BIHAREAN BIOLOGIST 15 (1): 39-47
Article No.: e201306

http://biozoojournals.ro/bihbiol/index.html

DNA barcoding (COI genetic marker) revealed hidden diversity of Cyprinid fish (*Barbonymus* spp.) from Aceh Waters, Indonesia

Agung S. BATUBARA[1], Zainal A. MUCHLISIN[1,2,*], Deni EFIZON[3], Roza ELVYRA[4], Nur FADLI[2], Syamsul RIZAL[1], Mohd N. SITI-AZIZAH[5] and Martin WILKES[6]

1. Doctoral Program in Mathematics and Sciences Application (DMAS), Graduate Program, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia.
2. Faculty of Marine and Fisheries, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia.
3. Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru, Indonesia.
4. Faculty of Sciences, University of Riau, Pekanbaru, Indonesia.
5. Institute of Marine Biotechnology, Universiti Malaysia Terengganu, Malaysia.
6. Centre for Agroecology, Water and Resilience, Coventry University, United Kingdom.
*Corresponding author, Z.A. Muchlisin, Email: muchlisinza@unsyiah.ac.id

Received: 29. April 2020 / Accepted: 13. August 2020 / Available online: 18. August 2020 / Printed: June 2021

Abstract. The objective of the present study was to barcode genus *Barbonymus* fish from the rivers of Aceh, Indonesia and establish their phylogenetic relationship. Three presumed taxa of the genus *Barbonymus,* referred here as Naleh, Lampam-a, and Lampam-b were investigated. The Naleh samples were collected from Nagan River in western Aceh. This presumed taxon is morphologically similar to the Javanese barb, *Barbonymus gonionotus*. The Lampam-a and Lampam-b, which were collected from Tamiang River in eastern Aceh, morphologically resembled the tinfoil barb, *B. schwanenfeldii*. Five random fin tissue samples from each taxon were taken for genetic analysis based on the Cytochrome oxidase subunit I (COI) gene. The study revealed that Lampam-a and Lampam-b are more closely related than to Naleh. Lampam-a and Lampam-b showed close identity with GenBank sequences of *B. schwanenfeldii* (99 – 100% identity) but Naleh did not closely match with any GenBank sequences. The inter-species divergence between (Lampam-a and Lampam-b) and Naleh was 5.0%, while the intra-specific genetic divergence within (Lampam-a and Lampam-b) and Naleh was 0.5% and 0.0%, respectively. Thus, the genetic data indicated at least two valid species of *Barbonymus* in Aceh waters i.e. *B. schwanenfeldii* and a taxonomically unknown species. We believe that the Naleh sample may be a cryptic species, morphologically similar to *B. gonionotus*.

Key words: *Barbonymus, B. schwanenfeldii*, COI gene, Genetic, Naleh, Lampam; *B. gonionotus.*

Introduction

The waters of the Aceh Province (Indonesia), lying within one of the global biodiversity hotspots, support a high diversity of fish species. Previous studies have reported 114 species of freshwater and brackish fishes from this region (Muchlisin & Siti-Azizah 2009, Muchlisin et al. 2015). Of these, 14 species have high economic value in food fish aquaculture, while 21 species have potential as ornamental fishes (Muchlisin 2013). Similar to most regions in Asia, Cyprinidae is the predominant family of freshwater fishes in the Aceh Province (Muchlisin et al. 2015, Defira & Muchlisin 2004, Dekar et al. 2018, Irhami et al. 2018, Timorya et al. 2018, Nasir et al. 2018). One of the most valuable genus within this family is *Barbonymus* (Kottelat et al. 1993).

Barbonymus comprises of ten species namely; *B. belinka*, *B. altus, B. balleroides, B. collingwoodii, B. gonionotus, B. mahakkamensis, B. platysoma, B. trigatus*, *B. sunieri*, and *B. schwanenfeldii* (Yang et al. 2012, Kottelat 2013, Zheng et al. 2016, Froese & Pauly 2018). The genus *Barbonymus* is commonly found in inland waters of Indonesia, Malaysia, Thailand, Kamboja, Vietnam, Myanmar, Laos and Philippine (Kottelat 2001, Cheng et al. 2004, Garcia 2010). Most members of the genus have colorful fins and body (Leunda 2010, Gante et al. 2008) and therefore high potential in the ornamental fish industry. Their fast growth rates and delicate taste make them highly favored by consumers (Mollah et al. 2011, Mondol et al. 2005, Hossain et al. 2016). Therefore, studies on the *Barbonymus* are very important to strategize management plans to ensure their continued conservation and sustainability. Several investigations on the group have been reported. Mondol et al. (2005), Sangpradub et al. (2015) and Gunawan et al. (2017) documented the feeding habit of the tinfoil barb, *B. schwanenfeldii* in Tamiang River, Aceh Tamiang waters and noted that the species has an omnivorous feeding habit. Kamarudin and Esa (2009) studied the phylogenetics and phylogeography of *B. schwanenfeldii* in Malaysian waters, while Akter et al. (2010) studied the genetic structure of the Javanese barb, *B. gonionotus* in Bangladeshi waters. In addition Pannusa et al. (2015) conducted a study on *B. gonionotus* in Thailand. However, no study has been conducted on the *Barbonymus* populations from the biodiverse Aceh waters, especially on genetic variation.

