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Genetic population structure of the Swordfish (*Xiphias gladius*) in the southwest Indian Ocean: Sex-biased differentiation, congruency between markers and its incidence in a way of stock assessment

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Abstract:

Genetic variation was surveyed at 11 microsatellite loci and at 517 bp of the mitochondrial control region to investigate the presence of genetic stock structure in swordfish (*Xiphias gladius*) in four proximal localities of the southwest Indian Ocean. One aim of this study was to serve as a preliminary examination for congruency of structure detected by these two genetic markers, prior to conducting a more comprehensive basin-wide survey of the Indian Ocean and nearby surrounding areas. Analyses of multilocus microsatellite genotypes and mitochondrial control region sequences both revealed a great homogeneity between samples. Genetic diversity detected at the regional scale was not significantly higher than detected at the local scale. Results suggest that the southwest Indian Ocean globally functions as a unique panmictic population. However, some discrete genetic differences appeared that could possibly indicate influence from a second genetic pool in the northern part of the Indian Ocean. This structure appeared to be sex-dependent with genetic differences higher among female than among male samples. This result may indicate a higher level of spawning area fidelity for females with a subsequent sampling bias tending to homogenise male genotypic distributions.

Keywords: Swordfish; Microsatellites; mtDNA; Population genetic; Indian Ocean

Introduction

The swordfish *Xiphias gladius* is one of the most widely distributed species of pelagic fish, commonly found in the tropical and temperate zones of the Atlantic, Indian and Pacific Oceans. This species is heavily exploited by commercial fisheries worldwide, mainly by drifting longline fisheries. Despite a constant increase of fishery effort, captures of swordfish have decreased since 2000 in the Indian Ocean (Indian Ocean Tuna Commission, 2008). On the basis of the 2008 stock indicators, the IOTC concluded that the recent catch level (about 319000 t from 2002-2006) have been around the current estimate of maximum sustainable yield (31500 t, 80% confidence limits: 24500t-34400t) and is so in acceptable limits. While the assessments indicate that the swordfish stock for the whole Indian Ocean is probably not currently overfished, catch rate data from the southwest Indian Ocean suggest that overfishing might occur in localised areas (IOTC, 2008). Consequently, management measures such as quotas introduction may be considered for a sustainable exploitation. However, in the absence of a clear definition of stock structure, determining the appropriate allocation of the resource will be impossible. In the case of quotas introduction, for example, the application of a unique quota on a mixed population is unfavourable for the species and may lead to stock depletion, whereas the application of several quotas on a unique population may penalize the fishing activity (Avise, 1998). The artificial spatial scale of stock assessment and management must match with the natural spatial scale of target populations (Francis *et al.*, 2007). In this context, improving knowledge on the population structure of swordfish is the first information needed by managers for defining relevant management measures.

Resolving stock structure in a highly migratory species such as the swordfish presents unique and interesting challenges especially considering observed sexually dimorphic life history strategies. As a general rule, adult female swordfish migrate to temperate areas for

feeding during summer, then move to warmer waters for spawning. In contrast males appear to remain more abundant in tropical regions and rarely undertake long distance migrations observed for females (De Martini *et al.*, 2000; Palko *et al.*, 1981). Tag-recapture experiments showed that even if some swordfish are able to undertake long-distance inter-ocean movements (at the scale of an ocean; Sedberry and Loefer, 2001; Takahashi *et al.*, 2003), 20% of tagged swordfish are recaptured close to the point of release, revealing important inter-individual plasticity in migrating behaviour and suggesting residency or homing behaviour for some individuals within specific regions (Sedberry and Loefer, 2001). Thus, considering dispersal of the swordfish, it may exist great disparities between the maximal dispersal range (mainly linked with feeding behaviour) and the reproductive effective dispersal range (more important in term of population replenishment and structure).

Genetic studies constitute an efficient mean to determine effective dispersal and delineate stock boundaries (Palumbi, 2003), even for highly migratory species. For example, whereas all tuna species possess similar high migration abilities, differentiation has been detected at various scale: within an ocean basin for bluefin tuna (in Mediterranean; Carlsson *et al.*, 2004), both within and among oceans for the yellowfin tuna (Diaz-Jaimes and Uribe-Alcocer, 2006; Ely *et al.*, 2005; Ward *et al.*, 1997) and for the bigeye tuna (Alvarado Bremer *et al.*, 1998; Durand *et al.*, 2005). On the other hand no differentiation was observed among oceans for skipjack tuna (Ely *et al.*, 2005). In the case of swordfish, structure appears quite important with populations subdivided on oceanic and infra-oceanic scales, in the Atlantic (Alvarado Bremer *et al.*, 2005; Alvarado Bremer *et al.*, 1996) and Pacific oceans (Lu *et al.*, 2006; Reeb *et al.*, 2000) and in the Mediterranean Sea (Kotoulas *et al.*, 1995). In a general way, swordfish structure has been less studied in the Indian Ocean. Some corridors seem to exist between oceans but these appear to be constrained and delineated by equatorial boundaries. For example, swordfish appear quite similar between South-Indian and South-Atlantic oceans, as well as between South Indian and Pacific oceans (Chow and Takeyama,

