LACK OF GENETIC DIFFERENTIATION
IN THE ALTANTIC DISTRIBUTION OF WAHOO

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SUMMARY

*In this study we present the analysis of the stock structure of Wahoo (*Acanthocybium solandri*) using the mitochondrial control region as genetic marker. We analyzed 276 individuals distributed in four locations int eh east and west Atlantic. Samples were from: Northeast Atlantic (AT-NE/BIL94B) (Canary Islands, Spain), from Southeast Atlantic (AT-SE/BIL97) (Côte d'Ivoire and Gabon) and Southwest Atlantic (AT-SW/BIL96). Four of individuals from Southwest Atlantic (AT-SW/BIL96) were genetically identified as* Scomberomorus cavalla. *The genetic comparison of the four locations failed to show genetic differences. This result suggests a single genetic pool of the Wahoo in the whole Atlantic. Based on these results, ICCAT should reconsider their management strategies for this species in the area studied.*

RÉSUMÉ

*Dans cette étude, nous présentons l'analyse de la structure du stock de thazard-bâtard (*Acanthocybium solandri*) en utilisant la région de contrôle de l'ADN mitochondrial comme marqueur génétique. Nous avons analysé 276 spécimens répartis dans quatre zones de l'Atlantique Est et Ouest. Les échantillons provenaient de : l'Atlantique Nord-Est (AT-NE/BIL94B) (îles Canaries, Espagne), l'Atlantique Sud-Est (AT-SE/BIL97) (Côte d'Ivoire et Gabon) et l'Atlantique Sud-Ouest (AT-SW/BIL96). Quatre spécimens provenant de l'Atlantique Sud-Ouest (AT-SW/BIL96) ont été génétiquement identifiés comme* Scomberomorus cavalla. *La comparaison génétique des quatre zones n'a pas révélé de différences génétiques. Ce résultat donne à penser à un patrimoine génétique du thazard-bâtard dans l'ensemble de l'Atlantique. En se fondant sur ces résultats, l'ICCAT devrait revoir ses stratégies de gestion pour cette espèce dans la zone à l'étude.*

RESUMEN

*En este estudio presentamos el análisis de la estructura del stock del peto (*Acanthocybium solandri*) utilizando la región de control mitocondrial como marcador genético. Hemos analizado 276 ejemplares distribuidos en cuatro localizaciones en el Atlántico este y oeste. Las muestras procedían de: Atlántico nororiental (AT-NE/BIL94B) (islas Canarias, España), del Atlántico suroriental (AT-SE/BIL97) (Côte d'Ivoire y Gabón) y del Atlántico sudoccidental (AT-SW/BIL96). Cuatro de los ejemplares del Atlántico sudoccidental (AT-SW/BIL96) fueron genéticamente identificados como* Scomberomorus cavalla. *La comparación genética de las cuatro localizaciones no mostraba diferencias genéticas. Este resultado sugiere un único acervo genético del peto en todo el Atlántico. Basándose en estos resultados, ICCAT debería reconsiderar la estrategia de ordenación para esta especie en la zona estudiada.*

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1. Introduction

The Wahoo (WAH) (*Acanthocybium solandri*), along with the Little tunny (LTA) (*Euthynnus alletteratus*) and the Atlantic Bonito (BON) (*Sarda sarda*), has been identified as key species in Small tuna working program in ICCAT to gather information for of growth, maturity and stock structure (Anon. 2019). Knowledge of these biological parameters are key to implement correct management strategies for the fisheries of these species.

One of the most useful and traditionally applied methods for inferring stock structure is based on population genetics (Ward 2000). This parameter is essential to infer the fishery stocks and finally to determine the correct measure of fishery management. For all the possible methodologies for inferring the population structure of the species, the use of mtDNA sequence variation is one of the most traditionally used in marine pelagic species. Although, nowadays other methods for inferring population structure such as microsatellites or Single Nucleotide Polymorphism (SNPs) are also widely used. The use of sequence variation of the Control Region (D-loop region) of mitochondrial DNA (mtDNA CR) is still extremely useful for inferring a preliminary assessment of the population's structure and a first step for subsequent application other methodologies with high power of resolution. One of the clear advantages of the mtDNA CR from other methods is that this methodology is already optimized for the majority of Scombridae species in (Allaya et al. 2015; Ollé et al. 2019; Viñas et al. 2010; Viñas et al. 2011). That implies that it could be applied to large number of samples with a relative low cost.

In this study, we have analyzed 272 individuals of Wahoo from four locations distributed between Northeast Atlantic (AT-NE/BIL94B) South Atlantic (AT-SE/BIL97) and South West Atlantic (AT-SW/BIL96) (**Table 1**). The samples have been acquired due to the participation of two three terms ICCAT contracts under the Small Tuna Year Program (SMTYP).

