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PENSOFT

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New report and range extension of smallmouth flounder, *Etropus microstomus* (Actinopterygii: Carangiformes: Cyclopsettidae), in the Gulf of Mexico

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Abstract

The smallmouth flounder, *Etropus microstomus* (Gill, 1864), is a species of benthic habits, associated with soft sandy bottoms, and distributed from Canada to the New Orleans coasts, and with specific reports in Corpus Christi, TX, USA. No records have been available from the Mexican coast, however. In the presently reported study, the first finding of this species, in three proximate localities, is described from the Mexican coast. This record constitutes a considerable expansion range in the Gulf of Mexico. Ten specimens were identified through traditional taxonomic characters, together with a *CO1* genetic sequence. The presence of this species in the Mexican coastal zone may be due to the dissemination of ichthyoplankton in the ballast water of commercial ships or to the ocean currents along the coasts of the Gulf of Mexico.

Keywords

CO1, distribution range, smallmouth flounder, taxonomy

Introduction

Cyclopsettidae (sand whiffs), is a recently classified family (Campbell et al. 2019), which is represented by four genera (Fricke et al. 2022), containing the genus *Etropus* represented by 10 species (Froese and Pauly 2022). They are mainly marine species and are very rarely found in freshwaters (McEachran and Fechhelm 2005). These fishes have benthic habits associated with soft sandy bottoms where they find shelter or food resources, although some larger species can emerge from the bottom to capture their prey (Richards 2006). The family Cyclopsettidae is considered ecologically important in the structure and function of the demersal fish community; its dominance is the result of its competitive capacity in complex trophic networks composed of species that occupy a similar niche (Sánchez-Gil et al. 2008). A representative of this family—*Citharichthys sordidus* (Girard, 1854) has commercial importance in the North American Pacific Ocean (He et al. 2016), and species of the genus *Cyclopsetta*—e.g., *Cyclopsetta querna* (Jordan et Bollman, 1890)—are part of the subsistence fishery from the Gulf of California to Peru (Froese and Pauly 2022).

The genus *Etropus* Jordan et Gilbert, 1882 has an Amphiamerican distribution (Castro-Aguirre et al. 1999) with six species that are distributed along the Atlantic coasts of North America. The species of this genus are characterized by having a small mouth, eyes always separated by a narrow bony ridge, and teeth that are found mainly on the blind side. *Etropus microstomus* (Gill, 1864) has been

recorded in the Atlantic with a distribution from Canada to the Mississippi delta (USA) (Gutherz 1967; Martin and Drewry 1978; Leslie and Stewart 1986). Froese and Pauly (2022) present a record for this species off the coasts of Texas and FishNet 2.0 (2022) reports a single record in the Gulf of Paria, Trinidad and Tobago (Fowler 1915).

Materials and methods

The specimens of *Etropus microstomus* were collected with a shrimp trawl (3.70 × 3.20 m and the mesh size of 3.5 cm) off the coast of Tamaulipas, Mexico (Fig. 1, Table 1) aboard an Oceanographic Cruise conducted in September 2018, between depths of 17 to 25 m, and 6.6 to 8.9 km from the coast, at coordinates:

(1) 23°35′24.28″N, 097°41′3.78″W
(2) 23°33′25.19″N, 097°41′51.87″W
(3) 23°30′13.13″N, 097°40′13.98″W

The specimens were frozen and taken to the Fish Taxonomy and Ecology Laboratory (CINVESTAV-Mérida) where they were identified through morphological and meristic characters, color patterns, and DNA barcodes using sequences of the *CO1* gene (cytochrome c encoded in the mitochondrial oxidase subunit 1), as a supplemental identification method (Norman 1934; Gutherz 1967; Richardson and Joseph 1973; Martin and Drewry 1978; Leslie and Stewart 1986; Robins and Ray 1986, Richards

Table 1. Specimens and the values of the morphometric and meristic characters for *Etropus microstomus* in the northern Gulf of Mexico.

Character	Specimen number									
	1	2	3	4	5	6	7	8	9	10
Standard length [mm]	45.0	48.1	43.3	41.9	41.2	45.5	43.7	46.0	43.7	50.5
Total weight [g]	2.51	2.84	2.50	2.47	2.38	2.52	2.51	2.54	2.42	2.83
Head length [mm]	11.3	12.3	10.7	10.9	11.0	12.2	11.5	11.4	11.0	12.9
As % of standard length										
Body depth	49.4	48.9	49.7	49.4	48.8	49.5	49.7	49.6	49.2	49.1
Head length	25.1	25.6	24.7	26.0	26.7	26.8	26.3	25.8	25.2	25.5
As % of head length										
Mandible	27.4	28	28.9	25.8	26.4	26.4	27.0	28.1	26.4	27.1
Lower eye	30.0	30.1	30.0	30.2	30.0	29.5	30.0	30.6	30.1	29.7
Dorsal-fin rays	74	72	69	71	73	73	74	75	75	73
Anal-fin rays	56	56	54	52	54	53	56	58	58	55
Pectoral-fin rays on blind side	9	8	9	8	9	8	8	9	8	8
Pectoral-fin rays on ocular side	10	9	9	9	9	9	9	10	9	9
Caudal-fin rays	17	17	17	17	17	17	17	17	17	17
Gill rakers on lower limb of 1st arch	5	5	5	6	5	5	5	5	4	5
Gill rakers on upper limb of 1st arch	3	4	4	3	4	4	4	4	3	4
Scales of the lateral line	38	37	38	38	38	37	37	36	36	38

