International Journal of Ecophysiology Vol. 04, No. 02, 2022 | 43 - 51

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Internasional Journal of Ecophysiology

Does Chitala Lopis Really Extinct? An Overview And DNA Barcode (COI) Comparison

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Abstract. The Asian Featherback Fish Or Ikan Belida Is One Of The Most Important Species Of Fish In Indonesia That Is Utilised By Locals As A Food Ingredient. Unfortunately, One Of The Species (*Chitala Lopis*) Has Already Been Reported Extinct By The IUCN. Nevertheless, Many Publications After That Still Claimed That *Chitala Lopis* Is Extant. We Study 28 COI Partial Sequences Of *Chitala Lopis* From NCBI. The Result Showed That Most Of These Sequences Were Not The Actual *Chitala Lopis*. It Is Probably Either *Chitala Hypselonotus* Or *Chitala Borneensis*. This Prediction Is Based On The Unmatch Between The Distribution Of *Chitala Lopis* COI Sample Origin And The Actual Habitat Of *Chitala Lopis*, Which Is Endemic To Java Island. However, There Is Also An Article That Reports If *Chitala Lopis* Is Tried To Be Cultivated. The Misinterpretation Is Perhaps Because *Chitala* Spp. Is A Complex Species, Which Has Very Similar Morphology. More Study On Molecular Chitala Sp. In Indonesia Is Still Needed To Complete The Unclear Status Of Extinct Chitala Lopis.

Keyword: *Chitala Lopis,* COI Sequence, Extinct, Genetic Distance, Phylogenetic Tree

Received [15 June 2022] | Revised [21 July 2022] | Accepted [18 August 2022]

1 Introduction

Asian featherbacks are unique freshwater fish that live in the river around southeast Asia, especially Indonesia. They also have morphology like a knife because of the modified ventral fin. In Indonesia, they are called 'Ikan Belida', the name was popularized by the people of Palembang city, Indonesia. These fish have much utilization specifically as food source. Local people of Palembang use these fish as ingredients to make their customary cuisine i.e. pempek, kerupuk, pindang, and kemplang [1]. Nevertheless, this fish habitat is widespread in the freshwater ecosystems across Sumatra, Java, Borneo, and the Malaya peninsula [2]. Ikan Belida

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DOI: 10.32734/ijoep.v4i2.11388
Journal Homepage: http://ijoep.usu.ac.id

lives especially in the river basin including the flooding area around the river periphery for mating and spawning [3].

Exploited for many centuries, the Asian featherback fish population decreased over decades. One of the causes of this phenomenon is environmental degradation either by pollution or development projects [4]. Overfishing is also a problem, local fishermen seem to have no sustainable way to harvest this fish [5]. Not only the river, but the flooding area suronding it should be protected if we want to conserved this fish because the flooding area are the most important for them to spawning [3]. Many try to cultivate this fish but still relatively difficult because the Asian featherbacks are one of the apex predators in freshwater ecosystems, so they need small fish or shrimp as feed [6]. Fortunately, all Ikan Belida species are protected in Indonesia based on the Decree of the Minister of Marine Affairs and Fisheries of the Republic of Indonesia Number 1 2021 concerning Protected Fish Species, also through Minister of Environment and Forestry Regulation No. P.106/MENLHK/SETJEN/KUM.1/12/2018 concerning Protected Plant and Animal Species [7].

Although local people know Ikan Belida as one kind of fish. Actually, there is a number of species of fish called 'Ikan Belida' in Indonesia [2]. However, the taxonomy study of 'Ikan Belida' in Indonesia is relatively few and then *Chitala lopis* already report as extinct species in Java [4]. After publishing as an extinct species by IUCN, *Chitala lopis* still reported in many articles as extant species but indeed not in Java [4] [6]. There is probably a misinterpretation among the fish scientist considering the taxonomy of Ikan Belida. Here we want to understand the misinterpretation between articles about *Chitala lopis*.

2 Materials and Methods

We collected all articles that mention *Chitala lopis* after the extinct status publication and molecular research on *Chitala lopis* outside Java island. We are using google scholar with the keyword as *Chitala lopis* or *Chitala lopis Cytochrome c oxidase subunit I* (*COI*) DNA barcode. We also search *Chitala lopis COI gene* in National Center for Biotechnology Information (NCBI) to make a comparison between them to other *Chitala* species using a phylogenetic tree. The genetic distance between *Chitala lopis* and other *Chitala* species is also analyzed. We are using maximum likelihood parsimony to construct the phylogenetic tree. Another Notopteridae member fish is used as an outgroup.

