Barcoding of Sparidae collected during east monsoon season in the eastern Indian Ocean south of Java, Indonesia

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Abstract. Previous studies have reported up to seven Sparidae species from Indonesia and Palabuhan Ratu Fishing Port, south of West Java. To our knowledge, no study has reported the diversity of Sparidae in the eastern Indian Ocean, south of Java, Indonesia, during the east monsoon season. Species diversity could be assessed through morphological identification and validated using molecular barcoding. Therefore, this study aimed to assess the diversity of Spariade from the eastern Indian Ocean, south of Yogyakarta to Palabuhan Ratu in West Java during the east monsoon season. Morphological identification resulted in three Sparidae species, which were fished by fishermen during the east monsoon season in the eastern Indian Ocean south of Yogyakarta to Pelabuhan Ratu, West Java. Genetic similarity, genetic identity, genetic distance, and monophyly of the observed morphotypes validated the morphological identification. Sparidae species collected during the east monsoon season in the eastern Indian Ocean south of Yogyakarta to Palabuhan Ratu were *Acanthopagrus pacificus*, *Argyrops bleekeri*, and *Dentex tumifrons*. Our findings prove that *D. tumifrons* is abundant in the eastern Indian Ocean south of Java. In conclusion, three Sparidae species were found in the eastern Indian Ocean south of Java during the east monsoon season.
Key Words: genetic distance, genetic identity, monophyly, morphology, snapper.

Introduction. The eastern Indian Ocean south of Java has productive fishing areas in Indonesia (Ma'mun et al 2017). Various economically important fish species are landed daily in the region throughout the year, including in the fishing ports and fish auction center from the southern region of Yogyakarta to Palabuhan Ratu, West Java (BPS-Statistics of Sukabumi Regency 2021; BPS-Statistics of Garut Regency 2021; BPS-Statistics of Pangandaran Regency 2021; BPS-Statisctics of Cilacap Regency 2021; BPS-Statistics of Bantul Regency 2021). Nevertheless, the eastern Indian Ocean at southern Java Island is affected by east and west monsoon seasons. The east monsoon occurs from June to August, while the west runs from December to February. There are also two transition seasons from August to November and March to May. The east monsoon is characterized by extreme weather, such as high tides, strong currents and wind (Ahmad et al 2019). This season is well known as the Indian Ocean Dipole phenomena, similar to El Nino in the Pacific Ocean (Ahmad et al 2019; Purba & Khan 2019). Therefore, the diversity of fish landed in the fishing ports in the eastern Indian Ocean south of Java is highly variable depending on seasons (Lumban-Gaol et al 2021) and on the different captured fish species (Imron et al 2021).

The Sparidae family is among the economically valuable fisheries commodities that has never been reported in the annual report of the district governments in the southern part of Java (BPS-Statistics of Sukabumi Regency 2021; BPS-Statistics of Garut Regency 2021; BPS-Statistics of Pangandaran Regency 2021; BPS-Statisctics of Cilacap Regency 2021; BPS-Statistics of Bantul Regency 2021). This fish family consists of five subfamilies, 39 genera, and 166 species (Parenti 2019). Indonesian marine waters have seven species of Sparidae (Iwatsuki et al 2010; Froese & Pauly 2022). A previous study reported that *Dentex tumifrons* is the only Sparidae species landed at the Palabuhan Ratu fishing port, West Java (Muchlis & Surachman 2015). *Acanthopagrus berda* was also

reported in Java (Dahruddin et al 2016). It has been also summarized by Froese & Pauly (2022) that only one individual of *D. tumifrons* was reported in the eastern Indian Ocean south of Bali, Indonesia. Moreover, Fricke et al (2022) stated that *D. tumifrons* are distributed in the Western Pacific from China and Taiwan north to Korea, Japan, and Russia. Therefore, there is no comprehensive information about the Sparidae diversity during the east monsoon season in the eastern Indian Ocean south of Java, specifically from southern West Java to Yogyakarta.

