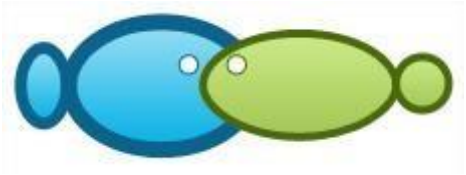

The mitochondrial 16S rRNA gene of *Ompok hypophthalmus* and *Ompok eugeneiatus* from Riau Province of Indonesia

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Abstract. The genus *Ompok* is one of the catfish which include local fishery resources from the Riau Province of Indonesia. Research on the genetic character of the genus *Ompok* in Indonesia is still very rare. This study aimed to determine genetic characterization and phylogenetic reconstruction of the genus *Ompok* from Riau Province of Indonesia using mitochondrial 16S rRNA gene sequences. A total of 15 fish samples were used in this study consisting of six samples of *Ompok eugeneiatus* and nine samples of *Ompok hypophthalmus*. The fish samples were collected from Kampar River, Tapung River and Indragiri River of Riau Province, Indonesia. Total genome was extracted from muscle tissue of fish using DNeasy Blood and Tissue Kit (Qiagen). The mitochondrial 16S rRNA gene was amplified using polymerase chain reaction (PCR) technique. The PCR reaction component follows protocol of the TopTaq Master Mix Kit (Qiagen). The analysis of genetic characterization and phylogenetic relationship was conducted using MEGA version 6.0 software. The results of multiple alignment of 16S rRNA gene sequences of *O. eugeneiatus* and *O. hypophthalmus* from Riau Province of Indonesia obtained 454 bp of nucleotides. The base composition on the 16S rRNA gene showed that the base composition was adenine (A) > thymine (T/U), and guanine (G) < cytosine (C). The composition of the nucleotide bases in the 16S rRNA gene showed that the A+T value was higher than that of G+C. Transition substitution mutations are more common than transversion substitution. The results of genetic distance to 16S rRNA sequences in *O. eugeneiatus* and *O. hypophthalmus* species found that interspecies genetic distance > intraspecies. The phylogenetic tree reconstruction using the 16S rRNA gene showed that this gene could differentiate interspecies between *O. eugeneiatus* and *O. hypohthalmus* from Riau Province, Indonesia. This accuracy is indicated by the formation of separate groups between the species *O. eugeneiatus* and *O. hypophthalmus* from Riau Province, with comparison *Ompok bimaculatus* and *Clarias gariepinus* from GenBank data.

Key Words: identification, phylogenetic, taxonomy.

Introduction. Indonesia is a country that has a very high diversity of fish species (Widayanti et al 2019). The fish of genus *Ompok* are one of the freshwater catfishes which includes local fishery resources that have important economic value in Riau Province of Indonesia. The genus *Ompok* belongs to the family Siluridae and the order Siluriformes (Kottelat 2013). The fish of genus *Ompok* are distributed in South and Southeast Asia (Ng 2013; Sudasinghe & Meegaskumbura 2016). The genus *Ompok* has been known to be paraphyletic consisting of four different clades, namely *Ompok bimaculatus*, *Ompok leiacanthus*, *Ompok hypophthalmus* and *Ompok eugeneiatus* groups (Ng 2003; Ng & Hadiaty 2009). Fish species *O. eugeneiatus* and *O. hypophthalmus* are consumed by people. The fishes *O. eugeneiatus* and *O. hypophthalmus* in Riau Province are known by the local names of selais kaporeh and selais danau, respectively.

Identification and characterization of species is an important step in taxonomic and phylogenetic studies (Elvyra & Afdizan 2021). The use of morphological characters has limitations for the identification of individuals of species which vary greatly in morphology and there are major changes in appearance during the developmental stages of fish (Yi et al 2017). Species identification with a genetic approach can be used to distinguish several species that have very similar external morphological characteristics; where it is difficult to distinguish only by morphological characteristics (Bingpeng et al 2018). Genetic identification

of species using DNA sequences can be applied for identifying stomach contents of piscivorous predators (Moran et al 2016) and is also useful for identifying species in ground meat products (Kane & Hellberg 2016). Approaches to genetic identification of species can help to overcome the limitations of morphological identification (Purnama et al 2019; Elvyra et al 2020; Batubara et al 2021).

