DNA Barcoding and Morphological Characters of Two Trevally Fish species (*Caranx* spp.) Collected from Youtefa Bay, Papua, Indonesia

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Abstract

Trevally fish (*Caranx spp.*) or Kuwe fish are small pelagic fish that are mostly caught in the waters of Youtefa Bay. There are two species of Kuwe fish that are difficult to distinguish due to morphological similarities. These Kuwe fish have differently scientific species identification so they need for correct naming. Accordingly, it is necessary to identify and find out the proper naming of the two Kuwe fish. DNA barcoding is a technique used to speed up and simplify the process of identifying organisms. The purpose of this research was to identify the proper scientific name of the two Kuwe species in Youtefa Bay waters, as well as the kinship between these two types by applying a DNA barcoding technique. Nineteen Kuwe fish were sampled at Youtefa Bay, Jayapura, Papua in July 2022. Samples were studied morphologically, then carried directly to the Integrated Laboratory of Diponegoro University for molecular studies. DNA extraction, amplification, and DNA sequencing were carried out to further validate the species' identification. The results showed that in the BLAST analysis that 10 samples were closely related to *C. sexfasciatus* (99%-100% in similarity) and 9 samples were similar to *C. tille* (97%- 99%). The genetic distance between individuals in *C. sexfasciatus and C. tille* has a value ranging from 0% - 0.2%, and the phylogenetic tree that the two species are included in different clades. This study revealed that there are two species of Kuwe fish (*C. sexfasciatus and C. tille*) in Youtefa Bay, Papua, Indonesia.

Keywords: DNA barcode, *Caranx sexfasciatus*, *Caranx tille*, morphometry, Youtefa bay, Papua

Introduction

Youtefa Bay is a semi-enclosed bay that lies on the coastline of Jayapura City which was designated as a Nature Tourism Park by Decree of the Minister of Forestry Number 714/Kpts-II/1996 dated 11 November 1996 with an area of 1,675 ha (BKSDA, 2007). Youtefa Bay is a coastal area that is rich in fisheries and marine resources (Tebaiy, 2014). Fisheries activities carried out by the community on the coast of the bay are the main activities to meet their daily needs. One of the fish productions caught from these fishing activities is small pelagic fish. The small pelagic fish caught have high economic value, ranging from the selling price to the benefits of these fish for consumers and the people living in the waters of Youtefa Bay.

Kuwe fish is one of the small pelagic fishes caught by fishermen which has high economic value and is consumed by the people around Youtefa Bay. There are various types of Kuwe fish that have the same morphology found in the bay, namely Gerong Kuwe Fish and Yellow Kuwe Fish. Kuwe Gerong fish has morphological characteristics, namely shapebody elongated, flattened, and slightly oval. There is color greenish or blue-gray above, silver-white below, both dorsal fins are grayish-white with a whitish margin, caudal fin dark or slightly yellow with a dark tip. The morphology of the Yellow Kuwe Fish is oval and flat. The color of the body varies from yellow-gray above and silver to whitish below. The body is covered with fine, cycloid-shaped scales. The scales are small with a branched lateral line. On the chest the scales are reduced or absent. Size of body weight from 6-8 kg can also reach 10 kg. Kuwe Gerong fish is the most commonly obtained fish. This is because the fish has several advantages, namely fast growth rates, being able to adapt to the environment, seeds are easy to find around seagrass beds and mangrove forests, and disease resistance. However, the existence of the Yellow Kuwe does not have a large number of catches compared to the Kuwe Gerong. This could happen because the results of the catch of these fish are obtained by fishermen who go to sea outside the waters of Youtefa Bay compared to those in the waters of Youtefa Bay.These two types of fish are often caught using drag nets, fishing rods, and spears or kalawai (Kainama *et al.,* 2019)

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The diversity of each species is shown by the differences in morphology of the existing fish species. This morphology is the result of phenotypic appearance which is the result of interactions between genetic factors and their habitat environment (Prehadi *et al*., 2015). According to Rafsanjani (2011), kinship relationships in a population or species are usually studied through a morphological approach. However, the morphological approach has a weakness, namely that some characters frequently overlap with nearby taxa (Rasmussen *et al.*, 2009). Morphological characters have long been used in many phylogenetic studies. Phylogenetic is a method most often used in systematics to understand the diversity of living things through phylogenetic relationships (Twindiko *et al.*, 2013; Insafitri et al., 2023). Therefore, DNA barcoding is carried out to be able to identify quickly and accurately. DNA barcoding is a technique used to speed up and simplify the process of identifying organisms using certain gene fragments (Bangola *et al.*, 2014). DNA barcoding techniques can provide a "biological bar code" from short DNA sequences that are standardized to identify a species (Hajibabaei *et al.*, 2009). The application of DNA barcoding has an important role in obtaining basic information on genes that have high diversity and is an effort to strengthen morphological identification so that it is useful for the process of identifying species (Arifin and Kurniasih, 2007).

