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RESEARCH NOTE

New record and range extension of the roughskin spurdog *Cirrhigaleus asper* in the Caribbean Sea

Nuevo registro y extensión de rango del tiburón espinudo *Cirrhigaleus asper* en el Mar Caribe

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Abstract.- This study constitutes the first record and a range extension of the roughskin spurdog (*Cirrhigaleus asper*) in the Caribbean Sea. A total of three mature female specimens were captured by artisanal fishermen between March and May of 2016 in the coastal community of El Quetzalito, Izabal, Guatemala. The total length of the sharks ranged from 1,110-1,280 mm, which is the largest total length so far reported for this species.

Key words: Deep-sea species, elasmobranch, Squalidae, Guatemala

INTRODUCTION

The family Squalidae (dogfish sharks) includes two genera: *Cirrhigaleus* (Tanaka, 1912) with three species; and *Squalus* (Linnaeus, 1758) with 26 species (Ebert *et al*. 2015). This family is distributed worldwide in temperate and tropical seas, with a preference for deep environments (>50 m) where they feed on benthic fishes and invertebrates (Compagno 2002, Compagno *et al*. 2005, Ebert *et al*. 2015). In the Atlantic, eleven species are reported (Compagno 2002, Ebert *et al*. 2015, Pfleger *et al*. 2018, Veríssimo *et al*. 2016, Viana *et al*. 2016, Finucci *et al*. 2020a).

The roughskin spurdog *Cirrhigaleus asper* Merrett, 1973 was first described by Merrett (1973) in the equatorial western Indian Ocean based on a 901 mm total length (LT) mature male holotype. This species has been reported in the South Atlantic Ocean, West and Central Indian Ocean and in the Central Pacific Ocean (Hawaiian Islands). In the Western Atlantic, *C. asper* has been reported from North Carolina to Florida, in the Gulf of Mexico and the South West of Brazil with no records in the Caribbean Sea (Compagno 2002, Castro 2011, Ebert *et al*. 2015, Rincon *et al*. 2017, Del Moral-Flores *et al*. 2018). According to Ebert *et al*. (2015) and Finucci *et al*. (2020b), *C. asper* inhabits continental shelves and insular slopes of warm temperate to tropical seas, ranging from 0-1,370 m. However, it can also be observed in river mouths and outer bays (Compagno 1984, Ebert *et al*. 2015).

The biological information of the *C. asper* is deficient. The age of maturity for this yolk sac viviparous species is unknown as well as longevity and gestation (Finucci *et al*. 2020b). However, size at maturity occurs between 89-118 cm for females, and 85-90 cm for males, with a size of 25-28 cm at birth, and a fecundity of 18-22 pups (Ebert *et al*. 2013, Finucci *et al*. 2020b). In terms of conservation status, *C. asper* is listed as "Data Deficient" due to the scarce information available to assess extinction risks based on their distribution and population trends by the International Union for Conservation of Nature (IUCN) (Finucci *et al*. 2020b). This report refers to a new record and range extension of *C. asper*, providing the first scientific evidence of the occurrence of *C. asper* off the coast of Guatemala, in the Caribbean Sea, based on three female specimens captured as bycatch of artisanal fisheries.

Materials and methods

Specimens examined and morphometrics

A total of three specimens of *C. asper* were incidentally captured by artisanal fishermen of the coastal community of El Quetzalito, Izabal, Guatemala (15°52.374 N, 88°18.712 W; Fig. 1). Two such specimens were captured on March 20th, 2016 with a 1,000 m long bottom trammel mesh net of 3.5 inches and one panel. Later, on May 18th, 2016 a new specimen of *C. asper* was captured with a 2,000 m long line with 200 hooks (#16). All three specimens were captured at approximately 200 m depth, based on the known length of the fishing gear deployed.

All specimens were examined and identified using identification guides (Compagno 1984, Compagno *et al.* 2005, Ebert *et al.* 2015). Regarding morphometry, a total of 76 measurements (following Compagno 2002) were registered for the specimen Id_215 (Table 1; Fig. 2a). Additionally, tissue sample was collected, for genetic identification, only for the specimen Id_235. Unfortunately, deposition of any specimen in a museum/academic collection was not possible due to the fisherman's decision to use the meat.

