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Morphological differentiation of *Pennahia aneus* (Bloch, 1793) populations from Northern Peninsular Malaysia using geometric morphometrics

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Abstract. *Kachi JB, Binashikhbubkr K, Naim DM. 2025. Morphological differentiation of* Pennahia aneus *(Bloch, 1793) populations from Northern Peninsular Malaysia using geometric morphometrics. Nusantara Bioscience 17: 30-38.* The investigation of geometric morphometrics offers evidence for the adaptive traits shown by fish in response to their aquatic environment, which may be attributed to evolutionary processes. The examination of body morphometry has significant value in assessing the structure of fish populations, which is a fundamental necessity for the implementation of conservation measures and the management of fisheries in a sustainable manner. The study used geometric morphometrics to differentiate three populations of *Pennahia aneus* from Northern Peninsular Malaysia. A sum of 110 samples were obtained from Pulau Pinang, Kedah, and Perak states in Malaysia. Photographs of fish samples were taken, and 13 homologous landmarks were digitized using the tpsUtil and tpsDig2 software. All data were analyzed using multivariate statistical tests such as canonical variate analysis, cluster analysis, principal component analysis, and discriminant function analysis. Results showed significant morphological variations among the population of *P. aneus* with two morphological groups: Batu Maung group and Pantai Remis and Kuala Kedah group. The overall reclassification grouping for the populations of *P. aneus* in this study was 67.3% successful. These findings are crucial for conservation and management efforts and contribute to formulating efficient conservation policies to maintain the species populations in Northern Peninsular Malaysia, highlighting the potential impact of this research on future conservation policies. Further investigations in terms of expanding the study scope and application of molecular tools will give a holistic understanding of *P. aneus* population structure in Malaysia. This study represents the first to compare morphological differences in *P. aneus* populations from Malaysian waters using geometric morphometric analysis.

Keywords: Geometric morphometrics, multivariate analyses, *Pennahia aneus*

INTRODUCTION

Fish, shaped by physical and biological selection in their aquatic environments, presents a diverse range of morphologies, forming the basis of observable biodiversity (Dwivedi et al. 2020; Martinez et al. 2021; De Brito et al. 2022; Thambithurai and Kuparinen 2024; Rincon-Sandoval et al. 2024). Morphological features, including meristic and morphometric characters, have a significant historical background in biology for population identification (Sidiq et al. 2021; Jawad et al. 2022; Binashikhbubkr et al. 2023; Ainsworth et al. 2024; Xiao and Yang 2024). Variations in species populations can indicate habitat and behavioral disparities, especially for aquatic organisms like fish. These variations underscore the need for fish to adapt to their environment to optimize their biological systems, making them crucial for survival (Jalili et al. 2015; Wilson et al. 2019; Mawer et al. 2023; Akther et al. 2024; Bernos et al. 2024).

Researchers employ various methods to identify stocks, including life-history stages, morphological characteristics, otolith chemistry, and molecular markers like protein allozymes, microsatellite DNA, and mitochondrial DNA (Imtiaz et al. 2016). Among these, morphological measurements are the most popular and economical technique for identifying stocks (Sibinamol et al. 2020; Binashikhbubkr et al. 2023). A recent addition to this tool is Geometric Morphometrics (GM) powerful and popular technique that quantitatively analyzes biological shapes using cartesian coordinates of anatomical landmarks, maintaining the geometric information of the specimen throughout the study (Shukri et al. 2024). Data from GM not only reveal the relationships between landmarks and species but also provide spatial correlations, further enhancing its relevance and popular technique today (Hoff et al. 2020; Iqbal et al. 2024; Shukri et al. 2024).

Pennahia aneus (Donkey croaker) is a commercially important demersal croaker species of the Sciaenidae found in Malaysia. It is widely distributed in the Indo-West Pacific, from the Persian Gulf east to western Indonesia, and occurs in shallow coastal waters to depths of 60 m (Froese and Pauly 2024). *Pennahia aneus* is one of the five species of *Pennahia* genus, collectively called Pennah croakers (Lim et al. 2021). This species is a valuable Asian seafood component due to its delicate flavor and high economic value, and it is marketed in fresh, dried, and salty forms. It predominates at fish landing sites, particularly from muddy coastal fishing areas, where it is often caught as a by-catch using a variety of fishing gear (Wagiyo et al.

2020). The International Union for the Conservation of Nature (IUCN) considers *P. aneus* a species of Least Concern (LC), but one of the major threats it faces is overfishing, especially as by-catch (Chao et al. 2020), which needs to be considered.

