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DNA Barcode Characterization of Chocolate Hind Grouper (*Cephalopholis boenak*) in Several Indonesia Waters with the New Sequences Record from Madura Island

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ABSTRACT

This study aims to describe the molecular characteristics of DNA barcodes in chocolate hind grouper (*Cephalopholis boenak*) in Indonesian waters with new sequence records from Madura Island waters. Partial sequences of the cytochrome c oxidase 1 (CO1) gene were successfully obtained from two samples of *C. boenak* in Madura Island waters. In contrast, the other sequences were obtained from the BOLD system database, covering the Islands of Bali, Lombok, and Ambon waters. The overall molecular analysis involved 10 *C. boenak* sequences with a length of 625 bp. The results indicated that the population of *C. boenak* in Indonesia waters has high genetic diversity, as evidenced by the value of haplotype diversity (Hd) = 0.956 and nucleotide sequence diversity (Pi) = 0.01746, in which the population is distributed into eight haplotypes. Results of phylogenetic tree reconstruction of neighbor-joining and maximum-likelihood indicate similar topology. The branching of NJ and ML phylogenetic trees of *C. boenak* in Indonesia waters is grouped into two geographical clades. Clade 1 with the subpopulations of the waters from Madura, Bali, and Lombok Islands. Clade 2 with the subpopulations of the waters from Ambon Island. The results of the median-joining network reconstruction depicted a similar topology with both phylogenetic trees.

1. Introduction

Chocolate hind grouper (*Cephalopholis boenak*) refers to a small grouper under the subfamily of Epinephelinae, widely distributed in Indopacific waters, including Indonesia waters (Heemstra and Randall 1993). The maximum length of this species is only 26 cm (Kuiter and Tonozuka 2001). Hong Kong and surrounding areas were the first consumers of *C. boenak*, previously considering the commercial importance in Hong Kong. However, due to the decline in the number of large groupers, the catch of *C. boenak* has increasingly becoming one of the most important species in the Hong Kong fish market, considered as a local grouper and most frequently obtained (Liu and Sadovy 2001). These species are exploited and sold for local human consumption in their respective territories, caught by using hooks, traps, and trawls (Heemstra and Randall 1993, Sadovy and Cornish 2000).

Grouper has become one of the primary commodities in fisheries captivity for the people of Madura Island, East Java, Indonesia in general (Sukandar *et al.* 2016). Therefore, in the future, there is potential where *C. boenak* originating from Indonesia waters, particularly from Madura Island, will be traded internationally, including for export to Hong Kong and its surroundings. This prospective is indicated by the FAO report (2020) regarding the landing of *C. boenak* in all Indonesia waters in the period of 2004-2018, reaching the highest number in 2018. Based on this potential, the collection and characterization of DNA barcodes become one of the efforts to assess the genetic diversity of this species.

Among the many species of groupers, specific DNA barcode to *C. boenak* has been limited in Indonesia, despite currently occupying an important position in the assessment of the genetic diversity of this species. The partial sequence of the CO1 gene is a DNA

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barcode that is widely selected to identify grouper in Indonesia waters, including the genus *Cephalopholis* (Fadli *et al.* 2021; Limmon *et al.* 2020; Razi *et al.* 2021; Tapilatu *et al.* 2021).

Application of partial sequence of CO1 gene as a DNA barcode of the *C. boenak* that has been reported only covers a few areas in Indonesia which are significantly limited, from the waters of Bali and Lombok Islands (CSIRO 2020) and Ambon Island (Limmon *et al.* 2020). All of these sequences are accessible in the BOLD system database (Ratnasingham and Hebert 2007), and only *C. boenak* sequences from Ambon Island are stored in GenBank (NCBI) (Benson *et al.* 2013). Hence, this study becomes the first to describe a DNA barcode of the partial sequence of CO1 gene in samples of *C. boenak* in the waters of Madura Island, exhibiting an important position to complete the DNA barcode of grouper in Indonesia in the available online databases.

2. Materials and Methods

2.1. Samples Collection and Preparation

Two samples of chocolate hind groupers *C. boenak* in the waters of Madura Island were obtained through local fishers at the Fish Landing Ports on Madura Island in October 2019. Interviews were also conducted with local fishers to ensure that samples of *C. boenak* were concisely obtained from local waters. The obtained species of *C. boenak* were identified using a morphological approach (Heemstra and Randall 1993; Kuiter and Tonozuka 2001). The sample of *C. boenak* was performed with a pectoral fin of approximately 15-20 grams for preservation in absolute ethanol (96%) and was prepared for molecular analysis. In detail, the two sequences of *C. boenak* from the waters of Madura Island were obtained in this study. The other eight *C. boenak* sequences in Indonesia waters were obtained from the BOLD system database (Ratnasingham and Hebert 2007), including the Islands of Bali, Lombok, and Ambon waters. Details of the sampling location, sequence identity, and data source are presented in Table 1, while the sample collection position map is illustrated in Figure 1.

