



Taiwanese Records of Oblong Large-Eye Seabream *Gymnocranius*oblongus (Teleostei: Lethrinidae) and Other Rare or Undetermined Large-Eye Seabreams

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The oblong large-eye seabream *Gymnocranius oblongus* Borsa, Béarez and Chen, 2010 has been described based on specimens from New Caledonia, the only locality where this species has been recorded up to now. Here, new records are reported from Taiwan, which is located over 7000 km from the previously known site of occurrence. Nucleotide sequences at the *cytochrome b* locus of the Taiwanese samples of *G. oblongus* were provided and compared to those of the conspecific New Caledonian samples. The results showed little intraspecific DNA variation among the sequences (only three segregating sites along the 1140-bp long aligned sequences). Further population genetic analysis revealed a relatively high level of genetic differentiation (ϕ st = 0.286) indicating limited gene flow between the two populations. The records thus available suggest a wide and antitropical distribution for this relatively rare and poorly known species. In addition to this finding, we report the first Taiwanese records of *Gymnocranius satoi* Borsa, Béarez, Paijo and Chen, 2013, a species widespread in the western Pacific, and of undetermined *Gymnocranius* specimens that potentially represent two new species.

Keywords: fishes, new record, new species, distribution, Taiwan, New Caledonia, diversity

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INTRODUCTION

Large-eye seabreams (Lethrinidae: Monotaxinae) comprise medium- to large-sized benthic fishes occurring over sandy or rubble substrates at or in the vicinity of coral reefs, at depths ranging from 1 to 180 m (Carpenter and Allen, 1989). Large-eye seabreams regularly occur at local fish markets throughout the tropical Indo-West Pacific (Carpenter and Allen, 1989; authors' personal observations). Despite the importance of several large-eye seabream species for human consumption, our knowledge on their biology, taxonomy, and biogeography is still limited. Currently, 14 species belonging to four genera are recognized in this fish group that is classified as one of two subfamilies of the perciform family Lethrinidae (Carpenter and Allen, 1989; Borsa et al., 2010, 2013; Eschmeyer et al., 2016). Gymnocranius is the most speciose genus, with 10 species described thus far. The oblong large-eye seabream, Gymnocranius oblongus Borsa, Béarez and Chen, 2010 is one of the three species that have

been described since the synopsis of Carpenter and Allen (1989); the other two are Gymnocranius satoi Borsa, Béarez, Paijo and Chen, 2013 and Gymnocranius superciliosus Borsa, Béarez, Paijo and Chen, 2013. The specimens used for the description of G. oblongus had all been collected from New Caledonia; its distribution was thought to be limited to the Coral Sea (Borsa et al., 2010). This fish is characterized by an oblong, fusiform body, a slightly rounded snout, an elongate tail with rounded tips and sub-horizontal, wavy blue lines or dashes on snout and cheeks (Borsa et al., 2010). It is distinct from Gymnocranius grandoculis (Valenciennes, 1830), regularly found at fish markets (e.g., in Taiwan), by a more slender body which is also more symmetrical dorso-ventrally and by a more elongated caudal fin. Another remarkable feature of G. oblongus is its prominent forehead, though less conspicuously so than its relative Gymnocranius microdon (Bleeker, 1851).

Two specimens purchased from local fish markets in Taiwan were identified as G. oblongus. The main purpose of the present work is to put the newly discovered Taiwanese specimens of this species on record. Cytochrome b gene sequences obtained from the Taiwanese specimens were compared to those from New Caledonia to evaluate the intraspecific genetic diversity and to estimate the level of genetic differentiation between the two populations. Additional records of rare or undetermined Gymnocranius species from Taiwan were also provided in this paper.