Prior reports on *P. aneus* in Malaysia are limited, but the most recent report only focused on their distribution and abundance in the Strait of Pulau Pinang (Hamid et al. 2023), where they were reported to be the third dominant species. In Indonesia, studies on *P. aneus* have been on the aspects of their biological characteristics, abundance index, and fishing in Tangerang waters (Wagiyo et al. 2020), and the discrimination between the males and females using the Truss morphometric network and meristic characters in Central Java's fish auction center (Utarini et al. 2021). The contributions of *P. aneus* to the marine fisheries sector in Malaysia make them susceptible to anthropogenic factors like overfishing, pollution, and habitat degradation (Lim et al. 2021). Understanding the population structure of this species is important to enable sustainable use, and morphological characterization provides a rapid assessment of this, which has not yet been fully explored and documented. As a preliminary to studying the sciaenid fish in Malaysia, this study aims to address the gap of little or no information on the body shape and size variations of these important species using geometric morphometric analysis. Also, this study seeks to validate the verity of geometric morphometric analysis in intraspecies population discrimination. Hence, this study, for the first time, used geometric morphometrics techniques to compare the morphological variations among *P. aneus* populations from Northern Peninsular Malaysia, with the belief that the results will help give foremost insights into their population structure for effective conservation plans and sustainable use.

MATERIALS AND METHODS

Area of study and sample collection

All fish samples were collected from designated fish landing sites in Northern Peninsular Malaysia. The sampling sites were the fishing Jetty locations at Batu Maung (Penang), Kuala Kedah (Kedah), and Pantai Remis (Perak) (Figure 1). In all, 110 fish samples were collected from the sampling sites (Table 1). Cool boxes with ice were used to transport samples from the landing sites to the Molecular Ecology Laboratory, School of Biological Sciences, Universiti Sains Malaysia. Upon arrival at the laboratory, samples were cleaned, drained, and placed on a black background surface to take clear photographs using a Nikon D90 Digital Camera. The proper origin and insertion of fins were captured and erected using pins. Every sample was labeled, photographed, and digitized. Geometric morphometric analyses used only purified images of the samples, and morphometric traits were measured using a digital caliper (Figure 2).

Table 1. Sampling locations and sample size description

Sampling location	Coordinates	No of samples (N)
Kuala Kedah	6º 6’ 25.98’’ N; 100º 17’ 36.56’’ E	43
Batu Maung, Penang	5º 16’ 60.00’’ N; 100º 16’ 60.00’’ E	42
Pantai Remis, Perak	4º 27’ 28.74’’ N; 100º 37’ 45.91’’ E	25
Total		110

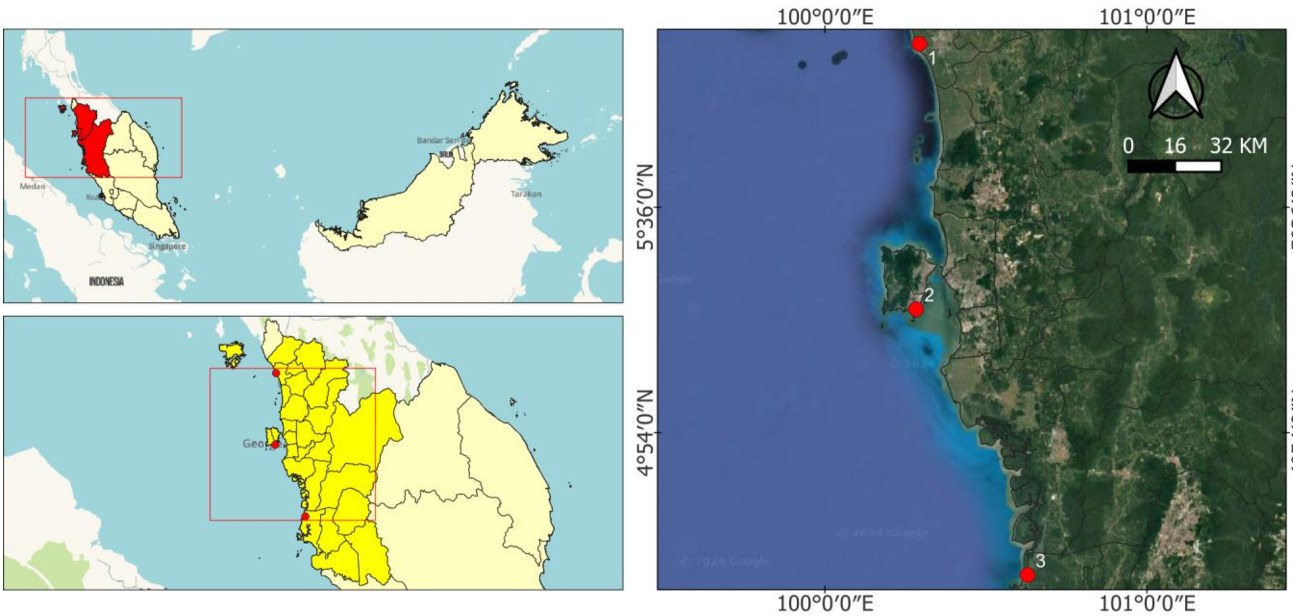

Figure 1. Map of the sampling sites of *Pennahia aneus* in Northern Peninsular Malaysia. Note: 1. Kuala Kedah (Kedah); 2. Batu Maung (Penang); 3. Pantai Remis (Perak)