2. Material and methods

Up to 276 Wahoo distributed in four different locations (see **Table 1**). Genetical variability of all individuals was assessed following the methodological procedure in Viñas et al. (2004). Briefly, once the samples arrived at the LIG-UdG total genomic DNA was isolated. Following extraction, DNA was resuspended in 100 µl of deionized water. We amplified approximately 450 base pairs (bp) of the first (left) domain of the mitochondrial control region (mtDNA CR) with the L-strand primer L15998 (5'-TAC CCC AAA CTC CCA AAg CTA-3'), in combination with the H-strand primer CSBDH (5'-TgA ATT Agg AAC CAg ATg CCA g-3'). Amplification was carried out in 12.5 µl reaction volumes using approximately 50 ng (0.5 µl) of the isolated DNA as the template. Each PCR reaction contained 1X Taq DNA polymerase buffer, 1.5–2 mM $MgCl_2$, 200 mM of each dNTP, 10 pmol of each primer, and 0.5 U Taq DNA polymerase. Thermal cycles involved an initial denaturing step of 5 min at 94°C, followed by 35 cycles of denaturing at 94°C for 45 s, annealing at 50°C for 45 s, and extension at 72°C for 1 min. Negative controls were included in all PCR runs to ascertain that no cross-contamination took place. Double-stranded DNA products were purified and subsequently were sequenced unidirectionally using the BigDye Kit v3.1 (Applied Biosystems) on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems). When sequencing results were ambiguous, the amplicon was sequenced in both directions. Sequence alignments were inspected using the Geneious v.R7. Sequence Phylogenetic tree was constructed using the Neighbor joining (Saitou and Nei 1987) procedure with the kimura 2-distance (Kimura 1980) with a resampling of 1000 bootstrap pseudoreplicates to assess the robustness of the branches in the tree. Haplotype (*h*) (Nei and Tajima 1981) and nucleotide diversity (*π*) (Nei 1987) were estimated from haplotype frequencies and haplotype divergence based on a pairwise distance matrix in ARLEQUIN v. 3.5 (Excoffier and Lischer 2010). The geographical structure for each species was estimated using analysis of molecular variance (AMOVA) (Excoffier et al. 1992) based on the pairwise matrix of distances between haplotypes. The haplotypic correlation measure (Φ_{ST}) was estimated for all possible permutations among regions for each species. The significance level of each haplotypic correlation was tested by conducting a non-parametric permutation procedure 10,000 times in ARLEQUIN.

3. Results

Analysis of the sequence variation revealed that four individuals were identified as *Scomberomorus cavalla*. These four samples were removed for th subsequent analysis. The sequence comparison of the 434 bp of the mtDNA-CR of the remaining 272 individuals revealed 186 variable sites. This variability resulted in 212 distinct haplotypes from the 272 sequences (**Table 1**). Accordingly, in all locations the haplotypic diversity was close to one, ranging from 0.993 to 1.000. Nucleotide diversity was also high and similar to the one observed in Atlantic bonito (Viñas et al. 2004; Viñas et al. 2010). This high sequence variation diversity is probably consequence of the presence of two highly divergent groups of sequences (haplogroups) (See **Figure 1**). These two haplogroups were

homogenously distributed among localities, ranging from a distribution of haplogroup 1 from 50% in Gabon to 67%. Therefore, this analysis failed to show genetic differentiation among locations with the overall $\Phi_{ST} = -0.009$ (*P-value* = 0.967). Accordingly, no differences were detected in the pairwise comparison among locations (**Table 2**).

4. Discussion

One surprising result is the genetic identification of the four individuals as Scomberomorus cavalla in the location of Brasil. This result could open the window of the presence of this species in the fishery of Wahoo. Nevertheless, additional analysis, including morphometric and other genetic markers are needed to confirm this result. The population genrtic anlysis of Wahoo presents a scenario of homogeneous distribution of genetic variation, which is expected in a species with high migratory potential and large effective population size. Similarly, a lack of genetic heterogeneity was previously observed in Wahoo in a more global study (although the sampling size was lower than the one carried out in this study), with a lack of genetic differentiation between Atlantic, Pacific and Indian locations (Theisen et al. 2008). The high mobile adult and the wahoo also have buoyant eggs and pelagic larvae, this dispersal during preadult stages by ocean currents may also facilitate genetic homogeneity (Collette et al. 1984; Wollam 1969). However, the lack of genetic differentiation within these areas should be taken in caution. It has to be kept in mind the presence of type II statistical error in the analysis of population structure (false negatives): actual presence of local structures, but not detected by the analysis (Waples et al. 2008). Thus, to confirm this putative lack of genetic heterogeneity, it should be validated with genetic that present higher power of resolution (ie, genomic makers such as SNPs, RadSeq) than the mtDNA- CR.

