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Gymnocranius oblongus, a new large-eye bream species from New Caledonia (Teleostei: Lethrinidae)

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Abstract

Gymnocranius oblongus is described as a new species of the subfamily Monotaxinae (Sparoidaea : Lethrinidae), a group of commercially important fishes distributed throughout the Indo-West Pacific, from six specimens collected in New Caledonia. It is characterized by an oblong, fusiform body, slightly rounded snout, elongate tail with rounded tips and sub-horizontal, wavy blue lines or dashes on snout and cheeks. It is distinct from sympatric *G. grandoculis* by a more slender body which is also more symmetrical dorso-ventrally, and a more elongated caudal fin. Both mitochondrial-DNA and nuclear-DNA markers provide a genetic basis to the distinction of *G. oblongus* from *G. grandoculis*.

Résumé

***Gymnocranius oblongus*, une nouvelle espèce de bossu blanc de la Nouvelle-Calédonie (Pisces: Lethrinidae).** *Gymnocranius oblongus* est décrit comme nouvelle espèce de la sous-famille des Monotaxinae (Sparoidaea : Lethrinidae), un groupe de poissons d'importance commerciale distribué dans tout l'Indo- Pacifique ouest, à partir de six spécimens récoltés en Nouvelle-Calédonie. Il se caractérise par un corps oblong et fusiforme, un museau légèrement arrondi, une queue allongée aux extrémités arrondies, des lignes ou des tirets bleus sub-horizontaux et sinueux sur le museau et les joues. Il se distingue de l'espèce sympatrique *G. grandoculis* par un corps plus allongé et plus symétrique dorso-ventralement et une nageoire caudale plus allongée. Les marqueurs mitochondriaux et nucléaires confirment la distinction des deux espèces.

Keywords : Monotaxinae; Taxonomy; Molecular genotyping; Reproductive isolation; Cryptic species
Mots-clés : Monotaxinae; Taxonomie; Génotypage moléculaire; Isolement reproductif; Espèces cryptiques

1. Introduction

Although most Lethrinidae (emperor fishes and large-eye breams) are of significant interest to fisheries in the Indo-Pacific region, research on their biology and fisheries is hampered by taxonomic confusion and difficulties in species identification [1, 2]. The taxonomy of large-eye breams of the genus *Gymnocranius* (Lethrinidae: Monotaxinae) is notoriously difficult. The two most recent revisions, made only three years apart [1, 2] disagreed on several points. Sato [1] recognised six species in the genus, whereas Carpenter and Allen [2] recognised 8, and some of the species recognised by both authors were ascribed different names, both [1] and [2] invoking the rules on nomenclature [3]. For instance, the name *G. grandoculis* (Valenciennes 1830) was not retained by Sato [1], who questioned the identity of the holotype of *Cantharus grandoculis* Valenciennes 1830. Sato [1] instead used the name *G. robinsoni* (Gilchrist and Thompson 1909), but the latter has been discarded by Carpenter and Allen [2] as a junior synonym of *G. grandoculis*. Among the problems pending is the description of a species first erroneously identified as *G. lethrinoides* (non Bleeker 1850) [4] and now widely referred to as "*Gymnocranius* sp." [1, 2, 5-8]. Agreement was reached by both [1] and [2] in recognising difficulties in the taxonomy of the genus, and on the need of further research.

Several *Gymnocranius* species regularly occur on the fish market in Noumea, including *G. elongatus*, *G. euanus* and *G. grandoculis* ([8]; P. Borsa pers. obs.). Between 2003 and 2008 we collected adult or sub-adult specimens of Lethrinidae to establish a reference genetic database from intron length polymorphisms and mtDNA (16S rDNA) single-strand conformation patterns [9]. The purpose of this database was to permit the identification of unknown Lethrinidae larvae. In the process, we had difficulties identifying to species a number of adult or sub-adult specimens of the genus *Gymnocranius* using the diagnoses provided by Sato [1] and Carpenter and Allen [2]. Multiple-locus genotype frequencies revealed three undescribed *Gymnocranius* species among our samples from New Caledonia [9]. Here, we describe one of these species, of which we also captured an early juvenile.