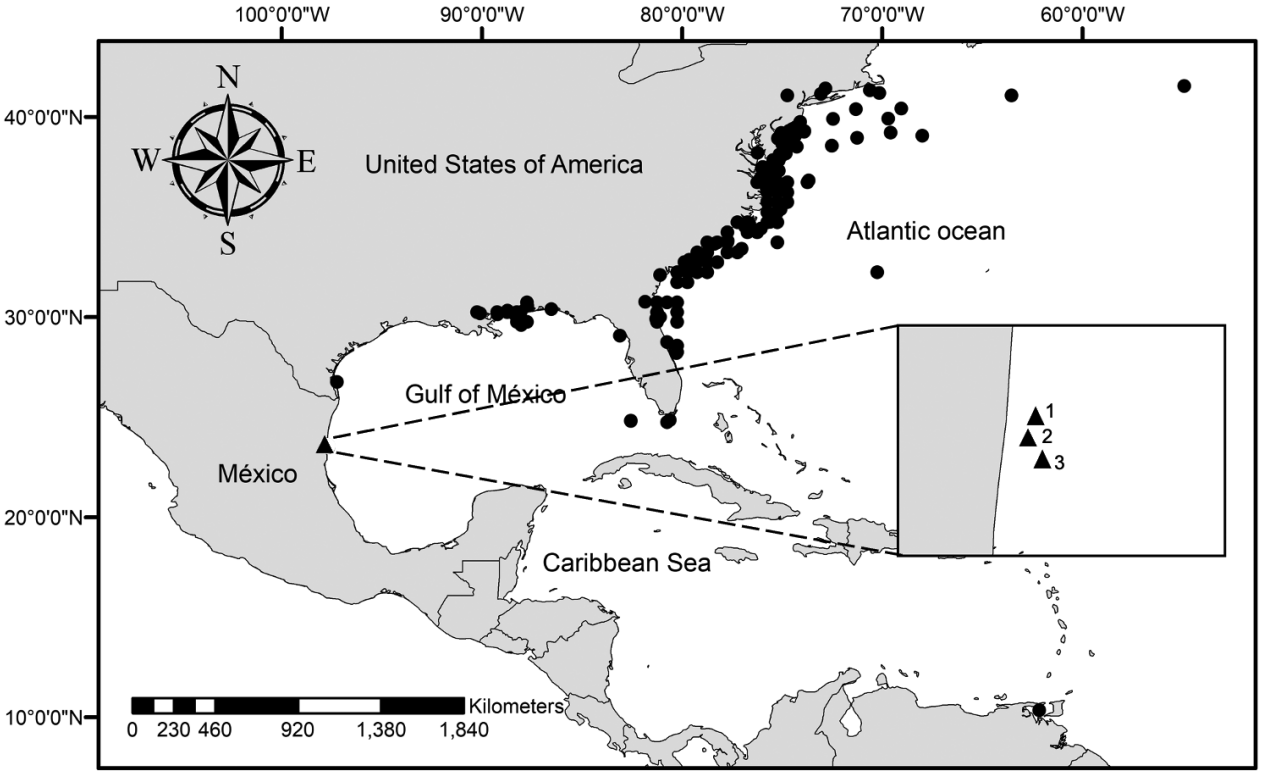

Figure 1. Sampling locations for *Etropus microstomus*, showing the historical distribution sites (circles) by FishNet 2.0 (2022) and distribution widening records (triangles) on the coast of Tamaulipas, México.

2006; Munroe 2016). Thereafter, they were preserved in 70% ethanol, cataloged, and deposited in the ichthyological collection (CINV-NEC) from CINVESTAV-Mérida (YUC-PEC.084.0999) (Fig. 2).

