BIODIVERSITAS
Volume 23, Number 9, September 2022
Pages: 4561-4569

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d230923

A genetic approach for authentication on morphological differences of cultivated pompano species in Indonesia

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Manuscript received: 10 August 2022. Revision accepted: 4 September 2022.

Abstract. *Hakim AA, Dikrurahman, Muhlis S, Astuti RP, Sari DW, Kamal MM. 2022. A genetic approach for authentication on morphological differences of cultivated pompano species in Indonesia. Biodiversitas 23: 4561-4569.* Due to the increasing demand for animal protein, the Batam Marine Cultivation Fisheries Center (BPBL Batam) developed pompano (*Trachinotus* spp.) for aquaculture. Because of the different external characteristics, local communities recognized three types of pompano, i.e., *bintang*, *emas*, and *hibrid* pompano. The study aimed to prove the authenticity of cultivated pompano (*Trachinotus* spp.) with morphological differences based on a DNA barcoding approach with 16S ribosomal RNA and cytochrome oxidase subunit I (COI) partial gene sequences. A total of 30 barcode sequences from COI and 16S rRNA genes were obtained from three types of pompano, and all samples were validated as *Trachinotus blochii* with percent identity of 99.85-100% by COI and 100% by 16S rRNA. *Emas*, *bintang*, and *hibrid* pompano also have the same or very similar GC content both based on COI and 16S rRNA sequences. The average genetic distances calculated between species in the genus *Trachinotus* were 0.09 and 0.02 among COI and 16S rRNA sequences, respectively. Phylogenetic analysis constructed from COI and 16S rRNA gene sequences of all types of pompano and related species segregated genus into one clade (*T. blochii*) and the outgroup (other species) as another clade. This study contributed essential genetic data to the NCBI database both COI and 16S rRNA gene sequences. Despite having morphological differences, all types of pompano were successfully authenticated into the same species because they have identical nucleotide base arrangements and can be presumed from the same ancestor.

Keywords: 16S rRNA, aquaculture, COI, DNA barcoding, *Trachinotus blochii*

INTRODUCTION

Due to the increasing demand for animal protein, especially in fish commodities, species domestication until cultivation activities need to be developed to supply these requirements. The continuous reliance on the capture fisheries sector is feared to have an impact on decreasing fish yield and automatically threatening natural stocks. Due to the high interest in pompano, the genus *Trachinotus* has become one of the marine fish developed for aquaculture by the Batam Marine Cultivation Fisheries Center (BPBL Batam) in Riau Islands, Indonesia. Pompano's advantages include rapid growth, high economic value, and consumer preference (Ariska et al. 2018). As a result, local communities recognized three types of pompano with morphological differences, which have folk names, i.e., *ikan bawal bintang* (star pompano), *ikan bawal emas* (golden pompano), and *ikan bawal hibrid* (hybrid pompano). The hybrid pompano comes from a cross between *bintang* pompano males and *emas* pompano females. Currently, hybrid pompano has been introduced by the government as *bawal sakti.*

In Indonesia, two varieties of pompano are widely known, long-finned and short-finned pompano, based on the dorsal fin size. The morphology of the two types can be distinguished by cultivators, exporters, and consumers. Based on taxonomic nomenclature, short-finned is a morphological feature of *T. carolinus* and *T. ovatus*, while long-finned is *T. blochii*. Fishbase (2022) released the common names for *T. carolinus* as Florida pompano, *T. ovatus* as pompano, and *T. blochii* as snubnose pompano. However, the common name used for the golden pompano is still confusing; most studies claim it for *T. ovatus* (Sun et al. 2013; Sun et al. 2014; Zhang et al. 2019; Qiao et al. 2021; He et al. 2022), while others state it for *T. blochii* (Cao et al. 2019; Weo et al. 2019; Zhang et al. 2020; Zhou et al. 2020; Zhang et al. 2022).

Usually, the community's folk name accorded to animals is based on understanding their physical characteristics to make them more accessible. Hidayati et al. (2022) state that folk names were created using traditional knowledge and linguistic mechanisms. Based on the folk name for pompano, *ikan* refers to fish in general, and *bawal* refers to pompano. Simply, to local understanding to distinguish the types of pompano, *ikan bawal bintang* has a star-like body shape, *ikan bawal emas* has a golden color, and *ikan bawal hibrid* is a cross between both pompanos. The general concord between the scientific taxonomies and folk name strengthens the reality used by the biological groups. An alternative to fisheries

management can be supported by ethno-taxonomy (folk identification and classification) (de Carvalho et al. 2018).

