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Shark fishing in fin soup

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Abstract

Shark DNA extracted from products used in trade (e.g. soup and dried fins) was amplified using PCR. A strategy is described that permits the identification of amplified material to species (for mtDNA Cytb and NADH2 loci) and the species-specific amplification of basking shark DNA from mixed templates (for the Cytb locus). These methods will be useful for forensic applications to govern trade in these heavily exploited species.

Introduction

While precise estimates are difficult, by some accounts the combined take of elasmobranchs worldwide (including direct fisheries and bycatch) has reached nearly 100 million per year (Watts 2000). More than 150 countries or territories are involved in the trade. Considered together with the typically slow population growth of elasmobranch species (a consequence of life history characteristics) their conservation and management has become a global concern (Rose 1997). At the CITES Eleventh Meeting of the Conference of the Parties in Nairobi (2000), several proposals were submitted for the listing of shark species on Appendix II (which restricts and regulates international trade). However, one of the difficulties in achieving control in trade for these species is the challenge of species identification from unlabelled and processed materials. Several methods have been proposed and implemented over the years, including isoelectric focusing of sarcoplasmic proteins from muscle samples (Weaver et al. 1999) and RFLP analysis of PCR amplified DNA (Heist and Gold 1999). In each case, however, certain classes of degraded or processed material would not be useful for the purpose of species identification. For example, shark fin soup would not provide material suitable to either of these methods due to contamination with other materials and degradation of the DNA. I present here a strategy that should permit the species identification of essentially any shark tissue sample, even if highly processed. The strategy is based on the recognition that the relatively ancient radiation of these species has led to deep branches in phylogenetic reconstructions using mtDNA markers. It is further facilitated by the tendency for low intra-specific variation in shark species at these loci (e.g. Heist et al. 1995, 1996).

Materials and methods

Samples for forensic analysis were digested in an isolated 'ancient DNA' lab with overnight digestion and constant rotation at 60 °C in 4 ml 10 mM Tris, 200 mM EDTA, 1% SDS, 0.6 mg/ml Proteinase K. DNA was extracted 2X with phenol, 1X with chloroform, and 120 *ul* purified on a Qiagen column (cat#28104). DNA was resuspended in 30 μl double-autoclaved H_2O. PCR reactions were set up in a laminar flow hood, plasticware was UV treated and double autoclaved, and surfaces were cleaned with bleach. No PCR was undertaken in the extraction lab, and all reactions were transferred in sealed tubes to a lab in a different building for PCR amplification. Both negative controls (no DNA) and 'extraction' controls (taken through the extraction process, but without tissue) were used.

Primers to amplify short (155 or 188 bp) segments from mtDNA NADH2 (bp 253 to 407 in U91428:

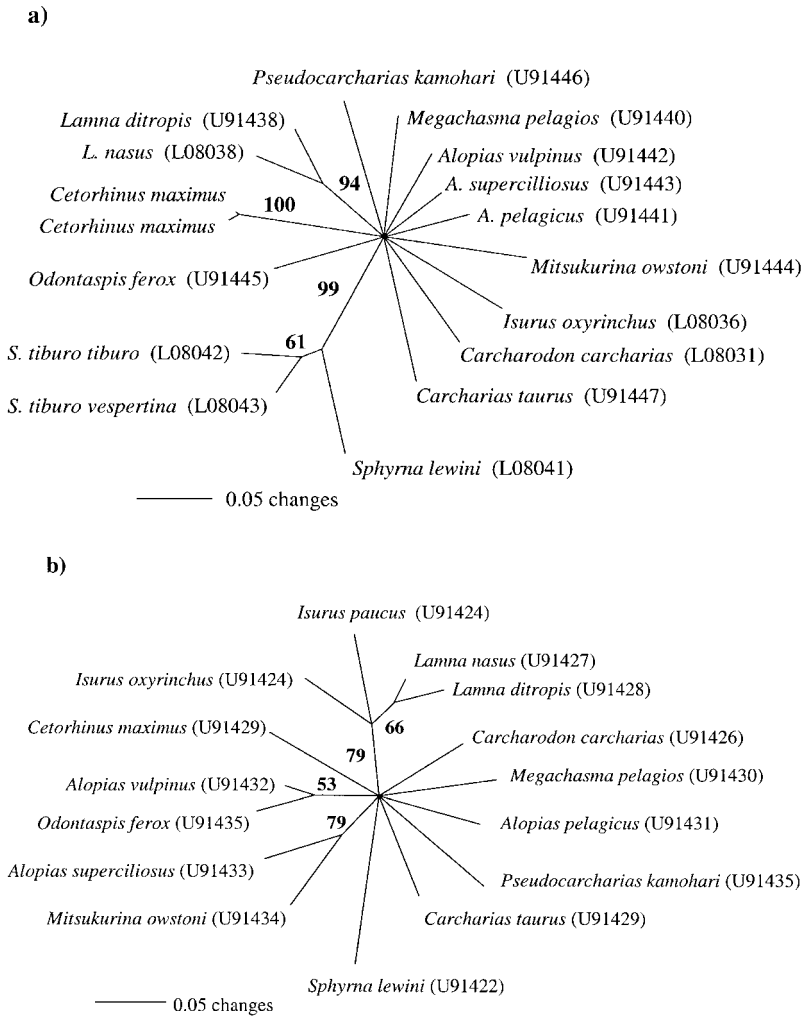

Figure 1. Unrooted neighbour joining trees based on 188 bp Cytb (a) or 155 bp NADH2 (b) mtDNA, and 1000 bootstrap replications (support shown in bold). All sequences from GenEMBL database with the exception of the Cytb *C. maximus* sequences (accession numbers given parenthetically).

