FINAL REPORT OF THE SHORT-TERM CONTRACT FOR ICCAT SMTYP FOR THE BIOLOGICAL SAMPLES COLLECTION FOR GROWTH, MATURITY AND GENETICS STUDIES – YEAR #2

J. Viñas[1]*, J. Ollé[1], G. Hajjej[2], D. Macias[3], S. Saber[3], P. J. Pascual-Alayón[4], P. G. Lino[5], R. Muñoz-Lechuga[4], S. Ahmed Baibbat[6], B. Meissa Habibe[7], F. Ngom Sow[8], N'G. C. Diaha[9], D. Angueko[10], G. Silva[11], F. Lucena Frédou[12]

SUMMARY

*This document is the final report of the second year of the short-term contract of the Small Tuna Year Program by ICCAT. In 2018, the Small Tuna Species Group decided to prioritize Little tunny (LTA) (*Euthynnus alletteratus*) Atlantic bonito (BON) (*Sarda sarda*) and Wahoo (WAH) (*Acanthocybium solandri*), based on their economic importance and the deficiency of knowledge of their biology. The objectives of the contract for three species were: i) Collect biological samples for estimating growth parameters, assessing the maturity and stock structure analysis (populations genetics), and ii) Conclude the analysis of the stock structure for at least one of the three species and provide preliminary results for the remaining. The obtained samples for growth, maturity and stock structure analysis was almost completed for Little tunny and Atlantic bonito, whereas for Wahoo the samples are scarce. The analysis of stock structure for Little tunny and Atlantic bonito revealed that the observed differentiated genetic pools do not comply with the ICCAT management areas of these species.*

RÉSUMÉ

*Le présent document est le rapport final de la deuxième année du contrat à court terme du Programme annuel sur les thonidés mineurs (SMTYP) de l'ICCAT. En 2018, le Groupe d'espèces sur les thonidés mineurs a décidé de donner la priorité à la thonine commune (LTA) (*Euthynnus alletteratus*), à la bonite à dos rayé de l'Atlantique (BON) (*Sarda Sarda*) et au thazard-bâtard (WAH) (*Acanthocybium solandri*), en raison de leur importance économique et de la méconnaissance de leur biologie. Les objectifs du contrat pour les trois espèces étaient les suivants : i) recueillir des échantillons biologiques pour estimer les paramètres de croissance, évaluer la maturité et analyser la structure du stock (génétique des populations) et ii) conclure l'analyse de la structure du stock pour au moins une des trois espèces et fournir des résultats préliminaires pour les autres. Les échantillons obtenus pour l'analyse de la croissance, de la maturité et de la structure du stock étaient presque terminés pour la thonine commune et la bonite à dos rayé, tandis que pour le thazard-bâtard, les échantillons étaient rares. L'analyse de la structure du stock de la thonine commune et de la bonite à dos rayé a fait apparaître que les patrimoines génétiques différenciés observés ne coïncident pas avec les zones de gestion de l'ICCAT pour ces espèces.*

[1]Laboratori Ictiologia Genètica, Departament de Biologia, Universitat de Girona. 17071, Girona, Spain.
*Corresponding author: jordi.vinas@udg.edu
[2]National Institute of Marine Science and Technology, Tunisie
[3]Instituto Español de Oceanografía, Centro Oceanográfico de Málaga, Spain
[4]Instituto Español de Oceanografía, Centro Oceanográfico de Canarias, Spain.
[5]Instituto Português do Mar e da Atmosfera, Portugal
[6]Laboratoire des Pêches (Dakhla), Morocco
[7]Laboratoire Evalutaion des Ressources Vivantes Aquatiques, Mauritania
[8]Centre De Recherches Oceanographiques de Dakar, Senegal
[9]Centrer of Oceanology Research,Côte d'Ivoire
[10]Direction General des Peches et de l'Aqualculture, Gabón.
[11]Universidade Federal Rural do Semiarido
[12]Departamento de Pesca e Aquilcutura – Universidad Federal Rural de Pernambuco, Brazil

RESUMEN

*Este documento es el informe final del segundo año del contrato a corto plazo del Programa año de pequeños túnidos de ICCAT. En 2018, el Grupos de especies de pequeños túnidos decidió conceder prioridad a la bacoreta (LTA) (*Euthynnus alletteratus*), el bonito (BON) (Sarda sarda) y el peto (WAH) (*Acanthocybium solandri*) basándose en su importancia económica y la ausencia de conocimientos sobre su biología. Los objetivos del contrato para las tres especies eran: i) Recoger muestras biológicas para estimar los parámetros de crecimiento, evaluar la madurez y el análisis de la estructura del stock (genética de las poblaciones), y ii) Concluir el análisis de la estructura del stock para al menos una de las tres especies y proporcionar resultados preliminares para las restantes. Las muestras obtenidas para el análisis del crecimiento, la madurez y la estructura del stock estaban casi completas en el caso de la bacoreta y el bonito, mientras que en el caso del peto las muestras eran escasas. El análisis de la estructura del stock de bacoreta y bonito reveló que los acervos genéticos diferenciados observados no se corresponden con las zonas de ordenación de ICCAT para estas especies.*

KEYWORDS

Small tunas, *Little tunny*, Euthynnus alletteratus, *Atlantic bonito*, Sarda sarda, *Wahoo*, Acanthocybium solandri, *growth*, *maturity*, *stock structure*, *genetics*

1. Introduction

The Small Tuna Year Program (SMTYP) has implemented a strategy for improving data about catches data (Task I) and catch-effort and size data (Task II). Several calls of tenders focusing these two tasks have been released during the last years. However, for the majority of species included in the Small tuna Species Group, biological data, in particular growth, maturity and stock structure are still uncertain, but are key for implementing appropriate fishery management strategies that ultimately will allow the preservation of stocks without compromising the viability of the natural populations.

In 2017, Small Tuna Species Group decided to prioritize three species based on their economic importance and the deficiency of knowledge of their biology. These species are: Little tunny (LTA) (*Euthynnus alletteratus*) Atlantic bonito (BON) (*Sarda sarda*) and Wahoo (WAH) (*Acanthocybium solandri*). Hence, in 2018, it was released a call of tenders in order to improve the biological knowledge of these priority species. The specific objectives of 2018 contract were: i) Collect biological samples for estimating growth parameters, assessing the maturity and stock structure analysis (populations genetics), and ii) Preliminary analysis of the stock structure for one of the three species.