2.2. Total DNA Isolation and Amplification of Partial Sequence of CO1 Gene

Total DNA isolation in two samples of chocolate hind groupers *C. boenak* from Madura Island waters was obtained using the Wizard® Genomic DNA Purification Kit (Promega Corporation) following the

Table 1. Samples of partial sequence of CO1 gene of *C. boenak* in this study

Location	Coordinates	Sequence identity	Data sources
Madura Island	-7°03′S-113°24′E	BRP017	This research
		DGK028	This research
Bali Island	-8°75'S-115°15'E	FOAK967-10	BOLD system database
		FOAM013-10	BOLD system database
Lombok Island	-8°8'S-116°48'E	FOAM144-10	BOLD system database
		FOAM145-10	BOLD system database
Ambon Island	-3°68'S-128°183'E	BIFZC433-17	BOLD system database
		BIFZC434-17	BOLD system database
		BIFZC435-17	BOLD system database
		BIFZC453-17	BOLD system database

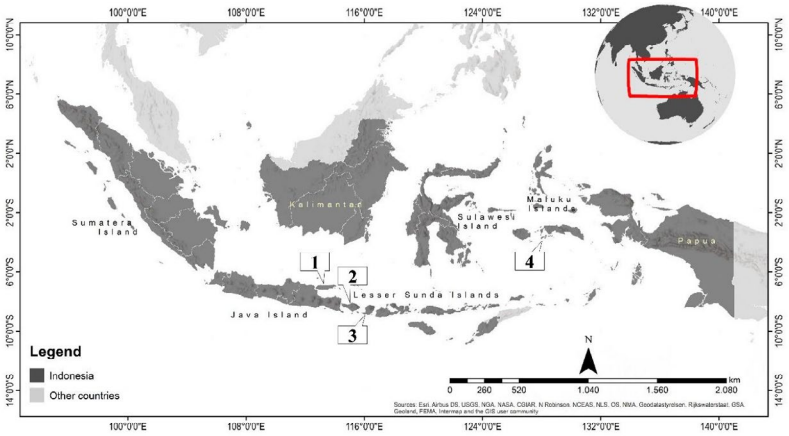

Figure 1. Illustration from samples position of *C. boenak*. Position (1) Madura Island, (2) Bali Island, (3) Lombok Island, and (4) Ambon Island. The Indonesia map was modified from Condro *et al.* (2020)

protocol. The universal primer for the CO1 gene used for amplification was developed by Ward *et al.* (2005) with the primers of a forward sequence of FishF1 and the reverse sequence of FishR1. Polymerase chain reaction (PCR) was processed by using MyTaq HS Red Mix (Bioline) with a detailed composition of 9.5 µl ddH_2O, 12.5 µl MyTaq HS Red Mix, 1 µl 10 M forward primer (FishF1), 1 µl 10 M reverse primer (FishR1), and 1 µl sample DNA template. The amplicons of the partial sequence of the CO1 gene were confirmed by using electrophoresis with 1% agarose gel at 100 volts for 25 minutes. The applied comparison marker was a 100 bp DNA ladder (Intron). Nucleotide sequencing was performed by utilizing the services of 1st BASE (Singapore).

2.3. Molecular Data Analysis

Partial sequence of CO1 gene of along 600-700 bp that have been successfully obtained from two samples of chocolate hind grouper *C. boenak* from Madura Island waters was firstly interpreted into amino acid sequences to examine and remove the stop codons in the middle of the sequence (Song *et al.* 2008). Sequence checking and editing were performed by using the software of BioEdit version 7.0.0 (Hall 1999). Further, a thorough manual check was also covered. Each sequence sample was validated using the online facilities, BLAST (NCBI) and BOLD System (Boratyn *et al.* 2013; Ratnasingham and Hebert 2007).

A total of eight accessions of *C. boenak* in Indonesia waters from the BOLD system were selected in group, and two accessions of *Cephalopholis fulva* were applied as an outgroup for the reconstruction of the phylogenetic tree. Multiple sequence alignment was calculated by using an algorithm of MUSCLE (Edgar 2004) run with the software of MEGA X version 10.2.6 (Kumar *et al.* 2018).

Analysis of the genetic diversity of *C. boenak* population from Indonesia waters was performed by using software of DnaSP 6 (Rozas *et al.* 2017) and MEGA X version 10.2.6. Genetic distance calculation was conducted by using the maximum composite likelihood method with 1000 bootstrap replications (Felsenstein 1985). Reconstruction of the phylogenetic trees was performed by applying the software of MEGA X version 10.2.6. The selected method for the phylogenetic tree reconstruction is through neighbor-joining (NJ) and maximum-likelihood (ML) with complete deletion gaps and missing data treatment (Saitou and Nei 1987; Truszkowski and Goldman 2016).