5′-AGTCTAATCGAAATTT CC; 5′-AGTTTTTGTC-AGGTAGAT) and Cytb (bp 387 to 574 in L08041: 5′-TAGGAGACGCTGAAAACT; 5′-AAGGTACTG-CTTCGTTGTTT) loci were designed as 'universal' primers (based on alignments of up to 60 species) and tested for their capacity to discriminate shark species. The segment positions were chosen to maximize discrimination. Short sequences were chosen to permit amplification even from highly degraded material. Basking shark-specific primers to amplify 142 bp of Cytb were also designed: 5′-CGTAGGCTAT-CTTTTGCC; 5′-TAGGGTGGCGTTGTCGAT, based on the same sequence alignments. For a population comparison of Cytb differentiation, 550 bp was amplified from 17 basking shark samples (6 from the South Pacific and 11 from the North Atlantic) using the following primers: 5′-ACCATGAGGACAAATATC; 5′-AATGGTTGTTCTACTGGT.

For the universal primers, DNA was amplified in 1.5 mM $mgCl_2$, 50 mM KCl, 10 mM Tris, 10 μg/ml of each primer, 200 μM dNTP and approximately 10 ng of template DNA in a 30 μl total volume, annealed at 50 °C, and direct sequenced using the ABI automated system. For the basking shark primers, samples were amplified in the same reaction conditions and sequenced as above, but annealing was at 55 °C (142 bp product) or 50 °C (550 bp product). For forensic tests using the universal primers, extractions

were amplified from both stock solutions and 50-fold dilutions in distilled, double-autoclaved water (in case inhibitory components had co-purified with the DNA).

Results and discussion

As a test case, 15 or 16 Lamniform species were compared for each locus (based primarily on database sequences, see Figure 1). These phylogenies illustrate the clear differentiation among Lamniform species at both the 188 bp Cytb locus, including con-generic species (Figure 1a) and the NADH2 locus (Figure 1b). Kimura 2-parameter (Kimura 1980) distances ranged from 0.11 to 0.27 between species for both segments (188 bp Cytb and 155 bp NADH2). Two subspecies (*Sphyrna tiburo tiburo* and *S. t. vespertina*) compared at the Cytb locus had a distance of 0.07. This illustrates that even though the sequences are short, there is sufficient differentiation detected for clear species identification. In addition, to assess the level of stereotypy within a species, a sample of 17 basking sharks (*Cetorhinus maximus*) representing the eastern and western North Atlantic, the North Sea, the Mediterranean and the western South Pacific was sequenced for 550 bp Cytb, including the 188bp region. Two haplotypes were found differing by 0.6% (1.1% for the 188 bp segment; a Kimura 2-parameter distance of 0.01), with no significant frequency difference among geographic regions (5 of haplotype 'A' and 1 of haplotype 'B' in the South Pacific, and 10 of A, 1 of B in the North Atlantic; Fisher exact test, $p > 0.5$).

The universal primers were chosen to facilitate general amplification from shark species. However, some processed material may include multiple species, or screening for a target species without sequencing may be desirable. To illustrate the potential for species-specific amplification, basking shark-specific primers were designed for Cytb, and shown to amplify preferentially from basking shark material (Figure 2).

Forensic tests using the universal primers were undertaken on dried shredded fin (sample 1), whole dried fin (sample 2), soup (sample 3; 'refined shark fin soup', Yeow Seng Co. Sea-food store), and cartilage pills (sample 4; Solgar Laboratories). Amplification was possible for all four samples (Figure 2), each sequence was different, and all were shark species. The soup and pill samples matched hammerhead shark (*Sphyrna lewini*) and basking shark, respectively. Neither fin sample found an exact match in the database, though each were within 10–12% of several Lamniform sharks.

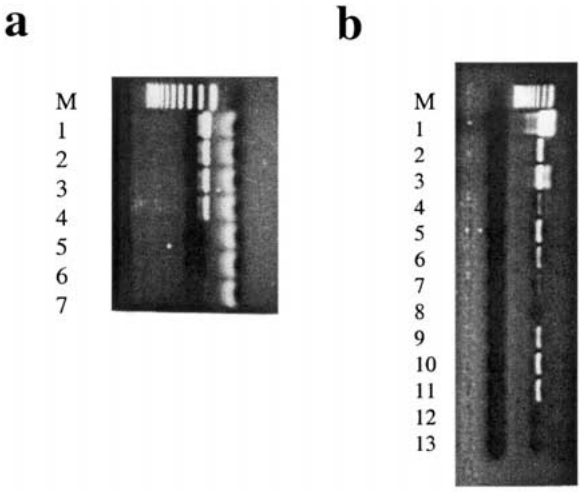

Figure 2. (a) Specificity of basking shark primers for Cytb locus. Samples 1–4 are basking sharks, 5 = *Carcharinus limbatus*, 6 = *Carcharinus sorrah*, 7 = *Rhincodon typus*, M = 100 bp ladder. (b) Amplification with universal Cytb primers from test samples after extraction. Samples 1–4 are test samples (see text), 5–8 test samples with DNA at 50-fold dilution, 9 = *Carcharinus sorrah*, 10–11 = *Cetorhynus maximus*, 12 = extract negative control (taken through all steps, but no tissue added), 13 = PCR negative control.

This method would permit the tracking of protected species in trade, and supports the recent proposals to have several shark species listed on CITES appendices. Ideally, matches for target species of conservation concern would be based on species-specific primer-sets for multiple loci. However, given the relatively ancient phylogenetic radiation of elasmobranch species, and consequent high levels of interspecific differentiation, 'fishing' with universal primers should also provide important data on the taxonomic classification of samples from trade.

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