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INDONESIAN FISHERIES RESEARCH JOURNAL
Volume 25 Nomor 1 June 2019
p-ISSN: 0853-8980
e-ISSN: 2502-6569
Accreditation Number RISTEKDIKTI: 21/E/KPT/2018

GENETIC CHARACTERIZATION OF KISSING GOURAMI (*Helostoma temminckii* Cuvier, 1829) IN OGAN RIVER, SOUTH SUMATRA INFERRED FROM *16S rRNA* AND *COI* MITOCHONDRIAL GENES

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Received; November 09-2018 Received in revised from March 28-2019; Accepted April 08-2019

ABSTRACT

Genetic characterization data of kissing gourami are important to understand historical lineage thus enhancing sustainability of the species and to establish regulation for sustainable management of the fish stock in their habitat. However, investigation of genetic characterization of kissing gourami, one of native Indonesian freshwater fishes has poorly understood. Therefore, the aim of this study was to examine genetic characterization of the fish species collected from Ogan River, South Sumatra using partial sequences of two mitochondrial genes, *16S rRNA* and *COI*. The results revealed that for the 621 bp determined in *16S rRNA* gene of the samples, five sites were variable, of which one was parsimony informative. Concatenate data revealed three haplotypes with an overall haplotype diversity of 0.833±0.222 and nucleotide diversity of 0.003±0.001. The genetic divergence varied from 0-0.49%. Next, sequence analysis of *COI* gene exhibited 609 bp which can be translated into 203 amino acids. For the 609 bp sequence determined in the fish samples, three haplotypes were revealed with nine variable sites and two parsimony informatives. Haplotype diversity and nucleotide diversity of the fish samples were 0.833±0.22 and 0.00794±0.0025, respectively. The haplotype divergence between the fish samples was also supported by three nonsynonymous codons. In addition, the genetic divergence varied from 0 % to 1.16 %. The results suggest that genetic variation of the kissing gourami has to be monitored and further studies are needed to compare the same species from different location to know the historical lineage and demography.

Keywords: Genetic variation; kissing gourami; mt-DNA; Ogan River

INTRODUCTION

Freshwater fishes in Indonesia constitute an important source of protein for the people. Like other countries over exploited wild fish stocks has meant that the demand for fish protein potentially can no longer be met from traditional fishing (Bogard *et al*., 2015). Thus appropriate aquaculture production and sustainable management of fish stocks are of critical importance for food security and enhanced income (Morin *et al*., 2010; Belton & Thilsted, 2014).

Genetic information gained from molecular markers is now considered essential for effective sustainable exploitation, management and conservation of wildlife and commercially important fish species and support the sustainable aquaculture (Casey *et al*., 2016; Lind *et al*., 2016). In addition molecular genetic markers have been especially useful for the identification of genetically divergent wild stock of conservation significance for a number of fish (Ji *et al*., 2014; Arisuryanti *et al*., 2016; Mahmood & Ahmad, 2017). Direct DNA sequencing has been used to analyse genetic diversity within many group of fish species such as swamp eel (Arisuryanti, 2016), marine eels (Peninal *et al*., 2017), asian red catfish (Syaifudin *et al*., 2017), mudskipper (Arisuryanti *et al*., 2018), and dwarf snakehead (Ilmi & Arisuryanti, 2018). The gene regions most commonly used in DNA sequencing studies of genetic characterization and genetic variation of freshwater fish has been the *16S rRNA* and *COI* mitochondrial genes. However, limited genetic data of kissing gourami has been investigated in previous studies using the mitochondrial genes. The previous studies focused on identification accuracy of kissing gourami using *COI* gene (Wibowo *et al*., 2015; Dahrudding *et al*., 2017) with limited sampling distribution. In addition, the complete mitochondrial DNA nucleotide sequence was described by Li *et al*. (2014) (GenBank accession number NC_022728).

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Unfortunately the origin of the specimen sequenced by Li *et al*. (2014) was not given or unknown. No more studies have been done for identifying genetic characterization and genetic variation of kissing gourami using mitochondrial genes.