2000; Lu *et al.*, 2006), more similar between neighbouring oceans than from the southern to the northern parts of the same ocean. While most of these genetic studies on the swordfish conducted up until now have involved only one genetic marker, conclusions based on nuclear and mitochondrial DNA data seemed concordant such as observed differentiation in Atlantic Ocean versus the Mediterranean Sea (Alvarado Bremer *et al.*, 2005). However, genetic structure defined in the Indian Ocean with two kinds of markers showed some discrepancies. Indeed, Jean *et al.* (2006)'s study failed to show population differentiation on the basis of microsatellite data in the southwest Indian Ocean in agreement with what could be suspected for a species displaying a high capacity of migration. On the other hand, Lu *et al.* (2006) showed with mitochondrial sequences also obtained in the southwest Indian Ocean that gene flow between adjacent populations appeared to be quite reduced or even absent. Even if the uniparental inheritance of mtDNA tends to accentuate genetic differences among population compared to nuclear genes, it does not capture the entire genetic history that is fundamental in the case of defining population structure as required for fisheries management. As such differences in conclusions can have drastic impact on stock assessment, it is imperative to determine whether these differences are due to sampling area, sample size, or to genetic marker discordance.

In order to further examine and clarify whether nuclear and mitochondrial markers are useful and complementary for swordfish stock discrimination, we examined variation at eleven DNA microsatellite loci and compared that with analysis of mitochondrial haplotype data (517-bp of the control region or d-loop). A total of 337 samples was examined from four different sites of the southwest Indian Ocean. The major objective of this study was to provide preliminary support in the form of a pilot study for using both DNA microsatellite and mtDNA markers to further assess population genetic structure of Indian Ocean swordfish in a global context (*i.e.* the Indian Ocean and its connections with the neighbouring oceanic basins) in an effort to assist better management of this important commercial species.

Materials and methods

Biological materials

Swordfish were sampled from four localities in the southwest Indian Ocean, respectively in fishery statistical square around the islands of Glorieuses (11°S, 46°E), Seychelles (5°S, 56°E), Reunion (21°S, 56°E) and in the south of Madagascar (31°S, 43°E), respectively called GLO, SEY, RUN and MADA (Figure 1). Muscle tissue biopsies from a total of 337 swordfish were collected onboard commercial fishing vessels between February 2005 and May 2006. Tissues were stored in ethanol 90% or in 20% Dimethylsulfoxide (DMSO) saturated salt solution (Dutton, 1996) and frozen until DNA was isolated. All the swordfish sampled were measured (LCK = Length from Cleithrum to Keel, *i.e.* fish length without head and caudal fin) and sexed (Table 1).

Genetic analysis

Total genomic DNA was extracted using DNAeasy Tissue Kit (Qiagen) following the manufacturers instructions. A 517 base pair (bp) fragment representing a small section of the mitochondrial d-loop of control region was amplified by PCR using the primers defined by Alvarado Bremer (1996; L15998: 5'-TACCCCAAACTCCCAAAGCTA-3'; H235: 5'-TGAATTAGGAACCAGATGCCA- 3'). Reactions were performed in 25 µl containing 1X PCR buffer, 2 mM $MgCl_2$, 20 µM of each dNTPs, 0.5 µM of each primer, 0.5 U of Advantage Polymerase Taq (Ozyme), 25 ng of genomic DNA. Cycling parameters were 93°C for 3 min, followed by 35 cycles of 93°C for 40 s, 60°C for 50 s, and 72°C for 40 s and a final elongation at 72°C for 2 min. PCR products were purified and sequenced on an ABI 3100 sequencer (Macrogen Inc.). Sequences were run forward and reverse. They were checked and edited using Chromas version 1.6 (McCarthy, 1997) and aligned using ClustalW (Thompson

et al., 1994) in BioEdit Sequence Alignment Editor (Hall, 1999). Sequences were submitted to GenBank (Accession number EU202452-EU202642).

Eleven microsatellite loci were used, eight from Reeb *et al.* (2003: Xg-55, Xg-56, XG-66, Xg-75, Xg-144, Xg-166, Xg-379 and Xg-396) and three newly developed loci (D2A, D2B and C8, with D2A and D2B linked loci on the same sequences). For isolation and characterization of these 3 new DNA microsatellite primers, first was total genomic DNA isolated from a single individual and then sent to Genetic Identification Services in California for development of four libraries enriched for sequences containing microsatellite repeats. Each library was enriched for the presence of a specific tetra repeat motif: CAGA, CATC, TAGA or TGAC. Sequences from these four enriched libraries were analysed for the presence of repeats and primers were designed for a few promising loci using Oligo software 6.8 (Molecular Biology Insights, Inc). Once primer pairs were chosen (Table 1), a CAG tag (5′-CAGTCGGGCGTCATCA-3′; see Schable *et al.*, 2002) was added to the 5′ end of one of each primer pair depending on which gave the least amount of secondary structure. This tag allows the use of a third primer in the PCR (CAG) that is fluorescently labelled for detection on the ABI capillary and gel based genotyping systems (Boutin-Ganache *et al.*, 2001). An additional sequence (GTTTC) was added on the other primer of each pair to promote a-tailing of the fluorescent strand. These 3 microsatellite loci were amplified using AmpliTaq Gold (Applied Biosystems, Inc.) in 50µL reaction using standard 1.5mM $MgCl_2$. Cycling was kept standard with one hold at 95^{o}C for 12 minutes followed by 40 cycles of (94^{o}C/15sec denature; then 50^{o}C/30sec anneal; and 72^{o}C/ 1minute extension) followed by a final extension at 72°C for 20 minutes. The 8 loci from Reeb *et al.* were amplified using the same PCR reaction for mtDNA, but with Red Gold Star DNA Polymerase (EuroGenTec), and using cycling parameters described in FitzSimmons *et al.* (1997). 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 GeneMapper4 (Applied Biosystems Inc.).

