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Congruency between microsatellite and mitochondrial DNA analyses of swordfish (*Xiphias gladius*) population structure in the southwest Indian Ocean: importance in a way of stock assessment

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Abstract

Genetic variation was surveyed at eleven microsatellite loci and the mitochondrial control region (517 bp) to investigate the presence of genetic stock structure in swordfish (*Xiphias gladius*) in four proximal localities of the southwest Indian Ocean. The aim of this study was to test for congruency of structure detected by these two genetic markers, in order to conduct a study at the scale of the whole ocean. 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 this detected at the local scale. Results suggest that the southwest Indian Ocean globally functioned as a unique panmictic population. However some discrete genetic differences appeared, possibly indicating the influence of, at least, a second genetic pool in the northern part of the Indian Ocean. In addition to the description of the genetic structure, we also discussed the importance of observed congruency of our markers in the aim of a management application.

Introduction

The swordfish *Xiphias gladius* is one of the most widely distributed species of pelagic fishes, commonly found in the tropical and temperate zones of the Atlantic, Indian and Pacific Oceans. This species has the greatest commercial value of the billfish resource and is so heavily exploited by commercial fisheries worldwide, mainly by longline fisheries. Despite a constant increase of fishery effort, captures of swordfish tend to decrease since 2000 in the Indian Ocean. On the basis of the 2006 stock assessments and indicators, the Indian Ocean Tuna Commission (IOTC) concluded that the level of catches in 2004 (about 32 000 t) was above the maximum sustainable yield (estimates range between 23 000 and 27 000 t). Furthermore, while the assessments indicated 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 of swordfish might occur in localised areas (IOTC, 2006). 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 and connectivity of swordfish is the first information needed by managers for defining relevant management measures.

The swordfish is a highly migratory species. As a general rule, adult swordfish, especially females, migrate to temperate areas for feeding during summer, then move to warmer waters for spawning where males appeared to be more abundant (Palko et al., 1981). Tag-recapture experiments showed that some swordfish are able to undertake long-distance movements (at the scale of an ocean; Sedberry and Loefer, 2001). On the other side, a large proportion of tagged swordfish recaptures are reported near from the point of release, suggesting residency or homing behaviour amongst components of population (Carey and Robinson, 1981; Sedberry and Loefer, 2001). Considering dispersal of the swordfish, there might be great disparities between the maximal dispersal range (rarely achieved but with a consequent impact on range expansion and colonisation of new areas) and the currently-achieved dispersal (that ensures population replenishment), with important consequences in term of effective structure observed within the species (Kinlan et al., 2005).

In this context, genetic studies generally constitute a precious help to determine effective dispersal and delineate stock boundaries (Palumbi, 2003), as it has already been the case for highly pelagic species. Thus the various species of tuna that all possess high migration abilities, present various levels of effective connectivity as estimated by genetic structure; differentiation is observed within ocean basin for the bluefin tuna (Broughton and Gold, 1997), among oceans for the yellowfin tuna (Ely et al., 2005) or even not observed between oceans for the skipjack tuna (Ely et al., 2005). In the case of swordfish, connectivity appeared quite reduced with populations subdivided on an 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 seemed to exist between oceans, with some swordfish migrating from South Indian to South Atlantic oceans (Alvarado Bremer et al., 2005), or between South Indian and Pacific oceans (Lu et al., 2006; Ward et al., 2001), more often than from the south to the north parts of the same ocean. Most of these genetic studies conducted up to now on the swordfish have involved only one genetic marker but conclusions based on nuclear and mitochondrial DNA data seemed concordant regarding differentiation in Atlantic Ocean and the Mediterranean Sea (Alvarado Bremer et al., 2005). On the opposite, 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 obtained in the same geographic area 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 population genetic history that is fundamental in the case of population structuring for fisheries management. As such differences in conclusions can have drastic impact on stock assessment, it appears important to determine whether they are due to sampling area, to sample size or to markers discordance.

In order to go deeper on that way and clarify whether nuclear and mitochondrial markers could be useful and complementary for swordfish stock discrimination, a microsatellite analysis (11 loci) combined to a mitochondrial control region haplotype analysis (517-bp sequences) was conducted on a total of 337 samples from four different sites of the southwest Indian Ocean. This step appeared necessary to be sure of the congruence of conclusions based on the two genetic markers for this species, for then conduct a genetic

study at a global scale, *i.e.* the Indian Ocean and its connections with the neighbouring oceanic basins.

Materials and methods

Biological materials

Sampling was realised in 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 samples from a total of 337 swordfish caught by commercial vessels were collected onboard between February 2005 and May 2006. All the swordfish sampled were sexed and measured (LCK = Length from Cleithrum to Keel, *i.e.* fish length without head and caudal fin). Samples indications are given in Table 1.

