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 steeped 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*.

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

**NOTE**

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

ALAN MARÍN1, *, RENATO GOZZER WUEST2, JORGE GRILLO-NUÑEZ2, IRINA B. ALVAREZ-JAQUE1 and JUAN CARLOS RIVEROS3

1Laboratorio de Genética, Fisiología y Reproducción, Facultad de Ciencias, Universidad Nacional del Santa, Av. Pacífico 508, Chimbote, Perú. 2REDES - Sostenibilidad Pesquera, Calle Porta N° 130, of. 608, 15074 - Lima, Perú. 3OCEANA - Perú, Av. del Ejército 250, of. 302, Lima, Perú. ORCID Alan Marín https://orcid.org/0000-0001-7842-133X, Renato Gozzer Wuest https://orcid.org/0000-0002-1156-4552, Jorge Grillo-Nuñez https://orcid.org/0000-0002-7501-6811, Irina Alvarez-Jaque https://orcid.org/0000-0001-5061-6485, Juan Carlos Riveros https://orcid.org/0000-0003-4051-181X

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, union snook, blackspot wasse, and steeped drum) y una especie de tiburón (el tiburón de hombro diente *Mustelus lunulatus*) que fue encontrada por error y podría ser desembarcada como el tiburón de hombro diente común *M. whitneyi*. Se deben realizar 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 Russell 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
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 *Mustelus lunulatus*, *Centropomus unionensis*, *Decodon melasma*, *Larimus acclivis*, and *Lutjanus argentiventris* (Table 1; supplementary material, Figure S1).
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 accession 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.

| 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 |
| *Centropomus unionensis* | Union snook | Centropomidae | *Robalo plateado* | Cancas, Tumbes | August 1, 2019 | 2 | MN880605 |
| *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 |
| *Larimus acclivis*       | Steeplined drum | Sciaenidae | *Bereche* | La Tortuga, Piura | July 30, 2019 | 1 | MN880602 |

Table 1. List of overlooked species from the SSF in northern Peru as identified through DNA barcoding.
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 (Johnson et al. 2017) could be applied to other Chondrichthyan 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 Ctenopoma viridis was actually the union snook Ctenopoma unionensis (supplementary material, Figure S3). Official landing records include only C. nigrescens and aggregated records to genus level of Ctenopomus spp. One individual identified as the blackspot wrasse Decodon melasma (supplementary material, Figure S4) and one sample of the steelpined 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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REFERENCES

AMOROS S, GOZZER R, MELGAR, V, ROVEGNO N. 2017. Peruvian mahi mahi fishery (Coryphaena hippurus): characterization and analysis of the supply chain. WWF (Marine Program of WWF) Peru. http://d2ouvy59p0dg6k.cloudfront.net/downloads/mahi_mahi_value_chain_en.pdf.

BIFFI D, LÓPEZ-MOBILIA A, KELEZ S, WILLIAMS DA, CHUMCHAL MM, WEINBURGH M. 2020. Mislabelling and high mercury content hamper the efforts of market-based seafood initiatives in Peru. Sci Rep. 10 (1): 1-12.

CORNEJO R, VÉLEZ-ZUAZO X, GONZÁLEZ-PESTANA A, KOURI C, MUCIENTES, G. 2015. An updated checklist of Chondrichthyes from the southeast Pacific off Peru. Check List. 11 (6): 1809.

DE LA PUENTE S, LÓPEZ DE LA LAMA R, BENAVENTE S, SUEIRO JC, PAULY D. 2020. Growing into poverty: Reconstructing Peruvian small-scale fishing effort between 1950 and 2018. Front Mar Sci. 7, 681.

EL PERUANO. 2016. Decreto supremo que establece medidas de ordenamiento para la pesquería del recurso tiburón. 2 de noviembre del 2016. Decreto Supremo Nº 021-2016-PRODUCE; [accessed 2021 September 16]. https://elperuano.pe/normaselperuano/2016/11/02/1448564-3.html.

ELLIOTT-RODRÍGUEZ W, PAREDES-BULNES, F, ZEVALLOS-FLOR J, JUÁREZ L, BARRETO-MEZA J. 1996. Nomenclatura actualizada de peces comerciales del Perú. Informe Progresivo Nº 37. Inf Inst Mar Perú. 12 p.

ESTRELLA-ARELLANO C, GUEVARA-CARRASCO R. 1998a. Informe Nº 131. Informe estadístico anual de los recursos hidrobiológicos de la pesca artesanal por especies, artes, caletas y meses durante 1996. Inf Inst Mar Perú. p. 215.

