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RESEARCH PAPER

Genetic Variation of Giant Gourami (*Osphronemus goramy* Lacepède 1801) in Indonesia: Insights from Morphometric and Mitochondrial DNA COI Analysis

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Abstract

Giant gourami (*Osphronemus goramy*) is one of the main aquaculture commodities in Indonesia, originally distributed across the waters of Java, Sumatra and Borneo. This study aims to determine the genetic variation of giant gourami in Indonesia through morphometric and molecular analyses using mitochondrial DNA marker Cytochrome Oxidase Subunit I (COI). Morphometric assessment revealed a low average coefficient of variance, ranging from 2% to 20%. Correlation analysis indicated that the forehead and caudal peduncle were key distinguishing characteristics of giant gourami. Discriminant and cluster analyses classified giant gourami into three geographical populations: Java, Sumatra, and Borneo. A similar clustering pattern was observed in the molecular analysis results. Our study concluded that the giant gourami populations in Indonesia exhibit distinct haplotypes and cluster according to their island of origin. The Sumatra and Java populations showed a closer genetic relationship compared to the Borneo samples, as reflected in both genetic distance and morphometric assessment.

Introduction

Giant gourami (*Osphronemus goramy* Lacepède, 1801) is an important freshwater fish commodity, particularly in the Southeast Asia region (Slembrouck et al., 2020). The species is highly valued both as an ornamental fish and for aquaculture (Amornsakun et al., 2014). As a well-established aquaculture species, giant gourami has the potential to become an emerging global commodity due to its resilience and adaptability (Caruso et al., 2019). As an anabantoid fish, it can thrive in low-oxygen environments by utilizing its labyrinth organ as an additional breathing apparatus (Sasmita et al., 2019). The species typically inhabits slow-moving waters such as swamps rich in aquatic vegetation, which serve as both feeding and spawning grounds.

Giant gourami is an omnivorous fish but is predominantly herbivorous, as its diet mainly consists of plant materials (Slembrouck, 2018). It is a voracious macrophyte feeder and has been utilized for aquatic plant control in waterways (Edwards, 1980), and as an ecological agent to manage the growth of waterborne weeds (Jayasree et al., 1990; Ismail et al., 2018). In aquaculture, giant gourami is commonly fed a mixture of plant materials, such as taro leaves (*Alocasia macrorrhizos*), combined with commercial pelleted feed

(Kristanto et al., 2019; Arifin et al., 2020). The use of plant-based feed significantly reduces feed expenses and minimizes farmers' reliance on costly commercial feeds. Since feed costs can account for approximately 70% of total aquaculture production expenses (Ardianto & Mudjahidin, 2022), incorporating affordable, locally available plant materials can lower production costs, making giant gourami a lucrative choice for commercial aquaculture.

Indonesia is one of the leading producers of giant gourami in Southeast Asia, with an annual production of 183,354 tonnes recorded in 2019 (FAO, 2022). This production contributes significantly to the country's freshwater aquaculture sector. Additionally, several morphotypes of commercially farmed giant gourami existed across different regions of Indonesia (Azrita & Syandri, 2015), increasing its market potential and offering diverse options to meet consumer demands. However, research on the genetic and morphological variations among giant gourami strains remains limited. Therefore, it is crucial to identify and characterize local strains to support the development of a more resilient and fast-growing giant gourami strain.

Morphological characteristics, particularly morphometry, are widely used to distinguish variations within fish species. In the case of commercially cultured giant gourami, different morphotypes have been identified based on body size, shape, and coloration. Several strains, such as Tambago, Palpah, Jepun, Krista, and Blusafir, have been classified based in morphometric traits (Azrita & Syandri, 2015; Suharyanto et al., 2016). However, morphological differences alone do not necessarily indicate genetic diversity. While classifying strains based on morphotypes facilitates identification, genetic divergence must be analyzed to confirm their distinctiveness. The presence of intraspecific strains and overlapping morphological traits in some populations highlights the need for molecular-level investigation to validate strain differentiation.

DNA barcoding is a powerful tool for molecular-level strain validation. Mitochondrial DNA (mtDNA) markers are commonly used to assess genetic variation due to their higher mutation rates compared to nuclear DNA, allowing for detection of genetic divergence over shorter evolutionary timeframes (Allio et al., 2017). DNA barcoding using mitochondrial gene markers has been successfully applied to identify species and phylogenetic relationships in fish (Parmaksiz et al., 2022; Sachitanandam and Mohan, 2020; Ude et al., 2020). Previous studies have utilized mtDNA markers to analyze genetic diversity within the Osphronemidae family (Degani et al., 2021; Arisuryanti et al., 2019; Syaifudin et al., 2019; Tan et al., 2019).

The mitochondrial cytochrome c oxidase subunit 1 (COI) gene is widely recognized as an effective DNA barcode for differentiating species of both vertebrates and invertebrates (Ahmed et al., 2022). In Osphronemidae, COI barcoding has been widely applied for genetic diversity analysis. Arisuryanti et al. (2019) investigated the genetic diversity of kissing gourami (*Helostoma temminckii*) using the COI and 16S rRNA genes, revealing multiple haplotypes and genetic divergence. Syaifudin et al. (2019) analyzed COI sequences of snakeskin gourami *Trichogaster pectoralis* and blue gourami *Trichogaster trichopterus*, showing high intraspecies similarity but distinct phylogenetic clustering. Degani et al. (2021) highlighted COI and other genetic markers in *T. trichopterus* as key tools for species differentiation within Anabantoidei.

Both morphometric and genetic approaches are essential for identifying and validating different varianst and strains of *Osphronemus goramy*. Establishing verified strains or species variants provides a foundation for breeding programs aimed at producing high-quality seeds while preventing inbreeding. In light of this, the present study aims to identify the genetic diversity of giant gourami in Indonesia through molecular analysis using mtDNA COI markers, complemented by a morphological approach utilizing truss morphometry. By validating the genetic differences among known strains, our study seeks to contribute to future selective breeding initiatives and the conservation of giant gourami strains in Indonesia, thereby safeguarding their genetic diversity for future sustainability.

Materials and Methods

Sample Collection

Samples were collected from three main islands of Indonesia: Java, Sumatra, and Borneo (Figure 1). Three strains were obtained from Yogyakarta, Java; one strain was collected from Padang, West Sumatra; and one strain was procured from Mandiangin, South Borneo. All fish samples were obtained from local aquaculture ponds.

The local strains of giant gourami used as samples included Cangkringan, Sendangsari, and Kapas giant gourami strains from Java; Sago giant gourami strain from Sumatra; and Mandiangin giant gourami strain from Borneo (Figure 2). A total of 92 fish samples were used, consisting of 15 Cangkringan giant gouramies, 18 Kapas giant gouramies, 20 Sendangsari giant gouramies, 20 Sago giant gouramies, and 19 Mandiangin giant gouramies. All samples had a body length ranging from 26 cm to 35 cm.

For consistency, the Cangkringan giant gourami strain, which tends to have a black-colored body, will be referred to as CB. The Sendangsari giant gourami strain, which also tends to have a black-colored body, will be referred to as SB. The Kapas giant gourami strain, which tends to have a white-colored body, will be referred to as KW. The Sago giant gourami strain, which also tends to have a white-colored body, will be referred to as SgW. Lastly, the Mandiangin giant gourami strain, which tends to have a black-colored body, will be referred to as MB.