MATERIALS AND METHODS

We examined 81 specimens of large-eye seabream species collected between 2011 and 2015 at several local fish markets in the eastern and southern parts of Taiwan and on Little Liuqiu and Penghu Islands in the Taiwan Strait (see Table S1). The two specimens of *G. oblongus*, which had been caught by line, were purchased from fish sellers, one by HCH in

Hengchun town, southern Taiwan on 05 August 2011, and the other one by WJC in Magong, Penghu Islands (Taiwan Strait) on 03 May 2015. The specimens were photographed shortly after collection. Standard length (SL), body depth at origin of first dorsal fin (BD), head length, snout length, eye diameter, inter-orbital width, and other parameters were measured to the nearest millimeter. Species identification followed Borsa et al. (2010). Other monotaxine specimens collected by us in Taiwanese waters were identified using diagnostic morphological traits, as provided in the relevant literature (Carpenter and Allen, 1989; Borsa et al., 2013; Nakabo, 2013) and/or assessed against the genetic library that we are currently building for the Lethrinidae.

Small pieces of muscle-tissue were excised from the specimens, preserved in 95% ethanol, and stored at −20°C in the Marine Biodiversity and Phylogenomics laboratory at Institute of Oceanography, National Taiwan University (NTU), Taipei with tissue identification numbers LET439 and LET1209, respectively. The Hengchun specimen was lost afterwards. The Penghu specimen was preserved in formaldehyde and deposited at the ichthyological collection of the NTU Museums, Taipei under registration no. NTUM 10819. In total, we accessed tissue samples from seven G. oblongus specimens including the two specimens from Taiwan and five specimens previously collected from New Caledonia (including the holotype and all three paratypes of the species), one G. satoi specimen, and two unidentified Gymnocranius specimens from Taiwan (Table 1). Genomic DNA was extracted using an automated DNA-extractor (LabTurbo 48 Compact System with LGD 480-220 kits: Taigene Bioscience Corporation, Taipei) following the manufacturer's protocol. Protocols for cytochrome b gene amplification and sequencing have been outlined in Borsa et al. (2013). The sequences were deposited in GenBank (http://www.ncbi.nlm.nih. gov/) as a genetic reference for future DNA-identification and research on large-eye seabreams.

TABLE 1 | List of Gymnocranius specimens referred to in present study, with sampling details, collection nos. and GenBank accession nos.

Species, collection site	Collection date	Field no.	Collection no.	MBPL no.	GenBank no.	Remark
Gymnocranius oblongus						
Nouméa fish market	12 Jan. 2005	IRDN z199	MNHN 2009-0007	LET12	KU597059	paratype
Nouméa fish market	01 Apr. 2006	IRDN 20060401	MNHN 2009-0008	LET5	KU597058	paratype
Pointe Maa, New Caledonia	31 May 2008	IRDN 20080531B	MNHN 2009-0009	LET90	KU597061	holotype
SW lagoon, New Caledonia	22 Nov. 2008	IRDN 20081122	MNHN 2009-0005	LET51	KU597060	paratype
Nouville, New Caledonia	10 April 2009	IRDN 20090410A	_	LET963	KU597063	-
Hengchun, S. Taiwan	15 Aug. 2011	20110815 HC006	_	LET439	KU597062	-
Penghu, Taiwan Strait	03 May 2015	Penghu050315-93	NTUM 10819	LET1209	KU597064	voucher
Gymnocranius satoi						
Little Liuqiu, Taiwan Strait	14Sept. 2013	_	NTUM 10760	LET992	KX357715	voucher
Gymnocranius sp. D						
Hengchun, S. Taiwan	19 Aug. 2011	20110819HC006	_	LET427	KX357714	-
Gymnocranius sp. E						
Hengchun, S. Taiwan	19 Aug. 2011	20110819HC004	NMMBA 21546	LET426	KX357713	voucher

IRDN, Institut de recherche pour le développement, Nouméa; MBPL, Marine Biodiversity and Phylogenomics Laboratory, Taipei; MNHN, Museum national d'histoire naturelle, Paris; NTUM, National Taiwan University Museums, Taipei; NMMBA, National Museum of Marine Biology and Aquarium, Pingtung, Taiwan.

