IEESE International Journal of Science and Technology (IJSTE), Vol. 3 No. 2, June 2014, 1-7
ISSN : 2252-5297

Genetic Variation Analysis of Snakeheads (Channidae) in Central Kalimantan Using Partial 16s rRNA Gene

Firlianty[1], E. Suprayitno[2], H. Nursyam[3], and Hardoko[4]

[1] Faculty of Agriculture, University of Palangkaraya, Central Kalimantan
[2,3,4] Faculty of Fisheries and Marine Science, University of Brawijaya, Malang
Indonesia

Email : firli_1980@yahoo.com

Abstract. Channidae Family is a predatory fish in Central Kalimantan. This fish also possesses high albumin and complete amino acid content that useful for health, particularly in wound healing. Channidae Family includes Gabus (*Channa striatus*), Toman (*Channa micropeltes*), Kerandang (*Channa pleurophthalmus*), and Mihau (*Channa maculata*). However, the phylogenetic relationship between these snakehead fishes in this area has not been determined yet. This study was aimed to assess the genetic variations of Channidae Family in Central Kalimantan using 16S rRNA gene. Fish samples were Toman, kerandang, gabus and mihau collected from Central Kalimantan. Results showed that Gabus, Kerandang, Toman and Mihau were grouped in *Channa* genus with varied genetic distance among species, and have a close relationship based on its mitochondrial DNA marker.

Key-Words: *Channidae, 16s rRNA gene, genetic variation*

1. Introduction

Channidae Family as a freshwater fish group distributed in Asia and Africa continents. This fish is naturally distributed in South Asia and South East Asia, including Sunda shelf [1]. *Channa* Genus is highly tolerant to poor water quality, survive in the mud during the drought and flood [2,3]. *Channa* Genus is a group of well-known freshwater fish called snakeheads due to its head similar to the head of snakes and this head profile distinguishes the fish from other fish species. There are several species of Channidae family in Central Kalimantan, i.e. Gabus (*Channa striata*), Toman (*Channa micropeltes*), Kerandang (*Channa pleurophthalmus*), and Mihau (*Channa maculata*). Each organism has a unique genetic structure, that genetically there are no two precisely same individuals. This uniquely reflect a batch of appropriate characteristics of certain individuals in the environments where they live.

Development of genetic studies has enabled to detect the genetic diversity or genetic variations in the snakeheads through blood plasmic protein polymorphism study, since there are many protein in the blood as other budy tissues. Protein polymorphism is a protein that could be categorized in several protein phenotypes and controlled by 2 alleles or more in certain gene locus. Blood protein polymorphism is genetically regulated by allele pairs or allele series without a dominance.

Mitochondrial DNA can be used in phylogenetic analysis due to strongly conserve and low sequence alteration rate [2]. Species identification using the mitochondrial DNA can utilize the 16s RNA gene sequence and Cytochrome B. The mutation rate of the mitochondrial DNA is higher than nucleic DNA, and it causes a high sequence difference in species level [4,5,6,7]. Gen mtDNA 16S rRNA is very effective to determine the fish evolutionary relationship [8]. This study was aimed to assess the genetic variations of Channidae family in Central Kalimantan using 16S rRNA gene.

2. Materials and Methods

2.1. DNA isolation and analysis

Fish samples comprised of Toman, Kerandang, Gabus and Mihau were collected from Central Kalimantan. DNA samples were extracted from the fish flesh using 460 µl buffer lysis (100 mM NaCl, 50 mM EDTA pH 8, 0.5% SDS, 10 mM Tris-Cl pH 8) and 5 µl Proteinase K (1mg/ml). The DNA was purified using phenol-chloroform and ethanol methods.

