BIODIVERSITAS
Volume 24, Number 11, November 2023
Pages: 6023-6030

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d241123

First record of red seabream, *Pagrus major* existence in the eastern Indian Ocean south of Java, Indonesia revealed by DNA barcoding

AGUS NURYANTO♥, DIAN BHAGAWATI, ELLY TUTI WINARNI, ASWI ANDRIASARI ROFIQOH
Faculty of Biology, Universitas Jenderal Soedirman. Jl. Dr. Soeparno No. 63, Banyumas 53112, Central Java, Indonesia.
Tel.: +62-281-638794, Fax.: +62-281-631700, ♥email: agus.nuryanto@unsoed.ac.id

Manuscript received: 16 October 2023. Revision accepted: 17 November 2023.

Abstract. *Nuryanto A, Bhagawati D, Winarni ET, Rofiqoh AA. 2023. First record of red seabream,* Pagrus major *existence in the eastern Indian Ocean south of Java, Indonesia revealed by DNA barcoding. Biodiversitas 24: 6023-6030.* The red seabream, *Pagrus major*, is widely believed to be mainly inhabited in the Northwest Pacific region. Although there are no previous studies reporting its existence in the Indian Ocean, a morphologically similar species of silver seabream, *P. auratus*, has been discovered. To comprehensively assess the geographical distribution of both species, there is a need to carry out a taxonomic study using DNA barcoding technique. Therefore, this study aimed to evaluate the taxonomic status of seabream (Sparidae) in the east Indian Ocean south of Java, Indonesia, using the cytochrome c oxidase 1 barcoding. Fish specimens were collected during the June to August 2023 field trips at fishing ports and auction centers along West Java and Banten southern coastlines. A total of 51 specimens were successfully barcoded and 99% genetic similarity was used as a species border. The results showed that three seabream species were identified, with intraspecific genetic similarity ranging from 99.77% to 100% and low genetic distance between 0.000 and 0.005 to one top hit conspecific. Furthermore, 21 specimens were identified as *P. major* with high genetic similarity ranging from 99.43% to 100% and genetic homology ranging from 99.44% to 100%. This discovery represented the first record of *P. major* in the east Indian Ocean, South Java, presenting essential data need for capture fisheries management in the region.

Keywords: Genetic distance, homology, similarity, *Sparus*

INTRODUCTION

The Sparidae is among valuable fisheries commodities in Indonesia (Nuryanto et al. 2023). There are 166 known species in the family Sparidae and divided into 39 genera (Parenti 2019). Seven sparid species have been described from Indonesia marine waters. These species are *Acanthopagrus pacificus*, *Argyrops bleekeri*, *Argyrops spinifer*, *Dentex spariformis*, *Pagrus auratus* (*Chrysophrys auratus*), *Rhabdosargus niger*, and *Rhabdosargus sarba* (Iwatsuki et al. 2010; Froese and Pauly 2023). Recently, Nuryanto et al. (2023) reported the presence of *Dentex tumifrons* (*Evynnis tumifrons*) in the Indian Ocean south of Java. No study has reported *Pagrus major* from the Indonesia marine waters.

Pagrus major (Temminck & Schlegel, 1843), a red seabream from the Sparidae, is widely distributed in the northwestern Pacific from the northeastern South China Sea to Japan, except the Philippines (Fricke et al. 2023, Froese and Pauly 2023). In Japan, it has been cultivated for over 60 years, gaining widespread popularity in the Mediterranean Sea since 1985 (Dulčić and Kraljevic 2007). However, discovery of *P. major* outside native geographic range is rare (Yannis et al. 2019) with only two studies reporting its occurrence, including in the Adriatic and Eastern Mediterranean Seas (Dulčić and Kraljevic 2007; Yannis et al. 2019).

A morphologically similar species of Sparidae, namely *P. auratus* (Fricke et al. 2023; Froese and Pauly 2023), has been reported to live in the Indo-West Pacific region. Due to their similarities, both species had been synonymized (Eggleston 1974), with *P. major* being identified as a subspecies of *P. auratus* (Tabata and Taniguchi 2000). This historical confusion has been dismissed (Fricke et al. 2023) and both species are regarded as two different species. A previous study using molecular character supported this placement into two distinct genetic species (Tabata and Taniguchi 2000).

The morphological similarities between *P. major* and *P. auratus* have posed a taxonomic challenge during species identification (Yannis et al. 2019), specifically for beginners. This challenge could be solved by applying molecular identification or DNA barcoding (von der Heyden et al. 2014; Chac and Thinh 2023). The partial sequence of the Cytochrome c oxidase 1 (COI) gene is accepted and commonly used as a molecular marker for animal barcoding (Al Amry et al. 2016; Dahruddin et al. 2016; Syaifudin et al. 2020). This technique has proven reliable for precise species identification (Ceruso et al. 2018; Abdalwahhab et al. 2020; Caputi et al. 2021; Ceruso et al. 2021; Mohammed-Geba et al. 2021) and successfully solved the problem in fish identification (Hubert et al. 2012; Nuryanto et al. 2023), including Sparidae (Ahmed et al. 2021), and product determination (Almerón-Souza et al. 2018; Hu et al. 2018; Ha et al. 2019). Furthermore, it was successfully used to unmask the presence of cryptic species (Becker et al. 2015; Bilgin et al. 2015; Muchlisin et al. 2017), unveiling undescribed and overlooked fish species, which might be eluded by morphological identification (Nuryanto et al. 2021).

The Eastern Indian Ocean in southern Java is among the most productive fishing areas in Indonesia, characterized by several fishing ports and auction centers (Nuryanto et al. 2023). Various fish species have become essential fisheries commodities in the regions, including the members of Sparidae (Dahruddin et al. 2016). Previous studies reported that four species of Sparidae was collected in the eastern of the Indian Ocean and traded in the fishing ports and auction center in South Java, including *A. berda*, *A. pacificus*, *A. bleekeri*, and *E. tumifrons* (Dahruddin et al. 2016; Nuryanto et al. 2023). Despite the numerous marine resources in the region, there is a lack of information regarding the presence of *P. auratus* or *P. major* in the eastern Indian Ocean south of Java, Indonesia. Therefore, this study aimed to evaluate the taxonomic status of seabream (Sparidae) in the east Indian Ocean south of Java, Indonesia, through the COI barcoding. The results are expected to provide valuable insight for further investigation to preserve the existence of *P. major* and enhance the management of marine fisheries in the region.