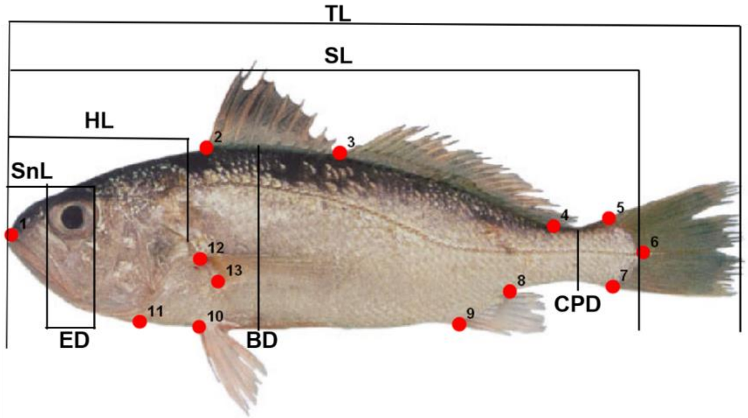

Figure 2. The 13 defined homologous landmark points used for body shape data extraction in populations of *Pennahia aneus*: 1. Anterior-most point of the snout; 2. Anterior end of dorsal fin base; 3. Notch between dorsal fin spines and rays; 4. Posterior end of dorsal fin base; 5. Posterodorsal end of caudal peduncle at its connection to caudal fin; 6. Posterior end of the body lateral line; 7. Posteroventral end of caudal peduncle at its connection to the caudal fin; 8. Posterior end of the anal fin base; 9. Anterior end of the anal fin base; 10. Anterior end of the pelvic fin; 11. Ventral edge of the head; 12. Anterior origin of pectoral fin; 13. Posterior end of pectoral fin. Source: Utarini et al. (2021)

Analyses of geometric morphometrics

Therefore, to describe the actual shape and dimension of every sample, twelve homologous landmarks were utilized (Figure 2). The input file for the data acquisition program was created using the tpsUtil software version 1.79. Images were retrieved to digitize landmarks that would later be used to determine the respective coordinates (x, y) of the landmarks using version 2.31 of the tpsDig2 software (Rohlf 2015). The data generated were analyzed using version 1.07 of the MorphoJ software, as described by Klingenberg (2011). MorphoJ minimizes dimension changes from digitalizing images by adjusting landmarks and creating a consensus configuration (Binashikhbubkr et al. 2022). Shape variations were analyzed using a wireframe designed by linking landmarks, which were measured and registered. The maximum amount of body shape variations was determined by conducting Principal Component Analysis (PCA) to estimate the differentiation in species (Klingenberg 2011). The Procrustes distances among the populations of *P. aneus* were utilized to conduct a Cluster Analysis (CA) based on the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and presented as Bray-Custis similarity, using the Paleontological Statistical (PAST) software version 4.03 (Hammer et al. 2001).

Seven morphometric traits were measured, including Head Length (HL), Snout Length (SnL), Eye Diameter (ED), Total Length (TL), Standard Length (SL), Body Depth (BD), and Caudal Peduncle Depth (CPD) (Figure 2). The mean Total Length (TL) in millimeters (mm) of the individual populations were Kuala Kedah (199.19 ± 15.53), Batu Maung (206.53 ± 16.58), and Pantai Remis (194.46 ± 8.40), respectively. The equation $Madj = M(Ls/L0)^b$ by Elliot et al. (1995) was used to normalize the morphometric variables accounting for variations in sizes across populations, where Madj equals the adjusted measurement in size, M equals the raw measurement, Ls equals the mean standard length of all samples, L0 equals the standard length for each sample. The value b was determined for each character from the observed data as the slope of log M on log L0 regression across populations for all fish species (Binashikhbubkr et al. 2024). Discriminant Function Analysis (DFA) was performed on the transformed morphometric characters to find a combination of traits that best maximizes population differentiations of the species. Discriminant function analysis was utilized to find the correct percentage classification for the populations using Wilks' lambda to distinguish between groups. Version 29 of the SPSS was used to determine the Discriminant Function Analysis (DFA).

RESULTS AND DISCUSSION

Data sampling

One hundred and ten samples of *P. aneus* were collected from three sampling sites (Batu Maung, Kuala Kedah, and Pantai Remis) within Northern Peninsular Malaysia (Table 1; Figure 1).

