Zoodiversity, **56**(3): 195–202, 2022
DOI 10.15407/zoo2022.03.195

UDC 597.551.2:57.06(594.41)

TAXONOMIC DIVERSITY OF THE GENUS *TOR* (CYPRINIDAE) FROM ACEH WATERS IN INDONESIA BASED ON CYTOCHROME OXIDASE SUB-UNIT I (COI) GENE

Z. A. Muchlisin[1,2*], N. Fadli[1,2], A. S. Batubara[3], F. M. Nur[1], M. Irham[1,2], A. A. Muhammadar[1], D. Efizon[4], Roza Elvyra[5], M. N. Siti-Azizah[6]

[1]*Faculty of Marine and Fisheries, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia*
[2]*Marine and Fisheries Research Center, Universitas Syiah Kuala, Banda Sceh 23111, Indonesia*
Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Medan, Indonesia
[4]*Faculty of Fisheries and Marine Sciences, Universitas Riau, Pekanbaru, Indonesia*
[5]*Faculty of Mathematic and Natural sciences, Universitas Riau, Pekanbaru, Indonesia*
[6]*Institute Marine Biotechnology, Universiti Malaysia Terengganu, Terengganu, Malaysia*
[*]*Corresponding author*
E-mail: muchlisinza@unsyiah.ac.id

Z. A. Muchlisin (https://orcid.org/0000-0002-0858-1853)
N. Fadli (https://orcid.org/0000-0001-6845-1383)
A. S. Batubara (https://orcid.org/0000-0001-8788-9567)
F. M. Nur (https://orcid.org/0000-0001-9142-0630)
M. Irham (https://orcid.org/0000-0002-2639-6723)
A. A. Muhammadar (https://orcid.org/0000-0002-8086-0099)
D. Efizon (https://orcid.org/0000-0003-3667-7796)
Roza Elvyra (https://orcid.org/0000-0002-9250-3611)

Taxonomic Diversity of the Genus *Tor* (Cyprinidae) from Aceh Waters in Indonesia Based on Cytochrome Oxidase Sub-Unit I (COI) Gene. Muchlisin, Z. A., Fadli, N., Batubara, A. S., Nur, F., Irham, M. M., Muhammadar, A. A., Efizon, D., Roza Elvyra, Siti-Azizah, M. N. — The mahseer or *keureling*, members of the genus *Tor* J. E. Gray, 1833 are the commercial freshwater fish. It has potency to be cultivated commercially. Presently, no studies have been conducted on the molecular taxonomy of these fish. Hence, the objectives of the present study were to complement this morphological identification using the DNA barcoding gene, cytochrome oxidase subunit I (COI). Samples were obtained from seven areas of Aceh Province, namely; Aceh Besar, Aceh Barat, Nagan Raya, Aceh Selatan, Aceh Tenggara, Gayo Lues and Pidie Districts. A total of 140 fish samples have been collected during the sampling, of these 37 samples have been successfully sequenced. Based on the results of the sequencing data and blasting to NCBI data, only two species of *Tor* occur in Aceh waters: *T. tambra* (Valenciennes, 1842) and *T. tambroides* (Bleeker, 1854); while *T. soro* (Valenciennes, 1842) and *T. douronensis* (Valenciennes, 1842) were not validated.
Key words: threatened fish species, freshwater fish, genetic, mahseer

Introduction

At least 114 species freshwater fishes were reported in the biodiverse Indonesian region of the Aceh Province (Muchlisin & Siti-Azizah, 2009). Of them, 15 species are highly valued commercially. The members of the genus *Tor* J. E.Gray, 1833 collectively referred to as the keureling, among the local people (Muchlisin 2013), or often having a vernacular name mahseer, are included on this list. Based on morphological variations, three taxa of keureling, *Tor soro* (Valenciennes, 1842), *T. tambra* (Valenciennes, 1842), and *T. tambroides* (Bleeker, 1854) were reported to occur in the Aceh waters. These morphologically identified species were recorded in the Alas River region (Southeast Aceh), Batee Iliek River (Biureun), Montala River (Aceh Besar), Meurebo River and Woyla River (Aceh Barat) and Nagan River (Muchlisin & Siti-Azizah, 2009; Muchlisin 2010).

However, it was suspected that the number of *Tor* species in Aceh waters was not accurate, because the identification was only used the morphological characters as the presence and size of the median lobe of lower lip.

