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SPECIES DIVERSITY AND PHYLOGENETIC RELATIONSHIPS OF SNAPPER (LUTJANIDAE: *LUTJANUS*) INFERRED FROM MITOCHONDRIAL DNA MARKERS

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SUMMARY

Snappers (Lutjaniformes: Lutjanidae) are commercially important fishes in tropical and subtropical waters. However, species complexes still exist due to low-level morphological differences. Additionally, current availability of molecular sequences has led to significant changes in fish taxonomy. Therefore, species diversity of lutjanids is still unclear. In this study, we applied two mitochondrial markers (16S rRNA and Cytochrome c oxidase subunit I, COI mtDNA) to investigate species diversity and phylogenetic relationships of lutjanid species collected from the coastal waters of Nghe An – Ha Tinh provinces, Northern Central, Vietnam. A total of 17 *Lutjanus* species have been identified using morphological and molecular methods. Combined with Genbank sequences, the phylogenetic tree was constructed using Neighbor Joining and Maximum Likelihood approaches based on 16S rRNA and COI mtDNA data set. Two main lineages have been detected with inconsistent basal clades between two topologies. Members of species complexes also showed a certain degree of closely relationships; however, conflicts between two topologies have also been recorded. These data contribute to the assessment of lutjanid biodiversity in Vietnam, and for resource management and conservation.

Keywords: Lutjanus, mitochondrial markers, phylogenetic relationships, species composition.

INTRODUCTION

Snappers (Lutjaniformes: Lutjanidae) are commercially important fishes in tropical and subtropical waters, consisting of 135 species, 21 genera. Etelinae, Apsilinae, Paradicichthyinae and Lutjaninae are currently recognized as four subfamilies of the family Lutjanidae (Betancur-R *et al.*, 2017). These fishes are considered long-lived, slow-growing and capable of occupying a wide range of habitats such as coral reefs or other associated structures. During early life cycle, some species, such as

Lutjanus argentimaculatus, L. griseus, may also occur in estuarine mangroves. Adult snapper plays an ecologically important role, acting as predators of a variety of food sources, which can modify the structure of their living environment (Nelson, 2006).

Among the family Lutjanidae, *Lutjanus* is by far the most speciose genus, with 73 known species (Andriyono *et al.*, 2019). Following the external coloration and diagnostic characters, lutjanids species were divided into several species groups, such as the black spot complex (6 species, Miller, Cribb (2007)), blue-lined complex (6 species, Barman (2014)), yellow-lined complex (7 species, Iwatsuki *et al.* (2015)), and red snapper (12 species, Rivas (1996)). Like other fish species, snappers are threatened by overfishing, habitat degradation, etc. (Gold, 2015). Thus, accurate species identification is fundamental for conservation efforts and the management of these valuable fish species.

Fish species identification is traditionally based on external morphological features, including body shape, color pattern, number of fin rays and spines, or various relative measurements of body parts (Allen, 1985). However, taxonomic identification of snapper species is difficult because of the similarities in their external morphology and overlap of diagnostic characters, as in case of *L. erythropterus* and *L. malabaricus* (Halim *et al.*, 2022). Additionally, species hybridization probably occurred (*L. erythropterus*×*L. sebae)* (Chen, 2006), leading to species misidentification. Presently, molecular markers provide useful and powerful tools to discriminate species. The generating genetic data can provide a valuable source of information for studies on phylogeny, phylogeographic, and evolutionary history (Afriyie, 2020). In the last few decade, molecular markers such as mitochondrial and nuclear genes (Miller, Cribb, 2007; Gold *et al.*, 2011; Chu *et al.*, 2013; Gold *et al.*, 2015; Wang *et al.*, 2015), and mitogenomes (Andriyono *et al.*, 2019) have been increasingly applied to investigate the diversity and phylogenetic relationships of the snapper species.

In Vietnam, based on 27 documents published during 1978-2010, Le (2013) confirmed 40 species belonging to 10 genera of family Lutjanidae, of which 26 species belong to the genus *Lutjanus*. Recently, Nguyen and Mai (2020) reported 26 species (4 genera), and 10 species (2 genera) of the families Lutjanidae and Caesionidae, respectively. Besides, phylogenetic relationships of lutjanids species have been explored using molecular data. Truong *et al.* (2015) used the 16S rRNA marker to investigate the molecular relationships of 12 species (*Lutjanus* and *Paracaesio*) collected from Kien Giang, Vung Tau, Khanh Hoa and Da Nang provinces. Their analyses showed that *Lutjanus* spp. species are monophyletic, and sister groups to *Paracaesio xanthura*. Pham *et al.* (2019) found 18 snapper species belonging to 6 genera. A phylogram from the barcode of COI mtDNA showed the paraphyly of *Lutjanus* species.

The research aims to examine the morphological and molecular characteristics of *Lutjanus* species collected from the coastal waters of Nghe An – Ha Tinh provinces based on 16S rRNA and COI mtDNA genes. The generated data will serve as scientific information, which could be applied for improving the monitoring program and conservation of fisheries resources.

MATERIAL AND METHODS

Study sites and fish sampling

A total of 55 snapper specimens were collected from local fishermen of Nghe An – Ha Tinh coastal waters (Lat. 18°06’33.24’’N-19°14’00.94’’N and Long. 105°44’49.31’’E -106°21’13.68’’E, Figure 1) during 2020-2022. All fishermen were interviewed to confirm that fish were caught within 10 nautical miles and no more than 12 hours. The fish were caught from a depth range of 5 to 50 m using traditional fishing gears, including gill nets, trawl nets (mesh size 40-60 mm), fishing fish, or fish traps. On the field, all specimens were photographed, and their body, fin coloration, and diagnostic features (spot, stripes or bars) were recorded.

