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Morphology and molecular studies to reveal the taxonomic status of endemic fish *Barbonymus belinka* of Lake Singkarak, West Sumatra, Indonesia

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Abstract. *Salis VM, Tjong DH, Syaifullah, Dahelmi, Aadrean, Roesma DI. 2025. Morphology and molecular studies to reveal the taxonomic status of endemic fish* Barbonymus belinka *of Lake Singkarak, West Sumatra, Indonesia. Biodiversitas 26: 536-550.* Species identification is very important in taxonomy and conservation. There are some cases where the distinctive morphological characters of a species show minor differences; for example, the Balingka fish (*Barbonymus belinka*), endemic to Lake Singkarak, West Sumatra, Indonesia and the Kapiek fish (*Barbonymus schwanefeldii*). The local community around Lake Singkarak gives two names to the Balingka fish based on size (the larger ones are called Balingka, and the smaller ones are called Kapiek), it can affect the taxonomy and validity of biodiversity data. The lack of comprehensive information on *B. belinka* has led to the species being classified as Data Deficient (DD) on the IUCN Red List. Additionally, the presence of a DNA barcode for Balingka fish in the BOLD system, listed under a different species name than in GenBank (NCBI), raises concerns about potential species misidentification and data inconsistencies. A thorough taxonomy review of Balingka fish (*B. belinka*) is necessary, utilizing both morphological and molecular approaches. This study aims to clarify the taxonomy and investigate the phylogenetic relationships of the two fish species through morphological and molecular analyses, utilizing Cytochrome Oxidase-I and Cytochrome b gene sequences. Individual samples were used for morphological identification, while liver tissue was used for molecular analysis. The results showed morphometric variations, but meristic traits confirmed that both fishes belong to the same species, *B. schwanefeldii*. Molecular analysis shows a genetic distance of 0-1.4% for Cytochrome Oxidase-I gene and 0-2.3% for Cytochrome b gene, indicating the same species. Two specific bases at 585 bp of the Cytochrome Oxidase-I gene and four specific bases at 599 bp of the Cytochrome b gene are also found unique to *B. schwanefeldii* from Lake Singkarak, suggesting that these two fishes may represent the same species.

Keywords: Cytochrome b gene, Cytochrome Oxidase-I gene, morphology, phylogenetic, taxonomy confirmation

INTRODUCTION

Lake Singkarak, the largest lake in West Sumatra and the second largest on Sumatra Island, Indonesia (Whitten et al. 2000), is an essential ecological and economic resource for the local population. The lake plays an important role in the local economy as a source of fisheries, a tourist destination, power generation, and irrigation (Ajiwibowo et al. 2019). One of the significant components of the lake water ecosystem is the freshwater fish group. Currently, the number of fish found in Lake Singkarak continues to decline (Roesma et al. 2023a). One of them is Balingka fish (*Barbonymus belinka* (Bleeker, 1860)) which is an endemic species of Lake Singkarak (Kottelat 2013; IUCN 2019; Dahruddin et al. 2021). Balingka fish (*B. belinka*), which is highly valued and endemic, is categorized as a Data Deficient (DD) species by the IUCN Red List (2019). This classification indicates a lack of adequate information to make a more precise evaluation of its conservation status. The Balingka fish's habitat is restricted to the fresh waters of Lake Singkarak, West Sumatra (Kottelat et al. 2013). Interestingly, the local community around Lake Singkarak gives two names to Balingka fish: larger fish are called Balingka, and smaller fish are known as Kapiek. However, Weber and de Beaufort (1916) reported that these two names refer to different species: Balingka for *B. belinka* and Kapiek for *Barbonymus schwanefeldii* (Bleeker, 1854). In contrast, Salis et al. (2024), through DNA barcoding using the Cytochrome Oxidase-I gene, showed that Balingka and Kapiek fish are the same species, *B. schwanefeldii*. The DNA sequence of *B. belinka* recorded in the BOLD System (BOLD:AED2516) was sourced from Aceh and was identified as *Barbonymus gonionotus* (Bleeker, 1849) in Genbank NCBI (MK978151). This discovery contradicts earlier reports that the *B. belinka* is endemic to Lake Singkarak. Balingka fish (*B. belinka*) has limited information, especially on its morphology, ecology, and molecular information. Therefore, further research is crucial to confirm the taxonomy and understand the unique characteristics of this species.

Precise species determination is an important step in conservation planning and management. Taxonomic uncertainty can hamper conservation efforts and increase the risk of losing important species (Peristiwady 2019). Species identification can be achieved through morphological and molecular studies. Morphological

studies involve the examination of physical traits such as meristic (countable features like fin rays and scales) and morphometrics (measurements of body proportions). These techniques are effective for identifying and classifying species (Hossen et al. 2016). Several morphological studies for taxonomic confirmation have been carried out, uncluding by Batubara et al. (2018). They reported that based on morphometrics, Naleh and Lampam fish in Aceh are *B. gonionotus* and *B. schwanefeldii*. Awas et al. (2023) also combined morphological and molecular approaches to validate nine fish species in the Poonch River, India, demonstrating the importance of integrating different methods in taxonomic studies.

In addition to morphological studies, accuracy in determining species also requires genetic information obtained through molecular studies. Molecular studies, which provide genetic information, are increasingly being used to resolve taxonomic ambiguities. DNA barcoding with the Cytochrome Oxidase-I gene has become a widely accepted method for species identification. Previous research on Balingka and Kapiek fish using the Cytochrome Oxidase-I gene (Salis et al. 2024) has shown promising results, but further genetic data is needed. In addition to the Cytochrome Oxidase-I gene, the Cytochrome b gene is also commonly used in genetic studies for species identification and phylogenetic analysis and studies such as Karlina et al. (2016) on *Puntius binotatus* (Valenciennes, 1842), Aksu and Bektaş (2019) on the genus *Gobio*, Alotaibi et al. (2020) on *Cyprinion*, and Roesma et al. (2023b) on *Cyclocheilichthys*. With the lack of comprehensive genetic information on *B. belinka* and the naming overlap between Balingka and Kapiek, further studies utilizing the Cytochrome Oxidase-I and Cytochrome b genes are necessary to confirm the taxonomic status, comparing the genetic sequences of *B. belinka*, *B. schwanefeldii*, and other related species within the *Barbonymus* genus. Combining the results from both morphological and molecular approaches will provide a clearer understanding of the systematic of these species, helping to resolve the confusion surrounding their identification. Resolving the taxonomic uncertainties surrounding Balingka fish is crucial for effective conservation planning and biodiversity management in Lake Singkarak. Conducting further research utilizing both morphological and molecular methods can ensure more accurate species identification, ultimately contributing to the conservation of these important freshwater fish.

MATERIALS AND METHODS

Study area

Sample collection was carried out in Lake Singkarak, West Sumatra, Indonesia (Figure 1). Sampling location surveys were conducted at 14 points in Lake Singkarak such as Paninggahan (PG), Tanjung Mutiara, Batu Taba (AB1), Batu Taba (AB2), Sumpur (AB3, AB4), Padang Laweh Malalo (AB5, AB6, AB10, AB12, AB13, AB14), Guguak Malalo (AB8), Sumani (SMN), and Ombilin (OMB). Only 5 points were successfully sampled: Paninggahan (PG), Sumpur (AB3), Padang Laweh Malalo (AB6), Sumani (SMN), and Ombilin (OMB). Morphological and molecular analyses were conducted at the Genetics and Biomolecular Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas, Padang, Indonesia.

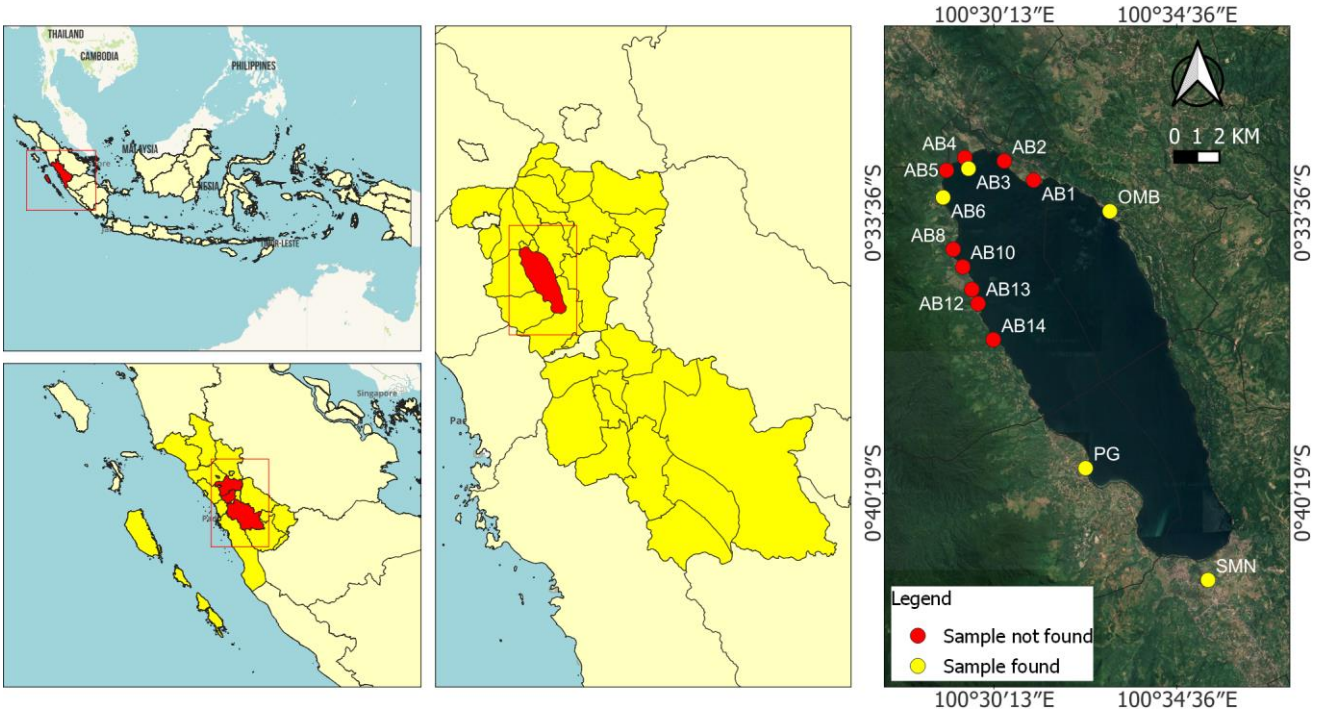

Figure 1. Map of sample collection sites in Lake Singkarak, Solok and Tanah Datar Districts, West Sumatra, Indonesia

