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

Nicko Amor Flores¹, Jade Tiffany Rey¹, Jeffrey T. Williams², Kent Carpenter³, and Mudjekeewis Santos¹*  

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), Virginia, 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).

Gymnocranius 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 I 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.

**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'-TCAACAYAATCAYAAAAGATATYGGCAY) and FISH-BCH (5'-TAATTCAGGTGACCAAAAATCA) 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).

![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).](image-url)
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 vermillion 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. superciliosus (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 Torao 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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Voucher specimen code</th>
<th>Standard Length (SL) in cm</th>
<th>Tissue specimen code</th>
<th>Collection sites in Philippines</th>
<th>Barcode of Life Database (BOLD) BIN ID</th>
<th>GenBank Accession Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>USNM 443548</td>
<td>18.1</td>
<td></td>
<td></td>
<td>BOLD:AAE3729</td>
<td>MZ921404</td>
</tr>
<tr>
<td>C</td>
<td>USNM:FISH:445411</td>
<td>20</td>
<td></td>
<td></td>
<td>BOLD:AAE3729</td>
<td>MZ921403</td>
</tr>
<tr>
<td>D</td>
<td>USNM 445413</td>
<td>22.5</td>
<td>LUZ 196 (PHILU196-18)</td>
<td>Tabaco City Market, Albay, Southeastern Luzon</td>
<td>BOLD:AAE3729</td>
<td>MZ921407</td>
</tr>
<tr>
<td>E</td>
<td>USNM 445415</td>
<td>23.8</td>
<td>LUZ-198 (PHILU198-18)</td>
<td>Tabaco City Market, Albay, Southeastern Luzon</td>
<td>BOLD:AAE3729</td>
<td>MZ921406</td>
</tr>
<tr>
<td>F</td>
<td>USNM:FISH:445417</td>
<td>19</td>
<td>LUZ-200 (PHILU200-18)</td>
<td>Tabaco City Market, Albay, Southeastern Luzon</td>
<td>BOLD:AAE3729</td>
<td>MZ921408</td>
</tr>
</tbody>
</table>

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.
respectively. We appreciate Lee Weigt, Amy Driskell and Jeff Hunt of the Laboratories of Analytical Biology (Smithsonian Institution) for providing support and assistance with logistics and molecular analysis of samples throughout the project. We also thank Carole Baldwin Jerry Finan, Diane Pitassy, Erika Wilbur, Shirleen Smith, Kris Murphy, David Smith and Sandra Raredon of the National Museum of Natural History for their support and assistance. Major funding for this food safety and security project was provided by the United States Food and Drug Administration (FDA) through a program administered by Jonathan Deeds under an ongoing Interagency Agreement between the FDA and the NMNH. This study was part of a project entitled “Collaboration on the Inventory and DNA Barcoding of Commercial Fishes of the Philippines for Food Safety and Biodiversity” made possible by a multi-year (2011 - 2019) Memorandum of Agreement (MoA) between the Philippine Department of Agriculture, Bureau of Fisheries and Aquatic Resources-National Fisheries Research and Development Institute (BFAR-NFRDI), and the National Museum of Natural History of the Smithsonian Institution-Department of Vertebrate Zoology, USA from .

Literature Cited


