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Composition of Mitochondrial DNA *16S* Nucleotide of Dwarf Snakehead (*Channa gachua* Hamilton, 1822) from Keji River, Magelang, Central Java

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A B S T R A C T

Indonesia has a high marine and freshwater biodiversity including freshwater fish biodiversity. One of freshwater fish which is commonly consumed by Indonesian people is dwarf snakehead (*Channa gachua* Hamilton, 1822). However, research on genetic characterization, especially the composition of mtDNA *16S* nucleotide of dwarf snakehead has poorly understood. Therefore, the aim of this study was to determine the composition of mtDNA *16S* nucleotide of dwarf snakehead as a part of genetic characterization of the fish species taken from Keji River, Magelang, Central Java which has not been previously examined. This study analyzed *16S* mt-DNA of two samples of dwarf snakehead from Keji River (KTS-01 and KTS-02). In addition, two sequences of *Channa gachua* with accession number KU986900, KU238074, and HM117234-HM117238 taken from GenBank were used as a comparison. A method used in this research was a PCR method and primers used in this research were 16Sar and 16Sbr. The results revealed that the average of nucleotide composition T, C, A and G of the fish species was 23.04%, 25.13%, 29.06% and 22.77% respectively whereas the average rate of nucleotide composition A+T and G+C was 52.10% and 47.90% respectively. The two dwarf snakehead had similar T and C composition but different in A and G composition. In addition, the G+C content in KTS-01 and KTS-02 had the highest frequency compared to other dwarf snakehead taken from GenBank. From this finding it could be assumed that there is genetic variation between the two dwarf snakehead from Keji River which is important genetic data for breeding program of the fish species in the future.

1. Introduction

Fish has important nutritional values especially protein, vitamin and minerals. Dwarf snakehead (*Channa gachua*) is one of freshwater fish which is commonly consumed by Indonesian people. The dwarf snakehead belonging to the family Channidae and considered as the important freshwater food fish in tropical Asia due to possess high economic value (Benziger *et al*., 2011; Kottelat, 2013). In addition, the fish are commonly used as ornamental fish in aquarium due to the beautiful colorization (Milton *et al*., 2011).

The fish is native to Asia and has widely distribution in Middle East and South Asia, including Indonesia (Berra, 2007; Kottelat, 2013). This species mainly inhabit most any type of wetland including streams, creeks and rivers with pH 6.0-7.0 and temperature 10-28^{o}C. The species can reach 28 cm in length and feeds on small fish, aquatic insects and crustaceans (Chaundhry, 2010).

In order to increase the production of the fish, research attention has to be focused on the fish genetic characterization using molecular approaches. Mitochondrial genes are widely used as efficient molecular tools not only for identification unambiguous species but also for examination genetic variation and biodiversity with high levels of accuracy (Pereira *et al*., 2008; Yang *et al*., 2014; Hammer *et al*., 2014; Satoh *et al*., 2016). One of the mitochondrial genes that can be used for molecular marker is *16S* mtDNA. The *16S* mt-DNA is often used for studies genetic characterization of the

species and genetic variation at inter-specific levels, such as *Coilia mystus* (Cheng *et al*., 2008), *Epinephelus lanceolatus* (Cheng *et al*., 2015), *Monopterus albus* (Arisuryanti, 2016), and *Labeo* spp. (Jahan *et al*., 2017). For the analysis, the *16S* mtDNA marker is first amplified by PCR using primers (universal or specific primers) and the amplicons are sequenced. Sequencing data are then aligned and compared using appropriate bioinformatic tools (Arif and Khan, 2009; Arif *et al.*, 2009).

Despite the importance of this species as a food consumption resource, little is known about genetic characterization and genetic diversity of dwarf snakehead in the Indonesian waters. The information of *16S* mtDNA varieties in *C. gachua* collected from Keji River (Magelang, Central Java) has never been reported. Failure to detect population units of the fish species coupled with local overfishing will ultimately lead to decrease in populations. In order to implement conservation and management strategies for a declining species, it is important to investigate composition of mitochondrial DNA *16S* nucleotide of dwarf snakehead as a part of the fish genetic characterization throughout its natural habitat. Therefore, the objective of this research was to find the basic nucleotide data of the *16S* mtDNA of dwarf snakehead from Keji River (Magelang, Central Java) to complete the genetic information of Indonesian dwarf snakeheads. It is expected that this finding of this study is able to give genetic information which is beneficial for improving the genetic quality of dwarf snakeheads in Indonesia through breeding program.