Procedures

Sample collection

Sample collection was carried following Cailliet et al. (1986). Sample collection was carried out using several types of fishing gear, namely tangguk and nets, centrum tools (electrofishing), and assistance from local fishermen. This multi-method approach ensured the robustness of our study. The total number of samples successfully collected was 32, consisting of 22 Kapiek fish and 10 Balingka fish. Individual samples that had been collected were then photographed and labeled. Individual samples were placed in a sample box containing 10% formalin. Tissue samples (liver) were stored in a sterile 1.5 mL Eppendorf microtube containing 96% ethanol. Individual and tissue samples were then taken to the Genetics and Biomolecular Laboratory, Department of Biology, Universitas Andalas, to be identified morphologically and molecularly. Individual samples were left for 3-4 weeks and then washed with running water for 5-6 hours to remove residual formalin. Furthermore, for long-term preservation, the samples were placed in a box containing 70% alcohol. Tissue samples (liver) were stored long-term in a refrigerator with a temperature of -20°C. The search for information regarding Balingka and Kapiek fish was conducted through brief interviews with fishermen or residents around the lake.

Morphological measurements

Morphological measurements were carried out on 23 morphometric characters (Table 1) and 8 meristic characters (Table 2) following Kottelat et al. (1993) and Suryaningsih et al. (2020) with two additional characters: Total Length (TL) and Standard Length (SL) (Figure 2). Morphological characters were measured using a digital caliper with an accuracy of 0.1 mm. The fish is placed on styrofoam, and truss distances are measured by connecting specific points. Each measured distance is compared to the total length of the fish to obtain the truss distance ratio. After completing all measurements, the data is analyzed using Kruskal-Wallis, Mann-Whitney, and PCA tests.

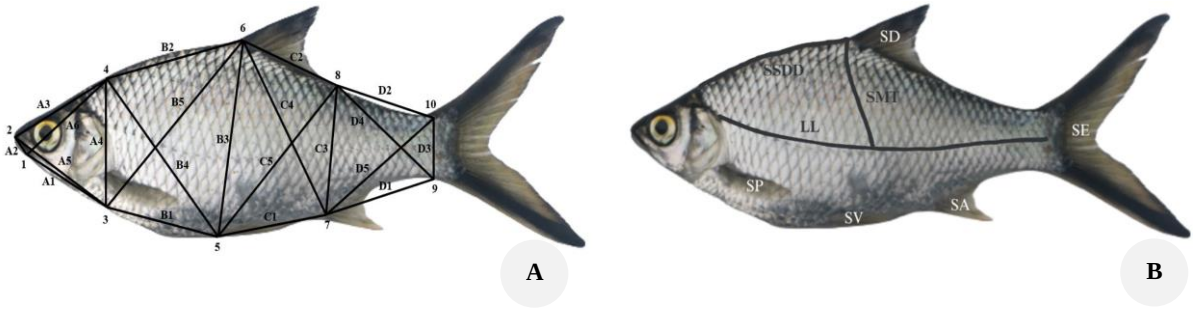

Figure 2. The morphological characters measurement of *Barbonymus* spp. in this study. A. Truss-morphometric; B. Meristic

Table 1. The truss-morphometric character codes and descriptions measured in this study

Character	Code	Characteristics description
Head	A1	The base of the lower jaw - border of the head and ventral body
	A2	The base of the lower jaw - the front end of the muzzle
	A3	The tip of the snout - the head and dorsal body boundary
	A4	The boundaries of the head and dorsal body-the boundaries of the head and ventral body
	A5	The tip of the snout - the boundaries of the head and ventral body
	A6	The boundaries of the head and dorsal body-based on the mandibular body
Anterior body	B1	The boundaries of the head and ventral body - front of pelvic fin
	B2	The boundaries of the head and dorsal body - the front of the dorsal fin
	B3	The front base of the dorsal fin - the front of the pelvic fin
	B4	The boundaries of the head and dorsal body - the front of the pelvic fin
	B5	The front base of the dorsal fin - the boundaries of the head and ventral body
Posterior body	C1	The front of the pelvic fin - the front of the anal fin
	C2	The front base of the dorsal fin - the back base of the dorsal fin
	C3	The back of the dorsal fin - the front base of the anal fin
	C4	The front base of the dorsal fin - the front of the anal fin
	C5	The back base of the dorsal fin - the front of the pelvic fin
Tail	D1	The front of the anal fin - folding of the ventral tail
	D2	The back base of the dorsal fin-folds the dorsal tail
	D3	Folding of the dorsal tail - folding of the ventral tail
	D4	The back of the dorsal fin - folding of the ventral tail
	D5	The front base of the anal-fold fin of the dorsal tail
	TL	Total length
	SL	Standard length

Table 2. The meristic character codes and descriptions measured in this study

Code	Characteristics description
LL	Lateral line
SMT	Scales between the lateral line and the beginning of the dorsal fin
SSDD	Scales in front of dorsal fin
SD	Dorsal fin
SV	Ventral fin
SP	Pectoral fin
SE	Caudal fin
SA	Anal fin

DNA extraction, DNA amplification, and sequencing

DNA extraction from liver tissue followed the protocol of Zymo Research Quick-DNA Miniprep Plus Kit (US). DNA amplification for Cytochrome Oxidase-I gene used a pair of universal primers (Fish F1 and Fish F2) based on Ward et al. (2005) (5' TCAACCAACCACAAAGACATT GGCAC 3' forward and 5' TAGACTTCTGGGTGGCC AAAGAATCA3' reverse). DNA amplification for Cytochrome b gene used a pair of primers based on Roesma (2011) (5' CGATTCTTYGCNTTCCAYTTCYT 3' forward and 5' CCTCCRATCTTCCG ATTACAAGAC 3' reverse). PCR was conducted in 25 µL volumes containing 11 µL MyTaq Hs Red Mix (Bioline), 1 µL forward and reverse primers, 3 µL DNA template, and 9 µL nuclear-free water. The temperature setting of amplification cycles follows the protocol by Roesma (2011) except at the annealing temperature is 54°C for Cytochrome b and the protocol by Ward et al. (2005) for Cytochrome Oxidase-I. The PCR products were visualized in 2% agarose at 100 Volt, 20 W for 50 min; good quality PCR products were used as samples for sequencing, which was carried out at FirstBase, Malaysia.

Data analysis

Morphology data analysis

Therefore, to address size variations across sample locations, all measurements were categorized based on fish length standards (Roesma and Santoso 2011) and then transformed using log10. The Kruskal-Wallis Test was applied to detect differences in morphometric characters among sample locations, followed by a Mann-Whitney Test for a more detailed analysis of the differences (Roesma and Santoso 2011). Both tests were conducted using the SPSS software. The Unweighted Pair Group Arithmetic Average (UPGMA) method was used to assess the relationships between characters across locations. Cluster analysis, performed with the PAST4.03 software, produced a phenogram based on character similarities. Taxonomic distances between locations were calculated using Euclidean distance (Rohlf 2009).

Molecular data analysis

The results of the forward and reverse DNA sequences were assembled and edited using the DNA STAR program (SeqMan) (Burland 2000). The similarity between Balingka and Kapiek sequences was checked against Genbank sequences using the online site (Basic Local Alignment Search Tool) available at http://blast.ncbi.nlm.nih.gov/Blast.cgi. DNA sequence data alignment using the Clustal X program (Larkin et al. 2007) with other *Barbonymus* populations sequences and other sequences taken from GenBank NCBI included outgroup species (Tables 3 and 4).

The alignment results were then saved in fasta format. Furthermore, it was checked using the BioEdit program (Hall 2011). Amino acid matching was carried out on the DNA sequence using the DNA to Protein Translation program available at http://insilico.ehu.es/translation. Polymorphism sequence analysis was carried out using the DnaSP 6.0 program (DNA Sequence Polymorphism) (Rozas et al. 2017) to evaluate the variation of nucleotide bases. Sequence divergence of all sequences was analyzed using the Kimura -2 parameter model (K2P) in the MEGA11 program (Tamura et al. 2021). The phylogenetic tree for both Cytochrome Oxidase-I and Cytochrome b genes was reconstructed using the Maximum Likelihood (ML) method in IQ-TREE (Minh et al. 2020).