Genetic identification

DNA was extracted using the Wizard Genomic DNA Purification Kit (PROMEGA®, Promega Inc., Madison, WI). The cytochrome oxidase subunit one (COI or CO_1) was amplified by PCR using the following primers FISH F2: 5TCGACTAATCATAAAGATATCGGCAC' and FISH R2: 5'ACTTCAGGGTGACCGAAGAATCAGAA3' (Ward *et al.* 2005). Amplifications were carried out in a PCR of 15 µL of volume with 10X PCR buffer, 25 mM $MgCl_2$, 10 mM dNTPs, 10 µM of each primer, 5 units of Dream Taq polymerase (Thermo Scientific™) and 1 µL of DNA. PCR was performed on a SimpliAmp cycler (Applied Biosystem, USA). An initial denaturing step was carried out at 95 °C for 2 min, followed by 30 cycles with 30 s at 94 °C, 30 s at 55 °C, and 1 min at 72 °C, followed by a final extension step of 10 min at 72 °C. The PCR products were sent to the molecular cloning laboratory (MCLAB) in the USA for Sanger sequencing on an ABI 3730 XL Genetic analyzer in both the forward and reverse directions. Forward and reverse sequences were edited and aligned using the GENEIOUS v10.2.3 software to solve ambiguities for the confirmation of nucleotide bases. Sequence divergences were calculated

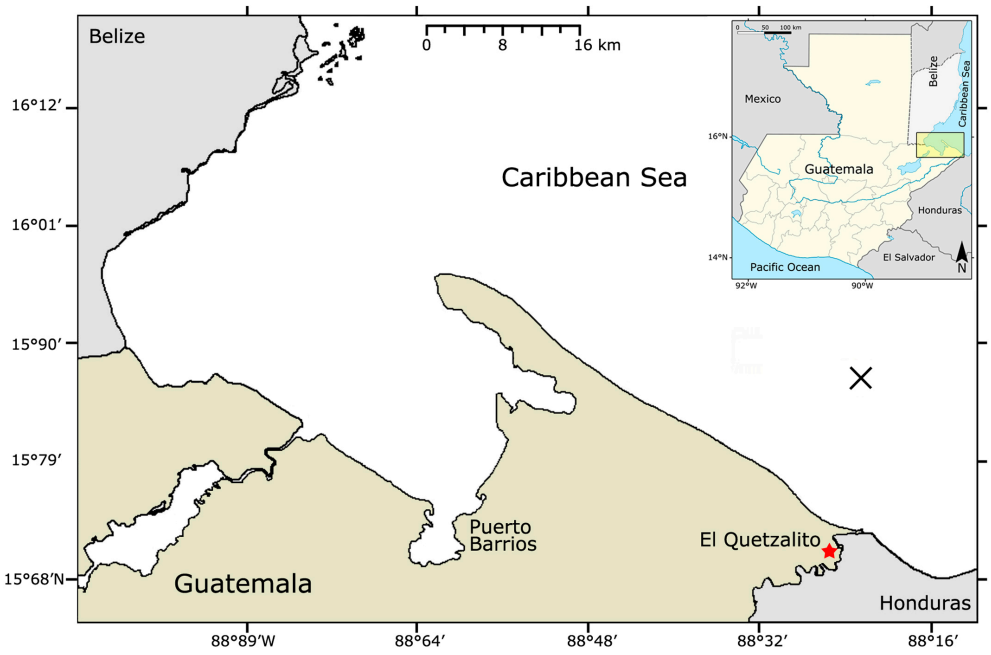

Figure 1. Study area and capture location (x) of *Cirrhigaleus asper* in the coastal zone of El Quetzalito, Izabal, Guatemala / Área de estudio y localidad de captura (x) de *Cirrhigaleus asper* en la zona costera de El Quetzalito, Izabal, Guatemala

[1]GenBank Overview. National Library of Medicine. National Center for Biotechnology Information (NCBI). <https://www.ncbi.nlm.nih.gov/genbank/>

Table 1. Morphometric measurements (mm) of one female specimen of *Cirrhigaleus asper* (Id_215) captured by artisanal fishermen between March and May of 2016 in the coastal zone off El Quetzalito, Izabal, Guatemala / Medidas morfométricas (mm) del espécimen hembra de *Cirrhigaleus asper* (Id_215) capturado por pescadores artesanales entre marzo y mayo de 2016 en la zona costera de El Quetzalito, Izabal, Guatemala