MATERIALS AND METHODS

Study site and fish specimen collection

Fish specimens were bought from collectors at several fishing ports which were selected randomly and auction centers along the south coastline of West Java and Banten Provinces, Indonesia, as illustrated in Figure 1. The name of sampling sites is presented in Table 1. The sampling efforts were carried out during the field trips from the middle of June to the end of August 2023. We bought all sparid specimens during each field trip. The member of Sparidae was selected based on their morphological characteristic, especially upper profile. According to Nuryanto et al. (2023), the member of Sparidae have steepened and deepened upper profile (Figure 2).

Total genomic DNA sources and barcoding process

Small clips of pectoral fin were excised from each fish, preserved in absolute ethanol, and stored at 4°C before molecular analysis. All the specimens were shipped to a company for molecular barcoding and Genomic DNA was extracted using a DNA Miniprep Kit (Zymo Research, D4081) following the manufacturer's protocol. Subsequently, the Polymerase Chain Reaction (PCR) amplification of the COI gene was performed using the 2x MyTaq HS Red Mix (Bioline, BIO-25048). The amplification settings were started with an initial denaturation at 95°C for 1 minute. The process was continued with denaturation at 95°C for 15 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 45 seconds, with total cycles of 35 times.

Table 1. Sampling site notation and name

Notation	Name
I	Binuangeun Fishing Port
II	Bayah Fish Auction Center
III	Cisolok Fish Auction Center
IV	Pelabuhan Ratu Fishing Port
V	Ciwaru Fish Auction Center
VI	Jayanti Fishing Port
VII	Santolo Fishing Port
VIII	Pamayangsari Fish Auction Center
IX	Legok Jawa Fish Auction Center
X	Bojongsalawe Fish Auction Center
XI	Pangandaran Fishing Port

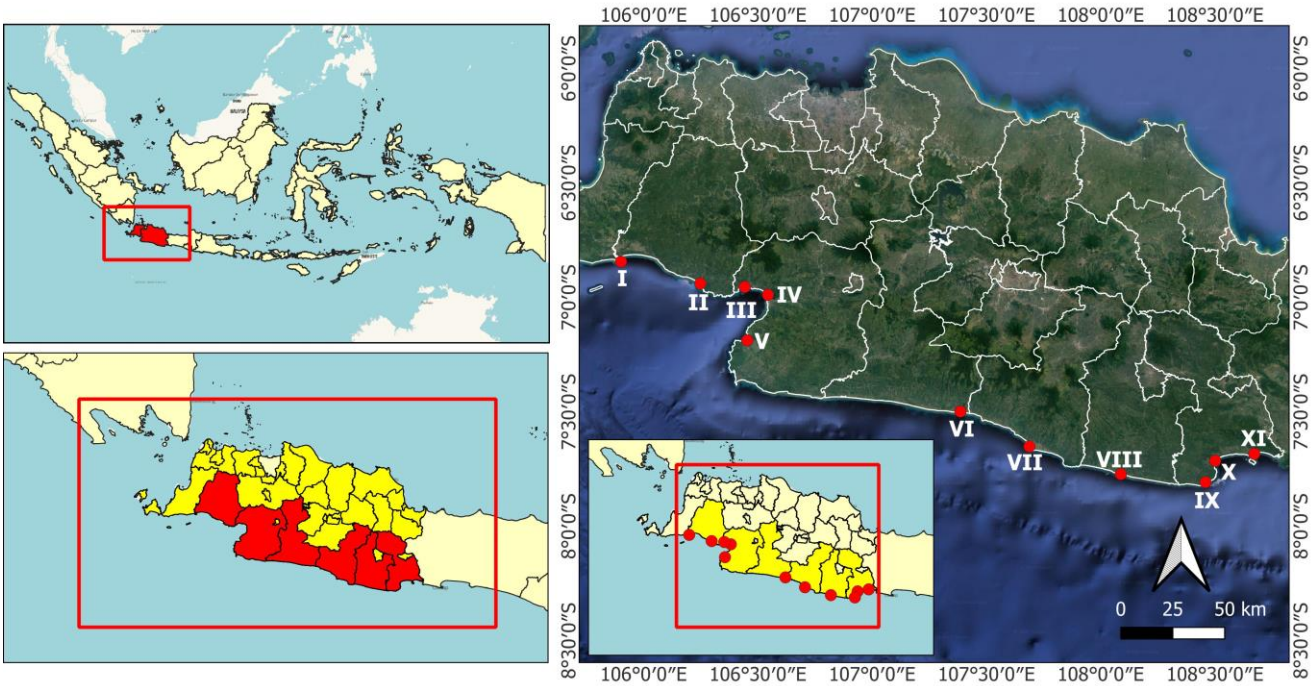

Figure 1. Indonesia map shows fishing ports and fish auction centers (sampling sites) along West Java and Banten coastline, Indonesia