Currently, species identification using mitochondrial genes as genetic markers has been widely applied (Chang et al 2017). Mitochondrial 16S rRNA gene that has some highly conserved regions in its sequence has been used as a molecular marker for identification studies of various animal species (Yang et al 2014). The rRNA gene is one of the most conserved genes, but the rRNA nucleotide sequence that varies between species supports its use as a genetic marker for species identification (Ranjithkumar et al 2018). The use of the mitochondrial 16S rRNA gene has been reported to be able to differentiate various fish species in previous studies (Singh et al 2015; Ilmi & Arisuryanti 2018; Arisuryanti et al 2019; Akhtar et al 2020; Andriyono et al 2020).

Research on the genetic characterization of the genus *Ompok* based on the mitochondrial 16S rRNA gene in Riau Province, Indonesia is still very rare. Therefore, it is important to study the 16S rRNA gene sequence in the *Ompok* genus, for the species *O. hypophthalmus* and *O. eugeneiatus* from Riau Province, Indonesia, to identify and determine the phylogenetic relationship.

Material and Method. The fishes used in this study were the species *Ompok eugeneiatus* and *Ompok hypophthalmus*. The fish samples were collected from Kampar River, Tapung River and Indragiri River of Riau Province, Indonesia. Morphological identification of fish was carried out according to Kottelat (2013), Ng (2003) and Kottelat et al (1993). A total of 15 fish samples were analysed, consisting of six samples of *O. eugeneiatus* and nine samples of *O. hypophthalmus*. Fish muscle tissue was taken (25-50 mg) and placed into a 96% ethanol solution, then stored in the freezer (Elvyra 2009). The research was conducted from December 2016 to June 2018. The molecular research was conducted in the Genetics Laboratory, Biology Department, Faculty of Mathematics and Sciences, University of Riau, Indonesia.

Total DNA isolation. The total genomic DNA was extracted from muscle tissue of fish using DNeasy Blood and Tissue Kit (Qiagen). The total DNA isolation process was conducted based on the kit protocol. The quality and quantity of DNA was detected using electrophoresis techniques on 1.2% agarose gel.

Amplification and sequencing. Total DNA amplification was carried out by the polymerase chain reaction (PCR) process. The PCR reaction follows protocol of the TopTaq Master Mix Kit (Qiagen). The primer pairs used to amplificate 16S rRNA gene, were the following: 16SF 5'AATGAAGACCTGTATGAATGGTGGAACGAGG3' and 16SR 5'GCATTACAGATAGAAACTGACCTGGATTGC3' (Vittas et al 2011).

The PCR process was conducted following Elvyra (2009) method with modifications. The PCR program includes the pre-PCR stage for 3 minutes at 94^{o}C. The PCR process was carried out with as many as 35 cycles, each cycle consisting of DNA denaturation at 94^{o}C for 30 seconds, annealing of primers at 55.2^{o}C for 1 minute and 30 seconds, and elongation at 72^{o}C for 1 minute. In the final stage, post PCR was performed at 72^{o}C for 10 minutes. The result of PCR amplification was detected by electrophoresis techniques on 1.2% agarose gel. Then, the PCR products are purified and sequenced at PT Genetica Science Indonesia. Sequencing was performed in one direction by using the same forward primer in the PCR process.

Data analysis. The sequence of 16S rRNA nucleotides from each sample was homologized, then multiple alignments were performed. The reference data used as a comparison are the complete 16S rRNA nucleotide sequence from GenBank namely *Ompok bimaculatus* (KY887474.1) and as an outgroup is *Clarias gariepinus* (NC027661.1) (NCBI 2020). All sample sequences have been registered to the National Centre for Biotechnology Information (NCBI) with accession numbers of MK503938-MK503943 for *O. eugeneiatus*, and MK503946-MK503954 for *O. hypophthalmus*. Data analysis was conducted using the MEGA software version 6.06 (Tamura et al 2013). Data analysed included the composition of nucleotides,

substitution of nucleotide, genetic distance using the pairwise distance calculation and phylogenetic trees using the Neighbour-Joining method with bootstrap test of 1000 replications.

