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ACTA ICHTHYOLOGICA ET PISCATORIA

First records of the seamoth, *Pegasus nanhaiensis* (Actinopterygii: Syngnathiformes: Pegasidae), from the southern South China Sea, with notes on fresh coloration

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Abstract

Three seamoth specimens (45.5–56.9 mm standard length; SL) (Syngnathiformes: Pegasidae), originally identified as *Pegasus laternarius* Cuvier, 1829, but now recognized as representing *P. nanhaiensis* Zhang, Wang et Lin, 2020, a species recently described from the northern South China Sea off Yangjiang and Beihai, China, were obtained at a local fish market in Maha Chai, Samut Sakhon Province, Thailand on 6 July 2012, having been caught in the northern Gulf of Thailand. In addition, single specimens, reported as *P. laternarius* or *Spinipegasus laternarius* from Bidong Island, South China Sea off the Malay Peninsula (46.1 mm SL) and from Ko Kradat, Trat Province, eastern Gulf of Thailand (66.1 mm SL), were re-identified here as *P. nanhaiensis*. Thai specimens and Malaysian record represent the first records of *P. nanhaiensis* from Thailand and Malaysia, respectively, and from outside Chinese coastal waters. Additionally, the Bidong specimen is the southernmost record for the species. The fresh coloration of *P. nanhaiensis* is described for the first time.

Keywords

Pegasus laternarius, distribution, southernmost record, Bidong Island, Malaysia, Gulf of Thailand

Introduction

The Indo-West Pacific genus *Pegasus* Linnaeus, 1758, characterized by having 11 tail rings, no spine on the dorsal surface of the last dorsal ring, and the eyes not visible in ventral view (Palsson and Pietsch 1989), includes five valid species, viz., *Pegasus lancifer* Kaup, 1861, *Pegasus laternarius* Cuvier, 1829, *Pegasus nanhaiensis* Zhang, Wang et Lin, 2020, *Pegasus tetrabelos* Osterhage, Pogonoski, Appleyard et White, 2016, and *Pegasus volitans* Linnaeus, 1758 (see Zhang et al. 2020; Fricke et al. 2021).

Pegasus nanhaiensis was originally described on the basis of 17 specimens from the northern South China Sea (off Yangjiang and Beihai) (Zhang et al. 2020), no further specimens having been recorded since. However, three specimens, collected from the northern Gulf of Thailand prior to that description, were re-identified here as *P. nanhaiensis*, two having been reported as *P. laternarius* by Matsunuma (2013). These three specimens, therefore, represent the first records of *P. nanhaiensis* from the Gulf of Thailand and the first records outside Chinese coastal waters. In addition, previous records of *P. laternarius* (or as *Spinipegasus laternarius*) from the eastern Gulf of Thailand and the eastern Malay Peninsula were re-identified here as *P. nanhaiensis*. As Zhang et al. (2020) described the coloration of dry specimens only, a fresh color description of *P. nanhaiensis* is provided here for the first time.

Methods

Counts and measurements followed Osterhage et al. (2016) and Zhang et al. (2020). Measurements were made to the nearest 0.1 mm with digital calipers under a dissecting microscope. Standard length is abbreviated as SL. Terminology of body parts and determination of sex followed Palsson and Pietsch (1989). The following description was based solely on the three specimens from the northern Gulf of Thailand (Figs. 1–3). Photographs of the lateral view of tail rings I–VI (Fig. 3) were taken with a Nikon D850 camera using the internal focus bracketing function (focus step width 1, number of shots 30); a set of multifocal images were then collated into an overall well-focused composite image using Combine ZP (free software: available at https://combinezp.software.informer.com). Institutional codes follow Sabaj (2020). Comparative material of *P. laternarius* (6 specimens, 24.5–55.1 mm SL, from Japan) examined in this study are as follows: KAUM–I. 420, female, 55.1 mm SL, east of Sakinoyama, Kataura, Kasasa, Minami-satsuma, Kagoshima, 31°25′44″N, 130°11′49″E, 27 m depth, set net, 6 Mar 2006; KAUM–I. 3234, female, 24.5 mm SL, KAUM–I. 3247, female, 28.0 mm SL, Kaihama Beach, Kasasa, Minami-satsuma, Kagoshima, 31°24′37″N, 130°11′32″E, 0.5 m depth, hand net, 17 Mar 2007; KAUM–I. 17604, female, 37.0 mm SL, off Kouzaki-yama, Kataura, Kasasa, Minami-satsuma, Kagoshima, 31°26′00″N, 130°10′05″E, 36 m depth, set net, 17 Mar 2007; KAUM–I. 31094, male, 36.6 mm SL, east of Sakinoyama, Kataura, Kasasa, Minami-satsuma, Kagoshima, 31°25′44″N, 130°11′49″E, 27 m depth, set net, 13 Mar 2010; KAUM–I. 105955, male, 49.0 mm SL, fish market in Tei, Yasu, Kounan, Kochi, 33°31′42″N, 133°45′14″E, 24 Oct 1981.

Results

Family Pegasidae Bonaparte, 1831
Pegasus Linnaeus, 1758

Pegasus nanhaiensis Zhang, Wang et Lin, 2020

Figs. 1–4; Table 1

Pegasus laternarius (not of Cuvier 1829): Palsson and Pietsch 1989: 23, fig. 11 (Ko Kradat, Trat Province, eastern Gulf of Thailand, southern South China Sea); Matsunuma 2013: 68, unnumbered figs. (northern Gulf of Thailand, southern South China Sea; incorrectly reported as Cuvier 1816).

Pegasus nanhaiensis: Zhang et al. 2020: 523, figs. 1, 3A, 3C, and 4A (type locality: off Yangjiang, China; paratype localities: off Yangjiang and Beihai, China, northern South China Sea).

Spinipegasus laternarius (not of Cuvier 1829): Hibino 2021: 14, unnumbered figs. (Bidong Island, off east coast of Malay Peninsula, southern South China Sea; incorrectly reported as Cuvier 1816).

Material examined. Three specimens from the northern Gulf of Thailand: KAUM–I. 47679, female, 45.5 mm SL, KAUM–I. 47680, male, 56.9 mm SL, KAUM–I. 47681, male, 49.7 mm SL, trawl, purchased at a fish market in Maha Chai, Samut Sakhon Province, Thailand, 6 July 2012 (originally deposited in Kasetsart University).

Description. Measurements are given in Table 1. Body depressed, encased in bony plates. Eyes not visible in ventral view. Rostrum of male long, club-shaped, with many small surface spines; that of female very short, pointed. Mouth small, inferior, toothless. Gill opening restricted to small dorsolateral hole behind head. Two rows each with two small tubercles on dorsum of head. Carapace comprising three pairs of dorsal plates ($d_{1–3}$), four pairs of dorsolateral plates ($dl_{1–4}$), paired superior pectoral-fin plates (pp.s.), and two paired extralateral plates ($el_{1–2}$); rounded hump-like tubercles on each dorsal plate ($d_{1–3}$); small posteriorly directed tubercles on lateral edges of each dorsolateral plate ($dl_{1–4}$). [KAUM–I. 47680 with hook-shaped tubercle between paired dorsal plates (d_2); absent in KAUM–I. 47679 and 47681]. Plastron comprising five paired ventrolateral plates ($vl_{1–5}$), paired gular plates (g), pectoral plates (p), ventral plates (v), anal plates (a) and inferior pectoral-fin plates (pp.i.) and an unpaired pre-anal plate (ip). Anus located between preanal plate and tail ring I. KAUM–I. 47679 with 6 inwardly directed spines (7 and

Table 1. Measurements of *Pegasus nanhaiensis*.

Character	This study		Zhang et al. (2020)		
	Northern Gulf of Thailand, southern South China Sea		China, northern South China Sea		
	Males	Female	Holotype	Paratypes	
	$n = 2$	$n = 1$	male	males	females
			$n = 1$	$n = 11$	$n = 5$
			[mm]		
Standard length (SL)	49.7–56.9	45.5	61.7	47.1–57.8	53.8–62.3
Precaudal length (PCL)	53.8–61.4	46.8	67.4	52.3–64.4	55.0–62.9
			[% of SL]		
Carapace length	47.5–49.3	53.9	47.1	48.6–53.2	51.4–54.5
Tail length	48.5–51.7	48.9	49.8	46.7–52.8	45.1–49.8
Prepectoral width	40.9–41.4	44.8	40.8	40.3–46.0	44.4–47.5
Interpectoral width	29.7–30.3	35.7	29.0	29.7–34.9	34.0–36.3
Carapace width	29.7–30.7	37.0	29.9	28.8–34.7	34.8–37.0
Body depth	18.7–19.7	19.8	17.9	16.5–20.9	16.1–20.0
Rostrum length	14.0–15.7	6.9	13.8	13.9–17.4	4.8–6.3
Rostrum width at tip	4.9–5.0	1.6	4.8	3.0–4.3	1.0–1.6
Orbit length	8.9–10.2	10.1	8.9	9.2–11.3	9.7–10.8
Interorbital width	9.5–11.2	10.7	9.7	9.1–11.2	8.7–10.7
Head width	26.6–27.7	30.5	26.4	26.7–31.8	30.2–32.4
Length of 5th pectoral ray	32.9–34.4	36.8	33.6	30.9–40.3	35.1–38.3
Height from dorsal to anal fins	10.4–10.7	10.2	11.3	10.2–12.8	10.0–12.2
Rostrum tip to pelvic fin length	50.9–51.2	45.8	49.6	49.5–53.6	41.2–46.6
Rostrum tip to anal fin length	66.8–67.0	65.3	65.3	67.0–66.4	67.0–73.1
Length from base of pectoral fin to pelvic fin	27.7–28.0	29.2	27.5	28.4–31.4	28.4–31.4

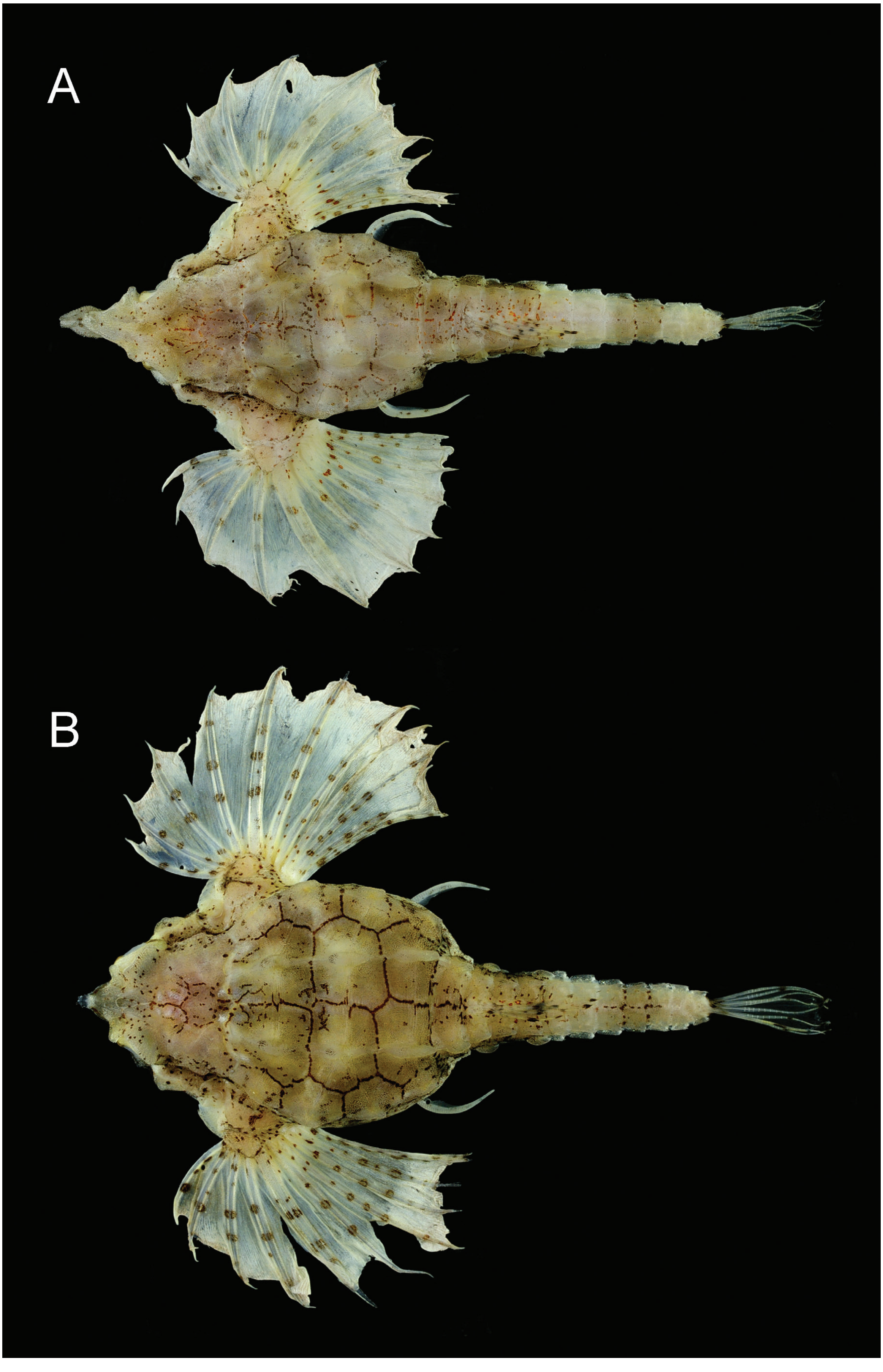

Figure 1. Dorsal views of *Pegasus nanhaiensis* from the northern Gulf of Thailand. **A**: KAUM–I. 47680, male, 56.9 mm SL; **B**, KAUM–I. 47679, female, 45.5 mm SL.

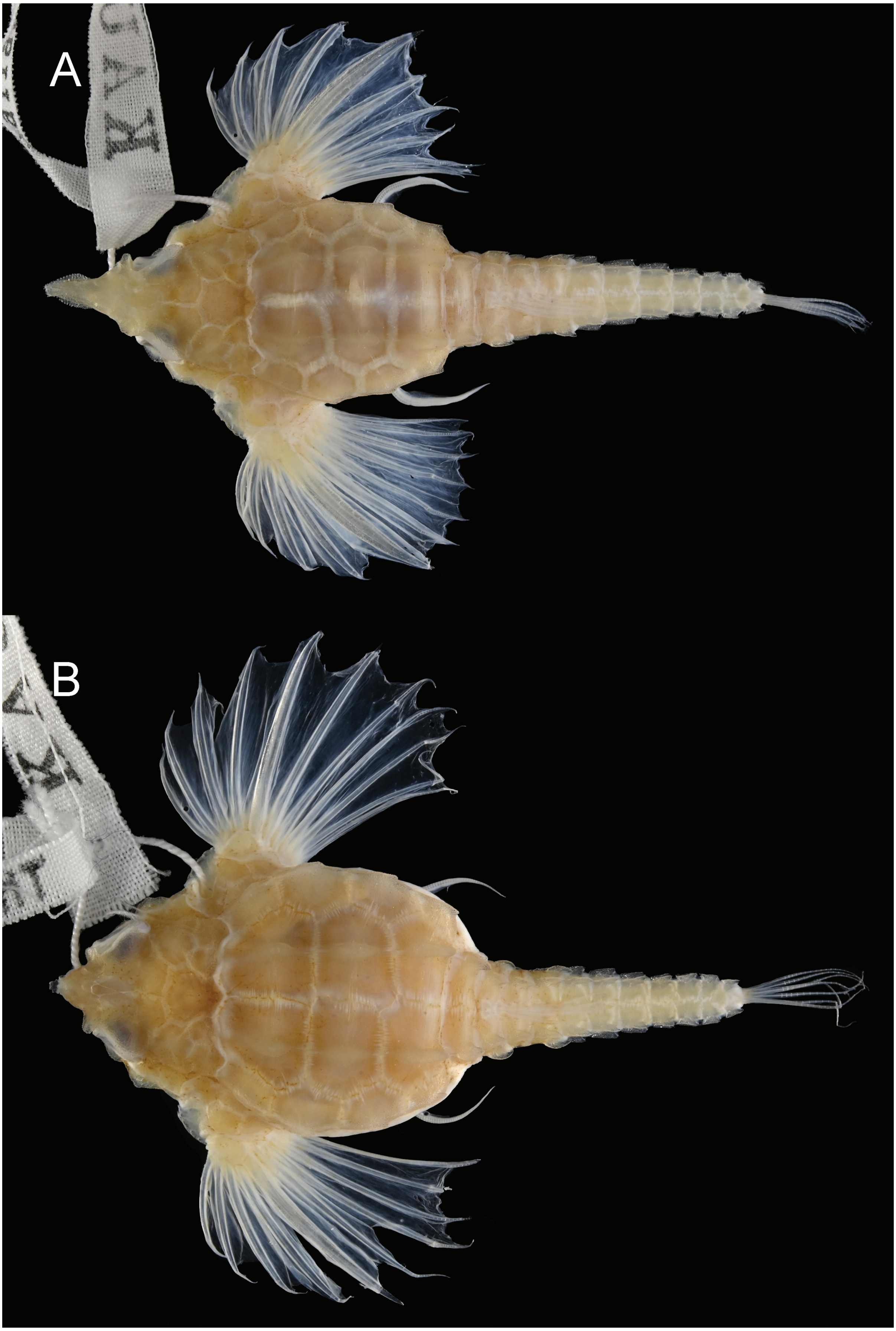

Figure 2. Dorsal views of preserved specimens of *Pegasus nanhaiensis* from the northern Gulf of Thailand. **A**: KAUM–I. 47680, male, 56.9 mm SL; **B**, KAUM–I. 47679, female, 45.5 mm SL.

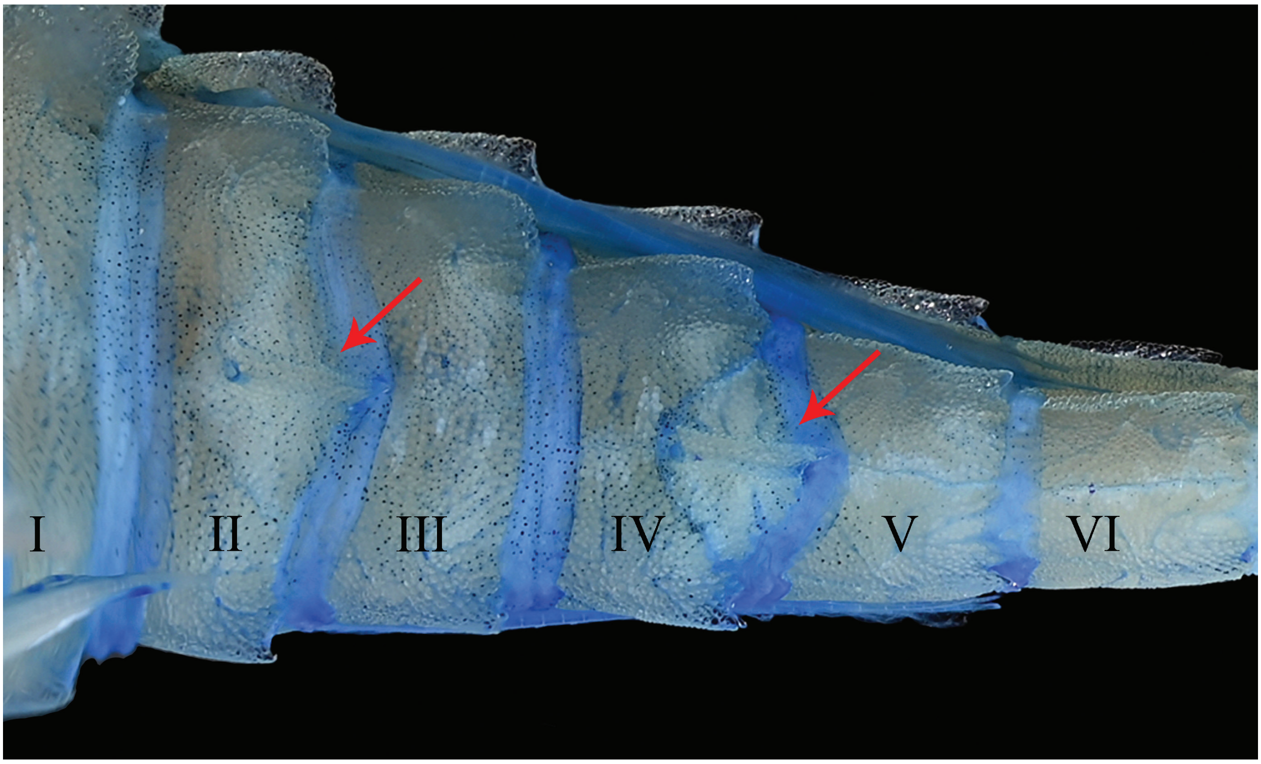

Figure 3. Lateral view of tail rings I–VI of *Pegasus nanhaiensis* (KAUM–I. 47680, male, 56.9 mm SL) stained with cyanine blue. Red arrows indicate caudolateral plates overlapping junctions between tail rings II and III and IV and V, respectively.

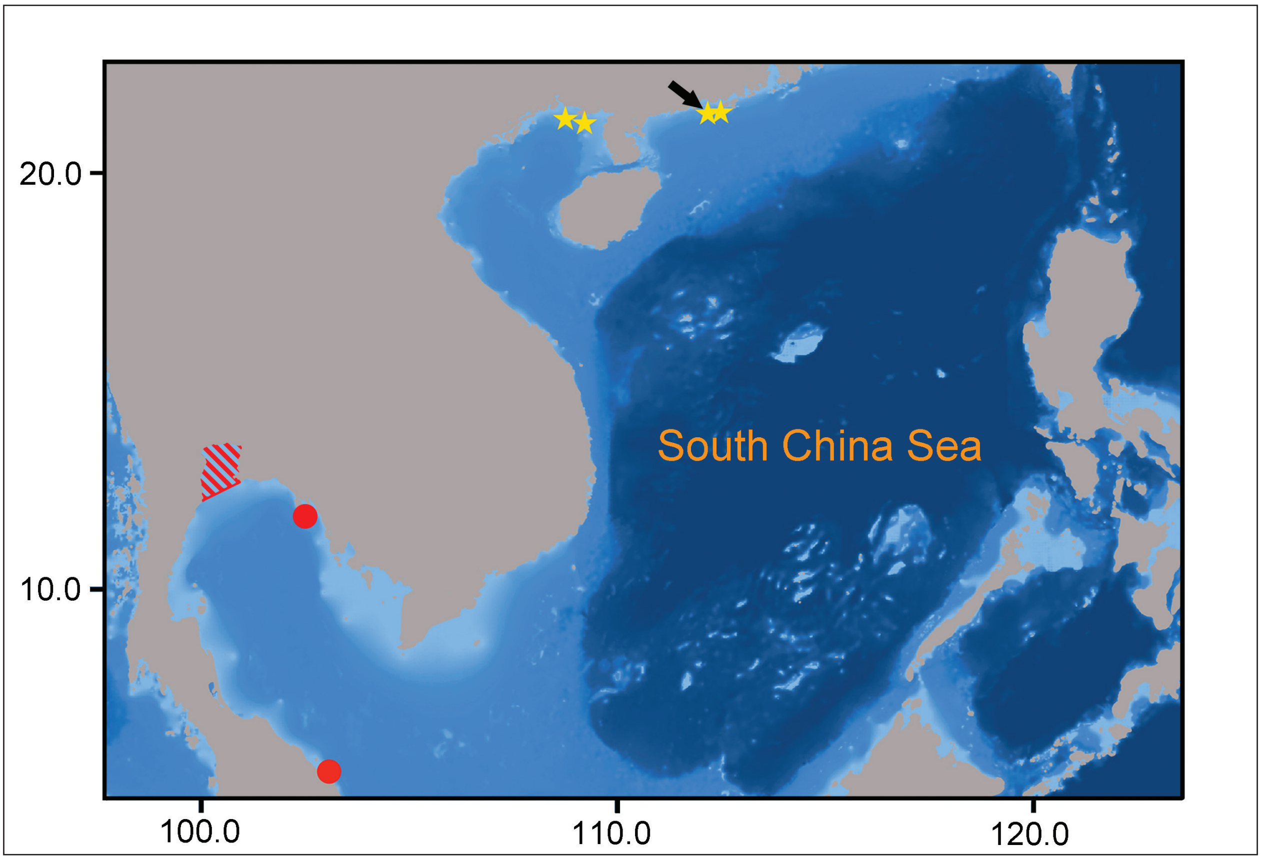

Figure 4. Distributional records of *Pegasus nanhaiensis*. Yellow stars: type series localities (black arrow: type locality); red circles and striped area: localities of presently reported specimens (specimens from northern Gulf of Thailand were obtained at a fish market; their approximate collection locality indicated).

5 in KAUM–I. 47680 and 47681, respectively) on dorsal surface of ventrolateral plate (vl_1). Small central tubercles on each pectoral and ventral plate; interventral and pre-anal plates with bulge, the latter plate with posteriorly directed tubercle; small, posteriorly directed tubercles on lateral edges of each vl_2–vl_4. Tail elongate, with 11 tail rings (I–XI); 9th and 10th tail rings fused together, anterior 8 rings mobile; small, posteriorly directed tubercles on corners of each tail ring, their tips sharply pointed; tubercles smaller on posterior tail rings; anteriorly directed spines on anterior of tubercles on tail rings IX, X, and XI; two paired caudolateral plates overlapping junctions between tail rings II and III and IV and V; dorsal surface of last tail ring lacking spine. Wing-like pectoral fins large, inserted horizontally, with 11 rays (10 and 12 rays on left and right side, respectively, in KAUM–I. 47679), 5th ray stout, thicker than other rays. Pelvic fins with 1 spine and 2 rays; each pelvic fin separated without membrane, inserted into an unpaired interventral plate; first spine very long, extended posteriorly. Dorsal and anal fins short, each with 5 soft rays, extending from center of dorsal and ventral tail ring II to center of tail ring IV, respectively. Caudal fin with 8 unbranched rays.

Coloration when fresh (Figs. 1A–1B). Dorsal body surface pale yellow to dark yellowish-brown with numerous reddish-brown to black spots; clear hexagonal patterns apparent on dorsal plate ($d_{1–3}$) and dorsolateral plate ($dl_{1–4}$), with distinct boundaries. Blurred black blotches on rounded hump-like tubercles on dorsal plates in KAUM–I. 47679 (absent in KAUM–I. 47680). Dorsal surface of ventrolateral plate (vl_1) whitish. Base color of dorsal surface tail rings I–IV, posterior half of VII and VIII brown; that of V–VI, anterior of VII and IX–XI white. Numerous reddish-brown spots on dorsal surface of tail rings I–IV, brown spots on dorsal tail rings VI–VII in KAUM–I. 47680 (reddish to brown spots on I–VIII in KAUM–I. 47679). Base color of pectoral-fins whitish; 2–9 brown blotches on each ray. Pelvic-fins white; small brown blotches on each ray. Dorsal and caudal fins translucent white, small brown blotches on each ray.

Color in alcohol (Figs. 2A–2B). Dorsal body surface and tail rings light reddish-yellow. Faint yellowish-brown spots on dorsal body and tail rings I–IV in KAUM–I. 47680 (dorsal body and tail rings I–VIII in KAUM–I. 47679). Margins of majority of dorsal plates white (some slightly yellowish-brown). Each fin translucent white.

Discussion

The presently reported specimens were consistent with the diagnosis of *Pegasus nanhaiensis*, provided by Zhang et al. (2020), all having a rounded hump-like tubercle on each of dorsal plates I, II, and III; clear, distinctly bounded hexagonal patterns on the dorsal plates (d_{1-3}), and dorsolateral plates (dl_{1-4}); two paired caudolateral plates overlapping the junctions between tail rings II and III and IV and V (Fig. 3); and a bulge on the margin of the ventral plate connecting with the paired pelvic fins. Although the rostrum length in the female and rostrum tip width in males in this study differed slightly compared with the original description (6.9% of SL and 4.9%–5.0% of SL, respectively, in the presently reported specimens vs. 4.8%–6.3% and 3.0%–4.8%, respectively, in the type series; Table 1), such minor differences were regarded here as intraspecific variations. *Pegasus nanhaiensis* is similar to *P. laternarius* in sharing 11 tail rings, thickened fifth pectoral-fin ray, the fused 9th and 10th tail rings, and a wider carapace (carapace width 28.8%–37.0% of SL in the former, 24.7%–35.8% in the latter), whereas other congeners have 12 (in *P. tetrabelos* and *P. volitans*) and 14 (in *P. lancifer*) tail rings, normal fifth pectoral-fin ray (not thickened; in *P. lancifer* and *P. volitans*), the posterior 3 (in *P. tetrabelos* and *P. volitans*) and 7 (in *P. lancifer*) tail rings fused together and a slender carapace (21.3%–28.1% of SL in *P. lancifer*, 13.5%–18.1% of SL in *P. tetrabelos* and 12.8%–15.5% of SL in *P. volitans*). *Pegasus nanhaiensis* can be distinguished from *P. laternarius* by the above-mentioned diagnostic characters (the latter with a pointed, roughly triangular tubercle on each of dorsal plates I, II, and III; no hexagonal pattern on dorsal plates; three paired caudolateral plates on tail rings II and III, III and IV, and IV and V) (Palsson and Pietsch 1989; Osterhage et al. 2016; Zhang et al. 2020; this study). In addition, 16S rDNA and COI analyses put *P. nanhaiensis* into a different clade from *P. laternarius*, separated by a genetic distance of 3.51–3.53 percentage points (Zhang et al. 2020).

Pegasus nanhaiensis was previously known only from the type specimens from the northern South China Sea, off Yangjiang and Beihai, China (Zhang et al. 2020), the three specimens described herein representing the first records of *P. nanhaiensis* from the Gulf of Thailand. In addition, a single specimen (ZMUC P 842, 66.1 mm SL), reported as *P. laternarius* by Palsson and Pietsch (1989: 23, fig. 11) from Ko Kradat, eastern Gulf of Thailand and a single specimen (FRLM 55093, 46.1 mm SL), reported as *Spinipegasus laternarius* (Cuvier, 1829) by Hibino (2021: 14, unnumbered figs.) from off Bidong Island, east off the Malay Peninsula, South China Sea, were re-identified here as *P. nanhaiensis*, based on clear, distinctly bounded hexagonal patterns on the dorsal plates (d_{1-3}) and dorsolateral plates (dl_{1-4}) from their photographs, respectively. The Bidong specimen represents the southernmost record of the species (Fig. 4), suggesting that *P. nanhaiensis* is widely distributed in coastal waters of the South China Sea.

The coloration of *P. nanhaiensis* was previously known only from dried specimens (Zhang et al. 2020), the fresh color description of the species being provided here for the first time. Although the dorsal and lateral body surfaces were dark brown and the first four segments of the tail rings darker than the remaining tail rings in the dried specimens (Zhang et al. 2020), the dorsal surface was yellow to dark yellowish-brown and tail rings I–IV and the posterior half of VII and VIII brown (remaining rings yellowish-white) in the presently reported fresh specimens from Thailand.

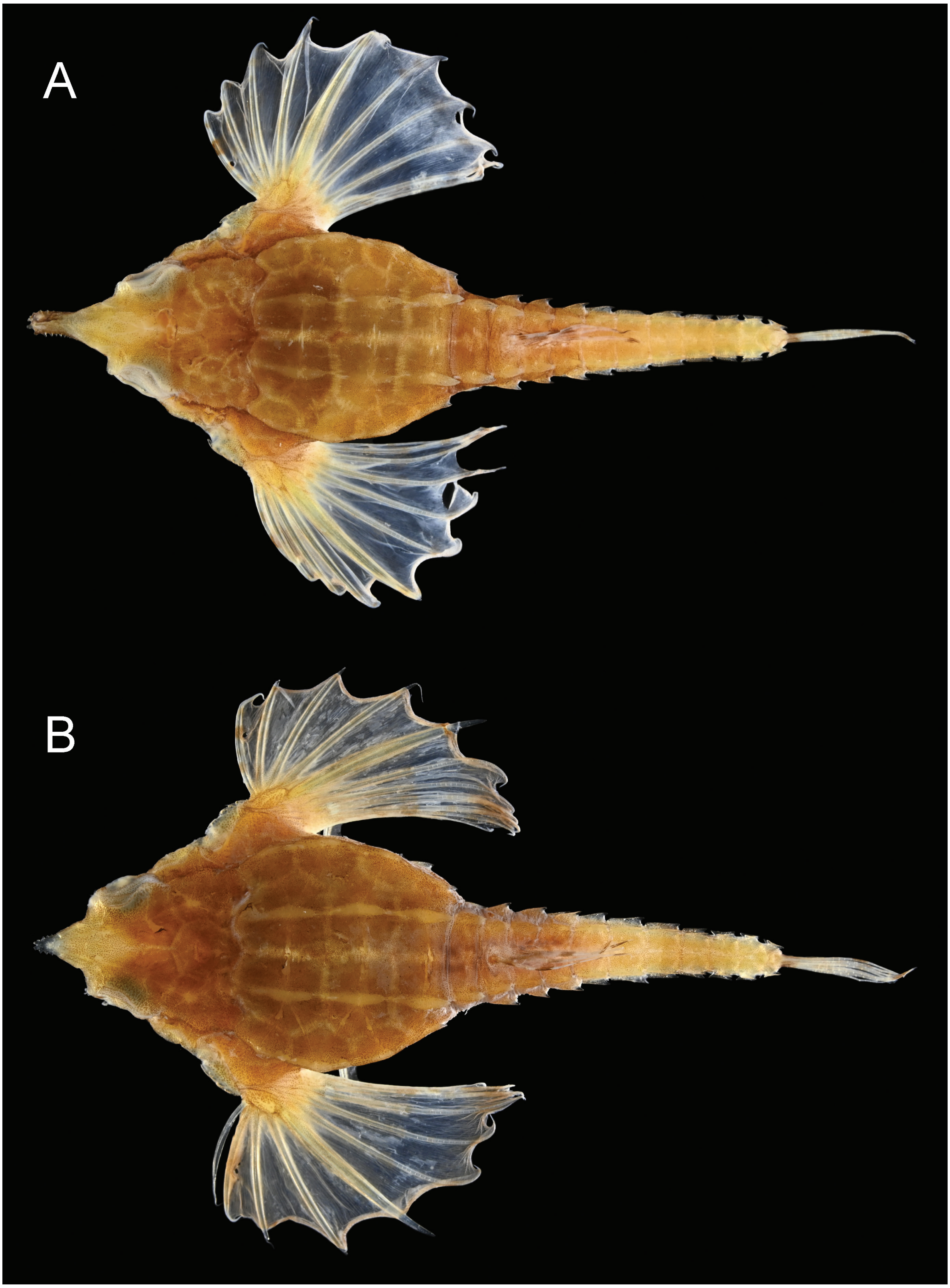

Figure 5. Dorsal views of preserved specimens of *Pegasus laternarius* from Japan. **A**: KAUM–I. 31094, male, 36.6 mm SL; **B**, KAUM–I. 17604, female, 37.0 mm SL.

The clear hexagonal patterns on the surface dorsal plate, found in fresh specimens of *P. nanhaiensis* (Fig. 1), was lost in preserved specimens (Fig. 2), which became indistinguishable from preserved *P. laternarius* on this basis (Figs. 2 and 5).

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Length–weight relations of 12 freshwater fish species (Actinopterygii: Cypriniformes) including two endangered species, *Cobitis choii* (Cobitidae) and *Gobiobotia naktongensis* (Cyprinidae), in the Geum River, South Korea

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Abstract

Length–weight relations (LWRs) of 12 freshwater fish species from the Geum River, South Korea were estimated. The following species representing the family Cobitidae, Xenocyprididae, Acheilognathidae, and Gobionidae were studied: *Cobitis choii* Kim et Son, 1984; *Opsariichthys uncirostris* (Temminck et Schlegel, 1846); *Zacco platypus* (Temminck et Schlegel, 1846); *Tanakia lanceolata* (Temminck et Schlegel, 1846); *Acheilognathus rhombeus* (Temminck et Schlegel, 1846); *Hemibarbus labeo* (Pallas, 1776); *Gobiobotia naktongensis* Mori, 1935; *Hemibarbus longirostris* (Regan, 1908); *Microphysogobio jeoni* Kim et Yang, 1999; *Pseudogobio esocinus* (Temminck et Schlegel, 1846); *Pseudorasbora parva* (Temminck et Schlegel, 1846); *Squalidus japonicus* (Sauvage, 1883). Parameter *b* ranged from 2.820 (*P. parva*) to 3.485 (*C. choii*), and parameter *a* ranged from 0.0015 (*C. choii*) to 0.0145 (*A. rhombeus*). The LWR for *C. choii* and *G. naktongensis*, endangered species in South Korea, was estimated for the first time. Our results could be useful as baseline information for evaluating population status.

Keywords

endangered species, fish stock management, Korean endemic species, LWRs, weight–length relations, WLRs

Introduction

The length–weight relations (LWRs) are derived from regression analysis using a paired dataset of length and weight of specific species that can be used to estimate the weight corresponding to a given length (Le Cren 1951), and parameters *a* and *b* of the LWRs are determined by the body shape and growth patterns of the species (Froese 2006). The LWR provides baseline information for stock management as indicators of stock status, including growth, sexual maturity, and food availability (Le Cren 1951; Al-Zibdah and Odat 2007; Karna et al. 2012). In addition, because the body shape and growth patterns of fish are related to their habitat status, including food availability and predation pressure (Brönmark and Miner 1992), LWRs are useful not only for fisheries research but also for ecological studies.

LWR estimations from various populations are required to estimate the relative weight index, which is a useful tool for comparing fish conditions across populations or species, unlike the condition factor and relative condition factor, which can only be used to compare conditions within a population (Froese 2006). FishBase currently provides LWR estimations for 6098 fish species, but LWR data are lacking for some species (Froese and Pauly 2021).

Cobitis choii Kim et Son, 1984 and *Gobiobotia naktongensis* Mori, 1935 are endemic Korean species with restricted distribution because they only inhabit streams with wide and clear sand streambeds with moderate water flow (Ko et al. 2012; Kim et al. 2014). *Cobitis choii* and *G. naktongensis* have both been identified as Class I endangered species by the Korean Ministry of Environment (NIBR 2018) because of their restricted distribution and small population size.

In the presently reported study, we provide LWR data for 12 freshwater fish species, including *C. choii* and *G. naktongensis*, which have not been previously reported.

Material and methods

The following species representing the families Cobitidae, Xenocyprididae, Acheilognathidae, and Gobionidae were studied: *Cobitis choii*; *Opsariichthys uncirostris* (Temminck et Schlegel, 1846); *Zacco platypus* (Temminck et Schlegel, 1846); *Tanakia lanceolata* (Temminck et Schlegel, 1846); *Acheilognathus rhombeus* (Temminck et Schlegel, 1846); *Hemibarbus labeo* (Pallas, 1776); *Gobiobotia naktongensis*; *Hemibarbus longirostris* (Regan, 1908); *Microphysogobio jeoni* Kim et Yang, 1999; *Pseudogobio esocinus* (Temminck et Schlegel, 1846); *Pseudorasbora parva* (Temminck et Schlegel, 1846); *Squalidus japonicus* (Sauvage, 1883).

The fishes were collected from the Geum River (36°27′14.89″N, 127°5′37.70″E) using a cast net (mesh 7 mm) and a kick net (mesh 4 mm) from March to October 2021. The total length (L) [cm] and weight (W, wet weight) [g] were measured immediately at the capture site. The fishes were examined after being anesthetized using 0.1 g · L^{-1} ethyl 3-aminobenzoate methanesulfonate salt (Sigma-Aldrich, Munich, Germany). The total length was measured using a digital caliper to the nearest 0.1 cm. The weight of *G. naktongensis* and *C. choii* was determined using a digital balance to the nearest 0.01 g, while other fishes were weighed to the nearest 0.1 g. After the examination and recovery, the fishes were released from the recovery tank (100 × 100 × 80 cm).

The LWR for each species was estimated using the regression equation

$$W = aL^b$$

where a and b are parameters of the equation (Le Cren 1951; Ricker 1973; Froese 2006). Before the regression analysis, outliers were removed by linear regression of the log-transformed equation (Froese 2006). Scientific names for all species and family assignments were based on Eschmeyer's Catalog of Fishes (Fricke et al. 2021).

Results

The LWRs of the 12 species were estimated, and the results are presented in Table 1. A total of 938 individuals were examined, and the sample size, ranges of total length, and weight of each species are presented. The total length ranges for 11 species were wide enough to include juveniles to adults, but *Hemibarbus labeo* only

Table 1. Summary of length-weight relations for 12 freshwater fish species in the Geum River, South Korea.

Species	***N***	**Total length [cm]**	**Weight [g]**	**Regression parameters**					
				a	**95% CL of a**	***B***	**95% CL of *b***	***r*²**	**BE of *b***
Cobitis choii	29	3.5–8.5	0.13–2.66	0.0015	0.0008–0.0029	3.485	3.139–3.831	0.938	3.05 (2.87–3.23)
Opsariichthys uncirostris	126	5.9–23.5	1.0–93.6	0.0035	0.0029–0.0042	3.231	3.154–3.309	0.982	3.08 (3.03–3.13)
Zacco platypus	91	4.8–14.8	0.9–29.0	0.0063	0.0052–0.0075	3.105	3.019–3.190	0.983	3.09 (3.05–3.13)
Tanakia lanceolata	90	5.8–11.2	1.9–16.6	0.0070	0.0051–0.0096	3.214	3.059–3.368	0.950	3.05 (2.92–3.18)
Acheilognathus rhombeus	31	6.9–9.6	4.2–11.7	0.0145	0.0107–0.0196	2.961	2.815–3.108	0.983	3.12 (2.98–3.26)
Hemibarbus labeo	165	6.6–20.0	2.1–53.6	0.0061	0.0051–0.0072	3.044	2.970–3.117	0.976	3.10 (3.05–3.15)
Gobiobotia naktongensis	94	2.4–6.0	0.08–1.30	0.0050	0.0044–0.0056	3.054	2.967–3.142	0.981	3.13 (2.96–3.30)
Hemibarbus longirostris	37	7.5–14.8	3.2–22.4	0.0065	0.0047–0.0092	3.021	2.873–3.169	0.979	3.15 (3.03–3.27)
Microphysogobio jeoni	45	4.7–9.5	0.5–5.4	0.0028	0.0019–0.0042	3.344	3.133–3.556	0.959	3.17 (3.02–3.32)
Pseudogobio esocinus	114	3.3–17.8	0.2–39.7	0.0042	0.0036–0.0050	3.153	3.078–3.228	0.984	3.12 (3.07–3.17)
Pseudorasbora parva	39	2.7–9.3	0.2–6.3	0.0113	0.0098–0.0130	2.820	2.745–2.895	0.993	3.12 (3.07–3.17)
Squalidus japonicus	77	6.1–11.3	1.9–14.9	0.0064	0.0044–0.0093	3.166	2.978–3.354	0.937	3.16 (3.03–3.29)

N = number of specimens studied; BE of b = Bayesian estimates of b (Froese et al. 2014); Bold font indicates South Korean endangered species; Weight was measured to the nearest 0.01 g; Text in shaded cells marks the species where only juveniles were included in the study.

included juveniles. Parameters *a* and *b* are presented with 95% confidence limits. The coefficient of determination (r^2) for all species was above 0.95, except for those of *C. choii* and *S. japonicus*. Parameter *b* for all species was within the expected range of 2.5–3.5 according to Froese (2006). Parameter *b* of *C. choii* was 3.485, which was the highest among the 12 species, and the other species showed small deviations from 3.0 for *b*. Parameter *a* for *C. choii*, the only species in this study representing the family Cobitidae, was 0.0015, which was the lowest observed, and *a* of *A. rhombeus* was 0.0145, which was the highest.

Discussion

Parameter *b* for *C. choii* was 3.485, which was high compared with that of other species, and such a high value of *b* could be caused by various reasons. One possibility is the narrow size range (Froese 2006). However, in the presently reported study, the total length range of *C. choii* was 3.5–8.5 cm, which was wide enough to cover juveniles through to fully grown adults. The second possibility was the small sample size, which contained a bias for large specimens. For example, large specimens could temporarily become heavier than usual because of the development of eggs or gonads during the spawning season or because large specimens could be in a much better nutritional condition than juveniles. The sample of *C. choii* in this study contained only 29 individuals, which is quite small, and could be the reason for the high value of *b*. The final possibility is that the fish changed their body shape as they grew. Several studies have reported positive allometric growth of *Cobitis* fish (Boroń et al. 2008; Patimar et al. 2011). In particular, *Cobitis keyvani* Mousavi-Sabet, Yerli, Vatandoust, Özeren et Moradkhani, 2012 from the Sefid-rud River of Iran showed a *b* value of 3.411 (Mousavi-Sabet et al. 2016), which is similar to our result. Nevertheless, more detailed investigations are needed to verify this result, and it seems reasonable to refer to our results as a case study from a specific fish population.

The LWR of *G. naktongensis* seemed to be reliable because the data used for the estimation satisfied the majority of the conditions proposed by Froese et al. (2011). The sample included approximately 100 specimens, which was adequate, and the length range was wide enough to cover juveniles to adults. The coefficient of determination (r^2) was higher than 0.950, indicating that the outliers were sufficiently removed. Parameter *b* was slightly higher than 3.0, which is common considering the tendency of the majority of fishes to increase in thickness as they grow (Froese 2006).

Parameter *a* is related to the body shape of fish (Froese 2006). In the presently reported study, the *a* of *C. choii* was the lowest and that of *A. rhombeus* was the highest. *Cobitis choii* was the only species representing the family Cobitidae, which consists of loaches that have an elongated body shape. *Acheilognathus rhombeus* is a representative of the family Acheilognathidae and has a relatively short and deep body shape compared to the other fish examined.

