

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 *COI* 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

COI, 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 *COI* 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 1 st arch	5	5	5	6	5	5	5	5	4	5
Gill rakers on upper limb of 1 st 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

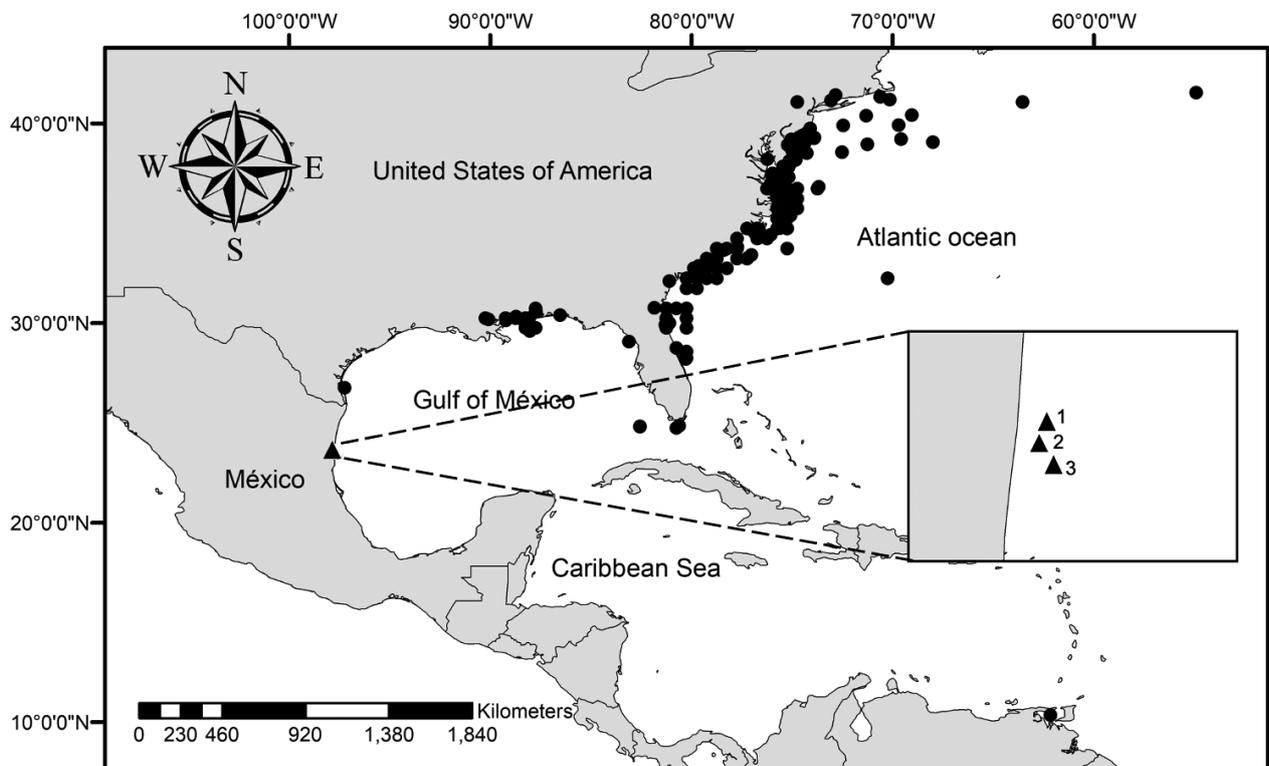


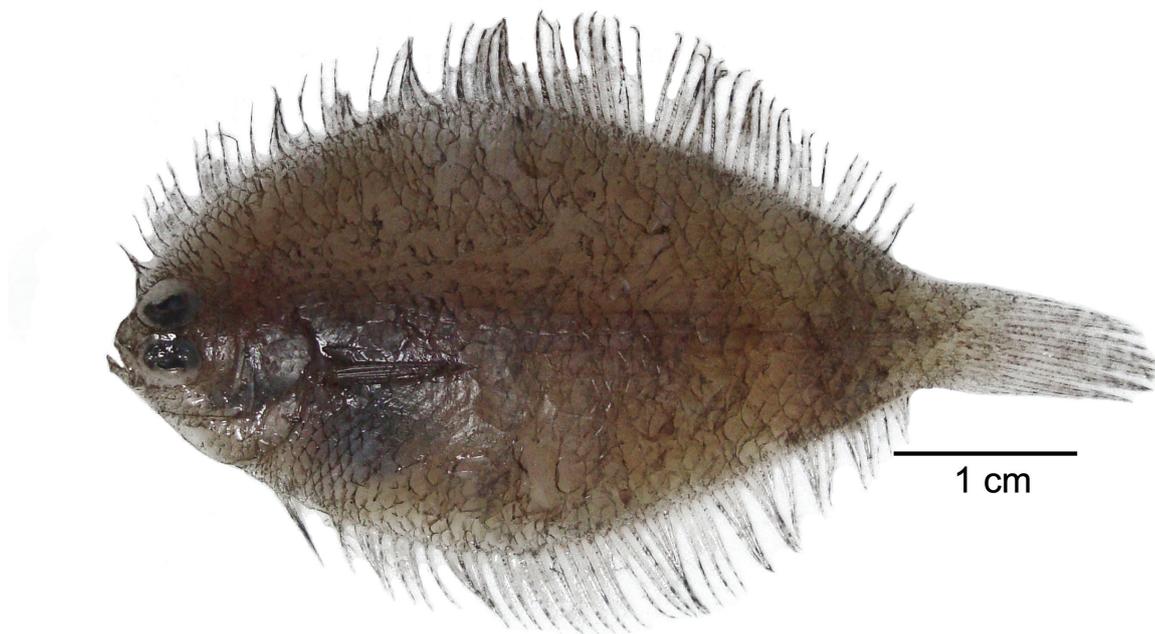
Figure 1. Sampling locations for *Etropus microstomus*, showing the historical distribution sites (circles) by FishNet 2.0 (2022) and distribution widening records (triangles) on the coast of Tamaulipas, México.

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 *COI* amplification. Partial fragments of the *COI* 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 $10\times$ 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 ($5\text{ U} \cdot \mu\text{L}^{-1}$), 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

A



B

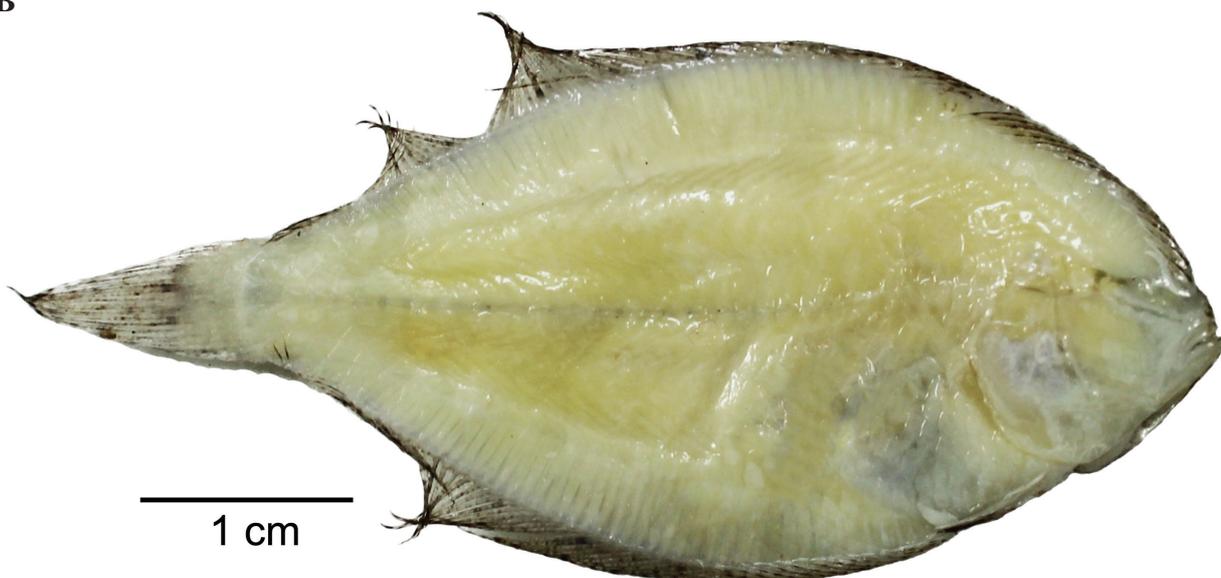


Figure 2. Ocular side (A) and blind side (B) of smallmouth flounder, *Etropus microstomus* (4.5 cm SL) from Tamaulipas, México.

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 *COI*, 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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Length–weight and length–length relations of 16 freshwater fish species (Actinopterygii) caught in Jiaxing section of the Beijing–Hangzhou Grand Canal, China

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Abstract

The length–weight (LWRs) and length–length (LLRs) relations were estimated for 16 fish species obtained from Jiaxing section of the Beijing–Hangzhou Grand Canal, China. One species represented Engraulidae: *Coilia nasus* Temminck et Schlegel, 1846; 11 species Cyprinidae: *Hypophthalmichthys molitrix* (Valenciennes, 1844); *Hypophthalmichthys nobilis* (Richardson, 1845); *Chanodichthys erythropterus* (Basilewsky, 1855); *Chanodichthys mongolicus* (Basilewsky, 1855); *Culter alburnus* Basilewsky, 1855; *Chanodichthys dabryi* (Bleeker, 1871); *Pseudobrama simoni* (Bleeker, 1864); *Hemiculter leucisculus* (Basilewsky, 1855); *Megalobrama terminalis* (Richardson, 1846); *Carassius auratus* (Linnaeus, 1758); *Cyprinus carpio* Linnaeus, 1758; one species Bagridae: *Tachysurus fulvidraco* (Richardson, 1846); two species Odontobutidae: *Odontobutis potamophilus* (Günther, 1861); *Micropercops swinhonis* (Günther, 1873); and one species Osphronemidae: *Macropodus ocellatus* Cantor, 1842. Fishes were collected using multipanel nylon gillnets with mesh sizes of 1–8 cm from November 2020 through August 2021. All fishes were measured for length (total length, TL; standard length, SL) to the nearest 0.1 cm and weight (*W*) to the nearest 0.1 g. The coefficients of determination R^2 of LWRs and LLRs were all over 0.950, and the 16 values of LWR parameter *b* were estimated from 2.505 to 3.364. Our study provides new information on LWRs for 2 species and LLRs for 7 species, as well as a new maximum total length recorded for 3 species for Fish-Base. This study would allow for the convenience of the conversion of TL–*W* and SL–TL in fish stock assessment and is expected to provide a useful baseline for further studies of population parameters to improve management decisions.

Keywords

growth coefficient, LLR, LWR, Beijing–Hangzhou Grand Canal

Introduction

The Beijing–Hangzhou Grand Canal, located in eastern China, is listed as a World Heritage Site and is one of the longest and oldest canals in the world, with a total

length of about 1797 km. It has played an important role in economic and cultural exchanges between the north and south regions of China. Fish stocks became seriously depleted and the reduction of the ichthyofauna biodiversity tended to be obvious in the 1980s due to overfishing,

water pollution caused by shipping, and other anthropogenic factors (Qin et al. 1988). Qin et al. (1988) collected 27 fish species in the Changzhou section of the canal from 1984 to 1985, accounting for only 26.73% of fish species inhabiting the Taihu Lake basin, and only 354 specimens were captured over the two-year period. Di et al. (2021) caught 14 fish species in the North Canal in 2019, showing characteristics of rare species/genus, high resistance to pollution, and a widely distributed type of fish population. However, limited data were available on the growth characteristics of fish species in the canal. In this study, length–weight (LWRs) and length–length (LLRs) relations were estimated for 16 species caught from the Jiaying section of the canal. Estimates would provide a useful reference for further studies of population parameters to improve management decisions.

Materials and methods

A total of 16 freshwater fish species was studied for their length–weight (LWRs) and length–length (LLRs) relations. One species represented Engraulidae: *Coilia nasus* Temminck et Schlegel, 1846; 11 species Cyprinidae: *Hypophthalmichthys molitrix* (Valenciennes, 1844); *Hypophthalmichthys nobilis* (Richardson, 1845); *Chanodichthys erythropterus* (Basilewsky, 1855); *Chanodichthys mongolicus* (Basilewsky, 1855); *Culter alburnus* Basilewsky, 1855; *Chanodichthys dabryi* (Bleeker, 1871); *Pseudobrama simoni* (Bleeker, 1864); *Hemiculter leucisculus* (Basilewsky, 1855); *Megalobrama terminalis* (Richardson, 1846); *Carassius auratus* (Linnaeus, 1758); *Cyprinus carpio* Linnaeus, 1758; one species Bagridae: *Tachysurus fulvidraco* (Richardson, 1846); two species Odontobutidae: *Odontobutis potamophilus* (Günther, 1861); *Micropercops swinhonis* (Günther, 1873); and one species Osphronemidae: *Macropodus ocellatus* Cantor, 1842. Fish samples were collected from Jiaying section (120°34′–120°69′E,

30°50′–30°96′N) of the Beijing–Hangzhou Grand Canal, China. Species were seasonally captured between November 2020 and August 2021, using multipanel nylon gillnets with a mesh size of 1–8 cm at about around 03:00–07:00 hours. Fish species identification was performed in accordance with the procedures of Mao et al. (1991). Species validation was confirmed with FishBase (Froese and Pauly 2022). Each specimen was measured to the nearest 0.1 cm (total length, TL; standard length, SL) and weighed to the nearest 0.1 g (weight, W) simultaneously.

The LWRs were determined using the formula

$$W = aTL^b,$$

where W was the weight [g], TL was the total length [cm], a was the intercept and b was the allometric coefficient/slope. The formula was equipped with a simple linear regression model based on log-transformed data. The 95% confidence interval (CI) for parameters a and b and the coefficients of determination (R^2) were also determined (Keys 1928; Froese 2006). A linear regression was used to determine the LLR,

$$TL = a + bSL$$

where SL was the standard length [cm] and other measurements are defined as above. For species with $R^2 < 0.95$, outliers were discarded and regression was recalculated. All statistical analysis was done in SPSS 16.0 (SPSS, Inc., Chicago, IL, USA).

The raw data are available as Suppl. material 1.

Results

LWRs and LLRs of 16 fish species were estimated. The descriptive statistics and the estimated LWR parameters are summarized in Table 1. In addition, similar parameters

Table 1. Length–weight relations of 16 fish species (Actinopterygii) sampled between November 2020 and August 2021 from Jiaying section of the Beijing–Hangzhou Grand Canal, China.

Species	N	TL range[cm]	W range [g]	a	a CL	b	b CL	R^2
<i>Coilia nasus</i> Temminck et Schlegel, 1846	51	14.5–25.7	7.6–34.9	0.004	0.003–0.007	2.791	2.626–2.956	0.959
<i>Hypophthalmichthys molitrix</i> (Valenciennes, 1844)	298	10.2–85.2	10.0–7150.0	0.010	0.009–0.011	3.019	2.981–3.057	0.988
<i>Hypophthalmichthys nobilis</i> (Richardson, 1845)	125	18.8–76.1	50.0–5513.2	0.010	0.008–0.013	3.058	2.992–3.124	0.986
<i>Chanodichthys erythropterus</i> (Basilewsky, 1855)	214	9.3–29.4	3.1–196.0	0.002	0.002–0.003	3.364	3.266–3.461	0.956
<i>Chanodichthys mongolicus</i> (Basilewsky, 1855)	107	13.5–49.2	10.0–905.0	0.003	0.002–0.004	3.234	3.124–3.343	0.970
<i>Culter alburnus</i> Basilewsky, 1855	311	7.3–78.5	3.0–2125.6	0.008	0.007–0.010	2.843	2.778–2.907	0.961
<i>Chanodichthys dabryi</i> (Bleeker, 1871)	400	6.7–40.1	1.2–442.7	0.003	0.002–0.003	3.284	3.215–3.352	0.957
<i>Pseudobrama simoni</i> (Bleeker, 1864)	50	11.5–21.5	12.6–90.2	0.004	0.002–0.007	3.310	3.098–3.522	0.954
<i>Hemiculter leucisculus</i> (Basilewsky, 1855)	274	8.2–22.5	3.3–68.5	0.009	0.007–0.011	2.846	2.768–2.924	0.950
<i>Megalobrama terminalis</i> (Richardson, 1846)	29	9.0–54.0	6.7–1915.1	0.008	0.005–0.011	3.123	3.015–3.231	0.992
<i>Carassius auratus</i> (Linnaeus, 1758)	263	6.7–29.8	3.6–455.6	0.014	0.012–0.018	3.025	2.954–3.095	0.965
<i>Cyprinus carpio</i> Linnaeus, 1758	159	14.4–70.0	51.2–4542.0	0.011	0.008–0.014	3.063	2.989–3.137	0.977
<i>Tachysurus fulvidraco</i> (Richardson, 1846)	79	12.0–27.0	23.7–172.0	0.045	0.035–0.059	2.505	2.414–2.596	0.975
<i>Odontobutis potamophilus</i> (Günther, 1861)	58	5.8–15.5	2.1–45.0	0.015	0.010–0.022	2.979	2.824–3.135	0.964
<i>Micropercops swinhonis</i> (Günther, 1873)^a	25	4.0–7.6	0.7–4.2	0.018	0.015–0.027	2.692	2.456–2.927	0.960
<i>Macropodus ocellatus</i> Cantor, 1842 ^a	24	4.5–7.9	0.8–6.0	0.008	0.005–0.014	3.215	2.925–3.505	0.960

N = sample size, TL = total length, W = weight, a and b = relation parameters in equation $W = aTL^b$, CI = 95% confidence interval, R^2 = determination coefficient. Species with new maximum size records are marked with **bold** font; ^a First record of LWR for the species.

are provided for the LLRs (TL vs. SL) in Table 2. All LWR and LLR estimates were statistically significant ($P < 0.05$), yielding $R^2 > 0.950$. Two new LWRs for *Micropercops swinhonis* and *Macropodus ocellatus*, and 7 new LLRs for *Chanodichthys mongolicus*, *Chanodichthys dabryi*, *Pseudobrama simoni*, *Megalobrama terminalis*, *Tachysurus fulvidraco*, *Odontobutis potamophilus*, and *Micropercops swinhonis*, were determined, as well as 3 new total lengths for *Pseudobrama simoni*, *Odontobutis potamophilus*, and *M. swinhonis* were recorded when compared with FishBase data (Froese and Pauly 2022).

Table 2. Length–length relations ($TL = a + bSL$) of 16 fish species sampled in the Jiaxing section of the Beijing–Hangzhou Grand Canal, China.

Species	LWR parameters		
	<i>a</i>	<i>b</i>	R^2
<i>Coilia nasus</i>	0.586	1.061	0.954
<i>Hypophthalmichthys molitrix</i>	0.084	1.186	0.987
<i>Hypophthalmichthys nobilis</i>	1.028	1.139	0.987
<i>Chanodichthys erythropterus</i>	0.814	1.146	0.978
<i>Chanodichthys mongolicus</i>	0.952	1.149	0.993
<i>Culter alburnus</i>	−0.171	1.223	0.981
<i>Chanodichthys dabryi</i>	0.734	1.166	0.981
<i>Pseudobrama simoni</i>	0.856	1.125	0.969
<i>Hemiculter leucisculus</i>	0.219	1.203	0.961
<i>Megalobrama terminalis</i>	0.296	1.202	0.993
<i>Carassius auratus</i>	0.656	1.202	0.991
<i>Cyprinus carpio</i>	1.076	1.178	0.971
<i>Tachysurus fulvidraco</i>	1.712	1.042	0.957
<i>Odontobutis potamophilus</i>	0.785	1.136	0.972
<i>Micropercops swinhonis</i>	0.530	1.129	0.961
<i>Macropodus ocellatus</i>	0.441	1.285	0.951

a = intercept, *b* = slope; R^2 = coefficient of determination. **Bold** font denoted first record of LLR for the species.

Discussion

The values of LWR parameter *b* were estimated from 2.505 to 3.364, which are consistent with the predicted range of 2.5–3.5 (Hile 1936; Froese 2006). Deviations of

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parameter *b* for some species in this study were identified when compared with values reported in FishBase (Froese and Pauly 2022). The LWRs are influenced by different growth stanzas, gender, fishing, and environmental factors, such as season, temperature, and food (Quasim 1973; Froese 2006; Rekha et al. 2021; Ni et al. 2022; Zhang et al. 2022). Since the specimens here were collected using multipanel nylon gillnets of mesh size from 1 to 8 cm, the inherent size biasedness might be expected. In this study, we estimated the LWRs and LLRs of 16 fish species inhabiting the Jiaxing section of the canal based on the long-term surveyed data, and the estimated parameters could be considered as the mean annual values (Guo et al. 2019; Ni et al. 2022). Our results provided the new data for FishBase (Froese and Pauly 2022), allow for the convenience of fish stock assessment, and are expected to provide a useful baseline for further studies of population parameters to improve management decisions in the Beijing–Hangzhou Grand Canal.

Conclusion

This study provides basic parameters on LWRs and LLRs for 16 fish species. The new information on LWRs for 2 species and LLRs for 7 species, as well as the new maximum total length recorded for 3 species, highlight the scarcity of information on the biological aspects of these fishes. These LWRs and LLRs allow for the conversion of TL (total length)–*W* (weight) and SL (standard length)–TL (total length) in fish stock assessment, and are useful for further studies of population parameters to improve management decisions in the Beijing–Hangzhou Grand Canal.

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Supplementary material 1

Original data

Authors: Aiju Zhang, Jiezhou Zhu, Qinqing Lian, Pengcheng Sheng, Aihuan Guo, Wei Luo, Zhiming Zhou, Julin Yuan

Data type: excel file

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Length–weight relation for seven Neotropical freshwater fish species (Actinopterygii) endemic to Central America

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Abstract

In the presently reported study, we estimated length–weight relation (LWRs) for seven species of freshwater fishes from Central America. Samples were collected using seines from 60 sites across Nicaragua, Costa Rica, and Panama during field expeditions conducted between 1997 and 2012. The fishes were preserved and transported to the lab, where their total weight (W) was measured (to nearest 0.0001 g) and standard lengths were taken (to nearest 0.01 mm). Data were collected from four livebearers (Poeciliidae), *Alfaro cultratus* (Regan, 1908), *Phallichthys amates* (Miller, 1907), *Poecilia gillii* (Kner, 1863), and *Priapichthys annectens* (Regan, 1907); the cichlids (Cichlidae), *Parachromis dovii* (Günther, 1864) and *Parachromis managuensis* (Günther, 1867); and a silverside (Atherinopsidae), *Atherinella hubbsi* (Bussing, 1979). Estimates of parameter b ranged from 2.936 (*A. hubbsi*) to 3.696 (*P. gillii*), while estimates of parameter a ranged from 1.7×10^{-6} (*P. gillii*) to 1.9×10^{-5} (*P. managuensis*). Parameter b estimates were greater than three, consistent with allometric growth, with the exception of *P. annectens*, *P. managuensis*, and *A. hubbsi*, for which t -tests failed to reject the null hypothesis of isometric growth. Our results provide the first LWR information for five (71%) of these species and may prove useful for data imputation or estimating the biomass of poeciliid, cichlid, and atheriniform fishes in Central American rivers in the future.

Keywords

Costa Rica, ecology, freshwater fishes, LWRs, Nicaragua, Panama

Introduction

Characterizing length–weight relation (LWRs) is an essential and routine task in fisheries science (Froese 2006). The resulting data and parameters are useful for predicting weight (W) from the length (L) of individuals (Clark 1928), determining and comparing the ‘condition’ or ‘robustness’ of individuals and populations (Le Cren 1951), and comparing relative weights of populations, species, or treatment groups (Froese 2006). Moreover, LWRs aid in estimating ecosystem parameters, e.g., cal-

culating species biomass from the length-frequency of a given sample. Recently, LWRs have also been applied for estimating fish length at first maturity (e.g., Hashiguti et al. 2019) and for building aquatic and marine ecosystem food-web models (e.g., Ecopath with Ecosim; Heymans et al. 2016). Once determined, LWRs also permit determining missing weight or length values from regression predictions (i.e., imputation); this is important, given that length can frequently be more readily and accurately measured than weight in field or laboratory studies of fishes.

With ~525 species, the freshwater fish assemblage of Central America (CA) is highly diverse relative to its drainage area and displays marked uniqueness, with 10 fish biogeographic provinces and up to 59.2% within-region endemism (Albert et al. 2011; Matamoros et al. 2014). While the CA ichthyofauna has been increasingly well characterized over the past 60 years (e.g., Myers 1966; Bussing 1976, 1998; Bermingham and Martin 1998; Bagley and Johnson 2014a, 2014b; Matamoros et al. 2014), basic ecological data on the fauna remains broadly lacking, and LWRs provide an illuminating case in point. To date, of 353 CA freshwater fishes that are represented on FishBase (www.fishbase.org), only 76 (21.5%) are listed as containing LWR records (Froese and Pauly 2022).