NJ reconstruction was calculated by using the substitution model of Kimura 2-Parameter (Kimura 1980), and rates among sites were performed by using Gamma distributed (G) (Susko *et al.* 2003). Meanwhile, the ML reconstruction applies the substitution model of Hasegawa-Kishino-Yano+Gamma distributed as recommended by the best-fit substitution model using the lowest scores on the BIC (Bayesian Information Criterion) and AICc (Akaike Information Criterion corrected) (Hasegawa *et al.* 1985; Nei and Kumar 2000). The reconstruction of the two phylogenetic trees was evaluated using 1000 bootstrap replications (Felsenstein 1985). The median-joining network was reconstructed by using the software of Network 10 (Bandelt *et al.* 1999; Kong *et al.* 2015; Fluxus Engineering 2020).

3. Results

Visualization on electrophoresis using 1% agarose gel indicated that the partial sequence of the CO1 gene had been successfully amplified with an estimated length of 650-700 base pairs (bp) for the sample of chocolate hind grouper (*C. boenak*) (Figure 2). Validation of partial sequence of CO1 gene related to species identity applying the online facilities of BLAST (NCBI) and BOLD system databases depicted a significantly high percentage of similarity in the range of 98-100%.

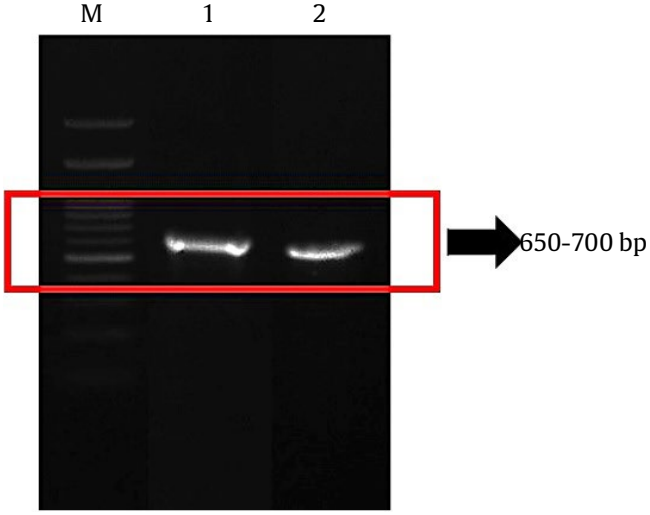

Figure 2. Electrophoresis gel visualization of partial sequence of CO1 gene of *C. boenak* from Madura Island waters

Based on multiple sequence alignment and manual analysis of the ten samples of partial sequence of CO1 gene, DNA barcode characterization in this study was conducted on the length of the sequence of 625 bp. Based on the length of the sequence, the percentage average of frequencies of nucleotide compositions T, C, A, and G were 30.86%, 25.78%, 26.09%, and 17.27% respectively (Table 2).

The population of *C. boenak* in Indonesia waters has high genetic diversity category, as evidenced by the value of haplotype diversity (Hd) = 0.956 and nucleotide diversity (Pi) = 0.01746 (Table 3). It is apparent that the probability of transitional substitution remains higher than that of transversion substitution (Table 4). A summary of the genetic distance of the population of chocolate hind grouper *C. boenak* in Indonesia waters are presented in Table 5.

The NJ and ML phylogenetic trees reconstruction results indicate similar topology, such as dividing the population of *C. boenak* in Indonesia waters

Table 4. Estimation of the nucleotide substitution pattern of *C. boenak* in Indonesia waters

	A	T	C	G
A	-	1.12	0.99	24.16
T	0.91	-	15.63	0.63
C	0.91	17.74	-	0.63
G	35.18	1.12	0.99	-

Each entry indicates the probability of substitution from one base (row) to another base (column). Rates of different transitional substitutions are presented in bold, and those of transversional substitutions are presented in italics

Table 5. Summary of genetic distance between subpopulations of *C. boenak* in Indonesia waters

Subpopulation	Madura Island	Bali Island	Lombok Island	Ambon Island
Madura Island	-			
Bali Island	0.004	-		
Lombok Island	0.012	0.008	-	
Ambon Island	0.027	0.023	0.026	-

Table 2. Nucleotide composition of partial sequence of CO1 gene of *C. boenak* from Indonesia waters

Sequence identity	Nucleotide frequencies (%)				G+C content
	T(U)	C	A	G	
BRP017 Madura Island	30.33	27.17	25.33	17.17	44.34
DGK028 Madura Island	30.93	25.64	25.96	17.47	43.11
FOAK967-10 Bali Island	30.62	25.84	26.16	17.38	43.22
FOAM013-10 Bali Island	30.62	25.84	26.16	17.38	43.22
FOAM144-10 Lombok Island	30,30	26.32	26.00	17.38	43.70
FOAM145-10 Lombok Island	30.78	25.68	26.16	17.38	43.06
BIFZC433-17 Ambon Island	31.25	25.32	26.44	16.99	42.31
BIFZC434-17 Ambon Island	31.26	25.36	26.32	17.07	42.43
BIFZC435-17 Ambon Island	31.26	25.36	26.32	17.07	42.43
BIFZC453-17 Ambon Island	31.26	25.36	26.00	17.38	42.74
Average	30.86	25.78	26.09	17.27	43.05