Statistical analyses

Mitochondrial DNA

Genetic variation among mitochondrial sequences was estimated as follow: for each population, the haplotype (H_d) and nucleotide (π) diversities, Tajima's (1989) D statistic and Fu's (1997) F statistic were examined using the DNAsp 4.0 software (Rozas *et al.*, 2003). Tajima's D and Fu's F statistics test for departures from equilibrium between mutation and drift; significantly negative values indicate population expansion or selective influence. Fu's F statistics is more sensitive to recent demographic expansion (Fu, 1997). Pairwise genetic distances (ϕ_{st}) were estimated between samples using Arlequin 2.000 (Schneider *et al.*, 2001). In all cases, critical significance levels for multiple testing were corrected using a sequential Bonferroni procedure (Rice, 1989). An AMOVA (Analysis of molecular variance; Excoffier *et al.*, 1992) was performed using Arlequin 2.000 (Schneider *et al.*, 2001). DNAsp 4.0 (Rozas *et al.*, 2003) was also used to estimate the nearest-neighbour statistic, *Snn* (Hudson, 2000). *Snn* is a measure of how often the 'nearest neighbours' (in sequence space) are from the same locality (in geographical space). *Snn* varies from 0 to 1: under 0.5, it is assumed that populations are in panmixia, and values closer to 1 indicate that populations are differentiated. *Snn* is particularly suitable when haplotype diversity is large (Hudson, 2000). Neighbour-joining trees, based on Kimura-2 parameter distance (Kimura, 1980), were constructed using the Mega 2.1 software (Kumar *et al.*, 2001).

Microsatellites

Allele frequencies, genetic diversity for each population, and genetic differentiation between populations were estimated from microsatellites following classical population estimators implemented in the Genepop 3.4 software (Raymond and Rousset, 1999). Values

estimated include the mean number of alleles per population (*Nall*), and the observed (*Hobs*), and expected (*Hnb*) heterozygosities (Nei, 1987). In addition, allelic richness (*Rs*) was estimated using Fstat 2.9.3.2 (Goudet, 1995). The null hypothesis of independence between loci was tested from statistical genotypic disequilibrium analyses using Genepop 3.4 (Raymond and Rousset, 1995). Deviations from Hardy-Weinberg equilibrium were examined for each population, at each locus, by calculating Wright's fixation index F_{is} as estimated by Weir and Cockerham's (1984) using the same software. Departure from Hardy-Weinberg equilibrium was then tested using exact tests. Overall levels of genetic differentiation were analysed by calculating the estimator θ of the Wright's F_{st} Statistic (Weir and Cockerham, 1984) for each locus, and differentiation was then tested using exact tests for the null hypothesis of identity of allelic distributions across populations. Effective population size (*Ne*) were estimated using changes in microsatellite allele frequencies with the software programme NeEstimator (Peel *et al.*, 2004). This software gave point estimation of *Ne* using linkage/gametic disequilibrium (Hill, 1981). NeEstimator was not used to estimate the actual long-term inbreeding effective population size but to compare *Ne* estimates as relative effective population sizes between samples. AMOVA analysis were performed using Arlequin 2.000 (Schneider *et al.*, 2001). A correspondence factorial analysis was performed on genotype frequencies with the Genetix 4.0 software (Belkhir *et al.*, 2000). To determine if the samples belonged to one or more populations, data were also analysed using the software Structure (Pritchard *et al.*, 2000) which uses iterative computation process to infer the most likely number of populations (*K*) represented in the total sample. For this analysis, an admixture model assuming independent allele frequencies was used and three replicates were run (each with 1.10^5 burn-ins and 5.10^5 iterations) at *K* values from 1 to 4.

Results

Genetic diversity and demographic stability

Mitochondrial DNA

A total of 117 variable sites, constituting 240 haplotypes was detected among the mtDNA control region sequences (517 bp) for the 337 swordfish sequenced. Compiling with Reeb *et al.*' (2000)s dataset previously published in GenBank (Accession number AF199616-AF200183), it appears that all these haplotypes belong to clade I with a divergence intra-clade lower than 1% (compared to a divergence between clades higher than 2.5%).

A similar high level of genetic diversity was encountered in each locality (Table 2). Mean haplotype diversity (H_d) and mean nucleotide diversity (π) were of the same order of magnitude between localities, with H_d near from 1, and π near from 0.02. Tajima's D and Fu's F values were negative but not significant (D = - 1.6 and F = - 2), except for Fu's statistics in RUN locality.

Microsatellites

Mean number of alleles and allelic richness were of the same order between the four localities respectively varying from 14.3 to 17.5 and from 13.3 to 14.6 (Table 1), with each time the lowest value in RUN and the highest value in GLO. The GLO population had the highest number of private alleles at 16 versus a range of 3 to 9 in the three other localities). *Ne* estimates varied from a low of 277 in RUN to 879 in GLO (Table 2). No loci were in disequilibrium ($p < 0.001$) over the whole dataset, supporting the independent assortment of alleles at different loci. Heterozygote deficiencies were highly significant in all samples with values ranging from a low of 0.079 for GLO to 0.176 for RUN (Tables 2 & 3).