Immediately after defrosting, using a sterilized scalpel and forceps a muscle tissue sample (<5 mm) was taken from behind the pectoral fin of each individual and immersed in a 1.5 mL vial with absolute ethanol and kept at –20°C for its preservation (Tiwary et al. 2016). The extracted DNA quality was determined by 1.2% agarose gel electrophoresis, and the DNA was used as a template for *CO1* amplification. Partial fragments of the *CO1* gene were amplified using four universal primers FishF1, FishF2, FishR1, and FishR2 (Ward et al. 2005). Amplification was performed via polymerase chain reaction (PCR) in a final volume of 25 µL containing 18.75 µL of distilled water, 2.25 µL of 10× Taq buffer, 0.8 µL of $MgCl_2$, 0.25 µL of each primer (0.01 mM), 0.25 µL of each of the dNTPs (nucleoside triphosphate), 0.1 µL of Taq Polymerase (5 U · μL^{-1}), 0.6–1.0 µL DNA template. The amplification conditions were an initial denaturation at 94°C for 2 min followed by 35 cycles of

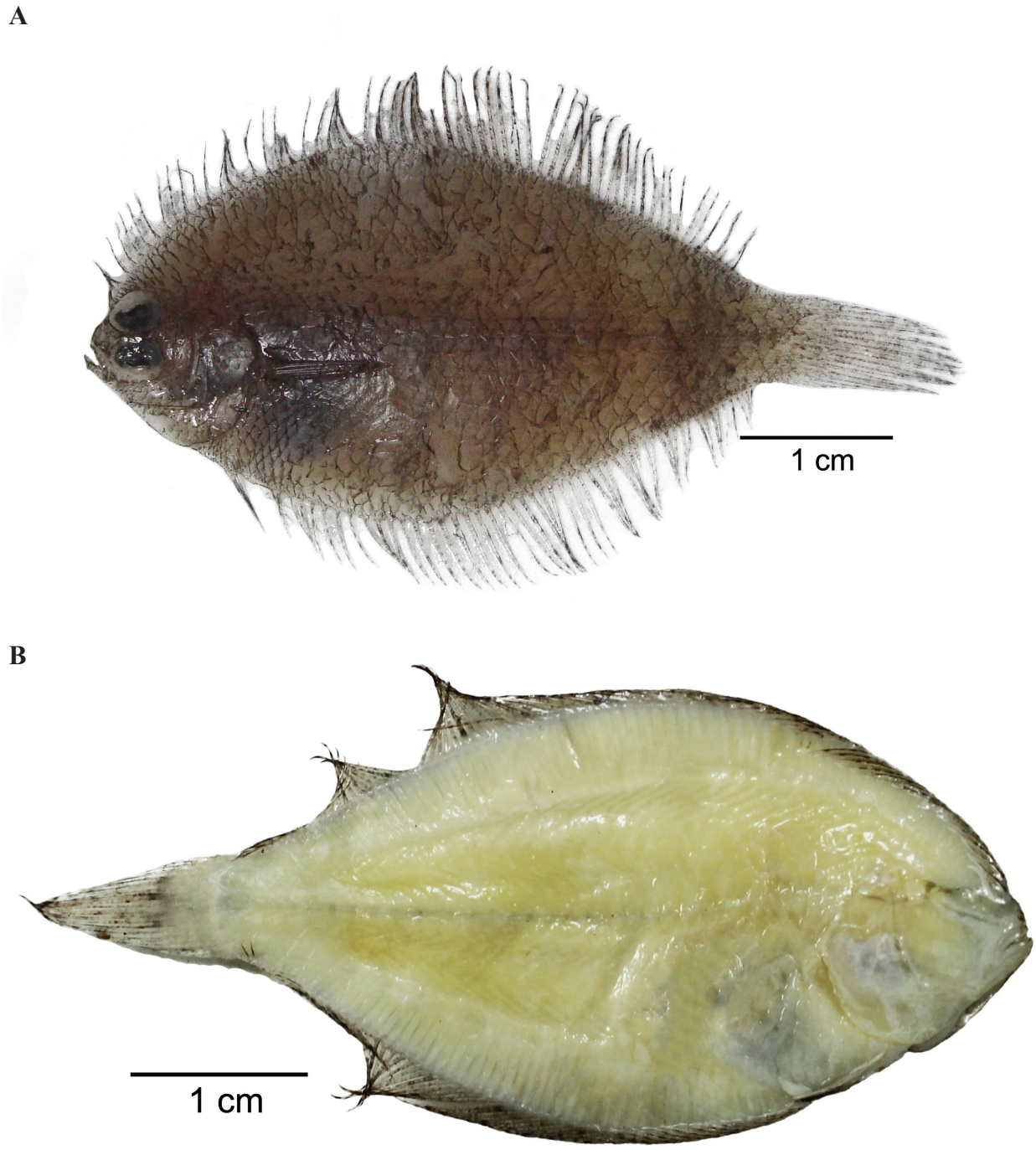

Figure 2. Ocular side (**A**) and blind side (**B**) of smallmouth flounder, *Etropus microstomus* (4.5 cm SL) from Tamaulipas, México.