Kissing gourami (*Helostoma temminckii*) belongs to order Perciformes and family Helostomatidae. The fish species is native to Indonesia and also widely distributed to other Southeast Asia countries such as Thailand, Philippines and Malaysia (Tan & Ng, 2005; Kottelat, 2013). The fish reaches about 17-30 cm and is earned the name for their 'kissing' behavior due to their grazing mechanism and aggression stance (Sulaiman & Daud, 2002). The fish species exhibits a preference for slow-moving or standing waters such as lakes, backwaters, rivers, canals and swamps with temperature 22-30^{o}C and pH 6-8. In its native habitat a single female kissing gourami spawns at the onset of the rainy season and is capable of producing several thousand eggs per spawning event. The fish is omnivorous, consuming algae and a variety of invertebrates such as chironomid larvae and insects (Vidthayanon, 2012).

Ministry of Maritime Affairs and Fisheries recorded that fish consumption of Indonesian citizen reached 31.64 kg/capita/ year in 2011 and increased significantly around 43 kg/capita/year in 2017. In addition, Directorate General of Fisheries Management and Marketing (P2HP) reported that 60% of fisheries production which have highest domestic market is freshwater fish including kissing gourami. Therefore, genetic data of the fish species should be gathered and applied for conservation management. This is due to overexploitation of the wild fish can affect diversity loss in their habitat. However, the study of genetic characterization and genetic variation of the fish has poorly understood. Even the information of mitochondrial diversity in kissing gourami collected from Ogan River (South Sumatra, Indonesia) has never been reported. Therefore, the aim of this study was to examine genetic characterization of the fish species collected from Ogan River, South Sumatra using partial sequences of two mitochondrial genes, *16S rRNA* and *COI*. The mitochondrial diversity data obtained from this study is important to understand historical lineage as one of important consideration establishing regulation related to implement conservation and management strategies of the species in their native habitat and supporting the sustainability of kissing gourami aquaculture. This is due to lacking of understanding genetic variation of the fish together with local overfishing will lead to decrease drastically in population as source of genetic properties for developing and sustaining kissing gourami aquaculture.

MATERIALS AND METHODS

Sample collection for 16S rRNA and COI mitochondrial sequencing

In this study, four samples of wild kissing gourami (code: TBK-01, TBK-02, TBK-03 and TBK-04) were collected from Ogan River, South Sumatra on 15th July 2016. The sampling location is shown in Figure 1 (3°2'53.57"S and 100°55'52.32"E). The 50-100 mg of muscle tissue of each fish sample was dissected with a sterilized surgical scissor, placed into a 1.5 ml screw top cryogenic vial, and preserved in 95% ethanol in the field and transported to Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada (Yogyakarta, Indonesia). All of the tissue samples were then stored at -20^{o}C until further processed.

Figure 1. Location of kissing gourami sampling site for this study in Ogan River (South Sumatra, Indonesia).

DNA Extraction, Polymerase Chain Reaction, and Sequencing

Total genomic DNA was extracted from the muscle tissue samples using the DNeasy tissue kit (QIAGEN, Valencia, USA) following the manufacture's instructions. A fragment of the 16S rRNA gene was amplified using the primers 16Sar (5'-CGCCTGTTTATCA AAAACAT-3') and 16Sbr (5'-CCGGTCT GAACTCAGATCACGT-3') (Palumbi, 1996), whereas a fragment of the cytochrome c oxidase subunit I (COI) was amplified using the

primers FishF2 (5'-TCGACTAA TCATAAAGATATCGGCAC-3') and FishR2 (5'-ACTTCAGGGTGACCGAAGAATCAGAA-3') (Ward *et al*., 2005). PCR amplification were conducted in 50 µL reaction volumes containing 10-100 ng of genomic DNA, 25 µL of KAPA2G Fast Ready Mix PCR kit (Kapa Biosystems), 0.6 µM of each primer, 2 mM of MgCl2, and 13 µL of ddH2O. The thermal cycle profile consisted of an initial step at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 50°C for 30 s, and elongation at 72°C for 30 s, with a final extension step at 72°C for 7 min. Next, the PCR products were migrated using 1% agarose gels containing staining 2 µL GelRed in 1xTBE buffer. The PCR products were then sent to First Base (Malaysia) for purification and sequencing service using the same primers with both direction as in the PCR.