In this study, the LWRs of *C. choii* and *G. naktongensis* were estimated, which are not currently available in FishBase (Froese and Pauly 2021). We expect that the results from this study will be useful as baseline information for evaluating the population status of these species in South Korea.

Author contribution

Conceptualization, JDY; methodology, SHB; software, SHB; validation, SHP, JHK, JHY, and JSM; formal analysis, JHK and DHK; investigation, SHP and JHK; resources, SHK, JHK and DHK; data curation, SHB and DHK; writing—original draft preparation, SHB and JDY; writing—review and editing, JHY; supervision, JDY; project administration, JHY and JSM; funding acquisition, JSM. All authors have read and accepted the final version of the manuscript.

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New records of recently described *Zebrus pallaoroi* (Actinopterygii: Gobiiformes: Gobiidae) with notes on its morphology, ecology, and molecular identification

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Abstract

The family Gobiidae represents the species-richest family of fishes in the Mediterranean Sea. However, many Mediterranean gobiid species are poorly known with just one or few species records in total. Among those species, the recently described *Zebrus pallaoroi* Kovačić, Šanda et Vukić, 2021 has been known only from the localities in the species description: the southern Adriatic, northern Ionian, and northern and western Aegean seas. The additional specimens of this species were collected in central and northern Adriatic Sea. The identification was confirmed by morphological and genetic methods. The morphological and preserved coloration characters were compared with the data from the limited type material and the alternative preserved coloration diagnostic character was suggested. The first data on alive and freshly dead coloration are reported. The phylogenetic analysis was performed on sequenced *COI*, cytochrome b, and rhodopsin genes. The analyzed molecular markers placed *Z. pallaoroi* in a distinct clade within *Zebrus*–*Millerigobius* group. The preference of this species for the very shallow habitat is confirmed and discussed.

Keywords

Adriatic Sea, *COI* gene, habitat, morphology, new records, *Zebrus pallaoroi*

Introduction

The family Gobiidae, with 48 species, represents the species richest family of fishes in the Adriatic Sea (Dulčić and Kovačić 2020; Kovačić et al. 2021). Gobies are also the species' richest family of fishes in the Mediterranean (Kovačić 2020). The known diversity and taxonomy of Mediterranean gobiid species was consolidated in the second half of the 20th century by a series of descriptions and redescriptions of species and genera and by a few review works and books (Kovačić 2020). However, since then a number of new species have been described for the area (Kovačić 2020). Some of these gobies still remain poorly known with only one to a few records in total (Patzner 2021). For those rare, or at least elusive species, every new record provides an increase of the limited knowledge on species geography, ecology, morphology, coloration, etc. The gobiid genus *Zebrus* de Buen, 1930 has been considered monotypic for nearly half of the century. The subgenus *Zebrus* de Buen, 1930, containing two species since establishing, was elevated to the rank of the genus by Miller (1966). *Gobius thori* de Buen, 1928, was synonymized later with *Thorogobius ephippiatus* (Lowe, 1839) (see Miller 1973). Miller (1977) provided the redescriptions of *Zebrus* as a monotypic genus and of its only species, *Zebrus zebrus* (Risso, 1827). Recently, Kovačić et al. (2021) described the second species of this genus, *Zebrus pallaoroi* Kovačić, Šanda et Vukić, 2021 based on the material from the southern Adriatic, northern Ionian, and northern and western Aegean seas, proving morphological and genetic differences between this cryptic species and *Z. zebrus*. *Zebrus pallaoroi*, so far known only from the localities mentioned in the original species description, has recently been found on two distant localities in the central and northern Adriatic.

The aim of the presently reported study was to reveal the additional records of recently described *Z. pallaoroi*, expanding its known range, with notes on its morphology, ecology, and molecular identification.

Materials and methods

Localities and sampling. The specimens were collected off the city of Split, the central Adriatic Sea, and off Cape Lipica, Oštro, the northern Adriatic Sea (Fig. 1). In order to collect specimens from Split, a 2.5% quinaldine and seawater solution was used to anesthetize specimens which were subsequently collected by the handnet. Both specimens were found by surveying a shallow and narrow coastal area during low tide in the vicinity of the Institute of Oceanography and Fisheries in Split, Croatia. Cryptic spaces below boulders and pebbles were visually examined in very shallow water. Specimens at Oštro were collected by the application of a rotenone solution in the intertidal bedrock hole. All collected fish specimens were killed by an overdose of quinaldine. The fin clips for DNA analysis were removed and specimens stored in 70% ethanol solution. The tissue samples for DNA analysis were preserved in 96% ethanol.

Material. Material collected (standard length + caudal fin length): 1 male, 26.66 mm, caudal fin damaged, PMR VP5043 (Fig. 2A) and 1 female, 21.65 + 5.56 mm, PMR VP5093, both Cape Lipica, Oštro, the northern Adriatic Sea, Croatia, 45°15′54″N, 14°33′46″E, 28 July 2021, collected by M. Kovačić; 1 male, 38.47 + 9.20 mm, PMR VP5094 (Fig. 2B) and 1 specimen of undetermined sex, 28.01 + 5.88, IOR-Zpallaoroi12021, collected on 29 March 2021, both from inside the port of the Institute of Oceanography and Fishery, Split, the Central Adriatic Sea, Croatia, 43°30′28″N, 16°23′19″E, 26 April 2021, collected by M. Pavičić and B. Dragičević.

Ecology and geographic distribution. Both individuals collected in Split were found below boulders of approx-

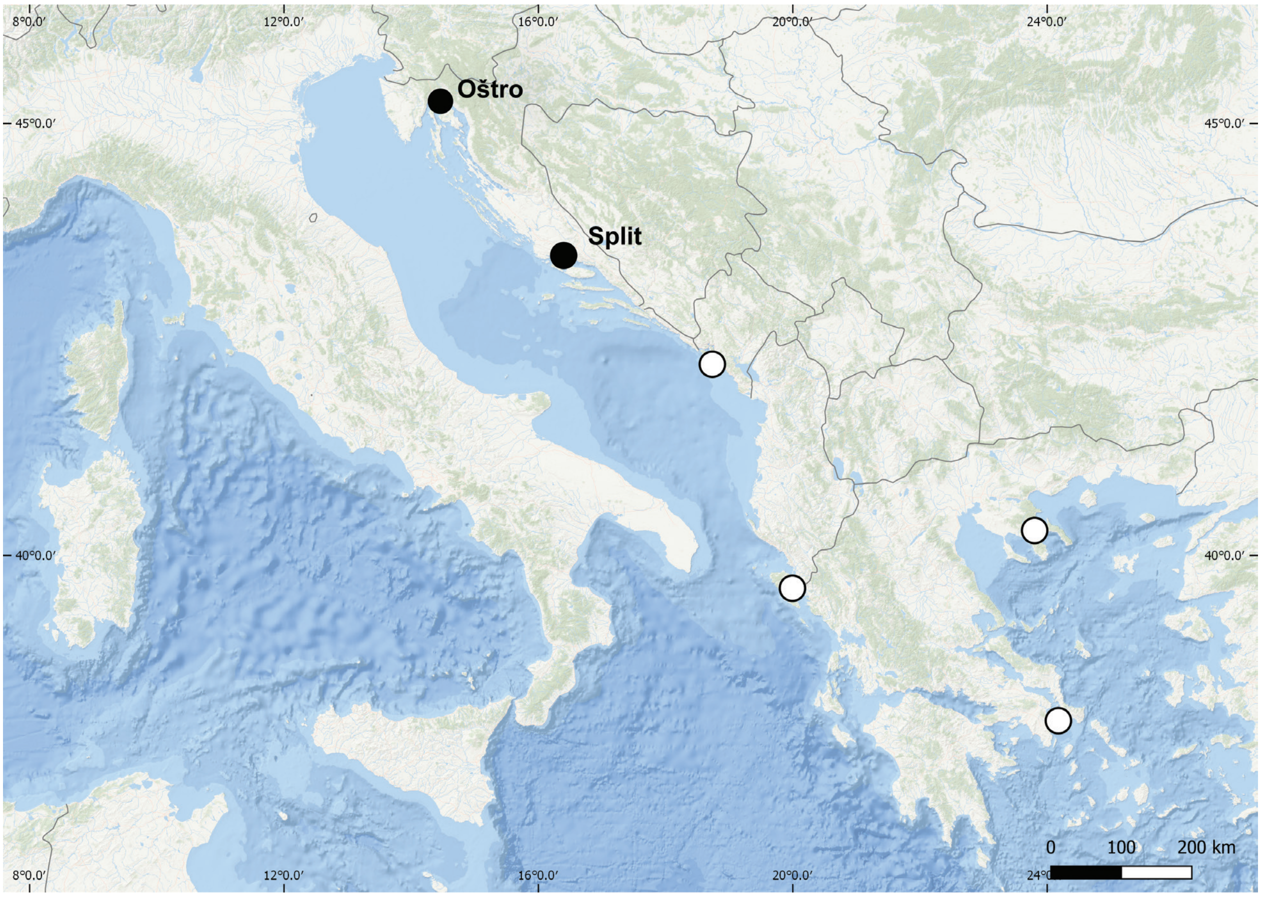

Figure 1. *Zebrus pallaoroi* present records (●) and the localities from Kovačić et al. (2021) (○). Map source: Esri Ocean Basemap by Esri, Garmin, GEBCO, NOAA NGDC, and other contributors.

Figure 2. *Zebrus pallaoroi*: (**A**) male, 26.66 mm, caudal fin damaged, PMR VP5043, Cape Lipica, Oštro, the northern Adriatic Sea, Croatia; (**B**) male, 38.47 + 9.20 mm, PMR VP5094, the port of the Institute of Oceanography and Fishery, Split, the Central Adriatic Sea, Croatia. Photos by M. Kovačić.

imately 20 cm diameter, which were sparsely overgrown by photophilic algae at a small beach consisting of boulders and cobblestone on top of a layer of medium and coarse gravel, at depths 0.0–0.2 m at low tide. During the search, several other individuals presumably of the same species have been observed but not collected. The beach was sheltered, situated inside the port (Fig. 3A). There have been no artificial alterations of the beach in terms of beach replenishment at least since the construction of the Institute in 1930. The two individuals from Oštro were found at the rocky cape in the bedrock hole with a shallow surface connection to the sea (Fig. 3B). The bedrock walls of the hole contained crevices and its bottom was covered in boulders. The hole was of about 0.5 m diameter and of visible depth 0.3 m to the bottom boulders at low tide, but the real thickness of bottom bolder layers is unknown. After the application of a rotenone solution in the hole, in addition to *Z. pallaoroi*, *Microlipophrys adriaticus* (Steindachner et Kolombatović, 1883) and *Parablennius sanguinolentus* (Pallas, 1814) were also found.

Morphological methods. The morphological data is a combination of characters that positively identify genus *Zebrus* and species *Z. pallaoroi* among family Gobiidae in the CLOFNAM area (Kovačić 2020; Kovačić et al. 2021). The terminology and the format style of head canal pores and head rows of sensory papillae followed Sanzo (1911) and Miller (1986). Morphometric and meristic methods followed methodology in Kovačić et al. (2021). The material was deposited in the Prirodoslovni muzej Rijeka (PMR) and Institute of oceanography and fisheries in Split (IOR).

Genetic methods. Molecular analysis was conducted on specimens PMR VP5043 and PMR VP5093 from northern Adriatic and specimen IOR-Zpallaoroi12021 from Central Adriatic. Total genomic DNA was isolated from fin clips preserved in 96% ethanol by proteinase K digestion, followed by a simplified DNA isolation procedure (Laird et al. 1991). After checking DNA quality and quantity by spectrophotometry (IMPLEN N50, Germany), DNA fragments were amplified using HotStarTaq DNA Polymerase kit (Qiagen, Germany) by PCR (Eppendorf Mastercycler Nexus Gx2 thermal cycler) with different primer combinations. A partial fragment of mitochondrial cytochrome c oxidase subunit I (*COI*) was amplified with primers FishF1 and FishR1 according to Ward et al. (2005). Primers reported by Kovačić et al. (2021) were used for the amplification of mt cytochrome b (ZzebF1; ZzebR1) and rhodopsin (RHSQF1; RHSQR1). PCR cycling conditions were as follows: an initial denaturing step at 94°C for 1 min; 35 cycles of denaturing at 94°C for 15 s, annealing at 50°C for *cyt b* (60°C for *rho*) for 15 s, and extending at 72°C for 10 s; and a final extending step of 72°C for 10 min.

Product purification and sequencing were performed by Macrogen Inc. (Amsterdam, the Netherlands) on an ABI 3730 automated sequencer. Sequencing was done

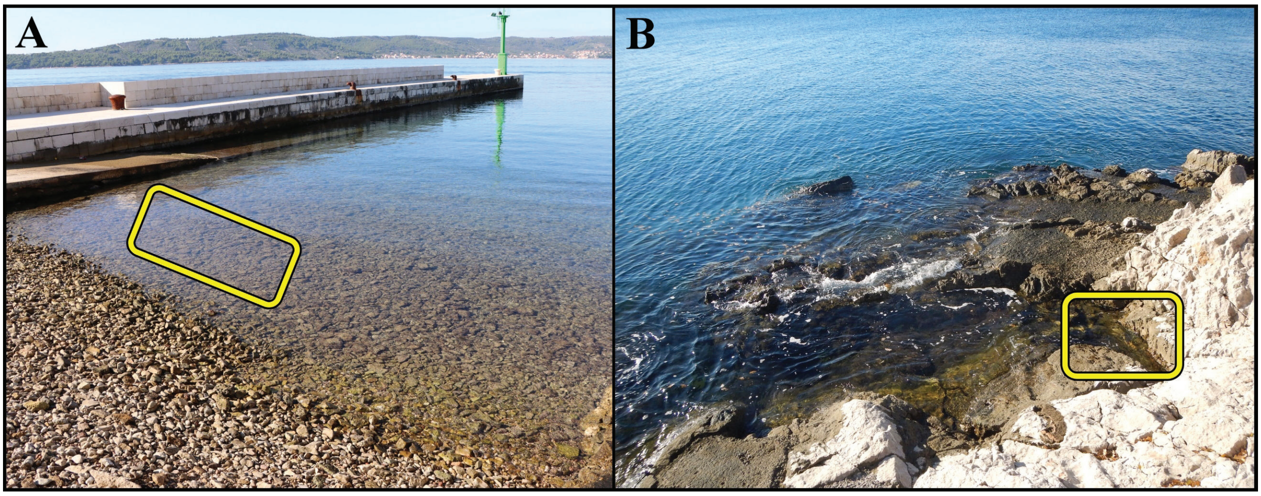

Figure 3. Habitats of collected *Zebrus pallaoroi* specimens: (**A**) Split, (**B**) Oštro. Exact positions where specimens were collected are marked with yellow frame.

from both directions. Chromas Pro 1.5 software (http://www.technelysium.com.au) was used to evaluate the quality of the chromatogram. BLASTn (NCBI, available online) was used for sequence identification. In addition, sequences of closely related species from GenBank and the Barcode of Life Database (BOLD; www.boldsystems.org) were used for phylogenetic analyses. Sequence alignment was run by the ClustalW tool in MEGA X software (Kumar et al. 2018). The best-fit substitution models in jModelTest (Posada 2008) according to the AIC criterion suggested K2, K2 + I and HKY + G + I for *rho*, *cyt b*, and *COI*, respectively. Therefore, Bayesian phylogenetic analysis was performed using the HKY model in Mr.Bayes 3.2.7a (Ronquist et al. 2012). Two million generations in two independent runs and 25% of sampled trees were cut as burn-in for the final tree construction. *Cheilodipterus quinquelineatus* Cuvier, 1828 for *COI*, *Knipowitschia caucasica* (Berg, 1916) for *cyt b*, and *Knipowitschia milleri* (Ahnelt et Bianco, 1990) for *rho* were species used as outgroups. The topologies of the trees were visualized in FigTree 1.4.2 (Rambaut and Drummond 2015). The obtained sequences were deposited in GenBank under the accession number (OM893816–OM893824).

Results

Morphological identification. Morphology was examined on specimens PMR VP5043 and PMR VP5093 from northern Adriatic and specimen PMR VP5094 from central Adriatic. The specimens were identified as *Zebrus* species by having (1) suborbital papillae of the lateral-line system without longitudinal row *a*; (2) all three head canals present; (3) predorsal area naked; (3) seven transverse suborbital rows; (4) two suborbital transverse rows below row *b*; (5) interorbital papillae absent.

The presently reported material matches *Z. pallaoroi* diagnosis and differs from *Z. zebrus* by all diagnostic morphological characters listed in Kovačić et al. (2021): snout longer than eye, its length 1.1–1.2 of eye diameter (same range as in Kovačić et al. 2021) vs. snout shorter than eye (Miller 1977), its length 0.8–0.9 of eye diameter in *Z. zebrus* (see Kovačić et al. 2021); posterior nostril short tube, height 0.9 of anterior nostril height in PMR VP5043 and PMR VP5093, but 0.7 of anterior nostril in PMR VP5094, extending the range 4/5–9/10 for *Z. pallaoroi* from Kovačić et al. (2021), still different from values for *Z. zebrus*: posterior nostril about 1/2 of anterior nostril (Miller 1977) or 1/4–2/5 of anterior nostril (Kovačić et al. 2021); eyes moderately small, eye diameter is 4.2–4.7 in head length slightly extending the range 4.3–4.7 for *Z. pallaoroi* from Kovačić et al. (2021), but still different from values for *Z. zebrus*: 3.4–4.1 in head length (Miller 1977), 3.1–4.1 in head length (Kovačić et al. 2021) in *Z. zebrus*; left and right ventrolateral head ridges transversally connected on anterior part by short transversal ridge vs. left and right ventrolateral head ridges disconnected anteriorly by midventral flat area in *Z. zebrus* (see Kovačić et al. 2021); anterior membrane in midline depth 0.6–0.7 of spinous ray (about 2/3 in Kovačić et al. 2021) vs. about 1/2 of spinous ray (Miller 1977) and 1/4–1/2 (Kovačić et al. 2021) in *Z. zebrus*; head canal pores large, pore α diameter 0.5 of the distance between pore ρ and ρ^1 (about half in Kovačić et al. 2021) vs. head canal pores of moderate size, distance between pore ρ and ρ^1 about three times or more longer than pore α diameter (Kovačić et al. 2021) in *Z. zebrus*; suborbital sensory papillae row *5i* going downwards to or near the level of row *d*, distance between row *5i* and row *d* much smaller than length of row *5i* (absent or much smaller in Kovačić et al. 2021) vs. suborbital sensory papillae row *5i* ends downwards distant from row *d*, row *5i* length equal or smaller than distance between row *5i* and row *d* in *Z. zebrus* (see Kovačić et al. 2021).

The diagnostic coloration character of preserved specimens in species description (Kovačić et al. 2021): body with 10 to 11 vertical dark brown bands present along lateral side, first in front of the first dorsal fin, last at end of the second dorsal fin, at upper edge about equal or narrower than pale interspaces in between vs. six to nine dark vertical bands at upper edge, broader or equal than pale interspaces in *Z. zebrus* was of variable occurrence in the presently reported material due to blurred lateral coloration pattern on posterior part. However, preserved specimens of *Z. pallaoroi* on all three morphologically checked preserved specimens had four dark lateral bars about equal or narrower than pale interspaces anteriorly to the second dorsal fin origin vs. three, or just two, dark lateral bars anteriorly to the second dorsal fin origin, broader or equal than pale interspaces, in *Z. zebrus*.

Coloration of alive under stress and freshly dead specimen. The coloration of the specimen from Split (IOR-Zpallaoroi12021) observed in situ was dark brown, almost blackish, with barely visible vertical bars on the flanks, slightly paler in comparison to the surrounding coloration and conspicuous curved pale band on the head. Very soon after capture, darker coloration subsided and the reticulated brownish and yellowish pattern became visible dorsally appearing as vertical bars laterally; conspicuous pale band visible on the head extended from pectoral fin bases and also partly from the base of the fin rays, over upper portions of operculum and connected at the coronal head section above the eyes.

Genetics. The nucleotide sequences of the *cyt b* gene and the *rho* were obtained to ensure species identification. Namely, *cyt b* (1141 bp) and *rho* (748 bp) fragments of the study specimens produced significant alignments with sequences of *Z. pallaoroi* from the GenBank with 99% and 98% similarity, respectively. Phylogenetic reconstruction inferred by *cyt b* gene and *rho* clearly aggregated our specimens with sequences of *Z. pallaoroi* in a separate clade, according to other sequences from the family Gobiidae (Fig. 4). Moreover, phylogenetic reconstruction of the 642 bp long *COI* fragment revealed a well-supported tree topology in which the studied species *Z. pallaoroi* from the Adriatic share the same haplotype and form a sister clade with *Z. zebrus* (Fig. 5). The number of base differences per site between the studied sequences and *Z. zebrus* was 0.17, with an overall mean distance of 0.19 between the 46 nucleotide sequences involved.

Discussion

The morphology of collected individuals matches Kovačić et al. (2021), except for the expanded range of posterior nostril height to 7/10–9/10 of anterior nostril height and eye diameter to 4.2–4.7 in head length. It could be expected that with the future increased sampling of *Z. pallaoroi* some diagnostic characters could overlap with the range of *Z. zebrus* changing or reducing the species diagnosis. There is always a risk that species descriptions based on a small sample may not encompass the entire variability of species qualitative character states or the entire range of quantitative characters. The majority of the recent Mediterranean Gobiidae species descriptions were not based on large series of specimens, and also many were collected at circalittoral or bathyal depths and were missing alive coloration data (the recent species description reviewed in Kovačić (2020)). However, in order to augment our ichthyological knowledge, and establish conservation needs, waiting sometimes for decades for new species descriptions for large samples, is simply not an acceptable alternative. *Didogobius bentuvii* Miller, 1966 after more than a half century, is still known only from the holotype, with a number of unknowns for

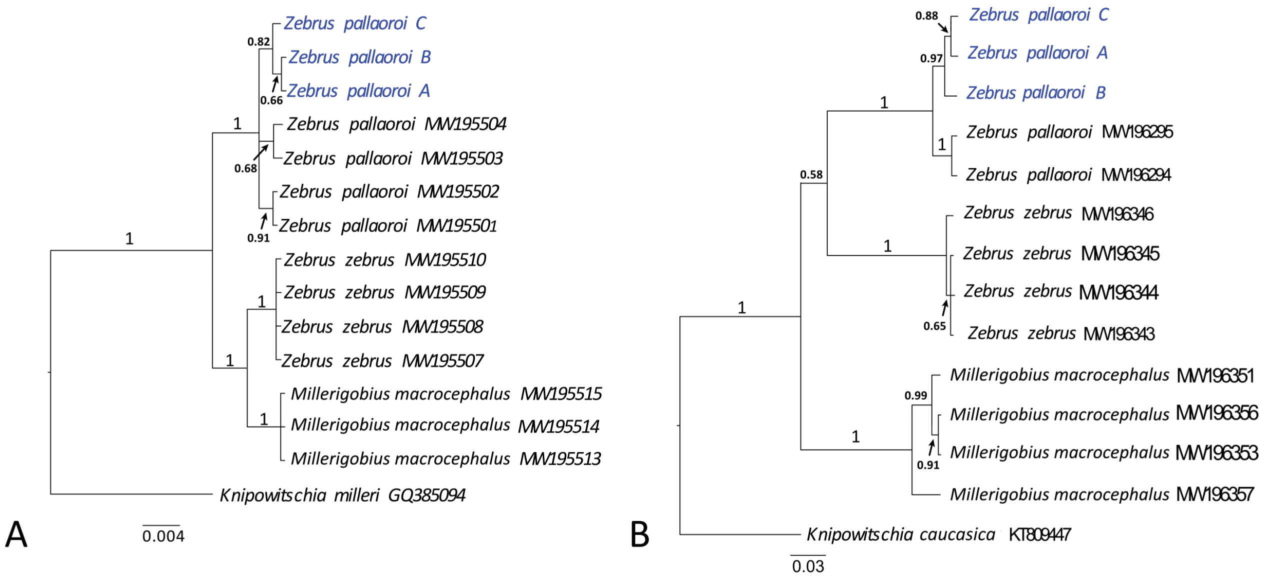

Figure 4. Bayesian phylogenetic tree for the taxa of the gobiid genus *Zebrus* and *Millerigobius*, reconstructed from nuclear rhodopsin DNA sequences (**A**) and mitochondrial cytochrome b (**B**). Numbers by nodes refer to posterior probabilities. Sequences obtained in this study are labelled with A (IOR-Zpallaoroi12021), B (PMR VP5043), and C (PMR VP5093), while sequences from GenBank are labelled according to accession number. Distance scale provided.

this species, including the live coloration (Miller 1966). In preserved coloration we suggest replacing the total count of lateral bands as the coloration character for identification of preserved specimens of *Z. pallaoroi* with the anterior count of lateral bands since the coloration pattern can be blurred backwards on lateral side and the bars are not always clearly visible posteriorly: *Z. pallaoroi* possesses four dark lateral bars about equal or narrower than pale interspaces anteriorly to the second dorsal fin origin vs. three, or just two, dark lateral bars anteriorly to the second dorsal fin origin, broader or equal than pale interspaces in *Z. zebrus*. The first data on the alive and freshly dead coloration of *Z. pallaoroi* are reported here. However, it should be noted that coloration of stressed individuals in their natural habitat and freshly dead coloration, as well as the coloration of live specimens kept in artificial containers on land, can be altered from the coloration of live, unstressed individuals in their natural habitat. The details of the alive coloration of *Z. pallaoroi* still have to be observed and described (Kovačić et al. 2021).

It is well known that the phylogenetic reconstruction of gobiid genera is complex. In this study, the molecular analysis corroborated the results presented by Kovačić et al. (2021) and successfully confirmed the identification of the specimens as *Z. pallaoroi*. Namely, all analyzed molecular markers successfully placed *Z. pallaoroi* in a distinct clade within *Zebrus–Millerigobius* group. The clade of the genus *Zebrus* is closely related to that of *Millerigobius*, confirming the interspecific relations observed in the *cyt b* and *rho* topologies (Fig. 5). At the time of writing, there was no *COI* sequence in public databases that refers to the species *Z. pallaoroi*. Phylogenetic reconstruction of *COI* fragments of *Z. pallaoroi* from Adriatic revealed that it forms a sister clade with *Z. zebrus*. Molecular analyses have well-distinguished these two species within the clade. In the future, it would be interesting to analyze the level of genetic differentiation of this species from various geographical areas. Therefore, we encourage additional research effort which could bring to light more records and possibly extend its known distribution further. The present study enabled the first *COI* sequence deposition of *Z. pallaoroi* in the GenBank.

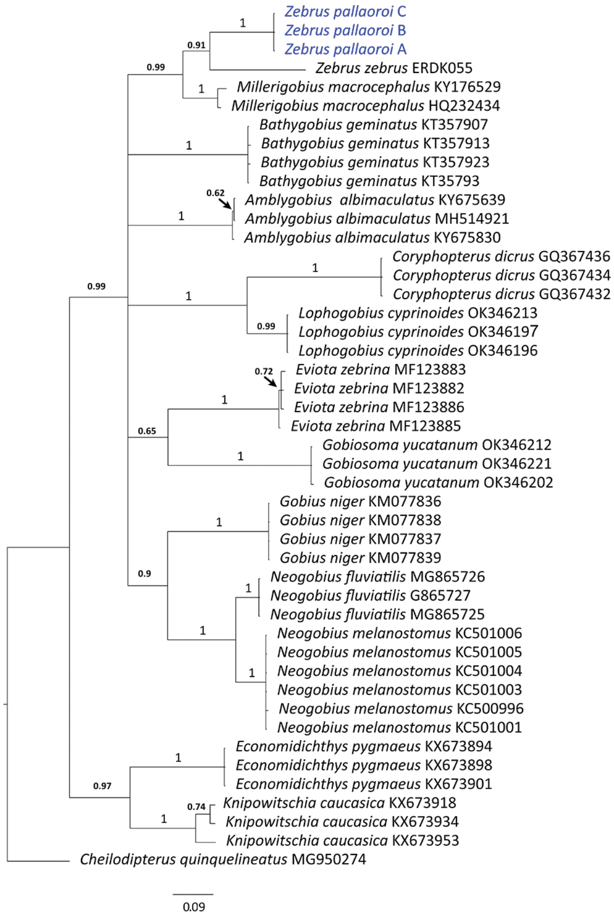

Figure 5. Bayesian phylogenetic tree for the several gobiid genus estimated by partial mitochondrial cytochrome c oxidase subunit I (*COI*). Sequences obtained in this study are labelled A (IOR-Zpallaoroi12021), B (PMR VP5043), and C (PMR VP5093), while Sequences from Barcode of Life Database (BOLD; www.boldsystems.org) database are labelled according to accession numbers. Numbers by nodes refer to posterior probabilities with distance scale provided.

The findings reported herein extend the known distribution of recently described *Z. pallaoroi* to the central and northern sections of the Adriatic Sea. This extension was the result of an increased research effort driven by the recent recognition of this cryptic species. The two presently reported collection sites were quite sheltered, which was not emphasized in the original description while the then noted preference for the very shallow water is confirmed (Kovačić et al. 2021). In the Adriatic intertidal zone, contrary to oceans and many seas, the small tide and mostly soft sediment or steep bedrock bottoms in the tidal zones rarely shape mediolittoral basins during low tide, so, unlike the coasts with large tides, only a few Adriatic fish species are present in the mediolittoral zone by surviving limited time above the water surface on an exposed rock or under the gravel or boulders. That has been known only for two amphibious blennies on rocks and for *Gouania* clingfishes living in the intertidal gravel (Wagner et al. 2021). The habitat of *Z. pallaoroi* collected in Split indicates that *Z. pallaoroi*, with some other fishes, also occurring in shallow infralittoral of 0–1 m depth inside the boulders or cobbles fields (e.g., *Chromogobius quadrivittatus* (Steindachner, 1863) and *Lepadogaster lepadogaster* (Bonnaterre, 1788) (see Patzner 1999, 2021)), could have its upper depth limit expanded to the mediolittoral zone and perhaps could be able to survive diurnal dried periods above water level at low tide. The preference of this species for shallow habitats is also important since those habitats are under heavy anthropogenic impact. Indeed, shallow habitats are under increased threat in Croatia mainly as a consequence of various habitat alterations at the coastline by embankments, beach nourishment, and marine infrastructure construction along the coast in recent decades (Matić-Skoko et al. 2020). About two-thirds of the entire Mediterranean coastline is urbanized, and more than half of the Mediterranean coasts are dominated by concrete (Airoldi and Beck 2007); data are not known for the Adriatic but probably are not better. In that sense, knowledge on the distribution of this and other cryptic species with a preference for shallow habitats is essential for the appropriate assessment of species conservation status and successful conservation actions.

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First records of the two gobies, *Cryptocentrus shigensis* and *Priolepis profunda* (Actinopterygii: Gobiiformes: Gobiidae), from the Andaman Sea

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Abstract

Single specimens of *Cryptocentrus shigensis* Kuroda, 1956 (41.1 mm standard length: SL) and *Priolepis profunda* (Weber, 1909) (24.4 mm SL) represent the first specimen-supported records of those species from the Andaman Sea. Notably, the specimen of *C. shigensis* represents the first Indian Ocean record, the species having been previously recorded only from southern Japan and Palau. Full descriptions of the specimens are provided.

Keywords

new records, description, distribution, R/V *Dr. Fridtjof Nansen*, trawl surveys

Introduction

Surveys at sea, carried out by the Norwegian R/V *Dr. Fridtjof Nansen*, are an important and integral part of the EAF-Nansen Programme (FAO) activities and Science Plan supporting the Programme's overall objective of promoting sustainable fisheries to improve food and nutrition security for partner countries. In 2013, 2015, and 2018, the Programme (in cooperation with the Myanmar Government) carried out three ecosystem surveys and one habitat survey off the coast of Myanmar to obtain biological and environmental information and identify species diversity as a basis for an FAO marine species identification guide intended for fishery purposes (see Psomadakis et al. 2019). Many unidentified gobiid specimens were collected during the 2018 survey from the Andaman Sea (northeastern Indian Ocean) that could not be examined in time to be included in the guide. Two of them, identified here as *Cryptocentrus shigensis* Kuroda, 1956 and *Priolepis profunda* (Weber, 1909) (all known primarily from the western Pacific Ocean), had not been previously recorded from the Andaman Sea. Detailed descriptions of the specimens are provided herein.

Methods

Counts and measurements generally followed Shibukawa et al. (2005), with the following descriptive modifications: longitudinal scale rows ("longitudinal scales" of

Shibukawa et al. 2005); anterior transverse scale rows (transverse scales from anal-fin origin upwards and forward to base of first dorsal fin); posterior transverse scale rows (transverse scales from anal-fin origin upwards and backwards to base of second dorsal fin); transverse scale rows from D2 (transverse scales from origin of second dorsal fin downwards and backwards to anal-fin base); and predorsal scale rows (predorsal scales). Measurements were made to the nearest 0.01 mm, except for standard length (abbreviated as SL), which was measured to the nearest 0.1 mm. Cephalic sensory canal pores and papillae were observed using versatile staining with cyanine blue (Saruwatari et al. 1997), their terminologies following Akihito (1984). Photographs of preserved specimens were taken with a Nikon D850 camera using an internal focus bracketing function; sets of multifocal images were then collated into an overall well-focused composite image, using Combine ZP (free software). Institutional codes follow Sabaj (2020).

Comparative material examined in this study was as follows: NSMT-P 45884, holotype of *Cryptocentrus shigensis*, 78.9 mm SL, Shige, Numazu, Shizuoka Prefecture, Japan, 25 Aug 1956.

Results

Family Gobiidae Cuvier, 1816
Cryptocentrus Valenciennes, 1837

Cryptocentrus shigensis Kuroda, 1956

[English name: Shige shrimp goby]
Fig. 1; Table 1

Material examined. SAIAB 208619, 41.1 mm SL, station 47 (14°41′27″N, 94°05′49″E), northeast of Coco Islands, Myanmar, Andaman Sea, Indian Ocean, 84 m depth, R/V. *Dr. Fridtjof Nansen* (P. N. Psomadakis), bottom trawl, 2 Sep 2018.

Description. Counts and measurements are given in Table 1 and general appearance in Fig. 1. Head and body slender, strongly compressed, width much less than depth. Anus located posteriorly, slightly separated from anal-fin origin. Snout short (much shorter than eye diameter), rounded; lateral profile steep, forming angle of ca. 60° with body axis. Eyes large, located dorsolaterally. Interorbital region very narrow (width much narrower than pupil diameter), flattened. Anterior and posterior nostrils close to each other; former located just before snout tip, with membranous tube; latter located posterodorsally of anterior nostril, small, circular. Mouth terminal, inclined anterodorsally, forming angle of ca. 50° with body axis. Lower jaw subequal to upper jaw, its posterior tip reaching vertical through posterior margin of pupil. Upper-jaw tip behind vertical through lower-jaw tip. Both jaws with irregular rows of small, pointed conical teeth, with tip of each slightly incurved posteriorly; teeth on outermost row on jaws spaced, distinctly larger than teeth on inner rows; 2 or 3 somewhat large canine-like teeth present on both sides of jaws; vomerine and palatine teeth absent. Gill membranes attached anteriorly to isthmus. Gill opening relatively narrow, anteroventral point extending slightly forward to vertical level of preopercle margin.

Table 1. Counts and measurements of specimens of two gobies, *Cryptocentrus shigensis* and *Priolepis profunda.*

	C. shigensis		*P. profunda*
	SAIAB 208619	**NSMT-P 45884 (holotype)**	**SAIAB 208454**
Standard length (SL) [mm]	41.1	78.9	24.4
Counts			
Dorsal-fin rays	VI-I, 10	VI-I, 10	VI-I, 10
Anal-fin rays	I, 9	I, 9	I, 8
Pectoral-fin rays	17	18	20
Pelvic-fin rays	I, 5	I, 5	I, 5
Longitudinal scale rows	ca. 55	ca. 60 (left) ca. 58 (right)	26
Anterior transverse scale rows	ca. 18	ca. 20	11
Posterior transverse scale rows	ca. 15	ca. 17	11
Transverse scale rows	ca. 15	ca. 18	11
Predorsal scale rows	ca. 25	ca. 26	18
Circumpeduncular scales	ca. 14	ca. 14	12
Gill rakers	3 + 14	broken	3 + 11
Measurements [% of SL]			
Head length	29.3	26.7	35.7
Snout length	5.9	5.7	9.6
Eye diameter	9.1	6.3	10.9
Interorbital width	1.2	0.8	3.3
Nape width	10.9	11.7	19.8
Head width	12.1	15.2	21.0
Head depth	18.9	16.9	26.4
Jaw length	13.5	14.8	15.9
Body depth	19.3	17.2	27.4
Body width	10.9	12.4	18.5
Predorsal length	37.5	32.2	40.5
Prepelvic length	33.9	31.1	36.0
Pre-anal length	65.1	60.1	67.0
Caudal-peduncle length	22.1	20.8	23.1
Caudal-peduncle depth	10.3	9.6	15.1
First dorsal-fin base length	15.1	15.8	16.0
Second dorsal-fin base length	26.7	27.6	21.4
Anal-fin base length	19.2	21.5	17.1
Pectoral-fin length	24.8	19.4	30.7
Pelvic-fin length	22.8	19.4	23.0
Caudal-fin length	38.2	44.0	23.0

Gill rakers of SAIAB 208619 and SAIAB 208454 were counted on the right side only.

Cephalic sensory system. A detailed pattern of cephalic sensory system given in Fig. 1B. Head sensory canals pores well developed; anterior oculoscapular canal with pores B’, C (single), D (single), E, F, G, and H’; posterior oculoscapular canal with pores K’ and L’; preopercular canal with pores M’ and O’. Head sensory papillae damaged, but following conditions confirmed: 4 transverse papillae rows extending from lower eye margin to upper jaw (anterior 2 rows) and cheek (posterior 2 rows); 2 longitudinal papillae rows present on cheek; single transverse papillae row present between longitudinal papillae rows.

Scales. Body covered with deciduous (almost all scales lost due to abrasion) cycloid scales, small anteriorly, becoming larger posteriorly. Pre-dorsal- and pelvic-fin regions covered with small cycloid scales, anterior scaled margins reaching vertical through between eye and preopercle and just behind anteroventral point of gill opening, respectively; lower margin of pre-dorsal scaled area

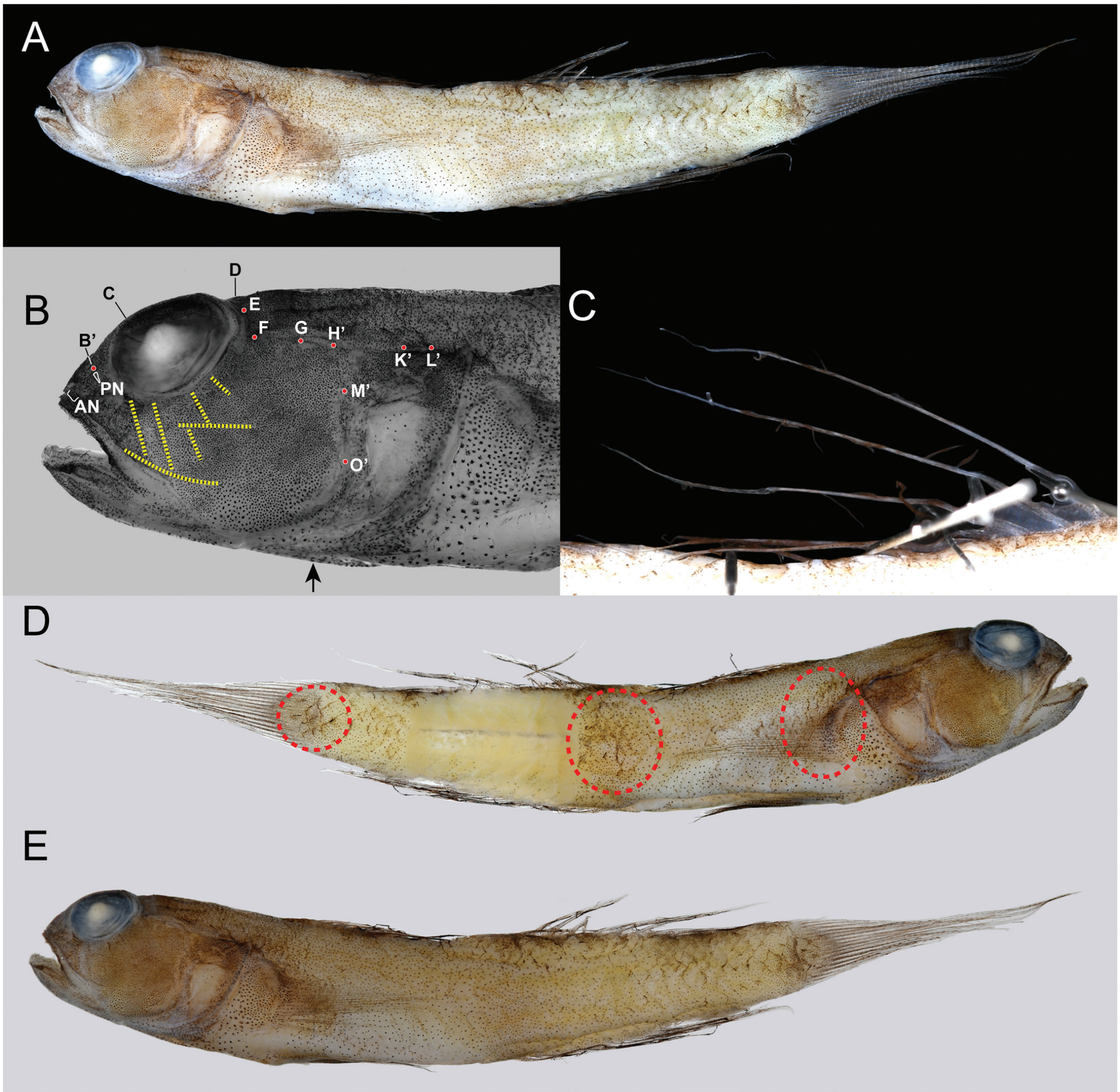

Figure 1. Preserved specimen of *Cryptocentrus shigensis* (SAIAB 208619, 41.1 mm SL). (**A**, **E**) and (**D**), left and right sides of body, respectively; (**B**), close-up of head, showing cephalic sensory system; (**C**): close-up of 1st dorsal fin (right side). Yellow dots and red circles indicate sensory papillae rows and brown blotches, respectively. AN and PN indicate anterior and posterior nostrils, respectively. Arrow head indicates anteroventral end of gill opening.

not reaching horizontal level of upper end of opercle. Entire head region (except for lateral surface of nape) naked.

Fins. All dorsal- and anal-fin spines slender, flexible. First dorsal fin triangular, all spines with very long filamentous tips, 2nd and 3rd spines longest (much longer than 1st dorsal-fin base) (Fig. 1C); dorsal-fin origin posterior to vertical through pectoral-fin base. Second dorsal and anal fins relatively long, origin of latter slightly posterior to vertical through 2nd dorsal-fin origin, posteriormost rays of both fins well separated from caudal-fin base. Pectoral fin moderately long, pointed, middle rays longest, tips extending posteriorly to a vertical line drawn between dorsal fins. Pelvic fins fused medially with connecting membrane (between innermost rays) and well developed frenum (between spines); posterior tips located vertically level with pectoral-fin tip; pelvic-fin origin just below ventral end of pectoral-fin base; posterior margin of pelvic frenum smooth, slightly emarginated; all segmented pelvic-fin rays branched. Caudal fin very long (subequal to predorsal length), lanceolate.

Coloration. Based on preserved specimen (Figs. 1A, D, and E). Head and body pale brown. Most pigmentation patterns lost, but three poorly defined brown blotches retained on right side of body, anteriormost blotch just behind opercle, middle blotch below 2nd dorsal-fin origin, posteriormost blotch on caudal-fin base (Fig. 1D). Dorsal, anal and pelvic fins blackish-brown; pectoral and caudal fins light gray.

Identification. Morphometric and meristic characters of the Andaman specimen (SAIAB 208619) agreed closely with the holotype of *C. shigensis* (Table 1) and the detailed description of the species provided by Akihito et al. (2013). In addition, the presently reported specimen conformed to other diagnostic characters for *C. shigensis* (e.g., pre-dorsal squamation and first dorsal- and caudal-fin shape; Figs. 1A, C, and E; see Remarks). Although head sensory papillae and body pigmentation patterns could not be completely determined due to damage, some characters [e.g., 4 transverse papillae rows extending from lower eye margin to upper jaw (anterior 2 rows) and cheek (posterior 2 rows) and position of three brown blotches on body; Figs. 1B and D] also matched those given by Akihito et al. (2013: 1591) and Kuroda (1956: fig. 1).

Distribution. *Cryptocentrus shigensis* was originally described on the basis of a single specimen collected from Shizuoka Prefecture, Japan (Kuroda 1956). Subsequently, Myers (1999) recorded the species from Palau [based on an unpublished photograph(s)], which remains the only record outside of southern Japan to date (Akihito et al. 2013). Accordingly, the presently reported specimen, collected from the Andaman Sea, represents the first Indian Ocean record of the species.

Remarks. Count of the longitudinal scale rows of the presently reported specimen (ca. 55) was much fewer than those given by the original description of *C. shigensis* (ca. 101; Kuroda 1956). However, re-examination of the holotype of the species revealed that its count was actually ca. 60 on the left side of the body (poor condition) and 58 on the right side (Table 1).