The sequences were aligned with homologous sequences from *Gymnocranius elongatus* Senta, 1973, *G. grandoculis*, and *Gymnocranius griseus* (Temminck and Schlegel, 1843) published by Lo Galbo et al. (2002) and retrieved from GenBank (accession nos.AF381260, AF381275, and AF381259, respectively), and those of *G. superciliosus* (from New Caledonia) and *G. satoi* (from West Papua) that have been produced previously (Borsa et al., 2013).

The softwares PopART (University of Otago, available from http://popart.otago.ac.nz) and PAUP* (Swofford, 2002) were used to compute number of haplotypes, minimum spanning network, pairwise p distances, nucleotide diversity, and segregating (polymorphic) sites to assess the intra- and inter-specific genetic diversity of the *cytochrome b* sequences. The level of genetic differentiation between populations was estimated by ϕ_{ST} , Excoffier et al.'s (1992) estimator of Wright's (1951) F_{ST} , the standardized among-population variance in allele frequency using ARLEQUIN v. 3.5 (Excoffier and Lischer, 2010). The nucleotide-substitution model chosen was TN93, the best model according to the Bayesian information criterion (MEGA6; Tamura et al., 2013) among the suite of models implemented in ARLEQUIN v. 3.5.

Estimates of genetic differentiation in G. oblongus were compared to those between populations of shore fishes from the Coral Sea and the northern South China Sea or adjacent locations. In blacktip grouper Epinephelus fasciatus and largescale mullet Planiliza sp. H (one of 10 cryptic species in the genus Planiliza; Durand and Borsa, 2015), genetic differences were estimated between populations from the Coral Sea or from nearby Fiji and the northern South China Sea and Philippines or nearby Ryukyu Islands, Japan, based on the nucleotide sequence data compiled or produced by Borsa et al. (2016) and Durand et al. (2012), respectively. For all other species examined, $F_{\rm ST}$ values or equivalents were taken directly from the relevant literature.

RESULTS AND DISCUSSION

The photographs of the two oblong large-eye seabream specimens from Taiwan are shown in Figure 1. The measurements on the single specimen kept as voucher (NTUM 10819) were the following: SL 241.4 mm, BD 94.5 mm, head length 67.5 mm, snout length 28.0 mm, eye diameter 21.0 mm, and inter-orbital width 28.5 mm; the count of pored scales on lateral line was 48. The ratio of SL to BD was 2.6, which was at the lower end of the range reported for six specimens from New Caledonia (2.6-2.8; Borsa et al., 2010). The SL/BD ratio in the Hungchen specimen (Figure 1A) was around 2.5. The combination of three features diagnose adult specimens of G. oblongus relative to all other species described to date in the genus Gymnocranius, namely, G. audleyi Ogilby, 1916, G. elongatus, G. euanus (Günther, 1879), G. frenatus Bleeker, 1873, G. grandoculis, G. griseus, G. microdon, G. satoi and G. superciliosus (Carpenter and Allen, 1989; Borsa et al., 2010, 2013). These are: (1) "sub-horizontal, wavy blue lines or dashes on snout and cheeks"; (2) "fusiform body"; and (3) "elongate tail" (Borsa

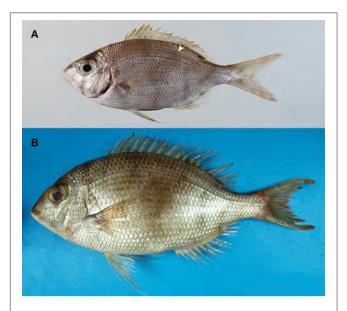


FIGURE 1 | *Gymnocranius oblongus* from Taiwanese waters. **(A)** Specimen from Hengchun, southern Taiwan, collected on 15 August 2011; standard length ~150 mm (photographed by HCH); arrow indicates oblique dark bar; **(B)** Specimen no. NTUM 10819 from Penghu Islands, Taiwan Strait, collected on 03 May 2015; standard length 244 mm (photographed by WJC).

et al., 2010, p. 245). The sub-horizontal wavy blue lines on snout were observed in the larger of our two Taiwanese specimens (**Figure 1B**), but not in the smaller specimen (**Figure 1A**). Both specimens had a fusiform body with remarkable dorso-ventral symmetry (**Figure 1**). Both possessed an elongate tail (**Figure 1**).