3 Result and Discussion

We found there are many *COI* sequences of *Chitala lopis* in NCBI, but most of them is partial sequence (see Table 1). We selected 28 *COI* sequences of *Chitala lopis* to process the genetic distance between them and other Chitala species. We used *Notopterus notopterus* from Java

island as an outgroup to make *Chitala lopis* phylogenetic tree. The genetic distance between them is relatively smaller (around 0,3%) than to other *Chitala* species (around 7,3%) (see Figure 1), indicating that all sequence comes from the same species. However, some sequence of *Chitala lopis* from Borneo (Belitung River, Empangau Lake, dan Soehait) has a relatively bigger genetic distance (around 3,1%). This probably indicated there are more than one species or subspecies between them. Still, we doubt if all of *Chitala lopis COI* sequences in NCBI came from the extinct *Chitala lopis* announced by IUCN that was endemic to Java island (Figure 3) [4]. This is because most of them were extracted from outside Java, the only sample from Java is from the Cisadane River in West Java but it looks like this sample was introduced to that river from Koto Panjang Reservoir, Riau Province because of no genetic distance between them (see Figure 1).

Table 1. List of Sequences Used

No	Species	Accession Number	Origin
1	*Chitala ornata*	HQ682678.1	Philippines: Laguna de Bay, Binangonan
2	*Chitala ornata*	KF805383.1	Thailand
3	*Chitala chitala*	MK577977.1	India: Assam
4	*Chitala chitala*	MK572123.1	Bangladesh: Dhaka
5	*Chitala blanci*	AP008921	Thailand
6	*Chitala lopis*	OP953267.1	Indonesia: Borneo, Kotawaringin Barat
7	*Chitala lopis*	OP953265.1	Indonesia: Borneo. Seruyan
8	*Chitala lopis*	OP953247.1	Indonesia: Borneo, Riam Kanan Reservoir
9	*Chitala lopis*	OP953260.1	Indonesia: Borneo, Katingan
10	*Chitala lopis*	OP953257.1	Indonesia: Borneo, Timpah
11	*Chitala lopis*	OP953251.1	Indonesia: Borneo, Buntok
12	*Chitala lopis*	KT001050.1	Malaysia
13	*Chitala lopis*	KM213054.1	Indonesia: Sumatra, Riau, Kampar River
14	*Chitala lopis*	OP953208.1	Indonesia: Java, Cisadae River
15	*Chitala lopis*	OP953294.1	Indonesia: Sumatra, WayKanan
16	*Chitala lopis*	OP953292.1	Indonesia: Sumatra, WayKiri
17	*Chitala lopis*	OP953279.1	Indonesia: Sumatra, Koto Panjang Reservoir
18	*Chitala lopis*	NC 012711.1[8]	Malaysia
19	*Chitala lopis*	AP008922.1[8]	Malaysia
20	*Chitala lopis*	OP953285.1	Indonesia: Sumatra, Taratak buluh
21	*Chitala lopis*	OP953214.1	Indonesia: Borneo, Tempura lake
22	*Chitala lopis*	OP953212.1	Indonesia: Borneo, Empangau lake
23	*Chitala lopis*	OP953221.1	Indonesia: Borneo, Embaloh River

24	*Chitala lopis*	OP953274.1	Indonesia: Sumatra, Musi River
25	*Chitala lopis*	OP953230.1	Indonesia: Borneo, Nibung River
26	*Chitala lopis*	OP953227.1	Indonesia: Borneo, Sintang
27	*Chitala lopis*	OP953291.1	Indonesia: Sumatra, Sekayu
28	*Chitala lopis*	OP953277.1	Indonesia: Sumatra, Muaro Belida
29	*Chitala lopis*	OP953209.1	Indonesia: Sumatra, Muaro Tebo
30	*Chitala lopis*	OP953216.1	Indonesia: Borneo, Belitung River
31	*Chitala lopis*	OP953211.1	Indonesia: Borneo, Empangau lake
32	*Chitala lopis*	OP953219.1	Indonesia: Borneo, Soehait
33	*Chitala lopis*	OP953273.1	Indonesia: Sumatra, Pedamaran
34	*Notopterus notopterus*	KU692675.1	Indonesia: Jawa Tengah, Ambarawa
35	*Notopterus notopterus*	KU692674.1	Indonesia: Jawa Timur, Mojokerto