During the 2019 Intersessional meeting of the ICCAT Small Tuna Species Group (SMT SG), it was reiterated the need and importance to proceed with the program. Thus, a new short contract was proposed with the same objectives proposed in the 2018 contract, namely:

I. Collect biological samples to fill the gaps aiming estimating growth parameters, assessing the maturity and stock structure analysis (populations genetics)

II. Conclude the analysis of the stock structure for at least one of the three species and provide preliminary results for the remaining.

Below, the results of this contract by objective

2. Objective I. Collect biological samples

In the contract and following the recommendations of the SMT SG, a sampling scenario was detailed involving several research teams from ICCAT contracting parties, that allow covering 4 (of the 5) ICCAT geographical areas defined for Small Tunas (NE Atlantic, SE Atlantic, SW Atlantic and Mediterranean).

The Consortium was unable to collect the number of samples targeted as summarized **Table 1**. The main difficulties concern Wahoo sampling.

The collection of samples was not homogenous along the months, species and ICCAT areas. Nevertheless, this issue was discussed in the meeting held in IEO-Fuengirola (Málaga-Spain) in February 2020, that concluded the current available samples seems to be enough for an analysis of growth and reproduction for some of the areas (See details in section 2.1).

A detailed summary of samples for each species by month is detailed in **Tables 2, 3 and 4** and also in the excel file attached (*Summary_Table_by_month_v20200316.xlsx).*

2.1 Comments about the sampling obtained:

Figure 1 provides histograms of the number of samples by size class (Fork length), by species and geographical area. Some species/areas have a good distribution with individuals' representatives for most length classes. However, it should be noted the lack of some specific lengths for all species and locations, especially small sized specimens. This is probably attributed to that the fact that the source of samples were mainly local fisheries, subjected to the fleet seasonality and gear size selectivity. Nevertheless, for Little tunny and Atlantic bonito, we can consider that we a good representation of size distributions for most of the locations (See F**igure 1**). On the contrary, for Wahoo, the sampling of AT-SW is still scarce.

2.1.1 Little tunny

- We have a good representation of samples of aging and growth for NE Atlantic and SE Atlantic. The Mediterranean sampling is still scarce.
- Reproduction. The best sampling is from SE Atlantic. For the other two areas more samples are needed.

2.1.2 Atlantic bonito

- Aging and Growth. We have enough samples to start the analysis in the SE Atlantic and Mediterranean. In the NE Atlantic the sampling should be completed.
- Reproduction. We have enough samples to start the analysis in the SE Atlantic and Mediterranean. In the NE Atlantic the sampling should be completed.

2.1.3 Wahoo

This is the species with the smaller sampling. But the analysis of Aging and growth and Reproduction can be started in SE Atlantic.

Files provided

1. Sampling_SMT_2018_2019_v20200325.xlsx.

Detailed description of the data associated of the collected samples can be found in this excel file. In the excel file, up to six different spreadsheets are included.

1. DATASET. All raw data. Validated for each partner up to Friday 13/03/2019
2. Reference. Codes in the Dataset
3. Summary Hard parts. Summary of Hard parts validated by the IPMA- Portugal. Only 2019 sampling.
4. Summary gonads. Summary of gonads validated by the IEO-Málaga. Only 2019 sampling.
5. Summary all Lab. Summary all samples, including the stock structure, validated by all partners, the coordinators of each part and the LIG-UdG. Only 2019 sampling
6. Summary of sampling 2019. A comparison of provided and samples to be committed.

2. Summary_Table_by_month_v20200325.xlsx

A detailed summary of samples for each species by month.

3. Objective II. Conclude the analysis of the stock structure

We analyzed for the stock structure all the new samples that arrived at the LIG-UdG before the end of January 2020. This new data was complemented with the data available from the previous contract. In addition, some new individuals of the sampling of the 2018 contract were also include during this period of time. In total, and for the three species, the LIG-UdG have analyzed 1438 individuals, of these, 845 were from the 2019 contract.

The genetics methods used were exactly the same that were already described in the previous contract. Using the same methodology facilitate merging all data sets regardless the timing of analysis. 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 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 sin 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.

3.1 Atlantic bonito (BON)

- Total individuals analyzed: 673. Total individuals included in the analysis: 615. A total of 58 samples were not included in the analysis given that the DNA extraction was not appropriate, the tissue was not in good conditions and the sequence obtained was not of high quality. Nevertheless, a 91% of success can be considered an excellent result.
- The individuals were distributed in ten different samplings. Some locations were repeated across the two years to assess fluctuation in the genetic variability along time.
- Of the 673 analyzed, 307 were from the 2019 contract.

3.1.1 Results

Genetic variation in Atlantic bonito was relatively high with 132 variable sites out of 392bp of the alignment. We identified up to 340 distinct haplotypes from 615 sequences (**Table 5**). Accordingly, in all locations, the haplotypic diversity was close to one, ranging from 0.974 to 0.996. Nucleotide diversity was also high compared to the ones observed in other species of *Sarda* (Viñas et al. 2010), but within the range observed in the Mediterranean Sea for the Atlantic bonito (Viñas et al. 2004). This high sequence variation diversity is probably consequence of the presence of two highly divergent groups of sequences (**Figure 2**), previously described in Viñas et al. (2004) as Clade I and Clade II. Clear genetic differentiation was observed, with a highly significant overall value of Φ_{ST}= 0.146 (*P-value* = 0.000). Pairwise comparisons of genetic differentiation among locations (**Table 6**) revealed a pattern of differentiation between the locations from NE Atlantic-Mediterranean locations (Spain, Portugal, Tunis) and the rest of locations (Morocco, Mauritania, Senegal and Cote D'Ivoire). In addition, there is a clear differentiation between the East Tropical Atlantic locations (Mauritania, Senegal and Cotê d'Ivoire). These locations are also differentiated among them. The location of Morocco seems to be situated in intermediate situation between the Atlantic- Mediterranean locations and the East Tropical locations. The clade distribution among locations (**Table 6**) seem to confirm these results. The two clades were highly heterogenous distributed (χ^2 = 41.25; df = 4; *P-value* < 0.0001), with individuals of Clade I being more abundant in all East Tropical Atlantic locations (more than 80%).