The diversity could be assessed on species or genetic level, but the data could only be achieved when the status of the specimens has been validly determined. The species status could be delineated based on conventional taxonomic studies using morphological and molecular identification of a barcode marker (Nuryanto et al 2021). Morphological identification is still a convenient and faster method of species identification. In a particular condition, the identification might lead to misidentification because specimens collected from different regions might present morphological variations (Khan et al 2019; Muslimin et al 2020). Moreover, specimens from different life stages might also present morphological differenced (Ko et al 2013). In such phenomena, molecular barcoding is a promising tool for highly precise species identification (Dahruddin et al 2016; Syaifudin et al 2020). This method can be used for species validation and to overcome the problem of morphological identification (Nuryanto et al 2021). The cytochrome c oxidase 1 (COI) gene is a standard molecular marker for animal barcoding (Ratnasingham & Hebert 2013), including fish (Hubert et al 2012; Karahan et al 2017; Guimaraes-Costa et al 2019) and successfully utilized for Sparidae barcoding (Ahmed et al 2021).

The COI gene showed variable intraspecific genetic distance among animal groups, from 0.00 to 0.085 (Pereira et al 2013; Diaz et al 2016; Ali et al 2020; Sholihah et al 2020). Therefore, previous studies used a convenient genetic threshold during species determination. Amatya (2019) used 97% genetic identity as a threshold value for species boundaries. Some studies used more strict values of 98% (Abdalwahhab et al 2020), 99% (Hanner et al 2011; Cote et al 2013; Aguilar et al 2017) and higher than 99% (Ha et al 2019) as a species border for genetic identity, while other studies used a genetic threshold higher than 3% (Candek & Kuntner 2015; Karanovic 2015; Bhagawati et al 2021; Setyaningrum et al 2022), although other considerations should be made (Hanner et al 2011).

This study aimed to assess the species diversity of Spariade with a special focus on the areas spanning from the eastern Indian Ocean south of Yogyakarta up to southern Palabuhan Ratu in West Java during the east monsoon season.

Material and Method

Description of the study sites. The fish were collected from fishing ports and auction centers on the southern coast of West Java to Yogyakarta. These fishing ports and auction centers receive fish collected from the eastern Indian Ocean south of Java. The samples were obtained from Palabuhan Ratu Fishing Port, Sukabumi, Pamayangsari Auction Centre, Tasikmalaya, Pangandaran Fishing Port, Pangandaran, in West Java, Jetis Fish auction center, Cilacap, and Logending Fishing Port, Kebumen Regency, Central Java, and Gunung Kidul Auction Centre, Gunung Kidul Regency, Yogyakarta. The sampling sites are illustrated in Figure 1.

Sample collections. Fish samples were collected purposively from each sampling site based on general body form. The 38 Sparidae's suspected members were collected during the field trips in the east monsoon season from June to August 2022. This study obtained complete specimens and tissue samples. The complete specimens were collected for conventional identification using morphological characteristics. Before chemical preservation in 70% ethanol, fresh specimens were photographed to obtain the original color. Tissue samples of approximately 0.5 cm^2 of the right pectoral fin were collected from each individual and preserved in 96% ethanol. Furthermore, tissue samples were utilized for barcoding and genetic diversity analysis.

Figure 1. Map of sampling sites on the southern coast of Java, from Palabuhan Ratu (west) to Gunung Kidul (east); source: google earth.

Morphological identifications. Morphological identification was conducted based on general body form, number of spines and rays on the dorsal fin and on the anal fin. Additional descriptive and morphometric characters were also examined (Table 1). Body parts measurements were carried out using a mattress thread to follow the curvatures of the body parts. The measurement results were calibrated using a caliper with an accuracy of 0.01 mm.

Molecular barcoding. A total of eleven specimens were shipped to a company for COI barcoding, and the procedures of COI barcoding were as follows. The genomic DNA extraction was analyzed with the gSYNC™ DNA Extraction Kit (Geneaid, GS300). Nucleic acid (genomic DNA) concentration was measured using Nanodrop™ 2000/2000c spectrophotometers. Molecular analysis was referred to Protocol Species Barcoding Fish GMS-165, Genetika Laboratory of Genetika Science Indonesia, in 2021.