The rest data sequences are unpublished.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1 C_ornata_COI_HQ682678.1_Philippines																																
2 C_ornata_COI_KF805383.1_Thailand	0,000																															
3 C_chitala_COI_MK577977.1_Assam_India	0,094	0,094																														
4 C_chitala_COI_MK572123.1_Dhaka_Bangladesh	0,083	0,083	0,014																													
5 C_blanci_COI_AP008921	0,109	0,109	0,074	0,059																												
6 C_lopis_COI_OP953267.1_KotawaringinBarat_Borneo	0,082	0,082	0,063	0,048	0,076																											
7 C_lopis_COI_OP953265.1_Seruyan_Borneo	0,082	0,082	0,063	0,048	0,076	0,000																										
8 C_lopis_COI_OP953247.1_RiamKananReservoir_Borneo	0,082	0,082	0,063	0,048	0,076	0,000	0,000																									
9 C_lopis_COI_OP953260.1_Katingan_Borneo	0,080	0,080	0,065	0,050	0,074	0,002	0,002	0,002																								
10 C_lopis_COI_OP953257.1_Timpah_Borneo	0,080	0,080	0,065	0,050	0,074	0,002	0,002	0,002	0,000																							
11 C_lopis_COI_OP953251.1_Buntok_Borneo	0,080	0,080	0,065	0,050	0,074	0,002	0,002	0,002	0,000	0,000																						
12 C_lopis_COI_KT001050.1_Malaysia	0,083	0,083	0,063	0,048	0,076	0,016	0,016	0,016	0,018	0,018	0,018																					
13 C_lopis_COI_KM213054.1_Kampar_Riau	0,083	0,083	0,063	0,048	0,076	0,016	0,016	0,016	0,018	0,018	0,018	0,000																				
14 C_lopis_COI_OP953208.1_Cisadane_Java	0,083	0,083	0,063	0,048	0,076	0,016	0,016	0,016	0,018	0,018	0,018	0,000	0,000																			
15 C_lopis_COI_OP953294.1_WayKanan_Sumatra	0,083	0,083	0,063	0,048	0,076	0,016	0,016	0,016	0,018	0,018	0,018	0,000	0,000	0,000																		
16 C_lopis_COI_OP953292.1_WayKiri_Sumatra	0,083	0,083	0,063	0,048	0,076	0,016	0,016	0,016	0,018	0,018	0,018	0,000	0,000	0,000	0,000																	
17 C_lopis_COI_OP953279.1_KutoPanjangReservoir_Sumatra	0,083	0,083	0,063	0,048	0,076	0,016	0,016	0,016	0,018	0,018	0,018	0,000	0,000	0,000	0,000	0,000																
18 C_lopis_COI_NC_012711.1_Malaysia	0,083	0,083	0,063	0,048	0,076	0,016	0,016	0,016	0,018	0,018	0,018	0,000	0,000	0,000	0,000	0,000	0,000															
19 C_lopis_COI_AP008922.1_Malaysia	0,083	0,083	0,063	0,048	0,076	0,016	0,016	0,016	0,018	0,018	0,018	0,000	0,000	0,000	0,000	0,000	0,000	0,000														
20 C_lopis_COI_OP953285.1_TaratakBuluh_Sumatra	0,083	0,083	0,063	0,048	0,076	0,016	0,016	0,016	0,018	0,018	0,018	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000													
21 C_lopis_COI_OP953214.1_TempuraLake_Borneo	0,090	0,090	0,065	0,050	0,078	0,018	0,018	0,018	0,020	0,020	0,020	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006												
22 C_lopis_COI_OP953212.1_EmpangauLake_Borneo	0,090	0,090	0,065	0,050	0,078	0,018	0,018	0,018	0,020	0,020	0,020	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,000										
23 C_lopis_COI_OP953221.1_EmbalohRiver_Borneo	0,090	0,090	0,065	0,050	0,078	0,018	0,018	0,018	0,020	0,020	0,020	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,000	0,000									
24 C_lopis_COI_OP953274.1Musi_Sumatra	0,090	0,090	0,065	0,050	0,078	0,018	0,018	0,018	0,020	0,020	0,020	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,000	0,000	0,000								
25 C_lopis_COI_OP953273.1_Pedamaran_Sumatra	0,090	0,090	0,065	0,050	0,078	0,018	0,018	0,018	0,020	0,020	0,020	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,000	0,000	0,000	0,000							
26 C_lopis_COI_OP953230.1_NibungRiver_Borneo	0,090	0,090	0,065	0,050	0,078	0,018	0,018	0,018	0,020	0,020	0,020	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,000	0,000	0,000	0,000	0,000						
27 C_lopis_COI_OP953227.1_Sintang_Borneo	0,090	0,090	0,065	0,050	0,078	0,018	0,018	0,018	0,020	0,020	0,020	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,000	0,000	0,000	0,000	0,000	0,000					
28 C_lopis_COI_OP953291.1_Sekayu_Sumatra	0,090	0,090	0,065	0,050	0,078	0,018	0,018	0,018	0,020	0,020	0,020	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,006	0,000	0,000	0,000	0,000	0,000	0,000	0,000				
29 C_lopis_COI_OP953277.1_MuaroBelida_Sumatra	0,088	0,088	0,067	0,052	0,080	0,020	0,020	0,020	0,022	0,022	0,022	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002				
30 C_lopis_COI_OP953209.1_MuaraTebo_Sumatra	0,088	0,088	0,067	0,052	0,080	0,020	0,020	0,020	0,022	0,022	0,022	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,008	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,002	0,000			
31 C_lopis_COI_OP953216.1_BelitungRiver_Borneo	0,096	0,096	0,065	0,050	0,093	0,030	0,030	0,030	0,033	0,033	0,033	0,018	0,018	0,018	0,018	0,018	0,018	0,018	0,018	0,018	0,020	0,020	0,020	0,020	0,020	0,020	0,020	0,020	0,020	0,022	0,022	
32 C_lopis_COI_OP953211.1_EmpangauLake_Borneo	0,096	0,096	0,065	0,050	0,093	0,030	0,030	0,030	0,033	0,033	0,033	0,018	0,018	0,018	0,018	0,018	0,018	0,018	0,018	0,018	0,020	0,020	0,020	0,020	0,020	0,020	0,020	0,020	0,020	0,022	0,022	0,000
33 C_lopis_COI_OP953219.1_Soehait_Borneo	0,096	0,096	0,065	0,050	0,093	0,030	0,030	0,030	0,033	0,033	0,033	0,018	0,018	0,018	0,018	0,018	0,018	0,018	0,018	0,018	0,020	0,020	0,020	0,020	0,020	0,020	0,020	0,020	0,020	0,022	0,022	0,000	0,000

