

SHORT COMMUNICATION

Phenotypic Variability in Dorsal-fin Rays of *Rachycentron canadum* (Linnaeus, 1766) from Western, Visayas, Philippines

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ABSTRACT

Cobia, *Rachycentron canadum*, is the only species in the family of Rachycentridae. Cobia is typically characterized as having 26–33 dorsal-fin rays. However, an investigation of specimens collected from Western Visayas waters revealed a wider variation in dorsal-fin ray count than previously reported, with some specimens having 35–36 dorsal-fin rays. Suspecting a possible cryptic species, we compared the mitochondrial cytochrome oxidase subunit 1 (COI) sequence for specimens with 35–36 dorsal-fin rays with those having the more typical 26–33 dorsal-fin rays. The sequences revealed no genetic differences between the two morphs. Morphometric measurements and meristic counting likewise found no significant differences. This is the first report of phenotypic variability in the dorsal-fin rays in Cobia. As a result of the phenotypic and genotypic characterization presented in this work, fisheries scientists and ichthyologists will be more aware of the existence of phenotypic variability in Cobia.

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Received: *September 5, 2022*
Accepted*: January 18, 2023*

Keywords: *COI, DNA barcoding, Cobia, meristic variation, phenotypic variability*

Rachycentron canadum (Linnaeus 1766), commonly known as Cobia, is a species in the monotypic family Rachycentridae. It is a dark-colored, elongated, migratory fish that occurs widely in tropical and subtropical seas (Briggs 1960; Shaffer and Nakamura 1989; Collette 1999), including a recent range expansion to the eastern Pacific (Fricke et al. 2022). Cobia can reach two meters in length (Collette 1999) and weigh up to 50–68 kg (Colette 1999; Shaffer and Nakamura 1989; Wheeler 1975). Cobia is an aggressive feeder on a wide range of prey throughout the water column (Freeman and Walford 1976; Shaffer and Nakamura 1989; Meyer and Franks 1996) and can reach depths of 1200 meters (Springer and Bullis 1956).

Cobia is highly valued and considered a deluxe food in some areas of Taiwan and Japan (Liao et al. 2004; Miao et al. 2008). Due to its high marketability in east Asia, several countries have developed Cobia aquaculture to supply the growing demand, despite its high capital requirement (Shaffer and Nakamura 1989). At present, China, Taiwan, Panama, and Vietnam are the top producers of cultured Cobia by volume (Benetti et al. 2021). In contrast, Cobia is not a popular food fish in the Philippines and has low market value and demand. In the past, there was an attempt to culture Cobia in the Southern Philippines (Surtida 2000); however, no reports on its success were made known. In Western Visayas, Philippines, this fish is locally known as "pandawan." This is seldom sold in fish markets within the region.

Cobia has been typically described to have only 26 to 33 soft dorsal rays (Collette 1999; Shaffer and Nakamura 1989); hence specimens that we found to have more than 33 dorsal rays were hypothesized to belong to a cryptic species. To address this hypothesis, we investigated phenotypic variability in *R. canadum* from various fishing grounds in Panay and

Guimaras Islands using an integrative morphological and molecular approach. This study provides insights into the phenotypic diversity exhibited by Cobia fish and generates COI genetic barcode reference data for Philippine Cobia, which may serve as a foundation for future genetic and taxonomic studies.

Sampling was conducted from August 2020 to February 2022 in Aklan, Capiz, Iloilo, and Guimaras provinces, as illustrated in the map (Fig. 1). All specimens were purchased from local fishers and fish markets. Curatorial protocols followed Motomura et al. (2013). The measurements were taken using a digital caliper to the nearest 0.1 mm following Molina et al. (2018), including total length (TL), upper jaw length, and lower jaw length. The two posteriormost soft rays of the dorsal and anal fins were counted as a single ray since they are associated with a single pterygiophore (Armbruster 2012). X-ray images were taken at a medical laboratory in Iloilo, Philippines, and the radiographs were utilized to count the vertebrae (Fig. 2). The total vertebrae number was counted from the postcranial region to the ural region (Jawad 2015).