Data Analysis

Chromatograms of the partial DNA sequences of *16S rRNA* and *COI* genes were initially assembled and inspected visually for reading errors using SeqMan and EditSeq (Lasergene, DNASTAR). The complete consensus sequences from each gene were checked manually and then they were converted to Fasta format. The sequencing data obtained from the *16S rRNA* and *COI* gene segment were verified using the BLAST program of NCBI. All of the sequences from each mitochondrial gene were then aligned using the opal (a multiple sequence alignment program) routine implemented by the MESQUITE 3.51 package (Maddison & Maddison, 2018) and ClustalW in MEGA 7.0 (Kumar *et al*., 2016). Nucleotide sequence divergences were calculated using the Kimura's two parameter (K2P) distance model (Kimura, 1980). In addition, mitochondrial DNA diversity of each gene was evaluated for number of haplotype, number of variable sites including parsimony informative, haplotype diversity (*h*) and nucleotide diversity (ð) using the software DnaSP ver.5.10 (Librado & Rozas, 2009). A neighbour joining (NJ) and a maximum likelihood (ML) trees were constructed using the MEGA 7 program with bootstrapping of 1,000 replications (Kumar *et al*., 2016). Sequences of *16S rRNA* and *COI* of kissing gourami from some accession number used to create phylogenetic trees were obtained from the GenBank.

RESULTS AND DISCUSSION

Results

16S rRNA mitochondrial sequence variation

Partial *16S rRNA* sequences with an aligned length of 621 bp were obtained from four individuals of the wild kissing gourami collected from Ogan River, South Sumatra. The BLAST analysis revealed that the samples exhibited a high level of *16S rRNA* similarity (98-100%) with *Helostoma temminckii* from GenBank database. A total of 3 haplotype sequences were obtained in this study, and deposited in the GenBank under the accession number MK120514 – MK120517.

As shown in Table 1, a total of 5 of the 621 (0.81%) polymorphic sites were variable with one was parsimony informative. There were two transitions and two transversion substitution changes in the variable sites, and one change involved insertion/deletion polymorphism. The haplotype diversity and nucleotide diversity were 0.833±0.222 and 0.003±0.001, respectively. The analysis revealed nucleotide frequencies of A, T, G, and C to be 30.92%, 22.97%, 21.97% and 24.14%, respectively. The average rate of nucleotide composition A+T (53.90%) was higher than the C+G base contents (46.10%) among the samples from Ogan River investigated in this study.

The K2P distance among all samples was quite variable ranging from 0.0 to 0.49% (mean=0.35%). The samples TBK-01 and TBK-02 have no nucleotide sequence divergence while TBK-04 has high nucleotide divergence with other kissing gourami samples (0.49%) investigated in this study. Level of nucleotide sequence divergences are shown in Table 2.

Table 1. Aligment of partial *16S rRNA* gene of four kissing gourami samples (only variable sites are reported)

Sample Code	Haplotype	Polymorphic Sites*				
		7	12	570	610	612
TBK-01	HT1	-	A	G	G	T
TBK-02	HT1	-	.	.	.	.
TBK-03	HT2	-	T	.	.	C
TBK-04	HT3	T	T	A	T	.

*The number corresponds to nucleotide base pair position
Dots (.) indicate identity in particular position with the one of kissing gourami (TBK-01)

Table 2. Percentages of nucleotide sequence differences in 621 bp fragment of the *16S rRNA* mitochondrial gene among four identified of kissing gourami in this study

	TBK-01	TBK-02	TBK-03	TBK-04
TBK-01	-			
TBK-02	0	-		
TBK-03	0.32	0.32	-	
TBK-04	0.49	0.49	0.49	-

The neighbour joining and maximum likelihood tree exhibited that all kissing gourami samples collected from Ogan River were clade together and have close genetic relationship with *H. temminckii* from China (accession number KX816037 and NC_022728) and were separated with other *H. temminckii* (accession number AY763696) with no specific location sampling (Figure 2) with genetic divergence ranged from 0.2% to 0.7%.