PCR amplification was conducted with (2x) MyTaq HS Red Mix (Bioline, BIO-25048) and KOD FX Neo (Toyobo, KFX-201). The components of the 1x25 µL PCR Master Mix were dd H2O 9.5 µL; MyTaq HS Red Mix, 2x12.5 µL; 10 µM VF2_t1 0.5 µL; 10 µM Fish F2_t1 0.5 µL; 10 µM Fish R2_t1 0.5 µL; 10 µM Fish FR1d_t1 0.5 µL; and DNA Template 1 µL. Primer sequences of PCR amplification were VF2-t1 5'-TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC-3', FR1d-t1 5'-CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA-3', FishR2_t1 5'-CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA-3', and FishF2_t1 5'-TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC-3' (Ivanova et al 2007).

The predenaturation phase initiated the PCR cycling for 1 minute (95°C). Subsequently, the actual PCR amplification, denaturation, annealing, and extension processes were conducted for 35 cycles, 15 seconds (95°C), 15 minutes (50°C), and 45 seconds (72°C). The PCR products (1 µL) were assessed by electrophoresis with 1% TBE agarose and Marker 100bp DNA ladder (loaded 2 µL). The quality and length of the PCR products were analyzed by agarose gel electrophoresis, and bi-directional sequencing was performed at 1st base Asia.

Data analysis. The morphospecies concept was applied during identification. Morphological data of each specimen were analyzed descriptively by comparing the characteristic of Sparidae available in scientific references (Iwatsuki et al 2007; Iwatsuki et al 2010; Yennawar & Tudu 2012; Siddiqui et al 2014; Froese & Pauly 2022), and the

comparison of results indicated the samples' taxonomic status. The genetic species concept was applied based on genetic identity through a basic local alignment search tool (BLAST) to conspecific sequences in the GenBank and genetic similarity test to the reference species in BOLDsystems based on biological identity index-BIN ID (Ratnasingham & Hebert 2013). In this study, genetic similarity and a homology of 97% were used as the threshold for species boundary. Genetic distance and phylogenetic relationships among samples with their conspecific species were estimated to support similarity and identity data. Furthermore, pairwise genetic distance was estimated based on the Kimura 2-parameter substitution model, which includes transition and transversion, assuming the substitution rate was uniform among sites. A similar calculation setting was utilized for overall or the average genetic distance among samples, and the analysis was performed in MEGA XI (Tamura et al 2021). The taxonomic tree was reconstructed using the maximum parsimony (MP) algorithm based on the K2P substitution model, and the tree was also developed in MEGA XI (Tamura et al 2021). Branching polarity was gained by 1000 bootstraps replication and outgroup comparison. The sample De-3, *Pomadassys argyreus* (GU673692, BOLD:AAD667, KY371983, and *P. maculatus* (MH230986) were utilized as the outgroup species.

Results. The 38 studied specimens have an oblong, deep, and compressed body form. The head is large with a steep upper profile anterior the dorsal fin. For a more detailed examination of the body form, the samples were divided in 3 morphotypes (Figure 1).