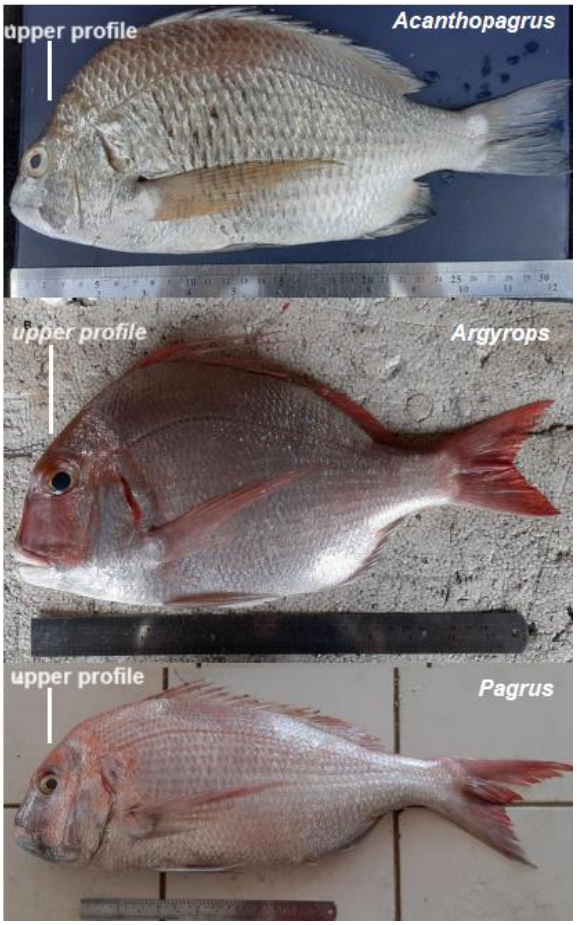

Figure 2. Sparid specimen obtained during the field trips

The final extension was carried out at 72°C for 3 minutes, completing one cycle. To amplify the target fragment of the COI gene, the following primer sets were used, as presented in Table 2. In each 25 µL PCR mixture, the chemical component was KOD FX Neo 1 µL, 2X PCR Buffer KOD FX Neo 12.5 µL, 2mM dNTPs 1 µL, 10pmol/µL of each primer was 1 µL, template DAN 1 µL, and ddH2O 9.5 µL. Total genomic DNA isolation and COI marker amplification were conducted at Genetika Laboratory (PT. Genetika Science Indonesia, Jakarta). The COI gene was sequenced using the bi-directional sequencing technique from Sanger methods at 1st BASE Asia in Kuala Lumpur, Malaysia (Nuryanto et al. 2023).

Sequence editing

Consensus sequence is a sequence DNA that represents the alignment of related sequences. In this study, consensus sequences were gained from the contig and trimming process of the forward and reverse sequences of COI gene in Bioedit 7.0 (Hall 1999). Functional COI gene sequences were obtained by altering raw contig sequences into open reading frame using the online ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder). The ORF Finder was configured with specific search parameters, including a minimal ORF length of 300 nucleotides, vertebrate mitochondrial genetic code, the activation of ATG as the ORF starts codon, and ignore nested ORF. The obtained ORF sequences were deposited in GenBank with the accession number OR512850-OR512881, OR527342-OR527344, OR591328-OR591343 (Table 2). The ORF sequences were aligned with conspecific sequences in GenBank to ensure the absence of gaps.

Data analysis

The genetic species concept was applied using a genetic similarity test to the reference species in barcode of life data systems (BOLDsystems) (Ratnasingham and Hebert 2007). This test was based on biological identity index-BIN ID (Ratnasingham and Hebert 2013) with search databases Species Level Barcode Records. The specimens were also subjected to genetic homology tests to reference species in GenBank using Basic Local Alignment Search Tool (BLAST). The search was set to standard database nucleotide collection and optimized for highly similar sequences. In this study, a genetic similarity and homology of 99% was used as the threshold for species boundary on Sparidae (Ahmed et al. 2021). Genetic distance among samples with their conspecific species were estimated to support similarity data. Furthermore, pairwise genetic distance was calculated using the Kimura 2-parameter substitution model, which included transition and transversion, while assuming a uniform substitution rate among sites. The calculation was performed in MEGA XI (Tamura et al. 2021).

RESULTS AND DISCUSSION

Genera assignment

A total of 51 Sparidae specimens were obtained during the field trips, with body weights ranging from 900 gr to 4600 gr, depending on the species available during the field trips. The identification process categorized the specimens into three genera by comparing their morphology with pictures available in the reference (Froese and Pauly 2023). As presented in Figure 2, the three genera were *Acanthopagrus*, *Argyrops*, and *Pagrus*.

DNA barcoding

Similarity tests of the ORF sequences showed genetic identity of samples ranging from 99.77% to 100% to the nearest three top reference species in BOLDsystems. The first top hit is presented in Table 2.

The genetic similarities presented in Table 2 showed that all Sparidae specimens obtained in this study were genetically identified at the species level, as their similarity value exceeded 99% of predetermined species border. These specimens were genetically barcoded into three species, namely *Acanthopagrus pacificus* (Iwatsuki et al. 2010), *Argyrops bleekeri* (Oshima, 1927), and *Pagrus major* (Temminck & Schlegel, 1843). Previous studies reported that the natural population of animal species showed broad range of genetic divergences or similarities. Moreover, the different intraspecific genetic similarity ranging from 92.5% to 99% (Pereira et al. 2013; Lim et al. 2016; Briñoccoli et al. 2020; Mohammed-Geba et al. 2021) has been used as species borders (Nuryanto et al. 2017; Abdalwahhab et al. 2020; Winarni et al. 2023). The standard value used in BOLDsustems was 97% (Ratnasingham and Hebert 2013; Nuryanto et al. 2018; Amatya 2019; Kusbiyanto

et al. 2020), while other reports utilized moderate genetic similarity ranging from 95% and 97%. However, reasonable genetic similarity during species delineation required logical and acceptable justification (Jeffery et al. 2011), such as geographic locality information between the sample and reference species (Higashi et al. 2011; Čandek and Kurtner 2015). Some investigations reported the use of stringent threshold values as species boundaries between 98% and 99% (Ha et al. 2019; Abdalwahhab et al. 2020; Salem et al. 2021; Abbas et al. 2022), particularly for newly diverged species. In this study, *P. major* and *P. auratus* showed similar morphology (Tabata and Taniguchi 2000; Froese and Pauly 2023), suggesting that they are newly diverged. Approximately, more than 99% of genetic similarity was reported in Sparidae specimens from the Mediterranean Sea (Ahmed et al. 2021). Consequently, 99% genetic similarity in this study was the most reliable threshold value as species border and could separate between *P. major* and *P. auratus*.