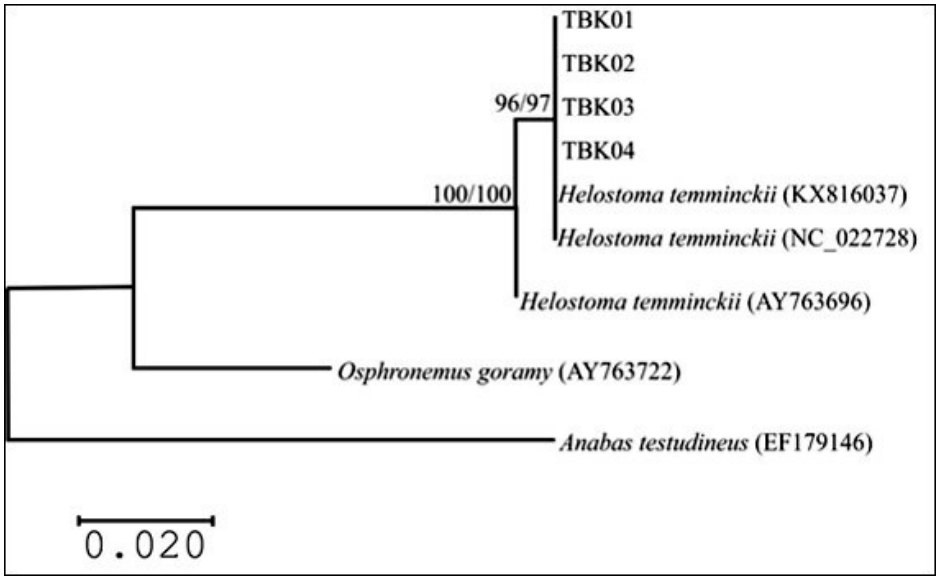

Figure 2. Neighbour joining (NJ) tree and maximum likelihood (ML) tree based on *16S rRNA* sequence data using Kimura 2 parameter (K2P) substitution model. Number at each node represent boostrap values (left node is for NJ and right node is for ML) and scale corresponds to substitution/site. Samples with accession number in brackets are taken from GenBank.

COI Mitochondrial Sequence Variation

The amplified partial sequences of *COI* had 609 bp which could be translated in to 203 amino acids. No insertions, deletions or stop codons were observed in all sequence samples. The identity of the four kissing gourami samples using BLAST program revealed 98-100% with *Helostoma temminckii* from GenBank database. Sequences representing each divergent *COI* haplotype have been deposited in GenBank with accession number MK120518 – MK120521.

The number of haplotype of *COI* sequences was 3 with 9 variable sites (1.48%) (Table 3). Among the 9 polymorphic sites observed, 7 were singleton variable sites and 2 were parsimony informative. The pattern of nucleotide substitution was 6 transition and 3 transversion changes. The two changes occurred in the third codon, resulting in synonymous substitutions. The other changes exhibited nonsynonymous substitutions. The average rate of nucleotide composition A+T (54.60%) was higher than the C+G base contents (45.40%) among the sequences examined. The mean number of nucleotide composition in the kissing gourami samples was A-23.93%, T-30.67%, C-26.72%, and G-18.68%.

Estimates of the genetic divergence among the samples, based on Kimura 2 parameter ranged between 0 – 1.16 % (Table 4). In addition, the samples examined in this study are characterized by low values of nucleotide diversity (0.00794 ± 0.00246) and mean haplotype diversity was found to be 0.833 ± 0.222. Next, the phylogenetic analysis conducted using the NJ and ML method revealed two lineages (Figure 3).

The first lineage showed that the three samples (TBK-01, TBK-02 and TBK-03) were clade together with *Helostoma temminckii* from GenBank database which was studied previously by Wibowo *et al*. (2015) and Dahruddin *et al*. (2017) whereas the other sample (TBK-04) was in the other lineage.

Table 3. Aligment of partial *COI* gene of four kissing gourami samples (only variable sites are reported)

Codon site*	1	2	4	140	142
				444	444
Nucleotide			111	112	222
Position**	123	456	012	890	456
TBK-01	**TCG**	**ACT**	**CAT**	TTC	CTG
TBK-02	...	...	...	...	...
TBK-03	...	...	**TC.**	..T	..A
TBK-04	**CGA**	**CT.**	**TC.**	...	...
Amino acid site***	1	2	4	140	142
TBK-01	**S**	**T**	**H**	F	L
TBK-02	.	.	.	.	.
TBK-03	.	.	**S**	.	.
TBK-04	**R**	**L**	**S**	.	.

