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The Mitochondrial D-loop Region of Kryptopterus apogon from Indragiri Hulu River of Riau Province

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ABSTRACT

The preliminary study on the D-loop region of mitochondrial DNA against *Kryptopterus apogon* has been done. This study aims to obtain sequence of D-loop region of *Kryptopterus apogon*.from Indragiri Hulu river of Riau Province. Primer of D-loop or control region used for Polymerase Chain Reactions process. The results of Polymerase Chain Reactions is partial D-loop region of 248 bp. The partial D-loop region can distinguish *Kryptopterus apogon* with other fish species.

Keywords: D-loop region, Indragiri Hulu river, *Kryptopterus apogon,* Riau

INTRODUCTION

Indragiri Hulu river is one of the major rivers in the province of Riau. Indragiri Hulu river has a lot of potential fishery resources of high economic value. One of the potential of fisheries in the Indragiri Hulu river is fish of *Kryptoterus apogon*. Research on the genetic resources of fish Indragiri Hulu river in particular or Riau province generally still very limited. Therefore it is necessary to do research on the genetic potential of fishery resources of the Indragiri Hulu the river especially against *K. apogon*. The study focused on the D-loop region of mitochondrial DNA region *K. apogon*. The D-loop region is part of the mitochondrial DNA genome does not encode a protein. Compared with protein-coding genes, the D-loop region have a faster rate of evolution. D-loop region is most interesting to study the mitochondrial genome of vertebrates because they have an conserved part of short and rapid rate of evolution (Guo *et al*. 2003). This study aims to determine the nucleotide sequences of the D-loop region *K. apogon* from Indragiri Hulu Riau Province.

METHODS

Fish of *K. apogon* obtained from Indragiri Hulu river of Riau Province. Research conducted at the Laboratory of Genetics Department of Biology, Faculty of Mathematics and Natural Sciences, University of Riau. Total DNA was isolated from fish muscle samples of *K. apogon*. Isolation and purification of total DNA was performed using DNeasy Blood and Tissue Kit of Qiagen. Isolation and purification methods of total DNA following the protocol of Qiagen Kit. Total DNA obtained from the isolation and purification of DNA is used as template for amplification process of D-Loop region by Polymerase Chain Reactions (PCR). Amplification of the D-loop region is done by using the primers D-loop or control region of the H00651 and L15926 (Kocher *et al*. 1989). PCR conditions following the procedures performed by Kocher *et al*. (1989). The composition of the PCR solution mixture following the protocol of the Top Taq Master Mix Kit of Qiagen. PCR products were used as template in sequencing reactions using primer H00651. Sequencing process is done in PT. Genetica Science Indonesia. Data of nucleotide sequences were analyzed using the program MEGA version 4.0 (Tamura *et al*. 2007). Nucleotide sequences of *K. apogon* from Indragiri Hulu river aligned and compared (multiple alignment) with the data D-loop region sequences of family Siluridae from GenBank (2011) is *Silurus glanis* (NC014261), *Silurus soldatovi* (NC022723) and *Wallago attu* (AY286130).

RESULTS AND DISCUSSIONS

Sequencing of PCR amplified products D-loop region of the sample *K. apogon* from Indragiri Hulu river obtained results of partial sequences along 248 bp. The results of the multiple alignment of sequences *K. apogon* from Indragiri Hulu river by using comparison D-loop region of Family Siluridae in GenBank is *S. glanis, S. soldatovi,* and *W. attu* obtained 248 nucleotides. Position of 248 nukleotide be in a position 325 to 572 (referred to the completed D-loop region of *S. glanis* (GenBank)).

Two hundred and forty-eight nucleotides of the D-loop region partial *K. apogon* from results of multiple alignment with the D-loop nucleotides of *S. glanis, S. soldatovi* and *W. attu* (GenBank) obtained nucleotide which vary as much as 152 sites. Based on the results of the study also found a specific marker of *K. apogon* from Indragiri Hulu river as much as 121 sites nucleotides that can distinguish *K. apogon* of Indragiri Hulu river with the *S. glanis, S. sodatovi* and *W. attu* of GenBank data.

The results of this study indicate that the D-loop region has a high variation in nucleotide sequences. D-loop region is often used in population genetics studies because of the high variation in the nucleotide sequences (Pereira 2000). There are four basic types of changes in DNA, they are substitution of a nucleotide for another nucleotide, deletion of nucleotides, insertion of nucleotides and inversion of nucleotides. Nucleotide substitutions can be divided into two classes, transitions and transversions (Nei and Kumar 2000).

Average nucleotide T and A are more common in nucleotide composition of the D-loop region of the fish family Siluridae (*K. apogon, S. glanis* and *W. attu*) is 32.0% and 25.2% (Table 1), followed by nucleotides C (22.9%) and an average of at least found is G (19.9%). Average nucleotide composition A + T on the species of fish family Siluridae more (57.2%) compared to the average composition of the nucleotide G + C (42.8%).