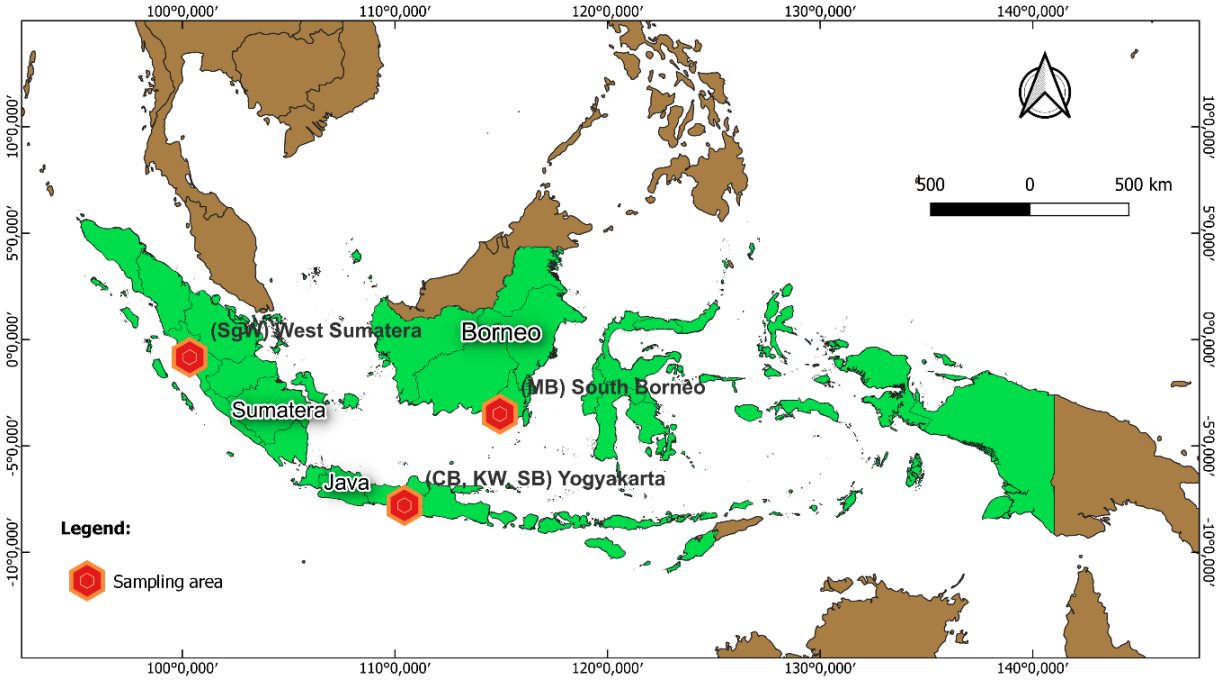

Figure 1. Map of the three sampling locations, marked with red icons. CB, KW, and SB represent giant gourami samples from Java; SgW represents samples from West Sumatra; and MB represents samples from South Borneo.

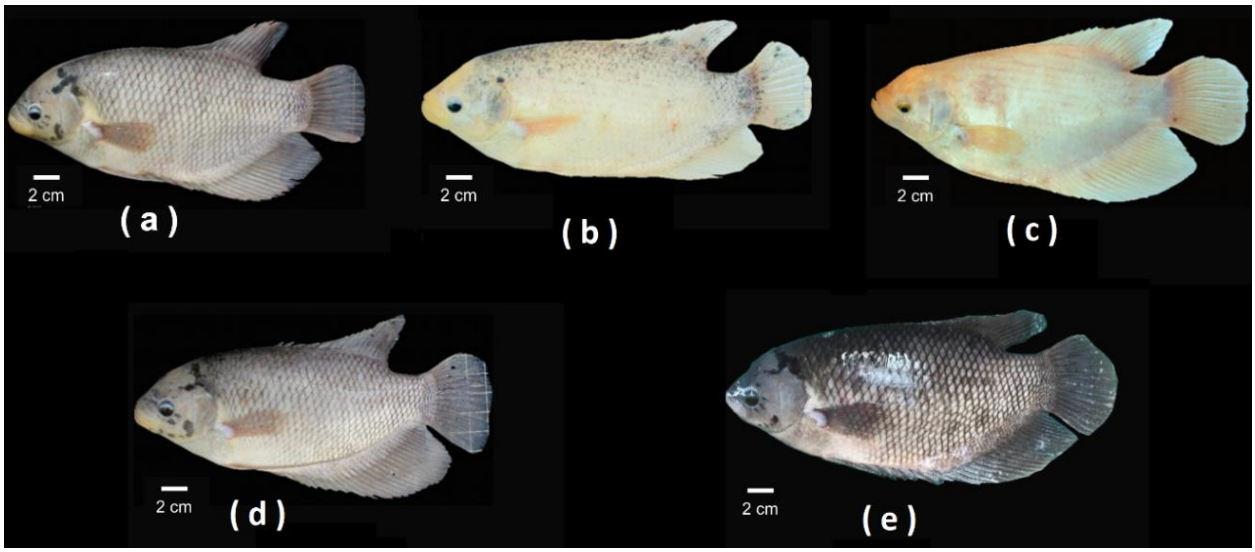

Figure 2. Local strains of giant gourami samples: (a) Cangkringan giant gourami from Java (CB), (b) Kapas giant gourami from Java (KW), (c) Sago giant gourami from Sumatra (SgW), (d) Sendangsari giant gourami from Java (SB), and (e) Mandiangin giant gourami from Borneo (MB). Scale bar = 2 cm.

Morphometrical Analysis

Giant gourami samples were measured on a grid paper, labeled, and photographed. Morphometric analysis was conducted using the truss morphometry method, with six truss areas and 13 morphological landmarks (Figure 3), based on the morphometry method described by Hardaningsih (2001). Twenty-eight truss lines were measured and analyzed using tpsUtil64 and tpsDig232 (Rohlf, 2015), as well as PAST 3 software (Hammer et al., 2001). The measurement results were standardized by dividing each value by the standard length of the respective sample. The obtained data represent the ratio between the characterized length and standard length.

The morphometric ratio data were subsequently analyzed using discriminant and cluster analyses. Discriminant analysis was performed using the stepwise method to determine population distribution, similarity indices, and distinguishing characteristics. Cluster analysis was conducted using the hierarchical method to generate a dendrogram depicting genetic relationships

between populations. Both analyses were performed using Microsoft Excel 2016 and SPSS 23 software.

Molecular Analysis

Molecular analysis was conducted using a total of 14 giant gourami samples. The CB, SB, and KW giant gourami populations each included two samples, while the SgW and MB populations each included four samples. A small portion of the fish caudal fin (1–2 cm^2) was cut, cleaned, and preserved in 95% ethanol for molecular analysis. DNA was extracted from the caudal fin samples using TNES method (Wasko et al., 2003). Primer3Plus was used to design primers for this study, based on the whole mitochondrial sequence from GenBank. The target for amplification was the COI partial gene. The primer sequences were as follows: TCA CAC GTT GAT TTC TCG ACT (forward); AAT AAG CGC GTG TGT CAA CG (reverse).