Table 3. List of comparison sequences and accession numbers/BIN ID (Cytochrome Oxidase-I gene)

Family, species	Location	Acc. No./ BIN ID
Cyprinidae		
Barbonymus belinka	Aceh	AED2516
Barbonymus schwanefeldii	Malaysia	NC_024274.1
Barbonymus schwanefeldii	Riau	AAU0688
Barbonymus schwanefeldii	West Kalimantan	AAU0688
Barbonymus schwanefeldii	South Sumatra	AAU0688
Barbonymus schwanefeldii	Aceh	MK978150.1
Barbonymus schwanefeldii	Malaysia 1	KT001006.1
Barbonymus schwanefeldii	Jambi	AAU0688
Barbonymus schwanefeldii	Malaysia 2	KT001008.1
Barbonymus schwanefeldii	Laos	JQ346171.1
Barbonymus schwanefeldii	Thailand 1	MK049364.1
Barbonymus schwanefeldii	Thailand 2	MK448176.1
Barbonymus gonionotus	Myanmar 1	LC189763.1
Barbonymus gonionotus	Myanmar 2	AAD1940
Barbonymus gonionotus	India 1	JX181874.1
Barbonymus gonionotus	India 2	JX181878.1
Barbonymus gonionotus	Fish market (Indonesia)	KP856760.1
Barbonymus gonionotus	South Sumatra	MZ636555.1
Barbonymus gonionotus	South Kalimantan	AAD1940
Barbonymus mahakkamensis	South Kalimantan	ADN2907
Barbonymus balleroides	Java	KU692330.1
Barbonymus altus	Malaysia	KT001010.1
Barbonymus altus	Laos	JQ346154.1
Barbonymus altus	Kamboja	JX066755.1
Barbodes lateristriga	Thailand	MT483454.1
Barbodes lateristriga	Malaysia	MW168729.1
Barbodes banksi	Jambi	ON668384.1
Barbodes banksi	West Sumatra	ON668382.1
Claridae		
Clarias batrachus		KU692432.1
Channidae		
Channa striata		KU692420.1

Table 4. List of comparison sequences and accession numbers/ BIN ID (Cytochrome b gene)

Family, species	Location	Acc. no./ BIN ID
Cyprinidae		
Barbonymus schwanefeldii	Malaysia	NC 024274
Barbonymus schwanefeldii	Malaysia	KU233186
Barbonymus schwanefeldii	Malaysia	KJ573467
Barbonymus schwanefeldii	Afrika	HM536796
Barbonymus schwanefeldii	Laos	JQ346151
Barbonymus schwanefeldii	Japan	AP011317
Barbonymus schwanefeldii	Afrika	KP712197
Barbonymus gonionotus	Thailand	NC 008655
Barbonymus gonionotus	Laos	JQ346136
Barbonymus altus		NC 031521
Barbonymus altus		AP011181
Barbonymus altus		JX066773
Barbonymus altus		JQ346133
Barbodes gonionotus	South Afrika	AF180822
Barbodes lateristriga		MT483249
Barbodes binotatus		MT483247
Barbodes banksi		MT483244
Poropuntius bantamensis		NC 031604
Poropuntius huangchuchieni		KC567011
Poropuntius huangchuchieni		MN723896
Puntius semifasciolatus		KC696521
Puntius semifasciolatus		AY856116
Systomus sarana		KP712217
Bagridae		
Hemibagrus guttatus		KU128417
Hemibagrus nemurus		AF499600

RESULTS AND DISCUSSION

Morphological analysis of Kapiek and Balingka Fish (*Barbonymus* spp.) in Lake Singkarak

Mann-Whitney U Test analysis on 23 morphological characters of Kapiek and Balingka fish showed that six characters were significantly different (Table 5). Characteristics that were significantly different were the distance between the lower jaw to the ventral head and body boundary (A1), the distance from the base of the lower jaw to the front tip of the snout (A2), the distance from the ventral head and body boundary to the front of the pelvic fin (B1), distance from the rear base of the dorsal fin to the base of the upper caudal fin (D2), distance from the rear base of the dorsal fin to the base of the lower caudal fin (D4), and Standard Length (SL). Morphological differences are mainly visible in the head, body, and tail peduncle, which shows that the head of the Kapiek fish is smaller than that of the Balingka. At the same time, the Balingka has a larger tail. The PCA plot shows a clear separation between Kapiek and Balingka (Figure 3). Kapiek is spread across all quadrants, with one individual being in quadrant III, while Balingka is only in quadrant III, showing significant morphometric differences.

Morphological analysis of Kapiek Fish in Lake Singkarak

Kruskal-Wallis analysis showed that of the 23 morphometric characters of Kapiek fish in Lake Singkarak, three characters showed significant differentiation ($p \leq 0.05$), namely, the distance between the lower jaw to the ventral head and body boundary (A1), the distance between the dorsal head and body boundary and the base of the dorsal fin (B2), and standard length (SL) (Table 6). The Mann-Whitney U Test showed that the location with the highest morphometric differentiation was between fish in the Sumpur and Sumani locations, with five significantly different characters (21.73% of the 23 characters measured) (Table 7).

Table 5. Results of the Mann-Whitney Test analysis on Kapiek and Balingka fish in Lake Singkarak, West Sumatra, Indonesia

Morphological characters	Kapiek (N: 22)	Balingka (N: 10)	Mann-Whitney U Test
A1	26.51 ± 3.25	40.04 ± 4.71	H: 0.00
	32.64 - 18.3	48.8 - 32.95	**P: < 0.001***
A2	9.92 ± 3.66	12.60 ± 1.16	H: 52.00
	18.19 - 5.79	14.09 - 10.47	**P: 0.018***
A3	24.98 ± 4.08	42.42 ± 5.14	H: 75.00
	31.61 - 18.41	48.21 - 34.08	P: 0.155
A4	31.91 ± 4.93	50.74 ± 8.12	H: 90.50
	37.67 - 22.41	63.07 - 39.72	P: 0.428
A5	31.60 ± 4.92	49.30 ± 7.53	H: 77.50
	40.67 - 21.36	59.08 - 37.21	P: 0.186
A6	24.19 ± 3.93	40.18 ± 5.96	H: 87.00
	31.51 - 18.92	49.33 - 29.08	P: 0.350
B1	31.68 ± 5.09	47.03 ± 6.22	H: 55.50
	43.64 - 21.27	53.22 - 38.58	**P: 0.027***
B2	35.50 ± 6.12	61.82 ± 9.17	H: 66.50
	47.56 - 24.33	72.4 - 46.67	P: 0.077
B3	46.50 ± 8.41	78.94 ± 9.03	H: 86.00
	57.48 - 30.73	90.51 - 67.39	P: 0.329
B4	49.34 ± 6.46	78.99 ± 10.37	H: 76.00
	59.87 - 38.11	89.78 - 64.16	P: 0.166
B5	48.22 ± 7.45	79.36 ± 12.62	H: 100.00
	59.14 - 35.41	92.43 - 62.79	P: 0.684
C1	28.32 ± 5.26	45.92 ± 7.59	H: 105.50
	40.29 - 20.33	54.71 - 35.35	P: 0.855
C2	19.95 ± 7.56	29.42 ± 4.40	H: 96.50
	49.53 - 11.55	35.07 - 23.07	P: 0.583
C3	34.29 ± 6.37	59.27 ± 7.67	H: 66.00
	41.37 - 22.55	67.36 - 49.75	P: 0.073
C4	49.02 ± 8.85	80.33 ± 9.79	H: 105.50
	63.14 - 33.33	91.9 - 68.64	P: 0.855
C5	45.97 ± 8.60	75.17 ± 10.00	H: 106.50
	56.1 - 30.36	86.86 - 62.83	P: 0.887
D1	33.50 ± 7.08	52.51 ± 7.06	H: 90.50
	51.03 - 22.16	63.63 - 41.98	P: 0.428
D2	33.46 ± 5.07	58.37 ± 7.29	H: 54.50
	40.47 - 26.31	71.67 - 47.58	**P: 0.024***
D3	16.35 ± 2.91	26.13 ± 2.97	H: 91.00
	20.26 - 9.85	29.36 - 22.27	P: 0.440
D4	40.26 ± 6.58	70.37 ± 9.36	H: 33.00
	49.29 - 29.31	82.82 - 58.45	**P: 0.002***
D5	41.21 ± 6.56	68.39 ± 8.79	H: 102.50
	51.14 - 27.89	79.7 - 57.85	P: 0.760
TL	153.73 ± 22.69	249.00 ± 28.02	H: 88.00
	182 - 104	281 - 213	P: 0.370
SL	109.94 ± 16.51	179.10 ± 24.43	H: 0.00
	134 - 76	206 - 150	**P: < 0.001***

Note: P significant ≤ 0.05; N: Number of samples; *: Significance of test results; the code for each character is explained in Figure 2

Table 6. Results of Kruskal-Wallis analysis of Kapiek fish at all locations in Lake Singkarak, West Sumatra, Indonesia