2. Materials and methods

The following specimens of *Gymnocranius oblongus* n. sp., all deposited at the Museum national d'histoire naturelle, Paris, were examined: MNHN 2009-0009 (Southern lagoon, New Caledonia, holotype), standard length (SL) 358 mm; MNHN 2009-0005 (Southern lagoon, New Caledonia, paratype), SL 220 mm; MNHN 2009-0007 (New Caledonia, paratype), SL 245 mm; and MNHN 2009-008 (New Caledonia, paratype), SL 226 mm. Two other specimens whose skull was preserved at Institut de recherche pour le développement (IRD), Nouméa (IRDN-20090410 A and B, from the Southern lagoon of New Caledonia), were examined fresh. Codes here follow [10] except IRDN, which refers to the specimens in the collections of IRD, Nouméa.

Gymnocranius oblongus n. sp. individuals had been initially identified by us as *G. grandoculis*, as they fell out with that species in the identification keys of both Sato [1] and Carpenter and Allen [2]. Comparative material thus included two *Gymnocranius grandoculis* specimens: MNHN 0000-8811 (Seychelles; holotype of *Cantharus grandoculis* Valenciennes, 1830), SL 186 mm; MNHN 2009-0006 (Northern lagoon, New Caledonia), SL 474 mm; IRDN-20080607 B (Kouaré Pass, New Caledonia), SL 342 mm. We also examined the type specimens of three species currently considered synonyms of *G. grandoculis* [2]: *Pentapus dux* Valenciennes, 1862 (Reunion Island; holotype, MNHN 0000-2951); *P. curtus* Guichenot, 1863 (Reunion Island; holotype, MNHN 0000-1317); and *Paradentex marshalli* Whitley, 1936 (Queensland; holotype, QM I.5284), SL 481 mm.

Meristics and measurements made on the specimens are listed in Table 1. *Gymnocranius oblongus* n. sp. specimens IRDN-20090410 A and B were gutted for analysis of stomach content and determination of sex. Molecular genotyping was performed on samples of 5 *G. grandoculis* and 6 *G. oblongus* n. sp., all from New Caledonia.

For molecular genotyping, whole genomic DNA of individuals was extracted from fin clips preserved frozen or in ethanol, using either the classical phenol-chloroform protocol [11], or the“DNeasy® Tissue Kit” of Qiagen GmbH (Hilden, Germany) according to the manufacturer’s instructions. DNA extracts were conserved in ultrapure water at -20°C. PCR amplification was done in 25 µl reaction mixture containing 1.5-2.0 mM $MgCl_2$, 0.64 mM dNTP mix, 0.1-0.3 µM of each primer and 0.03-0.2 U Taq DNA polymerase (Promega, Madison WI, USA). The primers for the *16S rRNA*

locus (forward: 5'-GCC CAA CCA AAG ACA TTA GGG CAG-3'; reverse: 5'-GAC CCG TAT GAA TGG CAT AAC GAG-3') were designed from the alignment of *Lethrinus ornatus*, *Lethrinus rubrioperculatus* and *Beryx splendens* homologous sequences (GenBank AF247446, AF247447 and AY141406). The primers used to amplify an intron of the metallothionein gene (forward: 5'-ATG GAY CCY TGH GAC TGC TC-3'; reverse: 5'-RCA GGA TCC WCC GCA GYT GC-3') were designed in the flanking exons, from the alignment of homologous metallothionein genes of *Carassius cuvieri*, *Dicentrarchus labrax*, *Takifugu rubripes* and *Salmo salar* (GenBank AY165048, AF199014, CA847265 and BG935118). The primers designed to amplify the other introns have been published previously [12-14]. PCRs were run in a RoboCycler Gradient 96 thermocycler (Stratagene, Cedar Creek TX, USA), with annealing temperature set to 50°C (*CK*), 51°C (*16S rRNA*) or 52°C (*Aldo-B*, *GnRH-1*, *Met*). Immediately before migration, 6 µl denaturing loading buffer (95% formamide, 10 mM NaOH, 84‰ bromophenol blue, 5% glycérol) was added to each well of the PCR plate and the mixture was heated for 5 min at 95°C.

