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Deteksi Polimorfime Gen Mitokondria *16S* Ikan Wader Bintik-Dua (*Barbodes binotatus* Valenciennes, 1842) dari Danau Lebo Taliwang, Nusa Tenggara Barat

Detection of *16S* Mitochondrial Gene Polymorphism on Barb Fish (*Barbodes binotatus* Valenciennes, 1842) from Lake Lebo Taliwang, West Nusa Tenggara

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Abstrak Ikan wader bintik-dua (*Barbodes binotatus*) merupakan ikan tropis yang melimpah di Danau Lebo Taliwang dan sering dikonsumsi oleh masyarakat karena memiliki gizi yang tinggi dan diperdagangkan sebagai ikan hias karena memiliki warna yang eksotis. Namun demikian, belum ada data informasi genetik yang berkaitan dengan variasi genetik intra-populasi ikan wader bintik-dua dari Danau Lebo Taliwang yang pernah dilaporkan. Padahal informasi genetik tersebut memiliki peranan yang penting dalam konservasi ikan wader bintik-dua di habitatnya. Oleh karena itu tujuan dari penelitian ini adalah mengamati polimorfisme pada gen mitokondria *16S* ikan wader bitnik-dua sebagai bagian dari deteksi variasi genetik intra-populasi ikan wader bintik-dua di Danau Lebo Taliwang. Metode yang digunakan pada penelitian ini adalah metode PCR dengan dua primer yaitu 16Sar dan 16Sbr. Data yang diperoleh selanjutnya dianalisis menggunakan program MESQUITE, MEGA, DnaSP dan PopART. Hasil pensejajaran yang dilakukan pada sampel ikan wader bintik-dua yang diteliti diperoleh panjang fragmen sekuen gen mitokondria *16S* sebesar 610 bp. Dari Panjang fragmen 610 bp tersebut terdeteksi 5 haplotipe dengan 9 situs polimorfik dan 2 situs *parsimony informative*. Nilai keragaman haplotipe dan keragaman nukleotida berturut-turut adalah 1,00 dan 0,0066. Nilai rata-rata jarak genetik antar sampel ikan wader bintik-dua yang diamati adalah sebesar 0,66%. Hasil penelitian ini memperlihatkan adanya polimorfime pada sekuen gen mitokondria *16S* dan mengindikasikan adanya variasi genetik intra-populasi ikan wader bintik-dua di Danau Lebo Taliwang.

Kata kunci: *Barbodes binotatus*; gen 16S; polimorfisme

Abstract The spotted barb fish (*Barbodes binotatus*), a tropical cyprinid fish, is one of the abundant fishes in Lake Lebo Taliwang commonly consumed due to high nutrition and utilized as an aquarium trade due to their exotic colour. However, no genetic information related to the intra-population genetic variation of the fish species in Lake Lebo Taliwang. Genetic information of the spotted barb fish can play an important role for the fish conservation. Therefore, the objective of this study was to detect *16S* mitochondrial gene polymorphism of the spotted barb fish which can be used to detect their genetic variation. The method used in this study was a PCR method with primers 16Sar and 16Sbr. The data was then analysed using MESQUITE, MEGA, DnaSP and PopART to identify *16S* mitochondrial gene polymorphism. This study obtained 610 base pairs after alignment of *16S* sequences of all samples. Five haplotypes were detected with 9 variable sites and 2 parsimony informative sites. Haplotype diversity and nucleotide diversity of the spotted barb fish were 1.00 and 0.0066 respectively. In addition, genetic distance among the samples was 0.66. This finding exhibited polymorphism on *16S* gene sequence among the fish samples and this data indicated genetic variation of the spotted barb from Lake Lebo Taliwang.

Key words: *Barbodes binotatus*; 16S gene; polymorphism

INTRODUCTION

Lake Lebo Taliwang is located at West Sumbawa, West Nusa Tenggara Province (Haryani, 2013). Many freshwater aquatic animals including freshwater fish inhabit in Lake Lebo Taliwang such as climbing perch (*Anabas testudineus*), three spot gourami (*Trichopodus trichopterus*), barb fish *(Barbodes* spp.), and swamp eel (*Monopterus* spp). One of freshwater fish commonly consumed and utilized as an aquarium trade is spotted barb fish (RIPED, 2008). The previous study investigated by Arisuryanti *et al.* (2019[b]) using *COI* mitochondrial gene as a DNA barcoding revealed that the spotted barb fish inhabited in Lake Lebo Taliwang was identified as *Barbodes binotatus*.