In the presently reported study, we describe LWRs for seven Neotropical fish species that are endemic to freshwater rivers and streams of CA. The majority of our focal species are non-game, ‘secondary’ fishes from families identified as having the capacity to disperse through marine environments (Myers 1966); of these families—Poeciliidae, Cichlidae, and Atherinopsidae, the former two make up the majority of species in the region. Data were collected from four livebearers (Poeciliidae), *Alfaro cultratus* (Regan, 1908), *Phallichthys amates* (Miller, 1907), *Poecilia gillii* (Kner, 1863), and *Priapich-*

thys annectens (Regan, 1907); the cichlids (Cichlidae), *Parachromis dovii* (Günther, 1864) and *Parachromis managuensis* (Günther, 1867); and a silverside (Atherinopsidae), *Atherinella hubbsi* (Bussing, 1979). Despite being important constituents of local CA freshwater fish communities (e.g., Bussing 1976, 1998), material for these species is globally rare. Species were selected due to their overlapping geographical distributions, and based on the zero to limited reference LWRs available for them in FishBase. Our study provides the first LWR data and modeling results for 5/7 (71%) of the focal species.

Materials and methods

The study area spans the CA Isthmus, from the Motagua Fault Zone in Guatemala, southeast to the Darién Isthmus, Panama (~523 000 km²; Fig. 1). We surveyed the CA ichthyofauna at 60 sites in Nicaragua, Costa Rica, and Panama during field expeditions conducted between 1997 and 2012. Fish specimens were sampled using 2 m × 1.7 m and 3.3 m × 1.7 m seines with 0.48–1.27 cm mesh size netting. Following field identification, specimens were preserved in 95% ethanol and then transported to the laboratory. Specimens were measured to the nearest 0.01 mm standard length (SL) using digital calipers



Figure 1. Map of the study area. The boundaries of Central America (Motagua Fault Zone to the north, Darién Isthmus at Panama’s connection with South America) and some major physiographic elements are shown. Geographical sampling localities ($n = 60$) for this study are shown (black circles with white outlines; see accompanying Mendeley Data accession for additional details).

and weighed to the nearest 0.0001 g on a Mettler-Toledo ME104TE/00 analytical balance. Data were collected from seven species listed in Table 1, including four live-bearing fishes (Poeciliidae), two cichlid fishes (Cichlidae) that typically are predators of the poeciliids, and one silverside (Atherinopsidae) species (Bussing 1998). Taxonomy, common names, geographical distributions, and prior LWR data for these species are summarized in Table 1.

The standard modern equation of weight (W ; body mass) in relation to length (L) takes the form

$$W = aL^b$$

where the scalar a and exponent b are constants. Beginning with Clark (1928), it was recognized that natural log-transformation of both sides resulted in a linearized form of the LWR model as

$$\log(W) = \log(a) + b \log(L)$$

(reviewed by Le Cren 1951; Froese 2006). We determined LWRs for each species (while pooling data across sexes and years) using the linearized equation above and custom scripts run in R v3.6.3 (R Core Team 2020), which drew partly on functions in the *FSA* and *FSAmisc* R packages (Ogle 2022; Ogle et al. 2022). Outliers were

determined by visual inspection of graphical plots in R and excluded prior to final analyses (cf. Froese 2006). We tested the null hypothesis (H_0) that $b = 3$, indicating ‘isometric’ growth, against the alternative hypothesis (H_A) of allometric growth ($b \neq 3$), using t -tests implemented in the ‘hoCoef’ function of *FSAmisc* (Ogle 2022). We also calculated 95% confidence intervals for the slope (b) and intercept [$\log(a)$] of linear models using the ‘confint’ function available in the *FSAmisc* package (Ogle 2022).

Raw length–weight data and collections data are archived in a Mendeley Data accession (archived version: <https://doi.org/10.17632/kphrvvgwwz.1>).

Results

The inferred length–weight relation are presented in Table 2, which lists family names, species names, sample sizes (n), size ranges (SL measurements in mm), length–weight parameter a and b estimates and their 95% confidence intervals (CIs), and the adjusted- R^2 values for each species LWR linear model. All LWR regressions were significant ($P < 0.001$), with R^2 values greater than or equal to 0.93. Estimates of parameter b ranged from 2.936 in *Atherinella hubbsi* to 3.696 in *Poecilia gillii*, while estimates of parameter a ranged from 1.7×10^{-6} in *P. gillii* to 1.9

Table 1. List of focal species examined in the presently reported study, with summaries of their taxonomic information, geographical distributions (Bussing 1976, 1998; Matamoros et al. 2014), and current state of knowledge of their length–weight relation (LWRs).

Family	Species name	Common name(s)	Geographical distribution	Current LWR n
Poeciliidae	<i>Alfaro cultratus</i> (Regan, 1908)	Knife-edged livebearer	N, CR, P	0
Poeciliidae	<i>Phallichthys amates</i> (Miller, 1907)	Merry widow livebearer	G, H, N, CR, P	0
Poeciliidae	<i>Poecilia gillii</i> (Kner, 1863)	Molly	G, H, N, CR, P	0
Poeciliidae	<i>Priapichthys annectens</i> (Regan, 1907)	Olomina	CR	0
Cichlidae	<i>Parachromis dovii</i> (Günther, 1864)	Guapote, wolf cichlid	H, N, CR	1
Cichlidae	<i>Parachromis managuensis</i> (Günther, 1867)	Jaguar cichlid	H, N, CR	2
Atherinopsidae	<i>Atherinella hubbsi</i> (Bussing, 1979)	Silverside	N, CR	0

CR = Costa Rica, G = Guatemala, H = Honduras, N = Nicaragua, P = Panama; n = sample size; Current LWR n indicates the number of LWR records available for the species on FishBase (Froese and Pauly 2022) before this study.

Table 2. Summary of length–weight relation for seven freshwater stream fishes from Central America.

Family	Species	n	n_r	Standard length [mm]	Weight [g]	a [95% CIs]	b [95% CIs]	R^2
Poeciliidae	<i>Alfaro cultratus</i>	102	92	22.24–66.33	0.1005–4.3930	2.3×10^{-6} [1.5×10^{-6} , 3.7×10^{-6}]	3.446 [3.321, 3.570]	0.971
Poeciliidae	<i>Phallichthys amates</i>	44	42	15.96–42.27	0.0604–2.3576	5.3×10^{-6} [2.4×10^{-6} , 1.2×10^{-5}]	3.439 [3.199, 3.680]	0.953
Poeciliidae	<i>Poecilia gillii</i>	49	48	19.33–53.91	0.0829–3.510	1.7×10^{-6} [8.5×10^{-7} , 3.6×10^{-6}]	3.696 [3.490, 3.902]	0.965
Poeciliidae	<i>Priapichthys annectens</i>	69	63	17.09–51.94	0.0780–2.5573	1.5×10^{-5} [8.9×10^{-6} , 2.6×10^{-5}]	3.0901 [2.926, 3.254]	0.958
Cichlidae	<i>Parachromis dovii</i>	22	—	12.87–96.80	0.0390–23.691	1.4×10^{-5} [9.9×10^{-6}, 2.1×10^{-5}]	3.1588 [3.057, 3.260]	0.995
Cichlidae	<i>Parachromis managuensis</i>	7	—	32.37–55.51	0.9260–4.8221	1.9×10^{-5} [6.1×10^{-6}, 5.7×10^{-5}]	3.1051 [2.811, 3.399]	0.992
Atherinopsidae	<i>Atherinella hubbsi</i>	14	—	32.82–57.43	0.2509–1.3047	9.7×10^{-6} [1.5×10^{-6} , 6.2×10^{-5}]	2.9359 [2.458, 3.414]	0.932

CIs = confidence intervals; n = sample size; n_r = size of reduced dataset after outlier removal, if deemed necessary, R^2 , adjusted- R^2 from linear regression. Species in **boldface** font are also represented in FishBase (Froese and Pauly 2022). Estimated values of $b < 3$, or whose 95% CIs overlapped $b = 3$, are set in *italic* font.

$\times 10^{-5}$ in *Parachromis managuensis*. Parameter b estimates were generally greater than three, consistent with allometric growth, and t -tests were statistically significant for the majority of species ($P < 0.05$). However, for *P. annectens*, *P. managuensis*, and *A. hubbsi*, t -tests failed to reject the null hypothesis of isometric growth ($P > 0.05$).

Discussion

The LWRs presented herein had exponent values within the expected range of $b = 2.5$ – 3.5 for fishes (Froese 2006). The majority of species had b estimates slightly greater than three indicating positive allometric growth, with fish growing plumper with increasing size (Blackwell et al. 2000). By contrast, three species had $b < 3$ or with 95% CIs overlapping $b = 3$, indicative of possible isometric growth, which is rare in fishes. However, two of those species had small sample sizes, thus we cannot rule out a possible sample-size effect. We consider LWRs for these species, *Parachromis managuensis* and *Atherinella hubbsi*, to be provisional, and we recommend limiting missing data imputation to the observed length classes. By contrast, the inference of isometry in *Priapichthys annectens* ($b = 3.0901$) based on robust sampling seems a rare exception but should be tested further to account for the full range of geographic, seasonal, and inter-annual variation in the species.

Our study provides the first LWR data and modeling results for 5 out of 7 (71%) of our species. For the two species with previously recorded LWRs on FishBase, our results improve the estimates greatly. The *Parachromis dovii* LWR record on FishBase was estimated by R. Froese in (1999) from a single maximum total length (TL) record and arbitrarily assigned $b = 3$. We more confidently estimate $b = 3.1588$ for this species based on 22 specimens (Table 2), although all size classes were not covered. By

contrast, the two existing *P. managuensis* LWR records on FishBase included one b estimate based on angling records of much larger specimens (26.7–54.6 cm TL), with no 95% CIs, and another much-improved estimate from Velázquez-Velázquez et al. (2015) with $b = 3.161$ based on 83 specimens. Our *P. managuensis* estimate of $b = 3.1051$ is within 2% of their estimate, with overlapping 95% CIs. All of our other LWRs and parameter estimates are new to science.

It is widely recognized that a number of different factors influence the growth and LWRs of fishes. Such factors include sex, health, the effect of gonad maturity on somatic growth, seasonality of resources, stomach fullness, sample size, and preservation techniques (e.g., Le Cren 1951; Wootton 1998), and these were not accounted for in our study. For example, samples were pooled across sexes and years, and thus LWR parameter estimates represent species mean values. Nevertheless, we followed Froese's (2006) guidelines for LWRs, which are recommended for standardizing LWR results across studies. Moreover, our results provide baseline information useful for calculating CA fish biomass or imputing missing data in future studies of this speciose and endemic fauna.

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First report of crested cusk-eel, *Ophidion josephi* (Actinopterygii: Ophidiiformes: Ophidiidae), in the southwestern Gulf of Mexico

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Abstract

The crested cusk-eel, *Ophidion josephi* Girard, 1858, is a coastal marine species that is distributed in the northwestern Atlantic, from the northern Gulf of Mexico to Georgia, USA. Eighteen specimens (177–230 mm in standard length) were caught by beach purse seine at a depth of about 5 m, in Veracruz, in the southwestern Gulf of Mexico. This is the first documentation from Mexican marine waters and the southernmost confirmed records of this species, showing that its range extends further south than previously known and that it shares faunistic similarities with other species in the northwestern Atlantic.

Keywords

distribution, marine fish, morphometric, Mexico, new record, range extension

Introduction

The family Ophidiidae comprises four subfamilies and 280 valid species. The subfamily Ophidiinae contains 65 species (Fricke et al. 2022). Ophidiid fishes are characterized by having supramaxillary, dorsal fin rays usually equal to or longer than opposing anal-fin rays, scales present on body, pelvic fins rarely absent, when they are present with one or two soft rays (Cohen and Nielsen 1978; Nelson et al. 2016). Cusk eels have a wide distribution around the world in tropical, subtropical and temperate seas. The majority of species are coastal, mainly in the continental shelf, although many are found in deep waters, reaching 7965 m (Gerringer et al. 2021). In the western Atlantic, this family is represented by 26 genera and about 68 species (Nielsen and Robins 2002;

Garrido-Linares and Acero-P. 2006). However, the limits of several species in both the southern and northern parts have not been clarified yet.

This genus *Ophidion* Linnaeus, 1758 is found nearly worldwide in warm-temperate and tropical coastal and shelf waters. The largest number of species is concentrated on both coasts of America (Matallanas and Casadevall 1999). A taxonomic revision of the genus *Ophidion* is necessary; there are several characters that are shared with other genera (Cohen and Nielsen 1978; Lea and Robins 2003). It is considered a paraphyletic group and its relation with other clades is unclear (Nielsen et al. 1999). This genus is diagnosed by the following combination of characters: scales absent from sides and top of head; scales on body elongated, arranged in a basketweave or anguilloid pattern; ethmoid spine absent,

or, if present, slender and anteriorly directed at its tip; total vertebrae 63 to 79; abdominal vertebrae 15 to 18; rays of each pelvic fin unequal in length; dorsal fin rays 129 to 150; anal fin rays 104 to 127; developed rakers on first gill arch 4 to 7 (Cohen and Nielsen 1978; Nielsen et al. 1999; Carnevale and Johnson 2015). Within them, the systematic and biogeographic review of the *Ophidion marginatum-josephi* complex is suggested and needed (Lea and Robins 2003), to understand the delimitation of species in their distribution areas. This group is characterized by having similarities of characters in early life history stages, such as an elongate body that becomes laterally compressed with development, the proportion of two or three pterygiophores per interneural space, the fact that the preanal length is short, and that the vertebrae ossify from both the anterior and posterior ends toward the middle (Fahay 1992; Fahay and Hare 2003).

In some species, there have been taxonomic problems. For example, Robins and Ray (1986) considered *Ophidion welshi* (Nichols et Breder, 1922) as a valid species. Later, Nielsen et al. (1999) considered it as a probable synonym. Finally, Nielsen and Robins (2002) indicate that it is a synonym for the crested cusk-eel, *Ophidion josephi* Girard, 1858. The latter is a coastal benthic species that is distributed in the northwestern Atlantic, from Georgia to northeastern Florida through the northern Gulf of Mexico (coastal bays to 55 m depth) (Nielsen et al. 1999; Fahay and Hare 2003). However, its southern distribution limit is unknown.

This work describes a new and thus far southernmost record of *O. josephi* based on morphometrics and meristic characters.

Materials and methods

During four fishing events (15 November 2018, 15 November 2019, 18 October 2020, and 27 September 2021), 18 specimens of *Ophidion josephi* were captured by an artisanal fisherman from Las Barrancas community (19°0'24"N, 095°57'52"W), in Veracruz, the southwestern Gulf of Mexico (Fig. 1). The specimens were captured by beach purse seine between about 3 to 5 m of depth. The specimens were identified following the keys of Hoese and Moore (1998) and McEachran and Fechhelm (1998). Morphometric measurements expressed in proportion to the standard (SL%) and cephalic lengths (HL %) were carried out on the fresh specimen with electronic calipers to the nearest 0.1 mm according to Nielsen et al (1999). Sex was determined by the presence of the typical supraoccipital crest in males and its absence in females (Courtenay 1971). The meristic count was conducted on five specimens, which were cleared and differentially stained with alizarin red S and Alcian blue (Kelly and Bryden 1983) and by X-ray. The samples were fixed in 10% buffered formaldehyde, preserved in 70% ethyl alcohol, and deposited in the Ichthyological Collection of the Facultad de Estudios Superiores Iztacala (CIFI).



Figure 1. Distribution of *Ophidion josephi* in the Western Atlantic, including previous records (circles) obtained from GBIF (2021), and new records (diamond).

Results

Systematic account

Order Ophidiiformes

Family Ophidiidae

Subfamily Ophidiinae

Genus *Ophidion* Linnaeus, 1758

Ophidion josephi Girard, 1858

Fig. 2; Table 1

New records. MEXICO • 7 specimens (177–212 mm SL); Las Barrancas, Alvarado, Veracruz; 19°00'24"N, 095°57'52"W; 15 Nov. 2018; Pedro Ramon Roman leg.; CIFI–222 • 5 specimens (198–213 mm SL); Las Barrancas, Alvarado, Veracruz; 19°00'24"N, 095°57'52"W; 15 Nov. 2019; Pedro Ramon Roman leg.; CIFI–1417 • 5 cleared

Table 1. Morphometrics measurements expressed as proportion to the standard length (SL) and head length (HL) of *Ophidion josephi* collected in the southwestern Gulf of Mexico.

Character	CIFI–222 (n = 7)	CIFI–1417 (n = 5)	CIFI–1907 (n = 1)
Weight [g]	40.0–67.5	43.5–60.2	55.3
Total length [mm]	180–215	201–218	201
Standard length [mm]	177–212	198–213	198
In %SL			
Body depth	13.3–16.3	12.7–15.9	13.5
Head depth	12.1–13.8	11.3–12.9	11.8
Head length	17.9–19.5	18.0–19.3	19.0
Depth at dorsal fin	13.6–16.1	13.0–15.7	13.0
Depth at anal fin	12.4–13.7	11.5–13.7	12.8
Preanal length	39.0–41.8	37.2–39.0	37.6
Predorsal length	24.7–27.4	25.7–27.0	26.4
Prepectoral length	15.4–17.8	15.4–17.1	16.0
Prepelvic length	5.0–6.9	4.7–6.0	5.5
Dorsal fin base	72.3–75.0	71.9–74.5	72.7
Anal fin base	57.6–61.3	59.1–62.1	62.1
Pectoral fin length	10.6–16.3	11.0–13.3	13.3
Pelvic fin length	6.6–15.4	13.9–16.1	16.1
Postorbital length	10.2–11.8	10.5–11.8	10.6
Eye length	3.3–4.3	3.2–4.0	3.5
In %HL			
Snout length	18.3–21.0	20.0–23.3	20.5
Interorbital width	16.9–20.9	15.2–16.6	16.5
Eye length	18.4–24.1	16.7–21.1	18.6
Upper jaw length	40.3–44.7	39.6–43.0	39.6

Table 2. Meristic counts of five specimens of *Ophidion josephi* (182–230 mm SL) collected in the southwestern Gulf of Mexico (CIFI–1649).

Character	CIFI–1649 (1) female	CIFI–1649 (2) female	CIFI–1649 (3) male	CIFI–1649 (4) male	CIFI–1649 (5) female
Caudal fin	9	9	9	9	9
Pectoral fin	23	21	21	22	22
Pelvic fin	2	2	2	2	2
Dorsal fin	142	139	139	147	142
Anal fin	115	108	112	117	115
Branchiostegal rays	7	7	7	7	7
Gill rakers (upper–lower)	2 + 4	2 + 4	2 + 4	2 + 4	2 + 4
Vertebrae (thoracic–caudal)	16 + 50	16 + 51	15 + 52	15 + 51	16 + 52

specimens (182–230 mm SL); Las Barrancas, Alvarado, Veracruz; 19°00'24"N, 095°57'52"W; 18 Oct. 2020; Pedro Ramon Roman leg.; CIFI 1649 • 1 specimen (198 mm SL); Las Barrancas, Alvarado, Veracruz; 19°00'24"N, 095°57'52"W; 27 Sep. 2021; Pedro Ramon Roman leg.; CIFI–1907.

Identification. The Mexican specimens of *Ophidion josephi* measured 177–213 mm SL and weighed 40.0–67.5 g (Fig. 2). The morphometric and meristic data are reported in Tables 1 and 2, respectively.

Description. Dorsal fin rays: 139–147; anal fin rays: 108–117; pectoral fin rays: 21–23; pelvic fin rays: 2; caudal fin rays: 9; branchiostegal 7; gill rakers total: 7; and trunk vertebrae total 16, and caudal vertebrae number 50 to 52. Elongate body covered with diminutive elongated cycloid scales, except for cephalic region. Identified by following combination of characteristics: short blunt snout (3.3%–4.4% of SL), slightly subterminal mouth, maxilla ending behind orbital margin. Moderate eye size, its length approximating that of snout (3.3%–4.3% SL). Five to four gill rakers on lower limb. Head length 17.9%–19.5% SL, with straight dorsal profile or in males strong crest, in straight line over preopercular region. Strong supraopercular spine covered by dermal fold. Pectoral fins shorter than head (57.5%–70% HL), round in shape, its coloration intense yellow, and in some cases, black. Distal margin of dorsal and anal fin black; that of anal fin wider. Predorsal fin length 24.7% to 27.4% in SL and its origin on middle part of pectoral fin. Yellow body coloration, some brown regions. Ventral part slightly lighter than dorsal one. Base of dorsal fin black along its entire length, becoming fainter near caudal fin. Three rows of dark spots on sides, upper one continuous, remaining ones interrupted and blurred. Body covered with cycloid and elongate scales, having basket-shaped pattern.

Diagnosis. This species is distinguished from its congeners by having three to four rows of spots usually fused into a solid stripe, with a distinct crest on the spots; and a swollen nape or crest.

Discussion

Ophidion josephi is a common species found in shallow coastal waters, which inhabits soft bottoms. It is the second most abundant cusk-eel in the bycatch of shrimp fishing (Hoese and Moore 1998). It has a narrow depth

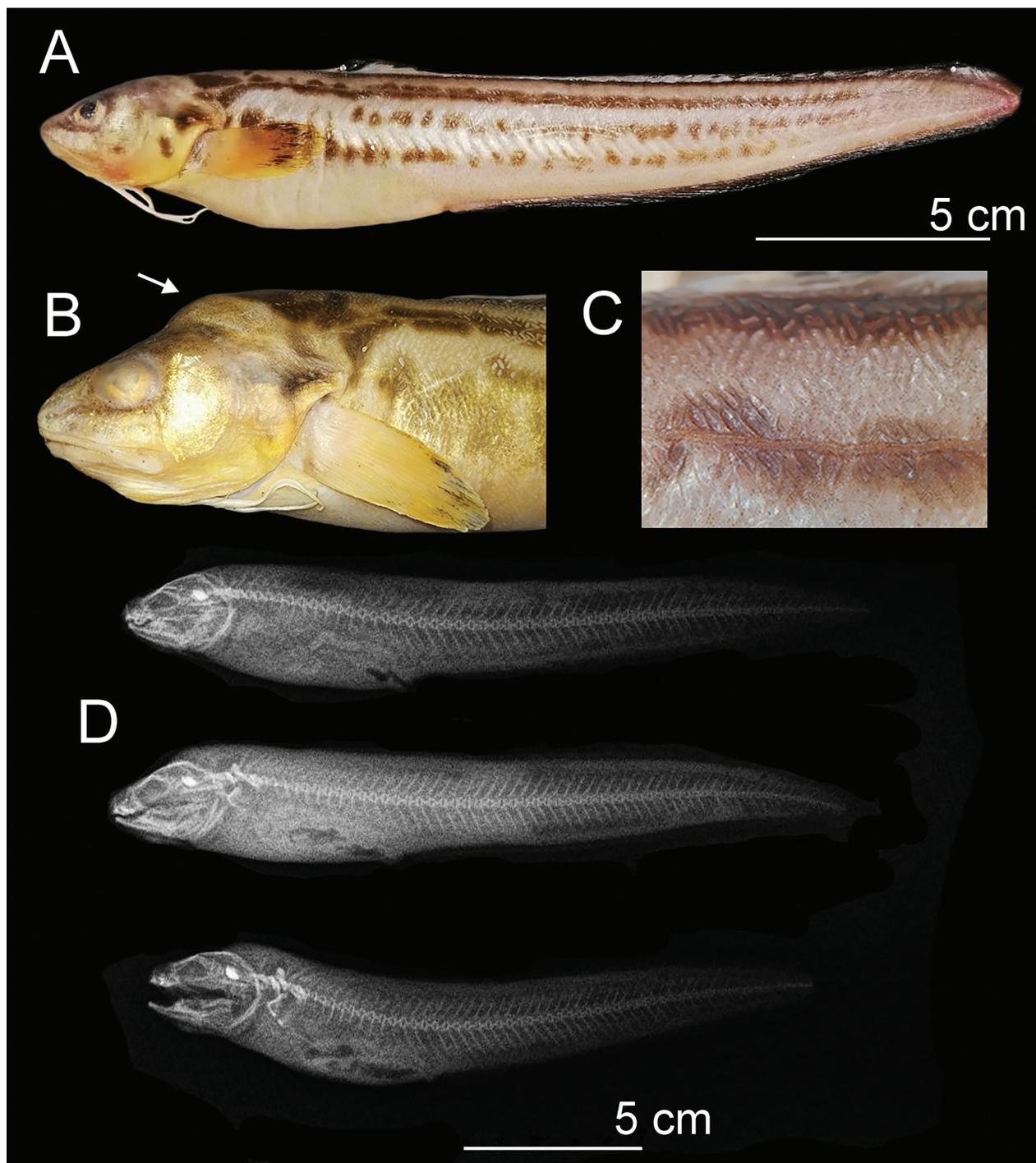


Figure 2. The Mexican specimen of *Ophidion josephi*, CIFI-222. **A)** An adult female, 181 mm SL. **B)** An adult male, 212 mm SL, the arrow points to the typical supraoccipital ridge of males. **C)** Arrangements of scales in body side. **D)** Radiographs of three specimens, 182–230 mm SL (CIFI-1949).

range of 9–55 m (Fahay and Hare 2003); spawn in the fall, has a rapid growth rate, and lives at least 27 months (Retzer 1991; Kells and Carpenter 2011).

