Genetic traceability of canned fish products manufactured in Cape Verde

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INTRODUCTION

Genetic traceability has been defined as the ability to identify the geographic origin and/or the essence of ingredients used in the elaboration of a food product or ingredient. The production of canned fish in the Cape Verde archipelago is supported by the local captures of the mackerel scad, *Decapterus macarellus* (Cuvier, 1833), and a number of scombrid species, including *Thunnus albacares* (Bonnaterre, 1788), *Auxis thazard* (Lacepède, 1800) and *Katsuwonus pelamis* (Linnaeus, 1758). In canned food, morphological features that allow the identification of taxa used in the elaboration process are removed. In this context, the genetic identification of tissues is a tool useful within the industry traceability standards, to verify the authenticity of seafood, and to resolve questions about fraud or accidental substitution with less valuable raw material.

Decapterus macarellus
(Cavala preta, Cabo Verde)

MATERIALS AND METHODS

Two sets of samples were analysed. The first one contains locally captured fish including *Decapterus macarellus* (Cuvier, 1833), *Thunnus albacares* (Bonnaterre, 1788), *Auxis thazard* (Lacepède, 1800) and *Katsuwonus pelamis* (Linnaeus, 1758). The second set includes a collection of canned products, and a variable number of replicates from each one can. DNA was isolated in all cases using Speedtools Food DNA Extraction kit (Bio-Tools). PCR was elaborated using GoTaq (Promega) and standard PCR cycling conditions. Concretely, the melting temperature was set at 50ºC and MgCl2 ranged from 1.5mM-3.5mM. The primers set used for partial COX1 amplification were FISHF2/FISHR2 (Ward *et al.* 2005), whereas for Cytochrome b amplifications were L14725/H15149 and L15424/H15573 (Kocher *et al.* 1989; Pääbo 1990) and L15998/CSBDH (Alvarado-Bremer 1994) and DCANF/DCANR (Quinteiro 2011) for the control region (CR). Sequences were obtained following ordinary sequencing protocols including PCR product purification with ExoSAP-IT (Amersham-Biosciences), sequencing with BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies) and separation of extension products in an ABI3500 sequencer (Life Technologies). Chromatograms were revised and aligned in BioEdit (Hall 1999). Species identification for obtained sequences was performed by i) BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and detection of 99-100% similarity match in GenBank records, ii) COX1 barcoding analysis en BOLD systems (http://www.boldsystems.org/index.php/IDS_OpenIdEngine) and iii) by clade assignation after phylogenetic reconstruction (Quinteiro *et al.* 1998) with MEGA software (Kumar *et al.* 2008).

RESULTS AND DISCUSSION

Barcoding of species captured in caboverdian waters

The locally sampled fishes in Cape Verde, theoretically belonging to the species considered in the present study and including *Decapterus macarellus* (Cuvier, 1833), *Thunnus albacares* (Bonnaterre, 1788), *Auxis thazard* (Lacepède, 1800) and *Katsuwonus pelamis* (Linnaeus, 1758) were genetically diagnosed using COX1 barcoding with FISHF2/FISHR2 primers. The sequences (N=5) belonging to the mackerel scad, "cavala preta" match (100% similarity) with *Decapterus macarellus* GENBANK records using BLAST algorithm and BOLD Systems. In particular the Cape Verde sample closely grouped with Caribean specimens **(Figure 2)** . Similarly, the tuna samples were genetically identified as *Thunnus albacares*, displaying >99% similarity values. Within the smallest tunas species were identified the bullet tuna, *Auxis rochei* (Risso, 1810) **(Figure 1)** and the frigate tuna *Auxis thazard* (Lacepède, 1800). Consequently, these genetically authenticated samples agree with the morphological diagnosis of captured fish and provide of reference tissues and sequences for the canned products traceability analysis.