The keureling fish has great potential in the aquaculture industry due to the economic demand as compared to other freshwater fish species in Aceh, Indonesia. Therefore, it is the major target of anglers using various fishing methods, including destructive fishing practices. This has led to a drastic population declines of members of the genus *Tor* in its natural habitat, not only in Indonesia but almost throughout its range worldwide. Several species of *Tor* have been listed in the Endangered Category of the IUCN Red List fairly early on (Raghavan & Ali, 2011; Jha et al., 2018; Pinder et al., 2018). Kottelat et al. (1993) and Singh (2007) attributed the decline and threatened extinction of mahseer due to overfishing, pollution, and environmental damage. To address this situation, a comprehensive information is critical encompassing various aspects of the genus. Several aspects of the mahseer from Aceh waters have been studied, for example the bio-ecology (Muchlisin et al., 2015 a), parasites infestation (Muchlisin et al., 2014, 2015 c), and nutritions requirement (Muchlisin et al., 2016 a, b; Muchlisin et al., 2017 a, b) However, information on its genetic diversity is not available. Genetic profile information is crucial in relation to planning a better fisheries management strategy and aquaculture development, for example in crossbreeding program.

The cytochrome oxidase sub-unit I (COI) mitochondrial gene commonly applied for DNA barcoding was used for identification of *Tor* species in the Aceh waters. DNA barcoding is one of the most reliable ways today to validate the taxonomic status of living organisms including fish (Tautz et al., 2003; Muchlisin et al., 2013). This genetic approach have been recognized for their usefulness in species identification (Prioli et al., 2002; Muchlisin et al., 2012), monitoring fisheries resources (Menezes et al., 2006), aquaculture program (Barriga-Sosa et al., 2004), especially for selective breeding. There have been several studies on DNA barcoding of the Mahseer. These include investigations of two and five species of Indian mahseer using COI gene (Sati et al., 2013), and Laskar et al. (2018), respectively, and the disentangling the taxonomy of the mahseers from Malaysian waters has been reviewed by Walton et al. (2017). Understanding the taxonomic status as well as species and genetic diversity are critical to the conservation and protection of a species (or a group of), particularly of an endangered one. However, so far the taxonomy of the genus *Tor* is still contentious. Although record of species diversity in Aceh waters is available, but this has been based on morphological identification with its associated difficulties for this genus (Muchlisin & Siti-Azizah, 2009; Muchlisin et al., 2015 b) Presently, the genetic diversity of the mahseer from Aceh waters, Indonesia has not been reported. Hence, the objective of the present study was to elucidate the taxonomic diversity of the genus *Tor* and genetic diversity within each taxon in the Aceh waters, Indonesia with the DNA barcoding method.

Materials and methods

Sampling

A total of 140 fish samples preliminarily identified to belong to four taxa of *Tor* have collected during the sampling at seven regions within Aceh Province namely; Aceh Besar, Aceh Barat, Nagan Raya, Aceh Selatan, Aceh Tenggara, Gayo Lues and Pidie during July to August 2016 (table 1 and table 2). Of them, 37 samples were successfully sequenced. Specific sampling sites were determined based on information from local anglers; the specimens were caught using casting nets and gill nets. Some samples also were purchased from local markets. Samples were morphologically identified based on Kottelat et al. (1993), Walton et al. (2017) and Desrita et al. (2018). Species identification was conducted based on the presence and size of median lobe of lower lip.

Sample collection and preparation

A total of 5–15 specimens of each preliminarily identified species of *Tor* were analysed for COI gene. Approximately 1 cm^2 of caudal fin tissue was taken from each specimen, placed into 2.0 ml tubes containing TNES-urea buffer, labeled and transported to the laboratory. There, the tissues were further minced into small pieces in order to enhance lysis activity. The samples were preserved at least two weeks prior to DNA extraction.

DNA extraction

Genomic DNA was isolated using Aqua Genomic DNA solution (Promega) following the manufacturer's protocol. Successfully extracted DNA was determined by electrophoresis conducted

Table 1. Total sample and sample code of presumed species based on sampling locations. The presumed species was identified based on the presence and size of median lobe of lower lip

No	Location	Sampling site	Sample code	Presumed taxa				Total
				T. tambra	*T. tambroides*	*T. douronensis*	*T. soro*	
1	Gayo Lues District	Blang Kejeren River	BLA	–	–	1	4	5
2	Aceh Tenggara District	Alas River	SAL	2	4	–	1	7
3	Aceh Besar District	Leupung River	SPL	4	–	–	–	4
		Jreu River	KJ	–	4	–	–	4
4	Nagan Raya District	Nagan River	KN	–	4	–	1	5
5	Pidie District	Geumpang River	KGM	–	–	–	3	3
6	Aceh Barat District	Ulee Raket River	UR	3	–	–	–	3
7	Aceh Selatan District	Manggamat River	KM	6	–	–	–	6
Total sample				15	12	1	9	**37**

on a 0.8 % agarose gel at 100 V for 45 minutes, stained by ethidium bromide and visualized on a gel documentation system (GENE FLASH, Syngene Bio-Imaging). The DNA extracts were kept at –20 °C prior to PCR amplification.