2.2. DNA amplification and analysis

The isolated DNA was amplified using Fast Start PCR master mix (Roche) and universal primer, 16SAR (5'-CGCCTGTTTAACAAAAACAT-3') and 16SBR (5'-CCGGTTTGAACTCAGATCA CGT-3') [9],

under the predenaturation cycle condition at 93ºC for 5 minutes followed with denaturation at 93ºC for 1 minute, annealing at 48ºC for 1 minute and extension at 72ºC for 1 minute as many as 30 cycles with final extension at 72ºC for 10 minutes. The sample of PCR output was then directly sequenced using ABI PRISM 3730 XL (Macrogen Inc., South Korea). The reference sequence obtained from the Gene Bank. The sequence of sequencing output was analyzed using the clustal W of MEGA 5.03. The phyligenetic tree analysis and ts/tv ratio of the taxa were obtained through Kimura-2 parameter by analyzing the confidence level value of each individual branch using the bootstrap 1000.

3. Result and Discussion

Four fish of family Channidae, i.e. Toman, Kerandang, Mihau, and Gabuswere aligned with 16s RNA sequence from the Gene Bank (Table 1). The pairwise was made from genetic distance data among the samples and the reference taxa of the Gene Bank (Table 2). It indicated that the genetic distance of the samples and the reference taxa were varied. The genetic distance was 0.009 for Toman (1.1; 1.2) and *Channa micropeltes* (DQ532852.1; KC200550.1); 0.053 for Kerandang (2.1;2.2) and *Channa micropeltes* (DQ532852.1;KC200550.1); 0.023 for Gabus (3.2) and *Channa striata* (HM117251.1; HM117250.1; HM117249.1; HM117223.1;HM117222.1); and 0.079 for Mihau (5.3;5.5) and *Channa maculata haplotype 1* (KC200548.1).

The mean of nucleotide composition data of all taxa resulted 20.16% of thymine base, 20.33% of cytosine, 25.91% of adenine and 31,33% of guanine. Meanwhile, A+T ratio is 51.56% and G+C of 48.43%. Measurement of ts/tv ratio using the Kimura-2 parameter resulted in R value of 2.46 (Fig. 1).

Table 1. Reference sequence of 16S rRNA gene from the Gene Bank

No	Name of Spesies	GeneBank Accession number
1.	*C. micropeltes*	DQ532852.1
2.	*C. micropeltes voucher UMT CM5*	JF900370.1
3.	*C. micropeltes haplotype 2*	KC200551.1
4.	*C. micropeltes haplotype 1*	KC200550.1
5.	*C. striata voucher NE-CS4*	HM117251.1
6.	*C. striata voucher NE-CS3*	HM117250.1
7.	*C. striata voucher NE-CS2*	HM117249.1
8.	*C. striata voucher NE-CS5*	HM117223.1
9.	*C. striata voucher NE-CS1*	HM117222.1
10.	*C. diplogramme voucher CARE CD2*	EU342188.1
11.	*C. diplogramme voucher CARE CD3*	EU342189.1
12.	*C. diplogramme haplotype ChdiH1*	KC835200.1
13.	*C. diplogramme voucher CARE CD1*	EU342187.1
14.	*C. striata haplotype 2*	KC200559.1
15.	*C. striata haplotype 1*	KC200558.1
16.	*C. maculata haplotype 2*	KC200549.1
17.	*C. maculata haplotype 1*	KC200548.1
18.	*C. argus haplotype H2*	JQ358711.1
19.	*C. argus haplotype H5*	JQ358714.1
20.	*C. argus haplotype H3*	JQ358712.1
21.	*C. argus haplotype H1*	JQ358710.1
22	*C.lucius haplotype 1*	KC200552.1
23	*C.lucius haplotype 2*	KC200553.1