Principal Component Analysis (PCA)

Principal Components Analysis (PCA) showed that 22 principal components were used to explain the differences in body shape and size of the 110 samples. Although the first Principal Component (PC1) had a low eigenvalue of 0.078% in comparison with the (>0.3) considered significant by Imtiaz and Naim (2018), it contributed a percentage variance of 45.84% of the total body shape and size variations among the study populations. However, the first four Principal Components (PCs) combine to account for 75.86% total variance of the two-dimension body shape and size differences with 12.57% (PC2), 9.8% (PC3), and 7. 87% (PC4) respectively (Figures 3 and 4).

The patterns of overlap in the PC1 and PC2 scatter plots (Figure 4) revealed limited body size and shape differences among the populations of *P. aneus* which was not significant to distinguish the populations based on the 0.078% eigenvalue obtained. In PC1, the body shape differences were evident in the head, body depth, pectoral, dorsal, anal, and caudal regions, while in PC2, the differences were reflected in the head, body depth and length, dorsal, pectoral, and caudal regions (Figure 5).

Canonical Variate Analysis (CVA)

Compared to PCA, the Canonical Variate Analysis (CVA) provides a better resolution for intra-genus discrimination of the study populations, as revealed in this study. The canonical variate analysis revealed that two canonical components were used to describe the body shape and size differences among the study populations of *P. aneus* species. The first canonical variate component (CV1) accounted for 58.37% of the variation with a significant eigenvalue of 0.81, while the second canonical variate (CV2) addressed the remaining 41.63% with an eigenvalue of 0.58 (Figure 6). The body shape and size variations among populations of *P. aneus* were demonstrated in the CVA analysis (Figure 6) with some degree of overlap.

The variations observed from the grid transformation graphs (partial warp) (Figure 7) showed maximum variation in the head region, entire body shape, dorsal, pectoral, anal, and caudal regions (CV1). The overall variations in CV2 were revealed in the head and entire body length, pectoral, anal and caudal parts. The Procrustes distances among the populations as generated by CVA were subjected to Cluster Analysis (CA) based on the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) (Figure 8), which further revealed two distinct morphological groups: Batu Maung group and Pantai Remis and Kuala Kedah group of *P. aneus* from Northern Peninsular Malaysia.

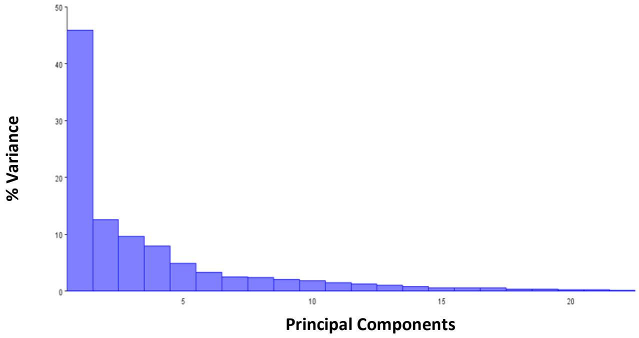

Figure 3. Shows a plot of all 22 principal components against the variance (%) that were involved in analyzing body shape variations among *P. aneus* populations from Northern Peninsular Malaysia

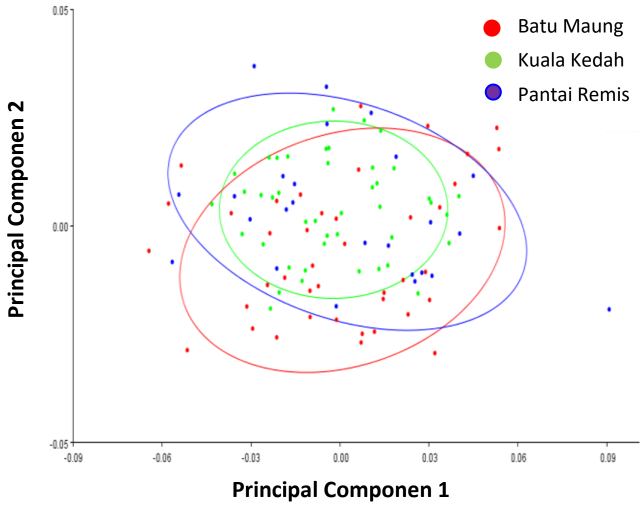

Figure 4. Principal components analysis of body shape and size variations among *P. aneus* populations with equal frequency ellipses shows PC1: 45.84%, PC2: 12.57%, PC3: 9.58%, and PC4: 7.87 % accounting for 75.86 % of the total variation