Population structure

Mitochondrial DNA

Pairwise ϕ_{st} estimates between localities are presented in Table 4. Mean value of ϕ_{st} was weak (= 0.01) with only one of the six values significant, between SEY and RUN samples. The two highest ϕ_{st} values were observed in the SEY sample. Interestingly, an AMOVA across the four samples demonstrated a small and non-significant Φ_{ST} value (0.005, p = 0.15). When hierarchical AMOVA analysis were undertaken with grouping schemes in agreement with significant pairwise ϕ_{st} estimates on the localities (*i.e.* SEY sample isolated from the three others or RUN sample isolated from the three others), more than 99% of the variance was observed within the samples ($\Phi_{ST} < 0.002$, $p > 0.05$) with a non-significant variance associated with the partition in two groups ($\Phi_{SC} < 0.001$, $p > 0.05$). To further test samples homogeneity, the nearest-neighbour statistic (*Snn*) was calculated on the mtDNA control region sequences. The test revealed a non-significant association between sequence similarity and geographical location (*Snn* = 0.288, p = 0.13). A neighbour-joining tree based on average pairwise distances estimated from the 517-bp mtDNA sequences between samples is presented in Figure 2. Samples from the four localities appeared well mixed. Adding our sequences to Lu *et al.* (2006)'s Indian Ocean sequences previously published in GenBank (Accession number DQ076502-DQ076643), pairwise ϕ_{st} estimates revealed no more differentiation. This could be due to the low sampling size of Lu *et al.* (2006)'s samples (four times lower than ours) or to shorter length of our sequences (517 pb against 819 pb).

Microsatellites

Pairwise multilocus θ values between localities are presented in Table 4. Mean value of θ was weak (= 0.02) with all the non-null θ values involving either SEY or GLO samples. Only one significant θ value was observed among six sample site comparisons. This was between SEY and GLO and was likely due to significant differentiation observed at 5 of the 11 loci tested. An AMOVA across the four samples demonstrated a small and non-significant Φ_{ST} value (0.000, p = 0.510). When hierarchical AMOVA analysis were undertaken with

grouping schemes in agreement with significant pairwise θ values between localities (*i.e.* SEY or GLO sample isolated from the three others), more than 99% of the variance was observed within the samples ($\Phi_{ST} < 0.001$, $p > 0.05$) with a non-significant variance associated with the partition in two groups ($\Phi_{SC} < 0.001$, $p > 0.05$). The analysis made with Structure suggested that the highest likelihood of obtaining such data was to consider that only one population ($K = 1$) existed. The likelihood decreased when estimates were made with one population to two (over three independent simulations: LnP(D) for $K = 1$ and $K = 2$ were −14217 and −14536, respectively) providing some evidence against subdivision. A correspondence factorial analysis was performed on genotype frequencies. Results of this multivariate analysis are presented on Figure 3; 81% of genetic variance was synthesized by the two first axis (respectively 39.3% and 31.7% by axis 1 and axis 2). Some groupings seem to exist in accordance with sampling locality, mainly due to the segregation of SEY against GLO on the first axis, whereas these two samples appeared opposed to MADA and RUN on the second axis. However the distribution of individuals along the axes showed a great disparity and a high degree of superposition.

Sex-dependant analysis

As sex identification was available for each swordfish, some statistics were re-estimated, for males and females, of each locality or of all localities. Levels of genetic diversities of both markers were of the same order within each sex category. Allelic richness was however always higher for female than for male but not in a significant way. Pairwise ϕ_{st} estimates between sex categories within each locality were weak. The previous significant value found between SEY and RUN disappeared (probably because of the very small number of females in RUN locality (n = 6)). Noteably, the previous pairwise multilocus θ value found between SEY and GLO was still significant indicating that significant differences were only due to females ($\theta = 0.01$, $p < 0.001$), as males from SEY and GLO showed no significant differences (see Table 5). Other pairwise differentiation involving females from GLO were

significant (*i.e.* with males from GLO or with females from MADA; see Table 5). In a global way, all pairwise differentiation values involving females were higher than the same values estimated between males. Two hierarchical AMOVA (one on mtDNA data, the second on microsatellites) were undertaken with partitioning in two sex groups. More than 99% of the variance was observed within the samples ($\Phi_{ST} < 0.001$, $p > 0.05$) with a non-significant variance associated with the partition of the two sex groups ($\Phi_{SC} < 0.001$, $p > 0.05$).

Discussion

This study aimed to yield results on two different levels. The first aim was to evaluate genetic differences between swordfish sampled in four distinct localities of the southwest Indian Ocean (SWIO). An other aim was to examine the amount of congruency between the two genetic marker types used to examine the population structure and its implication in the case of a regional swordfish multi-stock assessment program.

Genetic structure of the swordfish in SWIO

The analysis of mitochondrial sequences of *X. gladius* has revealed a very high level of mitochondrial diversity (nearly all individual displayed a distinct haplotype) as well as a high microsatellite polymorphism (some loci showing up to 40 alleles). Both markers showed a great genetic homogeneity between the four samples. *Snn* statistic as well as results obtained with the software Structure pointed out the existence of a unique pool of genes. In the same way, analysis of molecular variance mainly failed to find a genetic structure among the four localities sampled in the SWIO. The very high levels of genetic diversity and the lack of differentiation detected at a large spatial scale (*i.e.* about 2000 miles from North to South of the sampling area) is thus well in agreement with the assumption of a large population size that is not very sensitive to genetic drift (De Woody and Avise, 2000). This last point agrees with Tajima's D and Fu's F statistics that indicate these samples appear demographically

stable. The hypothesis of a large effective population size that do not fluctuate greatly over time is easily understandable for swordfish with fecundities of several millions of eggs per female (Palko *et al.*, 1981). Such a finding is also in agreement with the hypothesis of long range dispersal typical of large pelagic fishes (Waples, 1998).