3.2 Little tunny (LTA)

- Total individuals analyzed: 532. Total individuals included in the analysis: 504. A total of 28 samples were not included in the analysis given that the DNA extraction was not appropriate, the tissue was not in good conditions and the sequence obtained was not of high quality. Nevertheless, a 95% of success can be considered an excellent result.
- The individuals were distributed in nine different sampling locations. Some locations were repeated across the two years to assess fluctuation in the genetic variability along time.
- Of the 532 individuals analyzed, 351 were from the 2019 contract.

3.2.1. Results

Genetic variation in Little tunny was relatively low compared to one observed for Atlantic bonito. Of the 399 nucleotide positions in the mtDNA-CR alignment, 148 were variable. In addition, only 160 (out of 504 individuals) distinct haplotypes were detected (**Table 7**). Thus, the overall haplotypic diversity was relatively low with some locations presenting an haplotypic diversity as low as $h = 0.643$ in the Portugal location. The nucleotide diversity was low in all locations (range from 0.003 to 0.011), but the overall diversity was relatively high (0.052). These discrepancies between the intra and inter-locations nucleotide diversity is consequence of the high divergence of two groups of sequences in Little tunny (**Figure 3**) with a net average between groups of $D_A = 0.084 \pm 0.014$. A further inspection of the phylogenetic tree reveals a complete association of individuals from different locations to the two groups of haplotypes detected in the tree (**Figure 3**). All sequences from individuals from Senegal, Côte d'Ivoire and Gabon, are grouped together, and separated from the locations of Portugal, Tunis and Spain. Correspondingly, there is a clear genetic differentiation between these two groups of locations (**Table 8**). In this analysis, a sample of *E. affinis* from Vietnam (VNM) was include in the analysis in order to assess if the degree of genetic differentiation is at species levels. It can be observed that the levels of Φ_{ST}s between *E. affinis* and the rest of locations is similar to the level of differentiation between the samplings from the groups of locations described before. All these results seem to confirm the pattern observed before: a scenario of having two distinct species of Little tunny in the area analyzed.

3.3 Wahoo (LTA)

- Total individuals analyzed: 233. Total individuals included in the analysis: 213. A total of 20 samples were not included in the analysis given that the DNA extraction was not appropriate, the tissue was not in good conditions and the sequence obtained was not of high quality. Nevertheless, a 91% of success can be considered an excellent result.
- The individuals were distributed in five different samplings locations. Some locations were repeated across the two years to assess fluctuation in the genetic variability along the time.
- Of the 233 individuals analyzed, 187 were from the 2019 contract.

3.3.1 Results

Genetic variation in Wahoo was relatively high with 174 variable sites out of 434bp of the alignment. We identified up to 174 distinct haplotypes from 213 sequences (**Table 9**). Accordingly, in all locations the haplotypic diversity was close to one, ranging from 0.993 to 1.000. Nucleotide diversity was also high in a similar level observed in Atlantic bonito. Similar to the situation in Atlantic bonito, this high sequence variation diversity is probably consequence of the presence of two highly divergent groups of sequences (See **Figure 4**). To our knowledge this is the first time that these groups of sequences (haplogroups) were detected in Wahoo. These two haplogroups were homogenously distributed among localities, ranging from a distribution of haplogroup 1 from 50% in Gabon to 67% in Spain, and overall percentage of haplogroup 1 of 59%. This is the only species with no genetic differentiation among locations, overall $\Phi_{ST} = -0.009$ (*P-value* = 0.967). Accordingly, no differences were detected in the pairwise comparison among locations (**Table 10**). The result observed here conform with previously published results in Wahoo in a more global study (with a sampling size was lower than the one carried out in this study): No genetic heterogeneity was observed between Atlantic, Pacific and Indian locations (Theisen et al. 2008).

3.4 General comments of the stock structure analysis

The stock structure of all species is almost complete. We have results on the three species, with some minor questions to be resolved. In total, we were able to genetically analyze about 1438 individuals for the three species, of these 863 were analyzed during the 2019 contract. We had an overall success ratio of 92.6%, which can be considered an excellent result for this kind of methodology. One of the main conclusions is that all 3 studied species, the ICCAT management areas don't match with the observed stock structure.

We have different outcomes depending on the species. One of the main results is related to Little tunny, with a probable presence of two different species in the area studied. The boundary of these two putative species could be somewhere between Senegal and the south of Portugal. Thus, at least two different genetic pools are present in the ICCAT area of NE-Atlantic/BILL94B. On the contrary, no genetic differentiation has been found between the location of Senegal (NE-Atlantic /BILL94B) and the two locations of SE-Atlantic/BIL 97 (Côte D'Ivoire and Gabon). Similarly, in the northern area, several locations that belong to different ICCAT areas: Portugal from NE-Atlantic/BILL94B, and Tunis and Spain form from MD/BIL95, share the same genetic pool.

The Atlantic bonito also present a clear genetic differentiation, which is also in disagreement with the management areas adopted by ICCAT. In this case, however, the differentiation is at population level. The locations of the ICCAT area MD/BIL95 (Spain and Tunis) shares the same genetic pool with one location in the NE/BIL94B (Portugal). This genetic unit is clearly separated from Senegal (NE/BIL94B), and from Côte d'Ivoire (AT-SE/BIL97). There is also a clear differentiation between Senegal and Côte d'Ivoire. The locations of Morocco and Mauritania (NE/BIL94B) seem to be placed in an intermediate situation.

Finally, Wahoo did not show any genetic differentiation among the locations analyzed, although the samples proceed from only two ICCAT areas (NE/BIL94B and AT-SE/BIL97). However, there is a possibility of having a genetically different location in SW-ATL. The result of genetic homogeneity is in congruence with previous published results of lack of inter-oceanic genetic differentiation (Theisen et al. 2008).

