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First record of the blue-and-yellow grouper *Epinephelus flavocaeruleus* (Lacepède 1802) (Perciformes: Epinephelidae) from the Borneo waters, Malaysia

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Abstract

Groupers of the family Epinephelidae constitute a diverse and commercially valuable group of reef fishes globally. They comprise an assemblage of carnivorous marine fishes, comprising more than 177 species across 16 genera. The epinephelid genus *Epinephelus*, which consists of over 90 species, is found worldwide in the tropics and subtropics. To date, the ichthyofauna of Malaysia has documented a total of 43 epinephelid species. Apart from these, *Epinephelus flavocaeruleus* (Lacepède, 1802), commonly known as the blue-and-yellow grouper, is rarely reported in the Indo-Pacific Ocean. The present study extends the documented distribution range of *E. flavocaeruleus* eastwards from the Andaman Sea to the Borneo waters of Sabah, Malaysia. Five specimens of the blue-and-yellow grouper were collected from a local fish market. Species identification was confirmed by the color patterns and DNA barcoding of 630 base pairs of the cytochrome C oxidase I gene for all *E. flavocaeruleus* specimens, *Epinephelus cyanopodus* (Richardson, 1846), and 10 closely related *Epinephelus* species. The interspecies genetic distance ranged from 0.002–0.168. Results from the Templeton, Crandall, and Sing (TCS) haplotype network analysis and maximum likelihood phylogeny based on the COI marker indicate a close genetic relationship between *E. flavocaeruleus* and *E. cyanopodus*. However, we refrain from proposing any taxonomic revisions given that more in-depth studies using multiple molecular markers or phylogenomic analysis on a larger sample size are necessary to confirm the taxonomic status of both species. This study significantly contributes to a better understanding of the taxonomy, phylogenetic relationship, and genetic diversity of *E. flavocaeruleus*.

Key words: First records, taxonomy, DNA barcode, haplotype network, grouper

Introduction

The Perciform family Epinephelidae Bleeker, 1874 commonly known as groupers, are economically important members of marine ecosystems, particularly in Southeast Asia (Ma & Craig 2018). They were previously classified as the subfamily Epinephelinae within the Serranidae family (Craig & Hastings 2007). These groupers exhibit a wide distribution across various habitats in tropical and subtropical regions worldwide. According to Eschmeyer's Catalog of Fishes, the Epinephelidae family comprises 177 valid species belonging to 16 genera (Fricke *et al.* 2023). Six new grouper species were described in the last 10 years, between 2014 and 2023, including *Epinephelus insularis* Nakamura & Motomura, 2021; *Epinephelus tankahkeei* Wu, Qu, Lin, Tang & Ding 2020; *Epinephelus*

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fuscomarginatus Johnson & Worthington Wilmer 2019; *Epinephelus craigi* Frable, Tucker & Walker 2018; *Epinephelus kupangensis* Tucker, Kurniasih & Craig 2016; and *Hyporthodus griseofasciatus* Moore, Wakefield, DiBattista & Newman 2022. However, *Epinephelus tankahkeei* is currently accepted as a synonym of *Epinephelus japonicus* (Temminck & Schlegel 1843) by Nakamura and Motomura (2021).

Within the genus *Epinephelus* Bloch, 1793, 92 valid species are currently recognized (Craig *et al.* 2011; Tucker *et al.* 2016; Frable *et al.* 2018; Johnson *et al.* 2019; Wu *et al.* 2020; Nakamura & Motomura 2021; Moore *et al.* 2022; Fricke *et al.* 2023). However, the family of epinephelid fish encompasses approximately 97 species distributed among 16 genera in Malaysia (DOF 2009; Chong *et al.* 2010; Matsunuma *et al.* 2011; Ambak *et al.* 2012; Yusri *et al.* 2015; Nurnadia *et al.* 2016; Du *et al.* 2019; MyBIS 2023). Specifically, within the genus *Epinephelus*, there are 43 species recorded in Malaysia (MyBIS 2023; Froese & Pauly 2023a). The total landing of grouper in Malaysia has increased from 8,837 metric tonnes in 2002 to 11,842 metric tonnes in 2012, with a subsequent drop to 3,956 metric tonnes in 2022 (DOF 2002, 2012, 2022). However, the annual aquaculture production of grouper from farms in Malaysia has increased from 2002 to 2022, from 1,216 tonnes to 148,526 tonnes (DOF 2002, 2022).

