Phi_{CT} = 0.011$ ,  $p < 0.001$ ; see Table 2). This inter-ocean differentiation could be partly explained by the geographical distribution of haplotypes (shown on Figure 3). First, all areas except NAM (Atlantic Ocean) were dominated by the most common haplotype (#11) while NAM area was dominated by a secondary haplotype (#4; 33.3%), present in most areas but in a lower proportion. The proportion of the haplotype #4 in the AFS sample sets was highly variable according to sampling sets (from 0% in AFS\_10\_1 to 10% in AFS\_10\_3 with intermediate values of 2% in AFS\_10\_2 and 8% in AFS\_09\_1). Then, the COR area (Pacific Ocean) showed an absence of the haplotypes #4 and #41 and a higher proportion of haplotype #21 (Figure 3). The IND\_09\_01 sample set (Indonesia, the closest site to Pacific Ocean) was characterized by an absence of haplotype #4 only one haplotype #41 while the haplotype #21 is the most dominant one (10%) after haplotype #11.

**Microsatellites.** The Structure analysis suggested that the highest likelihood of obtaining such data was to consider that only one genetic pool existed ( $K = 1$ ). The likelihood decreases when estimates were made with more than one pool (over ten independent simulations:  $\ln P(D)$  for  $K = 1$  and  $K = 2$  were  $-109850$  and  $-110440$ , respectively). When considering two genetic pools, the mean posterior probability per individual is  $0.50 (\pm 0.04)$  providing more evidence against subdivision.

Overall  $F_{ST}$  was  $0.0028$  ( $p < 0.001$ ) when considering all the sample sets and  $0.0026$  ( $p < 0.001$ ) when considering only the swordfish sampled within the IO suggesting the same low level of structure within and between oceans. The neighbor-joining tree based on the pairwise  $F_{ST}$  estimates (Appendix F4b in File S1) failed to reveal any clear structure within the dataset. Of the 630 pairwise comparisons used in this NJ tree (see complete table in Appendix S1), 96 were significant ( $p = 0.000$ ) from which 39 concerned interoceanic comparisons (i.e. including at least one sample set from NAM, AFS or COR areas). Consistent with these lack of clear structure, values of Jost's  $D$  were very low (all  $D < 0.03$ ) even between oceans. However, an AMOVA analysis made with grouping done per ocean demonstrated a significant  $F_{CT}$  value ( $0.0008$ ,  $p < 0.01$ ; see Table 2), as for ND2 sequences. The mean  $F_{ST}$  values for the NAM, COR and AFS comparisons were higher than for intra-ocean comparisons ( $0.006$ ,  $0.004$  and  $0.004$  respectively while it was  $0.003$  among the Indian Ocean sample sets).

