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Morphological Distinction and Phenotypic Variance of *Mystus* (*Mystus gulio, M. nigriceps*, and *M. singaringan*) from Java Island, Indonesia

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ABSTRACT

Genus *Mystus* is widely distributed across Indonesia with eleven currently valid species. Three species are confirmed to inhabit Java Island: *Mystus gulio*, *M. nigriceps*, and *M. singaringan*. Due to their similar morphological appearances and relatively subtle diagnostic features, species identification and discrimination between these three species is somewhat challenging. This study evaluates the morphological differentiation of *M. gulio*, *M. nigriceps*, and *M. singaringan* at both interspecific and intraspecific levels using samples collected across Java Island. Morphological data including thirty morphometric and five meristic characters from 143 individuals in sampling sites were analyzed using Principal Component Analysis (PCA) and UPGMA clustering. PCA (75.32% total variance) effectively discriminated the three species, identifying maxillary barbel length and adipose-fin base length as the primary diagnostic characters. Intraspecific analyses revealed high morphological homogeneity across geographic sampling sites; however, significant phenotypic variance was observed, primarily attributed to ontogenetic allometric growth in juveniles and localized environmental plasticity. Despite testing for novel morphometric and meristic markers, this study reinforces the reliability of traditional adipose and barbel proportions as the definitive tools for field identification. These results provide a critical morphological baseline essential for the conservation, germplasm verification, and potential domestication of these indigenous aquatic resources.

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INTRODUCTION

Genus *Mystus* (Actinopterygii: Siluriformes) is classified in the family Bagridae and is widely distributed across the eastern Indian Ocean, including India, Pakistan, Indonesia, and Vietnam. Within the Indonesian archipelago, *Mystus* species are found on the islands of Sumatra, Borneo, and Java (Kumar et al.2019; Eschmeyer, 2025). To date, eleven valid species have been recorded in Indonesia: *M. abbreviatus, M. alasensis, M. bimaculatus, M. blekeeri, M. castaneus, M. gulio, M. impluviatus, M. nigriceps, M. punctifer*, *M. singaringan*, and *M. wolfii*. Among these, *M. gulio* (Hamilton, 1822), *M. nigriceps* (Valenciennes, 1840), and *M. singaringan* (Bleeker, 1846), are particularly widespread on Java Island (Paul & Chanda, 2017; Froese & Pauly, 2025; Herawati et al., 2025).These species are recognized locally by distinct vernacular names: *M. gulio* is commonly known as keting, *M. nigriceps* as ingir-ingir, and *M singaringan* as senggaringan. Ecologically, *Mystus* species inhabit various freshwater environments, including slow-flowing rivers, lakes, reservoirs, and docks. Based on dietary preferences, *Mystus* are considered omnivorous but tend to be carnivorous, primarily eating small fish, crustaceans, insects, zooplankton, and phytoplankton (Gupta, 2014; Kumar et al., 2019).

Beyond their ecological role, these three species in the genus *Mystus* have significant economic potential as food due to their affordability and year-round availability. The price of *Mystus* widely varies across Java Island, with averages Rp 26,800/kg in 2024, making them an important and accessible source of high-quality animal protein for local communities (Ministry of Marine Affairs and Fisheries, 2024). However, despite high market demand, aquaculture practices for *Mystus* species have not yet been established, resulting in continued dependence on wild-capture fisheries. Continued exploitation without the development of aquaculture strategies may lead to a decline in wild populations and increase the risk of local extinction in natural habitats (Rukayah et al., 2025; Rusdianto et al., 2025).

The three Javanese *Mystus* species (*M. gulio*, *M. nigriceps*, and *M. singaringan*) can be distinguished based on a combination of key morphological characteristics, including maxillary barbel length, head shape, and relative fin proportions. *M. gulio* exhibits a proportionally larger caudal region and an adipose fin smaller than the anal fin, while *M. singaringan* is characterized by maxillary barbels reaching the base of the caudal fin and an adipose fin extend nearly with dorsal fin. In contrast, *M. nigriceps* has a narrower and elongated head with an adipose fin larger than the anal fin (Hamilton, 1822; Hee, 2002; Kottelat, 1998; Roberts, 1993). Despite these diagnostic traits, strong morphological similarities among species frequently lead to misidentification, particularly between *M. nigriceps* and *M. singaringan* in rivers of Java due to historical misnaming and taxonomic misidentification (Pramono et al., 2017; Syafrialdi et al., 2020). Such misidentification can have serious consequences, including inaccurate biodiversity assessments, unreliable estimates of population size and distribution, misleading scientific conclusions, and failures in aquaculture domestication programs caused by mixed germplasm (Kirsch et al., 2018; Kürzel et al., 2025).

Despite the critical need for accurate species identification to support conservation and aquaculture, comprehensive morphological studies to resolve the frequent misidentification of *Mystus* species across Java Island remain limited. Therefore, this study aims to examine and evaluate the morphological characteristics of *Mystus* species inhabiting this region. Specifically, we characterize the morphology of *M. gulio*, *M. nigriceps*, and *M. singaringan* from nine sampling sites collected across Java Island to assess morphological diversity among species (inter-) and within populations (intraspecific). This approach will provide insights into their potential relationships with local environmental conditions. The results of this study are expected to provide essential baseline data to improve species identification accuracy and to support future domestication, selective breeding, and conservation management programs for Javanese *Mystus* species.

MATERIALS AND METHODS

Field sampling to collect *M. gulio*, *M. nigriceps*, and *M. singaringan* was conducted on Java Island from April to October 2025 encompassing both the dry and rainy seasons. Specimens were collected using both fishing rods and traditional fishing traps. A total of 143 individuals were collected from nine sampling sites across Java Island, including Situbondo, Pasuruan, Kediri, Pati, Wonogiri, Cilacap, Karawang, Bogor, and Banten (Figure 1). All collected individuals were deposited in the Depositorium Ichthyologicum Brawijayae, Faculty of Fisheries and Marine Science, Universitas Brawijaya, with voucher numbers (Table 1). All experimental procedures involving fish specimens in this study were approved by the Ethics Committee of the Faculty of Veterinary Medicine, Universitas Brawijaya (No. 98-KEP-FKHUB-2026).

Given the high morphological similarity among the three congeners, species identification was carried out with particular care following the key diagnostic characters described by (Hamilton, 1822; Roberts, 1993; Kottelat, 1998; Hee, 2002). Morphological key characters used for species identification a listed in Table 2. Sampling efforts yielded between one to sixteen individuals were successfully collected for each identified species from the fish encountered in the waters of Java Island.

Morphological observations were conducted on all specimens of *M. gulio*, *M. nigriceps*, and *M. singaringan*. Morphometric measurements and meristic counts were performed following (Ng & Kottelat, 2013). Morphometric data were obtained using a caliper with an accuracy of 0.01 mm. A total 30 morphometric characters were recorded, including standard length (SL), measured from the tip of the mouth to the end of the caudal base; maximum height of the adipose fin (MHAF), defined as its highest point, maximum length of the maxillary barbel (MBL), Body Depth at anus (BD) and several other morphometric features (Figure 2). Meanwhile, meristic characters were assessed by counting five traits, including number of dorsal, pectoral, pelvic, anal, and caudal fin rays.

