Universiti Malaysia Terengganu Journal of Undergraduate Research *eISSN: 2637-1138*
Volume 6 Number 2, April 2024: 41-53

MOLECULAR APPROACH FOR VALIDATION OF *Lutjanus lutjanus* AND *Lutjanus vitta* FROM TERENGGANU WATERS

NUR ALIYA RONHAKEM, MUHAMMAD SYAFIQ AIMAN MOHD NASIR AND NUR ASMA ARIFFIN*

Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.

**Corresponding author: nurasma@umt.edu.my*
https://doi.org/10.46754/umtjur.v6i2.366
Submitted final draft: 23 April 2024 *Accepted: 23 April 2024* *Published:30 April 2024*

Abstract: The Snapper is a fish that belongs to the family Lutjanidae and the order Perciformes. It can be discovered in tropical and subtropical mangrove habitats and reefs from depths ranging from 1 m to 500 m. *Lutjanus lutjanus* and *Lutjanus vitta* are two snappers found inhibiting Malaysia's coastal waters, including Terengganu. A total of 100 Lutjanid samples with the admixture of varieties of freshness grade were collected from 11 sampling sites that consist of landing ports and fish markets. Note that external morphological examination based on the body stripes' colour led to the identification of 30 samples of *L. lutjanus* and 30 samples of *L. vitta*. The samples were first identified via morphometric and meristic characterisation, with 39 morphological characters measured, and seven meristic characters were counted. 50 samples were successfully identified as *L. lutjanus* (n = 25) and *L. vitta* (n = 25) via morphometric and meristic identification. However, five samples of *L. lutjanus* with soft dorsal fin counts and five samples of *L. vitta* with the number of gill rakers did not follow the range stated by the previous study. These 10 samples were further analysed using molecular identification utilising the *CO1* gene. Consequently, the constructed phylogenetic tree using the Neighbour Joining method with the Kimura-2 Parameter (K2P) model indicates that both species were divided into clades. Furthermore, the molecular approach indicated five samples were validated as *L. Lutjanus* while another five samples were *L. vitta,* with a 99% identical percentage. For future research, it is recommended to use fresh samples, increase the number of samples from other states, and continue to validate the identity via DNA barcoding using the *CO1* gene.

Keywords: Molecular validation, morphological identification, cytochrome oxidase 1, *Lutjanus lutjanus*, *Lutjanus vitta*, Terengganu.

Introduction

Terengganu is an established tropical gateway with island destinations, urban, natural ecotourism, and coastal attractions (Mohd Noh *et al*., 2018; Ariffin *et al*., 2019; Zulkifli *et al*., 2020). East coast peninsular Malaysia, part of the South China Sea, was listed as one of the 17 biodiversity-rich countries, with an estimated 441 fish species recorded along the area (Matsunuma *et al*., 2011; Azahari *et al*., 2022). According to Aaron *et al*. (2018), well-managed fisheries stocks can provide economic benefits and prevent low genetic diversity populations. Around 497 metric tonnes (1.62%) from 30,647 metric tonnes of fish landings were recorded in Terengganu for *Jenahak* and *Remong/Kunyit-Kunyit* reported by the Department of Fisheries Malaysia (DOF, 2021). Snappers such as *Lutjanus lutjanus* and *Lutjanus vitta* are marine fishes, and they are from the family Lutjanidae and are ordered Perciformes. Small to big predatory fishes are normally found in tropical and subtropical mangrove habitats, reefs, and inshore at depths of 1 m to 500 m. Lutjanids are fishes with a robust, relatively elongated body, a single dorsal fin, and developed canine teeth in jaws (Bray, 2018; Motomura *et al*., 2021; Seah *et al*., 2021). *L. lutjanus* and *L. vitta* were two species from Family Lutjanidae found inhibiting Terengganu water. Iwatsuki *et al*. (2015) have reported several morphological characters between the two species, as in Table 1 below.

Table 1: Morphological characters between *L. lutjanus* and *L. vitta*

Characteristics	*Lutjanus lutjanus*	*Lutjanus vitta*
	Iwatsuki *et al*. (2015)	**Iwatsuki *et al*. (2015)**
Dorsal fin rays (D)	X-XII,12	X,12-13
Pectoral fin rays (P_1)	15	15
Pelvic fin rays (P_2)	I,5	I,5
Anal fin rays (A)	III,8	III,8
Gill rakers	24-26	17-22
Scale row above lateral line	4 or 5	8-10
Scale row on check	5-8	5-7
Preopercular flange	Scaled	Scaled
Posterior extension of vomerine tooth patch	Presence	Presence
Stripes insides	Thin stripes plus broad mid-lateral yellow stripes	Thin stripes plus broad mid-lateral dark stripes