PCR amplification

Partial sequences of the mitochondrial *COI gene were* amplified using the primer pair developed by Ward et al. (2005); FishF1)5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-3') and FishR1-5' (TAG ACT TCT GGG TGG CCA AAG AAT CA-3'). The 25 µL PCR reaction mix contained 17.65 µL of deionized water, 2.25 µL of 10X PCR buffer, 3.0 µL of $MgCl_2$ (25 mM), 0.25 µL of each primer (0.01 mM), 0.5 µL of mixed dNTP (0.05 mM), 0.1 µL of *Taq* polymerase, and 1.0 µL of DNA template. Amplifications were performed using a Mastercycler® Eppendorf gradient thermal cycler (Brinkmann Instruments, Inc). The thermal regime consisted of an initial step of 2 minutes at 95 °C followed by 35 cycles of 30 seconds at 94 °C, 30 seconds at 54 °C, and 1 minute at 72 °C, and finally 10 minutes of final extension at 72 °C (Ward et al., 2005). the PCR product were purified (PCR Clean-up System, Promega) and were then sent for sequencing to First BASE Laboratories Sdn Bhd (Selangor, Malaysia) using an ABI3730XL Genetic Analyzer (Applied Biosystems), and an ABI PRISM BigDye terminator cycle sequencing kit v3.1 (Applied Biosystems).

Data analysis

All obtained sequences were edited and aligned using MEGA 6.0 program (Tamura et al., 2013). Multiple sequence alignments were then performed on the edited sequences by Cluster *W* which is integrated into the MEGA 6.0 program. Nucleotide divergences among sequences were stimated based on Kimura 2-parameter (K2P) distances (Kimura, 1980) and neighbor joining (NJ) tree was conducted using K2P molecular evolutionary model in MEGA 6.0 program. Branch supports were estimated using 1000 bootstrap replications. *Neolissochilus hendersoni* (Accessing No. KT354858.1) sequence was used as outgroup.

Results

A final alignment of 655 bp of sequences among 37 individuals of *Tor* spp. from seven localities were obtained for the COI gene (table 1). The aligned sequences generated 9 sequences of *T. tambra* and 28 sequences of *T. tambroides* after BLAST analysis in the NCBI database with identical values ranging between of 98 % to 100 % (table 2). The higher number of samples were collected from Alas River (7 samples) consisting of *T. tambra* (4 samples) and *T. tambroides* (3 samples), while the least samples were recorded from Ulee Raket, Aceh Barat District where all sequences were *T. tambroides*.

A total of 5 haplotypes, consisting of 4 haplotypes of the *T. tambroides*, and one haplotype of the *T. tambra* were generated from 37 samples. Haplotype number two