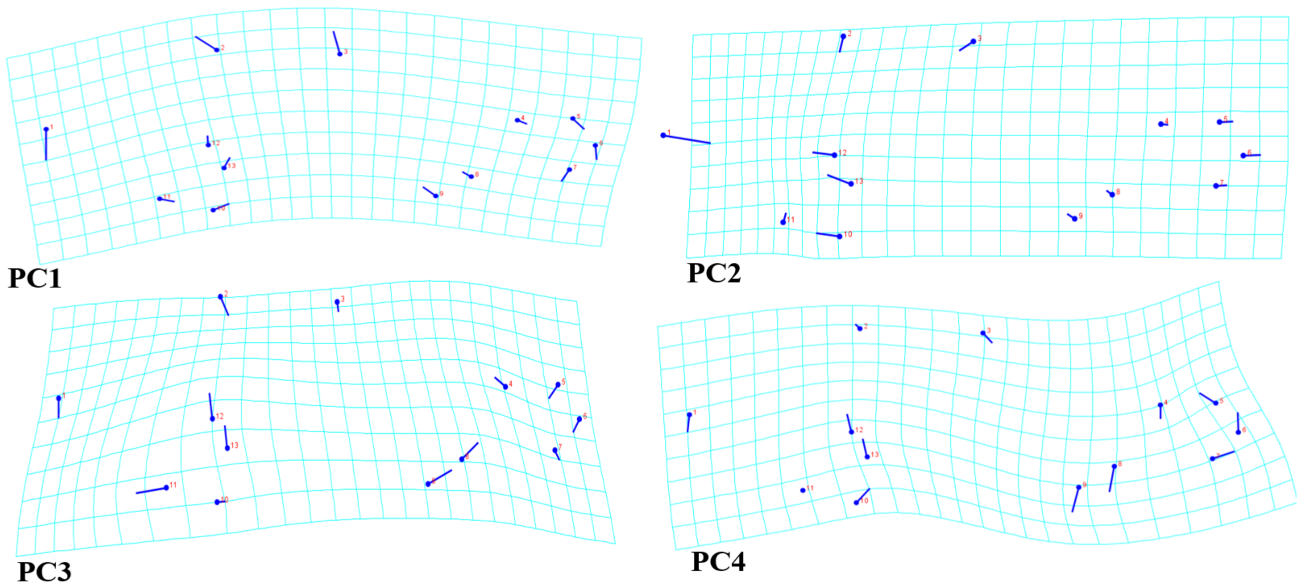

Figure 5. Visualization of body shape differences of *Pennahia aneus* populations via grid transformation graphs for PC1, PC2, PC3, and PC4. PC1 reveals variations in the head, body depth, dorsal, anal and caudal regions. PC2 reveals variations in the head, body depth, and length, dorsal, pectoral, and caudal regions. PC3 reveals variations in the head, body depth, dorsal, anal and caudal regions. PC4 reveals variations in the head, dorsal, pectoral, anal and caudal regions

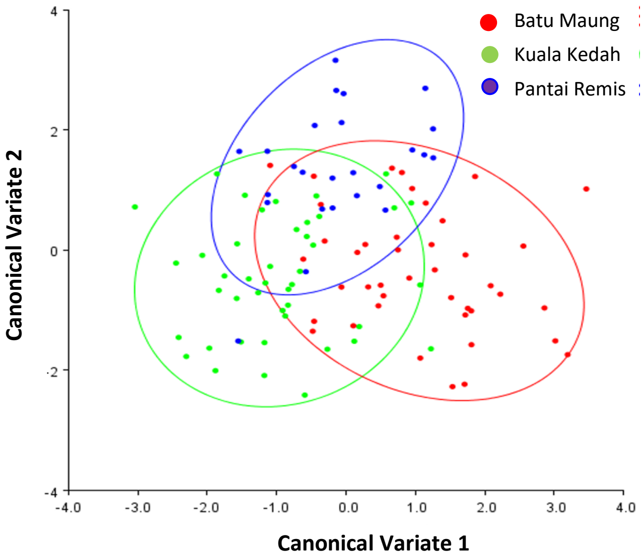

Figure 6. Canonical Variate Analysis (CVA) of body shape and size variations among *Pennahia aneus* populations with equal frequency ellipses showing (CV1: 58.37 %, eigenvalue: 0.81) and (CV2: 41.63 %, eigenvalue: 0.58) accounting for 100.00 % of the total variations among 110 individuals

Discriminant Function Analysis (DFA)

The DFA analyzed seven morphometric variables obtained from *P. aneus* populations. Only two canonical discriminant functions (1 and 2) were used for the analysis, with percentage variances of 73.9% and 26.1 %, respectively, accounting for 100% variations in morphometrics (Figure 9). In all, function 1 was the most contributor to identifying populations of *P. aneus* in the discriminant function analysis of the morphometric traits with an eigenvalue of 0.52. The best predicting morphometric variables for discriminating among *P. aneus* populations were the Standard Length (SL), Body Depth (BD), Total Length (TL), Snout Length (SnL), and Eye Diameter (ED) with correlations of -0.518, -0.471, -0.458, 0.338, and -0.137 respectively in function 1. The Caudal Peduncle Depth (CPD) and Head Length (HL) were the least predicting morphometric variables with 0.547 and -0.206 correlations, as shown in function 2 (Table 2).