Results and Discussion. The results of multiple alignment of 16S rRNA gene sequences of *Ompok eugeneiatus* and *Ompok hypophthalmus* from Riau Province, with comparison of *O. bimaculatus* (KY887474.1) from GenBank data obtained 454 bp of nucleotides. The nucleotide base composition of the 16S rRNA gene of *O. eugeneiatus* and *O. hypophthalmus* from Riau Province of Indonesia is presented in Table 1. The composition of the 16S rRNA gene sequentially has an average base of adenine (A) 31.1%, guanine (G) 22.1%, thymine (T/U) 22.5%, and cytosine (C) 24.3%. The results of the base composition in the 16S rRNA gene showed that the base composition was adenine (A) > thymine (T/U), and guanine (G) < cytosine (C). The nucleotide composition of bases A > T and G < C in the 16S rRNA gene was also obtained by Lakra et al (2009) and Cawthorn et al (2012).

Table 1

Nucleotide composition of 16S rRNA gene of *Ompok eugeneiatus* and *Ompok hypophthalmus* from Riau Province of Indonesia

Species	*T(U)*	*C*	*A*	*G*	*A+T*	*G+C*
1 *O. bimaculatus* (GenBank)	22.5	24.1	30.1	23.4	52.6	47.4
2 *O. eugeneiatus* (Indragiri 1)	22.8	23.7	31.3	22.2	54.1	45.9
3 *O. eugeneiatus* (Indragiri 2)	22.8	23.7	31.3	22.2	54.1	45.9
4 *O. eugeneiatus* (Indragiri 3)	22.8	23.7	31.3	22.2	54.1	45.9
5 *O. eugeneiatus* (Kampar 1)	22.8	23.7	31.3	22.2	54.1	45.9
6 *O. eugeneiatus* (Kampar 2)	22.8	23.7	31.3	22.2	54.1	45.9
7 *O. eugeneiatus* (Kampar 3)	22.8	23.7	31.0	22.4	53.9	46.1
8 *O. hypophthalmus* (Indragiri 1)	22.4	24.6	31.3	21.7	53.7	46.3
9 *O. hypophthalmus* (Indragiri 2)	22.4	24.6	31.3	21.7	53.7	46.3
10 *O. hypophthalmus* (Indragiri 3)	22.4	24.6	31.3	21.7	53.7	46.3
11 *O. hypophthalmus* (Kampar 1)	22.6	24.6	31.0	21.7	53.7	46.3
12 *O. hypophthalmus* (Kampar 2)	22.6	24.6	31.0	21.7	53.7	46.3
13 *O. hypophthalmus* (Kampar 3)	22.4	24.6	31.3	21.7	53.7	46.3
14 *O. hypophthalmus* (Tapung 1)	22.4	24.6	31.3	21.7	53.7	46.3
15 *O. hypophthalmus* (Tapung 2)	22.4	24.6	30.6	22.4	53.0	47.0
16 *O. hypophthalmus* (Tapung 3)	22.4	24.6	31.3	21.7	53.7	46.3
17 *C. gariepinus* (GenBank)	21.1	24.7	31.7	22.5	52.8	47.2
Average	22.5	24.3	31.1	22.1	53.6	46.4

Note: adenine (A), thymine (T/U), guanine (G), cytosine (C).

Overall composition of nucleotide bases A+T (53.6%) is greater than G+C (46.4%). Higher A+T values were found in *O. eugeneiatus* from the Indragiri River and Kampar River in Riau Province. Research of Tutar (2015) also found that the A+T content was higher than G+C in the 16S rRNA nucleotide composition of the fish species of *Mastacembelus mastacembelus*. Likewise, research of Singh et al (2015) obtained the composition of the 16S nucleotide base in the fish species *Barilius* spp. which has a higher A+T content. Ilmi and Arisuryanti (2018) also found that the average A+T was greater than that of G+C in the nucleotide composition of 16S rRNA of *Channa gachua*. Akhtar et al (2020) also found higher A+T than G+C in the nucleotide composition of 16S rRNA in fish species from the subfamily Schizothoracinae. Arisuryanti et al (2019) also found that the average A+T nucleotide composition was higher than the C+G base content in the fish species *Helostoma temminckii*. The results of the 16S rRNA gene sequence showed that the A+T content of *Tetraodon lineatus* and *Arothron hispidus* was higher than the C+G content (Mar'ie & Allam 2019).

Transitional substitution mutations in the 16S rRNA gene within intraspecies *O. eugeneiatus* from Riau Province occurred with as many as 0-3 nucleotides, while for intraspecies *O. hypophthalmus* from Riau Province there were 0-5 nucleotides (Table 2). The transitional substitution between the interspecies *O. eugeneiatus* and *O. hypophthalmus* is 8-13 nucleotides. The transitional substitution between *O. bimaculatus* and *Ompok* spp. from Riau Province was 17-21 nucleotides, while between *C. gariepinus* and *Ompok* spp. from Riau

Province was 18-23 nucleotides. The average transition substitution that occurred between *O*. *eugeneiatus*, *O. hypophthalmus*, *O*. *bimaculatus* and *C*. *gariepinus* was 9 nucleotides.

