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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).

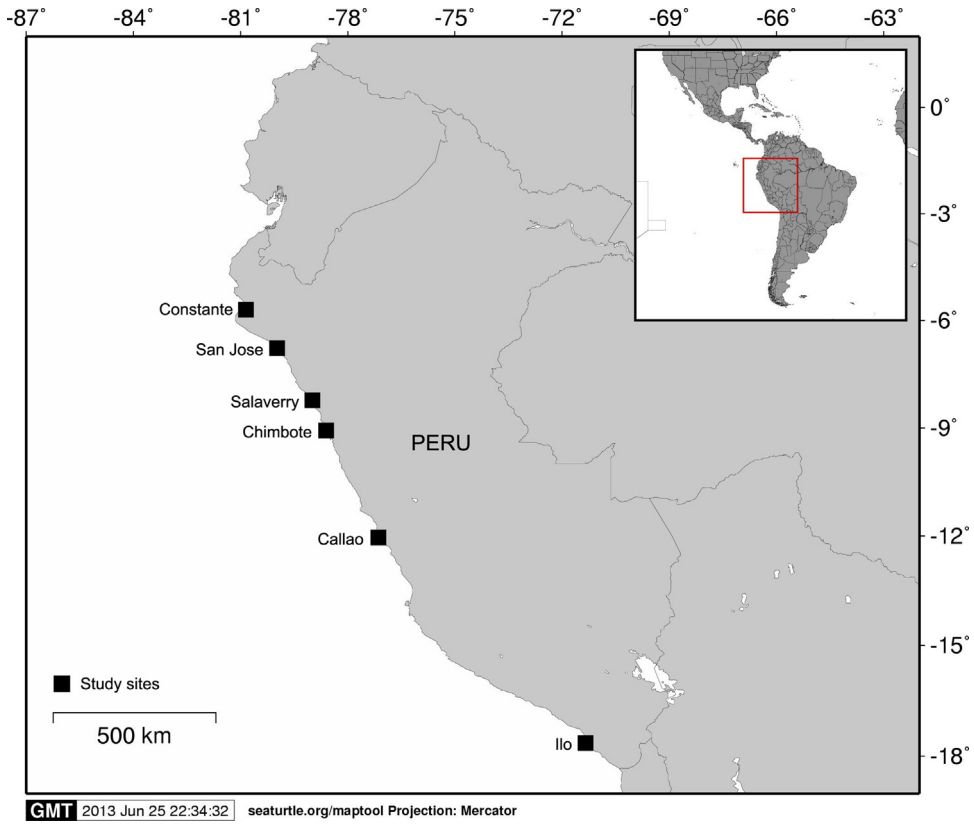

Fig. 1. Landing points where ProDelphinus has been collecting samples for elasmobranchs since 2004. Map created using SEATURTLE.ORG Maptool. 2002. SEATURTLE.ORG, Inc. http://www.seaturtle.org/maptool/ (2011).

The PCR had a total volume of 10 μl. The cocktail contained 0.1 μl of each primer (10 μM each), 5 μl of Taq MasterMix (Qiagen), 1 μl of genomic DNA (diluted 1:5) and ultrapure water. We used the following cycling conditions: An initial denaturing step (94 °C for 2 min) followed by 35 cycles of denaturing (94 °C, 30 s), annealing (52 °C, 40 s) and extension (72 °C, 1 min), and a final extension at 72 °C for 10 min. We visualized the PCR product in a 1% agarose gel and running 2 μl of Low DNA Mass Ladder (Invitrogen) to confirm amplification and for estimating the concentration of desired fragment. We used a combination of exonuclease (Fermentas) and shrimp alkaline phosphatase (Fermentas) to purify the PCR product before sequencing. Purified products were sequenced in both directions using M13 primers (Ivanova et al., 2007) with ABI Big-dye® terminator chemistry and run on the automated sequencer station ABI 3130xl (Applied Biosystems). We assembled and edited the forward and reverse sequences using Sequencher 4.8 (Gene Codes). Clean sequences were trimmed to the start and length (when possible) of baseline sequences from sharks downloaded from BOLD and finally exported as ASCCI files to conduct molecular evolutionary analyses. We aligned all sequences using ClustalW, with default parameters, implemented in Mega 5.05 (Tamura et al., 2011) and identified unique sequences using DNAsp 5.10 (Librado and Rozas, 2009). We estimated the frequency of mitochondrial haplotypes as well as haplotype and nucleotide diversity using DNAsp 5.10.