Authentication needs to conduct for identification, validation, and determination of the originality of species. Genetic markers are the highest-relevance method used to identify and certify species by comparing several methods (Raza et al. 2016). In fisheries, the application of a genetic approach is increasingly required for species authentication in Mediterranean marine fishes (Landi et al. 2014), saithe fish (Behrmann et al. 2015), and fish landings (Ardura et al. 2013). The genetic approach through DNA barcodes is becoming an invaluable instrument for fisheries managers and regulatory stakeholders for unambiguous identification, ecology assessment, evolutionary biology, and conservation management (Kress et al. 2015).

DNA barcoding technique utilizes short standard gene regions to identify species that successfully solve the problems of taxonomic ambiguities (Chu et al. 2019). Furthermore, Landi et al. (2014) stated that DNA based approach by DNA barcoding raises prospects for species-level identification widely using a standardized and authenticated DNA fragment. In its application, DNA barcoding is used to identify various kinds of organisms, such as fungi, viruses/prions, animals, and humans, through standardized pieces of DNA (Kaur 2015). Mitochondrial fragments of the cytochrome oxidase subunit I (COI) gene are applied globally in species identification and biodiversity studies (Bingpeng et al. 2018) which have been promoted by Hebert et al. (2003) and have been performed for species identification and classification. Not only COI, but we also explored 16S ribosomal RNA gene fragments in DNA barcodes. For example, the gene has been used in fish identification in Cynoglossidae (Soman et al. 2020).

Several studies on hybridization to obtain a new type of pompano have been conducted, especially in the recent study of Wu et al. (2022) which hybrid experimental performed on *Trachinotus ovatus* and *Trachinotus blochii*. Genetic approaches have also been established in previous studies, including sex-specific molecular markers (Zhang et al. 2022), polymorphic microsatellites (Sun et al. 2013), characterization and expression analysis (Qiao et al. 2021; He et al. 2022), chromosome-level genome assembly (Zhang et al. 2019), and complete mitochondrial genome sequencing (Sun et al. 2014; Zhang et al. 2014). An investigation of species authenticity based on a genetic approach needs to be conducted to determine taxonomic certainty, especially for Indonesia's three types of pompano. Therefore, the study aimed to prove the authenticity of cultivated pompano (*Trachinotus* spp.) with morphological differences based on a DNA barcoding approach with 16S ribosomal RNA and cytochrome oxidase subunit I (COI) partial gene. The record of generated DNA barcodes will provide a remarkable research tool to develop aquaculture and management of fisheries resources in this region.

MATERIALS AND METHODS

Sample collection

Fish specimens were collected in March 2021 from Batam Marine Cultivation Fisheries Center (BPBL), Indonesia, which is the result of the cultivation of marine commodities. Each type of pompano (*bintang*, *emas*, and *hibrid* pompano) was collected from five individuals, then transported in freezing conditions to the Laboratory of Aquatic Molecular Biology, IPB University. The specimens were placed at room temperature to defrost the fish and observe morphological characters. The tissue was collected from the part just below the dorsal fin and put into a 2 mL tube.

DNA extraction, PCR amplification, and sequencing

For each sample, 0.03 g of tissue were extracted using a Gene Aid kit with standard DNA barcoding methods. The total volume of the amplification reaction was 25 µL, including 4.5 µL double-distilled water, 12.5 µL *Taq* DNA polymerase buffer (My Tag HS Red Mix), 1.5 µL each primer at 10 mM, and 5 µL DNA template. The amplification process was focused on two gen partial markers, namely cytochrome oxidase subunit I (COI) (Hakim et al. 2020a) and 16S rRNA (Hakim et al. 2020b), with target sequences of approximately 600 base pairs. The conditions of polymerase chain reaction consisted of an initial step (pre-denaturing) of 5 min at 94°C followed by 30 cycles of denaturing (94°C, 45 s), annealing (54°C, 1 min for COI and 46°C, 45 s for 16S rRNA) and extension (72°C, 1 min), with a final extension (72°C for 5 min). The amplicon was tested for the quality of PCR products on a 1.2% agarose gel. Amplicons were sent to the service company for sequencing analysis using the Sanger method.