The blue-and-yellow grouper *Holocentrus flavo-caeruleus* Lacepède 1802, has been identified as a synonym of *Epinephelus flavocaeruleus* (Froese & Pauly 2023b). *E. flavocaeruleus* is an incredibly colorful grouper. The adults have yellow fins, a dark gray or purplish-brown body with yellow reduced to small areas on the head and margin of the spinous dorsal fin, and a black distal third of the pelvic area. Small juveniles are about half blue and half yellow in color (Randall & Whitehead 1985). It is a demersal species that inhabits the continental shelf over coral and rocky reefs at depths ranging from 10–150 meters. *E. flavocaeruleus* is a medium-sized fish that can grow up to a maximum total length (TL) of 90 cm (Heemstra & Randall 1986), with females reaching maturity at around 50 cm TL (Morgans 1982). However, little else is known about this naturally rare species. With regards to conservation status, *Epinephelus flavocaeruleus* (Lacepède 1802) is currently categorized as a Least Concern (LC) species (Samoilys 2018; IUCN 2019). The distribution of this species is widespread across the tropical waters of the Indian Ocean, spanning from the eastern coast of Africa to the western islands of Indonesia, and includes: South Africa, Mozambique, the Seychelles, Kenya, Egypt, Somalia, Namibia, Réunion, the Maldives, India, Belize, the Persian Gulf, Socotra, Madagascar, Mauritius, Yemen, Tanzania, India, Sri Lanka, and the Mascarenes east to the Andaman Islands, Papua New Guinea, western Thailand, Indonesia (Sumatra), and Singapore (Catania & Fong 2023).

The study expands the known distribution range of *E. flavocaeruleus* eastwards from the Andaman Sea to the waters of Borneo, Sabah, Malaysia. Consequently, this research significantly contributes to a better understanding of the taxonomy, phylogenetic relationship, and genetic diversity of *E. flavocaeruleus*.

Materials and Methods

Five specimens of the blue-and-yellow grouper were purchased from a local fish market, New Seng Kee (NSK) Trade City Sdn Bhd (KL), on 28 December 2020 and 19 February 2021. These specimens were captured using trawl nets operating in the Kota Kinabalu waters of Borneo, Sabah, Malaysia. Tissue samples obtained from these specimens were preserved in a 95% ethanol solution and deposited at the Institute of Ocean and Earth Sciences (IOES), University of Malaya (UM), Kuala Lumpur. Preliminary species identification was made based on the morphology of the whole fish specimens using the species identification keys and diagnostic characteristics reported in Heemstra and Randall (1993). Morphological measurements included total length (TL, mm), standard length (SL, mm), total weight (Wt, g), head length (HL, mm), pre-anal length (PreA, mm), body depth (BD, mm), eye diameter (ED, mm), and pre-orbital length (PreOL). Meristic counts of the spines and soft rays for dorsal, pectoral pelvic, and anal fins were also recorded.

Species identification was confirmed through a combination of color pattern and DNA barcoding of 630 base pairs (bp) of cytochrome C oxidase I. Total DNA extractions were performed on the tissue samples using 10% Chelex resin following the procedure in Hyde *et al.* (2005) with modifications. The primer combinations of the forward (FishF1, 5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-3') and the reverse (FishR1, 5'-TAG ACT TCT GGG TGG CCA AAG AAT CA-3') primer pairs followed Ward *et al.* (2005). A 25 µl PCR reaction mixture was prepared in a 0.2 ml tube containing 12.5 µl double distilled water (ddH$_2$O), 9.5 µl exTEN II PCR Master Mix 2X (1st BASE), 1 µl of each primer used, and 1 µl of total genomic DNA. An Eppendorf thermal cycler was used to run the following thermal cycle profile: initial denaturation at 94 °C for 5 minutes; 36 cycles of denaturation at 94 °C for 30s; annealing at 44 °C for 30s; extension at 72 °C for 1 minute; followed by a final extension at 72 °C for 5 minutes. The amplified PCR products were stained with loading dye and loaded onto wells of 1% agarose gel for gel electrophoresis. Successfully amplified PCR products were sent to Apical Scientific Sdn Bhd (Selangor, Malaysia) for sequencing using the same primers employed in the PCR reactions.