The lack of detailed taxonomic sampling and analysis of the ichthyofauna in Mexican littorals is a possible cause of the absence of valid records of the species in the study area. However, Bautista-Hernández et al. (2001) mentioned *Ophidion welshi*, a synonym of *O. josephi*, as a species that is usually caught as a part of the bycatch

of the sardine fishery practiced in the community of Las Barrancas, Veracruz. Chávez-López and Morán-Silva (2019) mentioned *O. josephi* as part of the incidental bycatch in shrimp fishing in the Campeche and estuarine zone or the Papalopan River. However, there is no valid record deposited in any collection. The presently reported study describes the finding of *Ophidion josephi* from the coast of Veracruz, Mexico which constitutes the southernmost record of this fish in the western Atlantic.

Information about their biology is unknown, but, like other species of *Ophidion*, they can be organisms that tend to bury themselves in the area during the day and leave their shelter at night to feed (Matallanas and Riba 1980). As the work of the fishermen begins during the day, it is unlikely that the organisms are captured.

Despite the similarity with the Caribbean, the biogeographic affinity of coastal and reef fish in the central-southern region of Veracruz also has a temperate affinity for the Carolinian Province (Del Moral-Flores et al. 2013). The warm-temperate Carolina Province contains a biogeographic area that is delimited into two sections: within the Gulf of Mexico, its limits are between Cabo Romano, Florida and Cabo Rojo, Mexico; and in the Atlantic section it is found between Cape Hatteras, North Carolina, and Cape Canaveral, Florida (Briggs and Bowen 2012). Larval dispersion can be a factor that does not allow to delimit a clear biogeographic barrier between the North and South of the Gulf of Mexico. In

the Veracruz Reef System, near the area of this record, the oceanographic conditions, such as the presence of “nortes” (north winds) and the Campeche gyre (Salas-Pérez and Granados-Barba 2008) have an influence on larval diversity and abundance (Ayala-Rodríguez et al. 2016). Besides, it allows the establishment and extension of the range of distribution of various species of marine fish. This finding is relevant to understanding the geographic ranges of the species, a component of the Mexicana fauna.

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Fish populations and biomass in headwater streams of the Lake Tumba Landscape, DR Congo, 2007–2011

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Abstract

The fish biodiversity in the Congo River and its tributaries is extremely rich but the information on fish communities in the headwaters in terms of catch and biomass estimates is rare. Fishes in the running and stagnant waters in this region are of vital importance as a food resource for local residents. This study aimed to describe the fish community, catch, and biomass in the three headwater streams Bambou, Lebomo, and Bongo in the Lake Tumba Landscape (LTL) of the Democratic Republic of the Congo. Such information is of vital importance as a benchmark to understand the sustainability of the fish population for future generations of residents of the LTL. The field data were collected from 2007 through 2011, including dry and wet seasons. Here we present the results of this systematic, multi-annual study which was the first for fishes in streams of this region. In total, 50 species of 15 families were found in the nutrient-poor brown waters of these streams where high concentrations of humic acids cause a low pH. Among abundant species occurring in all three streams were the cyprinid *Enteromius holotaenia* (Boulenger, 1904), the mormyrid *Marcusenius moorii* (Günther, 1867), the alestids *Clupeocharax schoutedeni* Pellegrin, 1926 and *Bryconaethiops boulengeri* Pellegrin, 1900, and the clariid *Clarias angolensis* Steindachner, 1866. Bongo Stream was distinguished from the others by a rich abundance of *Alestopetersius compressus* (Poll et Gosse, 1963). The presence of several species at low pH (between 5.0 and 5.5) is new information that lowers the bottom of the pH interval for these species compared to earlier reports. The maximum total length (TL) of some other species was by 5–20 percentage points higher than those reported earlier. The median weight per unit effort (WPUE) in the streams varied between 30 and 115 g per hour during the dry seasons and between 18 and 86 g per hour during the wet seasons. The fish biomass in the streams varied between 0.05 and 0.7 g · m⁻² with a median 0.14 g · m⁻². This relatively low value compared to other tropical headwaters may be a result of the low pH and dark color of these headwaters. The results of the study serve as a reference point to which future monitoring of fish fauna can be compared for sustainable management of the LTL.

Keywords

acidity, catch, Congo River, fish biomass, fish species, headwaters, humic water, multi-annual data

Introduction

The Congo River and its tributaries, covering an area of about 4 000 000 km², is the world's second-largest drainage basin after the Amazon River. It's ichthyofauna is

extremely rich—to a large extent incompletely known (Shumway et al. 2003; Harrison et al. 2016). In the central basin of the Congo River catchment, the UN Central African Forest Initiative (CAFI) has designated the 65 700 km² Lake Tumba Landscape (LTL) as one of twelve pri-

ority conservation areas in Africa (Twagirashyaka and Inogwabini 2009). The primary focus for the area is on the freshwater ecosystem, and the LTL is also a Ramsar site (Ramsar n.d.). The LTL has a tropical rainforest climate. Many of the waters in the LTL have high concentrations of dissolved organic matter with humic acids which color the water brown and lower the pH (Matthes 1964; Zanga et al. 2019).

Fishes in the running and stagnant waters are of vital importance as a food resource for the local residents of the LTL; 85% of the protein in their diet derives from fishes (Carpe n.d.). There are indications that the sustainability of the fish population in the landscape is compromised by changes in land use, and in some areas also by overfishing (Brummett et al. 2011). Freshwater ecosystems are sensitive to the clearing of forests for agriculture, overgrazing by livestock, and logging activity since these actions can all cause soil erosion and increase water turbidity (Bojsen and Barriga 2002; Chimwanza et al. 2006; Weijters et al. 2009). In tropical streams that are poor in nutrients, many fish species depend largely on food from terrestrial sources (Welcomme and de Merona 1988). Therefore, the destruction of forests may result in a decline in aquatic productivity (Lorion and Kennedy 2009). Another alarming issue is the use of nonselective fishing methods such as dynamiting, plant toxins, or mosquito nets. These practices have a basis in pervasive poverty, problematic governance, and lack of knowledge that could be overcome to improve the sustainable productivity of the fishery. Despite the high diversity of fish in the region, ichthyological studies are scarce, especially those with quantitative, multi-annual catch statistics. The majority of published results of ichthyological studies of the LTL have focused on Lake Tumba, and its species (e.g., Marlier 1958; Matthes 1964) but there was one focusing on quantitative aspects (Zanga et al. 2019).

Some important fish studies have been performed on other parts of the Congo River basin. For example, the fish fauna of the largest tributary, the Lualaba River, and its associated waters has been documented by several expeditions (e.g., Banister and Bailey 1979). Other examples include a comprehensive study on the ichthyofauna of three northeastern tributaries of the middle Congo and their headwaters (Decru et al. 2017), and studies on the lower Congo River (e.g., Hanssens 2009; Lowenstein et al. 2011). In some sections of the middle Congo River with a high proportion of acid brown waters, species in the Salonga and Luilaka rivers have been reported (Inogwabini 2005). In a later investigation, a number of sites along these rivers and the connecting Yenge River were more intensely inventoried together with water chemistry (Monsembula Iyaba and Stiassny 2013). An important article about fishes in streams was published by Matthes (1964), who for two months in 1959 fished 34 sites upstream of the Ruki River, a left bank tributary to the Congo River. Further fish studies have been conducted on growth rates of single species (Mbadu Zebe et al. 2010)

and the impact of environmental variables on fish growth in the Congo basin (Pwema Kiamfu et al. 2011). The streams of the LTL, situated in the middle Congo area, have received less attention although they are important for the livelihoods of local residents. A single survey carried out in August 2010 to identify fish species is the only previous study in Bambou and Lebomo streams (Stiassny and Mamonekene unpublished*).

The presently reported study was undertaken on three LTL headwater streams, Bambou, Lebomo, and Bongo, to improve knowledge of community structure and diversity of the LTL fish fauna. These streams were investigated systematically over a period of 3–5 years, with a special focus on the catch and biomass data. We describe the stream fish community composition and biomass with the aim to compare variation within and between streams over years and seasons. The relations to water quality are explored, and comparisons are made with previous studies from the Congo River basin. The information presented in this paper will increase our knowledge on the fish biomass in central African waters that are relatively poor in nutrients, low in pH, and high in dissolved organic matter.

Material and methods

Characteristics of investigated streams. The investigated streams Bambou, Lebomo, and Bongo are situated in the Mai-Ndombe province in the central part of the Democratic Republic of the Congo (Fig. 1). This area has a humid tropical climate with dry seasons (usually from mid-May to mid-September as well as shorter periods from January to March) as well as wet seasons that vary in onset dates and duration. In the Köppen climate classification system, this is Af, i.e., a tropical rainforest climate (Peel et al. 2007). Seasons were nominally classified as wet for months February to May, and dry for months May to July since it was not possible to measure water level frequently due to logistical reasons in more than one of the streams during two of the five study years. Small streams (catchments), like the three studied here, are affected by occasional, local heavy showers and dry periods which diminish seasonality. The mean annual precipitation in the province is about 1600 mm (DRC Ministry of Planning 2005).

The stream catchments were estimated from satellite images and their area ranged from 90 to 180 km² (Table 1). The topography of the area is rather flat and the agriculture practiced in the catchments is a small-scale but extensive type of slash-and-burn. The land adjacent to the streams is sparsely populated with villages at some distance. The area around Bambou and Lebomo streams is populated by some 15 000 people living in 15 villages. The riparian area around Bambou is an 80 m broad gallery forest with trees, roots, and stones on the riverbank. The riparian area of the Lebomo was formerly

* Stiassny MLJ, Mamonekene V (n.d.) Fish diversity in the Lake Tumba Landscape. (Study in August 2010). Unpublished report.

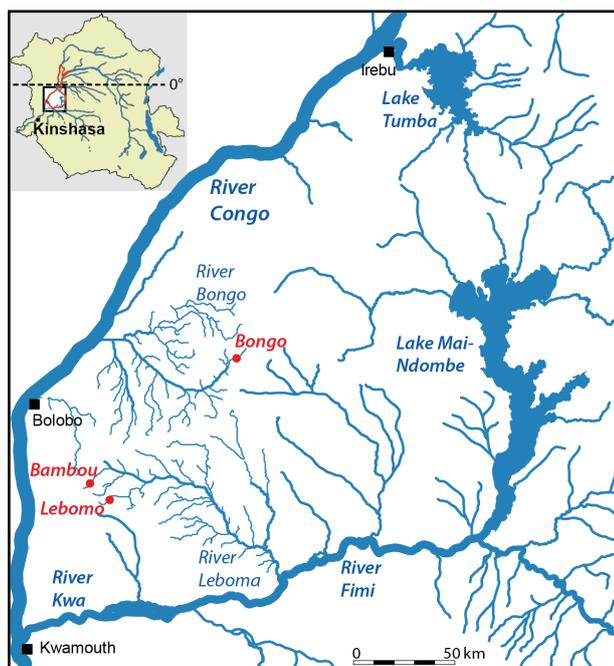


Figure 1. The sites investigated on the streams Bambo, Lebomo, and Bongo in the Lake Tumba Landscape, DR Congo. Based on a map from WWF (G. Tshimanga). The inserted map shows the Congo basin with the Ramsar area 1784 delimited in red.

used for cattle ranching. Mixed grassland savannah and forests cover the catchments of Bambo and Lebomo streams while the Bongo catchment is a mixed mature forest that is subjected to selective logging of blackwood (the endangered tree *Millettia laurentii*). This is evident from trails left by timber transport. The area close to the stream is very rugged with steep slopes. A waterfall of approximately 25 m in height lies in the highest section of the investigated part of Bongo Stream. The bottom substrate and the presence of aquatic vegetation were assessed in each transect by the use of a 1-m² wooden square frame. The bottom substrate of Bambo and Lebomo streams comprised sand and stones, while Bongo with its deeper water has clayey sediments in addition to stones.

Sampling and analysis. The study period was 2007–2011. In each stream 10 transects set approximately 200 m from each other were delineated. The total length covered was 1800 m. Five transects were placed upstream and five downstream of the existing bridges to facilitate subsequent sampling. In Bongo two transects became located upstream of the 25 m high waterfall and eight downstream. Width and depth were measured every meter by a stick at the ten fishing transects in each stream on eight occasions in Bambo and Lebomo, and six in Bongo. For each transect, a mean of three central depth values was used in order to avoid possible “pits”. Due to the heavy workload during fishing, this was done on other occasions close to fishing days.

An apparent visual observation of water color was recorded during a total of 53 days where the water was

Table 1. Basic physical and chemical data for the streams investigated in the headwater streams of the Lake Tumba Landscape, DR Congo. Drainage area data based on satellite images. Median width and depth with ranges in brackets. Chemical values presented as medians with ranges in brackets.

Character	Stream		
	Bambo	Lebomo	Bongo
Landscape type	Forest, savannah	Grassland, savannah	Mature forest
Land use	Natural	Ranching	Logging
Coordinates	2.29°S, 16.30°E	2.57°S, 16.55°E	1.95°S, 17.10°E
Bottom substrate	Sand, pebble	Sand, stone	Mud, boulder
Elevation [m]	440	380	340
Drainage area [km ²]	90	100	180
Number of measurements	8	8	6
Width [m]	7.6 (3.7–12.5)	7.7 (6.3–9.9)	8.5 (4.6–17.0)
Depth [m]	0.4 (0.17–1.4)	0.7 (0.38–0.97)	0.6 (0.18–1.15)
Number of measurements	10	10	4
pH	5.0 (4.8–5.8)	6.4 (6.0–6.8)	5.7 (5.5–5.8)
Color [mg Pt · L ⁻¹]	120 (30–120)	50 (10–90)	55 (40–60)
TOC [mg · L ⁻¹]	10.6 (4.5–12)	3.8 (0.8–8.9)	5.9 (4.5–6.5)
NO _{2,3} -N [μg · L ⁻¹]	70 (2–91)	52 (1–140)	300 (280–340)
Total P [μg · L ⁻¹]	41 (26–54)	35 (5–63)	69 (61–79)

Elevation is above the sea level. Pt = Platinum, TOC = Total organic carbon.

classified as white, clear, or brown as established for Amazonian waters by Sioli (1965).

Samples for water chemistry were collected at the bridge location on ten occasions in Bambo and Lebomo and on four occasions in Bongo. The samples were sent to the accredited water chemistry laboratory at the Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Uppsala, Sweden, and analyzed using standardized methods (International standards, ISO and European standards, EN).

Fishing was carried out in a way that allowed separation of catches between day (08:00–16:00 h or 18:00 h) and night (18:00–06:00 h). There was a variation in the times for setting and harvesting of the fishing gear which was set out up to five times a day at 08:00, 12:00, 14:00, 16:00, and/or 18:00 h. During the daytime, harvesting occurred two or four hours after nets were set. Night-time fishing nominally started at 18:00 h and continued until 06:00 h the following morning. The total number of fishing days was 176 of which 68 occurred during the dry seasons and 106 in wet seasons (Table 2). However, the number of fishing days varied between streams. Fishing was done more frequently on Bambo and Lebomo streams than on the more distant Bongo stream due to logistic problems with transport. Two sets of five different gillnets with mesh-size 1, 1.5, 2, 2.5, or 3 cm, 1 m high and 10 m long, were used on each 200 m transect. The nets were stretched perpendicular to the stream reach. Some transects (13%) were one to a few meters wider than 10 m, and thus not fully covered by the net. The nets were held up by ropes fastened to stones and anchored to the stream bed with sinks touching the bottom. Further-

more, to be able to catch fish of various sizes, lines with hooks of sizes 16, 14, and 12 were also used (the hook size decreased with increasing hook number). Lines with a total of 100 hooks baited with earthworms were set out from the riverbank between the nets crossing the river. However, hook sampling was used with less regularity than nets due to logistic reasons (Table 2). Out of a total of 176 fishing days, nets were used on 150 days, including occasions combined with hooks. Hooks were used separately or in combination with nets on 64 days. On site, each fish specimen was identified, weighed, and its total length (TL) was measured. The weighing was done up to the nearest 1 g (except for measurements before 2009, which were done up to 10 g accuracy), and TL was measured up to the nearest 1 mm. Every fish was assigned to a site, fishing time, and fishing gear. Events with no catch were also noted and included in the calculations of catch. After identification and measurement, live fish from hook fishing were in two cases returned to the stream whereas dead fish were used as food for the staff.

The number of sampling events varied between years but always included periods of the nominally dry and wet seasons. The streams were not fished during either the times of lowest or highest water levels as illustrated for Bambou Stream (Fig. 2). This was due both to transport difficulties at times of high precipitation and the need to avoid interfering with fishing by the local inhabitants. During the dry season when the water level drops in the streams, women were observed to use baskets to remove water from the pools and then use scoop baskets (ecopage) to catch fish.

An overview of the Congolese fish fauna has never been compiled and identification keys are lacking for the majority of regions and taxonomic groups (Decru et al. 2017). In the presently reported study, the first identifications of the specimens were made using FishBase (Froese and Pauly 2021) and local support. Specimens that were not identified in the field were given a vernacular name and photographed and three fish of each species were preserved in ethanol for later confirmation. Melanie Stiassny (American Museum of National History), during her visit to the station at Malebo (Bambou Stream), inspected the collection and helped to identify some specimens. Unfortunately, specimens kept at the station at Malebo (Bambou Stream) were inadequately preserved and later discarded. Based on photos, a number of species could later be verified or in some cases re-identified by specialists during a visit to the Royal Museum for Central Africa and the KU Leuven University in Belgium. Later, an additional check with the specialists was done. Photos may, however, not reflect all details that could be decisive for a correct determination. The procedures used may have had an impact on the species' determinations. In cases of apparent inaccuracy, specimens were only identified up to the genus level.

Weight (catch) was calculated as a daily sum of catches for all fishing gear and species. The Weight Per Unit Effort (WPUE) and number of fish per hour (NPUE) were

Table 2. Fishing days by stream, season, and gear for the headwater streams of the Lake Tumba Landscape, DR Congo.

Stream	Year	Season	Hook	Net	Net and hook	Total fishing days
Bambou	2007	Wet	—	12	—	12
Bambou	2008	Dry	—	5	—	5
Bambou	2008	Wet	4	14	—	18
Bambou	2009	Dry	—	2	—	2
Bambou	2010	Dry	—	7	3	10
Bambou	2010	Wet	4	6	—	10
Bambou	2011	Wet	3	7	—	10
Bambou	Total		11	53	3	67
Bongo	2008	Dry	1	7	—	8
Bongo	2008	Wet	—	7	1	8
Bongo	2009	Dry	1	6	—	7
Bongo	2009	Wet	—	6	—	6
Bongo	2010	Dry	—	2	4	6
Bongo	2010	Wet	—	3	5	8
Bongo	Total		2	31	10	43
Lebomo	2008	Dry	—	3	4	7
Lebomo	2008	Wet	—	7	—	7
Lebomo	2009	Dry	7	5	1	13
Lebomo	2009	Wet	—	5	—	5
Lebomo	2010	Dry	—	3	7	10
Lebomo	2010	Wet	7	7	1	15
Lebomo	2011	Wet	—	4	5	9
Lebomo	Total		14	34	18	66
Total			27	118	31	176

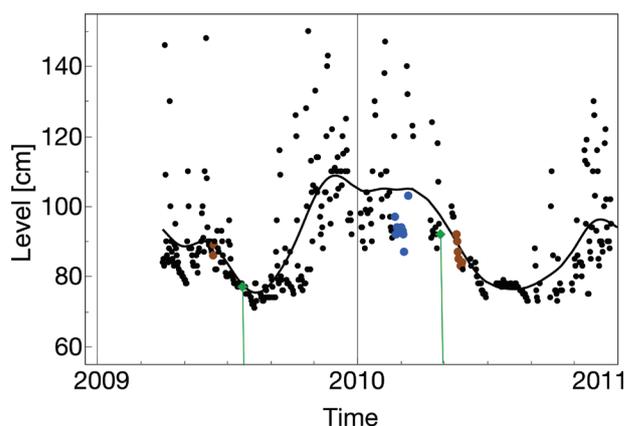


Figure 2. Water level recordings from Bambou Stream, the Lake Tumba Landscape, DR Congo. Fishing days indicated as blue markers for the wet season and brown markers for the dry season. Black markers indicate level recording days with no fishing. Line is a cubic smoothing. The green dots and vertical lines indicate the two dates with pH-measurements.

calculated with respect to actual fishing hours. Different subsets of fishing time and gear were used in order to obtain detailed, comparable subsets as specified for each statistical analysis.

In order to assess the total fish biomass in each stream, fishing was performed repeatedly during 4–12 days with the aim of achieving total fish depletion in the study area. The fishing gear was harvested 3–4 times per day and set up again immediately after each harvest, covering a 24-h period, until no or low catch was re-

corded, indicating complete or nearly complete removal of fish from the study area for the size of fish caught by the used mesh sizes.

Statistical analyses. Data handling and univariate statistical calculations were done in JMP® 14.0.0. Non-metric multidimensional scaling (NMDS) was used for visual representation and to compare the similarity in community composition for daytime (08:00–18:00 h) net-sampled fish abundances (NPUE). Only daytime catches were included in this analysis as not in all sites both day- and night-time catches were done. Differences between streams and seasons (groups) were tested with an analysis of similarities (ANOSIM, Clarke 1993), using the R statistic to test differences between groups ($R = 0$ means no difference, $R = 1$ means total dissimilarity between groups). The Bray–Curtis distance measure was used for separating groups. The ANOSIM and NMDS analyses were computed using the PRIMERv6 software (PRIMER-E Ltd, Plymouth, UK).

Results

Stream characteristics. Bambou Stream was the shallowest with a mean maximum depth of 0.5 m while the other streams were about 0.7 m deep on average (range in Table 1). The water depth did not vary significantly ($P > 0.05$) between dry seasons (0.5–1.8 m; mean 0.9 m) and rainy seasons (0.6–2.1 m; mean 1.1 m) when considering all the transects.

The streams were of similar width (Table 1) varying from 3.7 to 17 m along the study transects. The seasonal variation in the width was largest in Bongo where it could increase in width by 7.2 m to 9.8 m along the 1.8 km study reach during wet seasons (data not illustrated). The smallest seasonal variation in stream width of only 0.1 m was recorded in Lebomo. According to the visual water observations, white (turbid) water was always associated with the wet season while clear and brown waters were recorded during both wet and dry seasons.

Bambou and Bongo are tropical blackwaters with high concentrations of humic acids coloring the water deep brown (measured as total organic carbon (TOC) and water color) and causing a low pH of around 5 (Table 1). Lebomo Stream was slightly less colored and had a pH above 6. The groundwater level is likely higher during rainy seasons resulting in higher concentrations of humic matter reaching the streams from more superficial organic soils. Two pH values from Bambou Stream (green dots and vertical lines in Fig. 2); July 2009 with a pH of 5.8, and April 2010 with pH 4.8 illustrate events influenced by the water flow from the drainage area. The latter sample with the lower pH was also associated with a higher TOC concentration. Bambou and Lebomo were nutrient-poor streams with low concentrations of nitrogen and low to medium concentrations of Total-P (Table 1). Bongo was more nutrient rich with a maximum nitrogen concentra-

tion of $340 \mu\text{g} \cdot \text{L}^{-1}$ and a total phosphorus concentration of around $70 \mu\text{g} \cdot \text{L}^{-1}$.

Fish occurrence, abundance, size, and frequency. A total of 2028 fish individuals representing 50 species were caught in this study. The majority of specimens were identified up to the species level, however, ten could be identified up to genus only (Table 3). Sixteen species appeared in all streams while six or seven species were unique for each stream making a total of 19 unique species (Table 3). The 50 species were distributed over 15 families with species richness of 31 in Bambou, 36 in Lebomo, and 29 in Bongo. As the study period progressed, the cumulative species curves for each stream leveled off and approached a plateau (Fig. 3).