Table 1. Specimen locality of three species of *Mystus* from Java Island

No	Sampling Site	Spesies	Number of Sample	Voucher Number	Geography Coordinate	
					S	E
1.	Penarukan Pier, Situbondo, East Java	*M. gulio*	13	DIB.1.0463 .055-068	7°41'51.424"S	113°55'56.916" E
2.	Welang River, Gadingrejo, Pasuruan, East Java	*M. gulio*	10	DIB.1.0463 .045-054	7°37'58.85"S	112°53'46.697" E
		M. nigriceps	12	DIB.1.0464 .001-012		
		M. singaringan	6	DIB.1.0465 .001-006		
3.	Brantas River, Kediri, East Java	*M. nigriceps*	10	DIB.1.0464 .013-022	7°38'32.356"S	112°5'53.895"E
4.	Godi River, Juwana, Pati, Central Java	*M. gulio*	10	DIB.1.0463 .035-044	6°43'57.769"S	111°7'43.8"E
		M. nigriceps	7	DIB.1.0464 .032-038		
		M. singaringan	16	DIB.1.0465 .023-038		
5.	Gajah Mungkur Reservoir, Sendang, Wonogiri, Central Java	*M. singaringan*	5	DIB.1.0465 .018-022	7°51'29.977"S	110°54'32.8"E
		M. nigriceps	1	DIB.1.0464 .031		
6.	Tipar River, Kroya, Cilacap, Central Java	*M. gulio*	12	DIB.1.0463 .023-034	7°37'57.057"S	109°15'14.375" E
7.	Citarum River, Jayakerta Karawang, West Java	*M. gulio*	10	DIB.1.0463 .013-022	6°5'30.481"S	107°18'39.663" E
		M. nigriceps	8	DIB.1.0463 .013-022		
		M. singaringan	7	DIB.1.0465 .007-013		
8.	Ciliwung River, Bantarjati, Bogor, West Java	*M. singaringan*	4	DIB.1.0465 .014-017	6°34'46.72"S	106°48'6.972"E
9.	Cisadane River, Kresek, Tanggerang, Banten	*M. gulio*	12	DIB.1.0463 .001-012	6°7'21.935"S	106°22'45.704" E

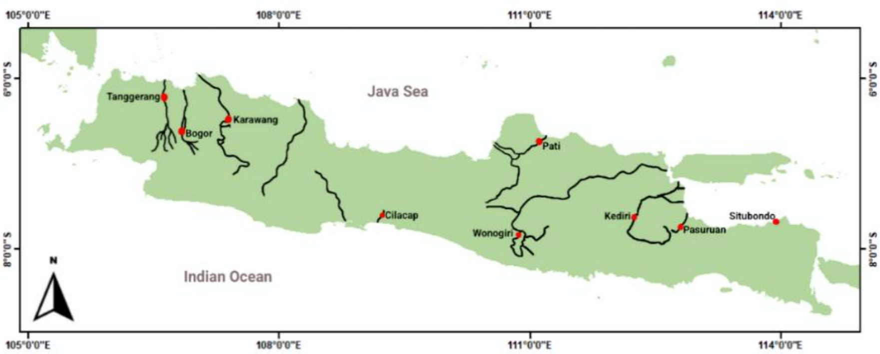

Figure 1. Map sampling localities of Javanese *Mystus* species

Table 2. Key morphological characters used for species identification of Javanese *Mystus* species

Character	*M. gulio*	*M. nigriceps*	*M. singaringan*
Maxillary barbel length	Maxillary barbels reach or extend beyond the pelvic-fin base	Maxillary barbels extending posteriorly to caudal fin base	Maxillary barbels are elongated, reaching to or extending beyond the caudal-fin base
Length of adipose-fin base	Adipose-fin base is shorter than the anal-fin base	Adipose-fin base is longer than the anal-fin base and contiguous with the dorsal fin.	Adipose fin is significantly longer than the dorsal fin and nearly with it.