Results obtained from both mtDNA and microsatellites are consistent with the idea that swordfish of the SWIO belong to a single unique panmictic population. However, some weak differentiation seems to exist within the SWIO, and thus despite the important within-sample diversity that has been noticed to considerably reduce the ability of detecting between-samples structure, either on mtDNA data (Charlesworth, 1998) or on microsatellites studies (Hedrick, 1999; O'Reilly *et al.*, 2004). Jean *et al.* (2006)'s study failed to demonstrate genetic structure of swordfish within the SWIO; increasing the sample size and the number of microsatellites loci (three times more samples, two times more microsatellites, between Jean *et al.* (2006)'s study and our) has thus permit to display heterogeneity within swordfish of the SWIO. The punctual estimation of effective population size (that is the spawning proportion of adults) tends to show higher values in the two northern localities (GLO and SEY), in parallel with higher allelic richness. This could be in agreement with the hypothesis of a reproductive aggregation in this northern zone (Mejuto *et al.*, 2006) whereas the two southern areas might better represent transition zones between feeding and spawning areas or feeding regions. Some swordfish collected in these northern localities (in GLO or SEY) could thus belong to a second genetic pool. The significant heterozygote deficits observed in all localities could partly be the signature of a Wahlund effect and could confirm the, more or less pronounced, influence of a second genetic pool. This is also in agreement with Lu *et al.* (2006)'s study which showed the existence of differences between swordfish sampled in the Indian Ocean (in this case, between north of Madagascar and other northern sampling sites). Swordfish within a locality may thus be a mixture from one dominant genetic pool and a second less influent one, with various level of homogeneity depending on the swordfish

behaviour, and thus explaining the weak structure observed. These results contrast with those obtained in Atlantic (Alvarado Bremer *et al.*, 2005) that support the homogeneity between spawning and feeding grounds, either in North and South Atlantic.

Moreover, examination of these populations indicates that there are some sex-biased intra-sample differences, that could also explain heterozygote deficits. For example, males in GLO differ from females of the same area and the genetic differences found between SEY and GLO was in fact only due to females. This species is known to migrate to temperate areas for feeding and then move to warmer waters to reproduce (Palko et al., 1981): differences in the level of genetic structure observed when only males or females are involved could thus be the signature of a more pronounced homing behaviour depending on sex (Keeney et al., 2005; Lee et al., 2007). In the present case, higher values for females indicate that they might present a higher fidelity to their reproduction areas than males (Prugnolle and de Meeus, 2002). A lower fidelity by males, means that the male fish are supposed to swim around more and are caught randomly with less structure when they are caught. This is supposed to homogenise the nuclear genetic signal for both sexes, and so the differentiation between females might be higher with the mtDNA. Actually it is not the case. So it could mean that a sampling biased exist, maybe without enough homogeneity in sampling periods. The sampling is not yet sufficient to deal further with sex-biased differences but open a very interesting perspective of research. Conclusions based solely on genetic data would be an insufficient method of defining population structure, without the light of some basic biological informational framework based on reproductive, feeding, and migrating strategies.

Congruency of molecular markers in a perspective of stock assessment

Many genetic studies have shown discrepancies in conclusions when using different markers types (Lemaire *et al.*, 2000; Nielsen *et al.*, 2006; Pogson *et al.*, 1995). In such cases,

result disparities may partly be explained by differences in mutation rates and in sensitivity to forces that promote population differentiation. Using two genetic markers was our initial option to limit errors, as ‘drawing conclusions from single genealogies can be problematic as each is only a single point in the space of all possible genealogies’ (Wakeley, 2003). In the present study, both markers seemed to support similar conclusions in the pattern of genetic differentiation. Despite the high genetic diversity levels and the consequent limits of interpretation both markers indeed showed a global pattern of panmixia within a unique population at the scale of the SWIO influenced by a putative second differentiated population in the equatorial region of the Indian Ocean. The putative existence of a second pool in a restricted area of the SWIO is important as this region is supposed to be more affected by stock declines due to high fishing pressures (IOTC, 2008). The existence of a distinct stock have first to be confirmed; but, if it is really the case, genetic data will provide a key information in the aim of swordfish stock assessment in Indian Ocean as decision have to be taken separately for each stock. This encourages further research. Sampling a more extensive area (within the Indian Ocean and neighbouring oceanic basins) may permit estimates of the number of independent gene pools of swordfish, their geographic boundaries, and the level of exchanges between them. The existence of a second putative spawning aggregation in the western part of the Indian Ocean as well as the specificity of bias due to dimorphic population patterns observed in the different sexes also warrants further investigation. Finally, a pertinent strategy for dealing with veracity of conclusions requires a good temporal sampling strategy that is replicated samples over a two to three year time frame to fully describe population dynamics for species like the swordfish that has the migratory potential to demonstrate high levels of gene flow (Waples, 1998). Temporal samples will also permit effective estimates of population size with higher precision (Fraser *et al.*, 2007) and help to challenge questions about overfishing.