Table 1
Common names used to label tissue samples collected at landing points and analyzed (*n*) and species represented after genetic analyses.

Local name	DNA-based species	*n*
Tiburon azul	*Prionace glauca*, *Sphyrna zygaena*, *Isurus oxyrinchus*	38
Cazon	*Carcharhinus obscurus*	1
Cazon o Pardo	*Carcharhinus brachyurus*	1
Tiburon cocodrilo	*Galeorhinus galeus*	1
Martillo	*Prionace glauca*, *Sphyrna zygaena*	18
Mako	*Isurus oxyrinchus*, other taxa*	6
NN	*Galeorhinus galeus*, *Lamna nasus*	5
Tiburon Pardo	*Carcharhinus brachyurus*, *Sphyrna zygaena*	5
Porbeagle	*Lamna nasus*	1
Tiburon chancho	*Lamna nasus*	1
Tiburon	*Prionace glauca*, *Isurus oxyrinchus*, other taxa*	17
Tiburon X	*Sphyrna zygaena*, *Isurus oxyrinchus*, *Lamna nasus*	3
Tollo gato	*Sphyrna zygaena*	1
Tollo mama	*Sphyrna zygaena*, *Mustelus* sp.	4
Vidrio	*Galeorhinus galeus*	2
Zorro	*Alopias pelagicus*, *Sphyrna zygaena*, *Isurus oxyrinchus*, other taxa*	17

* Sea turtle, fish, or ray species.

2.1. Distance-based species identification

We used the public database BOLD to identify all unique sequences at the species level, and Genbank for sequences not identified in BOLD. For identification of mtDNA *cox1*, BOLD has an interface that allows using four databases which differ in the length of the sequences it analyzes, the character of the data (i.e. public and/or private sequences), and the level of identification it provides for queried sequences. For our study, we used the option that analyzes sequences with minimum length of 500 bp and compares them with sequences identified at the species-level and species with interim taxonomy. For the identification of sequences to the species-level, BOLD uses a distance-based approach analysis using Kimura 2-parameter (K2P) model of nucleotide substitution (Hebert et al., 2004). The output of the analysis is provided in two

ways; a summary providing probability of placement at different taxonomic levels (i.e. Phylum to species), with a list with the best 100 matches and specimen similarity (%), and a tree-based identification depicting a neighbor-joining (NJ) tree that includes the queried sequence ("unknown specimen"). In our study we included the first two species listed in the list of 100 matches, along with percentages of specimen similarity.

2.2. Character-based identification

To create a character-based identification key we downloaded from BOLD all *cox1* sequences for the 31 shark species of commercial importance in Peru (Velez-Zuazo, 2012, Supplementary Data). Using ClustalW, we aligned all sequences and exported to DNAsp to identify unique sequences. We were aiming for as many unique *cox1* sequences as possible for each species larger than 500 bp, which would increase the probability of identifying individuals to species (e.g. Ekrem et al., 2007). From the list of 31 species, we were able to gather sequences for 26 species. To define pure unique identifying characters we used the Character Attribute Organization (CAOS) workbench (Sarkar et al., 2008, http://boli.uvm.edu/caos-workbench/caos.php). First, we used Mega 5.05 to align all unique sequences for the 26 shark species and to generate a NJ guide tree using K2P model, with 1000 replicates of bootstrapping. Using Mesquite (Maddison and Maddison, 2010) we generated a nexus file containing the alignment and NJ tree topology being careful to resolve any polytomy and used this file as input for CAOS-Analyzer for identifying the diagnostic characters. Diagnostic characters are different characteristic attributes (CAs) and can be simple-pure (sPu, characters find in a single position for all specimens in a given group but not in other group), compound-pure (cPu, two or more simple-pure CAs), simple-private (sPr, characters found in some members of a given group but not in other group), or compound-private (cPr, two or more simple-private CAs). After generating the file with CAs to differentiate all of the 26 species we used CAOS-Barcode to generate our own reference matrix of CAs, to be used as baseline for the identification of unknown specimens. Finally, we used CAOS-Classifier to identify at the species-level the sequences obtained in this study, using our reference matrix for 26 species of sharks of commercial importance. The output is a table that lists all queried sequences, its best-species hit and the match percentage, and also provides files for each individual query with a list of the best match and secondary matches.