Morphological Characters	Location					Kruskal-Wallis Test
	Paninggahan N: 3	Ombilin N: 4	Sumpur N: 6	Malalo N: 2	Sumani N: 7	
A1	26.22 ± 2.13	28.26 ± 0.15	27.96 ± 4.20	28.13 ± 0.34	23.94 ± 2.82	H: 12.932
	28.58 - 24.45	28.44 - 28.07	32.64 - 20.79	28.37 - 27.89	26.95 - 18.3	**P: 0.012***
A2	8.52 ± 0.92	10.14 ± 0.88	8.24 ± 2.04	9.65 ± 1.06	11.91 ± 5.84	H: 6.171
	9.51 - 7.69	11.31 - 9.27	10.62 - 5.79	10.4 - 8.9	18.19 - 6.6	P: 0.187
A3	27.03 ± 1.14	26.90 ± 2.24	27.22 ± 3.70	28.17 ± 0.57	20.18 ± 1.99	H: 5.048
	28.29 - 26.07	30.22 - 25.29	31.61 - 21.11	28.57 - 27.77	23.56 - 18.41	P: 0.282
A4	33.41 ± 1.87	36.52 ± 0.82	32.00 ± 6.32	34.65 ± 0.55	27.76 ± 3.50	H: 4.117
	34.87 - 31.3	37.49 - 35.69	37.67 - 22.41	35.04 - 34.26	31.78 - 23.45	P: 0.390
A5	34.18 ± 1.10	33.06 ± 3.21	31.64 ± 6.96	36.62 ± 4.72	28.19 ± 2.89	H: 4.388
	35.09 - 32.95	35.93 - 28.95	40.67 - 21.36	39.95 - 33.28	30.91 - 23.71	P: 0.356
A6	26.16 ± 1.12	24.78 ± 2.10	26.08 ± 4.70	28.59 ± 1.15	20.13 ± 1.22	H: 7.456
	26.97 - 24.89	26.5 - 21.86	31.51 - 19.32	29.4 - 27.78	21.85 - 18.92	P: 0.114
B1	33.71 ± 2.44	33.76 ± 5.39	33.94 ± 5.12	33.54 ± 2.55	27.14 ± 3.99	H: 2.118
	35.94 - 31.1	39.1 - 27.84	43.64 - 29.47	35.34 - 31.74	30.68 - 21.27	P: 0.714
B2	41.04 ± 2.66	42.37 ± 4.01	33.16 ± 6.20	37.79 ± 4.34	30.55 ± 1.66	H: 11.993
	43.32 - 38.12	47.56 - 37.79	41.52 - 24.33	40.86 - 34.72	32.77 - 27.8	**P: 0.017***
B3	51.74 ± 1.71	54.50 ± 2.02	46.68 ± 10.74	49.98 ± 1.22	38.54 ± 3.69	H: 4.329
	53.71 - 50.58	57.48 - 53.13	56.81 - 30.73	50.84 - 49.12	42.71 - 32.47	P: 0.363
B4	51.81 ± 1.01	54.90 ± 2.36	50.79 ± 6.78	54.80 ± 0.68	42.31 ± 3.35	H: 6.844
	52.5 - 50.65	57.5 - 52.04	59.87 - 40.85	55.28 - 54.32	46 - 38.11	P: 0.144
B5	51.63 ± 3.86	53.62 ± 3.00	50.29 ± 8.96	51.51 ± 0.27	40.97 ± 4.84	H: 3.269
	55.14 - 47.49	57.35 - 50.14	59.14 - 35.5	51.7 - 51.32	46.28 - 35.41	P: 0.514
C1	28.44 ± 3.46	30.69 ± 2.59	30.99 ± 6.14	34.07 ± 2.02	22.97 ± 1.43	H: 4.950
	30.61 - 24.45	34.2 - 27.96	40.29 - 22.63	35.49 - 32.64	24.7 - 20.33	P: 0.292
C2	20.90 ± 0.67	20.14 ± 1.20	25.23 ± 12.37	21.33 ± 3.90	14.52 ± 2.38	H: 6.128
	21.44 - 20.15	21.11 - 18.58	49.53 - 15.05	24.09 - 18.57	17.61 - 11.55	P: 0.190
C3	38.31 ± 1.37	39.94 ± 1.52	33.28 ± 8.66	38.74 ± 1.90	28.94 ± 2.58	H: 1.556
	39.89 - 37.48	41.35 - 37.95	41.37 - 22.55	40.08 - 37.39	31.58 - 25.06	P: 0.817
C4	52.98 ± 0.29	55.17 ± 2.47	51.27 ± 12.08	54.06 ± 0.86	40.43 ± 4.04	H: 2.474
	53.32 - 52.8	57.87 - 51.89	63.14 - 33.33	54.67 - 53.45	44.94 - 35.1	P: 0.649
C5	50.53 ± 3.25	53.83 ± 1.87	46.81 ± 10.63	50.74 ± 0.12	37.44 ± 3.82	H: 6.684
	54.27 - 48.37	56.1 - 51.56	54.94 - 30.36	50.82 - 50.65	41.13 - 31.6	P: 0.154
D1	34.77 ± 6.56	36.84 ± 3.37	34.21 ± 8.67	42.16 ± 8.05	27.97 ± 3.56	H: 2.534
	38.86 - 27.2	41.57 - 33.85	51.03 - 26.09	47.85 - 36.46	31.17 - 22.16	P: 0.639
D2	39.62 ± 0.93	37.27 ± 1.91	32.85 ± 3.15	38.66 ± 2.26	27.68 ± 1.37	H: 5.071
	40.47 - 38.63	39.18 - 34.82	37.31 - 28.76	40.25 - 37.06	30.09 - 26.31	P: 0.280
D3	18.17 ± 0.97	18.45 ± 1.28	17.07 ± 3.61	17.87 ± 0.14	13.33 ± 1.03	H: 3.017
	19.29 - 17.58	20.26 - 17.34	19.3 - 9.85	17.97 - 17.77	14.51 - 11.57	P: 0.555
D4	43.39 ± 3.34	46.24 ± 1.04	40.93 ± 6.36	47.37 ± 1.10	32.89 ± 2.04	H: 2.271
	47.03 - 40.45	47.48 - 45	49.29 - 33.57	48.15 - 46.59	35.48 - 29.31	P: 0.686
D5	44.50 ± 2.80	47.05 ± 2.82	40.77 ± 8.20	45.94 ± 0.03	35.48 ± 3.72	H: 2.999
	46.34 - 41.27	51.14 - 44.79	49.64 - 27.89	45.96 - 45.92	39.21 - 28.58	P: 0.558
TL	161.67 ± 8.02	172.8 ± 8.22	157.7 ± 25.76	171.5 ± 0.71	131 ± 14.06	H: 4.864
	170 - 154	182 - 162	182 - 115	172 - 171	142 - 104	P: 0.302
SL	116 ± 6.93	124.25 ± 7.14	114.77 ± 14.62	124.50 ± 0.71	90.86 ± 7.8	H: 12.932
	124 - 112	134 - 117	133 - 91.6	125 - 124	96 - 76	**P: 0.012***

Note: P significant ≤ 0.05; N: Number of samples; *: Significance of test results; the code for each character is explained in Figure 2

Table 7. The number of different characters between two Kapiek fish locations in Lake Singkarak, Indonesia. based on the Mann-Whitney Test

Location	Paninggahan	Ombilin	Sumpur	Malalo	Sumani
Paninggahan	-				
Ombilin	2	-			
Sumpur	1	3	-		
Malalo	-	-	-	-	
Sumani	3	3	5	2	-

The different characters include the distance between the lower jaw to the ventral head and body boundary (A1), the distance from the base of the lower jaw to the front tip of the snout (A2), the distance between the dorsal head and body boundary and the base of the dorsal fin (B2), the distance from the dorsal head and body boundary to the front of the pelvic fin (B4), and standard length (SL). Fish in the Sumani location showed more character differences (2-5 significant characters) compared to other locations. PCA analysis (Figure 4) showed that almost all individuals from five locations were in Quadrant II, with three individuals from Sumani being in Quadrant I. Figure 5

shows two main clusters: one cluster consisting of fish in Sumani and the other cluster consisting of fish in Malalo, Ombilin, Paninggahan, and Sumpur.

Morphological analysis of Balingka Fish in Lake Singkarak

The results of the Mann-Whitney U Test analysis showed that seven morphometric characteristics were significantly different from the 23 characters being measured, namely: the distance between the lower jaw to the border of the head and ventral body (A1), the distance from the base of the lower jaw to the front tip of the snout (A2), the distance from the front base of the dorsal fin to the front of the pelvic fin (B3), the distance from the front base of the dorsal fin to the front of the anal fin (C4), the distance from the dorsal tail fold to the ventral tail fold (D3), total length (TL), standard length (SL) (Table 8).