Table 2. Genetic identity of Sparidae specimens based on similarity value to their conspecific references in BOLDsystems

Sample code	Accession number	Similarity (%)	Reference species and BIN ID
PGN-Pa-01	OR512850	100	*Pagrus major* BOLD:AAC0553
PGN-Pa-02	OR512851	100	*Pagrus major* BOLD:AAC0553
PGN-Pa-03	OR512852	99.84	*Pagrus major* BOLD:AAC0553
PGN-Pa-04	OR512853	100	*Pagrus major* BOLD:AAC0553
PGN-Pa-05	OR591331	99.84	*Pagrus major* BOLD:AAC0553
PGN-Pa-06	OR591332	100	*Pagrus major* BOLD:AAC0553
PGN-Pa-07	OR591333	99.84	*Pagrus major* BOLD:AAC0553
PGN-Pa-08	OR591334	99.84	*Pagrus major* BOLD:AAC0553
PGN-Pa-09	OR591335	99.77	*Pagrus major* BOLD:AAC0553
PGN-Pa-10	OR591336	100	*Pagrus major* BOLD:AAC0553
PGN-Pa-11	OR591337	100	*Pagrus major* BOLD:AAC0553
BJS-Tb-01	OR512854	99.84	*Pagrus major* BOLD:AAC0553
BJS-Pa-02	OR591328	100	*Pagrus major* BOLD:AAC0553
BJS-Pa-03	OR591329	100	*Pagrus major* BOLD:AAC0553
BJS-Pa-04	OR591330	99.84	*Pagrus major* BOLD:AAC0553
GRT-01	OR591338	99.68	*Pagrus major* BOLD:AAC0553
TSK-01	OR591339	99.84	*Pagrus major* BOLD:AAC0553
JYT-01	OR591340	99.84	*Pagrus major* BOLD:AAC0553
JYT-02	OR591341	100	*Pagrus major* BOLD:AAC0553
JYT-03	OR591342	99.84	*Pagrus major* BOLD:AAC0553
JYT-04	OR591343	99.85	*Pagrus major* BOLD:AAC0553
BJS-APH-01	OR527342	100	*Acanthopagrus pacificus* BOLD:AAF1278
BJS-APH-02	OR512855	99.84	*Acanthopagrus pacificus* BOLD:AAF1278
BJS-APH-03	OR512856	100	*Acanthopagrus pacificus* BOLD:AAF1278
BJS-APH-05	OR512857	100	*Acanthopagrus pacificus* BOLD:AAF1278
BJS-APH-06	OR512858	100	*Acanthopagrus pacificus* BOLD:AAF1278
BJS-APH-07	OR512859	100	*Acanthopagrus pacificus* BOLD:AAF1278
BJS-APH-08	OR512860	100	*Acanthopagrus pacificus* BOLD:AAF1278
BJS-APH-09	OR512861	100	*Acanthopagrus pacificus* BOLD:AAF1278
BJS-APH-10	OR512862	100	*Acanthopagrus pacificus* BOLD:AAF1278
BJS-APH-11	OR512863	100	*Acanthopagrus pacificus* BOLD:AAF1278
BGN-Ap-01	OR512864	100	*Acanthopagrus pacificus* BOLD:AAF1278
BGN-Ap-02	OR512865	100	*Acanthopagrus pacificus* BOLD:AAF1278
BGN-Ap-03	OR512866	100	*Acanthopagrus pacificus* BOLD:AAF1278
BJS-Ap-01	OR527343	100	*Acanthopagrus pacificus* BOLD:AAF1278
BJS-Ap-02	OR527344	100	*Acanthopagrus pacificus* BOLD:AAF1278
BJS-AP-03	OR512867	99.84	*Acanthopagrus pacificus* BOLD:AAF1278
BJS-AP-04	OR512868	100	*Acanthopagrus pacificus* BOLD:AAF1278
BJS-AP-05	OR512869	100	*Acanthopagrus pacificus* BOLD:AAF1278
BJS-AP-06	OR512870	100	*Acanthopagrus pacificus* BOLD:AAF1278
BJS-AP-07	OR512871	100	*Acanthopagrus pacificus* BOLD:AAF1278
BJS-AP-08	OR512872	100	*Acanthopagrus pacificus* BOLD:AAF1278
BJS-AP-09	OR512873	100	*Acanthopagrus pacificus* BOLD:AAF1278
BJS-Tr-01	OR512874	100	*Argyrops blekkeri* BOLD:AAB3719
BJS-Tr-02	OR512875	100	*Argyrops blekkeri* BOLD:AAB3719
BJS-Tr-03	OR512876	100	*Argyrops blekkeri* BOLD:AAB3719
BJS-Tr-04	OR512877	100	*Argyrops blekkeri* BOLD:AAB3719
BJS-Tr-05	OR512878	100	*Argyrops blekkeri* BOLD:AAB3719
BJS-Tr-06	OR512879	100	*Argyrops blekkeri* BOLD:AAB3719
BJS-Tr-07	OR512880	100	*Argyrops blekkeri* BOLD:AAB3719
BJS-Tr-08	OR512881	100	*Argyrops blekkeri* BOLD:AAB3719

This study also used genetic distance as additional data during species delineation to support similarity data. Based on the results, low and slightly large genetic distances within species were observed in a genus. In contrast, high genetic distances were observed within a family, as presented in Table 3.

The intraspecific K2P genetic distance, presented in Table 3, supported the delineation of specimens into three different species as previously determined based on genetic similarity value. A low value below 0.010 was also reported in *Squalus graham* and *S. mitsukurii* (Ariza et al. 2022) as well as two genera of Haemulidae, namely *Brachygenys* and *Haemulon* (Cerqueira et al. 2021). Consequently, the use of 0.010 genetic distance as additional data for delineating specimens into species categories in this study was highly reliable (Ha et al. 2019). Other studies used considerable genetic distances from 0.02 to 0.05 as species borders during molecular barcoding (Čandek and Kuntner 2015; Karanovic 2015; Abdalwahhab et al. 2020; Boza et al. 2022) because animal group showed variable intraspecific sequence similarities (Díaz et al. 2016; Ali et al. 2020; Limmon et al. 2020; Sholihah et al. 2020). For example, the maximum K2P intraspecific genetic distance on Carangidae ranged from 0.00 to 0.048 (Mat Jaafar et al. 2012), while Neotropic fish had 0.000 to 0.085 (Pereira et al. 2013), with a higher value being of 0.148 being observed in freshwater *Hemiramphodon* (Lim et al. 2016).