In Aceh, based on morphological appearance, there are at least three presumed taxa of *Barbonymus*, referred here as as Naleh, Lampam-a and Lampam-b. The Naleh is distributed along the western region of Aceh, for example in Nagan River (Batubara et al. 2019a, Batubara et al. 2019b), while the Lampams are distributed in several main rivers of the eastern and central regions of Aceh, for instance in Tamiang and Peusangan Rivers. According to Batubara et al. (2018), Lampam-a and Lampam-b have high morphological similarity but Lampam-b can be distinguished from Lampam-a by the differences in snout and body depth, where the Lampam-a has a blunt snout, while the Lampam-b has a higher body depth and deeper nape (Figure 1). Based on morphological characteristics, Batubara et al. (2018) concluded that the Lampam-a and Lampam-b are synonymous to *B. schwanenfeldii*. However, there appear to be some slight variations among samples and therefore a more objective approach such as the utilization of genetic data is crucial to validate this previous findings. Batubara et al. (2018) also suggested that Naleh is synonymous with *B. gonionotus* based on similar morphological characteristics. According to Kottelat et al.

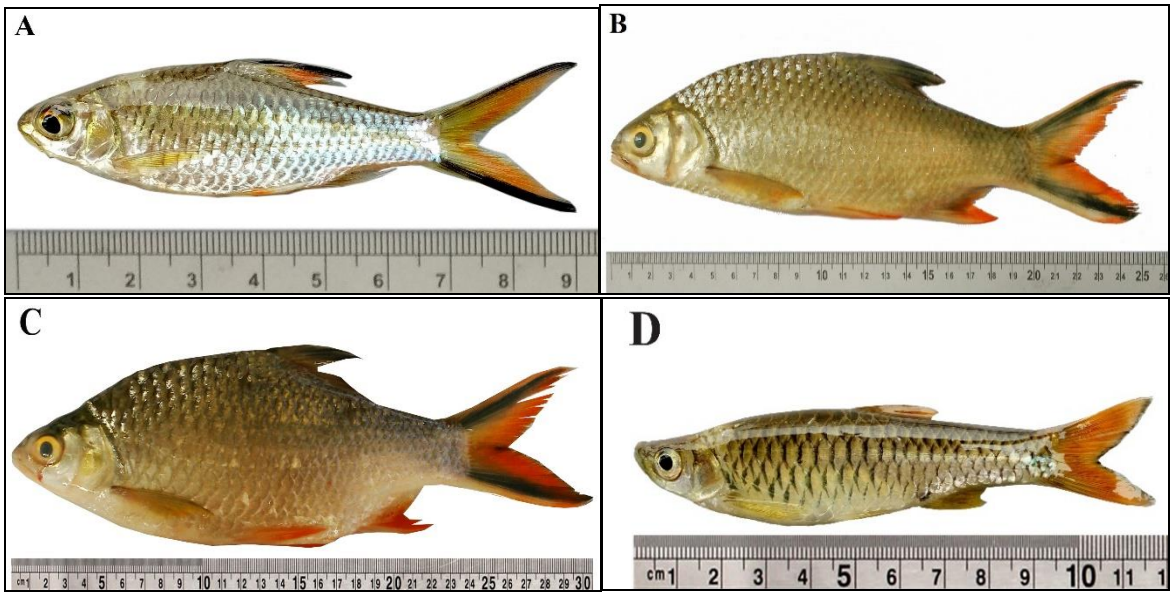

Figure 1. The presumed taxa of *Borbonymus*, (A) Naleh, (B) Lampam-a, (C) Lampam-b, (D) kedawah, *Rasbora sumatrana*.

(1993), the morphometric and meristic characters of *B. schwanenfeldii* have 13 predorsal scales; 8 scales between dorsal and lateral line; the body is silver or golden yellow; dorsal is red with a black blotch at tip; pectorals, pelvics, and anal fin are red; caudal is orange or red with white margin and black stripe along each caudal lobe, while *B. gonionotus* has 6 branched rays in anal fin and 3 scales between lateral line and pelvic fin.

It is well accepted that genetic data is an efficient technique for complementing morphological analysis in species identification (Prioli et al. 2002, Cheng et al. 2014, Dawnay et al. 2007) including of fish. Precise taxonomic identification is vital for the management of aquaculture as well as wild populations (Basheer et al. 2015). In the last decade, the molecular technique of DNA barcoding has become the golden standard for molecular identification of species (Hebert et al. 2003a, Hebert et al. 2003b). The technique is based on the premise that genetic variation of the DNA barcoding marker (Cytochrome oxidase subunit I in animals) within species is lower than between species. This approach has proven its universal efficacy in species identification including in freshwater fishes (Ward et al. 2005, Tan et al. 2012, Muchlisin et al. 2012, Muchlisin et al. 2013, Muchlisin et al. 2017, Farhana et al. 2018). Thus, to confirm the findings of Batubara et al. (2018), the objective of the study was to validate the taxonomic status of the *Barbonymus* fishes from Aceh waters using the DNA barcoding, COI gene.