Table 1. Composition of nucleotides in the partial D-loop region of *Kryptopterus apogon* (Indragiri Hulu river) with a comparison of data GenBank

Items	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	31.6	22.7	24.7	21.1	56.3	43.8	36.6	29.3	15.9	18.3	26.5	20.5	31.3	21.7	31.7	18.3	26.8	23.2
2	31.0	24.6	24.6	19.8	55.6	44.4	36.1	31.3	16.9	15.7	26.5	21.7	28.9	22.9	30.5	20.7	28.0	20.7
3	33.6	20.2	27.1	19.0	60.7	39.2	41.0	25.3	18.1	15.7	30.5	18.3	30.5	20.7	29.3	17.1	32.9	20.7
4	31.6	24.1	24.5	19.8	56.1	43.9	35.0	22.5	16.3	26.3	23.1	29.5	32.1	15.4	36.7	20.3	25.3	17.7
Avg	**32.0**	22.9	**25.2**	19.9	**57.2**	42.8	37.2	27.1	16.8	18.9	26.7	22.4	30.7	20.2	32.0	19.1	28.3	20.6

1. *Silurus glanis (GenBank)*; **2.** *Silurus soldatovi (GenBank)*; **3.** *Wallago attu (GenBank)*; **4.** *Kryptopterus apogon* (Indragiri Hulu); **Avg.** Average

Transition nucleotide substitutions of partal D-loop region in Table 2 shows the transition between *K. apogon* (Indragiri Hulu) with *S. glanis, S. soldatovi* and *W. attu* (GenBank) is 15-66 nucleotides, with an average substitution transition in Siluridae family as much as 40 nucleotides. Transversion nucleotide substitutions in the partal D-loop region between *K. apogon* (Indragiri Hulu) with other species in the family Siluridae show transversion rate is 6-79 nucleotides, with an average substitution tranversion of as much as 47 nucleotide (Table 2).

Nucleotide differences between *K. apogon* (Indragiri Hulu) with *S. glanis, S. soldatovi* and W. *attu* (GenBank) is 24-141 nucleotides, with an average difference of nucleotides in the family Siluridae as many as 87 nucleotides. Genetic distance (p-distance) between *K. apogon* with other species in the family

Siluridae is 0.1017 to 0.5975, with an average genetic distance of 0.3679 (Table 3). The difference in the number of nucleotides and genetic distance is related to the phylogenetic relationship between *K. apogon* (Indragiri Hulu) with *S. glanis, S. soldatovi* and *W. attu* (Siluridae, GenBank) based on 248 nucleotide sequences of partial D-loop region

Table 2. The number of nucleotide transition (below diagonal) and nucleotide transversion (above diagonal) in the partal D-loop region of *Kryptopterus apogon* (Indragiri Hulu river) with a comparison of data GenBank

	Type	[1]	[2]	[3]	[4]
1]	*Silurus glanis (GenBank)*	-	6	19	78
2]	*Silurus soldatovi (GenBank)*	18	-	23	74
3]	*Wallago attu (GenBank)*	19	15	-	79
4]	*Kryptopterus apogon (Indragiri Hulu)*	62	66	62	-
	Average of Transition			40	
	Average of Transversion			47	

Table 3. The number of nucleotide differences (below diagonal) and p-distance (above the diagonal) in the partial D-loop region of *Kryptopterus apogon* (Indragiri Hulu river) with a comparison of data GenBank

	Type	[1]	[2]	[3]	[4]
1]	*Silurus glanis (GenBank)*	-	0.1017	0.1610	0.5932
2]	*Silurus soldatovi (GenBank)*	24	-	0.1610	0.5932
3]	*Wallago attu (GenBank)*	38	38	-	0.5975
4]	*Kryptopterus apogon (Indragiri Hulu)*	140	140	141	-
	Average of nucleotide difference			87	
	Average of p-distance			0,3679	

The phylogenetic tree showed grouping and phylogenetic relationship between *K. apogon* (Indragiri Hulu river) with other genus in the family Siluridae (GenBank). *Kryptopterus apogon* of Indragiri Hulu river construct phylogeny group distinct with other species in family Siluridae (Figure 1). The formation of the group differences in the relationship because genus *Kryptopterus* different with genus *Silurus* and *Wallago*, but still form a large group of the same because both including family Siluridae.

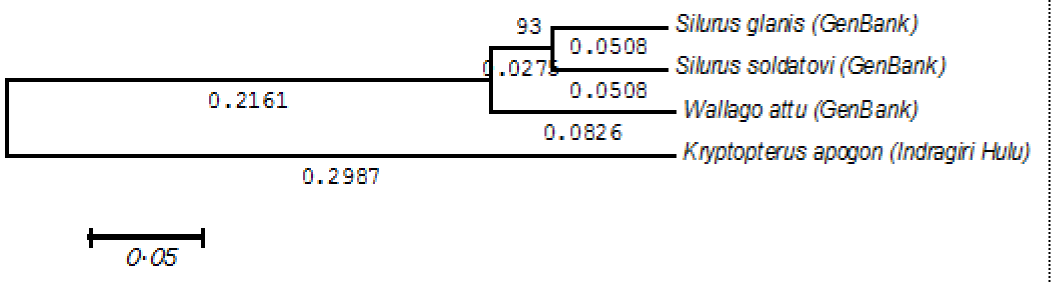

Figure 1. Phylogenetic relationship using Neighbor Joining method based on the genetic distance (p-distance) of the partial D-loop region nucleotide *Kryptopterus apogon* (Indragiri Hulu river) with a comparison other species in the family Siluridae (GenBank)

CONCLUSIONSS

The research can be concluded that D-loop region *Kryptopterus apogon* of Indragiri Hulu river in Riau Province amplified 248 bp. One hundred twenty-one specific nucleotides can distinguish *Kryptopterus apogon* of Indragiri Hulu river with other species in family Siluridae.

There are 15-66 nucleotide substitution transitions and 6-79 nucleotide transversions between *Kryptopterus apogon* with other fish species in family Siluridae. *Kryptopterus apogon* of Indragiri Hulu river construct phylogeny group distinct with other species in family Siluridae.

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