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FULL TITLE :
PHYLOGENETIC of MARBLE GOBY (*OXYELEOTRIS MARMORATA* BLKR.) in SOUTH SUMATRA based on *CYTOCHROME C OXIDASE SUBUNIT I (COI)* GENE

SHORT TITLE:
PHYLOGENETIC of MARBLE GOBY (*OXYELEOTRIS MARMORATA* BLKR.) in SOUTH SUMATRA

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ABSTRACT

Marble goby (*Oxyeleotris marmorata*) in Indonesia, spread across Sumatra, Kalimantan and Papua. The purpose of this study was to obtain the sequence of Cytochrome Oxidase Subunit I genes, the phylogenetic tree, genetic distance, and to determine the physical and chemical characteristics of the waters. DNA was extracted from fins sample, amplified using PCR (Polymerase Chain Reaction) and sequenced in the region of the COI gene. The domesticated samples were collected from Gandus Fish Seed Center (GFSC), Musi Banyuasin Regency, while the wild samples were captured from the Musi River in Beruge Village, Babat Toman District, Musi Banyuasin Regency. The COI gene sequencing of marble goby from this study produced a nucleotide length of 613 bp. Based on the BLAST (Basic Local Alignment Search Tool), domesticated marble goby (*O. marmorata*) (OMD2, OMD3) was in the same subcluster with *O. marmorata* from the Musi River (OMS2, OMS3). Domesticated marble goby and the wild had 99.35% similarity with *O. marmorata* from Cambodia and Indonesia (West Java) with a genetic distance of 0.02%. The water characteristics observed in the research were: temperature 29.1-30.9^{o}C, transparency 13-26 cm, dissolved oxygen 6.5-7.75 mgL^{-1}, pH 6, ammonia 0.01-0.08 mgL^{-1}, TDS 14-25 mgL^{-1} and total alkalinity 16-21 mgL^{-1} $CaCO_3$.

Keywords : Cytochrome C Oxidase Subunit I gene, *O. marmorata*, PCR, South Sumatra

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Author Contributions

The conception and design of the study: MSF; the acquisition of data, or the analysis and interpretation: RO, FHT; Funding Acquisition: MSF, writing-original draft: RO, DJ; wrirting-review and editing: MSF, FHT, DJ.

Conflict of Interest

The authors declare no competing interests

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Response to Reviewer:

1st reviewer:

Thank you very much for your great review to the article.

The official status of the marble goby (Oxyeleotris marmorata) is as a Vulnerable species even though it is a widespread and abundant fish, therefore the number of specimen collected were not many.

Furthermore, there is no genetic data in the NCBI from South Sumatra region, therefore it is pivotal to asses the DNA barcode of the species, even with small number of individuals.

Water qualities will affect on genetic study but in a long term, therefore we agree to delete this part due to less strong correlation at this stage.

Code of samples OMS1 and OMD1 did not mentioned in the text. It is now stated that OMS1 and OMD 1 have been isolated their DNA, but the PCR were not good enough for sequencing analysis.

2nd reviewer:

Many thanks for all your constructive feedback toward this paper, I have addressed the question and suggestion of this article.

The step of denaturation was mentioned in the DNA amplification section. Water qualities sections were deleted as suggested from another reviewer.

The number of samples were six, however OMS1, OMS2 and OMD1 were discarded from the analysis due to low quality of PCR product and sequencing result.

The similarity of Marble goby based on COI sequence has been revised according to code of sample, however the accession numbers are still waiting a review from BOLD system with sequence page of CLSP007-21 for OMD2, CLSP008-21 for OMD3 and CLSP006-21 for OMS3. In the phylogenetic construction, bootstrapping value was carried out in 1000 replications

Water qualities were deleted from the analysis due to low correlation to the study. The conclusion have also been rewrite as suggested by the reviewer.

Phylogenetic of Marble Goby (*Oxyeleotris marmorata* Blkr.) in South Sumatra Based on Cytochrome C Oxidase Subunit I (COI) Gene

Number of Pages, Tables, and Figures

Number of pages :12
Tables : 3
Figures : 2

ABSTRACT

Marble goby (*Oxyeleotris marmorata*) in Indonesia, spread across Sumatra, Kalimantan and Papua. The purpose of this study was to utilize a sequence of mitochondrial DNA Cytochrome Oxidase Subunit I gene, to analyze the phylogenetic tree and genetic distance between cultured and captured populations. This research was conducted on March-August 2020. The methods used in barcoding species were DNA isolation, amplification using PCR (Polymerase Chain Reaction) and sequencing of the COI mtDNA gene.. The domesticated samples (n=3) were collected from Gandus Fish Seed Center (GFSC), while the wild samples (n=3) were captured from the Musi River in Beruge Village, Babat Toman District, both in Musi Banyuasin Regency. The sequenced COI mtDNA gene fragments were obtained from the PCR method. Three samples performed good PCR results, while the other three didn't amplify properly. After the editing process, the COI gene sequencing produced a nucleotide length of 610 bp. Based on the BLAST (Basic Local Alignment Search Tool), domesticated marble goby (OMD2, OMD3) was in the same cluster with *marble goby* from the Musi River (OMS3). The genetic distance indicated that two specimens of domesticated marble goby were 100% identical, while the wild (OMS3) indicated a genetic distance of 0.0066 to domesticated species.