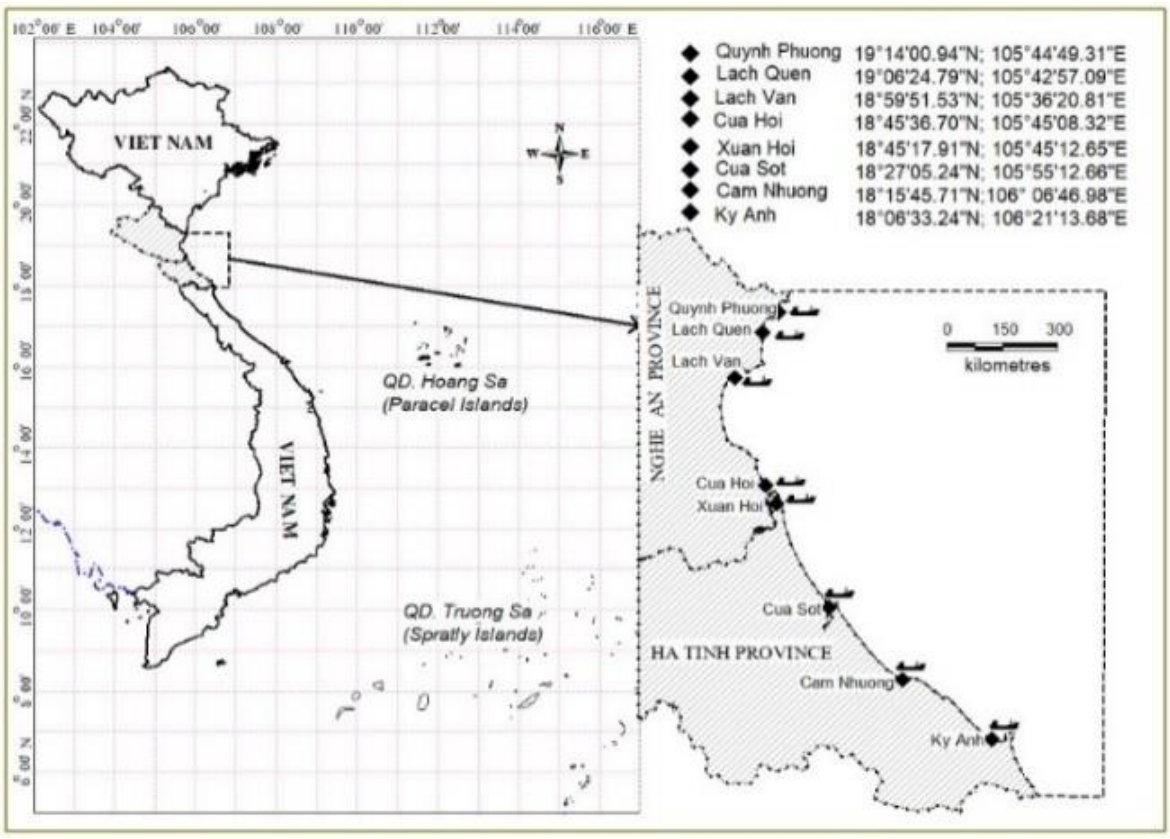

Figure 1. Sampling sites for the snapper specimens in Nghe An – Ha Tinh.

Muscle tissue samples (~1 cm^2) were excised from the right side of the fish specimens and put into 2 ml cap-screw tubes filled with 95% ethanol. All samples were kept on ice and transported to the Molecular Biology Laboratory of Nha Trang University for DNA analysis. Specimens were transported in an ice box to the Laboratory of the Coastal Branch of Joint Vietnam - Russia Tropical Science and Technology Research and kept at –20ºC until further study.

Morphological identification and voucher preservation

At the laboratory, specimens were defrosted under running water. All

specimens were identified based on taxonomic characteristics such as the coloration patterns on the head, body, and fins; presence of diagnostic features (spot, stripes or bars) on the body following the identification keys of Allen (1985). The number of spines and rays of the dorsal fin (D), anal fin (A), and pectoral fin (P) were counted (Carpenter, Allen, 1989) (Figure 2).

After the morphological analysis, one to three representative specimens of each species were preserved following Motomura, Ishikawa (2013). For fixation, the specimens were injected through the vent and dorsal musculature and completely soaked with 10% formalin. After 30 - 40 days, the specimens were transferred to a 3 – 5L wide-mouth glass jar filled with 99% ethanol for long-term preservation. All voucher specimens were labeled and kept at Coastal Branch of Joint Vietnam - Russia Tropical Science and Technology Research.

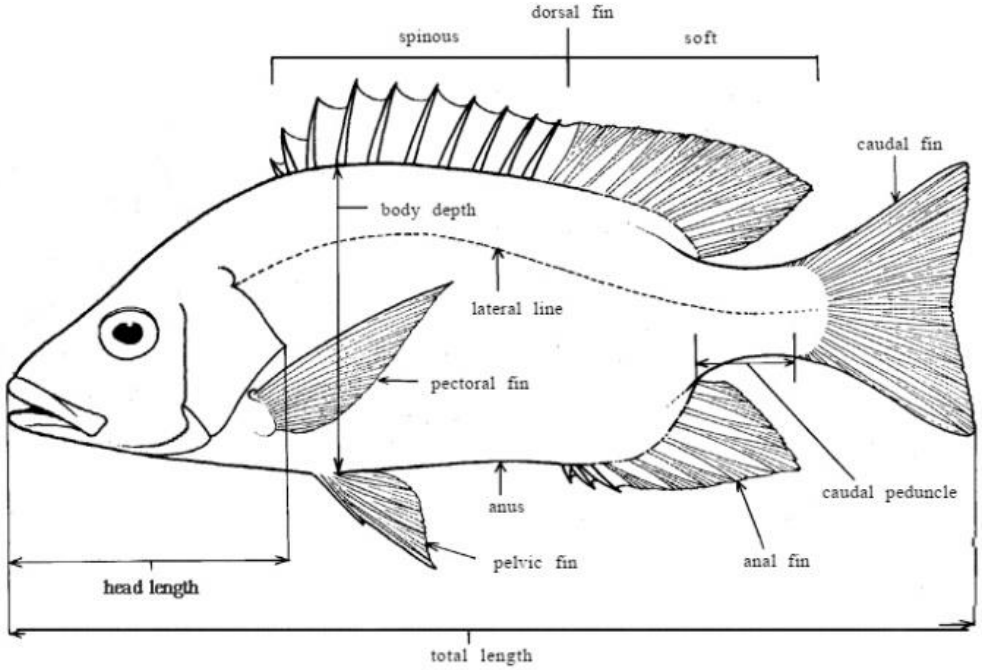

Figure 2. Morphometric and meristic characters (Carpenter, Allen, 1989).

Molecular identification

Ethanol-preserved tissue samples were extracted using Wizard® Genomic DNA Purification Kit (Promega, USA) following to the manufacturer's instructions. The extracted DNA samples (5 µl) were PCR amplified at partial fragments of the 16S rRNA and COI mtDNA genes using the primers 16Sar, 16Sbr (Palumbi, 1996) and FishF1, FishR1 (Ward *et al.*, 2005), respectively. The components and thermal cycle of the PCR reaction were performed following Vu *et al.* (2018). Successful PCR products were purified with the Promega PCR Purification Kit following the manufacturer's protocol. Sequences of both strands were performed at 1st Base Company (Malaysia) using ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, USA) with the amplification primers.

Forward and reverse sequences were assembled using Geneious Pro 5.5.7 (Kearse *et al.*, 2012). The Basic Local Alignment Search Tool (BLAST, https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to search for identical sequences. The obtained sequences were aligned, analysed using BioEdit 7.0.5.3 (Hall, 1999), and deposited to GenBank under the accession numbers OP316912-OP316928 (16S rRNA) and OP316932-OP316948 (COI mtDNA).