In summary, this is probably the most exhaustive analysis of stock structure done to any of this species. Where the genetic situation was observed, it can surely be considered for management purposes. For instance, in Little tunny and bonito the clear genetic heterogeneity among locations observed does not compels with the proposed ICCAT areas of managements for these species. It should be mentioned, however, that in the cases where we fail to detect genetic differentiation, we cannot confirm that there is a single genetic stock. There is always the peril of having a Class II statistical error (not detect differentiation but actually it exists, false negative).

Future analysis should be done in Atlantic bonito to determine the mix population off Mauritania and Morocco Atlantic coast. For the Little tunny samples from the Atlantic coast of Morocco and Mauritanian are needed to stablish the boundary of the putative species. For Wahoo, but also for the rest of species, the use of new and more powerful genetic markers could help in confirming these results.

4. General comments to the project

This contract is a continuation of the previous work realized under ICCAT-Small tuna group. These two contracts can be considered as extremely ambitious programs: three species with up to 13 institutions involved. The organization and execution have implied an extraordinary effort for all partners. The samples obtained up to date are probably the best sampling of any of these species obtained to date. But several comments about the present sampling and the future of the consortium is needed.

It is clear that the sampling is not complete but the analysis of aging and reproduction for the three species can be start for several regions for Little tunny and bonito and for one region in the case of Wahoo. Thus, if possible, the new contract should focus in starting these analyses on aging and growth in the laboratories responsible for these tasks and continuing filling the gaps of the sampling (see Section 2.1). The stock structure analysis is almost finished, although some minor questions still open. Relatively small effort is needed to have an even better picture of the stock structure of these species.

Acknowledgements

This study was conducted within the ICCAT Small Tunas Year Programme (SMTYP) and partially funded by the European Union (EU Grant Agreement No. SI2.796428 - Strengthening the scientific basis for decision-making in ICCAT).

References

Excoffier L, Lischer HEL. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Res. 10(3):564-567.

Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics. 131(2):479-491.

Kimura M. 1980 A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 16:111-120.

Nei M. 1987. Molecular evolutionary genetics. New York: Columbia University Press.

Nei M, Tajima F. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics. 97(1):145-163.

Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 4(4):406-425.

Theisen TC, Bowen BW, Lanier W, Baldwin JD. 2008. High connectivity on a global scale in the pelagic wahoo, Acanthocybium solandri (tuna family Scombridae). Mol Ecol. 17(19):4233-4247.

Viñas J, Alvarado Bremer JR, Pla C. 2010. Phylogeography and phylogeny of the epineritic cosmopolitan bonitos of the genus Sarda (Cuvier): inferred patterns of intra- and inter-oceanic connectivity derived from nuclear and mitochondrial DNA data. J Biogeogr. 37(3):557-570.

Table 1. Summary of target samples (No. samples), effective number of samples collected (Provided) and respective percentage.

Species	Research line	Area	CPCs involved	No. samples	Provided	%
Little tunny	Aging and growth	NE Atlantic	Senegal, EU-Spain, EU-Portugal, Mauritania, Maroc	250	238	95
		SE Atlantic	Cote d'Ivoire, Gabon, EU-Spain	150	146	97
		Med	Tunisia, EU-Spain	200	75	38
	Reproduction	NE Atlantic	Senegal, EU-Spain, EU-Portugal, Mauritania, Maroc	250	45	18
		SE Atlantic	Cote d'Ivoire, Gabon, EU-Spain	150	110	73
		Med	Tunisia, EU-Spain	200	27	14
	Stocks structure/	NE Atlantic	Senegal, EU-Spain, EU-Portugal, Mauritania, Maroc	250	263	105
		SE Atlantic	Cote d'Ivoire, Gabon, EU-Spain	150	150	100
		Med	Tunisia, EU-Spain	200	197	99
Bonito	Aging and growth	NE Atlantic	Senegal, EU-Spain, EU-Portugal, Mauritania, Marroc	250	42	17
		SE Atlantic	Cote d'Ivoire, Gabon, EU-Spain	150	77	51
		Med	Tunisia, EU-Spain	200	141	71
	Reproduction	NE Atlantic	Senegal, EU-Spain, EU-Portugal, Mauritania, Maroc	250	31	12
		SE Atlantic	Cote d'Ivoire, Gabon, EU-Spain	150	63	42
		Med	Tunisia, EU-Spain	200	142	71
	Stocks structure/	NE Atlantic	Senegal, EU-Spain, EU-Portugal, Mauritania, Maroc	250	132	53
		SE Atlantic	Cote d'Ivoire, Gabon, EU-Spain	150	79	53
		Med	Tunisia, EU-Spain	200	150	75
Wahoo	Aging and growth	NE Atlantic	EU-Spain	250	0	0
		SE Atlantic	Cote d'Ivoire, Gabon, EU-Spain	50	50	100
		SW Atlantic	Brazil	100	0	0
	Reproduction	NE Atlantic	EU-Spain	250	188	75
		SE Atlantic	Cote d'Ivoire, Gabon, EU-Spain	50	11	22
	Stocks structure/	NE Atlantic	EU-Spain	50	50	100
		SE Atlantic	Cote d'Ivoire, Gabon, EU-Spain	50	50	100
		SW Atlantic	Brazil	100	0	0

Table 2. Summary of sampling of Atlantic bonito by month, species and ICCAT area. For a more complete visualization please see the file: *Summary_Table_by_month_v20200325.xlsx*