The specimens examined in this study are deposited at the Museum of Natural Sciences, University of the Philippines Visayas, Miag-ao (UPVMI) as follows:

- UPVMI-01354, 263.31 mm SL, off Capiz, Panay Island, P. Aguilos Jr., 26 August 2020
- UPVMI-01355, 234.98 mm SL, off Aklan, Panay Island, M. Macavinta, 9 October 2020
- UPVMI-01356 (Fig. 3A), 194.21 mm SL, off Sibunag, Guimaras Island, M. Asgar, 6 November 2020
- UPVMI-01359, 226.43 mm SL, Estancia Fish Port, Iloilo, E. Delloro Jr., March 2021
- UPVMI-01357, 298.22 mm SL, Carles fish market, Iloilo, E. Delloro Jr., 13 February 2022
- UPVMI-01358 (Fig. 3B), 423.62 mm SL, Carles fish market, Iloilo, R. Babaran, February 2022

Muscle tissue was collected from the nape area of the fish and preserved in absolute ethanol. DNA extractions were carried out according

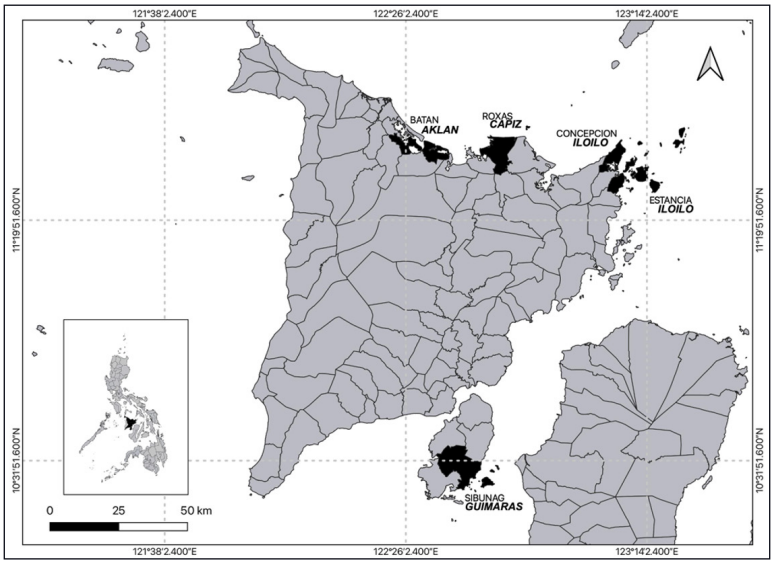

Figure 1. Map showing the provinces of Aklan, Capiz, Iloilo, and Guimaras where the Cobia samples used in this study were collected.

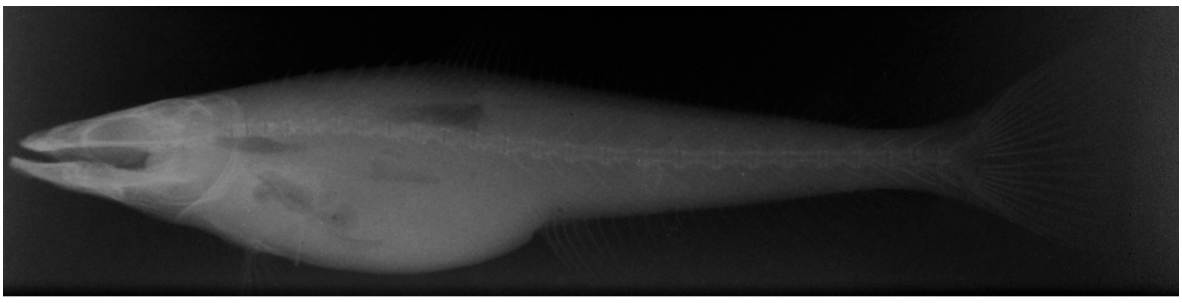

Figure 2. X-ray photograph of *Rachycentron canadum* (UPVMI-01359) from Western Visayas, Philippines.

