

Short Communication: Molecular characteristics and phylogenetic relationships of silurid catfishes (*Kryptopterus*, *Ompok* and *Phalacronotus*) from the Kampar River, Indonesia, based on the cytochrome b gene

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Abstract. Elvyra R, Solihin DD, Affandi R, Junior MZ, Suhendra M. 2020. Short Communication: Molecular characteristics and phylogenetic relationships of silurid catfishes (*Kryptopterus*, *Ompok*, and *Phalacronotus*) from the Kampar River, Indonesia, based on the cytochrome b gene. *Biodiversitas* 21: 3539-3546. The study of molecular characteristics and phylogenetic relationships among silurid catfishes (*Kryptopterus*, *Ompok*, and *Phalacronotus*) is very scarce. Existing data are mostly based on morphological characters. Genetic markers among *Kryptopterus*, *Ompok*, and *Phalacronotus* can be analyzed by exploring the nucleotide and amino acid sequences of the mitochondrial cytochrome b gene region (906 base pairs). This study aims to identify molecular characteristics and phylogenetic relationships of silurid catfishes from the Kampar River based on the cytochrome b gene. This research examined 14 silurids of four species from three genera in the Kampar River of Indonesia. Molecular phylogenetic trees were constructed using the neighbor-joining method. This study revealed that three amino acid sites can be used as specific genetic markers for characterizing *Kryptopterus limpok*. They are E (glutamic acid) at site 123, V (valine) at site 155, and M (methionine) at site 156. Two amino acid sites can be used as specific genetic markers of *Ompok* spp., A (alanine) at site 201 for *O. hypophthalmus*, and S (serine) at site 282 for *O. eugeneiatus*. Two amino acid sites, I (isoleucine) at site 197 and L (leucine) at site 284, can be used to identify *Phalacronotus apogon* from the Kampar River, Indonesia. The phylogram results based on genetic distance (p-distance) from nucleotide cytochrome b sequences generally showed that intraspecies of *K. limpok*, *O. eugeneiatus*, *O. hypophthalmus* and *P. apogon* from the Kampar River formed relationship groups within each species supported by high bootstrap values. Additionally, based on genetic distance, *K. limpok* data from the Kampar River samples and GenBank formed one relationship group within species of *K. limpok* with a high bootstrap value. This study proves that cytochrome b gene can be used to identify molecular characteristics and phylogenetic relationships of *K. limpok*, *O. eugeneiatus*, *O. hypophthalmus*, and *P. apogon* from the Kampar River, Indonesia.

Keywords: Catfish, Cytochrome b, Indonesia, Kampar River

INTRODUCTION

Phalacronotus, *Kryptopterus*, and *Ompok* are freshwater catfish belonging to the family Siluridae in order of Siluriformes. The members of the silurid catfish genus *Phalacronotus* and *Kryptopterus* are small- to medium-sized fishes, whereas *Ompok* is medium-sized. This catfish lives in freshwaters of South and Southeast Asia (Ng 2003; Ng and Tan 2004). The genus *Phalacronotus*, *Kryptopterus*, and *Ompok*, as currently understood, are probably paraphyletic based on morphological characters (Bornbusch 1995). The non-monophyly of the genus *Kryptopterus* comprises distinct clades (*K. bicirrhis*, *K. kryptopterus*, *K. limpok* and *K. schilbeides* groups) (Ng and Kottelat 2013), while the *K. apogon* and *K. hexapterus* groups are assigned to distinct

genus (*Phalacronotus* and *Micronema* respectively; Ferraris 2007). The genus *Ompok* has also been acknowledged as a non-monophyletic group, that it is comprised of four clades (*O. bimaculatus*, *O. leiakanthus*, *O. hypophthalmus*, and *O. eugeneiatus* group) (Ng 2013).

Approaches for identification are mostly based on morphological characteristics (Ng and Hadiaty 2009; Ng and Kottelat 2013; Sudasinghe and Meegaskumbura 2016; Batubara et al. 2018) because they are easier and not expensive. Yet, this approach has several boundaries or constraints. One of which is the body part of the object to be identified must be complete. Species morphological identification also has several limitations. For example, identification keys are often only suitable for certain stages of life or gender, so high expertise in taxonomy is needed (Hebert et al. 2003). At present, there is a new approach to

determining species, called DNA barcodes, using a short piece of DNA from the genome of the organism (Hebert et al. 2003; Stoeckle 2003; Patwardhan et al. 2014). DNA-based studies allow taxonomists to identify specimens even if they are incompletely, such as body fragments (Ivanova et al. 2007), larval stages (Azmir et al. 2017), and cryptic species (Ayudhaya et al. 2017). The morphology and molecular data can be used in cooperation of complement each other to resolve the taxonomic complication.