Data analysis

Forward and reverse sequences were spliced and edited using MEGA11 software (Tamura et al. 2021) combined with manual proofreading. After ensuring correct, species validations were performed using BLAST-n (Basic Local Alignment Search Tool-nucleotide). Furthermore, each sequence was submitted to GenBank (https://www.ncbi.nlm.nih.gov/) as a contribution to the genetic database of cultivated pompano. The sequences were analyzed using MEGA11 software, including sequence alignment using ClustalW (Larkin et al. 2007), nucleotide compositions (AT and GC content), calculation of genetic distances using the Kimura-2-parameter (K2P) model (Kimura 1980), and construct a phylogenetic tree using the neighbor-joining (NJ) method (Saitou and Nei 1987). The credibility of the branching tree was tested by bootstrap analysis with 1000 repeated sampling tests (Felsenstein 1985).

RESULTS AND DISCUSSION

Morphological characteristics

Morphologically, the three types of pompano (Figure 1) tend to be similar when viewed visually (Table 1). Identical

characteristics can be seen from body shape, coloring, the position of the mouth, tooth type, number of fin rays anterior to the first elongated dorsal fin, dorsal fin rays, anal fin rays, and dorsal fin lobe. But, all types of pompano showed variations, especially in the body color and second dorsal fin lobe. Compared to the others, the body color of *emas* pompano is gold only on the caudal fin as a specific characteristic of the species. However, the body color feature cannot be used as a morphology identifier because the character can change if the fish is not alive or fresh. According to the dorsal fin, only *emas* pompano has a shorter size than the other two types of pompano.

This study compared the morphological characteristics of three types of pompano (*bintang, emas,* and *hibrid* pompano) with *T. blochii*, *T. carolinus*, and *T. ovatus* derived from references (Bauchot 2003; Gothreaux 2008; FAO 2022). All types and three species of pompano have the same body shape and the same primary body color. *T. ovatus* has a striking feature among the other three species, with 3-5 vertically elongated black spots on the anterior half of the lateral line. However, in the samples, only *emas* pompano has a golden color of the caudal fin. Only *T. carolinus* had a small sub-terminal position of the mouth, while other species and samples had a terminal mouth type. Based on the number of fin rays anterior to the first elongated dorsal fin, dorsal fin rays and anal fin rays of the three pompanos have a match with *T. blochii*, *T. carolinus* and *T. ovatus* have more fin rays in all three characters. The dorsal fin lobe shows a difference where the *emas* pompano is similar to *T. carolinus* and *T. ovatus* (short). In contrast, *bintang* and *hibrid* pompano have similarities to *T. blochii* (long).

According to morphological characteristics, the three types of pompano were identified as *T. blochii*. However, it should be Highline that *T. blochii* has two variations in the size of the dorsal fin and color of the body and fin (long-finned and gray silvery; short-finned and golden). Two varieties of pompano, widely known by farmers, exporters, and consumers, have been identified as one species based on morphological characteristics. However, it is necessary to investigate the authenticity of species based on a genetic approach to determine taxonomic certainty.

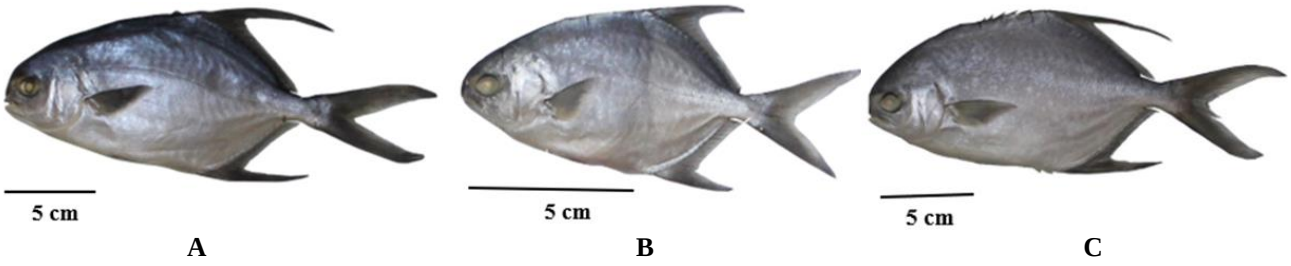

Figure 1. Three types of cultivated pompano in the Batam Marine Cultivation Fisheries Center (BPBL Batam). A. *bintang* pompano, B. *emas* pompano, C. *hibrid* pompano