Raw DNA sequences were checked and edited using Sequence Scanner v 1.0 (Applied Biosystem) to confirm the correct base calling. Then, the trimmed sequences were searched against GenBank (https://blast.ncbi.nlm.nih.gov/) and BOLD (http://www.boldsystems.org/) to find molecular matches. Reference sequences of *E. flavocaeruleus*, *Epinephelus cyanopodus* (Richardson, 1846), and some closely related *Epinephelus* species were obtained from GenBank or the Barcode of Life Database (BOLD) for alignment with sequences generated from this study using MEGA X (Kumar *et al.* 2018).

The raw sequences were first assembled and edited via ChromasPro version 1.42 (Technelysium Pty Ltd), subsequently aligned using Clustal X v. 2.0.8 (Larkin *et al.* 2007), and then manually adjusted with Bioedit v. 7.0.9.0 (Hall 1999). The COI gene of the five *E. flavocaeruleus* specimens was sequenced, along with the closely related species *Epinephelus cyanopodus*. An additional 10 related species available in GenBank, including *Epinephelus areolatus* (Forsskål, 1775), *Epinephelus bleekeri* (Vaillant, 1878), *Epinephelus coioides* (Hamilton, 1822), *Epinephelus epistictus* (Temminck & Schlegel, 1843), *Epinephelus erythrurus* (Valenciennes, 1828), *Epinephelus fuscoguttatus* (Forsskål, 1775), *Epinephelus heniochus* Fowler, 1904, *Epinephelus latifasciatus* (Temminck & Schlegel, 1843), *Epinephelus quoyanus* (Valenciennes, 1830) and *Epinephelus sexfasciatus* (Valenciennes, 1828), were also included in the analysis. The slender grouper, *Anyperodon leucogrammicus* (Valenciennes, 1828), was used as an outgroup (accession number GQ131336).

The maximum likelihood (ML) tree was reconstructed based on the best evolutionary model, namely the General Time Reversible model (GTR) with Gamma-distributed (G) distance and invariable sites (I). This model was selected using the lowest bias-corrected Akaike Information Criterion (AICc) value in a model test that included 1000 replications for bootstrap analysis. Both tree construction and model tests were completed in MEGA (Molecular Evolution Genetic Analysis) version X (Kumar *et al.* 2018). Similarly, a neighbor joining (NJ) tree was constructed based on the pairwise genetic distance using the Kimura 2-parameter (K2P) model with 1000 bootstrap resampling. The genetic distances of the sequences were calculated with the K2P model using MEGA X.

A Templeton, Crandall, and Sing (TCS) haplotype network analysis based on the COI marker of *E. flavocaeruleus* and *E. cyanopodus* was also constructed.

Results

Family EPINEPHELIDAE
Subfamily EPINEPHELINAE

Epinephelus flavocaeruleus (Lacepède, 1802)
Blue-and-yellow grouper
Fig. 1 a & b, Table 1

Specimens examined: The color pattern of the Malaysian specimens identified as *E. flavocaerules* was similar to the species description in Heemstra and Randall (1993). The five specimens were (n=4, G2, G3, G4, and G5), 470–540 mm SL (540–630 mm TL), and 2.66–4.82 g total weight, all males. G1, with only the head part and caught from the Borneo waters of Sabah, was purchased from a local fish market, NSK (KL), on 28 December 2021 and 21 February 2022.

Description of the Malaysian specimens. Description: *E. flavocaeruleus* is an incredibly colorful grouper. The adults have yellow dorsal fin, pectoral fin, pelvic fin, and anal fins (Fig. 1a), body dark gray or purplish brown with yellow reduced to small areas on the head and margin of spinous dorsal fin, distal third of pelvic blackish margin. Upper jaw lip often yellow (Fig. 1b). Large adults were losing all the yellow color, becoming deep blue to black, and yellow in the body. The dorsal and anal fins have a very narrow yellow margin for the fourth specimen, with 588 mm TL. Dorsal rays XI, 16 or 17; anal rays III, 8; pectoral rays 17 to 20. Morphometric and meristic traits of *E. flavocaeruleus* are given in Table 1. Body depth 2.27–2.57 in % SL; Head Length 2.46–2.83 in % SL; Pre-anal length 1.57–1.75 in % SL; Pre-orbital length 5.39–6.03 in % HL; Eye diameter 6.70–8.20 in % HL. Standard length 1.15–1.17 in % TL.