94°C for 30 s, 52°C for 40 s, and 72°C for 1 min, with an extension of 72°C for 10 min and a final lowering to 4°C. The extraction and amplification were carried out in the "Código de barras de la vida" laboratory in ECOSUR, Chetumal, México.

The visualization of the PCR products was performed through 1.2% agarose gel stained with ethidium bromide and run in a horizontal electrophoresis chamber (Bio-rad-Minicell primo) at 90 V for 35 min. Finally, they were placed in a UV light translucent (BioImagingSystems, miniBis Pro), where they were visualized and saved using the Gel Capture USB program. (Ward et al. 2005). The sequence data were analyzed using Sequencing Analysis v5.1 and SeqScape v2.5 (Applied Biosystems). Sequence data were submitted to the Barcode of Life Database (BOLD 2022). The sequencing was carried out by the company Eurofins Genomic (Canada).

Results

In total, ten specimens of *Etropus microstomus* with a mean size of 4.5 cm standard length (SL) were captured at three locations in the northern Gulf of Mexico. Table 1 shows the diagnostic morphological characters, which corroborate *E. microstomus*, such as small eyes in relation to the length of its head (29.5%–30.2%), lateral line with 36 to 38 scales, left pelvic fin below the lateral line, about a quarter down its body, the number of fin rays, a total of 7 to 9 gill rakers and a maximum length of 13 cm TL (Leslie and Stewart 1986; Munroe 2016). The result of taxonomical identification was validated by DNA barcoding *CO1*, which shows a 99.85% similarity to *E. microstomus* with the registered ID: FYPM297-20; the sequences were within 585–650 bp on the Barcode of life data system (BOLD 2022).

Discussion

The finding of presently reported specimens constitutes the first record of the *Etropus microstomus* in Mexican waters. FishNet 2.0 (2022), the global network of ichthyology collections, reported 226 records of *E. microstomus* from the Canadian coast to the southern coast of the United States, a few reports from Texas, Florida, and a single report from the Gulf of Paria. Leslie and Stewart (1986), in their review of the genus *Etropus*, concluded that some records from the Mississippi Delta (Borodin 1928) and the specimen from South America (Fowler 1915) were misidentifications of the *Etropus crossotus* Jordan et Gilbert, 1882. However, our results were consistent with traditional taxonomy and DNA barcoding.

The reports and distribution of *E. microstomus* are limited to New York to North Carolina with occasional strays as far south as Florida (Carolina Province) (Leslie and Stewart 1986; Munroe 2016), which presents a biogeographic barrier with the Mexican territory (Caribbean Province) that is generated through the Laguna Madre and the Delta of Rio Bravo (Toonen et al. 2016). This causes a diversity of different species between provinces (Briggs and Bowen 2012; Strongin et al. 2020). However, Ruiz et al. (2000) and Bailey et al. (2020) provide evidence of invasions of non-indigenous species through various routes, such as aquaculture, aquaria, biofouling, tsunamis, ballast water, and others, by means of vessels that travel for commercial purposes, using seawater from their area of origin as a ballast, which is released at the port of destination (Okolodkov and García-Escobar 2014). In Mexico, one of the busiest and most commercially active international ports in terms of trade with the east coast of the United States is the port of Altamira (Adams et al. 2004), which is the closest site to the *E. microstomus* sightings in this study. It is probable that the larvae of this species, along with other zooplankton organisms, have reached Mexican coasts by this route, adapting to local conditions, and it is possible due to the high survival of zooplankton in ballast water (93%–96%) in a period of one to two days (Okolodkov and García-Escobar 2014). Unfortunately, in Mexico, the transport of fish species through ballast water is inadequately studied, whereas many studies have focused on bacteria and pathogens that can affect commercially important organisms (Gollasch et al. 2015).

Another possible process of increasing the distribution of species is through larval dispersal by ocean currents, commonly called cyclonic eddies that move masses of water vertically along with organisms and nutrients (Albaina and Irigoien 2007), in addition, anticyclonic eddies move currents horizontally surface (within 10–100 km) (Durán-Campos et al. 2019), affecting surface planktonic organisms (Aldeco et al. 2009; Durán-Campos et al. 2019; Färber et al. 2019; Lara-Hernández et al. 2019). Currents off the coast of Texas and Louisiana go west during the months of September–March, and in Tamaulipas they go south during the same period (Zavala-Hidalgo et al. 2003). This process has possibly allowed the distribution, expansion, and colonization of *E. microstomus* from the north coast to the west of the Gulf of Mexico, due to its hydrological conditions being very similar to its habitat of origin (Day et al. 2013). Finally, the colonization process of a non-indigenous species can have many sources and its consequences are varied. In this study, *E. microstomus* does not present an invasion; however, we can identify processes that should be strictly controlled such as the treatment of ballast waters, although we could also be facing a process where ocean currents directly influence its distribution.

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