

*Research Articles*

## First record of a specimen of the shortbill spearfish *Tetrapturus angustirostris* Tanaka, 1915 in the Pacific coast of Mexico

José De La Cruz-Agüero<sup>1</sup>, Rogelio González-Armas<sup>1</sup>  
Francisco Javier García-Rodríguez<sup>1</sup> & Víctor Manuel Cota-Gómez<sup>1</sup>

<sup>1</sup>Departamento de Pesquerías y Biología Marina, Colección Ictiológica, Instituto Politécnico Nacional  
Centro Interdisciplinario de Ciencias Marinas (CICIMAR-IPN)  
La Paz, Baja California Sur, México  
Corresponding author: José De La Cruz-Agüero (jcruz@ipn.mx)

**ABSTRACT.** The shortbill spearfish *Tetrapturus angustirostris* Tanaka, 1915 is a widespread tropical and temperate species but is relatively rare, with very few published or cataloged records in museums and fish collections. We reported for the first time a specimen of *T. angustirostris* from the Pacific coast of Mexico, including meristic, morphometric and molecular data, which was caught in the vicinity of Los Cabos, Baja California Sur, Mexico, in November 2015.

**Keywords:** *Tetrapturus angustirostris*; voucher specimen; mitochondrial DNA; COI; mtCR; Cape San Lucas; Baja California

### INTRODUCTION

The shortbill spearfish *Tetrapturus angustirostris* Tanaka, 1915 is widely distributed throughout the tropical and temperate seas but rarely seen or captured compared to the rest of the billfishes. In the eastern Pacific, this species is found from California, USA, and the mouth of the Gulf of California to Peru, including all of the oceanic islands (Nakamura, 1985). This species is oceanic, epipelagic, offshore and deep-water, but is infrequently encountered by anglers in most parts of their range, or some cases are taken as by-catch by commercial longlines and secondarily in purse-seines and trolling (Kitchell *et al.*, 2006; Marín-Enríquez & Muhlia-Melo, 2018).

The primary distinguishing feature which separates all four spearfish species (*Tetrapturus* spp.) from marlins and sailfish is the position of the anus, or "vent." In all spearfishes, the anus is located well in front of the base of the first anal fin (at least as far forward of the anal fin as the longest spine of that fin), whereas in the other billfish the vent is located close to the anal fin (Pepperell, 2010). Other characteristics are a slender, lightweight body, short bill and a dorsal fin

that is highest anteriorly (greater than in marlin and lower than in the sailfish).

The shortbill spearfish *T. angustirostris* is listed in Annex I of the 1982 Convention on the Law of the Sea and in the Red List as Data Deficient (Collette *et al.*, 2011). However, there is little scientific information available about its biology and ecology (see Nakamura 1985).

According to with all available information (see below), despite its relatively small size among the billfish that would make it susceptible to being cataloged (normal length around 190 cm of total length (TL); Nakamura, 1985), there are scarce records of the shortbill spearfish for the eastern Pacific listed in museums or fish collections worldwide. In this regard, billfish specialists have claimed the lack of voucher specimens and their associated metadata (*i.e.*, molecular sequences, photographic images and GPS coordinates) which complicates the issue of species delineation, as some of these populations may also merit recognition as subspecies (Hanner *et al.*, 2011). In the same sense, given the strong morphological similarity of the billfishes, questionable identifications require reexamination with additional morphological and molecular data.

Particularly, in the Exclusive Economic Zone (EEZ) of Mexico (Pacific basin), the shortbill spearfish has been cited recently in several species' checklists (*e.g.*, for the Revillagigedo Islands: Castro-Aguirre & Balart-Páez, 2002; Del Moral-Flores *et al.*, 2016; Fourrière *et al.*, 2016). These works are based mainly on the results from the first ichthyological explorations date back to the late 19<sup>th</sup> and 20<sup>th</sup> centuries (*e.g.*, Jordan & McGregor, 1899; Snodgrass & Heller, 1905; Ricker, 1959) and museological online databases. However, in all those checklists the inclusion of the species, *i.e.*, *T. angustirostris*, has been made without confirmation of its presence and verification of voucher specimens. This is the same case of the observers aboard the fishing fleets where, due to the nature of the work, the registration of incidental fishing species (such as that of the shortbill spearfish) is made without the collection of specimens for their deposit and cataloging in museums or scientific collections (*e.g.*, Marín-Enríquez & Muhlia-Melo, 2018). In the present case, the existence and availability of a voucher specimen acquire greater relevance if one considers that the holotype of the species (formerly in the Zoology Department of Tokyo University Museum: ZUMT-4187) has been lost (Fricke *et al.*, 2019).