Small interspecific differences between the species, especially when the samples were not fresh, leading to the discolouration of the external marking and probably missing some important identification characteristics (Teletchea, 2009; Tahseen, 2014; Iwatsuki *et al*., 2015; Bakar *et al*., 2018). External morphological aspects have traditionally been used to identify fish species, especially in various developmental stages of fish, which are often hard to distinguish based on physical characteristics as subjected to environmental factors. Hence, the molecular approach must be used as a complementary method in the fish identification process to increase the accuracy of species validation. DNA barcoding with the use of the mitochondrial Cytochrome Oxidase 1 (*CO1*) gene is a molecular identification technique that is powerful in recognising newly registered species, including fish (Gao *et al*., 2011; Qin *et al*., 2013; Iwatsuki *et al*., 2015; Francisco *et al*., 2022; Jaonalison *et al*., 2022; Tsoupas *et al*., 2022). This is because the variability of the gene is very low among individuals of species and high among species, making it suitable for identification (Bingpeng *et al*., 2018; Olii *et al*., 2019). Bakar *et al*. (2018), Fadli *et al*. (2020), and Sala *et al*. (2023), who conducted a study on the DNA barcoding and genetic diversity on Lutjanidae, proved that the *Lutjanus* spp. can be differentiated accurately via molecular approach. The study aims to validate the samples of *L. lutjanus* and *L. vitta* from Terengganu based on the molecular approach.

Material and Methods

Sample Collection

A total of 100 Lutjanid samples with the admixture of varieties of freshness grade were collected from 11 sampling areas from four districts: Besut, Kuala Nerus, Kuala Terengganu, and Marang, Terengganu from August to October 2022. The sampling area consists of landing ports and fish markets. Figure 1 and Table 2 below show the sampling location map and information about the sampling area, respectively. All 100 samples of Lutjanids were initially identified based on the external body markings, which were the body stripes based on Matsunuma *et al*. (2011), Ambak *et al*. (2012), Motomura *et al*. (2021), and Seah *et al*. (2021). Some of the collected samples were not fresh, and their external marking was faded. However, the initial identification was still proceeded based on the visible external markings. Samples with the presence of a broad mid-lateral bright

yellow band were identified as *L. lutjanus,* and those with the presence of a dark brown to blackish band at the mid-lateral line from the eye to the upper half of the caudal peduncle were identified as *L. vitta*. The initial identification leads to the division of the Lutjanid samples into two groups, *L. lutjanus* (n = 30) and *L. vitta* (n = 30).

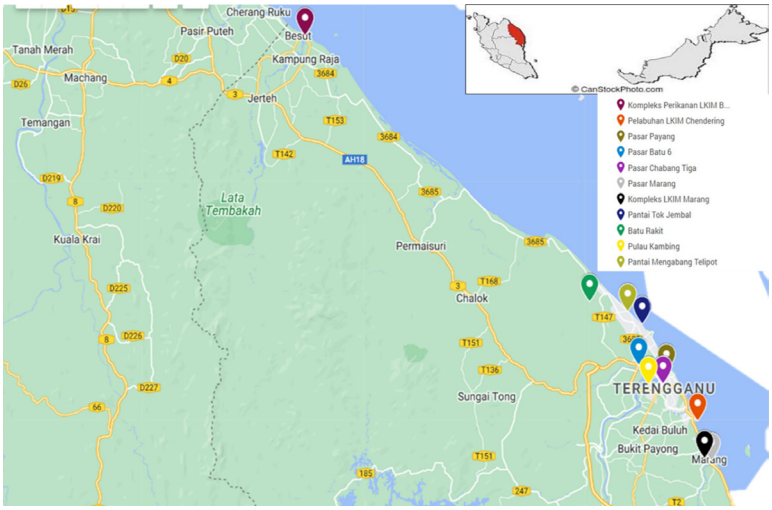

Figure 1: Map of sampling area

Table 2: Location of *L. lutjanus* and *L. vitta* collection

No.	Location	Latitude	Longitude	No. of Sample Collected
1	Mengabang Telipot	5°25’33.7”N	103°04’26.1”E	3
2	Batu Rakit	5°27’00.3”N	103°02’52.3”E	3
3	Pantai Tok Jembal	5°24’16.0”N	103°05’56.4”E	3
4	Pasar Chabang Tiga	5°10’24.24” N	103.1252° E	7
5	Kompleks Perikanan LKIM Marang	5°12’08.4”N	103°12’23.3”E	1
6	Pasar Bandar Marang	5°12’10.7”N	103°12’21.0”E	1
7	Kompleks Perikanan LKIM Pulau Kambing	5°19’19.7”N	103°07’42.8”E	28
8	Pasar Batu Enam	5°20’47.9”N	103°05’30.1”E	5
9	Kompleks Perikanan LKIM Besut	5°49’50.7”N	102°33’44.7”E	1
10	Kompleks Perikanan Chendering	5°16’16.1”N	103°09’54.4”E	6
11	Pasar Payang	5°20’15.4”N	103°08’09.5”E	2
	Total			**60**

Morphological Characteristic

Morphometric Measurements and Meristic Counts

There are 39 morphometric measurements measured for each fish based on Allen and Talbot (1985), Iwatsuki *et al*. (1993), and Aaron *et al*. (2018). All the characters were measured to the nearest 0.01 mm using an vernier calliper and ruler. Figure 2 and Table 3 below present some of the morphometric measurements taken for the *Lutjanus* sp. sample. This method of meristic count is referred to by Iwatsuki *et al*. (2015). According to Iwatsuki *et al*. (2015), *Lutjanus* species can be differentiated based on seven important characteristics, which are scale row on the cheek, scale row above the lateral line and gill rakers, dorsal fin rays (D), pectoral fins rays (P_1), pelvic fins rays (P_2) and anal fin rays (A). Apart from the stripes, other external morphology identifications were the body colour, presence of scaled preopercle flange, and position of the vomerine tooth. The body colour of *L. lutjanus* at the upper back was golden brown, along with yellowish fins. Meanwhile, the body colour of *L. vitta* was generally whitish or pink, along with yellowish fins, similar in colour to *L. lutjanus*. All samples of *L. Lutjanus* and *L. vitta* shall present a scaled preopercle flange with the vomerine tooth patch extended posteriorly. All the external morphological features observed on the samples were also reported by Matsunuma *et al*. (2011), Ambak *et al*. (2012), and Iwatsuki *et al*. (2015).