Distribution: *E. flavocaeruleus* was previously identified in the Indian Ocean, the Andaman Sea, and Sumatra, Indonesia. This study presents the first record of its presence in Borneo waters, extending the distribution range of *E. flavocaeruleus* eastwards from the Andaman Sea to the Borneo waters of Sabah, Malaysia (Fig. 2).

The 27 grouper specimens were classified as 13 species based on external morphology, which was consistent with the species names found in both the GenBank BLAST and BOLD databases. DNA sequences from the five specimens were submitted to GenBank with the accession numbers OR977058–977062 (Table 2). There were a total of 200/630 bp of variable sites and 163/630 bp of parsimony-informative sites after aligning the sequences. The average base composition obtained was 24.1% A bases, 28.5% C bases, 18.9% G bases, and 28.5% T bases. However, there were a total of 16/630 bp of variable sites and 9/630 bp of parsimony-informative sites for *E. flavocaeruleus* (n=10). The average base composition obtained was 23.8% A bases, 29.7% bases, 19.5% G bases, and 27.0% T bases. Figure 3 shows the Maximum Likelihood (ML) tree inferred from COI barcodes for some related *Epinephelus* species. Bootstrap values <50% are not shown, and the scale bar indicates percent divergence calculated under the GTR +G +I.

FIGURE 1. The blue-and-yellow grouper *Epinephelus flavocaeruleus* (a) Lateral view for specimen G5, (b) Head part for specimen G1.

TABLE 1. Comparison of morphometric measurements and meristic counts between the five specimens of *Epinephelus flavocaeruleus* collected from Malaysia and published studies.

	Present study						Heemstra & Randall (1993); Randall & Whitehead (1985)
Morphometric characters	1st specimen	2nd specimen	3rd specimen	4th specimen	5th specimen	(n=5)	
	G1	G2	G3	G4	G5		
Total weight (Wt)	N/A*	4820 g	3994 g	3934 g	2662 g	2662–4820 g	
Total length (TL)	N/A*	620 mm	630 mm	588 mm	540 mm	540–630 mm	
% in TL							
Standard length (SL)	N/A*	1.16	1.17	1.16	1.15	1.15–1.17	
% SL							
Head length (HL)	181 mm *	2.61	2.57	2.46	2.83	2.46–2.83	2.4–2.7
Pre anal length (PreA)	N/A*	1.75	1.64	1.62	1.57	1.57–1.75	
Body depth (BD)	N/A*	2.38	2.57	2.27	2.47	2.27–2.57	2.4–2.8
% HL							
Eye diameter (ED)	6.70	7.07	7.50	8.20	7.22	6.70–8.20	
Pre orbital length (PreOL)	5.66	5.39	5.68	6.03	5.53	5.39–6.03	6.8–8.8
Meristic counts							Randall & Whitehead (1985)
Dorsal spines, soft rays	N/A*	XI, 16	XI, 17	XI, 16	XI, 17	XI, 16–17	XI, 15–17
Pectoral fin	N/A*	18	17	17	17	17–18	18–19
Pelvic spines, soft rays	N/A*	I–5	I–5	I–5	I–5	I–5	
Anal spines, soft rays	N/A*	III, 8	III, 8	III, 7–8	III, 8	III, 7–8	III, 8

FIGURE 2. Map indicating the previously known distribution in the geographic range in the IUCN by Samoilys (2018) of *Epinephelus flavocaeruleus*; the red circle was the location of the landing site of samples in the current study.

TABLE 2. Accession numbers of sequences used in the analysis.

Species	Location	Accession number	Reference
Epinephelus areolatus	Egypt	MH707292	Mohammed-Geba *et al.* (*Unpublished*)
E. bleekeri	USA	JN021297	Shen and Ishida (*Unpublished*)
	Philippines	KU668653	Cabana (*Unpublished*)
E. coioides	Philippines	KJ013039	Marucot *et al.* (*Unpublished*)
E. cyanopodus	China	MF185487	Qu *et al.* (2018)
	Japan	MH707746	Driskell *et al.* (*Unpublished*)
	Indonesia	MN869938	Limmon *et al.* (2020)
	Japan	MH707749	Driskell *et al.*, (*Unpublished*)
	New Caledonia	JQ412502	Schoelinck *et al.* (*Unpublished*)
E. epistictus	Philippines	MK693007	Johnson *et al.* (2019)
E. erythrurus	India	MW810350	Mohapatra *et al.* (*Unpublished*)
E. flavocaerules	Mozambique	JF493441	Steinke *et al.* (*Unpublished*)
	India	KM226268	Vineesh *et al.* (*Unpublished*)
	China	MF185511	Qu *et al.* (2018)
	Madagascar	JQ349963	Hubert *et al.* (2012)
	South Africa	MH707753	Driskell *et al.* (*Unpublished*)
	Malaysia	OR977058	This study
	Malaysia	OR977061	This study
	Malaysia	OR977062	This study
	Malaysia	OR977060	This study
	Malaysia	OR977059	This study
E. fuscoguttatus	India	JX674997	Sachithanandam *et al.*, (*Unpublished*)