Phylogenetic relationships

Each sequence set of 16S rRNA and COI mtDNA (17 sequences from each gene) was separately tested for substitution oversaturation based on the concept of entropy information theory using DAMBE 6.4.101. Following Gontcharov *et al.* (2004), combined gene analysis enhanced phylogenetic resolution, 2 current sequence sets were combined with 8 available Genbank sequences using Geneious Pro 5.5.7. An incongruence-length difference (ILD) test (Farris *et al.*, 1995) was performed in PAUP*4.0b10 (Swofford, 2003) with 1,000 randomized replicates to estimate any difference in phylogenetic signal among the different molecular sections. ILD test (p-value = 0.01) indicating significant incongruence between the two data sets, therefore combination was not applied for further analysis.

Phylogenetic trees were constructed for 16S rRNA and COI mtDNA sequence alignments using the Neighbor-joining (NJ) and Maximum likelihood (ML) approaches in MEGA 11.0.11 (Kumar *et al.*, 2018). NJ analysis was conducted to determine the evolutionary relationships of all samples based on the Kimura 2-parameter model under 1,000 replicates. Prior to ML analysis, the best-fit model of nucleotide substitution was selected by the Akaike Information Criterion as implemented by Modeltest 3.7 (Posada, Crandall, 1998). General Time Reversible (GTR) and Hasegawa–Kishono–Yano (HKY) models with a proportion of invariable sites (+I) and rate of variation across sites (+G) were selected for 16S rRNA and COI mtDNA datasets, respectively. ML tree was applied under the selected best-fit models with 1,000 replicates. *Pristipomoides multidens* (Lutjaniformes: Lutjanidae) was chosen to root the ML and NJ tree constructions in this study.

RESULTS AND DISCUSSION

Morphological and molecular identification

Morphological identification

A total of 17 *Lutjanus* species were morphologically identified, and tentatively divided into four complexes (Rivas, 1996; Miller, Cribb, 2007). Their morphologic characters were presented in Table 1.

Black-spot snapper complex

Four species have been identified, the main taxonomic character is a black spot on the lateral line and below the soft part of the dorsal fin (Figure 3.A1-D1). *L. fulviflamma* is the most distinctive in possessing a series of six thin yellow stripes on the side (Figure 3.A2). *L. russellii* is quite similar to *L. monostigma,* however, only the dorsal and anal fins of *L. russellii* are yellow (Figure 3.B2), while all fins of *L. monostigma* are yellow (Figure 3.C2). *L. johnii* was distinguished from the others by the largest black spot and each scale with a black spot forming horizontal lines on the body (Figure 3.D2).

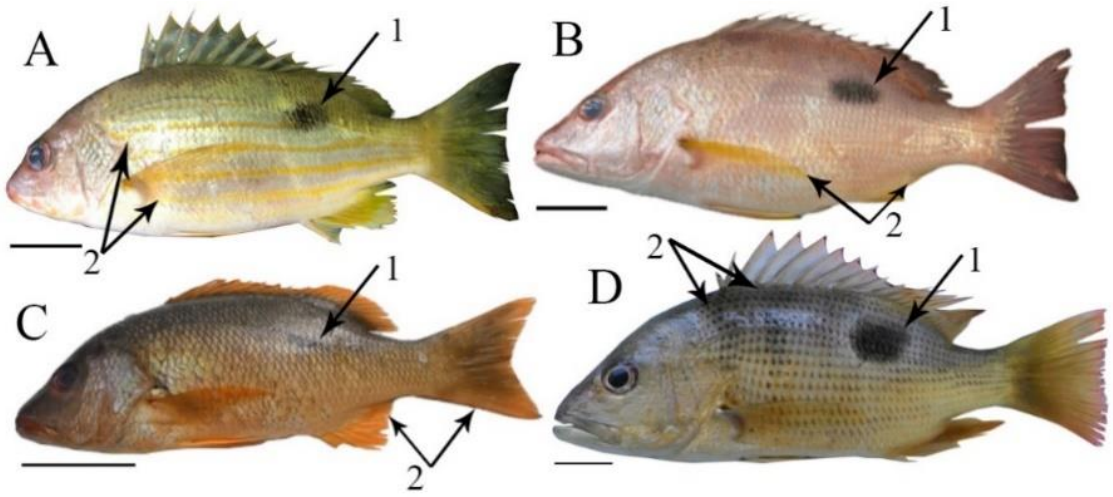

Figure 3. Images of four species belonging to black-spot snapper complex (Scale bar = 5 cm). (A) *L. fulviflamma;* (B) *L. russellii;* (C) *L. monostigma;* (D) *L. johnii.*

Blued-lined snapper complex

Two species have been found, and characterized in having conspicuous blue lateral bands on the body (Figure 4.A1-B1). *L. quinquelineatus* has two characters to distinguish it from *L. bengalensis*. Instead of four blue stripes on each side (Figure 4.A), it has five (Figure 4.B1). It also has a dark spot above lateral line and below soft dorsal fin (Figure 4.B2).

Yellowed-lined snapper complex

Three species have been recorded, the diagnosis character is numerous thin yellow to brownish stripes on the body and with a more prominent (usually wider or darker) yellow or brown stripe mid-lateral running along the side (Figure 4.C1-E1).

They can be differentiated by the coloration and size of the mid-lateral stripe. *L. vitta* most differs in having thin brown stripes plus a broad dark brown mid-lateral stripe (Figure 4.C2), whereas *L. lutjanus* and *L. xanthopinnis* have thin yellow stripes with a broad yellow mid-lateral stripe (Figure 4.D2-E2). *L. lutjanus* can be distinguished from *L. xanthopinnis* in having a broader mid-lateral stripe twice as wide as others vs. a darker mid-lateral stripe as others.

Red snappers

Five species have been reported, which possessing the reddish to pink body (Figure 5). Among them, *L. gibbus*, *L. sebae* and *L. timoriensis* have distinct characteristics from the other species. *L. gibbus* is characterized by a forked caudal fin with rounded lobes (Figure 5.A1) compare to slightly emarginated in *L. sebae* (Figure 5.B) or truncate in *L. malabaricus*, *L. erythropterus* and *L. timoriensis* (Figure 5.C-E). *L. sebae* differs in having three dark red bands on the body (Figure 5.B1) and 15–16 soft rays of dorsal fin and 10 soft rays of anal fin vs. 12–14 and 8–9 soft rays, respectively as others (Table 1). *L. timoriensis* is defined by elongation of posterior dorsal- and anal-fin rays (Figure 5.E1). *L. malabaricus* look very similar to *L. erythropterus*, and can be distinguished by its larger head and mouth (Figure 5.D2).