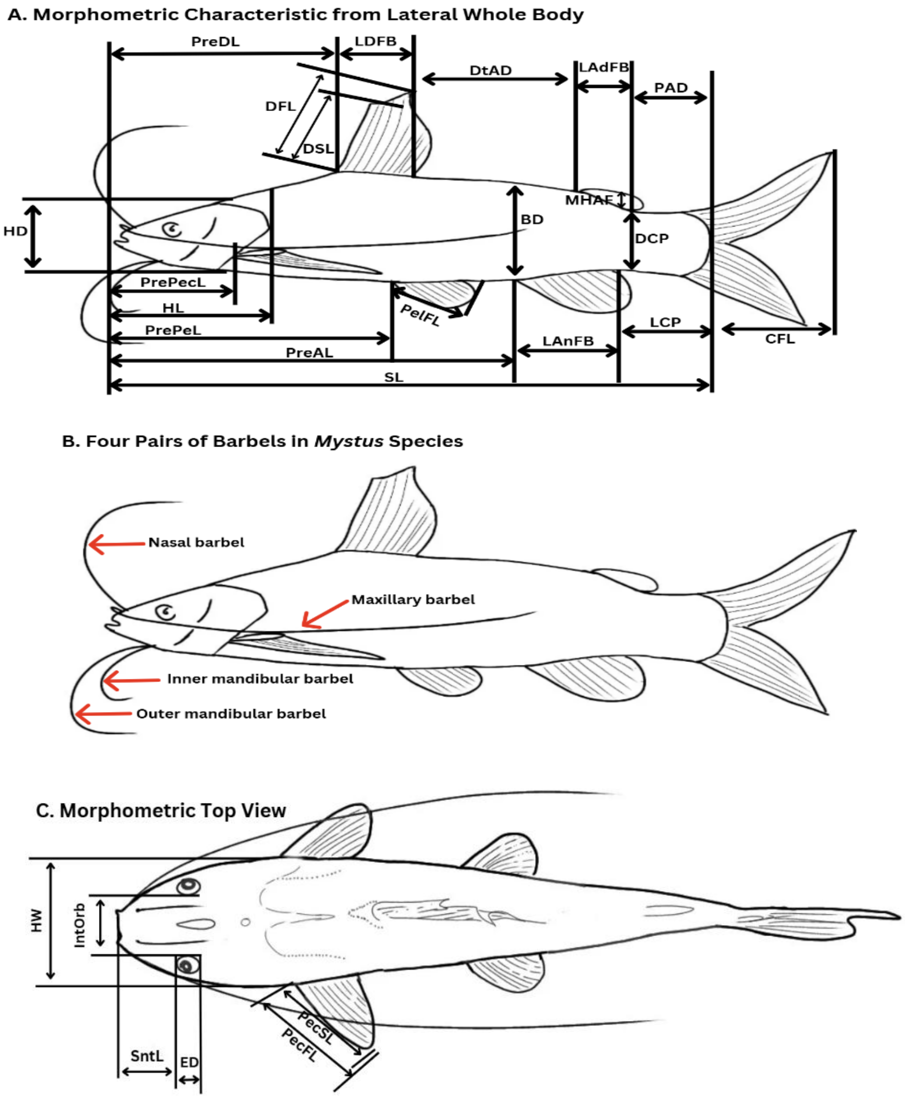

Figure 2. A. Morphometric characters from lateral whole body, B. Four pairs of barbels in *Mystus* species, C. Morphometrics from top view (Adapted from Ng&Kottelat, 2013). *SL (Standard Length), PreDL (Predorsal Length), PreAL (Preanal Length), PrePecL (Prepectoral Length), PrePeL (Prepelvic Length), DSL (Dorsal Spine Length), DFL (Dorsal Fin Length), LDFB (Length of Dorsal-Fin Base), PelFL (Pelvic Fin Length), PecSL (Pectoral Spine Length), PecFL (Pectoral Fin Length), DtAD (Dorsal to Adipose Distance), LAdFB (Length of Adipose-Fin Base), MHAF (Maximum Height of Adipose Fin), PAD (Post-Adipose Distance), LCP (Length of Caudal Peduncle), DCP (Depth of Caudal Peduncle), CFL (Caudal Fin Length), LAnFB (Length of Anal-Fin Base), HL (Head Length), HW (Head Width), HD (Head Depth), BD (Body Depth at anus), SntL (Snout Length), IntOrb (Interorbital Distance), ED (Eye Diameter), NBL (Nasal Barbel Length), MBL (Maxillary Barbel Length) IMBL (Inner Mandibular Barbel Length, OMBL (Outer Mandibular Barbel Length)

Data Analysis

Morphological analyses among species were conducted to identify novel diagnostic characters for discriminating among three target species, supplementing previously established keys. Additionally, morphological analyses within species were conducted to assess morphological variations among populations within each species. To minimize potential bias caused by growth and size effects, morphometric length was standardized by dividing each measurement by Standard Length (SL). While morphometric data related to the head were further standardized by dividing them by Head Length (HL) (Table 3). A dendrogram was constructed to illustrate the degree of similarity among species groups as well as among populations within species, with relatedness expressed in percentage units (Sabran et al., 2021). The dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) model and Euclidean similarity index. Furthermore, morphometric and meristic characters are further analyzed using Principal Component Analysis (PCA), to identify independent character correlations and evaluate group clustering. All multivariate analyses were performed using the Paleontological Statistics (PAST) software (Hammer & Harper, 2001). PCA was performed based on 10 morphometric and 5 meristic characters. The morphometric features included two established key characters as proposed in previous taxonomic literature (Table 2). Additionally, we incorporated eight candidate diagnostic features based on our preliminary observations: nasal barbel length (NBL), inner mandibular barbel length (IMBL), outer mandibular barbel length (OMBL), maximum height of adipose fin (MHAF), dorsal to adipose distance (DtAD), post adipose distance (PAD), head length (HL) and depth of caudal peduncle (DCP). All statistical analyses of morphological data using Multivariate Analysis of Variance (MANOVA) were carried out with IBM SPSS Statistics software version 31.0.2.0 (IBM Corporation, 2026).

RESULTS AND DISCUSSION

Morphometric and Meristic Data Among Javanese *Mystus*

Morphometric characterization revealed distinct interspecific variations among the three target species from Java Island (Table 3). For body proportions relative to SL, *M. gulio* exhibited the largest dorsal to adipose distance (DtAD) with a median of 26.5% (mean 26.4±2.9%), significantly exceeding those of *M. nigriceps* (median 7.8%, range 4.8±3.9%) and *M. singaringan* (median 2.9%, mean 4.2±3.9%). This indicates a more posterior placement of the adipose fin in *M. gulio* compared to its two congeners. Conversely, *M. singaringan* was characterized by the most extensive length of adipose-fin-base (LAdFB) with a median of 42.5% (mean 41.5±3.9%), followed by *M. nigriceps* (median 29.9%, mean 27.1±4.0%), and *M. gulio* (median 7.3%, mean 7.7±2.1%). Within the cephalic region, barbel length ratio (relative to HL) served as a notable diagnostic feature: *M. singaringan* possessed the longest appendages, specifically the maxillary (MBL, median 360.1%; mean 360.2±35.4%) and nasal barbels (NBL, median 64.7%; mean 63.6±16.0%). These values align closely with the ranges reported from the previous study e.g., by Hee (2002); Pramono et al. (2019); Muthukrishnan et al. (2021). Meanwhile, *M. gulio* consistently has the lowest median values for all types of barbels, including the inner (IMBL) and outer (OMBL) mandibular barbels compared to the other two species. Lateral side images of three Javanese *Mystus* species are shown in Figure 3.

Meristic counts further supported the taxonomic distinction of these species, as detailed in Table 4. Number of anal-fin ray in *M. singaringan* (median 10, range 8-13) were slightly higher than those in *M. nigriceps* (median 9, range 8-12) and *M. gulio* (median 9, range 8-14). Meanwhile, the pectoral-fin ray count for *M. singaringan* (median 10, range 5-13) was notably higher than for *M. gulio* (median 7, range 4-12) and *M. nigriceps* (median 6, range 4-8). The dorsal and pelvic fin ray counts remained constant across all species (7 and 6, respectively), the caudal-fin rays showed slight variation, with *M. gulio* displaying a median count of 18 compared to 17.5 in *M. nigriceps*, and 17 in *M. singaringan*. When compared to the original descriptions and literature research (Hamilton, 1822; Kottelat, 1998; Roberts, 1993; Hee, 2002; Pramono et al., 2025), the meristic ranges observed in this study generally conform to established holotypes; however, the lower pectoral ray count *M. nigriceps* and anal ray count *M. gulio* in Javanese suggests a degree of regional morphological divergence. Collectively, these morphometric and meristic discrepancies reinforce the biological separation and individual evolutionary trajectories of the *Mystus* genus within the Javanese river systems.