Intrageneric K2P genetic distances ranging from 0.028 to 0.030 was observed between *A. pacificus* and *A. berda* (Dahruddin et al. 2016). These low values were similar to the 0.0072 observed in *S. albicaudatus* and S. *cubensis* (Ariza et al. 2022). In this study, the maximum genetic distance was lower compared to previous reports which obtained 24.8% for Neotropical fish (Pereira et al. 2013), 27.03% for indigenous fish in Bangladesh (Ahmed et al. 2019), as well as 18.25% for ophichthid fishes (Xing et al. 2020), and engraulid fish (Afrand et al. 2018).

First record of *Pagrus major* in the eastern Indian Ocean

Based on the discovery in this study, more than 10 top hits among conspecific references in BOLDsystem and GenBank for 21 samples used were *P. major*. The genetic identities of specimens are provided in Table 4, which lists the five top reference species with the closest genetic identity of specimens. For BLAST parameters, the query cover and e-value for all samples were 100% and 0.00, respectively (Accession numbers for the conspecific taxa were MK560702, MK560705; MK560706; KY371849, KY371848; KU199066, KU199070).

Table 3. The K2P genetic distances

Category	Genetic distance
Intraspecific	0.000-0.005
Intrageneric	0.028-0.030
Intrafamily	0.117-0.199

Table 4 shows that all five top reference species in both BOLDsystems and GenBank were only *P. major*, with none corresponding to *P. auratus*. Based on those genetic identity values, this study provided the first taxonomic information about the presence of *P. major* in the eastern Indian Ocean south of Java. *P. major* is a seabream species widely distributed in the Western Pacific (Fricke et al. 2023; Froese and Pauly 2023). However, there is limited information about this species outside its natural geographic range, specifically in the Adriatic and Eastern Mediterranean Seas, respectively (Dulčić and Kraljevic 2007; Yannis et al. 2019). A previous study (Dulčić and Kraljevic 2007) only reported one individual from the Adriatic Sea, while Yannis et al. (2019) four individuals were obtained in the Eastern Mediterranean Sea. This scarcity indicated that the individual was unintentionally escaping from marine culture, which was started in 1985 (Yannis et al. 2019). Although, there is no study regarding the existence of *P. major* from Indonesia (Froese and Pauly 2023), a survey has been conducted about Sparidae, particularly from Java (Muchlis and Surahman 2015; Dahruddin et al. 2016; Nuryanto et al. 2023). *Dentex (Evynnis) tumifrons* were reported from Pelabuhan ratu, West Java (Muchlis and Surahman 2015) and *Acanthopagrus berda* from Java (Dahruddin et al. 2016). A recent investigation documented three species of Sparidae, namely *A. pacificus*, *Argyrops bleekeri*, and *Evynnis tumifrons* from the eastern Indian Ocean South of Java, Indonesia (Nuryanto et al. 2023) but only collected samples during the east monsoon season. Consequently, this study provided the first data about the existence of *P. major* on the east Indian Ocean south coast of Java, Indonesia. During the field trips at the end of August, this study observed a high abundance of *P. major*, and additionally fish collectors reported the highest *P. major* catchment occurs in September until beginning of October 2023 (Personal Communications with some collectors from Pangandaran to Jayanti, Cianjur). A total of three taxa were obtained during this investigation, which was in line with a report from this region (Nuryanto et al. 2023) but with a different species composition. Furthermore, *A. pacificus*, *Ar. bleekeri*, and *P. major* were discovered and the study in east monsoon season found *A. pacificus*, *Ar. bleekeri*, and *Evynnis tumifrons* (Nuryanto et al. 2023). This study also reported higher Sparidae species compared to Dahruddin et al. (2016) but was still lower than that summarized by Froese and Pauly (2023). Based on the results, only 21 specimens were obtained from the coastline of West Java and Banten Provinces, indicating the need for further study about the existence of *P. major* in the eastern Indian Ocean south of Java, to obtain a broader sampling coverage. This is because more sampling coverage will provide better information to support the wider geographical range of *P. major*, extending beyond the Northwest Pacific, excluding the Philippines.

Table 4. Genetic similarity and homology among sparidae specimens with their reference species in BOLDsystems and GenBank

Sample code	*Pagrus major*	
	Similarity (%) (BOLD)	**Homology (%) (GenBank)**
PGN-Pa-01	100	100
	100	100
	100	100
	100	100
	100	100
PGN-Pa-02	100	100
	100	100
	100	100
	100	100
	100	99.84
PGN-Pa-03	99.84	99.84
	99.84	99.84
	99.84	99.84
	99.84	99.84
	99.84	99.84
PGN-Pa-04	100	100
	100	100
	100	100
	100	100
	100	100
PGN-Pa-05	99.84	99.84
	99.84	99.84
	99.84	99.84
	99.84	99.84
	99.84	99.69
PGN-Pa-06	100	100
	100	100
	100	100
	100	100
	100	99.84
PGN-Pa-07	99.84	99.84
	99.84	99.84
	99.84	99.84
	99.84	99.84
	99.84	99.68
PGN-Pa-08	99.84	99.84
	99.84	99.84
	99.84	99.84
	99.84	99.84
	99.84	99.68
PGN-Pa-09	99.77	99.44
	99.43	99.44
	99.43	99.44
	99.43	99.44
	99.43	99.44
PGN-Pa-10	100	100
	100	100
	100	100
	100	100
	100	99.84
PGN-Pa-11	100	99.80
	99.80	99.80
	99.80	99.80
	99.80	99.80
	99.80	99.80
BJS-Tb-01	99.84	99.84
	99.84	99.84
	99.84	99.84
	99.84	99.84
	99.84	99.68
BJS-Pa-02	100	100
	100	100
	100	100
	100	100
	100	99.84
BJS-Pa-03	100	100
	100	100
	100	100
	100	100
	100	99.84
BJS-Pa-04	99.84	99.84
	99.84	99.84
	99.84	99.84
	99.84	99.84
	99.84	99.68
GRT-01	99.68	99.84
	99.68	99.68
	99.68	99.68
	99.68	99.68
	99.68	99.68
TSK-01	99.84	99.84
	99.84	99.84
	99.84	99.84
	99.84	99.84
	99.84	99.68
JYT-01	99.84	99.84
	99.84	99.84
	99.84	99.84
	99.84	99.84
	99.84	99.68
JYT-02	100	100
	100	100
	100	100
	100	100
	100	99.84
JYT03	99.84	99.84
	99.84	99.84
	99.84	99.84
	99.84	99.84
	99.84	99.68
JYT-04	99.85	99.85
	99.85	99.85
	99.84	99.85
	99.84	99.85
	99.84	99.69