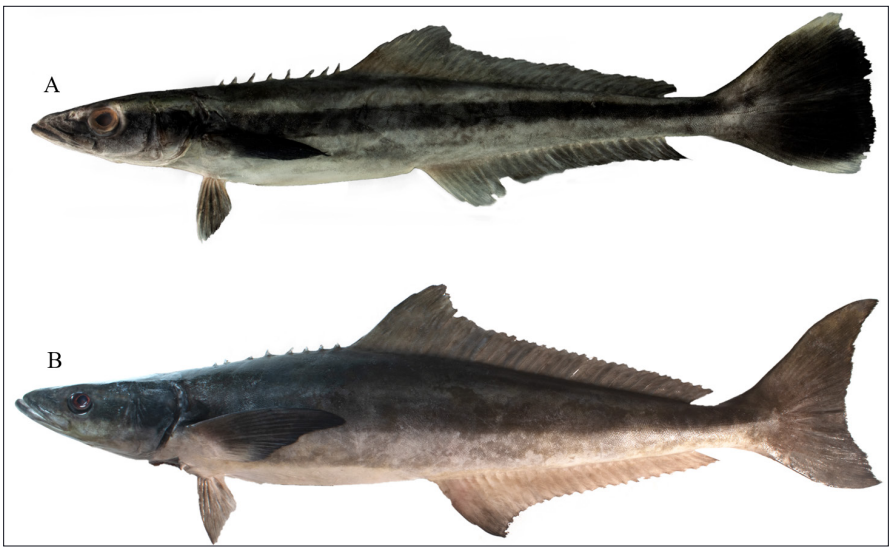

Figure 3. *Rachycentron canadum* (Linnaeus, 1766) collected from Western Visayas, Philippines. (A) UPVMI-01356 (smallest specimen) 194.21 mm SL, dorsal-fin ray count 32. (B) UPVMI-01358 (largest specimen) 423.62 mm SL, dorsal-fin ray count 35.

to the instructions of the GF-1 Nucleic Acid Extraction Kit (Vivantis Technologies Sdn. Bhd, Malaysia) and PureLinkTM Genomic DNA Mini Kit (Invitrogen). Polymerase chain reaction (PCR) amplification was performed using forward and reverse primers from Ward et al. (2005): FishF1-5'TCAACCAACCACAAAGACATTGGCAC3' and FishR1-5'TAGACTTCTGGGTGGCCAAAGAA TCA3'. The 25 µL PCR master mix consisted of 18.4 µL nuclease-free water, 2.25 µL 10x PCR buffer, 1.25 µL MgCl2, 0.5 µL dNTP mix, 0.25 µL 10 mM each primer, 0.1 µL Taq polymerase, and 2 µL DNA template. The thermocycler conditions were as follows: 2 mins at 95°C; 35 cycles of 0.5 min at 94°C, 0.5 min at 54°C, and 1 min at 72°C; and 10 mins at 72°C. The PCR products were visualized using 1% agarose gel. Purification of PCR products was carried out using GF-1 PCR Clean-up Kit and PureLinkTM PCR Purification Kit. The amplified DNA was quantified using a Genova nano spectrophotometer (Jenway, USA) or MultiSkan Skyhigh Microplate Spectrophotometer (Thermo Fisher Scientific). All PCR products were sent to Macrogen Inc. (South Korea) for sequencing. The forward and reverse sequences were checked, trimmed, and realigned using Unipro UGENE software (Okonechnikov et al. 2012). Sequences are deposited in GenBank (accession numbers OP268238 - OP268243).

The Basic Local Alignment Search TOOL (BLAST) of the National Center for Biotechnology Information (NCBI) was used to check the similarity of the nucleotide sequences from GenBank with our sequences. Selected Cobia, Remora, and Dolphinfish COI gene sequences available in GenBank were aligned with the six sequences obtained from the Panay samples using Multiple Sequence Comparison by Log-Expectation (MUSCLE) (Edgar 2004). The phylogenetic tree was constructed using the Maximum Likelihood (ML) employing the Kimura 2-parameter (K2P) model with 1000 bootstrap replications (Kimura 1980). The genetic distance between the samples was computed using the Kimura 2-parameter model (K2P) (Kimura 1980). All analyses were run in Mega X software program (Kumar et al. 2018).

Morphological Description: Body elongated, subcylindrical, and fusiform shape; body width ranges from 16.17 to 20.07 mm SL. Head broad and somewhat depressed on dorsal view; head length 23.38–26.41 mm SL. Snout broad and bluntly pointed. The mouth is large and terminal, with the lower jaw (42.68–43.79 mm HL) projecting beyond the upper jaw (40.53–42.45 mm HL). Mouth relatively wide when broadly opened, forming an oval in cross-section. Maxillary reaching the anterior margin of the small eye. Both jaws, roof of the mouth, and tongue have villiform, sharp teeth. Tongue short. Total gill rakers 12–13. Caudal fin is firm and emarginated; the upper lobe is longer than the lower lobe, rounded in the smaller specimen (194.21 mm). The first dorsal fin is composed of 8 sharp spines, almost equal in size and not connected by a membrane. Second dorsal fin 32–36, strongly connected by a membrane, anterior fin rays (3rd–13th) moderately elevated. Anal-fin rays (total) 24–25, anal-fin base shorter than the dorsal-