Species	MU_code	Year	month	INDIV_N	Muscle_N	Spine_N	Otolith_N	Head_N	Hard_Part_N	Stored_gonads_N	Weighted_gonads_N
BON	AT-NE	2018	mai	35	35	20	0	35	35	35	35
BON	AT-NE	2018	jun	15	15	0	0	15	15	15	15
BON	AT-NE	2018	jul	14	14	4	0	10	14	10	14
BON	AT-NE	2018	ago	15	15	15	0	4	15	15	15
BON	AT-NE	2018	set	51	51	51	0	42	51	51	51
BON	AT-NE	2018	out	47	47	47	0	0	47	7	47
BON	AT-NE	2018	nov	46	46	46	33	0	46	12	46
BON	AT-NE	2018	dez	27	27	27	0	0	27	27	26
BON	AT-NE	2019	jan	60	60	60	50	0	60	10	60
BON	AT-NE	2019	fev	51	51	51	34	0	51	16	51
BON	AT-NE	2019	mar	22	22	22	0	0	22	22	22
BON	AT-NE	2019	jun	10	10	10	0	10	10	10	10
BON	AT-NE	2019	jul	63	63	16	58	11	63	11	11
BON	AT-NE	2019	ago	20	20	20	20	20	20	20	20
BON	AT-NE	2019	set	8	8	8	0	0	8	0	0
BON	AT-NE	2019	out	1	1	1	1	1	1	0	1
BON	AT-NE	2019	dez	5	5	5	0	0	5	0	5
BON	AT-NE	2019	(Missing)	21	21	0	15	0	15	0	0
BON	AT-SE	2018	ago	28	28	28	25	0	28	28	28
BON	AT-SE	2018	set	32	32	32	24	0	32	32	24
BON	AT-SE	2018	out	16	16	16	0	0	16	16	0
BON	AT-SE	2018	nov	11	11	11	0	0	11	11	0
BON	AT-SE	2019	mai	10	0	0	0	0	0	5	10
BON	AT-SE	2019	jun	27	0	0	0	0	0	13	27
BON	AT-SE	2019	jul	6	0	0	0	0	0	5	6
BON	AT-SE	2019	out	79	79	73	18	0	73	54	79
BON	MD	2018	mai	10	10	10	10	0	10	4	10
BON	MD	2018	jun	23	23	23	23	0	23	19	23
BON	MD	2018	jul	20	20	20	16	4	20	20	20
BON	MD	2018	ago	41	41	41	7	41	41	36	34
BON	MD	2018	set	52	52	50	52	0	52	52	52
BON	MD	2018	out	13	13	13	0	13	13	13	13
BON	MD	2018	nov	6	6	6	6	0	6	6	6
BON	MD	2018	dez	23	23	23	3	20	23	23	23
BON	MD	2019	jan	10	10	3	3	0	3	10	0
BON	MD	2019	fev	3	3	2	2	0	2	3	0
BON	MD	2019	mar	57	56	56	4	41	57	57	41
BON	MD	2019	mai	37	37	37	14	0	37	37	0
BON	MD	2019	jul	31	30	31	10	1	31	31	1
BON	MD	2019	set	14	14	14	5	0	14	14	0
BON	MD	2019	out	31	31	31	4	8	31	31	8
BON	MD	NA	(Missing)	17	0	0	0	0	0	7	0

Table 3. Summary of sampling of Little tunny by month, species and ICCAT area. For a more complete visualization please see the file: *Summary_Table_by_month_v20200325.xlsx*

Species	MU_code	Year	month	INDIV_N	Muscle_N	Spine_N	Otolith_N	Head_N	Hard_Part_N	Stored_gonads_N	Weighted_gonads_N
LTA	AT-NE	2018	ago	68	68	68	64	18	68	48	67
LTA	AT-NE	2018	set	14	14	14	14	14	14	14	14
LTA	AT-NE	2018	out	48	48	48	48	48	48	48	48
LTA	AT-NE	2019	jan	1	1	1	1	1	1	1	1
LTA	AT-NE	2019	mai	38	38	38	37	0	38	20	38
LTA	AT-NE	2019	jun	50	50	50	50	0	50	0	50
LTA	AT-NE	2019	jul	5	5	5	5	0	5	0	5
LTA	AT-NE	2019	ago	52	52	45	48	4	49	11	45
LTA	AT-NE	2019	set	57	57	41	57	41	57	19	41
LTA	AT-NE	2020	jan	60	60	60	60	0	60	23	60
LTA	AT-NE	NA	(Missing)	5	0	0	0	0	0	0	0
LTA	AT-SE	2018	jul	70	70	24	25	0	25	25	25
LTA	AT-SE	2018	ago	24	24	0	0	0	0	0	0
LTA	AT-SE	2018	set	66	66	66	64	0	66	66	33
LTA	AT-SE	2018	out	23	23	0	0	0	0	0	23
LTA	AT-SE	2018	nov	17	17	17	17	0	17	17	0
LTA	AT-SE	2019	fev	23	23	23	0	23	23	23	23
LTA	AT-SE	2019	jun	3	3	3	0	3	3	3	3
LTA	AT-SE	2019	jul	4	4	4	0	2	4	3	4
LTA	AT-SE	2019	ago	9	9	9	0	0	9	9	9
LTA	AT-SE	2019	set	120	120	120	40	0	120	119	120
LTA	AT-SE	2019	out	10	10	10	0	0	10	10	10
LTA	AT-SE	2019	(Missing)	4	4	4	0	0	4	4	4
LTA	MD	2017	ago	15	15	15	15	0	15	7	0
LTA	MD	2017	set	7	7	7	7	0	7	1	0
LTA	MD	2018	jun	18	18	18	0	18	18	18	18
LTA	MD	2018	jul	18	18	18	0	18	18	18	18
LTA	MD	2018	ago	77	77	77	46	31	77	31	31
LTA	MD	2018	set	18	18	18	18	0	18	1	0
LTA	MD	2018	out	11	11	11	2	9	11	10	10
LTA	MD	2019	fev	21	21	21	0	21	21	21	21
LTA	MD	2019	jul	3	3	3	0	3	3	3	0
LTA	MD	2019	ago	10	10	10	0	10	10	10	0
LTA	MD	2019	set	41	41	41	19	21	41	32	1
LTA	MD	2019	out	53	53	53	30	22	53	38	8
LTA	MD	2019	nov	19	19	19	0	19	19	19	0
LTA	MD	2020	jan	16	16	16	0	16	16	16	16
LTA	MD	2020	fev	52	52	52	0	52	52	52	52
LTA	MD	2020	mar	3	3	3	0	3	3	3	3
LTA	MD	NA	(Missing)	35	0	0	0	0	0	0	0

Table 4. Summary of sampling of Wahoo by month, species and ICCAT area. For a more complete visualization please see the file: *Summary_Table_by_month_v20200325.xlsx*