The present study permits analysis of genetic structure observed in *X. gladius* in the SWIO and to address conclusions of previous studies (Jean *et al.*, 2006; Lu *et al.*, 2006). Our results also underline the necessity of compiling biological data to genetic information to examine swordfish stock structure. Stock estimates clearly require further investigations by increasing the spatio-temporal sampling, both within Indian Ocean and with the neighbouring oceanic basins.. As underlined by Francis *et al.* (2007) in the fourth of their Ten commandments for ecosystem-based fisheries scientists, 'continuing to rely on traditional stock assessments that either ignore or artificially delineate the true spatial structure of fish populations is clearly a recipe for disaster'. A project of this scale would provide much insight and constitute an excellent database for the CTOI and management authorities for both present and future management of swordfish fishery in the Indian Ocean.

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Tables

Table 1. Characteristics of 3 new microsatellite loci of Xiphias gladius. Repeat motif is derived from the sequenced clone

Locus Name	GenBank Accession no.	Repeat motif	Primer (5'−3')
D2A	not yet available…	(CCT)6	F-5'-CAGTCGGGCGTCATCACTCAAACTGAGACTTTCCAAGTAATCCT-3'
			R-5'-GTTTCACTTCCAGCCAAACTCTTGTTCGT-3'
D2B		(CAGT)8	F-5'-CAGTCGGGCGTCATCAAAGCAACAACATTGTCTTCTG-3'
			R-5'-GTTTCTGGCGTGAACGTGGCTCAATCC-3'
C7		(CTAT)22	F-5'-CAGTCGGGCGTCATCACCTTCAATGTAGAGATGGCAGG-3'
			R-5'-GTTTCAAATGTCGGTGGAGCTGTGGACAGA-3'

Table 2. Main characteristics of the four samples of *X. gladius*.

Sampling information are: sample size (N), mean length of fish (Length from Cleithrum to Keel in cm, ± Standard Deviation) and proportion of females estimated within each samples.

Genetic diversities are given for each markers successively. For mtDNA data, information are: number of haplotypes per population (h), haplotype diversity (H_d), nucleotide diversity (π), Tajima's D and Fu's F statistics. For microsatellites, information are: mean number of alleles (*Nall*), allelic richness (*Rs* as estimated for a common minimal sample size of 44 individuals), number of private alleles, unbiased (H_{nb}) and observed (H_{obs}) heterozygosities (Nei, 1987) and effective size estimates (*Ne*). Significant values are noticed by *p < 0.05 ; ***p < 0.001.

		GLO	MADA	RUN	SEY
Sampling	*N*	105	100	65	67
	Mean length (cm)	73.7 ± 19.0	90.1 ±9.4	87.2 ±21.5	75.2 ± 18.4
	Prop. of females	0.47	0.67	0.13	0.58
Mitochondrial diversity	*h*	90	90	59	60
	Hd	0.997	0.997	0.997	0.996
	π	0.020	0.019	0.021	0.019
	Tajima's D	-1.54	-1.45	-1.74	-1.41
	Fu's F	-1.52	-1.84	-2.54*	-2.04
Microsatellites diversity	*Mean Nall*	17.5	16.0	14.3	15.2
	Mean Rs	14.6	13.7	13.3	14.2
	Private Nall	16	8	3	9
	Hnb	0.784	0.780	0.776	0.771
	Hobs	0.720***	0.667***	0.640***	0.658***
	Ne estimate	879	585	277	506

Table 3. Mono - and multi-loci estimates of the fixation index F_{is} within each locality of *X. gladius*. Tests of significance were performed with Genetix 4.0 (Belkhir *et al.* 2000), *p < 0.05 ; ***p < 0.001. Allele size range (in base pairs) and number of alleles per locus are also given.

			Monolocus Fis			
Locus	*Size range*	*Nall*	**GLO**	**MADA**	**RUN**	**SEY**
X55	*79-191*	*47*	0.149***	0.330***	0.405***	0.170***
X56	*115-159*	*20*	0.025	0.110***	0.064	0.094*
X66	*110-140*	*12*	0.094*	0.115***	0.150***	0.080
X75	*142-276*	*53*	0.031	0.332***	0.343***	0.182***
X144	*151-172*	*8*	-0.005	0.033	0.076	0.140
X166	*120-144*	*9*	-0.007	0.079	0.078	0.048
X379	*100-142*	*15*	0.020	-0.013	0.099	0.127*
X396	*107-137*	*9*	0.182***	0.326***	0.381***	0.300***
D2A	*287-296*	*4*	0.079	0.048	0.064	0.230***
D2B	*142-202*	*16*	0.044	-0.007	0.019	0.060
C8	*136-240*	*28*	0.223	0.131	0.164	0.221
			Multiloci average Fis			
			0.079***	0.146***	0.176***	0.148***

Table 4. Pairwise values of genetic differentiation in *X. gladius* localities. Pairwise ϕ_{st} values obtained from the mtDNA sequences dataset are above the diagonal with test of significance performed with Arlequin 2.0 (Schneider *et al.*, 2001). Multiloci Weir and Cockerham's (1984) θ values obtained from the microsatellite dataset are below the diagonal with tests of significance performed with Genetix 4.0 (Belkhir *et al.* 2000). *p < 0.05 ; ***p < 0.001.

	GLO	**MADA**	**RUN**	**SEY**
GLO		0.000	0.000	0.001
MADA	0.001		0.001	0.001
RUN	0.000	0.000		**0.003***
SEY	**0.005*****	0.002	0.003	

Table 5. Pairwise microsatellite multiloci θ values of genetic differentiation between males and females swordfish with tests of significance performed with Genetix 4.0 (Belkhir *et al.* 2000) *p < 0.05 ; ***p < 0.001. Differentiation between females of two localities are below the diagonal (light grey), between males above the diagonal (dark grey) and between males and females from a same locality on the diagonal. Samples size are noticed into brackets.