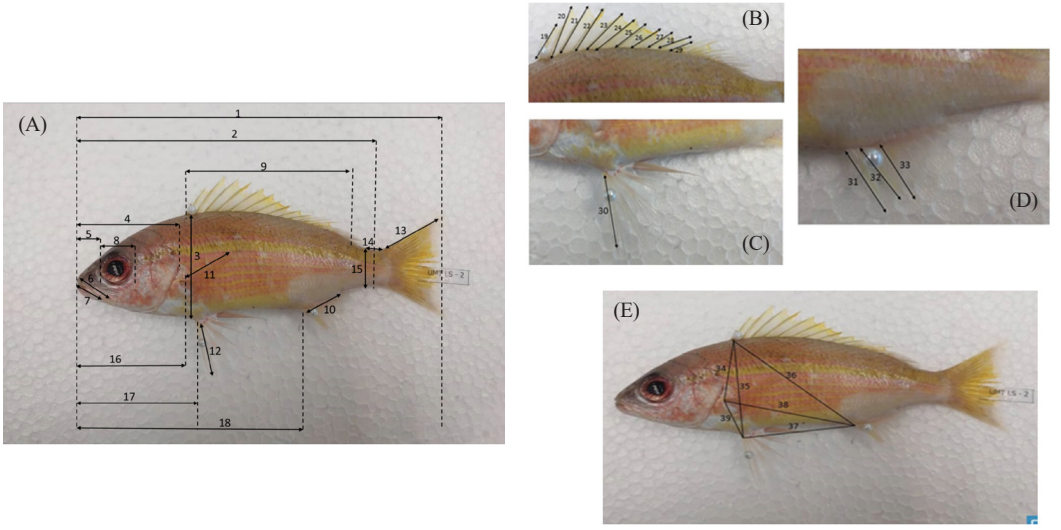

Figure 2: A total of 39 morphometric measurements that measured on the samples. (a) Characters 1 to 18, (b) Characters 19 to 29, (c) Character 30, (d) Characters 31 to 33, and (e) Characters 34 to 39

Table 3: Morphometric characters that were measured in this study

No.	Description	Characters
1	Total length	TL
2	Standard length	SL
3	Body depth	BD
4	Head length	HL
5	Snout length	SL
6	Upper jaw length	JLu
7	Lower jaw length	JLl
8	Eye diameter	ED
9	Dorsal fin length	DFL
10	Anal fin length	AFL
11	Pectoral fin length	PEC L
12	Pelvic fin length	PEL L
13	Caudal fin length	SFL
14	Caudal peduncle length	CPL
15	Caudal peduncle height	CPH
16	Pre-pectoral depth	PrPcD
17	Pre-pelvic length	PrpeD
18	Pre-anal length	PraD
19	First dorsal spine length	DSL1
20	Second dorsal spine length	DSL2
21	Third dorsal spine length	DSL3
22	Fourth dorsal spine length	DSL4
23	Fifth dorsal spine	DSL 5
24	Sixth dorsal spine	DSL6
25	Seventh dorsal spine	DSL7
26	Eighth dorsal spine	DSL 8
27	Ninth dorsal spine	DSL 9
28	Tenth dorsal spine	DSL 10
29	Eleventh dorsal spine	DSL 11
30	Pelvic spine length	PeSL
31	First anal spine length	ASL1
32	Second anal spine length	ASL2
33	Third anal spine length	ASL3
34	Pectoral fin insertion-dorsal fin origin	PCDD1
35	Pelvic fin insertion-dorsal fin origin	PEDD1
36	Anal fin origin-dorsal fin origin	ADD1
37	Pelvic fin insertion-anal fin origin	PeAD
38	Pectoral fin insertion-anal fin origin	PcAD
39	Pectoral fin insertion-pelvic fin insertion	PcPeD