Table 1. Morphological characteristics of *bintang, emas,* and *hibrid* pompano in the Batam Marine Cultivation Fisheries Center (BPBL Batam)

Characteristics	Type of pompano			Pompano species		
	Bintang	***Emas***	***Hibrid***	***T. blochii***	***T. carolinus***	***T. ovatus***
Body shape	Fusiform, oval shape	Fusiform, oval shape	Fusiform, oval shape	Fusiform, oval shape	Fusiform, oval shape	Fusiform, oval shape
Coloring	Body dull gray to silvery	Body dull gray to silvery, golden orange of caudal fin	Body dull gray to silvery	Dorsal side of body silvery blue grey, ventral pale; large adults golden orange, especially snout and ventral half of body	Body silvery with dark (bluish) back. Spinous dorsal fin low. Belly, anal fin, and caudal yellow	Dorsal side of body greenish-grey, sides silvery with 3-5 vertically elongated black spots on anterior half of lateral line; dorsal, anal, and caudal fin lobes black-tipped.
Position of mouth	Terminal	Terminal	Terminal	Terminal	Small sub-terminal	Terminal
Fin ray on anterior to the first elongated dorsal fin	5-6	6	5-6	5-6	6-7	7
Dorsal fin rays	18-20	19-20	18-20	18-23	23-25	23-27
Anal fin rays	17-18	17-18	17-18	16-20	21-22	22-25
Dorsal fin lobe	Long	Short	Long	Long	Short	Short
Reference	This study	This study	This study	Bauchot 2003; FAO 2022	Gothreaux 2008; FAO 2022	Bauchot 2003

By crossing *bintang* pompano males and *emas* pompano females, hybridization aims to obtain superior seeds to support aquaculture activities. The advantages of each parent are that the *bintang* pompano has a large body shape and attracts consumers, while the *emas* pompano has a thick meat (Puspita et al. 2021). They crossed to get a new type of pompano with a large body shape and a delicious meat texture. The protrusion of things you want to excel in and can attract consumers' interest is called hybridization in aquaculture (Sutarmat and Yudha 2013). The hybridization results show that the *hibrid* pompano can comply with increasing consumer demand with desired criteria such as delicious and soft meat texture and large size. In addition, the *hibrid* pompano also has a fast growth rate, resistant to disease, and adapts easily. Hybridization aims to obtain superior varieties and increase the production value of a cultured fish commodity (Rahman et al. 2013).

As one of the most promising candidate species for marine aquaculture, Nazar et al. (2012) stated that the cultivation of *T. blochii* has been successfully established in China, India, and Indonesia due to its fast growth, easy feed formulation, high consumer preference, and good meat quality. According to Gopakumar et al. (2012), *T. blochii* is widespread in the Indo-Pacific region. Still, the species in the wild have declined dramatically due to overfishing and environmental pollution, and recently can be caught infrequently in commercial fisheries. This species has good meat quality, high market demand, fast growth rate compared to other cultured fishes (Nazar et al. 2012), so aquaculture in Indonesia is more developed and creates hybrid species. Cultivation of this species is expected to meet market demand and reduce fishing pressure on wild natural resources.

Species validation

In this study, we successfully amplify cytochrome oxidase subunit I (COI) and 16S ribosomal RNA barcode sequences for three types of pompano using primer pairs that amplify target genes without insertion or deletion. Not only used in natural populations but molecular markers have also been used in cultured populations. The primers can be used as a global standard marker for DNA barcoding not only for fish (Hakim et al. 2020a) but also for crabs (Krisanti et al. 2020; Hakim et al. 2022), mollusks (Hakim et al. 2020b), mammals (Kamal et al. 2021), freshwater turtles (reptiles), amphibians, aquatic insects, and sea urchins (Echinoidea) (*in progress*). Editing sequence obtained base length from COI and 16S rRNA with a range of 612-653 and 606-615 base pairs (Table 2).

DNA barcoding proved effective for fish identification and transient cryptic diversity discovery (Hubert et al. 2012). Based on both gene markers, all samples from all types of pompano were validated as *Trachinotus blochii* that *emas*, *bintang*, and *hibrid* pompano samples were identified with a similarity level of 99.85-100% by COI and 99.20-100% by 16S rRNA. Specifically, for COI, the identical percent value of more than 97% indicates that the sample sequence can be confirmed to have a species name following the results from Genbank (Herbert et al. 2003). Although there is no clear standard range, the result percentage identical to the 16S rRNA gene is above 99%. Based on two genes, three types of pompano have been confirmed and validated as *T. blochii.* The results of this validation have closeness based on genetic data in Genbank on accession number KJ184305.1, which is the result of a study in China (Zhang et al. 2014).