......*Continued on the next page*

TABLE 2. (Continued)

Species	Location	Accession number	Reference
E. heniochus	China	MF185518	Qu *et al.* (2018)
E. latifasciatus	China	MF185521	Qu *et al.* (2018)
E. quoyanus	China	MF185570	Qu *et al.* (2018)
E. sexfasciatus	Philippines	KF809401	Marucot *et al.*, (*Unpublished*)
Anyperodon leucogrammicus	Taiwan	GQ131336	Lin *et al.* (*Unpublished*)

The ML phylogenetic tree reconstructed based on GTR +G +I is shown in Figure 3. All species are monophyletic, with bootstrap values between 62 and 100%. All five *E. flavocaeruleus* samples (OR977058– OR977062) were clustered together with the *E. flavocaeruleus* reference sequences from GenBank (KM226268 India, MF185511 China, MH707753 South Africa, JF493441 Mozambique, and JQ349963 Madagascar) with a 61–100% bootstrap value. Genetic distances between grouper species based on the 630 bp COI consensus sequences and the respective reference sequences with the K2P model are given in Table 3. The intraspecific nucleotide distances for *E. flavocaeruleus* ranged from 0.002–0.027. However, the specimens from Malaysia recorded low intraspecific genetic variation ranging from 0.000–0.002, whereas the samples from Madagascar (JQ349963) and South Africa (MH707753) were more genetically distant (0.011–0.025). The interspecific differences between other grouper species of the genus *Epinephelus* ranged from 0.002–0.168, with the largest genetic distance recorded between *E. heniochus* and *E. sexfasciatus* at 0.168.