Table 3. Summary of characteristics of the partial sequence of CO1 gene of *C. boenak* in Indonesia waters

Parameter	Analysis result
Haplotype diversity (Hd)	0.956
Number of haplotypes (nHap)	8
Variance of haplotype diversity	0.00353
Nucleotide diversity (Pi)	0.01746
Transition/transversion rate ratios (k)	38.528 (purines) 15.869 (pyrimidines)
transition/transversion bias (R)	12.206
Gamma distribution	0.0981
Number of polymorphic sites	28
Number of monomorphic sites	597
Number of informative sites	17
Position of informative sites	1, 2, 29, 35, 89, 143, 194, 242, 254, 293, 326, 338, 348, 422, 482, 548, 608
Number of singleton variable sites	11
Position of singletone variable sites	3, 17, 203, 257, 390, 404, 461, 491, 512, 599, 617

into two clades (Figure 3 and 4). Clade 1 consists of subpopulations of the Islands of Madura, Bali, and Lombok waters. Clade 2 only consists of the subpopulation of the waters of Ambon Island. The reliability of a phylogenetic tree is understood through the support of bootstrap values. Bootstrap support for the main branches of NJ and ML phylogenetic trees is generally in the moderate to high range, indicating that the reconstructed phylogenetic trees have a high level of reliability in the main branching section, which is beneficial to conclude.

A total of 10 partial sequences of CO1 gene of *C. boenak* in Indonesia waters involved in this analysis were distributed into eight haplotypes and grouped into two haplogroups (Figure 5).

4. Discussion

The partial sequence of the CO1 gene had been successfully amplified and proven valid based on the online database facilities. This result is in accordance with the explanation of Ward *et al.* (2005) that the developed primer generated partial CO1 gene sequences at 655 bp in length. Furthermore, the CO1 primer for the partial amplification of high-resolution with the accurate COI gene sequences for species-level identification in *C. boenak* samples.

The percentages of nucleotide frequencies of *C. boenak* are generally similar to that of Teleost, which were 29.38%, 28.75%, 23.58%, and 18.31%, respectively. This similarity indicates the calculation

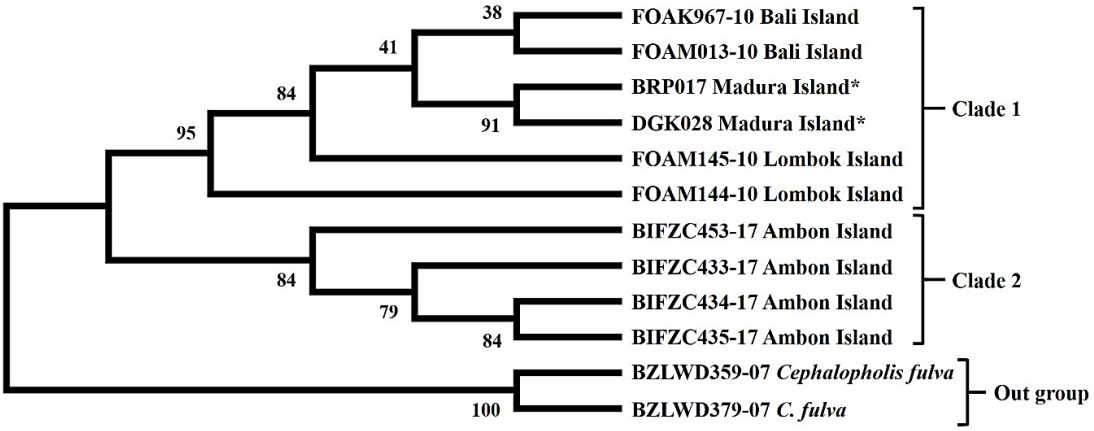

Figure 3. Phylogenetic tree reconstruction of neighbor-joining (NJ) of partial sequence of CO1 gene of *C. boenak* in Indonesia waters. The asterisk (*) denotes the sequence obtained in this study, and the number at the branching node indicates the percentage of bootstrap support