3. Results

Out of 292 samples a total of 130 samples provided sufficient quality and quantity of DNA to use for targeted amplification of *cox1*. The remaining samples ($n = 162$, 55%) gave spurious DNA yield (i.e. concentrations below 1 ng/μl and highly fragmented) and did not amplify our target *cox1* fragment after many trials. We think that preservation method and storage conditions (i.e. tabletop NaCl and room temperature) were primarily responsible for the degradation of genomic DNA of the samples that did not amplify our target.

3.1. Species identification and intra-specific variability

We obtained sequences that varied between 558 bp and 717 bp, with an average length of 669 bp. We identified a total of 328 variable sites that defined 28 distinct haplotypes (i.e. unique sequences). Sequences similarity to the BOLD barcodes database generated matches to shark species with accuracy equal to or higher than 96%, and an average best match of 99.89% for 111 out of the 130 samples (see Supplementary Data). The remaining 19 samples did not match shark species but three species of sea turtles (*Caretta caretta*, *Chelonia mydas*, and *Dermochelys coriacea*), one of ray (*Myliobatis* sp.), one of ray-finned fish and eight prokaryotic sequences respectively. These were removed from subsequent analyses as they likely reflect sample mislabeling (in the case of sea turtles, fish and ray) and amplification of associated marine microbes (Siddall et al., 2009). BOLD analysis on the 111 shark specimens provided information at the species level in 108 cases and at the genus level for the remaining three sequences. Unique shark species identified were the following: *Prionace glauca*, *Carcharhinus brachyurus*, *Carcharhinus obscurus*, *Sphyrna zygaena*, *Isurus oxyrinchus*, *Galeorhinus galeus*, *Lamna nasus*, and *Alopias pelagicus*. The sample identified as *C. obscurus* also had a 100% match to *Carcharhinus galapagensis*. The three samples for which only the genus was identified were assigned to *Mustelus* sp. with an average match of 98.14% to *Mustelus henlei*, but forming a separate cluster, thus suggesting they belonged to other species not included in the BOLD database. With these samples and with reference sequences from different species of *Mustelus* downloaded from BOLD we constructed a NJ-tree using K2P model and 1000 bootstrap replicates in Mega. All our samples clustered together (99% bootstrap support) and the genetic distance between them and the nearest sister cluster (*M. henlei*) was 0.021.

Comparing the common names assigned to samples with the genetic identification, we estimated that *I. oxyrinchus* ("mako") was identified correctly in 100% of samples. The other species that the fishermen identified correctly most of the time were *S. zygaena* ("martillo"), identified correctly 94.4% of the time, and *P. glauca* ("tiburon azul") identified correctly 92.1% of the time. Interestingly, all samples labeled as thresher shark corresponded to pelagic thresher shark (*A. pelagicus*); however, in Peru, the local name for threshers (i.e. "zorro") usually refers to the common thresher (*Alopias vulpinus*), the only species listed in official landing reports.

All shark species exhibited varying degrees of haplotypic (h) and nucleotidic diversity (π) (Table 2). In the 11 samples identified as *I. oxyrinchus*, 13 variable sites resulted in nine haplotypes and the highest molecular diversity ($h = 0.964$, $\pi = 0.008$), followed by *L. nasus* (3 haplotypes, $h = 0.8$, $\pi = 0.0069$) and *A. pelagicus* (5 haplotypes, $h = 0.727$, $\pi = 0.002$). The remaining species exhibited very low values, particularly *S. zygaena* ($n = 26$) whose sequences had two variable sites, defining two haplotypes with resulting low estimates of molecular diversity ($h = 0.077$, $\pi = 0.0003$).