Amplified 16S rDNA fragments were subjected to single-strand conformation polymorphism analysis after electrophoresis of the DNA fragments in vertical, non-denaturing polyacrylamide gels (MDE 1X, FMC corporation, Rockland, USA) overnight at 2 W at ambient temperature (25°C). Introns were separated according to size by vertical electrophoresis in denaturing, 0.4-mm thick polyacrylamide gels [6% acrylamide:bis-acrylamide (29:1) solution, TBE 1X, 7 M urea] at 50 W. DNAs were stained using silver nitrate.

3. Results

3.1. Diagnostic description

Gymnocranius oblongus Borsa, Béarez and Chen, n. sp. (Figs. 1-3; Table 1); *Gymnocranius* sp. A [15, 16]; *Gymnocranius* cf. *grandoculis* [17, in 16].

The following description of *G. oblongus* n. sp. is based on four specimens deposited at Museum national d'histoire naturelle, Paris [MNHN 2009-0009 (holotype), and MNHN 2009-0005, MNHN

2009-0007 and MNHN 2009-0008 (all paratypes)], and two individuals now preserved as skulls and tissue samples at Institut de recherche pour le développement, Nouméa, under reference numbers IRDN-20090410 A and B.

A species of Lethrinidae with the following combination of characters: four rows of scales on cheek; ten soft rays in dorsal fin; ten soft rays in anal fin; body oblong (hence the species epithet) and fusiform, ratio of standard length to body depth between 2.6 and 2.8, increasing with size (Table 1); other measurements in Table 1; dorsal and ventral profiles almost similarly convex; tip of snout only slightly below axis of body; snout slightly rounded; tail elongated with rounded tips; posterior part of jaws reaching to about level of nostril; pored scales on lateral line: 48; scales between middle portion of spinous dorsal fin and lateral line: 6. Lower edge of eye slightly (in the smaller individuals examined) to well above a line from tip of snout to middle of caudal fin fork; horizontal or sub-horizontal wavy blue lines or dashes on lower part of snout and on cheeks; pale blue speckles more or less visible on operculum. The lines or dashes become dark red or brown against paler background in preserved specimens; they do not extend up to the upper part of snout and their number slightly increases with size (Figs. 1, 2). Forehead, snout and upper lip of fresh specimens can be bright yellow, matched by similar yellow colouration of margin of operculum (Fig. 2); loosely defined vertical dark bar crossing the eye (e.g. Fig. 1 B-D); on fresh animals, dorsal, pectoral, anal and caudal fins drab, brownish or yellowish, with reddish to vermilion edges.

Each lower jaw has a raw of three small, slender canines on each side of one large canine at front, and lateral row of 8-10 conical teeth; numerous villiform teeth form a brush behind the front canines; each upper jaw has a front row of 6-10 small, slender canines followed by 4-5 conical teeth and a patch of villiform teeth.

3.2. Habitat and distribution

The type locality of *Gymnocranius oblongus* n. sp. is New Caledonia, from where all six specimens on which this description is based originate. Those four specimens from the southern lagoon of New Caledonia were captured using gillnets deployed on sandy bottom at ~8 to 15 m depth, close to the shore of Grande Terre. Two individuals were seen by P. Borsa at ~1 m depth, swimming above the

front of the northern fringing reef of Baie des Citrons, Noumea (22°17'S, 166°26'E; 14 June 2009). An early juvenile (Fig. 3) was captured by light-trapping [18] in Noumea's Grande Rade bay in January 2003. *G. oblongus* n. sp. was not found in several localities across the Indo-West Pacific, including Bali (Indonesia), Raja Ampat (West Papua) and Fiji, where other *Gymnocranius* species were sampled (P. Borsa, pers. obs.). *Gymnocranius oblongus* appears to occur from November to May at the Noumea fish market, although it is less abundant than *G. euanus* and *G. grandoculis*.

