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First record of blacknape large-eye bream *Gymnocranius satoi* (Perciformes: Lethrinidae) in the Philippines

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Abstract

The Philippines has been regarded as the center of the center of marine shorefish biodiversity, having the highest number of fish species per square area in the world. The blacknape large-eye bream, *Gymnocranius satoi*, has been reported to occur from Southern Japan, Taiwan to Northwestern Australia and to the Coral Sea, but has not previously been recorded from the Philippines. From 2011 - 2019, the National Fisheries Research and Development Institute (NFRDI) collaborated with the National Museum of Natural History of the Smithsonian Institution (NMNH/SI), USA, and the Old Dominion University (ODU), Virgina, USA, to inventory all commercial fish species sold in fish markets around the Philippines. During three (3) fish market surveys (Dumaguete City Market, Negros Oriental; Claveria Public Market, Cagayan, Northern Luzon; and Tabaco City Market, Albay, Southeastern Luzon in 2013, 2016 and 2017, respectively), we collected and eventually identified using morphological and DNA barcoding (COI) analysis, seven (7) specimens of *G. satoi*, representing the first records of this species from the country. Since the potential to discover new species and first records of fish species in the Philippines is high, further taxonomic study of the genus *Gymnocranius* is needed.

Keywords: Marine biodiversity, conservation, fisheries, DNA barcoding, taxonomy

Introduction

The Philippines is endowed with highly diverse marine species resulting in its being considered as the epicenter of the world's marine shorefish biodiversity (Carpenter & Springer 2005; Sanciangco *et al.*, 2013). However, the country has also been cited as a hotspot for marine biodiversity conservation (Roberts *et al*., 2002).

The genus *Gymnocranius* is one of the four genera of the large-eye seabreams or Monotaxinae subfamily of the Lethrinidae family (Sato, 1986; Carpenter & Allen, 1989). *Gymnocranius* currently includes 11 valid described species, and one undescribed species (Carpenter & Allen, 1989; Chen *et al.*, 2016, 2017) but the number could be as high as 15 with additional four (4) possibly undescribed species (Chen & Borsa, 2020).

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Gymonacranius satoi Borsa, Béarez, Paijo & Chen (Borsa *et al*., 2013) is known to occur from New Caledonia, West Papua (Raja Ampat), Southern Japan and Taiwan (Borsa *et al*., 2013; Chen *et al*., 2016, Chen *et al*., 2017; Chen & Borsa, 2020). It is possible that it also occurs in the Great Barrier Reef, Solomon Sea, Bismarck Sea, New Guinea and Lesser Sunda Islands (Fricke *et al*., 2021). A search of the Barcode of Life Data (BOLD) systems (https://v3.boldsystems.org/) yielded 2 published and released record of *G. satoi* from Taiwan (GenBank Accession Numbers: MT607083 and MT607084 with BIN Cluster ID BOLD:AAE3729).

The National Fisheries Research and Development Institute (NFRDI) collaborated with the National Museum of Natural History of the Smithsonian Institution (NMNH/SI) USA and the Old Dominion University (ODU), Virginia, USA from 2011 to 2019, to inventory and DNA barcode all fish species sold in fish markets around the country. The purpose of the study was to improve food safety and security by developing the capacity to identify fillets or other fish products that cannot be identified using normal taxonomic methods (Carpenter *et al*., 2017).

DNA barcoding is a method widely used to identify species using the mitochondrial cytochrome c oxidase subunit 1 gene (COI) (Hebert *et al*., 2003). It has been used in the

Philippines to describe novel fish species such as whitechin surgeonfish, *Acanthurus albimento* (Carpenter *et al*., 2017) and Pogi perchlet, *Chelidoperca santosi* (Williams & Carpenter, 2015). It has been utilized to identify new records of indigenous fishes like Pacific bluefin tuna, *Thunnus orientalis* (Sarmiento *et al*., 2016) and reef manta ray, Manta alfredi (Acebes *et al*., 2016) as well as those that are non-native to the country such as the Midas cichlid, Amphilophus citrinellus (Poniente *et al*., 2020) and Mayan cichlid Cichlasoma urophthalmus (Ordonez *et al*., 2015). The present paper reports the first record of blacknape large-eye bream, *G. satoi*, from the Philippines using morphological analysis and DNA barcoding.