Keywords : Cytochrome C Oxidase Subunit I gene, *O. marmorata*, PCR, South Sumatra

INTRODUCTION

The marble goby (*Oxyeleotris marmoratus*, Bleeker) also known as the sand goby is widely distributed in Southeast Asia, especially in Malaysia, Singapore, Thailand and Vietnam (Cheah, Senoo, Lam, & Ang, 1994; Mohsin & Ambak, 1983; Poh, Ving, & Shaliza, Ibrahim Vikineswary, 2015). It is also one of the indigenous species of freshwater fish in Indonesia. There are 17 species of marble goby, 11 of which are found globally and 8 species are found in Indonesia, which are *Oxyeleotris urophthalmoides*, *O. colasi*, *O. wisselensis*, *O. heterodon*, *O. paucipora*, *O. urophthalmus*, *O. marmorata* and *O. altipinna*. *Oxyeleotris marmorata*, commonly found in the Musi River (Kordi, 2013), is an introduction species from China since 1927 (Food and Agriculture Organization (FAO) of United Nation, 2005; Wargasasmita,

2012), either for ornamental or consumption. It is an important commercial fish because of its good taste, rich nutrition, large size and potential in aquaculture (Zhao et al., 2017). In Indonesia, the species is spread across the water resources of Sumatra, Kalimantan and Papua (Fishbase, 2021). However, the presence of introduced species could threaten the loss of indigenous freshwater species in Sempor Reservoir, Kebumen (Lestari, Rukayah, & Jamilatun, 2019).

Domestication efforts of marble goby have been carried out at the Mariana Public Aquatic Fisheries Research Institute (BRPPU) and Gandus Fish Seed Center (GFSC), South Sumatra. There are 260 genetic data for marble goby, one of them was marble goby from West Java, but none of the genetic data is originated from Sumatra (National Center for Biotechnology Information, 2021).

DNA barcoding is a system designed for precise and accurate identification of a species using a short and standardized gene region (Hebert et al., 2003). Research on DNA Barcode has been carried out on several freshwater fish, including featherbacks (Sodsuk. & Sodsuk, 2000), Asian Redtail Catfish (Syaifudin et al., 2017), tilapia (Syaifudin et al., 2017), striped snakehead and ocellated snakehead fish (Syaifudin et al., 2020). The most promising benefit of DNA barcoding for species authentication lies in the ability to identify early stages that cannot be done by using morphological descriptions. It has been proved to be effective for identifying species in juvenile and larvae of *Lutjanus cyanopterus* in Caribbean beach (Victor, Hanner, Shivji, Hyde, & Caldow, 2009) and 75% of coral reef fish larvae sampled (n=373) in the Pacific (Hubert, Espiau, Meyer, & Planes, 2014). Based on NCBI (2019), DNA barcode on marble goby fish has been carried out in Japan, Indonesia (West Java) (Dahruddin, Hadiaty, & Hubert, 2016), Australia (Ward, Zemlak, Innes, Last, & Hebert, 2005), China and Thailand (Panprommin, Iamchuen, Soontornprasit, & Tuncharoen, 2020).

This research is conducted to amplify nucleotide sequences of the COI gene, to identify species, determine genetic distances and analyze phylogenetic relationships of domesticated marble goby from Gandus Fish Seed Centre at Gandus, Palembang in South Sumatra and from the Musi River Banyuasin Regency, as a wild fish habitat in the region. Thus, the identification of the species using the COI gene is an effort to develop genetic information that can be used as the basis for the fish selection process through hybridization activities.

MATERIALS AND METHODS

Study Area

The study was conducted in the Musi River in Beruge Village, Babat Toman District, Musi Banyuasin Regency in South Sumatera, representing a wild habitat of marble goby (n=3); and GFSC Gandus, Palembang Regency, representing a domesticated fish (n=3)(Fig. 1).

Biological materials

Six specimens of marble goby were acquired from the wild and domesticated fish. The fishes were captured by local fisherman with approximate size of 0.309-0.326 kg for weight and 30 cm for length (code of sample OMS1, OMS2 & OMS3), meanwhile the domesticated fish size were 0.278-0.322 kg for weight and 21.5-30 cm for length (OMD1, OMD2 & OMD3). For each specimen, approximately 4 cm of a segment of the caudal fin was dissected with a sterile blade, then stored in 1.5 ml tubes containing 96% ethanol, labeled and kept in the freezer at -20ºC until DNA extraction.. Domesticated marble goby have been cultured in a rearing pond for a year, fed with live fish such as tilapia and nilem fry and spawned semi naturally at once.

