

Morphometric variations of *Kawakawa* (*Euthynnus affinis* Cantor, 1849) in Malaysian and Yemeni waters

KHALED BINASHIKHBUBKR^{1,2}, AHMAD DWI SETYAWAN³, DARLINA MD NAIM^{1,*}

¹School of Biological Sciences, Universiti Sains Malaysia. 11800 Pulau Pinang, Malaysia. Tel. +60-46534056, *email: darlinamdn@usm.my

²Department of Biology, Faculty of Science, Hadhramout University. Mukalla, Yemen

³Department of Environmental Science, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia

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Abstract. Binashikhbubkr K, Setyawan AD, Naim DM. 2023. Morphometric variations of *Kawakawa* (*Euthynnus affinis* Cantor, 1849) in Malaysian and Yemeni waters. *Nusantara Bioscience* 15: 269-278. *Euthynnus affinis* Cantor, 1849 (*Kawakawa*), a small epipelagic tuna that migrates, is one of the most commercially significant tunas in Indo-Pacific subtropical and tropical waters. Unfortunately, there is a lack of knowledge on the region's stock structure and management of the particular migratory species. The present investigation aimed to distinguish body shape differences among 20 populations of *E. affinis* across Malaysian and Yemen coastal waters. A total of 416 specimens of *E. affinis* were sampled from five central geographic regions: the South China Sea, Straits of Malacca, Sulu Sea, Celebes Sea, and the Arabian Sea. Multivariate analyses, such as Principal Component Analysis (PCA), Discriminant Function Analysis (DFA), and Cluster Analysis (CA) of 12 morphometric variables, were conducted to differentiate 20 populations of *E. affinis*. The findings revealed significant differences in the body form among 20 populations. Morphological homogeneity was observed among populations from Peninsular Malaysia and Malaysian Borneo (Sabah and Sarawak). As expected, Yemen's (Mukalla City) populations were clearly segregated and formed a separate cluster. The highest percentage of correctly classified specimens (100%) came from populations in Pulau Tenggol (Terengganu) and Kukup (Johor). In contrast, the highest percentage of misclassified specimens (100%) came from populations in Kota Kinabalu (Sabah), Bintawa (Sarawak), Lumut (Perak), and Endau (Johor). Based on the Mahalanobis distances, hierarchical cluster analyses revealed that 20 populations of *E. affinis* could be clustered into three distinct groups. This is the first report on *E. affinis* from Peninsular Malaysia, Malaysian Borneo (Sabah and Sarawak), and the waters of Yemen using morphometric techniques.

Keywords: DFA, *Euthynnus affinis*, morphometric, PCA, population discrimination

INTRODUCTION

The marine fisheries sectors in Malaysia and Yemen are poised for a significant growth phase, propelling them towards becoming prominent fishing hubs in Asia. Their strategic geographical locations strongly bolster this promising outlook, as both countries are surrounded by vital oceans, endowing them with abundant marine resources. In the case of Malaysia, the marine fisheries sector has made substantial contributions to the nation's economy. In 2021 alone, this sector yielded approximately 1.7 million metric tons of seafood, estimated at around RM 15 billion (Annual Fisheries Statistics 2021). Meanwhile, Yemen boasts a wealth of fisheries resources, as evidenced by a total production of 228,655 metric tons recorded in 2012 (Ministry of Fish Wealth 2015). This industry also provides several job possibilities and exports and protein supplies worldwide to Malaysians and other regional countries (Bakar et al. 2018).

The major Malaysian landing places exhibit a diverse range of marine fish species, estimated to be approximately 200-300. Daily, the fish landing site at these locations typically offers an average of 50-100 distinct species. During monsoon season, various species exhibit regular emergence patterns, with certain species potentially