3.2. Identification using diagnostic character attributes

We identified 284 diagnostic positions (184 with sPu and 100 with sPr) to distinguish all but one pair of species: *C. obscurus* from *C. galapagensis*. These two species were not resolved in the NJ tree and appeared in two clusters together with bootstrap node support of 87% and 71% while the whole cluster including all five sequences representing the two species had 99% of bootstrap node support. For the rest of the species, a combination of sPu and sPr CAs were identified. Example sets of sPu CAs are presented for seven shark species of commercial importance (Table 3) and the full set of diagnostic nucleotide positions are available upon request from the authors. The 26-species reference matrix was used to classify our sequences and test the reliability of this approach for species identification. Except for three barcodes, all unique sequences generated in our study were identified correctly to the species level and with an average match of 99.78% (min = 98.90, max = 100, SD ± 0.0027, Supplementary Data). Two of the three barcodes, identified in BOLD with confidence to genus level (*Mustelus* sp.), were identified as *M. henlei* with matches of 98% and 97.85%, the lowest percentage estimates obtained by CAOS-Classifier while one sample was identified

Table 2
Shark species identified using *cox1* barcodes. Information presented includes sample size (n), polymorphic sites observed (N_s), unique haplotypes (N_h), estimates of haplotypic (h) and nucleotide diversity (π) with values of standard deviation (±SD), current conservation status according to IUCN Red List (VU – Vulnerable, NT – Near Threatened), and GenBank accession number for unique haplotypes.

Species	n	N_s	N_h	h (±SD)	π (±SD)	IUCN status[a]	Accession number
Prionace glauca	45	2	3	0.1290 (0.0670)	0.0003 (0.0001)	NT	KJ146042-44
Carcharhinus brachyurus	2	–	1	–	–	NT	KJ146027
Carcharhinus obscurus	1	–	1	–	–	VU	KJ146021
Sphyrna zygaena	26	2	2	0.0770 (0.0700)	0.0003 (0.0002)	VU	KJ146045
Isurus oxyrinchus	11	13	9	0.9649 (0.0510)	0.0080 (0.0010)	VU	KJ146030-38
Galeorhinus galeus	7	1	2	0.2860 (0.1960)	0.0005 (0.0003)	VU	KJ146028-29
Lamna nasus	5	8	3	0.8000 (0.1640)	0.0069 (0.0018)	VU	KJ146039-41
Alopias pelagicus	12	4	5	0.7270 (0.0130)	0.0020 (0.0003)	VU	KJ146022-26
Mustelus sp.	3	–	–		–	–	N/A

[a] Accessed 5th September 2012.

equivocally as *C. galapagensis* and *C. obscurus* with 100% probability, in each case (Supplementary Data).

4. Discussion

This is the first and largest study using genetic barcodes to identify sharks in Peru, and builds upon previous international initiatives to identify shark species and determine their origin (e.g. Heist and Gold, 1999; Shivji et al., 2002; Clarke et al., 2006a,b; Sebastian et al., 2008; Holmes et al., 2009; Moore et al., 2012). We focused on the small-scale Peruvian shark fishery and isolated 28 different barcodes from nine species identified to species level. Results from our study include a new species report for Peru, the dusky shark (*C. obscurus*), and reinforce previous observations of taxonomic controversy between the dusky shark (*C. obscurus*) and the Galapagos shark (*C. galapagensis*, Wong et al., 2009; Naylor et al., 2012). Results also suggest a long-term misidentification between the common thresher shark (*A. vulpinus*) and the pelagic thresher shark (*A. pelagicus*) at landing points. We developed a character-based library of sharks in Peru that, in combination with a genetic-distance approach, improves shark species identification.