#### Within Indian Ocean Analysis

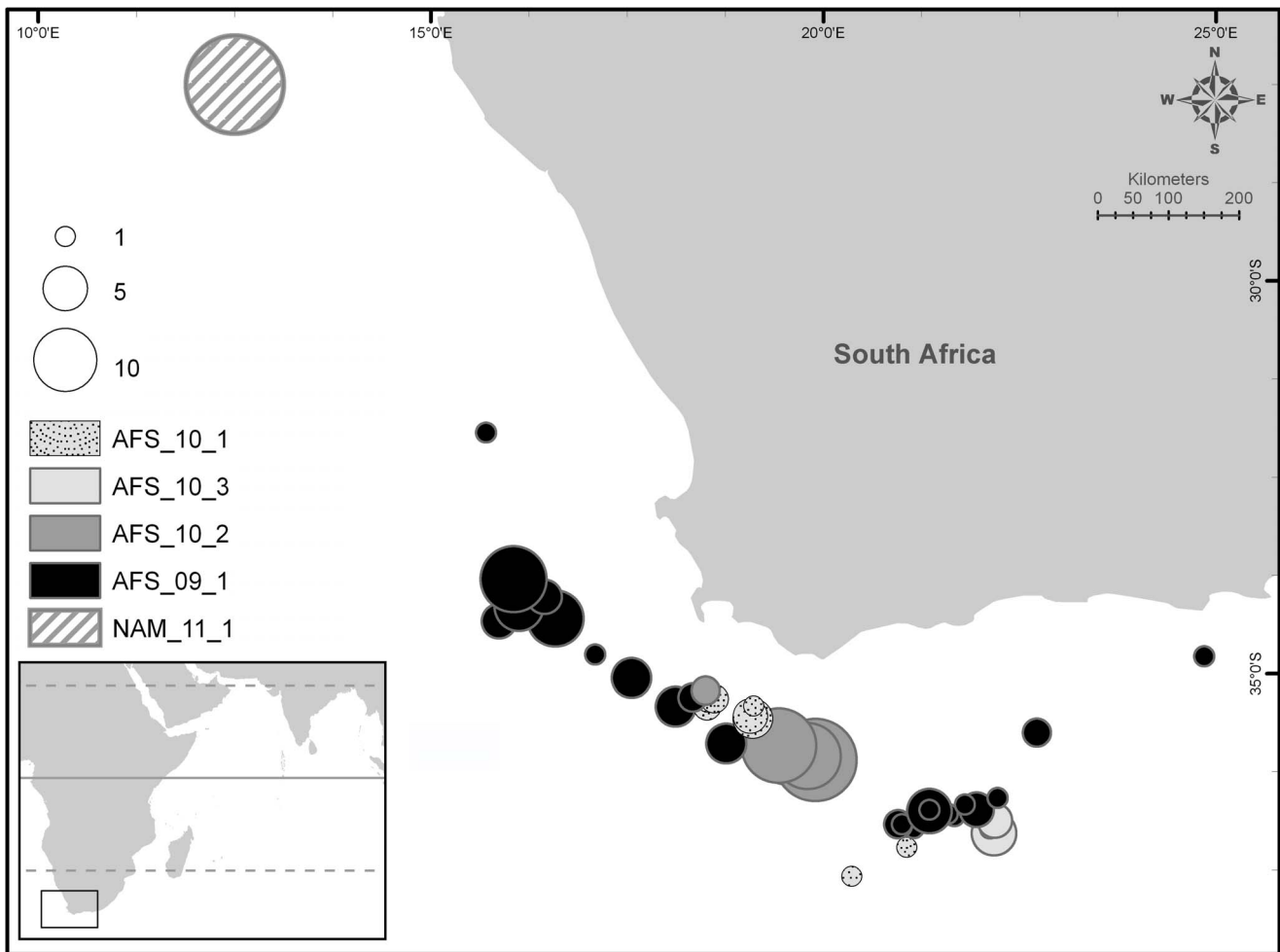
We considered only the IO sample sets, excluding the five ones from the Atlantic Ocean (NAM and AFS) and the two from Pacific Ocean (COR). Irrespective of which marker was examined, the AMOVA analysis conducted within the IO showed that more than 99% of the variance was observed within the samples with no



**Figure 4. Isolation-by-distance and Isolation-by-time graphs.** Graphs showed corrected pairwise genetic distances [ $\Phi_{st}/(1-\Phi_{st})$  for mtDNA and  $F_{st}/(1-F_{st})$  for microsatellite] plotted as a function of geographic distances (**4a**) or of time (**4b**) for Indian Ocean swordfish. Black diamonds are for mtDNA data and white squares for microsatellite data (all were not significant: Mantel tests with  $p > 0.05$ ). doi:10.1371/journal.pone.0063558.g004

variance significantly associated with the partition into any kind of grouping ( $\Phi_{CT}$  and  $F_{CT} < 0.001$ ,  $p > 0.05$ ; see Table 2). There is no clear partitioning between sample sets from a same site within

the same season or between different sites within a same season. Similarly, the SAMOVA analysis failed to demonstrate any population subdivision using any of the two markers (less than



**Figure 5. Sampling around South Africa and Namibia.** Exact sampling size and location of the South Africa (AFS) and Namibia (NAM) sampling sets (see table 1 for details). doi:10.1371/journal.pone.0063558.g005



1% of genetic variance,  $p > 0.05$ ); without any *a priori* geographic grouping, between-group variance was maximized when one sample set was considered isolated from all the others.