fin base, anterior anal-fin rays elevated similar to the dorsal fin (1st–6th). The pelvic fin thoracic is inserted just below the pectoral fin, composed of 1 spine and 5 rays. Pectoral fins pointed. Scales covering the body are smooth, very small, and adhesively embedded in the skin. Scales are absent in the head area. Lateral-line system is visible with small scales, irregularly wavy on the anterior part, while the posterior area slopes down evenly along the middle area of the body down to the caudal peduncle. Vertebral count 25–26. Color when fresh: body black with two sharp silver-white stripes, ventral side white; in larger samples (423.62 mm SL), white stripes not apparent on the body. Head black dorsally while ventral side is white. Caudal-fin rays black, tips on both lobes white. Dorsal, anal, and pectoral fins are brown or black, while the pelvic fin is black. Color when preserved: body brown, white stripes still prominent (in smaller samples). Tips on both the upper and lower caudal-fin lobe remain white. Morphological measurements are presented in Table 1.

Molecular Analysis. The COI gene sequences of specimens with more than 33 dorsal-fin rays match publicly available sequences in GenBank (100% percent identity). The six specimens collected in the Visayas region, as well as the six sequences retrieved from GenBank, clustered together as shown in the phylogenetic tree inferred by maximum likelihood (Fig. 4).

All the Cobia sequences used in this study have very low pairwise genetic distances (<0.001), as shown in Table 2. Based on the computed pairwise genetic distances, the Dolphinfish, *Coryphaena hippurus* (0.19), appears to be more closely related to the Cobia group than the Remora, *Echeneis naucrates* (0.26).

All Panay samples were recognized as *Rachycentron canadum* based on the separated dorsal-fin spines, body structure, and color pattern. However, the previous reports of dorsal-fin ray counts

Table 1. Meristic count and proportional measurements expressed as a percentage of standard length and head length (mm) of *Rachycentron canadum* from Western Visayas, Philippines.

	UPVMI 01354	UPVMI-01355	UPVMI-01356	UPVMI-01357	UPVMI-01358	UPVMI-01359
Standard length (SL)	263.31	234.98	194.21	298.23	423.62	226.43
Total length	306.61	271.97	223.48	346.35	524.63	274.95
Counts						
1st dorsal-fin spines	8	8	8	8	8	8
2nd dorsal-fin rays (total)	33	35	32	33	35	36
Anal-fin rays (total)	26	25	26	25	27	25
Pelvic-fin rays	6	6	6	6	6	6
Total gill rakers (total)	13	13	13	13	12	13
Vertebrae count	25	25	*	*	26	25
Measurement (% standard length)						
Body width	19.42	18.63	16.17	19.87	19.71	20.07
Pre-dorsal length	27.07	26.15	25.14	25.38	26.21	26.75
1st dorsal-fin base	15.90	15.13	16.77	15.67	16.93	16.40
2nd dorsal-fin base	44.42	43.99	43.23	44.80	46.89	45.86
Anal-fin base	35.83	34.91	33.81	36.11	36.40	36.97
Pectoral-fin base	5.02	4.58	4.14	5.11	5.43	5.24
Length of caudal peduncle	6.04	5.39	4.85	5.27	5.85	6.09
Head length	25.59	24.18	24.03	23.38	26.41	25.34
Measurement (% head length)						
Eye diameter	16.70	15.19	17.31	14.52	13.79	17.88
Upper jaw length	42.17	41.68	42.45	42.33	40.53	41.72
Lower jaw length	43.58	43.55	43.09	43.79	42.68	42.82

