NOTE

DNA barcoding reveals overlooked shark and bony fish species in landing reports of small-scale fisheries from northern Peru

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ABSTRACT. Species-level identification of commercially landed fish provides pivotal information for stock assessment and fishery management. However, there is a common lack of species determination in landing records from small-scale fisheries (SSFs) worldwide. Using DNA barcoding analyses, we detected four overlooked bony fish (yellow snapper, union snook, blackspot wrasse, and steeplined drum) and one shark species (the sicklefin smooth-hound) in official landing records of SSFs from northern Peru. Of particular concern is the sicklefin smooth-hound shark *Mustelus lunulatus* that was found to be overlooked and could mistakenly be landed as the humpback smooth-hound *M. whitneyi*. Increased efforts should be made to improve species identification capacities in Peruvian fishing landings. There is an urgent need to quantify the catch levels of members of the genus *Mustelus* to species level. This would contribute to a better understanding of the levels of exploitation in each particular species and to improved management decisions.

Key words: Smooth-hound, mitochondrial DNA, marine diversity, shark conservation, *Mustelus*.

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Código de barras de ADN revela tiburón y peces óseos no identificados en las estadísticas de desembarque de las pesquerías artesanales del norte del Perú

RESUMEN. La identificación a nivel de especie de los peces desembarcados comercialmente proporciona información fundamental para la evaluación poblacional y la ordenación pesquera. Sin embargo, es común tener dificultades para determinar la identidad de algunas de las especies de las pesquerías artesanales (SSF) en todo el mundo. Utilizando el análisis de códigos de barras de ADN, se detectaron cuatro especies de peces óseos (pargo amarillo, robalito, doncella y bereche) y una especie de tiburón (tollo) pasados por alto en los registros oficiales de desembarque de SSF del norte de Perú. Particularmente preocupante es la ocurrencia del tollo *Mustelus lunulatus*, que podría estar desembarcándose por error como tollo común *M. whitneyi*. Deben realizarse mayores esfuerzos para mejorar las capacidades de identificación de algunas de las especies de los desembarques pesqueros de Perú. Existe una urgente necesidad de cuantificar los niveles de captura de miembros del género *Mustelus* a nivel de especie. Esto contribuiría a una mejor comprensión de los niveles de explotación de cada especie en particular y a la mejora de las decisiones de gestión.

Palabras clave: Tollo, ADN mitocondrial, diversidad marina, conservación de tiburones, *Mustelus*.

Accurate species identification at landing points is pivotal to assessing exploited stocks' diversity to improve conservation planning and management. Information about catch estimates and species composition allows managers to determine a fishery's status. Small-scale fisheries (SSFs) play a crucial role in producing fishing landing data through fishing logbooks or landing declarations. However, management in various SSFs remains underserved and is characterized by a common lack of species determination in landing records, affecting the accuracy of official fish catch and stock estimates (Morgan and Burgess 2005).

In Peru, estimated total landings of SSF during 2018 were around 1.1 million tons (De la Puente et al. 2020). However, despite its social and economic importance, most Peruvian SSFs have weak management systems (De la Puente et al. 2020; Gozzer-Wuest et al. 2021), limited records and low taxonomic resolution on fisheries landings (Velez-Zuazo et al. 2015; Amorós et al. 2017). Landing data from Peruvian SSFs is gathered by the Instituto del Mar del Perú (IMARPE) through a network of field observers (Guevara-Carrasco and Bertrand 2017). They are tasked with the complex assignment of identifying several aquatic species, including highly similar congeneric species or individuals that have lost their distinctive morphological characteristics due to cellular and biochemical changes associated with postmortem (Ocaño-Higuera et al. 2009). Species identification of shark landings can be even more challenging because they are often landed headless and finless; albeit shark finning has been banned since 2016 (Supreme Decree Nº 021-2016 PRODUCE, El Peruano 2016).

The Peruvian sea is home to at least 67 shark species (Cornejo et al. 2015; Kelez et al. 2020), half of which interact with Peruvian SSFs (Gonzalez-Pestana et al. 2016). Currently, six *Mustelus* species occur in Peruvian waters: *M. californicus*, *M. dorsalis*, *M. henlei*, *M. lunulatus*, *M. mento*, and *M. whitneyi* (Cornejo et al.