24	*Parachanna obscura*	AY763726.1

Table 2. The Pairwise Genetic Distance with 16s rRNA Partial Gene

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1																															
2	0.002																														
3	0.004	0.002																													
4	0.004	0.002	0.004																												
5	0.092	0.090	0.092	0.092																											
6	0.095	0.092	0.095	0.095	0.002																										
7	0.092	0.090	0.092	0.092	0.000	0.002																									
8	0.092	0.090	0.092	0.092	0.000	0.002	0.000																								
9	0.025	0.023	0.025	0.020	0.082	0.085	0.082	0.082																							
10	0.027	0.025	0.027	0.023	0.082	0.084	0.082	0.082	0.002																						
11	0.082	0.079	0.082	0.082	0.032	0.034	0.032	0.032	0.077	0.077																					
12	0.084	0.082	0.084	0.084	0.034	0.036	0.034	0.034	0.079	0.079	0.002																				
13	0.082	0.079	0.082	0.082	0.032	0.034	0.032	0.032	0.077	0.077	0.000	0.002																			
14	0.084	0.082	0.084	0.084	0.034	0.036	0.034	0.034	0.079	0.079	0.002	0.004	0.002																		
15	0.090	0.087	0.090	0.090	0.077	0.080	0.077	0.077	0.080	0.082	0.075	0.077	0.075	0.077																	
16	0.087	0.085	0.087	0.087	0.075	0.077	0.075	0.075	0.077	0.080	0.072	0.075	0.072	0.075	0.002																
17	0.087	0.085	0.087	0.087	0.077	0.080	0.077	0.077	0.082	0.085	0.070	0.072	0.070	0.072	0.018	0.016															
18	0.087	0.085	0.087	0.087	0.077	0.080	0.077	0.077	0.082	0.085	0.070	0.072	0.070	0.072	0.018	0.016	0.000														
19	0.087	0.085	0.087	0.087	0.077	0.080	0.077	0.077	0.082	0.085	0.070	0.072	0.070	0.072	0.018	0.016	0.000	0.000													
20	0.087	0.085	0.087	0.087	0.077	0.080	0.077	0.077	0.082	0.085	0.070	0.072	0.070	0.072	0.018	0.016	0.000	0.000	0.000												
21	0.087	0.085	0.087	0.087	0.077	0.080	0.077	0.077	0.082	0.085	0.070	0.072	0.070	0.072	0.018	0.016	0.000	0.000	0.000	0.000											
22	0.090	0.087	0.087	0.090	0.080	0.082	0.080	0.080	0.080	0.082	0.077	0.080	0.077	0.080	0.013	0.011	0.023	0.023	0.023	0.023	0.023										
23	0.143	0.140	0.143	0.143	0.135	0.138	0.135	0.135	0.132	0.132	0.125	0.127	0.125	0.127	0.113	0.111	0.119	0.119	0.119	0.119	0.119	0.113									
24	0.143	0.140	0.143	0.143	0.135	0.138	0.135	0.135	0.132	0.132	0.125	0.127	0.125	0.127	0.113	0.111	0.119	0.119	0.119	0.119	0.119	0.113	0.004								
25	0.123	0.121	0.123	0.124	0.121	0.124	0.121	0.121	0.118	0.121	0.108	0.111	0.108	0.111	0.118	0.116	0.113	0.113	0.113	0.113	0.113	0.113	0.125	0.125							
26	0.115	0.113	0.115	0.110	0.070	0.073	0.070	0.070	0.100	0.100	0.053	0.056	0.053	0.055	0.101	0.098	0.101	0.101	0.101	0.101	0.101	0.103	0.136	0.136	0.132						
27	0.115	0.113	0.115	0.110	0.070	0.073	0.070	0.070	0.100	0.100	0.053	0.056	0.053	0.055	0.101	0.098	0.101	0.101	0.101	0.101	0.101	0.103	0.136	0.136	0.132	0.000					
28	0.089	0.087	0.089	0.089	0.089	0.092	0.089	0.089	0.079	0.082	0.092	0.095	0.092	0.095	0.087	0.084	0.082	0.082	0.082	0.082	0.082	0.087	0.132	0.132	0.105	0.113	0.113				
29	0.089	0.087	0.089	0.089	0.089	0.092	0.089	0.089	0.079	0.082	0.092	0.095	0.092	0.095	0.087	0.084	0.082	0.082	0.082	0.082	0.082	0.087	0.132	0.132	0.105	0.113	0.113	0.000			
30	0.087	0.084	0.087	0.087	0.027	0.029	0.027	0.027	0.077	0.077	0.009	0.011	0.009	0.011	0.072	0.070	0.077	0.077	0.077	0.077	0.077	0.075	0.127	0.127	0.106	0.058	0.058	0.087	0.087		
31	0.087	0.084	0.087	0.087	0.027	0.029	0.027	0.027	0.077	0.077	0.009	0.011	0.009	0.011	0.072	0.070	0.077	0.077	0.077	0.077	0.077	0.075	0.127	0.127	0.106	0.058	0.058	0.087	0.087	0.000	