An analysis of total landings for the past 61 years indicates that Peru is an important country for shark landings in the Pacific region (Velez-Zuazo, 2012). Fisheries managers have largely ignored this fact probably because the fishery of small pelagic fishes largely dominates the fishing industry in volume and revenues (Ñiquen et al., 2000; Jacquet et al., 2010). As a result, the shark fishery is not only poorly regulated but also poorly monitored, allowing for misidentification and underestimation of targeted species, and precluding estimations of the impact of fisheries on shark stocks. This was apparent from the variety of common names used to label the samples analyzed in this study.

4.1. Identifying common commercial species of sharks

The archive of samples maintained by ProDelphinus was characterized both by unequivocal common names (e.g. “tiburon azul” – *P. glauca*) and by generic common names (e.g. “tiburon”). Samples with easy-to-identify common names were more abundant but were restricted to two species (i.e. *P. glauca* and *I. oxyrinchus*). Other names like “martillo” and “zorro” were likely to be assigned to the most common species landed in Peru: smooth hammerhead

Table 3
Diagnostic characters (shaded in gray) for the identification of 26 shark species of commercial importance in Peru. We present an extract of species, representing five of the six most common species (*Alopias vulpinus*, *Isurus oxyrinchus*, *Prionace glauca*, *Sphyrna zygaena*, and *Squatina californica*), a species we believe is being misidentified (*A. pelagicus*), and a species also targeted by the Peruvian shark fishery (*Lamna nasus*). For each species, the number of sequences used to identify the characters is indicated in parenthesis.

Species	Nucleotide position																				
	43	53	64	70	76	91	94	97	100	103	124	166	178	208	211	235	259	262	274	275	277
Alopias pelagicus (5)	A/G	C	A	A	A	T	A	A	T	T	A	C	C	A	T	A	A	C	T	T	T
Alopias vulpinus (2)	A	C	A	A	A	T	A	A	T	T	A	C	T	A	T	G	A	A	T	T	T
Isurus oxyrinchus (17)	A	C	T	A	T	C	A	G	T	T	A	C	A	G	C	C	G	C	T	T	C
Lamna nasus (23)	A	C	C	G	T	C	A/G	C	C	T	A	T	G	A	T	T	A	C	T	T	T
Prionace glauca (4)	A	C	A	A	G	T	A	A	T	T	A	A	T	A	T	A	A	T	A	T	T
Sphyrna zygaena (5)	A	C	A	A	G/A	T	A	A	T	C	A	A	T	A	T	A	A	T	A	T	T
Squatina californica (5)	T	T	A	A	C	C	T	G	T	T	T	A	T	A	T	A	A	T	C	C	A
	280	284	286	289	304	307	308	319	331	334	343	349	361	373	376	388	409	418	421	425	484
Alopias pelagicus (5)	T/C	C	T	A	T	A	G	T	A	T	A	T	A	A	C	T	A	A	A	T	A
Alopias vulpinus (2)	T	C	T	G	T	A	G	C	A	T	C	G	A	A	C	C	A	A	A	T	A
Isurus oxyrinchus (17)	T	C	C	G/A	T	G	T	C	A	C	C	T	A	A	T	C	T	G	C	C	A
Lamna nasus (23)	C	C	C	A	C	A	G	T	G	T	C	T	A	C	C	T	G	T	A	T	G
Prionace glauca (4)	T	C	C	C	T	A	G	T	A	T	A	T	A	A	T	T	C	A	A	T	A
Sphyrna zygaena (5)	T	C	C	A	A	A	G	A	A	T	A	T	C	A	C	T	T	A	A	T	A
Squatina californica (5)	A	T	A	T	C	A	G	C	A	A	T	A	T	A	A	A	T	T	A	T	A
	490	499	508	511	517	526	527	529	530	535	544	556	559	564	565	583	604	607	616	619	625
Alopias pelagicus (5)	A	A	A	A	A	T	A	C/T	C	T	C	T	A	G	C	T	A	C	A	A	A
Alopias vulpinus (2)	A	A	A	A	A	T	A	C	C	T	C	C	A	G	T	T	A	C	G	A	G
Isurus oxyrinchus (17)	A	G	G	C	A	C	A	C	C	T	A	C	C	G	C	G	A	C	G/A	C	A
Lamna nasus (23)	G	A	A	C	A	T	A	T/C	C	T	C	T	A	G	C	T	T	C	A	A	G/A
Prionace glauca (4)	A	A	A	T	T	T	A	T	C	T	A	T	A	G	T	A	A	C	T	A	A
Sphyrna zygaena (5)	A	A	A	T	T	T	A	T	T	T	A	T	A	G	G	A	A	C	C	A	A
Squatina californica (5)	A	C	A	A	G	T	G	A	C	C	T	A	A	C	A	T	A	T	T	A	T