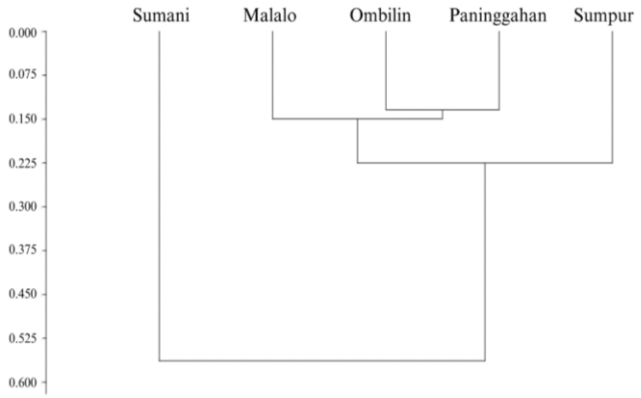

Figure 5. Dendrogram of Kapiek fish from several locations in Lake Singkarak, West Sumatra, Indonesia based on morphological characters with UPGMA character analysis

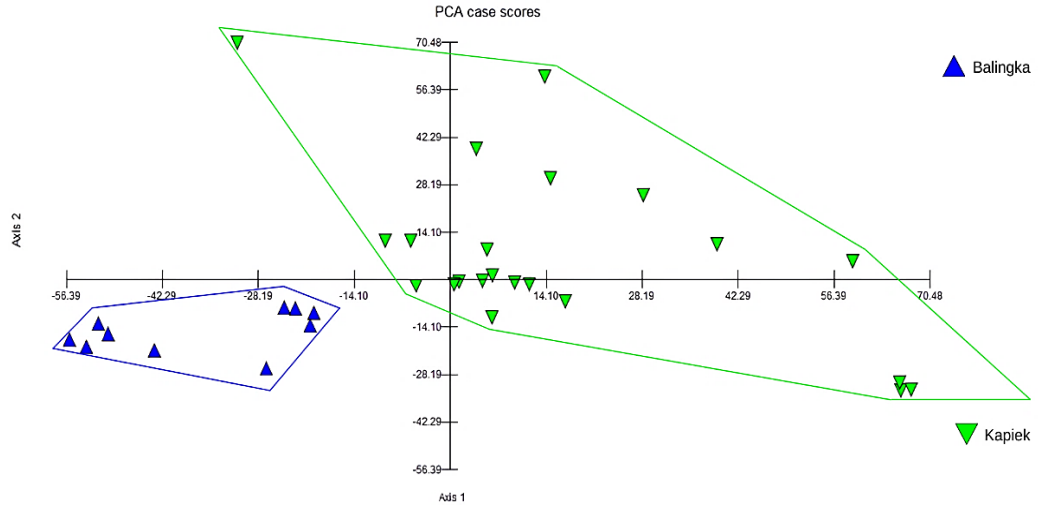

Figure 3. Location grouping pattern between Kapiek and Balingka in Lake Singkarak, West Sumatra, Indonesia based on PCA (Principal Component Analysis) analysis. PC1 is represented on the x-axis (Axis 1) and PC2 on the y-axis (Axis 2)

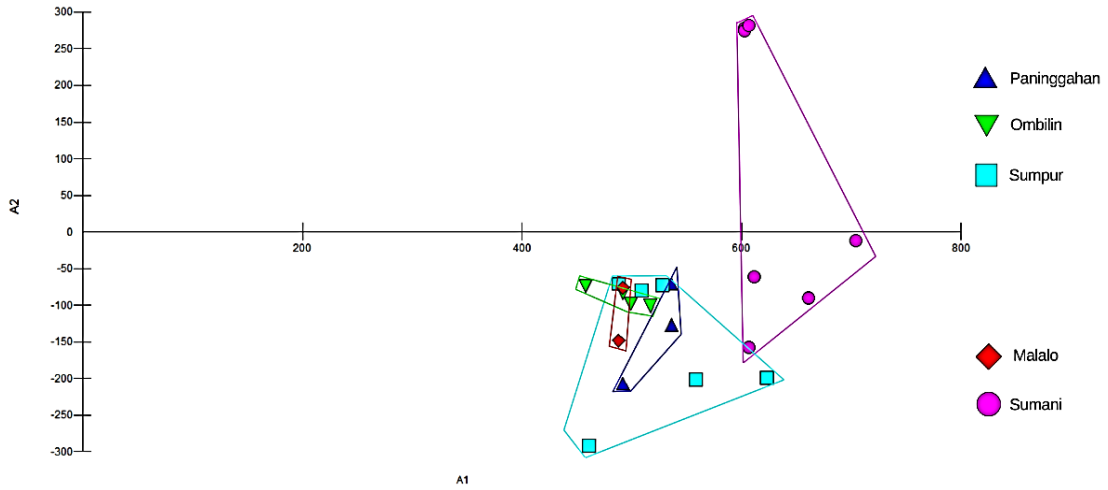

Figure 4. Grouping pattern of Kapiek fish from several locations in Lake Singkarak, West Sumatra, Indonesia based on PCA (Principal Component Analysis) analysis. PC1 is represented on the x-axis (A1) and PC2 on the y-axis (A2)

Table 8. Results of the Mann-Whitney Test analysis on Balingka fish in Lake Singkarak, West Sumatra, Indonesia

Morphological characters	Locations		Mann-Whitney U Test
	Paninggahan N: 4	Sumani N: 5	
A1	36.00 ± 3.29	43.31 ± 3.49	H: 0.00
	39.2 - 32.95	48.8 - 40.11	**P: 0.014***
A2	11.39 ± 0.61	13.46 ± 0.53	H: 1.00
	11.77 - 10.47	14.09 - 12.85	**P: 0.027***
A3	36.98 ± 2.95	46.20 ± 1.78	H: 6.00
	40.86 - 34.08	48.21 - 44.04	P: 0.327
A4	42.33 ± 3.02	57.18 ± 4.28	H: 8.50
	45.33 - 39.72	63.07 - 51.52	P: 0.712
A5	41.01 ± 2.59	55.31 ± 2.49	H: 7.50
	43.03 - 37.21	59.08 - 52.41	P: 0.539
A6	34.51 ± 3.86	44.78 ± 3.17	H: 7.00
	38.19 - 29.08	49.33 - 40.51	P: 0.462
B1	40.18 ± 2.34	51.33 ± 2.09	H: 7.50
	43.66 - 38.58	53.22 - 48.41	P: 0.539
B2	51.89 ± 4.64	68.99 ± 2.49	H: 9.00
	57.62 - 46.67	72.4 - 65.5	P: 0.806
B3	68.86 ± 1.49	86.45 ± 2.78	H: 0.00
	70.81 - 67.39	90.51 - 82.75	**P: 0.014***
B4	67.27 ± 2.45	87.63 ± 1.80	H: 7.500
	70.09 - 64.16	89.78 - 85.47	P: 0.532
B5	65.65 ± 2.56	90.69 ± 2.00	H: 3.00
	68.89 - 62.79	92.43 - 88.14	P: 0.085
C1	37.92 ± 2.55	52.65 ± 1.81	H: 4.00
	41.43 - 35.35	54.71 - 50.34	P: 0.142
C2	24.75 ± 1.39	33.19 ± 1.57	H: 8.00
	26.21 - 23.07	35.07 - 31.47	P: 0.623
C3	50.70 ± 0.90	66.02 ± 1.21	H: 5.50
	51.89 - 49.75	67.36 - 64.17	P: 0.266
C4	69.64 ± 1.17	88.85 ± 3.10	H: 2.00
	71.33 - 68.64	91.9 - 83.73	**P: 0.050***
C5	63.98 ± 1.18	83.80 ± 2.30	H: 9.00
	65.41 - 62.83	86.86 - 80.41	P: 0.806
D1	45.07 ± 2.11	58.07 ± 3.70	H: 9.00
	46.51 - 41.98	63.63 - 54.3	P: 0.0.806
D2	51.15 ± 2.54	64.02 ± 4.74	H: 5.00
	53.3 - 47.58	71.67 - 60.81	P: 0.221
D3	22.83 ± 0.58	28.63 ± 0.85	H: 0.00
	23.64 - 22.27	29.36 - 27.17	**P: 0.014***
D4	60.40 ± 1.75	78.39 ± 3.89	H: 1.50
	62.68 - 58.45	82.82 - 74.16	P: 0.176
D5	58.88 ± 0.89	76.17 ± 2.64	H: 7.00
	60.01 - 57.85	79.7 - 73.32	P: 0.462
TL	218.5 ± 4.93	273.8 ± 6.91	H: 1.50
	224 - 213	281 - 265	**P: 0.030***
SL	153 ± 2.58	201.40 ± 4.77	H: 0.00
	156 - 150	206 - 194	**P: 0.014***

Note: P significant ≤ 0.05; N: Number of samples; *: Significance of test results; the code for each character is explained in Figure 2

Characters A1, A2, B3, and C4 are related to the head and ventral body, while D3 is the tail. TL and SL measure the overall length of the fish and the standard length. Based on the measurement results, fish from the Sumani location (lotic waters) have higher body and tail diagonal heights (C4 and D3) than fish from Paninggahan (lentic waters). Overall, fish from Sumani have a higher average morphometric value than fish from Paninggahan. The distribution plot shows a clear separation between fish from the Sumani and Paninggahan locations even though both are in the same quadrant (quadrant II) (Figure 6).

Meristic analysis between Balingka and Kapiek in Lake Singkarak

Based on the meristic character calculation, both Kapiek and Balingka fish from Lake Singkarak have the following results: lateral line: 35-36, dorsal fins: 8-9, ventral fins: 8, pectoral fins: 12-14, caudal fins: 17-19, anal fins: 5-6, scales between the lateral line and the base of the dorsal fin: 8, scales in front of the dorsal fin: 13 (Table 9). This character calculation is in accordance with the identification key proposed by Weber and de Beaufort (1916) and Kottelat (1993), which distinguishes between *B. belinka* and *B. schwanefeldii*. In general, the results of this calculation indicate that both fish have the same key characteristics as *B. schwanefeldii*, namely the number of lateral line as many as 35-36 and the number of scales in front of the dorsal fin as many as 13. This similarity is also supported by the results of previous studies, as reported by Gante et al. (2008) and Radona et al. (2017).

Molecular analysis using Cytochrome Oxidase-I gene

The results of BLAST analysis of the Cytochrome Oxidase-I gene sequences showed that all Balingka and Kapiek fish samples from Lake Singkarak had a high level of similarity, namely 97%-99% with the *B. schwanefeldii* species recorded in the GenBank database, NCBI. The total sequences analyzed in the Cytochrome Oxidase-I gene were 62 sequences, 30 of which were obtained from the BOLD System and GenBank, NCBI. Based on the alignment results, the length of the Cytochrome Oxidase-I gene sequence analyzed was 585 bp.