Materials and methods

Sampling sites

The study was conducted from July to September 2016. Naleh samples were collected from Nagan River in western Aceh (4°16'25.25"N and 96°24'22.34"E; 4°17'4.73"N and 96°25'56.83"E; 4°16'48.49"N and 96°27'8.50"E), while Lampam samples were collected from Tamiang River in eastern Aceh (4°16'43.75"N and 98° 0'10.20"E; 4°17'48.32"N and 97°59'36.19"E; 4°16'41.68"N and 97°58'57.15"E) (Figure 2). Selection of sampling sites was guided by information from local fishermen. The sampling was performed from 8.00 AM to 6.00 PM through a period of 12 weeks. The fish were caught using gillnets with two mesh sizes (1.5 and 2.0 inches). Each sample was cleaned, then photographed for documentation. The samples were individually labeled, then preserved in crushed ice (4°C) during transportation to the laboratory for further analysis. The sampled fish were weighted and measured morphometric (mm) analysis using a digital balance (Toledo, AB-204. Error= 0.01 g) and digital calipers (Mitutoyo, CD-6CS. Error = 0.01 mm). The taxonomic identification was conducted based on Kottelat et al. (1993) and Batubara et al. (2018). The meristic and morphometric characters were referred to Keat-Chuan et al. (2017). The samples were kept in 75% alcohol and stored at the Laboratory of Ichthyology, Faculty of Marine and Fisheries, Universitas Syiah Kuala, Indonesia.

Tissue sample collection

Five samples of every presumed taxon were, respectively selected from the collection. Approximately 1 cm² of pectoral fin tissue was taken from each specimen. The tissue clipping from each individual was placed into 2.0 mL tubes containing 96% alcohol, while the remaining whole samples were preserved in 10% formalin and deposited at the Laboratory of Ichthyology, Faculty of Marine and Fisheries, Syiah Kuala University as reference samples (Museum Code: BAR-KNG and BAR-KU).

DNA extraction

Genomic DNA was isolated using Aqua Genomic (AG) DNA solution following the manufacturer's protocol and Muchlisin et al. (2013). The electrophoresis was conducted on a 0.8 % agarose gel at 100 volts for 45 minutes to assess the success of DNA extraction. After completion, the agarose gel was stained with ethidium bromide prior to visualization for the presence of the extracted DNA, indicated by the presence of a band in a gel documentation system (GENE FLASH, Syngene Bio-Imaging).

PCR amplification and purification

A 655-bp segment was amplified from the 5′ region of the mitochondrial cytochrome oxidase subunit I (COI) gene using the primer pairs (Ward et al. 2005):

FishF1 5′TCAACCAACCACAAAGACATTGGCAC3′
FishR15′TAGACTTCTGGGTGGCCAAAGAATCA3′

The 25 µL PCR reaction mix contained 16.25 µL of deionized water, 2.5 µL of 10X PCR buffer, 2.0 µL of $MgCl_2$ (25 mM), 0.5 µL of each primer (0.01 mM), 1.0 µL of mixed DNTP (0.05 mM), 0.25 µL of *Taq* polymerase, and 2.0 µL of DNA template. Amplifications were performed using a Mastercycler® Eppendorf gradient thermal cycler