Molecular Validation

DNA Extraction, COI Amplification, and Sequencing

DNA extraction was conducted using the salt extraction method, according to (Muhammad *et al*., 2016). Approximately 20 mg of fin clip tissue was used in the extraction. The extracted DNA was quantified using a 7415 Nano scanning micro-volume spectrophotometer (Jenway, United Kingdom). Amplification of the *CO1* gene fragments was carried out using the universal primers: FishF1 (5′-TCA ACC AAC CAC AAA GAC ATT GGC AC-3′) and FishR1 (5′-TAG ACT TCT GGG TGG CCA AAG AAT CA-3′) with a product size of approximately 650 bp (Ward *et al*., 2005). Consequently, polymerase chain reaction (PCR) amplification was conducted in 25 µL consisting of 13.0 µL of MyTaq® RedMix, 0.5 µL of 10 µM forward and reverse primer, respectively, 5.0 µL of DNA template, and 6.0 µL of ddH_2O. The PCR amplification was performed on SuperCycler® (Kyratec, USA). The cycling conditions consisted of three stages: (1) Initial denaturation at 95°C for 2 minutes, (2) 40 cycles for denaturation at 94°C for 30 seconds, annealing at 54°C for 30 seconds, and for an extension at 72°C at 1 minute, and (3) Final extension at 72°C for 10 minutes. The PCR products were then visualised on 2.0% agarose gel and viewed on Omega Lum™ Imaging System 81-12100-00 (Aplegen, USA). Correspondingly, successful PCR products were sent for unidirectional forward sequencing at Apical Scientific Sdn Bhd using BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems) and 3730xl Genetic Analyser (Applied Biosystems).

Data Analysis

The obtained sequences were aligned, trimmed to equal length, and cleaned from noisy ends and stop codons using Molecular Evolutionary Genetics Analysis version 11.0 (MEGA v11) software (Tamura *et al.*, 2021). The cleaned sequences were submitted to GenBank to identify and validate the sequence obtained via the nucleotide option of the Basic Local Alignment Search Tool (BLAST) tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The identity of the submitted sequences was confirmed when it matched ≥ 97% with the deposited Genbank sequence. The phylogenetic tree was constructed using the Kimura-2-Parameter (K2P) model via the Neighbour-Joining method with a bootstrap value of 1,000, including sequences from the sister group and outgroup.

Results and Discussion

Morphometric Measurements and Meristic Count

Among all the 30 samples of *L. Lutjanus* and 30 samples of *L. vitta* that were initially examined via stripes, meristic counts for 25 samples from each of the two sample groups fell within the ranges of 39 morphological characteristics for *L. Lutjanus* and 38 morphological characteristics of *L. vitta* that reported by Iwatsuki *et al.* (2015) (Table 1). The result of this study also revealed that the total length of *L. vitta* (17.30-35.00 cm) (22.28 ± 3.67 cm) was observed to have a bigger size than *L. Lutjanus* (14.00-18.90 cm) (16.9 ± 1.43 cm). Table 4 presents the morphometric measurements of *L. lutjanus* and *L. vitta* from this study, with 39 and 38 measured characters, respectively.

Table 4: Morphometric measurement of *L. Lutjanus* and *L. vitta*

No.	Descriptions	Characters	Mean ± SD (cm)		Range (cm)	
			Lutjanus lutjanus	***Lutjanus vitta***	***Lutjanus lutjanus***	***Lutjanus vitta***
1	Total length	TL	16.90 ± 1.43	22.28 ± 3.67	14.00 - 18.90	17.30 - 35.00
2	Standard length	SL	13.73 ± 1.29	18.21 ± 3.06	11.30 - 19.00	14.20 - 29.00
3	Body depth	BD	4.64 ± 0.43	6.97 ± 1.30	3.50 - 18.00	5.00 - 12.00
4	Head length	HL	4.62 ± 0.60	6.31 ± 1.08	2.14 - 2.94	5.00 - 10.50
5	Snout length	SL	1.28 ± 0.32	2.10 ± 0.61	1.00 - 5.50	1.30 - 4.00
6	Jaw length (upper)	Jlu	1.90 ± 0.24	2.73 ± 0.40	1.50 - 2.00	2.00 - 4.00
7	Jaw length (lower)	JLl	1.39 ± 0.26	1.99 ± 0.41	1.00 - 2.50	1.50 - 3.00
8	Eye diameter	ED	1.52 ± 0.17	1.59 ± 0.20	1.20 - 2.00	1.20 - 2.00
9	Dorsal fin length	DFL	6.89 ± 0.70	9.04 ± 1.79	5.70 - 2.00	6.50 - 14.50
10	Anal fin length	AFL	2.15 ± 0.38	3.16 ± 0.79	1.50 - 8.50	2.40 - 5.50
11	Pectoral fin length	PEC L	3.81 ± 0.46	4.76 ± 0.88	2.50 - 3.00	3.00 - 7.50
12	Pelvic fin length	PEL L	2.65 ± 0.36	3.46 ± 0.64	1.50 - 4.50	2.70 - 5.50
13	Caudal fin length	CFL	3.39 ± 0.39	4.54 ± 0.77	2.70 - 3.00	3.50 - 7.50
14	Caudal peduncle length	CPL	2.00 ± 0.33	2.91 ± 0.87	1.50 - 4.50	2.00 - 5.00
15	Caudal peduncle height	CPH	1.61 ± 0.25	2.32 ± 0.44	1.00 - 2.50	1.80 - 4.00
16	Pre-pectoral length	PrpcD	4.67 ± 0.51	5.24 ± 0.82	4.50 - 6.50	5.00 - 9.00
17	Pre-pelvic length	PrpeD	5.07 ± 0.17	6.20 ± 0.76	5.00 - 5.50	6.00 - 10.00
18	Pre-anal length	PraD	9.98 ± 0.09	10.87 ± 1 .67	9.50 - 10.00	10.50 - 19.50
19	First dorsal spine length	DSL1	0.23 ± 0.09	0.53 ± 0.13	0.20 - 0.50	0.40 - 1.00
20	Second dorsal spine length	DSL2	1.45 ± 0.15	2.00 ± 0.13	1.00 - 1.50	1.50 - 2.50
21	Third dorsal spine length	DSL3	1.97 ± 0.13	2.50 ± 0.13	1.50 - 2.00	2.00 - 3.00
22	Fourth dorsal spine length	DSL4	1.50 ± 0.00	2.08 ± 0.32	1.50 - 1.50	2.00 - 3.50
23	Fifth dorsal spine length	DSL 5	1.93 ± 0.17	2.05 ± 0.26	1.50 - 2.00	2.00 - 3.40
24	Sixth dorsal spine length	DSL6	1.93 ± 0.25	2.07 ± 0.25	1.00 - 2.00	2.00 - 3.00
25	Seventh dorsal spine length	DSL7	1.95 ± 0.20	2.00 ± 0.13	1.00 - 2.00	1.50 - 2.50
26	Eighth dorsal spine length	DSL 8	1.93 ± 0.25	1.98 ± 0.09	1.00 - 2.00	1.50 - 2.00
27	Ninth dorsal spine length	DSL 9	1.00 ± 0.00	1.05 ± 0.20	1.00 - 1.00	1.00 - 2.00