Materials and Methods

This research was carried out from December 2021 to February 2022. The sample collection in this study was located in the waters of Youtefa Bay, while data processing was carried out at the Diponegoro University Integrated Laboratory. The samples in this study were Gerong Kuwe Fish and Yellow Kuwe Fish, where the molecular identification samples were found in the mitochondrial DNA (mtDNA) of Gerong Kuwe and Yellow Kuwe.

A sampling of Kuwe Gerong Fish and Yellow Kuwe Fish were taken from the waters of Youtefa Bay based on the fresh catches of fishermen in the Hamadi Market TPI. The samples were preserved with 96% ethanol for molecular identification and morphology identification, where the samples that will be used for molecular identification are found in mitochondrial DNA (mtDNA) of Kuwe Gerong and Yellow Kuwe Fish which are found in the flesh of the tail of the fish. The sample is then placed in a coolbox so that the sample is not damaged and does not affect the DNA of the sample. Then stored in a frozen freezer at -20˚C.

DNA extraction and isolation

The tissue sample used in the extraction process is to cut the pectoral fin by 1-2 cm. DNA extraction using Chelex 10%, which aims to obtain sample DNA by isolating mtDNA in the sample (Walsh *et al*., 1991). The sample was heated at a state the exact temperature, in the presence of the resin contained in Chelex products that can be used to extract DNA (Marwayana, 2015; Kholilah *et al.,* 2021; Putri *et al*., 2022). Sample tissue was taken ±2 mm and then inserted into a tube containing 10% Chelex solution. The sample is vortexed for 10-15 seconds (Wijayanti, 2018), then heated using a heating block for 45 mins at a temperature of 95ºC. After being heated, the sample was vortexed again for 10-15 secs. Then centrifugation was carried out using a microcentrifuge with a speed of

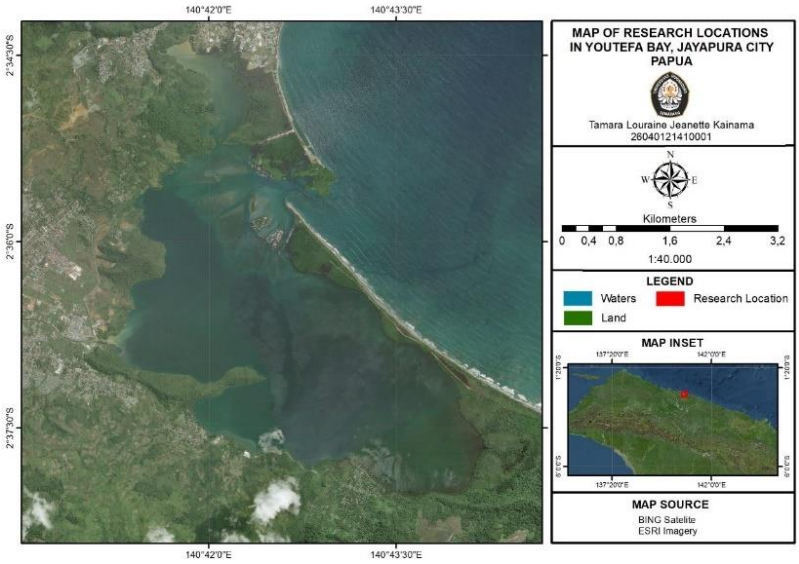

Figure 1. Map of Youtefa Bay

1000 rpm for 2 mins which aims to precipitate the sample tissue in the Chelex solution (Twindiko, 2013).

DNA amplification by PCR (Polymerase Chain Reaction)

The DNA extracted sample used for PCR was 2µL. DNA amplification was carried out with a target length of 587-589 base pairs. The primary design used in this amplification is 16S (Murakami, 2007). The base sequence of the primer that has been designed for the forward primer is 5'- CGC CTG TTT ATC AAA AAC AT-3', while the reverse primer is 5'- CCG GTC TGA ACT CAG ATC ACG T-3'. These two primers will be used in the DNA amplification process. The amplification process consists of denaturation, primer recognition of the target DNA (annealing), and primer elongation (extension).