DNA Extraction

DNA was extracted from fin clips of all specimens. Total genomic DNA was extracted using the Realpure Genomic DNA Extraction Kit (Durviz S.L) following the manufacturer's manual. DNA extraction consists of 5 stages including, cell lysis, RNAse treatment, DNA precipitation, washing and dissolution of DNA. Four µl of RNAse were added in the lysis step to minimise RNA contamination. Those samples that passed quality control (no observable RNA) based on DNA band visualization in the gel documentation were selected for used in PCR.

DNA Amplification

DNA of the four marble goby were used in PCR with primer pairs of FishF2-5'TCGACTAATCATAAAGATATCGGCAC3' and FishR2-5'ACTTCAGGGTGACCGAAGAATCAGAA3' to amplify 650 bp fragment (Ward et al., 2005). PCR was performed in a final volume of 50 µl using MyTaq^{TM} Red Mix (Bioline). Each reaction contained 1 µl of 10 µM each primer (F2 and

R2 primers) (Ward et al., 2005), 20 µl of nuclease-free water, 25 µl *myTaq polymerase red mix* and 3 µl of DNA template. The thermal cycling protocol was as follows: initial denaturation at 95°C for 1 min (1 cycle) followed by 35 cycles of 95°C for 15 sec, annealing at 50°C for 30 sec, extension or elongation at 72°C for 15 sec and a final extension at 72°C for 4 min. Furthermore, PCR products were run in electrophoresis 1% agarose gel at 75V for 35 minutes and visualized to determine the DNA bands using Gel Documentation. The size of PCR product was measured using 1 kb marker. DNA samples that were successfully amplified using PCR were then sequenced in the target area of the COI gene. PCR products were commercially sequenced (Sanger sequencing, GATC Biotech Ltd.) at 1st Base DNA Sequencing Service.

Data Analysis

Six sequences of COI gene (OMS3, OMD2 and OMD3) from forward2 and reverse2 were saved in Fasta format (OMS1, OMS2 and OMD1 were discarded from the analysis due to low quality of PCR product and sequencing result). The sequences were analyzed their identity using BLAST (Basic Local Alignment Search Tool) in NCBI (National Center for Biotechnology Information). For sequence comparisons, pairwise genetic distances were quantified based on the Kimura 2-parameter (K2P) distance model (Kimura, 1980). Voucher sequences from GenBank, and consensus sequences of each samples generated from this study were used to construct phylogenetic tree. The phylogenetic tree of marble goby was constructed using the Neighbor-Joining (NJ) method. Bootstrapping value was carried out in 1000 replications. In the phylogenetic construction, *Oreochromis niloticus* (KM438538.1) (Syaifudin, Bekaert, et al., 2017) was also added as species outgroup.

RESULTS AND DISCUSSION

The sequence Identity

The resultant fragments of sequences were approximately 680 - 698 base pairs (bp). After trimming process with MEGA (Molecular Evolutionary Genetics Analysis) version 7 (Kumar, Stecher, & Tamura, 2016), the length of sequences were 610 bp and no gaps within sequences. The BLASTn analysis showed that COI sequences of marble goby in this study

accordance with those in the GenBank database. The identity percentage was exhibited in Table 1.

The result of BLASTn analyses in Table 1 denoted that nucleotide sequences of the domesticated fish, OMD2 and OMD3 were identical, showing 99.51% matched to *Oxyeleotris marmorata* from Malaysia (KT022088.1) and USA (AY722177.1), meanwhile the wild marble goby (OMS3) indicated highest similarity (100%) to *Oxyeleotris marmorata* from Cambodia (EF609424.1), West Java, Indonesia (KU692726.1) and Vietnam (MH721190.1). Sequence similarity higher than 97% was the criterion for authentication at the species level (Wong & Hanner, 2008) and a similarity lower than that was used for recognition at the genus level. This result denoted that distinguishing species using DNA barcoding is very accurate. The COI is effectively used as species authentication method because intraspecific variation is low, but has high interspecific variation values especially in adjacent taxa (Ward et al., 2005). DNA barcoding validates to recognize species for international trading, either certifying fisheries products consumption or ornamental fish trade (Dahruddin et al., 2016). It has been successfully authorize species larval stages of marble goby in Thailand for sustainable fishery resource management (Panprommin et al., 2020).

Genetic Distance and Phylogenetic

Genetic distance at this study was also used to determine the genetic relationship between domesticated (OMD2 and OMD3) and wild stock (OMS3) marble goby in South Sumatra (Table 2). The genetic distance in Table 2 indicated that two specimens of domesticated marble goby were 100% identical, while the wild (OMS3) indicated a genetic distance of 0.0066 to domesticated species. The domesticated marble goby were also identic to the same species from Malaysia (KT001058.1), while OMS3 was identic to marble of goby from from Cambodia (EF609424.1), Thailand (MK448189.1) and West Java, Indonesia (KU692718.1 and KU692726.1). Hebert et al. (2003) stated that a genetic distance difference of less or equal to 3% indicates molecularly identical species. The smaller the genetic distance between individuals in a population, the more uniform the population is. Conversely, the greater the genetic distance of an individual in a population, the more diverse the population will be. The largest genetic distance (0.2082), was found between *O.*