Table 9. Meristic calculation results of samples from Lake Singkarak, West Sumatra, Indonesia with previous reports

Sample and references	LL	SD	SV	SP	SE	SA	SMT	SSDD
Kapiek	35-36	8-9	8	13-14	17-19	5-6	8	13
Radona et al. (2017)	31-36	9	8	11-13	15-17	6-8	-	-
Gante et al. (2008)	-	9	8	14	18-19	6	-	-
Weber and de Beaufort (1916)	35-36	8-9	8	14	19	5-6	8	13
Balingka	35-36	8-9	8	12-14	17-19	5-6	8	13
Kottelat (1993)	36	-	-	-	-	-	9	16
Weber and de Beaufort (1916)	36	9	8	14-16	19	6	9	16

Note: LL and SSDD is the key identification to distinguish *B. belinka* and *B. schwanefeldii* (Weber and de Beaufort (1916)

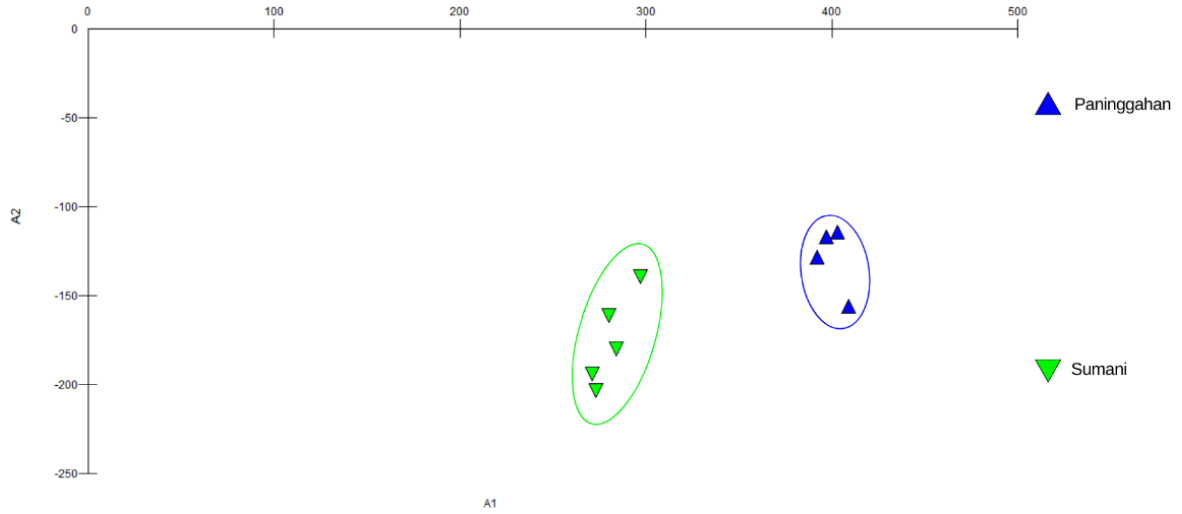

Figure 6. Grouping pattern of Balingka fish from several locations in Lake Singkarak, West Sumatra, Indonesia based on PCA (Principal Component Analysis) analysis

The nucleotide bases analyzed in the 62 Cytochrome Oxidase-I gene sequences consisted of 367 bp (62.73%) conserved sites and 218 bp (37.26%) variable sites. A total of 32 sequences from Lake Singkarak had Guanine+Cytosine (G+C) at 46.4% and Adenine+Thymine (A+T) at 53.6%. The high number of A+T bases compared to G+C was also found in other freshwater fish (Astuti et al. 2020; Kusuma et al. 2021; Dianiputri et al. 2022; Roesma et al. 2022). Haplotype analysis of 585 bp of Cytochrome Oxidase-I gene from 62 sequences, including samples from Lake Singkarak and reference sequences, identified 28 different haplotypes. A total of 32 samples from Lake Singkarak were divided into three haplotypes. The haplotype diversity (Hd) values of Cytochrome Oxidase-I gene of Kapiek and Balingka fish from three locations (Paninggahan, Ombilin, and Sumani) were classified as low based on Nei and Kumar (2000) and Hobbs et al. (2013) with values ranging from 0.4 to 0.476 (Table 10). The Sumpur location was the only location that showed a moderate haplotype diversity value with a value of 0.6 (Nei and Kumar 2000; Hobbs et al. 2013). In contrast, the Malalo location had a haplotype diversity value of 0 because all samples belonged to the same haplotype (identical DNA sequence). Table 10 also shows that all sequences from Lake Singkarak have low nucleotide diversity (π) (0.00081-0.00148) based on Stephan and Langley (1992) and Ouassal et al. (2021).

The analysis of nucleotide variations shows that 585 bp of the Cytochrome Oxidase-I gene from 62 sequences identified two distinct nucleotide bases among 32 samples from Lake Singkarak. Additionally, there were 42 nucleotide differences between these samples and *B. belinka* in the BOLD System. However, at the 231st position, *B. belinka* and eight Singkarak samples shared Adenine, while 24 others had Guanine. These 42 differences support previous findings (Salis et al. 2024), suggesting a strong alignment that the Singkarak samples align more with *B. schwanefeldii* rather than *B. belinka* from Aceh. There were also 16 base differences between the Singkarak samples and *B. schwanefeldii* from other populations, with transversion mutations at the 3rd and 31st positions and transition mutations at 14 others. The 3rd and 192nd positions can serve as markers specific to Singkarak samples, confirming earlier studies (Salis et al. 2024). Amino acid analysis of 42 sequences, including 32 Kapiek and Balingka samples and 10 *B. schwanefeldii*, showed consistent amino acids across all Singkarak samples.

Analysis of 62 Cytochrome Oxidase-I gene sequences resulted in a phylogenetic tree showing the position of 32 Kapiek and Balingka fish samples from Lake Singkarak, along with comparison sequences. Using IQ-TREE software (bootstrap 5000) and pairwise distance on MEGA 11, this phylogenetic tree was divided into two main clusters and one outgroup (Figure 7). The first cluster consisted of four subclusters, including samples from Lake Singkarak as well as *B. schwanefeldii*, *B. belinka*, *B. mahakkamensis* (Ahl, 1922), *B. altus* (Günther, 1868), *B. gonionotus*, and *B. balleroides* (Valenciennes, 1842). The second cluster consisted of other genera in the Cyprinidae family, while the outgroup included *Clarias batrachus* and *Channa striata*.

Table 10. Haplotype diversity (Hd) and Nucleotide diversity (π) of Kapiek and Balingka fish from Lake Singkarak, West Sumatra, Indonesia (Cytochrome Oxidase-I gene)

Locations	n	Hn	Hd	π
Paninggahan	7	2	0.476	0.00081
Ombilin	5	2	0.4	0.00068
Sumpur	6	3	0.6	0.00148
Malalo	2	1	0	0
Sumani	12	2	0.409	0.0007
All locations	32	10	1.885	0.00367

Note: n: Number of samples; Hn: Number of haplotypes; Hd: Haplotype diversity; π: Nucleotide diversity