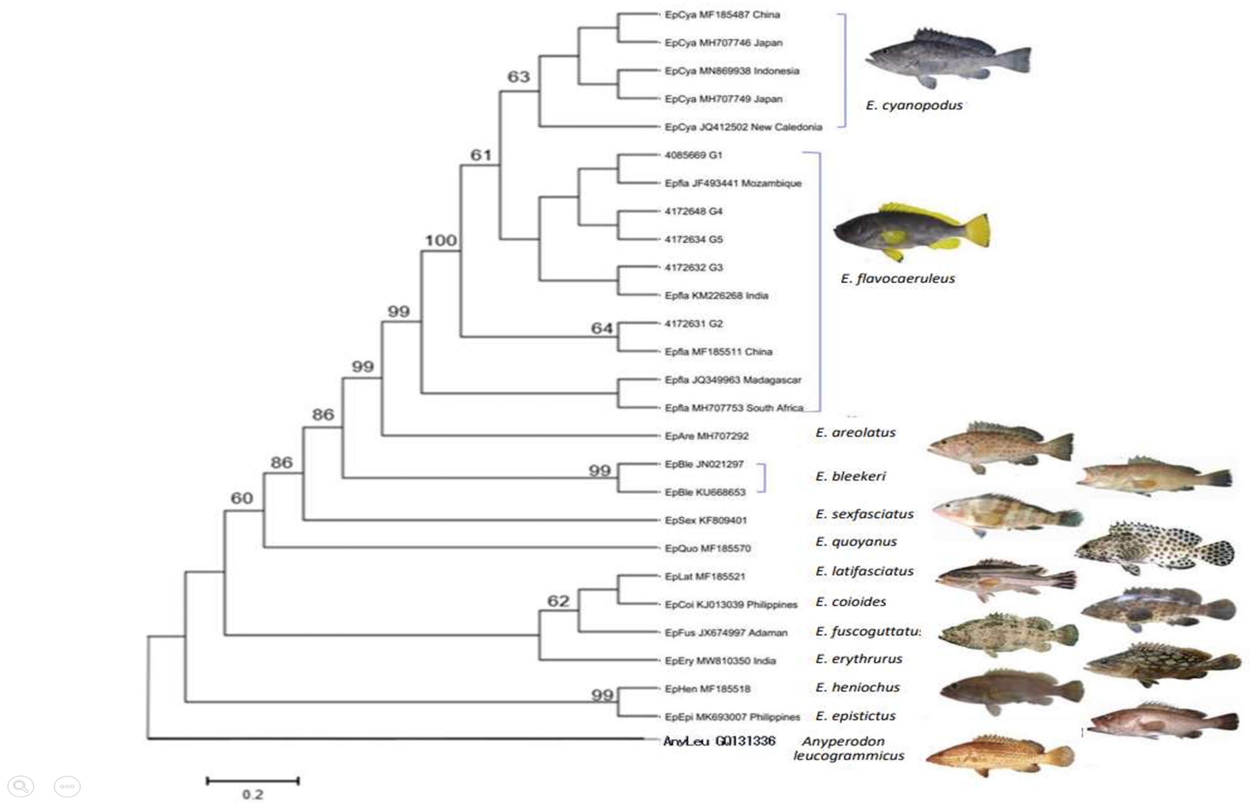

FIGURE 3. Maximum Likelihood (ML) phylogenetic tree inferred based on COI gene sequences (630 bp) and GTR +G +I model for the 12 *Epinephelus* species. Bootstrap values <50% are not shown at the tree nodes.

The TCS haplotype network analysis of *E. flavocaeruleus* and *E. cyanopodus* based on the COI marker is shown in Figure 4. The Malaysian samples of *E. flavocaeruleus* belong to two haplotypes, G1 and G2, with only a 1 bp difference between them. The *E. flavocaeruleus* haplotypes from South Africa (MH707753), Madagascar (JQ349963), and the Seychelles (SAIAB441-06) had more genetic variation. In contrast, another *E. flavocaeruleus*

TABLE 3. Pairwise comparisons of genetic distances (*d*) within all the grouper samples.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1																											
2	0.002																										
3	0.000	0.002																									
4	0.000	0.002	0.000																								
5	0.000	0.002	0.000	0.000																							
6	0.002	0.000	0.002	0.002	0.002																						
7	0.000	0.002	0.000	0.000	0.000	0.002																					
8	0.000	0.002	0.000	0.000	0.000	0.002	0.000																				
9	0.013	0.014	0.013	0.013	0.013	0.014	0.013	0.013																			
10	0.024	0.025	0.024	0.024	0.024	0.025	0.024	0.024	0.011																		
11	0.002	0.003	0.002	0.002	0.002	0.003	0.002	0.002	0.014	0.025																	
12	0.002	0.003	0.002	0.002	0.002	0.003	0.002	0.002	0.014	0.025	0.000																
13	0.002	0.003	0.002	0.002	0.002	0.003	0.002	0.002	0.014	0.025	0.000	0.000															
14	0.002	0.003	0.002	0.002	0.002	0.003	0.002	0.002	0.014	0.025	0.000	0.000	0.000														
15	0.002	0.003	0.002	0.002	0.002	0.003	0.002	0.002	0.014	0.025	0.000	0.000	0.000	0.000													
16	0.065	0.067	0.065	0.065	0.065	0.067	0.065	0.065	0.057	0.065	0.067	0.067	0.067	0.067	0.067												
17	0.102	0.103	0.102	0.102	0.102	0.103	0.100	0.102	0.098	0.102	0.103	0.103	0.103	0.103	0.103	0.103											
18	0.097	0.098	0.097	0.097	0.097	0.098	0.095	0.097	0.094	0.098	0.098	0.098	0.098	0.098	0.098	0.102	0.005										
19	0.146	0.148	0.146	0.146	0.146	0.148	0.145	0.146	0.143	0.144	0.148	0.148	0.148	0.148	0.148	0.152	0.130	0.130									
20	0.151	0.152	0.151	0.151	0.151	0.152	0.151	0.151	0.152	0.157	0.152	0.152	0.152	0.152	0.152	0.141	0.143	0.143	0.110								
21	0.133	0.135	0.133	0.133	0.133	0.135	0.132	0.133	0.137	0.135	0.135	0.135	0.135	0.135	0.135	0.132	0.124	0.124	0.111	0.129							
22	0.152	0.154	0.152	0.152	0.152	0.154	0.151	0.152	0.146	0.144	0.154	0.154	0.154	0.154	0.154	0.149	0.122	0.122	0.100	0.114	0.102						
23	0.149	0.151	0.149	0.149	0.149	0.151	0.148	0.149	0.152	0.157	0.151	0.151	0.151	0.151	0.151	0.151	0.144	0.144	0.124	0.073	0.138	0.132					
24	0.152	0.154	0.152	0.152	0.152	0.154	0.153	0.152	0.151	0.154	0.154	0.154	0.154	0.154	0.154	0.149	0.129	0.130	0.092	0.135	0.108	0.103	0.137				
25	0.127	0.129	0.127	0.127	0.127	0.129	0.127	0.127	0.119	0.130	0.129	0.129	0.129	0.129	0.129	0.133	0.132	0.130	0.140	0.149	0.141	0.146	0.168	0.144			
26	0.149	0.151	0.149	0.149	0.149	0.151	0.148	0.149	0.146	0.144	0.151	0.151	0.151	0.151	0.151	0.151	0.127	0.127	0.141	0.143	0.140	0.146	0.146	0.162	0.149		
27	0.152	0.151	0.152	0.152	0.152	0.151	0.151	0.152	0.149	0.151	0.154	0.154	0.154	0.154	0.154	0.154	0.140	0.140	0.121	0.125	0.144	0.122	0.135	0.135	0.146	0.146	