Electrophoresis

The DNA electrophoresis stage aims to determine the quality of DNA in PCR products. DNA fragments of different lengths are visualized using fluorescent dyes specific for DNA, such as ethidium bromide. The type of gel used is agarose which can show the band or size of the base pair fragment that can be seen with ultraviolet light (Klugs and Cumming, 1994). In the electrophoresis process of making agarose gel media by dissolving 0.75 g of agarose and 75 mL of buffered SB (sodium borate) solution in a 250 mL beaker, then heated using a microwave to dissolve agarose with SB buffer so that it becomes homogeneous. The 1% agarose solution is then poured into a mold that has been fitted with a well-drilling comb and allowed to stand for 30 mins until the agarose gel hardens. The PCR sample was then inserted into a well in agarose media as much as 2 L, then the electrophoresis tool was operated for 30 mins by moving from the - (negative) to + (positive) pole to determine the quality of the DNA from the PCR product by separating, identifying, and purifying the fragments. DNA. The electrophoretic media was then immersed in a solution of Ethidium Bromide (EtBr) for 15-20 mins to visualize the results with the help of UV (ultraviolet) light. DNA will bind to EtBr so that it will glow when exposed to UV light. The amplified DNA sample can be seen using the Transilluminator Gel – doc tool with the appearance of the DNA band according to the target.

Samples that have been amplified by the PCR method (amplicons), were then sequenced to obtain the nucleotide sequence. In the sequencing process, the method used is the chain termination method (Sanger method). The sequencing process is carried out by PT. Indonesian Genetics Science. The results of the sequence in the form of nucleotide base data that make up the DNA of organisms in ab1 format will be sent via email for further data analysis.

Data analysis

The readings of the nucleotide base sequences were then processed using the MEGA 5.05 (Molecular Evolutionary Genetic Analysis) program. The data were aligned using CustalW on the MEGA 5.05 program to see the diversity of nucleotide bases. Analysis of DNA sequences obtained by comparing the sample sequences of Kuwe fish with DNA sequences in the DNA database. The nucleotide alignment data obtained were then matched with the data available on the GeneBank at NCBI (National Center for Biotechnology Information) using the BLAST (Basic Local Alignment Search Tool) nucleotide facility. (http://blast.ncbi.nlm.nih.-gov).

Phylogenetic analysis is a method to determine the kinship and genetic distance of a species. Genetic distance is a measure of genetic differences between populations due to mutation, selection, random crossing, and gene drift that will lead to evolution. Reconstruction of the phylogenetic tree was carried out using the Neighbor-joining (NJ) tree method, the 2-parameter Kimura model, and the bootstrap method with 1000 repetitions. This method can produce the best estimate of the branch length that reflects the real distance between the sequences (Dharmayanti, 2011).

Morphometric analysis includes Body mass (BM), Fork length (FL), Standard length (SL), Head length (HL), Body length (BL), Body width (BW), Head width (HW), Tail stalk width (TSW), Body height (BH), Tail stem height (TSH), and Tail height (TH). After that the data will be processed using Ms. Excel.

Result and Discussion

Morphological and morphometric identification

The study of fish diversity and species identification is an important component of fisheries management. The data offered in this paper identifies two species of morphologically-similar *Caranx* species in Youtefa Bay, Papua. From the initial assessment of the 19 specimens using taxonomic keys and phenotypic appearance, 10 specimens were identified as *C. sexfaxiatus* and 9 specimens were identified as *C. tille*. These species that have similar morphology were used as an object in this study. Several color differences are portrayed in the dorsal fin, caudal fin, anal fin, and body of these species. The color of the caudal and anal fins of the *C. sexfaxiatus* is greyish yellow, while the color of the tail and anal fins of the *C. tille* is silver, and greyish white (Figure 2.).