*The number corresponds to codon site
** The number corresponds to nucleotide base pair position
*** The number correspond to codon site in relation to amino acid position
Dots (.) indicate identity in particular position with the one of kissing gourami (TBK-01)
Square in bold indicate nonsynonymous

Table 4. Percentages of nucleotide sequence differences in 609 bp fragment of the *COI* mitochondrial gene among four identified of kissing gourami in this study

	TBK-01	TBK-02	TBK-03	TBK-04
TBK-01	-			
TBK-02	0	-		
TBK-03	0.66	0.66	-	
TBK-04	1.16	1.16	1.16	-

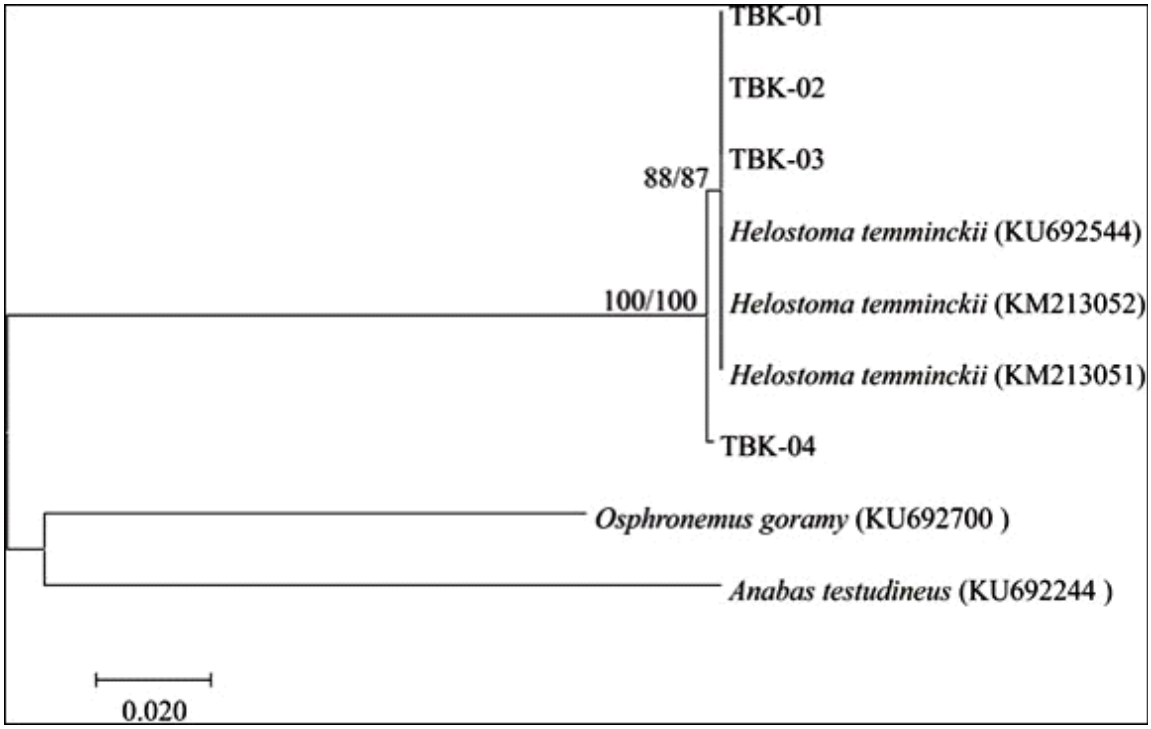

Figure 3. Neighbour joining (NJ) tree and maximum likelihood (ML) tree based on *COI* sequence data using Kimura 2 parameter (K2P) substitution model. Number at each node represent boostrap values (left node is for NJ and right node is for ML) and scale corresponds to substitution/site. Samples with accession number in brackets are taken from GenBank.

Discussion

DNA sequencing has become almost a routine for numerous kinds of biology research and has been used to measure and explore genetic diversity based directly or indirectly on DNA sequence difference among many groups of animals including freshwater fish. The findings of this study have been usefully applied to not only conservation fish stock management but also in supporting genetic improvement programmes.

