

Research Article

Genetic Diversity and Albumin Content of Striped Snakehead *Channa striata* in Indonesia: A Study on Farmed and Wild Fish from Five Main Islands

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ABSTRACT

Genetic characterisation and key traits of species are crucial in determining the direction of aquaculture development. The genetic diversity of the striped snakehead (*Channa striata*) across five main islands in Indonesia, both from aquaculture and wild populations, was explored through its partial mtDNA COI. Subsequently, this study examined the albumin content extracted from the meat and comprehensively aligned it with the variation in its genotype. Phylogenetic studies showed the Sumatran snakehead fish sample forms its own cluster and has a large genetic distance from other samples. Interestingly, the albumin content in the Sumatra samples was significantly higher (1.907 %) than in others (1.253-1.509 %). Further analysis of albumin content from the Borneo samples from the same taxa in the phylogenetic tree showed no significant difference between farmed and wild *C. striata*. The findings of this study are fundamental for initiating a selective breeding programme and developing conservation management.

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INTRODUCTION

More than forty species of the genus *Channa* are native to Malaysia, Indonesia, and Peninsular Asia (Courtenay & Williams 2004). One of them is the striped snakehead, *Channa striata* (Bloch 1793), which is well known in biomedical fields in many local Asian communities and is an important species in both fisheries and aquaculture. Froese and Pauly (2010) stated that *C. striata* is one of the most widely distributed snakehead species, with a native range covering southern China, Pakistan, most of India, southern Nepal, Bangladesh, Sri Lanka, and most Southeast Asian countries. A biodiversity assessment study in Bangladesh (Bashar et al. 2020) revealed that snakeheads decreased in the catch composition.

Albumin is an important component in biomedical applications and is abundantly found in *C. striata*. It is well known for its postoperative medicinal application to enhance wound healing and reduce postoperative pain and discomfort (Ab Wahab et al. 2015). In addition, local market demand for snakehead fish in Indonesia is greatly expanding due to its commercial value, its pleasant flavour in local food (especially in Borneo Island), and the biomedical benefits already known by local communities (Song et al. 2013). Due to high demand, striped snakehead has been cultured commercially in Indonesia since the 2000s.

The availability of *C. striata* in Indonesia mostly comes from the wild or capture due to the exploitation of open waters. The Ministry of Marine Affairs and Fisheries of the Republic of Indonesia (2018) stated that aquaculture contributes only 14 % of the total supply, although *C. striata* farming in Indonesia has already been carried out for a decade. There is limited genetic knowledge regarding the populations of *C. striata*, whereas this field is crucial to understanding and developing sustainable aquaculture, biotechnology, and conservation programmes. Studies conducted in Peninsular Malaysia (Ambak et al. 2006; Tan et al. 2012) and Mekong drainage (Adamson et al. 2010) highlighted that *C. striata* is a relatively non-migratory species, sharing common haplotypes among adjacent populations.

Hence, research on genetic variation and albumin content research in *C. striata* is needed to fundamentally support aquaculture activities, including domestication, breeding, and sustainable fisheries management. Furthermore, it can also contribute to pharmaceutical applications. This study focuses on *C. striata* samples of five main islands, spanning the northern to the western parts of Indonesia, examining the genetic characteristics and diversity inferred from the mtDNA cytochrome c oxidase subunit 1 (COI) gene. In addition, the albumin characteristics of *C. striata* from aquaculture and the wild are also covered in this study.

MATERIALS AND METHODS

Animal rights

The care and use of experimental animals complied with the UGM Ethics Committee animal welfare laws, guidelines, and policies as approved by the certificate No.00013/04/LPPT/I/2020.

Samples collection

Random samples of individuals (Table 1) were collected from a total of 13 locations distributed throughout Sumatra, Java, Borneo, Sulawesi, and Papua (Figure 1). Sampling locations were divided into five regions following the main islands of Indonesia.

