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Genetic Diversity of Pacific Seabream, *Acanthopagrus pacificus* in South-Coast Java, Indonesia

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Abstract. Pacific Seabream, *Acanthopagrus pacificus,* is a popular marine fish from the Sparidae family. This species is an economically essential fisheries commodity in the south-coast Java, Indonesia. However, limited studies are available on *A. pacificus* in Indonesia. Only two studies reported the presence of *A. pacificus* in South-Coast Java. No study has been carried out on the biology of *A. pacificus* from south-coast Java. Therefore, biological studies of *A. pacificus* are needed, including genetic diversity studies. This study aimed to evaluate the genetic diversity of *A. pacificus* in South-Coast Java using the cytochrome c oxidase gene. Fish samples were bought from Bantul Fishing Harbor, Yogyakarta, Panganadaran Fishing Port, Bojongsalawe Fish Auction Center, West Java, and Binuangeun Fishing Harbor in Lebak Regency, Banten. The genetic marker was processed in PT. Genetika Science Indonesia follows the company procedure. Haplotype and nucleotide diversity were calculated mathematically using Arlequin software. The result showed that polymorphic loci were only 1.3%, indicating low polymorphisms. *A. pacificus* showed haplotype and nucleotide diversities of 0.511±0.110 and 0.15%±0.12%, respectively. Those values indicated low genetic diversity. This study concluded that *the A. pacificus* population in South-Coast Java showed low genetic diversity. This study provides the first data about the genetic diversity of *A. pacificus* in South-Coast Java, which is essential data for fisheries management.

Keywords: diversity; haplotype; nucleotide; polymorphism; seabream.

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INTRODUCTION

Acanthopagrus pacificus Iwatsuki, Kume & Yoshino 2010, a Pacific seabream from the Sparidae, is widely distributed in the Western Pacific from Japan to Australia (Iwatsuki et al., 2010). However, no scientific data reported the presence of *A. pacificus* from South-Coast Java, Indonesia. A previous study reported that *Acanthopagrus* from Java was *A. berda* (Dahruddin et al., 2016). Nevertheless, recent studies by Nuryanto et al. (2023a) and Nuryanto et al. (2023b), who barcoded the member of Sparidae, proved that *A. pacificus* is highly abundance in the South-Coast Java, and none of the specimens were identified as *A. berda*.

Previous studies focused on exploring fish diversity in all fish taxa or the Sparidae Family. No in-depth biological study has been done on the *A. pacificus* from Indonesia's marine water (Dahruddin et al., 2016; Nuryanto et al., 2023a; 2023b), including genetic diversity study. Genetic diversity is essential information for fisheries management (Nuryanto & Pulungsari, 2017) and conservation strategies (Nuryanto et al., 2019). It is because genetic diversity data provide information about the capability of the population to cope with environmental changes (Ellegren & Galtier, 2016) or the impact of captures (Pinsky & Palumbi, 2014; Righi et al., 2020; Petit-Marty et al., 2022).

Population genetic diversity can be evaluated using a partial cytochrome c oxidase I (COI) sequence. Several studies have found that the COI gene is a reliable genetic marker for analyzing animal populations (Artamonova et al., 2018; Mohammed et al., 2020; Mustikasari et al., 2022; Nuryanto et al., 2022). This finding is because the COI gene has a greater mutation rate than other mitochondrial genes (Gupta et al., 2015; Nuryanto et al., 2017).

The large genetic variety of the population assessed using the COI gene was critical, ranging from invertebrate (Liu et al., 2017) to vertebrate

(Artamonova et al., 2018; Padmavathi & Srinu, 2019; Limmon et al., 2020). *A. pacificus* from natural world heritage in West Pacific, Taiwan, was found to have high haplotype diversity but moderate to medium nucleotide diversity (Islam et al. 2022). Previous research has shown that the COI gene is a highly variable genetic marker in a wide range of animal species, demonstrating its suitability (Padmavathi & Srinu, 2019; Limmon et al., 2020). As a result, the COI gene is predicted to be used to assess the genetic diversity of *A. pacificus* in South Coast Java.