Table 2. The *E-Value* dan *Identity* of *Tor* samples from seven locations

No.	Region	Sampling site	Code	Species	*E-Value*	*Identity*, %
1.	Gayo Lues	Blangkejeren River	SOR_BLA_01	*Tor tambra*	0.0	99
			SOR_BLA_07	*Tor tambra*	0.0	99
			SOR_BLA_08	*Tor tambra*	0.0	99
			SOR_BLA_06	*Tor tambra*	0.0	99
			DUR_BLA_02	*Tor tambra*	0.0	100
2.	Aceh Tenggara	Alas River	TAMB_SAL_01	*Tor tambra*	0.0	99
			TAMB_SAL_02	*Tor tambra*	0.0	99
			TAMB_SAL_03	*Tor tambra*	0.0	99
			TAMB_SAL_04	*Tor tambra*	0.0	99
			TAM_SAL_06	*Tor tambroides*	0.0	99
			TAM_SAL_09	*Tor tambroides*	0.0	99
			SOR_SAL_11	*Tor tambroides*	0.0	99
3.	Aceh Besar	Leupung River	TAM_SPL_03	*Tor tambroides*	0.0	98
			TAM_SPL_04	*Tor tambroides*	0.0	98
			TAM_SPL_05	*Tor tambroides*	0.0	98 %
			TAM_SPL_02	*Tor tambroides*	0.0	98
		Jreu River	KR_KJ_01	*Tor tambroides*	0.0	98
			KR_KJ_02	*Tor tambroides*	0.0	98
			KR_KJ_06	*Tor tambroides*	0.0	98
			KR_KJ_11	*Tor tambroides*	0.0	98
4.	Nagan Raya	Nagan River	TAMB_KN_01	*Tor tambroides*	0.0	98
			TAMB_KN_02	*Tor tambroides*	0.0	98
			TAMB_KN_04	*Tor tambroides*	0.0	98
			TAMB_KN_05	*Tor tambroides*	0.0	98
			SORO_KN_02	*Tor tambroides*	0.0	98
5.	Pidie	Geumpang River	SORO_KGM_06	*Tor tambroides*	0.0	98
			SORO_KGM_01	*Tor tambroides*	0.0	98
			SORO_KGM_07	*Tor tambroides*	0.0	98
6.	Aceh Barat	Ulee Raket River	TAM_UR_08	*Tor tambroides*	0.0	98
			TAM_UR_15	*Tor tambroides*	0.0	98
			TAM_UR_14	*Tor tambroides*	0.0	98
7.	Aceh Selatan	Manggamat River	TAM_KM_02	*Tor tambroides*	0.0	99
			TAM_KM_04	*Tor tambroides*	0.0	99
			TAM_KM_05	*Tor tambroides*	0.0	99
			TAM_KM_08	*Tor tambroides*	0.0	99
			TAM_KM_06	*Tor tambroides*	0.0	99
			TAM_KM_13	*Tor tambroides*	0.0	99

Note: SOR = presumed taxa of *Tor soro*, TAM = presumed taxa of *Tor tambra*, TAMB = presumed taxa of *Tor tambroides,* DUR = presumed taxa of *Tor douronensis*

belongs to *T. tambroides* and it was shared 15 samples from four different locations, namely; Nagan River, Leupung River, Geumpang River, and Jrue River. Haplotype number four is also belonging to *T. tambroides* and is common for 9 samples from two different locations of the Blangkejeren River, and Alas River. In addition, haplotype number five is belonging to *T. tambra*, this haplotype is shared by 9 samples from two different locations of the Blangkejeren River, and Alas River (table 3).Inter-specific variation calculated from 37 samples recovered two species (*T. tambra* and *T. tambroides*). The genetic distance between species was 3.1 % indicate these are the different species belonging in the same genus (table 4). The genetic distance value of the same species from different locations showed that the lowest genetic distance was found between *T. tambra* from Blangkejeren and *T. tambra* from Alas River (0.01 %), and the higher genetic distance occurred in the samples between *T. tambroides* from

the clade 1 (Geumpang River, Nagan River, Leupung River, Jreu River and Ulee Raket River) and *T. tambroides* from the clade 2 (Alas River and Manggamat River) had average genetic distance value of 2.4 %. The genetic three of 37 sequence samples were divided into three difference clades (fig. 1).