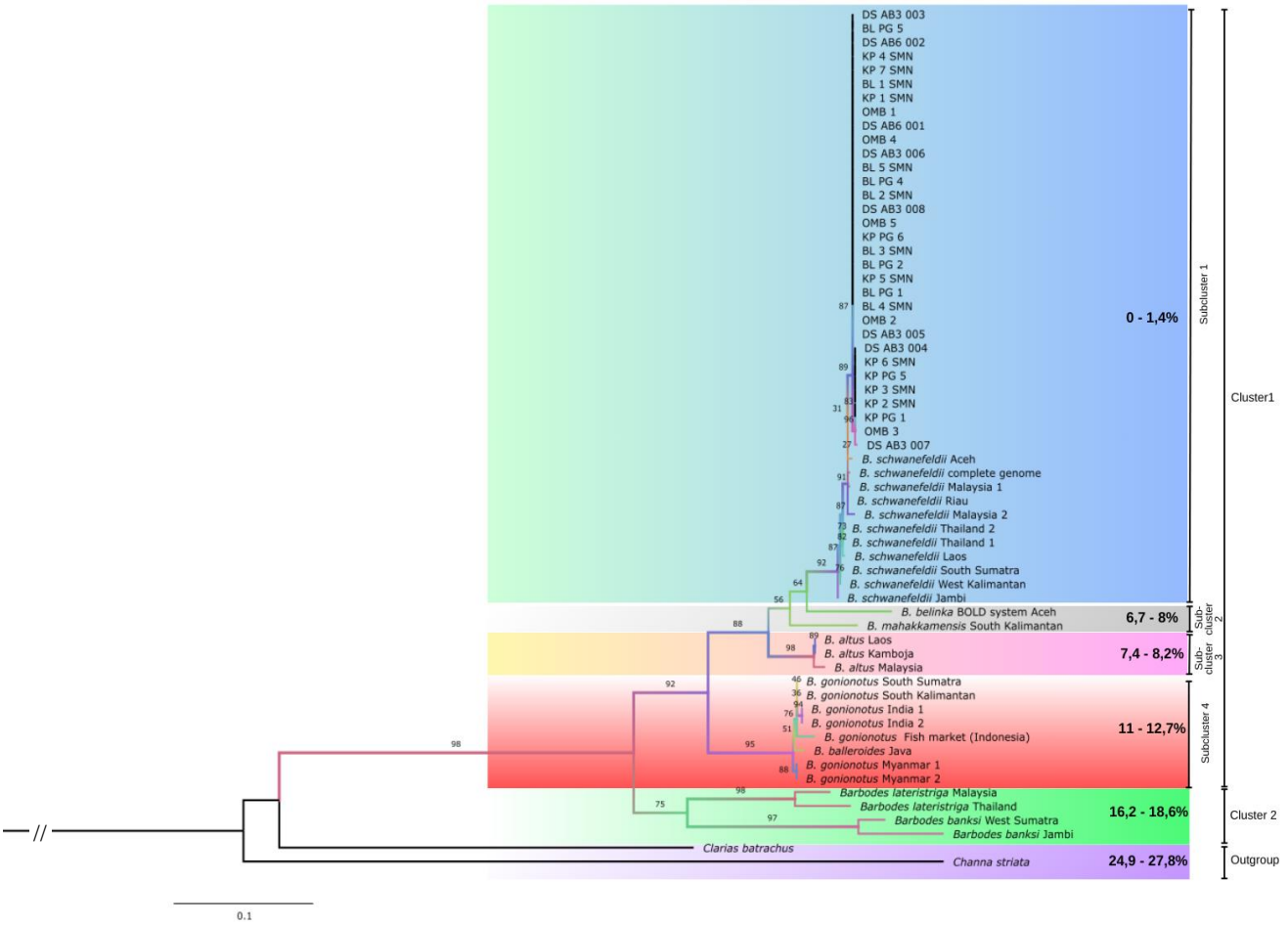

Figure 7. The phylogenetic tree based on Cytochrome Oxidase-I gene using the Maximum Likelihood (ML) algorithm in IQ-TREE with 5000 bootstraps

Molecular analysis using the Cytochrome b gene

BLAST results for the Cytochrome b gene sequence showed that the Kapiek and Balingka samples from Lake Singkarak had 91%-99% similarity to *B. schwanefeldii* in GenBank, NCBI. This indicates that all samples are *B. schwanefeldii*. Of the 57 Cytochrome b gene sequences analyzed, 25 were obtained from GenBank, NCBI, with the length of the Cytochrome b gene sequence analyzed being 599 bp. In the 57 Cytochrome b gene sequences, there were 400 bp (66.77%) conserved sites and 199 bp (33.23%) variable sites. The percentage of nucleotide bases was 44.6% Guanine+Cytosine (G+C) and 55.4% Adenine+Thymine (A+T). The high number of A+T bases compared to G+C was also found in other freshwater fish (Parmaksiz and Eksi 2017; Alam et al. 2021; Kuang et al. 2021; Fang et al. 2022). Haplotype analysis of 599 bp of Cytochrome b gene from 57 sequences showed 39 different haplotypes. A total of 32 samples of Lake Singkarak were divided into 20 haplotypes. The highest variation was found in Sumani, with 11 haplotypes, while Malalo had the lowest variation, with 2 haplotypes. These findings indicate individual variation in the genus *Barbonymus* in various locations of Lake Singkarak (Ombilin, Sumani, Paninggahan, Sumpur, Malalo). Haplotype diversity (Hd) of Kapiek and Balingka fish was classified as high, between 0.93-1 (Table 11), in accordance with the criteria (Nei and Kumar 2000; Hobbs et al. 2013). However, nucleotide diversity (π) was classified as low, ranging from 0.00338 to 0.00563, which was in accordance with the low criteria (Stephan and Langley 1992; Ouassal et al. 2021).

Table 11. Haplotype diversity (Hd) and Nucleotide diversity (π) of Kapiek and Balingka fish from Lake Singkarak, West Sumatra, Indonesia (Cytochrome b gene)

Location	n	Hn	Hd	π
Paninggahan	7	7	1	0.00418
Ombilin	5	5	1	0.00304
Sumpur	6	5	0.93	0.00563
Malalo	2	2	1	0.00338
Sumani	12	11	0.98	0.00532
All locations	32	30	4.91	0.02155

Note: n: Number of samples; Hn: Number of haplotypes; Hd: Haplotype diversity; π: Nucleotide diversity

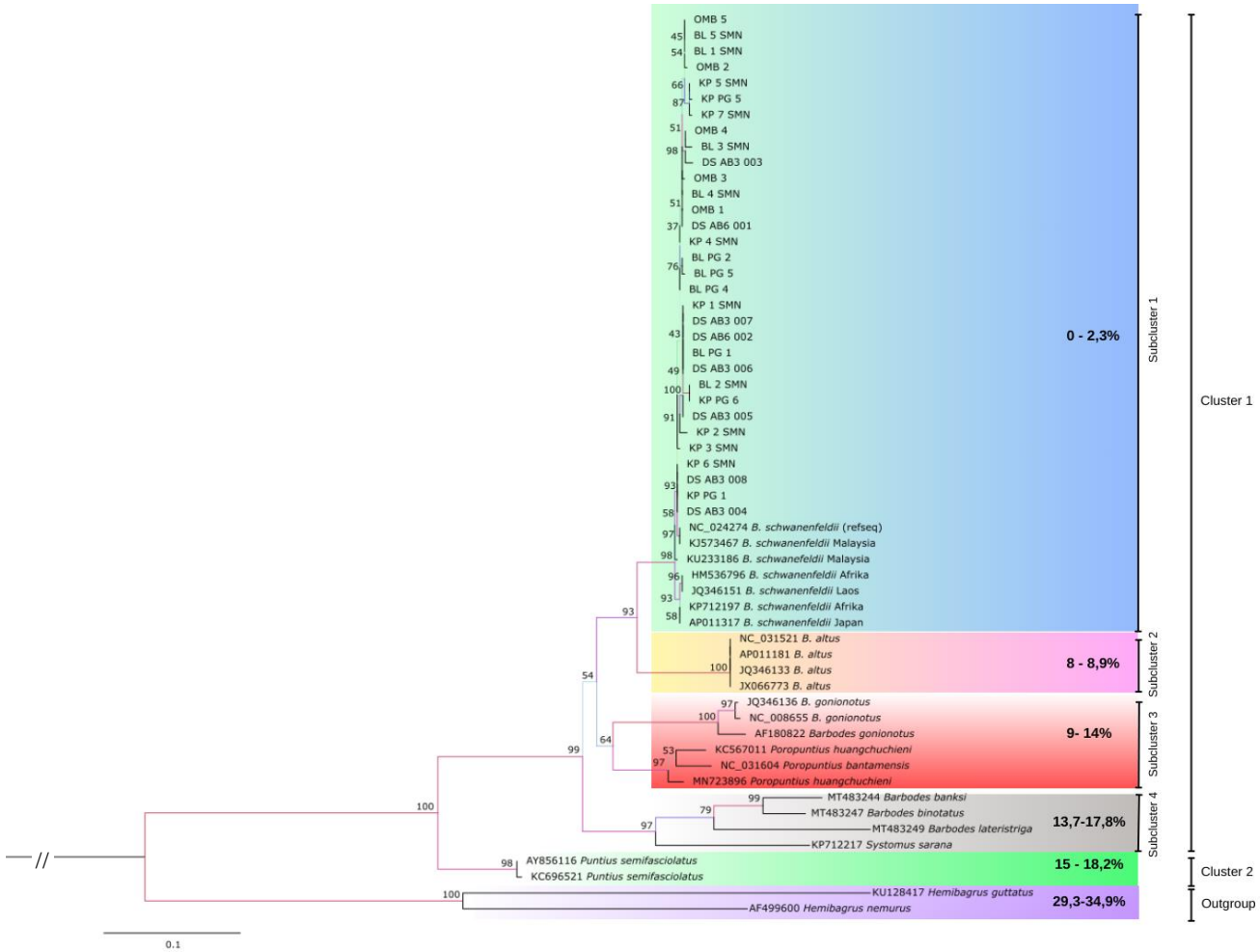

Figure 8. The phylogenetic tree based on Cytochrome b gene using the Maximum Likelihood (ML) algorithm in IQ-TREE with 5000 bootstraps

Analysis of nucleotide base variations in 55 Cytochrome b gene sequences of 599 bp were identified in 20 different positions among 32 samples from Lake Singkarak. A total of 32 samples from Lake Singkarak and *B. schwanefeldii* from various populations showed 29 base differences. These differences were caused by transversion mutations at five positions and transition mutations at 24 other positions. This analysis also found four specific sites in Lake Singkarak samples at positions 3, 5, 6, and 315. Based on the analysis of 599 bp of the Cytochrome b gene from 57 sequences, 32 samples from Lake Singkarak showed six variations of the 199 amino acids produced. Phylogenetic analysis of 57 Cytochrome b gene sequences showed a kinship relationship between Kapiek and Balingka fish (*Barbonymus* spp.) from Lake Singkarak with other species in the genus *Barbonymus* and the family Cyprinidae (Figure 8). The phylogenetic tree has two main clusters. The first cluster has three subclusters: *B. schwanefeldii*, *B. altus*, *B. gonionotus* and the genus *Poropontius*. The second cluster includes species from the genera *Barbodes*, *Systomus*, and *Puntius* in the family Cyprinidae, while the outgroup included the genus *Hemibagrus* (family Bagridae).