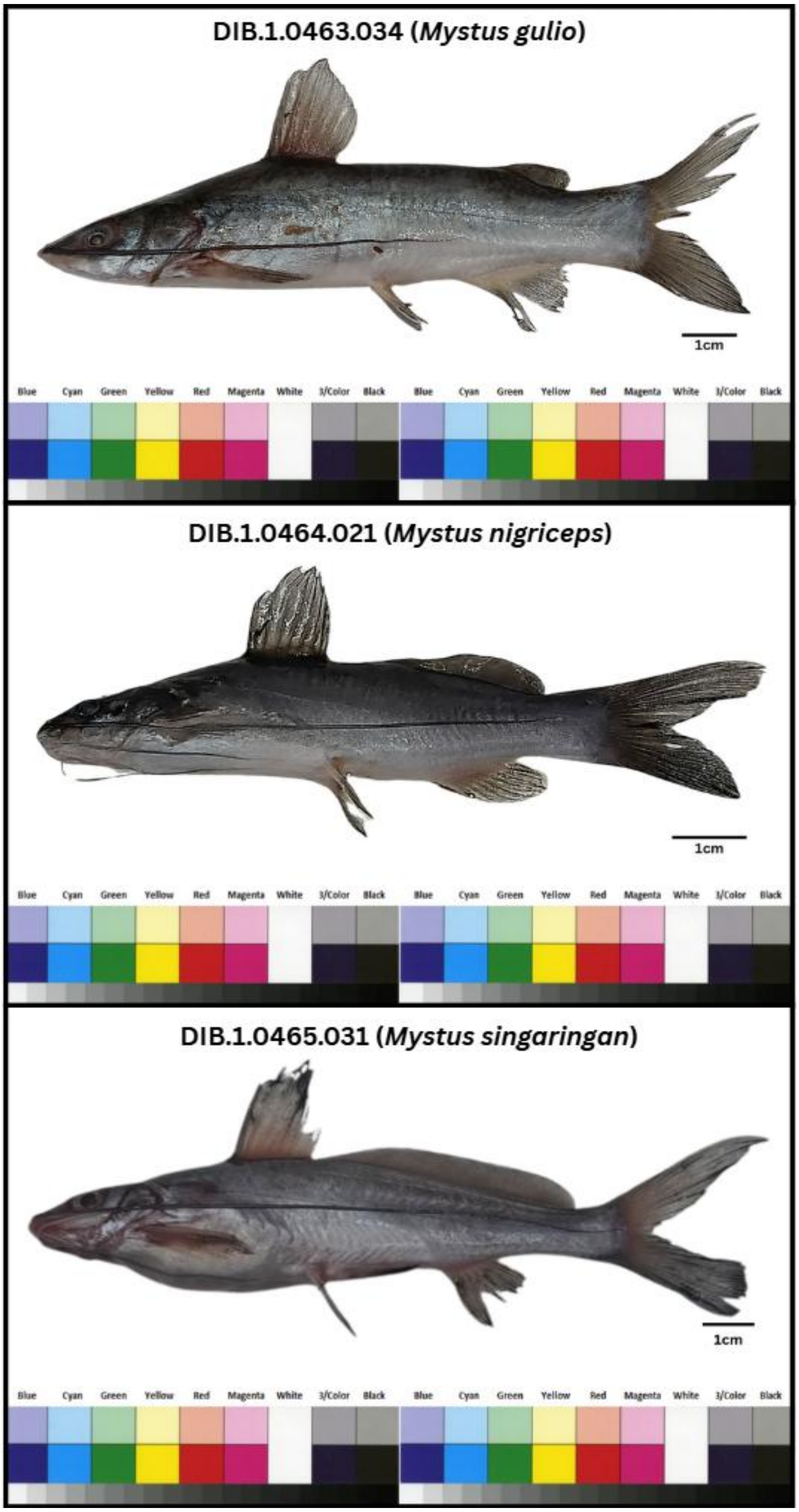

Figure 3. Lateral views of *Mystus* species collected from Java Island: *M. gulio* (top), *M. nigriceps* (middle), and *M. singaringan* (bottom). Scale bars below the specimen represent 1 cm.

Table 3. Morphometric data for three species of *Mystus* from Java Island

Character	*Mystus gulio* (n=67)		*Mystus nigriceps* (n=38)		*Mystus singaringan* (n=38)	
	Median	**Mean±St. Dev.**	**Median**	**Mean±St. Dev.**	**Median**	**Mean±St. Dev.**
SL	98.0	96.9±19.2	76.5	79.1±13.3	114.0	111.8±26.3
% In Standard Length						
PreDL	39.6	39.3±4.0	37.2	37.1±3.5	34.7	35.4±4.7
PreAl	71.8	71.5±4.6	70.8	71.0±3.8	69.8	68.9±5.4
PrePeL	55.3	54.9±4.3	50.0	51.0±3.3	47.4	47.0±4.1
PrePecL	25.3	25.8±3.3	22.3	23.7±7.1	21.2	21.4±3.5
DSL	15.5	15.5±2.2	11.9	12.0±2.6	15.6	15.1±2.3
DFL	22.3	21.8±4.0	21.7	22.2± 4.2	23.9	22.8±3.3
LDFB	10.2	10.2±1.8	13.4	2.2±2.6	12.6	12.6±1.6
LAnFB	12.0	12.1±2.1	11.6	13.6±1.9	8.1	8.7±3.0
PelFL	13.7	13.5±2.0	15.3	11.7±2.4	18.6	18.4±1.8
PecFL	20.8	21.3±3.2	21.3	14.9±4.2	19.8	20.7±4.0
PecSL	18.2	18.7±2.6	18.6	22.2±2.7	15.5	16.1±3.6
CFL	26.9	26.8±4.2	27.5	18.7±3.2	29.6	27.7±6.2
LAdFB	7.3	7.7±2.1	29.9	27.1±4.0	42.5	41.5±3.9
MHAF	4.2	4.4±1.0	4.6	4.8±1.0	6.6	6.7±1.0
DtAD	26.5	26.4±2.9	7.8	8.9±3.9	2.9	4.2±3.9
PAD	17.7	17.7±2.3	14.6	15.7±3.5	10.6	11.0±3.8
LCP	18.1	18.1±3.0	18.7	18.1±3.1	22.3	22.4±2.7
DCP	10.6	11.2±3.1	10.7	10.8±2.0	7.8	8.0±1.4
BD	16.8	16.5±2.6	17.6	17.6±3.1	15.1	14.8±1.9
HL	28.0	26.8±4.87	19.0	19.9±3.2	26.2	25.9±5.9
% in Head Length						
HW	75.0	76.9±11.2	73.4	73.7±8.5	67.9	68.7±5.3
HD	57.1	57.5±0.3	58.8	59.4±9.4	62.7	65.6±11.6
SntL	20.6	22.4±7.2	20.9	21.9±6.1	22.4	25.1±7.8
IntOrb	37.5	37.3±4.5	36.6	38.8±7.9	30.2	30.6±4.4
ED	16.1	16.4±0.6	17.2	18.4±3.6	22.2	22.3±5.1
NBL	44.4	45.2±9.5	58.5	59.4±12.5	64.7	63.6±16.0
MBL	217.8	214.5±40.0	308.6	310.9±36.7	360.1	360.2±35.4
IMBL	57.1	63.1±38.0	73.5	72.3±15.8	81.6	83.0±14.3
OMBL	100.0	100.0±15.2	129.1	126.5±29.8	148.3	148.9±19.9

*St. Dev. = Standard Deviation

Table 4. Meristic data for three species of *Mystus* from Java Island

Character	*Mystus gulio* (n=67)		*Mystus nigriceps* (n=38)		*Mystus singaringan* (n=38)	
	Median	**Range**	**Median**	**Range**	**Median**	**Range**
Dorsal Rays	7	6-8	7	6-8	7	6-9
Anal Rays	9	8-14	9	8-12	10	8-13
Pelvic Rays	6	5-11	6	5-7	6	5-7
Pectoral Rays	7	4-12	6	4-8	10	5-13
Caudal Rays	18	16-20	17,5	14-21	17	14-21

MANOVA results revealed significant multivariate morphological difference among the three *Mystus* species (Pillai's Trace = 1.933; F = 88.337; $p < 0.001$) (Table 5; Supplementary Tables S1-S2). Among 35 morphological characters tested, 16 characters differed significantly between species (p-value <0.05) while the remaining 19 characters were non-significant (p-value > 0.05). Of the 10 morphometric characters used in the PCA, seven characters exhibited statistically significant variation including head length (HL), maximum height of adipose fin (MHAF), length of adipose-fin base (LAdFB), dorsal to adipose distance (DtAD), and post adipose distance (PAD) (p-value < 0.05). Furthermore, on barbels dimension, two characters also showed significant variation in maxillary barbels (MBL) and outer mandibular barbels length (OMBL). Among the five meristic characters, anal, pelvic and pectoral ray count also differed significantly between the three species.