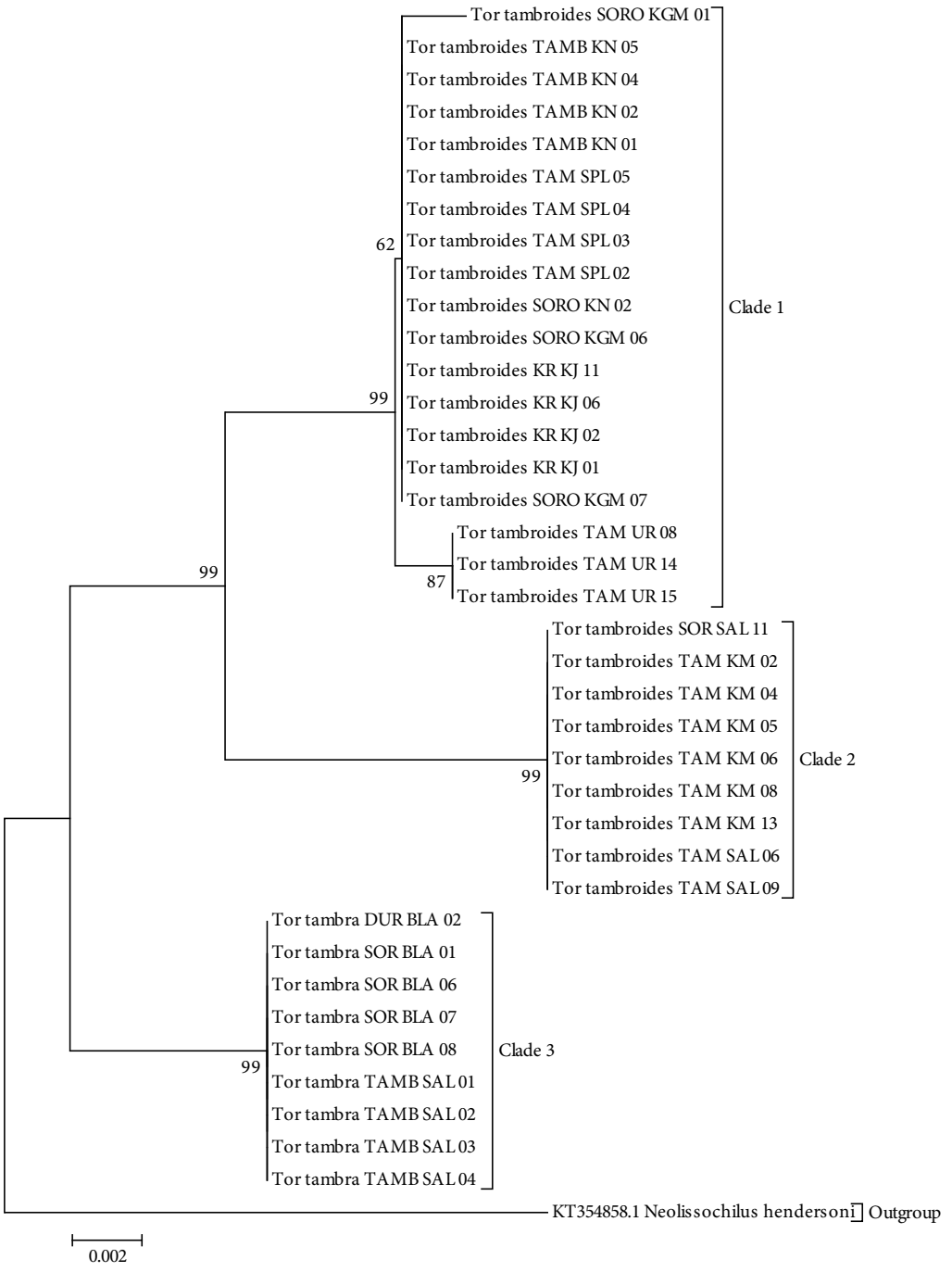

Fig. 1. The Neighbor Joining tree for 37 sequences of *Tor* from seven locations in Aceh Province estimated using 1000 bootstrap replications.

Table 3. Haplotype number and frequencies, specimen I.D, contributing morph and location

Haplotypes	Number of sequences	Specimen no. I.D	Contributing morph	Sampling Location
1	1	SORO KGM 01	*Tor tambroides*	Geumpang River
2	15	TAMB KN 01, TAMB KN 02, TAMB KN 04, TAMB KN 05, SORO KN 02, TAM SPL 02, TAM SPL 03, TAM SPL 04, TAM SPL 05, SORO KGM 06, SORO KGM 07, KR KJ 01, KR KJ 02, KR KJ 06, KR KJ 11	*Tor tambroides*	Nagan River, Leupung River, Geumpang River, Jrue River
3	3	TAM UR 08, TAM UR 14, TAM UR 15	*Tor tambroides*	Ulee Raket River
4	9	SOR SAL 11, TAM SAL 06, TAM SAL 09, TAM KM 02, TAM KM 04, TAM KM 05, TAM KM 06, TAM KM 08, TAM KM 13	*Tor tambroides*	Blangkejeren River, Alas River
5	9	DUR BLA 02, SOR BLA 01, SOR BLA 06, SOR BLA 07, SOR BLA 08, TAMB SAL 01, TAMB SAL 02, TAMB SAL 03, TAMB SAL 04	*Tor tambra*	Blangkejeren River, Alas River

Tabel 4. Inter and intra-specific variation among valid taxa/species of *Tor*

Species	COI				
	Inter-specific mean		Theta- prime mean	Intra-specific min.	Intra-specific max.
Tor tambra	n/a		0	0	0
Tor Tambroides	3.1		0.7	0	2.7
Neolissochilus hendersoni (outgroup)	3.8	4.5	0	0	0

Discussion

A total of 37 samples were succesflully amplified using 655 bp COI gene. Based on morphological characters, these samples were preliminarily identified as *T. soro* (9 samples), *T. douronensis* (1 sample), *T. brambroides* (12 samples), and *T. tambra* (15 samples). However, the BLAST result performed with MEGA 6.0 recovered only two species: *T. tambra* and *T. tambroides* with the E-Value 0.0. This value defines the absence of errors or no bias during blasting process and therefore the data in Genbank and this study was fit or suitable. In addition, the E-value of BLAST also shows the identity value in the two species ranged 98 % to 100 %, it means that these species have been well identified genetically. The presumed taxa *T. douronensis* from Blang Keujeren River is actually *T. tambra*, while the presumed *T. soro* from Blang Kejeren River is *T. tambra* and presumed taxa *T. soro* from Nagan River and Geumpang River are *T. tambroides.* Therefore, the presence and size of the median lobe of lower lip cannot be used as a sound character for identification of *Tor* in Aceh waters.