The PCR reaction was prepared using the ready-to-use MyTaq HS Red mix (Bioline), along with genomic DNA, primers, and nuclease-free water (1st Base Asia). The PCR amplification profile consisted of: initial denaturation at 94˚C for 5 minutes, 35 cycles of denaturation at 94˚C for 30 seconds, annealing at 50˚C for 30 seconds, elongation at 72˚C for 1 minute, final elongation at 72˚C for 5 minutes. DNA sequencing was outsourced to 1st Base Asia DNA sequencing service (Malaysia). All nucleotide sequences were checked and aligned using the ClustalW algorithm. Genetic diversity, genetic distance, and phylogenetic analyses were performed using MEGA 11 and DnaSP 6 software (Tamura et al., 2021).

Results

Morphometry Analysis

The distribution of giant gourami populations was assessed using discriminant analysis with morphometric characteristics as variables. Landmarks for truss network analysis are presented in Figure 3. A total of 27 out of 28 identified morphometric characteristics were statistically significant (Table S1). These findings suggest that the 27 features contributed to population differentiation.

Morphometric characteristics used to differentiate populations were sorted by calculating the wilks' lambda score. A score close to zero indicates that the variable plays a greater role in differentiation, whereas a higher score suggests a weaker influence (Suharyanto et al., 2018).

The existing characteristics were then further selected to obtain the best combination for population differentiation (Table S2). Wilks' lambda scores were compared, and nine variables were selected for population differentiation using the stepwise procedure. Five characteristics were further selected based on a Wilks' lambda score of >0.05. The variation in these five characteristics formed the basis of strain differentiation among giant gourami samples using discriminant analysis. The five morphometric characteristics with the highest Wilks' lambda values, in descending order, were B7 (body area), A4 and A12 (head area), and B6 and B4 (body area) (Figure 3, Table S2).

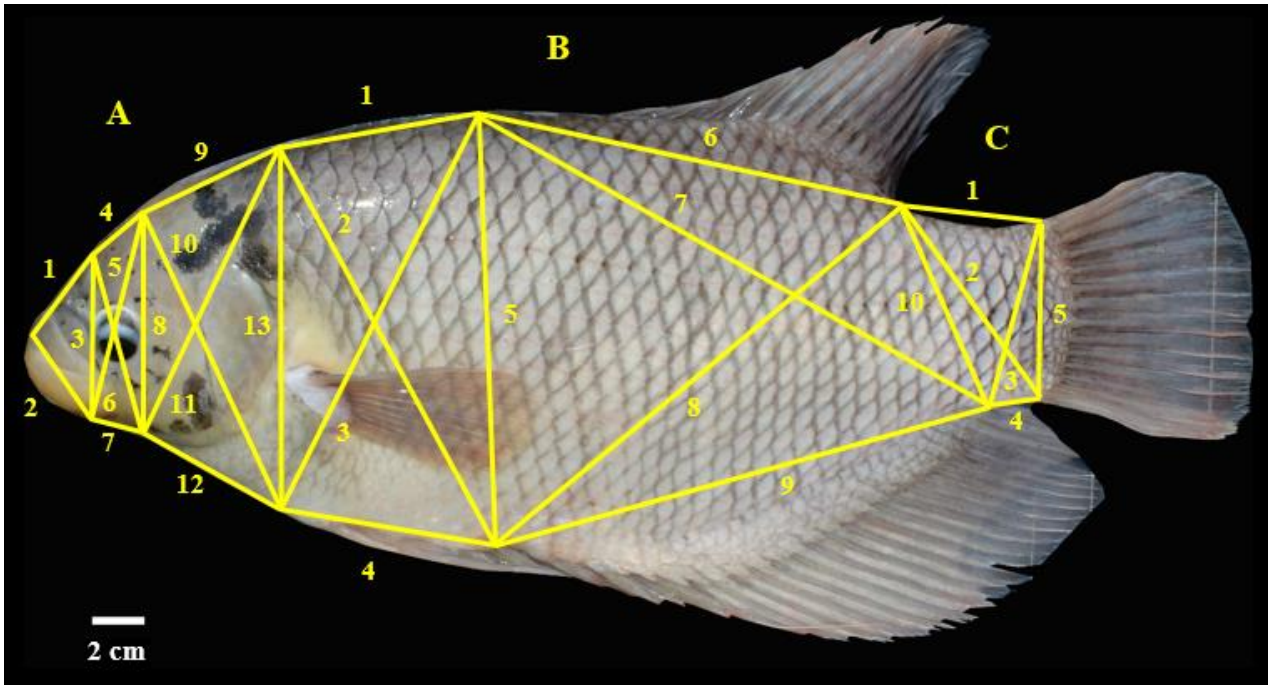

Figure 3. Landmark and truss line for morphometric analysis based on the three main body parts of giant gourami. Details are provided in Table S1.

A scatter plot was created using the results of the discriminant analysis (Figure 4). The plot represents population distribution based on four functions, further detailed in Table S3. The population distribution is classified into four quadrants: the first quadrant contains the SgW population, the second quadrant contains the KW population, the third quadrant contains both the CB and SB populations, and the fourth quadrant contains the MB population. The plot distance was calculated based on centroid distance (average discriminant value).

The differentiation between populations corresponds with the geographical origin of the fish. The SgW population was collected from Sumatra island, MB population from Borneo island, while CB, KW, and SB populations were obtained from Java island. The centroid position and the distribution of the KW population are adjacent to the CB and SB populations. The SgW and MB populations is more distinct from the other populations.

The similarity index measures the degree of similarity between two or more populations based on morphometric characteristics. It was calculated based on the distance and distribution between each population, as presented in Table S4. A total of 80% of the giant gourami CB population samples were correctly classified as CB, while 13% were classified as MB and 7% as SgW. Similarly, 83% of the giant gourami KW population samples were classified as KW, while 11% were classified as SB and 6% as CB. For the SB population, 80% were correctly classified as SB, while 20% were misclassified as CB. Meanwhile, all samples from the giant gourami MB and SgW populations were correctly classified as MB (100%) and SgW (100%), respectively. The MB and SgW populations were not cross-validated, indicating that they were significantly distinct.

The result of cluster analysis for each population are presented in a dendrogram (Figure 5). The SgW population clustered at a 25% distance, while the MB population clustered at a 20-23% distance. The CB and SB populations clustered at less than 5%.

The population clusters in the dendrogram showed a pattern like the results of the discriminant analysis. The SgW and MB giant gourami populations exhibited greater differentiation compared to the CB, KW, and SB populations. This pattern aligns with the geographical origin of the fish, where strains from Java (CB, KW, SB) formed a more contiguous distribution compared to those Borneo (MB) and Sumatra (SgW).

Molecular Analysis

A partial COI gene (809 sites) was used to assess genetic diversity and phylogeny among various strains of giant gourami (CB, SB, KW, SgW, and MB). Pairwise distance analysis was conducted to calculate genetic distances between samples, with the result presented in Table 1. The genetic distance between Java group samples (CB, KW, SB) and Sumatra samples (SgW) was 0.0025 (0.25%). Java giant gourami samples exhibited a 0.0101 (1.01%) genetic distance from Borneo giant gourami, while Sumatra giant gourami had a 0.0126 (1.26%) genetic distance from Borneo strain. Java and Sumatra giant gourami were found to be genetically closer to each other than to the Borneo samples (MB), indicating lower genetic divergence.