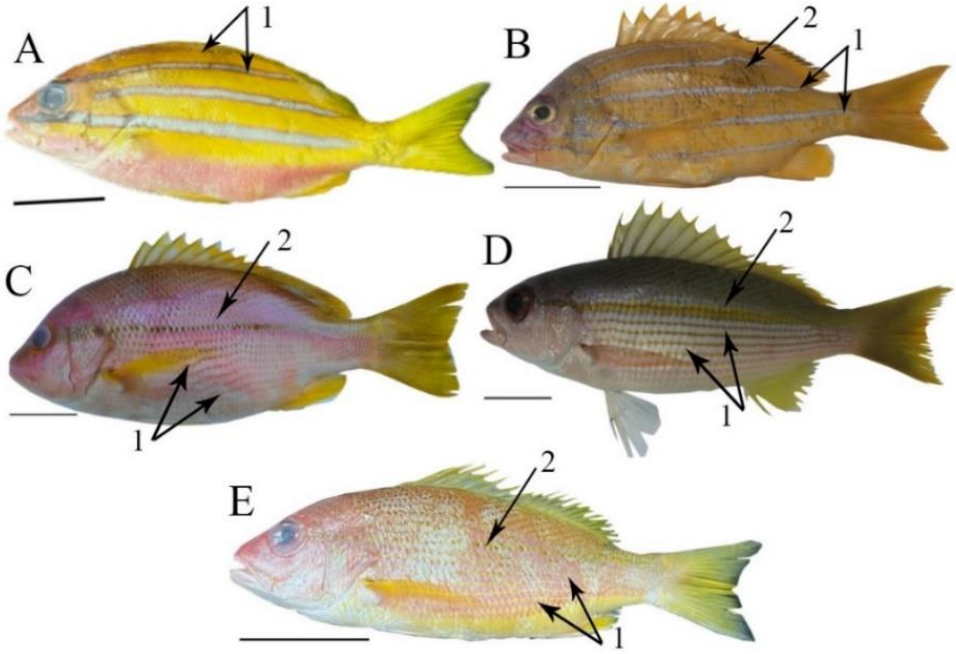

Figure 4. Images of two species belonging to blued-lined complex ((A) *L. quinquelineatus*, and (B) *L. bengalensis*), and three species belonging to yellowed-lined complex ((C) *L. vitta, (*D) *L. lutjanus,* and (E) *L. xanthopinnis*) (Scale bar = 5 cm).

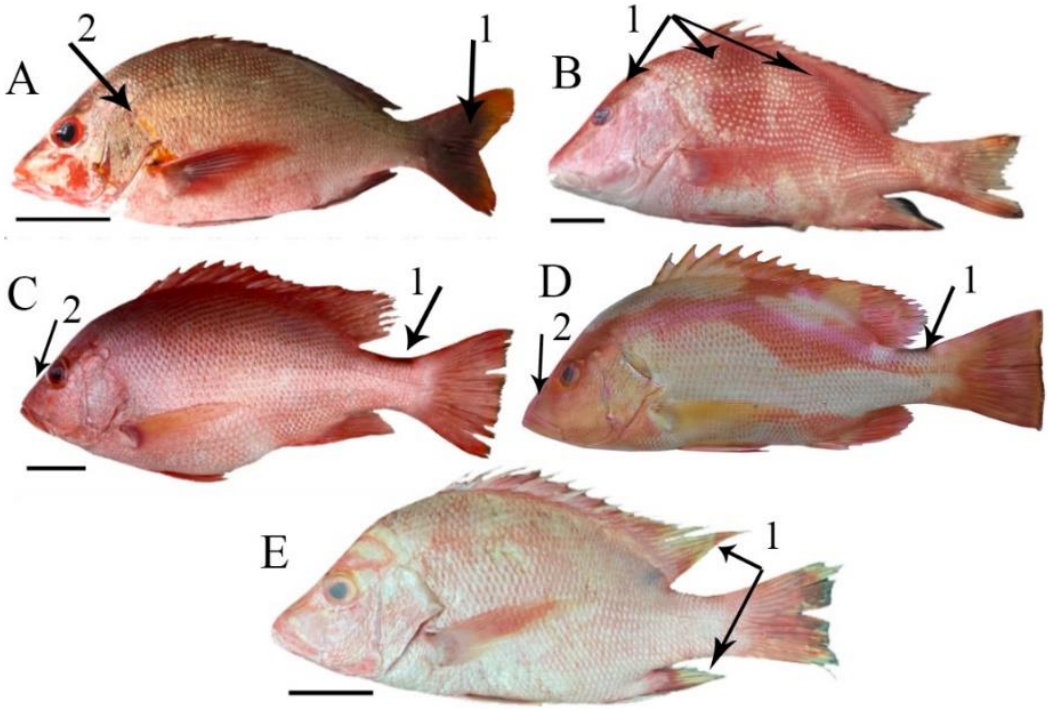

Figure 5. Images of five species belonging to red snapper (Scale bar = 5 cm). (A) *L. erythropterus,* (B) *L. malabaricus,* (C) *L. gibbus,* (D) *L. sebae,* (E) *L. timoriensis.*

Other snapper species

Three species have been classified. *L. argentimaculatus* differs most notably from others by scale rows on the back parallel to lateral line (Figure 6.A1). *L. bohar* is characterized by two whitish spots on upper back (Figure 6.B1). *L. fulvus* has a caudal fin and outer of soft dorsal fin blackish (Figure 6.C1).

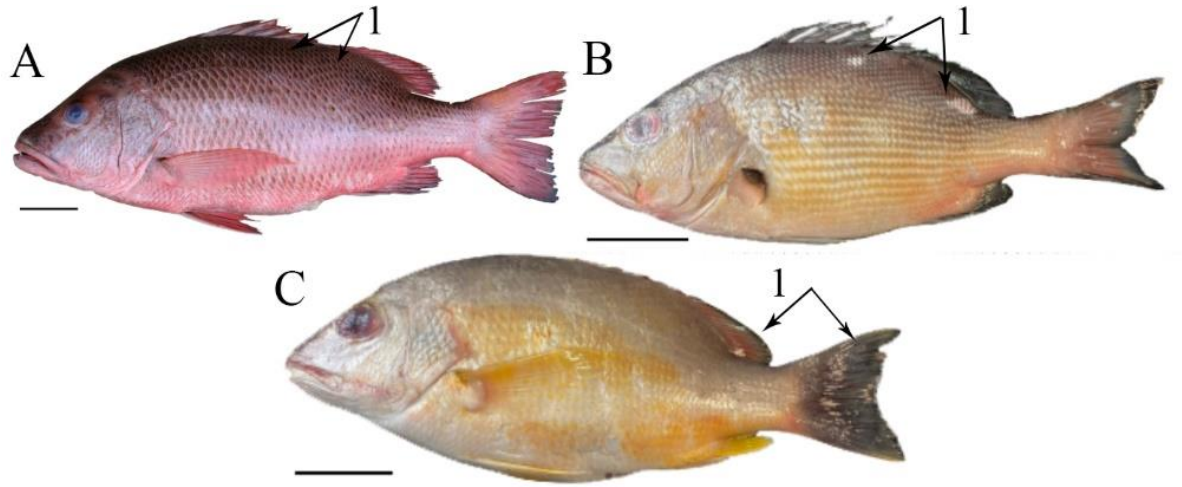

Figure 6. Images of other snapper species (Scale bar = 5 cm). (A) *L. argentimaculatus,* (B) *L. bohar,* and (C) *L. fulvus.*