shark (*S. zygaena*) and common thresher shark (*A. vulpinus*) but since there are four species of hammerheads and three of threshers interacting with the Peruvian fishery we were cautious about assuming that these common names corresponded only to these two species. Based on this assumption we expected to have more species than common names assigned to samples. The analyses of our sequences, however, notably reduced the 16 putative species to nine species of sharks.

In Peru, fishermen can use different names to identify shark species. While the common name hardly varies within a single landing point it can vary between landing points. We observed that, in general, species were identified by multiple common names (Table 1). For example, all the samples identified as *G. galeus* were labeled either as "crocodile P.", "NN" or "vidrio". The opposite occurs to, where a single common name is used to multiple species, thus reflecting the challenge to discriminate among shark species in the field. Indeed, all our samples labeled as "zorro" were identified as a single species of thresher, *A. pelagicus*, although in Peru, all three species of threshers are reported interacting with the fishery (Velez-Zuazo, 2012), but the landing statistics are exclusively reported for the common thresher and no landing information exist for the other two species. Based on our results we think a misidentification between *A. pelagicus* and *A. vulpinus* has been occurring at landing points and it is possible that reports pool together landing estimates of both species. These two species are hard to distinguish in the field and differ in coloration over the base of their pectoral fins (white for *A. pelagicus*) and alignment of posterior edge of dorsal fin with beginning of pelvic fins (not aligned for *A. pelagicus*, Compagno et al., 2005). Ongoing work includes the implementation of an identification guide for sharks of commercial importance that includes the common names used at landing points along the coast of Peru.

One sample labeled as "cazón" was identified equivocally as *C. obscurus* ("dusky shark") and as *C. galapagensis*, in both cases with 100% matching probability. The genetic and morphological similarity between these two species has been previously recognized (Holmes et al., 2009) with suggestions of a recent divergence of lineages (Naylor, 1992). The genetic similarity observed at the *cox1* region for these two species also has been reported for other mitochondrial molecular marker (i.e. NADH2, Naylor et al., 2012). As *C. galapagensis* has been already reported for Peru (Chirichigno and Cornejo, 2001) we abstained to consider this a new report for *C. obscurus* until further molecular evidence is obtained to distinguish them as different species.

4.2. Smooth-hounds in Peruvian in small-scale fisheries

Three samples representing two unique haplotypes were identified equivocally as *M. henlei* and *M. canis*. The lower matching probability observed, using both genetic distance and diagnostic character attributes, and the scarce information about smooth-hounds (*Mustelus* spp.) in Peru, however, prevented us from assigning them a species name. For the five species of smooth-hounds (*Mustelus* spp.) reported in Peru, the public repositories have barcodes available only for *M. henlei* and *M. lunulatus*. For the others species (*M. mento*, *M. dorsalis*, and *M. whitneyi*) barcodes exist only for *M. mento*, therefore, our comparisons, were only possible with *M. henlei*, *M. lunulatus* and *M. mento* and other available species of *Mustelus* sp. The mean genetic distance observed between our samples and *M. henlei* were 2.1%. A similar value was obtained with *M. canis* (2.3%), both of which were lower than the value proposed as the upper threshold to distinguish among species (3%, Hebert et al., 2004). For both species, BOLD had problems placing our samples with one or the other species. We believe, the samples are very likely from *M. whitneyi* for two reasons. First, in Peru, *M. whitneyi* is the species of smooth-hounds commonly landed and dominates the fishing reports (Velez-Zuazo, 2012). The other species are also landed, but are, by far, less abundant. Second, the samples of *Mustelus* spp. analyzed in our study were collected in San Jose, a point reporting the second highest landings for *M. whitneyi* (Velez-Zuazo, 2012). The challenge of identifying our samples to the species-level reflects the paucity of taxonomic and genetic studies about smooth-hounds in Peru, particularly for species like *M. whitneyi* that has a distribution range limited to Peru and Chile (Chirichigno and Cornejo, 2001; Compagno et al., 2005). From a conservation standpoint, the correct identification and discrimination of shark species is fundamental, particularly for species under threat, like *M. whitneyi*, which is currently considered as Vulnerable by the IUCN and with populations in decline (www.iucnredlist.org, accessed 27 September 2012). An on-going study is currently working toward the collection of a specimen of *M. whitneyi* to sequence the *cox1* region and report the barcode.