Materials and Methods

Sample collection and preservation

Commercial fish markets and fish landings in Dumaguete City market, Negros Oriental; Claveria Public Market, Cagayan, Northern Luzon; and Tabaco City Market, Albay, Southeastern Luzon were visited in 2013, 2016 and 2017, respectively, as part of the collaborative project of NMNH/SI, ODU and NFRDI to inventory species in fish markets around the country (Fig. 1).

Seven (7) voucher specimens of an unknown *Gymnocranius* species were observed and purchased from local market vendors and fishers (Table 1). Each specimen (voucher) was photographed to capture the fresh color pattern and their muscle tissues sampled in duplicate and preserved in ethanol and M2 buffer of the Autogen prep protocol for genetic analysis. The voucher specimens were then tagged with a unique identifying number, preserved in formalin and stored as reference vouchers at the NMNH/SI. All 7 specimens were labelled with place of origin/collection site and collection date.

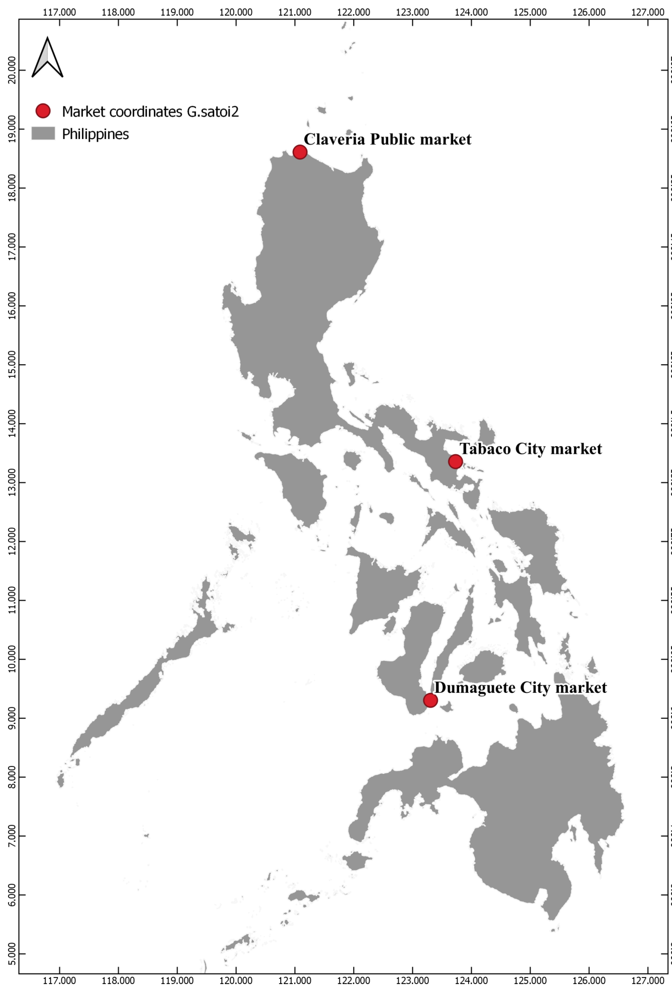

Figure 1. Map showing the collection sites of *Gymnocranius satoi* in Philippines. Inset map approximates the known distribution of the species in the Indo-West Pacific (IWP).

Sample identification

For morphological analysis, each of the 7 *Gymnocranius sp.* collected was identified based on identification keys in FAO Species Identification Guide for Fishery Purposes (Carpenter & Niem, 2001), in FishBase (Froese & Pauly 2010), the Market Fishes of Indonesia (White *et al*. 2013) and specifically the most recent compilation for identification purposes in Table 3 of Chen *et al*., (2017).