A specimen of *T. angustirostris* caught on the Pacific coast of Mexico was registered for the first time, including meristic, morphometric and molecular data.

## MATERIALS AND METHODS

On November 19, 2015, one specimen of *Tetrapturus angustirostris* (Fig. 1) was caught off Cabo San Lucas, Baja California Sur, Mexico (22°41'33"N, 109°50'33"W) aboard the "Shambala" 60 ft Hatteras yacht, captained by Ignacio "Nayo" Winkler. The fishermen used a trolling with a 30 lbs line and using circle hooks and Pacific sardine (*Sardinops sagax*) as bait. The shortbill spearfish was photographed *in situ* and then kept frozen within in the facilities of the "Pisces Fleet" at Cabo San Lucas, B.C.S. (<http://www.piscessport-fishing.com>). In December 2015, it was defrosted, weighted and identified firstly by both meristic and morphometric characters, which were taken following standard procedures, used in other descriptions of billfishes (Rivas, 1956; Robins, 1974; Nakamura, 1985).

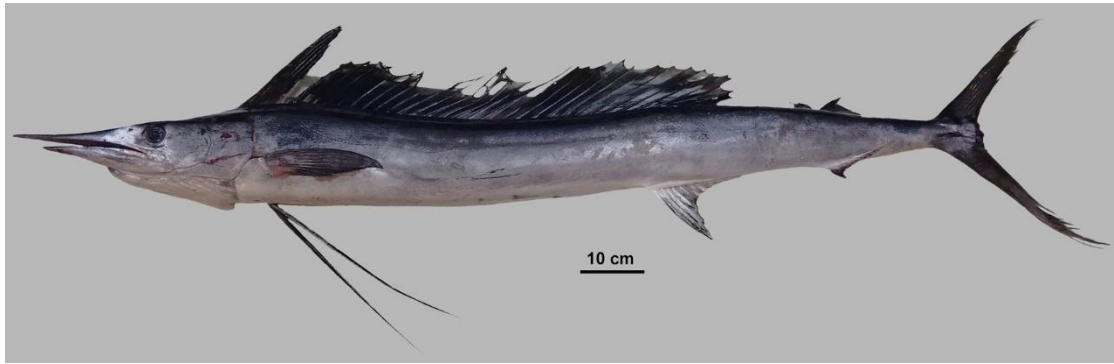
These counts and measurements are presented in Table 1, which were made on the left-hand side of the body the latter recorded to the nearest 0.1 cm. Before preservation, a small piece of muscle tissue from the pectoral left side was preserved in 96% ethanol for molecular analyses. After that, the specimen was fixed in formalin and later transferred to 70% ethyl alcohol

for preservation and deposited in the fish collection (CI) of the Centro Interdisciplinario de Ciencias Marinas (CICIMAR-IPN), in La Paz, B.C.S., Mexico, with the catalog number CICIMAR-CI 8328 (<http://coleccion.cicimar.ipn.mx>). Sex and maturity could not be established because it was not gutted for a better condition of conservation.

Three of the four recognized species of shortbill spearfish: *T. angustirostris* Tanaka, 1915, *T. belone* Rafinesque, 1810, and *T. pfluegeri* Robins & de Sylva, 1963, show highly similar DNA sequences and are not unambiguously distinguishable by barcodes alone (Hanner *et al.*, 2011). Consequently, to complete such metadata and recheck our identification, we supported the meristic and morphometric data using genetic information.

A total DNA was extracted from a muscle tissue sample using the QIAGEN<sup>®</sup> DNeasy Blood and Tissue kit (Catalogue N°Q01-69506, Hilden, Germany) following the manufacturer's protocol. Hanner *et al.* (2011) used the Cytochrome Oxidase subunit I (COI) to explore the *Tetrapturus* spp. complex. Besides, Collette *et al.* (2006) discovered that the control region (mtCR) could be used to support the discrimination and to recognize among these species. Thus, these two fragments of the mitochondrial DNA (mtDNA) were amplified by Polymerase Chain Reaction (PCR). For the COI, it was used the primers FishF2 and FishR2 (Ward *et al.*, 2005); and for the mtCR, the primers Pro-5 M13-F and D-loop (Collette *et al.*, 2006). Each reaction was made using a 35µL volume containing: 1X of PCR Buffer (MgCl<sub>2</sub>), 0.2 mM of dNTP mix, 0.48 µM of each primer, 4 mM of MgCl<sub>2</sub> and 0.05 U µL<sup>-1</sup> of Taq DNA polymerase (Invitrogen<sup>®</sup>, Carlsbad, CA, USA). An amount of 0.7 µL of total DNA was included in each reaction. Thermal cycler conditions for amplification consisted of an initial phase of 2 min at 94°C, followed by 35 cycles, and a final extension for 4 min at 72°C. Each of the 35 cycles consisted of three steps: a) 1 min at 94°C; b) 1 min at 58°C for 12 and 16 s; 1 min at 66.5°C for mtCR, and 30 s at 54°C for COI; and c) 2 min at 72°C. The PCR products were visualized on 1% agarose gels before purification and sequencing, using forward and reverse primers for each gene (Macrogen<sup>®</sup>, Seoul, Korea).