3.3. Other biological data

The stomach of specimen IRDN-20090410-A contained a broken fragment of bivalve shell with meat still partly attached to it; that of specimen IRDN-20090410-B contained a crushed Penaeid shrimp and bivalve meat (but no shell fragment). Specimen IRDN-20090410-A was a male with immature testicles; IRDN-20090410-B was a female, with empty gonads. Both specimens were captured in April 2009 at ~8 m depth on sandy bottom close to the shore of Nouville peninsula, Nouméa.

The two individuals sighted underwater in Baie des Citrons, Nouméa, had their whole body silvery to pale grey and the only noticeable colour patterns were the loosely defined vertical dark bar crossing the eye and the vermilion edges of the hind part of dorsal fin and of the caudal fin.

3.4. Comparison with G. grandoculis and assumed synonyms

Table 1 includes as *G. grandoculis* the type specimen of *Cantharus grandoculis* Valenciennes 1830 (Fig. 4A), in accord with [2], and that of *Paradentex marshalli* (Fig. 4B), in accord with [1] (under *G. robinsoni*) and [2]. *Gymnocranius oblongus* n. sp. is distinct from *G. grandoculis* by its ratio of standard length to body depth: for a given standard length, the body of *G. grandoculis* is higher (see Table 1). The head of *G. oblongus* is more symmetrical dorso-ventrally than that of *G. grandoculis*, the latter showing a higher profile above the tip of mouth than below. Four (*16S rRNA*, *Aldo-B*, *CK-6*, *Met*) out of five of the genetic loci scored in the present study were diagnostic (Table 2): any of those loci could be used to differentiate *G. oblongus* n. sp. from *G. grandoculis*. We concur with [1] in

questioning the identity of both *Pentapus curtus* and *P. dux* with *G. grandoculis*. The identity of *G. oblongus* with *P. dux* (410 mm SL) certainly can be rejected on the basis of general body shape (Table 3). The general body shape of *P. curtus* (Table 3) also differs from that of *G. oblongus* but the small size of the only type specimen of *P. curtus* (150 mm SL) relative to the type material of *G. oblongus* (Table 1) precludes meaningful comparisons.

4. Discussion

Molecular markers demonstrated that *Gymnocranius oblongus* n. sp. is reproductively isolated from all other *Gymnocranius* species sampled in New Caledonia. *Gymnocranius oblongus* is unique among species of the genus *Gymnocranius* by the combination of three features, which are the sub-horizontal, wavy blue lines or dashes on snout and cheeks, the fusiform body and the elongate tail. Two other species, namely *G. frenatus* and *G. grandoculis*, also have wavy blue lines on the cheek [2], but of shapes generally different from those of *G. oblongus*. *Gymnocranius frenatus*' markings are oblique, running from the lower part of snout towards the eye, and they are thicker than those of *G. oblongus*; *G. grandoculis*' markings are more continuous and appear to cover a wider area of snout and cheek than those of *G. oblongus*, especially in larger individuals. Another species, *G. microdon*, has blue markings on the snout: those are dots or dashes, either sub-vertical [2] or sub-horizontal (P.Borsa, pers. obs.). The more-markedly elongated and fusiform body of *G. oblongus* actually makes it distinct from all other described *Gymnocranius* species, except *G. microdon*. However, smaller individuals of *G. oblongus* appear to be bulkier than *G. microdon* according to the measurements reported for the latter species [2, 7]. Also, the forehead of *G. microdon* is distinctively prominent: the forehead of *G. oblongus* is moderately prominent and less steep than that of *G. microdon*. The tail of *G. elongatus* [19] is similar in shape to that of the smaller specimens of *G. oblongus* examined here, but the tips of the caudal fin are pointed in *G. elongatus* whereas they are rounded in *G. oblongus*, especially in larger individuals. In all cases, the caudal fin of *G. oblongus* is not as indented and elongate as that of *G. elongatus*. It is also different from that of *G. grandoculis*, which is broad, with a subtle middle notch (Fig. 4C).