This study aims to evaluate the genetic diversity of Pacific Seabream (*A. pacificus*) populations on Java Island's south coast to offer data for fisheries management, particularly for *A. pacificus*. The results of this study can be used as scientific references for fisheries management, especially for *A. pacificus* in South Coast Java, Indonesia.

METHODS

Sampling site and time

The *A. pacificus* samples were collected from four locations on the southern coast of Java, namely Bantul in Yogyakarta, Bojongsalawe Fish Auction Center and Pangandaran Fishing Port in West Java, and Binuangeun Fishing Port in Lebak Regency, Banten Province (Figure 1).

Fish specimens (Figure 2) were collected during the field trips from June to August 2022 and June to August 2023 (Nuryanto et al., 2023a; Nuryanto et al., 2023b).

Sequence source and editing

This study performed metadata analysis based on the COI gene sequences of *A. pacificus* retrieved from GenBank (Nuryanto et al., 2023a; Nuryanto et al., 2023b). The retrieved sequences are listed in Table 1.

Figure 1. Map of Java Island showing four sampling sites

Figure 2. Specimen of *A. Pacificus*

Table 1. Annotation of **sample** codes, accession number, and reference sources of the research object

No	Sample Code	Accession Number	Source
1	Dr1	OQ852695	Nuryanto et al. 2023a
2	PGN-SP-1-15	OQ852698	Nuryanto et al. 2023a
3	PGN-SP-1-16	OQ852699	Nuryanto et al. 2023a
4	PGN-SP-1-17	OQ852700	Nuryanto et al. 2023a
5	PGN-SP-1-18	OQ852701	Nuryanto et al. 2023a
6	PGN-SP-1-19	OQ852702	Nuryanto et al. 2023a
7	PGN-SP-1-20	OQ852703	Nuryanto et al. 2023a
8	BJS-APH-01	OR527342	Nuryanto et al. 2023b
9	BJS-APH-02	OR512855	Nuryanto et al. 2023b
10	BJS-APH-03	OR512856	Nuryanto et al. 2023b
11	BJS-APH-05	OR512857	Nuryanto et al. 2023b
12	BJS-APH-06	OR512858	Nuryanto et al. 2023b
13	BJS-APH-07	OR512859	Nuryanto et al. 2023b
14	BJS-APH-08	OR512860	Nuryanto et al. 2023b
15	BJS-APH-09	OR512861	Nuryanto et al. 2023b
16	BJS-APH-10	OR512862	Nuryanto et al. 2023b
17	BJS-APH-11	OR512863	Nuryanto et al. 2023b
18	BGN-Ap-01	OR512864	Nuryanto et al. 2023b
19	BGN-Ap-02	OR512865	Nuryanto et al. 2023b
20	BGN-Ap-03	OR512866	Nuryanto et al. 2023b
21	BJS-Ap-01	OR527343	Nuryanto et al. 2023b
22	BJS-Ap-02	OR527344	Nuryanto et al. 2023b
23	BJS-AP-03	OR512867	Nuryanto et al. 2023b
24	BJS-AP-04	OR512868	Nuryanto et al. 2023b
25	BJS-AP-05	OR512869	Nuryanto et al. 2023b
26	BJS-AP-06	OR512870	Nuryanto et al. 2023b
27	BJS-AP-07	OR512871	Nuryanto et al. 2023b
28	BJS-AP-08	OR512872	Nuryanto et al. 2023b
29	BJS-AP-09	OR512873	Nuryanto et al. 2023b

The sequences were aligned and edited manually using Bioedit package ver. 7.2.5 (Hall, 2017) for further analysis. Functional sequences were obtained by translating all the sequences into amino acid sequences, which was carried out in an online software ORFfinder (https://www.ncbi.nlm.nih.gov/orffinder/). The translation utilized mitochondrial vertebrate genetic code, ATG, and alternative initiation codon, ignoring nested codon as search parameters. Multiple sequence alignment was carried out using ClustalW implemented in Bioedit package ver. 7.2.5 (Hall, 2017).