Sequences were arranged and edited in Sequencher 4.5 software (Gene Code, Ann Arbor, MI). Basic Local Alignment Search Tool (BLAST, <https://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used to explore the similarity of our sequence with sequences found in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>). Since the highest molecular percent identity was found with the *Tetrapturus* complex, we used sequences of COI and mtCR deposited in GenBank to detect clades.



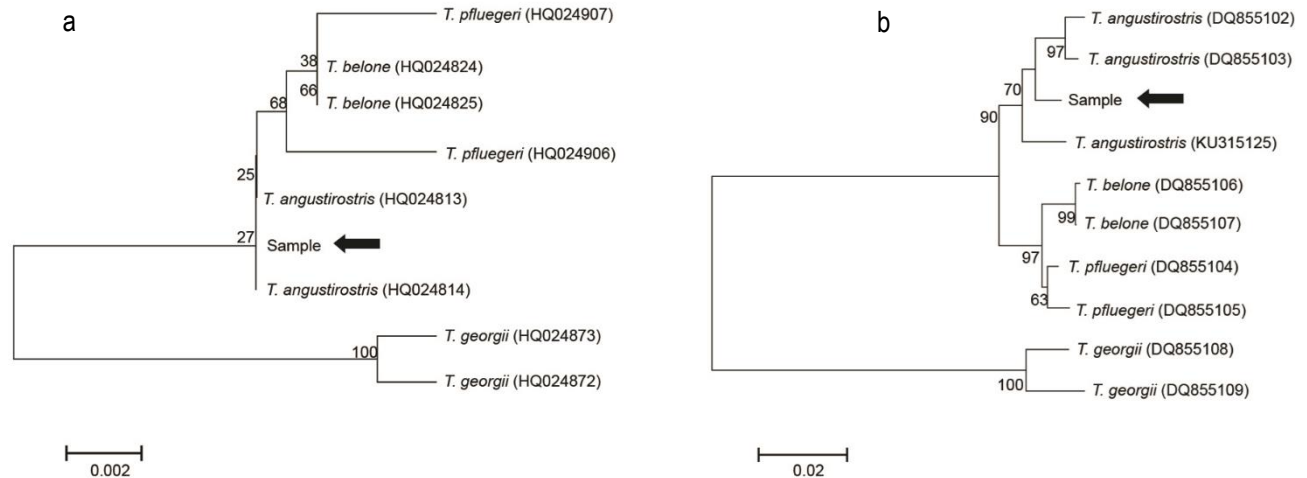
**Figure 1.** *Tetrapturus angustirostris* Tanaka, 1915 caught in November 2015, off Cabo San Lucas, Baja California Sur, Mexico. 153.7 cm body length and 11.650 kg total weight (CICIMAR-CI-8328).

**Table 1.** Morphometric and meristic characters of *Tetrapturus angustirostris* Tanaka, 1915 caught in November 2015, off Cabo San Lucas, Baja California Sur, Mexico (total weight 11.650 kg). Measurements expressed in centimeters are as defined by Rivas (1956).

Measurement/Count	Abbreviation	<i>T. angustirostris</i> CICIMAR-CI 8328
Body length	BL	153.7
Body girth	BG	41.4
Greatest body depth	GBD	21.2
Head length	HL	32.9
Snout length	SL	15.7
Bill length	BIL	20.3
Length of pectoral fin	PFL1	19.2
Length of pelvic fin	PFL2	36.3
Length of the second dorsal fin	DFL2	6.1
Length of second anal fin	AFL2	6.2
Depth of body at origin of first dorsal	DBD1	19.5
Depth of body at origin of first anal	DBA1	18.1
Width of body at origin of pectoral	WBP1	9.7
Width of body at origin of first anal	WBA1	9.2
Width of body at origin of second anal	WBA2	8.0
Width of caudal peduncle at the keel	WCP	3.8
Preopercular length	POL	26.5
Maxillary length	ML	15.5
Orbit diameter	OD	4.0
Interorbital width	IOW	7.6
Tip of the mandible to the tip of the bill	MLB	5.2
Length of upper caudal lobe	UCL	29.6
Length of lower caudal lobe	LCL	29.2
Caudal spread	CW	49.0
Caudal angle	CA	126°
Dorsal fin rays		46
Anal fin rays		12 (IV-8)
Pectoral fin rays		31-32