The genetic relationship was also analyzed in this study using the phylogenetic tree. The phylogenetic tree showed that the fish samples were divided into three different clades, where the clade 1 is monophyletic consisting *T. tambroides* from Leupung River (SPL_05, 04, 03, and 02), Ulee Raket (UR_08, 14 and 15), Nagan River (KN_05, 04, 02, 01 and 02), Geumpang River (KGM_01, 06 and 07) and Jrue River (KJ_11, 06, 02 and 01).

In the first clade seen that *T. tambroides* from five different locations formed the same clade. There is a close genetic relationship among *T. tambroides* samples, although the samples come from different locations, it can be seen from the constructed of the phylogenetic tree where the bootstrap (1000x bootstrapping) value was higher than 99 %. These values also strengthen the argument that the *T. tambroides* samples analyzed in this study are still from the same ancestor or monophyletic group, although the species *T. tambroides* from five locations grouping into synapomorphic form a clade based its closest genetic relationship. A total of 19 samples of tambroides clustered to form a clade in the clade first, where the higher samples incorporated in clade first comes from Nagan River with 5 individuals, of the all samples are formed synapomorphic identical.

As seen in the first clade, the *T. tambroides* in the second clade also comes from a different location but their genetic relationships are formed synapomorphic identical with the bootstrap value of 99%. In addition, the *T. tambroides* on the second clade has 9 individuals come from two locations; Manggamat River (KM_ 02, 04, 05, 06, 08 and 13) and the Alas River (SAL_ 11, 06 and 09). The third clade was formed by *T. tambra* from two locations i. e. Blangkejeren River (BLA_ 02, 01, 06, 07 and 08), and Alas River (SAL_ 01, 02, 03 and 04). The *T. tambra* in the third clade also comes from a different location but their genetic relationships are formed synapomorphic identical with the bootstrap value of 99 %.

In the phylogenetic tree also form an outgroup clade. The out-group was used to determine the accuracy of genetic distance obtained from the constructed phylogenetic tree. Outgroup species used in this analysis was *Neolissochilus hendersoni* retrieved from GenBank (Accessing No. KT354858.1). Relationship of life organisms is determined by the genetic distance. Two species of *Tor* found in this study had a genetic distance of 3.1 %. It means that they are two valid distinct species. According to Hebert et al. (2003) that the organisms can be regarded as the same species if it has a genetic distance of less than 3 %. Therefore, if the genetic distance is smaller or close to 0, it means an indication of the closer relationships, and vice versa.

Tor genetic distance of *T. tambra* from Blangkejeren and the Alas River has a value of 0.0 %. This value indicates that there are no significant differences (still from the same ancestor or monophyletic group) in genetic variability even though the location is different. This is because in Blangkejeren River is a tributary of the Alas Rivers so, that both rivers are connected. Beside, tambroides samples from these rivers produces one shared haplotype. While the genetic distance *T. tambroides* of clade 1 (Geumpang River, Nagan River, Leupung River, Jreu River and Ulee Raket River) and clade 2 (Alas River and Manggamat River) has a genetic distance between 2.4–2.7 % with average value of 2.4 %. These values also showed close genetic relationship or are still part of the same species that is *T. tambroides*. However, some of these rivers are not interconnected. The close genetic relationship between *T. tambroides* from some rivers is likely to happen in the past where these some rivers respectively interconnected (ancient rivers) (Tan et al., 2012).

Conclusions

Based on the COI gene showed only two valid species of *Tor* found in the waters of Aceh, namely *Tor tambra* and *T. tambroides*, while *T. soro* and *T. douronensis* were not detected in Aceh waters, Indonesia.

This study was supported by Kemenristek Dikti, the Republic of Indonesia through Unggulan Perguruan Tinggi (AUPT) scheme year 2016. Therefore, the author thanks the Kemenristek Dikti. The appreciation goes to Nurul Farhana Samsudin and Jamsari Amirul Firdaus Jamaluddin for their assistance with statistical analysis and critical review of the manuscript.