Table 5. Significance MANOVA test of 35 morphological characters three species *Mystus*

Characters	Sig.	Significance	Characters	Sig.	Significance
SL	0.003	Significant	DCP	1.000	Not Significant
PreDL	0.035	Significant	BD	0.315	Not Significant
PreAl	1.000	Not Significant	HL	0.046	Significant
PrePeL	0.001	Significant	HW	0.345	Not Significant
PrePecL	0.008	Significant	HD	0.538	Not Significant
DSL	0.124	Not Significant	SntL	1.000	Not Significant
DFL	1.000	Not Significant	IntOrb	0.544	Not Significant
LDFB	0.015	Significant	ED	0.226	Not Significant
LAnFB	1.000	Not Significant	NBL	1.000	Not Significant
PelFL	0.042	Significant	MBL	< 0.001	Significant
PecFL	0.566	Not Significant	IMBL	1.000	Not Significant
PecSL	0.246	Not Significant	OMBL	< 0.001	Significant
CFL	1.000	Not Significant	Dorsal Rays	0.586	Not Significant
LAdFB	< 0.001	Significant	Anal Rays	< 0.001	Significant
MHAF	0.047	Significant	Pelvic Rays	0.041	Significant
DtAD	< 0.001	Significant	Pectoral Rays	0.043	Significant
PAD	< 0.001	Significant	Caudal Rays	1.000	Not Significant
LCP	1.000	Not Significant			
Multivariate Test (Pillai Trace)					
Value	**F**	**Hypothesis df**	**Error df**	**Sig.**	
1.933	88.337	70.000	214.000	< 0.001	

Interspecific Morphological Characters Between *M. gulio*, *M. nigriceps*, and *M. singaringan*

Morphological observations can help identify species and reveal sampling sites differentiation. Morphology often features characteristic traits called key characters that distinguish one species from another (Astuti et al., 2020; Wilson et al., 2020). Ten morphometric and five meristic characters related to morphological key characters i.e., maxillary barbel length and length of adipose-fin base were used to conduct PCA analyses (Figure 4). These characters were previously suggested by (Hamilton, 1822; Roberts, 1993; Kottelat, 1998; Hee, 2002) which is able to effectively discriminate the three species.