Genetic distances were calculated based on the Kimura 2-parameter (K2P) model. 1= OR977058, 2= OR977059, 3= OR977060, 4= OR977061, 5= OR977062, 6= MF185511, 7= JF493441, 8= KM226268, 9= MH707753, 10= JQ349963, 11= MF185487, 12= MH707746, 13= MN869938, 14= JQ412502, 15= MH707749, 16= MH707292, 17= KU668653, 18= JN021297, 19= KJ013039, 20= MK693007, 21= MW810350, 22= JX674997, 23= MF185518, 24= MF185521, 25= MF809401, 26= MF185570, 27=GQ131336.

haplotype from the Seychelles (SAIAB149-06) is only 2 bp different from haplotype G1. Among the seven haplotypes of *E. flavocaeruleus*, haplotype G1 is the most common and is reported from Malaysia, South Africa, Mozambique, India, the Seychelles and Indonesia. Haplotype G2 is reported in two samples from Malaysia and the South China Sea, while the other five haplotypes are restricted to the western Indian Ocean. The network indicates a very close genetic relationship between *E. flavocaeruleus* and *E. cyanopodus*, with only a 1 bp difference between haplotype MF185487 (China, New Caledonia, Japan, India, and Australia) and G1 as summarized in Table 4.

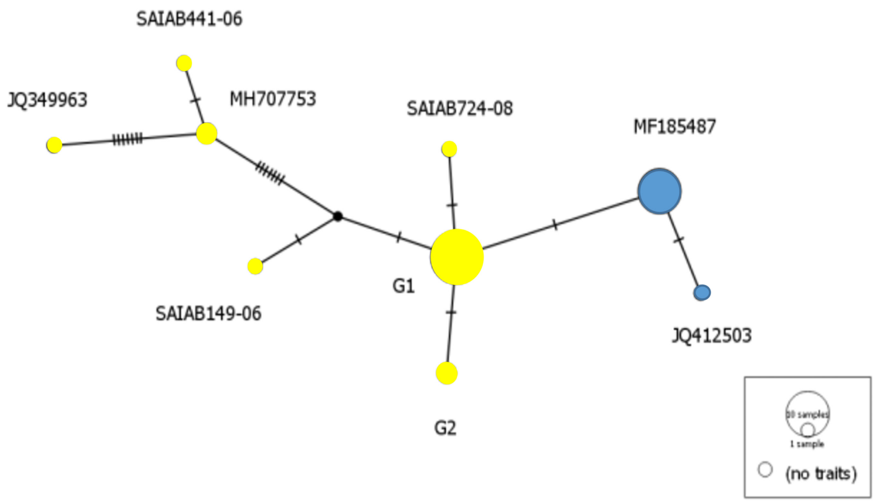

FIGURE 4. TCS haplotype network based on the CO1 marker for *E. flavocaeruleus* (yellow) and *E. cyanopodus* (blue). Each circle represents a haplotype. Each dash line between haplotypes indicates one mutation change. Missing haplotypes are indicated by small black circles.