*Samples without x-ray photograph

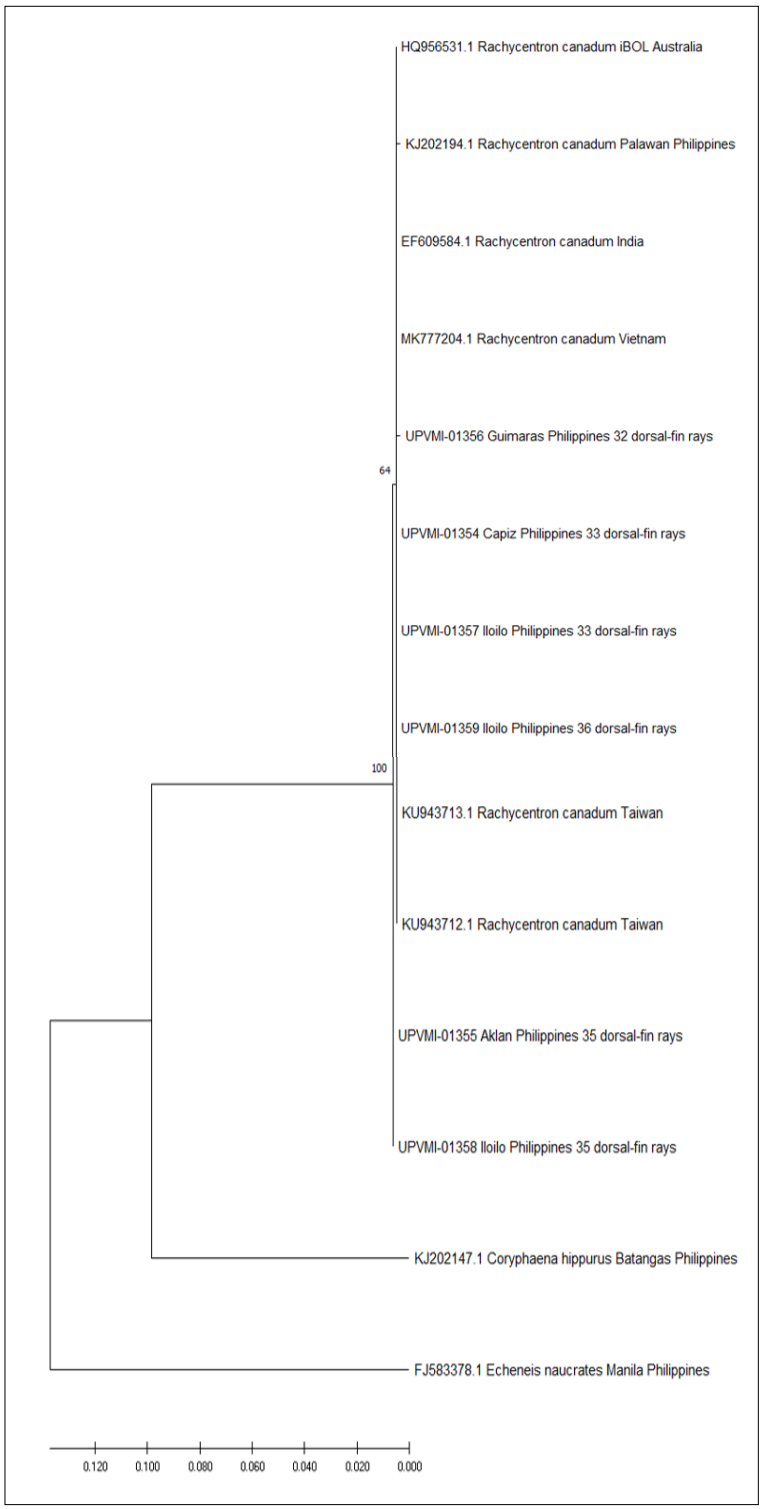

Figure 4. Maximum likelihood tree of six COI sequences of *Rachycentron canadum* collected in Western Visayas together with sequences downloaded from GenBank. Numbers on nodes represent bootstrap support values.

for Cobia are only 26–33 (Collette 1999; Shaffer and Nakamura 1989), but some of our samples have dorsal-fin ray counts of 35 (UPVMI-01355, UPVMI-01358) or 36 (UPVMI-1359).

Hence, to our knowledge, this is the first report of a higher number of dorsal rays in Cobia fish.

Fish dorsal-fin ray counts can be environmentally influenced, with experiments showing that higher temperatures can result in either higher or lower dorsal counts (Fahy 1980). Moreover, it has been experimentally established that different temperatures can cause variations in dorsal fins in the developmental stage of fish (Georgakopoulou et al. 2007; Sfakianakis et al. 2011). Given the strong migration activity of the Cobia, the intraspecific variation in the dorsal-fin rays we observed could be related to temperature. However, more research is needed to determine the relationship between the reported differences. There are also informal observations of variations in the number of dorsal-fin soft rays in numerous marine fish species, particularly in those with more than 15 dorsal-fin rays (H. Motomura, pers. comm.), suggesting that this observation may be a regular phenomenon.