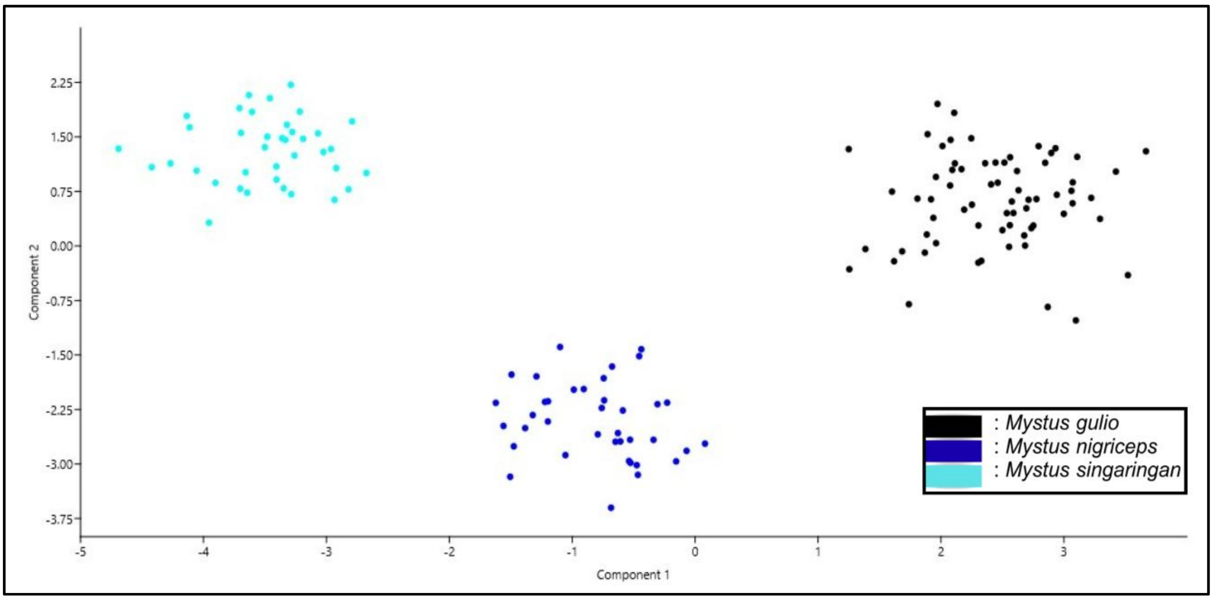

Figure 4. PCA scatter plot of 10 morphometrics and 5 meristics

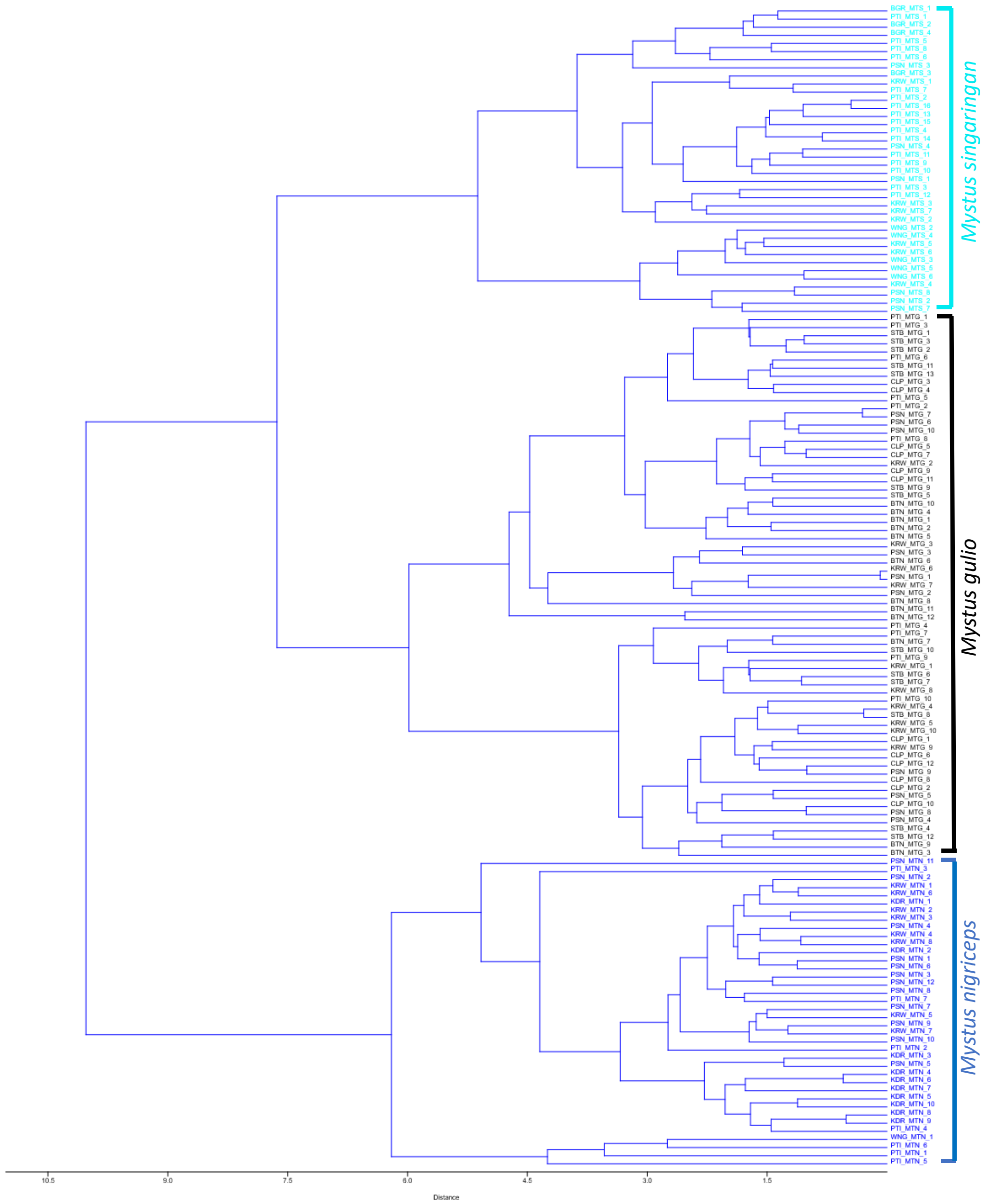

Figure 5. Dendrogram analysis for three species of *Mystus* based on 10 morphometric characters and 5 meristic characters

PCA revealed that the first four components (PC1-PC4) account for a cumulative variance of 75.32% (Supplementary Table S3). PC1 explains the largest portion of the variance (44.19%), primarily driven by length of adipose-fin base (LAdFB) and dorsal to adipose distance (DtAD), which likely reflect variations in overall body proportions. Meanwhile, PC2 (15.93%), PC3 (8.22%) and PC4 (6.99%), are dominated by anal fin ray counts depth of caudal peduncle (DCP), and inner mandibular barbel length (IMBL), respectively. These traits possibly reflect critical ecological adaptations; for instance, the adipose fin which varies significantly in tissue position among *Mystus* enhances swimming maneuverability by serving as a sensory structure or increasing thrust (Stewart, & Hale, 2013). Similarly, the elongated maxillary barbels observed in *M. singaringan* and *M. nigriceps* are essential tactile-chemoreceptive organs that facilitate prey detection and environmental exploration in turbid riverine habitats (Ikpegbu, & Nlebedum, 2015). On PC2, variation was largely influenced by head length (HL) and fin ray counts (AFR and PecFR). Such cephalic variation is frequently linked to resource utilization and water depth, where differences in food sources drive the development of the head region (Winkler et al., 2017).

The hierarchical clustering in the UPGMA dendrogram (Figure 5) corroborated the PCA results, clearly partitioning the specimens into three major groups with distances ranging from 0.5 to 10. Meanwhile, the clear separation of *M. singaringan* from *M. nigriceps* supports recent taxonomic misidentification, confirming that Javanese populations previously misidentified as *M. nigriceps* possess consistent and distinct morphological patterns (Hee, 2002; Syafrialdi et al., 2020). While MANOVA identified 16 morphometric variables with statistically significant differences (p-value < 0.05), these were not considered diagnostic. The high degree of character state overlap between species suggests these traits are secondary sexual characteristics or plastic responses to environment, rather than stable markers for species delimitation.

Intraspecific Morphological Characters Within Populations in *M. gulio, M. nigriceps,* and *M. singaringan*

Beyond interspecific comparisons, our study performed PCA at the intraspecific level to evaluate morphological diversity between populations and geographic sampling sites within the same species. These analyses revealed that while sampling sites of all three species share fundamental body proportions, significant individual and geographic variations exist, often driven by allometry and specific life stages (Figure 6).

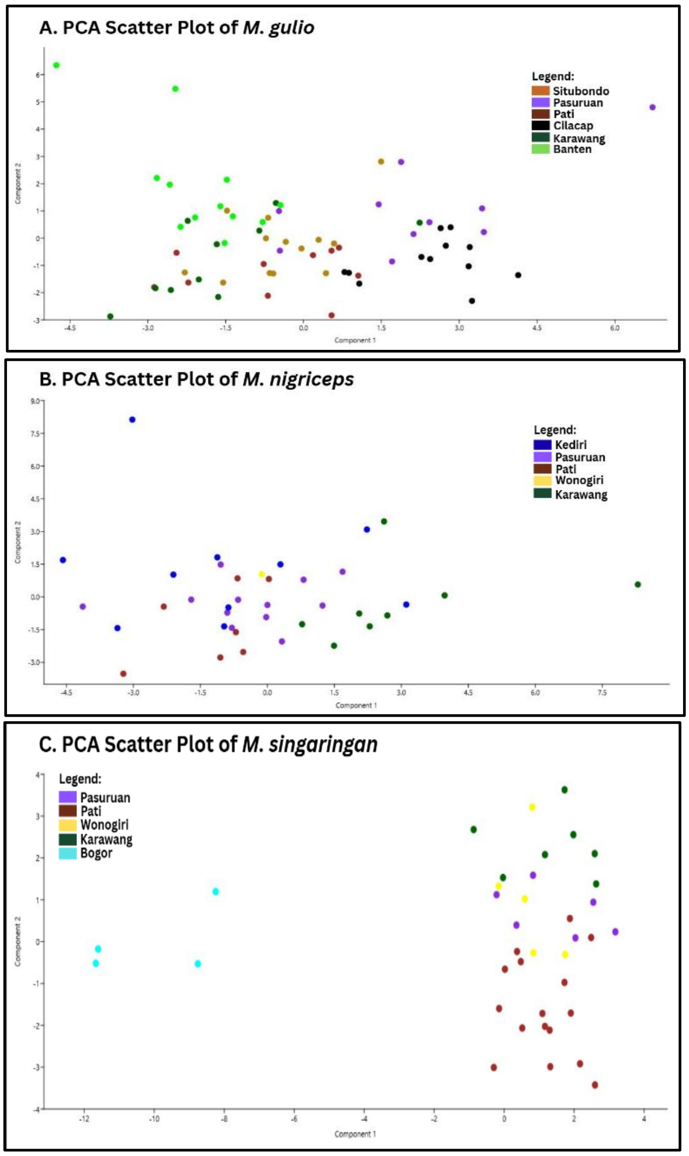

Figure 6. A. PCA scatter plot *M. gulio*, B. PCA scatter plot *M. nigriceps*, C. PCA scatter plot *M. singaringan*

For *M. gulio*, the first two components accounted for 26.3% of the total variation, with most sampling sites (Pati, Karawang, Pasuruan, Cilacap, and Situbondo) clustering in a central position in PC1, indicating high morphological homogeneity across these regions (Figure 6A). However, a divergence in cephalic traits, specifically head shape and barbel length (IMBL and MBL), was observed between the Banten (North/West Coast) and Cilacap (South Coast) sampling sites. Notable outliers in *M. gulio* from Pasuruan (one individual) and Banten (two individuals) were probably attributed to ontogenetic shifts; these smaller individuals occupied a distinct multivariate space due to allometric growth, where head dimensions and barbel lengths develop at different rates relative to somatic size (McMenamin & Parichy, 2013; Ismail, 2021).