The spotted barb fish (*Barbodes binotatus*) is kind of a cyprinid fish which have varies in colour from silvery grey to greenish grey with darker in dorsal and paler in belly. The fish can reach 20 cm in length. The fish has widely distribution in Southeast Asia such as Myanmar, Cambodia, Laos, Vietnam, Thailand, Brunei, Malaysia, and Indonesia (Kottelat 2013; Keat-Chuang *et al.*, 2018). The fish species is commonly found in stagnant water including sluggish-flowing canals and feed variety of zooplankton and insect larvae. The fish inhabit in a tropical climate and commonly prefer the freshwater with a temperature range of 24-26°C and a 6.0-6.5 pH (Kottelat, 2013). In addition, the fish species has high protein containing lysine (23.5 mg/g sample) which considered as an essential amino acid (Lim *et al.*, 2015).

No genetic information related to intra-population genetic variation of the spotted barb in Lake Lebo Taliwang. Genetic variation is important to be gained especially for brood stock conservation of the fish species and it can be explored by using *16S* mitochondrial gene. The *16S* mitochondrial gene is conserved, so change of few nucleotides within or among population might indicate substantial degree of genetic variation (Cawthorn *et al*., 2012; Yang *et al*., 2013). Therefore, in this study we use partial *16S* mitochondrial gene as a molecular marker to detect polymorphism in the spotted barb fish. The *16S* mitochondrial gene has been widely used to identify and detect genetic variation of freshwater fish such as carp fishes (Jahan *et al*., 2017) and kissing gourami (Arisuryanti *et al*., 2019[a]), Serranidae fish (Saad, 2019), and red sea parrotfish (Saad *et al*., 2019). The finding data can be also utilised to arrange *16S* mt-DNA library sequences of the spotted barb fish from Indonesia.

MATERIALS AND METHODS

Sampling and collection for 16S mitochondrial sequencing

Five samples of the spotted barb fish (code: WDR-01, WDR-02, WDR-03, WDR-04, and WDR-05) were collected from Lake Lebo Taliwang (Figure 1). Fish were caught using either baited fish traps or a seine net and documented afterward (Figure 2). Upon capture fish were placed on ice and then sent to the laboratory and frozen at -20°C. Subsequently, muscle tissue from each fish was dissected and placed into a tube containing 99% ethanol absolute and maintained at 4°C until required for DNA isolation.

Figure 1. Sample site of the spotted barb fish at Lake Lebo Taliwang.

Figure 2. The spotted barb fish collected from Lake Lebo Taliwang.

DNA extraction, amplification, and sequencing of 16S mitochondrial gene: procedure and analysis

Total DNA was extracted from a 50-100 mg preserved muscle tissue of the spotted barb fish using the DNeasy Blood and Tissue Kit (QIAGEN, Valencia, USA) following the manufacturer's protocol. The amplification reaction of the 16S mitochondrial gene was in 25 µl volumes. Amplification reaction mixture consisted of 10-100 ng DNA template, 12.5 µl MyTaq HS Red Mix PCR Kit (Bioline), 2 mM $MgCl_2$, 0.6 µM of each primer, with sterilized water added to make up to the final volume to 25 µl. The PCR profile can follow Arisuryanti *et al*. (2019a,b). Primer sequences for the partial 16S mitochondrial gene were 16Sar (5'-CGCCTGTTTATCAAAAACAT-3') and 16Sbr (5'-CCGGTCTGAACTCAGATCACGT-3') (Palumbi, 1996). The PCR products were electrophoresed on a 1% agarose gel (First Base) with Gel Red (Bioline) staining, and visualized under UV transilluminator (Daihan, Korea). Double stranded DNA products were purified using ExoSAP-ITTM (Applied Biosystems). Sequencing analysis was conducted using an Applied Biosystem Automated Sequencer. Sequence reactions followed the standard protocol using the Big Dye Terminator v.3.1. cycle sequencing kit (Applied Biosystems) on an ABI 3500 capillary sequencer according to the manufacturer's instructions. For each sample, sequence reactions were performed using both primers to allow complimentary DNA strands to be read in order to verify base sequences.