dominating the landing sites. In contrast, permanent resident species of estuaries, bays, and reefs can be found throughout the year (Bakar et al. 2018). Fish possess significant economic value and serve as a valuable source of animal protein for human consumption while also being crucial in maintaining biodiversity (Marchetti et al. 2020). The Thunnini tribe comprises the genera *Thunnus*, *Auxis*, *Katsuwonus*, and *Euthynnus*, which hold significant economic value (Pertiwi et al. 2017; Sato et al. 2020). *Euthynnus affinis*, *Euthynnus alletteratus*, and *Euthynnus lineatus* species belong to the *Euthynnus* Genus. *Kawakawa*, scientifically known as *E. affinis* (Cantor 1849), holds significant ecological importance as one species in the Scombridae family. This species is distributed in the Indo-Pacific region across tropical and sub-tropical waters (Yazawa et al. 2019; Khoa et al. 2021). Due to its migratory neritic epipelagic nature, this species has received significant attention as Malaysia's most significant tuna species with commercial value (Yazawa et al. 2015). In contrast, *E. alletteratus* and *E. lineatus* are found exclusively in the Atlantic and Pacific Oceans, respectively (Kumar and Kunal 2013; Sulistyaningsih et al. 2014).

Morphometric analysis of fish is very important in fisheries management, as it allows the calculation of

evolutionary characteristics and the identification of variations in form, ontogeny, function, and evolutionary relationships between individual animals. In this regard, identifying changes among populations of the same fish species across different geographical areas is the main function of morphometric characteristics (Meshram et al. 2021). Multiple multivariate techniques are commonly used to distinguish between populations of different fish species. These techniques include Principal Component Analysis (PCA), Discriminant Function Analysis (DFA), and Cluster Analysis (CA). Several researches have been conducted using DFA, PCA, and CA to evaluate morphometric variation between different fish species (e.g., Jalili et al. 2015; Pérez-Quinónés et al. 2017; Mounir et al. 2019; Aminan et al. 2020; Binashikhbubkr et al. 2022).

Previous research on *E. affinis* has mostly focused on various factors, including reproductive behavior, biological characteristics, stock assessment, genetic structure, and population dynamics (Santos et al. 2010; Rohit et al. 2012; Johnson and Tamatamah 2013; Sulistyarningsih et al. 2014; Nissar et al. 2015; Kumar et al. 2019; Binashikhbubkr et al. 2023). On the other hand, an investigation managed by Griffiths et al. (2017) focused on the morphological relationships of 4 fish species in the Scombridae family found in Australian waters. This study specifically determined the relationship between fork length and total length, as well as the relationship between length and weight for several species such as *E. affinis*, *Thunnus tonggol*, *Cybiosarda elegans*, and *Rastrelliger kanagurta*. However, there have been no morphometric investigations concerning *E. affinis* populations in many geographical areas, including Peninsular Malaysia, Malaysian Borneo (Sabah and Sarawak), and the Arabian Sea (specifically Mukalla City, Yemen). Therefore, to fill the gap in this field of study, this current study attempts to examine and differentiate morphological differences across 20 different populations of *E. affinis* found in Peninsular Malaysia, Borneo Malaysia, and Mukalla City, Yemen.

MATERIALS AND METHODS

Study area and sampling activities

Approximately 416 individuals of *Kawakawa* hailing from diverse regions, including Peninsular Malaysia, Malaysian Borneo, and Mukalla City (Yemen), were meticulously gathered directly by local fishermen at 20 distinct commercial fish landing sites between November 2019 and September 2021. These landing sites comprised 19 in Malaysia and one in Mukalla City (Figure 1). The specimens were obtained from 5 prominent geographical areas: South China Sea (SCS), Straits of Malacca (M), Sulu Sea (SS), Celebes Sea (CS), and Arabian Sea (AS). Specifically, within the Straits of Malacca, data were collected from 5 landing sites: Sungai Besar and Kuala Selangor in Selangor, Kukup in Johor, Lumut in Perak, and

Batu Maung in Penang. In the South China Sea region, 10 landing sites were chosen: Pasir Puteh and Tok Bali in Kelantan, Pantai Kijal and Pulau Tenggol in Terengganu, Kota Kinabalu in Sabah, Bintawa, Pulau Bruit, and Mukah in Sarawak, Kuantan in Pahang, and Endau in Johor. Additionally, the Sulu Sea was represented by 2 landing sites, Sandakan and Kudat in Sabah, while the Celebes Sea featured 2 sites: Lahad Datu and Tawau in Sabah. Finally, 1 landing site was selected from the Arabian Sea, specifically Mukalla City in Yemen (Figure 1).