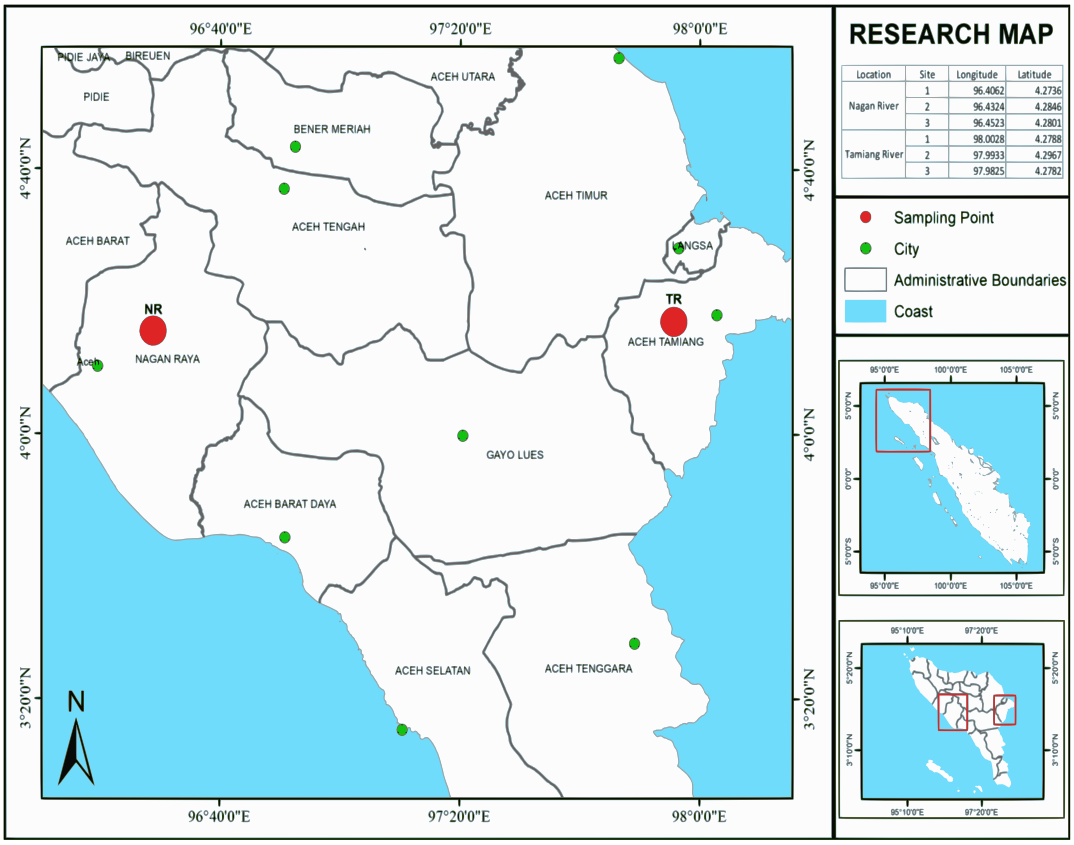

Figure 2. The Aceh Province showing location sampling (red dots) in Tamiang River (TR) in eastern part of Aceh and in Nagan River (NR) in western part of Aceh.

(Brinkmann Instruments, Inc). The thermal regime consisted of an initial step of 2 minutes at 94°C followed by 30 cycles of 45 seconds at 94°C, 45 seconds at 54°C, and 1 min at 72°C, followed by 8 min at 72°C and then held at 4°C (Ward et al. 2005). After amplification, the PCR products were run on a 0.8% agarose gel electrophoresis for 45 minutes and then visualized using GENEFLASH® Syngene Bio-Imaging.

Of the 15 samples, 14 were successfully amplified as indicated by the formation of clear bands, and one sample was failed amplified (BARB KU 02). The successfully amplified samples comprised of 5 sample of Naleh, 5 samples of Lampam-a, and 4 samples of Lampam-b (Figure 3). The most clarified products were selected for purification.

The 14 PCR products were purified using purification kits (PCR Clean-up System, Promega) by following the manufacturer's protocol standard. Then the purification products were run on 0.8% agarose gel electrophoresis, and clear band samples were sent for sequencing to a service provider (First Base Laboratories Sdn. Bhd, Malaysia).

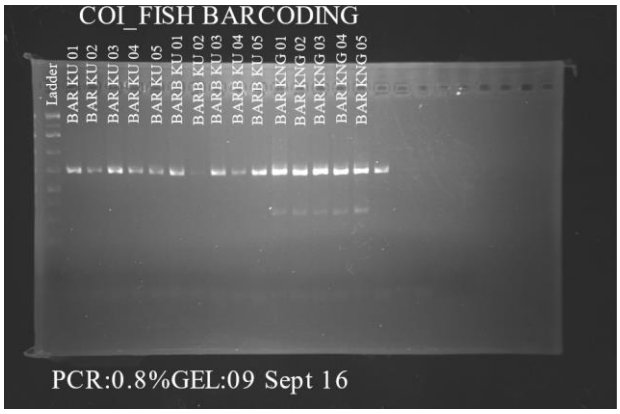

Figure 3. Cytochrome Oxidase-1 PCR run on 0.8 agarose gel with band appearing at approximately 582bp, where Naleh (BAR KNG), Lampam-a (BAR KU) and Lampam-b (BARB KU)

Cytochrome oxidase subunit I DNA data analysis

The 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* that is incorporated in the MEGA 6.0 program. Nucleotide divergences among sequences were estimated based on genetic distance using the Neighbour-Joining (NJ) method based on the Kimura 2 parameter. The genetic relationships among haplotypes were assessed by constructing phylogenetic trees through NJ method, while the confidence limits were assessed using the bootstrap procedure with 1000 pseudoreplicates for NJ (Felsenstein 1985). The haplotypes were produced using DnaSP Version 5.10.02 software (Rozas et al. 2003). The haplotype sequences from this study have been deposited in the GenBank with accession number: MK978148 - MK978151. The GenBank sequences of *Barbonymus* were included in the analysis (Table 1), and a single sequence of *Rasbora sumatrana* from Malaysia was also included in the analysis as an outgroup.

Results

Morphological descriptions

Based on the meristic characteristics, Naleh has 6.5 scales above the lateral line, 12-13 predorsal scales. The dorsal fin has 1 unbranched spinous ray and 9 branched soft rays (D.I.9), the anal fin had 1 unbranched spinous ray, and 6-7 branched soft rays (A.1.6-7), and the ventral fin had 1 un

branched spinous ray with 7-8 branched soft rays (V.I.7-8). The outer edge of the caudal fin and dorsal unbranched spinous ray are black while the inner edge of the caudal fin is yellow (Figure 1A). Body depth 0.3 times total length, caudal peduncle depth 0.1 times total length, caudal peduncle length 0.5 times total length, and the snout length 0.1 times total length.