Discussion

Morphological differences, especially in the head and tail, are closely related to the adaptation to the environment and living habits of both fish. According to Ellis and Miller (2016) and Taugbøl et al. (2020), differences in the head are related to feeding habits. Factors such as food sources, environment, water flow, depth, and water clarity also affect morphometric variations (Gonzalez-Martinez et al. 2020; Jan and Ahmed 2020; Jawad et al. 2022; Zakiya et al. 2023). Fish that live on the edge of the lake, such as Kapiek, have smaller heads, while fish that live in the middle of the lake with greater pressure, such as Balingka, have larger tails (Jůza et al. 2024). Habitat differences between adult and juvenile fish are also relevant; where adult fish, such as Balingka, are more often found in pelagic habitats (middle of the lake), while juvenile fish, such as Kapiek, are found on the edge of the lake (Desrita et al. 2022). The streamlined body shape, with a narrow tail peduncle, is thought to be adaptive for stable movement in open water for pelagic species (Friedman et al. 2020). In addition, closely related fish that share the same habitat often have morphometric similarities (Li et al. 1993; Tave 1993; Gonzalez-Martines et al. 2021; Zulfahmi et al. 2021; Quadroni et al. 2023). The fact that Balingka and Kapiek

fish are different in size and live in different habitats explains the clear separation in the PCA plot, where the greater the distance between individuals in the plot, the greater their morphometric differences (Modak et al. 2023).

Significant differences in morphometric characters, especially at the Sumani location, are thought to be related to different habitat conditions. Sumani is lotic waters (flowing), while the other locations are lentic waters (stagnant). These differences in water types have an impact on variations in fish morphology. Ribera and Vogler (2000) explained that lotic and lentic waters have different physical conditions, which affect fish morphology. Studies by Dunn et al. (2020) and Calazans et al. (2021) also show that variations in aquatic habitats can affect fish morphological characteristics. The absence of significant variations in several locations, such as Paninggahan-Malalo, Ombilin-Malalo, and Sumpur-Malalo (0%), may be due to environmental similarities between these locations, all of which are lentic waters. Esin et al. (2020), Sahami et al. (2020), and Yulianto et al. (2020), support that environmental and genetic conditions influence fish morphology. The separation of individuals from Sumani in quadrant I on the PCA plot indicates morphological changes in response to the lotic environment. According to Jacquemin and Pyron (2016), Shuai et al. (2018), Fox et al. (2019), and Amoutchi et al. (2023), morphological changes are often caused by adaptation to different environments. Figure 5 shows cluster differentiation, where fishes from Sumani form a separate cluster with the highest Euclidian distance (0.615) compared to fish from Malalo. Modak et al. (2023) explained that the greater the Euclidian distance, the higher the morphological variation between groups. The influence of different environmental conditions can also explain differences in morphological characters. According to Rainboth (1994) and Sahami et al. (2020), fish that live in different habitats, even though they are from the same species, will show significant morphological and genetic variations.

Significant differences in seven morphometric characters between fish from Paninggahan (lentic) and Sumani (lotic) locations are most likely caused by differences in water types. Roa-Fuentes et al. (2015) explained that the body shape of freshwater fish is greatly influenced by feeding habits and habitat types, such as flowing waters (lotic) and still waters (lentic). This is also supported by Calazans et al. (2021), who stated that there is a relationship between changes in fish morphology and their habitat types. The higher differences in body diagonal height and tail peduncle (C4 and D3) in fish from Sumani indicate adaptation to currents in lotic waters. Langerhans (2008), George and Westneat (2019), Sánchez-González et al. (2022) stated that the body morphology with a greater diagonal height and with a larger and narrower caudal peduncle depth factor helps fish swim more efficiently against the current by reducing friction and water pressure. Kelley et al. (2017) also support that morphological variations in fish occur in response to environmental conditions such as water flow. This morphological adaptation allows for more effective movement in habitats with strong currents. The differences in morphometric distribution in the plots also indicate that although fish from Sumani and Paninggahan are in the same quadrant, differences in environmental conditions trigger morphometric variations. According to Roesma and Santoso (2011) and Malik et al. (2020), environmental factors such as water flow and water type can affect morphometric variations in fish, even though they are from the same species. Adaptation to habitat differences results in significant changes in morphological characters. These seven morphometric character differences findings are of utmost importance, as they highlight the significant changes in morphological characters due to adaptation to habitat differences, thereby contributing to the field of biology or ecology.

The meristic characters of Kapiek and Balingka fish tend to be fixed, in contrast to the morphometric characters, which are more varied. According to Murta (2000), Bouzzammit and El Ouizgani (2019), and Uruku et al. (2021), meristic characters are more stable and do not change much throughout the life of the fish, while morphometric characters often change as the fish grows. This is also in line with Labidi et al. (2021), who explained that the meristic characters are discrete or fixed, while morphometric characters are continuous. Strauss (1985) added that the meristic characters do not depend on fish size and do not change during growth, which distinguishes them from morphometric characters, which are influenced by environmental and genetic factors.

Based on the results of genetic analysis using Cytochrome Oxidase-I gene and Cytochrome b gene, it was found that the Kapiek and Balingka fish samples from Lake Singkarak showed significant similarities to the *B. schwanefeldii* species recorded in GenBank and the BOLD System. These results are consistent with the previous findings stating that fish in Lake Singkarak is more suitable to be identified as *B. schwanefeldii* than *B. belinka* (Salis et al. 2024). The genetic differences found between samples from Lake Singkarak and *B. schwanefeldii* populations from other locations were mostly transition and transversion mutations, where transition mutations occurred more often than transversions, in line with previous studies (Roesma et al. 2020; Ude et al. 2020; Dianiputri et al. 2022). In general, haplotype variation and nucleotide diversity in samples from Lake Singkarak were relatively low (Nei and Kumar 2000; Hobbs et al. 2013). Analysis of nucleotide base variations showed that samples from Sumpur in Lake Singkarak had a higher genetic distance compared to other locations. This result indicates a greater genetic variation in fish in Sumpur, possibly due to differences in fishing systems and the presence of floating net cages. Based on interviews, Sumpur fishermen have strict rules regarding fishing time and methods, prohibiting electric devices and explosives. In addition, amino acid differences found in several samples from Lake Singkarak, especially at the Sumani location, may be an adaptive response to different environmental conditions (Cracraft and Helm-Bychowski 1991; Hussain et al. 2018). However, most of the nucleotide variations found did not affect the amino acid composition, indicating that the mutations that

occurred were silent mutations, which did not affect protein function (Fikiye et al. 2023).

Based on phylogenetic analysis of 62 Cytochrome Oxidase-I gene sequences and 57 Cytochrome b gene sequences, the kinship relationship between Kapiek and Balingka fish from Lake Singkarak with species in the genus *Barbonymus* and the family Cyprinidae was successfully identified. The phylogenetic tree generated from the Cytochrome Oxidase-I analysis showed that 32 fish samples from Lake Singkarak were grouped in one main cluster together with *B. schwanefeldii* and several other species in the same genus, such as *B. altus*, *B. gonionotus*, and *B. balleroides*. The genetic distance between samples from Lake Singkarak and *B. schwanefeldii* based on Cytochrome Oxidase-I gene was very low, namely 0-0.3% (Figure 7), which supports the conclusion that the Kapiek and Balingka fish are *B. schwanefeldii* (Batubara et al. 2021; Salis et al. 2024). Meanwhile, the results of Cytochrome b gene analysis also showed the suitability of Kapiek and Balingka fish with *B. schwanefeldii*, with the genetic distance between samples from Lake Singkarak and *B. schwanefeldii* populations from other countries only ranging from 0-2.3% (Figure 8). This distance is within the range of intraspecies divergence commonly found in Cyprinidae fish, which is usually below 3% (Kartavtsev 2011). Further nucleotide variation analysis showed differences in genetic distance between samples from various locations in Lake Singkarak, with higher variations found in the Sumpur area, which may be caused by environmental factors such as differences in fishing systems and floating net cage management practices.

Meanwhile, the *B. belinka* sequence from Aceh registered in the BOLD System has a genetic distance of 7.5-8%, with 32 sequences from Lake Singkarak. Based on this value, it can be concluded that the 32 Kapiek and Balingka sequences from Lake Singkarak are the same species as *B. schwanefeldii*, different from *B. belinka* from Aceh, in accordance with previous research (Salis et al. 2024). Information from personal communication with one of the researchers who reported the *B. belinka* sequence in the BOLD System revealed that the sequence was initially suspected to be *B. gonionotus*, but now it is suspected that it is *B. collingwoodii*. In addition to *B. belinka*, *B. mahakkamensis* is also in the second subcluster with a genetic distance of 6.7-6.9% from the Lake Singkarak sample. With a genetic distance of 6.7-8% between subclusters 1 and 2, it can be concluded that *B. belinka* in the BOLD System and *B. mahakkamensis* are sister taxa to Kapiek, Balingka, and *B. schwanefeldii* fish. The low genetic distance between samples from Lake Singkarak and *B. schwanefeldii* populations in Southeast Asia supports the hypothesis that the rivers in this area may have once been connected, allowing gene flow between fish populations in the region. Overall, these results indicate that the Kapiek and Balingka fish from Lake Singkarak are *B. schwanefeldii*, with a close monophyletic relationship with other populations in Southeast Asia. There has been no strong evidence to support the presence of *B. belinka* in Lake Singkarak, according to the results of the existing morphological and phylogenetic analyses.

Based on this morphological and molecular study, it is evident that the Kapiek and Balingka fish belong to the same species, *B. schwanefeldii*. The observed morphometric differences are attributed to phenotypic variation. Furthermore, both Kapiek and Balingka fish exhibit a monophyletic relationship with *B. schwanefeldii*, confirming that they are genetically the same species.

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