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What barcode sequencing reveals about the shark fishery in Peru

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ABSTRACT

Elasmobranchs are rapidly declining due to overfishing and bycatch, underlining the need for immediate protection. Critical baseline information on the diversity of targeted species is, however, often missing. Peru is a major country for shark fishery, an activity that has been under-regulated and poorly monitored, aggravated by the superficial taxonomic identifications at landing points across the country. Furthermore, most of the species landed by the shark fishery in Peru are listed as Vulnerable in the IUCN Red List. To assess the diversity of shark species targeted by fisheries in Peru we analyzed the variation of the cytochrome oxidase I (*cox1*) region of the mitochondrial DNA from 118 samples collected between 2004 and 2009, from six landing points. Our analysis revealed unambiguously that the 16 shark species classified by fishermen using meristic characters corresponded only to nine species. While some commonly landed species (e.g. *Prionace glauca*) were consistently correctly identified, for others species multiple inconsistent names were applied (e.g. *Galeorhinus galeus*). Our molecular characterization further allowed the identification of specimens with non-informative common names (i.e. "tiburon" = shark). In most cases the unknown specimens were *Isurus oxyrinchus* and *P. glauca*. Interestingly, all samples labeled as common thresher (*Alopias vulpinus*) were identified as pelagic thresher (*Alopias pelagicus*). Finally, one sample was equivocally identified as a dusky shark (*Carcharhinus obscurus*) and as a galapagos shark (*Carcharhinus galapagensis*) reinforcing the genetic similarity reported for these species. We generated a character-based identification library containing 26 of the 31 commercially important sharks landed in Peru and tested its performance as a species diagnostic. The library correctly identified 25 out of 28 barcodes tested, outperforming the distance-based approach. This is the first study sequencing barcodes of marine species in Peru and generated a genetic reference library of targeted shark species. We suggest that the molecular tools used are a quick and effective complement for the monitoring of the fishery of threatened shark species. A combined effort to obtain these data, by countries in the east Pacific region with an on-going shark fishery, would provide with the essential guiding information to promote the implementation of effective sustainable management plans.

1. Introduction

Overfishing and bycatch have severely reduced many populations of sharks around the globe (Baum et al., 2003; Dulvy et al., 2008; Hisano et al., 2011). The Food and Agriculture Organization of the United Nations (FAO) reports that between 1988 and 2002 more than 11 million tons of elasmobranchs (i.e. sharks and rays) were landed globally of which 60% were shark species (Bonfil, 1994). In 2002 around 850,000 tons of elasmobranchs were landed (Camhi et al., 2008) and in 2006, the fins of 38 million sharks were traded in Asian markets (Clarke et al., 2006a). Recently, initiatives are increasing to recover, protect and sustainably manage shark populations (Techera, 2012; Ward-Paige et al., 2012); however to implement meaningful conservation initiatives, biological and ecological baselines are required along with information of fishery dynamics (e.g. Köster et al., 2003). Some shark fisheries lack a basic understanding of species diversity,

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composition and population structure, which would greatly aid in setting conservation and management goals (Barker and Schluessel, 2005). One pervasive problem in shark fisheries is the deficient information collected at landing points due to, mainly, the difficult access to landing points, the superficial identification of species, and the limited number of inspectors at ports (e.g. Bonfil, 1994; Rose, 1996). A serious concern is the misidentification of species by fishermen and inspectors and the errors this can produce in landing reports of species composition and diversity (Camhi et al., 2008; Smale, 2008). A recent study assessing the skills of scientific observers at identifying shark species found that some taxonomic groups, regardless of the observer's experience, are problematic to correctly identify in the field (Tillet et al., 2012). In these situations, molecular analyses can play an important role in species identification.

Species diagnostics using molecular tools, like DNA barcodes, have high utility in species identification, including marine species (reviewed in Bucklin et al., 2011). For vertebrates, the identification of nucleotide substitutions of the mitochondrial gene cytochrome oxidase subunit I (*cox1*) has performed well as a species diagnostic tool and is now widely used (e.g. Hajibabaei et al., 2008; Zemlak et al., 2009). Furthermore, ongoing initiatives to barcode all living species of fishes using *cox1* (i.e. Fish Barcode of Life Project, www.fishbol.org) have isolated thousands of sequences available in a public repository of the Consortium for the Barcode of Life (i.e. Barcoding of Life Data Systems-BOLD, www.boldsystems.org, Ratnasingham and Hebert, 2007) as well as in the global public repository of genetic information (i.e. Genbank, http://www.ncbi.nlm.nih.gov/). These two repositories facilitate the identification of molecular information from parts or individuals not identified in the field, or known only from parts or remains. For shark species identification, barcodes have been used either for whole specimens or parts, for dry or fresh samples (reviewed in Dudgeon et al., 2012). For example, Ward et al. (2005) conducted the first study that included sharks and expanded it to include 945 specimens identifying putative new species (Ward et al., 2008). Likewise, Holmes et al. (2009) used barcodes sequencing approach to identify species from tissue samples obtained from shark fins confiscated from a vessel fishing illegally in Australian waters, resulting in 27 species of elasmobranchs identified including one species of shark considered Critically Endangered by the International Union for the Conservation of Nature (IUCN). In addition to the diversity of sharks, rays and skates have also been studied using a genetic barcodes (e.g. Spies et al., 2006; Coulson et al., 2011; Cerutti-Pereyra et al., 2012). Other techniques for rapid species diagnostics exist (i.e. multiplex PCR) and have demonstrated their utility (e.g. Shivji et al., 2002; Clarke et al., 2006b; Morgan et al., 2011; Pinhal et al., 2012), but they are still limited to a small number of species while the use of *cox1* offers the opportunity of identifying the broadest range of shark species.