The Lampam-a has 8 scales above the lateral line, 13-15 predorsal scales; orange-reddish dorsal fin with black spots on the tip; pectoral fins, abdominal fins, and anal fins are reddish. The dorsal fin has 3 unbranched spinous ray, and 9 branched soft rays (D.III.9), the anal fin has 3 unbranched spinous ray and 6 branched soft rays (A.III.6), and the ventral fin has 1 unbranched spinous ray and 8 branched soft rays (V.I.8) (Figure 1B). Body depth 0.3 times total length, caudal peduncle depth 0.1 times total length, caudal peduncle length 0.1 times total length, and the snout length 0.1 times total length.

On the other hand, Lampam-b has red pectoral, abdominal, and anal fins are red; orange, or red caudal fin with a black and white stripe along the lobe of the caudal fin. This fish has 9-10 scales above the lateral line; 15-16 predorsal scales. The dorsal fin has 3 unbranched spinous ray, and 9 branched soft rays (D.III.9), the anal fin has 3 unbranched spinous rays and 5-6 branched soft rays (A.III.5-6), the ventral fin has 1 unbranched spinous ray, and 8 branched soft rays (V.I.8). Body depth 0.4 times total length, caudal peduncle depth 0.1 times total length, caudal peduncle length 0.1 times total length, and the snout length 0.1 times total length. In general, the body and fin colors of Lampam-b are highly similar to Lampam-a (Figure 1C).

Molecular analysis

BLAST results with the GenBank database revealed 99-100 %

Table 1. The sequences of *Barbonymus* retrieved from the GenBank used for phylogenetic analyses.

Valid species	GenBank Accession number	Country of origin
Barbonymus altus	KU568766	South Africa
	JQ667501	India
	KT001010	Malaysia
	MK448137	Thailand
	KF410688	Thailand
	JQ346154	Laos
	NC031521	Japan
	AP011181	Japan
	MH119965	USA
Barbonymus schwanenfeldii	KT001006	Malaysia
	MK049360	Thailand
	MK448176	Thailand
	HM536894	Indonesia
	JQ346171	Laos
	Ap011317	Japan
Barbonymus gonionotus	NC008655	Japan
	KC242333	Thailand
	JN896651	Thailand
	JQ661377	Thailand
	KT001015	Malaysia
	INLE015-16	Myanmar
	LC189763	Myanmar
	JN896649	Thailand
	JQ346157	Laos
	JQ713846	India
	EU924631	India
	KX657718	Bangladesh
	KJ936769	India
Barbonymus balleroides	KU692330	Indonesia
	KU692324	Indonesia
	KU692323	Indonesia

Table 2. The E-value and identity values of 14 *Barbonymus* samples from Nagan Raya and Aceh Tamiang.

Presumed taxa (Origin)	Sample code	Closest Match (NCBI blast)	E-value	Identity
Naleh (From Nagan Raya)	BAR KNG 01	*B. gonionotus*	0.0	94%
	BAR KNG 02	*B. gonionotus*	0.0	93%
	BAR KNG 03	*B. gonionotus*	0.0	93%
	BAR KNG 04	*B. gonionotus*	0.0	94%
	BAR KNG 05	*B. gonionotus*	0.0	93%
Lampam-a (From Tamiang)	BAR KU 01	*B. schwanenfeldii*	0.0	100%
	BAR KU 02	*B. schwanenfeldii*	0.0	100%
	BAR KU 03	*B. schwanenfeldii*	0.0	100%
	BAR KU 04	*B. schwanenfeldii*	0.0	100%
	BAR KU 05	*B. schwanenfeldii*	0.0	99%
Lampam-b (From Tamiang)	BARB KU 01	*B. schwanenfeldii*	0.0	100%
	BARB KU 03	*B. schwanenfeldii*	0.0	100%
	BARB KU 04	*B. schwanenfeldii*	0.0	99%
	BARB KU 05	*B. schwanenfeldii*	0.0	100%

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

Haplotypes	Number of sequences	Specimen no. I.D	Contributing morph	Accession No.
1	7	BARB KU 01, BARB KU 03, BARB KU 05, BAR KU 01, BAR KU 02, BAR KU 03, BAR KU 04	Lampam-a and Lampam-b	MK978148
2	1	BARB KU 04	Lampam-b	MK978149
3	1	BAR KU 05	Lampam-a	MK978150
4	5	BAR KNG 01, BAR KNG 02, BAR KNG 03, BAR KNG 04, BAR KNG 05	Naleh	MK978151

species. However, Naleh was not closely matched with any of the GenBank *Barbonymous* sequences, the closest being with *B. gonionotus* at 93-94% similarity (Table 2). Three haplotypes of Lampam-a and Lampam-b (*B. schwanenfeldii*) and a single haplotype of Naleh (*B. gonionotus*) were generated and presented in the Table 3.