A phylogenetic tree (Figure 6) was constructed using the Neighbor-Joining (NJ) method with the Kimura-2 parameter. The best tree was selected from 1,000 bootstraps replicates. Sequences of *Osphronemus goramy* (AP006834) from Genbank were used for intraspecific comparison, with *Trichopodus pectoralis* (KY606168) as outgroup.

Phylogenetic tree analysis revealed that all markers produced similar tree patterns, forming three haplotypes. Interestingly, each haplotype corresponded with a distinct geographical location. Haplotype-1 (H1) consisted of strains from Sumatra Island (SgW), Haplotype-2 (H2) comprised of samples from Java Island (CB, KW, SB), and Haplotype-3 (H3) originated from Borneo Island (MB). The accession numbers for retrieving data from this study in NCBI GenBank are OQ363193-OQ363202 and OQ407537-OQ407540.

The population was divided into three haplotypes based on differences in 10 out of a total of 809 aligned base pairs. These differences occur at nucleotide positions 95, 176, 185, 354, 368, 443, 608, 668, 731, and 785 (Figure. S1). The MB giant gourami shows the most differences compared to other samples, specifically at nucleotide positions 95, 176, 185, 368, 443, 608, 731, and 785. Additionally, differences are found in the SgW giant gourami, which has nucleotide variations at positions 354 and 668.

Samples from Borneo consistently formed a separate branch from the Java and Sumatra groups, implying a higher level of genetic divergence. These results align well with our morphometric analysis, further confirming the presence of three distinct populations based on geographical origin.

Discussion

This study used morphometric methods to distinguish populations of known giant gourami strains in Indonesia, particularly those from Java, Sumatra, and Borneo. A truss morphometric approach was applied based on predetermined landmarks on three main parts of the fish's body: the head, body, and caudal. The truss network approach eliminates the need to identify specific distinguishing characteristics and the ideal number of traits, while also providing a comprehensive representation of fish shape (Turan, 2004).

Significant differences among the five examined strains were primarily observed in the head and body regions. Two of the most notable distinguishing features were the forehead area between the anterior and posterior frontal regions above the eye (A4) and the

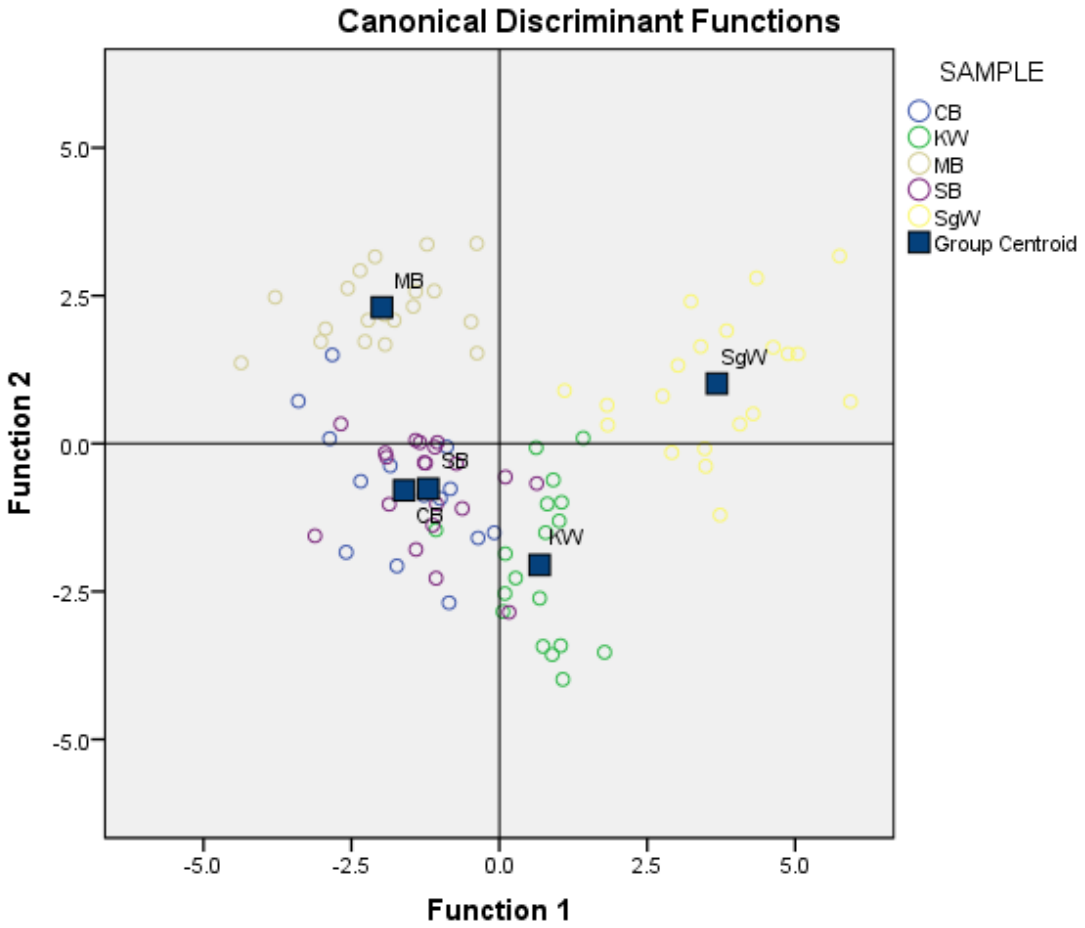

Figure 4. Scatter plot of the population distribution among the five giant gourami strains based on discriminant analysis: Cangkringan giant gourami from Java (CB), Kapas giant gourami from Java (KW), Sago giant gourami from Sumatra (SgW), Sendangsari giant gourami from Java (SB), Mandiangin giant gourami from Borneo (MB). The centroid represents the average position of each population.

measured distance between the origin of the dorsal fin to the posterior part of the anal fin (B7). The forehead region is known to be a key distinguishing feature for identifying sexual dimorphism, as male giant gouramies develop a hump in this area (Slembrouck et al., 2019). These traits were also identified in a previous study by Azrita & Syandri (2015).

Each of the five giant gourami strains clustered into distinct population group. Discriminant analysis revealed that the Borneo (MB) and Sumatra (SgW) populations were separate from the Java population, occupying different quadrants in Figure 3. Meanwhile, the Java strains (CB, KW, SB) were relatively close to each other, although the KW strain occupied a different quadrant from the CB and SB strains. This suggests that the Kapas strain (KW) is morphologically distinct from the other Java strains, namely Cangkringan (CB) and Sendangsari (SB) strains.

The genetic characteristics of each giant gourami population were further analyzed using mtDNA COI markers. Based on haplotype distribution patterns, the samples were grouped into three haplotypes corresponding to their geographical origins: the Sumatra haplotype (H1), Java haplotype (H2), and Borneo haplotype (H3) (Figure 6). The Sumatra haplotype included the Sago giant gourami population, the Java haplotype comprised the Cangkringan, Kapas, and Sendangsari populations, while the Borneo haplotype contained the Mandiangin population.