Neighbor-Joining (NJ) trees of Kimura two-parameter (K2P) distance (Kimura, 1980), were created to provide a graphical representation of the pattern of divergence between species and for supporting our identification

(Saitou & Nei, 1987). The robustness of the internal branches of the trees was supported by 1,000 bootstrap replications (Felsenstein, 1985). This analysis was performed with MEGA5 (Tamura *et al.*, 2011). It should



**Figure 2.** Neighbor-joining tree based on K2P distances of a) Cytochrome Oxidase subunit I (COI) and b) Control Region (CR) sequences of *Tetrapturus* complex. Numbers associated with the scientific name are the GenBank accession numbers. Arrows indicates our sample.

be noted that the tree depicted (Fig. 2) is not intended as a hypothesis of phylogenetic relationships but merely as a visual representation of haplotype groupings (Hanner *et al.*, 2011).

The specialized literature and several recent bibliographic published were consulted and reviewed deliberately to compare the record from this study with all available distribution information of the shortbill spearfish species (see references). In this way, a clear and cumulative database was compiled with all existing records for *T. angustirostris* within the eastern Pacific. This database was cross-referenced with information available online from biological collections and museums worldwide. Global databases consulted included the Ocean Biogeographic Information System (OBIS, 2019), the Global Biodiversity Information Facility (GBIF, 2019), and the FishBase Project (Froese & Pauly, 2019). The spellings in the citations of the common names of the species (English and Spanish, *sensu* Page *et al.*, 2013) follows Nelson *et al.* (2002). Finally, interviews with local fishers and fleet owners were conducted by the end of 2015, in Los Cabos, B.C.S. region, to assess if other shortbill spearfish specimens were captured in the same area.

## RESULTS

A picture of the shortbill spearfish is shown in Fig. 1. The specimen (153.7 cm body length, 11.650 kg total weight) was characterized by its body elongate and fairly compressed. The bill is short and slender, round in cross-section. Lower mandible shorter than the upper

jaw, but still projecting. Branchiostegal membranes completely united to each other, but free from isthmus. Two dorsal fins, the first with 46 rays and with a pointed anterior lobe, higher than body depth anteriorly; the base of the dorsal fin is long, extending from posterior margin of preopercle to near second dorsal fin origin. Second dorsal fin with six rays, its position slightly backward concerning the second anal fin. Two anal fins, the first with 12 rays (IV-8), and the second with six rays and very similar in size and shape to the second dorsal fin; pectoral fins with 31-32 rays. Pelvic fins slender, about twice the length of the pectorals. Caudal peduncle moderately compressed with double keels on each side and a shallow notch on both dorsal and ventral surfaces. The anus situated far anterior to first anal fin origin, at a distance usually longer than the height of the first anal fin. Lateral line single and well visible. Body densely covered with elongate bony scales, each scale with three to five posterior prolongations. Color: body dark blue dorsally, blue splattered with brown laterally, and silvery white ventrally, without dots or stripes. First dorsal fin dark blue and without dots or blotches; remaining fins brown or dark brown; bases of first and second anal fins often shading with silvery white. Meristic and morphometric characters are presented in Table 1. We successfully amplified a COI fragment up 677 bp and an mtCR fragment over 902 bp. The sequences were deposited in GenBank (accession numbers, COI: MF038038; mtCR: MF038039). The BLAST searches confirmed that one fragment belonged to COI and the other to mtCR. Finally, the exploration of the museological online databases revealed the existence of seven cataloged

**Table 2.** The voucher and reported specimens of the shortbill spearfish *Tetrapturus angustirostris* Tanaka, 1915, caught in the eastern Pacific Ocean. Data logged in the Global Biodiversity Information Facility (<http://www.gbif.org/>), the Ocean Biogeographic Information System (<http://www.iobis.org/>) and the FishBase Project (<http://www.fishbase.org/>). All accessed in February 2019. LACM: The Natural History Museum of Los Angeles County. CAS: Department of Ichthyology of the California Academy of Sciences. UF: The Florida Museum of Natural History Ichthyology Collection. SIO: Scripps Institution of Oceanography. \*Current record in Colección Ictiológica (CI) of Centro Interdisciplinario de Ciencias Marinas del Instituto Politécnico Nacional. B.C.: Baja California. B.C.S.: Baja California Sur.