These results, if confirmed, have a clear impact of the management of the Wahoo. Currently, ICCAT manages the Wahoo as three different stocks (AT-NE/BIL94B, AT-SE/BIL97 and AT-SW/BIL96) in the area studied, but the genetic results combined with other biological evidence suggest a single stock for this species in the whole Atlantic.

Acknowledgements

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Table 1. Results of Wahoo sampling and molecular diversity indices. Year, year of sampling. Samples with asterisks are the ones analyzed in the 2019 contract. N, number of individuals; M, number of haplotypes; h, haplotypic diversity; π, nucleotide diversity

Locality	ICCAT area	Code	Year	n	M	$h \pm SD$	$\pi \pm SD$	% haplgrp 1
Cotê d'Ivoire	AT-SE/BIL97	CIV	2018-2019	133	114	0.974 ± 0.011	0.077 ± 0.038	65%
Gabon	AT-SE/BIL97	GAB	2018	18	17	0.993 ± 0.021	0.082 ± 0.042	50%
Spain	AT-NE/BIL94B	ESP	2019	62	60	0.999 ± 0.003	0.077 ± 0.038	58%
Brazil	AT-SW/BIL96	BRA	2020	59	56	0.998 ± 0.003	0.079 ± 0.038	60%
All				272	212	0.998 ± 0.001	0.077 ± 0.037	59%

Table 2. Pairwise genetic differentiation among Wahoo samples. Below diagonal, Φ_{ST}s values. Above diagonal, P-values. Samples code as **Table 1**.

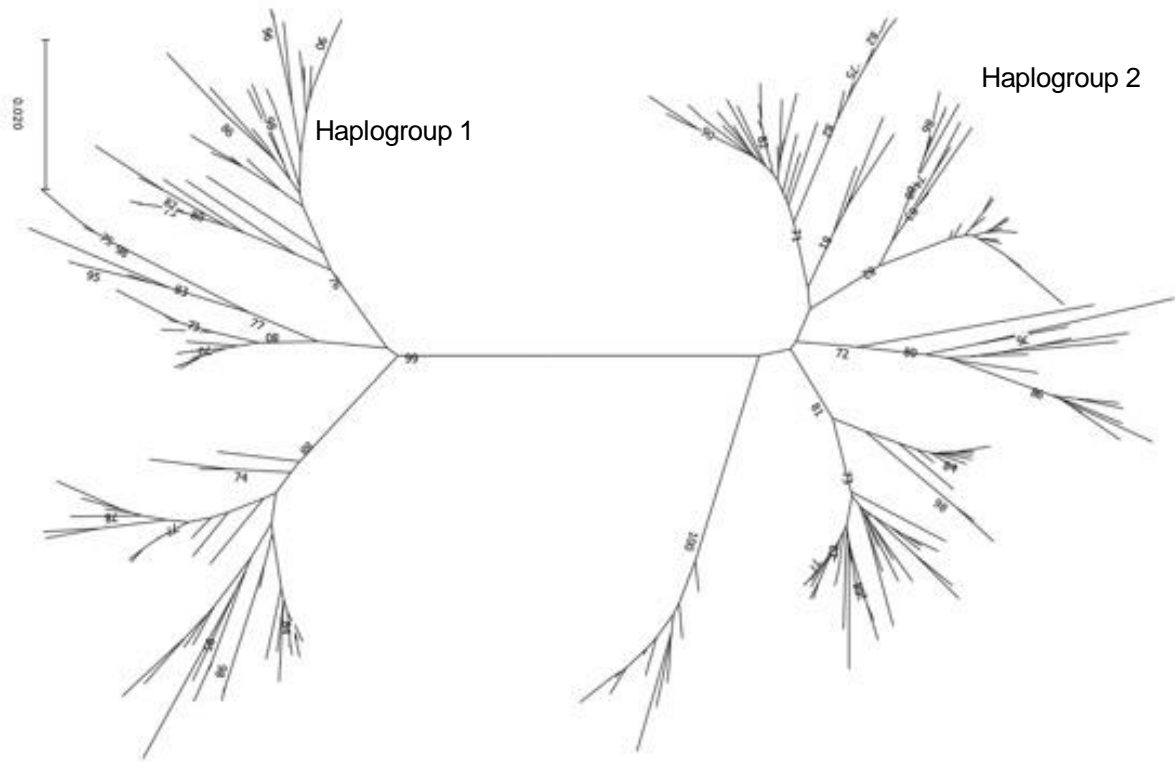|	CIV	GAB	ESP	BRA
CIV	--	0.449	0.995	0.678
GAB	-0.003	--	0.649	0.642
ESP	-0.006	-0.010	--	0.788
BRA	-0.003	-0.009	-0.007	--

Figure 1. Unrooted phylogenetic tree of the 212 Wahoo mtDNA-CR haplotypes. Values in branches are bootstrap percentages above 70% consistency