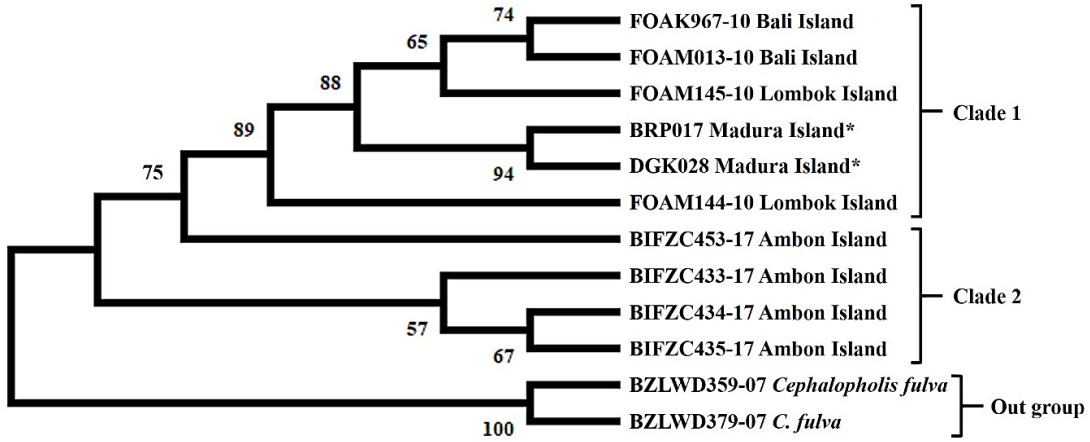

Figure 4. Phylogenetic tree reconstruction of maximum-likelihood (ML) of partial sequence of CO1 gene of *C. boenak* in Indonesia waters. The asterisk (*) denotes the sequence obtained in this study, and the number at the branching node indicates the percentage of bootstrap support

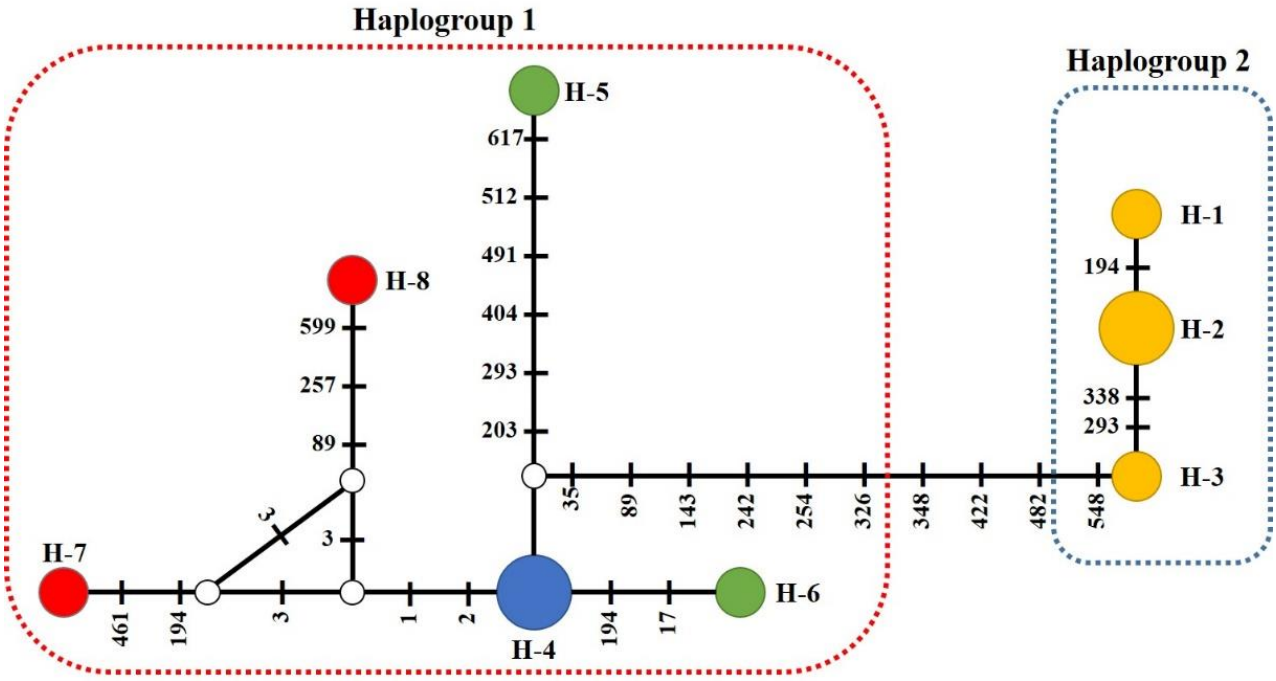

Figure 5. Median-joining network reconstruction based on partial sequence of CO1 gene of *C. boenak* in Indonesia waters. H-1 to H-8 indicate haplotypes 1 to 8; subpopulation color ● Madura Island, ● Bali Island, ● Lombok Island, and ● Ambon Island; the number on the line denotes the position of the nucleotide difference; the size of the circle denotes the number of haplotypes in it

accuracy of Ward *et al.* (2005) study, developing a universal primer for the partial sequence of the CO1 gene. The average percentage of G+C content in *C. boenak* is 43.05%, which is relatively far from Teleost, 47.1% (Ward *et al.* 2005). The percentage of the G+C content of the subpopulation of Ambon Island waters is lower than that of subpopulations of the Islands of Madura, Bali, and Lombok waters.