Measurements	Length (mm)	Proportion (%)	Measurements	Length (mm)	Proportion (%)
Total length (TL)	1,160	100.00	Pectoral-fin anterior margin (PIA)	152	13.10
Fork length (FL)	1,041	89.74	Pectoral-fin base (PIB)	65	5.60
Precaudal fin length (PCL)	960	82.76	Pectoral-fin inner margin (PLI)	132	11.38
Pre-second dorsal-fin length (PD2)	790	68.10	Pectoral-fin posterior margin (PIP)	133	11.47
Pre-first dorsal-fin length (PD1)	410	35.34	Pectoral-fin height (PIH)	140	12.07
Head length (HDL)	187	16.12	Dorsal caudal-fin margin (CDM)	240	20.69
Prebranchial length (PG1)	121	10.43	Preventral caudal-fin margin (CPP)	55	4.74
Prespiracular length (PSP)	78	6.72	Upper postventral caudal-fin margin (CPU)	175	15.09
Preorbital length (POB)	52	4.48	Lower postventral caudal-fin margin (CPL)	55	4.74
Prepectoral-fin length (PP1)	223	19.22	Caudal-fin fork width (CFW)	102	8.79
Prepelvic-fin length (PP2)	770	66.38	Caudal-fin fork length (CFL)	203	17.50
Snout-vent length (SVL)	696	60.00	Subterminal caudal-fin margin (CST)	39	3.36
Interdorsal space (IDS)	260	22.41	Subterminal caudal-fin width (CSW)	64	5.52
Dorsal caudal-fin space (DCS)	105	9.05	Terminal caudal-fin margin (CTR)	74	6.38
Pectoral-fin pelvic-fin space (PPS)	480	41.38	Terminal caudal-fin lobe (CTL)	76	6.55
Pelvic-fin caudal-fin space (PCA)	215	18.53	Pelvic-fin length (P2L)	124	10.69
Vent caudal-fin length (VCL)	453	39.05	Pelvic-fin anterior margin (P2A)	84	7.24
Prenarial length (PM)	42	3.62	Pelvic-fin base (p2b)	75	6.47
Preoral length (POR)	46	3.97	Pelvic-fin height (P2H)	80	6.90
Eye length (EYL)	45	3.88	Pelvic-fin inner margin [length] (P2I)	65	5.60
Eye height (EYH)	18	1.55	Pelvic-fin posterior margin [length] (P2P)	98	8.45
Intergill length (ING)	51	4.40	Head height (HDH)	137	11.81
First gill slit height (GS1)	30	2.59	Trunk height (TRH)	147	12.67
Second gill slit height (GS2)	29	2.50	Abdomen height (ABH)	165	14.22
Third gill slit height (GS3)	32	2.76	Tail height (TAH)	90	7.76
Fourth gill slit height (GS4)	34	2.93	Caudal-fin peduncle height (CPH)	65	5.60
Fifth gill slit height (GS5)	34	2.93	Pelvic midpoint -first dorsal base end (PDI)	180	15.52
First dorsal-fin length (DIL)	179	15.43	Pelvic midpoint - second dorsal origin (PDO)	195	16.81
First dorsal-fin anterior margin (DLA)	145	12.50	Second dorsal insertion - insertion anal (DAI)	91	7.84
First dorsal-fin base (DIB)	95	8.19	Mouth length (MOL)	91	7.84
First dorsal-fin height (DLH)	103	8.88	Mouth width (MOW)	42	3.62
First dorsal-fin inner margin (DLI)	76	6.55	Upper labial-furrow length (ULA)	25	2.16
First dorsal-fin posterior margin (DIP)	125	10.78	Lower labial-furrow length (LLA)	21	1.81
Second dorsal-fin length (D2L)	135	11.64	Nostril width (NOW)	44	3.79
Second dorsal-fin anterior margin (D2A)	134	11.55	Internarial space (INW)	45	3.88
Second dorsal-fin base (D2B)	85	7.33	Interorbital space (INO)	9	0.78
Second dorsal-fin height (D2H)	92	7.93	Spiracle length (SPL)	14	1.21
Second dorsal-fin inner margin (D2I)	63	5.43			
Second dorsal-fin posterior margin (D2P)	101	8.71			

using a Kimura two-parameter (K2P) distance model (Kimura 1980). Neighbor-joining (NJ) tree of K2P distances were estimated to provide a tree representation of the divergence between the *Cirrhigaleus* species for the CO_1 gene sequence obtained from from Barcode of Life DataSystems (BOLD) (Ratnasingham & Hebert 2007) and GenBank from the NCBI[1]. The NJ tree was performed in MEGAX (Kumar *et al*. 2018) with 1000 replications.