In *M. nigriceps*, the PCA (explaining 28.9% of variance) highlighted high intra-population variability, particularly within the Pati and Pasuruan (Figure 6B). PC1 was primarily influenced by fin dimensions (PecFL, DFL, and DSL), while PC2 captured cephalic diversity (SntL, ED, and HD). The wide distribution of individuals across the scatter plot suggests that *M. nigriceps* maintains a high degree of phenotypic plasticity. While most individual from several sampling sites overlapped, specific outliers from Kediri and Karawang displayed significantly elongated snouts and pectoral fins, respectively. This suggests that PCA in this genus captures not only size but also subtle shifts in relative proportions caused by localized habitat pressures or varying developmental rates.

For *M. singaringan* (26.6% total variance), a notable observation was the isolation of individuals from Bogor (BGR) from the Karawang, Pasuruan, Wonogiri, and Pati (Figure 6C). The Bogor specimens exhibited significantly shorter pre-anal and pre-pelvic proportions; a pattern strongly linked to the juvenile life stage of these individuals. This separation is consistent with the negative allometric growth patterns typical of bagrid catfishes, where early development of body shape and tactile-chemoreceptive organs (barbels) undergo substantial transformation during the transition from larval to juvenile stages (Pramono et al., 2025).

We conducted an extensive search for novel diagnostic morphometric and meristic characters beyond those established in historical literature, however, our analyses confirm that only maxillary barbel length and adipose-fin base length serve as effective morphological discriminators for Javanese *Mystus* (Figure 3). While qualitative observations initially suggested potential markers, such as the silvery coloration of *M. nigriceps* versus the darker hues of *M. gulio*, or the subtle variations in caudal peduncle thickness in *M. singaringan*, these traits failed to provide significant separation during quantitative morphometric assessment.

CONCLUSION

This study successfully resolved the morphological boundaries of three native Javanese *Mystus* species, achieving the primary objective of identifying reliable diagnostic markers amidst high phenotypic similarity. The integration of MANOVA and PCA confirms that *M. gulio*, *M. nigriceps*, and *M. singaringan* are morphologically distinct, with adipose-fin base length and maxillary barbel proportions serving as the most robust discriminators. Notably, the isolation of the Bogor (BGR) population within the *M. singaringan* cluster suggests that while species-level boundaries are clear, significant intraspecific variance exists, likely driven by localized environmental plasticity and ontogenetic allometric growth. These findings provide a necessary taxonomic baseline for the germplasm certification required to support the domestication of *M. singaringan* and the targeted conservation of *M. nigriceps*. Furthermore, a molecular taxonomic approach using COI barcoding is necessary to verify these morphological boundaries and investigate potential cryptic diversity within the Javanese populations. Ultimately, this work provides the foundational framework required for the sustainable management and genetic conservation of Java's indigenous *Mystus* resources.

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DECLARATIONS

Author Contributions : Author 1: Conceptualisation, Methodology, Writing - Original Draft; Author 2: Formal Analysis, Investigation; Author 3: Formal Analysis, Investigation; Author 3: Formal Analysis, Investigation; Author 4: Formal Analysis, Investigation; Author 5: Review, editing; Author 6: Review, Editing; Author 7: Conceptualisation, Methodology, Writing - Original Draft, Funding Acquisition, Project Administration. All authors have read and approved the final manuscript.

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Data Availability : The raw morphological data are available from the corresponding author upon reasonable request.

Ethical Approval : The study was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Universitas Brawijaya (*ethical clearance* reference number: 98-KEP-FKHUB-2026).

Declaration of AI Use : AI tools (ChatGPT and Gemini) were used for spelling checks and grammar corrections. The authors reviewed and take full responsibility for the final content.

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Supplementary Table S1. Results of normality, homogeneity, mean, and post-hoc tests of 35 morphological characters three species *Mystus* in Java

Character	Species	Shapiro-wilk (sig.)	Levene's Test (sig.)	F Statistic and Test of Between-Subjects	Mean ± SD and Post-Hoc Significance	Sig.
Morphometrics						
SL	1	0.009	0.261*	23.818; p < 0.001	96.947 ± 19.368[a]	0.003*
	2	0.032			79.144 ± 13.458[b]	
	3	< 0.001			110.477 ± 25.383[c]	
PreDL	1	< 0.001	0.069*	17.127; p < 0.001	0.394 ± 0.038[a]	0.035*
	2	< 0.001			0.370 ± 0.035[b]	
	3	< 0.001			0.339 ± 0.064[c]	
PreAL	1	0.119*	0.052*	8.441; p < 0.001	0.718 ± 0.039[a]	1.000
	2	0.480*			0.710 ± 0.038[b]	
	3	< 0.001			0.667 ± 0.102[b]	
PrePeL	1	0.004	0.161*	40.140; p < 0.001	0.548 ± 0.043[a]	0.001*
	2	0.016			0.510 ± 0.034[b]	
	3	< 0.001			0.452 ± 0.077[c]	
PrePecL	1	0.901*	0.563*	25.721; p < 0.001	0.258 ± 0.032	0.008*
	2	< 0.001			0.228 ± 0.058	
	3	< 0.001			0.199 ± 0.033	
DSL	1	0.758*	0.296*	25.704; p < 0.001	0.156 ± 0.021[a]	0.124
	2	0.494*			0.120 ± 0.026[b]	
	3	< 0.001			0.145 ± 0.026[a]	
DFL	1	0.448*	0.996*	0.118; p = 0.889	0.219 ± 0.040	1.000
	2	0.122*			0.222 ± 0.042	
	3	0.002			0.223 ± 0.040	
LDFB	1	0.243*	0.637*	28.500; p < 0.001	0.103 ± 0.017	0.015*
	2	< 0.001			0.135 ± 0.026	
	3	0.002			0.121 ± 0.022	
LAnFB	1	0.571*	0.689*	28.071; p < 0.001	0.121 ± 0.021[a]	1.000
	2	0.724*			0.117 ± 0.019[a]	
	3	< 0.001			0.086 ± 0.030[b]	
PelFL	1	0.742*	0.455*	34.754; p < 0.001	0.136 ± 0.020	0.042*
	2	0.007			0.148 ± 0.025	
	3	< 0.001			0.177 ± 0.030	
PecFL	1	0.012	0.036	5.552; p < 0.005	0.213 ± 0.030[a]	0.566
	2	0.031			0.221 ± 0.042[a]	
	3	0.483*			0.194 ± 0.039[b]	