28	Tenth dorsal spine length	DSL 10	1.00 ± 0.00	1.03 ± 0.18	1.00 - 1.00	1.00 - 2.00
29	Eleventh dorsal spine length	DSL 11	1.00 ± 0.00	NA	1.00 - 1.00	NA
30	Pelvic spine length	PeSL	1.05 ± 0.20	2.07 ± 0.25	1.00 - 2.00	2.00 - 3.00
31	First anal spine length	ASL1	0.24 ± 0.10	1.00 ± 0.00	0.20 - 0.50	1.00 - 1.00
32	Second anal spine length	ASL2	1.02 ± 0.09	1.98 ± 0.09	1.00 - 1.50	1.50 - 2.00
33	Third anal spine length	ASL3	1.02 ± 0.09	2.00 ± 0.13	1.00 - 1.50	1.50 - 2.50
34	Pectoral fin insertion-dorsal fin origin	PCDD1	3.07 ± 0.25	4.65 ± 0.65	3.00 - 4.00	4.50 - 8.00
35	Pelvic fin insertion-dorsal fin origin	PEDD1	4.57 ± 0.22	6.30 ± 1.15	4.50 - 5.50	6.00 - 12.00
36	Anal fin origin-dorsal fin origin	ADD1	6.05 ± 0.20	8.82 ± 1.30	6.00 - 7.00	8.50 - 15.50
37	Pelvic fin insertion-anal fin origin	PeAD	4.52 ± 0.09	5.20 ± 0.76	4.50 - 5.00	5.00 - 9.00
38	Pectoral fin insertion-anal fin origin	PcAD	5.60 ± 0.38	6.70 ± 0.92	5.50 - 7.50	6.50 - 11.50
39	Pectoral fin insertion-pelvic fin insertion	PcPeD	2.00 ± 0.00	2.20 ± 0.55	2.00 - 2.00	2.00 - 4.00

However, five Lutjanids samples were initially identified as *L. Lutjanus* and *L. vitta*, respectively, and were observed to have ambiguity in the number of soft fin rays and gill rakers. Samples UMT LS-2, UMT LS-37, and UMT LS-43 – UMT LS-45, initially identified as *L. Lutjanus,* were found to have two additional soft dorsal fin rays. Note that samples UMT LS-4, UMT LS-67, UMT LS-84 – UMT LS-86, which were initially identified as *L. vitta*, were discovered to have an absence of a gill raker. The locations of the 10 samples were taken were as follows: UMT LS-2 and UMT LS-37 from Batu Rakit; UMT LS-4 from Mengabang Telipot; and UMT LS-43 – UMT LS-45, UMT LS-67, UMT LS-84 – UMT LS-86 from LKIM Pulau Kambing. Table 5 presents the ambiguous meristic counts of the initially identified five *L. lutjanus* and five *L. vitta* samples from this study, compared with previously reported.

Table 5: Ambiguous meristic count of the initially identified *L. lutjanus* and *L. vitta* samples from this study and compared with the previous study

Characteristic	*Lutjanus lutjanus*		*Lutjanus vitta*	
	Iwatsuki *et al.* (2015)	**This study (UMT LS-2, UMT LS-37, UMT LS 43-UMT LS-45)**	**Iwatsuki *et al.* (2015)**	**This study (UMT LS-4. UMT LS-67. UMT LS-84-UMT LS-86)**
Dorsal fin rays (D)	X-XII,12	XI,14	X,12-13	X,13
Pectoral fin rays (P_1)	15	15	15	15
Pelvic fin rays (P_2)	I,5	I,5	I,5	I,5
Anal fin rays (A)	III,8	III,8	III,8	III,8
Gill rakers	24-26	24	17-22	16
Scale row above lateral line	4 or 5	5	8-10	8
Scale row on check	5-8	6	5-7	7

From the morphological characteristic, it can be confirmed that the identity of the 50 Lutjanid samples was identified correctly via morphometric and meristic as *L. Lutjanus* (n = 25) and *L. vita* (n = 25). The meristic count was more reliable between the two fish via morphological features as there were observable differences in the number of soft fin rays for the initially identified *L. lutjanus* samples and different gill rakers counts for initially identified *L. vitta* (Simon *et al*., 2010; Meshram *et al.*, 2021). Hence, the 10 Lutjanid samples having ambiguity in their morphological characters were further identified via the molecular approach method, which is crucial for accurate species validation.