Juvenile and pre-adult *Gymnocranius* species usually present several transversal dark bars on body sides (Carpenter and Allen, 1989). One of these dark bars runs from around the base of the second soft ray of the dorsal fin to the base of the anal fin spines (Carpenter and Allen, 1989). The equivalent transversal dark bar in the smaller *G. oblongus* specimen from Taiwan (indicated by an arrow on **Figure 1A**) ran forward and crossed with another transversal dark bar running from the base of 5th–6th dorsal spin to ventral part of body at approximately the place of the tip of the pectoral fin tip; a faint dark blotch was visible at the crossing position (**Figure 1A**). These pigmentation patterns also characterize an early juvenile specimen from New Caledonia (Borsa et al., 2010: Figure 3).

Seven new cytochrome *b* gene sequences of *G. oblongus* (GenBank nos. KU597058-KU597064) produced in this study resulted in three haplotypes. The nucleotide diversity was 0.18%. Individual LET439 from Taiwan possessed the same haplotype as 4/5 individuals from New Caledonia. The haplotype represented by the Penghu specimen (LET1209) diverged by two mutation steps from it. Three segregating sites were observed along the 1140-bp long aligned sequences of *G. oblongus*. A total of 323 variable nucleotide sites were observed among all included sequences of *Gymnocranius* species. The average interspecific nucleotide divergence (estimated from pairwise uncorrected *p*-distances) was 11.02% whereas the average intraspecific

TABLE 2 | Estimates of genetic differentiation (F_{ST} or equivalent) between populations of shore fishes from the Coral Sea and the northern South China Sea (SCS) (or adjacent location).

Species	Marker	Range	F _{ST}	References
Acanthurus triostegus	5 allozymes	NC-Batanga	0.065	Planes and Fauvelot, 2002
Cephalopholis argus	cytochrome b	GBR-Palawan	0.070	Gaither et al., 2011
Cephalopholis argus	multi-locus (nuclear)	GBR-Palawan	0.128	Gaither et al., 2011
Dascyllus aruanus	cytochrome b	6955-7528 km	0.060-0.117	Liu et al., 2014
Dascyllus aruanus	7 microsatellites	6955-7528 km	0.019-0.028	Liu et al., 2014
Epinephelus fasciatus	COI	GBR-SCS/Philippines	0.307	Borsa et al., 2016
Gymnocranius oblongus	cytochrome b	~7000 km	0.286	Present study
Myripristis berndti	cytochrome b	Lizard-Okinawa	-0.105	Craig et al., 2007
Naso hexacanthus	mtDNA control region	GBR-Philippines	0.029	Horne and van Herwerden, 2013
Naso hexacanthus	COI	GBR-Philippines	0.083	Horne and van Herwerden, 2013
Naso hexacanthus	EnoIN (nuclear)	GBR-Philippines	0.026	Horne and van Herwerden, 2013
Naso hexacanthus	CK (nuclear)	GBR-Philippines	-0.034	Horne and van Herwerden, 2013
Naso vlamingii	mtDNA control region	GBR-Philippines	-0.004	Horne et al., 2008
Planiliza sp. H	cytochrome b	NC/Fiji-Taiwan/Japan	-0.028	Durand and Borsa, 2015
Planiliza sp. H	COI	NC/Fiji-Taiwan/Japan	0.013	Durand and Borsa, 2015
Plectropomus leopardus	mtDNA control region	~7000 km	0.885	van Herwerden et al., 2009
Scarus psittacus	mtDNA control region	GBR-Taiwan	0.021	Winters et al., 2010
Scarus rubroviolaceus	9-15 microsatellites	GBR-Taiwan	0.010-0.020	Fitzpatrick et al., 2011
Scomberomorus commerson	mtDNA control region	~7000 km	0.332-0.675	Fauvelot and Borsa, 2011

NC, New Caledonia; GBR, Great Barrier Reef.