Table 1. Morphological and meristic characteristics of 17 *Lutjanus* species in Nghe An – Ha Tinh coast water (main taxonomic characters were bold, N: number of individuals)

No.	Species	N	Meristic characteristics			Morphological characteristics
			Dorsal-fin rays	Anal-fin rays	Pectoral-fin rays	
Black-spot complex						
1	*L. fulviflamma*	5	X, 13	III, 8	16-17	Color of back and upper sides brown, lower sides whitish or light brown, **a black spot on the lateral line below the soft part of the dorsal fin** (Figure 3.A1), **6 thin yellow stripes** on the sides (Figure 3.A2), and **longest stripe continuing through eye to snout** (Figure 3.A3). All fins are yellow.
2	*L. russellii*	3	X, 14	III, 8	16	Body color is grey. There is **a black spot on the lateral line below the soft part of the dorsal fin** (Figure 3.B1). Dorsal and caudal fins are dark maroon, and **other fins are yellow** (Figure 3.B2).
3	*L. monostigma*	3	X, 13-14	III, 8	16	Grey body on the upper sides and yellow ventrally. **A black spot on the lateral line below the soft part of the dorsal fin** (Figure 3.C1)**. All fins are yellow** (Figure 3.C2).

4	*L. johnii*	3	X, 13-14	III, 8	17	Body slight light brown, sides and below whitish. **A large black spot on the lateral line below the soft part of the dorsal fin** (Figure 3.D1). **Center of each scale with a black spot forming horizontal lines on the body** (Figure 3.D2).
Blue-lined complex						
5	*L. bengalensis*	3	X, 13-14	III, 8	16-17	Bright yellow with **4 blue stripes on sides (1 below eye)** (Figure 4.A1).
6	*L. quinquelineatus*	3	X, 13-14	III, 8	16-17	Generally bright yellow, including fins. **Five longitudinal blue stripes** on sides (Figure 4.B1), and **large black spot** mostly above lateral line below soft dorsal fin (Figure 4.B2).
Yellow-lined complex						
7	*L. vitta*	3	X, 12-14	III, 8-9	16-17	Body color is pinkish. **A series of thin brown horizontal stripes** below lateral line and oblique above lateral line (Figure 4.C1), with **a broad dark brown mid-lateral stripe running along the side from the eye to the caudal fin base** (Figure 4.C2). All fins are yellow except pelvic whitish.
8	*L. lutjanus*	3	X-XII, 12	III, 8	16-17	Body color is silvery white. **A series of yellow horizontal** lines below lateral line and oblique above lateral line (Figure 4.D1), with **a broad yellow stripe running along the side from the eye to the caudal fin base** (Figure 4.D2). All fins are yellow except pelvic whitish.
9	*L. xanthopinnis*	3	X, 13	III, 8	15-16	Color pinkish to silvery-grey on the dorsum with the whitish abdomen. **A series of yellow stripes** along sides (obliquely above lateral line, and horizontally below the lateral line) (Figure 4.E1), and **mid-lateral stripe slightly wider than others** (Figure 4.E2). All fins yellow except pelvic whitish.
Red snapper						
10	*L. gibbus*	3	X, 13-14	III, 8	17	Body red to greyish, fins red to dusky, narrow white margin to soft dorsal, caudal and anal fins**. Caudal fin forked** (Figure 5.A1). **An orange hue to lower part of the opercle and the pectoral fin axil** (Figure 5.A2)**.**
11	*L. sebae*	3	XI, 15-16	III, 10	17	Body red or pink, fins are red except the pectorals pink. **Three dark red bands** (from first dorsal spine through eye to tip of snout; from mid-dorsal fin to pelvic fin; from base of last dorsal spine to caudal peduncle) (Figure 5.B1).
12	*L. malabaricus*	5	XI, 12-14	III, 8-9	16-17	Body pinkish red, **a black band across caudal peduncle** (Figure 5.C1)**. A large mouth** (length of upper jaw equal to distance

						between bases of last dorsal- and anal-fin rays) (Figure 5.C2).
13	*L. erythropterus*	3	XI, 12-13	III, 8-9	16-17	Body pinkish red. Longitudinal scale rows above lateral line obliquely positioned. **A large black spot at the caudal peduncle** (Figure 5.D1)**. A small mouth** (length of upper jaw smaller than distance between base of last dorsal and anal rays) (Figure 5.D2).
14	*L. timoriensis*	3	XI, 14-15	III, 8	17	Body reddish. All fins reddish, and axil of pectoral fin black. **Posterior dorsal- and anal-fin rays elongate** (Figure 5.E1)**.**
Other snapper						
15	*L. argentimaculatus*	3	X, 13-14	III, 8	16-17	Color of the body dark reddish-brown on back, grading to a reddish belly. **Scale rows on the back parallel to lateral line** (Figure 6.A1).
16	*L. bohar*	3	X, 13-14	III, 8	16-17	Color of body dark brown with **two whitish spots on upper back** (one below last four dorsal spines and one under last six dorsal rays) (Figure 6.B1). Scale rows on the back rising obliquely above lateral line.
17	*L. fulvus*	3	X, 14	III, 8	16	Light yellow body. **Caudal fin and outer of soft dorsal fin blackish** (Figure 6.C1) and other fins are yellow.

Molecular identification

In this study, sequences were successfully generated from each gene region of 17 morphologically determined *Lutjanus* species. Most sequences exhibited more than 99% identity to the sequences of the same species available on Genbank database. Among that, 16S rRNA reference sequences are not available for three species (*L. timoriensis*, *L. xanthopinnis*, and *L. lutjanus*), however, the COI mtDNA sequence showed 100 % matching (Table 2).

In this study, genetic characteristics are very effective to verify the morphological identification of 17 *Lutjanus* species distributed in Nghe An - Ha Tinh provinces, Northern Central, Vietnam. Previous studies were mainly conducted in the Central and Southern regions of Vietnam. Using 16S rRNA molecular markers, Truong *et al*. (2015) also verified the morphological identification of 12 species belonging to 2 genera (*Lutjanus* and *Paracaesio*) at Kien Giang, Vung Tau, Khanh Hoa and Da Nang. Meanwhile, Pham *et al*. (2019) used COI barcode to identify 18 species (6 genera) in Ninh Thuan. Nguyen, Xuan (2020) investigated the *Lutjanus* species composition in the Central region (Quang Tri - Binh Thuan) based on morphological characteristics.