For genetic analysis, DNA extraction and amplification from the 7 muscle tissues were performed following Smithsonian protocols of Baldwin *et al*., (2011). Briefly, the CO1 sequences were amplified using primers FISH-BCL (5'-TCAACYAATCAYAAAGATATYGGCAC) and FISH-BCH (5'-TAAACTTCAGGGTGACCAAAAAATCA) at the following thermal cycling procedure: 1 cycle of 5 min at 95°C; 35 cycles of 30 s at 95°C, 30 s at 52°C, and 45 s at 72°C; 1 cycle of 5 min at 72°C; and a hold at 10°C. DNA sequences were obtained using an ABI 3730XL automated DNA sequencer and sequence files were exported into Sequencher 4.7 (GeneCodes, Ann Arbor, MI).

Reference CO1 sequences of *Gymnocranius spp*. (*G. satoi*. *G. superciliosus*, *G. oblongus*, *G. griseus* and *G. obesus*) and of *Lethrinus* spp. (*L. lentjan* and *L. atkinsoni*) as outgroups were obtained from GenBank and BOLD (Fig 3.) Alignment of all CO1 sequences were done using ClustalW. MEGA6 software (Tamura *et al*. 2013) was employed to determine the extent of genetic distance between species by calculating average pairwise comparisons of CO1 sequence differences across all individuals using Kimura 2-Parameter (K2P) method (Kimura, 1980). Maximum Likelihood (ML) method using K2P was done to generate the phylogenetic tree following Carpenter *et al*. (2017).

Results and Discussion

Morphological identification

Observations: The seven (7) unknown fish specimens (Table 1 and Fig. 2) were initially identified based on their distinct morphological characteristics as belonging to the genus *Gymnocranius* of the family Lethrinidae (Emperors). The specimens had naked cheeks versus scaly compared with the other Lethrinid genera (Carpenter & Niem, 2001). Other characteristics observed included the following: bumpy forehead and the area immediately above eye forms a distinctive brownish to blackish eyebrow; a vertical dark bar crossing the eye; the lower edge of eye is above the body axis; scales above lateral line have dark-grey patch; caudal fin is forked and the lobes are convex on inner side; scales in 3 rows below lateral line are darker. The characteristic reddish to bright vermilion red fins, reddish to red upper lip and white lower lip (Borsa *et al*., 2013) are unfortunately not visible in the photographs. Market specimens tend to lose color quickly.

Remarks: The morpho-anatomy of the unknown *Gymnocranius* specimens as mentioned above (Fig. 2) closely resemble the features of the holotype specimen of *G. satoi* as reported by Borsa *et al*. (2013)- with high body feature; bumpy forehead; shallowly forked caudal fin; flanks that are silvery; with distinctive brownish to blackish eyebrow and visible vertical dark bar crossing the eye. Taken together, these characteristics points to our specimens as *G. satoi*.

Genetic identification

The CO1 sequences of the seven (7) putative *G. satoi* samples formed a distinct monophyletic clade with the three (3) *G. satoi* reference sequences using Maximum Likelihood method (Fig. 3). Bootstrap value at the node between the nearest clade (*G. superciliosus*) is significant at over 50 similar to reports of Carpenter *et al*., 2017. In addition, pairwise genetic distance between the seven (7) putative *G. satoi* and three (3) reference *G. satoi* ranges from 0.002-0.003, which is significantly different from the nearest species *G. supeciliosus* (0.061-0.097) and *G. griseus* (0.067-0.069) (Table 2). Hence, both genetic analysis, like the morpho-anatomical features, strongly suggests that the 7 unknown *Gymnocranius* are in fact *G. satoi*.