Kissing gourami is a commercially important freshwater fish species due to its nutritional values. The population of wild kissing gourami will increase twice from the initial population within 15 months after the mating season that occurs between March-October (Sulaiman & Daud, 2002; Wibowo *et al*., 2015). However, overexploitation the wild kissing gourami can affect their loss in the habitat. This is due to demands of the freshwater fish including kissing gourami always increased every year to fulfil market needs. Data from Directorate General of Fisheries Management and Marketing (P2HP) revealed that 60% of fisheries production which have the highest domestic market is freshwater fish including kissing gourami. So, a better understanding of the fish population including their genetic information is important for their effective fisheries management.

The results showed that the two mitochondrial genes used in this study using BLAST program verified that the kissing gourami examined in this study is *Helostoma temminckii* with identity 98-100%. Despite of low nucleotide diversity, this study revealed intra-population variation based on the two mitochondrial genes examined, and was supported by nucleotide divergence and haplotype diversity (>80%). The fish genetic variation is also revealed by nucleotide substitution in *COI* partial sequences which affect amino acid translation. Three nonsynonymous and two synonymous were found in this study. In addition, the mitochondrial diversity found in this fish species indicated limited dispersal during life history stages of the fish species. This is due to the kissing gourami is considered as a non-migratory species. This life cycle is thought to be an important factor in accounting for high diversity of kissing gourami worldwide.

The phylogenetic analysis using NJ and ML method revealed that there are two lineages of the kissing gourami analysed in this study combined with the samples from GenBank database. Even though they are separated, they are still conspecific. This is due

to the nucleotide sequence divergence is still lower than 3.5%. According to Zemlak *et al*. (2009), the criterion of nucleotide sequence divergences used for discriminating species is higher than 3.5%.

These findings exhibited that the use of mitochondrial DNA genes (*16S rRNA* and *COI*) were effective not only to identify the kissing gourami samples examined in this study but also to characterize their genetic variation. The finding of mitochondrial diversity of the kissing gourami has important implication to establish regulation for sustainable management of the fish stock and their habitat. This is due to high level genetic variation of kissing gourami demonstrates potency of the fish as a brood stock which is important for aquaculture. Therefore, there is a clear need to preserve and take advantage of genetic variation of the kissing gourami to improve breeding programs.

CONCLUSION

This study reveals genetic information of kissing gourami collected from Ogan River, South Sumatra. The genetic data obtained from this study shows intra population variation based on two mitochondrial genes (*16S rRNA and COI*). From the results, it suggests that there are genetic variation of the kissing gourami in intra population level and further studies are needed to assess the extent of this variation using complete mitochondrial genome and population connectivity. The finding of mitochondrial diversity of the kissing gourami may be used as a reference to establish regulation for sustainable management of the fish stock and their habitat.

ACKNOWLEDGEMENT

We gratefully acknowledge the financial support by Faculty of Biology, Universitas Gadjah Mada (Yogyakarta Indonesia) through BPPTNBh-2018 funding with contract No. UGM/BI/1674/M/05/01.

REFERENCES

Arisuryanti, T. (2016). Molecular genetic and taxonomic studies of the swamp eel (*Monopterus albus* Zuiew 1793). *PhD Thesis*, Charles Darwin University, Australia.

Arisuryanti, T., Wei, N.-W.V. & Austin, C. (2016). Molecular evidence for determination cryptic species of Indonesian swamp eel populations using denaturing gradient gel electrophoresis (DGGE). *AIP Conference Proceedings,* 1744, 020060(1)-020060(6).

Arisuryanti, T., Hasan, R.L., & Koentjana, J.P. (2018). Genetic identification of two mudskipper species (Pisces: Gobiidae) from Bogowonto Lagoon (Yogyakarta, Indonesia) using *COI* mitochondrial gene as a DNA barcoding marker. *AIP Conference Proceedings*, 2002, 020068(1)-020068(7).

Belton, B., & Thilsted, S.H. (2014). Fisheries in transition: Food and nutrition security implications for the global South. *Global Food Security*, 3, 59-66.

Bogard, J.R. Thilsted, S.H., Geoffrey, C.M., Abdul Wahab, M., Hossain, M.A.R., Jacobsen, J., & Stangoulis, J. (2015). Nutrient composition of important fish species in Bangladesh and potential contribution to recommended nutrient intakes. *Journal of Food Composition and Analysis,* 42, 120-133.

Casey, J., Jardim, E., & Martinsohn, J.T. (2016). The role of genetics in fisheries management under the E.U. common fisheries policy. *Journal of Fish Biology*, 89, 2755-2767.