Table 2. Species composition and the comparison of sequences (16S rRNA and COI mtDNA) with Genbank database.

No.	Species studied	16S rRNA			COI mtDNA		
		Species identification	% similarity	Accession no.	Species identification	% similarity	Accession no.
1	*L. fulviflamma*	*L. fulviflamma*	100	DQ784731	*L. fulviflamma*	100	EU502683
2	*L. erythropterus*	*L. erythropterus*	99.7	NC_031331	*L. erythropterus*	100	GU673841
3	*L. gibbus*	*L. gibbus*	100	DQ784733	*L. gibbus*	100	OQ387116
4	*L. malabaricus*	*L. malabaricus*	100	NC_012736	*L. malabaricus*	99.9	ON394557
5	*L. sebae*	*L. sebae*	100	DQ784738	*L. sebae*	100	MN870188
6	*L. johnii*	*L. johnii*	100	NC_024572	*L. johnii*	100	KJ013052
7	*L. argentimaculatus*	*L. argentimaculatus*	100	LC508391	*L. argentimaculatus*	100	MN243478
8	*L. bohar*	*L. bohar*	100	DQ784729	*L. bohar*	99.7	GU673902
9	*L. russelli*	*L. russelli*	100	DQ784737	*L. russelli*	100	OQ387794
10	*L. fulvus*	*L. fulvus*	100	DQ784732	*L. fulvus*	99.7	KU176437
11	*L. vitta*	*L. vitta*	100	NC_042930	*L. vitta*	99.6	OQ385788
12	*L. timoriensis*	*L. malabaricus*	96.4	NC_012736	*L. timoriensis*	100	OQ387106
		L. sebae	95.5	NC_012737			
13	*L. xanthopinnis*	*L. ophuysenii*	99.5	NC_056806	*L. xanthopinnis*	100	JN311964
		L. ehrenbergii	98.6	OR123973			
14	*L. quinquelineatus*	*L. quinquelineatus*	100	DQ784736	*L. quinquelineatus*	100	KC970484
15	*L. lutjanus*	*L. ophuysenii*	98.1	NC_056806	*L. lutjanus*	100	MN870571
		L. carponotatus	97.6	NC_044104			
16	*L. monostigma*	*L. monostigma*	100	LC508471	*L. monostigma*	100	MN562555
17	*L. bengalensis*	*L. bengalensis*	100	NC_011275	*L. bengalensis*	100	OL512911

Although taxonomic literatures available for *Lutjanus* species (Allen, 1985; Nelson, 2006) misidentification was reported due to overlapping of morphometric and meristic characteristics, or the change during its development stages (Allen, 1985). For instance, *Lutjanus malabaricus* is often misidentified as *L. sanguineus* or *L. erythropterus* (Guo *et al.*, 2007; Halim *et*

al., 2022), *L. xanthopinnis* as *L. ophuysenii* (Iwatsuki *et al.*, 2015), *L. alexandrei* as either *L. griseus* or *L. apodus* (Moura, Lindeman, 2007). In our study, it is possible that the two species (*L. malabaricus* and *L. erythropterus*) may be morphologically confused because they both belong to the "red snapper" group. The outstanding feature to distinguish these two species is that species (*L. erythropterus*) has a "back spot", while species (*L. malabaricus*) has a "back band" at the caudal peduncle (Figure 5.C1&5D1). This characteristic can be easily distinguished when the sample is fresh, but can cause confusion in preserved samples. Fortunately, the sequence of the two genes has supported the identification of these two species with up to 99.7-100% sequence identity (Table 2). Clearly, DNA-based molecular markers are proved as effective tool to overcome the issue of morphological identification, and provided insight into the evolutionary relationship of species (Avise, 1994).

Phylogenetic relationships

The length of the aligned sequences was 627 bp and 652 bp for 16S rRNA and COI genes, respectively. All of the datasets passed the Xia test, indicating that there was no substantial substitution saturation. In both datasets, the topologies of NJ and ML were similar and thereby we only show the NJ topology. Only bootstrap values of two approaches above 70% are displayed at the nodes **(Figure 7)**.

For the 16S rRNA gene, the phylogenetic analysis displayed **two main lineages (Figure 7A)**. The **first lineage** included basal clade of three blue-lined species (*L. quinquelineatus*, *L. bengalensis*, and *L. kasmira*). The **second lineage** was divided into **two groups**. **Group 2.1** containing four species (*L. fulviflamma, L. monostigma*, *L. russelli*, and *L. carponotatus*), which clustered as a sister group of three yellow-lined species (*L. lutjanus*, *L. vitta*, and *L. xanthopinnis*). In **Group 2.2**, three species (*L. erythropterus*, *L. gibbus* and *L. bohar)* were clustered together, as a sister group to four species (*L. argentimaculatus, L. sebae, L. malabaricus* and *L. timoriensis*). Two species (*L. fulvus* and *L. johnii*) and *L. stellatus* from Genbank (Accession number NC-057609) performed the unidentified position in the phylogenetic tree.

In the COI phylogram, **two main lineages** were detected **(Figure 7B)**. A basal clade includes four red snapper species (*L. erythropterus, L. timoriensis*, *L. malabaricus*, and *L. sebae*) **(first lineage)**. In **second lineage,** the remaining lutjanids species were clustered into **three groups**. Members of **Group 2.1** are similar as in 16S rRNA phylogenetic tree. **Group 2.2** consisted of two species (*L. argentimaculatus*, and *L. stellatus)*, and placed a sister group to four species (*L. fulvus, and L. quinquelineatus*, *L. bengalensis*, and *L. kasmira*). **Group 2.3** is composed of two species (*L. bohar* and *L. gibbus).* The unidentified position of *L. johnii* also was shown.