When considering the number of specimens per family caught in the three streams, the pattern varied slightly. Alestids stand out markedly with 26% of the total number of caught fish specimens belonging to just four species (Table 3). Two of these species (*Clupeocharax schoutedeni* Pellegrin, 1926, and *Bryconaethiops boulengeri* Pellegrin, 1900) occurred frequently in all streams. The alestid, *Alestopetersius compressus* (Poll et Gosse, 1963) was only caught in Bongo, where it was the most common species (22% of total abundance, Table 4). In all streams, cyprinids, clariids, and mormyrids, with 18%, 16%, and 15% of the total fish abundance respectively, were also families with high specimen abundance. Some species among cyprinids and clariids were caught in large numbers and occurred in all streams such as *Enteromius holotaenia* (Boulenger, 1904) with 13% of the total catch, and *Clarias angolensis* Steindachner, 1866, with 9%. Seventy-five percent of all fish caught belonged to the aforementioned four families. An overview of species with an abundance of 20 specimens or more caught in each stream is provided in Table 4. These common species accounted for more than 80% of all fish caught in Bambou and Bongo, and over 70% in Lebomo. Species unique to a stream were usually caught in small numbers, and sometimes just a single specimen. The exception was the high abundance of *Alestopetersius compressus* in Bongo exclusively caught by net (22% of total catch). Several taxa caught by net were also caught by hook. There were, however, six species captured only by the hook and just as single individuals *Protopterus dolloi* Boulenger, 1900, *Mormyrops anguilloides* (Linnaeus, 1758), *Channalabes apus* (Günther, 1873), *Clarias gabonensis* Günther, 1867, *Malapterurus melanochir* Norris, 2002, and *Microctenopoma nanum* (Günther, 1896).

Compared to thirteen other studies of fish fauna found in the Congo River basin (Table 5) three more species for the basin were recorded: the mormyrid *Marcusenius dundoensis* (Poll, 1967), the anabantid *Ctenopoma multispine* Peters, 1844, and the clariid *Platyclarias machadoi* Poll, 1977. Of those, *Ctenopoma multispine* and *Platyclarias machadoi* were present in all of our study streams, with the latter found in large numbers in Bambou (9% of total catch).

Table 3. Summary table for species caught in the headwater streams of the Lake Tumba Landscape, DR Congo. Total number and measured maximum total length (TL) of each species. The FishBase data (FB) on maximum (TL) or standard length (SL) are provided for comparison (Froese and Pauly 2021).

FAMILY Species	Number of fish sampled			TL max [cm]	FB TL max [cm]	FB SL max [cm]
	Bambou	Lebomo	Bongo			
PROTOPTERIDAE						
<i>Protopterus dolloi</i> Boulenger, 1900	0	0	2	82	130	
POLYPTERIDAE						
<i>Polypterus ornatipinnis</i> Boulenger, 1902	5	0	0	40	60	
<i>Polypterus polli</i> Gosse, 1988	1	0	0	22.2		32.1
MORMYRIDAE						
<i>Brienomyrus</i> sp.	1	0	9	15.5		
<i>Campylomormyrus</i> sp.	0	4	2	22		
<i>Cyphomyrus psittacus</i> (Boulenger, 1897)	11	0	0	20.5	30	
<i>Gnathonemus petersii</i> (Gunther, 1862)	0	5	0	23.6	35	
<i>Heteromormyrus</i> sp.	0	0	5	20.5		
<i>Hippopotamyrus</i> sp.	16	2	2	17		
<i>Marcusenius dundoensis</i> (Poll, 1967)	4	3	0	21		12.3
<i>Marcusenius moorii</i> (Günther, 1867)	16	6	94	20		21.4
<i>Mormyrops anguilloides</i> (Linnaeus, 1758)	0	1	0	37	150	
<i>Mormyrops</i> sp.	16	3	25	17.1		
<i>Mormyrus caballus</i> Boulenger, 1898	3	0	0	18.5	50	
<i>Myomyrus macrodon</i> Boulenger, 1898	0	9	0	24	24	
<i>Petrocephalus christyi</i> Boulenger, 1920	1	4	3	13	7.7	
<i>Petrocephalus simus</i> Sauvage, 1879	0	2	1	12.1	12	
<i>Pollimyrus adspersus</i> (Günther, 1866)	0	1	0	8	9.8	
<i>Pollimyrus</i> sp.	2	1	35	15.5		
<i>Pollimyrus tumifrons</i> (Boulenger, 1902)	2	14	0	19	11.2	
ALESTIDAE						
<i>Alestopetersius compressus</i> (Poll et Gosse, 1963)	0	0	152	11.5		7.8
<i>Brycinus</i> sp.	0	0	1	6.5		
<i>Bryconaethiops boulengeri</i> Pellegrin, 1900	96	52	18	24	25	
<i>Clupeocharax schoutedeni</i> Pellegrin, 1926	127	84	3	20	25	
DISTICHODONTIDAE						
<i>Mesoborus crocodilus</i> Pellegrin, 1900	3	3	0	29.5	26.5	
<i>Phago boulengeri</i> Schilthuis, 1891	1	0	0	14	17	
HEPSETIDAE						
<i>Hepsetus microlepis</i> (Boulenger, 1901)	5	2	12	38	26.7	
CYPRINIDAE						
<i>Enteromius holotaenia</i> (Boulenger, 1904)	171	25	77	17.5		12
<i>Raiamas christyi</i> (Boulenger, 1920)	42	43	0	19	17.7	
CLARIIDAE						
<i>Channallabes apus</i> (Günther, 1873)	0	2	0	29	41.6	
<i>Clarias angolensis</i> Steindachner, 1866	22	7	146	40	35	
<i>Clarias gabonensis</i> Günther, 1867	1	3	0	21	36	
<i>Clarias jaensis</i> Boulenger, 1909	1	11	8	29	48,3	
<i>Clarias platycephalus</i> Boulenger, 1902	3	3	19	43	37.6	
<i>Clarias</i> sp.	0	1	0	14.5		
<i>Platyclarias machadoi</i> Poll, 1977	73	12	7	26.2	20.1	
MALAPTERURIDAE						
<i>Malapterurus melanochir</i> Norris, 2002	0	1	2	14		98
<i>Malapterurus</i> sp.	0	0	1	11		
CLAROTEIDAE						
<i>Parauchenoglanis balayi</i> (Sauvage, 1879)	117	5	0	19.5	39	31.7
<i>Parauchenoglanis pantherinus</i> (Pellegrin, 1929)	2	0	0	17		29.2
<i>Parauchenoglanis punctatus</i> (Boulenger, 1902)	12	58	0	38		41.0
SCHILBEIDAE						
<i>Schilbe marmoratus</i> Boulenger, 1911	0	69	35	21		21.7
MOCHOKIDAE						
<i>Euchilichthys royauxi</i> Boulenger, 1902	0	1	2	12.5	22	
<i>Synodontis</i> sp.	0	0	1	12.5		
ANABANTIDAE						
<i>Ctenopoma multispine</i> Peters, 1844	14	5	8	15	14	
<i>Microctenopoma nanum</i> (Günther, 1896)	0	0	1	9.2		6.7
CICHLIDAE						
<i>Hemichromis elongatus</i> (Guichenot, 1861)	44	14	13	22		18.7
<i>Hemichromis stellifer</i> Loisele, 1979	41	5	22	12.6		10
<i>Pelmatochromis nigrofasciatus</i> (Pellegrin, 1900)	0	2	0	7.3		11.6
MASTACEMBELIDAE						
<i>Mastacembelus congicus</i> Boulenger, 1896	1	5	0	36.5		43.5
Total number of species	31	36	29			

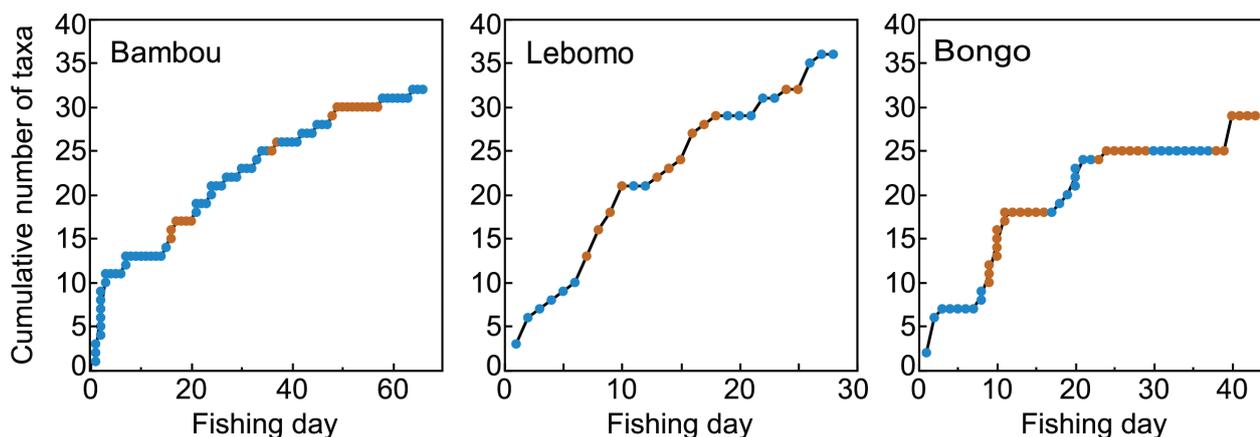


Figure 3. Species cumulative curves from the whole study period in three headwater streams of the Lake Tumba Landscape, DR Congo. Blue dots indicate wet season, brown dots dry season.

Table 4. Major species (caught in numbers ≥ 20) sorted by abundance for each stream for the headwater streams of the Lake Tumba Landscape, DR Congo. Percentages are compared to the total abundance in each stream.

Bambou		Lebomo		Bongo	
Species	[%]	Species	[%]	Species	[%]
<i>Enteromius holotaenia</i>	20	<i>Clupeocharax schoutedeni</i>	18	<i>Alestopetersius compressus</i>	22
<i>Clupeocharax schoutedeni</i>	15	<i>Schilbe marmoratus</i>	15	<i>Clarias angolensis</i>	21
<i>Parauchenoglanis balayi</i>	14	<i>Parauchenoglanis punctatus</i>	12	<i>Marcusenius moorii</i>	13
<i>Bryconaethiops boulengeri</i>	11	<i>Bryconaethiops boulengeri</i>	11	<i>Enteromius holotaenia</i>	11
<i>Platyclarias machadoi</i>	9	<i>Raiamas christyi</i>	9	<i>Schilbe marmoratus</i>	5
<i>Hemichromis elongatus</i>	5	<i>Enteromius holotaenia</i>	5	<i>Pollimyrus</i> sp.	5
<i>Raiamas christyi</i>	5			<i>Mormyrops</i> sp.	4
<i>Hemichromis stellifer</i>	5			<i>Hemichromis stellifer</i>	3
<i>Clarias angolensis</i>	3				

As expected, larger species tended to be caught with a hook rather than with a net. There was a difference in total length (TL) between catches with the two gears (Wilcoxon $P < 0.0001$). The median TL was 15.3 cm for hook fishing and 12.5 cm for nets. The largest species caught only by the hook was *Protopterus dolloi* in the Bongo stream with a maximum TL of 82 cm. Large species caught by net and reaching lengths of about 40 cm were *Polypterus ornatipinnis* Boulenger, 1902, *Hepsetus microlepis* (Boulenger, 1901), and *Parauchenoglanis punctatus* (Boulenger, 1902). There was a lack of small individuals due to the available fishing gear. The shortest fish caught was 6.3 cm TL.

The maximum TL of three species in this study exceeded the maximum TL recorded in FishBase (Froese and Pauly 2021) by up to a factor of 1.7 (Table 6). For one species, *Enteromius holotaenia*, only standard length (SL) was available in FishBase. We suggest a TL of 17.5 cm be added in FishBase for *E. holotaenia*, based on the maximum TL from this study (Table 6).

The high waterfall in Bongo most probably impeded some species from moving upstream. Only six species were found upstream from the 25 m high waterfall: *Alestopetersius compressus*, *Clarias angolensis*, *Clarias platycephalus* Boulenger, 1902, *Enteromius holotaenia*, *Marcusenius moorii* (Günther, 1867), *Mormyrops* sp. They were all found downstream of the waterfall as well.

Visual water color and fish occurrence. *Enteromius holotaenia*, *Hemichromis elongatus* (Guichenot, 1861), *Cyphomyrus psittacus* (Boulenger, 1897), *Bryconaethiops boulengeri*, and *Platyclarias machadoi* were species caught in the largest numbers during periods with brown water. Species almost totally lacking during brown water phases were *Alestopetersius compressus* in Bongo, *Schilbe marmoratus* Boulenger, 1911 and *Parauchenoglanis punctatus* in Bongo and Lebomo, as well as *Marcusenius moorii* in all streams. Species not caught during periods of brown water were roughly equally abundant during white and clear water phases.

Influence of pH on fish occurrence. Twenty-seven species in the streams occurred at pH 5, seven species were added when the pH was 5.5, and six species at pH 6 (Table 7). A comparison with pH ranges from FishBase (Froese and Pauly 2021) suggest that several species are present in much lower pH ranges than have previously been reported (Table 7).

Diurnal differences. Comparisons of NPUE and WPUE between day and night catches were inconclusive (Wilcoxon non-parametric test). The majority of species were caught during the daytime and *Hemichromis elongatus*, *Alestopetersius*

Table 5. Species list from the presently reported study compared with published records from other streams/ivers in the Congo River drainage area.

FAMILY Species	Reference study													Total No.
	1	2	3	4	5	6	7	8	9	10	11	12	13	
PROTOPTERIDAE														
<i>Protopterus dolloi</i>	–	x	x	–	x	x	x	–	–	–	–	–	x	6
POLYPTERIDAE														
<i>Polypterus ornatipinnis</i>	–	–	x	–	x	x	–	x	–	–	–	–	–	4
<i>Polypterus polli</i>	–	–	–	–	–	x	–	–	–	–	–	–	–	1
MORMYRIDAE														
<i>Cyphomyrus psittacus</i>	–	x	–	x	x	x	–	x	–	–	–	–	x	6
<i>Gnathonemus petersii</i>	L	x	x	–	x	x	–	x	x	–	x	–	x	9
<i>Marcusenius dundoensis</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	0
<i>Marcusenius moorii</i>	B L N	x	x	–	x	x	–	x	–	x	x	x	x	10
<i>Mormyrops anguilloides</i>	–	x	x	x	x	x	–	x	–	–	–	–	x	7
<i>Mormyrus caballus</i>	–	x	x	x	x	x	–	x	–	–	x	–	–	7
<i>Myomyrus macrodon</i>	–	–	–	x	–	–	–	–	–	–	–	–	–	1
<i>Petrocephalus christyi</i>	–	x	–	–	x	x	–	x	x	–	x	–	x	7
<i>Petrocephalus simus</i>	–	–	–	–	–	x	x	–	–	x	–	x	–	4
<i>Pollimyrus adpersus</i>	–	–	–	–	x	x	–	–	–	–	–	–	–	2
<i>Pollimyrus tumifrons</i>	–	–	–	–	–	x	–	–	–	–	–	–	–	1
ALESTIDAE														
<i>Alestopetersius compressus</i>	–	–	–	–	–	x	–	–	–	–	–	–	–	1
<i>Bryconaethiops boulengeri</i>	B	x	x	–	x	x	x	x	x	x	x	–	x	11
<i>Clupeocharax schoutedeni</i>	–	x	x	–	–	–	–	–	–	–	–	–	x	3
DISTICHODONTIDAE														
<i>Mesoborus crocodilus</i>	–	–	x	–	–	x	–	x	x	–	x	–	x	6
<i>Phago boulengeri</i>	–	x	–	–	x	x	x	x	–	–	x	–	x	7
HEPSETIDAE														
<i>Hepsetus microlepis</i>	–	–	x	–	x	x	–	–	x	x	x	–	x	7
CYPRINIDAE														
<i>Enteromius holotaenia</i>	–	–	–	–	–	x	–	–	x	x	x(cf)	–	–	4
<i>Raiamas christyi</i>	B(cf)	–	–	–	–	x	x	–	x	x	x	x	x	8
CLARIIDAE														
<i>Channallabes apus</i>	B	x	x	x	x	x	–	–	–	–	x	–	x	8
<i>Clarias angolensis</i>	–	x	x	x	x	x	–	–	–	–	x	x	x	8
<i>Clarias gabonensis</i>	–	x	x	x	x	x	–	–	x	x	–	x	x	9
<i>Clarias jaensis</i>	–	–	–	–	–	–	–	–	–	x	–	–	–	1
<i>Clarias platycephalus</i>	B	x	x	–	x	x	–	–	–	–	–	x	–	5
<i>Platyclarias machadoi</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	0
MALAPTERURIDAE														
<i>Malapterurus melanochir</i>	–	–	x	–	–	x	–	–	–	–	–	–	x	3
CLAROTEIDAE														
<i>Parauchenoglanis balayi</i>	–	–	–	x	x	x	–	–	–	x	–	–	–	4
<i>Parauchenoglanis pantherinus</i>	–	–	–	–	–	–	–	–	–	x	–	–	–	1
<i>Parauchenoglanis punctatus</i>	N	x	x	–	x	x	–	x	–	–	x	x	x	9
SCHILBEIDAE														
<i>Schilbe marmoratus</i>	–	x	x	–	x	x	–	x	x	–	x	–	x	8
MOCHOKIDAE														
<i>Euchilichthys royauxi</i>	–	–	–	–	–	x	–	–	–	–	–	–	–	1
ANABANTIDAE														
<i>Ctenopoma multispine</i>	–	–	–	–	–	–	–	–	–	–	–	–	–	0
<i>Microctenopoma nanum</i>	B L	x	x	–	x	x	x	–	–	x	x	–	x	9
CICHLIDAE														
<i>Hemichromis elongatus</i>	B N	–	x	x	x	x	–	x	x	x	x	x	–	10
<i>Hemichromis stellifer</i>	B	–	–	x	x	–	–	–	x	–	x	x	–	6
<i>Pelmatochromis nigrofasciatus</i>	–	–	x	–	x	x	–	–	–	–	x	–	x	5
MASTACEMBELIDAE														
<i>Mastacembelus congicus</i>	–	–	–	–	x	x	–	x	–	–	x	–	–	4
Total number	10	17	20	10	24	32	6	14	11	12	19	9	20	

Reference studies: 1 = Stiassny and Mamonekene unpublished (See footnote on page 196) Fish diversity in the Lake Tumba Landscape. (Study in August 2010). Unpublished report.) (Lake Tumba Landscape); 2 = Zanga et al. 2019 (Lake Tumba); 3 = Inogwabini 2005 (Salonga National Park); 4 = Wamuini Lunkayilakio et al. 2010 (Inkisi, Lower Congo); 5 = Monsembula Iyaba et al. 2013 (N'sele River); 6 = Decru et al. 2017 (NE tributaries to Congo River); 7 = Matthes 1964 (Ikela area); 8 = Mbimbi Mayi Munene and Stiassny 2011 (Kwilu River, Kasai region); 9 = Liyandja et al. 2019 (Musolo River catchment); 10 = Mamonekene and Stiassny 2012 (Ogowé and Kouilou rivers); 11 = Ibala Zamba et al. 2019 (Lefini River); 12 = Batiabo Mikembi et al. 2019 (Loua River); 13 = Stiassny et al. 2021 (Mfini River). X = species found; minus sign = species not found, B = found in Bambou Stream, L = found in Lebomo Stream, N = found in Ngampoko Stream, cf = the species was not fully identified in the article. Total No. denotes the total number of studies reporting a fish.

Table 6. Presently reported species from headwater streams of the Lake Tumba Landscape, DR Congo with lengths considerably larger than maximum total length (TL) recorded in FishBase (FB, Froese and Pauly 2021).

Species	No. of fish caught	Median TL [cm]	Max TL [cm]	FB TL (SL) [cm]	Max TL/ FB TL
<i>Clarias angolensis</i>	175	19.5	40	35	1.1
<i>Enteromius holotaenia</i>	273	12.0	17.5	na (12)	na
<i>Raiamas christyi</i>	85	15.6	23.8	17.7	1.3
<i>Pollimyrus tumifrons</i>	16	16.0	19	11.2	1.7

SL = standard length, na = not available.

Table 7. Fish species list with lowest recorded pH from the presently reported study from headwater streams of the Lake Tumba Landscape, DR Congo confronted with the data on pH range from FishBase (Froese and Pauly 2021).

FAMILY Species	Lowest pH	pH from FishBase
PROTOPTERIDAE		
<i>Protopterus dolloi</i>	5.5	na
POLYPTERIDAE		
<i>Polypterus ornatipinnis</i>	5.0	na
<i>Polypterus polli</i>	5.0	na
MORMYRIDAE		
<i>Cyphomyrus psittacus</i>	5.0	6.8–7.2
<i>Gnathonemus petersii</i>	6.0	6.0–8.0
<i>Marcusenius dundoensis</i>	5.0	na
<i>Marcusenius moorii</i>	5.0	na
<i>Mormyrops anguilloides</i>	6.0	na
<i>Mormyrus caballus</i>	5.0	na
<i>Myomyrus macrodon</i>	6.0	na
<i>Petrocephalus christyi</i>	5.0	na
<i>Petrocephalus simus</i>	5.5	na
<i>Pollimyrus adspersus</i>	6.0	na
<i>Pollimyrus tumifrons</i>	5.0	na
ALESTIDAE		
<i>Alestopetersius compressus</i>	5.5	na
<i>Bryconathrops boulengeri</i>	5.0	na
<i>Clupeocharax schoutedeni</i>	5.0	6.0–7.5
DISTICHODONTIDAE		
<i>Mesoborus crocodilus</i>	5.0	na
<i>Phago boulengeri</i>	5.0	na
HEPSETIDAE		
<i>Hepsetus microlepis</i>	5.0	na
CYPRINIDAE		
<i>Enteromius holotaenia</i>	5.0	6.0–6.5
<i>Raiamas christyi</i>	5.0	6.5–7.0
CLARIIDAE		
<i>Channallabes apus</i>	6.0	6.0–8.0
<i>Clarias angolensis</i>	5.0	7.0–9.0
<i>Clarias gabonensis</i>	5.0	7.0–9.0
<i>Clarias jaensis</i>	5.0	na
<i>Clarias platycephalus</i>	5.0	na
<i>Platyclarias machadoi</i>	5.0	na
MALAPTERURIDAE		
<i>Malapterurus melanochir</i>	5.5	na
CLAROTEIDAE		
<i>Parauchenoglanis balayi</i>	5.0	na
<i>Parauchenoglanis pantherinus</i>	5.0	na
<i>Parauchenoglanis punctatus</i>	5.0	na
SCHILBEIDAE		
<i>Schilbe marmoratus</i>	5.5	6.5–7.5
MOCHOKIDAE		
<i>Euchilichthys royauxi</i>	5.5	na
ANABANTIDAE		
<i>Ctenopoma multispine</i>	5.0	6.0–7.5
<i>Microctenopoma nanum</i>	5.5	na
CICHLIDAE		
<i>Hemichromis elongatus</i>	5.0	na–7.0
<i>Hemichromis stellifer</i>	5.0	na
<i>Pelmatochromis nigrofasciatus</i>	6.0	na
MASTACEMBELIDAE		
<i>Mastacembelus congicus</i>	5.0	na

na = not available.

compressus, *Mormyrops* sp., *Marcusenius moorii*, and *Platyclarias machadoi* were always day-time catches. Only one species was solely caught during the night, and this was *Myomyrus macrodon* Boulenger, 1898 in Lebomo. No other species were regularly caught in significant quantities during night-time in any stream, but all those caught at night belonged to the family Mormyridae.

Fish catches. A compilation of net catches in each stream between years and seasons with day-time fishing effort is given in Table 8. NPUE was significantly higher during dry seasons in Lebomo and Bongo (Wilcoxon $P = 0.04$ and 0.004 , respectively). For Bongo, WPUE was significantly higher also during dry seasons (Wilcoxon $P = 0.02$).

ANOSIM analysis of community composition based on NPUE data from daytime net fishing clustered the streams separately from each other (ANOSIM $P = 0.001$, Global $R = 0.583$, Fig. 4). ANOSIM did not indicate seasonal differences in community composition based on NPUE (seasons: $P = 0.693$, Global $R = 0.078$).

Estimation of fish biomass. The median fish biomass for all streams and occasions combined was $0.14 \text{ g} \cdot \text{m}^{-2}$. The biomass varied from 0.05 to $0.21 \text{ g} \cdot \text{m}^{-2}$ in Bambou, from 0.14 to $0.70 \text{ g} \cdot \text{m}^{-2}$ in Bongo, and from 0.05 to $0.43 \text{ g} \cdot \text{m}^{-2}$ in Lebomo (Table 9). Only in one stream was total fish depletion achieved, i.e. no fish caught on the last fishing day, while in the other streams up to 4% of the total catch was still added on the last day of fishing.

Discussion

Species distributions in the streams. During the presently reported study, we identified 31, 36, and 29 species in Bambou, Lebomo, and Bongo, respectively. Thanks to intense and prolonged fishing during both day and night these values are likely to be close to the total number of species present that could be caught with the net meshes and hook sizes used (Fig. 3). The count of species does, however, miss smaller-sized ones due to the fishing gear. The shortest fish caught was 6.3 cm.

Table 8. Compilation of comparable catch statistics of the presently reported study from headwater streams of the Lake Tumba Landscape, DR Congo during daytime net-fishing period (08.00–16.00/18.00) with all 10 nets (transects).