ESTRELLA-ARELLANO C, GUEVARA-CARRASCO R. 1998b. Informe Nº 132. Informe estadístico anual de los recursos hidrobiológicos de la pesca artesanal por especies, artes, caletas y meses durante 1997. Inf Inst Mar Perú. p. 412.

ESTRELLA-ARELLANO C, GUEVARA-CARRASCO R, AVILA-PÉREZ W, PALACIOS-LEÓN J, MEDINA-CRUZ A. 2000. Informe Nº 151. Informe estadístico de los recursos hidrobiológicos de la pesca artesanal por especies, artes, meses y caletas durante el segundo semestre de 1999. Inf Inst Mar Perú. 151: 1-194.

FAO. FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. 2020. Illuminating Hidden harvests study in the spotlight at COFI virtual dialogues. Voluntary guidelines for securing sustainable small-scale fisheries in the context of food security and poverty eradication online 17/07/2020; [accessed 2021 September 16]. http://www.fao.org/voluntary-guidelines-small-scale-fisheries/news-and-events/detail/en/c/1300712/

FERNÁNDEZ-RAMÍREZ F, VERA-DÍAZ S, MARCELO-PADILLA R, CHIRINOS E. 2000. Estadísticas de los desembarques de la pesquería marina peruana 1999. Inf Inst Mar Perú. 115.

FLORES M, VERA S, MARCELO R, CHIRINOS E.
1996. Estadísticas de los desembarques de la pesquería marina peruana durante 1995. Inf Prog Inst Mar Perú. 45. 35 p.

FLORES-PALOMINO M, VERA-DÍAZ S, MARCELO-PADILLA R, CHIRINOS E. 1994. Estadísticas de los desembarques de la pesquería marina Peruana 1983-1992. Inf Inst Mar Perú. 105. 202 p.

GONZALEZ-PESTANA A, KOURI C, VELEZ-ZUAZO X. 2016. Shark fisheries in the Southeast Pacific: a 61-year analysis from Peru. F1000Res. 3.

GOZZER-WUEST R, ALONSO-POBLETION E, TINGLEY G. 2021. Identifying priority areas for improvement in Peruvian Fisheries. Mar Policy. 129, 104545.

GUEVARA-CARRASCO R, BERTRAND A, editors. 2017. Atlas de la pesca artesanal del mar del Perú. Lima: Edición IMARPE-IRD. 183 p.

[IMARPE] INSTITUTO DEL MAR DEL PERÚ. 2015. Guía para la determinación de tiburones de importancia comercial en el Perú. Serie de divulgación científica. 1 (2). 80 p.

[IMARPE] INSTITUTO DEL MAR DEL PERÚ. 2019. Respuesta a la solicitud de acceso a la información pública: información sobre estimados de desembarque de los principales recursos hidrobiológicos registrados por la pesquería artesanal en las caletas de Tumbes y Piura, durante los años 2014-2018. Carta Nº 073 2019-IMARPE/OGA. Inst Mar Perú. 18 p.

[IMARPE] INSTITUTO DEL MAR DEL PERÚ. 2020. Respuesta a la solicitud de acceso a la información pública: información sobre estimados de desembarque de los principales recursos hidrobiológicos registrados por la pesquería artesanal en las caletas de Tumbes y Piura, durante los años 2010-2020. Oficios Nº 017, 018 y 019-2020-IMARPE/OGA. Inst Mar Perú. 18 p.

[IMARPE] INSTITUTO DEL MAR DEL PERÚ. 2021. Respuesta a la solicitud de acceso a la información pública: información sobre estimados de desembarque mensuales 2010-2020 en Piura y Tumbes de: Centropomus unionensis, Decodon melasma, Lutjanus argentiventris, Caulolatilus princeps, Pareques lanfeari, Larimus acclivis, Umbrina santi, Prionace glauca, Epinephelus analogus, Brotula orbivayi y Mustelus lunulatus. Oficios Nº 23 y 24-2021-IMARPE/OGA. Inst Mar Perú. 8 p.

JOHNSON GJ, BUCKWORTH RC, LEE H, MORGAN JAT, OVEN DEN JR, McM A HON CR. 2017. A novel field method to distinguish between cryptic carcharhinid sharks, Australian blacktip shark Carcharhinus tilstoni and common blacktip shark C. limbatus, despite the presence of hybrids. J Fish Biol. 90 (1): 39-60.