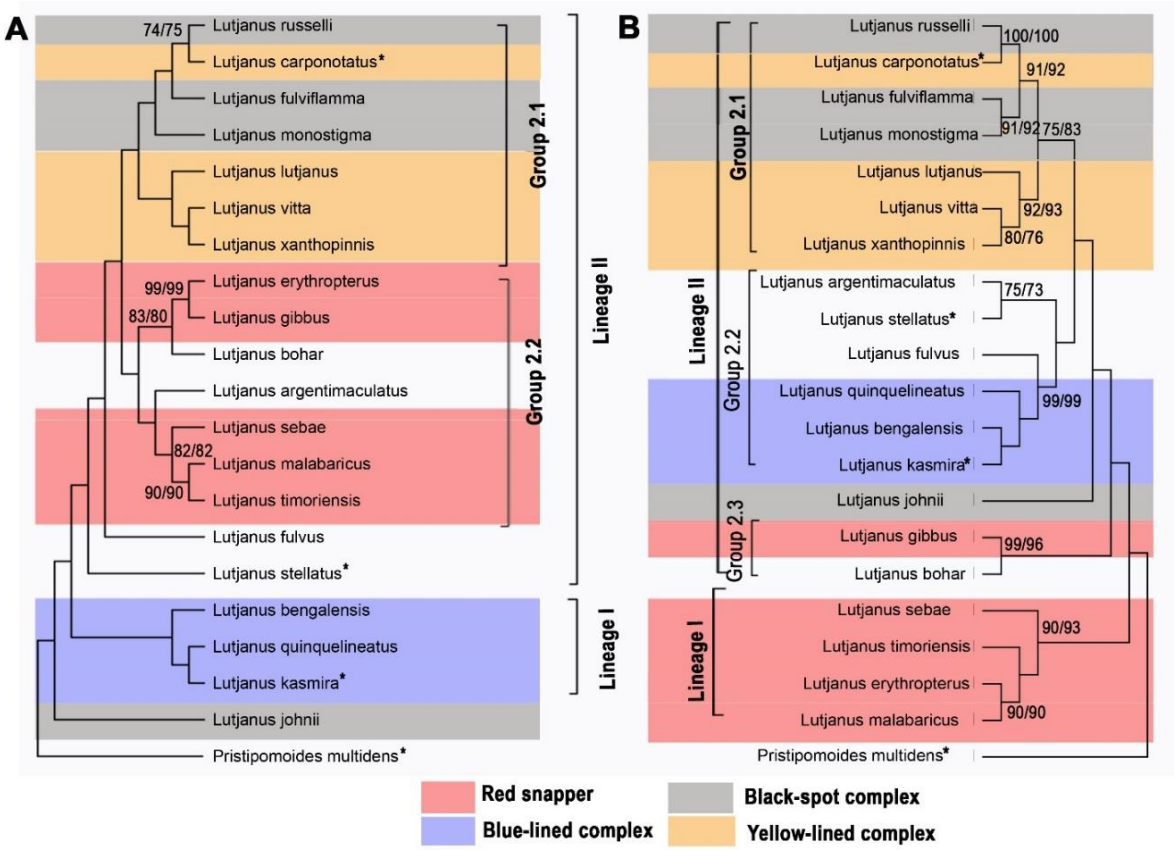

Figure 7. Phylogenetic tree of *Lutjanus* spp. species resulting from the Neighbor Joining analysis of 16S rRNA (A) and COI mtDNA (B) datasets. Bootstrap values (≥70%) from NJ/ ML along the branch (*sequences from Genbank).

In comparation, two phylogenetic trees showed the inconsistent basal clade, either three blue-lined species on the 16S tree **(Figure 7A)** or four red snapper species from the COI topology **(Figure 7B)**. Morphologically, the red snapper species consisted of five species. In COI phylogenies, *L. gibbus* is sister species to *L. bohar* (Group 2.3), while in 16S topology, they clustered in the Group 2.2, and also displayed the sister relationship to *L. bohar* and *L. argentimaculatus.*

In both analyses, *L. johnii* showed the unidentified position. This species has a black spot above the lateral line and below the anterior portion of the soft dorsal fin (Table 1, Figure 3.D). Based on those taxonomic characters, *L. johnii* should be subsumed within **Group 2.1** of the black-spot species. This unknown taxonomic position of *L. johnii* was also recorded in previous studies using the COI mtDNA (Velamala *et al*., 2019) and 16S rRNA (Ramadan *et al*., 2023) genes. Additionally,

L. fulvus also could not be determined on the 16S tree, while COI phylogeny placed it in the three blue-lined species group, which is consistent with other studies (Gold *et al*., 2015; Frédérich, Santini, 2017; Velamala *et al*., 2019).

Group 2.1 - contained two sister clades: i) three black spot species (*L. fulviflamma*, *L. monostigma*, and *L. russelli*) and *L. carponotatus* - yellow-lined species), and ii) three yellow-lined species (*L. lutjanus*, *L. vitta*, and *L. xanthopinnis*) - is consistent in both analyses. In this group, *L. carponotatus* has a series of eight or nine orange or yellow stripes on the sides, that is the diagnostic feature of the yellow-lined species complex (Allen, 1985). However, *L. carponotatus* performed closely related relationship to the black-spot species. This result has already been reported by previous studies based on 16S rRNA and cytb mitochondrial genes (Miller, Cribb, 2007) or mitogenomes (Kim *et al*., 2019). Miller, Cribb (2007) stated that the lack of the black spot in *L. carponotatus* may be a secondarily derived loss of this character.

In conclusion, based on the two phylogenetic trees, no species complex relationship was completely resolved. Members of species complexes also showed a certain degree of closeness; however, conflicts between two topologies have also been recorded. Thereby, we can see the necessity of applying multiple markers or complete genomes in phylogenetic analysis, along with investigate diagnostic characteristics in species identification, or/and determined species complexes.

CONCLUSION

In this study, 17 *Lutjanus* species distributed in Nghe An - Ha Tinh provinces, Northern Central, Vietnam were morphologically and molecular identified. Based on 16S rRNA and COI mtDNA markers, both phylogenetic trees detected two main lineages, and showed inconsistent basal groups. The morphologic species complex was not completely resolve, despite of their members showed a certain degree of closely relationships. Thereby, we highlight the necessity of applying multiple markers or complete genomes in phylogenetic analysis, along with investigate diagnostic characteristics in species identification, or/and determined species complexes.

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REFERENCES

Afriyie G, Wang Z, Dong Z, Ayisi LC, Asiedu B, Guo Y (2020) Complete mitochondrial genome and assembled DNA barcoding analysis of *Lutjanus fulgens* (Valenciennes, 1830) and its comparison with other *Lutjanus* species. *Ecol Evol* 10: 7971–7980.

Allen GR (1985) Snappers of the World. An annotated and illustrated catalogue of Lutjanid species known to date. *FAO Fish Synopsis* 6: 1–208.

Andriyono S, Sektiana SP, Alam J, Kim HW (2019) The complete mitochondrial genome of

black-spot snapper, *Lutjanus fulviflamma* (Perciformes: Lutjanidae). *Mitochondrial DNA Part B Resour* 4: 366–367.

Avise JC (1994) Molecular markers, Natural History and Evolution. Springer New York.

Barman RP, Das A, Mishra SS (2014) On the identity of blue striped snappers (Pisces: Perciformes: Lutjanidae) of India. *Rec Zool Surv India* 114: 177–183.