Currently, the generic position of *C. shigensis* is equivocal, the species being closer to *Myersina* Herre, 1934 rather than *Cryptocentrus* (the long filamentous tips on the 1st dorsal fin matching the former), according to Hoese and Lubbock (1982) and Winterbottom (2002). However, the pre-dorsal region covered with cycloid scales differs from the diagnosis of *Myersina* provided by Winterbottom (2002) (completely naked). In addition to the above-mentioned characters, *C. shigensis* can be easily recognized by the lanceolate caudal fin and four brown blotches on the body [3rd blotch (located under middle of 2nd dorsal fin; Kuroda 1956) of the presently reported specimen could not be determined] (Kuroda 1956; Allen and Erdmann 2012; Akihito et al. 2013; this study).

Priolepis Valenciennes, 1837

Priolepis profunda (Weber, 1909)

[English name: narrowbar reef goby]

Figs. 2 and 3; Table 1

Material examined. SAIAB 208454, male, 24.4 mm SL, station 143 (11°01′34″N, 97°56′32″E), north of Clara Island, Myanmar, Andaman Sea, Indian Ocean, 59 m depth, R/V. *Dr. Fridtjof Nansen* (P. N. Psomadakis), bottom trawl, 25 Sep 2018.

Description. Counts and measurements are given in Table 1 and general appearance in Fig. 2. Body somewhat stout, subcylindrical anteriorly, compressed posteriorly. Anus located just before anal-fin origin. Head relatively large, slightly depressed anteriorly. Snout moderate (slightly shorter than eye diameter), rounded. Eyes large, located dorsolaterally. Interorbital region narrow, flattened. Anterior and posterior nostrils close to each other; former located mid-way between anterior tip of snout and eye; latter located just before orbit, larger than former; both with membranous tube. Mouth terminal, inclined anterodorsally, forming an angle of ca. 60° with body axis. Lower jaw subequal to upper jaw, its posterior tip reaching to vertical through anterior margin of pupil. Upper-jaw tip behind vertical through lower-jaw tip. Both jaws with irregular rows of small, pointed conical teeth, with tip of each slightly incurved posteriorly; teeth in outermost row on jaws more widely spaced and distinctly larger than teeth in inner rows. Gill membranes attached anteriorly to isthmus. Gill opening relatively narrow, anteroventral point extending slightly forward to vertical level of preopercle margin.

Cephalic sensory system. Detailed pattern of cephalic sensory system is given in Figs. 3A–C. Head sensory canals pores absent. Head sensory papillae damaged, but following conditions confirmed: 5 transverse papillae rows present on suborbital region; 2 transverse papillae rows present on interorbital region, neither connecting in mid-line, anterior and posterior rows including 2 and 3 papillae, respectively; 2 longitudinal papillae rows present on chin and ventrolateral surface, each papillae row on chin well-spaced anteriorly, becoming gradually closer posteriorly, but not joining.

Scales. Body covered with ctenoid scales, except abdomen (covered with cycloid scales). Pre-dorsal region fully scaled (except just behind 1st dorsal-fin origin), anterior margin of scaled area rounded, reaching vertical through posterior margin of pupil. Pre-pelvic-fin region covered with ca. 6 rows of cycloid scales, anterior margin reaching just behind anteroventral point of gill opening. Entire head region (except for lateral surface of nape) naked. Pectoral-fin base with cycloid scales.

Fins. All dorsal- and anal-fin spines slender, flexible. First dorsal fin squarish, all spines without filamentous tips, 5th spine longest; dorsal-fin origin located just above dorsal origin of pectoral fin. Second dorsal and anal fins relatively short, origin of latter slightly posterior to vertical through 2nd dorsal-fin origin. Pectoral fin long, pointed, middle rays longest, tips reaching just above base of 2nd anal-fin ray. Pelvic fins weakly fused medially with connecting membrane (between ca. 1/5 innermost rays), pelvic frenum absent; posterior tip reaching anus; pelvic-fin origin just below ventral end of pectoral-fin base; all segmented pelvic-fin rays branched. Caudal fin relatively short, with rounded posterior margin.

Coloration. Based on Fig. 2. Head and body orange with many narrow white bars, all bars on each side connected mid-dorsally. Four bars on interorbital region; anterior two bars continuous with two bars under eye;

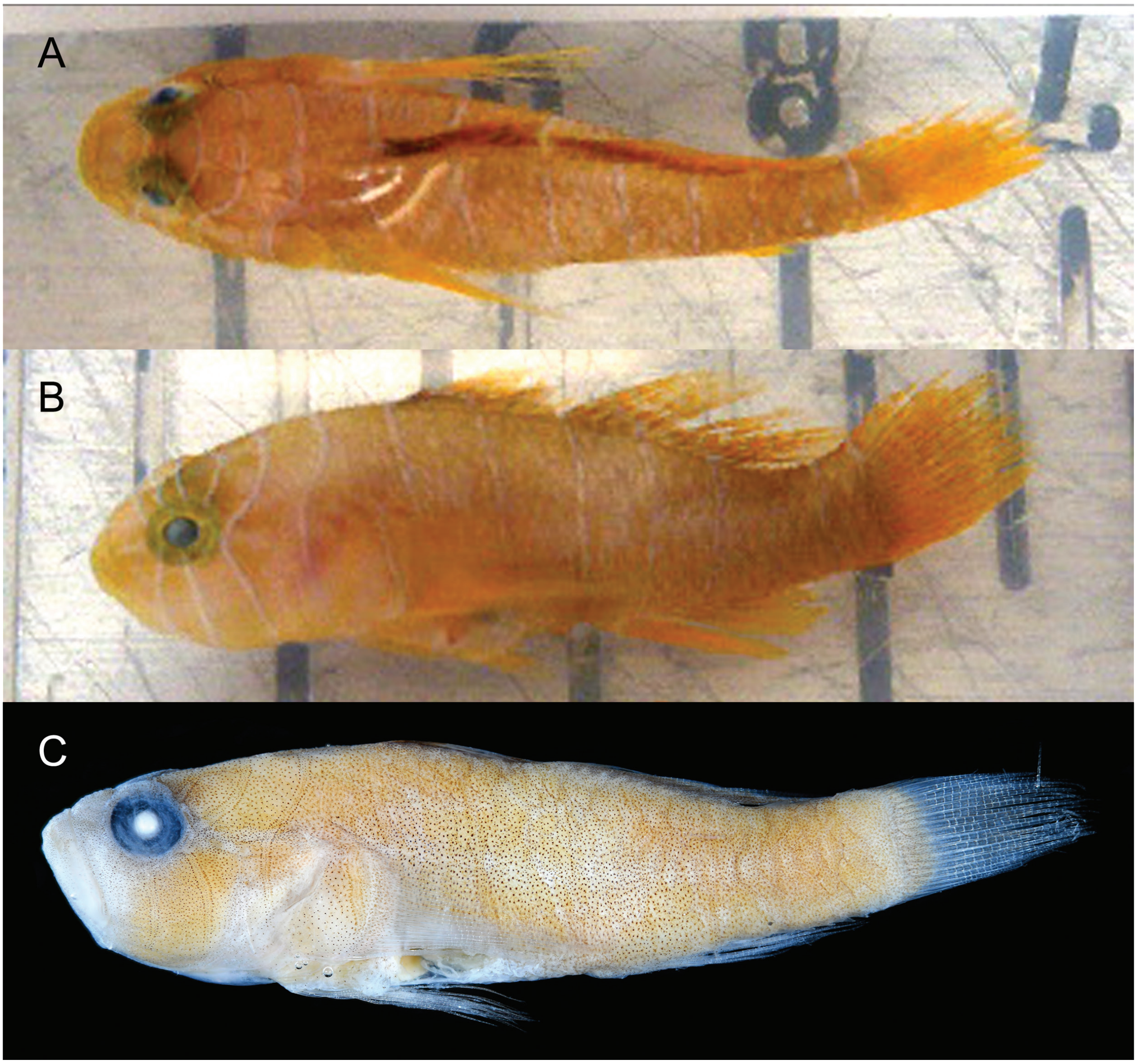

Figure 2. Fresh (**A**, **B**) and preserved (**C**) specimen of *Priolepis profunda* (SAIAB 208454, male, 24.4 mm SL).

posteriormost bar relatively curved posteriorly, more widely spaced from anterior bars. Two bars on postorbital region; former strongly curved, extending from middle of nape to lower edge of preopercle through posterior margin of eye; latter weakly curved, extending from posterior end of head to lower margin of opercle. Eight straight vertical bars along body; two anteriormost below origin and middle of 1st dorsal-fin base, respectively, middle three below origin, middle and posterior end of 2nd dorsal-fin base, respectively, three posteriormost on caudal peduncle (two) and caudal-fin base. All fins orange basally; anterior part of 1st dorsal fin with dark brown smudge; small reddish-orange spots on 2nd dorsal fin and upper part of caudal fin; a single short, pale white bar on pectoral-fin base; caudal fin lacking dark black blotches or bar.

Color in alcohol. Head and body pale brown. All bars visible in fresh specimen retained (pale white with brown edge), but those posteriorly on body somewhat indistinct. All fins translucent white basally, anterior part of 1st dorsal fin and 2nd dorsal-fin base dark brown.

Identification. The Andaman specimen (SAIAB 208454) agreed well with the detailed description of *P. profunda* provided by Hoese and Larson (2010), especially as follows: transverse papillae rows present on suborbital region (Fig. 3B); 6 papillae present on posterior part of interorbital region (Fig. 3A); anterior margin of pre-dorsal scales reaching to vertical through posterior margin of pupil (Figs. 3A and B); 8 narrow white bars on body (Fig. 2).

Distribution. *Priolepis profunda* has previously been recorded widely from the western Pacific Ocean (Japan, Philippines, Thailand, Indonesia, Papua New Guinea, and northwestern Australia; Hoese and Larson 2010; Allen and Erdmann 2012; Akihito et al. 2013). Recently, Ramachandran et al. (2020) recorded the species from India, being the first Indian Ocean record. However, because the inclusion of the Andaman Islands within the distributional range of *P. profunda* by Allen and Erdmann (2012) was not supported by underwater photographs or voucher specimens, the presently reported specimen represents the first reliable record of *P. profunda* from the Andaman Sea (Myanmar).

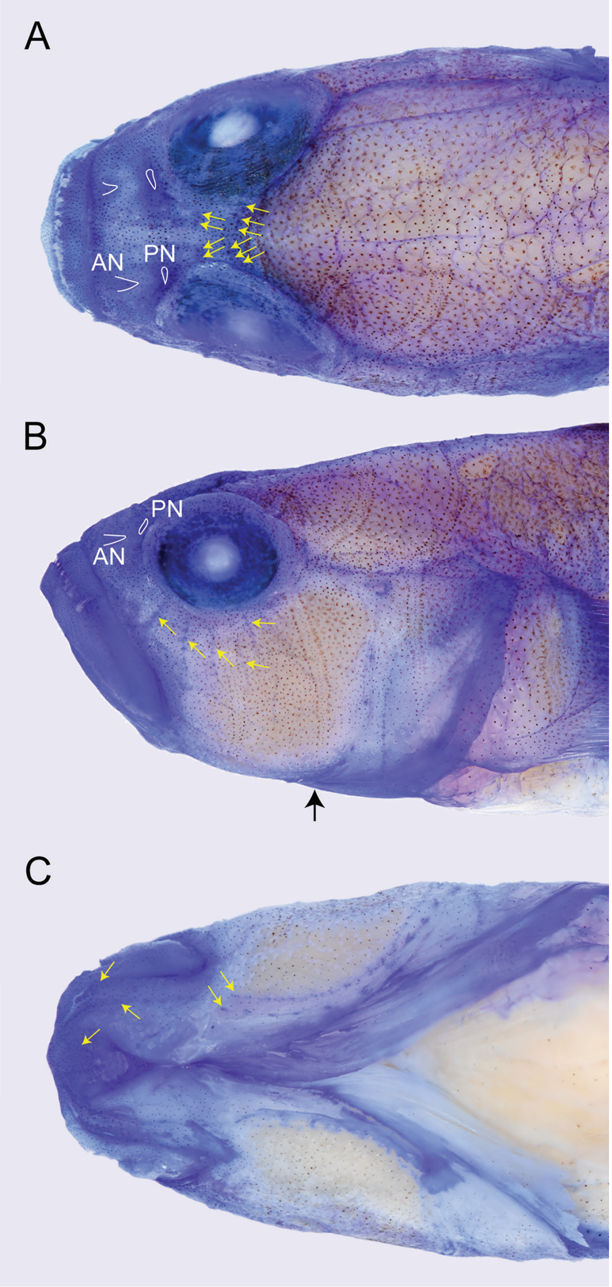

Figure 3. Head of *Priolepis profunda* (SAIAB 208454, 24.4 mm SL), showing cephalic sensory system. Yellow arrows indicate sensory papilla (**A**) and papillae rows (**B**–**C**). AN and PN indicate anterior and posterior nostrils, respectively. Black arrows indicate anteroventral end of gill opening.

Remarks. In addition to *P. profunda*, 11 species of *Priolepis* are known to have transverse papillae rows on the suborbital region [*P. profunda* grade *sensu* Winterbottom and Burridge (1993)] (Winterbottom and Burridge 1992; Nogawa and Endo 2007; Hoese and Larson 2010; Bogorodsky et al. 2016; Allen et al. 2018; Fujiwara et al. 2020; Koeda et al. 2021). *Priolepis profunda* and seven of the 11 species also share white bars on the body, although the number and width of the bars in *P. profunda* are relatively high (8 bars) and distinctly narrow, respectively. Moreover, the combination of squamation on the pre-dorsal region and number of papillae on the interorbital region of *P. profunda* (see Identification) is unique within the species complex.

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Length–weight relations and condition factors of 34 *Oxynoemacheilus* species (Actinopterygii: Cypriniformes: Nemacheilidae) from Turkish inland waters

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http://zoobank.org/C3F5D481-9341-4616-A527-44B894DFE0D9

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Abstract

This study aimed to provide the length–weight relations and condition factors of 34 *Oxynoemacheilus* species from the inland waters of Turkey: *Oxynoemacheilus anatolicus* Erk'akan, Özeren et Nalbant, 2008; *Oxynoemacheilus angorae* (Steindachner, 1897); *Oxynoemacheilus argyrogramma* (Heckel, 1847); *Oxynoemacheilus arsaniasus* Freyhof, Kaya, Turan et Geiger, 2019; *Oxynoemacheilus atili* Erk'akan, 2012; *Oxynoemacheilus banarescui* (Delmastro, 1982); *Oxynoemacheilus bergianus* (Derjavin, 1934); *Oxynoemacheilus* cf. *bureschi* (Drensky, 1928); *Oxynoemacheilus ceyhanensis* (Erk'akan, Nalbant et Özeren, 2007); *Oxynoemacheilus ciceki* Sungur, Jalili et Eagderi, 2017; *Oxynoemacheilus cilicicus* Kaya, Turan, Bayçelebi, Kalayci et Freyhof, 2020; *Oxynoemacheilus cyri* (Berg, 1910); *Oxynoemacheilus ercisianus* (Erk'akan et Kuru, 1986); *Oxynoemacheilus eregliensis* (Bănărescu et Nalbant, 1978); *Oxynoemacheilus euphraticus* (Bănărescu et Nalbant, 1964); *Oxynoemacheilus evreni* (Erk'akan, Nalbant et Özeren, 2007); *Oxynoemacheilus frenatus* (Heckel, 1843); *Oxynoemacheilus germencicus* (Erk'akan, Nalbant et Özeren, 2007); *Oxynoemacheilus hamwii* (Krupp et Schneider, 1991); *Oxynoemacheilus hazarensis* Freyhof et Özuluğ, 2017; *Oxynoemacheilus insignis* (Heckel, 1843); *Oxynoemacheilus kaynaki* Erk'akan, Özeren et Nalbant, 2008; *Oxynoemacheilus mediterraneus* (Erk'akan, Nalbant et Özeren, 2007); *Oxynoemacheilus namiri* (Krupp et Schneider, 1991); *Oxynoemacheilus nasreddini* Yoğurtçuoğlu, Kaya et Freyhof, 2021; *Oxynoemacheilus paucilepis* (Erk'akan, Nalbant et Özeren, 2007); *Oxynoemacheilus samanticus* (Bănărescu et Nalbant, 1978); *Oxynoemacheilus seyhanensis* (Bănărescu, 1968); *Oxynoemacheilus seyhanicola* (Erk'akan, Nalbant et Özeren, 2007); *Oxynoemacheilus simavicus* (Balik et Bănărescu, 1978); *Oxynoemacheilus theophilii* Stoumboudi, Kottelat et Barbieri, 2006; *Oxynoemacheilus tigris* (Heckel, 1843); *Oxynoemacheilus veyselorum* Çiçek, Eagderi et Sungur, 2018. Based on the results, the growth coefficient values (b) ranged from 2.770 (*O. argyrogramma*) to 3.285 (*O. theophilii*) with an R^2 estimate greater than 0.91. Fulton's condition factor (K_F) of the studied fishes ranged from 0.598 (*O. insignis*) to 1.07 (*O. nasreddini*). Relative conditions (K_R) were found to have a narrow distribution range (0.856–1.014 with a mean of 1.005). The form factors of these species were calculated between 0.006 and 0.14, with a mean and median value of 0.01. This study represents the first reports of LWRs parameters for 22 species, new maximum total length data were bigger than given in FishBase for 21 species, and first species listing for maximum total length for seven species. The findings of this study provide useful information for further fisheries management and fish population dynamic studies.

Keywords

condition factor, form factor, length–weight relation, loach, Nemacheilidae

Introduction

The Nemacheilidae are small fishes inhabiting the freshwaters of Asia, Europe, and northeastern Africa (Nelson et al. 2016; Sungur et al. 2017). This family has great diversity in Turkish inland waters with 48 species, of which 28 are endemics (Çiçek et al. 2015, 2018, 2020). They do not have commercial value but are important components for aquatic ecosystems (Kottelat 2012; Çiçek et al. 2015, 2018).

The study of the length–weight relation (LWR) of any fish species is a prerequisite for assessing its population characteristics (Le Cren 1951). As a result, LWRs provide fundamental knowledge in fisheries biology, which is required for management and conservation. Few Turkish nemacheilids have LWR data available (Gaygusuz et al. 2012; Erk'akan et al. 2013, 2014; Birecikligil et al. 2016; Özcan and Altun 2016; Yazıcıoğlu and Yazıcı 2016; İnnal 2019; Özdemir et al. 2019); hence, providing such data for these taxa is crucial for their management and conservation (Tabatabaei et al. 2015; Keivany et al. 2016; Jafari-Patcan et al. 2018).

Condition factor is computed using the weight and length of fish species to describe the condition or well-being of fish individuals in a particular water body (Froese 2006). It is assumed that the growth of fish in ideal conditions maintains equilibrium in length and weight and is a useful index for monitoring feeding intensity, age and growth rate, and assessing the status of the aquatic ecosystem where fish live (Radkhah and Eagderi 2015; Zamani-Faradonbe et al. 2015). Based on the above-mentioned background, the presently reported study was conducted to determine the LWRs parameters, condition factors, and form factors of 34 species of the genus *Oxynoemacheilus* inhabiting inland waters of Turkey. The following species were studied: *Oxynoemacheilus anatolicus* Erk'akan, Özeren et Nalbant, 2008; *Oxynoemacheilus angorae* (Steindachner, 1897); *Oxynoemacheilus argyrogramma* (Heckel, 1847); *Oxynoemacheilus arsaniasus* Freyhof, Kaya, Turan et Geiger, 2019; *Oxynoemacheilus atili* Erk'akan, 2012; *Oxynoemacheilus banarescui* (Delmastro, 1982); *Oxynoemacheilus bergianus* (Derjavin, 1934); *Oxynoemacheilus* cf. *bureschi* (Drensky, 1928); *Oxynoemacheilus ceyhanensis* (Erk'akan, Nalbant et Özeren, 2007); *Oxynoemacheilus ciceki* Sungur, Jalili et Eagderi, 2017; *Oxynoemacheilus cilicicus* Kaya, Turan, Bayçelebi, Kalayci et Freyhof, 2020; *Oxynoemacheilus cyri* (Berg, 1910); *Oxynoemacheilus ercisianus* (Erk'akan et Kuru, 1986); *Oxynoemacheilus eregliensis* (Bănărescu et Nalbant, 1978); *Oxynoemacheilus euphraticus* (Bănărescu et Nalbant, 1964); *Oxynoemacheilus evreni* (Erk'akan, Nalbant et Özeren, 2007); *Oxynoemacheilus frenatus* (Heckel, 1843); *Oxynoemacheilus germencicus* (Erk'akan, Nalbant et Özeren, 2007); *Oxynoemacheilus hamwii* (Krupp et Schneider, 1991); *Oxynoemacheilus hazarensis* Freyhof et Özuluğ, 2017; *Oxynoemacheilus insignis* (Heckel, 1843); *Oxynoemacheilus kaynaki* Erk'akan, Özeren et Nalbant, 2008; *Oxynoemacheilus mediterraneus* (Erk'akan, Nalbant et Özeren, 2007); *Oxynoemacheilus namiri* (Krupp et Schneider, 1991); *Oxynoemacheilus nasreddini* Yoğurtçuoğlu, Kaya et Freyhof, 2021; *Oxynoemacheilus paucilepis* (Erk'akan, Nalbant et Özeren, 2007); *Oxynoemacheilus samanticus* (Bănărescu et Nalbant, 1978); *Oxynoemacheilus seyhanensis* (Bănărescu, 1968); *Oxynoemacheilus seyhanicola* (Erk'akan, Nalbant et Özeren, 2007); *Oxynoemacheilus simavicus* (Balik et Bănărescu, 1978); *Oxynoemacheilus theophilii* Stoumboudi, Kottelat et Barbieri, 2006; *Oxynoemacheilus tigris* (Heckel, 1843); *Oxynoemacheilus veyselorum* Çiçek, Eagderi et Sungur, 2018.

Material and methods

A total of 1801 specimens of *Oxynoemacheilus* species were collected between May 2009 and September 2019 from Turkish inland water using an electrofishing device (SAMUS MP750). The sampling year of the species is given in Table 1. After anesthesia, the specimens were preserved in 4% buffered formalin and transported to the laboratory.

In the laboratory, the total length (L) and total weight (W) of each individual were determined using a digital caliper to the nearest 0.1 cm and 0.01 g, respectively. The LWRs were calculated by the method of least squares using the equation

$$W = aL^b$$

and logarithmically transformed (Froese 2006) into

$$\mathrm{Log}W = \log a + b \log L$$

where W is the whole-body weight [g], L is the total length [cm], a is the intercept, and b is the slope. Prior to regression analyses, log–log plots of the length–weight pairs were performed to identify outliers (Froese et al. 2011). Outliers perceived in the log–log plots of all species were evacuated from the regression. Fulton's condition factor (K_F) was estimated using the following formula (Ricker 1975; Froese 2006)

$$K_F = 100WL^{-3}$$

The relative condition factor (K_R) was calculated using the equation of Froese (2006)

$$K_R = W(aL^b)^{-1}$$

The mean condition factor (K_M) for a given length is derived from the respective WLR using the formula (Froese 2006)

$$K_M = 100aL^{b-3}$$

The form factor ($a3{:}0$) can be used to determine whether the body shape of a population or species differs significantly from that of others. It was calculated using the formula (Froese 2006)

Table 1. Sampling sites and descriptive statistics of length and weight for 34 *Oxynoemacheilus* species in Turkey.

Species	E	Habitat	Basin	Coordinates	SY	*n*	Total length [cm]				Total weight [g]				L_{max} in FishBase
							Min	Max	Mean	SD	Min	Max	Mean	SD	
Oxynoemacheilus anatolicus	+	Dalaman Stream	Western Mediterranean	37°08′17″N, 29°09′21″E	2018	11	4.5	**8.2**	6.04	1.28	0.87	5.03	2.33	1.52	5.20
Oxynoemacheilus angorae	+	Çubuk Stream	Kızılırmak	40°20′38″N, 33°02′21″E	2017	25	3.8	**8.9**	7.44	1.32	0.42	5.89	4.01	1.50	8.5
Oxynoemacheilus argyrogramma		Keysun Stream	Euphrates	37°30′39″N, 38°06′31″E	2009	54	4.5	**7.3**	5.65	0.81	0.74	3.49	1.71	0.69	6.20
Oxynoemacheilus arsaniasus	+	Haçlı Lake	Tigris	39°00′49″N, 42°20′11″E	2018	13	4.6	7.2	5.82	0.55	1.08	3.86	2.03	0.64	—
Oxynoemacheilus atili	+	Eflatun Pınarı	Konya closed	37°49′30″N, 31°40′27″E	2018	47	4.3	**8.3**	6.57	0.94	0.67	5.12	2.66	0.98	7.60
Oxynoemacheilus banarescui	+	Alaçam Stream	Kızılırmak	41°28′21″N, 35°45′57″E	2017	63	4.4	**7.8**	6.04	0.69	0.62	4.03	1.87	0.69	5.30
Oxynoemacheilus bergianus		Handere Stream	Aras	40°07′36″N, 42°14′55″E	2016	43	3.0	**8.4**	5.28	1.25	0.18	4.52	1.28	0.93	7.60
Oxynoemacheilus bergianus		Digor	Aras	40°23′38″N, 43°24′45″E	2016	74	3.3	**8.4**	6.12	1.30	0.26	3.98	1.83	0.93	7.60
Oxynoemacheilus cf. *bureschi*		Uluçay Stream	Sakarya	40°22′56″N, 31°46′06″E	2017	7	5.3	**7.7**	6.86	0.81	1.07	3.36	2.53	0.80	6.50
Oxynoemacheilus ceyhanensis	+	Ceyhan River	Ceyhan	38°05′35″N, 36°59′40″E	2017	50	3.3	7.8	5.56	1.08	0.21	4.56	1.66	0.95	8.80
Oxynoemacheilus ciceki	+	Sultan Marsh	Kızılırmak	38°23′23″N, 35°21′54″E	2009	103	4.3	**7.5**	5.34	0.52	0.42	2.61	0.95	0.34	5.80
Oxynoemacheilus cilicicus	+	Kızıl Stream	Eastern Mediterranean	36°51′27″N, 34°33′13″E	2018	105	3.4	9.9	5.60	1.02	0.45	9.10	1.81	1.38	—
Oxynoemacheilus cyri	+	Kura River	Kura	40°50′32″N, 42°48′57″E	2019	31	3.2	**8.6**	5.85	1.27	0.26	7.33	2.46	1.66	6.80
Oxynoemacheilus ercisianus	+	Ilıca Stream	Van Lake	39°00′15″N, 43°19′17″E	2016	103	2.9	7.7	4.09	0.91	0.21	4.53	0.74	0.76	—
Oxynoemacheilus eregliensis	+	Melendiz Stream	Konya closed	38°19′36″N, 34°14′20″E	2019	123	5.1	9.5	6.83	1.23	1.02	7.66	2.91	1.62	10.30
Oxynoemacheilus euphraticus		Aşkale	Euphrates	39°46′48″N, 40°26′55″E	2017	119	2.8	**9.3**	5.78	0.93	0.17	5.60	1.53	0.81	7.40
Oxynoemacheilus evreni	+	Ceyhan River	Ceyhan	38°15′17″N, 37°31′56″E	2016	17	3.1	6.5	5.07	1.05	0.25	2.72	1.44	0.78	9.40
Oxynoemacheilus frenatus		Arpaçay Stream	Tigris	38°01′21″N, 40°29′25″E	2012	31	4.1	**8.0**	5.56	1.02	0.68	5.83	1.96	1.36	7.50
Oxynoemacheilus germencicus	+	Kadın Stream	Küçük Menderes	38°18′27″N, 28°10′11″E	2017	19	5.1	**7.8**	6.61	0.70	1.18	4.49	2.73	0.86	6.30
Oxynoemacheilus hamwii		Orontes River	Orontes	36°58′33″N, 36°51′51″E	2015	63	3.1	**8.4**	5.14	1.07	0.22	5.36	1.30	0.96	6.20
Oxynoemacheilus hazarensis	+	Hazar Lake	Euphrates	38°27′08″N, 39°18′26″E	2013	13	4.1	**7.2**	5.85	0.88	0.54	3.23	1.88	0.74	6.50
Oxynoemacheilus insignis		Karasu Stream	Euphrates	37°22′35″N, 37°29′22″E	2017	29	3.4	6.5	5.23	0.82	0.18	1.72	0.91	0.38	12.00
Oxynoemacheilus kaynaki	+	Input of Dumluca Lake	Ceyhan	37°25′57″N, 40°06′45″E	2013	82	4.0	**7.5**	5.85	0.82	0.72	3.85	2.01	0.91	6.80
Oxynoemacheilus mediterraneus	+	Input of Sücüllü Dam Lake	Mediterranean	38°23′22″N, 31°07′56″E	2018	144	3.6	**7.6**	6.13	1.00	0.33	3.39	1.92	0.77	5.80
Oxynoemacheilus namiri		Orontes River	Orontes	38°23′22″N, 31°07′56″E	2016	88	4.1	8.7	6.21	1.02	0.50	7.02	2.64	1.43	—
Oxynoemacheilus nasreddini	+	Siyek Stream	Akarçay	38°35′10″N, 30°25′36″E	2009	77	4.7	9.9	6.99	1.01	0.99	9.45	3.88	1.69	—
Oxynoemacheilus paucilepis	+	Mancınık Stream	Euphrates	39°12′25″N, 37°12′04″E	2018	15	4.2	**8.2**	6.65	0.96	0.78	5.68	3.19	1.24	7.00
Oxynoemacheilus samanticus	+	Zamantı Stream	Seyhan	38°44′10″N, 36°24′46″E	2014	21	4.4	6.6	5.31	0.59	0.65	2.43	1.34	0.48	8.60
Oxynoemacheilus seyhanensis	+	Zamantı Stream	Seyhan	38°43′54″N, 36°22′46″E	2015	56	3.3	9.1	4.59	1.10	0.34	8.40	1.20	1.39	—
Oxynoemacheilus seyhanicola	+	Ceyhan River	Ceyhan	38°05′35″N, 36°59′40″E	2015	29	4.2	**9.5**	6.45	1.32	0.51	7.02	2.36	1.64	4.30
Oxynoemacheilus simavicus	+	Yağcılı Stream	North Aegean	39°19′47″N, 27°34′07″E	2017	10	4.8	**8.0**	6.44	0.87	1.13	5.62	2.93	1.32	7.10
Oxynoemacheilus theophilii		Sevişler Dam Lake	North Aegean	39°19′47″N, 27°34′07″E	2017	54	3.5	**7.7**	5.38	0.91	0.33	4.56	1.58	0.88	6.60
Oxynoemacheilus tigris		Seve Dam Lake	Euphrates	36°44′38″N, 37°14′56″E	2019	36	3.0	8.4	4.74	0.93	0.18	4.80	0.88	0.77	8.40
Oxynoemacheilus veyselorum		Bozkuş Stream	Aras	40°37′03″N, 42°47′04″E	2016	46	4.6	12.6	9.27	1.96	1.74	15.88	7.77	4.01	—

E = endemic (plus signs), SY = sampling year, *n* = number of individuals, SD = standard deviation; Values in **bold** font denote maximum length higher than given in FishBase; Text in shaded cells denotes maximum total length not given in FishBase.

$$a3{:}0 = 10^{\log a - S(b - 3)}$$

where S is the slope of the log$a$ vs. b regression, the mean slope $S = a - 1.358$ proxy for estimating the form factor (Froese 2006).

The degree of dependence between the variables was computed by the determination coefficient R^2. The significance level of R^2 was estimated by ANOVA. The Student's t-test was used to determine whether parameter b is significantly different from the expected or theoretical value of 3 (i.e., $b = 3$, $P < 0.05$). All statistical analyses were performed in MS Excel 2016 and Past 3.26.

Results and discussion

The presently reported study provides the LWRs and condition factors of 34 *Oxynoemacheilus* species. The descriptive statistics of length and weight with the parameters of the LWR; regression parameters a and b, the 95% confidence limits of b; the 95% confidence limits of a; correlation coefficient (R^2) and type of growth for the studied species are given in Tables 1 and 2. Based on our collected specimens, new maximum total lengths were recorded for 23 species.

The parameter b of the studied species ranged from 2.770 (*O. argyrogramma*) to 3.285 (*O. theophilii*) with the median value of 3.071 (Table 2 and Fig. 1). The value of b generally lies between 2.5 and 3.5 (Froese 2006) though the ideal value of b is 3.0 (Hile 1936). In LWRs, b-values that are higher and lower than 3 indicate positive and negative allometric growth, respectively. According to the b-value, 27 species are isometric; two are negative allometry and five are positive allometry (Table 2). The coefficient of determination (R^2) between length and weight varied from 0.91 for *O. ciceki* to 0.99 for *O.* cf. *bureschi*.

The values of K_F varied from 0.598 (*O. insignis*) to 1.070 (*O. nasreddini*). Clark (1928) showed the relation between K_F and the parameters of the respective WLR (Table 2 and Fig. 1). The K_M for a given length is derived from the respective WLR (Froese 2006) which ranged from 0.856 to 1.014 with a mean of 1.005. Clark (1928) also demonstrates that if b is not significantly different from 3, K_F can be compared directly. Le Cren (1951) proposed the relative condition factor (K_R), which accounts for changes in form or condition as length increases and

Table 2. Estimated parameters of the length–weight relations (LWR), condition factors, and form factor for 34 *Oxynoemacheilus* species in Turkey.

Species	LWR parameters in this study						LWRs in FishBase		Fulton's Condition				Relative Condition				*a*3:0
	a	*b*	R^2	SD of *b*	CI of *b*	GT	*a*	*b*	Min	Max	Mean	SD	Min	Max	Mean	SD	
Oxynoemacheilus anatolicus	0.007	3.168	0.981	0.1460	2.779–3.367	I	—	—	0.81	1.15	0.92	0.10	0.90	1.22	1.01	0.10	0.0115
Oxynoemacheilus angorae	0.011	2.903	0.979	0.0997	2.661–3.355	I	0.008	3.0	0.77	1.15	0.91	0.09	0.79	1.22	1.00	0.09	0.0081
Oxynoemacheilus argyrogramma	0.013	2.770	0.949	0.0895	2.607–2.929	–A	—	—	0.75	1.18	0.91	0.09	0.85	1.28	1.00	0.09	0.0065
Oxynoemacheilus arsaniasus	0.012	2.923	0.915	0.2693	2.485–3.360	I	—	—	0.91	1.13	1.01	0.08	0.90	1.13	1.00	0.08	0.0090
Oxynoemacheilus atili	0.011	2.879	0.978	0.0644	2.738–3.060	I	—	—	0.79	1.10	0.89	0.06	0.84	1.21	1.00	0.07	0.0077
Oxynoemacheilus banarescui	0.007	3.113	0.926	0.1130	2.975–3.305	I	—	—	0.64	1.07	0.81	0.09	0.79	1.32	1.01	0.11	0.0094
Oxynoemacheilus bergianus	0.007	3.052	0.983	0.0623	2.933–3.160	I	—	—	0.56	0.98	0.74	0.07	0.76	1.31	1.00	0.10	0.0080
Oxynoemacheilus bergianus	0.008	2.921	0.989	0.0367	2.830–3.013	–A	—	—	0.60	0.92	0.72	0.06	0.86	1.23	1.01	0.08	0.0064
Oxynoemacheilus* cf. *bureschi	0.006	3.095	0.990	0.1398	2.889–3.630	I	—	—	0.72	0.80	0.76	0.03	0.96	1.06	1.00	0.04	0.0085
Oxynoemacheilus ceyhanensis	0.007	3.152	0.950	0.1050	2.971–3.380	I	0.0056	3.13	0.58	1.22	0.86	0.13	0.74	1.41	1.01	0.15	0.0106
Oxynoemacheilus ciceki	0.005	3.129	0.910	0.0980	2.924–3.345	I	—	—	0.46	0.73	0.61	0.06	0.77	1.21	1.00	0.09	0.0073
Oxynoemacheilus cilicicus	0.006	3.274	0.946	0.0772	3.066–3.462	+A	—	—	0.60	1.44	0.91	0.13	0.70	1.69	1.01	0.14	0.0132
Oxynoemacheilus cyri	0.007	3.199	0.974	0.0980	3.045–3.419	+A	—	—	0.79	1.29	1.06	0.14	0.81	1.28	1.01	0.12	0.0138
Oxynoemacheilus ercisianus	0.009	3.041	0.981	0.0420	2.983–3.118	I	—	—	0.75	1.09	0.91	0.07	0.82	1.20	1.00	0.08	0.0098
Oxynoemacheilus eregliensis	0.009	2.961	0.973	0.0045	2.876–3.050	I	0.0050	3.20	0.63	1.01	0.83	0.07	0.76	1.22	1.01	0.09	0.0079
Oxynoemacheilus euphraticus	0.008	2.955	0.960	0.0561	2.821–3.082	I	0.0062	2.97	0.42	1.15	0.74	0.07	0.58	1.58	1.01	0.10	0.0069
Oxynoemacheilus evreni	0.008	3.159	0.988	0.0910	3.023–3.417	+A	0.0128	2.79	0.84	1.15	0.98	0.08	0.86	1.20	1.00	0.08	0.0125
Oxynoemacheilus frenatus	0.008	3.111	0.921	0.1695	2.747–3.529	I	—	—	0.61	1.60	1.02	0.16	0.62	1.64	1.02	0.16	0.0117
Oxynoemacheilus germencicus	0.007	3.114	0.979	0.1117	2.995–3.342	I	—	—	0.85	1.04	0.91	0.05	0.92	1.14	1.00	0.05	0.0106
Oxynoemacheilus hamwii	0.005	3.259	0.913	0.1483	2.939–3.614	I	0.0099	2.66	0.42	1.23	0.81	0.16	0.55	1.56	0.98	0.18	0.0121
Oxynoemacheilus hazarensis	0.011	2.889	0.935	0.2301	2.108–3.500	I	—	—	0.68	1.22	0.90	0.12	0.74	1.32	1.01	0.12	0.0076
Oxynoemacheilus insignis	0.006	3.048	0.915	0.1791	2.709–3.524	I	0.0150	2.95	0.46	0.79	0.60	0.10	0.79	1.33	1.00	0.16	0.0064
Oxynoemacheilus kaynaki	0.007	3.148	0.938	0.0902	2.944–3.335	I	—	—	0.73	1.14	0.94	0.11	0.79	1.27	1.01	0.11	0.0114
Oxynoemacheilus mediterraneus	0.009	2.944	0.974	0.0403	2.870–3.023	I	—	—	0.56	0.96	0.78	0.07	0.73	1.25	1.01	0.09	0.0072
Oxynoemacheilus namiri	0.007	3.188	0.924	0.0985	2.928–3.438	I	—	—	0.55	1.58	1.01	0.15	0.58	1.66	1.01	0.15	0.0128
Oxynoemacheilus nasreddini	0.011	2.981	0.932	0.0932	2.812–3.149	I	—	—	0.79	1.50	1.07	0.13	0.74	1.41	1.01	0.12	0.0104
Oxynoemacheilus paucilepis	0.009	3.048	0.979	0.1233	2.578–3.198	I	—	—	0.87	1.12	1.02	0.07	0.86	1.10	1.01	0.07	0.0108
Oxynoemacheilus samanticus	0.006	3.180	0.972	0.1230	2.824–3.376	I	0.0085	2.92	0.76	0.98	0.86	0.05	0.93	1.13	1.01	0.06	0.0111
Oxynoemacheilus seyhanensis	0.009	3.101	0.977	0.0654	2.962–3.292	I	—	—	0.78	1.28	1.00	0.10	0.79	1.24	1.00	0.10	0.0118
Oxynoemacheilus seyhanicola	0.005	3.239	0.975	0.1001	3.092–3.377	+A	—	—	0.55	0.95	0.76	0.09	0.71	1.22	1.00	0.10	0.0103
Oxynoemacheilus simavicus	0.007	3.239	0.976	0.1811	2.539–3.604	I	0.0044	3.26	0.92	1.15	1.03	0.08	0.91	1.09	1.00	0.07	0.0139
Oxynoemacheilus theophilii	0.006	3.285	0.954	0.0999	3.168–3.405	+A	—	—	0.75	1.33	0.92	0.13	0.83	1.50	1.02	0.13	0.0137
Oxynoemacheilus tigris	0.006	3.143	0.944	0.1318	2.898–3.455	I	0.0046	3.23	0.45	1.03	0.72	0.10	0.62	1.44	1.01	0.14	0.0089
Oxynoemacheilus veyselorum	0.011	2.887	0.972	0.0734	2.756–3.031	I	—	—	0.75	1.79	0.91	0.20	0.85	1.90	1.04	0.20	0.0079

Bold font indicates the first reported LWR value; *a* = intercept, *b* = slope, R^2 = correlation coefficient, SD = standard deviation CL = confidence intervals, GT = growth type, *a*3:0 = form factor; I = isometric growth; –A = negative allometric growth, +A = positive allometric growth.

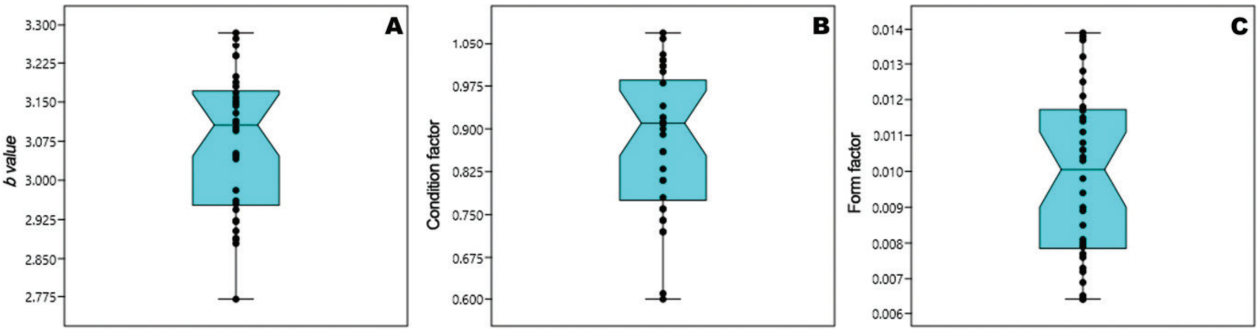

Figure 1. Box plot of (**A**) allometric co-efficient *b* values, (**B**) Fulton's condition factor (K_F), (**C**) form factor for 34 *Oxynoemacheilus* species from Turkey.

therefore assesses an individual's divergence from the sample's mean weight for length. To facilitate such comparisons, Le Cren (1951) introduced the relative condition factor, which compensates for changes in form or condition with an increase in length and thus measures the K_R. The values of K_R varied from 0.985 (*O. hamwii*) to 1.041 (*O. veyselorum*) (Table 2).

The condition factor is an index reflecting interactions between biotic and abiotic factors on the physiological condition of the fishes. Therefore, it can be used as an index to assess the status of the aquatic ecosystem in which fish live (Anene 2005). The results of the K_R value indicated good health and better environmental conditions for all the studied species.

The form factor *a*3:0 can be used to determine whether the body shape of a given population or species is significantly different from others (Froese 2006). The form factor varied from 0.006 to 0.014 for 34 species showing the fishes in the range of the elongated body shape (Table 2 and Fig. 1).

The LWRs of 22 species, provided in this paper, have not hitherto been available in FishBase (Froese and Pauly 2021). The results of this study provide useful information for fisheries management, fish population dynamic studies, and comparisons in future studies.

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PENSOFT.

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Revalidation of the genus *Ichthyocoris* Bonaparte, 1840 (Actinopterygii: Blenniiformes: Blenniidae)

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Abstract

Combtooth blennies belonging to the genus *Salaria* were known to have marine and freshwater species. However, recent molecular studies highlighted this genus as paraphyletic, clearly distinguishing both marine and freshwater species. In this paper, we revalidate the genus *Ichthyocoris*, which corresponds to the freshwater species: *Ichthyocoris atlantica* (Doadrio, Perea et Yahyaoui, 2011), new combination, *Ichthyocoris economidisi* (Kottelat, 2004), new combination, and *Ichthyocoris fluviatilis* (Asso y del Rio, 1801), new combination. It is distinguishable by the presence of brownish bars on the flanks not contrasted with black dots conferring a marble coat, a dorsal fin slightly notched between spined and soft rays (except for *I. atlantica*), 16–18 dorsal-fin soft rays, 16–20 anal-fin soft rays, 34–38 vertebrae, 8–9 circumorbital pores, 8–11 preopercular pores, and 3 supratemporal pores. The genus *Salaria* corresponds to the marine species *Salaria basilisca* (Valenciennes, 1836) and *Salaria pavo* (Risso, 1810).

Keywords

combtooth blennies, generic concept, integrative taxonomy, *Salaria*

Introduction

Combtooth blennies are small benthic fish belonging to the family Blenniidae with a worldwide distribution in the marine environment, but some taxa are also encountered in fresh or brackish waters. They inhabit coastal, intertidal, rocky areas, coral reefs, and mangroves. Blenniids are divided into five subfamilies, 57 genera, and almost 400 species (Hastings and Springer 2009). Blenniids' classification, based on morphological and anatomical data, was the subject of extensive discussions (Norman 1943; Springer 1968; Smith-Vaniz and Springer 1971; Springer and Smith-Vaniz 1972; Papaconstantinou 1977a, 1977b; Bath 1977, 1996, 2001; Zander 1978; Bock and Zander 1986; Williams 1990). However, the molecular advent has brought taxonomical changes (Stepien et al. 1997; Almada et al. 2005, 2009; Hundt et al. 2014; Vecchioni et al. 2019; Mehraban et al. 2021).