4.3. Use of combined genetic-based approaches for shark species identification

The distance-based approach used by BOLD allowed the identification of all our sequences. But we did as well used the library of character attributes for the 26 species of sharks in Peru to investigate its performance for identifying our sequences (see Supplementary Data). The character-based identification is based upon the assumption that species can be differentiated by a unique combination of characters (Sarkar et al., 2002) and for species with genetic distances lower than the cut-off value can perform better than the distance-based identification approach (e.g. Lowenstein et al., 2009). In our study, the use of diagnostic characters helped identifying all our samples as well. A good performance of the character-based approach over genetic distances for species identification has been reported before for tuna species, *Thunnus* spp. (Lowenstein et al., 2009), turtles (Reid et al., 2011), and marine gastropods (Zou et al., 2011), but their power for identification relies in a good representation of barcodes for each species. For sharks, some of the species had low representation (i.e. $\leq$2 barcodes) so an effort to increase the number of unique barcodes will improve this identification approach. Beyond reporting our results using both methods and avoiding advocating the use of one method over another, we suggest an integrative approach of methods for species identification. All the unique *cox1* sequences identified in our study and not repeated in BOLD and Genbank repositories have been submitted to Genbank to expand the barcode library of diagnostic characters for future identifications since more representations of singles species increases the power for species diagnoses (Rach et al., 2008).

With a shark fishery operating along the ~3000 km of coastline and with landings occurring in nearly 100 locations including ports, inlets and beaches, it is not surprising that this fishery is poorly monitored and managed (Alfaro-Shigueto et al., 2010; Velez-Zuazo, 2012). The great diversity and variability of common names used for sharks being landed challenges their identification by inspectors, particularly if inspectors have limited taxonomic skills for shark identification. The use of a barcode sequencing approach helped us to clarify the species composition and diversity in a subsample of the nearly 2000 samples collected over six years. Barcoding and barcode sequencing, in general, can greatly improve the current understanding of species diversity and should be incorporated in species inventories (e.g. Lowenstein et al., 2011) and, complemented with other methods, is useful for the identification of new species (DeSalle et al., 2005). The advent of new molecular techniques (i.e. Next generation sequencing – NGS) has, however, raised concerns about the validity of using ~650 bp of the *cox1* for species identification and the need for new approaches (Taylor and Harris, 2012). In spite of constant decreases in the costs of using NGS, this technique remains cost-prohibitive for many countries,

particularly developing countries like Peru, while Sanger sequencing is easily affordable and more likely to be funded. The conservation of sharks is gaining momentum and there is need for baseline information. Information at the molecular level is also being collected and could provide important insights for future fishery management of those species exploited at a commercial level. For shark species identification, *cox1* and now NADH2 (Naylor et al., 2012) are reliable molecular markers for identifying known species and "flagging" potential new ones (e.g. Naylor et al., 2012). In Peru, known shark species remain without barcodes (e.g. smoothhounds). This also applies to other elasmobranchs (i.e. rays). In this light, barcoding could be a good point from which to guide their conservation and for bringing attention to the myriad of other marine species that remain to be assessed, managed, and, even, discovered.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fishres.2014.06.005.

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