Figure 1. Estimates of Evolutionary Divergence between Sequences. The number of base substitutions per site from between sequences are shown. Analyses were conducted using the Maximum Composite Likelihood model [9]. The rate variation among sites was modeled with a gamma distribution (shape parameter = 1). This analysis involved 33 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 520 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [10]

The phylogenetic tree also supports the supposition of there is more than one species or subspecies within the *Chitala lopis* COI sequence from NCBI (see Figure 2). There are 4 species of Ikan Belida in Indonesia i.e. *Chitala borneensis* (Bornean Belida), *Chitala*

hypselonotus (Sumatran Belida), *Notopterus notopterus* (Java Belida), and *Chitala lopis* (Java lopis Belida) [2]. We predicted that all COI sequences of *Chitala lopis* in NCBI actually belong to *Chitala hypselonotus*, because the only *Chitala species* that lived in Sumatra, Borneo island, and Malaya peninsula is *Chitala hypselonotus* (see Figure 4) [11], *Chitala borneensis* only lived in Sumatra and Borneo island (see Figure 5) [12]. Some of the *Chitala lopis* COI sequences from Borneo make their own clade, perhaps indicating that is *Chitala borneensis*. unfortunately, neither *Chitala hypselonotus* COI sequence nor *Chitala borneensis* available in NCBI.