Chromatogram of each sample was viewed using SeqMan and edited using EditSeq program (Lasergene, DNASTAR). Consensus sequences of all samples were done manually and were then aligned with opal in MESQUITE v.3.51 (Madison & Madison, 2018). The partial 16S rRNA gene sequence of *Barbodes binotatus* obtained from GenBank (NC_034755), was used as a comparative purpose. The composition of mtDNA *16S* nucleotide of each sample sequence obtained in this study were calculated using DNA Statistics from EditSeq menu of Lasergene (DNASTAR). The *16S* mitochondrial variation was evaluated using number of haplotype, number of variable sites and parsimony informative, haplotype diversity, and nucleotide diversity using DnaSP ver.6 (Rozas *et al*., 2017). The genetic distances among the samples were estimated by using Kimura-2-parameter model in MEGA7 program (Kumar *et al*., 2016). Haplotype joining network was analysed using Population Analysis with Reticulate Trees (PopART) (Leigh & Bryant, 2015).

RESULTS AND DISCUSSION

The *16S rRNA* gene can all be amplified clearly in these five *B. binotatus* collected from Lake Lebo Taliwang. The *16S*

rRNA sequences were corrected and aligned, and 610 bp consensus sequences were obtained after excluding the ambiguous positions (Figure 3).

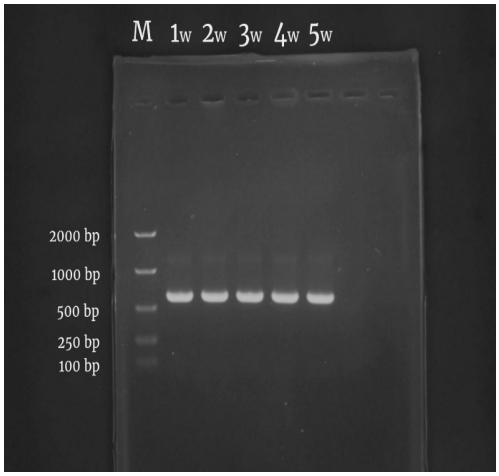

Figure 3. PCR amplification of 5 samples of *B. binotatus* from Lake Lebo Taliwang using the primers 16Sar and 16Sbr. Code 1w-5w refer to sample code WDR-01, WDR-02, WDR-03, WDR-04, and WDR-05; M is size marker 2.000 bp.

The number of *16S* nucleotide of all samples had no similar value (Table 1). This is due to some nucleotide deletion and insertion among the samples (Table 2). Among the five samples of *B. binotatus* investigated in this study with fragment length 610 bp, the divergences of nucleotide T, C, A, and G were between 0.01 and 0.35%, 0 and 0.24%, 0 and 0.2%, 0.02 and 0.16% respectively. The average rate of nucleotide composition A+T and G+C among the five samples was similar (0-0.26%). In addition, sample WDR-01 has the highest nucleotide C (24.08%) and A (32.58%) whereas sample WDR-03 has the highest nucleotide G (21.83%) and sample WDR-05 has the highest nucleotide T (22.01%). The divergences of *16S* nucleotide composition among the samples within the population of the *B. binotatus* from Lake Lebo Taliwang indicate *16S* genetic polymorphism at intrapopulation level.

Table 1. Average composition of *16S* nucleotide of the spotted barb (*B. binotatus* with code WDR 01-05) collected from Lake Lebo Taliwang with fragment length 610 bp.