Genome Isolation

Fin clips for DNA isolation were excised from the dorsal fin rays. These clips were approximately 2 cm² in size and preserved in 1.5 mL tubes containing




Figure 1. (a) Sampling sites of selected locations of farmed and wild *C. striata* from the five main islands in Indonesia and (b) Sampling sites of selected locations of Borneo Island. All sampling sites are indicated with .

Table 1. Sampling locality, coordinate, and collection date of *C. striata* in this study.

Island/Region	Location	Latitude (North)	Longitude (East)	Date Collected
Sumatra	Swamp - Palembang South Sumatra (1)	3° 04' 04"	104° 66' 48"	7.10.2019
Java	Lake - Ngawi, East Java (1)	7° 23' 55"	111° 34' 27"	20.8.2019
	Aquaculture - Yogyakarta Special Region (2)	7° 75' 42"	110° 43' 40"	19.10.2019
Borneo	Lake - Banjar, South Kalimantan (1)	3° 31' 11"	115° 00' 34"	6.8.2019
	Swamp - Hulu Sungai Selatan, South Kalimantan (2)	2° 66' 88"	115° 20' 17"	7.8.2019
	Estuary - Banjar, South Kalimantan (3)	3° 32' 44"	114° 77' 91"	8.8.2019
	Aquaculture Center - Banjar, South Kalimantan (4)	3° 29' 52"	114° 55' 58"	9.8.2019
	Aquaculture - Banjar, South Kalimantan (4.2)	3° 43' 12"	114° 88' 08"	10.8.2019
	Swamp - Barito Kuala South Kalimantan (5)	3° 00' 47"	114° 74' 67"	11.8.2019
	Littoral - Banjar South Kalimantan (6)	3° 45' 98"	114° 52' 84"	12.8.2019
	Swamp - Pulang Pisau, Central Kalimantan (7)	2° 64' 51"	114° 20' 63"	15.8.2019
Sulawesi	Swamp-Makassar South Sulawesi (1)	5° 08' 00"	119° 25' 04"	29.10.2019
Papua	Swamp -Merauke, Papua (1)	8° 28' 42"	140° 22' 51"	22.9.2019

95 % ethanol. Samples were stored at a temperature of -20 °C until use. The genome was isolated from fin clip samples using a TNES method (Wasko et al. 2003). A total of 24 samples were used for genome isolation, with 2 samples from each population (Borneo (7), Java (2), Sumatra (1), Sulawesi (1), and Papua (1)).

DNA amplification and sequencing

The COI gene from snakehead fish samples was amplified using combinations of the following forward and reverse primers: (COIF): 5'-

TCACACGTTGATTTTTCTCGACT-3' and (COIR): 5'-AATAAGCGCGTGTGTCAACG-3' (Hardaningsih et al. 2025). Amplification was conducted by Polymerase Chain Reaction (PCR) performed in a 50 mL reaction volume by using *My Taq* Extract-PCR Kit (Bioline®). Approximately 100 ng of genomic DNA, 1 mM of each primer, 2x *My Taq* Extract-PCR Kit, and Nuclease Free Water with a total reaction volume of 50 ml. The amplicons of approximately 906 bp were subsequently amplified under the following thermal conditions; initial denaturation at 94 °C (5 min), 35 cycles of 94 °C (30 sec), 50 °C (30 sec), 72 °C (1 min), and a final extension at 72 °C (5 min) before termination of the reaction at 12 °C (Hardaningsih et al. 2025). The PCR products were visualised on a 1 % agarose gel stained with Florosafe DNA stain (1st Base®). The PCR products were sent for DNA sequencing to 1st BASE Laboratories Sdn Bhd, Selangor, Malaysia. They were analysed using forward and reverse primers for the first batch, and only the forward primer for the next batch.

Data analysis

The sequences were aligned and compiled for editing using ClustalW implemented in MEGA v. 5.0 (Tamura et al. 2011). To ensure accuracy, the DNA strands were sequenced in both directions (COIF and COIR) for each individual in the first batch. Both DNA strands were checked for ambiguous bases by comparing them with chromatograms and editing them manually. BLAST (Basic Local Alignment Search Tools) analysis on the NCBI (National Center for Biotechnology Information) website (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_SPEC=GeoBlast&PAGE_TYPE=blastSearch) is used to confirm species. The phylogenetic tree was constructed using Maximum Likelihood (ML) methods with the Kimura 2-parameter evolution model and 1000× bootstrap replications (Tamura et al. 2011).