The alignment of Naleh nucleotide sequence (query sequence) with GenBank subject sequence of *B. gonionotus* gives a total identity score of 93% as shown below:

Score	Expect	Identities	Gaps	Strand
880 bits (975)	0.0	546/585 (93%)	0/585 (0%)	Plus/Plus

```
Query  1    GTGGGAACCGCCTTAAGCCTTCTTATTCGAGCTGAACTCAGTCAACCCGGATCACTTCTA  60
Sbjct  35   .....................................T..........G.........  94

Query  61   GGCGATGATCAAATTTATAATGTAATCGTTACCGCCCACGCTTTTGTAATAATTTTCTTT  120
Sbjct  95   .....C.............C.....................C...................  154

Query  121  ATAGTAATACCCATTCTCATTGGAGGATTCGGAAACTGGCTTGTACCCTTAATAATTGGA  180
Sbjct  155  ........G..A..........................A.........C.......C...  214

Query  181  GCCCCAGACATAGCATTCCCACGAATAAACAATATAAGCTTCTGATTACTACCCCCATCC  240
Sbjct  215  ........T..G.....................C..................G........A  274

Query  241  TTCCTACTACTACTAGCTTCTTCTGGTGTTGAAGCCGGTGCCGGGACAGGGTGAACAGTA  300
Sbjct  275  ...........G...........C.....................................  334

Query  301  TATCCACCTCTTGCAGGAAATCTAGCCCACGCAGGAGCATCAGTAGACCTAACGATTTTT  360
Sbjct  335  ........C...........C..G.............................A.....C  394

Query  361  TCACTTCATTTAGCGGGAGTATCATCAATTCTGGGGGCAATTAATTTTATTACCACAACC  420
Sbjct  395  .....C........A..T..G...........A..................C.........  454

Query  421  ATTAATATGAAACCCCCAGCCATCTCCCAATACCAAACACCATTATTTGTTTGATCCGTG  480
Sbjct  455  .....C...........................T...........................A  514

Query  481  CTTGTAACCGCCGTGCTACTACTCCTGTCACTACCTGTCCTAGCCGCTGGAATCACAATG  540
Sbjct  515  ..............A.....C.....................G.....C.....T......  574

Query  541  CTCCTAACAGATCGAAATCTTAACACCACATTCTTTGACCCGGCA  585
Sbjct  575  ..................C..C..................T......  619
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The alignment of query sequence of Lampam-a and Lampam-b with GenBank subject squence of *B. schwanenfeldi* gives a total identity score of 99% as given below:

Score	Expect	Identities	Gaps	Strand
1051 bits(1165)	0.0	584/585(99%)	0/585(0%)	Plus/Plus

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Query  1    GTGGGAACCGCCTTAAGCCTTCTTATTCGAGCTGAACTTAGTCAACCCGGGTCACTTCTA  60
Sbjct  1    ............................................................  60

Query  61   GGCGACGACCAAATTTATAACGTAATCGTTACCGCCCACGCCTTCGTAATAATTTTCTTT  120
Sbjct  61   ............................................................  120

Query  121  ATAGTAATGCCAATTCTCATTGGAGGATTCGGAAACTGACTTGTACCCCTAATAATCGGA  180
Sbjct  121  ............................................................  180

Query  181  GCCCCAGATATGGCATTCCCACGAATAAACAACATAAGCTTCTGATTACTGCCCCCATCA  240
Sbjct  181  ............................................................  240

Query  241  TTCCTACTACTGCTAGCTTCTTCCGGTGTTGAAGCCGGTGCCGGGACAGGGTGAACAGTA  300
Sbjct  241  ............................................................  300

Query  301  TATCCACCCCTTGCAGGAAACCTGGCCCACGCAGGGGCATCAGTAGACCTAACAATTTTC  360
Sbjct  301  ............................................................  360

Query  361  TCACTCCATTTAGCAGGTGTGTCATCAATTCTAGGGGCAATTAATTTTATCACCACAACC  420
Sbjct  361  ............................................................  420

Query  421  ATTAACATGAAACCCCCAGCCATCTCCCAATATCAAACACCATTATTTGTTTGATCCGTA  480
Sbjct  421  ............................................................  480

Query  481  CTTGTAACCGCCGTACTACTCCTCCTGTCACTACCTGTCCTGGCCGCCGGAATTACAATG  540
Sbjct  481  ............................................................  540

Query  541  CTCCTAACAGATCGAAACCTCAACACCACATTCTTTGATCCGGCA  585
Sbjct  541  .....................................A...  585
```

Table 4. Inter-specific variation among valid taxa/species of *Barbonymus*.

Species	COI						
	Inter-Specific mean				Theta- prime mean	Intra-specific min.	Intra-specific max.
Lampam ("B. schwanenfeldii")	n/a				0.2	0	0.5
Naleh	5.0				0	0	0
B. gonionotus	8.4	6.9			0	0	0
B. altus	6.8	5.8	5.1		0	0	0
B. balleroides	6.5	0.6	8.7	6.8	0	0	0

Although the sample size did not allow a comprehensive population genetics analysis to be performed, preliminary intra-specific variation based on the limited samples showed a 0.5% variation within the Lampam-a and Lampam-b (*B. schwanenfeldii*) while Naleh showed no variation (0.0%). The genetic distance between the two groups (Lampam a-b vs. Naleh) was 5.0 % (Table 4). The phylogenetic analysis (Neighbor-Joining Tree Analysis) revealed the monophyly of the (Lampam-a and Lampam-b) group and Naleh with bootstrap values of 100% (at 1000x bootstrap replicates), respectively for each clade. All GenBank *B. schwanenfeldii* sequences clustered within the (Lampam-a and Lampam-b) group. However, the Naleh group did not cluster with any of the other species. Two main clades were formed with 100% support. The first clade bifurcated into two highly supported monophyletic subclades of Lampam (100%) which also contained GenBank *B. schwanenfeldii* sequences and another subclade of Naleh (100%). GenBank sequences of outgroups *B. gonionotus*, *B. altus,* and *B. balleroides* (Accession numbers were presented in the Table 1) were clustered into well supported discrete subclades in the other major clade (Figure 4).