In this study, we focused on the genus *Salaria* Bath [ex Forsskål], 1977 (see Fricke 2008 for nomenclature changes in relation to *Salaria* Forsskål, 1775). This genus, belonging to the Salariinae subfamily and the Parableniini tribe (Hastings and Springer 2009), is characterized by a dorsal fin with XII to XIV spines and 15 to 25 rays, an anal fin with II spines and 18 to 26 rays, pectoral fins with 13 to 15 rays, absent or small supraorbital and nasal tentacles, a lateral line formed by anterious tubes more or less continuous, one row of teeth with one canine on each side of each jaw with 14 to 35 teeth on the upper jaw and 14 to 25 teeth on the lower jaw, a toothed vomer, a wide gill opening, a gill membrane not joined with isthmus and a sexual dimorphism, with the presence of a crest on the male's head (Norman 1943; Bath 1977, 2001; Krupp and Schneider 1989; Gharred and Ktari 2001; Orlando-Bonaca and Lipej 2010). It includes five species, two marine and three freshwater: *Salaria basilisca* (Valenciennes, 1836) occurring in the Mediterranean Sea, *Salaria pavo* (Risso, 1810) found in the eastern Atlantic (Bay of Biscay, Iberic coast) and the Mediterranean Sea (Almada et al. 2001), *Salaria fluviatilis* (Asso y del Rio, 1801) occurring in coastal catchments of the Mediterranean basin and the Atlantic Guadiana drainage in Spain (Perdices et al. 2000; Kottelat and Freyhof 2007), *Salaria economidisi* Kottelat, 2004 which is endemic to the lake Trichonis in Greece (Kottelat 2004), and *Salaria atlantica* Doadrio, Perea et Yahyaoui, 2011 which is endemic to the Sebou drainage in Morocco (Doadrio et al. 2011). All these species are distinguishable according to morphology, molecular and ecology data, as well as color patterning (Perdices et al. 2000; Kottelat 2004; Almada et al. 2009; Orlando-Bonaca and Lipej 2010; Doadrio et al. 2011; Geiger et al. 2014; Belaiba et al. 2019; Vecchioni et al. 2019; Tiralongo 2020; Wagner et al. 2021). Nuclear markers also highlighted that the two marine species *S. basilisca* and *S. pavo* can hybridize (Belaiba et al. 2019).

The genus *Salaria* has also a complex taxonomic history: these blennies species belonged first to the genus *Blennius* Linnaeus, 1758 (see Bath 1973) or as a *Salaria* subgenus (Norman 1943), which was later split into several genera by Bath (1977), thus revalidating *Salaria*. Using morphological data, Bock and Zander (1986) included *Salaria* within the genus *Lipophrys* Gill, 1896. Finally, molecular data strongly support the validity of the genus *Salaria* (see Almada et al. 2005, 2009; Wagner et al. 2021). However, both marine and freshwater species are well discriminated with a common ancestor estimated at the Miocene (around 35–5 Ma according to studies of Almada et al. 2009; Belaiba et al. 2019; Wagner et al. 2021). Moreover, this genus seems to be paraphyletic according to mitochondrial and nuclear markers (Hundt et al. 2014; Vecchioni et al. 2019, 2022).

The type species of the genus *Salaria* Bath [ex Forsskål], 1977, designated by Fricke (2008), is *Gadus salaria* Walbaum [ex Forsskål], 1792 and is a *nomen oblitum* synonym of *Salaria basilisca*. So, the genus *Salaria* corresponds to the marine clade. Bonaparte (1840) described the genus *Ichthyocoris* corresponding to freshwater blennies. Its type species, designated by Jordan (1919), is *Salarias varus* Risso, 1827 which is a junior synonym of *Salaria fluviatilis* (see Fricke et al. 2007). *Ichthyocoris* is currently considered as a *nomen oblitum* synonym of *Salaria* Bath [ex Forsskål], 1977 (see Fricke 2008).

The generic concept is widely discussed by Dubois (1982, 1988a, 1988b). According to him, a genus is considered as monophyletic, a genetic unit with the possibility of hybridization between species, and an ecological unit as genera occupy defined adaptative zones. Marine and freshwater *Salaria* fill these conditions, even if no hybridization between freshwater species was highlighted due to the absence of sympatry. According to Freyhof and Yoğurtçuoğlu (2020), a genus should be also diagnosable morphologically. In this paper, we bring a morphological diagnosis in order to validate the genus *Ichthyocoris* following an integrative taxonomy approach (e.g., Padial et al. 2010; Schlick-Steiner et al. 2010).

Material and methods

In order to distinguish genera, we did a bibliographical review of diagnoses published (Wirtz 1976; Bath 1977; Papaconstantinou 1977a, 1977b; Gharred and Ktari 2001; Kottelat 2004; Kottelat and Freyhof 2007; Doadrio et al. 2011; Kara and Quignard 2018; Keith et al. 2020) as well as the original descriptions of both genera *Salaria* and *Ichthyocoris* (see Bonaparte 1840; Bath 1977) and their type species respectively *Gadus salaria* and *Salarias varus* (see Walbaum 1792; Risso 1827). Color patterning characterization follows Orlando-Bonaca and Lipej (2010). We used available pictures on GBIF and Kottelat (2004) as well as our own pictures to characterize color patterning. X-ray pictures were also taken on specimens in collections. Notation for dorsal and anal-fin rays' counts follows Hubbs and Lagler (1947). The following characters were examined: color patterning, dorsal-fin rays, anal-fin soft rays, pectoral-fin rays, pelvic-fin rays, number of teeth on each jaw, number of vertebrae, the form of the supraopercular tentacle.

Abbreviations used. GBIF, Global Biodiversity Information Facility; **ICZN**, International Code of Zoological Nomenclature; **MHNG**, Muséum d'Histoire naturelle de Genève, Genève; **MNCN**, Museo Nacional de Ciencias Naturales, Madrid; **MNHN**, Muséum national d'Histoire naturelle, Paris; **SL**, standard length; **USNM**, Smithsonian Institution National Museum of Natural History, Washington DC.

Comparative material. *Salaria basilisca* (Valenciennes, 1836): Italy • 1; Syntype of *Blennius basiliscus*; Mediterranean Sea at Genoa; MNHN-IC-A-1779 • 1; Syntype of *B. basiliscus*; Mediterranean Sea in Sardinia; MNHN-IC-A-1829. FRANCE • 2; Syntypes of *B. basiliscus*; Mediterranean Sea at Toulon; MNHN-IC-A-1842. Greece • 4; Evoikos Gulf; MNHN-IC-1975-0497 •1; Adriatic Sea; USNM RAD 106716 • see Bath (1977).

Salaria pavo (Risso, 1810): France • 3; Mediterranean Sea at Nice; MNHN-IC-A-1851 • 1; Mediterranean Sea at Nice; MNHN-IC-A-1852 • 1; Mediterranean Sea at Nice; MNHN-IC-A-2137 • 1; Mediterranean Sea at Nice • 1; Mediterranean Sea at Nice; MNHN-IC-A-2138 • 1; Etang de Thau at Mèze; 43°25′09″N, 003°36′09″E; MNHN-IC-2012-0250 • 1; Atlantic Ocean at Trégunc, 47°51′24″N, 003°53′12″W; MNHN-IC-2012-0252. Spain • 1; Balearic Islands at Minorca; 39°48′53″N, 004°17′05″E; MNHN-IC-2012-0254 • see Bath (1977).

Systematic account

Family Blenniidae

Ichthyocoris Bonaparte, 1840

(Fig. 1, Table 1)
Feminine gender

Type species. *Salarias varus* Risso, 1827.

Synonyms. *Salariopsis* Vecchioni, Ching, Marrone, Arculeo, Hundt et Simons, 2022 (see remarks below).

Included species. Three species: *Ichthyocoris atlantica* (Doadrio, Perea et Yahyaoui, 2011), new combination; *Ichthyocoris economidisi* (Kottelat, 2004), new combination; *Ichthyocoris fluviatilis* (Asso y del Rio, 1801), new combination.

Material examined. *Ichthyocoris atlantica*: MOROCCO • 1; Ouerrha River at Ouazzane; MNCN 280135 (2, 48–61 mm SL) • see Doadrio et al. (2011).

Ichthyocoris economidisi: GREECE 10; Lake Trichonis; MNCN 120747–120756 • see Kottelat (2004) and Doadrio et al. (2011).

Ichthyocoris fluviatilis: FRANCE • 2; Têt River at Perpignan; 42°42′21″N, 002°54′04″E; Persat and Denys coll. leg.; MNHN-IC-2013-0674. SPAIN • 10; Ebro River at Zaragoza; MNCN 13657–13666 • see Kottelat (2004) and Doadrio et al. (2011).

Diagnosis. *Ichthyocoris* is distinguishable from *Salaria* by the presence of brownish bars on the flanks not contrasted with black dots conferring a marble coat (Fig. 1) (vs. brownish bars on the flanks very contrasted with blue stripes and dots conferring a marbled coat; Fig. 2); dorsal-fin slightly notched between spined and soft rays (Fig. 1) except for *I. atlantica* (vs. not notched; Fig. 2); 16–18 dorsal-fin soft rays (vs. 21–27); 16–20

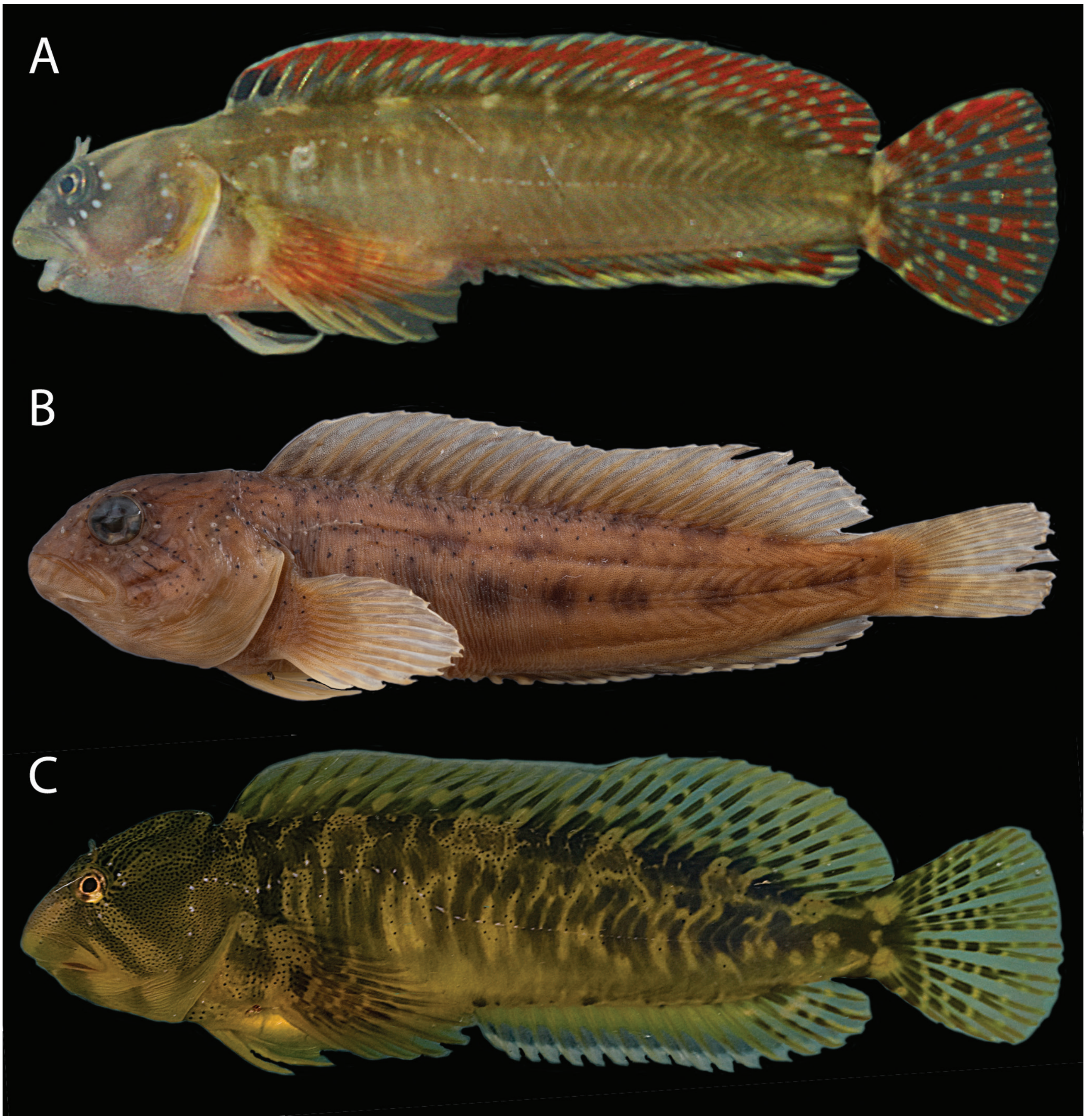

Figure 1. Lateral view of *Ichthyocoris* spp.: *I. atlantica*, MNCN 280135, 61 mm SL, Morocco, Ouerrha River (Sebou drainage) at Ouazzane (**A**; photo credits: I. Doadrio); *I. economidisi*, MHNG 2641.89, holotype, 60.8 mm SL, Greece, Lake Trichonis east of Panetolio (**B**; photo credits: R. Covain); *I. fluviatilis*, 89 mm SL, Spain, Jerea River (Ebro drainage) at Virués (**C**; photo credits: I. Doadrio).

Table 1. Meristic characters characterizing *Ichthyocoris* and *Salaria* species.

Character	*Ichthyocoris*			*Salaria*	
	I. atlantica	***I. economidisi***	***I. fluviatilis***	***S. basilisca***	***S. pavo***
Dorsal-fin rays	XII–XIII 16–17	XII–XIII 16–17	XII–XIII 16–18	XI–XIII 23–27	XI–XIII 21–24
Anal-fin rays	II 16–17	II 16–20	II 16–20	II 24–28	II 20–26
Vertebrae	34	36–37	34–38	40–44	38–42
Teeth upper jaw	13–15	25–30	16–24	25–33	19–28
Teeth lower jaw	14–16	20–27	16–20	22–28	16–23
Circumorbital pores	8–9	9	8	6–7	7
Preopercular pores	8–9	(9)10–11	9–10	6–8	6–8
Supratemporal pores	3	3	3	2	2

Figure 2. Lateral view of *Salaria* spp.: *S. basilisca*, MNHN-IC-A-1779, syntype, 138 mm SL, Italy: Mediterranean Sea at Genoa (**A**; photo credits: J. Pfliger); *S. pavo*, MNHN-IC-2012-0254, 74 mm SL, Balearic Islands at Menorca (**B**; photo credits: S. Iglesias).

anal-fin soft rays (vs. 20–28); 34–38 vertebrae (vs. 38–44); 8–9 circumorbital pores (vs. 6–7); 8–11 preopercular pores (vs. 6–8); 3 supratemporal pores (vs. 2) (Table 1).

Distribution. *Ichthyocoris* is present in drainages of the Mediterranean basin, in catchments of the Atlantic coast in Morocco and Spain as well as in the Black Sea.

Ecology. All *Ichthyocoris* species occur in freshwaters. However, due to their marine ancestry, *I. fluviatilis* has a one-month planktonic larval phase (Gil et al. 2010) and a high tolerance for salt water (Plaut 1998), allowing migration through marine waters (Perdices et al. 2000; Almada et al. 2009; Laporte et al. 2016; Méndez et al. 2019; Wagner et al. 2021).

Remarks. Comparing our diagnosis with the description of *Salarias varus* from Risso (1827), the three species do belong to the genus *Ichthyocoris*: the presence of dark dots on the body, 29 dorsal-fin spined and soft rays, and 19 anal-fin spined and soft rays. In the same fashion, comparing characters of *S. basilisca* and *S. pavo* with the diagnosis of *Gadus salaria* from Walbaum (1792), both species belong to the genus *Salaria*: the presence of 36 dorsal-fin spined and soft rays and 26 anal-fin spined and soft rays.

Other characters may discriminate between both genera: Papaconstantinou (1977a) distinguished *I. fluviatilis* from *S. basilisca* and *S. pavo* by the two lateral ethmoid bones between the median ethmoid and the vomer (vs. median ethmoid connected to the vomer). However, this character state is shared by other blenniids and needs to be checked within the two other *Ichthyocoris* species. Similarly, karyotype studies pointed out differences between *I. fluviatilis* and *S. pavo* (heterochromatin concentrated on the entire arm of two chromosome pairs like *Lipophrys* spp., vs. homogeneous distribution of heterochromatin like *Parablennius* spp. (Cataudella and Civitelli 1975; Unal et al. 2016). This character needs to be explored as well to characterize both *Ichthyocoris* and *Salaria* genera.

In a recent study, Vecchioni et al. (2022) also split the genus *Salaria*, distinguishing marine *Salaria* spp. from freshwaters species. They described a new genus *Salariopsis* with *Blennius fluviatilis* Asso y del Rio, 1801 as type species (mentioning erroneously the new combination *Salariopsis fluviatilis*) and grouping as a new combination *Salariopsis fluviatilis*, *Salariopsis economidisi*, and *Salariopsis atlantica*. This new genus is distinguished from *Salaria* by 16–17 dorsal-fin soft rays (vs. 22–27) and 16–19 anal-fin soft rays (vs. 23–28) from only bibliographical references (Bath 1977; Kottelat 2004; Doadrio et al. 2011; Tiralongo 2020). Their diagnosis is similar to ours for *Ichthyocoris* for these two characters. Thus, *Salariopsis* and *Ichthyocoris* designate the same taxa. However, the nomen *Ichthyocoris* Bonaparte, 1840 is older than *Salariopsis* Vecchioni, Ching, Marrone, Arculeo, Hundt et Simons, 2022. Thus, the principle of priority must be applied (art. 23.1 ICZN): *Ichthyocoris* must be the valid nomen and *Salariopsis*, its junior synonym.

Kottelat (2004) suspects a population from Lake Kinneret (Israel) to belong to a distinct species. Molecular studies confirm that populations of the Eastern Mediterranean basin form a distinct evolutionary lineage (Doadrio et al. 2011; Geiger et al. 2014; Belaiba et al. 2019; Wagner et al. 2021). Another evolutionary lineage in the Guadiana catchment of the Atlantic basin from Spain was also highlighted with molecular data (Perdices et al. 2000; Almada et al. 2009; Doadrio et al. 2011; Belaiba et al. 2019; Méndez et al. 2019; Wagner et al. 2021). These two evolutionary lineages may correspond to two new *Ichthyocoris* species if morphological characters were to be found.

Identification key of blenniids genera belonging to the Parablenniini tribe (adapted from Bath (1977), Chirichigno and Vélez (1998), Orlando-Bonaca and Lipej (2010), and Tiralongo (2020)

1 Gill opening wide, branchiostegal membrane not fused with the body..........2
– Gill opening restricted to the side of the head, branchiostegal membrane fused with the body..........11
2 Absence of supraorbital tentacles..........3
– Presence of supraorbital tentacles..........5
3 Triangular, fleshy skin flap in the anterior neck area. Canines only in the lower jaw.......... *Coryphoblennius*
– No triangular, fleshy skin flap in the anterior neck area. Canines in both jaws..........4
4 13 pectoral-fin rays. Body laterally compressed posteriorly. Relatively large mouth with a thick upper lip. General body color yellowish with dark brownish vertical bars. Dark brownish eyespot behind the eye. Mature males without bright coloration on cheeks.......... *Lipophrys*
– 12 pectoral-fin rays. Body well compressed laterally. Small mouth with thin lips. Absence of eyespot behind the eye. Mature males with bright yellow cheeks..........*Microlipophrys*
5 Canines in both jaws..........6
– Canines only in the lower jaw..........*Scartella*
6 Presence of teeth on the vomer..........7
– Absence of teeth on the vomer..........9
7 Supraorbital tentacles well developed, 3 mm in height minimum. Orbital canal with 2–3 rows of pores. I 4 pelvic fin rays. Male without any neck crest..........*Aidablennius*
– Supraorbital tentacles hardly visible, less than 3 mm in height. Orbital canal with only one row of pores. I 3 pelvic fin rays. Male with neck crest..........8
8 Presence of brownish bars on the flanks not contrasted with black dots conferring a marble coat. Dorsal fin notched between spined and soft rays (except for *I. atlantica*). 16–18 dorsal fin soft rays. 16–19(20) anal fin soft rays. 34–38 vertebrae. 8–9 circumorbital pores. 8–11 preopercular pores. 3 supratemporal pores......*Ichthyocoris*
– Brownish bars on the flanks very contrasted with blue stripes and dots conferring a marble coat. Dorsal fin not notched between spined and soft rays. 21–27 dorsal fin soft rays. 20–28 anal fin soft rays. 38–44 vertebrae. 6–7 circumorbital pores. 6–8 preopercular pores. 2 supratemporal pores..........*Salaria*
9 12 pectoral fin rays..........*Bathyblennius*
– 13–14 pectoral fin rays..........10
10 14 pectoral fin rays. Presence of tentacles on the anterior nostril..........*Parablennius*
– 13 pectoral fin rays. Absence of tentacles on the anterior nostril..........*Lupinoblennius*
11 Presence of canines in both jaws..........*Hypleurochilus*
– Absence of canines in either jaw..........12
12 Body skin loose and flaccid, encompassing dorsal and anal fins. XII–XIV dorsal fin pines.......... *Chalaroderma*
– Body skin not loose and flabby, not reaching over the fins. XI–XII dorsal fin pines..........13
13 The skin of the dorsal fin extends over to the proximal quarter of the caudal.......... *Chasmodes*
– The skin of the dorsal fin does not extend to the caudal..........14
14 IX–X 25 dorsal fin rays. 24 anal-fin soft rays. 15–16 pectoral fin rays..........*Parahypsos*
– XI–XII 15–18 dorsal fin rays. 12–20 anal fin soft rays. 13–15 pectoral fin rays..........*Hypsoblennius*

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Ecological status of fish fauna from Razim Lake and the adjacent area, the Danube Delta Biosphere Reserve, Romania

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Abstract

The aim of this study was to determine the ecological status of fish fauna of Razim Lake under the conditions of the water salinity changing from brackish, almost 70 years ago, into freshwater nowadays. The natural processes of siltation and organic deposits, characteristic of Danube Delta lake complexes, intensified in the last decades and included also Razim Lake. The presently reported study of Razim Lake and the adjacent area was undertaken in 2020 with intention to cover fish fauna collected with three different sampling methods (electrofishing, gillnetting, and seining). For each sampling method, Catch per Unit Effort (CPUE), relative abundance, and biomass were determined, as well as selected ecological parameters to determine ecological status of richness species in the area. Published data included 55 fish species, mainly marine and euryhaline, but in 2020 only 43 species were reported. Also, the species composition shifted from marine ones to freshwater or euryhaline ones. Of those 43 species captured in 2020 from Razim Lake and neighboring areas, 39 were native and four were non-native, including a newcomer, the Chinese sleeper, *Perccottus glenii* Dybowski, 1877. Few species were migratory, reophilous, or reophilous-stagnophilous which rarely enter Razim Lake, but the majority were limnophilous or stagnophilous-reophilous species. Four species were dominant in terms of the abundance; *Blicca bjoerkna* (Linnaeus, 1758); *Rutilus rutilus* (Linnaeus, 1758); *Alburnus alburnus* (Linnaeus, 1758); and *Carassius gibelio* (Bloch, 1782). In terms of the biomass the dominants were: *Cyprinus carpio* Linnaeus, 1758; *Carassius gibelio*; *Sander lucioperca* (Linnaeus, 1758); *Pelecus cultratus* (Linnaeus, 1758); and *Blicca bjoerkna*. Some differences between sampling methods used were observed. Eudominant, euconstant, and main species were *Blicca bjoerkna* and the majority of fish species were accessories, with differences amongst sampling methods used. Fish diversity parameters indicate a stable ichthyocoenosis, more stable along the lake shoreline. Ecological indicators of fish fauna from Razim Lake in 2020 grade the water lake quality as a moderate ecological class according to the Water Framework Directive of the European Union.

Keywords

fish species richness, abundance, biomass, fish ecology indicators, water ecological status

Introduction

The Razim–Sinoie lake complex is situated in the southern part of the Danube Delta Biosphere Reserve (DDBR) and formed in an old gulf of the Black Sea—Halmirys—with water surface of 86 770 ha. The largest lake in the complex is Razim Lake with 41 400 ha (Gâştescu 1971; Gâştescu and Ştiucă 2008;

Staras, unpublished[*]). The lake complex has two connections with the Black Sea from Sinoie Lake through the Periboina and Edighiol canals. These two openings to the sea maintain fish diversity and productivity of the entire lake complex (Staras, unpublished). Razim Lake is connected with Sinoie Lake through two canals (named Canal II and Canal V) that provide slightly brackish water for Razim Lake. The hydrotechnical works of the early 1970s transformed Razim Lake into a reservoir with 1 billion m^3 of freshwater (Staras, unpublished). Moreover, the salinity of Razim Lake changed over a short time, as proven by Leonte et al. (1956, 1960), from 2.5‰ in 1951 to 0.5‰in 1956 due to the freshwater influx from the Danube River. The Danube River, via the Sfântu Gheorghe arm as a major path, transports water and solids into Razim Lake via the Dunăvăț, Dranov, Mustaca, and Lipoveni canals. The mean monthly flows on Sf. Gheorghe arm indicate 9.66% of total flow ($135 \ m^3 \cdot s^{-1}$ liquid flows) and almost 2 million $t \cdot year^{-1}$ (solid flows) from the total flows of the arm by continuous lateral discharge to the Razim system (Driga 2004). The general water balance shows that the share of inputs is 90% from supply canals (Dranov, Dunăvăț, and Lipoveni canals), 9% from precipitation, and 1% from small rivers (Slava, Taița, Telița, Agighiol) and the exits from the system are represented by evapotranspiration (15%) and 85% irrigation and evacuation (Bondar cited by Staras, unpublished). The Danube River is the water supplier for all Danube Delta lakes including Razim Lake with which it has also an active exchange of fish fauna, especially at high river water levels because of the high degree of siltation of connecting canals in 2020. The diversity and structure of the fish community varies amongst lakes and can be regarded as a good indicator of the ecological state of the lakes. The aim of this study was to describe the ecological status of fish fauna from Razim Lake and the adjacent area, based on a fish survey conducted in 2020 and to discuss changes, based on earlier scientific publications.

Materials and methods

Study area, sampling period, fish, and water measurements. The study area was represented by five sectors of Razim Lake, a large-surface lake: Fundea Gulf (1), Holbina Gulf (2), southern lake (3), Mustaca sector north and south and Oaia Lake (4), west Lake Enisala (5), and canals (Dunăvăț, Mustaca, Dranov) (Fig. 1), with each sector being sampled at multiple sites. The ichthyofauna was sampled in Razim Lake and the adjacent area in July, August, and September of 2020. For biometric measurements, an ichthyometer with an accuracy of 1 mm per 50 cm for fish length and for weight, an electronic scale with an accuracy of 1 g per 5 kg were used. Geographical coordinates and physical-chemical parameters observed in the area were recorded with a Garmin device and Hach multiparameter, as well as a Secchi disc for water depth and transparency.

Figure 1. Investigation area from sectors of Razim Lake in the year 2020 (1 = Fundea Gulf, 2 = Holbina Gulf, 3 = South Lake, 4 = Mustaca sector north and south and Oaia Lake, 5 = west Lake Enisala).

Fish sampling. The fish sampling and Catch per Unit Effort calculation (CPUE) was done in accordance with EU recommendations by use of common methods:

- Electriofishing with SAMUS 1000 W electrofisher device, transect with multiple electric points during 10 min per site, the catch being standardized at individuals or $g \cdot h^{-1}$ of fishing effort (for shoreline or small canals from compact reed developed nearby lake).
- Passive gillnet fishing (stationary 12 h by night, the catch being standardized at 100 m^2 gillnets per night):

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[*] Staras M (1995) Studiul populațiilor piscicole din complexul de lacuri Razim–Sinoie și elaborarea strategiei de pescuit.[Study of the fish populations in the Razim-Sinoie lake complex and elaboration of the fishing strategy.] PhD thesis, University Dunărea de Jos, Galați, Romania. [In Romanian]

commercial gillnets or Nordic gillnets multi-meshes fishing tools (30 m length × 1.8 m high each). The Nordic gillnets have 12 randomly joined panels, each panel being 2.5 m in length, with multiples meshes: 6, 6, 8, 10, 12, 16, 20, 24, 30, 35, 45, and 55 mm (Nyberg and Degerman 1988; Năvodaru 2008) (main tools used in Razim Lake and adjacent area).

- Seine fishing with 2 wings of 100 m length each and a codend of 7 mm knot-to-knot mesh size. Standardization to one haul of active fishing (1 h).
- Directly observed species from angling and some traditional fishing tools (fyke net, hand cast net, fish landing) just for fish species identification, without other standardization.

Taxonomy and ecology. The fish species scientific names used are consistent with the Eschmeyer's Catalog of Fishes (Fricke et al. 2021). The specimens collected were identified after Antipa (1909), Cărăusu (1952), Bănărescu (1964), and taxonomic name and support knowledge after revision by some authors (Otel et al. 1992, 1993; Kottelat 1997; Otel 2001, 2007; Sindrilariu et al. 2002; Nelson 2006; Kottelat and Freyhof 2007; Năvodaru and Năstase 2011; Năstase et al. 2017, 2019a; Froese and Pauly 2021; Năstase, unpublished*). Relative abundance and biomass for each species and sampling methods were calculated as standard CPUE (Catch Per Unit Effort). The relative abundance or dominance (*D*) for each species and sampling methods was calculated as the proportion of species to total catch (Mühlenberg 1993; Sindrilariu et al. 2002). The relative abundance or dominance (*D*) for each species and sampling methods was calculated as the proportion of species to total catch ($D_i = n_i \cdot 100N^{-1}$ (%), where, D_i = dominance of species *i*, *ni* = individuals of the species *i*, and *N* = total number of individuals) (Mühlenberg 1993; Sindrilariu et al. 2002). The frequency of occurrence (*F*) or constancy (*C*) for each species and sampling method was calculated as the proportion of samples containing a species from the total number of samples ($C_i = b_i \cdot 100a^{-1}$ (%), where, C_i = frequency of occurrence of species *i*, b_i = the number of samples in which species *i* was observed and *a* = total number of samples) (Schwerdtfeger 1975; Sindrilariu et al. 2002). Ecological significance (*W*) is a relation between frequency (*C*) and dominance (*D*) ($W = D \cdot 100C^{-1}$). For frequency, five classes were used; six classes were used for abundance/dominance data analysis, and seven classes were used for ecological significance (Table 1).

To determine ecological status of the lake, some quantitative ecological parameters were chosen as most expressive for fish communities: Relative Abundance in Number per Unit Effort (NPUE), Relative Biomass in Biomass Per Unit Effort (BPUE), the biodiversity index according Shannon–Wiener Index H_s, and Equitability Index = Evenness index (*E*) as in Năstase et al. (2019a, 2021) (Table 2).

An ecological status classification matrix in accordance with the Water Framework Directive (WFD) is presented in Table 2 regarding the fish community. The Biodiversity Index (H_s), according to the Shannon–Wiener formulae, as well as maximal fish Diversity (H_{max}) and Equitability (Evenness) Index (*E*) were calculated. The Equitability Index describes the quantum of unequal distribution of different effective species proportion as an ideal community, ranges between 0 and 1. The Shannon–Wiener Index varies from values of 0 for communities with one species, to various other values for more mixed species (Odum 1975; Botnariuc and Vădineanu 1982; Gomoiu and Skolka 2001; Sârbu and Benedek 2004). Formulas used:

$$H_s = -\Sigma\, p_i \cdot \ln(p_i)$$

according Shannon–Wiener formulae

$$p_i = N_r \cdot N^{-1}$$

Table 1. Frequency (constancy), dominance, and ecological significance classification according to: Botnariuc and Vădineanu 1982; Gomoiu and Skolka 2001; Șindrilariu et al. 2002 Sârbu and Benedek 2004.

Category	Symbol	[%]
Dominance		
Sporadic	D1	<1
Subrecedent	D2	1–2
Recedent	D3	2–4
Subdominant	D4	4–8
Dominant	D5	8–16
Eudominant	D6	>16
Constancy		
Very rare	C1	0.0–10.0
Rare	C2	10.1–25
Widespread	C3	25.1–45.0
Frequent	C4	45.1–70.0
Very frequent	C5	70.1–100
Ecological significance		
Accidental-adventitious	W1A	<0.001
Accidental	W1	<0.1
Accessory	W2	0.1–1.0
Associate	W3	1.0–5.0
Complementary	W4	5.0–10.0
Characteristic	W5	10.0–20.0
Main, leading	W6	>20

Table 2. Ecological matrix class for fish parameters assessment in accordance with the WFD (expert judgement based) according to the "one out, all out" principle.

Status	Color	Class	NPUE (*n*)	BPUE [g]	H_s	*E*
Very bad	Red	I	< 25	< 500	< 1	< 0.2
Bad	Orange	II	25–100	500–2000	1.0–1.4	0.2–0.4
Moderate	Yellow	III	100–250	2000–5000	1.4–1.8	0.4–0.6
Good	Green	IV	250–500	5000–10000	1.8–2.2	0.6–0.8
Very good	Blue	V	>500	>10000	>2.2	>0.8

NPUE = Number Per Unit Effort, BPUE = Biomass Per Unit Effort, H_s = Shannon–Wiener Biodiversity Index, E = Evenness Index (Equitability Index).

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[*] Năstase A (2009) Cercetări asupra diversității ihtiofaunei din Delta Dunării pentru exploatarea durabilă a resurselor piscicole. [Researches of ichthyofauna diversity in the Danube Delta for sustainable management of fish resources.] PhD thesis, University Dunărea de Jos, Galați, Romania. [In Romanian]

where p_i is the dominance; N_r is the number of individuals belonging to a certain species; and N = total number of individuals in a sample.

$$E = H_s \cdot H_{max}^{-1}$$

According to the Water Framework Directive, it is desirable to test and apply known ecological parameters that could improve the methods of assessing the ecological status, using, when no other methods are available, even expert judgement analysis (this analysis from papers was thought of and used in a European project in 2014: Black Sea e-Eye **-** Innovative Instruments for Environmental Analysis in NW Black Sea Basin, to improve methodology after Moss et. al. (2003) and Ibram et al. (2015). The ecological lake classification matrix is in accordance with the Water Framework Directive. EU Water Framework) has five (I–V) limits classes marked with different colors. Actually, there are yet no developed statistical threshold limits classes (I–V) for those chosen ecological parameters (NPUE, BPUE, H_s, E) according to the WFD water quality regarding fish, but expert judgement was used as a future proposal. Class limits was proposed by the present authors, based on field experience and expert judgement in the Danube Delta (Năstase et al. 2019a, 2021). In the summer of 2020, sampling was conducted using 77 Nordic gillnets, totaling 2310 m of passive nets per night, 190 minutes of electric fishing, five seine active hauls and 48 commercial gillnets 1440 m in total of passive nets-nights–1 in total (Table 3).

Table 3. Fishing tools used in Razim Lake in 2020 and their yield and effort.

Sampling site		N gillnets		Electr.	C gillnets		Seine	Total catch	
Name	No.	No.	L [m]	[min]	No.	L [m]	H No.	*N*	[g]
Enisala	5	14	420	30	4	120	5	1537	68653.9
Fundea	1	12	360	30	3	90	0	2029	83557.5
Mustaca N	4	12	360	30	7	210	0	789	87343
Mustaca S	4	12	360	30	6	180	0	843	86432
Center	4	3	90	0	15	450	0	385	46174
Holbina	2	12	360	30	6	180	0	1029	70585
Periteasca S	3	12	360	30	3	90	0	1245	76443
Canal Mustaca	4	0	0	10	0	0	0	114	33403
Oaia mare	4	0	0	0	4	120	0	71	20826
TOTAL Fish		**77**	**2310**	**190**	**48**	**1440**	**5**	**8042**	**573417.4**
Crayfish								36	1446

N gillnets = Nordic gillnets, Electr. = electrofishing device, C gillnets = commercial gillnets, H No. = number of hauls, *N* = number of fish/crayfish.

Results

In the summer of 2020, we captured 8042 fish individuals with more than 573 kg of fish and 36 individuals weighting in a total of almost 1.5 kg of crayfish (Table 3).

Species richness. All captured individuals belong to 43 fish species and one crayfish species—*Pontastacus leptodactylus.* Overall, Razim's ichthyofauna is dominated by limnophilous or stagnophilic-rheophilic species, such as white bream, *Blicca bjoerkna* (Linnaeus, 1758) and roach, *Rutilus rutilus* (Linnaeus, 1758), followed by characteristic-complementary-associated species, such as ziege, *Pelecus cultratus* (Linnaeus, 1758); European perch, *Perca fluviatilis* Linnaeus, 1758; pike-perch, *Sander lucioperca* (Linnaeus, 1758); common bream, *Abramis brama* (Linnaeus, 1758); bleak, *Alburnus alburnus* (Linnaeus, 1758); and gibel carp, *Carassius gibelio* (Bloch, 1782), but the majority of species occur sporadically in the Lake, with a significant number of species being accidentally found here (Table 4). The numbers for the goby species—monkey goby, *Neogobius fluviatilis* (Pallas, 1814); round goby, *Neogobius melanostomus* (Pallas, 1814); racer goby, *Babka gymnotrachelus* (Kessler, 1857); bighead goby, *Ponticola kessleri* (Günther, 1861); syrman goby, *Ponticola syrman* (von Nordmann, 1840); mushroom goby, *Ponticola eurycephalus* (Kessler, 1874)—are worrying, as they are in a continuous decrease, being limited only to certain favorite places of the Lake, especially in the areas with submerged stones (used to avoid clogging of the mouths of the canals) and gravel areas, compared to the previous years when they dominated even sandy areas. It can be said that this phenomenon of numerical reduction of the gobies populations in Razim Lake is due to the obvious habitat changes which include increase of siltation, the mud of the Razim Lake transforming the lake into a pond, typical for lake complexes from the Danube Delta. Another question mark is the existence of percarina, *Percarina demidoffi* von Nordmann, 1840 (Percidae), a non-native not invasive, but sensitive species, first recorded 1986 (Otel and Bănărescu 1986). In recent years, it has not been found in Razim Lake, in the place where this species had formed vigorous populations in the past, even stronger populations than in its native range (Don River), the cause probably also being habitat change.

Out of the 43 fish species captured or observed in Razim Lake, nearly 1/3 are without commercial value (small fish) and 2/3 (30 fish species) have commercial value. From these 30 commercial fish species, more than 1/4 have high commercial value—pontic shad, *Alosa immaculata* Bennett, 1835; pike-perch, *Sander lucioperca*; Wels catfish, *Silurus glanis* Linnaeus, 1758; common carp, *Cyprinus carpio* Linnaeus, 1758; European eel, *Anguilla anguilla* (Linnaeus, 1758); and northern pike, *Esox lucius* Linnaeus, 1758). Almost half of the species have medium market value (like gibel carp, rudd, roach, tench, perch, bream, etc.) and almost 1/4 have low economic value (goby species). Of the 43 fish species, the majority are native and four are non-native species: Chinese sleeper, *Perccottus glenii* Dybowski, 1877; silver carp, *Hypophthalmichthys molitrix* (Valenciennes, 1844); grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844); pumpkinseed sunfish, *Lepomis gibbosus* (Linnaeus, 1758). While some of the species are migratory, reophilous or reofilous-stagnofilous, such as *Alosa immaculata*, *Anguilla anguilla*, and white-eye bream, *Ballerus sapa* (Pallas, 1814), occur rarely in the Lake, others are stagnofilous-reophilous or limnophilous species which are the majority. The stagnoph-

Table 4. Ecological significance of fish species from Razim Lake and the adjacent area (also included classes "Present = P" for species which could not be standardized, just observed).

Species	Nordic gillnets			Commercial gillnets			Electrofishing device			Seine			Other fishing gear
	D	C	W	D	C	W	D	C	W	D	C	W	
Abramis brama	D1	C2	W1	D4	C2	W3				D2	C3	W3	P
Alburnus alburnus	D4	C5	W4				D5	C5	W5	D4	C5	W4	P
Alosa immaculata	D1	C1	W1A										
Alosa tanaica	D2	C3	W2							D1	C2	W1	P
Pontastacus leptodactylus	D1	C3	W2	D1	C1	W1				D1	C3	W2	P
Atherina boyeri							D5	C2	W3				
Babka gymnotrachelus										D1	C3	W2	P
Ballerus sapa	D1	C1	W1A										
Blicca bjoerkna	D6	C5	W6	D2	C1	W1	D4	C4	W3	D6	C5	W5	P
Carassius carassius				D2	C1	W2							
Carassius gibelio	D1	C3	W2	D6	C5	W6	D4	C3	W3	D3	C5	W3	P
Clupeonella cultriventris	D6	C4	W4							D1	C2	W2	P
Cobitis tanaitica				D1	C1	W1							
Ctenopharyngodon idella										D1	C2	W1	P
Cyprinus carpio	D1	C1	W1A	D5	C3	W3	D4	C4	W3	D1	C2	W2	P
Esox lucius	D1	C1	W1	D1	C1	W1							P
Gymnocephalus cernuus	D1	C2	W2										
Hypophthalmichthys molitrix	D1	C1	W1A										
Knipowitschia caucasica							D2	C2	W2				
Lepomis gibbosus	D1	C1	W1A	D1	C1	W1	D1	C2	W1				
Leuciscus aspius	D1	C2	W1	D2	C2	W2				D1	C3	W2	P
Mugil cephalus	D1	C1	W1A										
Misgurnus fossilis	D1	C1	W1A										
Ponticola eurycephalus	D1	C1	W1A				D5	C2	W3				
Neogobius fluviatilis	D1	C1	W1				D1	C1	W1	D4	C4	W3	P
Ponticola kessleri							D2	C2	W2				
Neogobius melanostomus										D1	C2	W1	P
Pelecus cultratus	D5	C5	W5							D2	C5	W3	P
Perca fluviatilis	D4	C5	W4	D2	C1	W2	D4	C3	W3	D4	C5	W4	P
Perccottus glenii	D1	C1	W1A				D1	C1	W1				
Petroleuciscus borysthenicus	D1	C1	W1A										
Ponticola syrman	D1	C1	W1A							D1	C2	W1	P
Proterorhinus marmoratus							D1	C1	W1				
Pungitius platygaster							D1	C1	W1				
Rhodeus amarus	D1	C1	W1				D2	C2	W2				
Rutilus rutilus	D5	C5	W5				D6	C5	W6	D6	C5	W6	P
Sander lucioperca	D2	C4	W3	D4	C3	W3	D3	C3	W2	D5	C5	W5	P
Scardinius erythrophthalmus	D4	C4	W3	D1	C1	W1	D4	C3	W3	D5	C5	W4	P
Silurus glanis	D1	C1	W1A	D1	C1	W1	D1	C1	W1				
Syngnathus abaster	D1	C1	W1A							D1	C3	W2	P
Tinca tinca	D1	C1	W1	D4	C1	W2							
Umbra krameri							D1	C1	W1				
Vimba vimba	D1	C2	W1	D1	C1	W1							
Anguilla anguilla													P

ilous (limnophilous) species, like Caucasian dwarf goby, *Knipowitschia caucasica* (Berg, 1916) and mudminnow, *Umbra krameri* Walbaum, 1792, are very well represented in Razim Lake or the adjacent area.

Ecological status. The main species (eudominant, very frequent) in Razim Lake and adjacent waters are *Blicca bjoerkna*, *Rutilus rutilus* and *Alburnus alburnus*, but the majority of the species are accessory, as well as a significant percentage of species being accidental, with some differences between sampling methods (Table 4).

The parameters used in the ecological characterization of Razim Lake from the point of view of the ichthyofauna show that they fall into the moderate class, the majority of the indicators having moderate and good values, but according to the "one out, all out" principle there are some indicators in the moderate state class, which makes us assert that Razim Lake has a Moderate ecological status in 2020 (Table 5 and 6).

Some large fish individuals like *Sander lucioperca*, *Silurus glanis*, and *Abramis brama* were rarely found during our sampling campaign in Razim Lake, probably due to legal and illegal overfishing. Extensive poaching with nylon and small mesh-size gillnets fishing is one of the most dangerous practices in reducing the quality and size of fish populations in the area. There is no precise estimate of the extent of poaching in Razim Lake since 1990, but it is believed that poaching is threatening all animals, especially fishes. Razim Lake, the largest lake of Romania has always been fascinating for studies of fish fauna, especially due to the contact of freshwater with the brackish water, which make it a "natural biological laboratory" of living fish population species, with a lot of hybrid individuals or subspecies. The diversity indices of Razim Lake and adjacent water bodies indicate a stable ecosystem, so a stable fish coenosis, with values of equitability (E) more than medium 0.5 for each sampling method. Shannon–Wiener Index values are increased, the

Table 5. The ecological status of fish species from Razim Lake and the adjacent area according to Moss et al. (2003) (Pi = presence of locally native piscivores, Abex = absence of non-native species, Altd = either an absence of locally piscivores or presence of introduced species).