Subsequently, samples from Malaysian waters were transported in an icebox to the School of Biological Science at Universiti Sains Malaysia for investigation. Following the taxonomic guidelines provided by Collette and Nauen (1983), the collected specimens were morphologically identified and validated. Upon their arrival at the School of Biological Science, Universiti Sains Malaysia, the Malaysian samples underwent a thorough cleaning process with running water. Subsequently, they were gently tapped and systematically arranged on an even top with contrasting dark surrounding to ensure optimal clarity and visibility for further examinations. Conversely, the specimens originating from Mukalla City were subjected to measurements and photographic documentation within the confines of the Laboratory of Biology, Faculty of Science, Hadhramout University, Yemen. Each sample was meticulously labeled and snapshotted with a Nikon D90 digital camera. To assure precision, a digital caliper was employed to measure the morphometric variables, with measurements recorded to the nearest 0.1 cm (Figure 2).

Morphometric analyses

Moreover, the morphometric analysis of *E. affinis* involved the measurement of 12 characters using a digital caliper : Standard Length (SL), Fork Length (FL), Total Length (TL), Head Length (HL), Body Depth (BD), Snout length (Snl), Eye Diameter (ED), pectoral fin distance (DP), first dorsal fin distance (DD1), second dorsal fin distance (DD2), anal fin distance (DA), and ventral fin distance (DV) (Figure 2). Therefore, to mitigate the influence of size, all morphometric variables were normalized using the equation proposed by Elliott et al. (1995):

$$M_{adj} = M(L_s/L_0)^b$$

Where:

M : Original measurement

M_{adj}: Size-adjusted measurement

L_s : Overall mean of the standard length for all samples

L₀ : Standard length of each sample

b : Each character from the observed data as the slope of the log M on log L₀ regression, encompassing all fish from all populations.