marmorata and species outgroup *Oreochromis niloticus* from Stirling (KM438538.1), followed to *Palatogobius paradoxus* (0.1557) from America (MF049134.1). It is clearly denoted that within *O. marmorata* barcode variation was low in compare to the sequence variation between species in genus *Oxyeleotris*. Genetic distance indicates the ratio of a genetic distinction between species or populations (Dogan, Dogan, & Nurhan, 2016). Therefore, a smaller genetic distance value creates a more indistinguishable appearance partial sequence of CO1 gene compared between the two species (Basith, Abinawanto, Kusrini, & Yasman, 2021).

The phylogenetic tree of marble goby was presented in Figure 2. This study determined the level of evolution and kinship of a species, where the cluster *O. marmorata* was separated from O. selheimi (AY722166.1, AY722179.1) and O. lineolata (KJ669574.1, AY722165.1) (bootstrap value/bv of 97%). Within species, *O. marmorata* was separated into two sub-clusters (bv of 68%). The first sub-cluster consisted of *O. marmorata* from Thailand (MK448189.1, MK448069.1), Malaysia (KT001058.1), Cambodia (EF609424.1), Indonesia (KU692718.1, KU692726.1), Vietnam (MH721190.1) including samples of *O. marmorata* from the Musi River (OMS2, OMS3) and domesticated fish (OMD2, OMD3). The second sub-cluster consists of *O. marmorata* from Thailand (MK628379.1, KF410694.1) and Malaysia (KT022088.1). Meanwhile, O. marmorata from USA (AY722176.1 and AY722177.1 and AY722177.1) made distant subcluster to the same species afore mention.

The second cluster is occupied by species that are still in the same genus of marble goby. There were two species found in the second cluster, i.e. *O. selheimi* from Los Angeles (America) and *O. lineolata* (Los Angeles, USA) and *O. lineolata* (Australia). The third cluster was *P. paradoxus* (America), while, the fourth cluster was consisted of *O. niloticus* (Stirling), as an outgroup species. The Oxyeleotris was belong to Butidae family, while Palatogobius was belong to Gobiidae family. A previous study using sequence data of five molecular markers (two mitochondrial and three nuclear) indicated that Butidae being sister group to the Gobiidae clade, to the exclusion of Eleotrididae (Agorreta et al., 2013). A bootstrap value greater than 70% indicates that the data is relatively stable (Lemey, Selemi, & Vandamme, 2009). The phylogenetic tree construction resulted in a scale bar of 0.02. According to Wardani et al. (2017), a phylogenetic tree with a 0.01 scale bar shows a genetic distance with a change in nucleotides 1 time every 100 bp. So that the phylogenetic constructs obtained indicate a genetic distance with nucleotide changes 2 times in every 100 bp. DNA

barcoding technology has been utilized and validated for many aquatic species with detected more variation among congeneric species than among conspecific individual (Ward et al., 2005). Therefore it can effectively used to distinguish a complex of morphologically distinct species in the Indo-Pacific (Last, Manjaji, & Yearsley, 2005).

Conclusions

In summary, DNA barcoding have been developed for species authentication for pivotal aquaculture and wild species of marble goby. Thus, the DNA barcodes will support fish products inspection and regulation requirements by establishing correct labeling of domesticated and wild marble goby in the market.

References

Basith, A., Abinawanto, Kusrini, E., & Yasman. (2021). Genetic diversity analysis and phylogenetic reconstruction of groupers epinephelus spp. From madura island, indonesia based on partial sequence of co1 gene. *Biodiversitas*, *22*(10), 4282–4290. https://doi.org/10.13057/biodiv/d221020

Cheah, S. H., Senoo, S. Y., Lam, S. Y., & Ang, K. J. (1994). Aquaculture of a high-value freshwater fish in [Malaysia]: the marble or sand goby (Oxyeleotris marmoratus, Bleeker). *Naga*. Retrieved from https://search.proquest.com/docview/16308426?accountid=27308%0Ahttp://www.worldfishcenter.org/Naga/na_2082.pdf

Dahruddin, H., Hadiaty, R. K., & Hubert, N. (2016). DNA Barcoding: Foundations and Applications for Southeast Asian Freshwater Fishes. *Treubia*, *43*(December), 1–16.

Dogan, İ., Dogan, & Nurhan. (2016). Genetic Distance Measures: Review. *Turkiye Klinikleri Journal of Biostatistics*, *8*(1), 87–93. https://doi.org/10.5336/biostatic.2015-49517

Fishbase. (2021). Oxyeleotris marmorata Bleeker [online]. Retrieved 29 May 2021, from https: ://www.fishbase.se/summary/Oxyeleotris-marmorata.html

Food and Agriculture Organization (FAO) of United Nation. (2005). *International Mechanism for the Control and Responsible Use of Alien Species in Aquatic Ecosystem. Report of an ad hoc Expert Consultaion 27 - 30 August 2003.* Xishuangbanna, China.