Description: 1.*C. argus haplotype H1; 2. C. argus haplotype H2; 3. C. argus haplotype H3; 4. C. argus haplotype H5; 5. C. diplogramme haplotype ChdiH1; 6. C. diplogramme voucher CARE CD1; 7.C. diplogramme voucher CARE CD2; 8. C. diplogramme voucher CARE CD3; 9. C. maculata haplotype 1; 10. C. maculata haplotype 2; 11. C. micropeltes;* 12. *C. micropeltes haplotype 1; 13. C. micropeltes haplotype 2; 14. C.micropeltes voucher UMT CM5; 15. C. striata haplotype 1; 16. C. striata haplotype 2; 17. C. striata voucher NE-CS1; 18. C. striata voucher NE-CS2; 19. C. striata voucher NE-CS3;* 20. *C. striata voucher NE-CS4; 21.C. striata voucher NE-CS5; 22. Gabus (3.2); 23. C. Lucius haplotype 1; 24. C. lucius haplotype 2; 25. Parachanna obscura; 26. Kerandang (2.1); 27. Kerandang (2.2); 28. Mihau (5.3); 29. Mihau (5.5); 30. Toman (1.1); 31. Toman 1.2.*

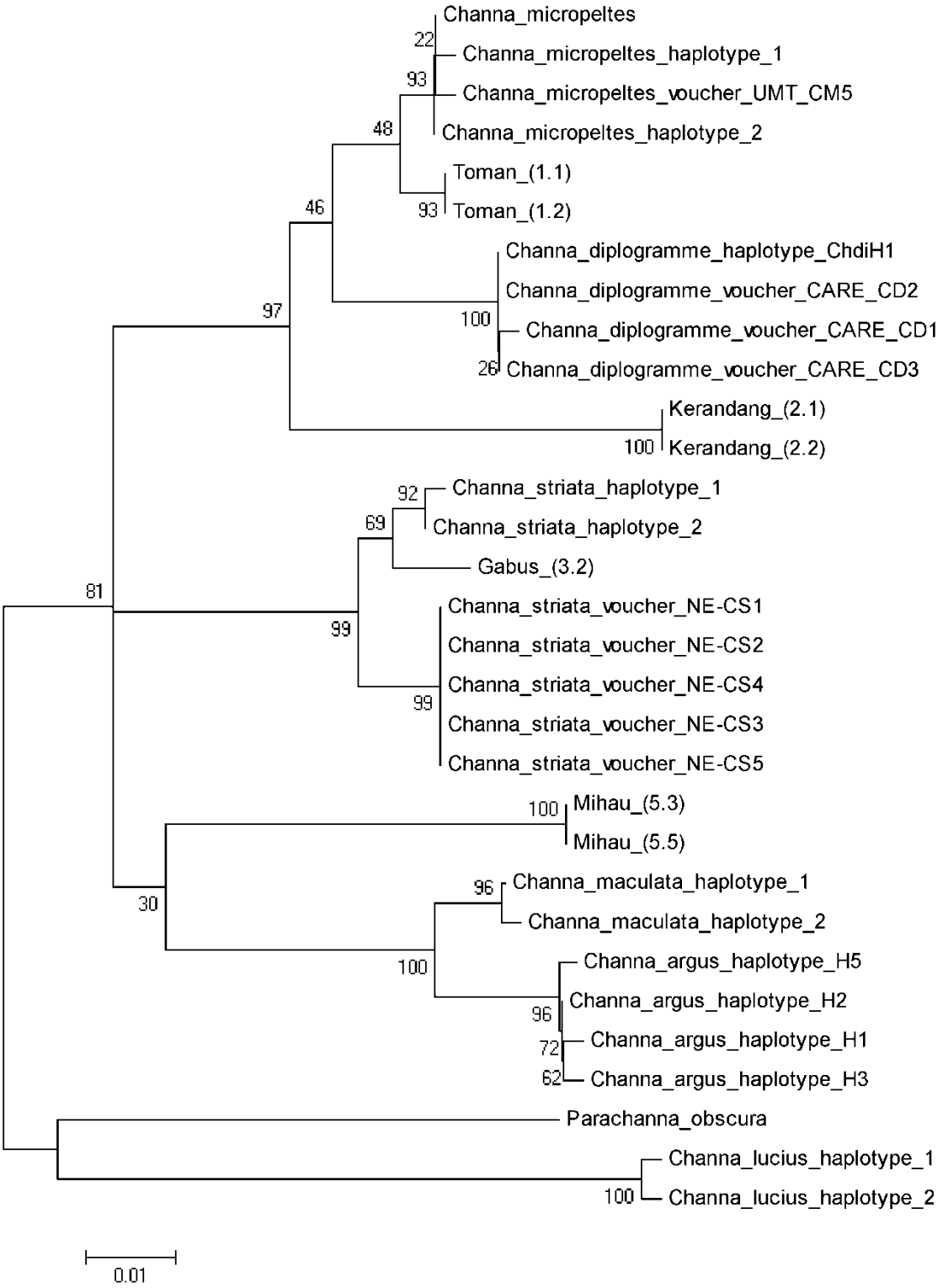

Figure 1. The phylogenetic tree of *Channidae* family using Maximum Likelihood with Kimura-2 parameter

Higher A+T% ratio than G+C% ratio reflects that adenine and timine base easily change and unstable (Xiaojuan et al., 2008). The different ratio in *Channa* previously recorded using Cytochrome b was C>T>A>G [1].

The ts/tv ratio of *Channa* genus using 16S RNA gene was higher than Chi-lin of *Varicorhinus* genus (1.7) and flatfish (1.25) [10 , 8]. The ts/tv ratio takes a very important role in determining the evolutionary distance [11]. Increase in ts/tv ratio could result from the mutagenic rate caused by free radicals during the

aerobic respiration of the mitochondria, bringing about change in ribosomal skeleton of the DNA and triggering the spontaneous deamination [12].