Molecular technique with the mitochondrial cytochrome b gene is often used in systematic and phylogenetic research at various fish taxonomic levels. Farias et al. (2001) investigated the utility of the cytochrome b gene as a genetic marker for concluding phylogenetic relationships of taxonomic levels within fish of the family Cichlidae. The phylogenetic utility of the cytochrome b gene has been studied at some taxonomic levels of fish taxa (e.g., Doadrio et al. 2002; Doadrio and Dominguez 2004; Peng et al. 2004; Hardman 2005; Ketmaier et al. 2004; Barat et al. 2012; Wilcox et al. 2004). Moreover, the cytochrome b gene has been used as a tool to differentiate various species of bullhead torrent catfish (Kartavtsev et al. 2007), European catfish (Vittas et al. 2010) and Anabantoidei fish (Degani 2013).

The cytochrome b gene contains the position of codons that develop slowly and rapidly, as well as more intraspecific variations, especially in the third position of codons that can be used to identify stocks (Habib et al. 2011). Therefore, this gene has been used for a diversity of systematic questions (Malakar et al. 2013). In this study, the mitochondrial cytochrome b gene region (906 base pairs) was used for genetic markers of 14 individuals from four species from three genera of silurid catfishes in the Kampar River, Indonesia. There are four species of catfishes from the genera *Kryptopterus*, *Ompok*, and *Phalacrotonotus* in the Kampar River: *K. limpok*, *O. eugeneiatus*, *O. hypophthalmus*, and *P. apogon*. Catfishes of these genera are consumed by humans and have a high economic value when prepared as smoked fish. The research on cytochrome b in Indonesia has been carried out on *Chanos chanos* (Adiputra et al. 2011), *Puntius* cf. *binotatus* (Karlina et al. 2016), *Osphronemus goramy* (Nugroho et al. 2019), Bagridae, Pangasiidae and Ariidae (Megarani et al. 2020). Research on the cytochrome b gene in silurid catfishes is very scarce in Indonesia; in fact, cytochrome b data for *O. eugeneiatus* and *O. hypophthalmus* have not been in GenBank until now (updated on December 9th of 2019). Hence, the aim of this research is to explore the utility of the cytochrome b gene as a genetic marker to identify molecular characteristics and phylogenetic relationships of *K. limpok*, *O. eugeneiatus*, *O. hypophthalmus* and *P. apogon* from Kampar River, Indonesia.

MATERIALS AND METHODS

Sample collection and preparation

A total of 14 individuals of silurids were used for analysis purposes. Four species, *K. limpok* (3 individuals), *O. eugeneiatus* (3 individuals), *O. hypophthalmus* (5

individuals), and *P. apogon* (3 individuals), were collected in the Kampar River (0°15'57.2"N 101°42'32.5"E), Riau Province, Indonesia. Caudal muscle tissue was taken from each sample to DNA analysis. The samples were preserved in 96% ethanol before the DNA extraction process.

DNA extraction and PCR amplification

Total DNA was isolated by a standard proteinase K and phenol/chloroform extraction method (Sambrook et al. 1989). Fragments of the cytochrome b gene (1104 bp) were amplified via polymerase chain reaction (PCR) from each DNA sample. The specific primers CBKR1 5'CCCGAAAACTCACCCCTTA3' and CBKR2 5'ATAGCCCGTTAGAGGGTTT3' were designed as cytochrome b primers (Elvyra 2009).

The PCR reaction mixture contained 100 ng of template DNA, 0.2 µM of each primer, 1x reaction buffer, 0.15 mM of MgCl₂, 0.2 mM dNTPS, 2.0 units Taq DNA polymerase and pure water until 50 µL total reaction. The amplification process was conducted as follows: an initial 94°C denaturation for 5 min, 35 cycles of 94°C denaturation for 30 s, 51°C annealing for 45 s, 72°C extension for 1 min, and a final 72°C extension for 5 min (Elvyra 2009). PCR products were electrophoresed for analysis on a 1.2% agarose gel. Nucleotide sequences of the cytochrome b gene were determined using purified PCR products. The same primers, i.e., CBKR1 and CBKR2, were used for sequencing. Strands were sequenced in both directions for each sample. Phenograms and alignments were visually checked and verified.