Dahruddin, H., Hutama, A., Busson, F., Sauri, S., Hanner, R., Keith, P., Hadiaty, R. & Hubert, N. (2017). Revisiting the ichthyodiversity of Java and Bali through DNA barcodes: taxonomic coverage, identification accuracy, cryptic diversity and identification of exotic species. *Molecular Ecology Resources*, 17(2), 288-299.

Ilmi, W., & Arisuryanti, T. (2018). Composition of mitochondrial DNA *16S* nucleotide of dwarf snakehead (*Channa gachua* Hamilton, 1822) from Keji River, Magelang, Central Java. *Journal of Tropical Biodiversity and Biotechnology*, 3(2), 57-61.

Ji, W., Zhang, G., Ran, W., Gardner, J.P.A., Wei, K., Wang, W., & Zou, G. (2014). Genetic diversity of and differentiation among five populations of blunt snout bream (*Megalobrama amblycephala*) revealed by SRAP markers: Implications for conservation and management. *PLoS One*, 9(9), e108967.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111-120.

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*, Supplement No. 27, 1-663.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: molecular evolutionary genetic analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33(7), 1870-1874.

Li, C.Y., Li, J.T., Xiao, G.Y., Hou, G.Y., & Sun, X.W. (2014). Complete mitochondrial genome of the kissing gourami, *Helostoma temminckii*. Mitochondrial DNA, 26(6), 1-2.

Librado, P., & Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25(11), 1451-1452.

Lind, C.E., Brummett, R.E., & Ponzoni, R.W. (2016). Exploitation and conservation of fish genetic resources in Africa: issues and priorities for aquaculture development and research. *Reviews in Aquaculture*, 4(3), 125-141.

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

Mahmood, H.M., & Ahmad, S.M. (2017). Advances in molecular markers and their applications in aquaculture and fisheries. *Genetics of Aquatic Organisms*, 1, 27-41.

Morin, P.A., Martien, K.K., Archer, F.I., Cipriano, F., Steel, D., Jackson, J., & Taylor, B.L. (2010). Applied conservation genetics and the need for quality control and reporting of genetic data used in fisheries and wildlife management. *Journal of Heredity*, 101(1), 1-10.

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

Peninal, S., Subramanian, J., Elavarasi, A., & Kalaiselvam, M. (2017). Genetic identification of marine eels through DNA barcoding from Parangipettai coastal waters. *Genomics Data*, 11, 81-84.

Sulaiman, Z.H., & Daud, H.K.H. (2002). Pond aquaculture of kissing gouramis *Helostoma temminckii* (Pisces: Helostomatidae) in Bukit Udal, Tutong: a preliminary investigation. *Bruneiana: Anthology of Science Articles*, 3, 34–41.

Syaifudin, M., Jubaedah, D., Muslim, M., & Daryani, A. (2017). DNA authentication of Asian Red Catfish *Hemibragus nemurus* from Musi and Penukal River, South Sumatra Indonesia. *Genetics of Aquatic Organisms*, 1, 43-48.

Tan, H. H., & Ng, P. K. L. (2005). The labyrinth fishes (Teleostei: Anabantoidei, Channoidei) of Sumatra, Indonesia. *Raffles Bulletin of Zoology Supplement,* 13, 115-138.

Vidthayanon, C. (2012). *Helostoma temminckii*. The IUCN Red List of Threatened Species 2012: e.T181326A1722150. http://dx.doi.org/10.2305/IUCN.UK.2012-1.RLTS.T181326A1722150.en. Downloaded on 08 October 2018.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., & Hebert, P.D. (2005). DNA barcoding Australia's fish species. *Philos Trans R Soc Lond B Biol Sci*, 360(1462), 1847-1857.

Wibowo, A., Sloterdijk, H., & Ulrich, P. (2015). Identifying Sumatran peat swamp fish larvae through DNA barcoding, evidence of complete life history pattern. *Procedia Chemistry*, 14, 76-84.

Zemlak, T.S., Ward, R.D., Connell, A.D., Holmes, B.H., & Hebert, P.D. (2009). DNA barcoding reveals overlooked marine fishes. *Molecular Ecolology Resources*, 9 Suppl s1, 237-242.