Institution	Catalog number	Geographic Location	Country	Locality	Date DD/MM/YY	Type of the record
LACM	25499	19°30'N, 156°00'W	USA	off Kona, Hawaii	13/08/1985	Preserved specimen
LACM	25478	19°30'N, 156°00'W	USA	off Kona, Hawaii	16/08/1985	Preserved specimen
LACM	25422	19°38'N, 156°00'W	USA	off Kailua-Kona, Hawaii	11/08/1988	Preserved specimen
LACM	25421	19°38'N, 156°00'W	USA	off Kailua-Kona, Hawaii	11/08/1988	Preserved specimen
CAS	88961	19°28'N, 155°58'W	USA	Kealakekua Bay, Hawaii	28/10/1973	Preserved specimen
FishBase	No Data	30°01'N, 113°31'W	Mexico	Upper Gulf of California	02/10/1996	Unknown
UF	76-341	31°10'S, 110°11'W	Chile	off Coquimbo	15/04/1958	Preserved specimen
SIO	208791	29°56'N, 71°19'W	International waters	154 miles off B.C.	15/10/1976	Preserved specimen
*CICIMAR-IPN	CI-8328	23°41'N, 109°50'W	Mexico	off Cabo San Lucas, B.C.S.	19/11/2015	Preserved specimen

specimens (plus one without data) from the central and eastern Pacific (Table 2). Although one of these specimens (SIO 76-341) is registered as caught off Baja California Norte, Mexico, that record does not correspond to the country, according to the delimitation of the EEZ of the Mexican Pacific (DOF, 1976), and none of the current specimens have been documented in any reference.

## DISCUSSION

All features of the captured specimen fitted well with the description of *Tetrapturus angustirostris* available in the literature (*i.e.*, Nakamura, 1985; Pepperell, 2010). *T. angustirostris* differs from the other Pacific billfishes (*e.g.*, *Xiphias gladius*, *Istiophorus platypterus*, *Kajikia audax*, *Makaira* spp.) by a number of main characters, such as pelvic fins present, first dorsal fin lower than body depth at level of midbody, not sail like; bill very short (less than 15% of body length), usually equal to, or shorter than head length, and the anus situated far anterior to first anal fin origin (Nakamura, 1985). The highest nucleotide identity values for the COI were obtained with the *T. angustirostris* or *T. pfluegeri* (100%) and for the mtCR only with *T. angustirostris* (100%). Considering genetic information of the species in the *Tetrapturus* complex, we support that our specimen belongs to the species *T. angustirostris* (Fig. 2).

However, it is not overlooked that the species-level (alpha) taxonomy of the spearfishes is not well resolved and species identification is difficult, especially in some areas of the eastern north Atlantic where three species may occur sympatrically (McDowell *et al.*, 2018). In this sense, the poor condition of some of the

type materials, incomplete descriptions, and the loss or non-designation of type materials have made it difficult to carry out taxonomic studies. Therefore, because of the loss of the holotype, the species *Tetrapturus angustirostris* Tanaka, 1915 it may require the designation of a neotype.

According to information provided by local fishermen in the south of the peninsula of Baja California, *T. angustirostris* is rare and uncommon in catches by the sports fishing fleet. The same applies to the tuna purse seine fleet in the tropical eastern Pacific, wherein 23 years and near 425,000 fishing sets, only the incidental catch of 687 individuals were recorded (Marín-Enríquez & Muhlia-Melo, 2018). So, it is not unusual that there are very few published or cataloged records in museums and fish collections, not only for the shortbill spearfish in the Mexican waters but all spearfishes worldwide, and particularly in the central and eastern Pacific (Table 2). Consequently, it can be said that within the istiophorid billfishes, the spearfishes (*Tetrapturus* spp.) yet they are the least studied due to their rarity (McDowell *et al.*, 2018). Because there are no particular fisheries for the shortbill spearfish, we expect that data from mitochondrial loci and morphological traits documented herein may be useful to compare to other regions inhabited by the species and for future studies on the relationships among spearfishes. The specimen is available for examination at CI-CICIMAR, and sample muscle or DNA aliquots may be obtained upon request.

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