The phylogenetic analysis results closely aligned with the discriminant analysis using the morphometric approach. Interestingly, the Kapas giant gourami strain displayed a different morphotype from the other Java strains, clustering in a separate quadrant in both the centroid plot and dendrogram. However, at the molecular level, the Kapas population did not exhibit genetic divergence from the other Java populations. The phylogenetic tree grouped all three Java populations into a single haplotype.

It is generally accepted that variations in morphotypes within a species are influenced by geographical location, environmental interactions, individual ontogeny, and genetic selection (Wang & Bradburd, 2014). In our study, the most distinct morphological trait among the strains was skin coloration. For instance, the Mandiangin giant gourami strain exhibited a significantly darker skin coloration compared to the other samples. This population was sampled from peatland rivers and swamps in Borneo, suggesting that environmental factors such as water

coloration, pH, and dissolved organic matter concentrations may have influenced its morphological features. These adaptations could be mediated through the melanocortin system (Cal et al., 2017).

Previous studies suggest that environments with lower luminosity promote deeper coloration to enhance visibility among individuals (Kelley et al., 2012). This phenomenon is commonly observed in peatland environments, where tea-colored water with low pH and high organic matter content is prevalent (Ishikawa & Gumiri, 2006). Nevertheless, our results only indicate that the Borneo population is genetically distinct from those in Java and Sumatra. Habitat fragmentation and geographical isolation may have contributed to the distinct genetic makeup and morphological characteristics of the Mandiangin strain.

A different pattern was observed in the Java and Sumatra populations. Although the three Java strains exhibited variations in coloration and pattern, the Kapas and Sago strains had the most distinct skin coloration, appearing white-silver rather than gray. The Sago strain originates from the highlands of West Sumatra (Azrita et al., 2021), while the origin of the Kapas strain remains unclear. However, our findings strongly suggest that the Kapas strain originates from Java, as it clusters with the Cangkringan and Sendangsari strains, both of which are well-established commercial strains from Java.

The unique morphology of the Kapas strain may be attributed to selective breeding of local strains. Coloration is genetically inherited and can only be modified gradually (Luo et al., 2021). It is likely that the Kapas strain was developed through selective breeding for aquaculture purposes, leading to morphological variations over time. Further studies using additional genetic markers would be beneficial in confirming the origins of this strain.

The Java strains, particularly Cangkringan and Sendangsari, exhibited similar hues, although differences in pattern were noted. Notably, the Sendangsari strain had a lighter shade on the ventral side. Cross-validation of several individuals further supported the high similarity index between the Cangkringan and Sendangsari populations, suggesting a shared parental lineage (Setijaningsih et al., 2007).

Our findings indicate low genetic divergence among the three Java populations, implying a common ancestral origin. Similarly, the genetic distance between the Java and Sumatra populations suggests that these haplotypes share a more recent common ancestor than the Borneo haplotype. However, morphometric analysis revealed that the Sago giant gourami strain was more morphologically divergent from the Java strains than from the Borneo strain. This suggests that morphological traits in the Sago strain exhibit greater variability. Both genetic and morphometric analyses displayed similar clustering patterns in relation to geographical location. From an evolutionary perspective, the genetic distance of this gourami follows different adaptation patterns, leading to different evolutionary pathways over time.

The study by Sularto et al. (2017) showed a similar pattern of genetic differences between geographically separated populations, indicating a consistent trend in this species. The morphometric analysis in this study also revealed that the Borneo gourami has a greater genetic distance compared to gourami from Jambi, Majalengka, and Tasikmalaya.

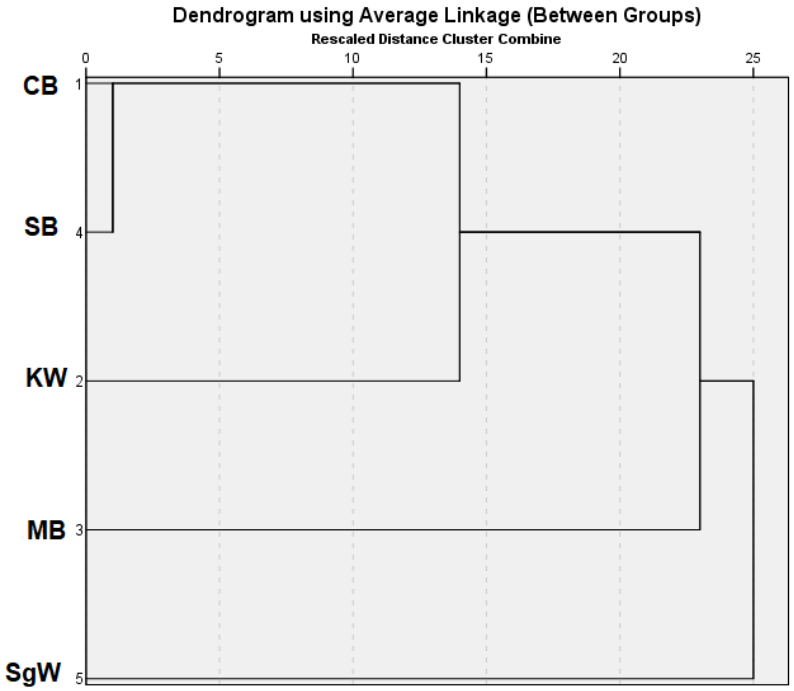

Figure 5. Dendrogram showing morphological similarity distances among the five giant gourami strains, calculated using cluster analysis: Cangkringan giant gourami from Java (CB), Kapas giant gourami from Java (KW), Sago giant gourami from Sumatra (SgW), Sendangsari giant gourami from Java (SB), and Mandiangin giant gourami from Borneo (MB).

Table 1. Genetic distances between samples and the outgroup sequences for the COI marker were calculated using the K2P model. The analyzed sequences included Cangkringan giant gourami from Java (CB), Kapas giant gourami from Java (KW), Sago giant gourami from Sumatra (SgW), Sendangsari giant gourami from Java (SB), Mandiangin giant gourami from Borneo (MB), *Osphronemus goramy,* and *Trichopodus pectoralis*.