Most ND2 pairwise values of differentiation (Appendix S1) were low and not significant, even between the most distant areas (e.g. AUS\_08\_1 versus RUN\_10\_1,  $\Phi_{ST} = -0.0064$ ,  $p > 0.05$ ). No significant differences were observed between years or seasons sampled at any site (e.g. AUS\_08\_1, AUS\_08\_2, AUS\_09\_1 and AUS\_11\_1;  $p > 0.05$ ) neither there was significant differences observed between different sites within a season (e.g. IND\_09\_1, MAD\_09\_1, ROS\_09\_1, MAY\_09\_1 and RUN\_09\_1;  $p > 0.05$ ). Looking at microsatellite pairwise  $F_{ST}$  values (Appendix S2), 139 upon the 240 comparisons appeared significant ( $p < 0.05$ ; 61 even highly significant,  $p < 0.000$ ). However, values of Jost's  $D$  were very low with only 19 values above  $D = 0.01$  (Appendix S2) at the intra IO level and no clear pattern of structure could be detected (Appendix F4b in File S1). For both sets of markers, there was no general trend for higher genetic divergence with increasing geographic or time separation (Figure 4). In other words, no isolation-by-distance nor isolation-by-time pattern were identified (all  $r < 0.23$  and all  $p > 0.05$ ).

Sex and maturity stage information were only available for localities in the South West Indian Ocean region (see Table 1). Most of the swordfish sexed were in stage maturity 1–2, i.e. 73% were immature individuals; analysis based only on mature swordfish could only be based on 114 individuals of the SWIO thus they were not done. Genetic structure partitioning was investigated by sex for sample sets where information was available (see Table 1; only in the South West Indian Ocean region). Overall  $\Phi_{ST}$  was 0.0018 (not significant;  $p > 0.05$ ) when considering all the samples from this area; it was still not significant and decreased to 0.0009 ( $p > 0.05$ ) when considering only females and increased to 0.0020 ( $p > 0.05$ ) when considering males. A similar trend but significant was observed with microsatellite data as overall  $F_{ST}$  was 0.0023 ( $p < 0.001$ ) when considering all the samples, decreased to 0.0010 ( $p < 0.05$ ) when considering only the females and increased to 0.0040 ( $p < 0.005$ ) when considering the males. This trend indicated a higher genetic homogeneity between females than between males at the scale of the SWIO.

## Discussion

### Homogeneity within the Indian Ocean

This study aimed as a first step to evaluate genetic structure of the swordfish *Xiphias gladius* within the Indian Ocean (IO). The results obtained from both ND2 sequences and microsatellites on multiple sampling areas over multiple seasons detected some level of genetic heterogeneity but failed to demonstrate evidence that swordfish have multiple discrete populations within the IO. All the analysis focusing on the important spatially and temporally stratified sampling done in the SWIO failed to identify any significant structure. These results are consistent with the existence of a single panmictic population in the Indian Ocean population and thus they contradict previous studies suggesting the presence of at least two potential genetic populations [16,17,23]. Previous studies were in fact conducted on small geographic areas, with few individuals opportunistically collected and often only one genetic marker was used, leading to a potential artifact genetic subdivision. Present results are more consistent with main swordfish life history traits, i.e. long distance migration observed for IO swordfish using tag-recapture approach [15,54] and fecundities of several millions of eggs per female [55]. The significant negative  $F_u$ 's values and the unimodal mismatch analysis could be interpreted as a pattern of recent demographic expansion. Together with the very high

levels of genetic diversity and the lack of differentiation detected at a large spatial scale, this might be well in agreement with the assumption of a large population size and long range dispersal typical of large pelagic fishes.

The existence of at least two distinct stocks observed for this species in the Atlantic and Pacific Oceans [8,20] could be partly explained by the existence of cold and productive waters in both North and South extremes of these oceans which are divided by warm oxygen poor waters of the equatorial region separating these two areas; admixture in same feeding areas of population breeding in different hemisphere was thus limited. Similarly, the IO is also divided into two hemispheres by the Equator, involving the known bio-ecological consequences in the marine realm driven by latitudes. This Ocean is also globally characterized by the westward South Equatorial Current (SEC; [56]) and around 12°S by the hydrochemical South Tropical Front which separated two large oligotrophic areas, the Indian Monsoon Gyre Province (MONS) in the north and the Indian South Subtropical Gyre Province (ISSG) [57] (see Appendix F1 in File S1). However, none of these physical and ecological geographic separations seem to impact on the observed genetic structure of the swordfish as they appear to do in the Atlantic and Pacific Oceans. One main difference between the IO and the two other oceans is that the first one could be defined as 'closed' by continent in the north, with consequently less latitudinal variation and most importantly no cold water in the north. However, the Arabian Large Marine Ecosystem (ALME - which bordered the north west of the MONS province; Appendix F1 in File S1) is one of the most intense large scale seasonal coastal upwelling [58], thus considered as a highly productive ecosystem [59] and even as one of the most important phytoplanktonic bloom systems in the world [60,61]. Such a specific oceanographic pattern makes ALME a serious candidate for a discrete feeding area; unfortunately, our sampling scheme did not allow us to identify whether this northwestern area is a specific foraging ground for some IO swordfish. Further exploration into the foraging behaviour of the swordfish, a deep-dwelling predator, with specialization on prey sizes and species [62,63,64], could be one of the key elements to explain the lack of genetic structure presently observed in the IO.