Table 2

Transitional substitution mutations in the 16S rRNA gene of *Ompok eugeneiatus* and *Ompok hypophthalmus* from Riau Province of Indonesia

Species	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*
1																	
2	18																
3	18	**0**															
4	20	**2**	**2**														
5	20	**2**	**2**	**0**													
6	18	**0**	**0**	**2**	**2**												
7	17	**1**	**1**	**3**	**3**	**1**											
8	18	8	8	10	10	8	9										
9	18	8	8	10	10	8	9	**0**									
10	18	8	8	10	10	8	9	**0**	**0**								
11	20	10	10	8	8	10	11	**2**	**2**	**2**							
12	20	10	10	8	8	10	11	**2**	**2**	**2**	**0**						
13	18	8	8	10	10	8	9	**0**	**0**	**0**	**2**	**2**					
14	18	8	8	10	10	8	9	**0**	**0**	**0**	**2**	**2**	**0**				
15	21	11	11	13	13	11	12	**3**	**3**	**3**	**5**	**5**	**3**	**3**			
16	18	8	8	10	10	8	9	**0**	**0**	**0**	**2**	**2**	**0**	**0**	**3**		
17	22	18	18	20	20	18	19	20	20	20	22	22	20	20	23	20	

Note: 1 *Ompok bimaculatus* (GenBank), 2 *Ompok eugeneiatus* (Indragiri 1), 3 *Ompok eugeneiatus* (Indragiri 2), 4 *Ompok eugeneiatus* (Indragiri 3), 5 *Ompok eugeneiatus* (Kampar 1), 6 *Ompok eugeneiatus* (Kampar 2), 7 *Ompok eugeneiatus* (Kampar 3), 8 *Ompok hypophthalmus* (Indragiri 1), 9 *Ompok hypophthalmus* (Indragiri 2), 10 *Ompok hypophthalmus* (Indragiri 3), 11 *Ompok hypophthalmus* (Kampar 1), 12 *Ompok hypophthalmus* (Kampar 2), 13 *Ompok hypophthalmus* (Kampar 3), 14 *Ompok hypophthalmus* (Tapung 1), 15 *Ompok hypophthalmus* (Tapung 2), 16 *Ompok hypophthalmus* (Tapung 3), 17 *Clarias gariepinus* (GenBank), average = 9 nucleotides.

There were no nucleotide transversion substitution mutations in the 16S rRNA gene within intraspecies *O*. *eugeneiatus* from Riau Province, while for intraspecies *O*. *hypophthalmus* from Riau Province there was 0-1 nucleotide substitution transversion (Table 3). The transversion substitution between the interspecies *O*. *eugeneiatus* and *O*. *hypophthalmus* is 1-2 nucleotides. The transversion substitution between *O*. *bimaculatus* and *Ompok* from Riau Province was 6-7 nucleotides, while between *C*. *gariepinus* and *Ompok* spp. from Riau Province was 8-9 nucleotides. The average transversion substitution between *O*. *eugeneiatus*, *O*. *hypophthalmus*, *O*. *bimaculatus* and *C*. *gariepinus* was 2 nucleotides.

Table 3

Transversional substitution mutations in the 16S rRNA gene of *Ompok eugeneiatus* and *Ompok hypophthalmus* from Riau Province of Indonesia

Species	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*
1																	
2	7																
3	7	**0**															
4	7	**0**	**0**														
5	7	**0**	**0**	**0**													
6	7	**0**	**0**	**0**	**0**												
7	7	**0**	**0**	**0**	**0**	**0**											
8	6	1	1	1	1	1	1										
9	6	1	1	1	1	1	1	**0**									
10	6	1	1	1	1	1	1	**0**	**0**								
11	7	2	2	2	2	2	2	**1**	**1**	**1**							
12	7	2	2	2	2	2	2	**1**	**1**	**1**	**0**						
13	6	1	1	1	1	1	1	**0**	**0**	**0**	**1**	**1**					
14	6	1	1	1	1	1	1	**0**	**0**	**0**	**1**	**1**	**0**				
15	6	1	1	1	1	1	1	**0**	**0**	**0**	**1**	**1**	**0**	**0**			
16	6	1	1	1	1	1	1	**0**	**0**	**0**	**1**	**1**	**0**	**0**	**0**		
17	8	9	9	9	9	9	9	8	8	8	9	9	8	8	8	8	