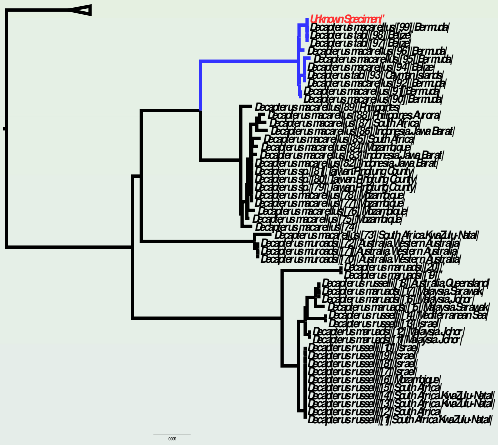

Figure 2. Tree based identification using BOLD Systems (http://www.boldsystems.org/). From an specimen (red), concretely DMAC29vB, it was obtained a partial COX1sequence and compared with the records in BOLD repository. This sequence shows a match of 100% similarity with *Decapterus macarellus* records. A midpoint rooted neighbor-joining tree displays graphically this similarity and the phylogenetic relationships respect to other taxa.

Species Identification of canned products

The samples of canned products were identified by standard procedures of genetic traceability (Mackie *et al.* 1999; Quinteiro 2011; Quinteiro *et al.* 2008; Quinteiro *et al.* 1998). Within the majority of samples containing tuna it was identified the skipjack, *Katsuwonus pelamis* based on CYTB and CR sequences. The yellowfin, *T. albacares*, is absent of the complete set of analysed products. Two products contained the frigate tuna *Auxis thazard.* An unexpected result was the presence, in diverse canned products, of the Atlantic mackerel, *Scomber scombrus* and Atlantic chub mackerel, *Scomber colias* (Table 1).

Only two products, with two replicates (4SU1-3, 5SU1-3), labelled as containing *D. macarellus* were successfully analysed by amplification with the L14524 and H15573 primers. Based in the sequencing results and the comparison with reference data from CytB, in these case a consistent result suggest the presence of *D. macarellus* tissue and the congruence with label indications. In addition, a PCR product located in the control region (DCANF/DCANR) was obtained from other 3 products (1SU1, 3SU3 and 4SU1). Although this set is not designed to this specie, the data also suggest the presence of *D. macarellus* in these products **(Table 1).**

However, the analysis involving the canned products putatively containing *D. macarellus* and the standard primers sets consistently failed. In order to solve this amplification difficulties in food products, it was designed a new set of primers located in both the cytb and control region sequences.

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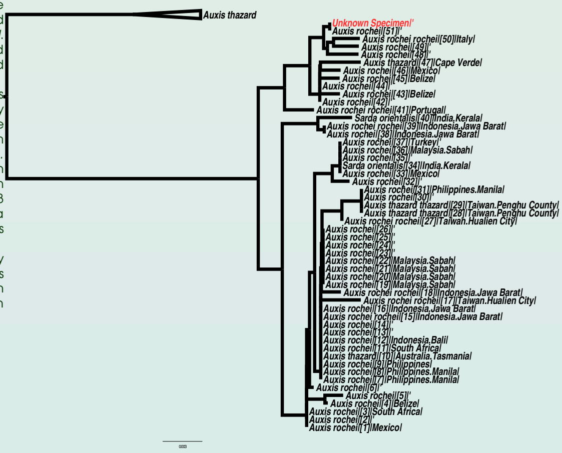

Figure 1. Tree based identification using BOLD Systems (http://www.boldsystems.org/). From an specimen (red), concretely ATHA07VV, it was obtained a partial COX1sequence and compared with the records in BOLD repository. This sequence shows a match of 100% similarity with Auxis rochei records. A midpoint rooted neighbor-joining tree displays graphically this similarity and the phylogenetic relationships respect to other taxa. All Auxis rochei records are monophyletically included in a weel supported clade, being A. thazard the sister clade.

Caracterization of partial mitochondrial sequences in *Decapterus macarellus* and primer design.

For the mackerel scad, *Decapterus macarellus* (Cuvier, 1833), a set of (N=16) sequences from the mitochondrial cytochrome b gene was obtained and flanked by the L14725 and H15573 primers. The alignment was 800 pb length from the initiation codon of the CytB gene. This sequence shows a 99-100% with partial cytochrome b haplotypes deposited in GenBank (EU349422, EU349423, EU349424). The alignment allows to design two set of primers in locations without intra-specific variability. The primers set Dmac-CB-1F/Dmac-CB-2R delimitated a 198bp length fragment (CB12) within the 5' portion of the Cytochrome b. Alternatively, the Dmac-CB-3F/Dmac-CB-4R primers flanks a 181 bp length fragment (CB34). Similarly, a 386 bp length alignment was elaborated for the control region, from 23 sequences amplified with the L15998 and CSBDH primers. Within this fragment the primers Dmac-CR-1F and Dmac-CR-2R were designed to amplify a 159 bp length fragment. The design take in consideration the high intra and inter-especific variability around this region, being considered highly specific for *D. macarellus* after "in silico" analysis.