Genetic diversity is generally based on the value of haplotype diversity (Hd) (Nei 1987) and nucleotide diversity (Pi) (Lynch and Crease 1990). The results of this study are in line with the analysis of Gaither *et al.* (2011) on *Cephalopholis argus* in the waters of the Pacific and Indian Oceans based on its mitochondrial DNA of cytochrome b gene and two nuclear introns (gonadotropin-releasing hormone and S7 ribosomal protein) with the value of Hd = 0.8 and Pi = 0.005. In addition, this study is in line with the report of Souza *et al.* (2015), which revealed high diversity in *C. fulva* based on its mitochondrial DNA of D-loop sequence with the value of Hd= 0.997 and Pi = 0.023.

Understanding nucleotide substitution patterns are considerably significant for studying the evolution of molecular sequences and for reliable estimation of phylogenetic relationships (Yang 1994). The probability of transitional substitution in the partial sequence of the CO1 gene of *C. boenak* in this study remains higher than that of transversion substitution. This probability is in line with the explanation of Keller *et al.* (2007) that mutations in animals tend to be more frequent through transitional substitution mechanisms than through transversion. This probability occurs because nucleotides with similar molecular structures and a number of bonds are more simply substituted through transitions. Payne *et al.* (2019) explained that mutation is not a completely random process but rather exhibits a bias towards changes in certain DNA sequences (nucleotide substitution patterns). As a result, mutations create genetic variation, thereby influencing evolution.

Genetic distance refers to the degree of gene difference (or genome difference) between species or populations (Dogan and Dogan 2016). The result of this study indicates that the genetic distance of *C. boenak* is in line with its geographical distance. The closest genetic distance values are seen between the subpopulations of the waters of Madura Island and Bali Island. In contrast, the farthest genetic distance values are seen between the subpopulations of the waters of Madura Island and

Ambon Island. This distance shows that the partial sequence of the CO1 gene has high accuracy for determination between subpopulations of *C. boenak* in Indonesian waters. Microsatellite molecular markers are required to detect genetic distances between subpopulations of *C. boenak* in more depth, as reported by Renshaw *et al.* (2010) on *C. fulva.*

Reconstruction of the phylogenetic tree indicated that partial sequences of CO1 gene were effective in determining between populations of *C. boenak* in Indonesia waters. This result is in line with the report of Ariyanti *et al.* (2015) that a partial sequence of CO1 gene can determine between populations of *C. urodeta* in the waters of Sulawesi, Indonesia, and the Andaman Islands, India. However, not all sequences of DNA barcode could used for geographic subpopulation determination, as Souza *et al.* (2015) reported that the application of a partial sequence of D-loop control region could not determine geographically the subpopulation of *C. fulva* in coastal Brazil.

The reconstruction of the phylogenetic trees structure is also likely to change as more sequences are collected in online databases in the future. However, it is reasonable to suspect that *C. boenak* from the geographical area of eastern Indonesia waters, including East Nusa Tenggara, Sulawesi, and Papua, will be grouped into one clade. In addition, it is perceived that the geographical area of western Indonesia waters has the potential to form a separate clade from the subpopulations of the waters from Madura, Bali, and Lombok Islands. In order to confirm such an assumption, the reconstruction results of the phylogenetic tree are combined with the reconstruction results of the haplotype network (Figure 5).

The results of the median-joining network reconstruction depicted a match with the number of clades formed in NJ and ML phylogenetic trees. The median-joining network in *C. boenak* in Indonesia waters was grouped into two haplogroups containing haplogroup 1 with subpopulations of Madura, Bali, and Lombok islands and haplogroup 2 with subpopulations of Ambon Island (Figure 5). The findings of this study serve as the basis for future studies of molecular phylogeography of *C. boenak.* However, additional sequence numbers are necessary to obtain a complete interpretation of the species' distribution, migration, and biogeography patterns, as conducted by Gaither *et al.* (2011) on *C. argus.*

Haplogroup 1 consists of 5 haplotypes, while haplogroup 2 consists of 3 haplotypes. This indicates a high genetic diversity in the population of *C. boenak* in Indonesian waters. While in haplogroup 1, it can be predicted that the subpopulation of Madura Island waters (haplotypes 7 and 8) has the potential to form a separate haplogroup. The distance between the two haplogroups is due to differences in 10 nucleotides, specifically nucleotides at positions 35, 89, 143, 242, 254, 326, 348, 422, 482, and 543.

A partial sequence of the CO1 gene are proved effective in determining species in the genus of *Cephalopholis* in Indonesia, with samples of *C. boenak* found in it (Fadli *et al.* 2021; Limmon *et al.* 2020; Tapilatu *et al.* 2021). In line with the results of previous studies, the partial sequence of the CO1 gene has indicated a high degree of accuracy in determining *C. boenak* species in this study, even in general grouping samples based on geographic position.