	CB1	CB2	SB1	SB2	KW1	KW2	SgW1	SgW2	SgW3	SgW4	MB1	MB2	MB3	MB4	*O. goramy*	*T. pectorali*
CB1																
CB2	0.0000															
SB1	0.0000	0.0000														
SB2	0.0000	0.0000	0.0000													
KW1	0.0000	0.0000	0.0000	0.0000												
KW2	0.0000	0.0000	0.0000	0.0000	0.0000											
SgW1	0.0025	0.0025	0.0025	0.0025	0.0025	0.0025										
SgW2	0.0025	0.0025	0.0025	0.0025	0.0025	0.0025	0.0000									
SgW3	0.0025	0.0025	0.0025	0.0025	0.0025	0.0025	0.0000	0.0000								
SgW4	0.0025	0.0025	0.0025	0.0025	0.0025	0.0025	0.0000	0.0000	0.0000							
MB1	0.0101	0.0101	0.0101	0.0101	0.0101	0.0101	0.0126	0.0126	0.0126	0.0126						
MB2	0.0101	0.0101	0.0101	0.0101	0.0101	0.0101	0.0126	0.0126	0.0126	0.0126	0.0000					
MB3	0.0101	0.0101	0.0101	0.0101	0.0101	0.0101	0.0126	0.0126	0.0126	0.0126	0.0000	0.0000				
MB4	0.0101	0.0101	0.0101	0.0101	0.0101	0.0101	0.0126	0.0126	0.0126	0.0126	0.0000	0.0000	0.0000			
O. goramy	0.0114	0.0114	0.0114	0.0114	0.0114	0.0114	0.0139	0.0139	0.0139	0.0139	0.0088	0.0088	0.0088	0.0088		
T. pectoralis	0.1766	0.1766	0.1766	0.1766	0.1766	0.1766	0.1799	0.1799	0.1799	0.1799	0.1749	0.1749	0.1749	0.1749	0.1799	

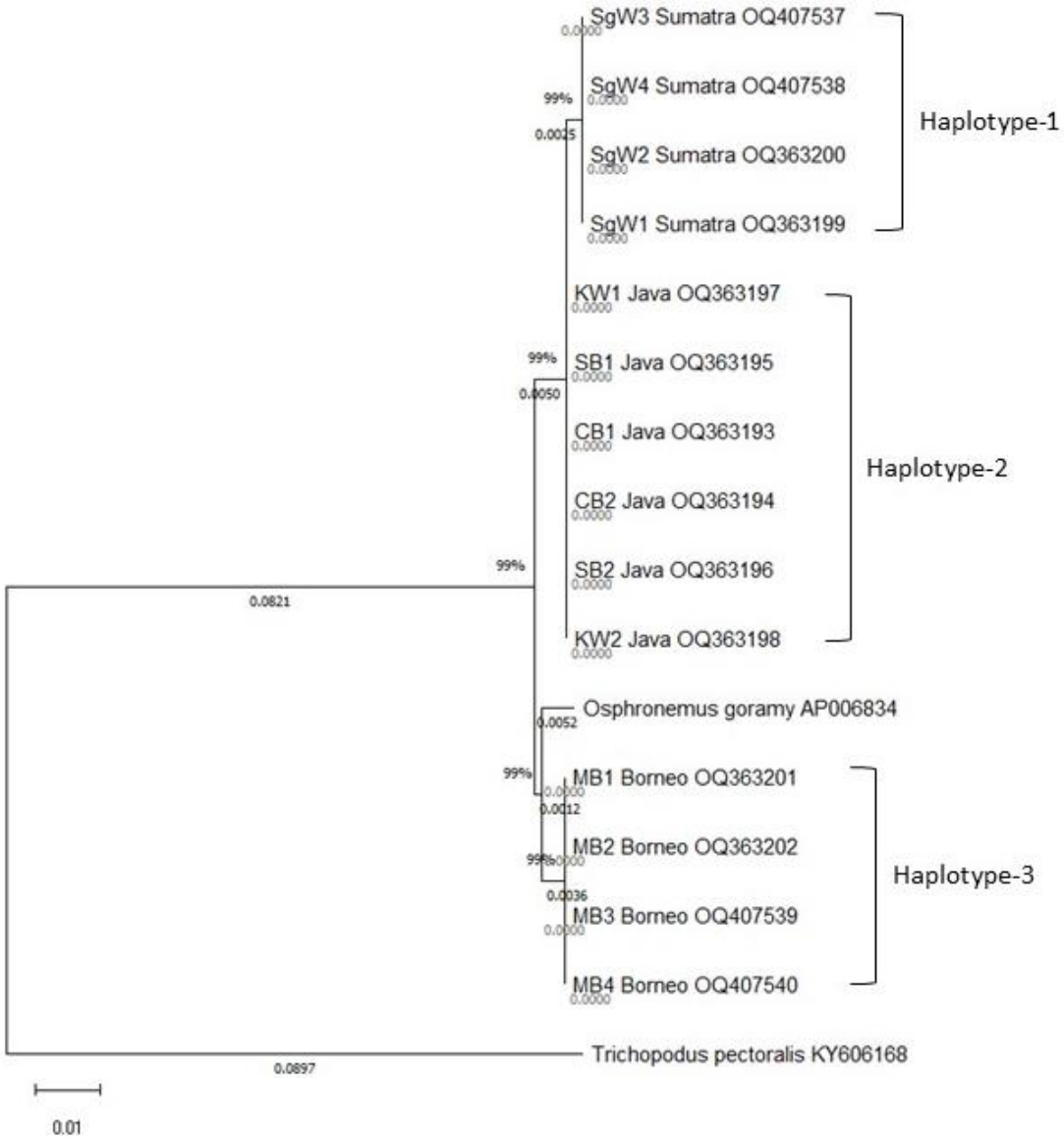

Figure 6. Phylogenetic tree of the COI mtDNA marker. The tree was constructed using the NJ method with the K2P model and 1,000 bootstrap replicates. The scale bar represents genetic distance among giant gourami strains, including Cangkringan giant gourami from Java (CB), Kapas giant gourami from Java (KW), Sago giant gourami from Sumatra (SgW), Sendangsari giant gourami from Java (SB), and Mandiangin giant gourami from Borneo (MB).

Geographical isolation generally contributes to intraspecific morphological and genetic variations within a species. Isolated habitats facilitate long-term interaction and adaptation to local environmental conditions while also limiting gene flow (Bourgain & Genin, 2005). Due to their high phenotypic plasticity, fish can adapt their physiology and behavior to their surroundings. These adaptive processes gradually modify their physiological and morphological characteristics (Mahfuj et al., 2019), leading to genetic changes associated with environmental adaptation (Xue et al., 2019).

Our findings, along with previous studies on giant gourami strains in Java, Sumatra, and Borneo (Azrita & Syandri, 2015; Nuryanto & Pulungsari, 2017; Nuryanto et al., 2019; Nugroho et al., 2019), highlight the extensive genetic diversity and distribution of this species within Indonesia. These results provide a foundation for the establishment of a germplasm inventory, validating the existence of distinct strains and haplotypes across different regions.

Given the significant commercial value of giant gourami, efforts have been made to selectively breed and hybridize the species to produce high-quality strains with desirable traits such as rapid growth (Sularto et al., 2017; Arifin et al., 2018). Identifying distinct strains is essential for germplasm preservation and contributes to the genetic conservation of the species. Future breeding programs can leverage the gene pool of existing strains to enhance desirable traits within cultivated populations.

The observed genetic differentiation has important implications for conservation and aquaculture breeding programs. Maintaining genetic diversity is crucial to ensure the sustainability and long-term adaptive capacity of fish populations, especially in the face of changing environmental conditions. Breeding programs relying on genetically similar stocks can unintentionally reduce genetic variation, potentially leading to decreased disease resistance, slower growth rates, or lower reproductive success (Muhajirah et al., 2021).

Anthropogenic factors, particularly breeding practices in aquaculture, can reduce genetic diversity in cultured populations. This is evident in studies such as Nugroho et al. (2013), which showed that continuous selective breeding of the Cangkringan red tilapia led to a reduction in genetic variation. Therefore, it is important to consider the potential impacts of selective breeding and inbreeding on the genetic health of gourami populations and to implement strategies that preserve genetic diversity, such as using genomic data to inform parent selection.