Neutrality and historical demography tests

The hypothesis of neutral evolution of the COI gene of *A. pacificus* was tested using Fu's Fs test. The test was selected because it was more sensitive than Tajima's D test. A non-significant Fu's Fs value indicates neutral evolution (Mohammed et al., 2022). The test used Arlequin 3.5 computer software (Excoffier & Lischer, 2015).

The demographic history of *A. pacificus* on the south coast of Java Island was estimated using mismatch distribution analysis (Roger & Harpending, 1992; Harpending, 1994) using two parameters. The Harpending's raggedness index (HRI) and sum square deviation (SSD) were calculated in Arlequin ver. 3.5 (Excoffier & Lischer, 2015) with 1000 pseudoreplicates (bootstrap) to estimate deviation from the expansion model.

Genetic diversity

Arlequin input and haplotype data files were generated in DnaSP6 (Rozas et al., 2017). This approach also gave haplotype information and individual numbers for each haplotype. Genetic diversity estimates for *A. pacificus* on Java's south coast were computed using polymorphism, haplotype diversity (*h*), and nucleotide diversity (π). The computation was carried out with Arlequin software version 3.5. (Escoffier &

Lischer, 2015).

Evolutionary relationships

The current study uses a haplotype network to assess the evolutionary links between individual *A. pacificus* haplotypes from South Coast Java, Indonesia. The network was formed by including *A. berda* (MW379787; MW379617) and *Pagrus major* (KY371849) as out-group species. The median-joining algorithm from the free program NETWORK ver. 10.2 was used to draw the network (Bandelt et al., 1999).

RESULTS AND DISCUSSION

Neutrality and historical demography

Neutrality of the genetic markers can be tested using Tajimas' D and Fus' Fs test. Tajimas' D test is calculated based on haplotype variations, while Fus' Fs is calculated based on nucleotide variations. This data shows that Fus' Fs are more sensitive than Tajimas' D (Mohammed et al., 2022). The results of neutrality analysis of the COI gene of *A. pacificus* on the south coast of Java, Indonesia, showed that the Fus' Fs value was -1.260 (p=0.141), indicating no significant value. Negative Fus' Fs value might indicate an excess number of alleles due to recent population expansion or genetic hitchhiking. Therefore, the used COI marker can be assumed as a neutral maker as indicated by the negative and non-significant Fus' Fs value, which means that the neutral hypothesis failed to be rejected even though not significant Fs might indicate a low number of samples or neutral evolution of the used COI marker. We were convinced to state the neutral evolution of the maker because we have a reliable number of samples (29 individuals). Several studies reported the neutral evolution of the COI gene in various animals (Nuryanto et al., 2019; Mohammed et al., 2020;). Therefore, COI gene utilization in genetic studies is reliable (Artamonova et al., 2018; Mustikasari et al., 2022).

It has been explained previously that Fus' Fs could not differentiate whether the population underwent recent population expansion or genetic hitchhiking. We did a historical demography analysis to access those two alternatives. The historical demography of *A. pacificus* on the south coast of Java was shown by a sum square deviation (SSD) value of 0.004 (p=0.333) and Harpending's raggedness index (HRI) value of 0.111 (p=0.641) indicated statistically non-significant. These results implied that the sudden expansion model was accepted. Simultaneously, non-significant SSD (p=0.158) and HRI (p=0.752) values were also observed during the spatial expansion model test. This finding also proved that the spatial expansion model was accepted. Therefore, the population of *A. pacificus* in south-coast Java underwent recent population expansion. Negative Fus' Fs value also supported this phenomenon. Recent population expansion phenomena were common in animal populations evaluated using the COI gene (Mohammed et al., 2022).