Results and discussion

The species of the genus *Cirrhigaleus* are distinguished from *Squalus* species by having a very elongated secondary lobe and by forming nasal barbels that extend to the anterior margin of the mouth while species of the genus *Squalus* have anterior nasal flaps with a short secondary lobe and, without forming nasal barbels (Compagno *et al*. 2005). The species of the genus *Cirrhigaleus* evidenced a similar length in both dorsal fins while in the *Squalus* species the second dorsal fin is smaller and lower than the first dorsal fin (Compagno *et al*. 2005). In *Cirrhigaleus* species, the second dorsal fin spine it is equal in length to the first dorsal fin spine. In contrast, *Squalus* species show a second dorsal fin spine larger than the first dorsal fin spine. In general, the body of the *Cirrhigaleus* species is robust and markedly humped dorsally, when compared with the genus *Squalus*, in which the members of this group evidenced a fusiform body arched dorsally throughout all of its length (Viana *et al*. 2016).

The specimens were identified as *Cirrhigaleus asper* due to the presence of a stocky body covered with a rough skin, a short-rounded snout and a broad flat head. The body showed a white pigmentation in the belly and a light brown color on the dorsal surface. The margins of the two dorsal fins were white and the second dorsal fin was about as large as the first, in which a strong and long spine was observed in both dorsal fins. The origin of the first dorsal was behind the pectoral fin rear tips (Compagno *et al*. 2005; Fig. 2a, b). Additionally, specimens examined had big nostrils and large-mouth upper labial furrows that were larger than the lower labial furrow length (Fig. 2a). Finally, the specimens presented anterior nasal flaps, with short barbels, a distinguished characteristic that differentiate *C. asper* from its congeners *Cirrhigaleus babifer* Tanaka, 1912 and *Cirrhigaleus australis* White, Last & Stevens, 2007 (Ebert *et al*. 2015). Specimens comprised the following morphometric measurements: Id_215: 1,160 mm total length (TL), 1,060 mm precaudal length (PCR), 960 mm fork length (FL) and mature female (Code number= Id_215) (Fig. 2a): Id_216: 1,100 mm TL, 1,030 mm PRC, 970 mm FL and mature female (Code number= Id_216); Id_235: 1,280 mm TL, 1,200 mm PRC, 1,080 mm FL and mature female (Code number= Id_235) (Fig. 2b).