Mitochondrial DNA data analysis

Homologous nucleotide or amino acid sequences in the cytochrome b gene of the mitochondrial DNA were obtained and then aligned (multiple alignments) in comparison with cytochrome b gene sequences of *Kryptopterus*, *Ompok* and *Micronema* species from GenBank data: *K. minor* (AY458895), *K. limpok* (DQ119431), *O. bimaculatus* (KJ646879), *O. miostoma* (DQ119435) and *Micronema apogon* (DQ119409). The cytochrome b gene sequence of *Ictalurus punctatus* (AB045119) from GenBank data was used as an outgroup for phylogenetic analysis.

Amino acids for coding followed the genetic code for vertebrate mitochondrial DNA. Analysis of genetic markers and relationships based on nucleotides and amino acid sequences were conducted using the MEGA program version 6.0 (Tamura et al. 2013) with the bootstrapped neighbor-joining method with 1000 replicates. All sequences were submitted to the *National Center for Biotechnology Information* (NCBI) with accession numbers of MH745411-MH745413 for *K. limpok*, MH793319-MH793321 for *O. eugeneiatus*, MH793322-MH793326 for *O. hypophthalmus* and MH745414-MH745416 for *P. apogon*.

RESULTS AND DISCUSSION

Cytochrome b gene amplification using the forward primer CBKR1 and reverse primer CBKR2 produced a 1104 bp cytochrome b gene fragment in all species of

catfish. Based on the complete cytochrome b gene sequence (1141 bp) of *K. minor* (GenBank, 2009, access code AY458895; Wilcox et al. 2004), which was used as a reference, this fragment is located from base 8 to 1111. The DNA sequence from the multiple alignments of 906 nucleotides is located at position 115 to 1020 (for the whole 1141-bp reference based on the complete cytochrome b gene of *K. minor* from GenBank data).

The overall nucleotide composition of cytochrome b was obtained (Table 1); on average, the nucleotide C is the most dominant (32.1%), while the least frequent nucleotide is G (14.3%). Regarding codon positions, the most common nucleotide in the first codon position is C (26.4%), while the least nucleotide in the first codon position is A (23.5%). The most common nucleotide in the second codon position is T (41.1%), while the least nucleotide in the second codon position is G (13.3%). Finally, the most common nucleotide in the third codon position is C (44.5%), while the least nucleotide in the third codon position is G (3.6%). In this study, low G values found in the second and the third codon position. Results of this study have the lowest G values in the nucleotide composition of the cytochrome b gene. Low G values are commonly found in the mitochondrial DNA of the Iberian Barbels fishes (Doadrio et al. 2002) and Glyptosternoid fishes (Peng et al. 2004). This lower G value is also found in *O. bimaculatus* (Malakar et al. 2013). The pattern of differences between A, T, C, and G can show genetic signs and characteristics of a species (Afreixo et al. 2009).

It is also found that the greatest variation of the nucleotide composition was in the position of the third codon from codon triplets of the cytochrome b gene. Doadrio and Perdices (2005); Ketmaier et al. (2004); Peng et al. (2004) also found the greatest variation at the third codon position of codon triplets on the whole cytochrome b gene. This is because the first and the second positions have a low substitution rate, while the third position has a higher substitution value (Farias et al. 2001).

Overall, composition nucleotide showed a similar condition, which is A+T, was greater than G+C. The average A + T nucleotide composition of *K. limpok*, *Ompok* spp. and *P. apogon* (53.5%) was greater than the average G + C nucleotide composition (46.5%). The highest nucleotide A + T composition was found in *K. limpok* (53.8%), and the lowest nucleotide A + T composition was found in *O. hypophthalmus* (52.0%). A greater A + T composition than G + C composition was also found by Ketmaier et al. (2004) and Malakar et al. (2013). The nucleotide sequence pattern of cytochrome b rich in A + T nucleotide base composition was generally shown for taxa of fishes (Johns and Avise 1998).