Muscle tissues were stored in ethanol 90% or in 20% Dimethylsulfoxide (DMSO) saturated salt solution (Dutton, 1996) and frozen until DNA was isolated.

Genetic analysis

Total genomic DNA was extracted using DNAeasy Tissue Kit (Qiagen). PCR amplification of control region gene were made 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 (D2A, D2B and C8; Grewe, unpublished data). These loci were amplified using the same PCR reaction for mtDNA, but with Red Gold Star DNA Polymerase (EuroGenTec), and using cycling parameters described in FitzSimmons (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; under influence of selection, and significantly negative values indicate population expansion or selective influence. Fu's F statistics seemed to be more sensitive to recent demographic expansion. 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 (Holm, 1979). 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 more the value is near from 1, more the 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): the mean number of alleles per population (*Nall*), and the observed (*Ho*) and expected (*Hnb*) heterozygosities (Nei, 1987). In addition, allelic richness (*Rs*) was estimated with 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 spawning numbers (*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. A similar high level of genetic diversity was encountered in each locality (Table 1). 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. This should indicate that population showed a relative demographic stability.

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. Highest allelic richness in GLO could mainly be explained by the highest number of private alleles (16 against 3 to 9 in the three other localities). Consequently, *Ne* estimates (Table 1) also varied from 277 in RUN to 879 in GLO (which simply means that the spawning proportion of adults is supposedly more important in GLO than in the three other localities). 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 (Tables 1 & 2). Values ranged from 0.079 for GLO to 0.176 for RUN.

Population structure

Mitochondrial DNA. Pairwise ϕ_{st} estimates between localities are presented in Table 3. 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 involved SEY sample. On the other side, 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. Combining our sequences to those of Lu *et al.* (2006) previously published in GenBank, 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 multiloci θ values between localities are presented in Table 3. Mean value of θ was weak (= 0.02) with all the non-null θ values involving either SEY or GLO samples. The only one significant of the six θ values was between SEY and GLO, induced by differentiation on 5 of the 11 loci. On the other side, 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. Only allelic richness was always, but not in a significant way, higher for female (from 1 point). 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, respectively N=XX and N=YY). On the opposite, the previous pairwise multiloci θ value found between SEY and GLO was still significant meaning that significant differences are only due to females (θ= 0.01, $p < 0.001$), as males from SEY and GLO did not show significant differences (see Table 4). Other significant differences were identified involving females from GLO, *i.e.* with males from GLO or with females from MADA (see Table 4). Two hierarchical AMOVA (one on mtDNA data, the second on microsatellites) were undertaken with grouping schemes in agreement with sex identification. 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 sex groups ($\Phi_{SC} < 0.001$, $p > 0.05$).

Discussion

This study aimed to yield results on two different topics. The first aim was to evaluate the level of connectivity between swordfish sampled in four different localities of the southwest Indian Ocean (SWIO). The second one was to provide some evidence of congruency between the two genetic markers used in order to validate conclusions that can be useful on stock structure for a regional swordfish multi-stock assessment.

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 and pairwise estimates of differentiation mainly failed to find a strong genetic structure between 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 is in agreement with Tajima's D and Fu's F statistics which estimates that samples are in a situation of demographic stability. The hypothesis of a large effective population size that did not fluctuate so much over time is easily understandable for the swordfish which presents fecundities of several millions of eggs by female (Palko et al., 1981). Such a finding is also in agreement with first hypothesis of great dispersal capabilities for large marine pelagic fishes (Waples, 1998). Considering results obtained both on mtDNA and microsatellites, swordfish of the SWIO might be considered to belong to a same and unique panmictic population.

However some weak differentiation seems to exist within the SWIO. As important within-sample diversity have been noticed to considerably reduce the ability of detecting between-samples structure, either on mtDNA data (Charlesworth, 1998) or in the case of microsatellites studies (Hedrick, 1999; O'Reilly et al., 2004), the detection of these weak differentiations but with both markers, is relevant et this stage. Even if Jean *et al.* (2006) study failed to found genetic structure of swordfish within the SWIO, the increase of the sample size and the number of microsatellites loci between Jean *et al.* (2006)'s study and our (respectively from 86 to 337 and from six to 11) have thus permitted to display heterogeneity within swordfish of the SWIO that have not been observed previously. In fact, some swordfish collected in northern localities (in GLO or SEY) seems to belong to a second genetic pool. In parallel, the point estimation of effective population size tends to show higher values in the two northern localities according to sample size. This is 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 at least hypothetical feeding regions. The significant heterozygote deficits observed in all localities could partly be the signature of a Wahlund effect and should confirm the 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 north of Madagascar and other northern sampling sites in the Indian Ocean. Going deeper on our data, the influence of these two supposed genetic pools seems different for each sex category