Species	MU_code	Year	month	INDIV_N	Muscle_N	Spine_N	Otolith_N	Head_N	Hard_Part_N	Stored_gonads_N	Weighted_gonads_N
WAH	AT-NE	2018	jun	5	5	5	0	0	5	5	5
WAH	AT-NE	2018	ago	21	21	21	21	21	21	5	5
WAH	AT-NE	2018	set	87	87	87	60	60	87	38	32
WAH	AT-NE	2018	out	48	45	45	38	41	48	7	7
WAH	AT-NE	2019	ago	16	16	0	0	0	0	16	16
WAH	AT-NE	2019	set	59	59	0	0	0	0	59	59
WAH	AT-NE	2019	out	41	41	0	0	0	0	41	41
WAH	AT-NE	2019	nov	23	23	0	0	0	0	23	23
WAH	AT-NE	2019	dez	14	14	0	0	0	0	14	14
WAH	AT-NE	2020	jan	15	15	0	0	0	0	15	15
WAH	AT-NE	2020	fev	22	22	0	0	0	0	22	22
WAH	AT-SE	2018	jul	14	14	0	0	0	0	0	0
WAH	AT-SE	2018	ago	9	9	2	2	0	2	2	2
WAH	AT-SE	2018	set	10	10	10	10	0	10	5	10
WAH	AT-SE	2018	out	16	16	16	16	0	16	6	16
WAH	AT-SE	2018	nov	7	7	7	7	0	7	4	7
WAH	AT-SE	2018	dez	5	5	5	5	0	5	2	5
WAH	AT-SE	2018	(Missing)	35	35	35	0	0	35	35	0
WAH	AT-SE	2019	mar	50	50	50	0	50	50	0	50
WAH	AT-SE	2019	jul	15	15	14	0	0	14	0	15
WAH	AT-SE	2019	ago	3	3	0	0	0	0	0	3
WAH	AT-SE	2019	set	21	21	21	15	0	21	21	21
WAH	AT-SE	2019	out	1	1	1	0	0	1	1	1
WAH	AT-SE	2019	(Missing)	18	18	18	0	0	18	0	18
WAH	AT-SW	2018	jan	1	1	0	0	0	0	0	0
WAH	AT-SW	2019	jan	5	5	0	0	0	0	0	0
WAH	AT-SW	2019	fev	4	4	0	0	0	0	0	0
WAH	AT-SW	2019	abr	20	20	0	0	0	0	0	0
WAH	AT-SW	2020	jan	8	0	0	0	8	0	8	0

Table 5. Results of Atlantic bonito sampling and molecular diversity indices by 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. Distribution of clades along locations according the phylogenetic tree in **Figure 2**.

Location/	ICCAT area	Code	Year	N	*M*	*h*±*SD*	π±*SD*	Clade distribution		
								I	***II***	*%Clade I*
Spain	MD/BIL95	ESP2018	2018	108	76	0.985 ± 0.005	0.070 ± 0.034	49	59	45.4
Spain	MD/BIL95	ESP2019	2019*	96	54	0.960 ± 0.013	0.066 ± 0.032	45	51	46.9
Portugal	AT-NE/BIL94B	PRT2018	2018	65	46	0.975 ± 0.010	0.069 ± 0.034	31	34	47.7
Portugal	AT-NE/BIL94B	PRT2019	2019*	38	28	0.979 ± 0.012	0.067 ± 0.034	20	18	52.6
Tunisia	MD/BIL95	TUN2018	2018	49	30	0.974 ± 0.010	0.066 ± 0.033	28	21	57.1
Morocco	AT-NE/BIL94B	MAR2018	2018	40	28	0.968 ± 0.016	0.048 ± 0.024	31	9	77.5
Mauritania	AT-NE/BIL94B	MRT2019	2018*	48	45	0.996 ± 0.005	0.047 ± 0.024	40	8	83.3
Senegal	AT-NE/BIL94B	SEN2018	2018	49	43	0.990 ± 0.009	0.039 ± 0.020	43	6	87.8
Cotê d'Ivoire	AT-SE/BIL97	CIV2018	2018	50	38	0.975 ± 0.013	0.017 ± 0.009	48	2	83.3
Cotê d'Ivoire	AT-SE/BIL97	CIV2019	2019*	72	51	0.975 ± 0.010	0.032 ± 0.016	65	7	90.3
All				615	340	0.988 ± 0.002	0.064 ± 0.031	400	215	65.0

Table 6. Pairwise genetic differentiation among Atlantic bonito samples. Below diagonal, Φ_{ST}s values. Above diagonal, *P*-values. In bold, *P*-values significant after multiple testing.

	ESP2018	ESP2019	PRT2018	PRT2019	TUN2018	MAR2018	MRT2019	SEN2018	CIV2018	CIV2019
ESP2018	--	0.013	0.905	0.060	0.178	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
ESP2019	0.031	--	0.036	0.493	0.012	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
PRT2018	-0.009	0.031	--	0.100	0.341	0.003	**0.000**	**0.000**	**0.000**	**0.000**
PRT2019	0.031	-0.007	0.026	--	0.058	0.004	0.001	**0.000**	**0.000**	**0.000**
TUN2018	0.011	0.057	-0.001	0.037	--	0.040	**0.000**	**0.000**	**0.000**	**0.000**
MAR2018	0.129	0.182	0.115	0.148	0.055	--	0.001	0.025	0.004	**0.000**
MRT2019	0.213	0.174	0.207	0.133	0.171	0.140	--	**0.000**	**0.000**	0.041
SEN2018	0.221	0.265	0.219	0.250	0.167	0.058	0.178	--	**0.000**	**0.000**
CIV2018	0.323	0.379	0.332	0.390	0.274	0.093	0.279	0.113	--	**0.000**
CIV2019	0.286	0.250	0.289	0.219	0.248	0.176	0.034	0.205	0.269	--

Table 7. Results of Little tunny sampling and molecular diversity indices by 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.