2. Materials and Methods

2.1. Materials

The materials used were 100 mg fillet of two samples of dwarf snakehead fish from Keji River, Magelang (Figure 2), 99% ethanol, aquades, aquabides, Qiagen DNA isolation kits (Qiagen, USA), agarose (1st Base, Singapore), double distilated water (ddH2O, 1st Base, Singapore), DNA ladder (Bioline), Tris-EDTA (TE, 1st Base, Singapore), Tris-Borate-EDTA buffer (TBE, 1st Base, Singapore), DNA ladder (Bioline), MyTaq HS Red mix PCR, $MgCl_2$, FluoroSafe (1st Base, Singapore), DNA template, 16Sar primer (5'-CGCCTGTTTATCAAAAACAT-3') and primer 16Sbr (5'- CCGGTCTGAACT CAGATCACGT-3') (Palumbi, 1996).

2.2. Methods

2.2.1. Sample collection and storage

The dwarf snakehead samples were collected from Keji River, Magelang, Central Java (7°35'34.85"S 110°16'19.48"E) (Figure 1). The fish were caught by net and then documented (Figure 2). Approximately 100 mg of muscle tissue from two individuals (KTS-01 and KTS-02) was preserved in 99% ethanol and each was placed into 1.5 ml tube. Fish samples were then brought to Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada and stored at 4°C until processed.

Figure 1. Map of sampling collection site for *C. gachua* samples in Keji River, Magelang, Central Java

2.2.2. DNA isolation, PCR amplification and electrophoresis

Total genomic DNA was extracted from the ethanol preserved muscle tissue using the DNeasy blood and tissue kit (Qiagen, Valencia, CA, USA) following manufacture's protocols. The MyTaq HS Red Mix PCR kit (Bioline) was used for the polymerase chain reaction (PCR) and the primers used in this study were 16Sar (5'-CGCCT GTTTATCAAAAACAT-3') dan primer 16Sbr (5'- CCGGTCTGAACTCAGATCACGT-3') (Palumbi, 1996). The total volume of each PCR reaction was 50 µL. The reaction mixture consisted 10-100 ng of genomic DNA, 25 µL MyTaq HS Red Mix PCR, 2 mM $MgCl_2$, 0.6 µM of each primer and 11 µL double distilated water (ddH_2O). A negative control was set up by omitting template DNA from the reaction mixture to evaluate the reliability of the DNA amplification. The reaction mixture was initially pre-denatured at 95°C for 5 min followed by 35 cycles of denaturation at 94°C for 35 s, annealing at 50°C for 30 s, and extension at 72°C for 30 s. Reaction was then subjected to a final extension at 72°C for 7 minutes. All of PCR products were visualized using 1% agarose gel electrophoresis buffered with Tris-Borate-EDTA (TBE), stained with FlouroSafe nucleic acid stain, and visualized under UV light. All samples were

then sent to First Base Sdn Bhd (Malaysia) through P.T. Genetika Science (Jakarta) for purification and sequencing.

2.2.3. Data analysis

Chromatograms of the two dwarf snakeheads were checked and assembled using SeqMan, and edited using EditSeq Pro Program Lasergene DNASTAR software package (DNASTAR Inc., Madison, USA). Consensus sequences of the *16S* mtDNA were checked from forward and reverse. The sequence of each sample was verified using BLAST. The composition of mtDNA *16S* nucleotide of each fish sequence obtained in this study were then calculated using DNA Statistics from EditSeq menu. The composition of C+G was validated using DnaSP v.5.10.01 (Librado and Rozas, 2009). The comparison nucleotide composition of dwarf snakehead fish used was taken from GenBank (accession number KU986900, KU238074, and HM117234-HM117238).

Figure 2. *C. gachua* collected from Keji River, Magelang, Central Java (1) KTS-01 and (2) KTS-02

3. Results and Discussion

The amplification product for the *16S* mitochondrial gene of the two dwarf snakeheads investigated in this study (KTS-01 and KTS-02) generated 573 bp in fragment length (Figure 3). The analysis using BLAST showed that the two dwarf snakeheads investigated in this study have 98% similarity with *C. gachua* deposited at GenBank.

Tabel 1. Percentage composition of 16S mtDNA nucleotide of *C. gachua* (KTS-01 and KTS-02) collected from Keji River, Magelang, Central Java

Sample	T(U)	C	A	G	A+T	C+G
KTS-01	23.04	25.13	28.97	22.86	52.00	47.99
KTS-02	23.04	25.13	29.14	22.69	52.18	47.82
$\bar{x}$	23.04	25.13	29.06	22.77	52.10	47.90