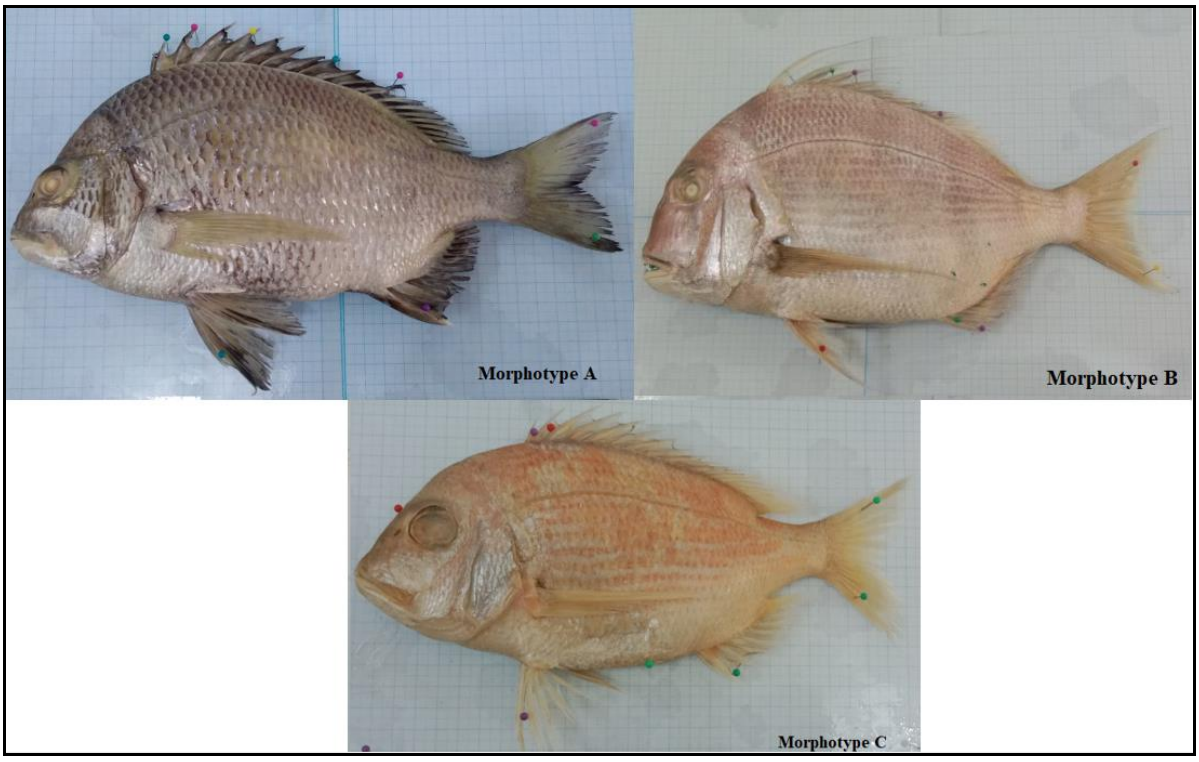

Figure 1. Three Sparidae morphotypes observed during east monsoon season in the south of Palabuhan Ratu to Yogyakarta fishing ports.

Morphotype A consisted of 20 individuals, morphotype B consisted of 13 individuals, and morphotype C consisted of 5 individuals. The morphological features of each morphotype are summarized in Table 1.

Table 1

Morphological features of studied Sparidae's morphotypes

Morphological characteristic	*Morphotype A*	*Morphotype B*	*Morphotype C*
Body form	Moderately oblong	Oblong	Moderately oblong
Upper profile	Steep	Deep	Steep
Preopercula	Scaly	Scaly	Scaly
Opercula	Scaly	Scaly	Scaly
Cheek	Scaly	Scaly	Scaly
Interorbital distance	Scaly	Scaly	Scaly
Dorsal spine	XI	X	XI-XII
Dorsal rays	11	10-11	10
Anal spine	III	III	III
Anal rays	8-9	8-9	8
Scale	Ctenoid, moderately large	Ctenoid, large	Ctenoid, moderately large
Standard length (SL) (cm)	20.14±2.79	26.19±1.80	22.58±2.34
Total length (TL) (cm	25.46±3.35	33.23±2.28	28.30±2.66
Head length (HL) (cm)	6.91±0.76	8.69±0.84	8.16±1.47
Head height (HH) (cm)	9.23±2.12	13.98±2.69	10.46±2.38
Eye diameter (ED) (cm)	1.32±0.22	2.08±0.46	2.24±0.34
TL:SL	1.26±0.45	1.27±0.70	1.25±0.47
HL:SL	0.34±0.12	0.33±0.18	0.36±0.15
ED:HL	0.07±0.08	0.08±0.04	0.09±0.04

Similarity test. For the COI gene fragment, eleven samples of Sparidae were successfully sequenced. The sequence test for the reference species in the BOLDsystems database showed that the samples have genetic similarities ranging from 99.31% (between sample KB-JT5 and *D. tumifrons*) to 100% (between some specimens and their references). The test was based on two top hits conspecific references. The exact similarity values of the three species are presented in Table 2.