Genetic diversity

A length of 596 base pairs (bp) of the COI gene were analyzed from 29 sequences of *A. pacificus*, resulting in eight polymorphic loci. The polymorphism value was 1.34%, meaning the most abundant loci have a frequency over 95% (98.66%). The values indicate low polymorphism. A gene is polymorphic if the maximum frequency of the most common allele is 95% (Nuryanto et al., 2019a; Nuryanto et al., 2022). The current results were similar to previous studies, which showed low genetic polymorphism in the COI gene (Nuryanto et al., 2018). However, the current study showed a different result from that of Nuryanto et al. (2017), who reported high polymorphism in the COI gene of *Aguilla bicolor*. The difference could be due to the different species under study. The current study used *A. pacificus*, while Nuryanto et al. (2022) used *A. bicolor* as the object of study. Previous studies proved that different species might show various levels of polymorphisms in their COI gene (Righi et al., 2020; Petit-Marty et al., 2022)

Haplotype diversity (h) was 0.468±0.107, and nucleotide diversity (π) was 0.134%±0.111%. The obtained haplotype and nucleotide diversity values indicated that the *A. pacificus* population on Java's south coast has minimal genetic diversity. According to Nuryanto et al. (2019), haplotype diversity varies from 0.00 to 0.4, signifying low genetic diversity, and ranges from 0.5 to 7 are considered medium genetic diversity levels. *A. bicolor* (Nuryanto et al., 2020; Nuryanto et al., 2022) and *Channa striata* (Setyaningrum et al., 2022) have similarly shown little genetic diversity. However, considerable genetic variety was found in the COI genes of lanternfishes (Pappalardo et al., 2015) and *Osphronemus goramy* (Nuryanto et al., 2018). Similarly, substantial genetic diversity of the COI gene has been reported in *C. striata* (Baisvar et al., 2019) and Bivalvia (Liu et al., 2017). These findings suggest that complex patterns of genetic variety based on the COI gene can be detected between or within species. According to a prior study, a complicated pattern of genetic diversity was identified in *Capoeta trutta*, which was collected from various local populations (Parmaksiz & Eksi, 2017). Thus, it is not surprising that, while being examined using the same genetic markers, various species may exhibit varying degrees of genetic variety (Abbas et al., 2017; Achrem et al., 2017; Gouskov et al., 2016). The latest discovery adds to the knowledge regarding the complexity of genetic diversity among species subjected to COI gene analysis.

Different levels of genetic diversity were identified in the current investigation compared to a survey conducted by Islam et al. (2022), who gathered samples of *A. pacificus* from the Philippines and Japan. In contrast to Islam et al. (2022), the current investigation discovered minimal haplotype diversity in *A. pacificus* from South Coast Java, Indonesia. There are two possible causes for the discrepancy. Islam et al.'s (2022) study was conducted in protected areas with a lower rate of exploitation than ours, which was carried out in unprotected areas with high exploitation rates. Previous studies proved that high exploitation rates might significantly impact lowering the genetic diversity of populations (Barasa et al., 2014; Tan et al., 2015; Baisvar. et al., 2019).

The comparison between this study and Islam et al. (2022) was not truly equivalent because both studies used different genetic markers. The current study used the COI gene, while Islam et al. (2022) utilized control region sequences as the genetic marker. Therefore, it is reasonable that both studies observed different genetic diversity levels in *A. pacificus* populations. Previous studies reported that populations might show complex patterns of genetic diversity levels depending on the used markers (Liu et al., 2017).

The evolutionary relationships

Five haplotypes were obtained from 29 individuals of *A. Pacificus*. This haplotype number also indicated that *the A. Pacificus* population in South-Coast Java showed low genetic diversity. Low genetic diversity is also demonstrated by low mutation steps (2-6) between Haplotype-2 and the remaining haplotypes. Six mutation steps were observed between haplotype-2 and haplotype-1 (Figure 3).

Haplotype-1 and haplotype-3 consisted of one individual, respectively. Twenty-one individuals formed Haplotype-2, while haplotype-4 and haplotype-5 consisted of 3 individuals. Haplotype-2 was found in three out of four locations. The Haplotype network showed a star-like network with Haplotype-2 as the center of the network. This network type indicates that *the A. pacificus* population in South-Coast Java evolved from a single ancestor (H2). Therefore, Haplotype-2 is referred to as the most primitive haplotype. These decisions were made based on the fact that haplotype 2 was the dominant and widely distributed haplotype. Previous studies reported that primitive haplotype can be inferred from its abundance and distribution across sampling sites (Barasa et al., 2014; Nuryanto et al., 2022). Star-like haplotype networks were also reported in other fish species (Baisvar et al., 2018; Baisvar et al., 2019; Setyaningrum et al., 2022).