Stream	No. of fishing days		NPUE [no. · h ⁻¹]		WPUE [g · h ⁻¹]	
	Dry	Wet	Dry	Wet	Dry	Wet
Bambou	12	18	1.4	2.6	30	86
Lebomo	26	25	0.9	0.5	38	18
Bongo	19	17	2.8	1.0	115	69
Total	57	60				

Dry and Wet refer to the respective seasons. Number of fishing days and median values for number of fish per hour (NPUE) and weight of catch per hour (WPUE) for seasons classified as dry or wet.

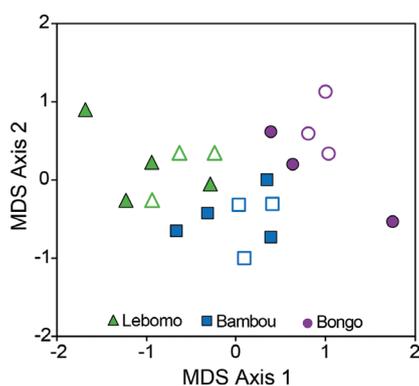


Figure 4. Non-metric multidimensional scaling (MDS) ordinations showing differences in community structure between streams and seasons of the presently reported study in three headwater streams of the Lake Tumba Landscape, DR Congo. Filled markers represent wet seasons, open markers dry seasons.

Stiassny and Mamonekene (unpublished*) also fished in two of these streams and caught only two species of smaller size: *Aphyosemion cognatum* Meinken, 1951 with a maximum recorded TL of 5 cm, and *Epiplatys multifasciatus* (Boulenger, 1913) with a TL of 6 cm (Froese and Pauly 2021). Their fishing gear involved rotenone which kills all specimens independent of size and therefore covers also the smallest species. Compared to the number of species found in earlier studies in the Congo River tributaries, the species richness of our study was relatively low. For example, in the Lualaba River and connected waters of Upemba National Park, 131 species were recorded (Banister and Bailey 1979), three Congo Basin tributaries recorded 187 to 246 species (Decru et al. 2017), in streams of Salonga National Park 56 (Inogwabini 2005) to 152 species (Monsembula Iyaba and Stiassny 2013) were found. The reason for the low richness in our study streams is most likely that these streams are short headwater streams compared to the other studies that investigated larger sections of each river. Headwater streams do often have lower richness than downstream sections of a river (Matthews 1986; Decru et al. 2017) which we suggest to be the main reason for the relatively low richness in our study together with the finding that they were very acidic which we discuss further below.

Many of the fish species of our study have been found in similar streams in the region (Table 5). One of the most abundant species in our study, *Enteromius holotaenia* is recorded as widespread in the Congo River area in FishBase (Froese and Pauly 2021). This species was, however, not regularly noted in the river fish stocks from the study areas summarized in Table 5 except in the northeastern tributaries of the Congo River and in the Republic of Congo (Mamonekene and Stiassny 2012; Decru et al. 2017; Ibala Zamba et al. 2019). Another species frequently found in our study,

Table 9. Estimation of fish biomass of the presently reported study from three headwater streams of the Lake Tumba Landscape, DR Congo.

Stream	Year	Season	Gear	Fishing days	Fishes caught	Stream width [m]	Biomass [g·m ⁻²]
Bambou	2010	Dry	n	4	40	7.23	0.115
Bambou	2010	Wet	n	5	44	8.03	0.212
Bambou	2011	Wet	n	4	22	8.79	0.045
Bongo	2010	Dry	nh	5	166	7.22	0.707
Bongo	2010	Wet	nh	8	33	9.37	0.135
Lebomo	2008	Dry	n	7	105	8.02	0.253
Lebomo	2009	Dry	nh	5	55	7.75	0.137
Lebomo	2009	Wet	n	10	22	7.56	0.066
Lebomo	2010	Dry	nh	10	31	8.01	0.083
Lebomo	2010	Wet	n	5	21	8.18	0.054
Lebomo	2011	Wet	nh	9	90	7.79	0.425
Median					40		0.135

Fishing gear n = net, nh = net and hook.

Clupeocharax schoutedeni, is classified as vulnerable in the IUCN Red List of threatened species, due to its continuing decline in areal distribution and its only occasional appearance in parts of the Congo River basin where it is considered endemic (IUCN 2021). The rich occurrence of *Clupeocharax schoutedeni* in our study streams is therefore valuable information along with additional records from Salonga National Park and Lake Tumba (Monsembula Iyaba and Stiassny 2013; Zanga et al. 2019). The dominant occurrence of *Alestopetersius compressus* in Bongo has not been recorded in the other streams of the Lake Tumba Landscape (Table 5) but it occurred in many of the northeastern tributaries of the Congo River that includes both headwaters and lower reaches (Decru et al. 2017).

Platyclarias machadoi, present in all of our study streams, but in particularly large numbers in Bambou, needs a special comment as it is not recorded in the studies compiled in Table 5. Due to the small amount of information on its distribution, it is stated as DD-species (data deficient) in the IUCN Red List (IUCN 2021). There are also two more species *Marcusenius dundoensis* and *Ctenopoma multispine* which were found in our streams that were not recorded in the other river investigations listed in Table 5. Both species have, however, been reported previously from the nearby Kasai River basin (Froese and Pauly 2021). As many of the Congo River tributaries are poorly sampled it would not be surprising to find the presence of species new to the region after (more) intensive sampling.

A species caught in all three streams although in a smaller number as well as in several other rivers of lower and middle Congo is *Hepsetus microlepis*. In the investigations of the Salonga area and N'sele region it is named *Hepsetus odoe* as it precedes the revision of the genus and the revalidation of *Hepsetus microlepis* (see Monsembula Iyaba and Stiassny 2013; Stiassny et al.

* See footnote on page 196.

2021). The fish names in our study follow those of Decru et al. (2015). *Hepsetus microlepis* is reported from large parts of the lower and middle Congo basin in rivers as well as in large lakes (Decru et al. 2015). The species *Myomyrus macrodon* was the only one caught only during the night. Mormyridae, the family of *Myomyrus macrodon* and all the other species caught at night in this study, are nocturnally active and noted for large cerebellums as well as for use of electricity and sound that are beneficial when searching for food at night (Froese and Pauly 2021).

Fish size. Headwater streams are usually environmentally impoverished in relation to downstream watercourses as suggested by the river continuum concept (Welcomme 1974; Doretto et al. 2020). A smaller diversity of habitats and limited food resources probably affect the growth and occurrence of fish in our acid headwater streams. Information on fish size distribution is not commonly available but information on maximum TL can be used as a comparison. The common fish *Parauchenoglanis balayi* (Sauvage, 1879) is such an example. Its maximum TL noted in FishBase (Froese and Pauly 2021) is 39 cm but the longest single specimen in our study was 32 cm, with all others reaching lengths < 20 cm. *Clupeocharax schoutedeni* is another abundant species with > 200 caught specimens with a maximum length of 20 cm in our study compared to the FishBase TL maximum of 25 cm.

Some of the common species, however, in the presently reported study, reached larger sizes than recorded in FishBase (Froese and Pauly 2021). *Clarias angolensis* and *Raiamas christyi* (Boulenger, 1920) are such cases where the longest individuals caught in our study exceeded the FishBase maximum values by 5 and 20 percentage points, respectively. The most frequent TL of *Clarias angolensis* varied between 16.5 and 23.5 cm with a median of 19.5 cm while the corresponding values for the *Raiamas christyi* were 11.5–19.0 with a median of 15.6 cm. *Pollimyrus tumifrons* (Boulenger, 1902) in Bongo had several specimens with TL larger than the max TL listed in FishBase. The large data set of this study is probably the reason for finding large specimens of species that were not usually so well studied.

Tolerance of acidity. The majority of fish thrive at a narrow range of a near neutral pH (Kwong et al. 2014). As fish are sensitive to low pH which affects their survival and reproduction (Kwong et al. 2014), it is remarkable that a majority of the captured species were found in water with a pH between 5.0 and 5.5 (Table 7). There are, however, other areas in the central Congo basin with fish studies in highly acid waters. In three rivers of Salonga National Park in the Equateur Province, pH values of 4.8 and 4.9 were recorded when fish were caught at 15 sites (Monsembula Iyaba and Stiassny 2013). Nineteen of the

species found in our three streams were also present in the Salonga waters. Sometimes these fish were just found at single sites in Salonga, but *Bryconaethiops boulengeri*, *Mesoborus crocodilus* Pellegrin, 1900, *Schilbe marmoratus*, and *Hemichromis elongatus* had a wider regional occurrence. In recent studies of Lake Tumba, where the median pH of the water was 4.3, reported species that were also found in our study were *Mormyrops anguilloides* and *Clarias angolensis* (see Zanga et al. 2019). This indicates a high tolerance to the low pH of these species. Taken together with the findings in our study, we suggest that the minimum pH listed in FishBase (Froese and Pauly 2021) needs to be adjusted for some species from this region where brown, acid waters are so common.

Fish biomass aspects. Three earlier publications from Africa with areal fish biomass data were found (Loubens 1969, 1970; Kapetsky unpublished*). However, those dealt with relatively large rivers and seasonally flooded ones (Kafue River and a tributary to Lake Chad). Therefore, we compared our data with some tropical streams from South America. These originate from Brazil (two catchments), Venezuela, and Ecuador where the Ecuadorian streams are defined as headwaters (Angermeier and Karr 1983; Penczak and Lasso 1991; Agostinho and Penczak 1995; Bojsen and Barriga 2002). These streams were situated in forested landscapes with stream widths and depths similar to those of the streams in our study. Biomass values in the South American streams varied from 0.5 to 1.9 g · m⁻² in streams of central Panama, from 1.5 to 17.9 g · m⁻² in Venezuela, 2.0–8.3 g · m⁻² in small tributaries of the Paraná River, Brazil, 1–10 g · m⁻² in the Ecuadorian Amazon, and 13.4–63 g · m⁻² in a stream in Brazil. Median values for these studies varied between 1.5 and 25 g · m⁻². The biomass values from the three investigated streams in our study (Table 9, median 0.14 g · m⁻²) were thus considerably lower than those from these South American streams.

One cause of lower biomass in our study is the lack of small specimens in the catch. Da Silva Gonçalves and de Souza Braga (2012) published a table relating the weight of caught species and SL. Based on this study we calculate that we may have missed 70% of fish biomass. That would raise the median biomass from 0.14 to 0.20 g · m⁻². But this only explains a minor part of the difference. An important feature of all the South American streams, in addition to a different fish fauna, was a circumneutral pH and generally less colored water. Most likely the low pH values of our study streams affect the fish especially when it comes to species presence and physiological responses to a low pH. A secondary effect may be the brown-colored, nutrient-poor water (cf. Table 1). Such dark water affects the food chain and especially prevents the development of phytoplankton, periphyton, and submerged macrophytes, i.e., primary production in the water is reduced due to the

* Kapetsky JM (1974) Growth, mortality and production of five fish species of the Kafue River floodplain, Zambia. PhD dissertation, University of Michigan, 194 pp.

low light conditions (e.g., Sanches et al. 2011). Several of the dominant species, such as *Clarias angolensis* and *Enteromius holotaenia* caught in the study streams, feed on aquatic insects, shrimps, and fishes (Froese and Pauly 2021). As the streams in our study have low to very low pH and were often strongly colored, the constraints on fish biomass will be considerable, in comparison to the South American streams.

Only one previous study of the fish biomass for African headwaters has been found. Malaisse (1976) reported values of 1.3, 26.1, and 31.7 kg · km⁻¹ (i.e., not per area) in the reaches of a tributary to the Kwa River (Welcomme and de Merona 1988). A comparable median value for the presently reported study would be 1.1 kg · km⁻¹ based on all streams and years. This value is similar in magnitude to that from the most upstream stretch in Malaisse's study, 1.3 kg · km⁻¹. A biomass value for a stream distance is, of course, influenced by the stream width, which was not included in Welcomme and de Merona (1988). Since we have not found a study with a similar design as ours for African headwaters, it is, therefore, possible that the biomass data presented in our study are unique for African headwaters.

Overfishing risk. Overfishing could be a major threat to the sustainability of continued fishing of the fish populations (Brummett et al. 2011). This would result in reduced catches and changes in fish size and relative abundance. Over the course of our study, no sign of overfishing in the streams could be discerned since there were no statistically significant changes in fish size (TL) or relative abundance over time. If a fish gear discriminates on the basis of size, this could lead to a pronounced decrease in the abundance of some species. The fishing gear used by local people such as plaited basket dips (ecopage) and poisons are not likely to be size discriminating. Our study was, however, not designed to detect the effects of earlier overfishing, for example, species already lost.

Welcomme (1976) attempted to evaluate the importance of small rivers (first order streams) and estimated those with a mean length of 1.6 km to have a mean fish yield of 8 kg · year⁻¹. Recalculated for the stream's length in this study, the yield would be 9 kg · year⁻¹. Annual fish catches in our study were well below this value in Bam-bou and Lebomo (4.4 and 5.7 kg · year⁻¹, respectively), but slightly higher in Bongo (11.5 kg · year⁻¹). This result together with no signs of reductions in catches between the years indicates that the streams were not severely overfished during the study period. No information was available about the local people's fishery, but due to an increasing population, this risk is likely to increase. Any effect of the research fishing during the study period was most likely temporary. The finding of species with larger TL than previously reported also suggests that overfishing is not yet an issue.

Conclusions

This study presents a comprehensive investigation of the fish community composition for several years in three headwater streams in the Congo basin drainage area. Alestids, cyprinids, clariids, and mormyrids were the most common fish families and, in total, 50 species representing 15 families were found. The reason for the low richness compared to other tributaries is most probably because they are small headwater streams. Ten species were found in lower pH waters than those reported in FishBase (Froese and Pauly 2021), and for 18 species we provide the first information on the lowest tolerated pH found. For several species, the lowest end of the pH range should be revised to be as low as 5–5.5. From the LTL area, where ichthyological information has been limited, we now have the first estimates of fish biomass in headwater streams ranging from 0.05 to 0.7 g · m⁻² with a median of 0.14 g · m⁻². This relatively low value is probably caused by the extremely acidic and humic water. We did not find evidence of overfishing despite the local residents having their main protein source from local fish. Fish were in some cases larger than previously reported which also suggests overfishing not to be as devastating (yet) as might have been feared. Fish monitoring is proposed as an integral part of effective fisheries management especially when local fish are an important food resource. To be successful, such a project should involve representatives from local communities for which sustainable fishing is important.

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First report of abnormal body coloration in *Sebastes koreanus* (Actinopterygii: Perciformes: Sebastinae)

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Abstract

This study reports the first record of abnormal coloration in *Sebastes koreanus* Kim et Lee, 1994. The specimen (163.4 mm standard length and 197.3 mm total length) was collected from the Yellow Sea in South Korea in July 2021. The whole body of the specimens was red, and all fins also had red coloration while the slight dark red pattern under the eyes and dark spot on the opercula were similar to normal *S. koreanus*. It also showed a similar dotted pattern on the pectoral fins with a normal specimen.

Keywords

abnormal coloration, new maximum size, *Sebastes koreanus*, Yellow Sea

Introduction

Color variations have been reported in several fish species. They appear in both natural and aquaculture fishes (Muto et al. 2016) but are frequently found in flatfishes reared in aquaculture environments (Venizelos and Benetti 1999; Macieira et al. 2006; Burton 2010). In the natural environment, several fish species, such as pricklebacks (*Dictyosoma rubrimaculatum* Yatsu, Yasuda et Taki, 1978), skates (*Raja montagui* Fowler, 1910; *Raja brachyura* Lafont, 1871; and *Raja clavata* Linnaeus, 1758), angel sharks (*Squatina californica* Ayres, 1859), and rockfishes (*Sebastes trivittatus* Hilgendorf, 1880; *Sebastes chrysomelas* (Jordan et Gilbert, 1881); and *Sebastes pachycephalus* Temminck et Schlegel, 1843)} have also been reported to have sporadic albinism (Ball et al. 2013; Lewand et al. 2013;

Muto et al. 2013; Escobar-Sánchez et al. 2014; Kwun et al. 2016; Muto et al. 2016) while *Cephalopholis fulva* (Linnaeus, 1758) and *Lethrinus nebulosus* (Forsskål, 1775) have shown melanisms of body coloration (Simon et al. 2009; Jawad et al. 2013). Albinism is a genetic abnormality that reduces melanin biosynthesis, resulting in partial or total lack of body color, typically producing a golden-orange color. Melanism is the presence of excessive amounts of pigment in tissues and skin, and it is a phenomenon in which dark patterns appear on the body color (Simon et al. 2009; Jawad et al. 2013; Escobar-Sánchez et al. 2014).

Sebastes koreanus Kim et Lee, 1994 belongs to the genus *Sebastes*, of which approximately 110 species are known worldwide, with more than 30 species found in the North Pacific (Yu et al. 2015; Nelson et al. 2016). Rockfishes (subfamily Sebastinae) often show variations

in body coloration according to their habitat, which causes difficulties in species identification based only on body morphology (Kai et al. 2011; Kai and Nakabo 2013). Recently, genetic analysis was further developed to reduce errors in taxonomic classifications and to find patterns of various intraspecific body colorations (Narum et al. 2004; Hawkins et al. 2005). It has also become possible to distinguish taxonomic variations of rockfishes at the species level by combining body color and genetic analyses. For example, *S. pachycephalus* has been divided into two species according to color pattern and the presence or absence of scales under the dorsal fin (Kai et al. 2011; Kai and Nakabo 2013). In addition, *Sebastes inermis* Cuvier, 1829 has been divided into three morphotypes according to the pattern of body color and the number of pectoral fin rays (Kai and Nakabo 2002, 2008). As described above, body color is often used as a taxonomic key to distinguish species in the subfamily Sebastinae. The normal body color of *S. koreanus* is speckled with dark brown and light ivory, and there is no significant color variation among individuals (Kim and Lee 1994; Fang et al. 2015). This species is distributed in the Yellow Sea off the Korean and Chinese coasts, with a relatively narrow distribution range (Choi and Yang 2008; Murdy 2010; Fang et al. 2015). Biological and ecological studies of *S. koreanus* inhabiting Korean waters have only reported on the morphological development of egg and juvenile fishes (Park et al. 2015; Yu et al. 2015).

In this study, a single specimen of *S. koreanus* was collected by fishing on the western coast of Korea, but its body size was considerably larger, and its color was unusually different from usual *S. koreanus* specimens. We aimed to confirm if the specimen was *S. koreanus* and to study whether there are intraspecific color variations in *S. koreanus* through morphology and molecular analyses. Consequently, this study reports the first abnormal body coloration *S. koreanus* collected from the western waters of Korea.

Methods

A *Sebastes koreanus* specimen with a total length (TL) of 197.3 mm was collected from Ongdo (36°38'49.81"N, 126°0'30.56"E) in the coastal waters of Taean Province, western Korea (Fig. 1). Sampling was conducted at a depth of approximately 13 m during the daytime via fishing on 16 July 2021. Immediately after capture, the sample was frozen with seawater and transported to the laboratory. In the laboratory, an image of the specimen was taken. Body measurements were recorded following Hubbs et al. (2004), measuring both standard length (SL) and TL. The specimen was measured to the nearest 0.1 mm using a digital Vernier caliper. The specimen was then preserved in 5% formalin for

24 h and was later transferred to 70% ethanol for further analyses.

To compare molecular data, total genomic DNA was extracted from muscle tissue using 10% Chelex resin (Bio-Rad, Hercules, CA, USA). A portion of the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified using universal primers (Ward et al. 2005). PCR was performed in a 30 µL reaction tube containing 3 µL genomic DNA, 3 µL 10 × PCR buffer, 2.4 µL 2.5 mM dNTP, 1 µL of each primer, 0.3 µL Ex-Taq DNA polymerase, and 19.3 µL sterile distilled H₂O using a thermal cycler (MJmini PTC-1148, Bio-Rad, Hercules, CA, USA). The PCR profile consisted of initial denaturation at 95°C for 5 min, followed by 34 cycles of denaturation at 95°C for 1 min, annealing at 50°C, extension at 72°C for 1 min, and a final extension at 72°C for 5 min. PCR products were purified using ExoSAP-IT (United States Biochemical Corporation, USA) and were sequenced using an ABI PRISM BigDye Terminator v.3.1 Ready Reaction cycle sequencing kit (Applied Biosystems Inc., USA) on an ABI 3730xl DNA Analyzer (Applied Biosystems Inc.). We compared our molecular data with the mtDNA COI sequences from other *Sebastes* species and one outgroup, *Sebastiscus marmoratus* (Cuvier, 1829) obtained from the GenBank (National Center for Biotechnology Information, www.ncbi.nlm.nih.gov). Sequences were aligned using ClustalW (Thompson et al. 1994) in BioEdit version 7 (Hall 1999). Genetic divergences were calculated using the Kimura 2-parameter (K2P) model (Kimura 1980) with Mega 6 (Tamura et al. 2013). Phylogenetic trees were constructed using the neighbor-joining method (Saitou and Nei 1987) in Mega 6 (Tamura et al. 2013), with confidence assessed based on 1000 bootstrap replications.

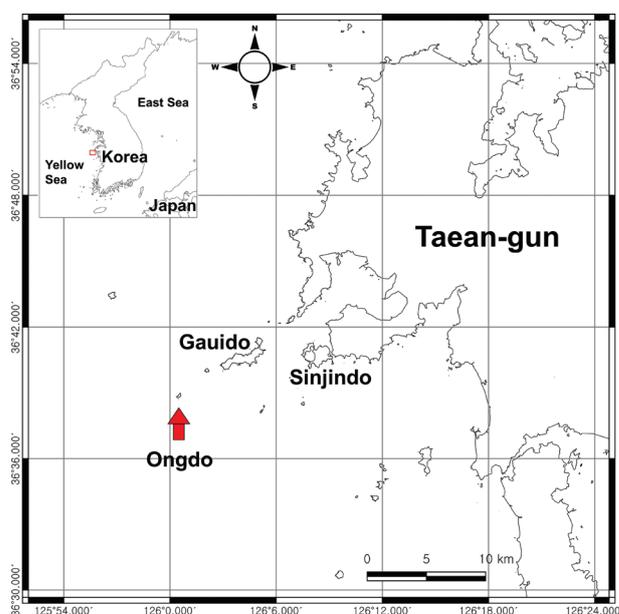


Figure 1. Map showing the sampling area of *Sebastes koreanus* in Ongdo, Taean-gun, Korea.

Results

Subfamily: Sebastinae Kaup, 1873 *Sebastes* Cuvier, 1829

Sebastes koreanus Kim et Lee, 1994

Korean common name: 황해볼락

Suggested English common name: yellow Korean rockfish

Figs 2, 3

Description. Body moderately compressed (Fig. 2). Strong spines on head. Large mouth and eyes large. Maxilla not reaching posterior margin of eye. Dorsal fin continuous, notched between 13th spine and 14th spine; soft part of dorsal fin length similar to spinous part length. Origin of anal fin same as origin of soft part of dorsal fin. Pectoral fin large; upper half rounded; rays of lower half thickened; origin of pectoral fin located on second spine of dorsal fin; posterior margin located on 11th spine of dorsal fin. Pelvic fin short; origin of pelvic fins located behind origin of pectoral fins. Pectoral and pelvic fins covered with skin and ctenoid scales near base. Caudal fin truncated. Lateral line sloping moderately downward above pectoral fin.

Dorsal fin rays XIV, 12; anal fin rays III, 6; pectoral fin rays 16; pelvic fin rays I, 5; lateral line pores 31. Proportions as percentage (%) of SL (163.4 mm): head length

37.6; head width 22.0; head depth 31.0; snout length 11.1; orbit diameter 9.1; interorbital width 6.5; body depth 39.0; body width 21.3; upper jaw length 16.8; pre-dorsal fin length 35.6; pre-anal fin length 74.4; pectoral fin length 29.9; pelvic fin length 24.5; 1st dorsal fin spine length 5.3; 2nd dorsal fin spine length 7.8; 3rd dorsal fin spine length 11.4; longest dorsal fin ray length 15.7; 1st anal fin spine length 7.0; 2nd anal fin spine length 16.1; 3rd anal fin spine length 14.0; caudal peduncle length 18.3; caudal peduncle depth 11.1.

Body generally orange, upper part of head faded yellow, with two slight dark red stripes behind and under eye (Fig. 2). Anterior and posterior parts of eyes faded yellow with bit of red. Dorsal side of body red and ventral side pale yellow. Membrane of spine dorsal fin interspersed with red dots on pale yellow background. Membrane of soft dorsal fin orange and tip pale yellow. Pelvic and anal fins red. Caudal fin dark red interspersed with tiny black dots. Front of pectoral fin red, but in middle and rear, red dots scattered on faded red background.

Remarks. Based on the analysis of the COI gene sequence (577 bp) of the presently reported specimen and the mtDNA COI region of *S. koreanus* registered in the NCBI, the genetic distance between the two individuals was found to be 0.002. The other four species in the genus *Sebastes* had genetic distances of 0.032–0.051 (Fig. 3).