Large-eye breams of genus *Gymnocranius* are large-size, commercial fishes abundantly distributed throughout the tropical Indo-West Pacific [1, 2, 7] where they are sold on fish markets; their flesh is prized [1, 2, 20]. Therefore, the discovery of a new *Gymnocranius* species is a significant event: why this species and several other *Gymnocranius* species awaiting description [15, 16] have been unnoticed up to now can be explained by several factors, including the rarity of well-preserved specimens in museum collections [2], the difficulties generally encountered for separating *Gymnocranius* species on the single basis of morphology [2], allometry in growth (present work), and the apparent lability of blue markings on snout and cheeks, which turn out to be one of the most helpful characters in identification to species ([2]; present work).

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Table 1

Measurements (in mm) and other parameters on specimens of *Gymnocranius oblongus* n. sp. and comparison with *G. grandoculis* specimens of a range of sizes. *MNHN* Museum national d'histoire naturelle, Paris; *IRDN* Institut de recherche pour le développement, Noumea; *QMI* Queensland Museum, Brisbane; *SL* standard length; *BD* body depth at dorsal-fin origin

Parameter	*G. oblongus*						*G. grandoculis*			
	MNHN 2009-0005 (paratype)	MNHN 2009-0008 (paratype)	MNHN 2009-0007 (paratype)	IRDN 20090410A	IRDN 20090410B	MNHN 2009-0009 (holotype)	MNHN 0000-8811 (holotype)	IRDN 20080607 B	MNHN 2009-0006	QMI 5284 [a]
Measurement (mm)										
SL	221	226	245	265	350	358	186	342	474	481
BD	85	87	88	101	133	130	77	135	177	187
Body depth at anal-fin origin	76.5	84	85	95	121	126	78	119	169	166
Head length	70	68	73	79	105	109	64	102	140	147
Snout length	28	27	30	31.5	43	46	24	38	66.5	72
Eye diameter	23	22	23.5	23	39	28.5	23	31	36	34
Inter-orbital width	26.5	27	29	29	40.5	42	-	-	49	-
Predorsal length	77	80	90	95	114	130	61	109	147	172
Prepelvic length	79.5	77	86	99	122	122	72	122	159	174
Preanal length	139	141	152	172	218	223	104.5	224	277	309
Other										
Ratio of SL to BD	2.60	2.60	2.78	2.62	2.63	2.75	2.42	2.53	2.68	2.57
Pored scales on lateral line	48	48	48	48	48	48	-	48	48	48

[a] from photograph courtesy by the Queensland Museum

Table 2

Haplotype frequencies at four DNA loci in *Gymnocranius oblongus* n. sp., compared to those of *G. grandoculis*. Haplotypes at three other *Gymnocranius* species sampled in New Caledonia [15, 16] are given for an overview of genetic polymorphism in the genus. *n*, number of haplotypes sampled (in brackets)

Locus, Haplotype	Species				
	G. oblongus n. sp. [a]	*G. grandoculis*	*G. euanus*	*Gymnocranius* sp. B	*Gymnocranius* sp. C
16S rRNA					
A"	1.00	-	1.00	0.94	1.00
H	-	1.00	-	-	-
H3	-	-	-	0.06	-
(*n*)	(6)	(5)	(12)	(17)	(2)
Aldo-B					
1007	-	1.00	1.00	1.00	1.00
1017	1.00	-	-	-	-
(*n*)	(12)	(10)	(20)	(32)	(4)
CK-6					
140	1.00	-	-	-	0.25
142	-	0.38	-	1.00	0.75
144	-	-	1.00	-	-
145	-	0.63	-	-	-
(*n*)	(12)	(8)	(24)	(34)	(4)
GnRH-1					
968	-	0.20	-	0.12	-
984	-	0.10	-	0.06	-
992	0.42	0.10	-	0.18	0.50
1000	-	0.50	0.50	0.26	-
1008	0.58	0.10	0.50	0.38	0.50
(*n*)	(12)	(10)	(20)	(34)	(4)
Met					
926	-	-	1.00	-	1.00
935	-	1.00	-	1.00	-
949	1.00	-	-	-	-
(*n*)	(12)	(10)	(24)	(34)	(4)