Betancur-R R, Wiley EO, Arratia G, Acero A, Bailly N, Miya M, Lecointre G, Ortí G (2017) Phylogenetic classification of bony fishes. *BMC Evol Biol* 17:162.

Carpenter KE, Allen GR (1989) Emperor fishes and large-eye breams of the world (Family Lethrinidae). An annotated and illustrated catalogue of lethrinid species known to date. Biological Sciences Faculty Books.

Chen GH, Yin S, Lei C (2006) Artificial reproduction and rearing of snapper hybrids (*Lutjanus erythropterus*×*L. sebae*). *Fish Sci* 25: 1–4.

Chu C, Rizman-Idid M, Ching CV (2013) Phylogenetic relationships of selected genera of Lutjanidae inferred from mitochondrial regions, with a note on the taxonomic status of *Pinjalo pinjalo*. *Ciencias Mar* 39: 349–361.

Cunningham CW (1997) Can three incongruence tests predict when data should be combined? *Mol Biol Evol* 14: 733–740.

Drummond AJ, Rambaut A, Shapiro B, Pybus OG (2005) Bayesian coalescent inference of past population dynamics from molecular sequences. *Mol Biol Evol* 22: 1185–1192.

Frédérich B, Santini F (2017) Macroevolutionary analysis of the tempo of diversification in snappers and fusiliers (Percomorpha: Lutjanidae). *Belgian J Zool* 147: 17–35.

Gold JR, Voelker G, Renshaw MA (2011) Phylogenetic relationships of tropical western Atlantic snappers in subfamily Lutjaninae (Lutjanidae: Perciformes) inferred from mitochondrial DNA sequences. *Biol J Linn Soc* 102: 915–929.

Gold JR, Voelker G, Renshaw MA (2011) Phylogenetic relationships of tropical western Atlantic snappers in subfamily Lutjaninae (Lutjanidae: Perciformes) inferred from mitochondrial DNA sequences. *Biol J Linn Soc* 102: 915–929.

Gold JR, Willis SC, Renshaw MA, Buentello A, Walker HJ, Puritz JB, Hollenbeck CM, Voelker G (2015) Phylogenetic relationships of tropical eastern Pacific snappers (Lutjanidae) inferred from mitochondrial DNA sequences. *Syst Biodivers* 13: 916–927.

Gontcharov AA, Marin B, Melkonian M (2004) Are combined analyses better than single gene phylogenies? A case study using SSU rDNA and rbcL sequence comparisons in the Zygnematophyceae (Streptophyta). *Mol Biol Evol* 21: 612–624.

Guo Y, Wang Z, Liu C, Liu L, Liu Y (2007) Phylogenetic relationships of South China Sea snappers (genus *Lutjanus*; family Lutjanidae) based on mitochondrial DNA sequences. *Mar Biotechnol* 9: 682–688.

Halim LJ, Rahim I, Mahboob S, Al-Ghanim KA, Amat A, Naim MDD (2022) Phylogenetic relationships of the commercial red snapper (Lutjanidae sp.) from three marine regions. *J King Saud Univ Sci* 34: 101756.

Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows95/98/NT. *Nucleic Acids Symp Ser* 41: 95–98.

Iwatsuki Y, Tanaka F, Allen GR (2015) *Lutjanus xanthopinnis*, a new species of

snapper (Pisces: Lutjanidae) from the Indo-west Pacific, with a redescription of *Lutjanus madras* (Valenciennes 1831). *J Ocean Sci Found* 17: 22–42.

Kearse M *et al*. (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649.

Kim G, Lee JH, Alam MJ, Lee SR, Andriyono S (2019) Complete mitochondrial genome of Spanish flag snapper, *Lutjanus carponotatus* (Perciformes: Lutjanidae). *Mitochondrial DNA Part B Resour* 4: 568–569.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 35: 1547–1549.

Le TTT (2011) A checklist of the Snappers (Family Lutjanidae) in Vietnam, Proceedings of the 4th National Scientific Conference on Ecology and Biological Resources, 362–368.

Miller TL, Cribb TH (2007) Phylogenetic relationships of some common Indo-Pacific snappers (Perciformes: Lutjanidae) based on mitochondrial DNA sequences, with comments on the taxonomic position of the Caesioninae. *Mol Phylogenet Evol* 44: 450–460.

Motomura H, Ishikawa S (2013) Fish collection building and procedures manual. The Kagoshima University Museum, Kagoshima and the Research Institute for Humanity and Nature, Kyoto, 70 pp.

Moura RL, Lindeman KC (2007) A new species of snapper (Perciformes: Lutjanidae) from Brazil, with comments on the distribution of *Lutjanus griseus* and *L. apodus*. *Zootaxa* 1422: 31–43.

Nelson JS (2006) Fishes of the world. 4th Edition, John Wiley & Sons, Hoboken, 601 pp.

Nguyen LV, Xuan DM (2020) Reef fish fauna in the coastal waters of Vietnam. *Mar Biodivers* 50: 1-10.

Palumbi SR (1996) Nucleic Acids II: The polymerase chain reaction, in Molecular Systematics, Hillis DM, Moritz C, Mable BK, Eds., Sinauer, Sunderland, Massachusetts.

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

Quyen VDH, Oanh TT, Phuong TTL, Thuoc TL, Binh DT (2018) Molecular phylogeny of catfishes (Teleostei: Siluriformes) inferred from mitochondrial markers - Implications for Lower Mekong River Basin. *Eur J Adv Res Biol Life Sci* 6: 1–12

Ramadan A, Almaaty AHA, Al-Tahr ZM, Allam M (2023) Phylogenetic diversity of some snappers (Lutjanidae: Perciformes) inferred from mitochondrial 16S rRNA sequences. *Egypt J Aquat Biol Fish* 27: 307–317.

Rivas LR (1996) Review of the *Lutjanus campechanus* complex of red snappers. *Q J Florida Acad Sci* 29: 117–136.

Swafford D (2002) PAUP*: Phylogenetic analysis using parsimony (*and other methods), Version 4.0b10. Sinauer Associates, Sunderland.

Thu PT, Huang WC, Chou TK, Van QN, Van CP, Li F, Shao KT, Liao TY (2019) DNA barcoding of coastal ray-finned fishes in Vietnam. *PLoS One* 14: 1–13.

Truong OT, Doan TV, Dang BT (2015) Distribution and phylogenetic relationships of snappers (Lutjanidae) based on mitochondrial

DNA sequences. *J Fish Sci Technol* Special issue: 160–166.

Wang ZD, Guo YS, Tan W, Li L, Tang EP, Liu CW, Liu Y (2010) DNA barcoding, phylogenetic relationships and speciation of snappers (genus *Lutjanus*). *Sci China Life Sci* 53: 1025–1030.

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN (2005) DNA barcoding Australia's fish species. *Philos Trans R Soc B Biol Sci* 360: 1847–1857.