Distribution of G. satoi

G. satoi was previously reported as "*G. lethrinoides*" by Masudaka *et al*. (1984). It was Torao Sato, a Japanese ichthyologist who first recognized that the said specimen was distinct from the other *Gymnocranius* species (Sato, 1986). The specimen was finally described as a new species named after Tarao Sato (Borsa *et al*, 2013; Borsa *et al*., 2010a, 2010b). *G. satoi* is known to be distributed in the southern lagoon of New Caledonia, Raja Ampat in western West Papua, Southern Japan, Northwestern Australia, Coral Sea and Taiwan (Borsa *et al*. 2013; Chen *et al*, 2016; Chen *et al*., 2017; Chen & Borsa 2020). It could also be present in the Great Barrier Reef, Solomon Sea, Bismarck Sea, New Guinea and Lesser Sunda Islands (Fricke *et al*., 2021). This study provides the first definitive evidence of *G. satoi* in the Philippines, completing its range distribution in the Indo-West Pacific (IWP). This information is valuable for the species' global and regional IUCN Red List assessment, conservation and management.

Conclusion

The discovery of the occurrence of *G. satoi* in the Philippines fills the large geographic gap distribution between Southern Japan and West Papua to Coral Sea, leading to the conclusion that *G. satoi* exhibits continuous distribution and is recurring between Southern Japan and Western Pacific Ocean. Preliminary analysis of COI sequences for fishes we have collected at Philippine fish markets suggest that there may be hundreds of yet to be described new species and first records of fishes occurring in the Philippines. Moreover, rarefaction and extrapolation species curves after 258 sampling events over 6 years indicates that around 1,500 species could eventually be sampled from fish markets in the Philippines (Carpenter *et al*., 2017). Hence, further taxonomic study of fishes in the genus *Gymnocranius* is needed.

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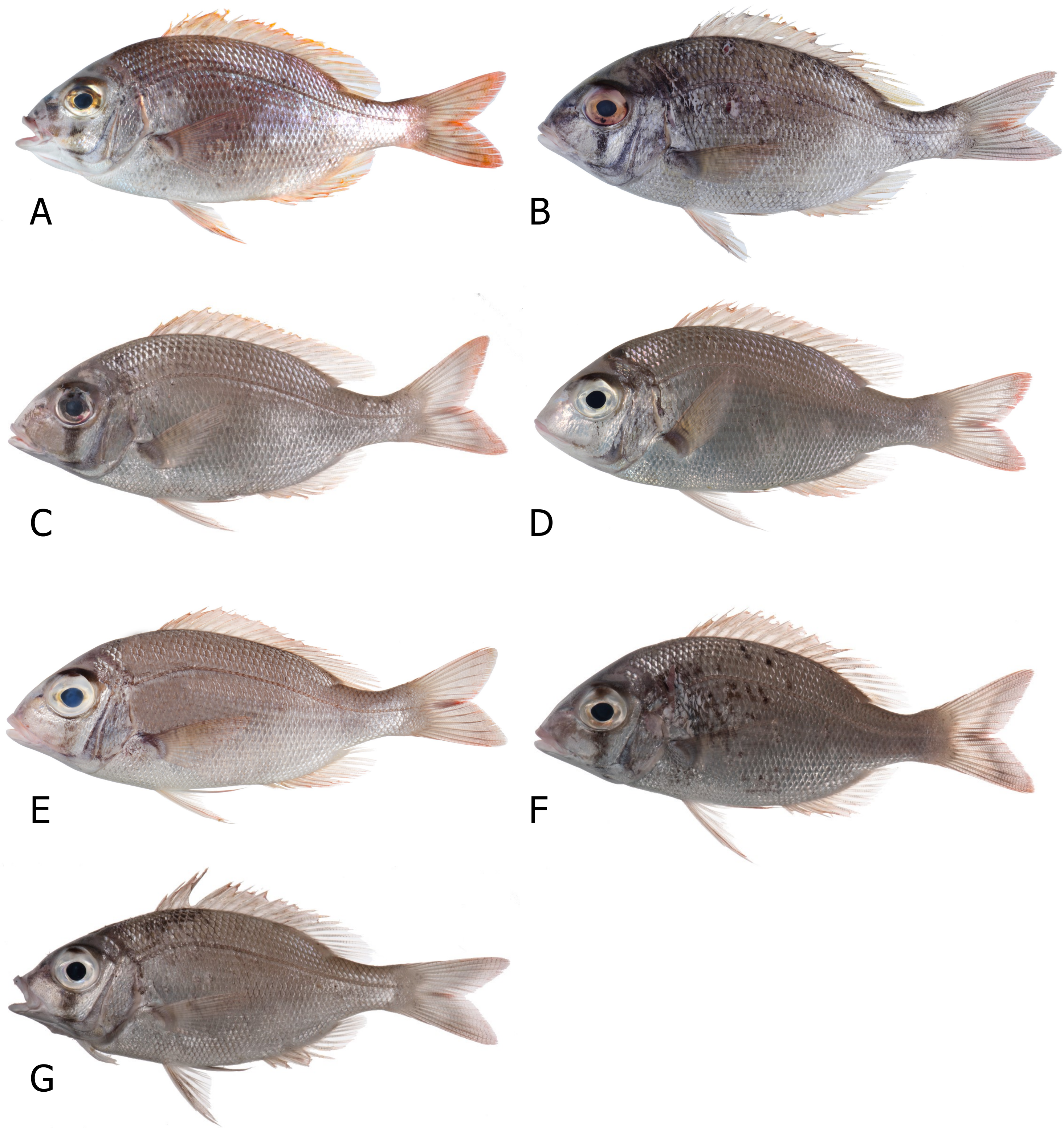