Based on the 380 amino-acid references of the *K. minor* cytochrome b gene (GenBank data, access code AY458895), 906 nucleotides were coded for 302 amino acids in the region from amino acid position 39 to 340. Amino acid sites can distinguish species of *K. limpok* (Table 2), *P. apogon* (Table 3), and *Ompok* spp. (Table 4) from the Kampar River. Intraspecies markers of *K. limpok*

from the Kampar River can be characterized by specific amino acid sites. Three amino acid sites have a marker pattern specific to the species *K. limpok*; of these sites, i.e., V (valine) at site 155 and M (methionine) at site 156, are characterizing *K. limpok* from both the Kampar River and GenBank data. The amino acid at site 123 is E (glutamic acid), characterizing the population of *K. limpok* only from Kampar with the codon GAA.

Intraspecies markers of *P. apogon* from the Kampar River also can be characterized by specific amino acid sites. Species of *P. apogon* from Kampar river have two amino acid sites, i.e., sites 197 and 284 as a specific marker. Two amino acids are I (isoleucine) and L (leucine), which are coded by the codons of ATC and CTC (Table 3). These amino acids can be used as markers to distinguish *P. apogon* from other species.

Nucleotide sequences of cytochrome b gene of *O. hypophthalmus* and *O. eugeneiatus* are not found in the GenBank data. Therefore, data for *O. hypophthalmus* and *O. eugeneiatus* were obtained from this research. Intraspecies markers in the genus *Ompok* from the Kampar River can be characterized by specific amino acid sites. There are two amino acid sites that can be used as specific genetic markers for species in the genus *Ompok* from the Kampar River (Table 4). One amino acid site, i.e., S (serine) at site 282, has a pattern specific to *O. eugeneiatus* from the Kampar River with the codon TCC. Catfish of *O. hypophthalmus* can also be differentiated from other species. One amino acid, i.e., A (alanine) at site 201 can be used as a marker for *O. hypophthalmus* from the Kampar River with the codon GCA.

This research has been able to determine the genetic markers of *K. limpok*, *P. apogon*, and *Ompok* spp. This was not found before in Elvyra et al. (2009), which amplified the cytochrome b gene using a universal primer only 373 bp, with the results of multiple alignments of 159 nucleotides and translating 53 amino acids at sites 62-114 based on the reference of complete cytochrome b gene.

A genetic distance matrix of the nucleotide sequences of *K. limpok*, *Ompok* spp. and *P. apogon* is shown in Table 5. The genetic distance between the nucleotide sequences of *K. limpok* from the Kampar River with that of *K. limpok* from GenBank data is 0.03, while the genetic distance within the group of *K. limpok* from the Kampar River is 0.00-0.01. The genetic distance among the nucleotide sequences of *P. apogon* from the Kampar River is 0.00-0.01.

The genetic distance based on the nucleotide sequence within the *O. eugeneiatus* group from the Kampar River is 0.00. The genetic distance within *O. hypophthalmus* group is similar to that within *O. eugeneiatus* group from the Kampar River, which is 0.00. The cytochrome b gene can be used to analyze genetic distance and phylogenetic relationships of *Kryptopterus*, *Ompok*, and *Phalacrotonotus*. This was also found in research of *Schizothorax* and *Schizopyge* species in the subfamily of Schizothoracinae (Barat et al. 2012); also in strains of *Osphronemus goramy* (Nugroho et al. 2019).

Table 1. Compositions of the four nucleotide bases from the partial cytochrome b gene