Table 2

Genetic similarity between samples and their conspecific references in BOLD systems

Sample code	*Similarity (%)*	*Reference species*	*BIN ID*
Dr1	99.33	*Acanthopagrus pacificus*	BOLD: AAF1278
	99.33	*Acanthopagrus pacificus*	
JT1-Trisi	100	*Argyrops bleekeri*	BOLD: AAB3719
	100	*Argyrops bleekeri*	
KB-JT3	99.31	*Dentex tumifrons*	BOLD:AAD0508
	99.31	*Dentex tumifrons*	
PGN-SP-1-15	100	*Acanthopagrus pacificus*	BOLD:AAF1278
	100	*Acanthopagrus pacificus*	
PGN-SP-1-16	100	*Acanthopagrus pacificus*	BOLD: AAF1278
	100	*Acanthopagrus pacificus*	
PGN-SP-1-17	100	*Acanthopagrus pacificus*	BOLD: AAF1278
	100	*Acanthopagrus pacificus*	
PGN-SP-1-18	99.84	*Acanthopagrus pacificus*	BOLD: AAF1278
	99.84	*Acanthopagrus pacificus*	
PGN-SP-1-19	99.84	*Acanthopagrus pacificus*	BOLD: AAF1278
	99.84	*Acanthopagrus pacificus*	
PGN-SP-1-20	100	*Acanthopagrus pacificus*	BOLD: AAF1278
	100	*Acanthopagrus pacificus*	
PL-SP-5-01	100	*Argyrops bleekeri*	BOLD: AAB3719
	100	*Argyrops bleekeri*	
TSK-SP-3-01	100	*Argyrops bleekeri*	BOLD: AAB3719
	99.84	*Argyrops bleekeri*	

Identity test. The taxonomic status of the samples was also checked through a genetic identity test using the basic local alignment search tool (BLAST). Furthermore, the species identity was determined based on percentage and expected value to two top hits reference species. The samples' genetic identity ranged from 99.32% to 100%. The complete genetic identity and expected value for each sample are presented in Table 3.

Table 3

Genetic identity and expected value of the samples compared to the conspecific reference in GenBank

Sample code	*E-value*	*Identity (%)*	*Reference species and accession number*
Dr1	0.00	99.33	*Acanthopagrus pacificus* GU673695
	0.00	99.16	*Acanthopagrus pacificus* GU673694
JT1-Trisi	0.00	99.84	*Argyrops bleekeri* KU682551
	0.00	99.84	*Argyrops bleekeri* KJ012283
KB-JT3	0.00	99.32	*Dentex tumifrons* KY371533*
	0.00	99.32	*Dentex tumifrons* KJ012365*
PGN-SP-1-15	0.00	100	*Acanthopagrus pacificus* GU673695
	0.00	99.84	*Acanthopagrus pacificus* GU673694
PGN-SP-1-16	0.00	100	*Acanthopagrus pacificus* GU673695
	0.00	99.84	*Acanthopagrus pacificus* GU673694
PGN-SP-1-17	0.00	100	*Acanthopagrus pacificus* GU673695
	0.00	99.84	*Acanthopagrus pacificus* GU673694
PGN-SP-1-18	0.00	99.68	*Acanthopagrus pacificus* GU673695
	0.00	99.52	*Acanthopagrus pacificus* GU673694
PGN-SP-1-19	0.00	99.84	*Acanthopagrus pacificus* GU673695
	0.00	99.68	*Acanthopagrus pacificus* GU673694
PGN-SP-1-20	0.00	100	*Acanthopagrus pacificus* GU673695
	0.00	99.84	*Acanthopagrus pacificus* GU673694
PL-SP-5-01	0.00	99.33	*Argyrops bleekeri* KU682551
	0.00	99.16	*Argyrops bleekeri* KJ012283
TSK-SP-3-01	0.00	99.69	*Argyrops bleekeri* KU682551
	0.00	99.52	*Argyrops bleekeri* KJ012286

Note: * - synonym of *Evynnis tumifrons* (Fricke et al 2022).

Genetic distance. Genetic distance between morphotypes and their relatives available in GenBank ranged between 0.000 and 0.025. Meanwhile, the pairwise genetic distance between each morphotype and closed related taxa from GenBank ranged between 0.159 and 0.218. Pairwise genetic distances for all samples with species references in the GenBank are presented in Table 4.