Design of a PCR-RFLP analysis for species identification

In accord with this preliminary results about the species presence in canned products within tunas and relatives the main problematic question is to discriminate among *T. albacares/T.obesus*, *K. pelamis* and *Auxis* spp. and *Scomber* spp. The digestion of the B126 fragment produces a highly species-specific RFLP pattern for tuna (Quinteiro 2011) (Table 2). In the case of *D. macarellus*, a digestion strategy of the CB12 and CB34 fragments was selected to produce a different RFLP pattern from those expected in related species **(Table 2).**

These PCR protocol will be evaluated for consistency in the available sample set. Constitutes a rapid and easy to perform protocol for species identification without the need of DNA sequencing (Quinteiro *et al.* 1998).

Table 1. Samples of canned products

Product Code	Replicate	Gene	Primers set	Species
KP 1SU	1	CYTB	L15424/H15573	*Katsuwonus pelamis*
	2			*Katsuwonus pelamis*
	3			*Katsuwonus pelamis*
KP 4FR	1	CYTB	L15424/H15573	*Katsuwonus pelamis*
	2			*Katsuwonus pelamis*
	3			*Katsuwonus pelamis*
	4			*Katsuwonus pelamis*
KPEL 02SU	1	CYTB	L15424/H15573	*Katsuwonus pelamis*
	2			*Katsuwonus pelamis*
	3			*Katsuwonus pelamis*
KPEL 03SU	1	CYTB	L15424/H15573	*Katsuwonus pelamis*
	2			*Katsuwonus pelamis*
	3			*Katsuwonus pelamis*
KPEL 09FR	1	CYTB	L15424/H15573	*Katsuwonus pelamis*
	2			*Katsuwonus pelamis*
	3			*Katsuwonus pelamis*
	4			*Katsuwonus pelamis*
	5			*Katsuwonus pelamis*
	6			*Katsuwonus pelamis*
	7			*Katsuwonus pelamis*
	8			*Katsuwonus pelamis*
	9			*Katsuwonus pelamis*
	10			*Katsuwonus pelamis*
KPEL 01SU	2	Control Region	DCANF/DCANR	*Katsuwonus pelamis*
KPEL 08FR	3	Control Region	DCANF/DCANR	*Thunnus obesus*
	5			*Thunnus obesus*
KPEL 09FR	6	Control Region	DCANF/DCANR	*Thunnus obesus*
	7			*Thunnus obesus*
	8			*Thunnus obesus*
	9			*Thunnus obesus*
ATHA 01SU	3	CYTB	L15424/H15573	*Auxis thazard*
ATHA 02SU	3	CYTB	L15424/H15573	*Auxis thazard*
1SU	1	Control Region	DCANF/DCANR	*Decapterus macarellus*
3SU	3	Control Region	DCANF/DCANR	*Decapterus macarellus*
4SU	1	Control Region	DCANF/DCANR	*Decapterus macarellus*
2FR	1	Control Region	DCANF/DCANR	*Scomber scombrus*
	2			*Scomber scombrus*
	3			*Scomber scombrus*
4FR	3	Control Region	DCANF/DCANR	*Scomber scombrus*
8FR	8	Control Region	DCANF/DCANR	*Scomber japonicus*
9FR	3	Control Region	DCANF/DCANR	*Scomber japonicus*

	HphI, AsuHPI GGTGA	AciI, BspACII, SsiI CCGC	MboI, Sau3AI ^GATC	*MnI* CCTC	*Nla*III ^CATG
Fragment CB12					
Decapterus macarellus	57/141		71/127		
Fragment CB34					
Decapterus macarellus		115/66	157/24		
Fragment B126 L15424/H15573					
T. albacares			47/129	21/25/35/43/52	176
T. obesus			176, 47/129[1]	21/25/35/43/52	135+41
K. pelamis			176	60/49/43/24	135+41
Auxis thazard			146/30	103/52/21	135+41
Auxis rochei			146/30	78/73/25	135+41

[1] Atypical pattern

Table 2. Expected size fragments after enzymatic digestion of diverse PCR products from canned products manufactured in Cape Verde.

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