FIGURE 2 | Gymnocranius sp. D from Taiwanese waters. Specimen from Hengchun, southern Taiwan, collected on 19 August 2011; standard length 148 mm (photographed by HCH).



FIGURE 3 | Gymnocranius sp. E from Taiwanese waters. Specimen no. NMMBA 21547 from Hengchun, southern Taiwan, collected on 19 August 2011; standard length 183 mm (photographed by HCH).

nucleotide divergence was 0.08% in *G. oblongus* and 0.4% in *G. satoi* (see below).

The estimate of genetic differentiation (ϕ_{ST}) between the New Caledonian and Taiwanese populations of G. oblongus was 0.286 (P=0.057; permutation test; ARLEQUIN), suggesting limited gene flow. This was the fourth highest-ranking value in **Table 2**. The three species with higher F_{ST} were E. fasciatus, the coral trout Plectropomus leopardus and the narrow-barred Spanish mackerel Scomberomorus commerson. The high value in E. fasciatus reflected the occurrence of a divergent mitochondrial lineage within a sample (Borsa

et al., 2016). The high value in *P. leopardus* was caused by the occurrence of two mitochondrial clades with allopatric distribution (van Herwerden et al., 2009). The high values reported for *S. commerson* stemmed from differences between multiple divergent mitochondrial clades (Fauvelot and Borsa, 2011). The phylogeographic structure uncovered in all three cases suggests the occurrence of cryptic species. Homologous estimates of genetic differentiation in all other species listed in **Table 2** were <0.200. The high $\phi_{\rm ST}$ value observed here suggests geographically isolated populations, hence an antitropical distribution.

Among the other Monotaxinae collected from Taiwan, four specimens were identified as G. satoi and 15 others could only be identified to genus Gymnocranius based on the available morphological keys (Table S1). These undetermined Gymnocranius specimens could be classified into two different morphological types based on their transversal dark bar patterns on body sides and fin ray colors (reddish or yellowish; Figures 2, 3). They also showed substantial genetic divergence (p-distance >10.7%) with all other known species in the genus for which cytochrome b sequence data were available, indicating potential new species. These two species have been mentioned for the first time by Borsa et al. (2013) as Gymnocranius sp. D (Figure 2) and Gymnocranius sp. E (Figure 3). G. satoi is the red-finned "Gymnocranius sp." depicted previously (Sato, 1986; Carpenter and Allen, 1989; Nakabo, 2013) and formally described in Borsa et al. (2013). Its distribution includes New Caledonia, Japan and Raja Ampat in West Papua, and possibly the Great Barrier Reef, the Solomon Sea, the Bismarck Sea, the Pacific coast of New Guinea and the Lesser Sunda Islands (Borsa et al., 2013, and references therein). The present record from Taiwan is new.

CONCLUDING REMARKS

The oblong large-eye seabream likely has an antitropical distribution in the western Pacific. Thus, most monotaxine species, now including *G. oblongus*, have a relatively wide distribution in the Indo-West Pacific (Carpenter and Allen, 1989;

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Borsa et al., 2013; this study). The new records and species reported in this study make up to 10 known and two undescribed monotaxine species in Taiwanese waters (Shao, 2016; this study).