KELEZ S, NAPURI RM, PFENNING AM, MARTINEZ OC, CARRASCO AT. 2020. First reports of Megamouth Shark, Megachasma pelagios Taylor, Compagno & Struhsaker, 1983 (Lamniformes, Megachasmidae), in Peru. Check List. 16: 1361.

KUMAR S, STECHER G, TAMURA K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 33 (7): 1870-1874.

LÓPEZ DE LA LAMA R, DE LA PUENTE S, RIVEROS JC. 2018. Attitudes and misconceptions towards sharks and shark meat consumption along the Peruvian coast. PLoS ONE. 13 (8): e0202971.

LUQUE C. 2007. Estudio de la diversidad hidrobiologica en Tumbes. Informe Anual 2007. Tumbes: Inst Mar Perú. 36 p.

MARÍN A, SERNA J, ROBLES C, RAMÍREZ B, REYES-FLORES LE, ZELADA-MÁZMELA E, SOTIL G, ALFARO R. 2018. A glimpse into the genetic diversity of the Peruvian seafood sector: Unveiling species substitution, mislabeling and trade of threatened species. PLoS ONE. 13 (11): e0206596.

MEDINA-MORALES SA, CORRO-ESPINOYA D, ESCOBAR-SÁNCHEZ O, DELGADO-ÁLVAREZ CG, RUELLAS-INZUNZA J, FRÍAS-ESPERICUETA MG, et al. 2020. Mercury (Hg) and selenium (Se) content in the shark Mustelus henlei (Triakidae) in the northern Mexican Pacific. Environ Sci Pollut Res. 27 (14): 16774-16783.
MORGAN A, BURGESS GH. 2005. Fishery dependent sampling: total catch, effort and catch composition. In: MUSICK JA, BONFIL R, editors. Elasmobranch fisheries management techniques. FAO Tech Rep. 454. p. 182-200.

[NOAA] NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION. 2020. International affairs. Shark conservation. https://www.fisheries.noaa.gov/national/international-affairs/shark-conservation.

OCAÑO-HIGUERA VM, MARQUEZ-RÍOS E, CANIZALES-DÁVILA M, CASTILLO-YÁÑEZ FJ, PACHECO-AGUILAR R, LUGO-SÁNCHEZ ME, et al. 2009. Postmortem changes in cazon fish muscle stored on ice. Food Chem. 116 (4): 933-938.

PASTOR R, ZAVALAGA F, PALACIOS J. 2018. Caracterización de la comunidad bentodermeral que cohabita con la merluza Merluccius gayi peruanus Ginsburg entre la frontera norte del dominio marítimo peruano y Huarmey. 2015. Inf Inst Mar Perú. 45 (1): 104-121.

PEDROSA-GERASMIO IR, BABARAN RP, SANTOS MD. 2012. Discrimination of juvenile yellowfin (Thunnus albacares) and bigeye (T. obesus) tunas using mitochondrial DNA control region and liver morphology. PLoS ONE. 7 (4): e35604.

PÉREZ-JIMÉNEZ JC, NISHIZAKI OS, CASTILLO-GENIZ JL. 2005. A new eastern North Pacific smooth-hound shark (genus Mustelus, family Triakidae) from the Gulf of California. Copeia. (4): 834-845.

PÉREZ-JIMÉNEZ J, WHITE CF, RUIZ C, CARLISLE AB, LOWE CG. 2016. Mustelus lunulatus. The IUCN Red List of Threatened Species 2016: e.T161640A80672480.

[PRODUCE] MINISTERIO DE LA PRODUCCIÓN. 2020. Anuario estadístico pesquero y acuícola 2019. Lima: Ministerio de la Producción. 181 p.

ROMERO MA, ALCÁNTARA PF, VERDE K, editors. 2015. Guía de campo para la determinación de tiburones en la pesca artesanal del Perú. Lima: Inst Mar Perú. 15 p.

SAMBROOK J, RUSELL D. 2001. Molecular cloning: a laboratory manual. 3rd ed. New York: Cold Spring Harbor.

VELEZ-ZUAZO X, ALFARO-SHIJUETO J, MANGEL J, PAPA R, AGNARSSON I. 2015. What barcode sequencing reveals about the shark fishery in Peru. Fish Res. 161: 34-41.

WOSNITZA-MENDO C, ESPINO M, VELIZ M. 1988. La pesquería artesanal en el Perú durante junio de 1986 a junio de 1988. Inf Inst Mar Perú. 93. 142 p.