within each locality: for example, the male swordfish in GLO differ from the females of the same area. This species is known to migrate to temperate areas for feeding and then move to warmer waters for reproducing (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, or on site (Keeney et al., 2005; Lee et al., 2007), the north of the SWIO being a reproduction site for swordfish (Mejuto et al., 2006). Swordfish within a locality may thus be a mixture from one dominant genetic pool and a second one less influent, 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. It appears thus evident that all the conclusions based on genetic data would not be sufficient without the light of some basic biological data on reproductive, feeding and migrating strategies in the case of a highly migratory species. Furthermore, it seems evident that temporal sampling iterations are also needed to validate such conclusions.

Congruency of molecular markers in a perspective of stock assessment

Many genetic studies had shown discrepancies in conclusions when using two kinds of markers (Lemaire et al., 2000; Nielsen et al., 2006; Pogson et al., 1995) which could partly be explained by differences in evolutionary rates and in sensitivity to evolution forces. In the present study, some evidences of congruency exist in the pattern of genetic differentiation given by each marker. 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 second genetic pool in the equatorial area. Using two genetic markers was our initial option to limit errors, as ‘drawing conclusions from single genealogies can be problematic because each is only a single point in the space of all possible genealogies’ (Wakeley, 2003). The fact that, in the present case, both markers seemed to draw conclusions in a similar way is all the more interesting in a global aim of stock assessment and encourages to conduct a study at the scale of the whole Indian Ocean. Sampling a more extensive area (within the Indian Ocean and neighbouring oceanic basins) may allows to estimate the number of independent genetic pool of swordfish, their geographic boundaries and the level of connectivity between them. The existence of a second genetic pool in the northern part of the West Indian Ocean as well as the specificity of each sex category should thus be confirmed. In the same way, Ward *et al.* (2001) suggest that

swordfish of the West coast of Australia constitutes an isolated pool from the rest of the Indian and Pacific oceans. Finally, a pertinent strategy for dealing with veracity of conclusions should be to replicate samples over time, all the more for a species displaying high levels of gene flow (Waples, 1998). Temporal samples will also allow to estimate effective population size accurately (Fraser et al., 2007) and help to challenge questions about overfishing. *In fine*, more the genetic data will be exhaustive and the genetic conclusions homogeneous and more the decisions taken by politics and managers will be right and easier.

The present study thus permit to discuss pattern of genetic structure observed in *X. gladius* in the SWIO and to reconcile structures previously observed (Jean et al., 2006; Lu et al., 2006). Although these results on the genetic stock structure of swordfish in the Indian Ocean clearly needs to be further investigated by increasing the number and the size of samples, as well as it evolution through time, they allow to test congruence of the results obtained by the two genetic markers used and thus comfort to conduct a study on swordfish biology at a global scale, *i.e.* the Indian Ocean and its connections with the neighbouring oceanic basins, using at least these two markers. 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'. Such a larger project might constitute a great help for the CTOI to deal with the present and future state and management of swordfish stock in the Indian Ocean.