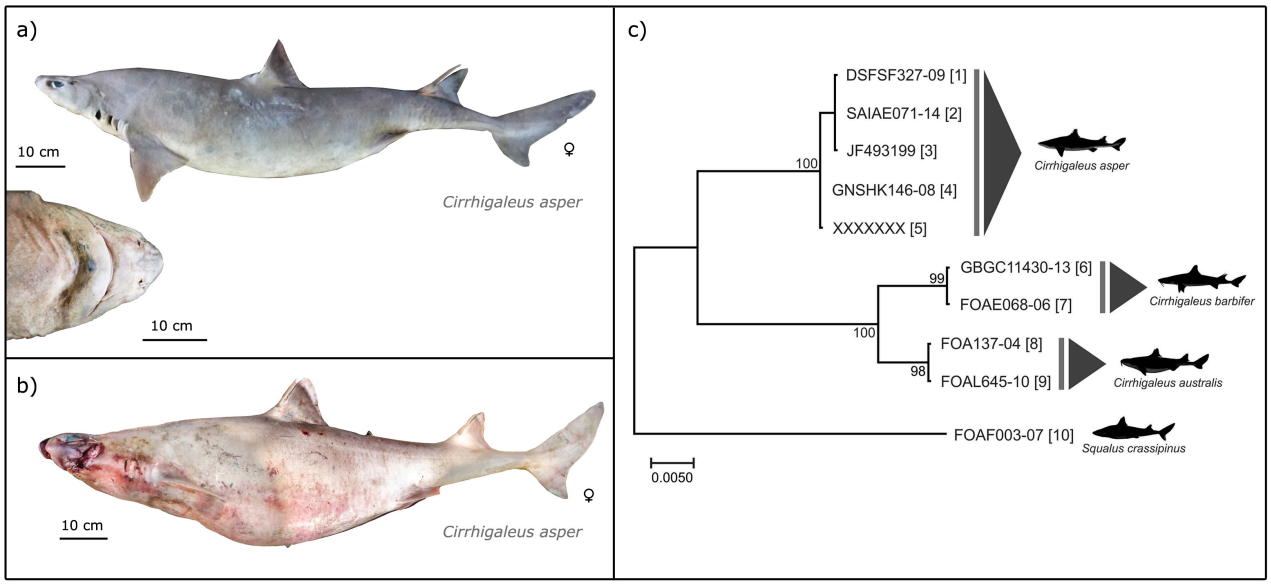

Figure 2. *Cirrhigaleus asper* specimens: a) Id_215 (1,160 mm TL, mature female); b) Id_235 (1,280 mm TL, mature female); c) Id_235, neighbour-joining phylogenetic tree of *C. asper* and related species, inferred from gene sequence (COI) / Especímenes de *Cirrhigaleus asper*. a) Id_215 (1.160 mm LT, hembra madura); b) Id_235 (1.280 mm LT, hembra madura); c) Id_235, árbol filogenético generado por el método de unión de vecinos de *C. asper* y sus especies relacionadas, inferida a partir de la secuencia del gen (COI)

Edited COI sequences were 573 bp long from the specimen identified as Id_235 from Guatemala, in the Caribbean coast (GenBank Accession number MN982926). The sequence is identical to an available voucher specimens of *C. asper* (DSFSF327-09), and only differing by one bp from the other sequences identified as *C. asper* obtained from GenBank (JF43139) and from two other voucher specimens of *C. asper* (SAIAE071-14 and CNSHK146-08). However, the CO_1 sequence of Id_235 differed by 23 substitutions from those of two voucher specimens of *C. australis* (White *et al*. 2007) (FOA137-04 and FOAL645-10), and by 24 bp substitutions from one specimen of *C. barbider* (Kempster *et al*. 2013) (FOAE068-06). One sequence (KC349854) of *Squalus crassispinus* Last, Edmunds & Yearsley, 2007 (FOAFOO3-07), it was used as an out-group. The percentages of identity are represented and supported for each branch in the NJ tree (Fig. 2c).

These specimens confirm the first record of the roughskin spurdog *C. asper* in the Caribbean Sea, off Guatemala. The species has been reported in the Western North Atlantic, the Gulf of Mexico (Campeche), on the southern coast of Brazil and the western coast of Venezuela (Península de Paraguaná) (Castro 1983, Compagno 2002, Fischer *et al*. 2006, Rincon *et al*. 2017, Del Moral *et al*. 2018, Ehemann *et al*. 2019). The roughskin spurdog is captured incidentally as a result of trawling, longline fishery, and tilefish fishery in the Southeastern coast of the United States (Compagno 2002). According to Compagno (1984) and Castro (2011), *C. asper* has no commercial importance to date. However, *C. asper* is captured incidentally with trammel net and longline in the Caribbean, off Guatemala, and in recent decades the fishers have sold its meat and liver oil to local markets, despite the low quality and price of the meat (Hacohen-Domené *et al*. 2020).

Regarding body size, the maximum size reported for *C. asper* is 1,235 mm TL (Fischer *et al*. 2006). However, frequent sizes for males and females are 710 mm TL and 690 mm TL respectively (Fischer *et al*. 2006). In this manner, two out of three individuals reported in the present study coincide with the size range reported for this species (specimen Id_215 and Id_216). However, one specimen (Id_235) was longer (1,280 mm TL) than the maximum size reported; which constitutes the largest specimen recorded to date.

Information on the reproduction of this species varies according to the locality. In the southwestern equatorial Atlantic Ocean, Fischer *et al*. (2006) observed that females of *C. asper* reached sexual maturity at 1,100 mm TL, while males reached it at 910 mm TL. In contrast, the population from the eastern coast of North America reaches its sexual maturity at 710-850 mm TL for males, and 880-900 mm TL for females (Castro 2011). During this study, female reproductive tracts could not be kept for analysis due to the fisherman's decision to sell the meat and organs. However, based on previous studies (Fischer *et al*. 2006, Castro 2011) and the TL of the three specimens, all specimens reported in this study were likely sexually mature.

The relevance of this study resides in the fact that it represents the first record for *C. asper* in the Caribbean Sea. Finucci *et al*. (2020b) observed that its range of distribution could be wider, but the information was limited. In this manner, this record constitutes a significant range extension of its distribution to the Caribbean Sea. More information on the biology and ecology of *C. asper* is needed for the proper evaluation of the demography in this region. Finally, this study and the record of several deep-sea elasmobranch records in the area (Hacohen-Domené *et al*. 2016, 2017, 2020; Polanco-Vásquez *et al*. 2017, Ehemann *et al*. 2019) highlights the need for comprehensive deep-sea research surveys to have a better assessment of the region's deep-water elasmobranchs. Such records in deep-sea species have important implications for future research and conservation actions for these poorly known sharks and ray species, which could favor present and future management plans regarding local fisheries.

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