Hubert, N., Espiau, B., Meyer, C., & Planes, S. (2014). Identifying the ichthyoplankton of a coral reef using DNA barcodes. *Molecular Ecology Resources*, *15*(1), 57–67.

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol*, *15*, 111–120.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol Biol Evol*, *33*, 1870–1874.

Last, P. R., Manjaji, B. M., & Yearsley, G. K. (2005). Pastinachus solocirostris sp. nov., a new species of Stingray (Elasmobranchii: Myliobatiformes) from the Indo-Malay Archipelago. *Zootaxa*, *1040*, 1–16. https://doi.org/https://doi.org/10.11646/zootaxa.1040.1.1

Lemey, P., Selemi, M., & Vandamme, A. M. (2009). *The Phylogenetic Handbook: A Practical Approach to Phylogenetic Analysis and Hypothesis Testing*. Cambridge-UK: Cambridge University Press.

Lestari, W., Rukayah, S., & Jamilatun, T. (2019). Ikan Introduksi Oxyeleotris marmorata, Bleeker (1852): Populasi, Laju Exploitasi dan Pengendaliannya di Waduk Sempor, Kebumen. *Majalah Ilmiah Biologi Biosfera*, *36*(3), 132–138. https://doi.org/10.20884/1.mib.2019.36.3.823

Mohsin, A., & Ambak, A. (1983). *Freshwater fishes of Peninsular Malaysia*. Serdang, Malaysia: University Pertanian publishing house.

National Center for Biotechnology Information. (2021). Oxyeleotris marmorata Bleeker [online].

Panprommin, D., Iamchuen, N., Soontornprasit, K., & Tuncharoen, S. (2020). The utility of DNA barcoding for the species identification of larval fish in the lower ing river, Thailand. *Turkish Journal of Fisheries and Aquatic Sciences*, *20*(9), 671–679. https://doi.org/10.4194/1303-2712-v20_9_02

Poh, L. L., Ving, C. C., & Shaliza, Ibrahim Vikineswary, S. (2015). Manipulating Culture Conditions and Feed Quality to Increase the Survival of Larval Marble Goby Oxyeleotris marmorata. *North American Journal of Aquaculture*, *77*(2). https://doi.org/https://doi.org/10.1080/15222055.2014.987932

Sodsuk., P. K., & Sodsuk, S. (2000). Genetic diversity of featherback fish in Thailand. *Kasetsart Journal (National Sciences)*, *34*, 227–233.

Syaifudin, M., Bekaert, M., Taggart, J., Hulata, G., D'Cotta, H., Baroiller, J., … McAndrew, B. (2017). DNA typing across ten tilapia species using cytochrome C oxidase subunit I (COI). *Aquaculture*, *472*(4/1), 125–125.

Syaifudin, M., Jubaedah, D., Muslim, M., & Daryani, A. (2017). DNA authentication of asian redtail catfish Hemibagrus nemurus from Musi and Penukal River, South Sumatra Indonesia. *Genetics of Aquatic Organisms*, *1*(December), 43–48. https://doi.org/10.4194/2459-1831-v1

Syaifudin, M., Wijayanti, M., Dwinanti, S. H., Muslim, Mahendra, M., & Marliana, S. (2020). Short Communication : DNA barcodes and phylogenetic of striped snakehead and ocellated snakehead fish from South Sumatra , Indonesia, *21*(3), 1227–1235. https://doi.org/10.13057/biodiv/d210350

Victor, B. C., Hanner, R., Shivji, M., Hyde, J., & Caldow, C. (2009). Identification of the larval and juvenile stages of the Cubera Snapper, Lutjanus cyanopterus, using DNA barcoding. *Zootaxa*, *36*(August), 24–36.

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. N. (2005). DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *360*(1462), 1847–1857. https://doi.org/10.1098/rstb.2005.1716

Wargasasmita, S. (2012). Ikan air tawar Sumatera yang terancam punah. *Jurnal Iktiologi Indonesia*, *2*(2), 41–49.

Zhao, C., Zhu, X., Gu, Y., Wang, Q., Li, Z., & Yin, S. (2017). Population genetic diversity of marble goby (Oxyeleotris marmoratus) inferred from mitochondrial DNA and microsatellite analysis. *Journal of Genetics*, *96*(s1), 65–71. https://doi.org/10.1007/s12041-017-0850-3

291 Table 1. Identity percentage of nucleotide of marble goby

No.	Species	Origin	Accession no.	Identity (%)
Samples code: OMD3 and OMD2				
1	*Oxyeleotris marmorata*	Malaysia	KT022088.1	99,51
2	*Oxyeleotris marmorata*	USA	AY722177.1	99,51
3	*Oxyeleotris marmorata*	Indonesia	KU692726.1	99,35
Sample code: OMS3				
	Oxyeleotris marmorata	Indonesia	KU692726.1	100
	Oxyeleotris marmorata	Cambodia	EF609424.1	100
	Oxyeleotris marmorata	Vietnam	MH721190.1	99