Supplementary Table S1. Continued

Character	Species	Shapiro-wilk (sig.)	Levene's Test (sig.)	F Statistic and Test of Between-Subjects	Mean ± SD and Post-Hoc Significance	Sig.
PecSL	1	0.175*	0.050*	19.811; p < 0.001	0.185 ± 0.024[a]	0.246
	2	0.729*			0.195 ± 0.021[a]	
	3	0.383*			0.155 ± 0.042[b]	
CFL	1	0.059*	0.057*	0.528; p = 0.591	0.269 ± 0.041	1.000
	2	0.929*			0.271 ± 0.032	
	3	0.005			0.278 ± 0.056	
LAdFB	1	0.004	0.050*	1412.977; p < 0.001	0.075 ± 0.021[a]	< 0.001*
	2	0.002			0.302 ± 0.032[b]	
	3	< 0.001			0.408 ± 0.046[c]	
MHAF	1	< 0.001	0.401*	48.538; p < 0.001	0.044 ± 0.011[a]	0.047*
	2	0.031			0.050 ± 0.009[b]	
	3	0.125*			0.066 ± 0.012[c]	
DtAD	1	0.329*	0.078*	1042.837; p < 0.001	0.269 ± 0.027[a]	< 0.001*
	2	< 0.001			0.081 ± 0.039[b]	
	3	0.063*			0.028 ± 0.010[c]	
PAD	1	0.396*	0.082*	120.232; p < 0.001	0.176 ± 0.023[a]	< 0.001*
	2	0.128*			0.154 ± 0.030[b]	
	3	0.888*			0.099 ± 0.019[c]	
LCP	1	0.210*	0.886*	15.224; p < 0.001	0.182 ± 0.029[a]	1.000
	2	0.452*			0.181 ± 0.032[a]	
	3	0.002			0.215 ± 0.033[b]	
DCP	1	< 0.001	0.961*	13.191; p < 0.001	0.111 ± 0.027[a]	1.000
	2	0.008			0.115 ± 0.022[a]	
	3	0.042			0.089 ± 0.018[b]	
BD	1	0.232*	0.876*	14.927; p < 0.001	0.166 ± 0.025[a]	0.315
	2	0.007			0.176 ± 0.031[a]	
	3	< 0.001			0.141 ± 0.030[b]	
HL	1	0.044	0.098*	106.633; p < 0.001	28.398 ± 3.182[a]	0.046*
	2	0.004			20.210 ± 2.429[b]	
	3	0.031			26.994 ± 2.464[c]	
HW	1	< 0.001	0.903*	13.147; p < 0.001	0.770 ± 0.113[a]	0.345
	2	0.429*			0.736 ± 0.086[a]	
	3	< 0.001			0.661 ± 0.105[b]	

Supplementary Table S1. Continued

Character	Species	Shapiro-wilk (sig.)	Levene's Test (sig.)	F Statistic and Test of Between-Subjects	Mean ± SD and Post-Hoc Significance	Sig.
HD	1	0.013	0.010	6.925; p = 0.001	0.574 ± 0.075	0.538
	2	0.305*			0.593 ± 0.095	
	3	0.048			0.646 ± 0.123	
SntL	1	< 0.001	0.542*	0.165; p = 0.848	0.223 ± 0.073	1.000
	2	< 0.001			0.219 ± 0.062	
	3	< 0.001			0.229 ± 0.087	
IntOrb	1	0.002	< 0.001	25.951; p < 0.001	0.373 ± 0.046[a]	0.544
	2	< 0.001			0.388 ± 0.080[a]	
	3	0.089*			0.299 ± 0.053[b]	
ED	1	0.008	0.010	10.568; p < 0.001	0.164 ± 0.033[a]	0.226
	2	0.002			0.184 ± 0.037[b]	
	3	0.653*			0.202 ± 0.056[b]	
NBL	1	0.142*	0.087*	22.782; p < 0.001	0.451 ± 0.096[a]	1.000
	2	0.263*			0.594 ± 0.127[b]	
	3	0.024			0.577 ± 0.145[b]	
MBL	1	< 0.001	0.312*	270.197; p < 0.001	2.081 ± 0.379[a]	< 0.001*
	2	0.181*			3.053 ± 0.263[b]	
	3	0.383*			3.621 ± 0.331[c]	
IMBL	1	< 0.001	0.248*	3.833; p = 0.024	0.620 ± 0.378	1.000
	2	0.491*			0.724 ± 0.101	
	3	0.182*			0.767 ± 0.175	
OMBL	1	0.027	0.067*	66.136; p < 0.001	0.985 ± 0.143[a]	< 0.001*
	2	< 0.001			1.170 ± 0.266[b]	
	3	0.927*			1.430 ± 0.172[c]	
Meristics						
Dorsal-fin rays	1	< 0.001	0.127*	6.548; p = 0.002	7.194 ± 0.583	0.586
	2	< 0.001			6.973 ± 0.544	
	3	< 0.001			6.815 ± 0.392	
Anal-fin rays	1	< 0.001	0.287*	331.358; p < 0.001	10.925 ± 0.784[a]	< 0.001*
	2	< 0.001			8.578 ± 0.500[b]	
	3	< 0.001			12.763 ± 0.751[c]	
Pelvic-fin rays	1	< 0.001	0.059*	658.525 < 0.001	9.656 ± 0.686[a]	0.041*
	2	< 0.001			5.868 ± 0.577[b]	
	3	< 0.001			6.210 ± 0.413[c]	

Supplementary Table S1. Continued

Character	Species	Shapiro-wilk (sig.)	Levene's Test (sig.)	F Statistic and Test of Between-Subjects	Mean ± SD and Post-Hoc Significance	Sig.
Pectoral-fin rays	1	< 0.001	0.758*	285.487; p < 0.001	6.910 ± 0.965[a]	0.043*
	2	< 0.001			6.473 ± 0.796[b]	
	3	< 0.001			10.657 ± 0.745[c]	
Caudal-fin rays	1	< 0.001	0.081*	77.460; p < 0.001	19.641 ± 0.792[a]	1.000
	2	< 0.001			17.578 ± 0.826[b]	
	3	< 0.001			17.631 ± 1.344[b]	

Note: Species 1 is *M. gulio*, species 2 is *M. nigriceps*, and species 3 is *M. singaringan*. In the Shapiro-Wilk normality test and Levene's Test for homogeneity, an asterisk (*) indicates that the data are normally distributed and homogeneous. In Levene's Test, data (p < 0.05) will be examined further with the Post-Hoc Games-Howell test, while if (p > 0.05), the data will be examined further with the Bonferroni test. The notation [abc] on the mean ± SD indicates that different letters in the row show significant differences based on the Post-Hoc Test; if no notation is present, it indicates no significant differences in the Post-Hoc Test.