Several previous studies on *Caranx* sp. demonstrated the correlation between the physicochemical parameters of their habitat with their morphological and morphometric measures to determine the differentiation of the *Caranx* population. In this study, the average length of the samples for C. *sexfaciatus* and *C. tille* was 21.87± 1,4cm and 23.82±12.3 cm, respectively. For fork length, an average of 19.21±0.91 cm and 21.54±12.4 cm was obtained from *C. sexfasciatus* and *C. tille*, respectively. Meanwhile, Santos and Torres (2019) reported an average length of 21.70 cm *Caranx* sp. in the Philippines. For fork length, an average of 15.43 cm was obtained from *C. sexfasciatus*. *Caranx* sp. usually has a body size that ranges from 4–7.5 cm, while adults usually have an average total length of 16-29 cm (Ruiyana *et al*., 2016). Food factors affect the external and internal morphology of Kuwe Fish. In addition, for optimal fish growth, sufficient quantity and quality of food are required and accompanied by water conditions (Maherung, 2018).

The results of the range analysis in Table 1 show that there is a difference between the Kuwe Gerong (*C. sexfasciatus*) and the Yellow Kuwe (*C. tille*). The difference in the size range appears to be seen in all morphometric characteristics in the two species, but has the same average size in body weight (BW) with a value of 27± 2.4 cm. *C. sexfasciatus* has a higher average range of morphometric characteristics than *C. tille*. Even though they have different average sizes, these two species have almost the same habitat, namely in coastal waters and around coral reefs and the distribution of these two species is also usually found in the waters of the Indo East Pacific and in the Indo West Pacific in coastal waters. beaches and around coral reefs (Allen, 1999). But, this difference can occur when fishermen catch fish regardless of the size and weight of the two fish. Communities in the waters of Youtefa Bay usually catch Kuwe fish using small shrimp. To get the Kuwe, the fishing gear used by the community are fishing rods and trawl nets.

(A) (B)

Figure 2. The Kuwe fish of Youtefa bays, Papua (A: *C. tille*; B: *C. sexfasciatus*)

Table 1. Morphometric characters of C. *sexfasciatus* and *C. tille* of Youtefa Bay

Morphometric characters	Mean±SD (cm)	
	C. *sexfasciatus*	*C. tille*
Total length (TL)	218.7±10,4	238.2±12.3
Standard length (SL)	171.2±11.1	183.4±10.7
Fork length (FL)	192.1±9.1	215.4±12.4
Head length (HL)	50.9±11.5	61.3±4.0
Head width (HW)	30.2±2.8	30.5±2.0
Body length (BL)	109.8±4.4	120.1±6.4
Body width (BW)	27±2.4	27±2.4
Body height (BH)	59.7±13.4	70.5±4.4
Snout length (SNL)	13,6±2.0	16±3.3
Orbit/eye diameter (ED)	15.1±1.0	17.1±2.2
Tail stem height (TSH)	7.6±1.0	9.1±1.0
Tail stalk width (TSW)	12.8±2.4	13.4±1.1
Tail height (TH)	59.8±8.3	73.4±7.5

Molecular identification, phylogenetic studies, and genetic distance

16S gene sequence analysis in a total of 19 samples representing two species and one genus of *Caranx* were sequenced in our study. The partial 16S sequence length of the 19 sequences ranged from 616 bp to 658 bp, and after being aligned with the other 9 sequences obtained from GenBank, the length was cut to 617 bp. There were no insertions, deletions, or stop codons in the sequences after translating DNA sequences into amino acid sequences using vertebrate mitochondrial code, indicating that all of the sequences represented the functional mitochondrial 16S gene.

The results of the Kuwe Fish Samples that were successful in BLAST identified 19 samples with the percentage of similarity values ranging from 97% - 100%.The sequence of the 16S gene demonstrated that IKG1 to IKG10 samples were closely related to *C. sexfasciatus,* and IKK1 to IKK9 samples were closely related to *C. tille* (Table 2.). The data used comes from Hawaii with accession no. DQ427055.1 (Murakami, 2007) for *C. sexfasciatus* species and from Japan with accession no LC646863.1 (Kimura, 2021. In this study, a phylogenetic tree was built by using BLAST and species reference Genebank to determine the closeness between species. Based on the Neighbor-joining (NJ) tree, distinct clusters of *C.* were formed with high bootstrap values and separated the highly similar *C. sexfasciatus* and *C. tille*. The phylogenetic - tree showed that there were four clades and the bootstrap value for the Neighbor-Joining method ranges from 48-99 % (Figure 3.).