In conclusion, this study discovered three species of Sparidae from the eastern Indian Ocean south of Java, Indonesia, namely *Acanthopragrus pacificus*, *Argyrops bleekeri*, and *P. major*. The discovery of *P. major* in the fishing ports and auction center along the West Java coastline provided the first record of its existence. The results were expected to provide valuable insight for sustainable fisheries management in this region. However, this study only sampled specimens from West Java and Banten Provinces, resulting in 21 samples of *P. major* being barcoded. To establish statistically reliable data for management and expand the geographical range map, further study should include representatives from other locations and more sequenced specimens.

ACKNOWLEDGEMENTS

The authors are grateful to the Directorate of Research and Community Services, Ministry of Education, Culture, Research and Technology of Indonesia for funding this study through the Regular Fundamental Research grant (*Penelitian Fundamental Reguler*; Contract No. 3.52/ UN23.35.5/PT.01/VII/2023). Furthermore, the authors are grateful for the administrative support received from the Research and Society Service Institute of the University of Jenderal Soedirman, Banyumas, Indonesia and the assistance provided by all parties who contributed to the fieldwork and collectors from the sampling sites.

REFERENCES

Abbas EM, Kato M, Sharawy ZZ. 2022. Phylogenetic relationship and systematic identification of different shrimp and prawn species in Egypt. Egypt J Aquat Biol Fish 26 (3): 565-578. DOI: 10.21608/EJABF.2022.244300.

Abdalwahhab O, Galal-Khallaf A, Saber SAE-L, Osman AGM, Mohammed-Geba H. 2020. A case study for the application of DNA barcoding in identifying species and genetic diversity of fish from the Suez city market, Egypt. Aquat Living Resour 33: 11. DOI. 10.1051/alr/2020012.

Afrand M, Sourinejad I, Fazeli SAH, Akbarzadeh A. 2018. Identification of Engraulids (*Encrasicholina punctifer* and *E. pseudoheteroloba*) using the COI gene as a DNA barcoding marker. J Aquat Ecol 7 (3): 39-47.

Ahmed MS, Chowdhury MMK, Nahar L. 2019. Molecular characterization of Small Indigenous fish Species (SIS) of Bangladesh through DNA barcodes. Gene 684: 53-57. DOI: 10.1016/j.gene.2018.10.048.

Ahmed SME, Fiteha YG, Elhifnawy HT, Magdy M, Mamoon A, Hussien N, Rashed MA-S. 2021. DNA barcoding for identification of some fish species (Sparidae) in Mediterranean Sea area. J Sci Res Sci 38 (2): 168-182. DOI: 10.21608/jsrs.2021.210686.

Al Amry W, Al-Marzouqi A, Al-Sinadi R, Al-Anbory I. 2016. DNA barcoding of *Cheimerius nufar* (Valenciennes, 1830) (Perciformes: Sparidae) from the Sultanate of Oman. J Aquac Mar Biol 4 (4): 00091. DOI: 10.15406/jamb.2016.04.00091.

Ali FS, Ismail M, Aly W. 2020. DNA barcoding to characterize biodiversity of freshwater fishes of Egypt. Mol Biol Rep 47 (8): 5865-5877. DOI: 10.1007/s11033-020-05657-3.

Almerón-Souza F, Sperb C, Castilho CL, Figueiredo PICC, Gonçalves LT, Machado R, Oliveira LR, Valiati VH, Fagundes NJR. 2018. Molecular identification of shark meat from local market in southern Brazil based on DNA barcoding: Evidence for mislabeling and trade of endangered species. Front Genet 9: 138. DOI: 10.3389/fgene.2018.00138.

Amatya B. 2019. DNA barcoding of cyprinid fish *Chagunius chagunio* Hamilton, 1822 from Phewa Lake, Nepal. Intl J Biol 11 (4): 88-100. DOI: 10.5539/ijb.v11n4p88.

Ariza AA, Adachi AMCL, Roque P, Hazin FHV, Vianna M, Rotundo MM, Delpiani SM, de Astarloa JMD, Delpiani G, Oliveira C, Foresti F, Cruz VP. 2022. DNA barcoding and species delimitation for dogfish sharks belonging to the *Squalus* Genus (Squaliformes: Squalidae). Diversity 14 (7): 544. DOI: 10.3390/d14070544.

Becker RA, Sales NG, Santos GM, Santos GB, Carvalho DC. 2015. DNA barcoding and morphological identification of neotropical ichthyoplankton from the Upper Paraná and São Francisco. J Fish Biol 87 (1): 159-168. DOI: 10.1111/jfb.12707.

Bilgin R, Utkan MA, Kalkan E, Karhan SU, Bekbölet M. 2015. DNA barcoding of twelve shrimp (Crustacea: Decapoda) from Turkish sea reveals cryptic diversity. Mediterr Mar Sci 16 (1): 36-45. DOI: 10.12681/mms.548.

Boza BR, Cruz VP, Stabile G, Rotundo MM, Foresti F, Oliveira C. 2022. Mini DNA barcodes reveal the details of the foraging ecology of the largehead hairtail, *Trichiurus lepturus* (Scombriformes: Trichiuridae), from São Paulo, Brazil. Neotrop Ichthyol 20 (2): e210166. DOI: 10.1590/1982-0024-2021-0166.

Briñoccoli YF, Garrido GG, Alvarez A. 2020. DNA barcoding identifies three species of croakers (Pisces, Sciaenidae) in the ichthyoplankton of the High Paraná River. An Acad Bras Cienc 92 (1): e20180783. DOI 10.1590/0001-3765202020180783.

Čandek K, Kuntner M. 2015. DNA barcoding gap: Reliable species identification over morphological and geographical scales. Mol Ecol Resour 15 (2): 268-277. DOI: 10.1111/1755-0998.12304.