	T(U)	C	A	G	A+T	G+C	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
[1]	26.6	31.9	27.2	14.3	53.8	46.2	24.2	25.8	23.5	26.5	41.1	25.5	20.2	13.2	14.6	44.4	37.7	3.3
[2]	26.7	31.8	27.2	14.3	53.9	46.1	24.2	25.8	23.5	26.5	41.4	25.2	20.2	13.2	14.6	44.4	37.7	3.3
[3]	26.6	32.0	27.0	14.3	53.6	46.4	24.2	26.2	23.2	26.5	41.4	25.2	19.9	13.6	14.2	44.7	38.1	3.0
r	26.6	31.9	27.1	14.3	53.8	46.2	24.2	25.9	23.4	26.5	41.3	25.3	20.1	13.4	14.5	44.5	37.9	3.2
[4]	27.3	31.8	26.2	14.8	53.4	46.6	24.5	26.2	23.5	25.8	41.1	25.5	20.2	13.2	16.2	43.7	34.8	5.3
[5]	27.3	31.8	26.2	14.8	53.4	46.6	24.5	26.2	23.5	25.8	41.1	25.5	20.2	13.2	16.2	43.7	34.8	5.3
[6]	27.4	31.8	26.0	14.8	53.4	46.6	24.8	26.2	23.2	25.8	41.1	25.5	20.2	13.2	16.2	43.7	34.8	5.3
r	27.3	31.8	26.1	14.8	53.4	46.6	24.6	26.2	23.4	25.8	41.1	25.5	20.2	13.2	16.2	43.7	34.8	5.3
[7]	23.8	34.5	28.1	13.5	52.0	48.0	22.8	27.8	23.5	25.8	40.7	25.8	20.2	13.2	7.9	50.0	40.7	1.3
[8]	24.0	34.5	28.0	13.5	52.0	48.0	22.8	27.8	23.5	25.8	40.7	25.8	20.2	13.2	8.3	50.0	40.4	1.3
[9]	23.7	34.7	28.1	13.5	51.9	48.1	22.8	27.8	23.5	25.8	40.4	26.2	20.2	13.2	7.9	50.0	40.7	1.3
[10]	23.8	34.5	28.1	13.5	52.0	48.0	22.8	27.8	23.5	25.8	40.7	25.8	20.2	13.2	7.9	50.0	40.7	1.3
[11]	23.8	34.5	28.1	13.5	52.0	48.0	22.8	27.8	23.5	25.8	40.7	25.8	20.2	13.2	7.9	50.0	40.7	1.3
r	23.8	34.6	28.1	13.5	52.0	48.0	22.8	27.8	23.5	25.8	40.7	25.9	20.2	13.2	8.0	50.0	40.7	1.3
[12]	25.6	32.2	26.9	15.2	52.5	47.5	23.8	26.5	23.2	26.5	41.4	24.8	20.2	13.6	11.6	45.4	37.4	5.6
[13]	25.7	31.9	27.4	15.0	53.1	46.9	23.8	26.5	23.5	26.2	41.4	25.2	20.2	13.2	11.9	44.0	38.4	5.6
[14]	25.7	32.2	27.0	15.0	52.8	47.2	23.8	26.5	23.5	26.2	41.4	25.2	20.2	13.2	11.9	45.0	37.4	5.6
r	25.7	32.1	27.1	15.1	52.8	47.2	23.8	26.5	23.4	26.3	41.4	25.1	20.2	13.4	11.8	44.8	37.7	5.6
[15]	29.1	29.0	27.4	14.5	56.5	43.5	25.8	24.2	23.8	26.2	40.7	25.8	20.2	13.2	20.9	37.1	38.1	4.0
[16]	26.6	31.8	26.8	14.8	53.4	46.6	23.5	26.2	23.2	27.2	41.4	25.2	20.2	13.2	14.9	44.0	37.1	4.0
[17]	26.7	30.0	29.2	14.0	56.0	44.0	25.2	25.2	23.2	26.5	41.1	25.5	20.2	13.2	13.9	39.4	44.4	2.3
[18]	27.7	29.7	28.6	14.0	56.3	43.7	24.8	25.8	23.8	25.5	41.1	25.5	20.2	13.2	17.2	37.7	41.7	3.3
[19]	25.5	33.5	26.1	14.9	51.6	48.4	24.2	26.5	22.2	27.2	40.7	25.8	20.2	13.2	11.6	48.2	35.9	4.3
[20]	29.4	28.1	28.0	14.5	57.4	42.6	23.8	25.8	25.8	24.5	42.4	24.5	19.5	13.6	21.9	34.1	38.7	5.3
R	26.2	32.1	27.4	14.3	53.5	46.5	24.0	26.4	23.5	26.1	41.1	25.5	20.1	13.3	13.4	44.5	38.5	3.6

Note: [1] *K. limpok* (Kampar 1); [2] *K. limpok* (Kampar 9); [3] *K. limpok* (Kampar 16); [4] *O. eugeneiatus* (Kampar 2); [5] *O. eugeneiatus* (Kampar 3); [6] *O. eugeneiatus* (Kampar 11); [7] *O. hypophthalmus* (Kampar 5); [8] *O. hypophthalmus* (Kampar 12); [9] *O. hypophthalmus* (Kampar 13); [10] *O. hypophthalmus* (Kampar 14); [11] *O. hypophthalmus* (Kampar 15); [12] *P. apogon* (Kampar 6); [13] *P. apogon* (Kampar 7); [14] *P. apogon* (Kampar 10); [15] *K. minor* (GB); [16] *K. limpok* (GB); [17] *O. bimaculatus* (GB); [18] *O. miostoma* (GB); [19] *M. apogon* (GB); [20] *I. punctatus* (GB); r. The average of each species from the Kampar River; R. Overall average.