Albumin extraction

The extraction method used in this study was carried out on raw fish meat using cold extraction (Asfar et al. 2019). A total of 39 samples were used for albumin extraction, with 3 samples from each population: Borneo (8), Java (2), Sumatra (1), Sulawesi (1), and Papua (1). Specimens weighing 200–500 g each were weighed, cleaned, and filleted. A total of 40 grams of meat from each fish was used for albumin extraction and stored at -25 °C until use. The fish fillets were then cut into small pieces, weighed, and blended using 0.9 % NaCl solution at a ratio of 1:1. Next, the homogenate was centrifuged at 2500 g at 4 °C for 40 minutes. The supernatant obtained was adjusted to a pH of 4.6 by adding HCl or NaOH, which is known to be the isoelectric point of fish albumin (Ramdhani et al. 2019). The homogenate was centrifuged at 2500 g at 4 °C for 45 minutes to form a precipitate, then incubated at 5-10 °C. The precipitate obtained was then dried by heating at 45 °C until it reached a constant weight. The albumin obtained was crushed to form a powder. The extraction was replicated three times for each sample.

Albumin content analysis

All values of albumin content were presented as means for each group. Albumin datasets were analysed statistically using Analysis of Variance (ANOVA), followed by the Tukey test at $\alpha = 0.05$. SPSS v. 20 was used for statistical analysis. An independent T-test was used to determine a statistically significant differences between the wild (Borneo 1, 2, 3, 6, 7) and farmed (Borneo 4.1 and 4.2) samples from the same haplotype.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was conducted to analyse the albumin protein molecular masses of the extracted *C. striata* according to Laemmli (1970) with modifications.

larities can be explained by common ancestry. Although the Sumatra samples were in a different clade from the other samples from main islands in Indonesia, they still share a common ancestor. Two sequences from NCBI were used as an outgroup. High bootstrap values supported the phylogenetic tree in each group branch. These data show that the phylogenetic tree construction has a high accuracy level, which confirming that *C. striata* from Indonesia shares a common ancestor.