TABLE 4. TCS haplotype network based on the CO1 marker for *Epinephelus flavocaeruleus* and *E. cyanopodus*.

Species	Haplotype	N	Distribution
E. cyanopodus	**JQ412503**	**1**	**NCL**
	MF185487	**10**	**CHN (2), NCL (1), JPN (4), IDN (2), AUS (1)**
E. flavocaeruleus	**SAIAB724-08**	**1**	**SYC**
	G1	**14**	**MYS (4), ZAF (1), MOZ (2), IND (4), SYC (1), IDN (2)**
	G2	**2**	**MYS (1), *South China Sea* (1)**
	SAIAB149-06	**1**	**SYC**
	JQ349963	**1**	**MDG**
	MH707753	**2**	**ZAF (2)**
	SAIAB441-06	**1**	**SYC**

NCL: New Caledonia, CHN: China, JPN: Japan, IDN: Indonesia, AUS: Australia, SYC: Seychelles, MYS: Malaysia, ZAF: South Africa, MOZ: Mozambique, IND: India, MDG: Madagascar.

Discussion

Epinephelus flavocaeruleus was first reported from the Gulf of Mannar, located between the southeastern tip of India and the west coast of Sri Lanka (Varghese *et al.* 2006). To date, the distribution of this species has ranged from the Andaman Islands in western Thailand (Monkolprasit 1997) to Meulaboh, Tapak Tuah, and Simeulue in Sumatra, Indonesia (Fadli *et al.* 2022), the Philippines (BFAR 2019), and Singapore (Catania & Fong 2023), which are all in close proximity to Malaysia. The present study marks the first report of *E. flavocaeruleus* in the Borneo waters of Sabah, Malaysia. However, the data recorded from Singapore by the CAS Ichthyology (Catania & Fong 2023), which includes a specimen preserved in alcohol (75% EtOH) collected in May 1937 with the field number

AWH V-37A and catalogue number 14094 in the California Academy of Sciences (CAS), requires more information to refine this locality.

A recent study placed groupers into the family Epinephelidae sensu Smith and Craig (2007) and supports the inclusion of both *E. flavocaeruleus* and *E. cyanopodus* in the *Epinephelus* genus, based on the aligned COI, 12S, 16S, and TMO4C4 sequences, which contained 654 bp, 424 bp, 594 bp, and 507 bp, respectively, totaling 2179 bp as reported by Ma and Craig (2016, 2018). At the species level, the status of *E. flavocaeruleus* has not been disputed. The phylogenetic relationship between *E. cyanopodus* and *E. flavocaeruleus*, as shown in both the ML tree and Bayesian Inference (BI), has high bootstrap values, consistently at 100%, and posterior probabilities (PP) = 1 (Ma & Craig 2016, 2018). Moreover, *E. cyanopodus* and *E. flavocaeruleus* were possible conspecifics, as suggested by Qu *et al.* (2018). The genetic distance between *E. cyanopodus* and *E. flavocaeruleus* (0.0030 for both COI and ND2) was significantly less than the average interspecific genetic distance (COI: 0.1661, ND2: 0.2123) and even lower than the average intraspecific distance (COI: 0.0034, ND2: 0.0054) (Qu *et al.* 2018). The only difference between *E. flavocaeruleus* and *E. cyanopodus* lies in their color pattern: the former is dark blue or grayish blue with bright yellow fins and upper jaws, while the latter is blue gray with scattered black spots, although juveniles typically have yellow fins and faint dark spots. Interestingly, the distribution of these two species is adjacent to each other without any overlap (Qu *et al.*, 2018). Present findings reported that both groupers, *E. flavocaeruleus* and *E. cyanopodus*, have the closest genetic distance, with a value of 0.002–0.025. At this stage, we refrain from proposing any taxonomic revisions, given that more in-depth studies using multiple molecular markers or phylogenomic analysis on a larger sample size are necessary to confirm the taxonomic status of both species.

Conclusion

Epinephelus flavocaeruleus was previously known in the Indian Ocean, the Andaman Sea, and Sumatra, Indonesia. The present study extends the distribution range of the blue-yellow grouper, *E. flavocaeruleus*, eastwards from the Andaman Sea to the Borneo waters of Sabah, Malaysia. This study contributes to a better understanding of the taxonomy, phylogenetic relationship, and genetic diversity as well as distribution of this species, which would be helpful for sustainable management of this grouper species in Malaysia.

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