The plot of the canonical discriminant function 1 against function 2 (Figure 9) revealed that the populations of *P. aneus* from Kuala Kedah, Batu Maung, and Pantai Remis formed a clustered group with overlapping morphometric characteristics. This attests to their homogeneity in morphology and further confirms the results of the PCA and CVA.

The DFA concluded by predicting the correct classification group of *P. aneus* populations (Table 3). Overall, 67.3% of the original group membership was correctly classified based on the morphometrics, while the remaining 32.7% could have been due to factors other than morphology. The Kuala Kedah population had the best classification success of 79.1%, followed by 69.0% of the Batu Maung population, while the Pantai Remis population had the lowest classification success of 44.0%.

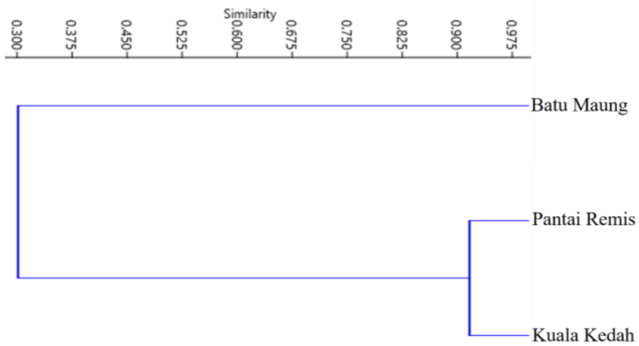

Figure 8. Bray-Curtis similarity cluster analysis of body shape and size variation among populations of *Pennahia aneus* based on UPGMA from the generated Procrustes distances

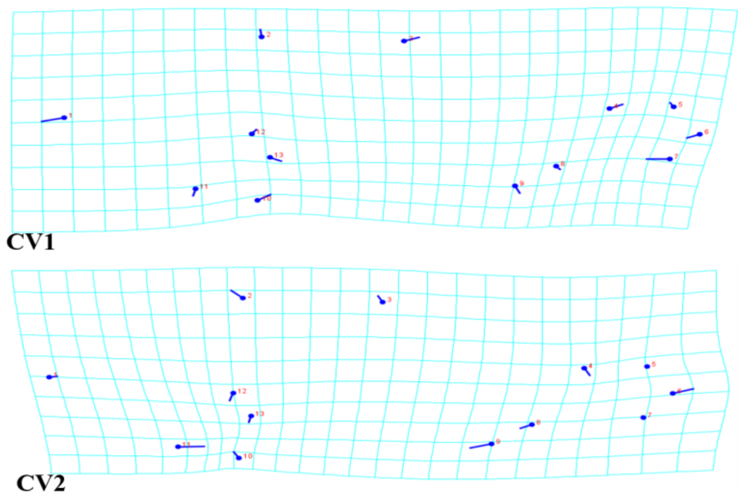

Figure 7. Visualization of body shape differences among *Pennahia aneus* populations via transformation grid graphs for CV1 and CV2. CV1 reveals variations around the head, body depth and length, and pectoral, anal, and caudal regions. CV2 reveals variations in the head, body depth and length, and pectoral, anal, and caudal regions

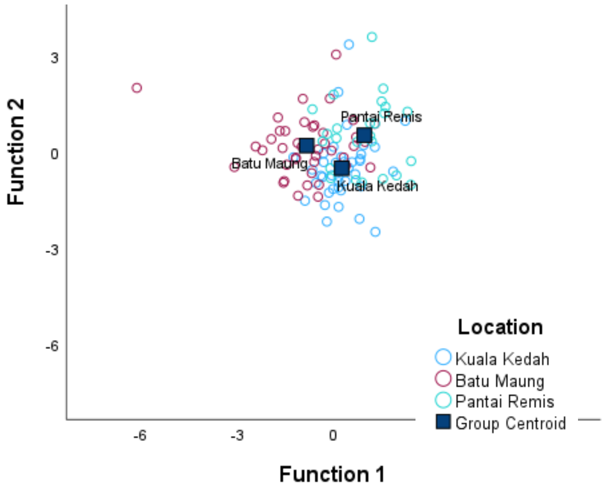

Figure 9. Discriminant function analysis shows (function 1: 73.9 %, eigenvalue: 0.52) and (function 2: 26.1 %, eigenvalue: 0.18) accounting for 100% total discrimination among *Pennahia aneus* populations from Northern Peninsular Malaysia based on morphometric measurements

Table 2. Distributed correlation structure matrix among morphometric variables with discriminant functions among *Pennahia aneus* populations