Areas and locations	Code	Year	N	*M*	*h*±*SD*	π±*SD*
Mediterranean (MD/BIL95)						
Tunisia	TUN2018	2018	46	12	0.644 ± 0.077	0.005 ± 0.003
Spain	ESP2019	2018*	71	27	0.710 ± 0.060	0.004 ± 0.003
NE Atlantic (AT-NE/BIL94B)						
Portugal	PRT2018	2018	32	9	0.643 ± 0.094	0.003 ± 0.002
Portugal	PRT2019	2019*	90	24	0.633 ± 0.059	0.003 ± 0.002
Senegal	SEN2018	2018	50	31	0.958 ± 0.018	0.010 ± 0.006
SE Atlantic (AT-SE/BIL97)						
Côte d'Ivoire	CIV2018	2018	47	36	0.981 ± 0.010	0.011 ± 0.006
Côte d'Ivoire	CIV2019	2019*	100	55	0.972 ± 0.008	0.009 ± 0.005
Côte d'Ivoire (Spain)	ESP-CIV	2018*	23	20	0.984 ± 0.019	0.009 ± 0.005
Gabon	GAB2019	2018-2019*	45	33	0.981 ± 0.010	0.008 ± 0.005

Table 8. Pairwise genetic differentiation among Little tunny samples. Below diagonal, Φ_{ST}s values. Above diagonal, *P*-values. In bold, *P*-values significant after multiple testing. VNM, a sample from Vietnam (*E. affinis*) it is included in the analysis to test the possible differentiation at species level.

	PRT2018	TUN2018	PRT2019	ESP2019	SEN2018	CIV2018	CIV2019	ESP-CIV	GAB2019	VNM*
PRT2018	*	0.202	0.572	0.443	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
TUN2018	0.008	*	0.563	0.487	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
PRT2019	-0.004	-0.004	*	0.426	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
ESP2019	-0.001	-0.002	0.000	*	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
SEN2018	0.939	0.936	0.950	0.940	*	0.232	0.551	0.279	0.419	**0.000**
CIV2018	0.933	0.931	0.947	0.937	0.005	*	0.163	0.632	0.942	**0.000**
CIV2019	0.927	0.926	0.939	0.931	-0.002	0.005	*	0.454	0.170	**0.000**
ESP-CIV	0.949	0.943	0.958	0.947	0.005	-0.005	-0.001	*	0.438	**0.000**
GAB2019	0.946	0.942	0.955	0.945	0.000	-0.010	0.005	0.000	*	**0.000**
VNM	0.973	0.965	0.973	0.965	0.927	0.921	0.920	0.933	0.935	*

Table 9. 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* ± *SD*	π ± *SD*	*% haplgrp 1*
Cotê d'Ivoire 2018	AT-SE/BIL97	CIV2018	2018	26	26	1.000 ± 0.011	0.077 ± 0.039	65%
Spain (CIV)	AT-SE/BIL97	ESP-CIV	2018*	39	38	0.999 ± 0.006	0.078 ± 0.039	67%
Cotê d'Ivoire 2019	AT-SE/BIL97	CIV2019	2019*	68	62	0.997 ± 0.003	0.077 ± 0.038	56%
Gabon	AT-SE/BIL97	GAB2019	2018*	18	17	0.993 ± 0.021	0.082 ± 0.042	50%
Spain	AT-NE/BIL94B	ESP2019	2019*	62	60	0.999 ± 0.003	0.077 ± 0.038	58%
All				213	174	0.998 ± 0.001	0.077 ± 0.037	59%

Table 10. Pairwise genetic differentiation among Wahoo samples. Below diagonal, Φ_{ST}s values. Above diagonal, *P*-values.

	CIV2018	ESP-CIV	CIV2019	GAB2019	ESP2019
CIV2018	*	0.997	0.754	0.633	0.935
ESP-CIV	-0.022	*	0.605	0.315	0.788
CIV2019	-0.009	-0.004	*	0.647	0.936
GAB2019	-0.012	0.003	-0.009	*	0.693
ESP2019	-0.014	-0.007	-0.008	-0.010	*