Molecular Validation

All 10 extracted samples were successfully amplified at 634 bp with no amplification at the negative control. Phylogenetic tree analysis (Figure 3) via the Neighbour-Joining tree with a K2P model with 1,000 replicates demonstrated a distinct clustering between the two species. UMT LS-2, UMT LS-37, and UMT LS-43 to UMT LS-45 formed a single cluster with other *L. lutjanus* sequences extracted from GenBank. Meanwhile, UMT LS-4, UMT LS-67, and UMT LS-84 to UMT LS-86 were clustered into another cluster with *L. vitta* sequences taken from GenBank. The analysis proved that samples UMT LS-2, UMT LS-37, and UMT LS-43 to UMT LS-45 sequences were confirmed as *L. Lutjanus,* while samples UMT LS-4, UMT LS-67, and UMT LS-84 to UMT LS-86 were confirmed as *L. vitta*.

However, Genbank extracted sequence, *L. lineatus* (EU 600134.1), that clustered together with the study sequences and other GenBank *L. Lutjanus* sequences might happen possibly because of wrongly named *L. Lutjanus* that deposited into GenBank. The presence of misidentification of Genbank sequences can be proven due to the previous reports by Meiklejohn *et al.* (2019) and Pentinsaari *et al.* (2020). The 10 samples were submitted to GenBank and their accession number were as follows: UMT LS-2 and UMT LS-37 (OQ439411 – OQ439412), UMT LS-43 – UMT LS-45 (OQ439414-OQ439416), UMT LS-4 (OQ439413), UMT LS-84 – UMT LS-87 (OQ439417-OQ439420). The sample ID and their respective accession number were also tabulated in Table 6 below.

Table 6: Sample ID and their respective NCBI accession number

Sample ID Number	NCBI Accession Number
UMT LS-2	OQ439411
UMT LS-37	OQ439412
UMT LS-4	OQ439413
UMT LS-43	OQ439414
UMT LS-44	OQ439415
UMT LS-45	OQ439416
UMT LS-84	OQ439417
UMT LS-85	OQ439418
UMT LS-86	OQ439419
UMT LS-87	OQ439420

Results from the NCBI BLASTn indicated that five samples were *L. lutjanus* while another five individuals were validated as *L. vitta*, with the identical percentage from 99% to 100%, respectively. Thus, it can further confirm that the samples UMT LS-4, UMT LS-67, and UMT LS-84 to UMT LS-86 were identified as *L. Lutjanus* while UMT LS-4, UMT LS-67, and UMT LS-84 to UMT LS-86 as *L. vitta*.

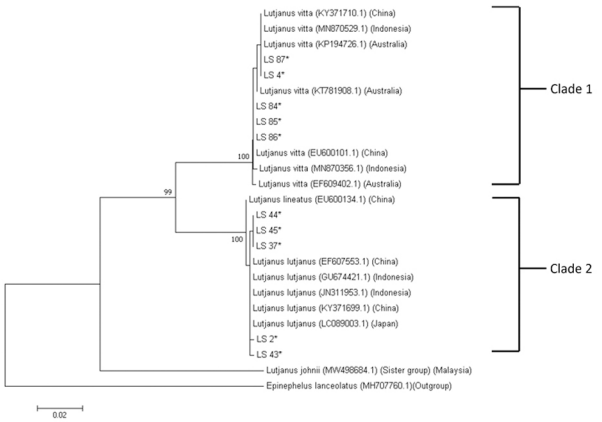

Figure 3: Neighbor-Joining Phylogenetic tree using Kimura-2 Parameter model on the of 10 samples. Samples from this study were labeled as LS---*, and the remaining sample sequences were taken from NCBI Genbank

Defects in important morphological features, such as discolouration of body colour and damage of fins and gill rakers, can cause the morphological identification to become inaccurate and complicated (Wang *et al*., 2018; Fadli *et al*., 2020; Sawalman & Maduppa, 2020). Although DNA barcoding was a better tool for species identification, it was not meant to replace traditional morphological identification. DNA barcoding was more of a complementary method for morphological identification, which can increase the accuracy of a species identified (Wang *et al*., 2018; Liu *et al*., 2022).