EcT	*T* [°C]	Ar [km²]	Geo	*C*	EcS	Fc	Fb	P:Z	FcR	FbR	P:Z/R
17	10–25	<100	Peat	101–800	High	Pi + Abex	5–20	>1			
					Good	Pi + Abex	5–20	>1			1.4
					Mod.	Pi or Abex	>20	0.5–1	Yes	68	
					Poor	Altd	>20	<0.5			
					Bad	Altd	<5	<0.5			

EcT = ecotype number, *T* = temperature of warmest month, Ar = area, Geo = catchment geology, *C* = conductivity [µS· cm⁻²], EcS = ecological status, Fc = fish community, Fb = fish biomass [g · m⁻²], P:Z = Piscivores:zooplanktivoures (ratio by biomass), FcR = fish community of Razim Lake, FbR = fish biomass of Razim Lake [g · m⁻²], P:Z/R = Piscivores/zooplanktivoures (ratio by biomass) of Razim Lake; Mod. = moderate, Pi = presence of locally native piscivores, Abex = absence of non-native species, Altd = either an absence of locally piscivores or presence of introduced species.

boundaries are more than 1.955 with the maximum on the shorelines or canals from the reed band on the shoreline of the Lake (Fig. 2).

Relative abundance and biomass. Relative abundance (CPUE) is dominated by bream species (especially white bream), roach, giebel carp, perch, bleak, rudd, and ziege, but for the majority of fish species, it has low values, with some differences between sampling methods (Fig. 3). Relative biomass (CPUE) was dominated by common carp, gibel carp, roach, white bream, ziege, perch, pikeperch, and rudd with some differences between sampling methods (Fig. 4).

Table 6. Ecological status of Razim Lake and the adjacent area (according to WFD) using the "one out, all out" principle for fish biological parameters.

Parameter	NPUE (A)	BPUE (B)	H_s	*E*
Nordic gillnets (NG)	158.6	6843.6	1.955	0.564
Ecological status NG	Moderate	Good	Good	Moderate
Electrofishing (E) values	126	18853.5	2.320	0.774
Ecological status E	Moderate	Very Good	Very Good	Good
Seine (S) values	136.8	7249.5	2.082	0.695
Ecological status S	Moderate	Good	Good	Good
TOTAL	**Moderate**	**Good**	**Good**	**Moderate**

A = relative abundance, NPUE; *B* = relative biomass, BPUE; H_s = Shannon–Wiener Biodiversity Index; *E* = Evenness Index; by type of sampling methods; Nordic gillnets (NG) values [No. of individuals (or grams) per 100 m² of nets per night]; Electrofishing (E) values individuals or [g · h⁻¹]; Seine (S) values [individuals (or grams) per haul].

Physico-chemical parameters of water. Geographical coordinates in some sites and physico-chemical parameters of water are presented in Table 7. Sampled water body points had depth between 25 and 250 cm, transparency 20–35 cm, conductivity 369–1183 µS · cm⁻¹, salinity did not exceed 0.5‰, dissolved oxygen 4.45–16.06 mg · L⁻¹, and oxygen saturation 55.1%–174.6% (Table 7).

Discussion

Since the 19th century, when Grigore Antipa drew attention to the decline in fish production in Razim Lake, reaching less than 1/3 of what it was 15 years before his studies

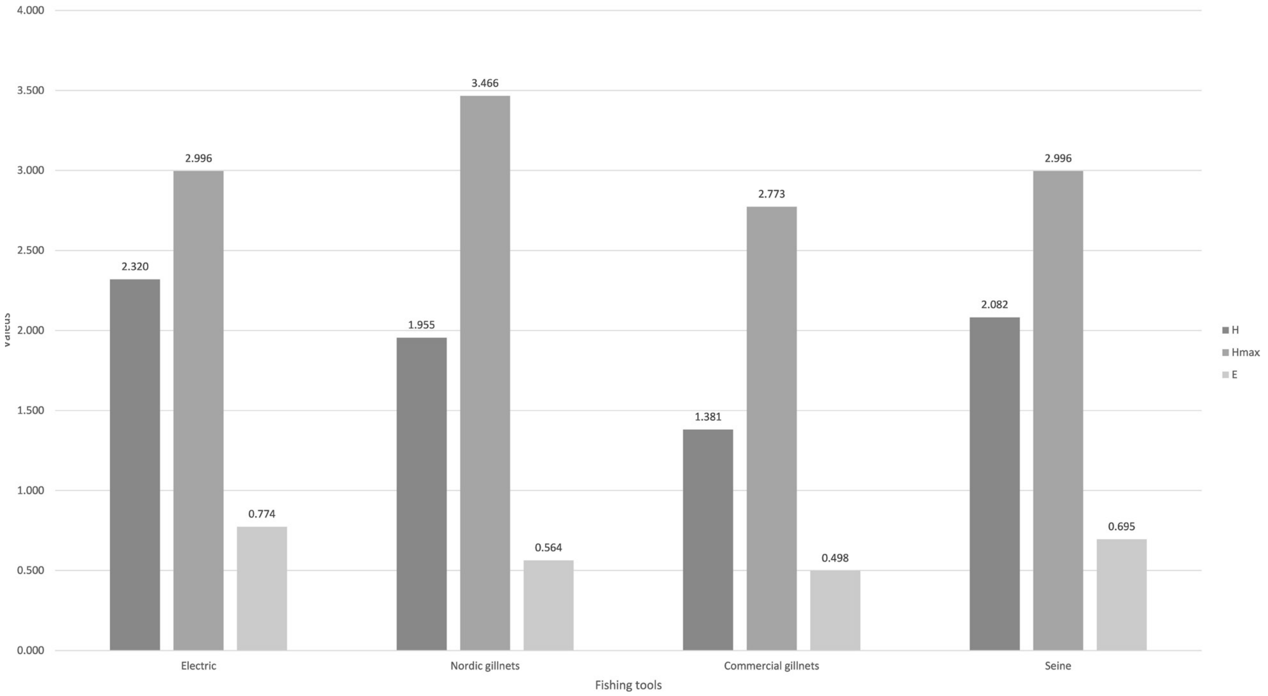

Figure 2. Comparative biodiversity indices between sampling methods in Razim Lake in 2020 (H_S = Shannon–Wiener Index, H_{max} = the maximal diversity, *E* = Evenness Indices.

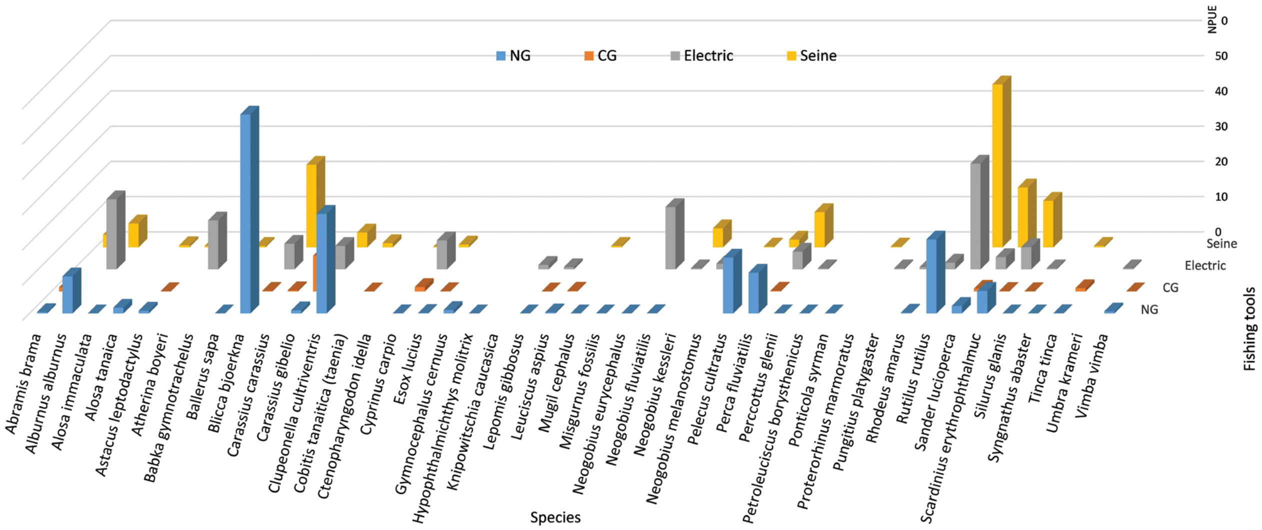

Figure 3. Relative abundance (CPUE = Catch per Unit Effort) in Razim Lake in 2020 (NG = Nordic gillnets, CG = commercial gillnets).

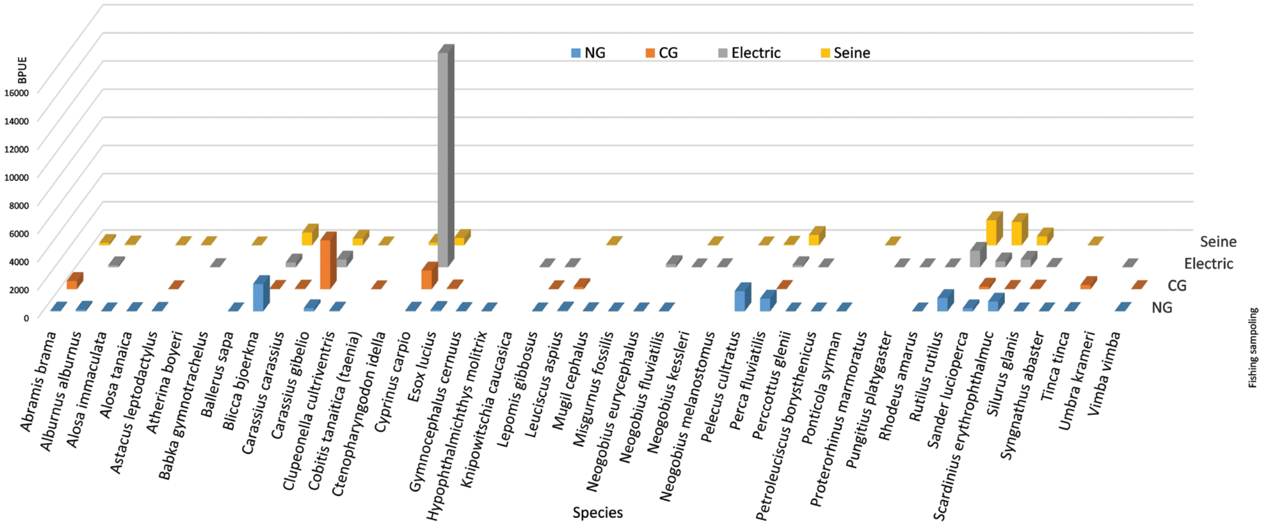

Figure 4. Relative biomass (BPUE = Biomass catch per Unit Effort) in Razim Lake in 2020 (NG = Nordic gillnets, CG = commercial gillnets).

(Antipa 1894), the trend in 2020 remains the same, mainly due to legal and illegal overexploitation, even with the appearance (1895 first fishing permit) and periodic updating of fishing laws. Even at the beginning of the 21st century, contravention of the fishing laws is usually not considered a serious offence in courts of law. In the past, the marine species entering Razim Lake in significant quantities were: blunt-snouted mullet, *Mullus ponticus* Essipov, 1927; Volga pikeperch, *Sander volgensis* (Gmelin, 1789); European flounder *Platichthys flesus* (Linnaeus, 1758); Black Sea turbot, *Scophthalmus maeoticus* (Pallas, 1814); beluga, *Huso huso* (Linnaeus, 1758); Danube sturgeon, *Acipenser gueldenstaedtii* Brandt et Ratzeburg, 1833; starry sturgeon, *Acipenser stellatus* Pallas, 1771; fringebarbel sturgeon, *Acipenser nudiventris* Lovetsky, 1828 (which is currently an extinct species in the Danube delta); garfish, *Belone belone* (Linnaeus, 1760); big-scale sand smelt, *Atherina boyeri* Risso, 1810; Mediterranean sand smelt, *Atherina hepsetus* Linnaeus, 1758; *Chelon auratus*; leaping mullet, *Chelon saliens* Risso, 1810; flathead grey mullet, *Mugil cephalus* Linnaeus, 1758; black goby, *Gobius niger* Linnaeus, 1758; knout goby, *Mesogobius batrachocephalus* (Pallas, 1814); *Alosa immaculata*; Black Sea shad, *Alosa tanaica* (Grimm, 1901); Atlantic mackerel, *Scomber scombrus* Linnaeus, 1758; bluefish, *Pomatomus saltatrix* (Linnaeus, 1766); *Anguilla anguilla*; and European anchovy, *Engraulis encrasicolus* (Linnaeus, 1758) (Antipa 1894; Leonte et al. 1960; Otel et al. 1992, 1993; Staras, unpublished), but succession of species happens due to changes in water salinity. Namely, in 2020, only rare, accidental entry of *Alosa immaculata*, *Anguilla anguilla* and some mullets species was observed, with higher presence of *Alosa tanaica*, *Atherina boyeri* and freshwater species. Historic data (Leonte 1969 cited by Staras, unpublished) cite around 55 fish species, a considerable number being marine and euryhaline. In 2020, 43 fish species were described in Razim Lake, with 39 native and four non-native (*Perccottus glenii*;

Table 7. Geographical coordinates and physical-chemical parameters observed in some fishing points from Razim Lake and the adjacent area in summer 2020.

Site code	Geographical coordinates	*T* [°C]	WD [cm]	TR [cm]	Sal [‰]	*C* [μS·cm⁻¹]	Ox [mg·L⁻¹]	OxS [%]
Raz_iul_20_N1	44.90654°N, 028.86275°E	28.7	160	35	<0.5	495	8.58	112.4
Raz_iul_20_N2	44.90374°N, 028.86633°E	30.2	180	35	<0.5	492	9.01	121
Raz_iul_20_N3	44.89632°N, 028.86646°E	28.7	140	35	<0.5	495	8.58	112.4
Raz_iul_20_N4	44.86862°N, 028.88374°E	23.0	130	30	<0.5	505	8.01	92.7
Raz_iul_20_N5	44.85979°N, 028.89621°E	22.8	180	30	<0.5	508	8.18	94.5
Raz_iul_20_SN1-12	44.893994°N, 028.865412°E	23.0	150	30	<0.5			
Raz_iul_20_Ave	44.898313°N, 028.871662°E	23.0	150	30	<0.5			
Raz_iul_20_E1	44.88736°N, 028.83898°E	26.6	80	20	<0.5	1142	14.06	174.6
Raz_iul_20_E2	44.88985°N, 028.84497°E	25.4	110	20	<0.5	531	9.72	118.8
Raz_iul_20_E3	44.89308°N, 028.82632°E	28.3	110	20	<0.5	1183	13.85	173
Raz_aug_20_SN1-12		25.0	150	30	<0.5			
Raz_aug_20_Ave		25.0	200	30	<0.5			
Raz_aug_20_E1	44.89899°N, 029.09472°E	25.3	250	20	<0.5	398	6.58	80.1
Raz_aug_20_E1	44.89899°N, 029.09472°E	25.9	250	20	<0.5	369	6.08	75.3
Raz_aug_20_E2	44.86952°N, 029.09857°E	25.6	50	25	<0.5	388	8.07	99.5
Raz_aug_20_E3	44.85786°N, 029.11197°E	26.0	80	35	<0.5	426	8.93	110.8
Raz_aug_20_E4	44.84264°N, 029.09601°E	25.8	120	35	<0.5	388	8.65	107.8
Raz_aug_20_E5	44.82828°N, 029.07246°E	25.7	130	30	<0.5	466	10.8	124.4
Raz_aug_20_E6	44.85986°N, 029.04191°E	25.5	140	30	<0.5	435	8.96	110.1
Raz_aug_20_E7	44.88725°N, 029.03616°E	25.7	90	35	<0.5	440	11.6	143.3
Raz_DrMus_aug_20_E1	44.90084°N, 029.03267°E	26.7	110	25	<0.5	438	11.8	147.7
Raz_Est_aug_20_E2	44.91323°N, 029.03304°E	25.8	90	25	<0.5	443	10.15	124.1
Raz_Duna_aug_20_E3	44.94065°N, 029.03714°E	26.1	25	25	<0.5	383	6.53	81.5
Raz_GoFu_aug_20_E4	44.94658°N, 029.05917°E	26.3	45	25	<0.5	445	12.81	159.2
Raz_GoFu_aug_20_E5	44.96377°N, 029.09998°E	26.9	80	20	<0.5	426	10.74	134.4
Raz_GoFu_aug_20_E6	44.98711°N, 029.09542°E	26.6	50	35	<0.5	431	9.1	113.6
Raz_Peri_aug_20_E1	44.78973°N, 029.13181°E	27.0	40	25	<0.5	424	10	126
Raz_Peri_aug_20_E2	44.80348°N, 029.13816°E	26.5	40	25	<0.5	394	10.33	130
Raz_Peri_aug_20_E3	44.83177°N, 029.1365°E	26.1	80	25	<0.5	381	4.45	55.1

T = water temperature, WD = water depth, Tr = transparency, Sal = salinity, *C* = conductivity, Ox = oxygen content, OxS = oxygen saturation (%).

Hypophthalmichthys molitrix; *Ctenopharyngodon idella*; and *Lepomis gibbosus*), compared to 44 fish species with seven non-native species—*Percarina demidoffi*; *Hypophthalmichthys molitrix*; *Ctenopharyngodon idella*; *Lepomis gibbosus*; stone moroko, *Pseudorasbora parva* (Temminck et Schlegel, 1846); black carp, *Mylopharyngodon piceus* (Richardson, 1846); and bighead carp, *Hypophthalmichthys nobilis* (Richardson, 1845)—found by Otel et al. (1993) and Staras (unpublished) in the Razim–Sinoie Lake complex in the 1990s. *Carassius gibelio* and *Cyprinus carpio* are given as native species from Central Europe to Siberia (Kottelat and Freyhof 2007; Otel 2019). The current living conditions favor the development of freshwater eutrophic species with less than 0.5‰ salinity, large variations in dissolved oxygen and increased quantities of nutrients in water. A new non-native fish species was recently recorded in the natural environment of the Lower Danube River Basin, *Perccottus glenii*, first recorded in the Romanian River Suceava (Nalbant et al. 2004). It was first recorded in DDBR by Năstase (2007). Its range has expanded to Razim Lake, being first recorded in 2016 in Holbina Gulf of Razim Lake (Năstase et al. 2019a). Its population has increased in the Danube Delta (Năstase et al. 2019b) also in the Razim–Sinoie Lake complex, having a strong invasive behavior (Vilizzi et al. 2021), well adapted to new biotope conditions in Razim Lake. Qualitative and quantitative decreases in species numbers and abundance is undesirable throughout the DDBR, not only for Razim Lake. For that reason, the absence of *Percarina demidoffi* is worrying, as well as the reduction in the number of goby species (*Ponticola syrman*, *Neogobius melanostomus*). Future studies and new actions to avoid their population collapse are necessary, in conditions of habitat change. Species, such as *Anguilla anguilla*; *Acipenser stellatus*; three-spined stickleback, *Gasterosteus aculeatus* Linnaeus, 1758; golden grey mullet, *Chelon auratus* (Risso, 1810); *Platichthys flesus*; schraetzer, *Gymnocephalus schraetser* (Linnaeus, 1758); and white-finned gudgeon, *Romanogobio albipinnatus* (Lukasch, 1933) were present in the Razim–Sinoie Lake complex in the 1990s (Otel et al. 1992, 1993; Staras, unpublished), some of them in considerable quantities. However, in 2020, only a few species in Razim Lake are migratory, reophilous or reofilous–stagnofilous, such as *Alosa immaculata*, *Anguilla anguilla*, and *Ballerus sapa*, which occur rarely in the Lake and the majority are stagnofilous–reophilous or limnophilous species.

Conclusions

The main species (eudominant, very frequent) in Razim Lake and adjacent waters were white bream, *Blicca bjoerkna*; roach, *Rutilus rutilus*; and bleak, *Alburnus alburnus*, but mostly are accessory, also a significant percentage of species being accidental, with some differences between sampling methods. Relative abundance (CPUE) was dominated by bream species (especially white bream), roach, gibel carp, perch, bleak, rudd, and ziege with low values for the majority of fish species, but relative biomass (BPUE) is dominated by common carp, gibel carp, roach, white bream, ziege, perch, pike-perch, and rudd with some differences between sampling methods. The diversity indices of Razim Lake and the adjacent area point to a more than medium stable fish coenosis, with the most stable being the shoreline area. The parameters used (according to Moss et al. 2003) and four selected ecological parameters used according to the WFD) in the ecological status characterization of Razim Lake from the point of view of the fish fauna, categorise Razim Lake into the moderate class, using the "one out, all out" principle of the WFD. The ecological indicators have not completely captured a decreasing trend in commercial fishing. This aspect is studied for fisheries resources using stock estimations from fishery landings. However, the absence of large fish (pike-perch, wells catfish, common bream) is a sign of overfishing, especially when adult individuals are missing or an insignificant number is spawning, that could have negative repercussions on future generations, such as for pike-perch). The investigation of Razim Lake has always been a challenge for researchers and this paper aims to be a benchmark for future fish ecological studies. From another perspective, monitoring of fish fauna from Razim Lake is vital because it represents the main reservoir of some commercial fish species like pike-perch, common bream, common carp, but also for some important ecological species, such as *Percarina demidoffi*, *Ponticola syrman*, and *Umbra krameri*, as well as to adjust ecological parameters as support for the determination of conservation status.

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Many immature individuals and largest size classes lacked females for three coral reef fishes (Actinopterygii) in Fiji market surveys: Implications for fishery management

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Abstract

Data-limited fisheries benefit from using life-history traits as biological indicators of targeted stocks. We used histology-based reproductive analyses to estimate size at maturity, per capita egg production, and the number and biomass of immature individuals in the catch for three common coral reef fishes in Fiji market surveys during 2010–2019. We studied *Lutjanus gibbus* (Forsskål, 1775), *Parupeneus indicus* (Shaw, 1803), and *Chlorurus microrhinos* (Bleeker, 1854), which represent three families: Lutjanidae, Mullidae, and Scaridae, respectively. Fork length comprising 50% mature individuals for females of *L. gibbus* was 22.7 cm, that of *P. indicus* was 25.9 cm, attaining 38.0 cm for *C. microrhinos*. Females were rare or absent in the largest size classes of all three species. Immature fish represented up to 50% by number and 41% by biomass of the catch in market surveys, with *P. indicus* having the greatest immature number (8%–50%) and biomass (6%–41%), followed by *C. microrhinos* (20%–30% by count, 11%–18% by biomass) and *L. gibbus* (9%–28% by count, 5%–14% by biomass). Individuals ≤ 30 cm for *L. gibbus* and *P. indicus* and ≤ 45 cm for *C. microrhinos* were responsible for ≥ 90% of egg production per spawning. Skewed size-specific sex ratios suggested that exploitation of the largest size classes had minimal effect on overall egg production. Decreased catches of immature fishes would increase the reproductive population sizes for these species.

Keywords

Chlorurus microrhinos, histology, *Lutjanus gibbus*, *Parupeneus indicus*, per capita egg production, size at maturity, weight–length relation

Introduction

Coral reef fisheries supply protein to more than half of the people living in tropical coastal areas and support jobs, recreational and cultural activities. However, balancing long-term conservation of the coral reef resources with the cultural, food security, and monetary needs of coastal communities is difficult due to increasing fishing efforts, reinforced by access to new technologies and driven by expanding markets. As a result, overall decline and/or overexploitation of coral reef fish stocks has been consistently reported since the 1940s (Fenner 2012; Lachs and Oñate-Casado 2020).

Fiji's subsistence and artisanal marine fisheries generate annual landings of 27 000 t (Gillett 2016), and prior to COVID-19, was the third largest natural resource sector in the country after sugarcane and other crops (Hand et al. 2005). Per capita annual consumption rates are estimated between 20.7 and 36.8 kg, and therefore its fish are crucial to Fiji's food security and nutrition (Bell et al. 2009; Gillett and Tauati 2018). However, decades of poor management means that many fisheries are heavily exploited (Mangubhai et al. 2018), with many species sold in markets below reproductive size, and many species have spawning potential ratio estimates below 20% (Prince et al. 2019).

Two less obvious causes of mismanagement potentially leading to overexploitation are poorly defined size restrictions and the deficiency of fish reproductive biology information. A common size restriction is the minimum size limit, where fishers can only retain the fishes above a certain size—usually, the estimated length at or near maturity. This approach typically relies on the assumption that each fish has reproduced at least once in its life before being caught. This way, each fish has contributed recruitment to at least one cohort of a fished population. However, reproduction can only be achieved when both sexes are present and mature, which does not happen at the same size in sequential hermaphroditic fishes. Eleven of the fourteen families where sex exchange is known in at least one species, inhabit coral reefs (Warner 2011). A size limit based on the sex that matures first has cascading negative effects on protogynous and protandrous fished populations, as only a reduced number of individuals will survive until maturity in the other sex. Knowledge of size at maturity for both sexes is therefore required for many coral reef fishes—this information can be used either to set a minimum and a maximum size limit (i.e., a slot limit), or to push the minimum limit to the bigger of the two sizes.

The identification of basic reproductive biology information (e.g., size at maturity) for each population of a coral reef fishery, where catches may contain up to 200 species whose abundance change seasonally (Dalzell et al. 1996), requires time and economic efforts that are often unattainable (Roberts and Polunin 1993; Johannes 1998). When size at maturity estimates are not available for local populations, researchers may use estimates generated at other locations. However, the practice necessarily assumes that the "borrowed" estimates are representative of the local fish populations. That assumption, if not valid, may lead to mismanagement. Temporal and geographic variability in life history parameters is known for several coral reef fish species (Gust 2004; DeMartini et al. 2014). In addition, estimates of reproductive parameters should be obtained via histological examination, when possible, to minimize biases (Grandcourt et al. 2006, 2011; Vitale et al. 2006; Longenecker et al. 2017). For example, empirical relations (Froese and Binohlan 2000) that underly the FishBase life history tool (Froese and Pauly 2019) increasingly overestimate female size at maturity as the maximum size of a species increases (Longenecker and Langston 2016; Longenecker et al. 2017). Also, macroscopic evaluation of gonads results in misclassification of sex and/or reproductive status of up to half of the inspected specimens (Longenecker et al. 2013a, 2013b, 2020), with the misclassifications tending to overestimate the number of mature females (Longenecker et al. 2013a, 2013b), underestimate female size at maturity (Grandcourt et al. 2006, 2011), and overestimate female spawning biomass (Vitale et al. 2006). Finally, histological analysis is required to diagnose sex change (Sadovy and Shapiro 1987).

In this case study from Fiji, we used rapid, low-cost, onsite, histology-based reproductive analysis (Longenecker et al. 2020), to assess the reproductive parameters including size at maturity and per capita egg production of three coral reef fishes. Each is vital to Fiji's subsistence and/or artisanal fisheries, has been identified by Fiji's Ministry of Fisheries as vulnerable to overexploitation, and is common across Pacific Island countries and territories: the humpback red snapper, *Lutjanus gibbus* (Forsskål, 1775), the Indian goatfish, *Parupeneus indicus* (Shaw, 1803), and the steephead parrotfish, *Chlorurus microrhinos* (Bleeker, 1854). We combine the reproductive analyses with data from fish market surveys to determine the size composition of the three species relative to size at maturity to determine the proportion of immature fish in the catch.

Materials and methods

This research was approved by Fiji's Ministry of Education, Heritage and Arts (research permit RA 19/18) and conducted in accordance with relevant guidelines and regulations. No live animals were used. The methods we used for reproductive analysis followed established, statistical methods (Longenecker et al. 2020), summarized below, to describe weight–length relations (WLR), size at maturity, sexual pattern, and sex ratios.

Specimen acquisition and whole specimen processing. All specimens for reproductive analysis originated from Labasa in Vanua Levu and were purchased between 9 March and 25 June 2018 from fishers in fish markets (Fig. 1). Length was determined for each specimen by measuring to 0.1 cm the distance from the front of the head, with mouth closed, to the distal end of the middle caudal ray (called fork length, L_F, for consistency although the caudal fin of *C. microrhinos* is not forked), and weight was determined with the smallest-possible capacity hanging spring-scale (100, 1000, 2500, or 10 000 g capacity, with 1, 10, 20, or 100 g increments, respectively). Gonads were excised, then examined macroscopically to evaluate sex and maturity status (for comparison with histological results). From each gonad a small, cuboid subsample (approximately 3 mm in each dimension) was excised and fixed in Dietrich's solution for at least 24 h pending histological analysis. The subsample was a partial cross section that included the most central part of the gonad adjacent to the lumen (if ovarian) where oocytes were most likely to be mature.

Size-at-maturity and sexual pattern. The Dietrich's-fixed gonad subsamples were trimmed to approximately 2 mm

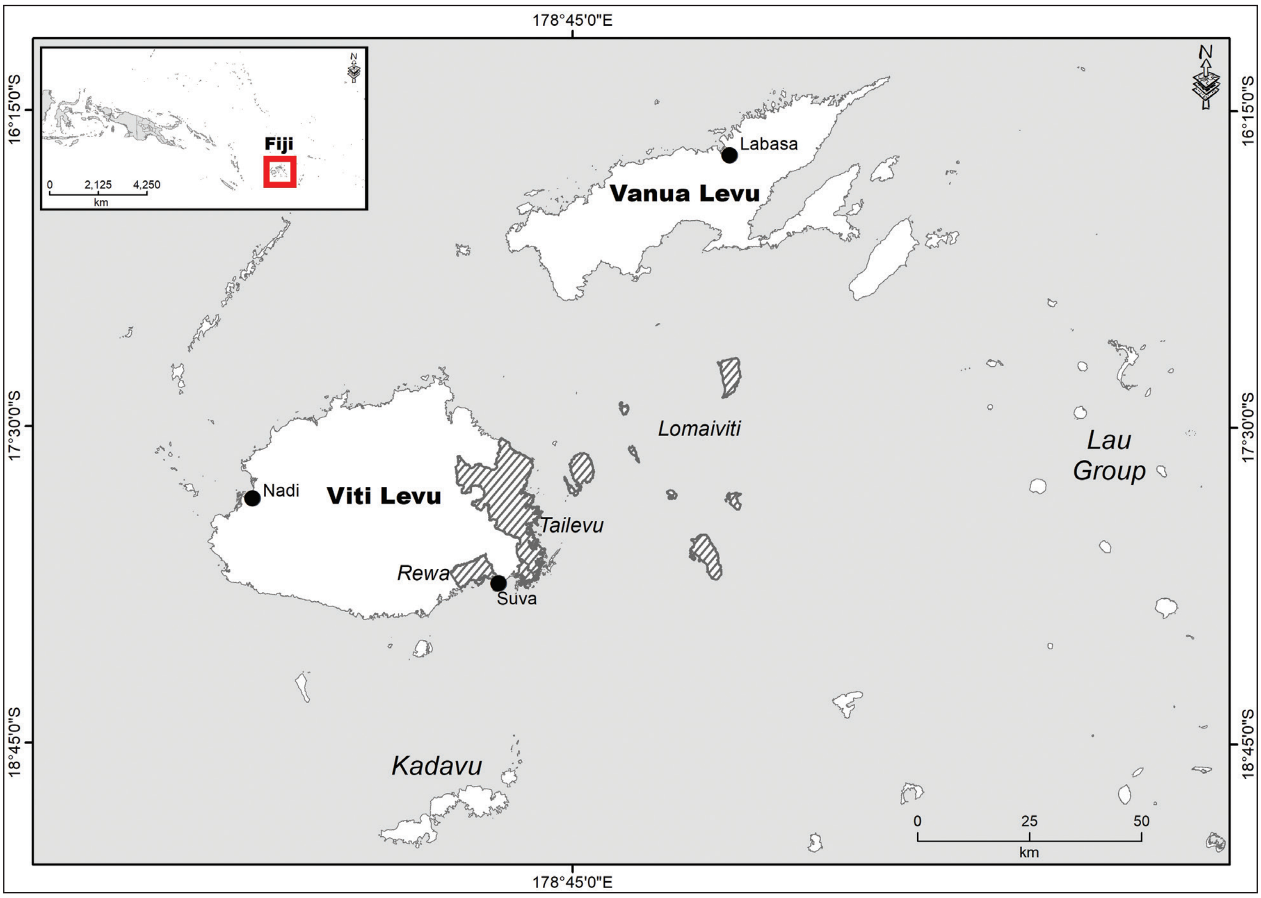

Figure 1. Map of the Republic of Fiji. Specimens were obtained in Labasa from fish-market vendors and from individual fishers. Additional specimens, also originating from the northern island of Vanua Levu, were purchased from vendors at Bailey Bridge market in Suva. Shaded areas represent locations referenced in the description of market survey data.

in each dimension, then dehydrated in alcohol (60 min in each of 50%, and two changes of 95% ethanol). Using glycol methacrylate embedding kits (HistoResin, Leica Biosystems; or JB-4, Electron Microscopy Sciences) and following manufacturer instructions, gonad subsamples were infiltrated with two changes of infiltration solution (1 h and > 8 h, respectively), transferred to embedding capsules (BEEM®, size 00), then embedded in catalyzed resin. Tissue blocks were then dehydrated for 12 h in a watertight case containing silica gel packets. Ten tissue sections (approximately 7 µm thick), distributed evenly throughout each tissue subsample, were obtained from each tissue block by serial sectioning with an MT1 Porter–Blum ultramicrotome fitted with a glass knife. Tissue sections were affixed to glass microscope slides, then stained with Toluidine Blue. Ovary sections were examined at 100× and testis sections at 400× for evidence of reproductive maturity. Guides to gamete development were used to classify ovaries (Wallace and Selman 1981) and testes (Nagahama 1983); females were considered mature when vitellogenic oocytes or post-ovulatory follicles were observed in ovary sections, and males were considered mature when spermatozoa were observed in testis sections.

Data analysis. We constructed WLR using log-transformed data and following established statistical protocols (Froese et al. 2011). We considered all data points with a residual >0.1 to be outliers. We used analysis of covariance (ANCOVA) to analyze data from histologically sexed individuals to evaluate whether WLRs varied between sexes, and for regional comparisons of grouped-sex WLRs for *L. gibbus* and *P. indicus*. We report size-at-maturity as L_{50}, the length predicted to comprise 50% mature individuals (L_{50}) of a given sex. We also report L_{95}, the length comprising 95% mature individuals. Here, we used logistic regression analysis of the dependent variable, proportion of mature individuals, and the independent variable, the midpoint of each 2-cm size class, to produce a maturation curve. Our regression model was:

$$p_M = (1 + e^{((-ln(19)(L - L_{50})/(L_{95} - L_{50})))})^{-1}$$

where p_M is the predicted proportion of mature individuals at a given length (L), L_{50} is the length comprising 50% mature individuals, and L_{95} is the length comprising 95% mature individuals. We report size of transition for protogynous species as the length predicted to comprise 50% males (X_{50}). We used logistic regression analysis of the dependent variable, proportion of males, and the independent variable, the midpoint of each 2-cm size class, to produce a sexual transition curve for protogynous species. Our regression model was:

$$p_{\text{♂}} = (1 + e^{((-ln(19)(X - X_{50})/(X_{95} - X_{50})))})^{-1}$$

where $p_{♂}$ is the predicted proportion of males at a given length (X), X_{50} is the length comprising 50% males, and X_{95} is the length comprising 95% males. We used chi-square (χ^2) analysis to test whether overall or operational sex ratios differed from 1:1. We described size-specific sex ratios of mature-sized individuals, by plotting the percent of mature females within a size class as a function of mean length within each size class. We then used exploratory regression analysis to evaluate whether sex ratios of mature individuals varied predictably with length.

Market survey data collection. Fish market surveys were conducted between 2010–2013 at Laqere, Bailey Bridge and Suva fish markets and within 2016–2018 at Bailey Bridge. All markets are located in Suva on the island of Viti Levu. In general, the majority of the fishes sold at Bailey Bridge were most likely from Labasa, with a much smaller amount from Lomaiviti, those sold at Laqere were most likely from the Rewa and Tailevu areas on Viti Levu, and those sold in Suva were most likely from a diversity of locations, ranging from Labasa in the North to Kadavu in the South, with no one area being the dominant supplier (Gillett and Musadroka 2020) (Fig. 1). Data were collected on fish species abundance and size (fork length, to the nearest centimeter) and pooled to determine the size class distribution patterns for the three study species. Within 2010–2013, we surveyed 180 specimens of *C. microrhinos*, 443 specimens of *L. gibbus*, and 215 specimens of *P. indicus*. In 2016–2018, we surveyed 65 specimens of *C. microrhinos*, 169 specimens of *L. gibbus*, and 12 specimens of *P. indicus*.

Per capita egg production. We used size at maturity and size-specific sex ratios to estimate per capita egg production: the number of eggs produced per spawning event by an individual fish, regardless of sex. Fecundity should, in theory, be proportional to the cube of female body length (Leary et al. 1975; Healey and Heard 1984). We therefore assumed that batch fecundity (F_B) is a cubic function of fork length (i.e., $F_B = L_F^3$) and used the midpoint of each size class to estimate batch fecundity for a female in that size class. We multiplied that estimate by the proportion of females in that size class (from size-specific sex ratio formulae), then multiplied the product by the proportion of females in that size class that were mature (from female size-at-maturity relations). We compared the result with what per capita egg production would be estimated to be under the common assumption of an equal, invariant sex ratio (i.e., the proportion of females in all size classes is 0.5).

Immature individuals in fisheries catch. We used size at maturity and weight–length relations with the length composition data for the three species from market surveys to estimate the number and biomass of immature fish in the catch. We calculated the proportion of immature fish in each 1-cm size class from the specimens collected during the 2019 market survey and used them for reproductive analysis for each market survey data set (i.e., 2010–2013, 2016–2018, 2019). The product of the proportion of immature fish by length and the weight at that length from the WLR provided an estimate of the immature biomass in the catch for each size class, which were summed to give a total immature biomass. We calculated percentages of the number and biomass of immature fish relative to the total market catch of both immature and mature fish for each species.

Results

We estimated reproductive parameters for *Lutjanus gibbus*, *Parupeneus indicus*, and *Chlorurus microrhinos* using histology-based methods (Table 1). For all three species, total body weight (W) in g was an approximately cubic function of fork length (L_F) in cm. The ovaries of mature females of all three species contained several discrete stages of oocytes, indicating group-synchronous oocyte development (Wallace and Selman 1981). We therefore classify all of them as batch spawners. Species-specific information is presented in separate sections, below.

***Lutjanus gibbus*.** The weight–length relation (WLR) regression parameters a and b had 95% confidence intervals of $4.90 \cdot 10^{-6}$–$1.16 \cdot 10^{-5}$ and 3.10–3.26, respectively ($r^2 = 0.977$, $n = 155$, L_F range: 17.2–35.4 cm, W range: 100–960 g). Per ANCOVA results, there was no significant sex-based difference in WLRs ($F = 0.60$, df = 1, $P = 0.441$).

We examined the gonads of 49 male and 61 female *L. gibbus*. Photomicrographs of immature and mature gonads of both sexes are presented as supplementary information (Fig. 2). Vitellogenic oocytes were seen in females as small as 21.7 cm L_F and all females ≥ 26.3 cm L_F were mature. We estimated female L_{50} at 22.7 cm L_F (Fig. 3A) and L_{95} at 27.2 cm L_F.

Table 1. Summary of weight–length relations and reproductive information for four exploited reef fishes from Fiji. L_m is the length of the smallest mature individual. L_{50} and L_{95} are the lengths comprising 50% and 95% mature individuals, respectively. Lengths are in cm.

	Lutjanus gibbus	***Parupeneus indicus***	***Chlorurus microrhinos***
Weight–length (overall)	$W = 7.55 \cdot 10^{-6}(L_F)^{3.18}$	$W = 1.04 \cdot 10^{-4}(L_F)^{2.71}$	$W = 1.01 \cdot 10^{-5}(L_F)^{3.13}$
Male weight–length	—	$W = 1.09 \cdot 10^{-4}(L_F)^{2.70}$	—
Female weight–length	—	$W = 3.35 \cdot 10^{-4}(L_F)^{2.48}$	—
Male L_m	22.5	21.0	33.7
Female L_m	21.7	20.5	36.2
Male L_{50}	23.1	24.2	—
Female L_{50}	22.7	25.9	38.0
Male L_{95}	25.3	30.0	—
Female L_{95}	27.2	32.5	47.8
Oocyte development	Batch synchronous	Batch synchronous	Batch synchronous
Sexual pattern	Gonochore	Gonochore	Protogynous (presumed)
Size of transition (X_{50})	n/a	n/a	40.0
Overall sex ratio (M:F)	1:1.25	1:0.51	1:0.87
Functional sex ratio (M:F)	1:0.95	1:0.27	1:0.49
Size specific sex ratios	See Fig. 3B	See Fig. 5B	See Fig. 7C

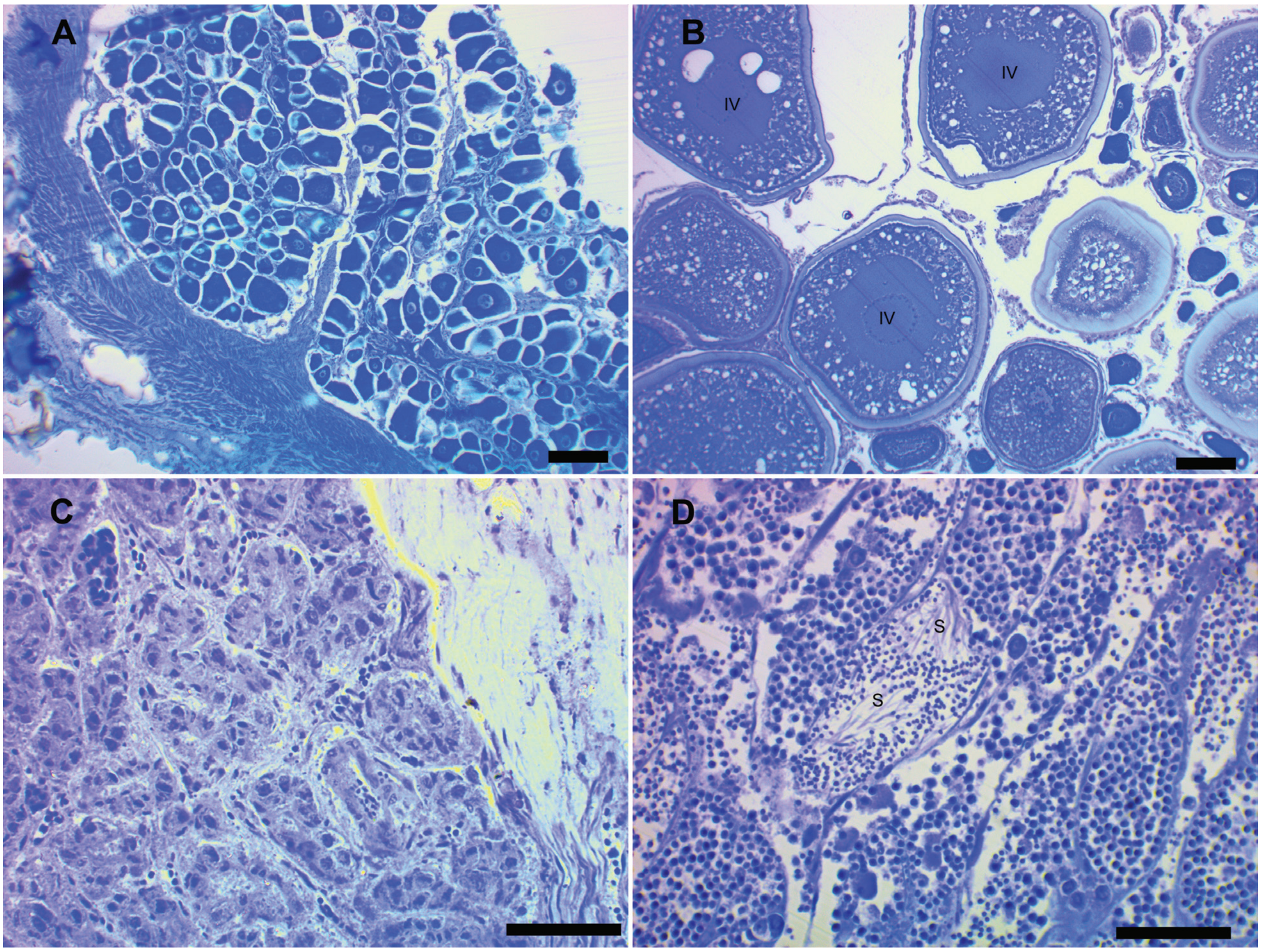

Figure 2. Histological sections of gonads of *Lutjanus gibbus* from Fiji. (**A**) Ovary of immature female (21.6 cm L_F) containing only primary-growth oocytes; (**B**) ovary of mature female (27.3 cm L_F) containing a mixture of oocyte stages including final maturation (IV); (**C**) testis from an immature male (22.6 cm L_F) containing no tailed spermatozoa; (**D**) testis of a mature male (29.8 cm L_F:) with tailed spermatozoa (S); scale bars = 100 µm (**A** and **B**) or 50 µm (**C** and **D**).