Sample	T(U) (%)	C (%)	A (%)	G (%)	A+T (%)	C+G (%)
WDR-01	21.67	24.08	32.58	21.67	54.25	45.75
WDR-02	21.92	23.84	32.48	21.76	54.40	45.60
WDR-03	21.83	23.92	32.42	21.83	54.25	45.75
WDR-04	21.66	24.04	32.48	21.82	54.14	45.86
WDR-05	22.01	23.92	32.38	21.69	54.39	45.61
Average	21.82	23.96	32.47	21.75	54.29	45.71

The sequence analysis of the five spotted barb fish from Lake Lebo Taliwang were compared and multiple sequence alignment revealed 5 distinct haplotypes. There were between 1 and 12 variable nucleotide sites among the haplotypes, 6 of which differed by transitional substitutions, 3 by transversional changes and 3 insertion/deletions (Table 2). Transitional changes are commonly found in animal mitochondrial genomes compared to tranversional changes (Malakar *et al*., 2009; Fišer Pečnikar & Buzan, 2013). In addition, 9 variable sites with 2 parsimony informative sites were detected. Haplotype diversity and nucleotide diversity were 1.00 and 0.0066 respectively. This data revealed low genetic variation even though the haplotype diversity was high. This is due to the polymorphism based on *16S* mitochondrial gene of the *B. binotatus* within the population is just 1.47%. However, *16S* mitochondrial gene is a conserved gene and the divergences of some nucleotides of the samples within population may indicate genetic variation within this population (Cawthorn *et al*., 2012; Yang *et al*., 2013). In addition, genetic distance among the *B. binotatus* samples from Lake Lebo Taliwang was between 0.16%-1.50% (0.66%) which supported intra-population genetic variation of the *B. binotatus* at Lake Lebo Taliwang.

Table 2. Summary of nucleotide variations found in the partial *16S* mitochondrial gene. Only variable sites are shown. Dots indicate identity with WDR-01 (Hap-1) haplotype sequence. Dash indicate insertions/deletions. The numbers shown above the reference sequence indicate the nucleotide positions in the alignment.

Samples	Haplotype	Polymorphic sites											
				2	1	2	3	4	4	4	4	6	6
		4	5	9	7	1	1	3	3	4	4	0	1
					1	4	1	8	9	0	2	8	0
WDR-01	Hap-1	A	T	T	C	T	A	-	-	-	C	T	C
WDR-02	Hap-2	.	.	.	.	.	.	-	-	-	.	G	T
WDR-03	Hap-3	T	.	.	.	.	.	-	-	-	.	G	T
WDR-04	Hap-4	T	A	C	T	C	G	C	A	A	T	G	T
WDR-05	Hap-5	.	.	.	.	.	.	-	-	-	.	.	T

The polymorphism of *16S* mitochondrial sequences of *B. binotatus* in this study was also supported by Median Joining Network which revealed that among the haplotypes were separated by 1 and 6 mutation points (Figure 4). This mutation points showed that intra-population genetic variation may occur in *B. binotatus* within the population of Lake Lebo Taliwang.

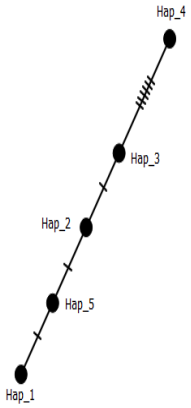

Figure 4. Median Joining Network from 610 bp of *16S* mitochondrial sequences of *B.binotatus* from Lake Lebo Taliwang.

The *16S* mitochondrial sequence polymorphism detected in our investigation indicated the potential usefulness of this sequence to determine genetic variation of *B. binotatus* within population in Lake Lebo Taliwang. This genetic information data is not only important to be implemented for conservation management of the fish species in its habitat but also can be used to arrange *16S* DNA library for biodiversity data of *B. binotatus* in Indonesia.

CONCLUSION

The usage of *16S* mitochondrial gene can be used to detect polymorphism on *B. binotatus* from Lake Lebo Taliwang. Five haplotypes were detected with 9 variables sites and 2 parsimony informative sites. Haplotype diversity and nucleotide diversity were 1.00 and 0.0066 respectively. The divergences on the *16S* mitochondrial sequence among the *B. binotatus* samples within the population may indicate genetic variation at intra-population level.