		Males			
		GLO (57)	**MADA (15)**	**RUN (31)**	**SEY (26)**
Females	**GLO (49)**	**0.005*** **	0.005	**0.005***	0.001
	MADA (33)	0.005	0.000	0.006	0.000
	RUN (6)	0.009	0.000	0.010	0.005
	SEY (25)	**0.013*** **	0.004	0.006	0.003

Figures

Captions:

Figure 1. Geographic location of the four IOTC fishery statistical square (5° x 5°) wherein *X. gladius* were sampled for this study.

Figure 2. Neighbour-joining tree based on pairwise number of differences between haplotypes of *X. gladius* from the four localities of the southwest of Indian Ocean. Samples are respectively represented by black squares for Glo, grey circles for Mada, white squares for Run and white triangles for Sey.

Figure 3. Correspondence factorial analysis, performed on genotype frequencies of *X. gladius*. Each point represents a given individual whose symbol corresponds to its sampling locality.

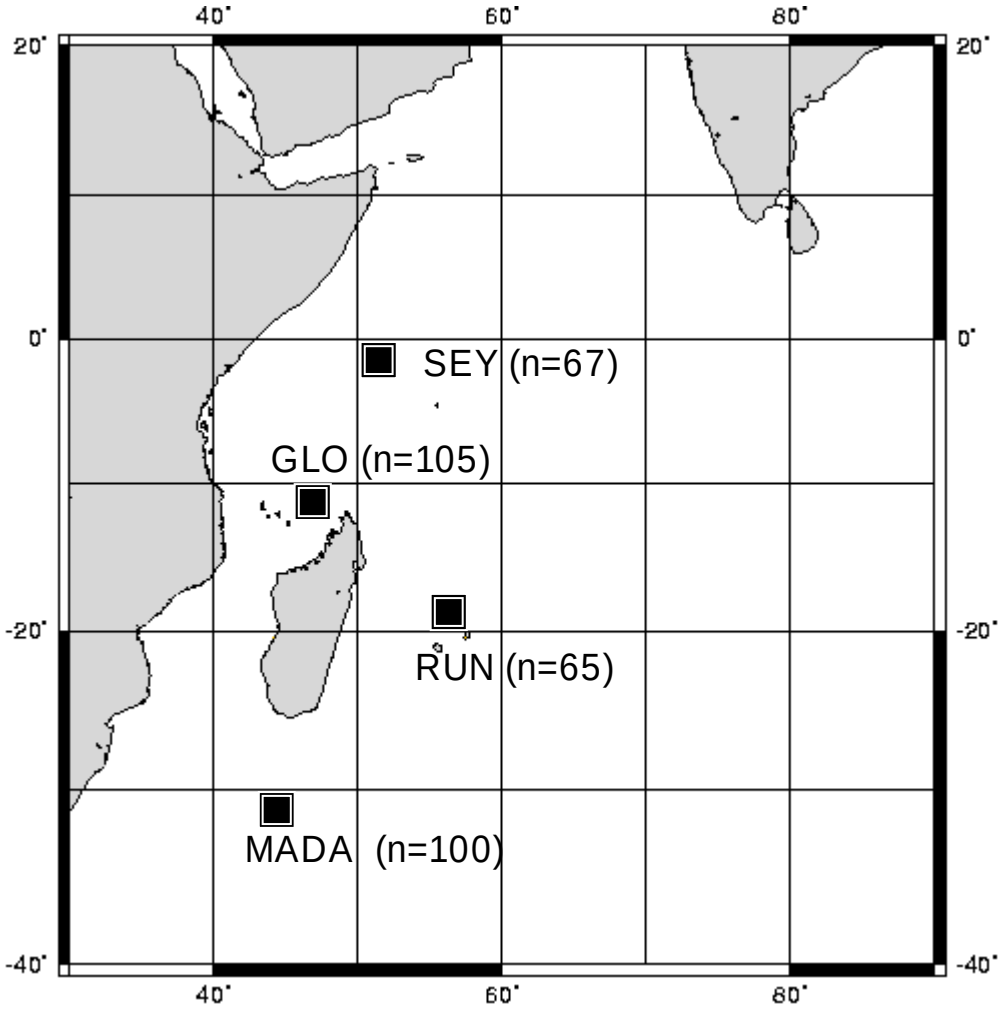

Figure 1. Geographic location of the four IOTC fishery statistical square (5° x 5°) wherein *X. gladius* were sampled for this study, in the vicinity of Glorieuses (GLO), in south of Madagascar (MADA), around Reunion island (RUN) and in Seychelles (SEY).