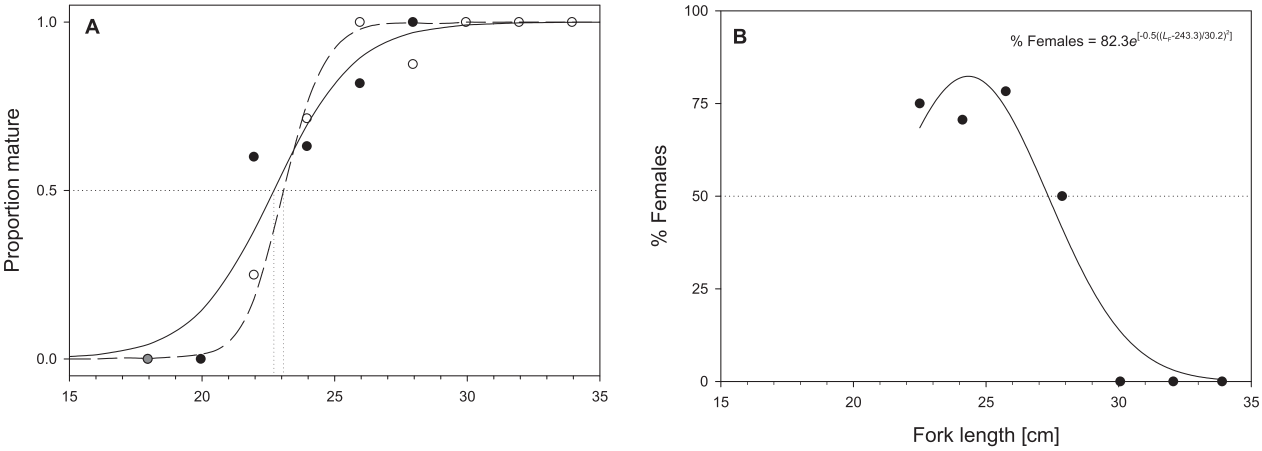

Figure 3. Reproductive information for *Lutjanus gibbus* from Fiji. (**A**) size at maturity (L_{50}); (**B**) percentage of mature females, relative to all mature individuals, versus length. Females are represented by closed circles and the solid curves, males are represented by open circles and the dashed curve.

Spermiated testes were seen in males as small as 22.5 cm L_F and all males ≥ 27.5 cm L_F were mature. We estimated male L_{50} at 23.1 cm L_F (Fig. 3A) and L_{95} at 25.3 cm L_F.

A *t*-test detected a sex-based bimodal size distribution in *L. gibbus*. The mean length of males was significantly greater than that of females ($t = -6.218$, df = 83, $P < 0.001$). However, there was no histological evidence of hermaphroditism in *L. gibbus*; testes did not contain a central membrane-lined lumen, and we did not detect a mixture of ovarian and spermatogenic tissue in any gonad. We classified *L. gibbus* as a gonochore.

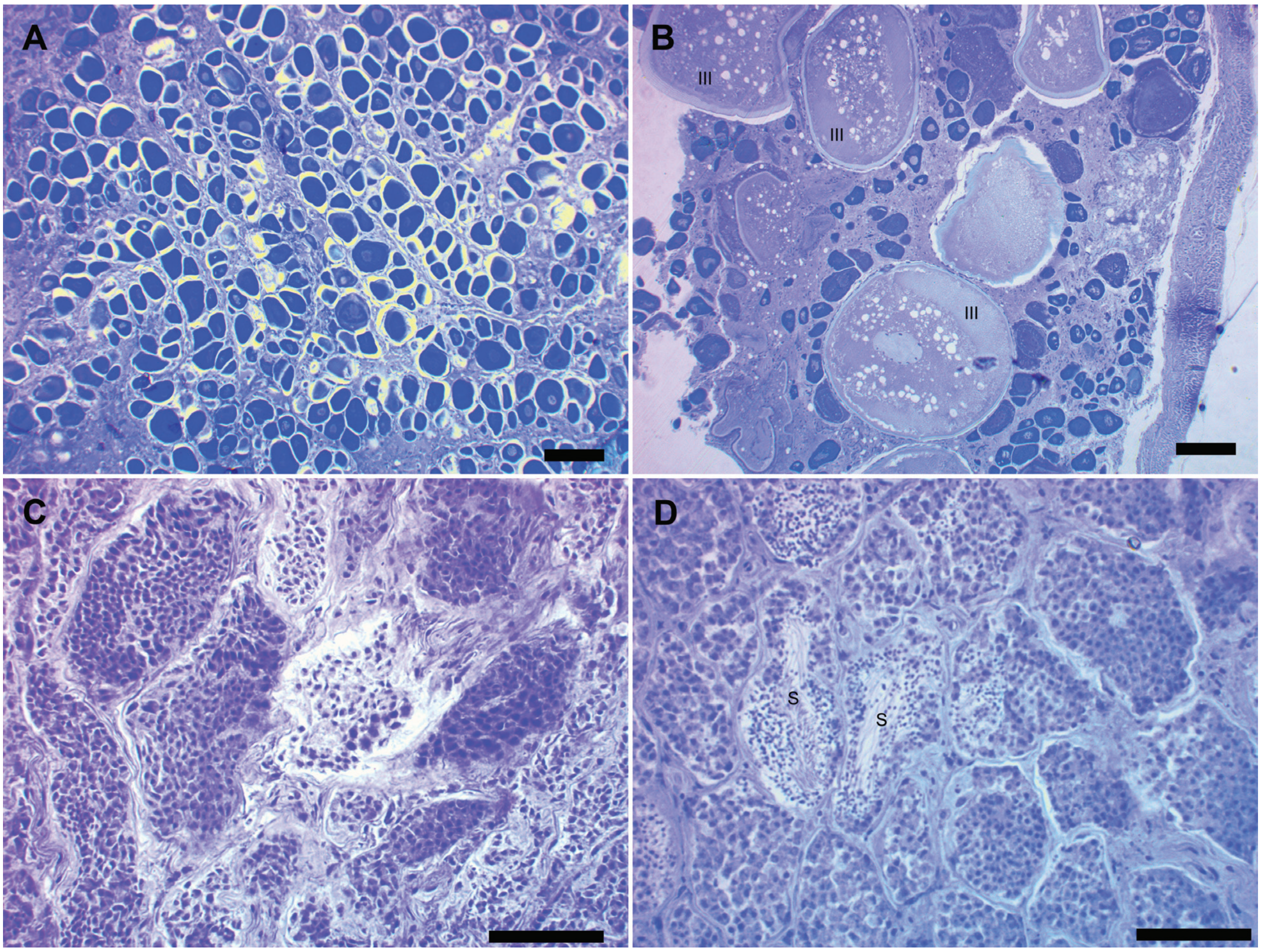

Figure 4. Histological sections of gonads of *Parupeneus indicus* from Fiji. (**A**) ovary of immature female (22.2 cm L_F) containing only primary-growth oocytes; (**B**) ovary of mature female (23.6 cm L_F) containing a mixture of oocyte stages including vitellogenesis (III); (**C**) testis from an immature male (31.5 cm L_F) containing no tailed spermatozoa; (**D**) testis of a mature male (26.3 cm L_F) with tailed spermatozoa (S); scale bars = 100 µm (**A** and **B**) or 50 µm (**C** and **D**).

Overall sex ratio in this *L. gibbus* population was female-biased, but not statistically different from 1:1 (Table 1, $\chi^2 = 1.309$, df = 1, $P = 0.253$). Operational sex ratio (considering only mature individuals) was essentially 1:1 ($\chi^2 = 0.049$, df = 1, $P = 0.825$). However, sex ratios varied predictably throughout the size range of mature individuals (Fig. 3B). Curvilinear regression analysis of the percent of mature females (%$_♀$) versus L_F (Table 1, $r^2 = 0.946$) indicates that the smallest mature individuals were female biased. The percentage of females reached a maximum at 24.3 cm L_F, then decreased as length increased; sexes were present in approximately equal numbers at 27.4 cm L_F but males dominated at larger sizes (Fig. 3B). All mature individuals ≥ 28.4 cm L_F were male.

***Parupeneus indicus*.** The WLR regression parameters *a* and *b* had 95% confidence intervals of $4.63 \cdot 10^{-5}$–$2.36 \cdot 10^{-4}$ and 2.56–2.85, respectively ($r^2 = 0.908$, $n = 134$, L_F range: 18.9–34.1 cm, W range: 150–730 g). Per ANCOVA results, there was a significant sex-based difference in WLRs ($F = 14.50$, df = 1, $P < 0.001$). Sex-specific WLRs are presented in Table 1. For males, the WLR regression parameters *a* and *b* had 95% confidence intervals of $3.86 \cdot 10^{-5}$–$3.06 \cdot 10^{-4}$ and 2.51–2.89, respectively ($r^2 = 0.938$, $n = 57$, L_F range: 18.9–31.5 cm, W range: 150–670 g). For females, the WLR regression parameters *a* and *b* had 95% confidence intervals of $6.92 \cdot 10^{-5}$–$1.62 \cdot 10^{-3}$ and 2.19–2.77, respectively ($r^2 = 0.917$, $n = 30$, L_F range: 19.4–27.9 cm, W range: 160–420 g).

We examined the gonads of 61 male and 32 female *P. indicus*. Photomicrographs of immature and mature gonads of both sexes are presented as supplementary information (Fig. 4). Vitellogenic oocytes were seen in females as small as 20.5 cm L_F. Inactive/immature females (range 19.4–27.0 cm) were scattered throughout the size range of mature females (range 20.5–27.9 cm). We estimated female L_{50} at 25.9 cm L_F (Fig. 5A) and L_{95} at 32.5 cm L_F. Spermiated testes were seen in males as small as 21.0 cm L_F. Inactive/immature males (range 18.9–33.3) were scattered throughout the size range of mature males (range 21.0–30.6 cm). Ignoring the two largest individuals, which were immature, we estimated male L_{50} at 24.2 cm L_F (Fig. 5A) and L_{95} at 30.0 cm L_F.

A *t*-test detected a sex-based bimodal size distribution in *P. indicus*. The mean length of males was significantly greater than that of females ($t = -3.536$, df = 80,

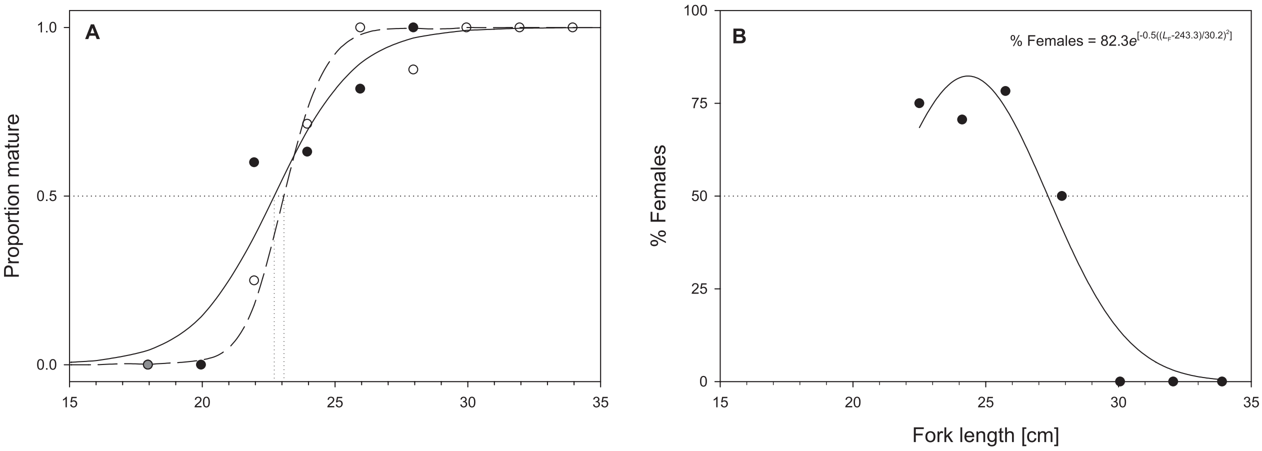

Figure 5. Reproductive information for *Parupeneus indicus* from Fiji. (**A**) size at maturity (L_{50}); (**B**) percentage of mature females, relative to all mature individuals, versus length. Females are represented by closed circles and the solid curves, males are represented by open circles and the dashed curve.

$P < 0.001$). However, there was no histological evidence of hermaphroditism in *P. indicus*; testes did not contain a central membrane-lined lumen, and we did not detect a mixture of ovarian and spermatogenic tissue in any gonad. We classified *P. indicus* as a gonochore.

Overall sex ratio in this *P. indicus* population was significantly male-biased (Table 1, $\chi^2 = 9.783$, df = 1, $P = 0.002$). Operational sex ratio was also significantly male-biased ($\chi^2 = 15.511$, df = 1, $P < 0.001$). Sex ratios varied predictably throughout the size range of mature individuals (Fig, 5B). Curvilinear regression analysis of the percent of mature females ($\%_{♀}$) versus L_F (Table 1, $r^2 = 0.814$) indicates that males were more abundant than females at all sizes. However, smaller size classes had a higher percent of females than larger size classes. The percentage of females reached a maximum at 23.2 cm L_F, then decreased as length increased (Fig. 5B). All mature individuals ≥ 28.0 cm L_F were male.

***Chlorurus microrhinos*.** The WLR regression parameters *a* and *b* had 95% confidence intervals of $4.98 \cdot 10^{-6}$–$2.05 \cdot 10^{-5}$ and 3.01–3.24, respectively ($r^2 = 0.966$, $n = 100$, L_F range: 29.6–52.2 cm, W range: 660–3300 g). Per ANCOVA results, there was no significant sex-based difference in WLRs ($F = 0.00$, df = 1, $P = 0.982$).

We examined the gonads of 47 male and 41 female *C. microrhinos*. Photomicrographs of immature and mature gonads of both sexes are presented as supplementary information (Fig. 6). Vitellogenic oocytes were seen in females as small as 36.2 cm L_F. We estimated female L_{50} at 38.0 cm L_F (Fig. 7A) and L_{95} at 47.8 cm L_F. Spermiated testes were seen in males as small as 33.7 cm L_F. We could not reliably estimate male L_{50} because of the low number of immature males (Fig. 7A).

A *t*-test detected a sex-based bimodal size distribution in *C. microrhinos*. The mean length of males was significantly greater than that of females ($t = -5.471$, df = 81, $P < 0.001$). There was some histological evidence of sex change in *C. microrhinos*; 17 testes contained a central membrane-lined lumen. However, we did not detect a mixture of ovarian and spermatogenic tissue in any gonad. We provisionally classify *C. microrhinos* as a protogynous hermaphrodite. Assuming we correctly evaluated its sexual pattern, the size of transition (X_{50}) for *C. microrhinos* in Fiji was 40.0 cm L_F (Fig. 7B). All individuals ≥ 44.3 cm L_F were male.

Overall sex ratio in this *C. microrhinos* population was not statistically different from 1:1 (Table 1, $\chi^2 = 0.409$, df = 1, $P = 0.522$). However, operational sex ratio was significantly male-biased ($\chi^2 = 8.471$, df = 1, $P = 0.004$). Sex ratios varied predictably throughout the size range of mature individuals (Fig. 7C). Curvilinear regression analysis of the percent of mature females ($\%_{♀}$) versus L_F (Table 1, $r^2 = 0.750$) indicates that the population was female biased from 38.7–41.4 cm L_F. The percentage of females reached a maximum at 40.1 cm L_F, with males dominating ≥ 41.5 cm L_F (Fig. 7C). All mature individuals > 43.0 cm L_F were male.

Per capita egg production. When considering size-specific sex ratios, peak per capita egg production per spawning event was, for all species, estimated to be within a few centimeters of female L_{50} and less than 2/3 of the maximum length observed during market surveys (Fig. 8). For the gonochores *L. gibbus* and *P. indicus*, peak per capita egg production was in the 26 cm size class (Fig. 8A, B). For the likely protogynous *C. microrhinos*, the peak was in the 41 cm size class (Fig. 8C), one cm larger than the size at sexual transition (X_{50}). Greater than 90% of cumulative per capita egg production per spawning event was represented by individuals < 30 cm L_F for *L. gibbus* and *P. indicus*, and by individuals < 45 cm L_F for *C. microrhinos*. For all three species, larger individuals were exclusively male (Figs. 3B, 5B, 7C). These estimates of egg production are vastly different from those obtained when sex ratios are assumed to be 1:1 and invariant across size classes. Assuming equal sex ratios, the size at which 90% of cumulative per capita egg production per spawning event is predicted to occur is at greater lengths, from an additional 10.7 cm L_F for *P. indicus* to 22.8 cm L_F

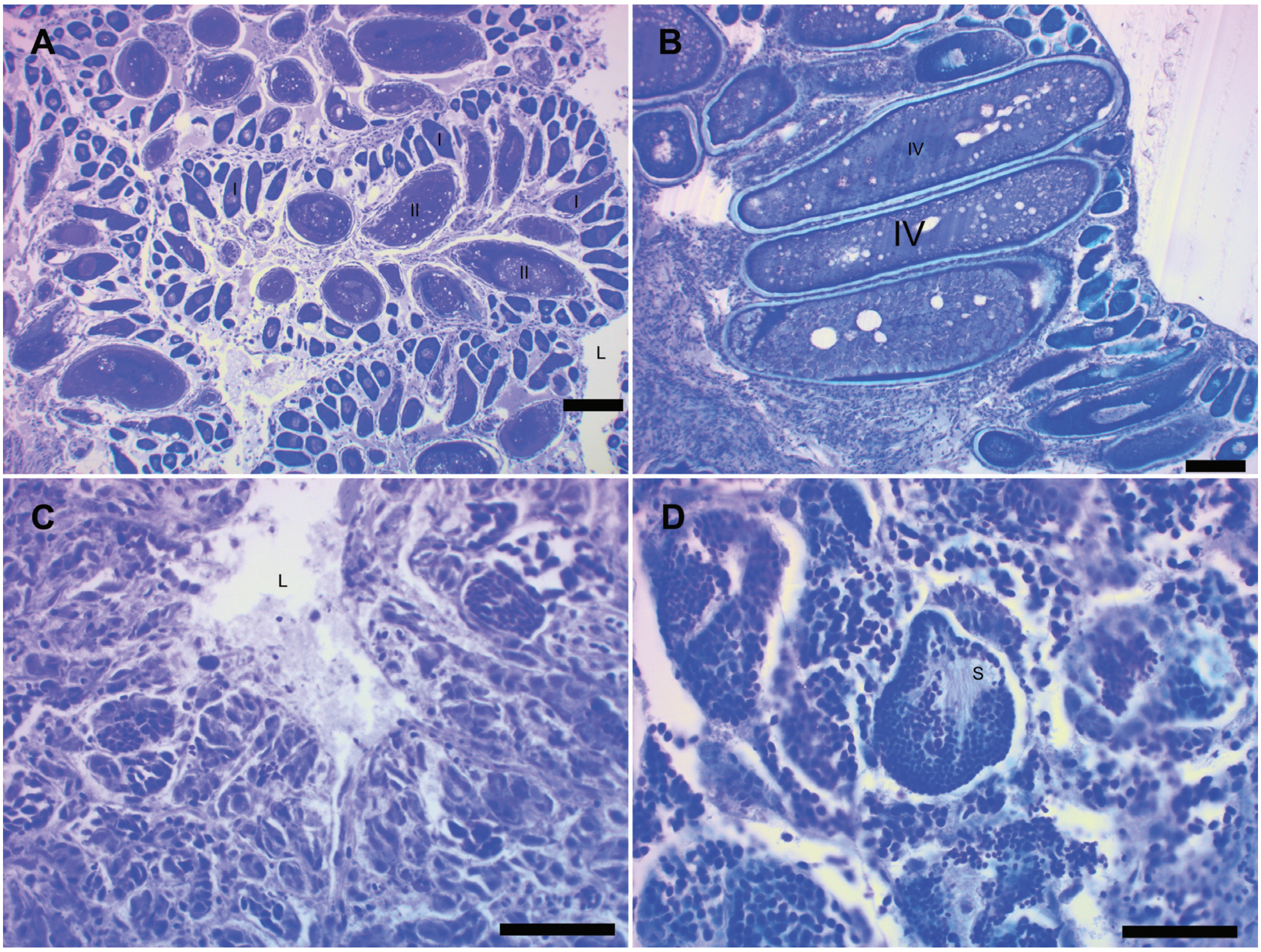

Figure 6. Histological sections of gonads of *Chlorurus microrhinos* from Fiji. (**A**) ovary of immature female (36.3 cm L_F) containing primary-growth (I) and cortical vesicle (II) oocytes, plus a conspicuous lumen (L); (**B**) ovary of mature female (37.1 cm L_F) containing a mixture of oocyte stages including final maturation (IV); (**C**) testis from an immature male (38.0 cm L_F) containing no tailed spermatozoa, but with a conspicuous lumen (L); (**D**) testis of a mature male (38.7 cm L_F) with tailed spermatozoa (S); scale bars = 100 µm (**A** and **B**) or 50 µm (**C** and **D**).

for *L. gibbus* (Fig. 8). Further, assuming equal sex ratios overestimates peak per capita egg production from 365% (*C. microrhinos*) to 1745% (*P. indicus*) per spawning event (Fig. 8).

Immature individuals in fisheries catch. Immature fish of the three species represented between 8%–50% by number and 5%–41% by biomass of the catch in market surveys (Table 2). Of the three species, *P. indicus* had the greatest immature number of fish (28% in 2010–2013 and 8% in 2016–2018 from market surveys, 50% in 2019 from fish sampled for histology) and biomass (17%, 6%, 41%, respectively). For *C. microrhinos*, 30%, 20%, and

Table 2. The number and estimated weights of immature and mature fishes for three reef fish species from Fijian fish market surveys (years 2010–2013 and 2016–2018). The fish number and weights of the reproductive samples (year 2019) were measured.

Parameter	*Lutjanus gibbus*			*Parupeneus indicus*			*Chlorurus microrhinos*		
	2010	**2016**	**2019**	**2010**	**2016**	**2019**	**2010**	**2016**	**2019**
Total fish No.	443	169	163	215	12	141	180	65	101
Female No.	314	79	104	44	1	50	97	27	48
Males No.	129	90	59	171	11	91	83	38	53
Total immature No.	123	16	44	60	1	71	54	13	22
Immature Female No.	113	13	38	29	1	35	54	13	22
Immature Male No.	10	3	6	31	0	36	0	0	0
Total W [kg]	119.4	56.8	41.4	203.6	11.4	92.8	214.8	84.7	121.9
Female W [kg]	62.8	19.7	20.0	25.4	0.6	27.8	84.0	24.9	45.5
Male W [kg]	56.6	37.1	21.3	178.2	10.8	65.0	130.8	59.8	76.4
Total immature W [kg]	17.1	2.8	6.0	33.7	0.6	38.0	38.4	9.0	18.0
Immature Female W [kg]	15.4	2.3	5.0	14.8	0.6	18.0	38.4	9.0	18.0
Immature Male W [kg]	1.7	0.5	1.0	18.9	0.0	20.0	0.0	0.0	0.0

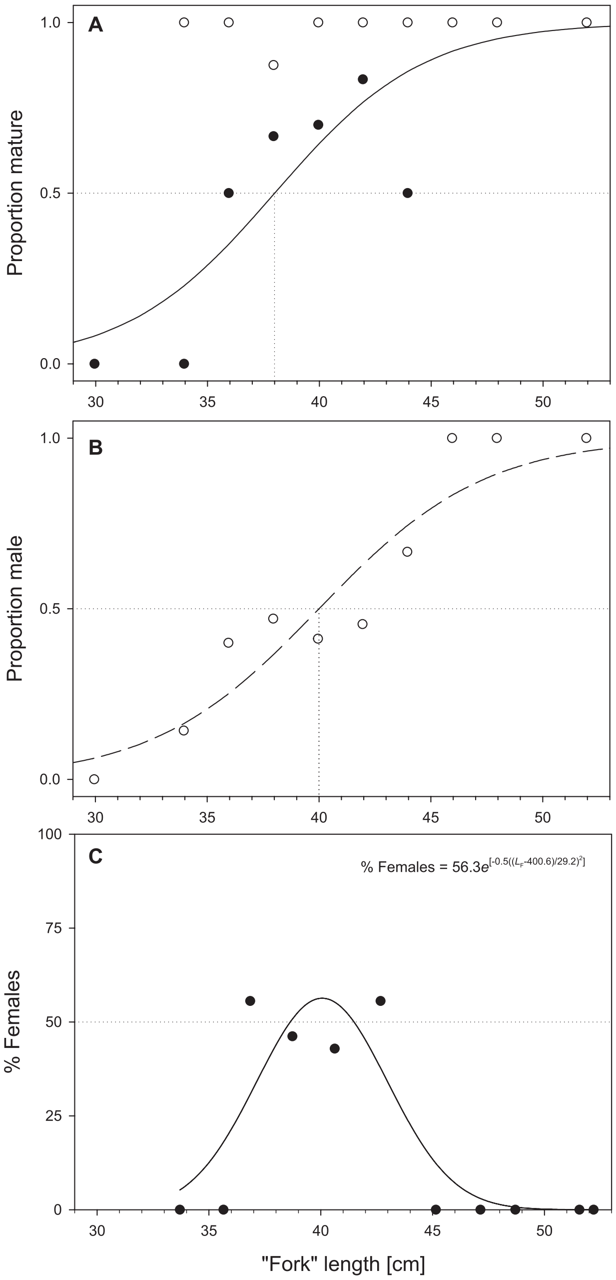

Figure 7. Reproductive information for *Chlorurus microrhinos* from Fiji. (**A**) size at maturity (L_{50}); (**B**) size of transition (X_{50}); (**C**) percentage of mature females, relative to all mature individuals, versus length. Females are represented by closed circles and the solid curves, males are represented by open circles and the dashed curve.

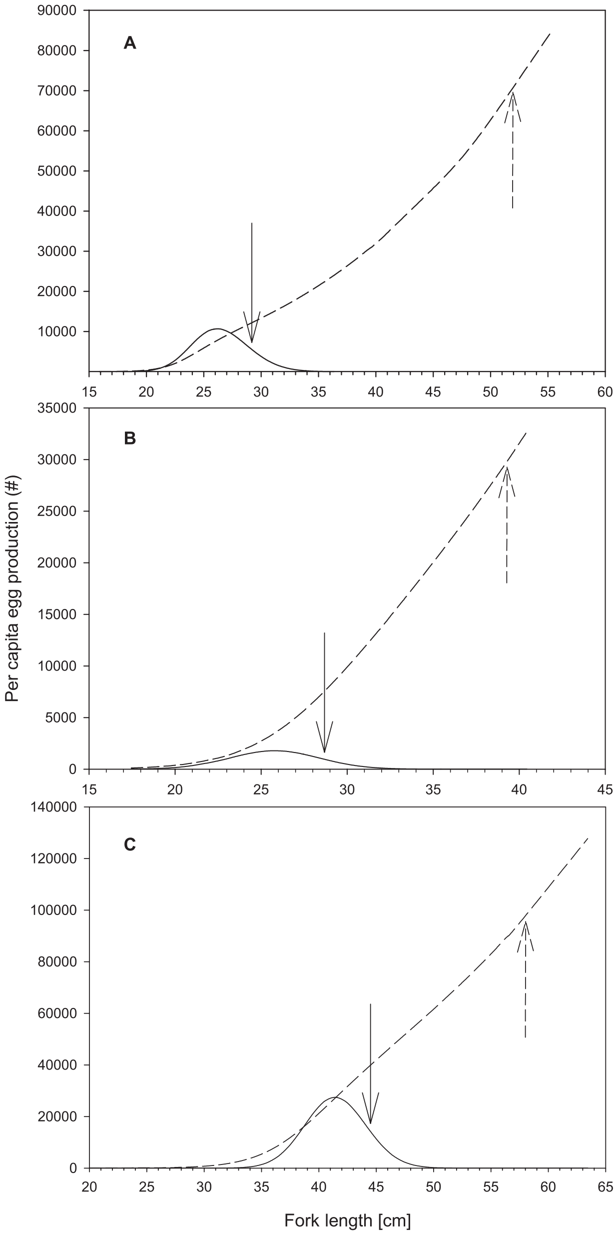

Figure 8. Estimated per capita egg production per spawning event. The solid curve represents estimates when size-specific sex ratios are considered, the dashed curve represents estimates when sex ratios are assumed to be equal and invariant. (**A**) *Lutjanus gibbus*; (**B**) *Parupeneus indicus*; (**C**) *Chlorurus microrhinos*. Arrows indicate 90% cumulative egg production. Note that *x*- and *y*-axis scales vary.

22% of fish were immature and 18%, 11%, and 15% of biomass was immature, respectively. Immature *L. gibbus* contributed 28%, 9%, or 27% by number, and 14%, 5%, or 14% of the biomass.

Discussion

We used rapid, histological methods to estimate a suite of reproductive parameters for three coral reef fishes. We acknowledge that estimates of life history parameters for high value fishery species are typically based on hundreds to thousands of specimens, often collected and compared annually or spatially. For small-scale tropical fisheries, a similar production-scale effort for life history studies is rarely feasible. The approach that we present follows a scientifically valid methodology working with relatively smaller sample sizes that provides important life history information for conservation and management guidance on coral reef species in data limited fisheries.

Our results allow comparisons of method (different methods used in the same location) and location effects

Table 3. A comparison of macroscopic (from Prince et al. 2019) and histology-based (present report) estimates of L_{50} (cm) for three reef fishes from Fiji.

	Lutjanus gibbus	*Parupeneus indicus*	*Chlorurus microrhinos*
Macroscopic L_{50}	29.8	32.5	37.5
Histological L_{50} male	23.1	24.2	—
Histological L_{50} female	22.7	25.9	38.0

(the same methods used in different regions). Table 3 presents the species-level (i.e., grouped sexes) L_{50} estimates produced during a three-year-long macroscopic analysis of Fijian reef fishes (Prince et al. 2019) and the histology-based, sex-specific L_{50} estimates we produced. Our histology-based results indicate that males mature from >1 cm (*P. indicus*) to nearly 7 cm (*L. gibbus*) shorter than suggested by macroscopic methods. For females, the different methods produced similar results for two species; histology-based results suggest that *C. microrhinos* matures about 0.5 cm larger than was reported for macroscopic analysis. Conversely, our histology-based results suggest that *Lutjanus gibbus* and *Parupeneus indicus* mature ~7 cm shorter than was indicated by macroscopic analysis. These differences likely result from inaccuracy in macroscopic evaluation. For instance, in the presently reported study, we misclassified sex and/or maturity status in 20.0%–60.2% of specimens (*L. gibbus* and *P. indicus*, respectively). Similar differences between histological and macroscopic results have been reported elsewhere (Vitale et al. 2006; Grandcourt et al. 2011; Longenecker et al. 2013a, 2013b, 2020; Longenecker and Langston 2016). Although the accuracy of macroscopic staging improves as personnel gain experience, the misclassification rate exceeds 40% for many gonad stages, even when workers are experienced (Mackie and Lewis 2001).

In the absence of a robust understanding of geographic patterns of size at maturity, it would be prudent to use site-specific estimates of reproductive parameters when developing management strategies. Two of the species we analyzed in this study were the subject of histology-based reproductive analysis at other locations. Similar histology-based methods were used to study *P. indicus* in Papua New Guinea (Longenecker et al. 2016) and *L. gibbus* in the Federated States of Micronesia (Longenecker and Langston 2016). For the majority of parameters that we could statistically compare, there were significant regional differences. WLRs, and overall and operational sex ratios differed for both species. When we used the data from Papua New Guinea (Longenecker et al. 2016) and Federated States of Micronesia (Longenecker and Langston 2016) to estimate L_{50} with the regression model employed in the presently reported study, we found that *P. indicus* females mature at a much larger size in Fiji than in Papua New Guinea (25.9 vs. 18.4 cm L_F, respectively). Additionally, *L. gibbus* females mature at a slightly larger size in Fiji than in the Federated States of Micronesia (22.7 vs. 21.0 cm L_F, respectively). Although of biological and management importance, neither of these differences could be demonstrated to be statistically significant.

The use of rapid histology-based methods allows the identification of emergent patterns in reproduction during processing for each set of specimens. For instance, for the three species analyzed in the presently reported study, mature females are more abundant in the lower range of size classes containing mature individuals and become increasingly rare, then absent, as length increases (Figs. 3B, 5B, 7C). This pattern has been reported elsewhere; however, its impact on the size classes overwhelmingly responsible for population-level egg production is under-recognized (but see Longenecker et al. 2014, 2016, 2017, 2020). The common pattern of large females producing exponentially more eggs than small females has led to a longstanding assumption that large fish are disproportionately responsible for population-level egg production (Roberts and Polunin 1993; Allison et al. 1998; Halpern 2003; Froese 2004; Sale et al. 2005; Birkeland and Dayton 2005). However, the assumption may not be valid if females are rare in the largest size classes and is invalid if females are absent. Considering the size-specific sex ratios reported here, the decreasing proportion of females in the largest size classes overwhelms length-related increases in batch fecundity, resulting in peak per capita egg production well below maximum observed length (Fig. 8). That we report this pattern for the likely protogynous hermaphrodite, *C. microrhinos*, may not be surprising. However, the impact of detecting the same pattern for the gonochores, *L. gibbus* and *P. indicus*, cannot be overstated.

Size-specific sex ratios such as those we report here have two major implications for fishery conservation and management. First, with females absent from the largest size classes, fishing at or near the maximum size will have little impact on population-level egg production. Second, imposing slot limits to protect the largest individuals would direct fishing pressure toward smaller size classes which, because they comprise the highest proportions of females, are the major source of population-level egg production.

Our results suggest that protection of the individuals overwhelmingly responsible for the majority of per capita egg production per spawning event could be achieved by establishing minimum size limits of 30 cm L_F for *L. gibbus* and *P. indicus*, and 45 cm L_F for *C. microrhinos*. These minimum limits would also protect all immature individuals of *L. gibbus* and *C. microrhinos* (Fig. 9A, C), and all immature females of *P. indicus* (Fig. 9B). However, implementation of minimum size limits should also consider other factors, such as monitoring compliance and subsistence fishing needs, to achieve effective management of coastal reef fishes.

Our analyses of per capita egg production do not consider potential maternal effects, such as the possibility that larger females may spawn more often or produce higher quality eggs. Nor do they consider the typical population size structure of fishes, comprising many more small individuals than large individuals. The impact of the latter has been demonstrated by genetic parentage analysis showing that highly abundant mature fish contribute disproportionately to population replenishment

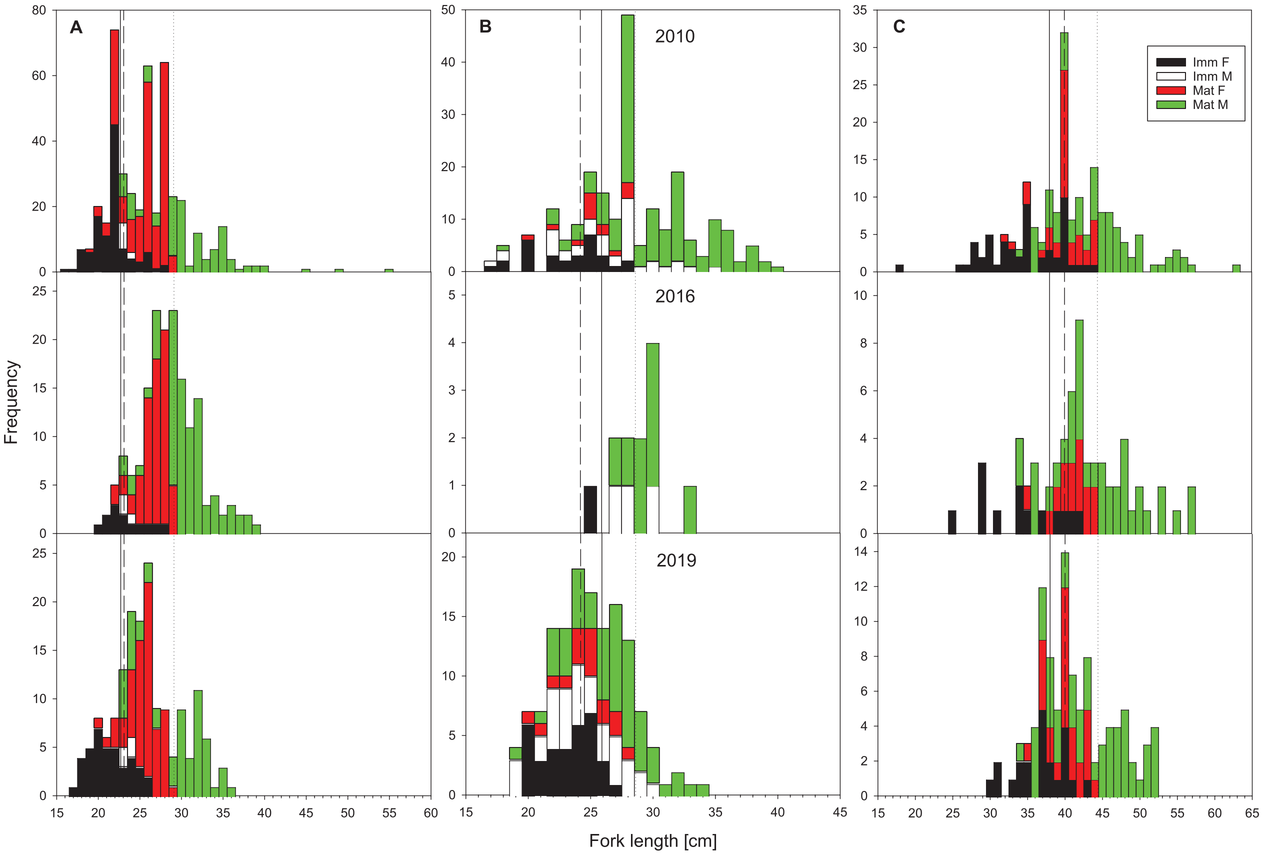

Figure 9. Size class distribution of three exploited reef fishes from three market surveys in Fiji. **A** *Lutjanus gibbus*; **B** *Parupeneus indicus*; **C** *Chlorurus microrhinos*. Solid line = female L_{50}; dashed line = male L_{50}; dotted line = 90% cumulative per capita egg production. Note that *x*- and *y*-axis scales vary.

(Lavin et al. 2021). At the population level, the typical size structure would magnify the per capita egg production patterns described above.

Conclusions

We used rapid, histology-based methods to estimate Fiji-specific reproductive parameters for three reef fishes. We then used the parameter estimates to describe the reproductive characteristics of fish observed in Suva fish markets. The absence of females in the largest size classes of all three species suggests that, at the population level, individuals well below maximum size are responsible for the majority of egg production. Minimum size limits of 30 cm L_F for *Lutjanus gibbus* and *Parupeneus indicus*, and 45 cm L_F for *Chlorurus microrhinos* may enhance population-level egg production while also protecting almost all immature individuals of all three species.

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Triploid forms' karyotypes of spined loaches from the genus *Cobitis* (Actinopterygii: Cypriniformes: Cobitidae) of the upper Dnieper and Western Dvina rivers: Analysis of the triploids' origin

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Abstract

Unisexual forms of lower vertebrates (fish, amphibians, and reptiles) reproduced by parthenogenesis, gynogenesis, or hybridogenesis are represented by diploids, triploids, or tetraploids, whose origin is associated with interspecific hybridization. Among fish species, the highest variability of unisexual polyploids was found in the genus *Cobitis*. The structure of their genomes and putative parental species holds great interest for the investigation in association with questions about possible evolutionary success. In particular, it serves to elucidate the possible high colonization properties of a few polyploid forms, in contrast to the local history of rather numerous hybrid forms with a limited distribution. Therefore, the aim of this study was to describe the karyotype structure of two newly discovered triploid forms of the genus *Cobitis*, to analyze their origin and putative parental species. The karyotype structure of 182 spined loach individuals from the Western Dvina River and 91 individuals from the upper Dnieper River of the Smolensk District of Russia was studied. A total of 121 studied individuals from the Western Dvina comprised triploid females with a chromosome number 74 and karyotype consisting of 13 meta-, 39 submeta-, and 22 subtelo-acrocentric chromosomes. Among loaches collected in the upper Dnieper River, 42 triploid females were found with 74 chromosome number including 23 meta-, 26 submeta-, and 25 subtelo-acrocentric chromosomes. Other individuals from both localities were karyologically identified as *Cobitis taenia* Linnaeus, 1758 with $2n = 48$. The triploid form of spined loaches of the Western Dvina River most likely arose as a result of the hybridization of *Cobitis tanaitica* Băcescu et Mayer, 1969 and *C. taenia*. The range of *C. tanaitica*, whose karyotype is characterized by an evolutionarily fixed Y-autosomal translocation, is limited to the rivers of the northern coast of the Black Sea. Therefore, hybridization probably happened in late Pleistocene in the Dnieper River system, where both parental species occur. The triploid form that arose here is unique for the Baltic Sea basin. Probably, it colonized the Western Dvina through the artificial Berezinskaya water system (Berezina Canal = Daugava–Dnieper Canal), but at the same time it was forced out of its area of origin by other triploid forms which are now widespread there. According to the karyotype structure, the triploid form, common for both the upper and lower reaches of the Dnieper, has a trihybrid origin, with probable hybridization of *Cobitis elongatoides* Băcescu et Mayer, 1969, *C. tanaitica*, and yet unidentified species *Cobitis* sp. Both studied triploid forms are parts of unisexual-bisexual complexes, in which their putative diploid maternal species (*C. tanaitica* and *C. elongatoides*) are absent, and the role of the host species involved in reproduction belongs to *C. taenia*.

Keywords

hybrid origin, parental diploid species, polyploidy, unisexual-bisexual complexes

Introduction

About 90 unisexual forms reproducing by parthenogenesis, gynogenesis, or hybridogenesis are known among fish, amphibians, and reptiles at present. These forms can be diploid, triploid, and tetraploid; however triploid unisexual forms are much more widespread, while tetraploids are extremely rare. The genesis of unisexual forms is associated with interspecific hybridization, while triploid and tetraploid forms can have not only dihybrid, but even trihybrid origin (see Vasil'ev et al. 1989, 2011; Vrijenhoek et al. 1989).

In contrast to parthenogenesis in several reptiles (see Vrijenhoek et al. 1989), natural gynogenetic reproduction of unisexual forms requires males whose sperm stimulates egg development without fertilization. Therefore, gynogenetic fishes and amphibians are found together with one or two related bisexual species, the hybridization of which led to their origin. Rarely, clonal gynogenes reproduce with less related bisexual species. Thus, gynogenetic reproduction of unisexual forms results in the formation of unisexual-bisexual (clonal-bisexual, diploid-polyploid) complexes. Such complexes have been found in fish from the genera *Poecilia*, *Poeciliopsis* (Poeciliidae), *Fundulus* (Fundulidae), *Menidia* (Atherinopsidae), *Chrosomus*, *Carassius*, *Squalius* (Cyprinidae), *Misgurnus* and *Cobitis* (Cobitidae) (see Vasil′ev 1985; Vrijenhoek et al. 1989; Dawley et al. 2000; Lamatsch and Stöck 2009; Arai and Fujimoto 2013).

The first clonal-bisexual (diploid-polyploid) complex in the genus *Cobitis* was noted in 1981 in the Volga River basin (Vasil′ev and Vasil′eva 1982; Vasil′ev et al. 1989). Further studies revealed the wide distribution of diploid-polyploid loach complexes in the Volga River basin, as well as in the rivers of the Black, Baltic, and North seas (Vasil′ev 1990; Boroń 1992, 1995, 1999, 2003; Ráb and Slavik 1996; Vasil′eva and Vasil′ev 1998; Boroń and Danilkiewicz 1998; Bohlen and Ráb 2001; Bohlen et al. 2002; Vasil′ev et al. 2005, 2007, 2011; Janko et al. 2007, 2012; Choleva et al. 2008; Buj et al. 2008; Mezhzherin et al. 2022). The structure of these complexes varies, but their obligatory elements are at least one all-female form and one diploid bisexual species (host), which is a sperm donor for gynogenetic reproduction. The most common complexes include a diploid and/or triploid unisexual form and one diploid bisexual species (Vasil′ev et al. 2011). The high variability of unisexual forms in *Cobitis* observed in ploidy levels and in karyotype structure is the result of different combinations of haploid sets from several diploid bisexual species distributed in European waters. According to various genetic studies, the presumed parental species for the unisexual forms are as follows: *Cobitis taenia* Linnaeus. 1758, distributed in the North Atlantic basin from France and Great Britain to Russia; *Cobitis melanoleuca* Nichols, 1925 widespread from the Volga River system to the waters of the Western Pacific in the Far East and China; the Black Sea species *Cobitis tanaitica* Băcescu et Mayer, 1969, *C. taurica* Vasil′eva, Vasil′ev, Janko, Ráb et Rábová, 2005, and *C. pontica* Vasil′eva et Vasil′ev, 2006; *Cobitis elongatoides* Băcescu et Mayer, 1969, common in the Danube basin; *C. strumicae* Karaman, 1955, recorded in the basins of the Aegean and southwestern Black Seas (Vasil′ev et al. 1989, 2011; Ráb et al. 2000; Bohlen et al. 2002; Boroń 2003; Janko et al. 2005a, 2007, 2018; Choleva et al. 2008). It is assumed that their ranges fluctuated during the Quaternary (Janko et al. 2005a; Culling et al. 2006) and overlapped in Central Europe, the Lower Danube basin, and southern Ukraine (Janko et al. 2007). Hybrid unisexual forms that arose in the mentioned hybrid zones achieved outstanding evolutionary successes and colonized most of the European continent (Janko et al. 2018). However, the observed diversity of unisexual genotypes suggests a local history of bisexual-unisexual complexes (Bohlen and Ráb 2001; Vasil′ev et al. 2011). That is why further work on the discovery of new complexes, the study of their origin and distribution in our opinion holds great interest for the investigation.

In this study, we describe the karyotypes of two triploid forms found in the Western Dvina and Upper Dnieper rivers, respectively, and analyze their origin and putative parental species.