Figure 2. Photographs of the seven (7) *Gymnocranius satoi* collected from 3 areas in the Philippines (Table 1). **A.** USNM 403315, **B.** USNM 443548, **C.** USNM:FISH:445411, **D.** USNM 445413, **E.** USNM 445415, **F.** USNM:FISH:445417 and **G.** USNM:FISH:445419. Photos by J.T. Williams.

Table 1. *Gymnocranius satoi* samples from Philippines with corresponding specimen codes, standard lengths, sampling location, BOLD Barcode Index Numbers (BIN) Identification and GenBank Accession Numbers.

Sample	Voucher specimen code	Standard Length (SL) in cm	Tissue specimen code	Collection sites in Philippines	Barcode of Life Database (BOLD) BIN ID	GenBank Accession Number
A	USNM 403315	17	PI-0315 (PHILA318-13)	Dumaguete City Market, Negros Oriental	BOLD:AAE3729	MZ921405
B	USNM 443548	18.1	PHISH-708 (PHILA-2899-18)	Claveria Public Market, Cagayan, Northern Luzon	BOLD:AAE3729	MZ921404
C	USNM:FISH:445411	20	LUZ-194 (PHILU194-18)	Tabaco City Market, Albay, Southeastern Luzon	BOLD:AAE3729	MZ921403
D	USNM 445413	22.5	LUZ 196 (PHILU196-18)	Tabaco City Market, Albay, Southeastern Luzon	BOLD:AAE3729	MZ921407
E	USNM 445415	23.8	LUZ-198 (PHILU198-18)	Tabaco City Market, Albay, Southeastern Luzon	BOLD:AAE3729	MZ921406
F	USNM:FISH:445417	19	LUZ-200 (PHILU200-18)	Tabaco City Market, Albay, Southeastern Luzon	BOLD:AAE3729	MZ921408
G	USNM:FISH:445419	12.4	LUZ-202 (PHILU202-18)	Tabaco City Market, Albay, Southeastern Luzon	BOLD:AAE3729	MZ921409

Table 2. Computed pairwise distances between the samples and reference sequences from GenBank and BOLD databases using the Kimura 2-parameter model. The number of base substitutions per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal.