The nucleotide composition of all types of pomfret based on the COI and 16S rRNA gene sequences is shown in Table 3. GC contents of *emas*, *bintang*, and *hibrid* pompano are obtained sequentially as follows 43.4-43.7, 43.3-43.6, and 43.5-43.6% by COI while 45.1-45.2, 45.1-45.3, 45.1-45.2% by 16S rRNA. The results of this study are by the study of complete genome sequences by Zhang et al. (2014); the overall base composition is estimated to be 15.74% for G, 29.21% for A, 28.56% for C, 26.49% for T, respectively, with a low GC content (44.30%). The GC content has 3 hydrogen bonds while the AT content has 2 hydrogen bonds (Jusuf 2001), where pompano is dominated by AT content. The nucleotide base composition of *T. blochii* indicates that the bonds are easily separated, so the possibility of mutations in *T. blochii* is higher. The DNA barcoding technique provides accurate species identification results by utilizing DNA sequence fragments in organisms with significant species differences (Bingpeng et al. 2018). DNA barcoding in pompano identification has reduced the dependence on taxonomists with the ability and personal experience to perform traditional morphological classifications. Applied DNA barcode marker sequences to specific taxonomic organisms are invaluable for understanding species diversity, community ecology, trophic interactions, the evolution of functional traits, and conservation (Kress et al. 2015). Complete mitochondrial genome sequences can provide essential data for understanding molecular systematics, population structure, stock evaluation, and conservation genetics (Iguchi et al. 2012). The data also helps develop appropriate policies to formulate fisheries resource management strategies, especially *T. blochii.*

Genetic distances and phylogenetic tree

In this study, we use two markers to calculate genetic distance. Based on the genetic distance value (Table 4 and 5), the fifteen sequences of COI and fifteen sequences of 16S rRNA observed have the same genetic distance between samples with *Trachinotus blochii* (KJ184305.1), which is 0.000. It can be assumed all samples are one species because they have a low genetic distance. The genetic distance of the sample with the in-group used has the closest relationship with *T. blochii* compared to *T. carolinus* and *T. ovatus*. The genetic distance value indicates that the sample belongs to one species, *T. blochii*. In contrast to other species, all samples had a genetic distance of 0.09 for two species by COI while a genetic distance of 0.01 for *T. carolinus* and 0.02 for *T. ovatus* by 16S rRNA.

Table 2. The result of species validation using COI and 16S rRNA partial gene on three types of pompano

Samples	COI result			16S rRNA result		
	Base length (base pair)	Molecular identification	Percent identic (%)	Base length (base pair)	Molecular identification	Percent identic (%)
Emas Pompano 1	640	*Trachinotus blochii* (Accession: KJ184305.1)	100	606	*Trachinotus blochii* (Accession: KJ184305.1)	100
Emas Pompano 2	645		100	615		100
Emas Pompano 3	638		100	615		100
Emas Pompano 4	645		100	615		100
Emas Pompano 5	646		100	615		100
Bintang Pompano 1	653		99.85	611		100
Bintang Pompano 2	653		99.85	614		100
Bintang Pompano 3	627		100	613		100
Bintang Pompano 4	642		100	614		100
Bintang Pompano 5	612		100	614		100
Hibrid Pompano 1	644		100	614		100
Hibrid Pompano 2	650		99.85	614		100
Hibrid Pompano 3	642		100	614		100
Hibrid Pompano 4	646		100	614		100
Hibrid Pompano 5	643		100	615		100

Table 3. Nucleotide composition and GC content of all types of pompano based on the COI and 16S rRNA gene sequences

Base	Nucleotide compositions (%)		
	***Emas* pompano**	***Bintang* pompano**	***Hibrid* pompano**
Cytochrome Oxidase Subunit I (COI)			
T	30.7-30.9	30.6-31.2	30.7-30.9
C	25.3-25.7	25.2-25.6	25.5-25.7
A	25.5-25.6	25.5-25.8	25.5-25.7
G	18.0-18.2	18.0-18.5	17.9-18.1
GC content	43.4-43.7	43.3-43.6	43.5-43.6
16S ribosomal RNA			
T	23.5-23.7	23.3-23.8	23.3-23.6
C	24.6-24.9	24.6-24.8	24.6-24.7
A	31.2-31.6	31.1-31.5	31.2-31.6
G	20.3-20.6	20.5-20.6	20.5
GC content	45.1-45.2	45.1-45.3	45.1-45.2