[a] Includes one larva (Fig. 3) a posteriori identified to species by its multiple-locus DNA barcode

Table 3

Measurements (in mm) and other parameters on the holotypes of *Pentapus curtus* and *P. dux*. Same abbreviations as in Table 1.

Parameter	Species	
	Pentapus curtus, MNHN 0000-1317	*Pentapus dux*, MNHN 0000-2591
Measurement (mm)		
SL	150	410
BD	56	133
Body depth at anal-fin origin	58	124
Head length	48	117
Snout length	18	53.5
Eye diameter	16	32
Inter-orbital width	16	40.5
Predorsal length	51	133
Prepelvic length	51	133
Preanal length	94	245
Other		
Ratio of SL to BD	2.68	3.11
Pored scales on lateral line	48	47

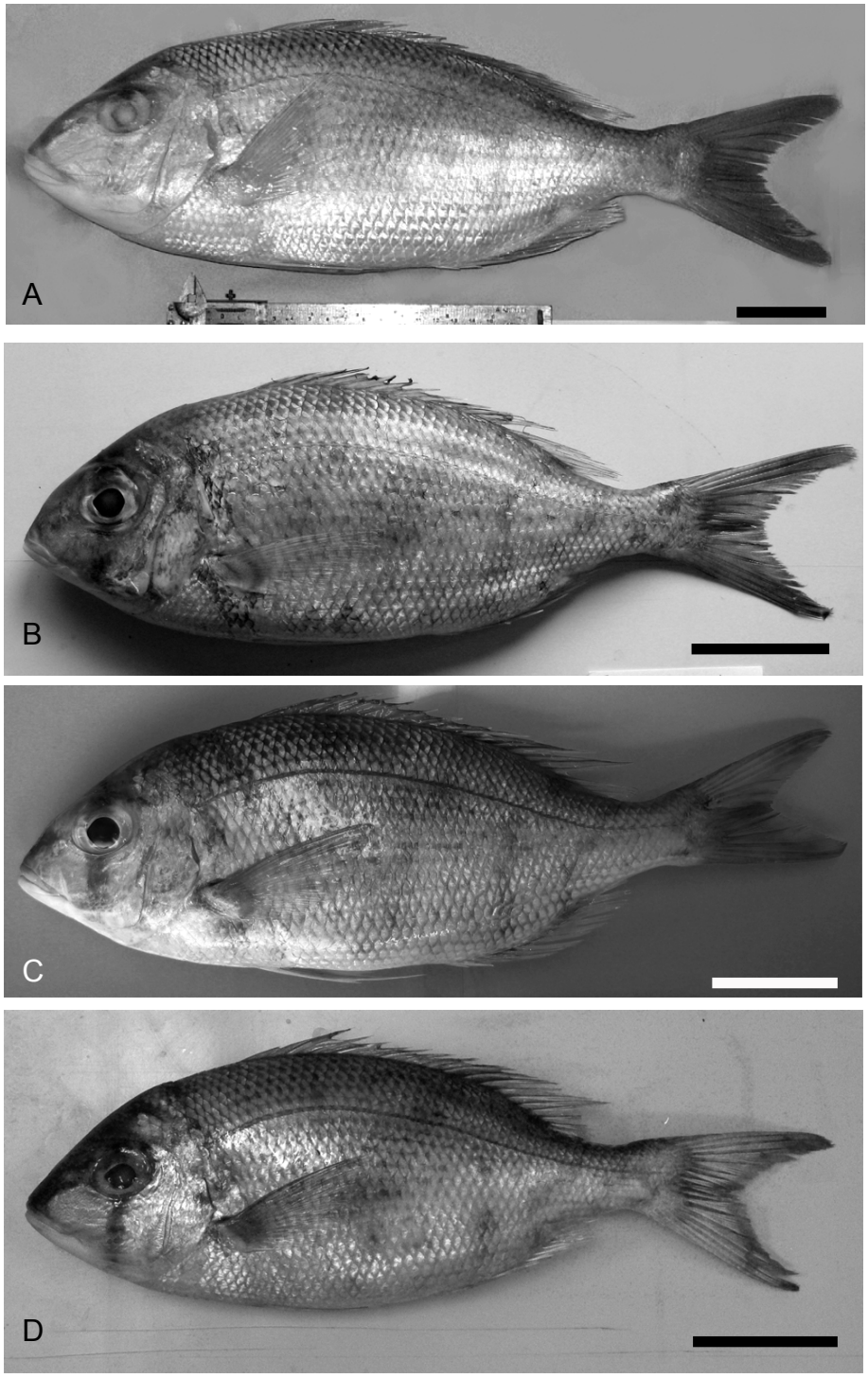
FIGURE CAPTIONS

Figure 1. Type material of *Gymnocranius oblongus* n. sp. (A) MNHN 2009-0009 (holotype, SL 358 mm); (B) MNHN 2009-0005 (paratype, SL 221 mm); (C) MNHN 2009-0007 (paratype, SL 245 mm); (D) MNHN 2009-0008 (paratype, SL 226 mm). Scale bar: 5 cm.