AUTHOR CONTRIBUTIONS

WJC and PB contributed to the conception and design of the work, analyzed, and interpreted the data, and wrote the paper; WJC and HCH collected and managed the samples; HCH provided precious photographies taken from the fresh fish specimens. WJC collected the molecular data and led the project including the search of funding support for completing this study.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmars. 2016.00107

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary Table 1. List of 81 Taiwanese *Gymnocranius* spp. samples collected during the present survey

thodentex a	aureolineatus	Taitung, Taiwan	ASIZP0801861	-
		O-		NMMBP13833
thodentex a			20110815 HC004	_
thodentex c			20110815 HC005	_
enocranius e			-	NTUM10758
			-	NTUM10807
			-	NTUM10807
			-	NTUM10807
	_		-	NTUM10773
			-	NTUM10773
			-	-
			-	NTUM10742
			-	NTUM10742
			-	NTUM10726
			B4	-
			_	NTUM10720
			_	NTUM10701
			_	NTUM10767
			_	NTUM10767
			_	NTUM10809
_			-	NTUM10809
_			_	NTUM10809
			_	NTUM10809
			_	NTUM10809
			-	NTUM10809
			_	NTUM10809
			-	NTUM10809
			-	NTUM10809
_			-	NTUM10809
			-	NTUM10740
			-	NTUM10713
_			-	NTUM10800
_			20110722佳樂水02	NMMB13837
_		-	-	_
			_	NTUM10746
			20110919HBH001	NMMBP14071
				_
			-	NTUM10765
_	,		_	NTUM10765
	,		_	NTUM10765
			Penghu050415-1	NTUM10817
				_
				_
			_	NTUM10768
	*		_	NTUM10768
_			_	NTUM10768
			_	NTUM10768
_			-	NTUM10768
_			_	NTUM10768
			_	NTUM10808
			B2	_
_			-	NTUM10722
_			-	NTUM10759
			-	NTUM10772
			-	NTUM10772
			-	NTUM10772
		0 -	-	NTUM10804
			-	NTUM10702
			Penghu050315-93	NTUM10819
			20110815 HC006	-
	0		-	NTUM10834
			20110919HBH002	NMMBP21547
		Little Liuqiu, Taiwan	-	NTUM10760
enocranius s	uioi			
		Hengchun, Taiwan	20110513HC002	NMMB12114
	thodentex abodentex abodentex abodentex amocranius amoc	athodentex: aureolineatus alongatus aureoranius alongatus aureoranius alongatus aureoranius alongatus aureoranius alongatus aureoranius alongatus aureoranius arandoculis aureoranius arandoculis aureoranius arandoculis aureoranius arandoculis aureoranius arandoculis aureoranius aureoranius arandoculis arandoculis arandoculis arandoculi	thodentex aureolineatus thengchun, Taiwan thogenius thodentex aureolineatus thogenius thengchus, Taiwan thengchun,	thodontex aurolineatus

LET1362	Gymnocranius	sp. D	Hengchun, Taiwan	-	NTUM10835
LET1363	Gymnocranius	sp. D	Hengchun, Taiwan	-	NTUM10835
LET1211	Gymnocranius	sp. D	Penghu, Taiwan	Penghu050415-2	NTUM10818
LET427	Gymnocranius	sp. D	Hengchun, Taiwan	20110819HC006	-
LET998	Gymnocranius	sp. E	Fugang Taitung, Taiwan	-	NTUM10766
LET999	Gymnocranius	sp. E	Fugang Taitung, Taiwan	-	NTUM10766
LET1004	Gymnocranius	sp. E	Fugang Taitung, Taiwan	-	NTUM10766
LET1005	Gymnocranius	sp. E	Fugang Taitung, Taiwan	-	NTUM10766
LET1006	Gymnocranius	sp. E	Fugang Taitung, Taiwan	-	NTUM10766
LET890	Gymnocranius	sp. E	Hengchun Taiwan	-	NTUM10741
LET891	Gymnocranius	sp. E	Hengchun Taiwan	-	NTUM10741
LET892	Gymnocranius	sp. E	Hengchun Taiwan	-	NTUM10741
LET1192	Gymnocranius	sp. E	Hengchun, Taiwan	-	NTUM10827
LET426	Gymnocranius	sp. E	Hengchun Taiwan	20110819HC004	NMMBP21546
LET1360	Monotaxis	grandoculis	Hengchun, Taiwan	-	NTUM10833
LET1384	Monotaxis	grandoculis	Little Liuqiu, Taiwan	-	-