Conclusions

In conclusion, among the 50 Lutjanid samples, 25 were confirmed as *L. lutjanus,* while another 25 samples were identified as *L. vitta* via morphological identification. Molecular validation has confirmed the five Lutjanid samples (LS-2, LS-37, LS-43-45) the presence of ambiguity in the number of soft dorsal fin rays as *L. lutjanus*. Another five samples that present ambiguity in the number of gill rakers were also confirmed as *L. vitta* based on the molecular approach. This concludes that 30 samples of *L. lutjanus* and 30 samples of *L. vitta* were identified in this study. Hence, identifying the species is crucial as it could be added to the vast and intricate taxonomic status of the Lutjanidae family. A molecular approach via DNA barcoding has also proven to be very helpful in validating species with ambiguous morphological characteristics and admixture of freshness grade. For the next study, it is recommended that the samples be taken from other states so the morphological comparison between the individuals can be more accurate. Besides, species validation of the molecular approach via *CO1* should be continued to enable the fish species to be validated at the highest accuracy possible.

Acknowledgements

The author would like to thank the staff of the Fisheries Biosystem Laboratory, Fisheries Science Laboratory, Faculty of Fisheries and Food Science, and Universiti Malaysia Terengganu for providing the research facilities.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

References

Aaron, C. C., Aziz, A. H. A., Meriam, S. A. S. T., Seah, Y. G., & Asma, A. N. (2018). Morphological and molecular identification of mullet species (Mugilidae) from Setiu Wetland, Terengganu, Malaysia. *Aquaculture, Aquarium, Conservation & Legislation*, *11*(2), 429-438.

Allen, G. R., & Talbot, J. H. (1985). Review of snappers of the genus *Lutjanus* (Pisces: Lutjanidae) from the Indo-Pacific, with the description of a new species. *Indo-Pacific Fish*, *11*, 1-87.

Ambak, M. A., Isa, M. M., Zakaria, M. Z., & Ghaffar, M. A. (2012). *Fishes of Malaysia* (2nd ed.). Terengganu: Penerbit UMT, Universiti Malaysia Terengganu.

Ariffin, E. M., Sedrati, M., Daud, N. R., Mathew, M. J., Akhir, M. F., Awang, N. A., Yaacob, R., Siddiqui, N. A., & Hussain, M. L. (2019). Chapter 5 Shoreline evolution under the influence of oceanographic and monsoon dynamics: The case of Terengganu, Malaysia. In M. Ramkumar, R. Arthur James, M. David & K. Kumaraswamy (Eds.), *Coastal zone management global perspective, regional processes, local issue* (pp. 113-130). ElSevier Inc.

Azahar, M. A., Rosli, N. A., Ab Rahman, N. S., Badrul Hisham, N. S., Solehin, S. N., Rehan, A. M., Zainal, M., Abdul-Latiff, M. A., Seah, Y. G., & Kamarudin, K. R. (2022). Species checklist and DNA barcoding of marine fishes from Pulau Tinggi, Johor, Malaysia. *Journal of Sustainability Science and Management*, *17*(11), 45-55.

Bingpeng, X., Heshan, L., Zhilan, Z., Chunguang, W., Yanguo, W., & Jianjun, W. (2018). DNA barcoding for identification of fish species in the Taiwan Strait. *PLOS ONE*, *13*(6), e1098109.

Bray, D. J. (2018). *Tropical snappers, fusiliers, Lutjanidae in fishes of Australia.* Accessed May 16, 2022, from https://fishesofaustralia.net.au/home/family/308

DOF Department of Fisheries Malaysia. (2021). *Annual Fisheries Statistics 2021.* Department of Fisheries, Ministry of Agriculture and Food Industry. Accessed February 10, 2023, from https://www.dof.gov.my/en/resources/fisheries-statistics-i/.

Fadli, N., Mohd Nor, S. A., Othman, A. S., Sofyan, H., & Muchlisin, Z. A. (2020). DNA barcoding of commercially important reef fishes in Weh Island, Aceh, Indonesia. *PeerJ Publishing*, *8*, e9641.

Francisco, S. M., Lima, C. S., Moreira, I., Shahin, A. A., & Ben Faleh, A. (2022). DNA barcoding of commercially relevant marine fish species in Tunisian waters. *Journal of the Marine Biological Association of the United Kingdom*, *102*(3-4), 178-185.

Gao, T., Ji, D., Xiao, Y., Xue, T., Yanagimoto, T., & Setoguma, T. (2011). Description and DNA barcoding of a new Sillago species, *Sillago sinica* (Perciformes: Sillaginidae), from coastal waters of China. *Zoological Studies*, *50*(2), 254-263.

Iwatsuki, Y., Akazaki, M., & Yoshino, T. (1993). Validity of lutjanid fish, *Lutjanus ophuysenii* (Bleeker) with a related species, *L. vitta* (Quoy et Gaimard). *Japanese Journal of Ichthyology*, *40*(1), 47-59.

Iwatsuki, Y., Tanaka, F., & Allen, G. R. (2015). *Lutjanus xanthopinnis*, a new species of snapper (Pisces: Lutjanidae) from the Indo-west Pacific, with a redescription of *Lutjanus madras* (Valenciennes 1831). *Journal of the Ocean Science Foundation*, *17*, 22-42.

Jaonalison, H., Durand, J.-D., Mahafina, J., Valade, P., Collet, A., Cerqueira, F., & Ponton, D. (2022). Application of DNA barcoding for monitoring Madagascar fish biodiversity in coastal areas. *Diversity*, *14*(5), 377.