Morphometric traits	Function	
	1	2
SL	-0.518*	-0.153
BD	-0.471*	-0.087
TL	-0.458*	0.000
SnL	0.338*	-0.013
ED	-0.137*	-0.020
CPD	0.257	0.547*
HL	-0.192	-0.206*

Note: *: Largest absolute correlation between each variable and any discriminant function. SL: Standard Length; BD: Body Depth; TL: Total Length; SnL: Snout Length; ED: Eye Depth; CPD: Caudal Peduncle Depth; HL: Head Length

Table 3. Reclassification of group membership of *Pennahia aneus* populations from Northern Peninsular Malaysia with 67.3% correctly classified

		Predicted group membership			
	Location	Kuala Kedah	Batu Maung	Pantai Remis	Total
Original count	Kuala Kedah	34	4	5	43
	Batu Maung	9	29	4	42
	Pantai Remis	9	5	11	25
%	Kuala Kedah	**79.1**	9.3	11.6	100.0
	Batu Maung	21.4	**69.0**	9.5	100.0
	Pantai Remis	36.0	20.0	**44.0**	100.0
The total percentage of correct reclassification is					67.3%

Discussion

Geometric morphometrics has shown that body size and shape are vital for assessing the relationship between populations of similar taxa (same species) or different taxa, especially by revealing morphological variations (Imtiaz and Naim 2018; Shukri et al. 2024). This study, using Procrustes superimposition, has successfully correlated landmark coordinate variables, revealing subtle shape variation patterns (Shukri et al. 2024). Two morphologically distinct groups were identified in this study.

The PCA results revealed some degree of homogeneity in morphology among the populations of *P. aneus* (Figure 4). The first four Principal Components (PCs) accounted for more than 50 % variation in body shape and size of the 110 individuals considered. This similarity in morphology was also reported by Asaduzzaman et al. (2024) in their study of body divergence in populations of *Polynemus paradiseus* Linnaeus, 1758 from southern Bangladesh's coastal habitats, providing a reassuring alignment with previous studies. Other past reports of Imtiaz and Naim (2018) on *Nemipterus* species within Peninsular Malaysia and surrounding Seas, Moreira et al. (2020) on *Trachurus picturatus* (Bowdich, 1825) (blue jack mackerel) populations in the North Atlantic and Binashikhbubkr et al. (2022) on *Euthynnus affinis* (Cantor, 1849) populations in Peninsular Malaysia agree with the morphological homogeneity observed in this study. Specifically, the 75.86% of the total variance accounted for by the first four PCs observed in this study corroborates the 75.3% body shape variations reported in reef fishes (Claverie and Wainwright 2014), 71.3% body shape variation reported in *Euthynnus alletteratus* and *Thunnus thynnus* (Karakulak et al. 2016), and the 81.3% body shape variation reported in *Barbus* species (Geiger et al. 2016) from combining the first four PCs. The findings of Imtiaz and Naim (2018) and Binashikhbubkr et al. (2022) also reported relatively high percentages in the first four PCs of body shape variation of 80% in *Nemipterus* species and 65.69 % in *E. affinis*, respectively, from Peninsular Malaysia. The morphological variations observed around the body length, depth, and head region of *P. aneus* populations in this study are in congruence with the findings of (Bilici et al. 2015), who asserted that variations observed in the cyprinid fish *Cyprinion macrostomus* Heckel, 1843 revolved around the fish's body size and head region. The body shape variations discovered and reported in *Nemipterus* species (Imtiaz and

Naim 2018) and *E. affinis* (Binashikhbubkr et al. 2022) were all attributed to changes around the head region and the whole body which agrees with our findings.

The morphological variations among populations of *P. aneus* were well-detailed in the Canonical Variate Analysis (CVA) (Figure 6) with some intersections. In this study, the grid transformation graphs of CV1 and CV2 (Figure 7) showed that variations were pronounced around the head region and general body shape. This was consistent with the morphometric research undertaken by Kasinath et al. (2024), who revealed morphological differentiation around the head region among *Rastrelliger kanagurta* (Cuvier, 1816) (Indian mackerel) populations from the eastern Indian Ocean. Our findings also supported the geometric morphometric discrimination analysis conducted by Imtiaz and Naim (2018) on Nemipterid fish species (*Nemipterus* spp) found in Malaysia and the neighboring Seas. Their study demonstrated that body depth, body length, and head orientation were critical factors in the identification of *Nemipterus* species. The body shape variations observed in this study's CVA align with previous reports comparing populations of *Alburnus filippii* Kessler, 1877 in rivers Aras and Ahar-Chai in Iran (Jalili et al. 2015), *Capoeta trutta* (Heckel, 1843) in the Tigris River basin (Iran) (Keivany and Arab 2017), *Squalius namak,* an endemic fish in Iran's Namak inland basin (Saleh et al. 2017), and *Planiliza abu* (Heckel, 1843) (Abu Mullet) in Iran's Bushehr basin (Shabaninejad et al. 2021), using geometric morphometric techniques.