	SAMPLES	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	PHILU196-18_*Gymnocranius sp.*		0.002	0.000	0.002	0.002	0.002	0.002	0.002	0.002	0.000	0.011	0.011	0.011	0.011	0.012	0.010	0.010	0.011	0.022	0.022
2	PHILU198-18_*Gymnocranius sp.*	0.003		0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.011	0.011	0.011	0.011	0.012	0.010	0.010	0.011	0.022	0.022
3	PHILA2899-18_*Gymnocranius sp.*	0.000	0.003		0.002	0.002	0.002	0.002	0.002	0.002	0.000	0.011	0.011	0.011	0.011	0.012	0.010	0.010	0.011	0.022	0.022
4	PHILA318-13_*Gymnocranius sp.*	0.002	0.002	0.002		0.002	0.002	0.000	0.002	0.002	0.002	0.010	0.010	0.011	0.011	0.012	0.010	0.010	0.011	0.022	0.022
5	PHILU194-18_*Gymnocranius sp.*	0.003	0.003	0.003	0.002		0.002	0.002	0.002	0.002	0.002	0.011	0.011	0.011	0.011	0.012	0.010	0.010	0.010	0.022	0.022
6	PHILU200-18_*Gymnocranius sp.*	0.003	0.003	0.003	0.002	0.003		0.002	0.002	0.002	0.002	0.011	0.011	0.011	0.011	0.012	0.010	0.010	0.011	0.022	0.022
7	PHILU202-18_*Gymnocranius sp.*	0.002	0.002	0.002	0.000	0.002	0.002		0.002	0.002	0.002	0.010	0.010	0.011	0.011	0.012	0.010	0.010	0.011	0.022	0.022
8	GBMNC14689-20_*G. satoi*	0.003	0.003	0.003	0.002	0.003	0.003	0.002		0.000	0.002	0.011	0.011	0.011	0.011	0.012	0.010	0.010	0.011	0.022	0.022
9	MT607083.1_*G. satoi*	0.003	0.003	0.003	0.002	0.003	0.003	0.002	0.000		0.002	0.011	0.011	0.011	0.011	0.012	0.010	0.010	0.011	0.022	0.022
10	MT607084.1_*G. satoi*	0.000	0.003	0.000	0.002	0.003	0.003	0.002	0.003	0.003		0.011	0.011	0.011	0.011	0.012	0.010	0.010	0.011	0.022	0.022
11	MT607076.1_*G. griseus*	0.069	0.069	0.069	0.067	0.069	0.069	0.067	0.069	0.069	0.069		0.000	0.011	0.011	0.011	0.011	0.011	0.012	0.022	0.022
12	MT607075.1_*G. griseus*	0.069	0.069	0.069	0.067	0.069	0.069	0.067	0.069	0.069	0.069	0.000		0.011	0.011	0.011	0.011	0.011	0.012	0.022	0.022
13	MT607082.1_*G. oblongus*	0.075	0.075	0.075	0.073	0.075	0.075	0.073	0.073	0.073	0.075	0.071	0.071		0.002	0.013	0.012	0.012	0.013	0.023	0.022
14	MT607080.1_*G. oblongus*	0.077	0.077	0.077	0.075	0.077	0.077	0.075	0.075	0.075	0.077	0.069	0.069	0.002		0.013	0.012	0.012	0.013	0.023	0.022
15	MT607079.1_*G. obesus*	0.080	0.080	0.080	0.078	0.080	0.080	0.078	0.076	0.076	0.080	0.076	0.076	0.089	0.087		0.013	0.013	0.013	0.021	0.021
16	MT607085.1_*G. superciliosus*	0.063	0.063	0.063	0.061	0.060	0.063	0.061	0.063	0.063	0.063	0.067	0.067	0.080	0.082	0.091		0.002	0.003	0.022	0.022
17	MT607088.1_*G. superciliosus*	0.065	0.065	0.065	0.063	0.061	0.065	0.063	0.065	0.065	0.065	0.069	0.069	0.082	0.084	0.093	0.002		0.003	0.022	0.022
18	MT607087.1_*G. superciliosus*	0.069	0.069	0.069	0.067	0.065	0.069	0.067	0.069	0.069	0.069	0.073	0.073	0.086	0.088	0.097	0.005	0.007		0.022	0.022
19	MN870430.1_*Lethrinus lentjan*	0.224	0.227	0.224	0.227	0.229	0.224	0.227	0.224	0.224	0.224	0.237	0.237	0.243	0.241	0.211	0.224	0.222	0.224		0.016
20	MN870331.1_*Lethrinus atkinsoni*	0.216	0.219	0.216	0.219	0.221	0.221	0.219	0.216	0.216	0.216	0.216	0.216	0.223	0.225	0.203	0.223	0.225	0.218	0.122	