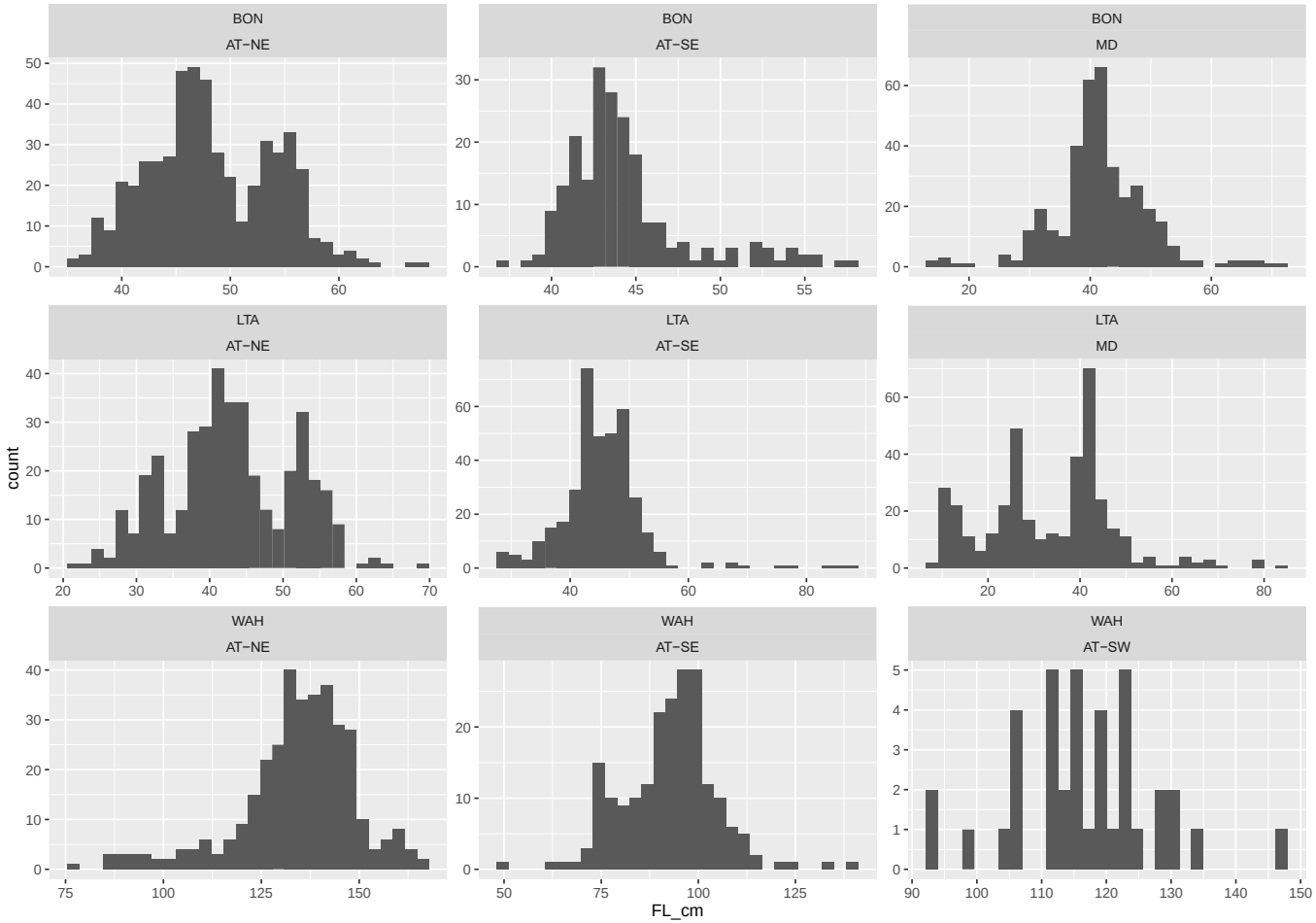

Figure 1. Histograms of sampled specimens, by size classes (fork length), species and locations.

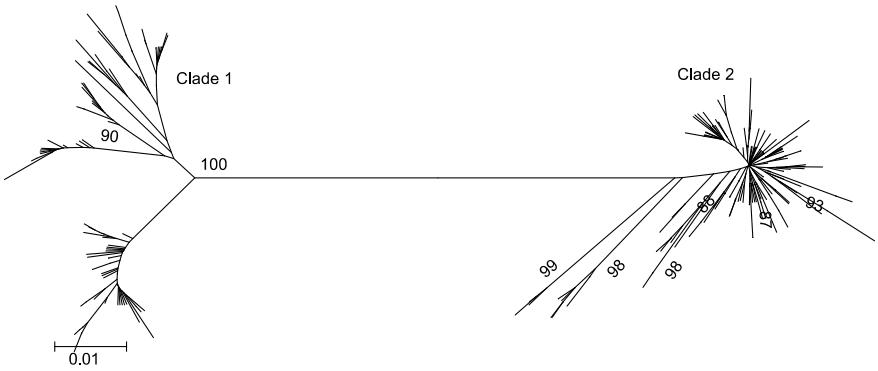

Figure 2. Unrooted phylogenetic tree of the 340 Atlantic bonito mtDNA-CR haplotypes. Values in branches are bootstrap percentages above 80% consistency.

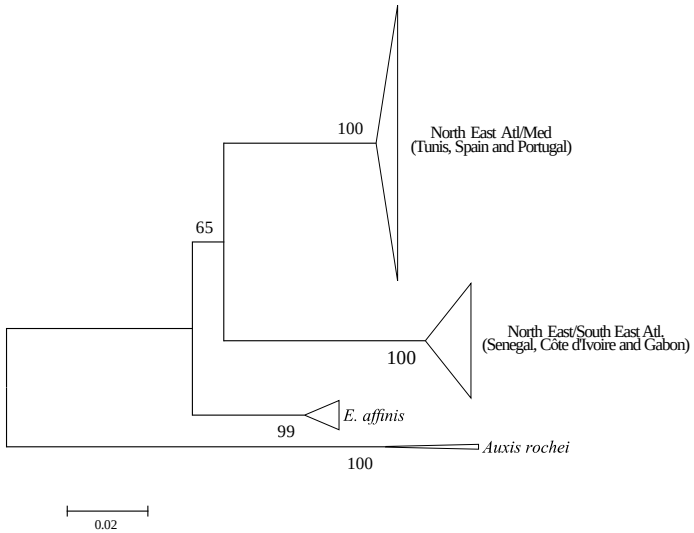

Figure 3. Rooted phylogenetic tree of the 160 Little tunny mtDNA-CR haplotypes. Tree is rooted with an outgroup (*Auxis rochei*). *E. affinis* from Vietnam were also included in the analysis. To facilitate visualization individuals from the respective areas described in **Table 7** were collapsed together (grey triangles). Values in branches are bootstrap percentages above 65% consistency.

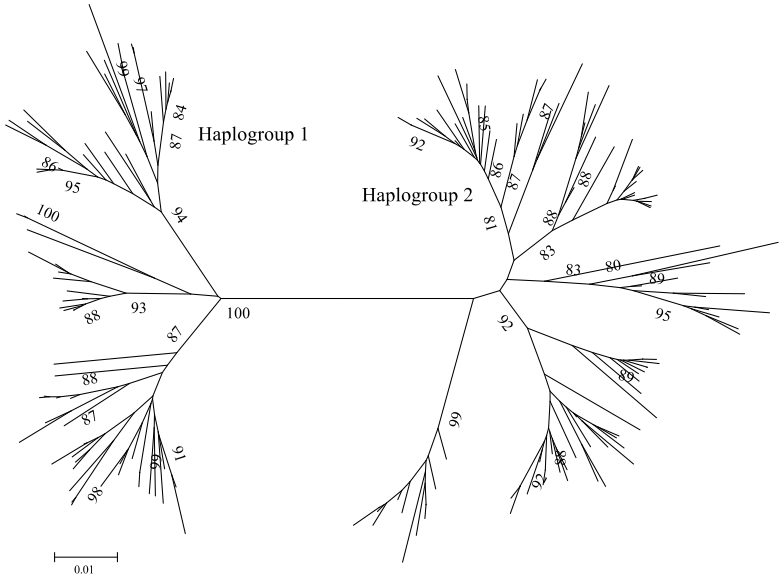

Figure 4. Unrooted phylogenetic tree of the 174 Wahoo mtDNA-CR haplotypes. Values in branches are bootstrap percentages above 80% consistency.