Genetic distance has been frequently adopted in assessing the species taxonomy of closely related taxa and evolutionary studies (Doğan and Doğan 2016). The highest genetic distance indicates that the samples are very distantly related and have many differences in nucleotide bases (Hebert et al. 2003). Tallei et al. (2016) added that the low genetic distance between two different individuals displayed a very close kinship. This result can be proven in the study by Achmad et al. (2019) that individuals from the exact location and parental origin will have close kinship relationships. In this study, all types of pompano were successfully authenticated into the same species because they have identical nucleotide base arrangements and can be presumed from the same ancestor.

The data matrix of genetic distance was constructed into a phylogenetic tree for COI and 16S rRNA genes (Figure 2). All samples formed one significant clade with *T. blochii* and were separate from *T. carolinus* and *T. ovatus*. The use of COI and 16S rRNA gene has succeeded in confirming and proving that the three types of pompano cultivated in Indonesia are *T. blochii*.

Evolutionary relationships between samples and other species can be explained by constructing a phylogenetic tree. Pigot and Etienne (2015) stated that evaluating historical impacts often requires phylogenetic data that can provide information about the history of diversity and past dispersal that may have created contemporary species assemblages. This study's results indicate a clear separation between *T. blochii*, *T. carolinus*, and *T. ovatus*, which shows the evolution of the three species compared. Intense species dispersal will result in a lack of phylogenetic community structure as species come from different branches and have different evolutionary histories (Dexter et al. 2017).

Previous studies on DNA barcoding of carp cultured resulted in more beneficial COI than 16S rRNA (Mohanty et al. 2013). In this study, COI and 16S were equally applicable and successfully revealed the taxonomy of the three types of pompano. COI and 16S rRNA origin from mtDNA are commonly inherited maternally, non-recombination, and have a higher mutation rate than nuclear DNA (DeSalle et al. 2017). mtDNA has been widely used for phylogenetic analysis for species identification and specification (Jaafar et al. 2021). The effect of hybridization is difficult to describe by morphological characters when considered individually. Still, when viewed together in multivariate analyses, it can mean that they have a dominant expression or that there is a material effect that makes observing the separation in offspring difficult (Bacilieri et al. 1996). The hybridization of pompano in Indonesia by crossing *bintang* pompano males and *emas* pompano females resulted in a combination of characters, i.e., long-finned and large-sized from *bintang* pompano and thick meat from *emas* pompano.

Table 4. Pair-wise genetic distances of *emas*, *bintang*, and *hibrid* pompano and related species based on cytochrome oxidase subunit I (COI) gene

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Bintang Pompano 1																		
Bintang Pompano 2	0.00																	
Bintang Pompano 3	0.00	0.00																
Bintang Pompano 4	0.00	0.00	0.00															
Bintang Pompano 5	0.00	0.00	0.00	0.00														
Emas Pompano 1	0.00	0.00	0.00	0.00	0.00													
Emas Pompano 2	0.00	0.00	0.00	0.00	0.00	0.00												
Emas Pompano 3	0.00	0.00	0.00	0.00	0.00	0.00	0.00											
Emas Pompano 4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00										
Emas Pompano 5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00									
Hibrid Pompano 1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00								
Hibrid Pompano 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00							
Hibrid Pompano 3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00						
Hibrid Pompano 4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00					
Hibrid Pompano 5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
Trachinotus blochii	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
Trachinotus carolinus	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09		
Trachinotus ovatus	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.12	-

Table 5. Pair-wise genetic distances of *emas*, *bintang*, and *hibrid* pompano and related species based on 16S ribosomal RNA gene