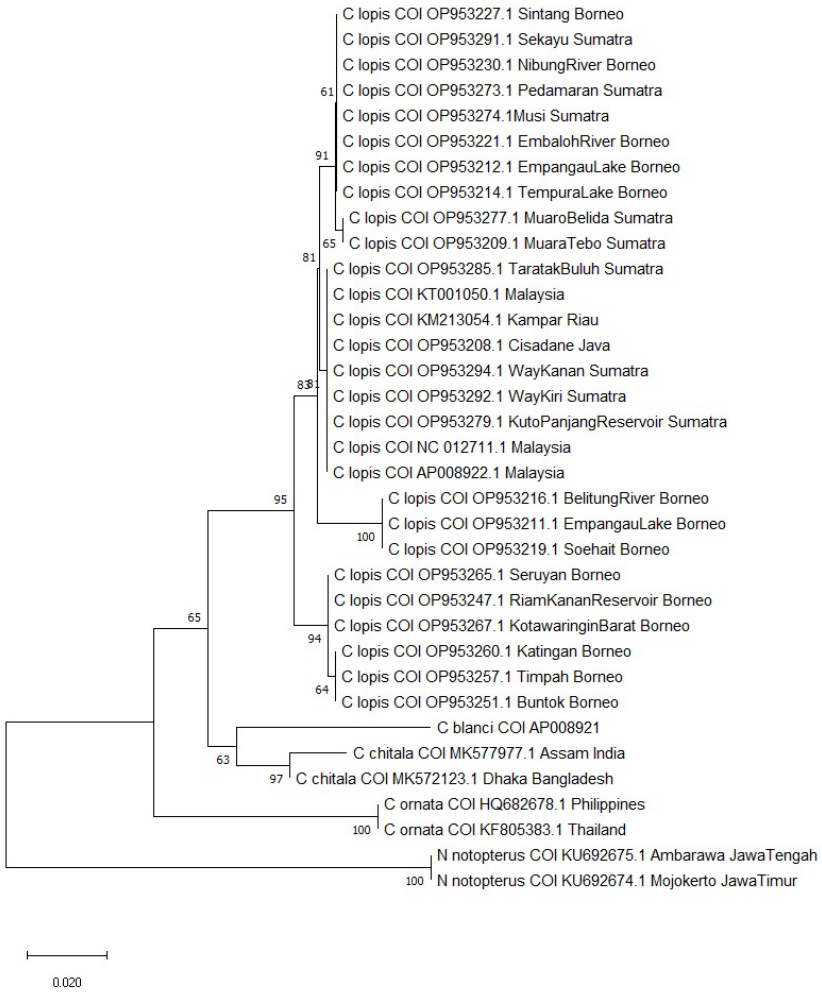

Figure 2. Evolutionary analysis by Maximum Likelihood method .The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model [13]. The tree with the highest log likelihood (-1413.39) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.2802)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 35 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 520 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [10]

Another study using COI DNA barcode to the Chitala lopis samples also supports our prediction, that there are two species that are mistaken for Chitala lopis. The samples of Chitala lopis are collected from South Sumatra Province in the Musi River and PLN cultivation ponds, which should not be the habitat of Chitala lopis. Their phylogenetic tree has the same pattern as ours, there are 2 clades separated. The correct species should be Chitala hypselonotus and/or Chitala borneensis [14].

All species of Ikan Belida in Indonesia are in stable populations with the Least concern status from IUCN (*Chitala borneensis* [12], *Chitala hypselonotus* [11], *Notoptorus notoptorus* [15]), except for *Chitala lopis* which is already extinct [4]. The research that explains the difference between them is still only based on morphology [16]. Moreover, *Chitala* spp. is a complex species that have very similar morphology and overlapping in habitat [17]. Nevertheless, the isolation could still separate them, either through geography or behaviour. Research about the extinct species of *Chitala lopis* still remains unclear because many articles still claim to have that species extant [4] [18], even try to cultivate them [6].

Figure 3. *Geographic Range of Chitala lopis* [4]

Figure 4. Geographic Range of *Chitala hypselonotus* [11]

Figure 5. Geographic Range of *Chitala borneensis* [12]

4. Conclusion

The misinterpretation between fish scientists regarding the status of *Chitala lopis* looks like come from the NCBI database which presumably uses the incorrect species name for the gene sequence. This statement is supported by the unmatch distribution pattern between the actual *Chitala lopis* and *Chitala lopis* sample from the NCBI database. We predict the actual species name of *Chitala lopis* from NCBI is either *Chitala hypselonotus* or *Chitala borneensis*. Fortunately, there is news reported that a BRIN scientist has success to collect the actual *Chitala lopis* and for now the sample is kept in an aquarium of Taman Mini Indonesia Indah (TMMI) [19]. We suggest an immediate molecular study on at least which claimed to be the last specimens of Chitala lopis, better using Next Generation Sequencing (NGS) to collect the whole genome of the species.

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