Table 4

Pairwise genetic distances

Sample	*Reference species*		
	Acanthopagrus pacificus	*Argyrops bleekeri*	*Dentex tumifrons*
Morphotype A	0.000-0.025		
Morphotype B	0.186-0.196	0.000-0.005	
Morphotype C	0.199-0.218	0.159-0.171	0.000-0.007

Phylogenetic tree. The phylogenetic tree that shows the evolutionary relationships among samples and their conspecific references in the database was reconstructed using maximum parsimony algorithm with Kimura 2 parameters (K2P) substitution model. The K2P maximum parsimony tree is presented in Figure 2.

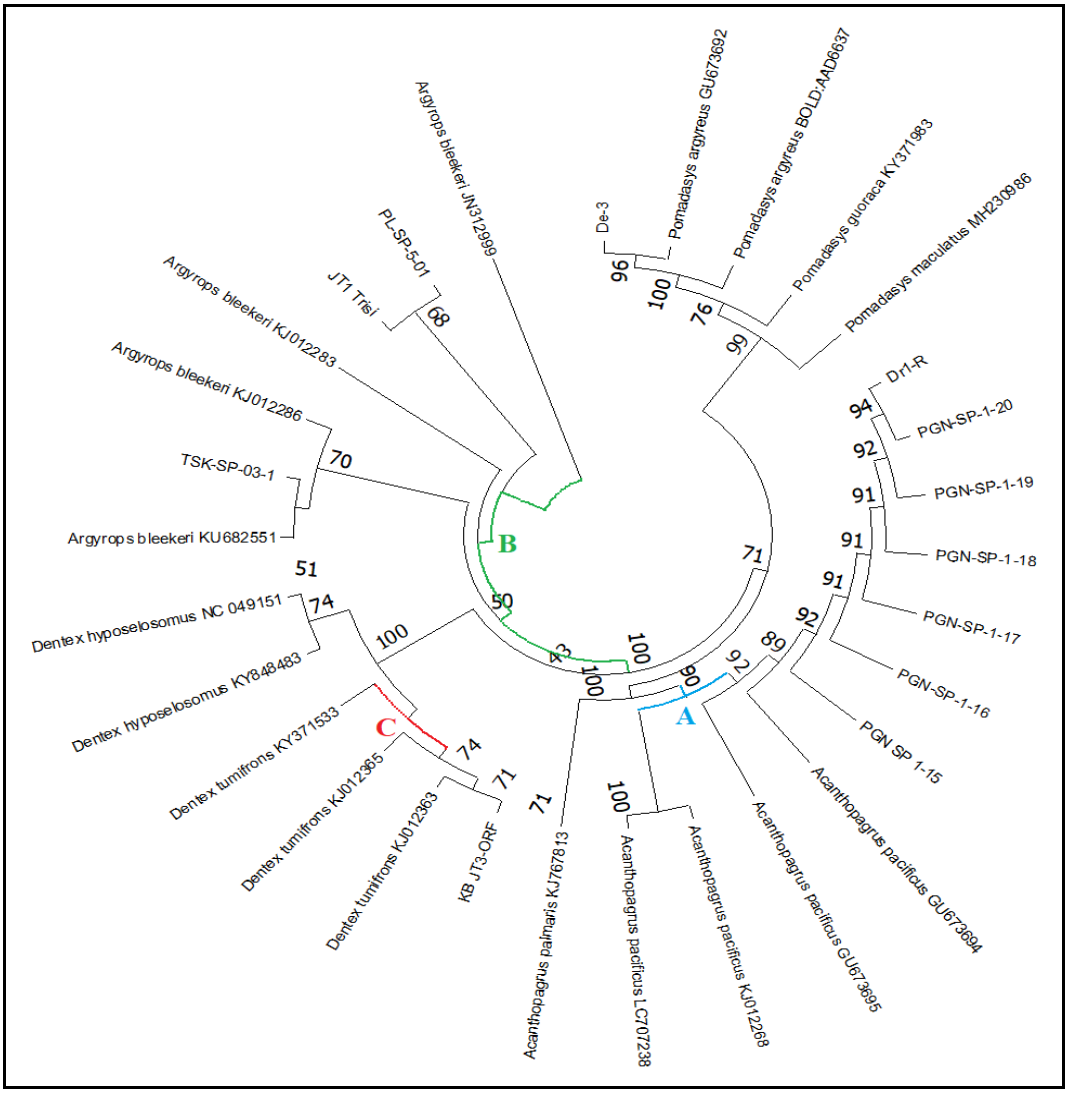

Figure 2. The K2P maximum parsimony tree showing monophyly between samples and their conspecific taxa retrieved from the GenBank.