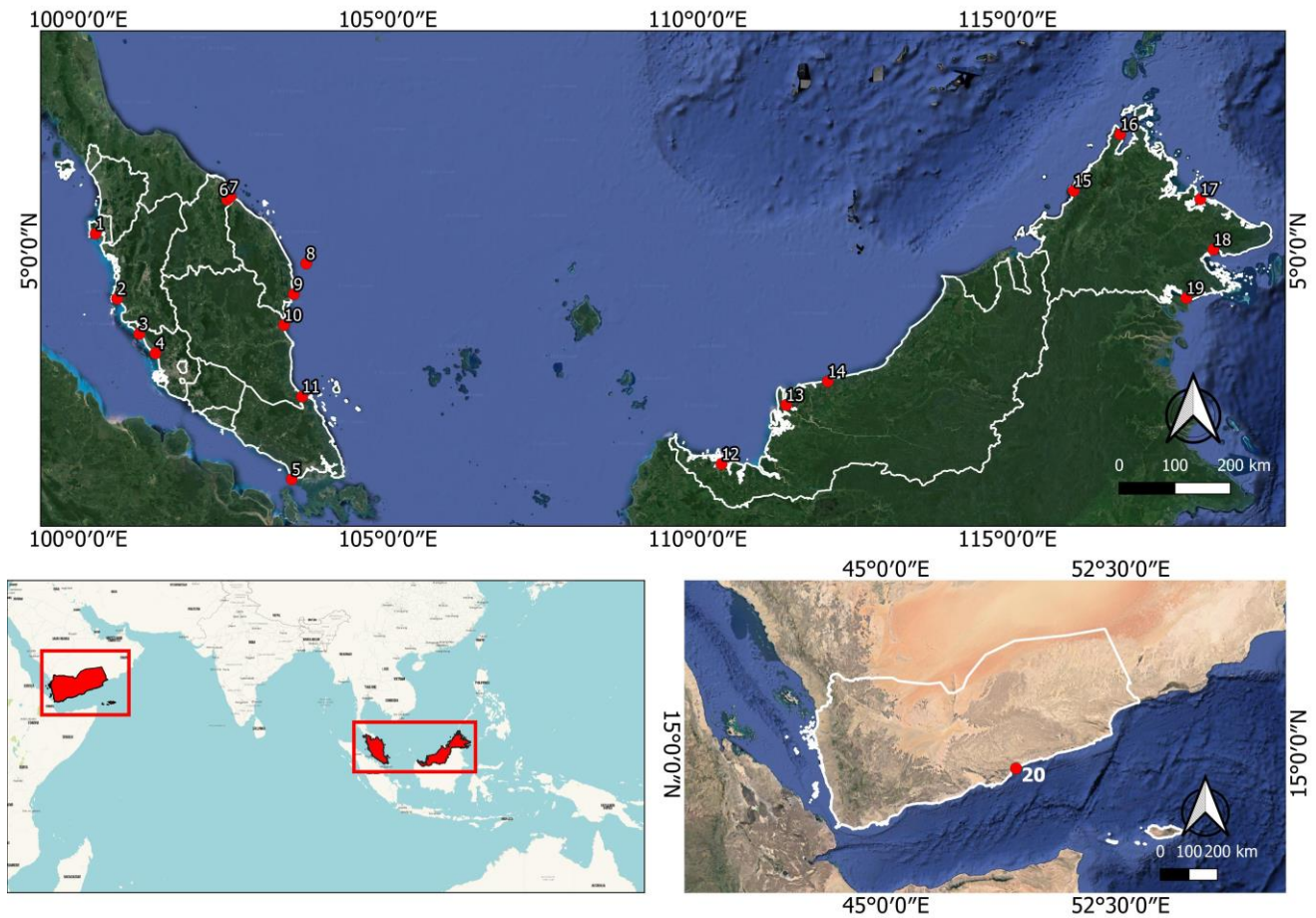


Figure 1. Sampling locations of *Euthynnus affinis* specimens collected from the Straits of Malacca, South China Sea, Sulu Sea, Celebes Sea, and Arabian Sea regions are as follows: 1. Batu Maung, Penang; 2. Lumut, Perak; 3. Sungai Besar, Selangor; 4. Kuala Selangor, Selangor; 5. Kukup, Johor; 6. Pasir Puteh, Kelantan; 7. Tok Bali, Kelantan; 8. Pulau Tenggol, Terengganu; 9. Pantai Kijal, Terengganu; 10. Kuantan, Pahang; 11. Endau, Johor; 12. Bintawa, Serawak; 13. Pulau Bruit, Serawak; 14. Mukah, Serawak; 15. Kota Kinabalu, Sabah; 16. Kudat, Sabah; 17. Sandakan, Sabah; 18. Lahad Datu, Sabah; 19. Tawau, Sabah; 20. Mukalla, Yemen

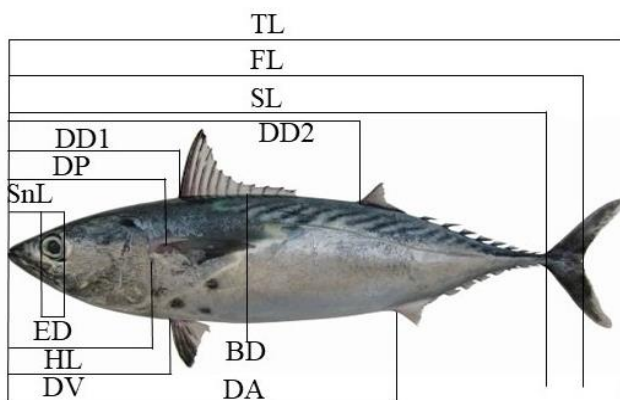


Figure 2. Morphometric measurements of *Euthynnus affinis* (*Kawakawa*). TL: Total Length, FL: Fork Length, SL: Standard Length, HL: Head Length, BD: Body Depth, ED: Eye Diameter, SnL: Snout length, DP: Distance of pectoral fin, DD1: Distance of the first dorsal fin, DD2: Distance of the second dorsal fin, DV: Distance of ventral fin, DA: Distance of anal fin.