References

Barriga-Sosa, I. D. L. A., Jimenez-Badillo, M. D. L., Ibanez, A. L., Arredondo-Figuuero, J. L. 2004. Variability of tilapias (*Oreochromis* spp.) introduced in Mexico. Morphometric, meristic and genetic characters. *Journal of Applied Ichthyology,* **20**, 7–14.

Desrita, D., Muhtadi, A., Tamba, I. S., Ariyanti, J. 2018. Morfometrik dan meristik ikan Tor (*Tor* spp.) di DAS Wampu Kabupaten Langkat, Sumatera Utara, Indonesia. *Tropical Fisheries Management Journal*, **2** (2), 68–74.

Hebert, P. D. N., Ratnasingham, S., deWaard, J. R. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. Royal Soc. B,* **270** (Suppl. 1), 96–99.

Jha, B. R., Rayamajhi, A., Dahanukar, N., Harrison, A., Pinder, A. 2018. *Tor putitora*. The IUCN Red List of Threatened Species 2018: e.T126319882A126322226. https://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T126319882A126322226.en. Downloaded on 28 March 2020.

Kottelat, M., Whitten, A. J., Kartikasari, S. N., Wirjoatmodjo, S. 1993. *Freshwater Fishes of Western Indonesia and Sulawesi*. Periplus Editions Limited, Singapore.

Laskar, B.A., Kumar, V., Kundu, S., Tyaqi, K., Candra, K. 2018. Taxonomic quest: validating two mahseer fishes (Actinopterygii: Cyprinidae) through molecular and morphological data from biodiversity hotspots in India. *Hydrobiologia*, **815**, 113–124.

Menezes, M. R., Ikeda, M., Taniguchi, N. 2006. Genetic variation in skipjack tuna Katsuwanus pelamis (L) using PCR-RFLP analysis of the mitochondrial DNA D-loop region. *Journal of Fish Biology*, **68** (supplement A), 156–161.

Muchlisin, Z. A., Siti-Azizah, M. N. 2009. Diversity and distribution of freshwater fishes in Aceh waters, northern Sumatera, Indonesia. *International Journal of Zoological Research*, **5** (2), 62–79.

Muchlisin, Z. A. 2010. The diversity of freshwater fishes in Aceh with emphasis on several biological aspects of the depik (*Rasbora tawarensis*) an endemic species in Lake Laut Tawar. Ph.D. Thesis, Universiti Sains Malaysia, Penang, 1–275.

Muchlisin, Z. A., Fadli, N., Siti-Azizah, M. N. 2012. Genetic variation and taxonomy of Rasbora group (Cyprinidae) from Lake Laut Tawar, Indonesia. *Journal of Ichthyology*, **52** (4), 284–290.

Muchlisin, Z. A. 2013. Study on the potency of freshwater fishes in Aceh waters as a basis for aquaculture and conservation development programs. *Jurnal Iktiologi Indonesia*, **13** (1), 91–96.

Muchlisin, Z. A., Thomy, Z., Fadli, N., Sarong, M. A., Siti-Azizah, M. N. 2013. DNA barcoding of freshwater fishes from Lake Laut Tawar, Aceh Province, Indonesia. *Acta Ichthyologica et Piscatoria*, **43** (1), 21–29.

Muchlisin, Z. A., Munazir, M., Fuadi, Z., Winaruddin, W., Sugianto, S., Adlim, M., Fadli, N., Hendri, A. 2014. Prevalence of ectoparasites on mahseer fish (*Tor tambra* Valenciennes, 1842) from aquaculture ponds and wild population of Nagan Raya District, Indonesia. *Human and Veterinary Medicine*, **6** (3), 148–152.

Muchlisin, Z. A., Batubara, A. S., Siti-Azizah, M. N., Adlim, M., Hendri, A., Fadli, N., Muhammadar, A. A., Sugianto, S. 2015 a. Feeding habit and length-weight relationship of keureling fish, *Tor tambra* Valenciennes, 1842 (Cyprinidae) from the western region of Aceh Province, Indonesia. *Biodiversitas*, **16** (1), 89–94.

Muchlisin, Z. A., Fuadi, Z., Munazir, M., Fadli, N., Winaruddin, W., Defira, C. N., Hendri, A. 2015 b. First report on Asian fish tapeworm (*Bothriocephalus acheilognathi*) infection of indigenous mahseer (*Tor tambra*) from Nagan Raya district, Aceh province, Indonesia. *Bulgarian Journal of Veterinary Medicine*, **18** (4), 361–366.