Discussion. *A. pacificus* present scales on the preopercula, dorsal fins rays have the formula D XI, 11 (holotype, NSMT-P 93818), D XI, 11 (paratypes), and non-type specimens have D XI, 10-11 (usually XI, 11). Anal fin rays have the formula A III, 8 (holotype NSMT-P 93818), A III, 8 (paratypes), and A III, 8-9 usually A III, 8 (non-type). *A. berda* present no scales on the preopercula, dorsal fin rays have the formula D XI, sift rays are missing (holotype ZMUC P50555), while non-type specimens have the formula D XI-XII, 10-12 (usually D XI, 11) (Iwatsuki et al 2010). The samples of morphotype A had scales on the preopercula, dorsal-fin rays had the formula D XI, 11 and anal fin rays had the formula A III, 8-9. The morphotype A could be differentiated from *A. berda* by having scales on their preopercula. In contrast, the presence of scales on the preopercula of morphotype A was similar to *A. pacificus* as described by Iwatsuki et al (2010). Hence, morphotype A was morphologically identified as *A. pacificus*.

A. bleekeri have a deep body, highly compressed laterally. The formula of the dorsal fin rays is D XI, 11 and for anal fin rays it is A III, 8 (Yennawar & Tudu 2012). This is summarized by Froese & Pauly (2022), with *A. bleekeri* have D X-XI, 9-11 and A III, 8-9. *Argyrops spinifer* are characterized by D XII, 10 and A III, 8 (Siddiqui et al 2014) and it has been summarized that *A. spinifer* have D XI-XIII, 9-10 and A III, 7-8. The samples of morphotype B present dorsal fin rays D X, 10-11 and A III, 8-9. These characteristics were more similar to the characters of *D. tumifrons* that to those of *D. spinifer*. Therefore, the current study identified morphotype B as *D. tumifrons*.

D. tumifrons have the dorsal fins rays formula of D XII 10 and anal fin rays A III, 8 (Akazaki & Seret 1999; Iwatsuki et al 2007). The geographic distribution of *D. tumifrons* includes China and Taiwan northward to Korea, Japan, and Rusia (Fricke et al 2022; Froese & Pauly 2022). Acording to Iwatsuki et al (2007), *D. spariformis* have D XII, 10 and A III, 8 (lectotype), D XII, 10 and A III, 8 (paralectotype), and D XII, 10 and III, 8 (non-type). *D. spariformis* are distributed from Bali eastward to Queensland (Fricke et al 2022; Froese & Pauly 2022). The samples of morphotype C have dorsal fin rays D XI-XII, 10 and anal fin rays A III, 10. This is similar either to *D. tumifrons* or *D. spariformis*. However, since the samples were collected from eastern Indian Ocean south of Java from Yogyakarta to Palabuhan Ratu West Java, we believe the morphotype C is *D. tumifrons*. *D. tumifrons* was also reported in Palabuhan Ratu fishing port (Muchlis & Surachman 2015).

This study has confirmed the existence of *D. tumifrons* in the eastern Indian Ocean outside Bali. According to Iwatsuki et al (2007), the existence of *D. tumifrons* is questionable because only 1 specimen was found in Bali. *D. tumifrons* was abundant in the Auction Center of Gunung Kidul, Yogyakarta, and Jetis, Cilacap, Central Java, which receives commodities collected at the eastern Indian Ocean south off Java. The presence in Southern Java was also reported in Palabuhan Ratu (Muchlis & Surachman 2015). The determined taxonomic status might be incorrect, because *D. tumifrons* is similar to *D. spariformis,* which is also found in some parts of eastern Indian Ocean (Iwatsuki et al 2007). Therefore, the findings need to be validated through molecular identification to ensure the species status of the fish samples.