Note: 1 *Ompok bimaculatus* (GenBank), 2 *Ompok eugeneiatus* (Indragiri 1), 3 *Ompok eugeneiatus* (Indragiri 2), 4 *Ompok eugeneiatus* (Indragiri 3), 5 *Ompok eugeneiatus* (Kampar 1), 6 *Ompok eugeneiatus* (Kampar 2), 7 *Ompok eugeneiatus* (Kampar 3), 8 *Ompok hypophthalmus* (Indragiri 1), 9 *Ompok hypophthalmus* (Indragiri 2), 10 *Ompok hypophthalmus* (Indragiri 3), 11 *Ompok hypophthalmus* (Kampar 1), 12 *Ompok hypophthalmus* (Kampar 2), 13 *Ompok hypophthalmus* (Kampar 3), 14 *Ompok hypophthalmus* (Tapung 1), 15 *Ompok hypophthalmus* (Tapung 2), 16 *Ompok hypophthalmus* (Tapung 3), 17 *Clarias gariepinus* (GenBank), average = 2 nucleotides.

Based on substitution mutations that occur in the 16S rRNA gene within intraspecies *O*. *eugeneiatus* and *O*. *hypophthalmus*, it is shown that the mutations that occur are relatively very small compared to interspecies. Overall, the results of the nucleotide substitution analysis obtained show that the number of transition substitutions > transversion substitutions. The more frequent transitional substitution rates were also found in the 16S rRNA gene of the fish family Sciaenidae (Lakra et al 2009) and family Salmonidae (Dudu et al 2011). Singh et al (2015) found that transition substitution occurred more frequently than transversion in the 16S rRNA gene of *Barilius* spp. Akhtar et al (2020) also obtained research results regarding more transitional substitution than transversion substitution in the 16S rRNA gene of fish subfamily Schizothoracinae.

The genetic distance between *O*. *eugeneiatus* and *O*. *hypophthalmus* from Riau Province, with *O*. *bimaculatus* and *C*. *gariepinus* from GenBank data are presented in Table 4 (NCBI 2020). The results of genetic distance (p-distance) against 16S rRNA sequences in species of *O*. *eugeneiatus* and *O*. *hypophthalmus* from Riau Province, with comparison sequences from *O*. *bimaculatus* (GenBank) and *C*. *gariepinus* (GenBank) data, obtained genetic distances among species, that ranged from 0.00-0.007. The genetic distance within intraspecies *O*. *eugeneiatus* from Riau Province was 0.00-0.01, as well as the genetic distance within intraspecies *O*. *hypophthalmus* was 0.00-0.01. The genetic distance between the interspecies *O*. *eugeneiatus* and *O*. *hypophthalmus* was 0.02-0.03. The value of the genetic distance between *O. bimaculatus* and *Ompok* spp. from Riau Province was 0.05-0.06. The genetic distance value between *C*. *gariepinus* (outgroup) and *Ompok* spp. from Riau Province was 0.06-0.07.

Table 4

Genetic distance of 16S rRNA gene of *Ompok eugeneiatus* and *Ompok hypophthalmus* from Riau Province of Indonesia

Species	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*
1																	
2	0.06																
3	0.06	**0.00**															
4	0.06	**0.00**	**0.00**														
5	0.06	**0.00**	**0.00**	**0.00**													
6	0.06	**0.00**	**0.00**	**0.00**	**0.00**												
7	0.05	**0.00**	**0.00**	**0.01**	**0.01**	**0.00**											
8	0.05	0.02	0.02	0.02	0.02	0.02	0.02										
9	0.05	0.02	0.02	0.02	0.02	0.02	0.02	**0.00**									
10	0.05	0.02	0.02	0.02	0.02	0.02	0.02	**0.00**	**0.00**								
11	0.06	0.03	0.03	0.02	0.02	0.03	0.03	**0.01**	**0.01**	**0.01**							
12	0.06	0.03	0.03	0.02	0.02	0.03	0.03	**0.01**	**0.01**	**0.01**	**0.00**						
13	0.05	0.02	0.02	0.02	0.02	0.02	0.02	**0.00**	**0.00**	**0.00**	**0.01**	**0.01**					
14	0.05	0.02	0.02	0.02	0.02	0.02	0.02	**0.00**	**0.00**	**0.00**	**0.01**	**0.01**	**0.00**				
15	0.06	0.03	0.03	0.03	0.03	0.03	0.03	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**			
16	0.05	0.02	0.02	0.02	0.02	0.02	0.02	**0.00**	**0.00**	**0.00**	**0.01**	**0.01**	**0.00**	**0.00**	**0.01**		
17																	
	0.07	0.06	0.06	0.07	0.07	0.06	0.06	0.06	0.06	0.06	0.07	0.07	0.06	0.06	0.07	0.06	