Caputi L, Osca D, Ceruso M, Venuti I, Sepe RM, Anastasio A, D'Aniello S, Crocetta F, Pepe T, Sordino P. 2021. The complete mitochondrial genome of the white seabream *Diplodus sargus* (Perciformes: Sparidae) from the Tyrrhenian Sea. Mitochondrial DNA B Resour 6 (9): 2581-2583. DOI: 10.1080/23802359.2021.1915209.

Cerqueira NNCD, Rotundo MM, Marceniuk AP, da Cruz VP, Foresti F, Oliveira C. 2021. Molecular identification of *Brachygenys* and *Haemulon* species (Perciformes: Haemulidae) from the Brazilian coast. Neotrop Ichthyol 19 (2): e2001009. DOI: 10.1590/1982-0224-2020-0109.

Ceruso M, Mascolo C, De Luca P, Venuti I, Biffali E, Ambrosio RL, Smaldone G, Sordino P, Pepe T. 2021. *Dentex dentex* Frauds: Establishment of a new DNA barcoding marker. Foods 10 (3): 580. DOI: 10.3390/foods10030580.

Ceruso M, Mascolo C, Palma G, Anastasio A, Pepe T, Sordino P. 2018. The complete mitochondrial genome of the common dentex, *Dentex dentex* (Perciformes: Sparidae). Mitochondrial DNA B Resour 3 (1): 391-392. DOI: 10.1080/23802359.2018.1450675.

Chac LD, Thinh BB. 2023. Species identification through DNA barcoding and its applications: A review. Biol Bull Russ Acad Sci 50 (6): 1143-1156. DOI: 10.1134/S106235902360229X.

Dahruddin H, Hutama A, Busson F, Sauri S, Hanner R, Keith P, Hadiaty R, Hubert N. 2016. Revisiting the ichthyodiversity of Java and Bali through DNA barcodes: Taxonomic coverage, identification accuracy, cryptic diversity and identification of exotic species. Mol Ecol Resour 17 (2): 288-299. DOI: 10.1111/1755-0998.12528.

Díaz J, Villanova GV, Brancolini F, del Pazo F, Posner VM, Grimberg A, Arranz SE. 2016. First DNA barcode reference library for the identification of South American freshwater fish from the Lower Paraná River. PLoS ONE 11 (7): e0157419. DOI: 10.1371/journal.pone.0157419.

Dulčić J, Kraljevic M. 2007. On the record of red seabream *Pagrus major* (Temminck and Schlegel, 1843) (Osteichthyes: Sparidae) in the Adriatic Sea. Sci Mar 71: 15-17. DOI: 10.3989/scimar.2007.71n115.

Eggleston D. 1974. Sparidae. In Fischer W, Whitehead PJP (eds). FAO species identification sheets for fishery purposes. Eastern Indian Ocean (Fishing Area 57) and Western Central Pacific (Fishing Area 71). FAO, Rome.

Fricke R, Eschmeyer WN, Van der Laan R. 2023. Eschmeyer's catalog of fishes: Genera, species, references. http://researcharchive. calacademy .org/research/ichthyology/catalog/fishcatmain.asp [5 September 2023]

Froese R, Pauly D. 2023. FishBase. World Wide Web Electronic Publication. www.fishbase.org.

Ha TTT, Huong NT, Hung NP, Guiguen Y. 2019. Species identification using DNA barcoding on processed panga catfish products in Viet Nam revealed important mislabeling. Turk J Fish Aquat Sci 18: 457-462. DOI: 10.4194/1303-2712-v18_3_11.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41: 95-98.

Higashi R, Tsukagoshi A, Kimura H, Kato K. 2011. Male dimorphism in a new interstitial species of the genus *Microloxoconcha* (Podocopida: Ostracoda). J Crust Biol 31 (1): 142-152. DOI: 10.1651/09-3234.1.

Hu X, Huang SY, Hanner R, Levin J, Lu X. 2018. Study of fish products in Metro Vancouver using DNA barcoding methods reveals fraudulent labeling. Food Control 94: 38-47. DOI: 10.1016/j.foodcont.2018.06.023.

Hubert N, Meyer CP, Bruggeman HJ, Guérin F, Komeno RJL, Espiau B, Caussee R, Wiliams JT, Planes S. 2012. Cryptic diversity in Indo-Pacific coral reef fishes revealed by DNA barcoding provides new support to the center of overlap hypothesis. PLoS ONE 7 (3): e28987. DOI: 10.1371/journal.pone.0028987.

Iwatsuki Y, Kume M, Yoshino T. 2010. A new species, *Acanthopagrus pacificus* from the Western Pacific (Pisces, Sparidae). Bull Natl Mus Nat Sci Ser A 36 (4): 115-130.

Jeffery NW, Elías-Gutiérrez M, Adamowicz SJ. 2011. Species diversity and phylogeographical affinities of the Branchiopoda (Crustacea) of Churchill, Manitoba, Canada. PLoS ONE 6 (5): e18364. DOI: 10.1371/journal.pone.0018364.

Karanovic I. 2015. Barcoding of ancient lake Ostracods (Crustacea) reveals cryptic speciation with extremely low distances. PLoS ONE 10 (3): e0121133. DOI: 10.1371/journal.pone.0121133.

Kusbiyanto, Bhagawati D, Nuryanto A. 2020. DNA barcoding of crustacean larvae in Segara Anakan, Cilacap, Central Java, Indonesia using cytochrome c oxidase gene. Biodiversitas 21 (10): 4878-4887. DOI: 10.13057/biodiv/d211054.

Lim H-C, Abidin MZ, Pulungan CP, de Bryun M, Nor SAM. 2016. DNA barcoding reveals high cryptic diversity of the freshwater halfbeak Genus *Hemirhamphodon* from Sundaland. PLoS ONE 11 (9): e0163596. DOI: 10.1371/journal.pone.0163596.

Limmon G, Delrieu-Trottin E, Patikawa J, Rijoly F, Dahruddin H, Buson F, Steinke D, Hubert N. 2020. Assessing species diversity of coral triangle artisanal fisheries: A DNA barcode reference library for the shore fishes retailed at Ambon harbor (Indonesia). Ecol Evol 10 (7): 3356-3366. DOI: 10.1002/ece3.6128.