Materials and methods

Materials for karyological research were collected in June 2003 and June 2005 in the Western Dvina River (vel Zapadnaya Dvina vel Daugava) at Velizh City, Smolensk District of Russia, 55°36′N, 031°12′E, and in June 2003 and June 2005 in the Upper Dnieper at Bilino village, Smolensk District of Russia, 55°13.4′N, 033°28.7′E (Fig. 1). In total, 182 spined loach individuals were studied karyologically from the first locality, and 91 individuals from the second. The voucher specimens were deposited in the collection of the Zoological Museum of Moscow State University under the numbers P-21434, P-21800, P-21801, P-21805, P-23738, P-24010. All individuals were injected with about 0.05 mL colchicine solution (0.3%–0.4%). After 4 h cells of anterior part of kidney were used for chromosomal slides prepared according to Vasil′ev (1978). The study was performed using anesthesia of fishes with a solution of MS-222. Metaphase chromosomes stained in 4% Giemsa solution (pH 6.8) were counted with PC software Quick Photo Micro. From 3 to 5 metaphase plates were analyzed for each studied fish. The chromosomes were classified according to Levan et al. (1964). Photos of metaphase chromosomes were taken with a Leica DFC 295 digital camera. For a comparative analysis, we used previously published data on loaches collected in the Lower Dnieper and Dnieper estuary (Vasil′ev 1990).

Results

Among the karyotyped spined loaches of the Western Dvina River, 44 (63.8%) were identified as triploid females

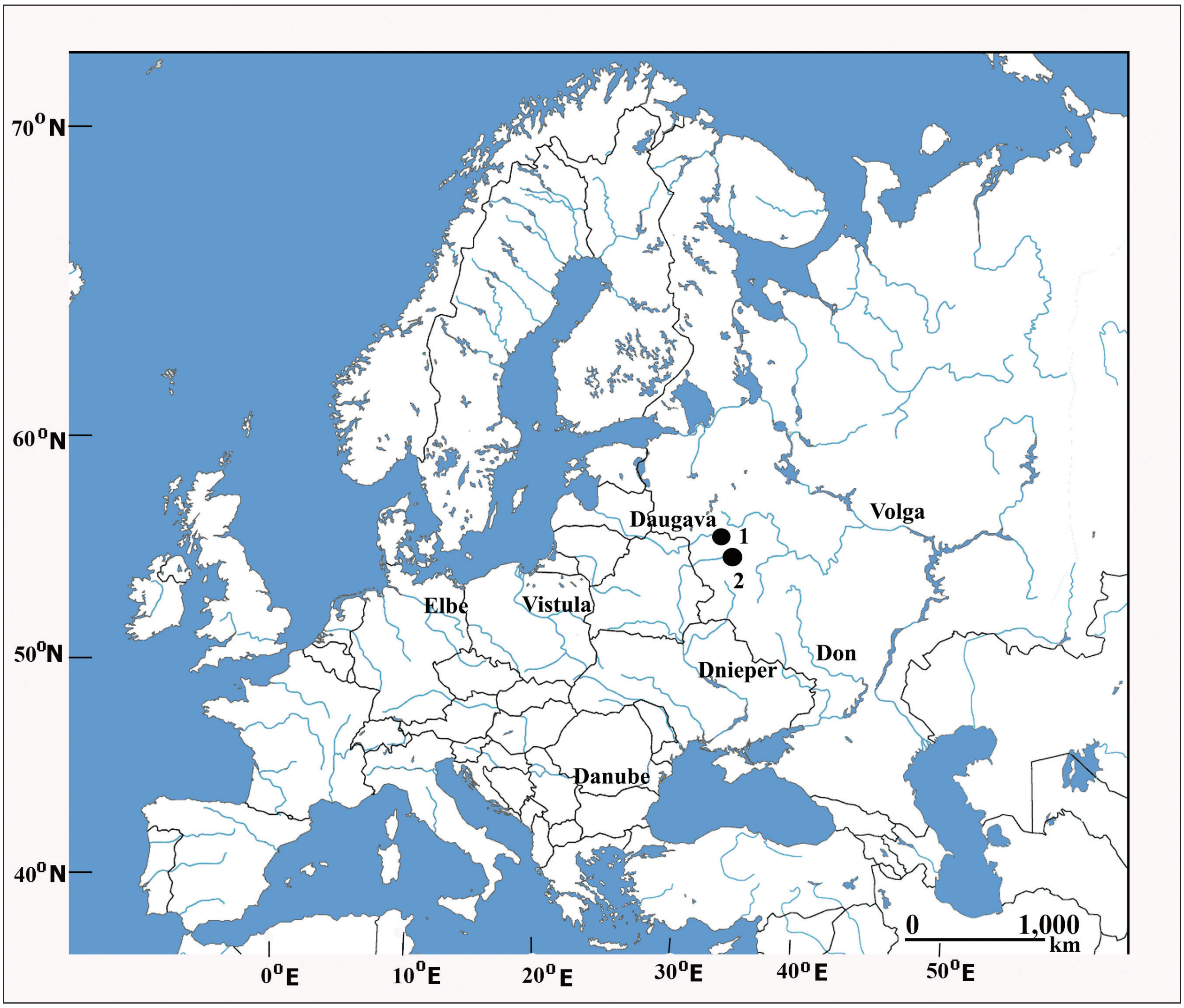

Figure 1. Localities of sampling of triploid loaches of the genus *Cobitis*: 1 = Western Dvina (=Daugava), 2 = upper Dnieper.

in the sample collected in 2003 and 77 (68.1%)—in the sample collected in 2005. These triploids had 74 chromosomes, including 13 meta-, 39 submeta- and 22 subtelo- and acrocentrics (Table 1, Fig. 2). Other fishes were karyologically identified as *C. taenia*. The karyotype of this species was described earlier (Vasil′ev and Vasil′eva 1982; Vasil′ev et al. 1989) and is shown in Table 2. Karyotyped loaches of the Upper Dnieper included 22 triploid females (46.8%) in the sample collected in 2003 and 20 (45.9%)—in the sample collected in 2005. Their karyotype included 74 chromosomes: 23 meta-, 26 submeta- and 25 subtelo- and acrocentic (Table 1, Fig. 3). Other spined loaches found in this location belonged to *C. taenia*.

Discussion

Triploid form in the Western Dvina basin. According to the number of chromosomes (Fig. 2A), the karyotype of this triploid form includes two haploid sets of 50-chromosome species and one haploid set of 48-chromosome species. *Cobitis taenia* Linnaeus, 1758 is the only 48-chromosome species among the karyotyped European spined loaches (Table 2). After removing the haploid set of *C. taenia* with 5 meta- (m), 9 submeta- (sm) and 10 subtelo- and acrocentric (sta) chromosomes (Fig. 2B) from the triploid karyotypes, the remaining chromosomes of the triploid form of the Western Dvina (8 m + 30 sm + 12 sta) correspond fairly well to the sum of

Table 1. The karyotype structure of the studied triploid forms, their putative parental diploid species of *Cobitis* and diploid species coexisting with triploids.

River system	Chromosome number	m	sm	sta	Putative parent species	Coexisting diploid species
Western Dvina	74	13	39	22	2 *C. tanaitica*–*C. taenia*	*C. taenia*
Upper Dnieper	74	23	26	25	*C. elongatoides*–*C. tanaitica*–*Cobitis* sp.	*C. taenia*

m = meta-, sm = submeta-, sta = subtelo- and acrocentric chromosomes.

Table 2. The karyotype structure of diploid spined loach species of the genus *Cobitis* apparently participated in the origin of polyploid forms.

Species	River system	Chromosome number	m	sm	sta	Reference
C. taenia	Volga, Dnieper, Dniester, south Bug, Elbe, Weser, Vistula, Odra	48	10	18	20	Vasil′ev and Vasil′eva 1982; Vasil′ev et al. 1989; Vasil′eva and Vasil′ev 1998; Ráb and Slavík 1996; Ráb et al. 2000; Boroń 2003
C. taenia	Odra	48	10	20	18	Bohlen et al, 2002; Janko et al. 2007
C. taenia	Vistula	48	12	18	18	Boroń 1995, Boroń 1999, Boroń 2003
C. elongatoides	Elbe, Danube, Odra, Tisza	50	30	16	4	Ráb et al. 2000; Boroń and Kotusz 2000 (as *C. danubialis*); Lusk et al. 2003
C. elongatoides	Odra	50	28	18	4	Boroń 2003
C. elongatoides	Danube, Kamchya, Elbe, Odra	50	22	26	2	Janko et al. 2007
C. tanaitica, females	Dnieper estuary	50	8	24	18	Vasil′ev 1990
C. tanaitica, males		49	9	24	16	
C. tanaitica, females	Don, Dnieper, Dniester, Kuban	50	8	28	14	Vasil′ev 1995; Vasil′eva and Vasil′ev 1998; Vasil′ev et al. 2007
C. tanaitica, males		49	9	28	12	
C. tanaitica^A p. 395	Danube, Odra, Sinoe, Don	50	10	24	16	Janko et al. 2007
C. tanaitica^A Fig. 1c		50	10	26	14	
C. tanaitica^A	Danube, Sinoe	50	10	26	14	Majtánová et al. 2016
C. tanaitica^A	Danube	50	10	22	18	Bohlen et al. 2002
C. taurica	Crimea	50	10	30	10	Janko et al. 2005b
C. pontica	Veleka	50	10	30	10	Janko et al. 2005b; Vasil′eva and Vasil′ev 2006
C. taurica^A	Crimea, S. Bug, Veleka	50	10	30	10	Janko et al. 2007
C. melanoleuca	Volga	50	8	18	24	Vasil′ev and Vasil′eva 1982; Vasil′ev et al. 1989
C. strumicae	Kamchya, Jantra	50	10	20	20	Janko et al. 2007

^{A} Species identification is provided by Bohlen et al. 2002 and Janko et al. 2007 (see comments in the text); m = meta-, sm = submeta-, sta = subtelo- and acrocentric chromosomes.

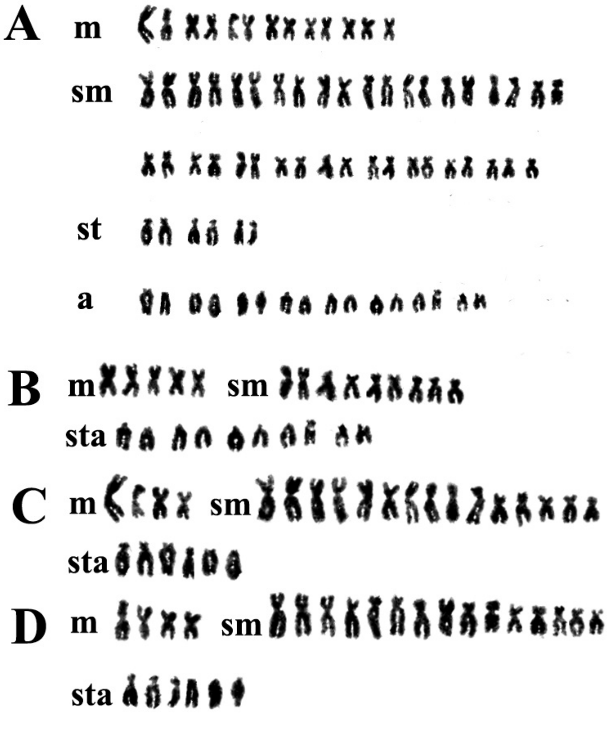

Figure 2. Karyotype of triploid form of the genus *Cobitis* from the Western Dvina River (**A**) and haploid sets of the putative parental species involved: **B**, *C. taenia*; **C**, *C. tanaitica*, female; **D**, *C. tanaitica*, male; m = metacentric, sm = submetacentric, st = subtelocentric, a = acrocentric chromosomes.

the haploid set of *C. tanaitica* females (4 m + 15 sm + 6 sta) and the male of the same species (4 m + 15 sm + 6 sta) (Fig. 2C, D). Differences in the ratios of submeta- / subtelocentrics may be associated with a difference in the classification associated with various degrees of spiralization of chromosomes in the analyzed triploids or diploid species. Other karyotyped 50-chromosome species are unsuitable as putative parental species: they have a higher number of metacentrics and differ in sm/sta ratio (Table 2). Accordingly, the proposed scheme of the origin of this triploid form should include the following stages: 1) hybridization of a female *C. tanaitica* and a male *C. taenia*; 2) hybridization between a hybrid *C. tanaitica* × *C. taenia* and a male *C. tanaitica*. This scheme is due to the unique karyotype of *C. tanaitica*, characterized by an evolutionarily fixed Y-autosomal translocation (Vasil′eva and Vasil′ev 1998), leading to differences in the number of chromosomes in males and females (Table 2). It is also confirmed by our preliminary results that demonstrated a close relation between the triploids of the Western Dvina and *C. tanaitica* from the Don River based on the variability of the cytochrome *b* gene (Vasil′ev et al. 2005).

In this line, it is necessary to discuss the differences observed in the structure of the karyotype of *C. tanaitica* in our studies and in publications of other authors (Bohlen et al. 2002; Janko et al. 2007). Previously, we studied the karyotype of *C. tanaitica* (the species was originally described from the Don River basin) based on materials, collected from 7 populations of the Black Sea and Sea of Azov basins: Don River at Rogozhkino (47°10.367′N, 039°20.8′E), Obitochnaya River (about 46°40.217′N, 036°11.8′E), Beisug (46°2.533′N, 038°34.717′E), Kalka River, tributary of the Kal'chik River (47°7′N, 037°36.75′E), Kazachii Erik, Kuban River system (45°18.467′N, 037°14.567′E), Dniester River at Mayaki (46°24.733′N, 030°16.383′E), Dnieper estuary (46°32.333′N, 032°3.967′E) (Vasil′eva and Vasil′ev 1998). A total of 50 individuals were studied, all of them had a characteristic karyotype with 2*n* = 50 with 8 metacentrics in females and 2*n* = 49 with 9 metacentrics (an unpaired large chromosome is noticeably prominent) in males (Vasil′ev 1990, fig. 1a–b; Vasil′ev 1995, fig. 1). All studied specimens, as well as non-karyotyped fishes from the same samples and fishes from the south Bug system (Savranka River) were morphologically identified and described as a new species *Cobitis rossomeridionalis* Vasil′eva et Vasil′ev, 1998, a junior synonym of *C. tanaitica*. This species differed from *C. taenia* sympatrically found in the Kalka and Obitochnaya rivers, Dnieper estuary, and Dniester River at Mayaki both in the karyotype structure (36 individuals were karyotyped) and morphological features (Vasil′eva and Vasil′ev 1998).

Bohlen et al. (2002) presented the karyotype of *C. tanaitica*, which they isolated from the chromosome set of the tetraploid specimen (4*n* = 100) from the Danube River in Germany. They proposed that the genome of this tetraploid includes 3 haploid sets of *C. elongatoides* and 1 set of *C. tanaitica*. Janko et al. (2007) indicated that they karyotyped or examined by flow cytometry (?) 8 specimens of *C. tanaitica* from the Odra River, 4 specimens from the Danube River, 6 specimens from the Lake Sinoe, and 9 specimens from the Don River (Janko et al. 2007, Table 1). They stated that a biotype corresponding to *C. tanaitica* (2*n* = 50, 10 m + 24 sm + 8 st + 8 a) was found in both sexes in the lower Danube River, upper Odra River, and in the Don River (p. 395); this karyotype was presented by the authors in Fig. 1C. However, the presence of diploid bisexual *C. tanaitica* specimens has never been confirmed in the Odra basin despite the fine-scale intensive researches (Janko et al. 2012; Kotusz et al. 2014). The karyotypes proposed by Bohlen et al. (2002) and Janko et al. (2007) are similar in structure, but differ significantly from the karyotype described by us from the confirmed range of *C. tanaitica* not only in the absence of sex differences, but also in the higher number of metacentric (Table 2) and acrocentric chromosomes: 8 acrocentrics versus 4–6 in males and females from the Sea of Azov basin. Janko et al. (2003, 2007) have never confirmed that the studied fish belong to *C. tanaitica* according to the accepted morphological diagnostic characters (Vasil′eva and Vasil′ev 1998; Vasil′eva 2004; Kottelat and Freyhof 2007). Most likely, Janko et al. (2007) studied the karyotype of some other spined loach species, found only in the lower reaches of the Danube and in Lake Sinoe, a lagoon in northern Dobrudja, Romania, in the Danube Delta. Indeed, Bohlen and Ráb (2001) noted that only specimens from Sinoe Lake were karyotyped. It should also be noted that during two years of our field work in the Danube Delta and in the adjacent territories of Ukraine (2010–2011) we did not find a single specimen of *C. tanaitica*. The spined loaches in our samples from the Danube system were mainly represented by triploids (3*n* = 75), a small number of tetraploids (4*n* = 100), and diploid males and females with 2*n* = 50, which were identified as *C. elongatoides* according to our preliminary DNA studies (unpublished). Therefore, in association with the foregoing, in this paper we designate the diploid loach species found in the lake Sinoe, in the Odra and the lower reaches of the Danube with 2*n* = 50, 10 m, 22–26 sm, 14–18 sta as *Cobitis* sp.

Among the described triploid karyotypes, the fishes of the Western Dvina are most similar to the fishes of the Oława (Odra River system) and Bug (Vistula River system) rivers. They also have 3*n* = 74 with a low number of metacentric and a high number of submetacentric chromosomes (Table 3). It has been suggested that the

Table 3. The karyotype structure of previously studied triploid forms, their putative parental diploid species of *Cobitis* (as identified in the cited publication) and diploid species coexisting with triploids.

River system	Chromosome number	m	sm	sta	Putative haploid sets of parental species	Coexisting diploid species	Reference
Odra (Oława)	74	16	36	22	2 *Cobitis* sp. (2*n* = 49)–*Cobitis* sp. 1 (2*n* = 50)	2*n* = 49 (19 m + 18 sm + 12 sta)	Boroń and Kotusz 2000
Vistula, Bug	74	18	33	23	*C. taenia*–2 *Cobitis* sp. (2*n* = 50, 12 m + 24 sm + 14 sta)	*C. taenia*	Boroń 1999, 2003
Lower Dnieper	74	23	25	26	*C. taenia*–2 *Cobitis* sp.	*C. tanaitica*^A	Vasil′ev 1990
Volga	74	23	27	24	*C. taenia*–2 *Cobitis* sp. or *C. taenia*–*C. tanaitica*–*Cobitis* sp. 1 or *C. taenia*–*C. elongatoides*–*Cobitis* sp. 2	*C. taenia*, *C. melanoleuca*	Vasil′ev and Vasil′eva 1982; Vasil′ev 1990; Vasil′ev et al. 2011
Vistula, Odra	73	24	27	22	2 *C. taenia*–*C. elongatoides*	*C. taenia*	Boroń 2003
Odra (Polska Woda)	74	24	36	14	2 *C. elongatoides*–*C. taenia*	*C. elongatoides*^AA	Boroń and Kotusz 2000
Vistula, Odra	74	21	31	22	*C. taenia*–2 *Cobitis* sp.	*C. taenia*, *C. taenia* × *C. elongatoides*	Boroń 2003
Elbe (Pšovka creek)	74	35	25	14	2 *C. elongatoides*–*C. taenia*	*C. elongatoides*, *C. taenia*	Ráb et al. 2000
Odra	74	33	27	14	*C. taenia*–2 *C. elongatoides*	*C. elongatoides*	Boroń 2003
Danube (Dyje River)	75	38	29	8	2 *C. elongatoides*–*Cobitis* sp.1	*C. elongatoides*	Ráb et al. 2000
Tisza basin	75	35	27	13	2 *C. elongatoides*–*C. tanaitica*	*C. elongatoides*	Lusk et al. 2003
Odra (Polska Woda)	75	24	36	15	2 *C. elongatoides*–*C. taenia* or *Cobitis* sp.	*C. elongatoides*	Boroń and Kotusz 2000; Boroń 2003
Odra (Polska Woda)	75	24	35	16	2 *C. elongatoides*–*C. taenia* or *Cobitis* sp.	*C. elongatoides*	Boroń 2003

^AIdentified as *Cobitis* sp., but not the same as suggested in the triploid genome; ^AA identified as *C. danubialis* Nalbant, 1993. m = meta-, sm = submeta-, sta = subtelo- and acrocentric chromosomes.

genome of triploids of the Vistula basin includes a haploid set of *C. taenia* and two haploid sets of an unknown species with 2*n* = 50, 12 m + 24 sm + 14 sta (Boroń 1999). Taking into account the different degree of chromosome spiralization, we can assume the already mentioned *Cobitis* sp. as the second parental species for this triploid. The same dihybrid origin can be assumed for the triploids of the Odra River. Boroń and Kotusz (2000) revealed in the Odra River a diploid spined loach with 2*n* = 49 (19 m + 18 sm + 12 sta) and primarily identified it as a separate undescribed species, parental to sympatric triploids. However, according to the described karyotype structure, this diploid form should be considered as a hybrid of *C. taenia* and *C. elongatoides* (see Table 2), as Boroń (2003) later concluded; both species are known from the Odra system (Boroń 2003).

Thus, the triploid form of the Western Dvina differs from other triploids found in the Baltic Sea basin (Odra, Vistula) by the karyotype structure and the supposed origin; this form also differs from other triploids revealed in various European river systems (Table 3). Judging by the present-day distribution of its parental species, *C. taenia* and *C. tanaitica*, the triploid form did not originate from the Baltic Sea basin; its origin, of course, should be associated with the Black Sea basin and dated to the Late Pleistocene according to Culling et al. (2006).

It is well known that the recent freshwater fish fauna of the Baltic Sea is one of the youngest. The Baltic Sea depression was covered with ice during the last glacial advance in the Pleistocene and was filled with fresh water at the end of the Quaternary after the retreat of the ice sheet, starting about 13 thousand years ago (Reid and Orlova 2002). Berg (1949) associated the genesis of southern fish forms in the Baltic zoogeographic province with their northward dispersal after the retreat of the ice. However, Reid and Orlova (2002) concluded that much of the current biological diversity in the Baltic Sea reflects relatively recent colonization of non-native species, intentionally or unintentionally introduced by human activities. In our case, an important transport artery connecting the Western Dvina with the Dnieper basin was the Berezinskaya water system (Berezina Canal)—a former artificial waterway that connected the Berezina River (tributary of the Dnieper) with the Western Dvina on the territory of the Vitebsk region of (the present day) Belarus, built within 1797–1805. The total length of this system was 169 km, it had 14 locks and connecting and bypass canals with a length of about 24 km. The system was opened to traffic in 1805; timber rafting continued until the early 1950s; but at present, the canals are impassable due to blockages (Snytko et al. 2016). As follows from the current distribution of polyploid forms of spined loaches in the river systems of Europe, after penetrating the Baltic Sea basin (about 13 000–200 years ago), the analyzed triploid form disappeared from its natural range. Probably, it was replaced by another triploid form, which is now widespread in the upper Dnieper basin.

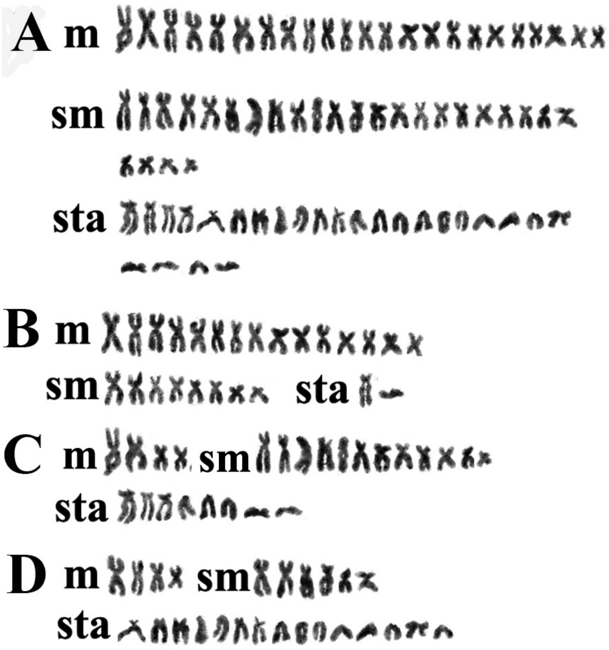

Figure 3. Karyotype of triploid form of the genus *Cobitis* from the upper Dnieper River (**A**) and haploid sets of the putative parental species involved: **B**, *C. elongatoides*; **C**, *C. tanaitica*, male; **D**, *Cobitis* sp.; m, metacentric, sm, submetacentric, sta, subtelo- and acrocentric chromosomes.

Triploid form in the upper Dnieper. The karyotype structure of triploid females in the upper Dnieper basin indicates its trihybrid origin (Fig. 3A). A similar karyotype structure was found in triploid forms from the Lower Dnieper and Volga systems (Table 3). Since their genomes consist of 74 chromosomes, one of the parental species must have 24 chromosomes in the haploid set. According to the first hypothesis of the origin of studied triploids, *C. taenia* can be such a parental species. After the removal of its haploid set, the remaining chromosomes of triploids (18 m + 16–17 sm + 15–16 sta) suggest that the other parental species was a diploid spined loach with a high number of metacentrics and a low number of uni-armed chromosomes; the only corresponding karyotyped species is *C. elongatoides* (Fig. 3B, Table 2). The rest of the chromosomes (4–7 m + 4–8 sm + 13–15 sta) are similar to haploid sets of spined loach species common in the Caucasus (Eastern Black Sea region and Caspian basin) (Vasil′ev 1995; Vasil′eva et al. 2021): *Cobitis saniae* Eagderi, Jouladeh-Roudbar, Jalili, Sayyadzadeh et Esmaeili, 2017, *Cobitis satunini* Gladkov, 1935, *Cobitis derzhavini* Vasil′eva, Solovyeva, Levin et Vasil′ev, 2020, and populations requiring identification. It should be noted that our long-term studies in the Caucasus showed the absence of polyploid loaches in this area (Vasil′eva et al. 2021). *C. taenia*, *C. elongatoides* and unknown species *Cobitis* sp. were previously assumed as parental species for the triploid form, widespread in the Volga River basin (Vasil′ev et al. 2011). *C. taenia* was confirmed as its maternal species by our preliminary study of the variability of the cytochrome *b* gene (Vasil′ev et al. 2005). At the same time, triploids from the Lower Dnieper basin showed pronounced divergence from both *C. taenia* and triploids of the Volga system and were closer to *C. tanaitica* and triploids from the Western Dvina basin (Vasil′ev et al. 2005).

According to the second hypothesis of the origin of triploids from the Dnieper, the parental diploid with 24 chromosomes in the haploid set may be a male of *C. tanaitica*. After removing its haploid set (Fig. 3C), the remaining genome of triploids should include 19 m, 14 sm, 17 sta. And after further removal of the haploid set of *C. elongatoides* (Fig. 3B), the resulting set with 4 m + 6 sm + 15 sta (Fig. 3D) will be similar to haploid sets of spined loaches from the Caucasus, as in the previous hypothesis. Based on our preliminary mtDNA data (Vasil′ev et al. 2005), we suggest that the most probable origin of the Dnieper triploids is via hybridization of *C. elongatoides*, *C. tanaitica*, and *Cobitis* sp., with the first species as the maternal form. Anyway, this triploid form undoubtedly arose in the water bodies of the northwestern part of the Black Sea basin, where *C. elongatoides*, currently found in the Danube basin, could meet the Caucasian *Cobitis* sp., as well as *C. tanaitica* (or *C. taenia*), living in northeastern Europe. The strong competition and deep northward dispersion observed for this triploid form may explain the absence of the putative maternal species in diploid-triploid complex, where the role of the host species belongs to *C. taenia*.

Conclusions

The triploid form of spined loaches of the Western Dvina River most likely arose as a result of the hybridization of *Cobitis tanaitica* and *C. taenia*. Since the range of *C. tanaitica*, whose karyotype is characterized by an evolutionarily fixed Y-autosomal translocation, locally distributed in the rivers of the northern coast of the Black Sea, hybridization probably happened in the Dnieper River system, where both parental species occur. The triploid form that arose here, unique for the Baltic Sea basin, probably colonized the Western Dvina through the artificial Berezinskaya water system (Berezina Canal), but at the same time it was forced out of its area of origin. According to the karyotype structure, the triploid form, common both in the upper and lower reaches of the Dnieper, has a tri-hybrid origin, with probable hybridization of *C. elongatoides*, *C. tanaitica* and an unknown species *Cobitis* sp. Both studied triploid forms are parts of unisexual-bisexual complexes, in which their putative diploid maternal species (*C. tanaitica* and *C. elongatoides*) are absent, and the role of the host species involved in reproduction belongs to *C. taenia*. This phenomenon can be explained by the strong ecological competition of the unisexual form and its maternal species, as well as the deep northward dispersion observed for the triploid forms of the Western Dvina and upper Dnieper basins, where maternal species cannot exist due to their more southerly origin.

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PENSOFT

ACTA ICHTHYOLOGICA ET PISCATORIA

Diet seasonality and food overlap of *Perca fluviatilis* (Actinopterygii: Perciformes: Percidae) and *Rutilus rutilus* (Actinopterygii: Cypriniformes: Cyprinidae) juveniles: A case study on Bovan Reservoir, Serbia

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Abstract

European perch, *Perca fluviatilis* Linnaeus, 1758 and roach, *Rutilus rutilus* (Linnaeus, 1758) are the most common species present in mesotrophic and eutrophic lakes throughout Europe. Their biomass, especially in juvenile stages, contributes the most to the fish production of these ecosystems. In Bovan Reservoir, these two species constitute the bulk of the juvenile fish biomass. This study aimed to investigate the feeding composition of these two species in order to evaluate their niche overlap due to the availability of resources during different seasons. Traditional diet analysis indices and Kohonen artificial neural network (i.e., a self-organizing map, SOM) were used to investigate the diet of 158 individuals of both species and evaluate their food niche overlap. The indicator value (IndVal) was applied to identify indicator food categories based on which the contents of their alimentary tracts were grouped first into neurons and then into clusters on the SOM. Our results showed that juvenile fish used zooplankton and benthic prey in their diet. Roach often fed on nonanimal prey, while perch of age 0+ used fishes in the diet. Additionally, four clusters of neurons were isolated on the SOM output network. The distribution of perch and roach alimentary tracts in neurons indicated no high degree of competition between them. While diet analyses indices show which food category is generally important in specimens' diet, the SOM recognizes those specimens and arranges them together into the same or adjacent neurons based on dominant prey. Understanding fish feeding habits is critical for the development of conservation and management plans. Since Bovan is a eutrophic reservoir, our knowledge of fish feeding habits needs to be considered for stocking strategies in the future.

Keywords

feeding overlap, IndVal index, perch, roach, self-organizing map

Introduction

Dietary analysis has been used for decades in biological and ecological studies of different fish species (Manoel and Azevedo-Santos 2018). Fishes live in quite variable environments where the availability of resources varies in time and space (Nurminen et al. 2010). The feeding spectrum and share of actively feeding specimens depend to a great extent on the season (Gerasimov et al. 2018), so the seasonal differences are evident in the diet of the majority of fish species (Specziár and Erős 2014). Considering juvenile fish, seasonal shifts in the diet are usually a trade-off between prey abundance and increasing body size, which allows individuals to target larger prey (Gopalan et al. 1998). It is widely accepted that the ecology of fish feeding in the first year of life is a critical period in fish life histories (Bogacka-Kapusta and Kapusta 2010). Fishes change habitats or prey types during their ontogeny, and they are often exposed to the selection pressure on important morphological and behavioral traits at different life stages (Werner 1988). Juvenile fish are particularly susceptible to fluctuations in food availability. Thus, Dinh et al. (2017) noted that the study on the variation of food types consumed by fish at different seasons and sizes is critically important for improving our understanding of fish adaptations to their environment and habitat changes.

Studies of diet in fish assemblages at a certain location allow us to recognize distinctive trophic guilds and make inferences about their structure, the degree of importance of the different trophic levels, and the relations among their components (Novakowski et al. 2008). The ecological theory predicts that species belonging to the same ecological guild can coexist only if there are differences in their responses to the limited availability of resources. This theory also suggests that competition is an important interaction between species when the resources are scarce (Begon et al. 1996). That can affect patterns of habitat selection, niche overlap, and diet activity (David et al. 2007). Understanding the biological mechanisms, such as trophic relations, through which species interact with one another is the basis of many ecological studies, from dietary research to the elaboration of food web models (Costalago et al. 2014).

Perch, *Perca fluviatilis* Linnaeus, 1758, and roach, *Rutilus rutilus* (Linnaeus, 1758), are two fish species cohabiting the littoral zone in many European lakes (Syväranta and Jones 2008). They were selected for this study as they constitute the bulk of the young-of-the-year fish biomass in Bovan Reservoir and play a significant role in the food chain since they are intermediates between the lower stages of the food chain and predatory fish (Persson and De Roos 2012). This study aimed to investigate the feeding composition of these two species to evaluate their niche overlap due to the availability of resources during different seasons. A further aim was the assessment of the efficiency of combining the Kohonen unsupervised artificial neural network, i.e., a self-organizing map (Kohonen 1982) and IndVal index (Dufrêne and Legendre 1997) for the analysis of data regarding perch and roach diets. Self-organizing maps and IndVal index, which are widely used in biocenology, have previously been applied only twice (Dukowska et al. 2013, 2014) in ecological studies of a fish diet. This is the first study that presents fish diet assessment combining traditional diet analysis indices (Hyslop 1980; Hickley et al. 1994) and self-organizing maps.

Methods

Study area and fish sampling. Bovan is an artificial reservoir situated in the middle flow of the Sokobanjska Moravica River near the municipality of Aleksinac in southeast (43°38′46″N, 021°42′28″E) (Fig. 1). Its surface area is 4 km^2, maximum depth 50 m, and maximum width 500 m. The reservoir was formed from 1978 to 1984 in Bovanska Gorge as a multifunctional system, with the primary aim to regulate the Morava River basin and protect the Đerdap I reservoir. Its important functions are to maintain sludge and flooding waves, enrich small waters, as well as produce hydro-energy. Initially, it was not planned for a water supply. However, due to its great potential, the water treatment plant was added, and the reservoir nowadays supplies drinking water to the population of the region (Zlatković et al. 2010). Bovan is a eutrophic reservoir (Simić et al. 2006), and the fish community consists mainly of common bream, *Abramis brama* (Linnaeus, 1758); perch; pikeperch, *Sander lucioperca* (Linnaeus, 1758); roach; and Prussian carp, *Carassius gibelio* (Bloch, 1782) (see Pavlović et al. 2015). Detailed qualitative and quantitative analyses of zooplankton and bottom fauna, which represents available food for fishes in the study area, were given by Ostojić (2006) and Simić et al. (2006). The authors stated that analysis of zooplankton composition established the presence of taxa from groups Protozoa, Rotatoria, Cladocera, and Copepoda. On the other hand, the greatest number of species in the bottom fauna was recorded for groups Oligochaeta and Chironomidae.

The field-work was conducted in May and September of 2011 and 2012. Fish were sampled using gillnets of mesh size 10 mm. For each analyzed fish, the total length (TL) was measured to the nearest mm and then weighted (W) to the nearest g. Studies of fish diet, feeding ecology, and food habits are carried out commonly through dissection and examination of alimentary tracts (Hynes 1950; Hyslop 1980). Immediately after the capture and measuring, fish were preserved in 4% formalin and transported to the laboratory, where alimentary tracts were removed, transferred to a Petri dish, and analyzed under binoculars. Prey items were identified to the lowest possible taxonomic level, counted under binoculars, and preserved in 70% ethanol.

Alimentary tract content analysis. Shannon's diversity index (H) was used to assess the prey diversity of the dietary contents in each fish species during all seasons. The index was calculated as

$$H = -\Sigma(p_i)(\ln p_i)$$

where p_i is the proportion of individuals belonging to the ith species relative to the total number of individual prey items recovered for a fish species (Magurran 1988).

To determinate the most important prey in the diet, the Prominence Value (PV) of the dietary component was calculated using the following formulas (Hickley et al. 1994; Lorenzoni et al. 2002):

$$\mathrm{PV} = \%N\sqrt{(\%\mathrm{FO})}$$

$$\%\mathrm{PV} = 100\mathrm{PV} \cdot \Sigma\mathrm{PV}^{-1}$$

where %FO is the frequency of occurrence (the number of alimentary tracts containing each food item in relation to the total number of alimentary tracts with food), and %*N* is relative abundance (the number of individuals of each food item with respect to the total number of individuals). The vacuity index (%VI) was used to express a number of empty alimentary tracts (Hyslop 1980).

To interpret the species' feeding strategy, the Costello (1990) graphical method modified by Amundsen et al. (1996) was applied, in which prey-specific abundance of each food category is plotted against the frequency of occurrence (%FO) on a two-dimensional graph. In this approach, prey-specific abundance was calculated as

$$P_i = 100\Sigma S_i \cdot \Sigma S_{\mathrm{t}i}^{-1}$$

where P_i is the prey-specific abundance of prey *i*; S_i is the alimentary tract content (by number) comprised of prey *i*, and $S_{\mathrm{t}i}$ is the total alimentary tract content in only those fish with prey *i* in their alimentary tracts. In the graph, prey items positioned in the upper part of the graph show a specialist feeding strategy of the fish, and those positioned in the lower part indicate a generalist feeding strategy of the fish. Besides, the diet specialization was estimated by the diet evenness index (*E*)

$$E = H \cdot H_{\max}^{-1}$$

where $H_{\max} = \ln S$, and *S* is the total number of preys in the sample. According to Oscoz et al. (2005) values close to zero mean a stenophagous diet and those closer to one represent an euryphagous diet. The evenness index was employed together with modified Costello's graphical method.

Diet similarity among different species of fish, or the same species during different seasons, was assessed using Schoener's overlap index (*α*). It was evaluated using the Prominence value (PV) of each food item (Lorenzoni et al. 2002) according to the following formula (Schoener 1970):

$$\alpha = 1 - 0.5(\Sigma|\mathrm{PV}_{xi} - \mathrm{PV}_{yi}|)$$

where PV_{xi} is prominence values of food item *i* in species *x*, PV_{yi} is prominence values of food item *i* in species *y*. The index has a minimum of 0 (no overlap), and a maximum of 1 (complete overlap). According to Wallace (1981), a value 0.6 or higher may be considered to be evidence of significant overlap.

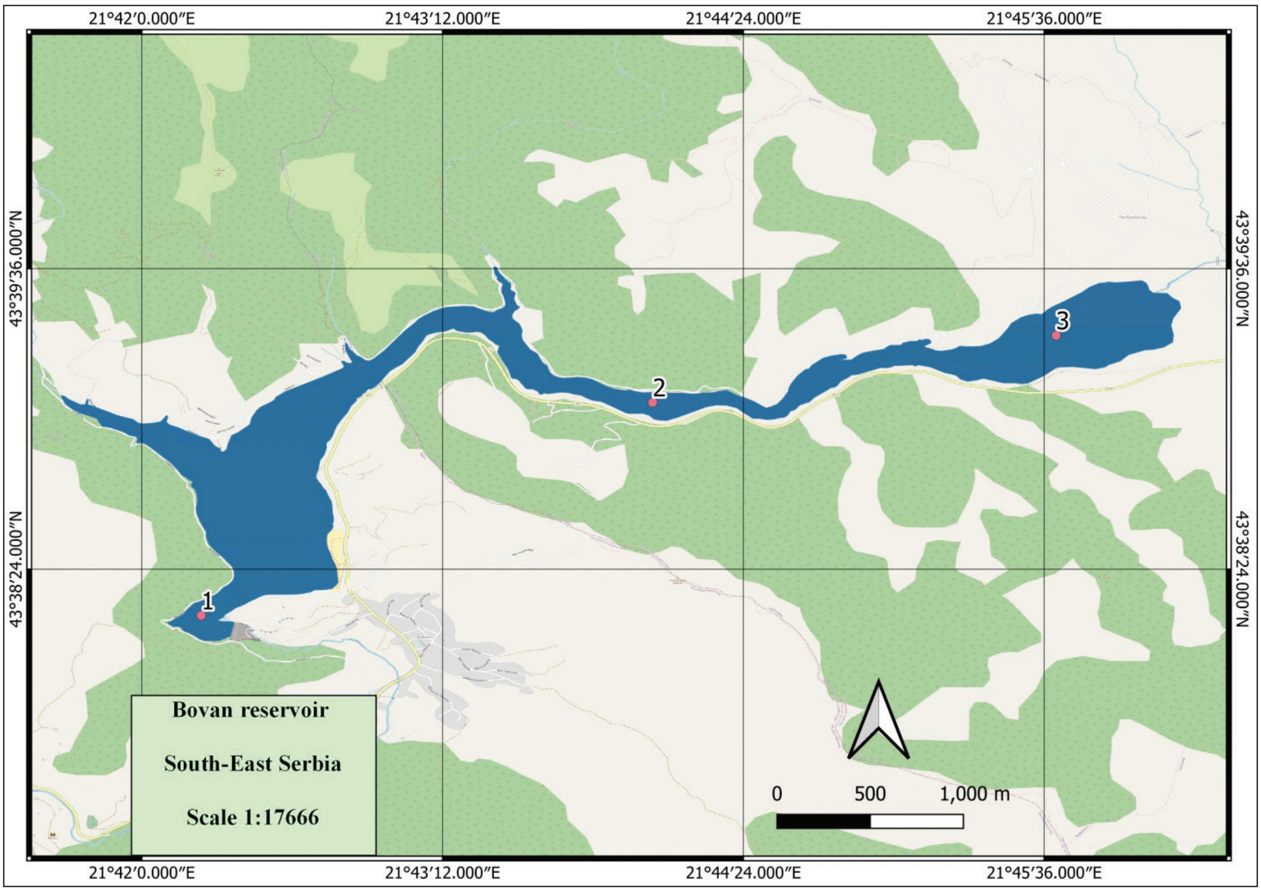

Figure 1. Map of Bovan Reservoir, southeast Serbia. Numbers on the map represent sampling sites, 1 = dam, 2 = middle part of the reservoir, and 3 = lower part of the reservoir.

Statistical data analysis. Analysis of alimentary tract content allows us to determine species' diet composition and further understand their feeding habits and trophic role in the ecosystem (Cailliet et al. 1986). On the other hand, data obtained from alimentary tracts could be noisy because many fragmented and/or digested elements cannot be identified. Moreover, it is rare that the amount of a given food category recorded in alimentary tracts equals the amount of a given food category eaten (Dukowska et al. 2013). Kohonen's unsupervised artificial neural network (i.e., a self-organizing map, SOM) (Kohonen 1982) is resistant to the noise in data (Lek and Guégan 1999; Park et al. 2006). In this work, we used them to determine patterns in the content of the alimentary tracts. The SOM technique is a useful method for the clustering and visualization of large data sets (Penczak et al. 2012; Stojković et al. 2013). It can visualize and explore linear and nonlinear relations in the high-dimensional data set.

The network structure of the SOM is composed of two layers, the input and output, each consisting of data processing units, i.e., neurons (Kohonen 1982, 2001). The input for the SOM is the input matrix. In our study, it consisted of 130 columns (one column represented one alimentary tract) and 26 rows (one row represented one prey taxa). The relative abundance data of prey taxa from the alimentary tracts of fish were log-transformed ($\log(x + 1)$), normalized, and scaled from 0 to 1. Each input neuron was sent through the network throughout the learning process. During the learning process of the SOM network, an alimentary tract content was created in each output neuron. All these neurons present the output layer represented by a codebook matrix. It consists of two-dimensional grids, where the differences between neurons, i.e., models carried by the neurons, increased in accordance with mutual distance increase. The total variability observed in the data set was covered by models from all neurons (Penczak et al. 2006). To distinguish subsets of neurons and subdivide them into clusters on the SOM map, the *k*-means method was used (Jain and Dubes 1988). The map resolution (number of output neurons) is an important parameter for the detection of deviation in the data. If the resolution is wrong, for example, too low or too high, the differences are too small for a plausible interpretation (Céréghino and Park 2009). Since there is no conventional theoretical method for determining the best optimal map resolution, we used the two most recommended methods. The first method, proposed by Vesanto et al. (2000), implies that the optimal number of neurons in the map should be close to 5 square roots of 5 where n is the number of training samples. The alternative method (Park et al. 2003) indicates that the optimal resolution is determined by considering the local minimum quantization error (QE) and topographic error (TE). Using these methods and trying to avoid a large number of empty output neurons (Penczak et al. 2012), we found that a 7 × 7 grid is most appropriate for our study. The SOM Toolbox also generated a visualization of the associations of food categories with SOM regions (sub-clusters of neurons) represented by shades of gray but not for the statistical verification of those associations (Lek et al. 2005). The SOM analysis was carried out using the Matlab ver. 6.1.0.450 algorithm interface (http://www.cis.hut.fi/projects/somtoolbox).

Since SOM is a visualization technique without any statistical indication, the indicator value (IndVal) by Dufrêne and Legendre (1997) was used to identify indicator food categories significantly associated with each cluster of SOM output neurons. An IndVal of the food category (i) in all alimentary tracts of each SOM cluster (j) was calculated as the product of A_{ij} (the relative abundance in % calculated as the mean mass of the food category (i) in the alimentary tracts of cluster (j) divided by the sum of the food category mean masses in all the clusters in the study) and F_{ij} (the relative frequency of occurrence of the food category (i) in the alimentary tracts of cluster (j) also expressed as a %), as follows:

$$A_{ij} = M_{ij} \cdot M_i^{-1}$$

$$F_{ij} = \mathrm{NAT}_{ij} \cdot \mathrm{NAT}_j^{-1}$$

$$\mathrm{IndVal}_{ij} = 100 A_{ij} F_{ij}$$

where M_{ij} is mean value of mass of food category (i) in the alimentary tracts of cluster (j), M_i is mean value of mass of food category (i), NAT_{ij} is the relative frequency of occurrence of food category (i) in the alimentary tracts of cluster (j), NAT_j is the relative frequency of occurrence of all food categories of cluster (j), A_i is the relative abundance in percentage (%), and F_{ij} is the relative frequency of occurrence in percentage (%) of food category (i) in the alimentary tracts of cluster (j).