Limmon *et al.* (2020) explained that the trend of fish landings for species from the family of Serranidae (*Epinephelus* and *Cephalopholis,* including *C. boenak*) and Lutjanidae (*Lutjanus*) ranks first and second regarding the number of species landed which indicates a trend of increasing market demand. Thus, although the genetic diversity of *C. boenak* was high in this study, information on the biological aspects of *C. boenak* in Indonesia has been limited, where the decisions made regarding the conservation management of this species could contain an inaccuracy. As a final remark, the results of this study are generally expected to contribute significantly to the genetic diversity of *C. boenak* in Indonesia waters. In this study, a partial sequence of the CO1 gene of *C. boenak* in the waters from Madura Island will immediately be submitted to the GenBank and BOLD system databases, although limited biological data is expected to facilitate the improved monitoring, conservation and management of fisheries in Indonesia waters.

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References

Ariyanti, Y., Farajallah, A., Arlyza, I.S., 2015. Phylogenetic analysis of the darkfin hind, *Cephalopholis urodeta* (Serranidae) using partial mitochondrial CO1 gene sequences. *Ilmu. Kelaut. Indones. J. Mar. Sci.* 20, 38-44. https://doi.org/10.14710/ik.ijms.20.1.38-44

Bandelt, H.J., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* 16, 37-48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

Benson, D.A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Sayers, E.W., 2013. GenBank. *Nucleic. Acids. Res.* 41, D36-D42. https://doi.org/10.1093/nar/gks1195

Boratyn, G.M., Camacho, C., Cooper, P.S., Coulouris, G., Fong, A., Ma, N., Madden, T.L., Matten, W.T., McGinnis, S.D., Merezhuk, Y., Raytselis, Y., Sayers, E.W., Tao, T., Ye, J., Zaretskaya, I., 2013. BLAST: a more efficient report with usability improvements. *Nucleic. Acids. Res.* 41, 29-33. https://doi.org/10.1093/nar/gkt282

Condro, A.A., Setiawan, Y., Prasetyo, L.B., Pramulya, R., Siahaan, L., 2020. Retrieving the national main commodity maps in Indonesia based on high-resolution remotely sensed data using cloud computing platform. *Land.* 9, 377. https://doi.org/10.3390/land9100377

[CSIRO] Commonwealth Scientific and Industrial Research Organisation, 2020. Available at https://www.csiro.au. [Date accessed: 20 December 2021]

Dogan, I., Dogan, N., 2016. Genetic distance measures: review. *Turk. Klin. J. Biostat.* 8, 87-93. https://doi.org/10.5336/biostatic.2015-49517

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic. Acids. Res.* 32, 1792-1797. https://doi.org/10.1093/nar/gkh340

Fadli, N., Muchlisin, Z.A., Siti-Azizah, M.N., 2021. DNA barcoding of commercially important groupers (Epinephelidae) in Aceh, Indonesia. *Fish. Res.* 234, 105796. https://doi.org/10.1016/j.fishres.2020.105796

[FAO] Food and Agriculture Organization, 2020. Available at www.fao.org/fishery/statistics/software/fishstatj/en. [Date accessed: 20 December 2021]

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evol.* 39, 783-791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Fluxus Engineering, 2020. Available at http://www.fluxus-engineering.com. [Date accessed: 20 December 2021]

Gaither, M., Bowen, B., Bordenave., T.-R., Rocha, L., Newman, S., Gomez, J., Herwerden, L., Craig, M., 2011. Phylogeography of the reef fish *Cephalopholis argus* (Epinephelidae) indicates Pleistocene isolation across the Indo-Pacific barrier with contemporary overlap in the Coral Triangle. *BMC. Evol. Biol.* 11, 189. https://doi.org/10.1186/1471-2148-11-189

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic. Acids. Symp. Ser.* 41, 95-98. https://doi.org/10.14601/Phytopathol_Mediterr-14998u1.29

Hasegawa, M., Kishino, H., Yano, T., 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* 22, 160-174. https://doi.org/10.1007/BF02101694

Heemstra, P.C., Randall, J.E., 1993. Grouper of the world: (family Serranidae, subfamily Epinephelinae): an anonated and illustrated catalogue of the grouper, rockcod, hind, coral grouper, lyretail species: known to date. Food and Agriculture Organization of the United Nations, Rome (IT).