2015). In Peru, the humpback smooth-hound *M. whitneyi* is listed among the top six most landed shark species (Gonzalez-Pestana et al. 2016), representing the only member of *Mustelus* genus reported to species-level in recent landing records from northern Peru (i.e. the regions of Piura and Tumbes). Landing records from the same regions reported two other *Mustelus* species (*M. mento* and *M. dorsalis*) to species level only during the mid-1990s. On the other hand, the sicklefin smooth-hound *M. lunulatus* has never been reported to species-level in Peruvian landing records, despite being listed as a commercial species by IMARPE in the '90s decade (Elliot-Rodriguez et al. 1996). Furthermore, two independent molecular studies found *M. lunulatus* in retail markets and supermarkets from Lima and Tumbes (Marín et al. 2018; Biffi et al. 2020). *Mustelus* species are traded in domestic markets simply as *tollos* (López de la Lama et al. 2018). They often look similar and display partially overlapping characters within species, making their identification difficult even for experts (Morgan and Burgess 2005; Pérez-Jiménez et al. 2005). Aiming to reveal economically important species that could be overlooked in official landing reports, a DNA barcoding approach for the authentication of fish landings of SSFs from northern Peru was used. The results of genetic identification were contrasted against official landing records from Tumbes and Piura obtained over the past three decades.

A total of 95 fish samples were collected in twelve landing sites from northern Peru, including the regions of Piura (n = 8) and Tumbes (n = 4) (Figure 1; supplementary material, Table S1) from May to August 2019. Fin clip samples were preserved in ethanol 96% at the moment of landing and stored at -15 °C until DNA extraction. Genomic DNA was isolated using the phenol-chloroform method (Sambrook and Rusell 2001). We used two mitochondrial markers: cytochrome c oxidase subunit I (COI) and the control region (D-Loop); the latter was used only to discriminate
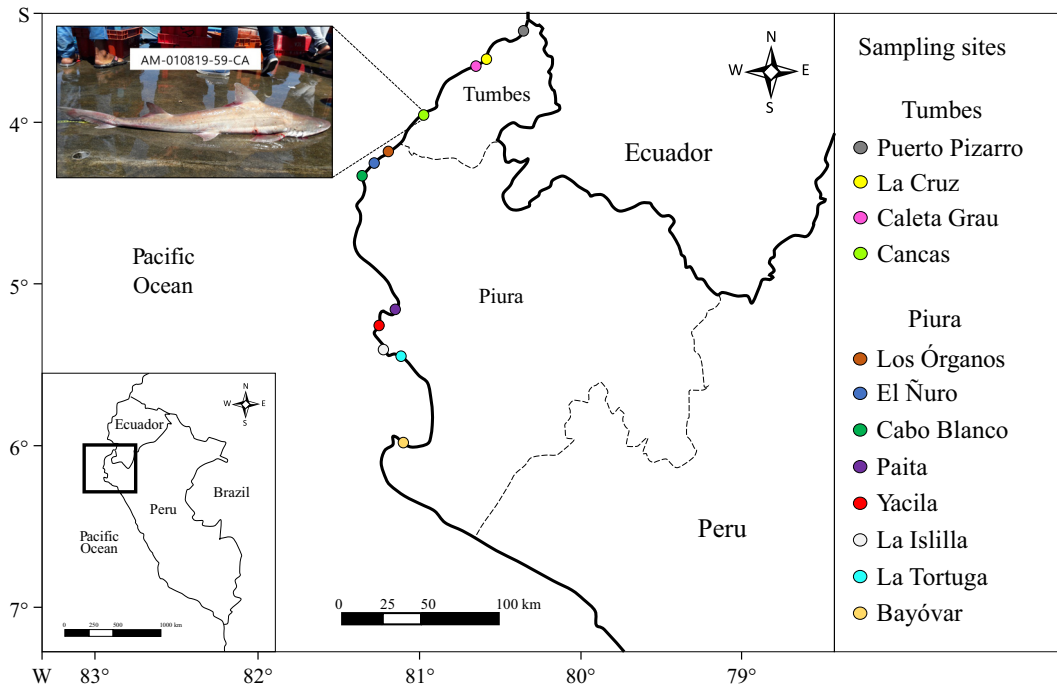
Figure 1. Sampling locations from this study. Fish landing sites from Tumbes region: Puerto Pizarro, La Cruz, Caleta Grau, and Cancas. Fish landing sites from Piura region: Los Órganos, El Ñuro, Cabo Blanco, Paita, Yacila, La Islilla, La Tortuga, and Bayóvar. The inset picture depicts the landing of *Mustelus lunulatus* collected at Cancas fish landing site (Tumbes).