Discussion

Both genetic distance (5.0%) and phylogenetic analyses strongly indicated that the Naleh and Lampam groups are two discrete taxa. According to Hebert et al. (2003a) and Hebert et al. (2003b), intra-species divergence values are typically <3% and this argument supports the validity of Naleh and Lampam as two distinct species. The BLAST results precisely identified Lampam-a and Lampam-b as synonymous to *B. schwanenfeldii* with genetic variation (0.5%), well below the threshold distance, further supporting their monophyletic status. This is also supported by the similar morphological features mainly the spines and fin rays in dorsal and anal fins and number of pre -dorsal scales in both the species. Kamarudin and Esa (2009) reported slightly higher genetic divergence (1.01%) among two populations of *B. schwanenfeldii* but these were based on two geographically distant populations, Peninsular Malaysia versus Kalimantan populations. Our DNA barcoding study is in agreement with the findings based on morphometric analysis of Batubara et al. (2018), although they observed slight morphological differences between Lampam-a and Lampam-b for several characteristics - caudal peduncle length (CPL), caudal peduncle depth and snout length (SNL) - both taxa were identified as *B. schwanenfeldii*. These differences could be attributed to environmental plasticity and based on the current genetic data the two groups appeared to be morphological variants of the species. Morphometric differences may be affected by sex differentiation, population, geographical distribution, physiology, and food sources (Sedaghat et al. 2012, Aktas et al. 2006, Khan et al. 2012), trophic niche and water depth (Clabaut et al. 2017). A strong relationship between morphology and the ecological environment of fishes is a common occurrence (Cavalcanti et al. 1999). Thus, further studies are needed to ascertain the correlations (if any) and their underlying factors with the morphological differences observed between the two groups.

Batubara et al. (2018) had previously classified Naleh as *B. gonionatus* based on morphological characteristics. However, genetic analysis of the Naleh group in the current study did not support its classification as *B. gonionotus*. The GenBank *B. gonionotus* sequences were found be genetically distant from Naleh by 8.4%. This is much higher than the 3% threshold intra-species distance (Hebert et al. 2003a, Hebert et al. 2003b) and even exceeds the genetic distance between Naleh and Lampam which indicate a closer relationship between these two taxa. Thus, based on this weight of evidence, we conclude that the samples of Naleh analysed in the current study are not *B. gonionotus*. Several factors could be attributed to this discrepancy with Batubara et al. (2018). The most obvious explanation is that our Naleh samples represent as cryptic species which are morphologically closely related with the *B. gonionotus* samples of Batubara et al. (2018) or both groups are cryptic. Considering the high diversity of this region (Muchlisin & Siti-Azizah 2009, Muchlisin et al. 2015), it is not surprising that there are many undocumented species still to be discovered. This species could be new to science since apart from *B. collingwoodii* (with no available sequence), the remaining four species in this genus were included in our analysis but returned no satisfactory match at species level. *Barbonymus collingwoodii* has only been documented in Borneo (Kottelat 2013) and there has been no report of its occurrence in Aceh (or Sumatra). This also rules out hybridisation with currently documented species to explain the close resemblance of *B. gonionotus*, since all (except *B. collingwoodii*) were analyzed in this study. Interestingly, Esa et al. (2012) reported a genetic divergence of 13.0% between *B. schwanenfeldii* and *B. gonionotus* from Malaysian waters which is clearly much higher than the 5.0% between Naleh and Lampam, further supporting the fact that the Naleh samples in the current study is not *B. gonionotus*. Regardless of the reasons, this study clearly shows that there exist ambiguities with the taxonomic classification of the Naleh. Thus, we recommend complementing the genetic data obtained with a more detailed morphological examination of the specimens and comparisons with type specimens and consultation with experts in *Barbonymus* taxonomy.

As the study was limited in sampling population and sampling sizes, it will be not be appropriate to make any