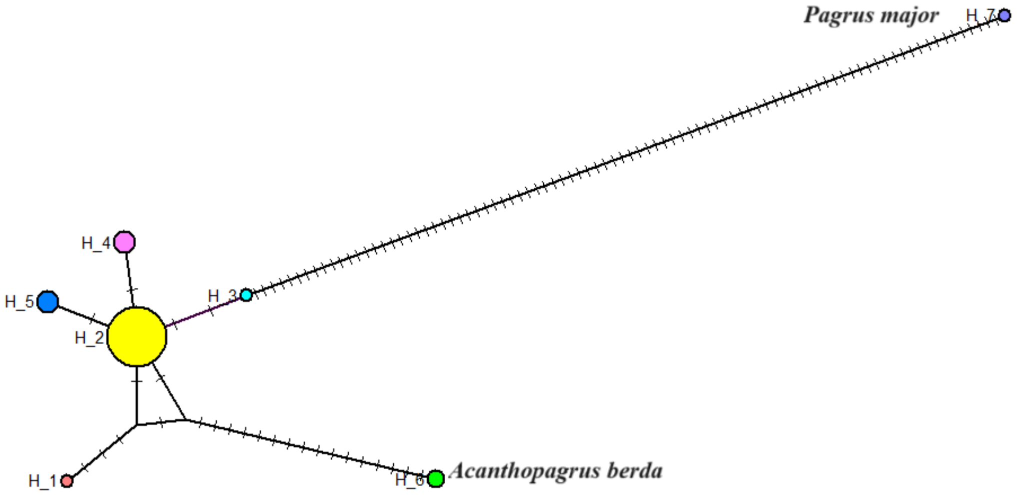

Figure 3. Star haplotype networks indicate *that A. pacificus* evolved from a single primitive ancestor and low genetic diversity

The haplotype network in Figure 3 also clearly showed the separation of our samples from *A. berda* (H6) by at least 19 mutation steps. This data strengthens that our samples belong to other species, instead of *A. berda*. The current samples were barcodes as *A. pacificus* (Nuryanto et al., 2023a; Nuryanto et al., 2023b). This result proved that two seabream species inhabit South-Coast Java, *A. berda* (Dahruddin et al., 2016; Nuryanto et al., 2023a; Nuryanto et al., 2023b).

Several molecular studies have been carried out on *A. pacificus*. However, these studies were focused on taxonomic studies, such as barcoding (Renxie et al., 2018; Zheng et al., 2022; Nuryanto et al., 2023a; Nuryanto et al., 2023b) and mitochondrial genome sequencing (Kawai et al., 2022). Only one study reported the genetic diversity of *A. pacificus*. However, the study was conducted in the Philippines and Japan and utilized the control region sequence as a genetic marker (Islam et al., 2022). Therefore, this report is the first study of the genetic diversity of *A. pacificus* in Indonesia and uses the COI gene as a genetic marker. This study provides additional data about the biology of Indonesian black seabream (*A. pacificus*), essential information for fisheries management, especially for *A. pacificus* in South-Coast Java, Indonesia.

CONCLUSION

This study concluded that *A. pacificus* in South-Coast Java showed low genetic diversity, as indicated by polymorphisms, haplotype and nucleotide diversity values, and low mutation among haplotypes. This study provides the first data about the genetic diversity of *A. pacificus* in Indonesian marine water, which is essential for fisheries management.

This study only collected limited samples (29 individuals) from South-Coast Yogyakarta to Banten Provinces. A broader sampling area and more samples are necessary to provide more comprehensive and reliable data about the genetic diversity of *A. pacificus* in South-Coast Java or even Eastern Indian Ocean, Indonesia.

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