Comparison of genetic distance to 16S rRNA sequences showed that there was lower genetic distance variation within the same species population (intraspecies), with genetic distance values ranging from 0.00-0.01. The interspecies genetic distance in the 16S rRNA sequence between *O*. *eugeneiatus* and *O*. *hypophthalmus* from Riau Province, which are part of the *Ompok* genus, ranged between 0.02-0.03. The results of genetic distance to 16S rRNA sequences in *O*. *eugeneiatus* and *O*. *hypohthalmus* species found that interspecies genetic distance > intraspecies. A low level within intraspecies variation (0.00-0.02%) was also found by Singh et al (2015). The significant genetic distance in the 16S rRNA sequence was shown in the comparison of *C*. *gariepinus* species (outgroup) to *Ompok* spp. from Riau province with the farthest genetic distance value of 0.06-0.07. In addition to coming from different genera, the high genetic distance is also caused by the very separate geographical location between these species which does not allow genetic flow to occur (Elvyra & Afdizan 2021).

Phylogenetic reconstruction using the 16S rRNA gene between *O*. *eugeneiatus* and *O*. *hypophthalmus* from Riau Province, with *O*. *bimaculatus* and *C*. *gariepinus* from GenBank data is presented in Figure 1 (NCBI 2020).

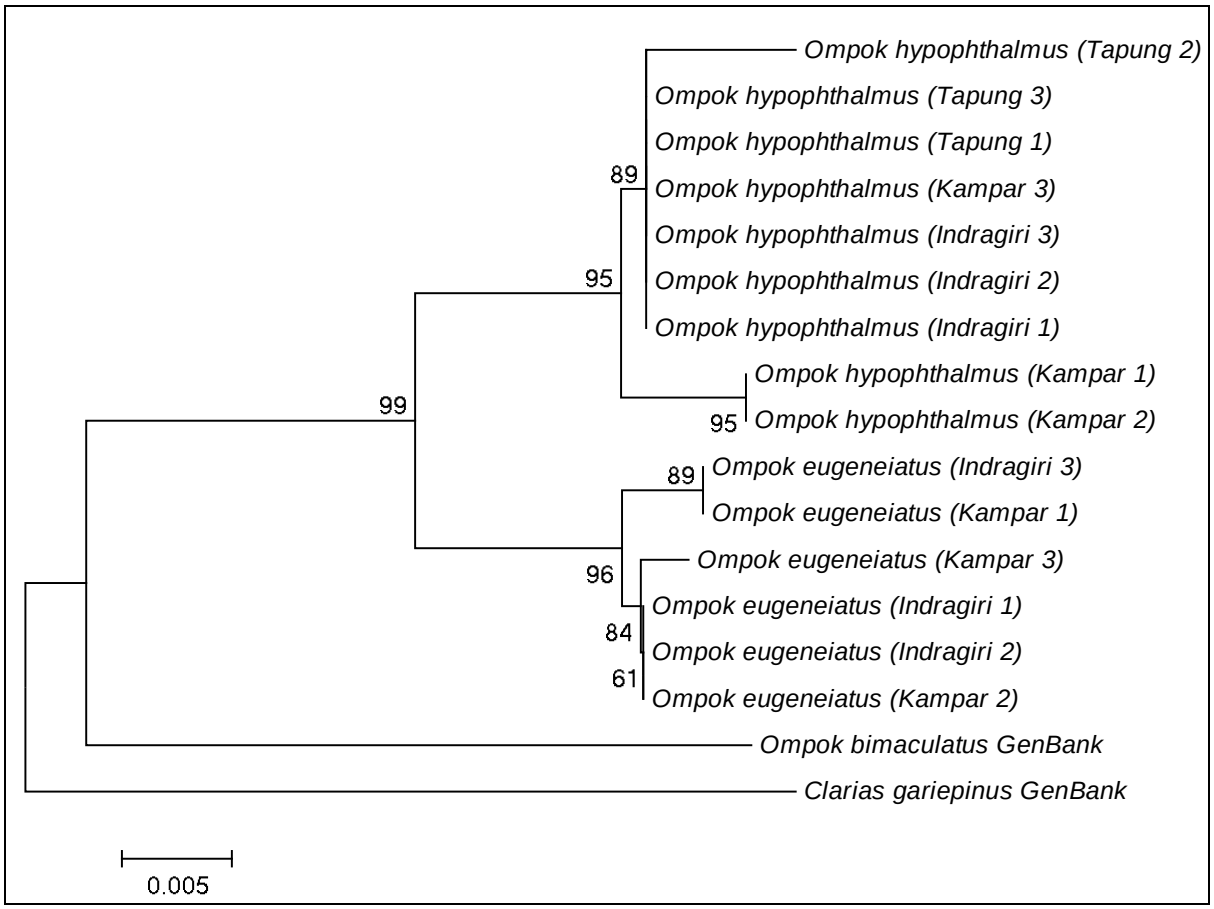

Figure 1. Phylogenetic tree of *Ompok eugeneiatus* and *Ompok hypophthalmus* from Riau Province of Indonesia based on 16S rRNA gene.

The results of the phylogenetic tree reconstruction using the 16S rRNA gene obtained a phylogenetic topology with bootstrap values between 61-99%. *O*. *hypophthalmus* originating from the Tapung, Kampar and Indragiri rivers formed a group with a bootstrap value of 95%. *O*. *eugeneiatus* originating from the Kampar and Indragiri rivers formed a relationship group with a bootstrap value of 96%. Species of *O*. *eugeneiatus* and *O*. *hypophthalmus* which are both from Riau Province have a close genetic relationship because although they are divided according to their respective species, they still form one group with a bootstrap value of 99%. The comparison species *O*. *bimaculatus* forms a large group with *O*. *eugeneiatus* and *O*. *hypophthalmus* because they are both included in the same genus, namely *Ompok* and the family Siluridae. The