Figure 2. Specimens of *Sebastes koreanus* with abnormal and natural body colorations between presently reported and previous studies, 197.3 mm total length, Ongdo, Yellow Sea, Korea. Scale bar = 5 cm.

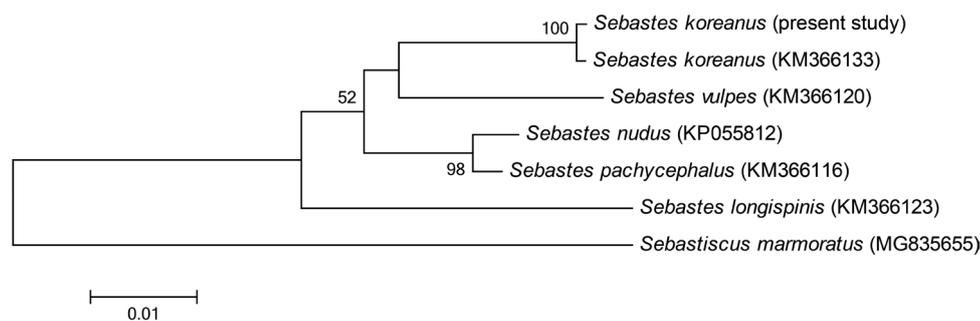


Figure 3. A neighbor-joining tree based on the partial mitochondrial DNA COI gene region using *Sebastes koreanus* (KI-OST_22_001) showing the relations among the four species of *Sebastes* and one outgroup (*Sebastiscus marmoratus*). Numbers at the branches indicate bootstrap probabilities in 10 000 bootstrap replications. Scale bar equals 0.01 of Tamura and Nei's distance (1993) with K2 parameter model. Abbreviation: COI, cytochrome oxidase subunit I.

Discussion

Sebastes koreanus is generally dark brown with dark stripes and tiny dark spots, four to five vertical patterns on the body side, small brown spots scattered on each fin, two stripes behind and below the eyes, one dark blotch on the opercula, interspersed black dots on the pectoral fins, and 14 dorsal fin spines (Kim and Lee 1994; Fang et al. 2015). In this study, a single specimen of rockfish showed a difference in body coloring from the normal *S. koreanus*. Its body was overall red without a black blotch on the opercula. The specimen was morphologically similar to that of *S. koreanus*, especially in the number of dorsal fin spines (i.e., 14 dorsal fin spines). The specimen also showed similar patterns on the body with two lines under the eyes and red dots on the pectoral fins, although the color was different from *S. koreanus* (i.e., red vs. black). The mtDNA COI region further demonstrated that the genetic difference between the presently reported specimen and *S. koreanus* was 0.002, indicating within-species variation. Therefore, this species can be identified as *S. koreanus*.

The abnormal body colorations of fishes are mainly due to the lack or excess of melanin, which causes albinism and melanism, respectively (Jawad et al. 2013; Muto et al. 2013). Such abnormal body color patterns of rockfish-

es have also previously been reported in *S. trivittatus* (see Muto et al. 2016). Overall, morphometrics were identical between the abnormal and normal specimens, and only the color form combinations were different. In the presently reported specimen, the black and brown colors of *S. koreanus* appeared as red and yellow, especially in the two lines under the eyes and in the scattered dots on the pectoral fins, which are important morphological traits of *S. koreanus*. Such abnormal body color in the presently reported specimen appears to be a form of albinism.

Sebastes koreanus showed a relatively narrow distribution range, inhabiting only the Yellow Sea off the Korean and Chinese coasts. This study collected a single specimen of *S. koreanus* and reported a color variation in *S. koreanus*, with the body having an unusually orange color.

Acknowledgments

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New and interesting records of marine fishes (Actinopterygii) from the Maltese Islands (central Mediterranean)

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<https://zoobank.org/850A82A7-B7C7-44A6-95E0-7583DA61EB11>

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Abstract

The occurrence of three bony ray-finned fishes, *Thorogobius ephippiatus* (Lowe, 1839), *Chlopsis bicolor* Rafinesque, 1810, and *Grammonus ater* (Risso, 1810) is reported for the first time in the scientific literature from Maltese coastal waters. The leopard-spotted goby, *T. ephippiatus*, was mostly recorded within the 8–32 m depth range on soft sediment and rocky bottoms within caves, but one individual was recorded on a rocky bottom with a thin layer of silt at a depth of 117 m where no cave was present. The bicour colour eel, *C. bicolor*, was recorded within the 318–518 m depth range on rocky bottoms covered with a thin layer of muddy-detrilic sediment; and the cusk-eel *G. ater* was recorded at a depth of 10 m within a cave. Notes on these three species as well as on another bony fish, the Azores rockling, *Gaidropsarus granti* (Regan, 1903), including new information on their bathymetric range and habitat association, are presented.

Keywords

bathymetric distribution, demersal fish, fish-habitat associations, Malta, marine caves, marine surveys, new records

Introduction

Knowledge of the indigenous marine fish fauna of the Maltese Islands has improved in recent years, especially following new investigations of previously poorly known habitats such as cobble habitats, marine caves, and deep-sea habitats (e.g., Kovačić et al. 2013; Castriota and Deidun 2014; Evans et al. 2016; Kovačić and Schembri 2019; Tsagarakis et al. 2021). Surveys made in 2015 and 2016 during the LIFE BaHAR for N2K project (<https://lifebahar.org.mt>) have contributed new records of fishes as well as provided information on some interesting species that have been recently recorded but whose ecology in the central Mediterranean is not well known.

Methods

The presently reported study is based on surveys using SCUBA diving and underwater photography, and on videography using a Remotely Operated Vehicle (ROV; Saab Seaeye Falcon DR) undertaken within the 25-nautical mile Fisheries Management Zone around the Maltese Islands during the summer of 2015 and 2016 as part of the LIFE BaHAR for N2K project. The SCUBA dives were carried out within the 0–40 m depth range in fully submerged caves along the southwestern, western, and northern coasts of the Maltese Islands where the shore is characterized by sheer cliffs. The ROV surveys were made at depths between 50 m and 1030 m in coastal areas characterized by rhodolith/maerl beds, offshore along the edges of the

southeastern end of the Malta Graben, and off the western, northwestern, and northern parts of the island of Gozo.

Apart from images and geographical coordinates, data on the benthic habitats and water depth were collected. Photographs and videos were analysed in the laboratory to identify the imaged fish. Identification was based on morphological and coloration characters visible in the imagery. The Kovačić and Svensen (2018) stringent version of Bello et al. (2014)'s best practice protocol for first records, which includes species diagnosis, was applied. The diagnoses correspond to the minimum combination of characters that differentiate the species from confamilials in the area (Nielsen 1986; Kovačić et al. 2022; also, the photographs of Bythitidae from figures in Dulčić and Kovačić 2020). Taxonomic nomenclature follows Fricke et al. (2022).

Results and discussion

Thorogobius ephippiatus (Lowe, 1839)

Figs 1, 2

Diagnosis. Base coloration greyish with blue-green sheen on back; covered with large, round dark spots. Head, including predorsal area, covered with brown to dark orange, smaller round spots, usually lighter in colour and more reddish than blotches on body. Five to six large, dark brown or brown-purple to black midlateral blotches. Dark blotches above midline smaller than midlateral blotches.

Remarks. In the SCUBA surveys there were six occurrences of this species from depths of between 8 and 32 m within caves, with four occurrences on soft sediment bottoms and two on rock (see Table 1). An additional single record from the ROV surveys was from a depth of 117 m on a rocky bottom having a thin layer of silt (Table 1 and Fig. 2). No cave or rock fissure or any other similar feature was present in the vicinity of this individual. Given that the literature indicates that *T. ephippiatus* is typical of cave habitats, crevices, overhangs or deep gullies (Bussotti and Guidetti 2009; Bussotti et al. 2015; Ragkousis et al. 2021; Kovačić et al. 2022), the occurrence of this species outside this type of habitat is unusual. *Thorogobius ephippiatus* is a new record for Maltese coastal waters; it is a NE Atlantic and Mediterranean species. In the Mediterranean its distribution appears to be mostly along the northern shores of both west and east basins including the Aegean Sea, Cyprus, and Israel (Kovačić et al. 2022). According to Froese and Pauly (2022), the depth range for *T. ephippiatus* is 6–40 m, with the fish being commonest in the 6–12 m range. The record from a depth of 117 m is therefore noteworthy, especially as the present authors are aware of only one previous deep-water record of this species, by Stern et al. (2018), who recorded it from a depth of 156 m off the north Israeli coast. Stern et al. (2018) attribute the deep-water occurrence of *T. ephippiatus* to its requirement for dark and cold conditions, which in the eastern Levantine Sea only occur in deep water. However, the species is present



Figure 1. Photograph of *Thorogobius ephippiatus*, taken on 27 June 2016 at a depth of 24 m within a cave (36°04.879'N, 014°14.110'E). Photograph: OCEANA/Carlos Minguell© LIFE BaHAR for N2K.

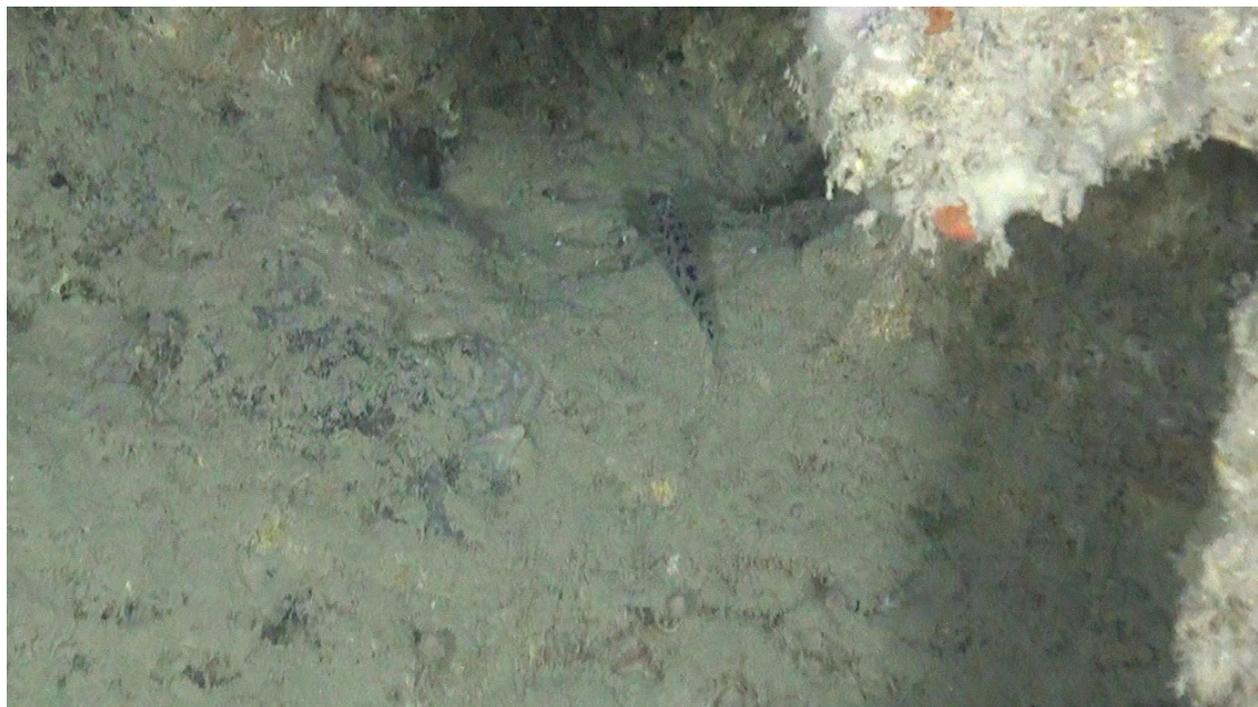


Figure 2. Image grab from ROV footage collected on 7 July 2016 at a depth of 117 m (36°00.776'N, 014°50.517'E) showing an individual of *Thorogobius ephippiatus*. Image: OCEANA® LIFE BaHAR for N2K.

in shallow water in Cyprus (Gerovasileiou et al. 2017), which raises doubts about this hypothesis. On the other hand, the present deep-water record from Malta confirms that *T. ephippiatus* has a much wider bathymetric range than previously thought. It may be significant that both the Maltese and Israeli deep-water records were from open rocky bottoms and not from caves, suggesting that the association with caves in shallow water is due to the fish's requirement for a dark habitat, while in deep water the species can occur in the open. In fact, Kovačić (1997) had already noted that this species occurs deep within caves in very shallow waters, but closer to the mouth at the deeper end of the depth range studied by this author (32 m). It therefore also occurs outside of caves in deeper water.

Chlopsis bicolor Rafinesque, 1810

Fig. 3

Diagnosis. Body highly elongated. Snout rounded, slightly projecting beyond tip of lower jaw. Anterior nostril tubular, located near tip of snout. Eyes well developed. Pectoral and pelvic fins absent; dorsal and anal fins confluent with caudal fin. Body distinctly bicoloured, with grey-brown dorsal region and cream-white ventral section; in head region boundary between two-colour bands located at lower edge of pupil.

Remarks. There were five occurrences of this species, all from the ROV surveys and within the 318–528 m depth range (Table 2). This newly recorded species appears

Table 1. Individuals of *Thorogobius ephippiatus* recorded from the LIFE BaHAR for N2K marine benthic surveys.

Survey method	Date	Geographical coordinates	Water depth [m]	Habitat type
SCUBA diving	8 June 2015	35°52.021'N, 014°21.174'E	32	Rock covered with a thin layer of detritus within cave
SCUBA diving	10 July 2015	36°00.813'N, 014°15.883'E	8	Soft sediment covered with a thin layer of detritus within cave
SCUBA diving	12 June 2016	36°04.879'N, 014°14.110'E	24	In rock fissure within cave
SCUBA diving	12 June 2016	36°04.879'N, 014°14.110'E	24	Soft sediment bottom within cave
SCUBA diving	27 June 2016	36°04.879'N, 014°14.110'E	24	Soft sediment bottom within cave
ROV	7 July 2016	36°00.776'N, 014°50.517'E	117	Rock covered with a thin layer of detritus
SCUBA diving	25 July 2016	35°50.969'N, 014°23.050'E	20	Soft sediment bottom within cave

Table 2. Individuals of *Chlopsis bicolor* recorded from the LIFE BaHAR for N2K marine benthic surveys.

Survey method	Date	Geographical coordinates	Water depth [m]	Habitat type
ROV	2 June 2016	35°34.354'N, 014°30.444'E	486	Muddy bottom
ROV	2 June 2016	35°34.369'N, 014°30.755'E	494	Muddy bottom
ROV	26 June 2016	35°32.060'N, 014°13.220'E	458	Rock covered with a thin layer of sediment
ROV	28 July 2016	36°13.090'N, 013°47.860'E	318	Rock covered with a thin layer of muddy-detritic sediment
ROV	28 July 2016	36°10.496'N, 013°52.855'E	528	Rock covered with a thin layer of muddy-detritic sediment



Figure 3. Image grab from ROV footage collected on 26 June 2016 at a depth of 458 m, (35°32.060'N, 014°13.220'E) showing an individual of *Chlopsis bicolor*. Image: OCEANA® LIFE BaHAR for N2K.

to predominantly prefer rocky bottoms covered with a thin layer of muddy-detritic sediment. Froese and Pauly (2022) give a depth range of 80–365 m for *C. bicolor*, while Erguden and Bayhan (2015) recorded a single individual from a depth of 513 m from off Mersin Bay, Turkey, which is the same depth as the majority of our records (Table 2). This suggests that the species habitually occurs in waters that are deeper than previously reported. *Chlopsis bicolor* is native to the Mediterranean, where it is widely distributed and reported to occur on muddy bottoms (Froese and Pauly 2022). Our records show that the species also occurs on bottoms of muddy sediment intermixed with patches of hard substrata (Table 2).

***Grammonus ater* (Risso, 1810)**

Fig. 4

Diagnosis. Head not strongly depressed, eyes directed mainly laterally. No sharp spines at lower angle of preopercle. Opercle triangular, posterior edge angled and pointed only at upper edge. Snout blunt. Posterior angle of jaws ending behind vertical of eye posterior margin. Body and head uniformly dark brown.

Remarks. A single individual of this species was recorded from the SCUBA surveys at a depth of 10 m within a cave (Fig. 4 and Table 3). Froese and Pauly (2022) give the depth range for this species as 5–30 m and in-

Table 3. Individuals of *Grammonus ater* and *Gaidropsarus granti* recorded from the LIFE BaHAR for N2K marine benthic surveys.

Species	Survey method	Date	Geographical coordinates	Water depth [m]	Habitat type
<i>Grammonus ater</i>	SCUBA diving	20 June 2016	36°01.106'N, 014°14.730'E	10	Rocky wall within cave
<i>Gaidropsarus granti</i>	ROV	23 June 2015	35°52.880'N, 014°07.351'E	871	Muddy bottom and rocky outcrops
<i>Gaidropsarus granti</i>	ROV	9 July 2016	36°00.185'N, 013°59.860'E	748	Muddy bottom and rocky outcrops

dicating that *G. ater* is associated with reef habitats. The present record, as well as two other local records reported in social media*, refer to this species as occurring within caves in shallow water. The occurrence of *G. ater* in the Maltese Islands has not been previously reported in the scientific literature. The majority of authors (e.g., Bussotti and Guidetti 2009; Bussotti et al. 2015) consider this species to be speleophilic, while Ragkousis et al. (2021) commented that it is only found in exclusively dark conditions. Froese and Pauly (2022) state that *G. ater* occurs in the eastern Atlantic, and from the Balearic Islands to the Adriatic in the Mediterranean. On the other hand, Ragkousis et al. (2021) note that its occurrence in the Azores Archipelago remains unconfirmed, while recent studies give records for this species from Crete and Cy-

* https://www.naturamediterraneo.com/forum/topic.asp?TOPIC_ID=174828;
<https://www.facebook.com/photo/?fbid=10226466313230014&set=pcb.10226466366111336>



Figure 4. Image grab from ROV footage collected on 20 June 2016 at a depth of 10 m (36°01.106'N, 014°14.730'E) showing an individual of *Grammonus ater*. Image: Enrique Talledo/OCEANA® LIFE BaHAR for N2K.

prus. The distribution of *G. ater*, which is considered to be a Mediterranean endemic, has therefore been updated to extend from Spain to Cyprus, having been reported from 23 marine caves in Spain, France, Italy, Croatia, Greece, and Cyprus (Ragkousis et al. 2021), and from at least one cave in Malta (Table 3).

***Gaidropsarus granti* (Regan, 1903)**

Fig. 5

Diagnosis. Dorsal part of head reddish-brown with cream reticulations. Rest of body with three longitudinal brown bands (one dorsal and one dorsolateral band on either side) separated by thin, undulating cream stripe. Dorsolateral brown bands interrupted by thin cream stripes in the posterior region, breaking up into spots towards the caudal peduncle. Lower surfaces including head, ventral part of flank, and belly, all cream-coloured.

Remarks. This species has recently been recorded from Malta by Tsagarakis et al. (2021), based on a single individual caught off west Gozo (coordinates: 36.00°N, 014.10°E) at a depth of 290 m. Bello (2018) gives another record as “*W of Malta (ANDALORO et al. 2011)*” but this online article (Anonymous, not dated) does not include details of the record apart from a point on a map. From this map, it appears that the fish originated from waters some 75 km west of the island of Gozo. Two individuals of *G. granti* (Fig. 4) were recorded from the presently reported ROV surveys, one in 2015 and one in 2016, which antedate the record by Tsagarakis et al. (2021).

Both individuals occurred on a muddy bottom with rocky outcrops, one at a depth of 748 m and the other at 871 m. Froese and Pauly (2022) give the typical depth range for *G. granti* as 20–250 m but there are many Mediterranean records of this fish from deeper waters (e.g., Orsi-Relini and Relini 2014; Bello 2018; Spinelli and Castriota 2019; Tsagarakis et al. 2021). However, the depth of the presently reported findings (748 m and 871 m, Table 3) far exceeds almost all previous Mediterranean depth reports, and the 871 m depth is substantially greater than the deepest known record for the species, from the Galician Bank (Atlantic), where this species occurred at 823 m (Bañón et al. 2002). *Gaidropsarus granti* is native to the eastern and central Atlantic and its first record (cf. Bello 2018) from the Mediterranean in 1995 (Zachariou-Mamalinga 1999) sparked a debate as to whether it is an overlooked native or an Atlantic species that had recently expanded its range. The species' status in the Mediterranean is best given as cryptogenic, although the prevailing opinion is that it is an Atlantic range-expanding species (Orsi-Relini and Relini 2014; Bello 2018).

The present note adds knowledge to the fish faunal diversity of the Maltese Islands: *Thorogobius ephippiatus* and *Chlopsis bicolor* are new records for Malta; *Grammonus ater* has not yet been reported from Malta in the scientific literature; and *Gaidropsarus granti* has only been reported once from close to the Maltese islands in the published literature. In addition, the present findings include the deepest records of *G. granti* and *C. bicolor*,

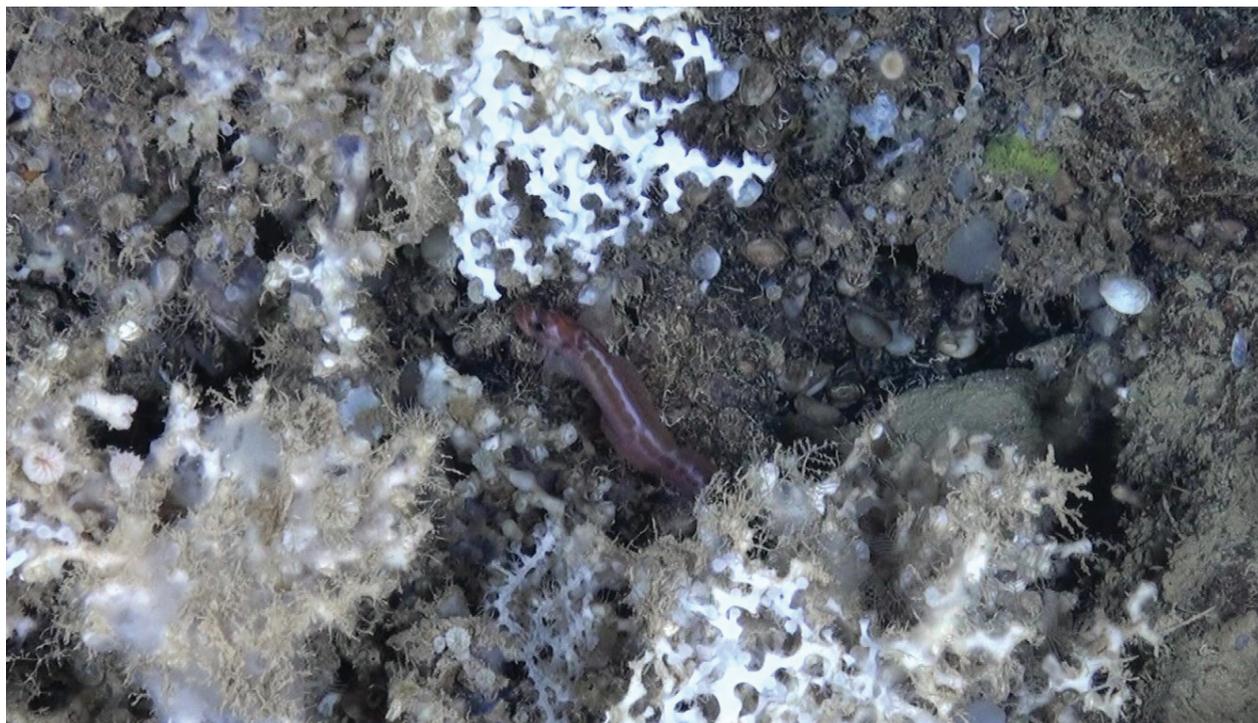


Figure 5. Image grab from ROV footage collected on 23 June 2015 at a depth of 871 m (35°52.880'N, 014°07.351'E) showing an individual of *Gaidropsarus granti*. Image: OCEANA © LIFE BaHAR for N2K.

and the second deepest record of *T. ephippiatus* for the entire Mediterranean Sea.

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New finding and description of the Galapagos batfish, *Ogcocephalus darwini* (Actinopterygii: Lophiiformes: Ogcocephalidae), in marine waters of Manabi, Ecuador

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<https://zoobank.org/121D71C8-55CF-4A54-B7BE-067B55A1D0E3>

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Abstract

The finding of a specimen of the Galapagos batfish, *Ogcocephalus darwini* Hubbs, 1958 in marine waters of continental Ecuador was recorded. The specimen was captured by the artisanal fishing fleet that operates with bottom longlines in Las Piñas fishing cove, Manta Municipality, Manabí province, Ecuador. The specimen was transferred to the Biology Laboratory of the Faculty of Marine Sciences of the Universidad Laica Eloy Alfaro de Manabí, where morphometric data were taken for its identification. Until now, the species was considered endemic to the Galapagos, but it had been reported off northern Peru and now from the Ecuadorian continental shelf.