Similar to other areas in the world, shark fisheries in the eastern Pacific include both pelagic and coastal fisheries that target different species, but their contribution to total global and regional landings is poorly understood (Camhi et al., 2008). Whereas pelagic fishermen target species of oceanic habits (e.g. *Isurus* spp., *Prionace glauca*) coastal fishermen target benthic and demersal sharks (e.g. *Mustelus* spp., *Squatina* spp.). In the southeast Pacific, the fishery targeting shark species is poorly regulated and, in some areas, largely unmonitored (Gilman et al., 2008; Jacquet et al., 2008). Until very recently, the practice of finning was common in countries like Costa Rica, Ecuador and Chile, resulting not only in an underestimation of the real number of sharks taken, but also of the diversity of species captured (e.g. Jacquet et al., 2008). Moreover, for many countries, insufficient monitoring of the landing process coupled with limited taxonomic identification at ports has resulted in a poor understanding of the diversity of species caught. In Peru, 58 species of sharks are reported (Chirichigno and Cornejo, 2001) and of these, 31 species are identified as commercially important (Velez-Zuazo, 2012). Nevertheless, official reports of shark landings at the species level are deficient (Estrella Arellano and Swartzman, 2010). For example, smooth-hounds (*Mustelus* spp.) and houndsharks (*Triakis* spp.) are reported under a single common name ("tollo") that most likely includes the eight species reported in Peru. A recent analysis of six decades of shark landings suggests that Peru stands as the country with the highest accumulated landings of sharks in the entire Pacific region (Velez-Zuazo, 2012). In this light, an accurate identification of the species targeted in Peru is necessary if one is to propose actions for these fisheries currently under-managed or toward the development of a National Plan of Action, as recommended by the FAO (1999).

Since 2004, the local NGO ProDelphinus has been collecting tissue samples from sharks and rays landed by small-scale fisheries operating at six ports along the coast of Peru (Fig. 1). All species were identified and labeled using their common name. For some species of sharks, however, a single common name (e.g. "tiburon") can represent many species. For other species, like thresher sharks (*Alopias* sp.), distinction of species based on subtle morphological characteristics can be difficult to assess at the port, particularly if only parts of individuals are being landed. While finning (i.e. the landing of shark fins while carcass are discarded at sea) is not practiced in Peru, only shark trunks are typically landed, which makes challenging the identification of species with diagnostic morphological features located on the head. We used barcode sequencing to identify, at the species-level, the sharks landed at six ports along the coast of Peru from 2004 to 2009 (Fig. 1). We isolated genetic barcodes from Peruvian sharks and generated a character-based identification library for the commercially important shark species in Peru. Identification of species using diagnostic nucleotide characters has proved to be reliable for different species (e.g. Rach et al., 2008; Reid et al., 2011) including sharks (Wong et al., 2009), and it can be used in combination with distance-based approaches as a species diagnostic (e.g. Lowenstein et al., 2009). Our main goals in this study were to generate a genetic-based taxonomic list of shark species of commercial importance in Peru and to provide an integrative approach for their rapid identification using genetic diagnostic characters and genetic distances.

2. Methods

Tissue samples were previously collected by ProDelphinus from 2004 to 2009, mostly from fins and muscle of specimens landed at six locations (see Fig. 1), and stored with tabletop salt at room temperature. The tissue collection comprises nearly 1902 samples from putatively 16 different shark species (based on common name assigned during collection) but for the purpose of this study, we analyzed 292 samples covering all the diversity of putative shark species (see Table 1). From the most commonly landed species, like blue shark, mako shark, and hammerhead (*Sphyrna* spp.), we analyzed 50 samples whereas from the uncommon species (based on local names) we analyzed all samples available ($n = 60$).

We isolated whole genomic DNA using DNAeasy (Qiagen) following manufacture instructions and eluted in 30 μl of AE buffer. To confirm DNA isolation and to measure its concentration (ng/μl) we run a 0.8% agarose gel along with a Lambda DNA marker at different DNA concentrations (i.e. 15 and 30 ng/μl). We compared eye-estimates of DNA concentration with values obtained using a spectrophotometer (NanoDrop-Thermocientific). We targeted and amplified a 679 base-pair (bp) fragment of the mitochondrial DNA cytochrome oxidase I gene by the polymerase chain reaction (PCR) and using the M13-tailed cocktail primers Fish-F1t1(FishF2_t1 and VF2_t1) and Fish-R1t1 (Fish R2-t1 and FR1d_t1; Ivanova et al., 2007).