Liu, B., Yan, Y., Zhang, N., Guo, H., Liu, B., Yang, J., Zhu, K., & Zhang, D. (2022). DNA barcoding is a useful tool for the identification of the family Scaridae in Hainan. *Journal of Marine Science and Engineering*, *10*(12), 1915.

Matsunuma, M. (2011). *Fishes of Terengganu: East Coast of Malay Peninsula, Malaysia*. National Museum of Nature and Science.

Meiklejohn, K. A., Damaso, N., & Robertson, J. M. (2019). Assessment of bold and GenBank – Their accuracy and reliability for the identification of biological materials. *PLOS ONE*, *14*(6), e0217084.

Meshram, M. M., Mridula, R., Rajesh, K. M., & Suyuni, N. K. (2021). Morphological measurements, length weight relationship and relative condition factor (kN) of obtuse Barracuda Sphyraena obtusata (Cuvier, 1829) from South-Eastern Arabian Sea. *Indian Journal of Geo-Marine Sciences*, *50*(06), 481-488.

Mohd Noh, A. W., Shuib, A., Tai, S., & Mohd Noh, K. (2018). Indicators of governance of marine ecotourism resources: Perception of communities in Pulau Perhentian, Terengganu. *International Journal of Business and Society*, *19*(S1), 17-25.

Motomura, H., Kimura, S., Seah, Y. G., Sheikh Abdul Kadie, S. T., & Mazlan, A. G. (2021). *Reef and shore fishes of Bidong Island, off East Coast Malay Peninsula*. The Kagoshima University Museum.

Muhammad, H., Iqbal, Z., Iqbal, M. U., Younas, T., & Bashir, Q. (2016). An efficient method for DNA isolation from fish fin. *Pakistan Journal of Agricultural Sciences*, *53*(4), 843-850.

Olii, A. H., Sahami, F. M., Hamzah, S. N., & Pasisingi, N. (2019). Molecular approach to identify gobioid fishes, "Nike" and "Hundala" (local name), from Gorontalo Waters, Indonesia. *OnLine Journal of Biological Sciences*, *19*(1), 51-56.

Pentinsaari, M., Ratnasingham, S., Miller, S. E., & Hebert, P. D. (2020). Bold and genbank revisited – Do identification errors arise in the lab or in the sequence libraries? *PLOS ONE*, *15*(4), e0231814.

Qin, Y., Song, N., Zou, J., Zhang, Z., Cheng, G., Gao, T., & Zhang, X. (2013). A new record of a flathead fish (Teleostei: Platycephalidae) from China based on morphological

characters and DNA barcoding. *Chinese Journal of Oceanology and Limnology*, *31*(3), 617-624.

Sala, R., Kusuma, A. B., & Pranata, B. (2023). Phylogenetic of red snapper (Lutjanidae) in Yapen Island waters, Papua, Indonesia. *Biodiversitas*, *24*(2), 716-723.

Sawalman, R., & Madduppa, H. (2020). The analysis of morphological and genetic characteristics of yellowstripe scad from Muara Baru modern fish market in North Jakarta. *Jurnal Ilmiah Perikanan dan Kelautan*, *12*(2), 308-314.

Seah, Y. G., Ali, M. S., Mazlan, A. G., & Mat Jaafar, T. N. A. (2021). *Marine fishes of Kuantan: Malaysian Biodiversity Information System (MYBIS)*. Terengganu: Penerbit UMT.

Simon, K. D., Bakar, Y., Temple, S. E., & Mazlan, A. G. (2010). Morphometric and meristic variation in two congeneric Archer Fishes *Toxotes Chatareus* (Hamilton 1822) and *Toxotes Jaculatrix* (pallas 1767) inhabiting Malaysian Coastal Waters. *Journal of Zhejiang University SCIENCE B*, *11*(11), 871-879.

Tahseen, Q. (2014). Taxonomy-the crucial yet misunderstood and disregarded tool for studying biodiversity. *Journal of Biodiversity & Endangered Species*, *02*(03), 1-9.

Tamura, K., Stecher, G., & Kumar, S. (2021). Mega11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, *38*(7), 3022-3027.

Teletchea, F. (2009). Molecular identification methods of fish species: Reassessment and possible applications. *Reviews in Fish Biology and Fisheries*, *19*(3), 265-293.

Tsoupas, A., Papavasileiou, S., Minoudi, S., Gkagkavouzis, K., Petriki, O., Bobori, D., Sapounidis, A., Koutrakis, E., Leonardos, I., Karaiskou, N., & Triantafyllidis, A. (2022). DNA barcoding identification of Greek freshwater fishes. *PLOS ONE*, *17*(1), e0263118.

Wang, L., Wu, Z., Liu, M., Liu, W., Zhao, W., Liu, H., & You, F. (2018). DNA barcoding of marine fish species from Rongcheng Bay, China. *PeerJ*, *6*, e5013.

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. (2005). DNA barcoding of Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*(1462), 1847-1857.

Zulkifli, N. H., Ibrahim, P. H., & Zakariya, K. (2020). The significance of coastal landscape characters among locals and tourists: A case study of Gebeng – Kuala Terengganu tourism route. *Journal of Architecture, Planning and Construction Management*, *10*(1), 44-54.