tuna species (Pedrosa-Gerasmio et al. 2012) (primer sequences are shown in supplementary material, Table S2). PCR products were amplified in a local private laboratory using the same conditions described in Marín et al. (2018). PCR products were purified and sequenced at the Macrogen Inc. sequencing facilities in Korea. Sequences were manually edited using MEGA 7 (Kumar et al. 2016). For species identification, both the Identification Engine at the Barcode of Life Data System and the Basic Local Alignment Search Tool (BLAST) at the National Center for Biotechnology Information were used. Species identification obtained by DNA barcoding analyses was compared with the corresponding scientific and common/market names included in the IMARPE reports from Tumbes and Piura landing sites. These reports were issued from 1986 to 1988 (Wosnitza-Mendo et al. 1988), from 1996 to 2000 (Estrella et al. 1998a, 1998b, 1999 and

2000), and from 2010 to 2020 (Sistema de Captación de Información de la Pesca Artesanal del IMARPE) and were kindly provided by IMARPE (IMARPE 2019, 2020, 2021). DNA sequences obtained in this work were submitted to the GenBank database under the accession numbers MN880503 to MN880608.

DNA barcoding results revealed the presence of 40 fish species belonging to 15 families (supplementary material, Table S3). Among these species, five were found to be overlooked in the IMARPE landing reports by location or at least were not explicitly mentioned in the data provided by IMARPE (i.e. some species could be implicitly included in the statistics as aggregated records such as 'others' or registered to the genus level only). These five species were *M. lunulatus*, *Centropomus unionensis*, *Decodon melasma*, *Larimus acclivis*, and *Lutjanus argentiventris* (Table 1; supplementary material, Figure S1).

Table 1. List of overlooked species from the SSF in northern Peru as identified through DNA barcoding.

Species match (BOLD/NCBI)	Common name	Family	Label	Sampling site	Sampling date	n	GenBank ID accession
Mustelus lunulatus	Sicklefin smooth-hound	Triakidae	*Tollo mama*	Cancas, Tumbes	June 25, 2019	2	MN880526
					August 1, 2019		MN880605
Centropomus unionensis	Union snook	Centropomidae	*Robalo plateado*	Cancas, Tumbes	June 27, 2019	1	MN880528
Decodon melasma	Blackspot wrasse	Labridae	*San pedrano*	La Islilla, Piura	May 23, 2019	1	MN880543
Lutjanus argentiventris	Yellow snapper	Lutjanidae	*Paramo rojo*	Cancas, Tumbes	June 25, 2019	2	MN880525
			Paramo muelon		July 30, 2019		MN880602
Larimus acclivis	Steeplined drum	Sciaenidae	*Bereche*	La Tortuga, Piura	June 11, 2019	1	MN880556

Among the total number of smooth-hound shark samples collected in this study (n = 8), six samples (75%) were identified as *M. whitneyi* (100% sequence identity in BOLD). In comparison, two samples (25%) were identified as *M. lunulatus*. (99.7-99.8% sequence identity in BOLD, GenBank access MN880526 and MN880605). The two *M. lunulatus* samples were collected at Cancas landing site (Tumbes, collection date June and August 2019, supplementary material, Figure S1) and mislabeled as *tollo mama* (common name for *M. whitneyi*). Since this species is not reported in statistics, its landings could often be misidentified as *M. whitneyi* at landing points, or just listed in the IMARPE annual statistics landing reports under the category *tollo*. The latter may include different species such as *M. whitneyi*, *M. lunulatus*, *Triakis maculata* and *Schroederichthys chilensis* (Flores-Palomino et al. 1994; Flores 1996; Fernández et al. 2000). Additionally, the Peruvian Ministry of Production, which compiles official statistics, considers *tollo* as one single species despite being a common name for several species, as previously mentioned (PRODUCE, 2020).