Genetic analysis made by sex also failed to reveal any structure, however there was an indication of a higher genetic homogeneity between females than between males at the scale of the SWIO (significant using the nuclear marker but not using the mtDNA one). The fact that the genetic information given by the two genders is not the same could indicate a sex-biased dispersal in which gene flow between populations is accomplished primarily by one gender [14,65]. In the present case, one could speculate there was a higher dispersal for females than males. However, this contradicts results from both a previous study undertaken in the SWIO which showed more pronounced homing behavior in females [23], and with the common pattern of higher dispersal abilities recognized for male swordfish [66]. The discrepancy in conclusions between these two SWIO studies, associated with the low level of structure observed and the unclear genetic structure found using microsatellites may thus be interpreted as an indication of a lack of structure within the SWIO and *in extenso* is another argument suggesting a homogeneous single panmictic population in the IO. In the present case, the more pronounced structure observed with mitochondrial data could also underline the fact that our microsatellite loci were not as discriminating as they were expected to be [22]. Our study therefore highlighted a strong limitation of identifying population structure based on a single genetic marker and the obvious advantages of using combined molecular approaches. The additional use of genes

supposedly under selection, *e.g.* SNPs (single nucleotide polymorphisms), or scan genomics approaches and detection of outlier loci [67], may be more relevant for future examination of swordfish - or more generally to large pelagic fish - population identification [68]. Detailed information about reproductive strategies, spawning areas and population dynamics in different areas within the IO are also needed to determine if the genetic homogeneity coincides with a demographical connectivity or only with evolutionary connectivity [69]. These general recommendation may be more pronounced when studies have concrete conservation implications such as fishery management of overexploited stocks by implementing Management Units and dedicated management measures.

### Interoceanic Isolation

Analyses of mitochondrial ND2 sequences and microsatellite polymorphism indicated significant isolation between oceans. Both molecular markers showed a significant level of genetic variance associated when comparing samples by Oceans, with a level of differentiation of the IO higher with the Atlantic Ocean (AO) than the Pacific Ocean (PO).

Indo-Pacific swordfish was until now considered to belong to a unique stock [15,16,53]. The fact that the IO was sampled more intensively than for previous studies and the use of the ND2 mitochondrial marker [22] could explain why this differentiation between Indian and Pacific samples was not detected in previous studies. Even if most of the water in the PO is circulated within the Pacific itself [70], some enters the Indonesia Seaway creating the Indonesian Throughflow current (ITF) flowing westward into the IO, supplying, to a large part, the global westward South Equatorial Current [56]. That current pattern therefore potentially transports swordfish larvae and juveniles from the important known spawning ground of western tropical Pacific [71], and could in turn be homogenizing the Indian and Pacific swordfish populations and justifying a unique Indo-Pacific population [15,16,20]. The absence in the PO and Indonesia of the haplotype #4 (very frequent in Atlantic, present at 5% in the IO) associated with the higher proportion of haplotype #21 in PO and Indonesia than in the rest of the IO (Figure 4) and the consequent high  $\Phi_{ST}$  values observed between PO sample sets and the Indian sample sets are elements that strongly indicated an Indo-Pacific differentiation. Our results also showed that a significant part of genetic variance is associated with the Indo-Pacific differentiation (as well as low but still significant values of  $\Phi_{ST}$  and  $F_{ST}$ ). One of the criteria previously used to discuss the interoceanic differentiation and consider the swordfish from Indo-Pacific as one population was the shared absence of Clade II in both oceans [53]. In our study, Clade II was not observed in the PO and Indonesia but was observed in the IO (at the low frequency of 2% but in all the IO areas); this could be viewed again as an argument against a unique Indo-Pacific population. A mark-recapture study around Australia also suggested this interoceanic disruption as the swordfish were recaptured in the ocean they have been released [54]. Therefore it should be more appropriate to consider the Indian and Pacific swordfish as belonging to separate stocks.