Figure 2. *Gymnocranius oblongus* n. sp. Colour patterns of head, specimen no. IRDN-20090410B from the southwestern lagoon of New Caledonia.

Figure 3. *Gymnocranius oblongus* n. sp. early juvenile from the southwestern lagoon of New Caledonia, SL 21.8 mm (composite photograph, courtesy of D. Ponton, IRD).

Figure 4. Specimens of *Gymnocranius grandoculis*. (A) MNHN 0000-8811, holotype of *Cantharus grandoculis* Valenciennes, 1830, Seychelles, SL 186 mm; (B) QMI 5284, holotype of *Paradentex marshalli* Whitley, 1936, Queensland, SL 481 mm (photograph courtesy of the Queensland Museum, Brisbane); (C) MNHN 2009-0006, voucher, Northern Lagoon of New Caledonia's Grande Terre, SL 474 mm.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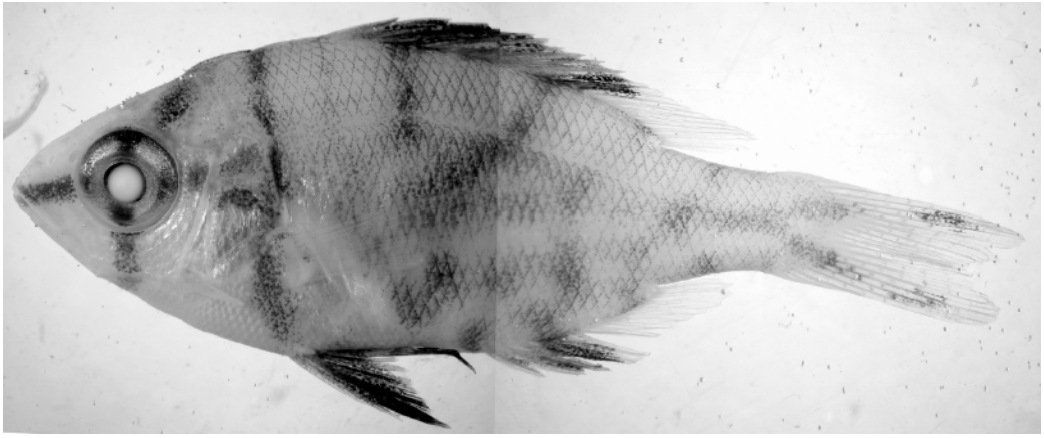

A

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