Keywords

continental shelf, distribution area, eastern Pacific Ocean, morphology

Introduction

Batfishes (Ogcocephaloidei: Ogcocephalidae) are members of the order Lophiiformes, which is composed of some 402 extant valid species and includes deep-sea frogfish (Antennarioidei and Ceratioidei), anglerfish (Lophioidei), and marine toadfish (Chaunacioidei) (Fricke et al. 2022). The classification followed van der Laan et al. (2022). Fricke et al. (2022) counted 90 valid species in ten genera within the family Ogcocephalidae. Except for a limited number of species of commercial interest, there is a distinct paucity of published information on the life history, biology, and ecology of the majority of these species (Bradbury 1980). The bulk of the primary literature on the family Ogcocephalidae is dominated by descriptions of individuals and lists of fish from

specific locations (Grove and Lavenberg 1997; Endo and Shinohara 1999).

Out of 13 valid species of the genus *Ogcocephalus* only two can be found in the Pacific Ocean: *Ogcocephalus porrectus* Garman, 1899 and *Ogcocephalus darwini* Hubbs, 1958. The latter can be easily distinguished from *O. porrectus* by having a pair of dark stripes on the dorsal disc that extends to the lateral sides of the tail (Hubbs 1958; Bradbury 1980). *Ogcocephalus darwini* is found in relatively shallow waters, from 3 to 76 m (Humann and Deloach 1993), although specimens have been observed down to 120 m (Long 1999).

Bradbury (1980) considered both species as island endemics of the eastern Pacific, *O. darwini* in the Galapagos Archipelago and *O. porrectus* off Cocos Island, Costa Rica. However, there are reports of specimens of *O. darwini* being collected off Puerto Pizarro and Manco-

ra Bank, south of Tumbes on the northern coast of Peru (Chirichigno 1978; Bradbury 2003). The report by Zeballos et al. (2000) on this species in Peruvian Pacific waters, between latitudes 3° and 6° South at depths of 90 to 180 m, together with *Zalieutes elater* (Jordan et Gilbert, 1882) seems doubtful since they call both species as batfish with 2 ocelli, but *O. darwini* lacks them.

Materials and methods

One specimen, a female, 21.5 cm SL, was captured in Las Piñas fishing cove, southeast of Manta, Manabi Province, Ecuador, eastern Pacific Ocean (Fig. 1; 01°05'46"S, 080°53'51"W), on 23 April 2022. It was caught by fishermen operating from a fiberglass boat in the artisanal fishery using bottom longlines, 4 NM off the coast and at a depth of 30 m, with a J-type hook number 10.

The specimen was stored in an ice box and taken to the laboratory of the Faculty of Marine Sciences of the Universidad Laica Eloy Alfaro de Manabí. There, the specimen was weighed, and the morphometric parameters were measured using a Fluke 4190 digital ichthyometer with a precision of 1 mm, or a caliper to the nearest 1 mm. The meristic characteristics, sex, and gonadal maturation were determined. The scale of gonadal maturation followed Brown-Peterson et al. (2011). The description of the taxonomic and morphometric characteristics followed Hubbs (1958) and Robertson and Allen (2015).

Results

Family Ogocephalidae Gill, 1893

Ogocephalus Fischer, 1813

Ogocephalus darwini Hubbs, 1958

English common name: Galapagos batfish

Spanish common names: pez murciélago de las Galápagos, pez murciélago de labios rojos.

Figs 2, 3; Tables 1, 2

Description. Head depressed, raised above disc; disk triangular; snout pointed, with horn-like rostrum projecting well in front of eyes; horn with few short hairs; fish-lure with 3 fleshy tips, in small cavity below horn; spine of lower rear corner of operculum blunt, and poorly developed; gill rakers oval plates covered with small teeth; eyes on sides of head; gill opening high, above pectoral base; pectoral and pelvic fins arm-like; pectorals completely separated from body; small dorsal and anal fins on tail; skin with few projecting small bony plates; flank without fringe of hairs; belly completely covered with bony, pointed scales; under tail densely covered with small spines, and sometimes few conical spines on medial line. Light brown to grayish above, white below; snout and horn dark brown to reddish brown; bright red lips; dark brown stripe (sometimes discontinuous) from top of head to base of tail fin on each side of body. Meristic characters shown in Table 1.

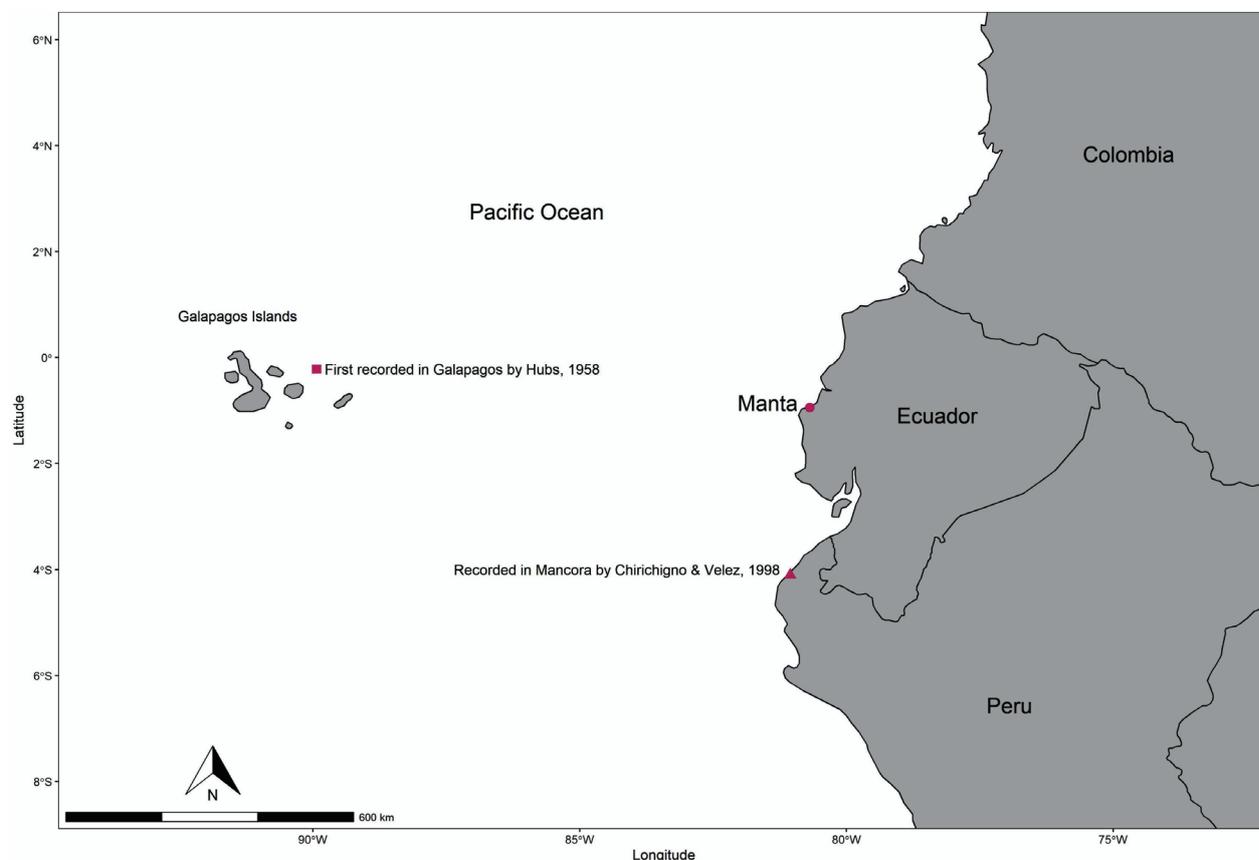


Figure 1. Map of the capture area of the individual of *Ogocephalus darwini* off the coast of the fishing cove Las Piñas, Ecuador, and the areas reported in Peru.

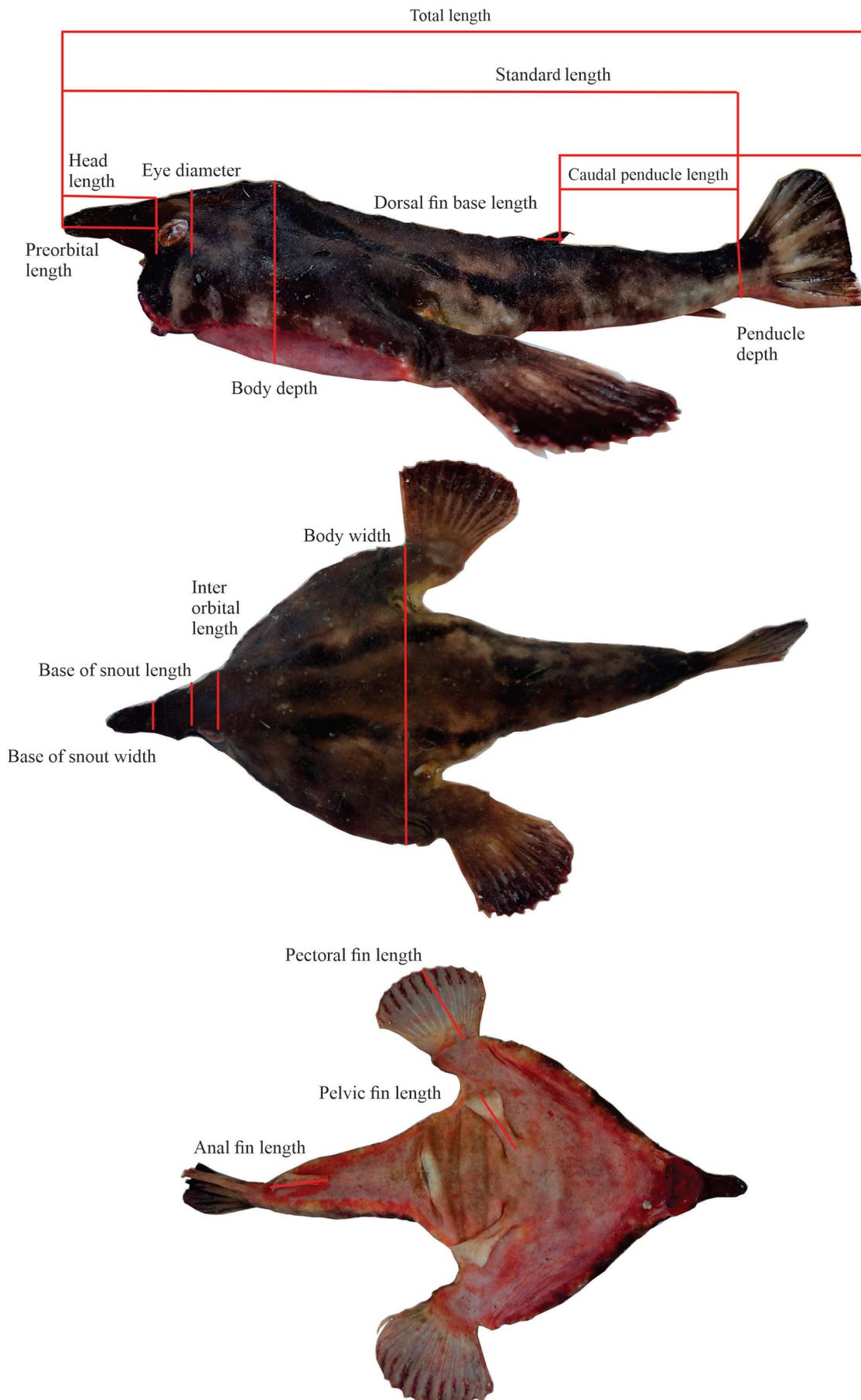


Figure 2. Lateral (a), dorsal (b) and ventral (c) view of the individual of *Ogocephalus darwini* captured off the coast of the fishing cove Las Piñas, Ecuador, with its respective morphometric measurements.

Table 1. Meristic characters of the fins in the specimen of *Ogcocephalus darwini* found in the fishing cove Las Piñas, Manta, Ecuadorian Pacific.

Character	Value
Rays in pectoral fins	14
Rays in pelvic fins	5
Rays in caudal fin	9
Rays in anal fin	3
Rays in small dorsal fin	3

Remarks. The total weight of the studied specimen was 278.4 g while its total length reached 26 cm. The morphometric characteristics are shown in Table 2 and Fig. 2A, 2B, 2C. After evisceration, it was determined that it was a mature female in stage III. The liver weighed 21.4 g and the gonads 9.7 g. The stomach had fish remains of *Opisthonema* spp., most probably from the bait used in the fishery. The specimen still had the hook attached to the mouth. There were numerous parasitic Nematoda inside the intestine, and in the coelomic cavity near the liver and gonads; no parasites were observed in the gills, mouth, or external surface of the body.

The sagitta otoliths measured 5.69×3.44 mm (Fig. 3). The shape of the otoliths was oval, with an irregular dorsal margin and crinated ventral margin. The acoustic groove was heterosulcoidal, in supramedial position. Ostium, funnel-shaped, larger than cauda. Cauda, tubular, slightly curved. The ridges run the entire cauda. Anterior side blunt, with rostrum prominent and round shape; anti-rostrum, small and pointed. There were at least 17 sets of hyaline and opaque rings counted in the sagitta otoliths, which may suggest that the specimen was 17 years.

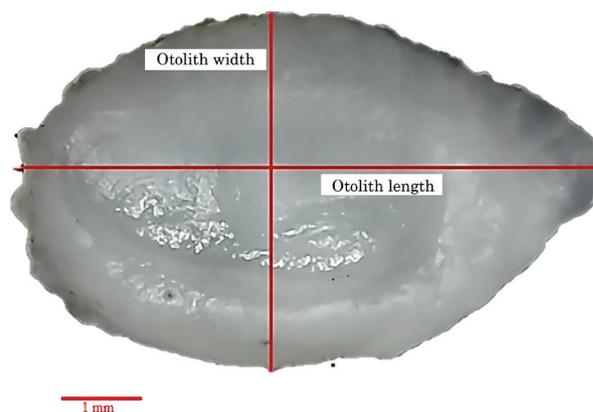
Discussion

In continental Ecuador, there was a previous record of the presence of *Ogcocephalus darwini* published by Massay (1983), without a description of the individuals or indication of the place of finding. This author also reported two other species of Ogcocephalidae, *Zalieutes elater* and *Dibranchius spinosus* (Garman, 1899). This record of *O. darwini* was later reported by Béarez (1996) and Jiménez and Béarez (2004), without further verification of the presence of the species. However, Coello and Herrera (2010), in four cruises made between 2003 and 2007 along the continental shelf of Ecuador, at depths of 10 to 120 m, did not report the presence of *O. darwini*, but did find *Z. elater*. Similar results were reported by Garcia et al. (2014), who did not find the presence of *O. darwini* in the cruises made for 8 months (April to December 2013) in the fishing grounds of the fleet targeting *Merluccius gayi* (Guichenot, 1848) located in the central and southern Ecuadorian coast with trawls at depths of 20–500 m.

The paucity in the detection of fish species typical from the Galapagos Archipelago (GA), like *O. darwini*, in the continental shelf of Ecuador and northern Peru, could be associated with the occasional arrival of fish

Table 2. Morphometry of the specimen of *Ogcocephalus darwini* found in the fishing cove Las Piñas, Manta Municipality, Manabi, Ecuador.

Character	Value [cm]
Total length	26.00
Standard length	21.50
Body width	10.88
Body depth	5.41
Length at mean width of horn	1.03
Depth at mean base of horn	1.82
Dorsal fin base length	0.58
Pectoral fin length	2.66
Pelvic fin length	3.26
Base of pelvic fins length	2.21
Base of pectoral fin length	3.26
Caudal peduncle length	6.05
Caudal peduncle depth	1.83
Snout length	2.64
Caudal lobe length	3.20
Anal fin length	2.43
Pre-orbital length	2.97
Maximum body height	5.47
Interorbital length	1.89
Ocular diameter	1.10

**Figure 3.** Description of the internal face of the right sagittal otolith of the specimen *Ogcocephalus darwini* landed in the artisanal fishing cove Las Piñas, Ecuador.

larvae from this archipelago. Larval movement could be either in ballast water from vessels traveling between GA and the continent or in water masses loaded with plankton from the surroundings of this archipelago. The area between GA and the South American continental shelf is affected by several oceanic currents, the Southern Equatorial (surface) Current which moves west after receiving the flow of the Humboldt Current, and the Cromwell or Equatorial Undercurrent, moving eastward at depths from 100 to 400 m (Knauss and Garfield 2016). Changes in the flow of these currents may cause eddies that pinch off the main current and could move toward the continental shelf transporting fish larvae from the GA.

It seems that the occasional arrival of larvae of *Ogcocephalus darwini* has permitted its recruitment to

the continental shelf, but not its dispersal within this new territory for the species. The fact that old fishermen from Las Piñas Cove described that they had not observed this species before, which is characterized not only by its brilliant colors but also its large size, in comparison with other local batfish, reveals that the presence of *O. darwini* in continental waters is a rare phenomenon and could be related to recent environmental changes occurring worldwide.

It should be noted that the records reported a maximum total length of 25 cm (Merlen 1988; Robertson and

Allen 2015), so this report extends the maximum total length of *O. darwini* to 26 cm.

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Supplementary material 1

Ogcocephalus darwini images

Authors: Jesus Briones-Mendoza, Klever Mendoza-Nieto, José Alió-Mingo, Erik Muñoz-Cedeño, Marcela García-Zambrano, Joshue Flores-Bailón

Data type: images

Explanation note: Photos of the morphology, organs and parasites of the individual *Ogcocephalus darwini*.

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Development and characterization of microsatellite markers for *Chaeturichthys stigmatias* (Actinopterygii: Gobiiformes: Gobiidae) based on restriction site-associated DNA sequencing (RAD-seq)

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Abstract

Chaeturichthys stigmatias Richardson, 1844, a fish species of the family Gobiidae, is an offshore warm-temperate fish species and a dominant component of estuarine ecosystems. In this study, restriction site-associated DNA sequencing was adopted to analyze the traits of candidate microsatellite markers for *C. stigmatias*, and 30 polymorphic loci were developed. A total of 5631 microsatellites with primer fragments were detected, among which trinucleotide repeats (57.56% of the total simple sequence repeats) were the most abundant, followed by di- (23.30%), tetra- (11.79%), penta- (4.14%), and hexa- (3.21%) nucleotide repeats type. The numbers of alleles per locus ranged from 6 to 14 with the mean value of 10.4. The mean value of observed heterozygosity and the expected heterozygosity were 0.349 and 0.870, respectively. The microsatellite locus with the lowest polymorphic information content (PIC) was 0.749, which indicated that all sites were highly polymorphic (PIC > 0.50). This is the first microsatellite development and characterization of this species to be reported.

Keywords

Chaeturichthys stigmatias, DNA sequences, microsatellite, polymorphic sites, primer detection, RAD-seq

Introduction

The branded goby, *Chaeturichthys stigmatias* Richardson, 1844 (also known as 矛尾鰕虎魚, finespot goby, or Cá Bống râu mắt nhỏ), is a warm-temperate nearshore benthic fish which is widely distributed in the coastal areas of China, Korea, and Japan (Sun et al. 2015). *Chaeturichthys stigmatias* expresses strong phenotypic

plasticity and can adapt to the changes of a variety of environmental factors, such as the bottom temperature and salinity (Liu et al. 2015). Although the important ecological values of *C. stigmatias* have been determined by biologists, the related studies mainly focused on the resource survey, community structure, feeding ecology, and fishery biological characteristics (Zhang et al. 2016; Meng et al. 2017; Li et al. 2018; Feng et al. 2019;

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Kindong et al. 2020; Han unpublished*). The information, however, about its genetic diversity and molecular markers is limited.

Exploring genetic diversity and population genetic structure may lead to a better understanding of the ecological importance of Gobiidae (see Yuan et al. 2012; Meng et al. 2017). Among molecular markers, microsatellites, or simple sequence repeats (SSR), is a simple repeat that is uniformly distributed in eukaryotic genomes and consists of tandem repeats of 2–6 nucleotides (Edwards et al. 1991). Given its codominance, high reproducibility, rich polymorphism, wide distribution, high stability, and easy detection (Dou et al. 2015; Song et al. 2016; Parthiban et al. 2018; Li et al. 2019), microsatellite markers have been used in a wide range of applications in population genetics, genetic breeding, evolutionary studies, identification of relations and individual identification (Lu et al. 2005; Hayes et al. 2007; Queirós et al. 2015), which can provide data reference and research guidance for them. In addition, microsatellite markers are greatly effective tools in population genetic studies because they could reveal the distinct population segments even in fine-scale genetic structure studies (Gandomkar et al. 2021). Therefore, it is essential to use microsatellite markers more conveniently and efficiently. However, the traditional methods for developing microsatellite markers are usually expensive, time-consuming, and cumbersome steps, with low coverage of loci in the genome, a long development cycle, and low versatility. Especially for non-model species with insufficient genetic information, the development of microsatellite markers is still difficult. In recent years, with the further maturity of the new generation of high-throughput sequencing technology and the rapid reduction of sequencing costs, a large number of plastid genomes, transcriptomes, and even genomes of non-model organisms have been sequenced, and the sequencing data in NCBI or other databases have increased significantly. As a reliable tool, high-throughput sequencing technologies optimize the field of discovery and development of molecular markers by generating large amounts of data (Shendure and Ji 2008; Stapley et al. 2010; Ekblom and Galindo 2011; Duan et al. 2017). In fact, high-throughput sequencing for developing SSR does not require sequencing depth as high as genome assembly and annotation, so the cost of this method is relatively low. Recently, high-throughput sequencing has been used to develop microsatellite markers in many fish, such as *Ctenopharyngodon idella* (Valenciennes, 1844) (see Yu et al. 2014), *Coilia nasus* Temminck et Schlegel, 1846 (see Fang et al. 2015), *Colossoma macropomum* (Cuvier, 1816) (see Ariede et al. 2018), *Genypterus chilensis* (Guichenot, 1848) (see González et al. 2019), and *Capoeta aculeata* (Valenciennes, 1844) (see Gandomkar et al. 2021).

Restriction site-associated DNA sequencing (RAD-seq) is a powerful tool to characterize the microsatellite

and single nucleotide polymorphism (SNP) markers, which was based on the second-generation sequencing technology (Khoshkholgh and Nazari 2020; Gandomkar et al. 2021). The reads generated by RAD-seq are grouped according to the enzyme recognition sequence, which could improve the precision and accuracy of contigs assembly, and improve the success rate of developing polymorphic microsatellite markers (Wei et al. 2014). In this study, RAD-seq was used to obtain preliminary data, and these data were applied to develop the *Chaeturichthys stigmatias* microsatellite primers, and finally, the validity of polymorphic primers was verified. The presently reported results may lay the foundation and provide references for the management and conservation of fishery resources.

Material and methods

Sampling and DNA extraction

A specimen of *Chaeturichthys stigmatias* was collected from the coast of Qingdao, China in November 2018, and sent for high-throughput sequencing. Dozens of *C. stigmatias* were collected from Zhoushan (August 2019), Qingdao (December 2019), Yantai (December 2019), and Weihai (October 2020), and 24 of them were used for polymorphism detection and genetic diversity analysis in this study. The samples were quickly dissected, and part of the muscle tissues on the caudal peduncle were collected and preserved in 95% alcohol in ice box, and then stored in -80°C for DNA extraction. The traditional phenol-chloroform method was used to extract genomic DNA. The total DNA was treated with RNase, and the DNA with high purity and without RNA contamination was obtained for the detection of SSR primers polymorphism. The extracted DNA was measured using a Nanodrop 2000 (Thermo Scientific, USA) and a Qubit 2.0 (Invitrogen, USA) bioanalyzer system.

RAD library construction and sequencing

After DNA quality inspection, library construction and sequencing were conducted. The steps of RAD library construction (Baird et al. 2008) were as follows: (1) Genomic DNA from the sample was digested at specific sites with a restriction enzyme, and the adapter P1 was ligated to the digested product. The P1 adapter contains forward amplification and Illumina sequencing primer sites, and an individual-specific nucleotide barcode; (2) The adapter-ligated fragments were then pooled, randomly sheared, and size-selected; (3) DNA was then ligated to a second adapter (P2), a Y adapter containing the reverse complement of the reverse amplification primer site, which en-

* Han DY (2013) [Study on feeding ecology of dominate gobiid fishes in Jiaozhou Bay.] Dissertation, Ocean University of China, Qingdao, China. [In Chinese]

sequences that lacking P1 adapter-ligated genomic fragments could not be amplified; (4) RAD tags with P1 adapter will be selected and amplified, and the 300–700 bp sequences were recovered. Agilent 2100 and Q-PCR were used to detect the size of library fragments and library quantification to determine whether the library meets the sequencing standard and then sequenced using the Illumina HiSeq2000 platform following the manufacturer's protocol. To obtain the clean reads, the reads with more than 10% N bases or low-quality bases ≤ 5 , adapter sequences, and duplicated sequences were discarded. The clean reads were used for subsequent analysis.