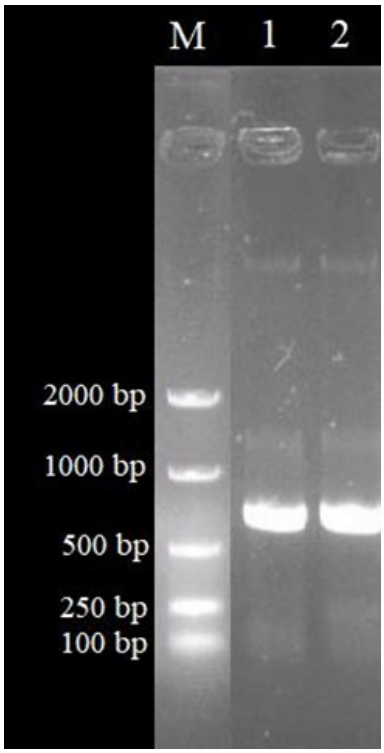

Figure 3. The DNA band profiles of *16S* mitochondrial DNA of *C. gachua* (1) KTS-01 and (2) KTS-02. M = 1 kb DNA ladder (Bioline).

Based on the range of the analysis 573 bp nucleotide, the result of nucleotide parallelization of the two dwarf snakehead taken from Keji River (Magelang, Central Java) is shown on the Table 1. From the Table 1, it can be seen that the average of nucleotide T, C, A and G was 23.04%, 25.13%, 29.06% and 22.77% respectively whereas the average rate of nucleotide composition A+T and G+C was 52.10% and 47.90% respectively. Both dwarf snakeheads had similar nucleotide T and C but different A and G. The nucleotide divergence between the two *C. gachua* taken from Keji River indicated that there is intra-population genetic variation This is due to the 16S mitochondrial gene is considered as a highly conserved and usually used for interspecies or intergeneric level of identification and diversity (Arif and Khan, 2009). Therefore, the finding of 16S mtDNA nucleotide divergence between the two samples indicate that there is intra-population genetic variation which is important genetic data for breeding program of the fish species in the future.

Next, the *16S* mtDNA sequence data of *C. gachua* collected from Keji River (Magelang, Central Java) were compared to *C. gachua* taken from GenBank (KU986900 from Malaysia, KU238074 from China and HM117234-HM117238 from India) and it can be shown in Table 2. From the Table 2, it can be seen that the composition of nucleotide C from *C. gachua* examined in this study has the highest frequency compared to the two other *C. gachua* from Malaysia and China. Similarly, the composition of C+G of *C. gachua* from Keji River is also higher than that of C+G from Malaysia and

Tabel 2. Percentage composition of mtDNA *16S* nucleotide of *C. gachua* (KTS-01 and KTS-02) collected from Keji River, Magelang, Central Java compared to other *C. gachua* samples taken from GenBank.

Sample	T(U)	C	A	G	A+T	C+G
KTS-01	22.76	25.10	29.57	22.57	52.33	47.66
KTS-02	22.76	25.10	29.77	22.37	52.53	47.47
KU986900*	23.54	24.31	30.35	21.79	53.89	46.11
KU238074*	23.15	24.51	29.96	22.37	53.11	46.89
HM117234*	22.42	25.40	30.55	21.63	52.98	47.02
HM117235*	22.42	25.20	30.55	21.83	52.98	47.02
HM117236*	22.42	25.20	30.55	21.83	52.98	47.02
HM117237*	22.42	25.20	30.55	21.83	52.98	47.02
HM117238*	22.42	25.20	30.55	21.83	52.98	47.02

*Sample taken from GenBank

China which was previously examined and deposited to GenBank but unfortunately it has not been published. In addition, the composition of nucleotide T and G from *C. gachua* examined in this study has the highest frequency compared to the other five *C. gachua* from India. Similarly, the composition of C+G of *C. gachua* from Keji River is also higher than that of C+G from India which was previously examined by Lakra *et al.* (2010).

Based on the *16S* mtDNA sequence data, the dwarf snakeheads (*C. gachua*) from Keji River (Magelang, Central Java, Indonesia) have had a specific nucleotide bases that distinguish them from *C. gachua* outside Indonesia, so it can be location-specific genetic markers from Keji River. This finding is important for developing policies regarding the conservation of the fish species and their habitat. Further research has to be examined on this fish species especially investigating the fish genetic variation through hyper variable mitochondrial gene (D-loop) and also microsatellite.

4. Conclusions

The average of nucleotide composition T, C, A and G of the *C. gachua* from Keji River (Magelang, Central Java) was 23.04%, 25.13%, 29.06% and 22.77% respectively whereas the average rate of nucleotide composition A+ T and G+ C was 52.10% and 47.90% respectively. The two *C. gachua* had similar T and C composition but different in A and G composition. In addition the *C. gachua* have had specific nucleotide composition compared to other *C. gachua* from outside Indonesia which can be used as a molecular marker for the fish species.