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Bintang Pompano 1																		
Bintang Pompano 2	0.00																	
Bintang Pompano 3	0.00	0.00																
Bintang Pompano 4	0.00	0.00	0.00															
Bintang Pompano 5	0.00	0.00	0.00	0.00														
Emas Pompano 1	0.00	0.00	0.00	0.00	0.00													
Emas Pompano 2	0.00	0.00	0.00	0.00	0.00	0.00												
Emas Pompano 3	0.00	0.00	0.00	0.00	0.00	0.00	0.00											
Emas Pompano 4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00										
Emas Pompano 5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00									
Hibrid Pompano 1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00								
Hibrid Pompano 2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00							
Hibrid Pompano 3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00						
Hibrid Pompano 4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00					
Hibrid Pompano 5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
Trachinotus blochii	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
Trachinotus carolinus	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01		
Trachinotus ovatus	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	-

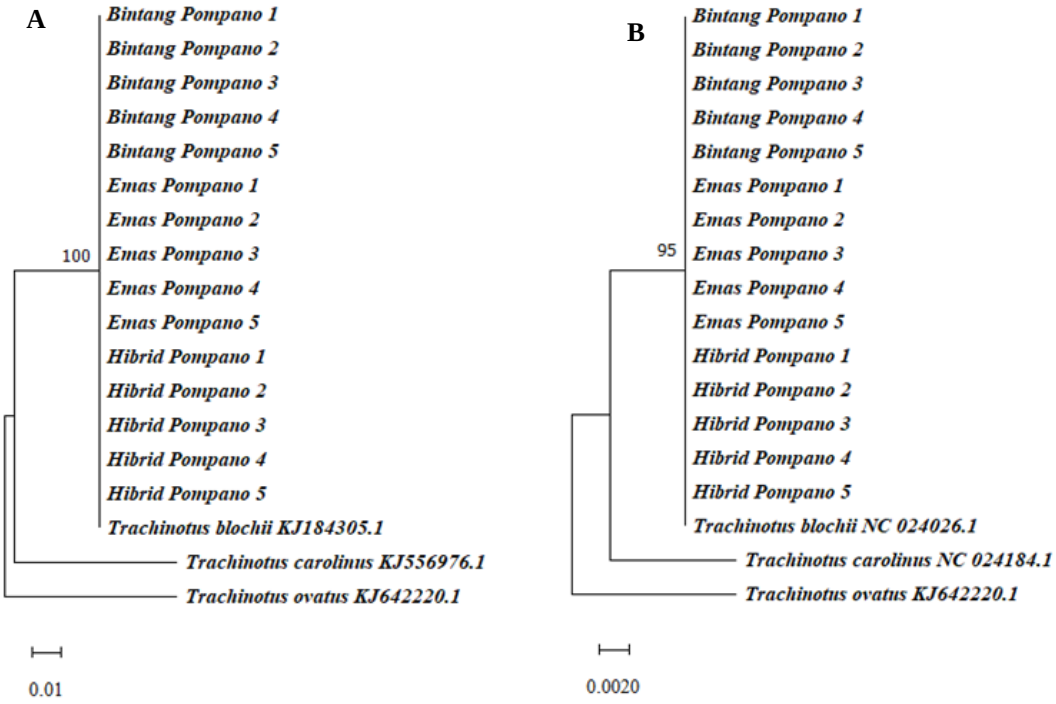

Figure 2. Neighbor-joining tree of COI gene sequences of *emas*, *bintang*, and *hibrid* pompano and related species as outgroup constructed with bootstrap value 1000. A. Based on COI gene sequences, B. Based on 16S rRNA gene sequences

In conclusion All DNA barcode sequences of three types of pompano (*emas, bintang,* and *hibrid* pompano) were validated as *Trachinotus blochii* with a range of 99.85-100% by cytochrome oxidase subunit I (COI) and 100% by 16S ribosomal RNA. This study contributed essential genetic data to the NCBI database by both gene sequences. Despite morphological differences, three pompano types were successfully authenticated into the same species because they have identical nucleotide base arrangements and can be presumed from the same ancestor.

ACKNOWLEDGEMENTS

Writers would like to thank the Batam Marine Cultivation Fisheries Center (BPBL Batam) for their support in obtaining cultivated pompano specimens and giving a research grant.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in [NCBI] at https://www.ncbi.nlm.nih.gov/, reference number [OP046714 OP046715 OP046716 OP046717 OP046718 OP046719 OP046720 OP046721 OP046722 OP046723 OP046724 OP046725 OP046726 OP046727 OP046728 OP047720 OP047721 OP047722 OP047723 OP047724 OP047725 OP047726 OP047727 OP047728 OP047729 OP047730 OP047731 OP047732 OP047733 OP047734].

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