Mat Jaafar TNA, Taylor MI, Mohd Nor SA, de Bruyn M, Carvalho GR. 2012. DNA barcoding reveals cryptic diversity within commercially exploited Indo-Malay Carangidae (Teleostei: Perciformes). PLoS ONE 7 (11): e49623. DOI: 10.1371/journal.pone.0049623.

Mohammed-Geba K, Yousif A, Younis AM, Harby-Rashed A. 2021. DNA barcoding and molecular phylogeny confirm the presence of the cryptic *Penaeus japonicus* Form II (*P. pulchricaudatus*) in the Egyptian demersal fisheries of the Gulf of Suez and the Bitter Lakes. Egypt J Aquat Biol Fish 25: 615-627. DOI: 10.21608/ejabf.2021.192469.

Muchlis N, Surahman A. 2015 Komposisi jenis dan laju tangkap gillnet dasar di Palabuhan Ratu. Prosiding Seminar Nasional Ikan ke 8. [Indonesian]

Muchlisin ZA, Batubara AS, Fadli N, Muhammadar AA, Utami AI, Farhana N, Siti-Azizah MN. 2017. Assessing the species composition of tropical eels (Anguillidae) in Aceh Water, Indonesia, with DNA barcoding gene cox1. F1000Res 6: 258. DOI: 10.12688/f1000research.10715.1.

Nuryanto A, Bhagawati D, Rofiqoh AA. 2023. Barcoding of Sparidae collected during east monsoon season in the eastern Indian Ocean south of Java, Indonesia. AACL Bioflux 16 (2): 805-817.

Nuryanto A, Kusbiyanto, Bhagawati D. 2021. Molecular barcoding of marine ornamental fish from the southern coast of West Java validates conventional identification. E3S Web Conf 322: 01004. DOI: 10.1051/e3sconf/202132201004.

Nuryanto A, Pramono H, Kusbiyanto, Ghifari MI, Andareswari N. 2018. Barcoding of fin clip samples revealed high exploitation of *Plectropomus leopardus* in the Spermonde Archipelago. Biosaintifika 10 (3): 629-635. DOI: 10.15294/biosaintifika.v10i3.16142.

Nuryanto A, Pramono H, Sastranegara MH. 2017. Molecular identification of fish larvae from East Plawangan of Segara Anakan, Cilacap, Central Java, Indonesia. Biosaintifika 9 (1): 33-40. DOI: 10.15294/biosaintifika.v9i1.9191.

Parenti P. 2019. An annotated checklist of the fishes of the family Sparidae. FishTaxa 4 (2): 47-98.

Pereira LHG, Hanner R, Foresti F, Olivera C. 2013. Can DNA barcoding accurately discriminate megadiverse Neotropical freshwater fish fauna? BMC Genet 14: 20. DOI: 10.1186/1471-2156-14-20.

Ratnasingham S, Hebert PDN. 2007. BOLD: The Barcode of life data system (www.barcodinglife.org). Mol Ecol Notes 7 (3): 355-364. DOI: 10.1111/j.1471-8286.2006.01678.x.

Ratnasingham S, Hebert PDN. 2013. A DNA-based registry for all animal species: The Barcode Index Number (BIN) system. PLoS ONE 8 (7): e66213. DOI: 10.1371/journal.pone.0066213.

Salem MA, Abdel-Maogood SZ, Abdelsalam M, Mahdy OA. 2021. Comparative morpho-molecular identification of *Clinostomum phalacrocoracis* and *Clinostomum complanatum* metacercaria coinfecting Nile tilapia in Egypt. Egypt J Aquat Biol Fish 25 (1): 461-475. DOI: 10.21608/ejabf.2021.145698.

Sholihah A, Delrieu-trottin E, Sukmono T et al. 2020. Disentangling the taxonomy of the subfamily Rasborinae (Cypriniformes, Danionidae) in Sundaland using DNA barcodes. Sci Rep 10: 2818. DOI: 10.1038/s41598-020-59544-9.

Syaifudin M, Wijayanti M, Dwinanti SH, Muslim, Mahendra M, Marliana S. 2020. DNA barcodes and phylogenetics of striped snakehead and ocellated snakehead fish from South Sumatra, Indonesia. Biodiversitas 21 (3): 1227-1235. DOI: 10.13057/biodiv/d210350.

Tabata K, Taniguchi N. 2000. Differences between *Pagrus major* and *Pagrus auratus* through mainly mtDNA control region analysis. Fish Sci 66 (1): 9-18. DOI: 10.1046/j.1444-2906.2000.00032.x.

Tamura K, Stecher G, Kumar S. 2021. MEGA11: Molecular evolutionary genetic analysis version 11. Mol Biol Evol 38 (7): 3022-3027. DOI: 10.1093/molbev/msab120.

Von der Heyden S, Beger M, Toonen RJ, van Herwerden L, Juinio-Meñez MA, Ravago-Gotanco R, Fauvelot C, Bernardi G. 2014. The application of genetics to marine management and conservation: Examples from the Indo-Pacific. Bull Mar Sci 90 (1): 123-158. DOI: 10.5343/bms.2012.1079.

Winarni ET, Simanjuntak SBI, Nuryanto A. 2023. DNA barcoding of crustacean larvae with two new records of *Caridina gracilipes* and *Ptychognathus altimanus* in the western region of Segara Anakan lagoon in Indonesia. Biodiversitas 24 (1): 341-348. DOI: 10.13057/biodiv/d240141.

Xing B, Chen X, Zhang Z, Sun R, Xiang P, Lin M, Wang C. 2020. Genetic identification of ophichthid fishes through DNA barcoding. Mitochondrial DNA B Resour 5 (2): 1970-1974. DOI: 10.1080/23802359.2020.1756940.

Yannis C, Kallia S, Vangelis D, Vlasis K-R, Costas B, Constantin K. 2019. Presence confirmation of non-native species *Pagrus major* (Temminck and Schlegel, 1843) in the eastern Mediterranean. Oceanogr Fish 9: 555756. DOI: 10.19080/OFOAJ.2019.09.555756.