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References

Arif, I.A., Khan, H.A., Bahkali, A.H., Al Homaidan, A.A., Al Farhan, A.H., Shobrak, M. & Al Sadoon, M., 2009, Comparison of neighbor-joining and maximum parsimony methods for molecular phylogeny of *Oryx* species using 12S rRNA and 16S rRNA gene sequences, *Animal Biology Journal*, 1(2), 117-125.

Arif, I.A. & Khan, H.A., 2009, Molecular markers for biodiversity analysis of wildlife animals: a brief review, *Animal Biodiversity and Conservation*, 32(1), 9–17.

Arisuryanti, T., 2016, *Molecular Genetic and Taxonomic Studies of the Swamp Eel (Monopterus albus Zuiew 1793)*. Ph.D. Thesis, Charles Darwin University, Australia.

Benziger A., Philip, S., Raghavan, R., Ali, P.H.A., Sukumaran8, M., Tharian, J.S., Dahanukar, N., Baby, F., Peter, R., Devi, K.R., Radhakrishnan, K.V., Haniffa, M.A., Britz, R. & Antunes, A., 2011, Unraveling a 146 years old taxonomic puzzle: Validation of Malabar snakehead, species-status and its relevance for Channid systematics and evolution, *Validation of Malabar Snakehead Species-Status*, 6(6), 1-12.

Berra, T.M., 2007*, Freshwater Fish Distribution*, The University of Chicago Press, Chicago.

Chaundhry, S., 2010, '*Channa gachua',* in *The IUCN Red List of Threatened Species 2010*, viewed 05 January 2018, from http://www.iucnredlist.org/details/166123/0.

Cheng, Q., Ma, C., Cheng, H. & Zhang, Q., 2008, Mitochondrial DNA diversity of *Coilia mystus* (Clupeiformes: Engraulidae) in three Chinese estuaries, *Environmental Biology of Fishes*, 83(3), 277-282.

Cheng, S.S., Senoo, S., Siddiquee, S. & Rodrigues, K.F. 2015. Genetic variation in the mitochondrial genome of the giant grouper *Epinephelus lanceolatus* (Bloch, 1790) and its application for the identification of brood stock, *Aquaculture Reports*, 2, 139-143.

Hammer, M.P., Unmack, P.J. & Adams, M., 2014, A multigene molecular assessment of cryptic biodiversity in the iconic freshwater blackfishes (Teleostei: Percichthyidae: *Gadopsis*) of south-eastern Australia, *Biological Journal of the Linnean Society*, 111, 521-540.

Jahan, H., Akter, M., Begum, R.A. & Shahjahan, R.M., 2017, Identification and comparison of three carp fishes based on mitochondrial 16S rRNA gene. *Journal Biological Science,* 26(2), 167-174.

Kottelat, M., 2013, The fishes of the inland waters of Southeast Asia: A Catalogue and core bibliography of the fishes known to occur in freshwaters, mangroves and estuaries, *The Raffles Bulletin of Zoology*, 27, 1-663.

Lakra, W.S., Goswami, M., Gopalakrishnan, A., Singh, D.P., Singh, A., Nagpure, N.S., 2010, Genetic relatedness among fish species of Genus *Channa* using mitochondrial DNA genes. *Biochemical Systematics and Ecology*, 38(6), 1212-1219.

Librado, P. & Rozas, J., 2009, DnaSP v5: A Software for Comprehensive Analysis of DNA Polymorphism Data, *Bioinformatics*, 25, 1451-1452.

Milton, J., Bhat, A.A., Haniffa, M.A., Hussain, S.A., Rather, I.A., Al-Anazi, K.M., Hailan, W.A.Q. & Farah, M.A., 2017, Ovarian development and histological observations of threatened dwarf snakehead fish, *Channa gachua* (Hamilton, 1822), *Saudi Journal of Biological Sciences*, 1(25), 149-153.

Palumbi, S.R., 1996, 'Nucleid acids II : The Polymerase Chain Reaction', in D.M. Hillis, C. Moritz, B.K. Mable (eds.), *Molecular Systematic*, pp. 205-247, Sinauer Associates, Massachusetts.

Pereira, F., Carneiro, J. & Amorim, A., 2008, Identification of species with DNA-Based Technology: Current progress and challenges. *Recent Patents on DNA and Gene Sequences*, 2, 187-200.

Satoh, T.P., Miya, M., Mabuchi, K. & Nishida, M., 2016, Structure and variation of the mitochondrial genome of fishes. *Biomed Central Genomics*, 17(719), 1-20.

Yang, L., Tan, Z., Wang, D., Xue, L., Guan, M., Huang, T. & Li, R., 2014, Species identification through mitochondrial rRNA genetic analysis. *Scientific Reports*, 4, 4089.