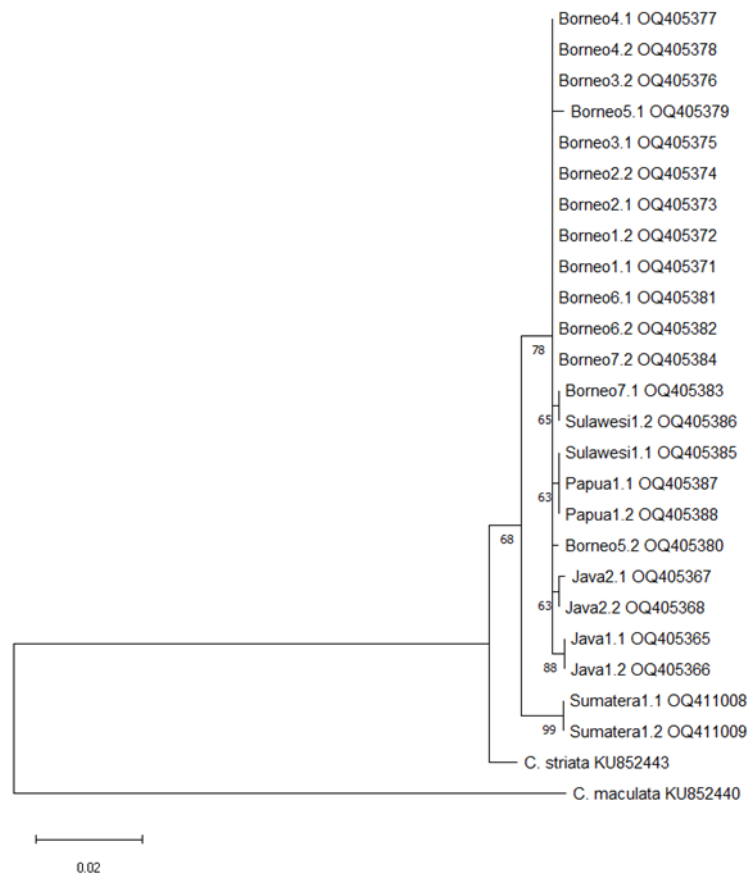


Figure 3. Phylogenetic tree of *C. striata* from the five main islands in Indonesia using Maximum Likelihood inferred from DNA sequences of the mitochondrial gene COI.

Albumin Content

Characterisation of albumin by SDS-PAGE showed that the albumin isolate consisted of seven protein bands with two major bands and five minor bands (Figure 4a). These protein bands ranged from 14 to 133 kDa. Two major bands were observed at 27 and 133 kDa, with one minor band at 14 kDa, and the three minor bands in the 25–39 kDa range. One minor band corresponds to the size of BSA at 66 kDa. This result aligns with Nugroho (2013), who conducted albumin isolation from *C. striata* by Bromocresol Green (BCG) and generated the same electrophoretic result band profiles, with two minor bands and five major bands in the range of 14–133 kDa. Meanwhile, other studies on albumin isolation in *Salmo salar* (Maillou & Nimmo 1993) resulted in a broad-spectrum fraction ranging from 46–145 kDa. Proteomic analysis of *C. striata* muscle tissue by Gam et al. (2006) found that the protein profiles of fish with different lengths and catch months did not differ significantly and were evenly distributed across a molecular masses from 10 to 205 kDa, detected in 16 protein bands. In addition, the identification of crude protein from *C. pleurophthalmus* by Aryani et al. (2020) showed 27 protein bands with molecular weights ranging from 10.202 to 134.432 kDa. Andreeva (2010)

stated that albumin identification in fish is rather difficult due to the atypical location of mobile plasma fractions in electropherograms, which do not correspond with the mobility of mammalian albumin. Low-molecular-weight fractions are often represented in electrophoresis by multiple bands or spots with unclear borders, overlapping the fractions. This statement leads to confusion in terminology, so some authors use the term albumin or albumin-like protein. All bands or fractions found in this study were considered total albumin, based on the broad spectrum of albumin in fish.

Figure 4b shows that the albumin content obtained in this study ranged from 1.253 to 1.907 % with an average value of 1.45 ± 0.15 %. The highest value was found in the Sumatra samples (1.907 ± 0.11 %), and the lowest in the Java 2 sample (1.253 ± 0.15 %). The Sumatra sample had the highest albumin content and was significantly different ($P < 0.05$) from the Borneo 4.1, Borneo 4.2, Borneo 6, Borneo 7, Java 2, Papua, and Sulawesi samples.

Genetic variation and albumin content analyses continued at the same taxonomic level, involving both wild and farmed *C. striata*. Based on the genetic variation and reconstruction of phylogenetic trees using mtDNA marker, all *C. striata* from the Borneo samples belonged to the same taxon, except for the sample labelled Borneo 5. Figure 4c shows that the albumin content of *C. striata* among the samples was not significantly different. The average albumin content of farmed *C. striata*, as represented by Borneo 4.1 and Borneo 4.2, was lower (1.20 ± 0.01 %) than that of wild *C. striata* (1.28 ± 0.05 %) but this difference was not statistically significant. This study indicates that *C.*