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Figures and Tables

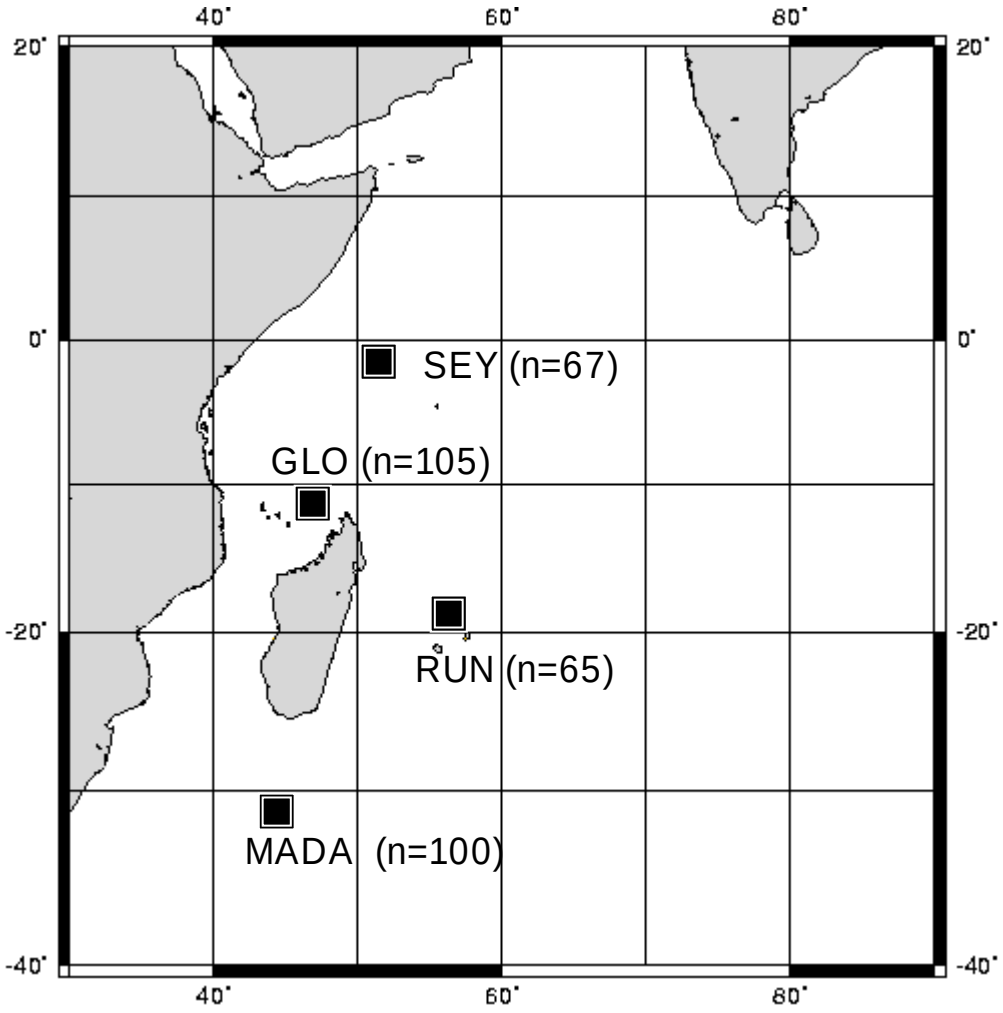

Figure 1. Geographic location of the four fishery statistical square wherein *X. gladius* were sampled for this study.

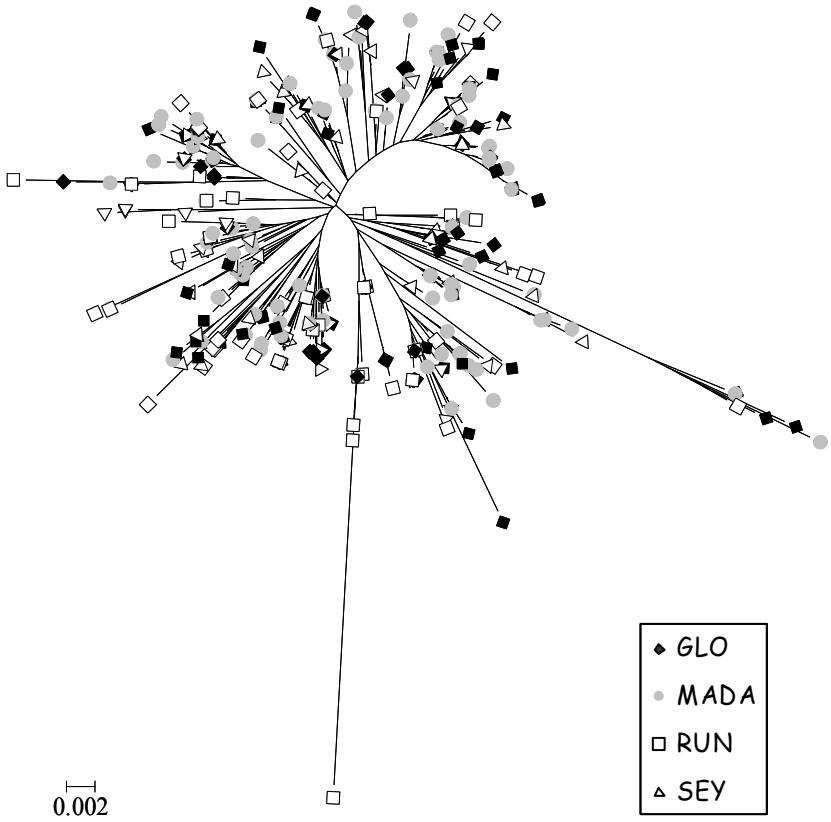

Figure 2. Neighbour-joining tree based on pairwise number of differences between haplotypes of *X. gladius* from the four localities of the southwest of Indian Ocean. Samples are respectively represented by black squares for Glo, grey circles for Mada, white squares for Run and white triangles for Sey.