Keller, I., Bensasson, D., Nichols, R.A., 2007. Transition-transversion bias is not universal: a counter example from grasshopper pseudogenes. *PLoS. Genet.* 3, 185-191. https://doi.org/10.1371/journal.pgen.0030022

Kimura, M.A., 1980. Simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* 16, 111-120. https://doi.org/10.1007/BF01731581

Kong, S., Sánchez-Pacheco, S.J., Murphy, R.W., 2015. On the use of median-joining networks in evolutionary biology. *Cladistics.* 32, 691-699. https://doi.org/10.1111/cla.12147

Kuiter, R.H., Tonozuka, T. 2001. Pictorial guide to Indonesian reef fishes. Part 1. Eels- Snappers, Muraenidae-Lutjanidae. Zoonetics, Australia.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 35, 1547-1549. https://doi.org/10.1093/molbev/msy096

Limmon, G., Delrieu-Trottin, E., Patikawa, J., Rijoly, F., Dahruddin, H., Busson, 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, 3356–3366. https://doi.org/10.1002/ece3.6128

Liu, M., Sadovy, Y., 2001. Embryos and early larvae of *Cephalopholis boenak* (Bloch 1790) (Serranidae), in: Hendry, C.I., Van Stappen, G., Willie, M. and Srgeloos, P. (Eds.), Larvi'01-Fish and Shellfish Larviculture Symposium 30. Oostende: European Aquaculture Society. pp. 322-325.

Lynch, M., Crease, T.J., 1990. The analysis of population survey data on DNA sequence variation. *Mol. Biol. Evol.* 7, 377-394. https://doi.org/10.1093/oxfordjournals.molbev.a040607

Nei, M., 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Nei, M., Kumar, S., 2000. Molecular evolution and phylogenetics. Oxford University Press, New York

Payne, J.L., Menardo, F., Trauner, A., Borrell, S., Gygli, S.M., Loiseau, C., Gagneux, S., Hall, A.R., 2019. Transition bias influences the evolution of antibiotic resistance in Mycobacterium tuberculosis. *PLoS. Biol.* 17, e3000265. https://doi.org/10.1371/journal.pbio.3000265

Ratnasingham, S., Hebert, P.D.N., 2007. BOLD: The barcode of life data system (http://www.barcodinglife.org). Mol. Ecol. Notes. 7, 355-364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

Razi, N., Muchlisin, Z., Maulida, S., Ramadhaniaty, M., Firman, M.N., Damora, A., Manalu, S., Fadli, N. 2021. Grouper DNA barcoding studies in Indonesia: a short review. *Depik.* 10, 186-193. https://doi.org/10.13170/depik.10.2.21255

Renshaw, M.A., Portnoy, D.S., Gold, J.R., 2010. PCR primers for nuclear-encoded microsatellites of the groupers *Cephalopholis fulva* (coney) and *Epinephelus guttatus* (red hind). *Conserv. Genet.* 11, 1197-1202. https://doi.org/10.1007/s10592-009-9918-9

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., Sánchez-Gracia, A., 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Mol. Biol. Evol.* 34, 3299-3302. https://doi.org/10.1093/molbev/msx248

Sadovy, Y., Cornish, A.S., 2000. Reef Fishes of Hong Kong. Hong Kong University Press, Hong Kong.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4, 406-425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Song, H., Buhay, J.E., Whiting, M.F., Crandall, K.A., 2008. Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. *Proc. Natl. Acad. Sci.* 105, 13486-13491. https://doi.org/10.1073/pnas.0803076105

Souza, A., Júnior, E., Galetti, Jr.P., García-Machado, E., Pichorim, M., Molina, W., 2015. Wide-range genetic connectivity of Coney, *Cephalopholis fulva* (Epinephelidae), through oceanic islands and continental Brazilian coast. *An. Acad. Bras. Cienc*. 87, 121-136. https://doi.org/10.1590/0001-3765201520130411

Sukandar, Handayani, M., Dewi, C.S.U., Harsindhi, C.J., Maulana, A.W., Supriyadi, Bahroni, A., 2016. Profile of coastal villages in East Java Province volume III (Madura Islands). Marine, Coastal, and Supervision of the Department of Fisheries and Marine Affairs of East Java Province, Surabaya.

Susko, E., Field, C., Blouin, C., Roger, A.J., 2003. Estimation of rates-across-sites distributions in phylogenetic substitution models. *Syst. Biol*. 52, 594-603. https://doi.org/10.1080/10635150390235395

Tapilatu, R., Tururaja, T., Sipriyadi., Kusuma, A., 2021. Molecular phylogeny reconstruction of grouper (Serranidae: Epinephelinae) at Northern Part of Bird's Head Seascape-Papua inferred from COI gene. *Fish. Aquatic. Sci.* 24, 181-190. https://doi.org/10.47853/FAS.2021.e18

Truszkowski, J., Goldman, N., 2016. Maximum likelihood phylogenetic inference is consistent on multiple sequence alignments, with or without gaps. *Syst. Biol.* 65, 328-333. https://doi.org/10.1093/sysbio/syv089

Ward, R., Zemlak, T., Innes, B., Last, P., Hebert, P., 2005. DNA Barcoding Australia's fish species. P*hilos. Trans. R. Soc. Lond. B. Biol. Sci.* 360, 1847-1857. https://doi.org/10.1098/rstb.2005.1716

Yang, Z., 1994. Estimating the pattern of nucleotide substitution. *J. Mol. Evol.* 39, 105-111. https://doi.org/10.1007/BF00178256