On phylogenetic tree, Mihau gets nearer to *Channa maculata*. Taxonomically, Channidae family is very difficult to be grouped since it has very high morphological diversity [1]. The phylogenetic relationship of Channidae family, particularly genus of *Channa* in South Asia, is strongly affected by the phylogeographic patterns [13]. A wide genetic distance of Mihau and *Channa* genus could be resulted from the geographic barrier that could influence individual morphological characteristic and mtDNA. Geographic barrier could affect the individual development and character of the mtDNA in a population which would also influence the individual morphological configuration [14,15]. Mihau is included in *Channa lucius* [16] and *Channa melasoma* [17]. Based on the 16S rRNA analysis, Mihau is close to *Channa maculata* taxa. It could result from its bigger mutation rate in the mtDNA so that it would influence the nucleotide base alteration in the mtDNA sequence of the fish. Beside that, the genetic distance of *Channa lucius* is closer to *Parachanna obscura* that was used as an outgroup, and therefore, Mihau cannot be included in *Channa lucius* taxa.

4. Conclusion

Snakeheads, Mihau, Toman and Kerandang distinguished by 16s rRNA gene, belonged to the genus of *Channa*. The genetic distance of the fish varied for each *Channa* species. The variation was highly affected by environment, morphological characteristics, and behavior, which could raise the genetic variations in Channidae family.

References

[1] Kottelat M., 2001, *Fishes of Lao*, WHT Publications, Colombo.

[2] Adamson E.A.S., Hurwood D.A., Baker A.M., Mather P.B., 2009, Population subdivision in Siamese mud carp *Henicorhynchus siamensis* in the Mekong River basin: implications for management, Journal of Fish Biology, Vol. 75, pp. 1371-1392.