Table 2. Amino acid sites that are specific genetic markers in the partial cytochrome b gene (302 aa) of *Kryptopterus limpok* from the Kampar River with GenBank data for comparison

Species	Site positions					
	123 (161)		155 (193)		156 (194)	
	Codon triplet	Amino acid	Codon triplet	Amino acid	Codon triplet	Amino acid
<i>K. minor</i> (GB)	CAA	Q	GCC	A	ATC	I
<i>K. limpok</i> (GB)	CAA	Q	<u>GTA</u>	V	<u>ATA</u>	M
<i>K. limpok</i> (Kampar 1)	<u>GAA</u>	E	<u>GTA</u>	V	<u>ATA</u>	M
<i>K. limpok</i> (Kampar 9)	<u>GAA</u>	E	<u>GTA</u>	V	<u>ATA</u>	M
<i>K. limpok</i> (Kampar 16)	<u>GAA</u>	E	<u>GTA</u>	V	<u>ATA</u>	M

Note: Number in parentheses () indicates site positions based on complete cytochrome b gene of *Kryptopterus minor* from GenBank data (GB); Q (glutamine); E (glutamic Acid); A (alanine); V (valine); I (isoleucine); M (methionine)

Table 3. Amino acid sites that are specific genetic markers in the partial cytochrome b gene (302 aa) of *Phalacrotonotus apogon* from the Kampar River with GenBank data for comparison

Species	Site positions				
	197 (235)		284 (322)		
	Codon triplet	Amino acid	Codon triplet	Amino acid	
<i>K. minor</i> (GB)		CTC	L	TTC	F
<i>M. apogon</i> (GB)		CTC	L	TTC	F
<i>P. apogon</i> (Kampar 6)		<u>ATC</u>	I	<u>CTC</u>	L
<i>P. apogon</i> (Kampar 7)		<u>ATC</u>	I	<u>CTC</u>	L
<i>P. apogon</i> (Kampar 10)		<u>ATC</u>	I	<u>CTC</u>	L

Note: Number in parentheses () indicate site positions based on complete cytochrome b gene of *Kryptopterus minor* from GenBank data (GB); L (leucine); I (isoleucine); F (phenylalanine)

Table 4. Amino acid sites that are specific genetic markers in the partial cytochrome b gene (302 aa) of *Ompok* spp. from the Kampar River with GenBank data for comparison

Species	Site positions			
	201 (239)		282 (320)	
	Codon triplet	Amino acid	Codon triplet	Amino acid
<i>O. bimaculatus</i> (GB)	ACA	T	GCC	A
<i>O. miostoma</i> (GB)	ACA	T	GCC	A
<i>O. eugeneiatus</i> (Kampar 2)	ACA	T	<u>TCC</u>	S
<i>O. eugeneiatus</i> (Kampar 3)	ACA	T	<u>TCC</u>	S
<i>O. eugeneiatus</i> (Kampar 11)	ACA	T	<u>TCC</u>	S
<i>O. hypophthalmus</i> (Kampar 5)	<u>GCA</u>	A	GCC	A
<i>O. hypophthalmus</i> (Kampar 12)	<u>GCA</u>	A	GCC	A
<i>O. hypophthalmus</i> (Kampar 13)	<u>GCA</u>	A	GCC	A
<i>O. hypophthalmus</i> (Kampar 14)	<u>GCA</u>	A	GCC	A
<i>O. hypophthalmus</i> (Kampar 15)	<u>GCA</u>	A	GCC	A

Note: Number in parentheses () indicates site positions based on complete cytochrome b gene of *Kryptopterus minor* from GenBank data (GB); T (threonine); A (alanine); S (serine)

Table 5. Matrix of genetic distances (p-distance) based on the nucleotide bases in the partial cytochrome *b* gene of *K. limpok*, *Ompok* spp. and *P. apogon* from the Kampar River with GenBank data for comparison