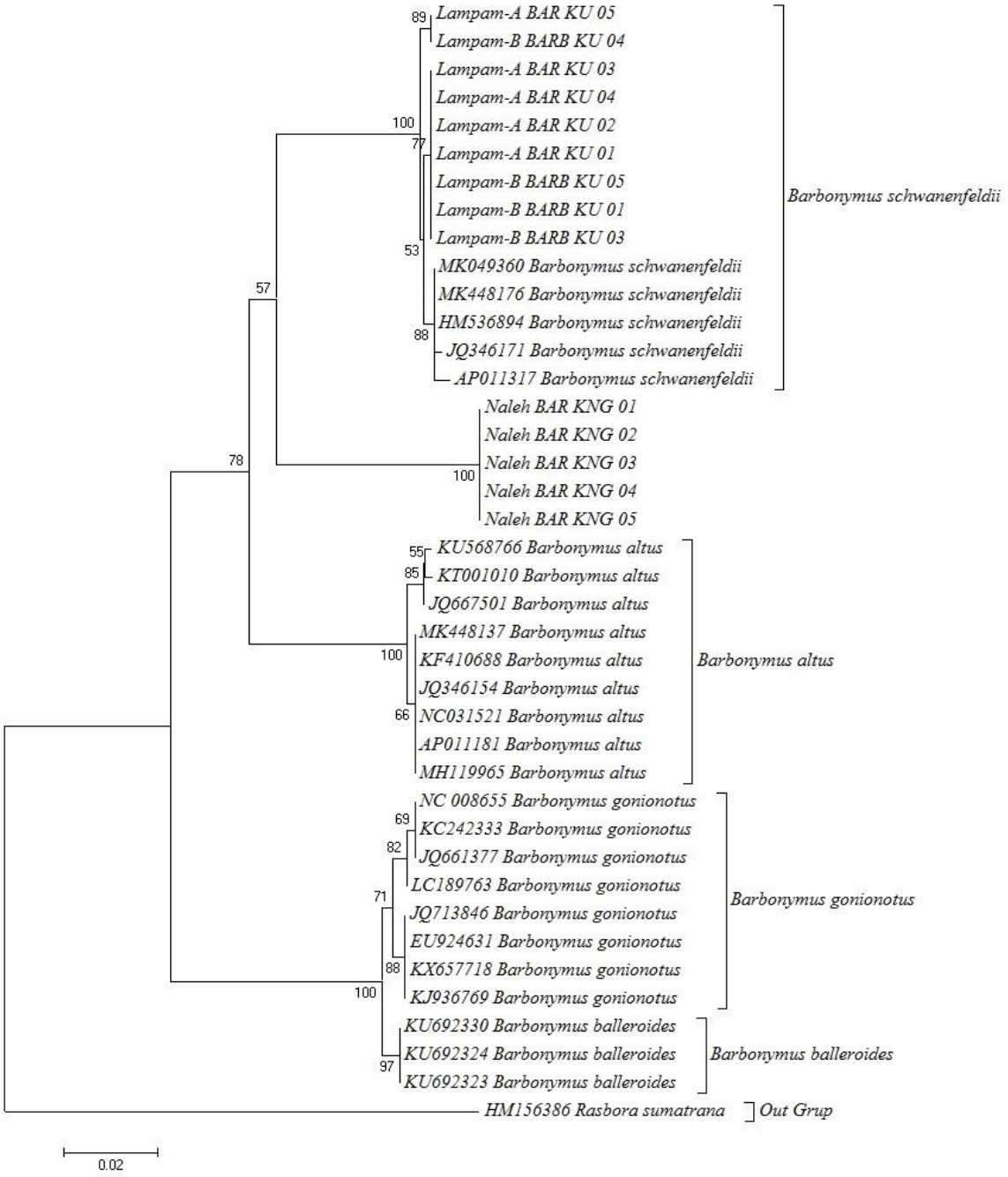

Figure 4. The phylogenetic tree (cladogram) of 14 individuals of *Barbonymus* and the related genera retrieved from the GenBank constructed using NJ methods, where *Rasbora sumatrana* was out-group

firm conclusion on the genetic variability of the species. However, despite the small sample sizes (9 samples) and number of populations, the Lampam harboured three haplotypes. The previous study by Kamarudin and Esa (2009) on the phylogenetics and phylogeography of *B. schwanenfeldii* from Malaysian waters revealed six haplotypes comprising of five haplotypes from Peninsular Malaysian and a single haplotype from Sarawak. Comparing the geographical coverage between the current (within a single river) and their study, it suggests that the Aceh populations are still at a healthy level despite its commercial importance. However, we must take steps to ensure the sustainability of this species in our waters. In contrast, Naleh was monomorphic. However, this observation could be due to inadequate sampling and may not reflect the true genetic variability of the taxon. More extensive studies are required to determine the true genetic variability of this species in Aceh waters.

This DNA barcoding study confirms that the Lampam-a and Lampam-b of the Tamiang River, Aceh are synonymous and belonged to the same species, *B. schwanenfeldii*. The Naleh specimens formed another species with a genetic divergence of 5.0% from the Lampam group. Naleh did not form any close match from the voucher sequences with GenBank database and we believe that it is a cryptic species. Therefore, further detailed study is crucial to clarify this finding.

Acknowledgments. Kemenristik Dikti supported this study through the PMDSU 2016 scheme. Therefore, the authors would like to thank Kemenristek Dikti for supporting this study. The authors would also like to express appreciation to Mr. Khaidir, and Ms. Afrita for their assistance during field and laboratory works. We would also like to extend our thanks to members of the Molecular Ecology Laboratory at Universiti Sains Malaysia Penang for assistance with the analysis. We would also like to thank Bhaba Amatya (Prithvi Narayan Campus, Pokhara, Tribhuvan University, Nepal) for corrections and suggestions during the publication process.

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