Furthermore, expanding similar genetic diversity studies to other regions in Southeast Asia would be highly beneficial. As giant gourami is endemic to this region, strains from different geographical locations within Southeast Asia may exhibit unique characteristics. Further exploration of giant gourami diversity would enrich our understanding of the species and provide valuable insights for its management and conservation.

Conclusion

Our study confirms the distinct morphometric and genetic differences among giant gourami strains in Indonesia, with a clear association to their native geographical origins. Discriminant and phylogenetic analyses revealed the uniqueness of the Cangkringan, Sendangsari, and Kapas strains from Java Island, while the Sago giant gourami strain was identified as part of the Sumatra haplotype. Notably, the Borneo strain formed a distinct haplotype group, separate from both the Java and Sumatra strains. These findings suggest that geographical isolation has likely contributed to the genetic divergence observed among the strains. As we conclude our study, we emphasize the importance of further research to assess the genetic diversity of giant gourami in other countries, particularly within Southeast Asia. Expanding our understanding of the species' genetic variation across different geographic regions will provide valuable insights for conservation and management efforts.

Future research should integrate genome-wide approaches, such as Single Nucleotide Polymorphism (SNP) analysis, to validate the observed genetic differentiation with higher resolution. Further studies using more specific markers can be employed to characterize the advantageous traits of each giant gourami strain as a basis for hybridization programs.

Ethical Statement

All fish specimens were sourced from local farmers with the approval of the Universitas Gadjah Mada Ethical Clearance Commission (certificate No. 00058 / 04 / LPPT / XI / 2020). Researchers prioritized minimizing any discomfort experienced by the animals during the study.

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Author Contribution

Conceptualization: IH and DWKS; Project Administration and Supervision: IH, M, and DWKS; Funding Acquisition: IH and DWKS; Formal Analysis: DWKS, HB, and RA; Investigation: RA, RH, and SA; Methodology: IH and DWKS; Resources: RH, SA and RA; Visualization: HB and RA; Writing orginal draft: DWKS, SA, HB and RA; Writing review and editing: HB, DWKS, and RA.

Conflict of Interest

The authors state that they have no known conflicts that could have influenced the work presented in this paper.

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Table S1. Truss dimensions measured to indicate morphological variations among the collected giant gourami samples. A13 was not statistically significant and was therefore excluded from the discriminant analysis

No.	Area	Code	Description	Mean ratio	*Wilks' Lambda* score	F	Sig.
1.	Head	A1	Anterior of the upper lip - upper frontal area above the eye	0.09	0.74	7.65	0.00
2.		A2	Anterior of the upper lip - posterior lower jaw below eye	0.12	0.58	15.74	0.00
3.		A3	Vertical line between upper frontal and lower jaw adjacent to eye	0.16	0.62	13.52	0.00
4.		A4	Anterior - posterior part of frontal area above eye	0.06	0.55	17.85	0.00
5.		A5	Anterior of frontal above eye - posterior part of angular area below eye	0.20	0.57	16.16	0.00
6.		A6	Anterior of angular below eye - posterior part of frontal area above eye	0.20	0.69	9.92	0.00
7.		A7	Anterior to posterior part of angular area below eye	0.06	0.64	12.39	0.00
8.		A8	Vertical line between frontal area and angular area posterior of eye	0.23	0.64	12.33	0.00
9.		A9	Frontal area above the eye - Posterior frontal above upper operculum	0.15	0.68	10.14	0.00
10.		A10	Frontal area above the eye - pelvic fin base below lower operculum	0.34	0.64	12.16	0.00
11.		A11	Angular area below the posterior eye - frontal area above operculum	0.31	0.68	10.34	0.00
12.		A12	Angular area below the posterior eye - pelvic fin base below operculum	0.15	0.55	18.04	0.00
13.		A13	Vertical line between the frontal part and pelvic fin adjacent to operculum	0.36	0.95	1.08	0.37
14.	Body	B1	Edge of upper operculum to anterior edge of dorsal fin	0.21	0.72	8.61	0.00
15.		B2	Edge of upper operculum to the anterior edge of anal fin	0.43	0.81	5.23	0.00
16.		B3	Edge of lower operculum to anterior edge of dorsal fin	0.45	0.85	3.74	0.01
17.		B4	Edge of lower operculum to anterior edge of anal fin	0.17	0.67	10.62	0.00
18.		B5	Anterior edge of dorsal fin to anterior edge of anal fin	0.44	0.84	4.24	0.00
19.		B6	Anterior edge of dorsal fin to posterior end of dorsal fin	0.40	0.64	12.08	0.00
20.		B7	Anterior edge of dorsal fin to posterior end of anal fin	0.54	0.48	23.57	0.00
21.		B8	Anterior edge of anal fin to posterior edge of dorsal fin	0.55	0.79	5.97	0.00
22.		B9	Anterior edge of anal fin to posterior edge of anal fin	0.53	0.88	2.99	0.02
23.		B10	Posterior edge of dorsal fin to posterior edge of anal fin	0.20	0.58	15.67	0.00
24.	Caudal	C1	Posterior end of dorsal fin to anterior edge of upper caudal fin	0.12	0.72	8.31	0.00
25.		C2	Posterior end of dorsal fin to anterior edge of lower caudal fin	0.22	0.74	7.49	0.00
26.		C3	Posterior end of anal fin to anterior edge of upper caudal fin	0.18	0.87	3.33	0.01
27.		C4	Posterior end of anal fin to anterior edge of lower caudal fin	0.05	0.72	8.69	0.00
28.		C5	Anterior edge of upper caudal fin to anterior edge of lower caudal fin	0.17	0.90	2.44	0.05

Table S2. Morphological characteristics selected through stepwise discriminant analysis. Bolded characters indicate significant differentiation