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Tabel 2. Genetic distance between species marble goby based on COI gene

No	Species	Genetic Distance																				
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	OMD3																						
2	OMD2	0.0000																					
3	OMS3	0.0066	0.0066																				
4	Oreochromis_niloticus_Stirling	0.2082	0.2082	0.2098																			
5	Oxyeleootris_marmorata_USA_AY722177.1	0.0049	0.0049	0.0082	0.1997																		
6	Oxyeleotris_lineolata_Australia_KJ669574.1	0.1311	0.1311	0.1328	0.1917	0.1319																	
7	Oxyeleotris_lineolata_Los_Angeles_AY722165.1	0.1295	0.1295	0.1311	0.1907	0.1203	0.0046																
8	Oxyeleotris_marmorata_Cambodia_EF609424.1	0.0066	0.0066	0.0000	0.2031	0.0092	0.1319	0.1298															
9	Oxyeleotris_marmorata_Indonesia_KU692718.1	0.0066	0.0066	0.0000	0.2040	0.0092	0.1319	0.1304	0.0000														
10	Oxyeleotris_marmorata_Los_Angeles_AY722176.1	0.0049	0.0049	0.0082	0.1982	0.0016	0.1304	0.1187	0.0076	0.0077													
11	Oxyeleotris_marmorata_Malaysia_KT001057.1	0.0000	0.0000	0.0077	0.2146	0.0057	0.1360	0.1341	0.0077	0.0077	0.0057												
12	Oxyeleotris_marmorata_Malaysia_KT022088.1	0.0049	0.0049	0.0082	0.1967	0.0282	0.1304	0.1455	0.0076	0.0077	0.0268	0.0057											
13	Oxyeleotris_marmorata_Thailand_KF410694.1	0.0119	0.0119	0.0154	0.2104	0.0064	0.1389	0.1346	0.0146	0.0147	0.0064	0.0096	0.0064										
14	Oxyeleotris_marmorata_Thailand_MK448069.1	0.0067	0.0067	0.0033	0.2095	0.0095	0.1330	0.1317	0.0048	0.0032	0.0095	0.0077	0.0095	0.0162									
15	Oxyeleotris_marmorata_Thailand_MK448189.1	0.0068	0.0068	0.0000	0.2115	0.0080	0.1343	0.1314	0.0000	0.0000	0.0080	0.0077	0.0080	0.0146	0.0048								
16	Oxyeleotris_marmorata_Sukabumi_IDN_KU692726.1	0.0066	0.0066	0.0000	0.2040	0.0092	0.1319	0.1304	0.0000	0.0000	0.0077	0.0077	0.0077	0.0147	0.0032	0.0000							
17	Oxyeleotris_marmorata_Thailand_MK628379.1	0.0050	0.0050	0.0084	0.2073	0.0016	0.1320	0.1295	0.0080	0.0081	0.0016	0.0057	0.0016	0.0080	0.0096	0.0080	0.0081						
18	Oxyeleotris_marmorata_Vietnam_MH721190.1	0.0083	0.0083	0.0017	0.2077	0.0096	0.1294	0.1278	0.0016	0.0016	0.0096	0.0096	0.0096	0.0164	0.0048	0.0016	0.0016	0.0097					
19	Oxyeleotris_marmorata_Malaysia_KT001058.1	0.0071	0.0071	0.0000	0.2199	0.0089	0.1365	0.1348	0.0000	0.0000	0.0089	0.0077	0.0089	0.0161	0.0035	0.0000	0.0000	0.0089	0.0018				
20	Oxyeleotris_selheimi_Los_Angeles_AY722166.1	0.1230	0.1230	0.1246	0.1832	0.1227	0.0706	0.0765	0.1267	0.1273	0.1211	0.1226	0.1427	0.1298	0.1270	0.1266	0.1273	0.1248	0.1230	0.1259			
21	Oxyeleotris_selheimi_Los_Angeles_AY722179.1	0.1295	0.1295	0.1311	0.1907	0.1203	0.0046	0.0000	0.1298	0.1304	0.1187	0.1341	0.1455	0.1346	0.1317	0.1314	0.1304	0.1295	0.1278	0.1348	0.0765		
22	Palatogobius_paradoxus_USA_MF049134.1	0.1557	0.1557	0.1623	0.1730	0.1531	0.1813	0.1807	0.1562	0.1567	0.1516	0.1609	0.1516	0.1596	0.1597	0.1613	0.1567	0.1557	0.1565	0.1702	0.1746	0.1807	