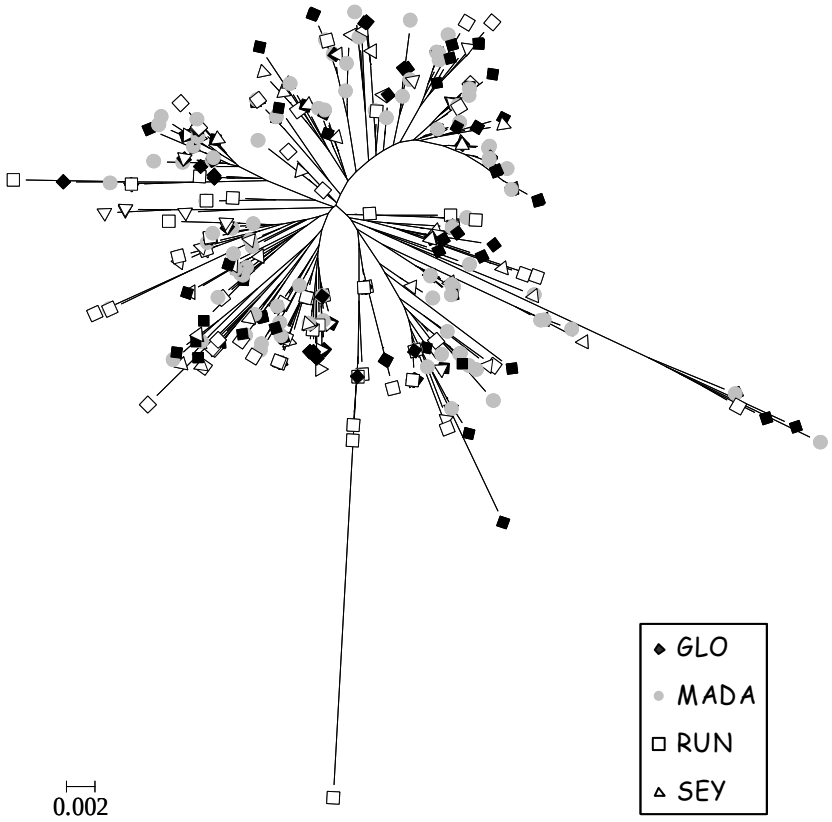

Figure 2. Neighbour-joining tree based on pairwise number of differences between haplotypes of *X. gladius* from the four localities of the southwest of Indian Ocean. Samples are respectively represented by black squares for Glorieuses, grey circles for Madagascar, white squares for Réunion and white triangles for Seychelles.

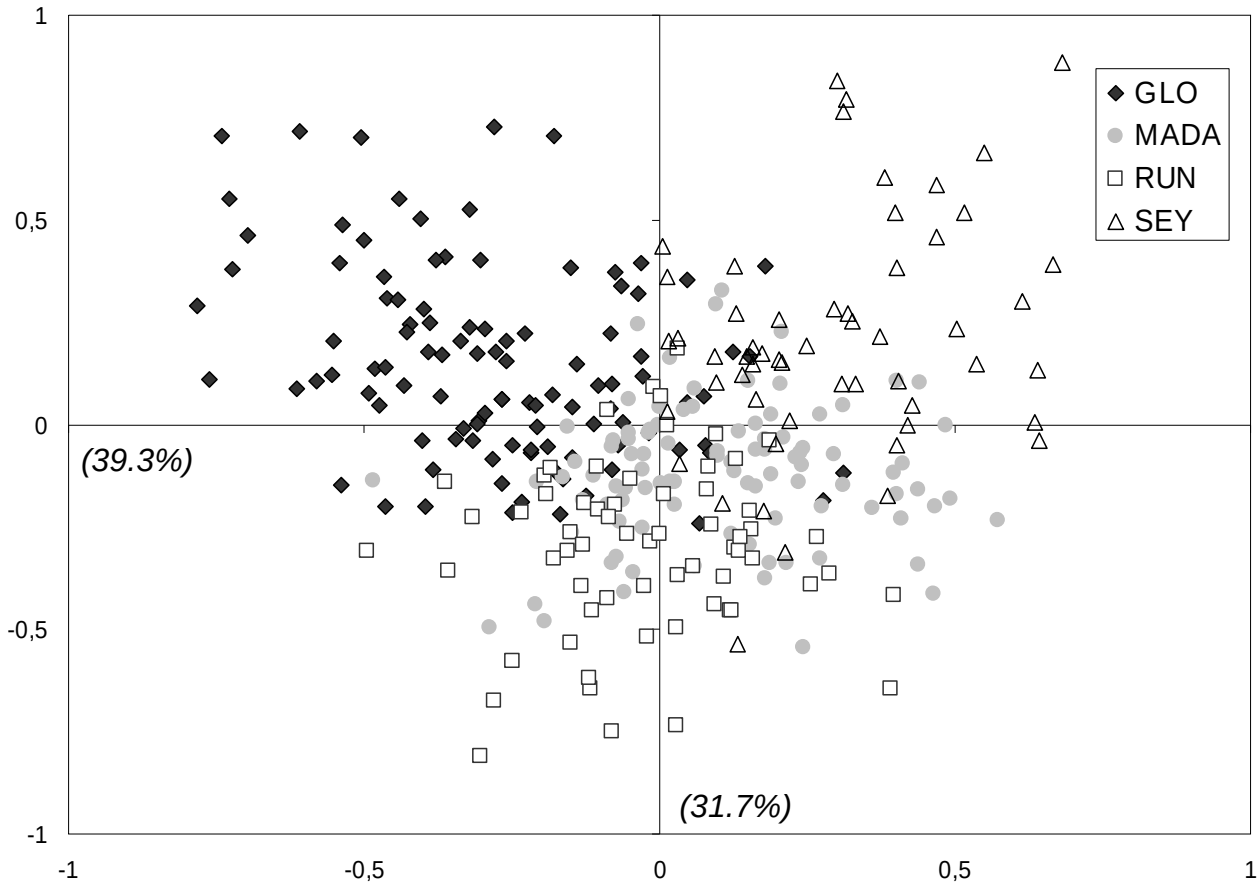

Figure 3. Correspondence factorial analysis, performed on genotype frequencies with Genetix 4.0 (Belkhir *et al.* 2000). Each point represents a given individual whose symbol corresponds to its sampling locality.