	[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	[10]	[11]	[12]	[13]	[14]	[15]	[16]	[17]	[18]	[19]	[20]
[1]	–																			
[2]	0.15	–																		
[3]	0.15	0.03	–																	
[4]	0.15	0.03	0.00	–																
[5]	0.15	0.03	0.01	0.00	–															
[6]	0.16	0.16	0.15	0.15	0.15	–														
[7]	0.16	0.15	0.15	0.15	0.15	0.13	–													
[8]	0.16	0.12	0.12	0.12	0.12	0.16	0.16	–												
[9]	0.16	0.12	0.12	0.12	0.12	0.16	0.16	0.00	–											
[10]	0.15	0.12	0.12	0.12	0.12	0.15	0.16	0.00	0.00	–										
[11]	0.14	0.11	0.11	0.11	0.11	0.13	0.15	0.13	0.13	0.12	–									
[12]	0.14	0.11	0.11	0.11	0.11	0.13	0.15	0.13	0.13	0.12	0.00	–								
[13]	0.14	0.11	0.11	0.11	0.11	0.13	0.15	0.13	0.13	0.12	0.00	0.00	–							
[14]	0.14	0.11	0.11	0.11	0.11	0.13	0.15	0.13	0.13	0.12	0.00	0.00	0.00	–						
[15]	0.14	0.11	0.11	0.11	0.11	0.13	0.15	0.13	0.13	0.12	0.00	0.00	0.00	0.00	–					
[16]	0.17	0.12	0.12	0.12	0.12	0.14	0.16	0.14	0.14	0.14	0.10	0.11	0.11	0.10	0.10	–				
[17]	0.16	0.12	0.12	0.12	0.12	0.15	0.17	0.13	0.13	0.13	0.11	0.11	0.11	0.11	0.11	0.12	–			
[18]	0.16	0.11	0.11	0.12	0.12	0.15	0.17	0.13	0.13	0.13	0.11	0.11	0.11	0.11	0.11	0.12	0.01	–		
[19]	0.16	0.11	0.11	0.12	0.12	0.15	0.17	0.13	0.13	0.13	0.11	0.11	0.11	0.11	0.11	0.11	0.00	0.00	–	
[20]	0.19	0.19	0.20	0.20	0.20	0.18	0.19	0.21	0.21	0.21	0.18	0.19	0.19	0.18	0.18	0.20	0.21	0.21	0.21	–

Note: [1] *K. minor* (GB); [2] *K. limpok* (GB); [3] *K. limpok* (Kampar 1); [4] *K. limpok* (Kampar 9); [5] *K. limpok* (Kampar 16); [6] *O. bimaculatus* (GB); [7] *O. miostoma* (GB); [8] *O. eugeneiatus* (Kampar 2); [9] *O. eugeneiatus* (Kampar 3); [10] *O. eugeneiatus* (Kampar 11); [11] *O. hypophthalmus* (Kampar 5); [12] *O. hypophthalmus* (Kampar 12); [13] *O. hypophthalmus* (Kampar 13); [14] *O. hypophthalmus* (Kampar 14); [15] *O. hypophthalmus* (Kampar 15); [16] *M. apogon* (GB); [17] *P. apogon* (Kampar 6); [18] *P. apogon* (Kampar 7); [19] *P. apogon* (Kampar 10); [20] *I. punctatus* (GB).