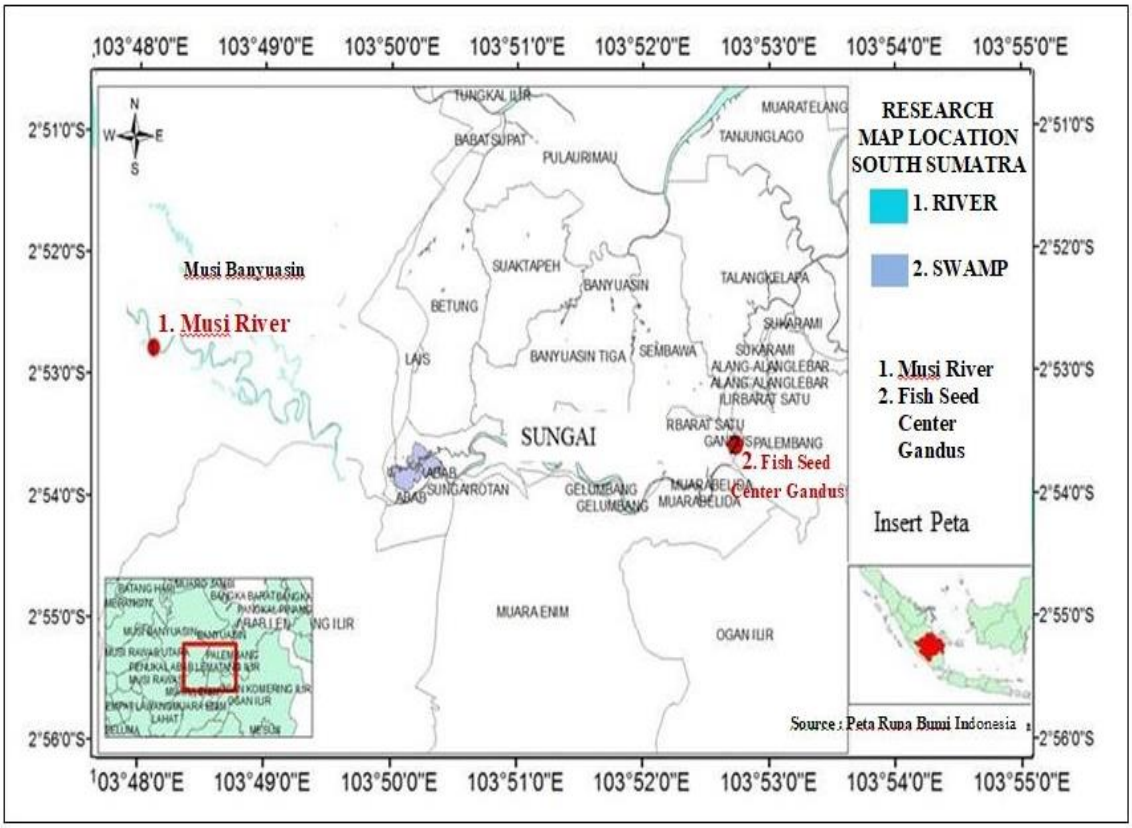
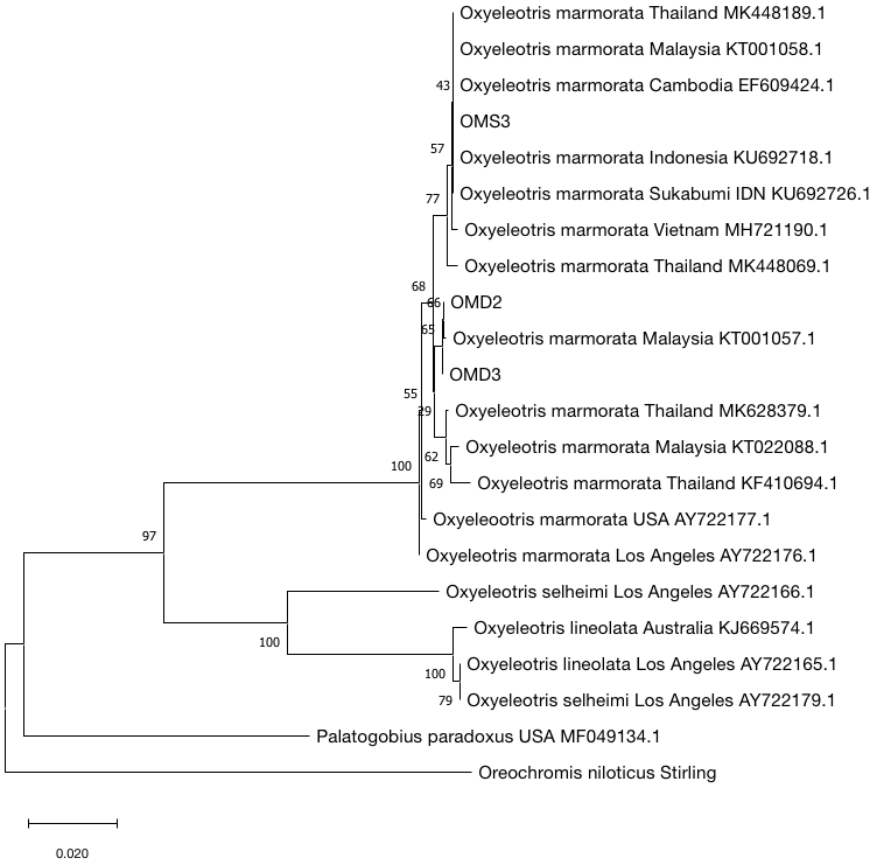