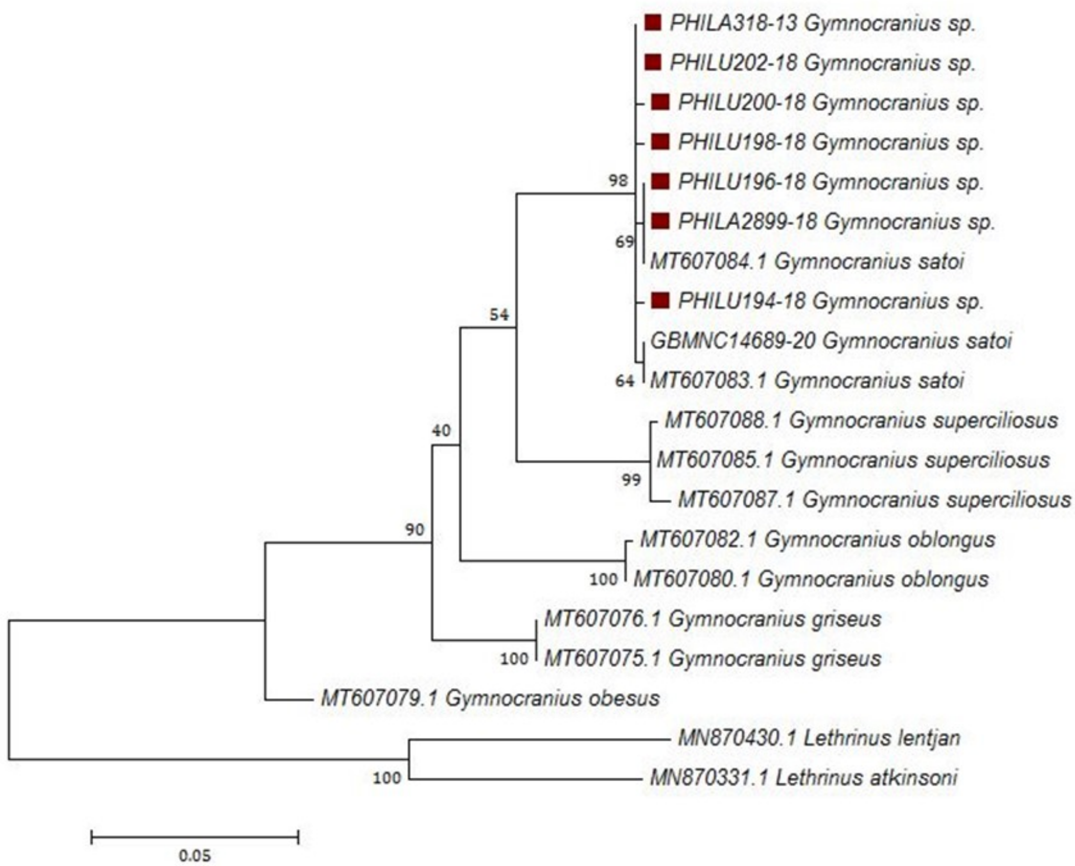

Figure 3. Maximum-likelihood (ML) Tree of CO1 sequences of *Gymnocranius spp* including the (7) specimens (red box) collected from this study (Table 1) and other species from GenBank (*G. satoi*. *G. superciliosus*, *G. oblongus, G. griseus and G. obesus*) with corresponding Accession Numbers. GenBank CO1 sequences of *Lethrinus lentjan* and *L. atkinsoni* (also under family Lethrinidae) were included as outgroup. Scale bar refers to substitution per site.

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