Nonetheless, observations on intraspecific phenotypic variations in marine fishes, specifically regarding meristic counts, should be regularly reported to alert other researchers that such variations occur in order to avoid confusion when identifying specimens. In addition, in light of the changing climate and human activities that adversely impact fish habitats, reports like this will hopefully encourage scientists to explore further the relationship between environmental conditions and changes in fish morphology.

Table 2. Pairwise genetic distances between six sequences of Rachycentron canadum from the Visayas region and GenBank sequences calculated using the K2P model. The number of base substitutions per site is given between sequences. The standard error estimate(s) indicated above the diagonal were obtained using a bootstrap procedure (1000 replicates).

UPVMI-01354 Capiz Philippines		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.26	0.19
UPVMI-01355 Aklan Philippines	0.002		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.26	0.19
UPVMI-01356 Guimaras Philippines	0.002	0.002		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.27	0.19
UPVMI-01357 Iloilo Philippines	0.000	0.002	0.002		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.26	0.19
UPVMI-01358 Iloilo Philippines	0.002	0.000	0.003	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.26	0.19
UPVMI-01359 Iloilo Philippines	0.000	0.002	0.002	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.27	0.19
KU943713.1 R. canadum Taiwan	0.000	0.002	0.000	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.27	0.19
KU943712.1 R. canadum Taiwan	0.000	0.002	0.000	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.27	0.19
HQ956531 R. canadum Australia	0.000	0.002	0.000	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.26	0.19
EF609584 R. canadum India	0.000	0.002	0.000	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.26	0.19
MK777204.1 R. canadum Vietnam	0.000	0.002	0.000	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.26	0.19
KJ202194 R. canadum Palawan Philippines	0.001	0.003	0.003	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.26	0.19
FJ583378 E. naucrates Manila Philippines	0.262	0.260	0.269	0.262	0.260	0.266	0.271	0.271	0.262	0.262	0.263	0.265		0.27
KJ202147 C. hippurus Batangas Philippines	0.188	0.185	0.190	0.187	0.185	0.191	0.187	0.187	0.188	0.189	0.192	0.187	0.267	

ACKNOWLEDGMENTS

We are very grateful to Professor Hiroyuki Motomura of the Kagoshima University Museum for providing suggestions to improve the manuscript. We thank the Provincial Government Units, the Office of Provincial Agriculture (PAO) from the provinces of Aklan, Antique, Capiz, Iloilo, and Guimaras, especially Ms. Grace-an Villareal Perlas (PAO staff, Aklan), Ms. Glenda Sanchez (MAO staff, Tangalan, Aklan) and Ms. Ramie Lyn G. Bañares (PAO staff, Capiz), the Local Government Units (Guimbal, Tangalan, Batan, Tibiao, Pandan, San Jose, Sibunag, Cabalagnan, Batad, and Roxas) for their assistance and support. Contributions of our field enumerators (M.C. Macavinta, S. Sucgang, S. Gelera, A. Abisan, P. Aguilos Jr., M. Batay, M. Asgar, and J. Tubillara) are gratefully acknowledged. We are also grateful to the Philippine Genome Center-Visayas Satellite Facility (PGC-VSF), the National Institute of Molecular Biology and Biotechnology (UPV-NIMBB), and the UPV Museum of Natural Sciences (UPV-MNS). We thank and appreciate L. Mooc, N. Ylaron, E. Delloro Jr., E.A. Obar, K.D. Barnuevo for their generous help throughout the project. This study was funded by the UP System Emerging Inter-Disciplinary Research Program (OVPAA-EIDR-C08-011-R) and Leverage fund from the Office of the Vice Chancellor for Research and Extension (OVCRE), University of the Philippines Visayas.

AUTHOR CONTRIBUTIONS

Cabebe R: Conceptualization, Formal Analysis, Investigation, Writing - Original draft. **Penuela D**: Formal analysis, Investigation, Writing - Review and editing. **Mediodia D:** Writing -Review and editing. **Babaran P:** Writing - Review and editing, Project Administration, Funding Acquisition. **Malay M:** Conceptualization, Writing - Review and editing, Supervision, Project Administration.

CONFLICTS OF INTEREST

The authors indicate no conflicts of interest.

ETHICS STATEMENT

There were no ethical concerns during the research because the fish were purchased from fish markets and landing sites.

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