Figure 1. Two sampling site of marble goby from South Sumatra
1. Musi River; 2. Fish Seed Center Gandus



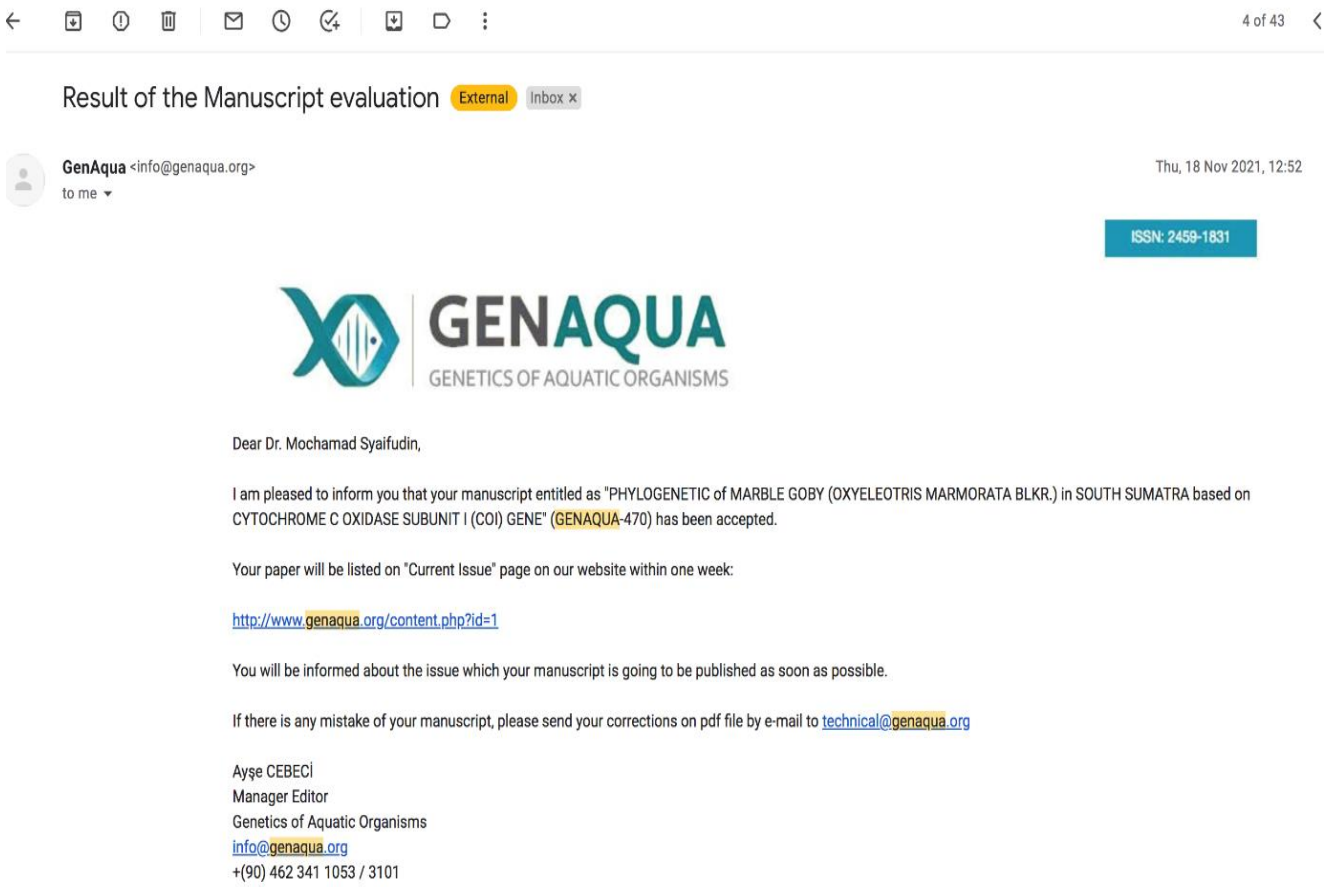
OMD2, OMD3 and OMS3 indicated the specimens of this study

Figure 2. Phylogenetic tree of marble goby from South Sumatra

Highlights

- Four speciemens include domesticated marble goby (OMD2, OMD3) and the wild stock from the Musi River (OMS2, OMS3) were 99.35% matched to *Oxyeleotris marmorata* from Cambodia (EF609424.1) and West Java, Indonesia (KU692718.1 and KU692726.1).
- The phylogenetic tree indicated that the specimens of *O. marmorata* were formed in one sub-cluster (bootstrap value of 96%) with the same species, consisted of *O. marmorata* from Thailand (MK448189.1) and Malaysia (KT001057.1).

Bukti accepted artikel (18 November 2021)



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