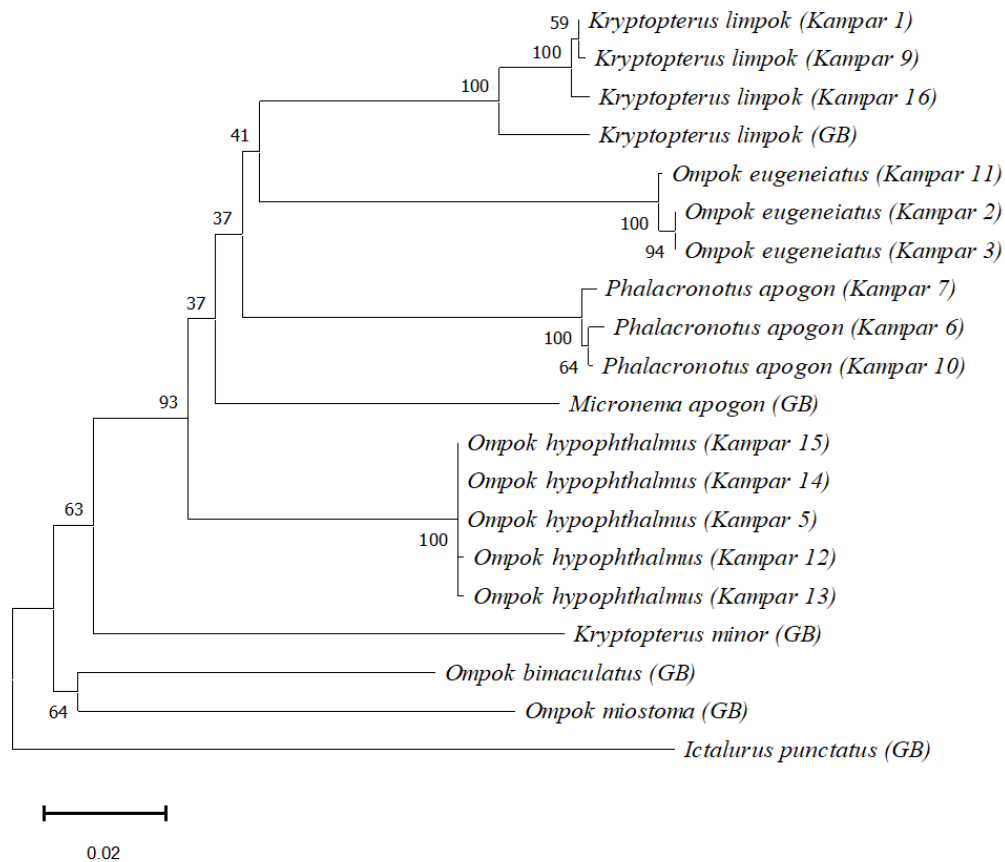


Figure 1. Phylogram using bootstrapped Neighbour-Joining method with 1000 replicates based on 906 cytochrome b gene nucleotide of *Kryptopterus limpok*, *Ompok* spp. and *Phalacronotus apogon*

A reconstruction of phylogenetic relationships of *Kryptopterus*, *Ompok* and *Phalacronotus* based on the genetic distances of nucleotide sequences is shown in Fig. 1. The phylogram results based on the cytochrome b nucleotide sequence showed that intraspecies of *K. limpok*, *O. eugeneiatus*, *O. hypophthalmus* and *P. apogon* from the Kampar River have relationship groups supported by a bootstrap value of 100%. Catfish of *K. limpok* from the Kampar River and *K. limpok* from GenBank data formed a group with 100% bootstrap value.

The results of genetic distance (p-distance) based phylogram from nucleotide cytochrome b sequences generally showed that intraspecies samples of *K. limpok*, *O. eugeneiatus*, *O. hypophthalmus* and *P. apogon* from the Kampar River formed relationship groups within each species supported by high bootstrap values. Based on genetic distances from nucleotide sequences, *K. limpok* from the Kampar River formed a relationship group with *K. limpok* from GenBank data with high bootstrap values. This phylogenetic relationship is caused by a small value of genetic distance within the intraspecies of *K. limpok*, *O. eugeneiatus*, *O. hypophthalmus*, and *P. apogon*. The relationship of the groups of *K. limpok*, *O. eugeneiatus*, *O. hypophthalmus*, and *P. apogon* forms a large group because they are together in one family, namely Siluridae, which

separates from the outgroup of *I. punctatus* from the family of Ictaluridae, but all belong to the same order of Siluriformes. This study supports the utility of the cytochrome b gene in phylogenetic relationships between *Kryptopterus*, *Ompok*, and *Phalacronotus*. This is in accordance with the results of molecular phylogenetic research that has been carried out on various species in the genus *Puntius* (Pallavi et al. 2012), Pangasiidae and Schilbidae (Karinthyakrit and Jondeung 2012) and also catfish species in the family of Bagridae, Pangasiidae, and Ariidae (Megarani et al. 2020).

The cytochrome b gene is suitable as a marker from the intraspecies to the family levels and it has been shown to be useful for classifying relationships in a variety of fish. Based on codon position, cytochrome b gene has conserved and varied regions. Conserved regions can be used as a genetic marker to accurately identify the genetic originality of a species and also as a genetic marker to determine the origin of a species. In contrast, varied regions can be used to determine phylogenetic relationships. This research has successfully identified the grouping of catfish species molecularly.

This work is a preliminary study of the molecular data of catfishes in the Kampar River, Indonesia. It can be concluded that the validity of the use of cytochrome b as a

genetic marker supports the species identification of catfish. The cytochrome b gene can be used as a genetic marker to identify molecular characteristics and phylogenetic relationships of *K. limpok*, *O. eugeneiatus*, *O. hypophthalmus*, and *P. apogon*. This research provides an important contribution to enhance the database of silurid catfishes of *K. limpok*, *O. eugeneiatus*, *O. hypophthalmus* and *P. apogon* from the Kampar River, Indonesia.

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