Phylogenetic tree is created by adding ingroups and outgroups taken from GeneBank data. The ingroups used were *C. sexfasciatus* and *C. tille*, while the outgroup was taken from *Caranx* sp. also has a presentation identification value of 96.24%, namely *C. crysos* which functions to polarize the specimen (Figure 3.). The genetic distance between individuals in the species *C. sexfasciatus* and *C. tille* has values ranging from 0-0.2%. Morphologically, the two Kuwe species are almost the same but have some color differences in several parts, namely the caudal fin, anal fin, dorsal fin and body. But based on genetic analysis by looking at the phylogenetic tree formed, the two species have separate clades.

Intraspecific and interspecific genetic distance

In this study, the genetic distance among individuals of *C. sexfasciatus* and *C. tille* ranged from 0-0.02% and 0-0.2%, respectively. The farthest genetic distance is in clade 2 with a value of 0.0023 and the closest genetic distance is clade 4 which is 0.0188 (Table 3.). The smaller the genetic distance, the closer the similarity, and may indicate that a particular species may have similar origins (Tapilatu *et al*., 2021; Dwifajri *et al*., 2022).

Table 2. BLAST analyses of 16S DNA sequences

Sample	Closely related	Acc. no. reff.	length (bp)	Similarity (%)
IKG1	*Caranx sexfasciatus*	DQ427055.1	618	100%
IKG 2	*Caranx sexfasciatus*	DQ427055.1	618	100%
IKG 3	*Caranx sexfasciatus*	DQ427055.1	618	100%
IKG 4	*Caranx sexfasciatus*	DQ427055.1	618	100%
IKG 5	*Caranx sexfasciatus*	DQ427055.1	616	100%
IKG 6	*Caranx sexfasciatus*	DQ427055.1	619	99,83%
IKG 7	*Caranx sexfasciatus*	DQ427055.1	617	99,83%
IKG 8	*Caranx sexfasciatus*	DQ427055.1	630	100%
IKG 9	*Caranx sexfasciatus*	DQ427055.1	636	100%
IKG 10	*Caranx sexfasciatus*	DQ427055.1	631	99,83%
IKK 1	*Caranx tille*	LC646863.1	618	98,54%
IKK 2	*Caranx tille*	LC646863.1	619	100%
IKK 3	*Caranx tille*	LC646863.1	633	100%
IKK 4	*Caranx tille*	LC646863.1	658	100%
IKK 5	*Caranx tille*	LC646863.1	618	98,54%
IKK 6	*Caranx tille*	LC646863.1	618	100%
IKK 7	*Caranx tille*	LC646863.1	618	100%
IKK 8	*Caranx tille*	LC646863.1	618	100%
IKK 9	*Caranx tille*	LC646863.1	618	97,37%

Table 3. Average genetic divergence within and between *Caranx* species based on 16S gene.

IKG_1																		
IKG_2	0,0000																	
IKG_3	0,0000	0,000																
IKG_4	0,0000	0,000	0,000															
IKG_5	0,0000	0,000	0,000	0,000														
IKG_6	0,0023	0,002	0,002	0,002	0,002													
IKG_7	0,0000	0,000	0,000	0,000	0,000	0,002												
IKG_8	0,0000	0,000	0,000	0,000	0,000	0,002	0,000											
IKG_9	0,0000	0,000	0,000	0,000	0,000	0,002	0,000	0,000										
IKG_10	0,0023	0,002	0,002	0,002	0,002	0,005	0,002	0,002	0,002									
IKK_1	0,0188	0,019	0,019	0,019	0,019	0,016	0,019	0,019	0,019	0,021								
IKK_2	0,0188	0,019	0,019	0,019	0,019	0,016	0,019	0,019	0,019	0,021	0,005							
IKK_3	0,0188	0,019	0,019	0,019	0,019	0,016	0,019	0,019	0,019	0,021	0,005	0,000						
IKK_4	0,0188	0,019	0,019	0,019	0,019	0,016	0,019	0,019	0,019	0,021	0,005	0,000	0,000					
IKK_5	0,0188	0,019	0,019	0,019	0,019	0,016	0,019	0,019	0,019	0,021	0,005	0,000	0,000	0,000				
IKK_6	0,0188	0,019	0,019	0,019	0,019	0,016	0,019	0,019	0,019	0,021	0,005	0,000	0,000	0,000	0,000			
IKK_7	0,0188	0,019	0,019	0,019	0,019	0,016	0,019	0,019	0,019	0,021	0,005	0,000	0,000	0,000	0,000	0,000		
IKK_8	0,0188	0,019	0,019	0,019	0,019	0,016	0,019	0,019	0,019	0,021	0,005	0,000	0,000	0,000	0,000	0,000	0,000	
IKK_9	0,0215	0,021	0,021	0,021	0,021	0,019	0,021	0,021	0,021	0,024	0,007	0,002	0,002	0,002	0,002	0,002	0,002	0,002