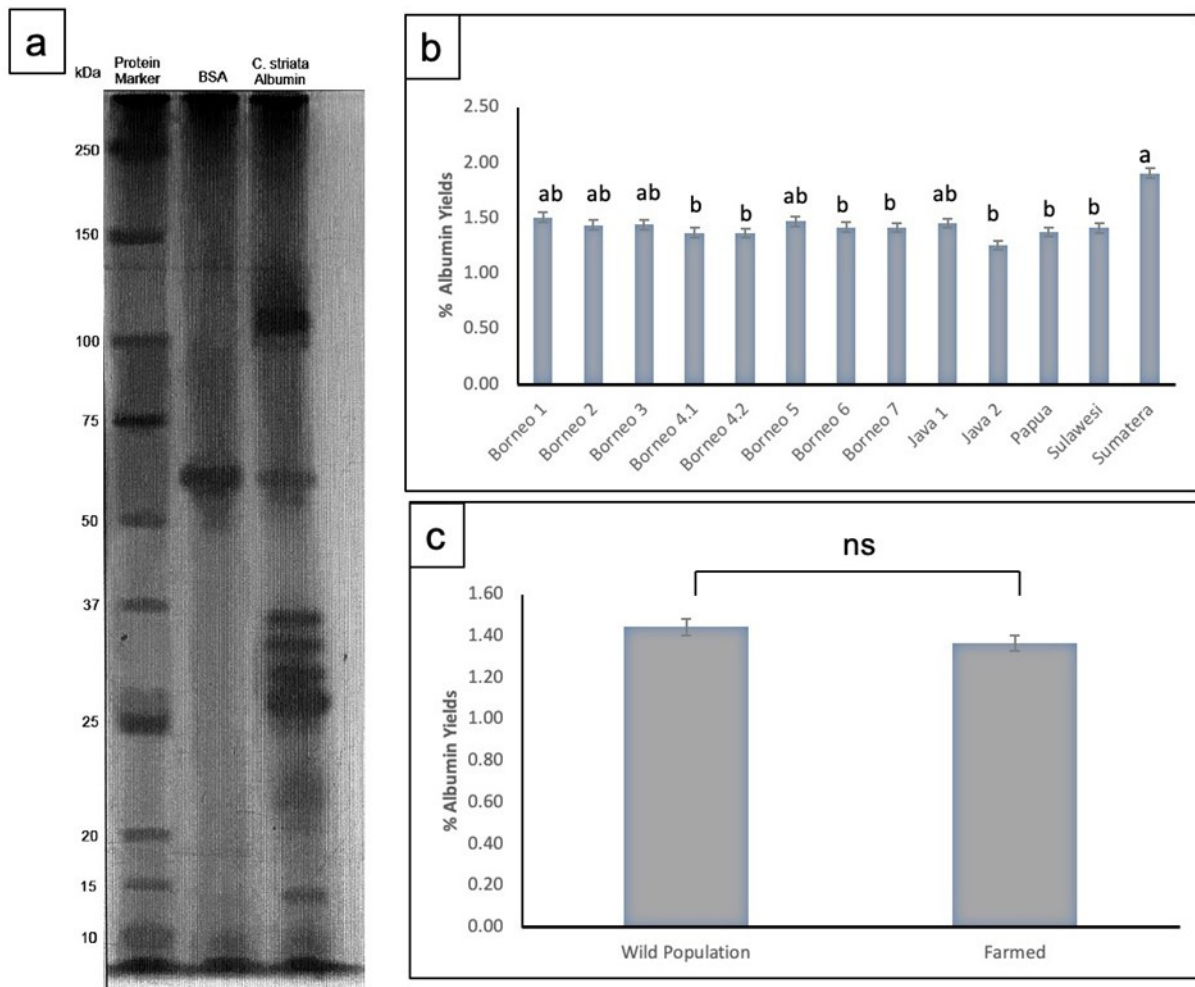


Figure 4. Albumin content of *C. striata* from the five main islands in Indonesia. (a) Analysis by Polyacrylamide Gel Electrophoresis of Albumin extracted from *C. striata* muscle compared with Bovine Serum Albumin and protein marker; (b) Albumin yield of *C. striata* from all locations. Significant differences ($P < 0.05$) shown by different superscripts; (c) Albumin concentration of *C. striata* from wild (Borneo 1, 2, 3, 6 and 7) and farmed (Borneo 4.1 and 4.2) under the same taxa samples ($P < 0.05$).

striata of the same genetic taxon, whether from aquaculture or the wild, have albumin contents that do not differ significantly. In addition, based on the proximate analysis (Table S1), the average total protein of *C. striata* meat was 17.07 ± 2.25 %, with the highest protein (21.3 %) found in the swamp sample from Borneo Island (Borneo 2). Furthermore, albumin in *C. striata* contributed 8.62 ± 1.45 % of the total protein.