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REFERENCES

Arisuryanti, T., G.A. Pratama, L. Hakim, J.P. Koentjana & F.K. Nazira. 2019a. Genetic characterization of kissing gourami (*Helostoma temminckii* Cuvier, 1829) in Ogan River, South Sumatra inferred from *16S rRNA* and *COI* mitochondrial genes. Indones. Fish. Res. J. 25 (1): 37-44

Arisuryanti, T., R.L. Hasan, K.L. Ayu, N. Ratman & L. Hakim. 2019b. Genetic identification of freshwater fish species from Lake Lebo Taliwang, West Nusa Tenggara through DNA barcoding. J. Tropical Biodiversity Biotechnology 4 (3): 107-112

Cawthorn, D.M., H.A. Steinman & R.C. Witthuhn. 2012. Evaluation of the 16S and 12S rRNA genes as universal markers for the identification of commercial fish species in South Africa. Gene 491 (1): 40-48

Fišer Pečnikar, Ž. & E.V. Buzan. 2013. 20 years since the introduction of DNA barcoding: from theory to application. J. Appl. Genet. 55 (1): 43-52

Haryani, G. 2013, Kondisi danau di Indonesia dan strategi pengselolaannya. Prosiding Pertemuan Ilmiah Tahunan Masyarakat Limnologi Indonesia. I: 1-19.

Jahan, H., M. Akter, R.A. Begum & R.Md. Shahijahan. 2017. Identification and comparison of three carp fishes based on mitochondrial 16S rRNA gene. Dhaka Univ. J. Biol. Sci. 26 (2): 167-174

Keat-Chuang, C., P. A. Ooi, W. L. Wong, & G. Khoo. 2018. Ichthyofauna checklist (Chordata: Actinopterygii) for indicating water quality in Kampar River catchment, Malaysia. Biodiversitas 19 (6): 2252-2274

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. Raffles B. Zool. Supplement. 27: 1-663

Kumar, S., G. Stecher & K. Tamura. 2016. MEGA7: molecular evolutionary genetic analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33 (7): 1870-1874

Leigh, J.W. & D. Bryant. 2015. POPOART: full-feature software for haplotype network construction. Methods Ecol. Evol. 6: 1110-1116

Lim, L.S., W.K. Chor, R.F. Firdaus, L. Malitam, J. Ransangan & R. Shapawi. 2015. Proximate and amino acid compositions of the pond-cultured spotted barb, *Puntius binotatus*. Malaysian Journal of Science. 34 (2): 168-171

Maddison, W.P. & D.R. Maddison. 2018. Mesquite: A modular system for evolutionary analysis. Version 3.51 (http://www.mesquiteproject.org)

Malakar, A.K., W.S. Lakra, M. Singh, M. Goswami & R.M. Mishra. 2009. Genetic characterization of three Ompok species using mitochondrial DNA sequences. J. Indian Fish Assoc. 36: 65-82

Palumbi, S.R. 1996. Nucleic acids II: The polymerase chain reaction. In: Molecular systematics, (Eds.) D.M. Hillis, C.Moritz, B.K. Mable, Sinauer Associates. Sunderland. Massachusetts. p. 205

RIPED, 2008, Lebo Taliwang, kekayaan flora dan fauna Sumbawa Barat, viewed 27 February 2019, http://konservasi4lebotaliwang.blogspot.com.

Rozas, J., A. Ferrer-Matta, J.C. Sanchez-DelBarrio, S. Guirao-Rico, P. Librado, S.E. Ramos-Onsins & A. Sanchez-Gracia. 2017. DnaSP 6: DNA sequence polymorphism analysis of large datasets. Mol. Biol. Evol. 32 (12): 3299-3302

Saad, Y.M. 2019. Analysis of 16S mitochondrial ribosomal DNA sequence variations and phylogenetic relations among some Serranidae fishes. S. Afr. J. Anim. Sci. 49(1): 80-89

Saad, Y.M., A.K.M. Shaikh Omar & W.M. Gharbawi. 2019. Evaluation of molecular diversity in some Red sea parrotfish species based on mitochondrial 16S ribosomal RNA gene sequence variations. Res. J. Biotechnol. 14 (12): 8-21

Yang, L., T. Zongqing, D. Wang, L. Xue, M. Guan, T. Huang & R. Li. 2013. Species identification through mitochondrial rRNA genetic analysis. Sci. Rep. 14: 1-11

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