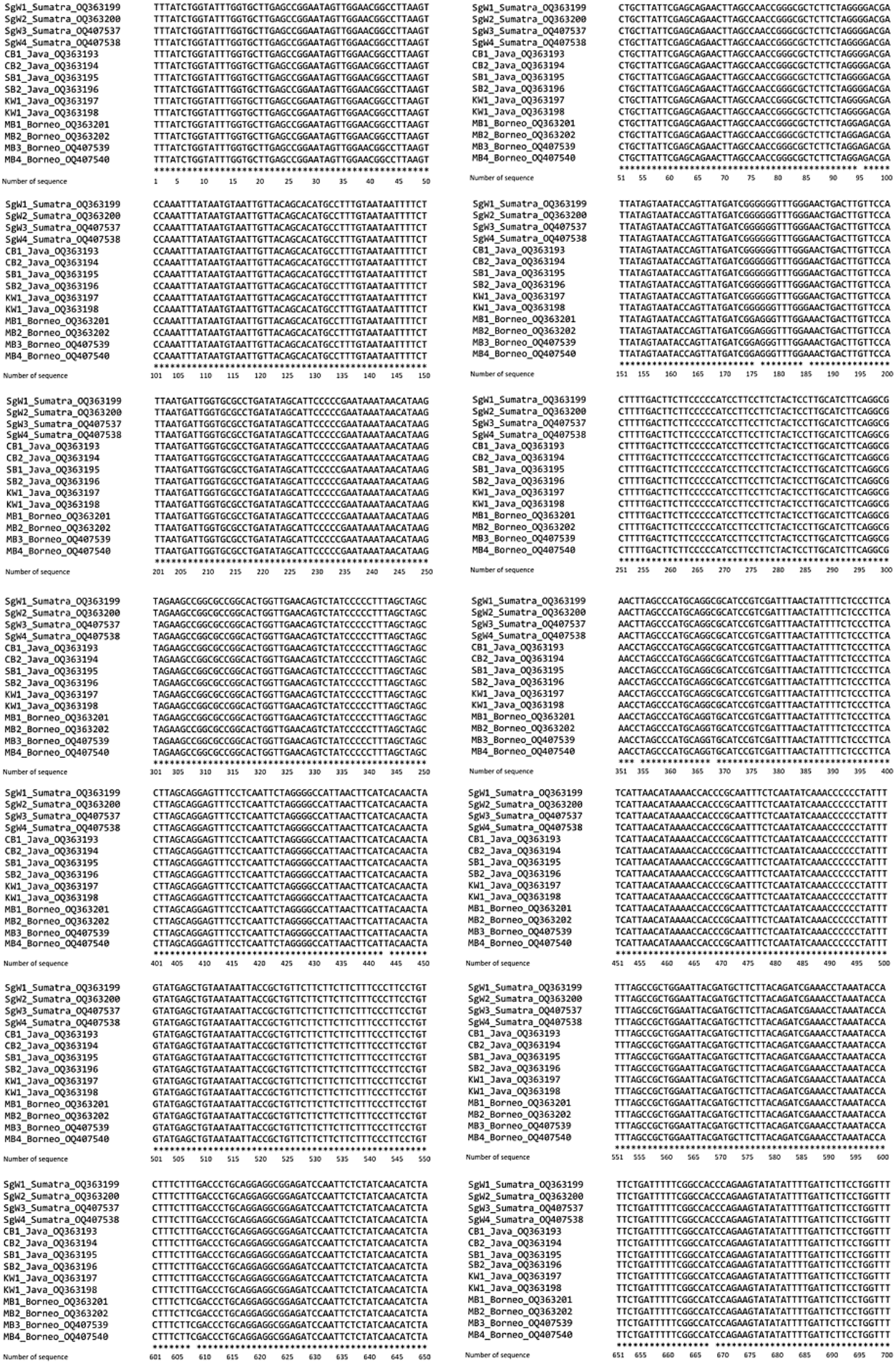
Step	Character	*Wilks' Lambda*	F	df1	df2	df3	Sig.
1	**B7**	**0.48**	23.57	1	4	87	0.00
2	**A4**	**0.27**	19.96	2	4	87	0.00
3	**A12**	**0.16**	18.66	3	4	87	0.00
4	**B6**	**0.10**	17.65	4	4	87	0.00
5	**B4**	**0.07**	17.25	5	4	87	0.00
6	B9	0.04	17.66	6	4	87	0.00
7	A5	0.03	17.23	7	4	87	0.00
8	A11	0.02	16.93	8	4	87	0.00
9	B1	0.02	16.09	9	4	87	0.00

Table S3. Results of the discriminant analysis with four functions

Function	*Eigenvalue*	*% of Var.*	*Cum. %*	*Canonical Correlation*
1	4.84	54.5	54.5	0.91
2	2.51	28.2	82.6	0.85
3	1.36	15.3	97.9	0.76
4	0.18	2.1	100	0.40

Table S4. Similarity index among the five giant gourami population strains: Cangkringan giant gourami from Java (CB), Kapas giant gourami from Java (KW), Sago giant gourami from Sumatra (SgW), Sendangsari giant gourami from Java (SB), and Mandiangin giant gourami from Borneo (MB)

Population	*Sharing Component*					Total
	CB	KW	MB	SB	SgW	
CB	12	-	2	1	-	15
KW	1	15	-	2	-	18
MB	-	-	19	-	-	19
SB	4	-	-	16	-	20
SgW	-	-	-	-	20	20
CB	80%	-	13%	7%	-	100%
KW	6%	83%	-	11%	-	100%
MB	-	-	100%	-	-	100%
SB	20%	-	-	80%	-	100%
SgW	-	-	-	-	100%	100%


SgW1_Sumatra_OQ363199
SgW2_Sumatra_OQ363200
SgW3_Sumatra_OQ407537
SgW4_Sumatra_OQ407538
CB1_Java_OQ363193
CB2_Java_OQ363194
SB1_Java_OQ363195
SB2_Java_OQ363196
KW1_Java_OQ363197
KW1_Java_OQ363198
MB1_Borneo_OQ363201
MB2_Borneo_OQ363202
MB3_Borneo_OQ407539
MB4_Borneo_OQ407540
Number of sequence

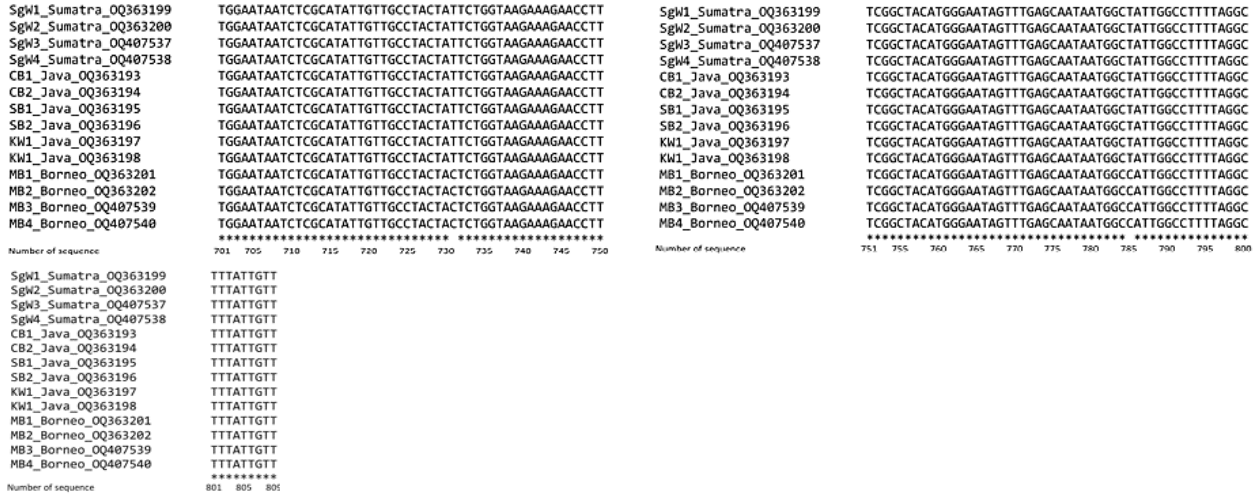


Figure S1. The distinct nucleotide sequences from the BLAST results of 14 giant gourami samples include six from Java (CB1, CB2, SB1, SB2, KW1, KW2), four from Sumatra (SgW1, SgW2, SgW3, SgW4), and four from Borneo (MB1, MB2, MB3, MB4). Sequences without an asterisk indicate nucleotide sequence variations.