Our DFA findings corroborated the reports by Verma et al. (2014), Binashikhbubkr et al. (2022), and Rahayu et al. (2023), revealing that the first two functions (1 and 2) sufficed to separate among the populations of *Clupisoma garua* (Hamilton, 1822), *E. affinis,* and snapper species (Lutjanidae) in their respective studies. The Standard Length (SL), Body Depth (BD), and Total Length (TL) were shown to be the morphometric traits that significantly contributed to discrimination among *P. aneus* populations (Table 2), which further confirmed the general body form variation in the PCA and CVA results. However, our findings did not align with the reports of Karakulak et al. (2016) and Aminan et al. (2020), who showed that the head length was among the best two predicting morphometric traits to distinguish among populations of *Thunnus thynnus* (Linnaeus, 1758), *Euthynnus alletteratus* (Rafinesque, 1810), and *Rasbora* species, respectively. The DFA was concluded by the Jackknifed cross-validation reclassification of the original group membership of *P. aneus* populations, revealing moderate percentage (67.3%) of the original group members were classified correctly (Table 3). The analysis utilized the significant resemblance among the examined components to predict the group membership data (Aminan et al. 2020). Our findings did not conform with the high accuracy rates reported in previous studies. For instance, Binashikhbubkr et al. (2022) achieved a correct classification rate of 88.6% for seven populations of *E. affinis* in Peninsular Malaysia. Similarly, Moreira et al. (2020) achieved a correct classification rate of 83% for six populations of *Trachurus picturatus* (Bowdich, 1825) in the North-East Atlantic. Additionally, Hoff et al. (2020) reported a reclassification rate of 79% for five populations of the Sciaenid, *Isopisthus parvipinnis* (Cuvier, 1830), in the South-West Atlantic Ocean. Recently, Shahana et al. (2024) reported a reclassification rate of 88.01% for the crescent perch (*Terapon jarbua* (Forsskål, 1775)) population along the Indian coast, which was higher than that obtained in our result. This could be due to overlapping or similar habitat requirements for the different populations, which encourage the inhabiting of wider habitat ranges, bringing about population mixing.

Generally, fish are more prone and sensitive to environmental changes that alter their morphological features than other vertebrates (Verma et al. 2014). Although it is difficult to comprehend how these variations in morphology come about, previous researchers have opined that interactions between genetic and environmental factors could have aided the emergence of such morphological traits (Verma et al. 2014; Bilici et al. 2015; Aminan et al. 2020; Binashikhbubkr et al. 2022; Binashikhbubkr et al. 2024). According to Adibah et al. (2015), the Malay Peninsular specifically poses a significant physical challenge for various species, including fish, whose movement relies on ocean currents. Habitat stratification along the Strait of Malacca is believed to have elicited specific fish morphological responses in the strategic feeding patterns and modified body shapes for maneuvering their habitats (Jagerroos 2016; Simbolon et al. 2019). The morphological changes observed around the head, body length, depth, and caudal regions, as depicted (Figures 5 and 7; Table 2), could have been influenced by a combination of factors like food availability, temperature, radiation, salinity, water depth, and current velocity (Mustikasari et al. 2020). These variations bordering on body size and shape could contribute to their balance and movement, ultimately adapting them to navigating the water currents (Cantabaco et al. 2015). Also, the observed differences in the head morphological pattern could be due to prey types and the variety of ecological niches available in the respective habitats (Tripathy 2020; Binashikhbubkr et al. 2022). The Batu Maung population forming a distinct morphological group from those of Pantai Remis and Kuala Kedah (Figure 8) is not much of a surprise because Batu Maung is on the Island of Penang state, whose shorelines have been reported to be subjected to different anthropogenic activities, which eventually result in loss of habitat, pollution, and siltation (Hamid et al. 2023). We reasoned that these factors could have aided the observed morphological variations.

In conclusion, our study is a pioneering use of geometric morphometric techniques to study morphological variations among populations of *P. aneus* from Northern Peninsular Malaysia. The application of multivariate analyses (PCA, CVA, CA, and DFA) has shown significant morphological variations among *P. aneus* populations. Most importantly, GM has unveiled that the three populations of *P. aneus* species from Northern Peninsular Malaysia form two distinct groups morphologically. This study has demonstrated the potential of GM as a powerful tool for uncovering morphological differences within populations of the same species. Looking ahead, we are excited about the future of this research. To further our understanding of the stock structure of *P. aneus*, we plan to extend our research to other coastal areas within Peninsular Malaysia and incorporate molecular characterization of the species. We believe that these future steps will significantly advance our knowledge in this area.

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