[3] Abol-Munafi A.B., Ambak M.A., Ismail P., Tam B.M., 2007, Molecular data from the Cytochrome B for the Phylogeny of Channidae (*Channa* sp.) in Malaysia, Biotechnology, Vol. 6, No. 1, pp. 22-27.

[4] Eleanor E.A.S., 2010, Influence of historical landscapes drainage evolution and ecological traits on patterns of genetic diversity in South East Asian freshwater snakehead fishes, PhD thesis, Queensland University of Technology.

[5] Siti-Balkhis A.B., Jamsari A.F.J., Hwai T.S., Yasin Z., Siti-Azizah M.N., 2011, Evidence of geographical structuring in the Malaysian Snakehead, *Channa striata* based on partial segment of the CO1 gene, Genetic and Molecular biology, Vol. 34, No. 3, pp. 520-523.

[6] Hellberg M.E., 2006, No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation, BMC evolutionary Biology, Vol. 6, No. 24.

[7] Elmogy M., Sarhan O.M., Elgendy A.M., Alamodi W.M., 2013, Morphological and molecular identification of some *Uromastyx species* (Reptilia; Agamidae) in Makkah, Saudi Arabiah by forensically informative nucleotide sequencing (FINS) of 16S rRNA gene and electrophoretic protein patterns, Life Science Journal, Vol. 10, No. 4.

[8] Yang L., Tan Z., Wang D., Xue L., Guan M., Huang T., Li R., 2014, Species identification through mitochondrial rRNA genetic analysis, Scientific report 4:4089.

[9] Pardo B.G., Machordom A., Foresti F., Porto-Foresti F., Azevedo M.F.C., Banon R., Sanchez L., Martinez P., 2005, Phylogenetic analysis of flatfish (order Pleuronectiformes) based on mitochondrial 16s rDNA sequences, Scientia Marina, Vol. 69, No. 4, pp. 531-543.

[10] Palumbi S.R., Benzie J., 1991, Large mitochondrial DNA differences between morphologically similar penaeid shrimp, Molecular Marine Biology and Biotechnology, Vol. 1, pp. 27-34.

[11] Xiaojuan Q., Bosheng Z., Yuan W., 2008, Mitochondrial partial DNA sequences analysis of the Chi-lin fish from Tai Mountain: implication for its systematic and evolution. http://www.paper.edu.cn/en_releasepaper/content/18195.

[12] Strandberg A.K.K., Salter L.A., 2004, A comparison of methods for estimating the transition:transversion ratio from DNA sequences, Molecular Phylogenetics and Evolution, Vol. 32, pp. 495–503.

[13] Belle E.M.S., Piganau G., Gardner M., Eyre-Walker A., 2005, An investigation of the variation in the transition bias among various. Animal mitochondrial DNA, Gene, Vol. 355, pp. 58 – 66.

[14] Tan M.P., Jamsari A.F.J., Siti Azizah M.N., 2012, Phylogeographic pattern of the striped Snakehead, *Canna striata* in Sunda Land: ancient river connectivity, geographical and anthropogenic signatures, Plos One, Vol. 7, No. 12, e52089. Doi:10.1371/journal.pone. 0052089.

[15] Dodson J.J., Colombani F., Ng. P.K.L., 1995, Phylogeographic structure in mitochondrial DNA of a South-East Asian Freshwater fish, *Hemibagrus nemurus* (Siluroidei;Bagridae) and Pleistocene sea-level changes on the Sunda shelf, Molecular Ecology, Vol. 4, pp. 331-346.
[16] Asmawi S., 2011, Strategy of fisheries management to actualize sustainable fish resource of terrestrial open water in South Kalimantan, Center of Documentation and Scientific Information. Indonesian Institution of Science, PD II – LIPI.
[17] Handayani T., Lilia, Ardianor, 2009, Correlation of water quality parameter with Ichtiofauna in Lutan Lake, Central Kalimantan, Journal of Tropical Fisheries, Vol. 4, No. 2, pp. 416-430.