The high frequency of the haplotype #4 in the Namibia area (NAM; 30%) that decreased to less than 5% in the IO and absent in the PO and the consequent high  $\Phi_{ST}$  values observed between NAM and Indian sample sets are elements that strongly indicated an Indo-Atlantic differentiation. Such differentiation observed is consistent with previous studies [15,53] as well as with phylogeographic pattern reported for the bigeye tuna *Thunnus obesus* [72], the sailfish *Istiophorus platypterus*, and the blue marlin *Makaira nigricans* [73]. The Clade I was supposed to originate in the Pacific

and the Clade II in the Atlantic. The co-occurrence of these two clades previously observed only in the Atlantic could be explained by unidirectional gene flow from the Indo-Pacific into the South Atlantic [55]. The fact that Clade II was now observed in the IO (at the low frequency of 2% but in all the IO areas) tends therefore to indicate that a flux of Atlantic swordfish into the IO could also occur. Such dispersal events from the Atlantic into the Indian Oceans were observed in only few species, because it necessitated strong swimming capacities to go against the Agulhas Current; it is the case of the hammerhead shark *Sphyrna lewini* [74], the green turtle *Chelonia mydas* [75] or the leatherback turtle *Dermochelys coriacea* [76], all being active swimmers in most of their life stages. The Agulhas Current [77] is one of the strongest currents in the world [78], with large westward current rings pinching off and transcending into the Atlantic, where it is also created the Agulhas Current Retroflexion (ACR) that flows back into the IO [77]. The greater warm and saline Agulhas system influences temperature and salinity of the AO over the full depth of the water column [79], creating an important gradient of temperature/salinity in short distance (up to 6° in less than 20 km; [78]), and therefore being an environmental front in different pelagic habitat characteristics. Based on the fact that this front is highly variable in space and time [80], we suspect that the variability observed in mitochondrial signature of AFS samples (see Figure 5 for detailed localisation) could be attributed to specific pelagic habitats respectively used by Indian and Atlantic swordfish around South Africa, rather than an ontogenetic migration of individuals from the AO to the IO. The four sample sets from South Africa indeed showed contrasted mitochondrial signature, even if no significant genetic difference could be identified among them ( $p > 0.05$  for both  $\Phi_{ST}$  and  $F_{ST}$ ). The sample sets AFS\_09\_1 (July - November 2009) and AFS\_10\_2 (April 2010) showed a mtDNA signature more related to the AO population while the two others sets (AFS\_10\_1 and AFS\_10\_3) were more similar to IO genetic signature (see details in Appendix F4a in File S1 and S5; congruently with a frequency of the haplotype #4 varying between 0 and 10%). This indicates that the boundary between Atlantic and Indian swordfish populations is not so strict and might be more considered as a transition zone between 17° and 23° east that is spatio-temporarily driven by the Agulhas Current activity. This last point engendered great perspectives in terms of management as the Atlantic stock is managed by the International Commission for the Conservation of Atlantic Tunas and the Indian stock by the Indian Ocean Tuna Commission, both legally being separated by the 20° east meridian. As already suggested for the bigeye tuna [71,81], it might be very interesting to investigate the patterns of habitat use of IO and AO of large pelagic fishes in the South African waters, with a special focus on the sex-biased dispersal.

### Supporting Information

**File S1** containing: Appendix F1: Geographic location of sampling areas and of the biogeographic provinces (Longhurst, 1998). Appendix F2: Swordfish sampling schedule. Appendix F3: Mismatch distribution for swordfish based on 1007 bp of the ND2 sequences. Appendix F4: Neighbor-joining trees showing the relationship between sample sets on the basis of pairwise genetic distances. Appendix S1: ND2 sequences pairwise  $\Phi_{ST}$  and D Jost's values. Appendix S2: Microsatellites pairwise  $F_{ST}$  and D Jost's values.  
(DOCX)

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## Author Contributions

Conceived and designed the experiments: DM PG JB. Performed the experiments: DM SLC HE. Analyzed the data: DM SLC JB. Wrote the paper: DM PG JB.

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