A previous study by Marín et al. (2018) reported this species' incidence in a fillet sample bought in a supermarket in Lima (collection date June 2017). Similarly, using the DNA barcode approach, Biffi et al. (2020) identified four *M. lunulatus* samples (two came from retail markets in Tumbes and two from supermarkets in Lima) collected between May and June 2017. Before our study, there were no records of *M. lunulatus* collected from Peruvian landings and authenticated by DNA analysis. Together, these identifications support the hypothesis of regular catches of this species in northern Peru.

Globally, there is a general lack of data reporting on sharks' catch particularly species-specific data, which makes fisheries conservation and management challenging (NOAA 2020). Furthermore, most life history patterns of shark species (including *Mustelus* species) display slow growth, long gestation times, low fecundity, and late sexual maturity (Medina-Morales et al. 2020), which makes them especially vulnerable to exploitation. Therefore, there is an urgent need to quantify catch levels of members of the genus

Mustelus to species level. This would contribute to a better understanding of the exploitation level in each particular species, so as to improve management decisions (Pérez-Jiménez et al. 2016). Misidentifications of other *Mustelus* species, such as *M. whitneyi*, also raise concerns over its catch landing estimates, which may be biased by the landings of *M. dorsalis* and *M. mento* (reported to species level only during the years 1996, 1997, and 1999).

Further studies are needed to determine to what extent Peruvian landings of *M. lunulatus* (or other *Mustelus* species) are misidentified as *M. whitneyi*. Additionally, the IUCN Red List of Threatened Species listed *M. whitneyi* and *M. lunulatus* as critically endangered and least concern, respectively. Inaccurate species identifications may also have an impact on global IUCN listings. In this regard, a molecular survey of *Mustelus* landings could be a good starting point to obtain accurate baseline data. However, more substantial efforts must also be made to find more practical and cheaper species identification solutions. For instance, the possibility of developing field identification techniques based on diagnostic morphometric measurements is required, which should be developed alongside DNA barcoding to obtain more voucher specimens of *Mustelus* species. Additionally, multivariate and machine learning methods successfully applied in Carcharhinidae shark species should also be evaluated, as this approach could be applied to other Chondrichthyan species (Johnson et al. 2017). It is also highly recommended that field observers receive more intense training by recognized experts on identification of the *Mustelus* species. There are two identification guides for commercially important Peruvian shark species (IMARPE 2015; Romero et al. 2015), but none of these include *M. lunulatus*.

Our results also detected four landed Osteichthyes species that were not included in landing reports. Two yellow snappers *Lutjanus argentiventris* (supplementary material, Figure S2) were sampled at Cancas landing site (Tumbes, collection date June and July 2019), and one was misidentified as spotted rose snapper *L. guttatus* by fishermen. Official landing records only include two species of genus *Lutjanus*: *L. guttatus* and *L. jordani*. In another case, a sample taken in Cancas (Tumbes, collection date June 2019) landed as white snook *Centropomus viridis* was actually the union snook *Centropomus unionensis* (supplementary material, Figure S3). Official landing records include only *C. nigrescens* and aggregated records to genus level of *Centropomus* spp. One individual identified as the blackspot wrasse *Decodon melasma* (supplementary material, Figure S4) and one sample of the steeplined drum *Larimus acclivis* (supplementary material, Figure S5) collected in La Islilla and La Tortuga landing sites (Piura), respectively, were found to be overlooked in the official landing reports. Three other overlooked species detected in this study (*C. unionensis*, *L. acclivis*, and *L. argentiventris*) have been described as part of the marine diversity of Tumbes (Luque 2007), whereas the blackspot wrasse *D. melasma* has been reported in a survey cruise by IMARPE (Pastor et al. 2018). Nevertheless, there is still a lack of knowledge about the population status and landing estimates of these species. Further studies are needed to determine whether catches are significant or negligible from the point of view of the impacts of fishing on the health of the stocks.

Finally, this note emphasizes the usefulness and importance of DNA barcoding analyses in detecting species that may have been overlooked in official statistics. Accordingly, we recommend that proper attention should be paid to these events. More specialized training on how to identify morphologically similar species should be given to field observers and fishermen, which would significantly improve landing data and, consequently, could trigger future management actions to preserve Peruvian marine diversity.

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