outgroup used is *C*. *gariepinus* which belongs to the family Clariidae, where both Siluridae and Clariidae are members of the order Siluriformes.

Reconstruction of the phylogenetic tree using the 16S rRNA gene showed that this gene could differentiate *O*. *eugeneiatus* and *O*. *hypophthalmus* species at the interspecies level. This accuracy is indicated by the formation of separate relationship groups between the species *O*. *eugeneiatus*, *O*. *hypophthalmus*, *O*. *bimaculatus* and *C*. *gariepinus* (outgroup). The separation that occurs is supported by bootstrap values ranging between 95-99%. Hillis and Bull (1993) stated that a bootstrap value of more than 70% is the minimum value to be able to produce a precise phylogram accuracy, and a bootstrap value of 95% is an indication that the resulting phylogram has high accuracy or is a form of an actual cluster.

Phylogenetics within the intraspecies level with the 16S rRNA gene resulted in overlapping branching, this was shown within the grouping of *O*. *eugeneiatus* and *O*. *hypophthalmus* species from different river habitats into the same group. Phylogenetics at the intraspecies level using the 16S rRNA gene were shown in the grouping of *O*. *eugeneiatus* and *O*. *hypophthalmus* species from different river habitats into the same group. These phylogenetic results indicate that the 16S rRNA gene has a low ability to reconstruct a phylogenetic tree at intraspecies level. However, the 16S rRNA gene was able to separate the interspecies groups *O*. *eugeneiatus* and *O*. *hypophthalmus* from Riau Province, with *O*. *bimaculatus* and *C*. *gariepinus* from GenBank data (NCBI 2020).

Conclusions. Based on the results of research on genetic distance and reconstruction of phylogenetic tree using the 16S rRNA gene, it can be concluded that this gene can distinguish *O*. *eugeneiatus* and *O*. *hypophthalmus* species at the interspecies level. At the intraspecies level, it showed overlapping branching in each population of *O*. *eugeneiatus* and *O*. *hypophthalmus* from different river habitats but included in the same group. The results of this study indicate that the 16S rRNA gene has a low ability to distinguish groups of habitat origin at the intraspecies level but has a high ability to distinguish between species.

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