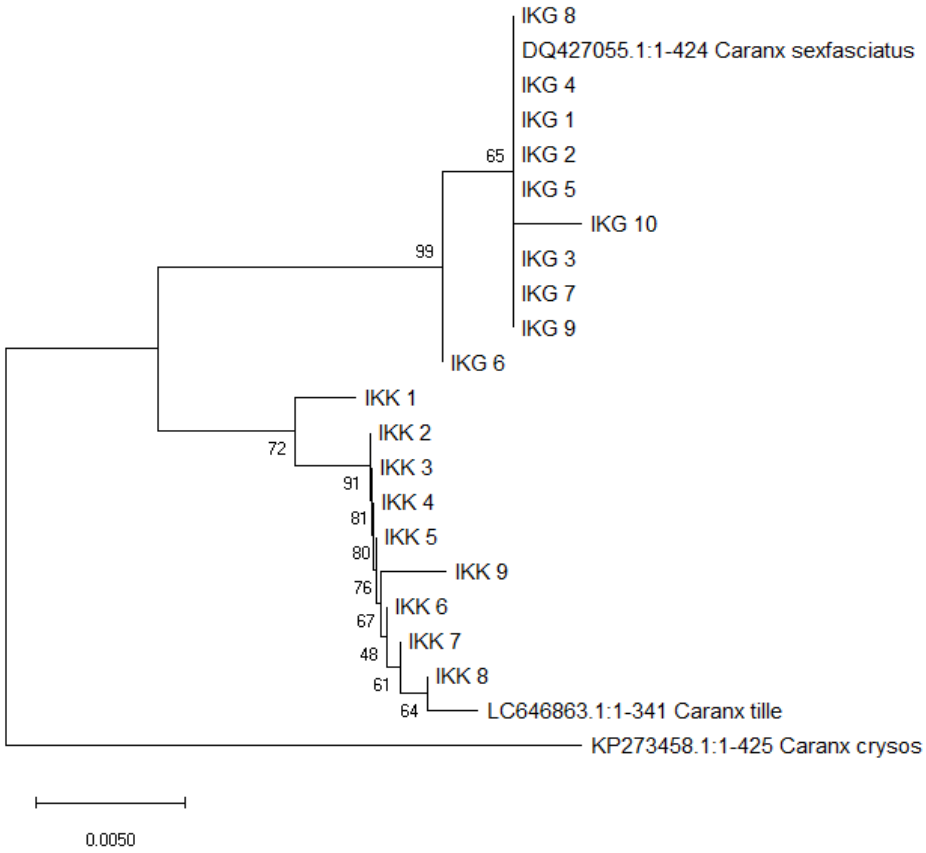

Figure 3. Phylogenetic-tree of two trevally fish species (*Caranx spp.*) from Youtefa bay, Papua,

The genetic distance between individuals in *C. sexfasciatus* and *C. tille* has a value ranging from 0%-0.2%. Morphologically, the two species of Kuwe Fish are almost the same but have some color differences in some parts, namely the caudal fin, anal fin, dorsal fin and body. But based on genetic analysis by looking at the phylogenetic tree formed, the two species have separate clades. This shows that these two species are indeed different but have almost the same morphology.

Research on this species has not been done much, this is supported by the lack of *C. sexfasciatus* and *C. tille* sequences in GenBank, until before this research was conducted there were only 2 sequences in GenBank. These sequences came from different loci, namely 1 sequence from the 16S ribosomal RNA locus (Murakami *et al*., 2007) for *C. sexfasciatus* and 1 sequence from the 16S ribosomal RNA locus (Kimura, 2021) for *C. tille*.

Conclusion

In conclusion, this research successfully identified *Caranx spp.* Youtefa Bay, Papua. The data presented in this paper identifies two species of morphologically-similar *Caranx* species. Phylogenetic analysis shows that the Gerong Kuwe Fish (*Caranx sexfasciatus)* and Yellow Kuwe Fish (*Caranx tille)* are separated. The utilization of both morphological and genetic analysis for species identification would be ideal for future studies to accurately identify species.

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