Muchlisin, Z. A., Akyun, Q., Rizka, S., Fadli, N., Sugianto, S., Halim, A., Siti-Azizah, M. N. 2015 c. Ichthyofauna of Tripa Peat Swamp Forest, Aceh province, Indonesia. *Check List*, **11** (2), 1560.

Muchlisin, Z. A., Arisa, A. A., Muhammadar, A. A., Fadli, N., Arisa, I. I., Siti Azizah, M. N. 2016 a. Growth performance and feed utilization of keureling (*Tor tambra*) fingerlings fed a formulated diet with different doses of vitamin E (alpha-tocopherol). *Archives of Polish Fisheries*, **24**, 47–52.

Muchlisin, Z. A., Afrido, F., Murda, T., Fadli, N., Muhammadar, A. A., Jalil, Z., Yulvizar, C. 2016 b. The effectiveness of experimental diet with varying levels of papain on the growth performance, survival rate and feed utilization of *keureling* fish (*Tor tambra*). *Biosaintifika*, **8** (2), 172–177.

Muchlisin, Z. A., Nazir, M., Fadli, N., Hendri, A., Khalil, M., Siti-Azizah, M. N. 2017 a. Efficacy of commercial diets with varying levels of protein on growth performance, protein and lipid contents in carcass of Acehnese mahseer, *Tor tambra*. *Iranian Journal of Fisheries Sciences*, **16** (2), 557–566.

Muchlisin, Z. A., Murda, T., Yulvizar, C., Dewiyanti, I., Fadli, N., Afrido, F., Siti-Azizah, M. N., Muhammadar, A. A. 2017 b. Growth performance and feed utilization of keureling fish *Tor tambra* (Cyprinidae) fed formulated diet supplemented with enhanced probiotic. *F1000Research*, **6**, 137.

Pinder, A., Katwate, U., Dahanukar, N., Harrison, A. 2018. *Tor remadevii*. The IUCN Red List of Threatened Species 2018: e.T56096394A56717605. https://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T56096394A56717605.en. Downloaded on 28 March 2020.

Prioli, S. M. A. P., Prioli, A. J., Julio, Jr. H. F., Avanelli, C. S., de Oliveira, A. V., Carrer, H., Carraro, D. M., Prioli, L. M. 2002. Identification of *Astyanax altiparanae* (Teleostei, Characidae) in the Iguacu River, Brazil, based on mitochondrial DNA and RAPD markers. *Genetics and Molecular Biology*, **25** (4), 421–430.

Raghavan, R., Ali, A. 2011. *Tor malabaricus*. The IUCN Red List of Threatened Species 2011: e.T172457A6895822. https://dx.doi.org/10.2305/IUCN.UK.2011-1.RLTS.T172457A6895822.en. Downloaded on 28 March 2020.

Sati, J., Sah, S., Pandey, H., Ali, S., Sahoo, P.K., Pande, V., Baratz, A. 2013. Phylogenetic relationship and molecular identification of five Indian Mahseer species using COI sequence. *Journal of Environmental Biology*, **34** (5), 933–939.

Singh, A. K. 2007. Biological and reproductive diversity in reverie as well as lacustrine golden mahser, *Tor putitora* (Hamilton 1822) in central Malayas, India. *In*: Siraj, S. S., Christianus, A., Kiat, N. C., De Silva, S. S., eds. *Mahser, the biology, culture and conservation*. Proceeding of the International Symposium on the Mahseer. 29–30 March, 2006. Kuala Lumpur, Malaysia. Malaysian Fisheries Society. 79–97.

Tan, M. P., Jamsari, A. F., Siti-Azizah, M. N. 2012. Phylogeographic pattern of the striped snakehead, *Channa striata* in Sundaland: ancient river connectivity, geographical and anthropogenic signatures [corrected]. *Plos One*, **7** (12), e52089.

Tautz, D., Arctander, P., Minelli, A., Thomas, R. H., Vogler, A. P. 2003. A plea for DNA taxonomy. *Trends in Ecology & Evolution*, **18**, 70–74.

Walton, S. E., Gan, H. M., Raghavan, R., Pinder, A. C., Ahmad, A. 2017. Disentangling the taxonomy of the mahseers (*Tor* spp.) of Malaysia: an integrated approach using morphology, genetics and historical records. *Reviews in Fisheries Science & Aquaculture*, **25** (3), 1–13.

Ward, R. D., Zemlak, T. S., Ines, B. H., Last, P. R., Hebert, P. D. N. 2005. DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **360**, 1847–1857.

Received 2 August 2021
Accepted 5 June 2022