Detecting and verifying microsatellite primers

The software of detecting sequence repeats is “SSR search”, which is a Perl program written by Novogene (Beijing). The detection software is divided into three modules. The first module is used to detect all simple repeats of DNA sequence, the second module is to filter the results of the first module to remove the simple repeats that are too close. The detection criteria were as follows: the length of the SSR repeat unit ranges from 2 to 6 bp; the minimum length of the SSR sequence was 12 bp; the length of the upstream and downstream

sequences of the SSR was 100 bp, and 12 bp was the minimum distance between two SSR sequences. The third module is to use Primer3 (a software that designs primers under Linux or UNIX systems) to design primers (Rozen and Skaletsky 2000). The detected microsatellite primer sequences were further screened, and the screening criteria were as follows: the SSR units were repeated more than 6 times; the length of the SSR units ranges from 3 to 10 bp; the expected length of the PCR product was between 130 and 300 bp; the sequence of four consecutive bases was excluded. The selected primers were suitable for synthesizing SSR primers to verify primer polymorphism.

Microsatellites were verified through PCR and electrophoresis. Each 25 μ L PCR amplification system contained the following reagents: 17.25 μ L ultrapure water, 2.5 μ L $10 \times$ PCR buffer, 2 μ L dNTPs, 1 μ L each primer ($5 \mu\text{mol} \cdot \text{L}^{-1}$), 0.25 μ L Taq polymerase, and 1 μ L template DNA. The PCR reactions ran for 5 min at 94°C, followed by 38 cycles of 45 s at 94°C, 45 s at the annealing temperature (Table 1), and 45 s at 72°C in a thermal cycler. Cycling was followed by a final extension step at 72°C for 10 min. The PCR product was incubated at 4°C. The amplified PCR product was electrophoresed on an 8% non-denaturing polyacrylamide gel at 14 W for 3–4 h, and it was shown by silver staining (Lin et al. 2015). The allele size was identified according to the 20 bp DNA ladder.

Table 1. Characteristics of microsatellite loci in *Chaeturichthys stigmatias* from China.

Locus	Primer sequence (5'→3')		Repeat motif	T_a [°C]	Expected product length [bp]	N_a	H_o	H_e	PIC
	Forward	Reverse							
MW29	ACTAATTAGCATTTCAGCACCAGC	GTCATGCACAGTGACACCATAAT	(TG)15	58.3	135	7	0.000	0.823	0.778
MW31	TGATCGACAATGAAATGTAATG	TATTCTATAGCCACAGCTGCCT	(TG)7	56.4	145	13	0.292	0.895	0.866
MW32	TAAAGTGCCTGAACAAGTTGGAT	CGTCATGATTTCAGGAAGTAACA	(TA)10	55	144	9	0.042	0.870	0.836
MW34	AAGTGTCTATTCTGAGCGCACTT	TTGCAGTGTGAATCAAACATTC	(GAT)8	58.3	153	9	0.292	0.878	0.844
MW40	TCTGCATCTTCTGAACCTCACCT	CTCTGAAACACACAGTACACACT	(GC)7	56.4	156	11	0.333	0.894	0.862
MW54	ATAGAAGGGACTTCAGTTGGACC	CCATTAAACTCTGTCCAGACCCA	(AT)7	56.4	138	6	0.167	0.796	0.749
MW56	TGTATTCTCGCTTACTGCAGCTC	TCATTCTCAGCATTGACTCTCAT	(ATA)7	56.4	132	14	0.375	0.883	0.854
MW66	AGAGTGAAGAAGACGACTGACC	GACCTTAGTGAGAGTGTGCGTGT	(CA)9	58.3	140	8	0.458	0.855	0.819
MW72	TGCAAACTGCTTGTGTAGTT	TGAGCTGATTGTGTAGTTTGTCA	(TA)7	56.4	150	11	0.458	0.903	0.873
MW77	CTGTCTGTGTTTACTCAGATG	TATCAAGGGCTCACTAAAGGACA	(GAG)7	58.3	137	6	0.250	0.821	0.774
MW79	GAAGAGGGAAGAGAGAACCAAAAG	TTCTGTCCCAAATTCACCTCTC	(GA)9	56.4	160	10	0.417	0.834	0.793
MW80	TTAGACAGGACAGCGTTAGCATT	CACAGCAAAGGCTCTGAATACTT	(GA)7	56.4	147	13	0.417	0.867	0.834
MW83	GAGACACTGTCAGAGCAGATCCT	TAATCAACAGCATGAAGAGCAGA	(GCT)7	56.4	148	10	0.500	0.840	0.801
MW86	AAATCCTTCTGCAATTGACTCTG	GAGAGGGAGGAAGAGATAATGGA	(CT)8	55	139	7	0.167	0.816	0.769
MW87	ACTGCTGTAGATTTACTGGTGC	TATCCTTATCCTCCTCTTTCACA	(TAC)8	60.2	157	11	0.417	0.876	0.842
MW88	TTGAGTATATTCAGCCCGTCTC	GCCGTTTGCTCATAACATAAACT	(AT)8	56.4	133	13	0.250	0.912	0.884
MW92	TTTGAAAAGGTGCAGGAGATG	TGAACTCCACTGCTGTGTAAA	(CT)15	53.1	136	12	0.708	0.908	0.879
MW97	CACAGCAAACAAGAAACAACAC	TATTACGGAAAAGGGTAGGACCAT	(TAC)7	58.3	138	12	0.417	0.857	0.826
MW100	TCCCACCACAGAAGTTAAACAGT	GCATGTTCTTACAAAGGTTTAC	(TAT)7	55	148	8	0.042	0.840	0.800
MW103	CTTTCTACTTTCCCGCTCTCTT	CATGGAAATGGATAGAAATGGAA	(CT)7	55	133	11	0.375	0.889	0.858
MW104	AGGCAAGAAATATCACAGGGACT	TCGTGACTCATGGAATACCAAT	(AT)7	55	147	10	0.333	0.860	0.824
MW111	CAGGCCTGTTAGCTTAGCTGTAG	CACTGGCACACACAACCTAAATA	(AT)12	58.3	139	12	0.542	0.893	0.862
MW113	GTATTTATCCGAGCACGACTAC	TAAACGCACGAACAGTATCGTAA	(TG)12	55	156	12	0.833	0.898	0.868
MW115	TTATTTGCCAGTATTGACCCAGT	CCAAGCCTCTAAGAGTGTCTGAA	(CA)11	49.6	150	9	0.000	0.876	0.841
MW117	TGACGTGTGAACATTCGTGAGT	GAGGGAATGATGTCTGTGATTC	(ACA)8	58.3	151	13	0.417	0.931	0.905
MW118	TTATGGCCCTCAGTGTGTTAAT	CCTCGAGGAAATATCAGAGTATCG	(TAA)10	55	157	10	0.333	0.874	0.840
MW119	AAATGACGAGACAATTACAACCTGAT	TTCTTTGTGTATTATGGAAGTTCA	(TA)15	58.3	139	11	0.833	0.886	0.854
MW120	TTTCAGATACACTCATTGGACC	GAAACAACAGCAGTTGCACAAT	(AAT)7	60.2	140	13	0.500	0.897	0.867
MW121	TCTGTTGTGACAGTGACAGAGT	CCTCCAGAAAAGGACTCATCAT	(TGC)7	58.3	132	12	0.167	0.903	0.873
MW123	TCCATCTAAACTGAACCAAATG	TGAAATGTAGTCAATCTTTGCCA	(TTA)7	58.3	154	9	0.125	0.835	0.795

T_a = optimized annealing temperature, N_a = number of alleles, H_o = observed heterozygosity, H_e = expected heterozygosity, PIC = polymorphism information content.

Data analysis

After statistical analysis, the results were input into Genepop 4.0 (Rousset 2008). The parameters of SSR primers were calculated, including the mean value of effective allele number (N_e), polymorphism information content (PIC), observed heterozygosity (H_o) and expected heterozygosity (H_e), and Hardy–Weinberg equilibrium was also performed.

Ethics statement

We have read the policies relating to animal experiments and confirmed this study complied. All procedures performed in this study were approved by the Institutional Animal Care and Use Committee of the Ocean University of China.

Result and discussion

High-throughput sequencing and quality estimation.

A total of 4.682 Gb high-quality data was obtained, and the Q20 and Q30 values were 97.31% and 92.52%, respectively. The RAD-Tag capture rate was 98.03%, and the GC content was 39.43%. Genomic GC content had a significant effect on the randomness of second-generation genome sequencing. Too high (>65%) or too low (<25%) GC content will lead to sequencing bias and seriously affect the results of genomic analysis. The GC content of *Chaeturichthys stigmatias* was normal, and the sequencing quality was qualified, indicating that the sequencing of the database was successful (Zerbino and Birney 2008).

The sequences were clustered and assembled. The total contig base was 113 171 723 bp, and the total contig number was 337 800. The mean value of contig length of the assembly sequences was 335 bp, and N50 length was 393 bp. The GC content of the assembly result was 39.04%, which was consistent with the GC content of the sequencing clean data, indicating that the assembly result was true and reliable (Wang et al. 2017; Gao et al. 2018). Subsequently, the variation detection was carried out on

the assembly results. The number of heterozygous SNPs in the detected SNPs was 142 307, and the heterozygous rate was 82.47%. The high heterozygous SNP and the low homozygous SNP values also indicated the reliability of the assembly results.

Characterization of microsatellite loci

Based on the RAD-seq, the total number of identified microsatellites was 5829. Among them, there were 5631 microsatellite loci containing primer fragments (Table 2). The trinucleotide repeats were dominant (57.56%), followed by dinucleotide repeats (23.30%), tetranucleotide repeats (11.79%), pentanucleotide repeats (4.14%), and hexanucleotide repeats (3.21%).

The previous studies showed that the dominant repeating unit was discrepant. Some fish species were dinucleotide, such as *Megalobrama amblycephala* Yih, 1955 and *Larimichthys crocea* (Richardson, 1846) (see Wang et al. 2012; Zeng et al. 2013; Li unpublished*), while some fish were trinucleotide, such as *Acanthogobius hasta* (Temminck et Schlegel, 1845) and mollusk, *Ruditapes philippinarum* (see Yan et al. 2015; Song et al. 2019). The previous studies have suggested that enzymes and other proteins involved in various aspects of DNA processing and chromatin remodeling may be responsible for the taxonomic specificity of microsatellite abundance. This was manifested in that not only the repetitiveness of the genome varies, but also the dominant microsatellite types are different. This might indicate that SSRs play an important role in genome evolution, and the process responsible for the generation and fixation of SSR has also changed during evolution (Toth et al. 2000). In this study, trinucleotide repeats have absolute quantitative advantages, and the number of dinucleotide repeats was less than half. We speculate that a genetic mutation might occur during the evolution of *Chaeturichthys stigmatias*. Further comparative investigations including more species are needed to clarify this point.

The distribution and frequency of microsatellite motifs were presented in Fig. 1. The AT repeat motif (300) was the most frequent among all 11 types of dinucleotide repeat, whereas GC was the least frequent, with only one microsatellite locus. The AAT repeat motif (396) was the most frequent among all 60 types of a trinucleotide repeat. The AAAT repeat motif (55) was the most frequent among all 104 types of tetranucleotide repeat. The AATTG repeat motif (56) was the most frequent pentanucleotide repeat, and the ATTCTG (35) was the most frequent hexanucleotide repeat. Because the repeat types of trinucleotide, tetranucleotide, pentanucleotide, and hexanucleotide were too dispersed, only the top 30 types of loci were selected for illustration in order to show the results more clearly. All detailed data was provided in Suppl. material 1: Appendix 1 (online resource).

Table 2. Simple sequence repeat (SSR) distribution statistics for *Chaeturichthys stigmatias* from China.

Nucleotide repeat type	Statistics	
	SSR number	Percentage
Di-	1312	23.30
Tri-	3241	57.56
Tetra-	664	11.79
Penta-	233	4.14
Hexa-	181	3.21

* Li HM (2014) [New microsatellite satellite markers development based on whole genome sequencing information and its application in population genetics in large yellow croaker.] Dissertation, Zhejiang Ocean University, China. [In Chinese]

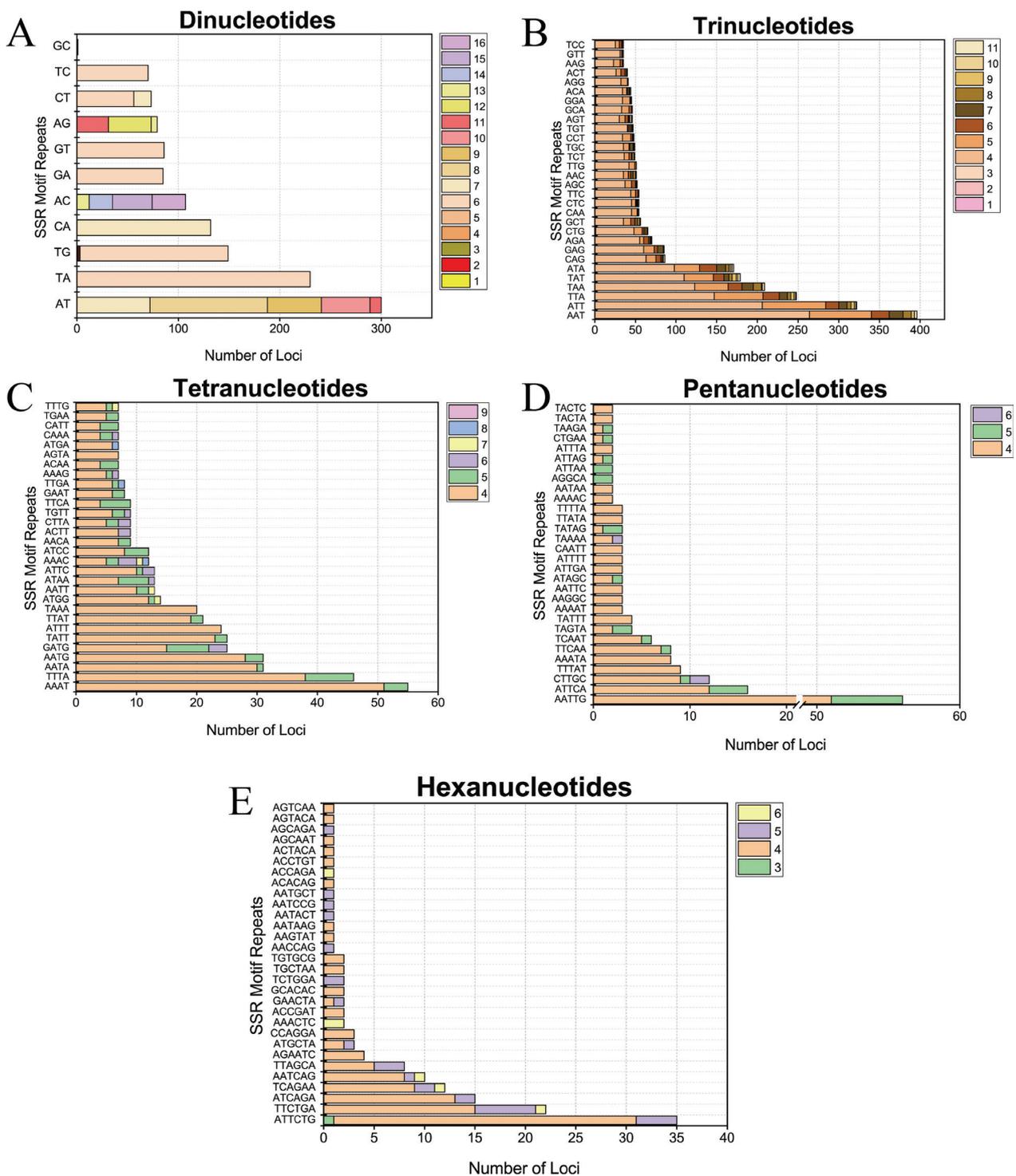


Figure 1. The distribution and frequency of microsatellite motifs of *Chaeturichthys stigmatias* from China. (A) Frequency of different dinucleotide microsatellite motifs; (B) Frequency of different trinucleotide microsatellite motifs; (C) Frequency of different tetranucleotide microsatellite motifs; (D) Frequency of different pentanucleotide microsatellite motifs; (E) Frequency of different hexanucleotide microsatellite motifs.

In terms of the frequency of repeating units, there were only four distinct types of repeats detected in pentanucleotide and hexanucleotide, and all of them were predominant at a frequency of a 4-fold repeat. Seven types were identified and 4-fold repeat was predominant in all tetranucleotide repeats. The types of repetition frequency detected in dinucleotide and trinucleotide were not less

than 10 types, and 5-fold repeat and 6-fold repeat were the main components in dinucleotide and trinucleotide respectively (Figs 1, 2).

In this study, the frequency distribution of the repetition units of dinucleotide, trinucleotide, tetranucleotide, pentanucleotide, and hexanucleotide microsatellite were mainly 4–10 times, 4–7 times, 4–5 times, 4 times, and 4

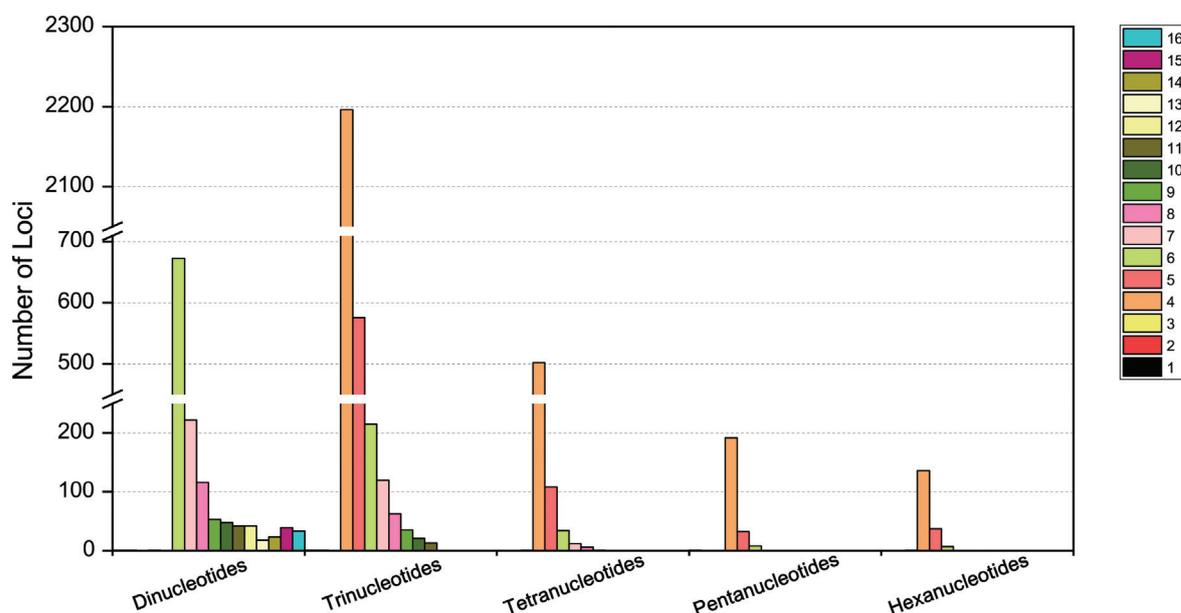


Figure 2. Frequency of repeating units of different types of microsatellites of *Chaeturichthys stigmatias* from China.

times, respectively (Fig. 2). This result showed that the frequency of tandem repeats decreased exponentially with the increase of repetition unit length, and this was consistent with the conclusion proposed by Chen et al. (2010) that the number of repeating units was negatively correlated with the length of repeating units. According to previous studies, slipped-strand mispairing is a major mechanism for DNA sequence evolution (Levinson and Gutman 1987), and the results of this study can be explained as microsatellites with a large number of repetitions may be more unstable due to the increase of sliding possibility (Ellegren 2004). It was generally believed that there was a certain positive correlation between the variation frequency of SSR sites and the number of repetition units (Schlötterer 2000). Katti et al. (2001) also reported that the mutation rate increases gradually with the increase of the length of repeating units in eukaryotes.

Detection of primer polymorphism

A gradient PCR experiment was performed on the synthesized 148 pairs of primers, and the optimal temperature of each pair of primers was screened. The results showed that a total of 97 pairs of primers were successfully amplified. Then after the PCR product was subjected to polyacrylamide gel electrophoresis experiments, a total of 30 primers with polymorphism were screened out. A total of 312 alleles were detected for 24 individuals at 30 polymorphic loci, and the number of alleles per locus ranged from 6 to 14, with the mean value of effective alleles was 10.4. The mean value of expected heterozygosity was 0.870, the observed heterozygosity was 0.349, and the mean value of polymorphic information content was 0.836 (Table 1). All the polymorphic sites deviated significantly from Hardy–Weinberg equilibrium ($P < 0.05$). The PIC was between

0.749 and 0.905, and all loci showed high polymorphism ($PIC > 0.5$) (Botstein et al. 1980).

In this study, 30 primers with polymorphism were screened out as dinucleotide and trinucleotide repeats, without tetranucleotide, pentanucleotide, and hexanucleotide repeats. Kong et al. (2019) observed that compared with trinucleotide and tetranucleotide repeats, dinucleotide repeats had a higher screening efficiency and polymorphism (Kong et al. 2019). However, in recent years, it has also been found that the trinucleotide and tetranucleotide repeats have higher screening efficiency and polymorphism than dinucleotide repeats in *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix* (Valenciennes, 1844), and *Cyprinus carpio* Linnaeus, 1758 (see Fang et al. 2018). The higher repetition unit length has the disadvantages of lower repeats, lower sequence richness, and lower mutation rate. The better trinucleotide and tetranucleotide repeats polymorphisms obtained in other experiments may be related to the genome doubling in the long-term evolution of this species (Lu et al. 2009; Fang et al. 2018). The different results may be related to the specificity of species, the randomness of the number and type of primers selected in the experiment, and the number of samples. The above-mentioned results indicated that the SSR repeats which had higher screening efficiency and polymorphism may be species-dependent, and the most probable SSRs were dinucleotide and trinucleotide repeats. In terms of polymorphism, the mean PIC values of dinucleotide and trinucleotide repeats microsatellite primers screened in this study were 0.836 and 0.835, respectively, with little difference in polymorphism. Therefore, differences in screening efficiency and polymorphism may be caused by species differences or other factors (Kong et al. 2019).

The higher heterozygote ratio reflects the stability of the genetic structure of the population. We found that the observed heterozygosity (H_o) of 30 polymorphic sites was lower than the expected heterozygosity (H_e), show-

ing a relative lack of heterozygosity. It was generally believed that the loss of heterozygosity was caused by geographical isolation, decreased gene exchange between populations, and increased inbreeding (Zhao et al. 2009). The samples used in this study were collected from the Yellow Sea, the Bohai Sea, and the East China Sea. The low heterozygosity of *Chaeturichthys stigmatias* may be due to geographical isolation and excessive intraspecific hybridization. It was commonly accepted that the expected heterozygosity (H_e) was a more accurate reflection of the genetic diversity of a population than the observed heterozygosity (H_o) (Nei 1978). Therefore, the mean value of observed heterozygosity of 0.870 in this study showed a high population diversity.

At the same time, according to Hardy–Weinberg equilibrium analysis, all the 30 microsatellite loci discussed in this study showed significant imbalance, which was a common phenomenon in fish populations, such as *Siniperca scherzeri* Steindachner, 1892 and *Lutjanus peru* (Nichols et Murphy, 1922) (see Dou et al. 2015; Paz-García et al. 2017). This result also confirmed that these populations did not mate randomly, and non-random sampling was also the reason for the deviation of Hardy–Weinberg equilibrium. It was worth noting that inbreeding, subgroup structure, genetic drift, overfishing, Wallund effect, and ineffective alleles should also be considered (Bergh and Getz 1989; Lu et al. 2017; Song et al. 2018). The above results indicated

that the microsatellite markers identified in this study have high polymorphisms and can be used as effective molecular markers to analyze the genetic diversity and phylogenetic relations among *C. stigmatias*.

Conclusion

This study was conducted in combination with high-throughput sequencing, which also marks the first analysis of the microsatellite characteristics of *Chaeturichthys stigmatias*. In summary, a total of 4.682 Gb high-quality sequence data was obtained and 5631 SSRs were identified based on RAD-seq, indicating the high efficiency of the primer development of this technology. The 30 pairs of polymorphic primers obtained in this study will provide an effective basis for the future comparative analysis of the genetic structure and genetic characteristics of *C. stigmatias*, and also provide a significant basis for the development of microsatellite primers using high-throughput sequencing technology in the future.

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Supplementary material 1

Appendix 1

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Data type: excel file

Explanation note: Detailed data for types of trinucleotide, tetranucleotide, pentanucleotide, and hexanucleotide loci.

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