Genetic similarity values summarized in Table 2 proved that all samples had high genetic similarities (99.31% to 100%) to their reference species in BOLDsystems. Similarly, the genetic identity test to conspecific references the in GenBank proved that all specimens have a high percentage (99.16 to 100%) of genetic identities (Table 3). Both genetic similarity and identity were higher than the predetermined value of 97% as species border. The values could become strong indicators that the samples belong to the same species as their conspecific references in the BOLDsystems and GenBank. Previous studies reported that genetic similarity and identity of 97% were commonly used in species determination during molecular barcoding of animal species (Ratnasingham & Hebert 2007; Nuryanto et al 2018; Amatya 2019; Riani et al 2021). Even a genetic similarity and identity as low as 95% are also acceptable as a species boundary in species determination in some cases (Karanovic 2015; Bhagawati et al 2022; Setyaningrum et al 2022). In such cases, additional consideration is needed (Higashi et al 2011; Candek & Kuntner 2015; Lin et al 2015). It has been well known that several genetic similarity and identity values have been used in a wide range of animal species (Cote et al 2013; Aguilar et al 2017; Ha et al 2019; Abdalwahhab et al 2020), because COI genetic distances are high among animal groups (Pereira et al 2013; Diaz et al 2016; Ali et al 2020; Sholihah et al 2020).

Genetic distances between samples and their reference species ranged from 0.000 to 0.025 (Table 4). These values were below the genetic distance value commonly used as a species limit in animal molecular identification, i.e., 0.03 (Pereira et al 2013; Kusbiyanto et al 2020; Mohammed et al 2021). Pereira et al (2013) reported that genetic distance within fish species might reach 0.085. The finding proved that genetic distances among conspecific individuals might be higher than 0.03 and within species genetic distance might vary greatly in fish groups (Puckridge et al 2013; Diaz et al 2016; Ali et al 2020; Sholihah et al 2020). Therefore, the predetermined threshold of 0.03 genetic distance is the reliable value for species determination in this study and strengthens the placement of samples into three genetic species.

The phylogenetic tree (Figure 2) showed that all samples and their conspecific references form monophyletic clades compared to the outgroup species. A detailed examination of the tree proved that all samples formed monophyletic clades with each of their close relatives with high bootstrap support. Each specific clade was separated from other clades, forming 3 different clades (A, B, and C). The monophyly of Sparidae samples with their conspecific references strengthened the placement of the samples into the same species. The use of monophyly as a basis for species determination was also

reported in several other studies (Xu et al 2015; Kusbiyanto et al 2020; Palecanda et al 2020).

According to genetic similarity and identity, genetic distance, and monophyly data, morphotype A was identified as *A. pacificus*. Morphotype B was genetically identified as *A. bleekeri*. Morphotype C was identified as *D. tumifrons*. These results confirmed that COI barcoding is a reliable method for species level identification (Hubert et al 2012; Karahan et al 2017), with some exception in closely related species (Bhattacharjee et al 2012). COI barcoding is a reliable method to reveal cryptic species (Dasmahapatra et al 2010; Guimaraes-Costa et al 2019) and can be used to validate morphological identifications (Song et al 2013; Yi et al 2017).

Previous studies reported two species of Sparidae from Java, i.e., *D. tumifrons* (Muchlis & Surahman 2015) and *A. berda* (Dahruddin et al 2016). The current study reported more Sparidae species by adding a new record, of *A. pacificus*. More species of Sparidae could be observed in the eastern Indian Ocean south of Java if the sampling is carried out throughout the year and covers the entire southern Java from East Java to Banten, rather than only from Palabuhan Ratu West Java to Yogyakarta. Our assumption is that fish diversity and production in the eastern Indian Ocean south of Java are significantly affected by the monsoon seasons (Lumban-Gaol et al 2021; Imron et al 2021). Additionally, Iwatsuki et al (2007, 2010) reported that Indonesian marine waters host seven species of Sparidae.

Conclusions. Based on morphology and molecular barcoding, this study has successfully documented three Sparidae species: *Acanthopagrus pacificus*, *Argyrops bleekeri*, and *Dentex tumifrons* during the east monsoon season of 2022 from the eastern Indian Ocean, south of Java, from Yogyakarta to Palabuhan Ratu, West Java. Molecular barcoding validates morphological identification on the taxonomic status of the Spariade species.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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