In this study (Table S2), albumin extraction was also carried out on tilapia *Oreochromis niloticus*. The albumin yield of *O. niloticus* was lower than that of *C. striata* (0.57 ± 0.04 %). A previous study on the albumin concentration of various species by Fatma et al. (2020) found that *C. striata* had a higher albumin yield than *Decapterus russelli* and *Lates calcarifer*. This result suggests that albumin is abundantly found in *C. striata* and is higher than in certain fish species commonly found in brackish water and marine environments. Based on the evidence, this study proves that, using the same method and under the same circumstances, albumin extracted from *C. striata* muscle tissue is higher than that of *O. niloticus*, a common freshwater aquaculture commodity.

CONCLUSIONS

Based on the profile of genetic variations and albumin contents of *C. striata* in this study, it was found that *C. striata* from Sumatra are genetically formed in a different clade on the phylogenetic tree. The albumin content of *C. striata* in Indonesia varies; significant differences were found between the Sumatra samples and most Indonesian samples from Borneo, Java, Sulawesi, and Papua. There were no significant differences in the albumin content of *C. striata* from farmed and wild individuals of the same taxon. This research could serve as a foundational study and be considered in the development of aquaculture, e.g., broodstock selection, and the improvement of Indonesia's fisheries resource management. Furthermore, further studies on functional genomics can be developed from these findings. It is necessary to build up an understanding of the gene networks underlying important traits such as albumin content.

AUTHOR CONTRIBUTIONS

R.H., P., and D.W.K.S. performed experiments and/or data analyses. D.W.K.S. conceived the project and designed the experiments. R.H. and D.W.K.S. wrote the manuscript. All authors reviewed the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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APPENDICES

Table S1. Nutritional value of *C. striata* based on proximate analysis with the following methods: Kjeldahl for total protein; Soxhlet for total lipid, Gravimetry for ash and moisture; and by difference for carbohydrate

Samples	Ash (%)	Total Fat (%)	Carbohydrate (%)	Total Protein (%)	Moisture (%)
Borneo 1	1.37	0.8	2.8	18.06	76.97
Borneo 2	1.08	0.97	2.68	21.3	73.97
Borneo 3	0.91	1.65	2.23	16.38	78.83
Borneo 4.1	0.9	0.58	0.81	18.88	78.83
Borneo 4.2	0.7	0.47	1.21	17.31	80.31
Borneo 5	0.97	1.53	0.6	16.18	80.72
Borneo 6	1.03	0.73	1.54	18.59	78.11
Borneo 7	1.18	0.48	1.64	17.18	79.52
Java 1	1	0.54	1.55	18.64	78.27
Java 2	0.44	0.38	1.4	14.47	83.31
Papua	1.11	0.83	6.46	12.08	79.52
Sulawesi	1.23	0.18	2.65	16.65	79.29
Sumatra	0.72	0.53	0.67	16.24	81.84

Table S2. Yield of albumin extracted from *C. striata* muscle tissue. W0: initial weight; X Wt: Yield of albumin.

Samples	W0 (g)	X Wt (g)	% Yields
Borneo 1	40	0.604	1.509
Borneo 2	40	0.576	1.439
Borneo 3	40	0.576	1.441
Borneo 4.1	40	0.548	1.370
Borneo 4.2	40	0.548	1.369
Borneo 5	40	0.588	1.471
Borneo 6	40	0.567	1.418
Borneo 7	40	0.566	1.416
Java 1	40	0.583	1.457
Java 2	40	0.501	1.253
Papua	40	0.550	1.376
Sulawesi	40	0.565	1.412
Sumatra	40	0.763	1.907
Tilapia	40	0.228	0.570