The Monte Carlo significance test with 1000 permutations was applied to identify significant prey taxa with the use of PC-ORD statistical software (McCune and Mefford 2011). All indicator species with an IndVal score over 25 were interpreted as representative prey taxa of a particular group, with a relative frequency and abundance of at least 50%.

Results

A total number of 130 individuals, with 7.4–11.2 cm in TL, were used to examine diet composition. The number of analyzed specimens by season was as follows: 23 specimens for perch in spring 2011, 20 specimens in autumn 2011, then 17 specimens in spring 2012, and 12 specimens in autumn 2012. The number of analyzed specimens of roach was the same in the spring of both years (18 specimens), then in autumn of 2011 (15 specimens), and finally in the autumn of 2012 (7 specimens). Fish with empty alimentary tracts (28 individuals) were excluded (%VI = 17.72).

Values of the frequency of occurrence (%FO), relative abundance (%*N*), and prominence value (%PV) for each

Table 1. Assessment of diet composition of perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) collected in 2011 from Bovan Reservoir, Serbia, expressed as relative abundance (%*N*), frequency of occurrence (%FO), and prominence value (%PV) of food.

Taxon or group	Spring 2011						Autumn 2011					
	Perch			Roach			Perch			Roach		
	%*N*	%FO	%PV	%*N*	%FO	%PV	%*N*	%FO	%PV	%*N*	%FO	%PV
Protozoa	2.06	26.08	1.22	—	—	—	0.83	20.00	0.43	—	—	—
Rhizopoda	—	—	—	2.63	11.11	1.14	—	—	—	5.61	20.00	3.32
Rotatoria	0.51	4.34	0.12	2.63	5.55	0.81	—	—	—	3.57	6.66	1.22
Bryozoa	6.92	30.43	4.45	—	—	—	7.61	30.00	4.88	4.08	6.66	1.39
Hydracarina	0.07	4.34	0.01	—	—	—	1.39	25.00	0.81	—	—	—
Ostracoda	2.35	43.47	1.81	10.52	55.55	10.24	5.47	75.00	5.55	6.12	53.33	5.92
Anostraca	—	—	—	—	—	—	—	—	—	—	—	—
Conchostraca	0.88	13.04	0.37	—	—	—	0.18	5.00	0.04	—	—	—
Notostraca	—	—	—	—	—	—	—	—	—	—	—	—
Cladocera	0.22	4.34	0.05	—	—	—	0.37	5.00	0.09	—	—	—
Daphnia sp.	5.15	26.08	3.07	14.73	88.88	18.14	1.11	10.00	0.41	11.73	86.66	14.46
Bosmina sp.	6.84	73.91	6.86	25.78	88.88	31.75	8.72	80.00	9.14	24.48	93.33	31.32
Leptodora kindtii	0.88	17.39	0.42	—	—	—	0.09	5.00	0.02	—	—	—
Calanoida (Copepoda)	27.54	100.0	32.17	12.63	55.55	12.29	21.63	85.00	23.38	14.28	66.66	15.44
Cyclopoida (Copepoda)	35.42	95.65	40.46	20.00	55.55	19.47	43.63	90.00	48.53	18.87	66.66	20.40
Isopoda	0.07	4.34	0.01	—	—	—	—	—	—	—	—	—
Amphipoda	5.59	73.91	5.61	1.57	5.55	0.48	4.82	65.00	4.55	—	—	—
Gammaridae	0.07	4.34	0.01	—	—	—	—	—	—	—	—	—
Insecta (other)	—	—	—	—	—	—	0.09	5.00	0.02	—	—	—
Diptera (other)	—	—	—	—	—	—	0.27	5.00	0.07	—	—	—
Chironomidae	3.97	34.78	2.73	1.05	11.11	0.45	3.24	25.00	1.89	0.51	6.66	0.17
Plecoptera	0.58	8.69	0.19	—	—	—	—	—	—	—	—	—
Ephemeroptera	—	—	—	—	—	—	0.18	5.00	0.04	—	—	—
Trichoptera	0.07	4.34	0.01	—	—	—	0.09	5.00	0.02	—	—	—
Oligochaeta	0.07	4.34	0.01	8.42	22.22	5.18	0.18	5.00	0.04	10.71	20.00	6.34
Fishes	0.66	17.39	0.32	—	—	—	—	—	—	—	—	—
Detritus	—	94.44	—	—	33.33	—	—	—	—	—	100.0	—

food category found in alimentary tracts of analyzed fish are presented in Tables 1 and 2. Prey items included 27 different taxa, but they were not all represented as prey in both species during different seasons. Additionally, detritus was excluded from the calculation because the remains of animal and plant materials have degraded to a large extent, so it was not possible to put them into any category. Small crustaceans belonging to Ostracoda, Calanoida, Cyclopoida, and Cladocera were food categories consumed by both analyzed species throughout the studied seasons, but to a different extent.

The most varied diet was recorded in perch caught in the spring of 2011 ($H = 2.05$), with even 21 different prey categories detected, while the perch caught in the autumn of 2012 had the least varied diet (15 different prey categories, $H = 1.63$). Organisms categorized as Protozoa, Bryozoa, Ostracoda, *Bosmina* sp. and *Daphnia* sp. cladocerans, Calanoida, and Cyclopoida copepods, then Amphipoda, and Chironomidae, were the most common prey of all perch, but their proportion in the diet varied from season to season. Calanoid copepods were present in all analyzed perch alimentary tracts caught in spring 2011 and 2012, while cyclopoid copepods were present in all analyzed perch samples caught in autumn 2012. Only perch specimens caught in the spring of 2011 used fish fry in their diet as well as detritus and isopod crustaceans. The similarity in the diet of the analyzed perch was suggested by the high values of Schoener's overlap index (α from 0.87 to 0.95, Table 3).

Roach did not have a varied diet as perch, and, within species, they had quite a uniform diet during different seasons. Out of, in total, 12 identified prey categories in the diet of roach caught in spring 2011 and 2012, and in autumn 2011, there were as many as 11 prey categories ($H = 1.75–1.9$). Roach caught in autumn 2012 had the least diverse diet (seven prey categories, $H = 1.55$). Rhizopoda was the only prey present in the roach diet, but not in the perch diet. The most frequent food categories in the roach diet were members of the class Ostracoda, Calanoida, and Cyclopoida, as well as *Daphnia* sp. and *Bosmina* sp. (%FO ≥ 50 in all studied seasons) (Tables 1 and 2). In autumn 2012, *Daphnia* sp. and *Bosmina* sp. were present in all analyzed alimentary tracts of roach. Schoener's overlap index showed that the roach had a very similar diet during all seasons. However, roach (sampled in spring 2012) had significant index values with all other analyzed specimens of roach as well as perch from other seasons (Table 3).

The modified Costello graphic showed mostly a generalized feeding strategy in studied fish including some specimens that specialized on certain prey items (Fig. 2). In perch, the graphic analysis revealed that the feeding strategy of this species was a generalist feeder as all of the prey items were positioned in the lower part of the graph. Only Cyclopoida stood out according to the higher frequency of occurrence and prey-specific abundance values in relation to other prey items. Rare preys are also

Table 2. Assessment of diet composition of perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) collected in 2012 from Bovan Reservoir, Serbia, expressed as relative abundance (%*N*), frequency of occurrence (%FO), and prominence value (%PV) of food.

Taxon or group	Spring 2012						Autumn 2012					
	Perch			Roach			Perch			Roach		
	%*N*	%FO	%PV	%*N*	%FO	%PV	%*N*	%FO	%PV	%*N*	%FO	%PV
Protozoa	2.73	35.29	1.84	—	—	—	0.83	25.00	0.48	—	—	—
Rhizopoda	—	—	—	2.95	16.66	0.84	—	—	—	—	—	—
Rotatoria	0.91	5.88	0.25	1.68	5.55	0.47	—	—	—	—	—	—
Bryozoa	6.66	35.29	4.49	2.95	5.55	0.83	8.22	25.00	4.82	—	—	—
Hydracarina	0.10	5.88	0.02	—	—	—	0.97	16.66	0.46	—	—	—
Ostracoda	1.82	29.41	1.12	7.59	50.00	6.46	4.87	50.00	4.03	6.25	71.43	5.74
Anostraca	0.10	5.88	0.02	—	—	—	—	—	—	—	—	—
Conchostraca	—	—	—	—	—	—	0.69	8.33	0.23	—	—	—
Notostraca	—	—	—	—	—	—	0.97	8.33	0.32	—	—	—
Cladocera	—	—	—	—	—	—	1.11	8.33	0.37	—	—	—
Daphnia sp.	3.23	17.64	1.54	18.98	94.44	22.21	2.08	8.33	0.70	16.66	100.0	18.11
Bosmina sp.	4.54	70.58	4.33	18.98	94.44	22.21	5.29	83.33	5.66	36.45	100.0	39.62
Leptodora kindtii	0.20	5.88	0.05	—	—	—	—	—	—	—	—	—
Calanoida (Copepoda)	26.36	100	29.94	17.72	77.77	18.81	23.67	91.66	26.58	9.37	71.43	8.61
Cyclopoida (Copepoda)	42.93	94.12	47.30	25.32	77.77	26.88	39.97	100.0	46.89	22.92	85.71	23.06
Isopoda	—	—	—	—	—	—	—	—	—	—	—	—
Amphipoda	5.85	94.12	6.44	—	—	—	5.57	83.33	5.96	—	—	—
Gammaridae	—	—	—	—	—	—	—	—	—	—	—	—
Insecta (other)	0.10	5.88	0.02	—	—	—	—	—	—	—	—	—
Diptera (other)	0.10	5.88	0.02	—	—	—	—	—	—	—	—	—
Chironomidae	4.04	29.41	2.48	1.68	11.11	0.67	4.45	33.33	3.01	—	—	—
Plecoptera	0.30	5.88	0.08	—	—	—	0.83	8.33	0.28	—	—	—
Ephemeroptera	—	—	—	—	—	—	—	—	—	—	—	—
Trichoptera	—	—	—	—	—	—	—	—	—	—	—	—
Oligochaeta	—	—	—	2.11	5.55	0.59	0.42	8.33	0.14	8.33	28.57	4.84
Fishes	—	—	—	—	—	—	—	—	—	—	—	—
Detritus	—	—	—	—	100.0	—	—	—	—	—	100.0	—

Table 3. Schoener's overlap index (α) for the whole sample of perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) collected in 2011 and 2012 from Bovan Reservoir, Serbia. The codes provided include P or R for fish species (perch or roach, respectively), the year (2011 and 2012) and the season (S for spring and A for autumn).

α	P2011S	R2011S	P2011A	R2011A	P2012S	R2012S	P2012A	R2012A
P2011S	—	0.54	0.87	0.58	0.93	0.65	0.94	0.65
	R2011S	—	0.31	0.93	0.49	0.84	0.54	0.86
		P2011A	—	0.61	0.89	0.68	0.95	0.54
			R2011A	—	0.56	0.84	0.57	0.83
				P2012S	—	0.61	0.93	0.46
					R2012S	—	0.54	0.83
						P2012A	—	0.50

present in the perch diet, which are located at the lower-left corner on the graph. Similarly, the graphic analysis indicated the generalist feeding strategy of roach as most prey items were at the lower part of the graph, with two exceptions of Rotatoria (autumn 2011) and Oligochaeta (autumn 2011, and spring 2012) at the upper left corner of the graph. Evenness index confirmed these results (perch 0.49 ± 0.01; roach 0.38 ± 0.01).

Four clusters of neurons (A, B, C, and D) were isolated on the SOM output network (Fig. 3). The alimentary tracts of all analyzed roach were distributed in clusters A and B. Cluster A contained two samples of perch (both sampled in autumn 2011), and cluster B had four samples of perch (without any specimen in spring 2011). Clusters C and D exclusively contained perch alimentary tracts. Cluster B had the largest number of neurons, while cluster D had the largest number of samples. In cluster A, the most numerous were alimentary tracts of the roach sampled in spring 2011 (ten samples), while the least numerous were alimentary tracts of the roach sampled in the autumn of 2012, with only one sample. According to samples within, cluster B was the most diverse. In that group, the most numerous were alimentary tracts of roach, sampled in spring 2012. Clusters C and D contained the alimentary tracts of perch sampled in spring and autumn during both study years. In both clusters, the most numerous were the alimentary tracts sampled in spring 2011, while the least numerous were those sampled in autumn 2012.

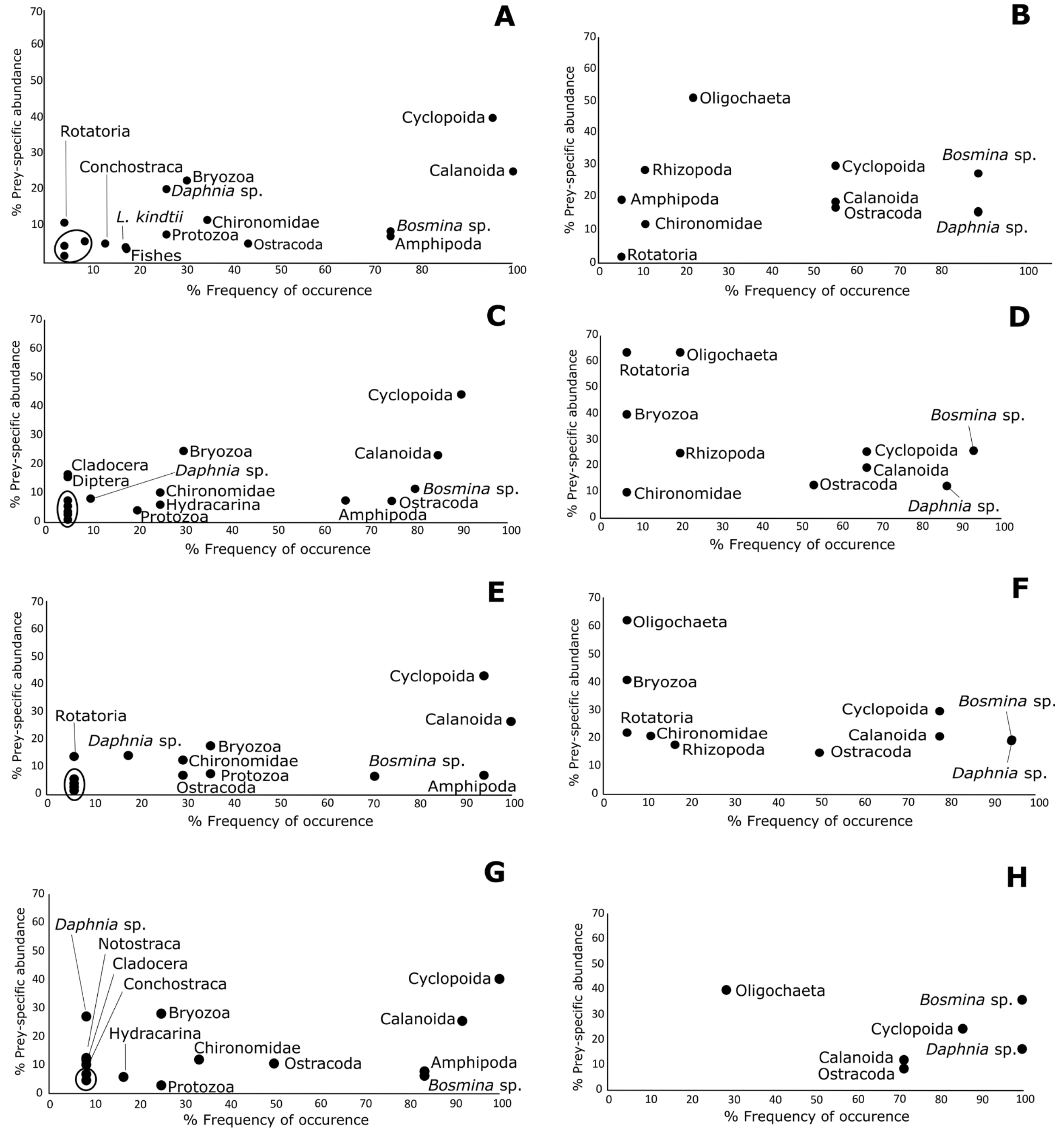

Figure 2. Costello graph. Prey-specific abundance vs. frequency of occurrence the diet of perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) collected in 2011 and 2012 from Bovan Reservoir, Serbia. (**A**) perch spring 2011, (**B**) roach spring 2011, (**C**) perch autumn 2011, (**D**) roach autumn 2011, (**E**) perch spring 2012, (**F**) roach spring 2012, (**G**) perch autumn 2012, (**H**) roach autumn 2012. Rare preys are encircled.

Significant IndVal values were recorded for 10 out of 26 food categories (Table 4, Fig. 4). One food category was significantly associated with alimentary tracts assigned to cluster A, two food categories for alimentary tracts of cluster B, six food categories for cluster C and five food categories for cluster D. Three out of 10 food categories (Cyclopoida, Calanoida, and Amphipoda) were significant for specimens whose alimentary tracts were assigned to clusters C and D, while *Bosmina* sp. were significant for specimens in clusters B and D. Oligochaeta were significant prey for specimens from cluster A and *Daphnia* sp. for specimens from cluster B. Nevertheless, they both were completely absent in the alimentary tracts of specimens assigned to cluster C. Also, Protozoa and Chironomidae were significant prey for specimens in cluster C, whereas they were absent in the alimentary tracts of specimens distributed in cluster B. On the other hand, *Bosmina* sp. were present in all the alimentary tracts of specimens assigned to cluster B, whereas Cyclopoida were also present in all the alimentary tracts of specimens assigned to clusters C and D (Table 4).

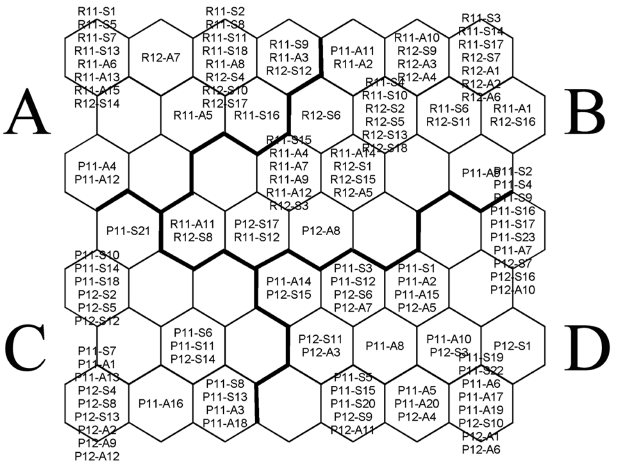

Figure 3. The 130 alimentary tracts of perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) collected in 2011 and 2012 from Bovan Reservoir, Serbia, assigned to 49 (7 × 7) SOM output neurons within clusters **A**, **B**, **C**, and **D**. The code for each alimentary tract consists of one letter for the fish species (P or R), two digits for the year of sampling 11 (2011) or 12 (2012), one letter for sampling season (S = spring or A = autumn) and the ordinal number of the individual.

Discussion

In this study, we have analyzed the food interactions between perch and roach juveniles. Although general food categories consumed by perch and roach were similar, each species had its own predominant prey items during different seasons. In general, perch changes diet during ontogeny by feeding on zooplankton, macroinvertebrates, and fish (Rezsu and Specziár 2006). In contrast, roach does not undergo notable ontogenetic dietary shifts and is considered a more efficient planktivore than perch (Werner and Gilliam 1984). There have been many papers on juvenile perch and roach diet with, in general, contradictory opinions. Persson et al. (2000), and Estlander et al. (2010) claimed that these two species have the same preferences for zooplankton, while Rezsu and Specziár (2006) and Schleuter and Eckmann (2008) stated that they have different food preferences.

Zooplankton is the essential diet of fish fry (Karus et al. 2014), and this was observed in our research. Based on the Prominence values, the food categories presented in the diet of both species throughout the entire study period were Ostracoda, *Daphnia* sp., *Bosmina* sp., Calanoida, and Cyclopoida, but in different proportions. The Prominence value showed that only roach caught in autumn 2012 had in each alimentary tract *Bosmina* sp. and *Daphnia* sp. It is noticeable in our study that perch in each of the studied seasons more often used *Bosmina* sp. than *Daphnia* sp. in the diet. This result is similar to the findings of Mehner et al. (1995, 1998), who noted that perch tend to consume small cladocerans. Frankiewicz and Frankiewicz-Wojtal (2012) and Evtimova et al. (2015) had the opposite opinion and stated that perch more often use large cladocerans such as *Daphnia* sp. in their diet. Despite these opposing views, the reason for perch consuming smaller rather than large cladocerans may be the significantly higher number of cladocerans of the genus *Bosmina* than the genus *Daphnia* in Bovan Reservoir (Ostojić 2006). According to Tarvainen et al. (2002), Vašek et al. (2006), and Peterka and Matěna (2009), zooplankton is the main food of 0+ roach. This statement agrees with our results, but among zooplankton *Bosmina* sp. stood out as the most dominant prey of roach during all studied seasons.

In Bovan Reservoir, consumption of cladocerans was higher in roach than in perch and, in contrast, perch was more likely to feed on amphipods and copepods (Cyclopoida and Calanoida) than roach. This is also indicated by Okun and Mehner (2005). Zapletal et al. (2014) reported that roach consumed far fewer copepods, while Kornijów et al. (2005) noted that copepods were not part of roach

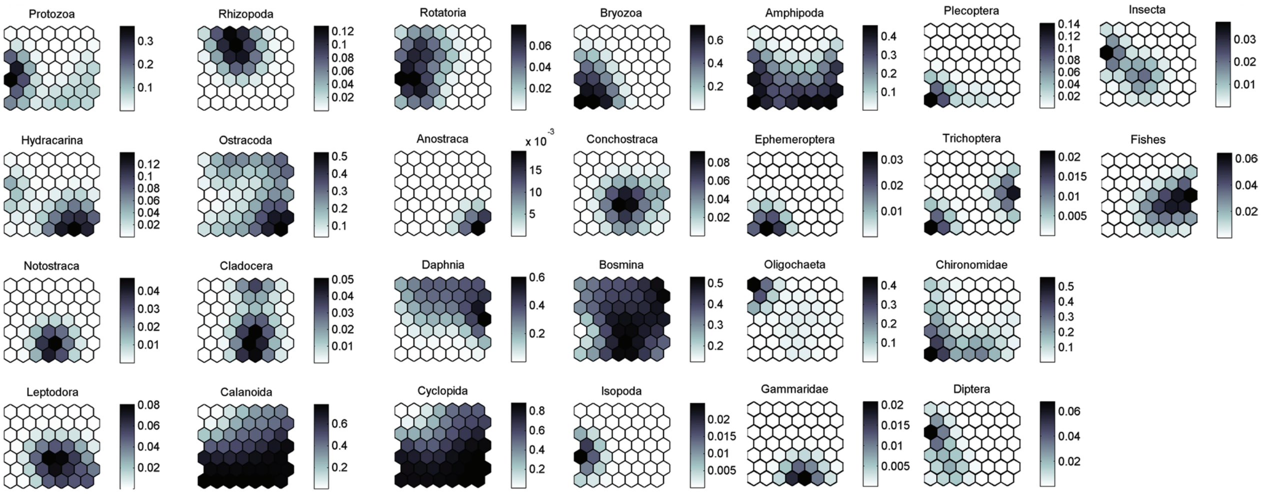

Figure 4. Distribution pattern for 26 food categories represented in the diet of perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) collected in 2011 and 2012 from Bovan Reservoir, Serbia. The shading is scaled independently for each food category. The shade of black for each food category is highly correlated with the values of the IndVal index. The degree of shading decrease is also indicated by a decline in the values of the IndVal index.

Table 4. Relative frequency (%FO), relative abundance (%*N*), and indicator values (IndVal) for food categories of perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) collected in 2011 and 2012 from Bovan Reservoir, Serbia. The highest (at $P \leq 0.05$) IndVal in a given cluster (A, B, C, D) are in bold (exact significance levels are presented in Fig. 3) (modified according to Dukowska et al. 2013, 2014).

Fish diet group	A			B			C			D		
	%FO	%*N*	IndVal	%FO	%*N*	IndVal	%FO	%*N*	IndVal	%FO	%*N*	IndVal
Protozoa	4	3	0	0	0	0	43	73	**32**	18	23	4
Rhizopoda	17	51	9	10	0	5	0	0	0	0	0	0
Rotatoria	4	27	1	3	9	0	9	64	6	0	0	0
Bryozoa	4	2	0	5	3	0	87	91	**79**	2	4	0
Hydracarina	4	7	0	0	0	0	4	7	0	16	85	14
Ostracoda	54	20	11	59	18	11	26	14	4	59	48	**28**
Anostraca	0	0	0	0	0	0	0	0	0	2	100	2
Conchostraca	0	0	0	0	0	0	0	0	0	11	100	11
Notostraca	0	0	0	0	0	0	0	0	0	2	100	2
Cladocera	0	0	0	3	29	1	0	0	0	5	71	3
Daphnia sp.	75	25	19	92	32	**29**	0	0	0	25	43	11
Bosmina sp.	83	18	15	100	32	**32**	43	18	8	93	33	**31**
Leptodora kindtii	0	0	0	0	0	0	0	0	0	14	100	14
Calanoida	17	1	0	95	10	9	96	43	**42**	98	46	**45**
Cyclopoida	17	0	0	97	10	10	100	39	**39**	100	50	**50**
Isopoda	0	0	0	0	0	0	4	100	4	0	0	0
Amphipoda	8	5	0	5	1	0	83	45	**37**	77	48	**37**
Gammaridae	0	0	0	0	0	0	0	0	0	2	100	2
Insecta (other)	4	65	3	0	0	0	0	0	0	2	35	1
Diptera (other)	4	5	3	0	0	0	4	74	1	0	21	0
Chironomidae	21	5	1	0	0	0	57	74	**42**	20	21	4
Plecoptera	0	0	0	0	0	0	13	97	13	2	3	0
Ephemeroptera	0	0	0	0	0	0	4	100	4	0	0	0
Trichoptera	0	0	0	0	0	0	0	0	0	5	100	5
Oligochaeta	38	85	**32**	3	9	0	0	0	0	7	6	0
Fishes	0	0	0	0	0	0	0	0	0	9	100	9

diet. Copepods rarely occur in planktivorous fish diets, such as roach, because of their ability to escape from predators (Peterka and Matěna 2009; Karus et al. 2014). Also, Prominence values are higher for Cyclopoida than for Calanoida, although all perch specimens from the spring of both years had Calanoida in their alimentary tract content.

The large cladoceran *Leptodora kindtii* is also an important food component in the roach and perch diet (Vašek and Kubečka 2004; Vašek et al. 2006). This does not coincide with our results since *L. kindtii* has not been found in any of the alimentary tracts of the roach, and perch rarely used it in the diet. For perch as a visually oriented predator (Persson and Greenberg 1990), it is difficult to catch because of its transparency due to its extremely reduced body elements (predator defense strategy) (Liu and Uiblein 1996). However, even with the low Prominence values, it was detected in the perch diet in all studied seasons, except autumn 2012.

In general, our results showed that macroinvertebrates constituted a minor fraction of the food items found in the perch and roach alimentary tracts. The majority of juvenile perch fed on chironomids (Mehner et al. 1995, 1998), while roach fed on chironomids and Odonata larvae (Bogacka-Kapusta and Kapusta 2007). Adamczuk and Mieczan (2015) noted that juvenile specimens of both species showed the same high preference for chironomids. Our results supported this statement because chironomids were the prey of both species during all studied seasons (except roach in autumn 2012). According to Simić et al. (2006) chironomids are very abundant in Bovan Reservoir bottom fauna. Also, Oligochaeta were not recorded in the perch diet only in the spring of 2012 and throughout the research, the Prominence value was low. According to Kornijów et al. (2005), only a few roach included macroinvertebrates (mainly ephemeropteran and trichopteran larvae, seldom chironomid larvae) in their diet despite the high biomass of these prey. It could be concluded that only a few perch included macroinvertebrates such as Plecoptera, Ephemeroptera, and Trichoptera larvae, in their diet. A small and sporadic presence of these organisms in the perch diet can be assumed from the Prominence value.

During the investigated seasons, detritus was also present in the diet of juvenile perch, but to a much lower extent than in the juvenile roach diet. It was possible to detect its presence in the diet but not to quantify it, except with frequency of occurrence, the values of which were high. The importance of detritus in the roach diet has been noted by Kornijów et al. (2005) and Zapletal et al. (2014). According to Matěna (1995, 1998), the roach diet changes according to the ontogenetic stage, with the proportion of macrophytes and detritus increasing as the fish gets older. On the contrary, Lyagina (1972) and Vøllestad (1985) referred that a high proportion of detritus in the roach diet indicates the low availability of animal prey.

Also, according to Brandl (1994), roach consumed detritus before the increase of cladoceran abundance.

This study showed that the roach has better competitive abilities for cladocerans than juvenile perch. It results in a shift in feeding preferences of juvenile perch and thereafter increased competition with older perch and additionally decreased growth and recruitment to the piscivorous stage (Persson and Greenberg 1990). This is not rare, and during this research, the occurrence of 0+ perch feeding on fish was recorded. This was recorded only in the spring of 2011. Perch can feed on increasingly larger prey as gape size increases (Romare 2000) and can reach their piscivorous niche in their first growing season (Borcherding et al. 2000; Rezsu and Specziár 2006; Schleuter and Eckmann 2008). This phenomenon is useful because it is known that piscivorous juvenile perch have one of the key roles in contributing to water transparency in many lakes and reservoirs (Shapiro 1980; Gulati et al. 2008; Jacobsen et al. 2014).

The modified Costello's method suggests that some of the analyzed specimens specialized on certain types of prey, whereas the entire sample seems to have a generalized feeding strategy. This can be deduced from the fact that a few prey items have a high prey-specific abundance (%P_i) and low frequency of occurrence (%FO). Roach is considered a generalist feeder with the exception of specialization on Oligochaeta and Rotatoria. According to Costello's graph, for some roach specimens, Oligochaeta were of great importance during the whole investigation, with the exception of autumn 2012 (%$Pi < 50$). The explanation for this is the dominance of Oligochaeta in Bovan Reservoir bottom fauna (Simić et al. 2006). The generalist feeding strategy in perch is likely associated with its opportunistic feeding behavior that feeds on the most available and abundant prey in a given time and place (Gerking 1994). According to Costello's graph, Cyclopoida are positioned nearest the upper right corner during all seasons, while *Daphnia* sp. (autumn 2012) approached the upper left corner. Also, in the lower-left corner rare or unimportant preys are placed (Amundsen et al. 1996).

Due to the different degrees of digestion, information on the alimentary tracts' contents may consist of only general food categories (i.e., higher taxonomic levels) or may be identified to the lowest possible taxonomic level. If we decide to uniform the data and present the alimentary tracts' contents "roughly" or on the other hand in detail this would result in losing information on a large part of the alimentary tracts' content (Marszał et al. 1996, 1998), and could result in methodological errors, too (Dukowska et al. 2013). For these reasons, self-organizing maps could be useful in fish feeding analysis because they easily deal with nonlinear variables that are related in a complex way and that exhibit normal or skewed distributions (Lek et al. 2005; Dukowska et al. 2013).

First, there were two groups of roach specimens assigned to clusters A and B, and two groups of perch specimens assigned to clusters C and D. Those in cluster A benefited from Oligochaeta, which were used during the whole study as reflected in significant IndVal. Specimens in cluster B during all study periods most often fed on cladocerans *Bosmina* sp. and *Daphnia* sp., which is proved by significant IndVal values. All perch and roach specimens from the most diverse cluster B had *Bosmina* sp. in their alimentary tracts. Perch assigned to cluster C focused on Chironomidae and zooplankton, including Protozoa and Bryozoa (IndVal significant only for cluster C), while those in cluster D ate mostly zooplankton. Also, it is visible in cluster C that no specimens consumed *Daphnia* sp. Copepods played an important role in the diet of perch, as indicated by significant IndVals. Additionally, each specimen distributed in clusters C and D had Cyclopoida in its alimentary tract. Protozoa, Bryozoa, Ostracoda, and Amphipoda are good examples of the advantage of self-organizing maps and IndVal in relation to traditional index Prominence value. IndVal for these groups is significant only for cluster C, only for cluster D, or both, while the Prominence value for these preys is low throughout the whole research. This distribution of specimens' alimentary tracts in neurons indicates that there was no high degree of competition between perch and roach, and the segregation between them was strict. The value of Schoener's niche overlap index found in this research was indicating an almost total diet overlap within the species, as also visually shown by the results obtained using self-organizing maps, where all roach and only six specimens of perch were classified into clusters A and B. All other specimens of perch were in clusters C and D. Low trophic overlap is expected for these two species that seem to use this strategy to allow their coexistence in high abundance in Bovan Reservoir. Seasonality significantly affected both species' diet composition, indicating the different proportions of food resources between periods because similar food categories were present during all seasons, but IndVal singles out certain food categories as significant.

Self-organizing maps have proven to be most suitable for application over complex and nonlinear ecological data and are particularly suitable for application over large data sets (Kruk et al. 2007; Chon 2011; Penczak et al. 2012). Compared to various methods of linear ordination, self-organizing maps provide a better overview of community planning in ecological studies (Giraudel and Lek 2001). As Dukowska et al. (2013, 2014) stated, the diet analysis presented in this way increases the credibility of the obtained data. This is important because there were food categories used in both species' diets but represented to a lesser extent or only represented in single specimens. Presentation of fish diet in this way provided a clearer picture of the trophic relations within and between species in Bovan Reservoir.

This study shows the diet analysis based on traditional indices, which have been used for decades, and the diet analysis presented using self-organizing maps and IndVal. Comparing the results obtained in these two ways, the impression is that results are very similar or even identical. The high Prominence values and separation of certain preys on Costello's graph (upper right corner) show which preys are dominant. This is confirmed by significant IndVal. Also, there are preys like Protozoa, Bryozoa, Ostracoda, Amphipoda, and Chironomidae that are positioned in the middle of Costello's graph all the time, and the Prominence values are not particularly high or low. For these preys IndVal values are significant, and the specimens that consume them are together in a cluster on the SOM map, which means that these preys are important only for certain specimens, and not for the whole population. Oligochaeta are a good example, too. They are important prey for certain roach specimens based on Costello's graph, and IndVal is significant for them. All these specimens are arranged in cluster A. Also, there are, in the perch diet, rare or unimportant preys, for which the Prominence values are low, and on a graph, they are in the lower-left corner. Consequently, these specimens are arranged in the same cluster, and IndVal values are insignificant. Likewise, the SOM output network visually shows the results of Schoener's niche overlap index too, where the separation between species is clearly seen. It appears that the IndVal shows the same results as the Prominence value and Costello's graph, while the SOM output network shows whether there is an overlap in diet between specimens or species, as do the Schoener's niche overlap index.

Conclusions

Our results showed that juvenile fish used in diet both zooplankton and macrozoobenthos specimens; roach often fed on nonanimal prey, while perch of age 0+ also used fish in their diet. However, both species play an important role in the food web of ecosystems. Thus, the presented study provides a basis for further research on the feeding biology of these two species. Moreover, integrating these results with those previously published could be used to draw up a common strategy for managing the reservoir fish stock.

In summary, this study offers valuable insights into the dietary strategies of perch and roach. However, fish feeding analysis using self-organizing maps provides a more complete insight into the fish feeding habits, and thus the similarities and differences between them. Because as the distance in the network increases, the differences in models assigned to the neurons also increase. One neuron can contain data from several samples (i.e., specimens), and therefore there is certainly a high degree of their dietary similarity. In the end, it should be mentioned that with the identification of the alimentary tract contents, which is a complex and time-consuming process, especially in juveniles, self-organizing maps in combination with the IndVal index represents an adequate and time-saving analysis.

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Length-to-weight and length-to-length relations of 15 freshwater fish species (Actinopterygii: Cypriniformes) from the Oujiang River, China

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Abstract

Length-to-weight and length-to-length relations were estimated for 15 freshwater fishes belonging to the order Cypriniformes from Yuxi power station to Kaitan reservoir dam trunk stream of the Oujiang River, Zhejiang Province, China. The following species were studied: *Distoechodon tumirostris* Peters, 1881, *Xenocypris davidi* Bleeker, 1871, *Plagiognathops microlepis* (Bleeker, 1871), *Hemibarbus labeo* (Pallas, 1776), *Hemibarbus maculatus* Bleeker, 1871, *Chanodichthys erythropterus* (Basilewsky, 1855), *Culter alburnus* Basilewsky, 1855, *Chanodichthys dabryi* (Bleeker, 1871), *Opsariichthys bidens* Günther, 1873, *Zacco platypus* (Temminck et Schlegel, 1846), *Sinibrama macrops* (Günther, 1868), *Hemiculter leucisculus* (Basilewsky, 1855), *Pseudohemiculter hainanensis* (Boulenger, 1900), *Rhodeus sinensis* Günther, 1868, and *Squalidus argentatus* (Sauvage et Dabry de Thiersant, 1874). The determination coefficients r^2 of LWRs were all over 0.96, and the 15 values of parameter b were consistent with the predicted range of 2.5–3.5. The total length-to-standard length relations were also calculated with $r^2 \geq 0.97$. Our study provides new information on LWR for 1 species and LLRs for 8 species, as well as new maximum total length recorded for 4 species (i.e., *Distoechodon tumirostris*, *Opsariichthys bidens*, *Pseudohemiculter hainanensis*, and *Rhodeus sinensis*) in FishBase. This study is expected to provide a useful baseline for further studies of population parameters to improve management decisions on the Oujiang River.

Keywords

growth coefficient, length-length relation, length-weight relation, Oujiang River

Introduction

The Oujiang River (118°45′–121°00′E, 27°28′–28°48′N) is the second major river in Zhejiang Province, China, with a basin area of about 18 028 km^2. Other than drinking, it also has power generation, flood control, irrigation, and tourism functions. The river had a history of rich fish resources with 111 species in the 1970s. However, fish stocks have continued to decline in recent decades due to overfishing, biological invasion, environmental damage, and hydroelectric dam construction (Guo et al. 2019; Kim et al. 2020; Lin et al. 2021), particularly the cascading development of power plants. It destroyed the integrity of the ecosystem, dividing it into units of discontinuous structure and increasing habitat fragmentation. Statistics show that more than 90 reservoirs with a storage capacity of more than 1 million m^3 were constructed in the river. Habitat fragmentation is becoming one of the most

Table 1. Descriptive statistics and estimated parameters of LWR ($W = a\mathrm{TL}^b$) for 15 freshwater fish species sampled in the Oujiang River, southeast China.

Species	Total length [cm]			Total weight [g]		LWR parameters					
	N	Min	Max	Min	Max	*a*	95% CL of *a*	*b*	95% CL of *b*	r^2
Distoechodon tumirostris ^{A}	555	14.5	42.0	23.2	740.0	0.007	0.006–0.008	3.041	2.997–3.085	0.97
Xenocypris davidi	184	7.5	39.7	4.0	491.0	0.011	0.010–0.015	2.884	2.816–2.952	0.97
Plagiognathops microlepis	287	10.7	38.2	13.4	474.6	0.016	0.014–0.020	2.770	2.713–2.827	0.97
Hemibarbus labeo	287	10.2	42.5	8.2	695.6	0.009	0.008–0.010	2.976	2.934–3.017	0.99
Hemibarbus maculatus	19	6.3	29.5	2.0	241.3	0.015	0.008–0.029	2.877	2.635–3.119	0.97
Chanodichthys erythropterus	30	14.6	26.2	22.3	152.7	0.005	0.003–0.010	3.096	2.903–3.289	0.97
Culter alburnus	189	11.0	46.5	6.0	480.5	0.006	0.005–0.008	2.951	2.891–3.010	0.98
Chanodichthys dabryi	76	11.5	31.6	7.2	209.0	0.002	0.002–0.003	3.286	3.194–3.378	0.99
Opsariichthys bidens	30	9.2	21.2	6.4	105.5	0.003	0.002–0.006	3.402	3.194–3.609	0.98
Zacco platypus	206	6.4	16.3	2.6	45.7	0.006	0.006–0.008	3.157	3.083–3.232	0.97
Sinibrama macrops	399	7.1	22.5	3.8	119.1	0.003	0.003–0.004	3.354	3.292–3.417	0.97
Hemiculter leucisculus	49	10.4	23.0	6.8	107.4	0.003	0.002–0.006	3.231	3.061–3.401	0.97
Pseudohemiculter hainanensis	98	10.3	26.3	6.0	126.2	0.003	0.002–0.004	3.267	3.177–3.357	0.98
Rhodeus sinensis ^{A}	29	5.3	7.6	2.3	6.6	0.015	0.010–0.024	2.934	2.700–3.167	0.96
Squalidus argentatus	189	6.0	14.0	1.3	25.2	0.003	0.003–0.004	3.412	3.319–3.504	0.97

N = sample size, Max = maximum, Min, minimum, *a* and *b* = constant parameters in equation $W = a\mathrm{TL}^b$, CI = confidence interval, r^2 = coefficient of determination. Species with new maximum size records are marked with **bold** font; ^{A} First record of LWR for the species.

important factors influencing biodiversity and is also a major reason for species extinction. However, little data was available on the growth characteristics of fish species in the river. In this study, length-to-weight (LWRs) and length-to-length relations (LLRs) were established for the 15 species captured from Yuxi power station to Kaitan reservoir dam trunk stream of the Oujiang River, in order to provide a useful reference for further studies of population parameters to improve management decisions.

Materials and methods

Fish samples were collected from Yuxi power station to Kaitan reservoir dam trunk stream of the Oujiang River, Zhejiang Province, China (28°17′–28°27′N, 119°44′–119°53′E), which is a relatively complete structural unit with original ecological preservation of the river's valley features. Sampling was conducted seasonally from the section between March and November 2019. Multipanel nylon gillnets ranging in size from 1 cm to 8 cm were deployed to collect the fish at 05:00–07:00 h. All fish caught were identified to species (Mao et al. 1991). 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 for 15 species were calculated using the formula

$$W = a\mathrm{TL}^b$$

where *W* is the weight [g], TL is the total length [cm], *a* and *b* are the intercept and slope of the power equation, respectively. 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 similar linear regression was used to determine the LLR

$$\mathrm{TL} = a + b\mathrm{SL}$$

where SL is the standard length and other measurements are defined as above. For species with $r^2 < 0.95$, outliers were discarded and regression was recalculated. All statistical analyses were performed using SPSS 16.0 (SPSS, Inc., Chicago, IL, USA).

Results

A total of 2627 individuals were examined. The descriptive statistics and the estimated LWR parameters are summarized in Table 1, providing the regression parameters *a* and *b* along with the estimated 95% confidence intervals and the coefficient of determination (r^2). Additionally, similar parameters are given for the length-to-length relations (Total length versus Standard length) in Table 2.

Table 2. Length–length relations ($\mathrm{TL} = a + b\mathrm{SL}$) of 15 freshwater fish species sampled in the Oujiang River Basin, southeast China.

Species	LWR parameters		
	a	*b*	r^2
Distoechodon tumirostris	1.137	1.976	0.98
Xenocypris davidi	1.342	–0.944	0.98
Plagiognathops microlepis	1.219	0.208	0.99
Hemibarbus labeo	1.118	1.973	0.98
Hemibarbus maculatus	1.196	0.292	0.99
Chanodichthys erythropterus	1.155	0.750	0.99
Culter alburnus	1.163	1.529	0.98
Chanodichthys dabryi	1.192	0.607	0.99
Opsariichthys bidens	1.171	0.451	0.99
Zacco platypus	1.227	–0.009	0.99
Sinibrama macrops	1.222	0.395	0.97
Hemiculter leucisculus	1.223	–0.131	0.99
Pseudohemiculter hainanensis	1.211	–0.073	0.99
Rhodeus sinensis	1.186	0.373	0.97
Squalidus argentatus	1.152	0.507	0.98

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

Discussion

As a result, all LWR and LLR estimates were highly significant ($P < 0.01$), yielding $r^2 > 0.96$. One new LWR for *Distoechodon tumirostris* was found in comparison with the FishBase database (Froese and Pauly 2021) (Table 1). The values of parameter b for 15 species were consistent with the predicted range of 2.5–3.5 (Hile 1936; Froese 2006). The LLRs of the 15 species were updated, among which 8 new LLRs were discovered. Additionally, the LLR parameters of b for *D. tumirostris*, *Hemibarbus labeo*, *Culter alburnus*, *Zacco platypus*, *Sinibrama macrops*, and *Hemiculter leucisculus* here were not within the ranges from prior studies which are listed in FishBase (Froese and Pauly 2021), different growth stanzas and environmental factors can explain some of the reasons (Froese 2006; Lin et al. 2018; Yang et al. 2017). The new maximum values of total length for 4 species were also recorded, such as *D. tumirostris*, *Opsariichthys bidens*, *Pseudohemiculter hainanensis* and *Rhodeus sinensis*.

Froese et al. (2011) suggested that the individual number (sample size) for the LWR analysis of each species should be greater than 100 to meet the need for sampling statistics. Here, sample sizes for 7 species including *Hemibarbus maculatus*, *Chanodichthys erythropterus*, *Chanodichthys dabryi*, *O. bidens*, *H. leucisculus*, *P. hainanensis* and *R. sinensis*, were all less than 100, indicating an inadequate number of specimens to estimate an adequate LWRs equation. Therefore, we recommend using LWRs of these species from this study as baseline information for future studies.

Conclusion

This study provides basic information on LWRs and LLRs for 15 fish species. The new LWR for *D. tumirostris*, new LLRs for 8 species, and the new maximum size recorded for 4 species highlight the scarcity of information on the biological aspects of these fishes. These LWRs and LLRs should assist fisheries scientists and managers to complement their further studies of population parameters to improve management decisions on the Oujiang River.

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Supplementary material 1

Table S1

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Data type: Data of length and weight

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