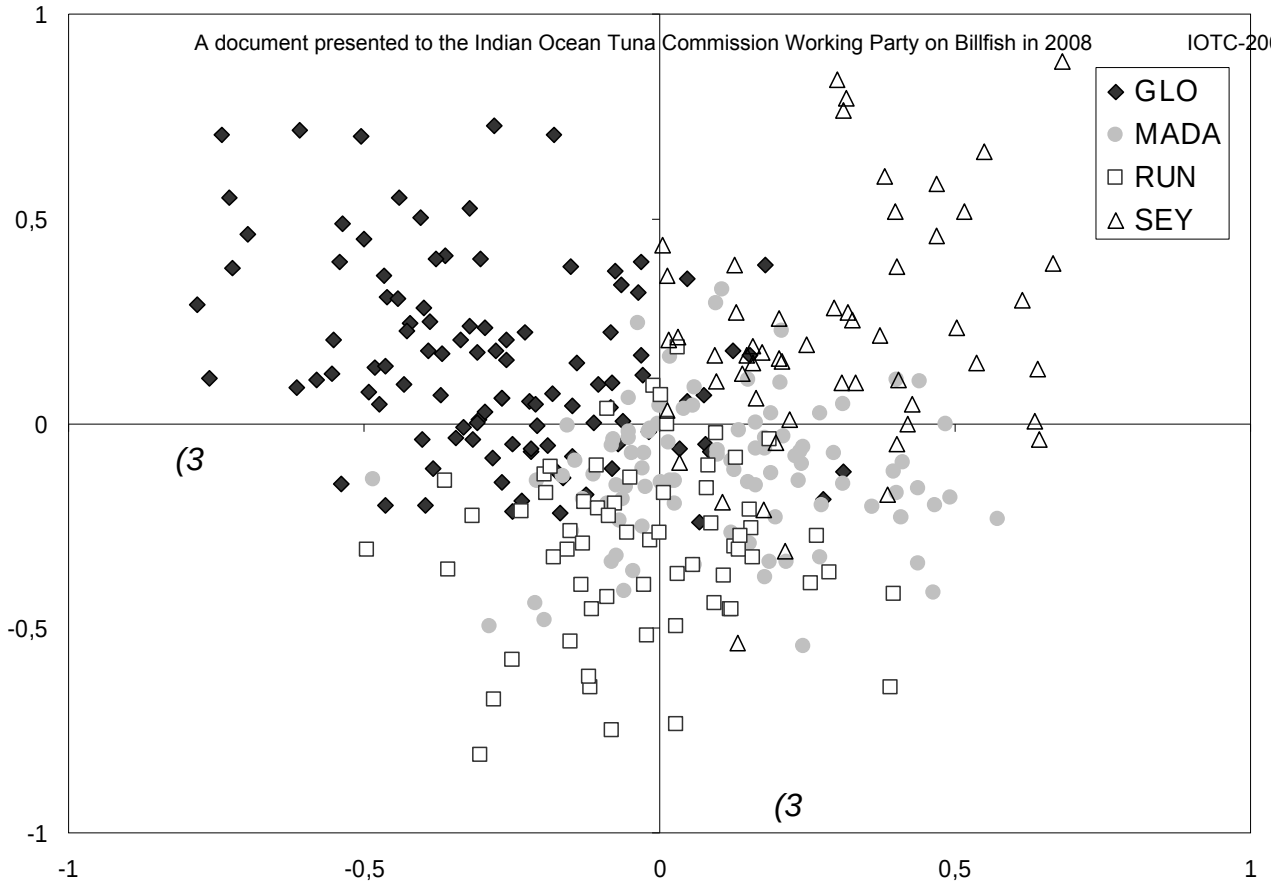

Figure 3. Correspondence factorial analysis, performed on genotype frequencies with Genetix 4.0 (Belkhir *et al.* 2000). Each point represents a given individual whose symbol corresponds to its sampling locality.

Table 1. Main characteristics of the four samples of *X. gladius*.

Sampling information are: sample size (N), mean size 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 size (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 2. 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*	*16*	0.020	-0.013	0.099*	0.127*
X396	*107-137*	*9*	0.182***	0.326***	0.381***	0.300***
D2A	*287-286*	*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 3. Pairwise values of genetic differentiation in *X. gladius* localities. 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). 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). *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 4. Pairwise 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.

	GLO	MADA	RUN	SEY
GLO	**0.005*****	0.005	**0.005***	0.001
MADA	0.005	0.000	0.006	0.000
RUN	0.009	0.000	0.010	0.005
SEY	**0.013*****	0.004	0.006	0.003