A multivariate Discriminant Function Analysis (DFA) was employed to distinguish among 20 *E. affinis*

populations by identifying a combination of features that maximized population divergence. The discriminant analysis used the results to determine correctly differentiated characters' proportions. Wilks' lambda analysis was utilized to distinguish variations among the populations. In this study, Discriminant Function Analysis (DFA) and Principal Component Analysis (PCA) were conducted using SPSS software version 25 to pinpoint the most influential differences in body shape for distinguishing between populations. To assess population relationships among 20 *E. affinis* populations, hierarchical cluster analysis was carried out based on Mahalanobis distances, employing the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) (Hammer et al. 2001).

RESULTS AND DISCUSSION

Sampling data

Moreover, 416 samples of *E. affinis* were collected from 20 distinct locations within 5 primary geographic regions: Straits of Malacca, South China Sea, Sulu Sea, Celebes Sea, and Arabian Sea (AS).

Table 1. Eigenvalue and Wilk's lambda of each function of DFA of *Euthynnus affinis*

Function	Eigenvalue	% of variance	Cumulative %	Canonical correlation	Wilks' Lambda	Sig.
1	2.897 ^a	47.9	47.9	0.862	0.026	0.000
2	1.500 ^a	24.8	72.7	0.775	0.102	0.000
3	0.667 ^a	11.0	83.7	0.632	0.254	0.000
4	0.501 ^a	8.3	92.0	0.578	0.423	0.000
5	0.188 ^a	3.1	95.1	0.398	0.635	0.000
6	0.176 ^a	2.9	98.0	0.387	0.755	0.001
7	0.076 ^a	1.3	99.2	0.267	0.888	0.649
8	0.028 ^a	0.5	99.7	0.165	0.956	0.994
9	0.010 ^a	0.2	99.9	0.099	0.982	0.999
10	0.008 ^a	0.1	100.0	0.089	0.992	0.977

Note: ^a) indicates the first 10 discriminant functions used in the analysis

Table 2. The structure matrix represents the distributed correlations among measurement characteristics and functions for morphometric characteristics.

Variables	Structure Matrix									
	1	2	3	4	5	6	7	8	9	10
DP	.969*	.004	.026	.039	.014	-.043	-.051	-.224	-.058	-.005
DD1	.912*	-.038	.010	.150	.115	-.031	.020	-.299	-.035	-.002
DV	.876*	-.024	.005	-.080	.119	-.049	.159	-.386	.134	.132
DA	.798*	.128	-.099	.400	.222	-.040	.006	-.273	-.080	.213
HL	.711*	.401	-.178	.387	-.281	.147	.136	.033	.070	.166
DD2	.686*	.144	-.031	.564	.168	.150	.061	-.362	-.044	-.046
TL	.683*	.598	-.050	.290	-.006	.078	-.079	-.152	-.115	.144
FL	.672*	.577	-.105	.298	.013	.093	-.006	-.218	-.197	.146
SL	.660*	.610	-.116	.293	.051	.083	-.079	-.236	-.103	.110
BD	.541	.620*	.322	.409	-.045	-.189	-.006	.066	-.018	.095
ED	.526	.572*	-.256	.262	.286	.280	.211	.184	-.096	-.121
SnL	.422	.508*	.461	.210	-.017	.247	-.291	.131	.196	.326

Note: *Largest absolute correlation between each variable and any discriminant function

Table 3. Correct and misclassifications for 20 populations of *Euthynnus affinis* collected from Peninsular Malaysia, Malaysian Borneo, and Mukalla City (Yemen)

Sampling Locations	N	% of Correctly Classified	% of Misclassified	Total (%)
Pasir puteh, Kelantan	18	38.9	61.1	100
Tok Bali, Kelantan	14	71.4	28.6	100
Pantai Kijal, Terengganu	8	62.5	37.5	100
Pulau Tenggol, Terengganu	21	100	0	100
Sungai Besar, Selangor	14	92.9	7.1	100
Kuala Selangor, Selangor	24	37.5	62.5	100
Kukup, Johor	15	100	0	100
Mukalla, Yemen	18	94.4	5.6	100
Sandakan, Sabah	20	25	75	100
Lahad Datu, Sabah	24	4.2	95.8	100
Tawau, Sabah	24	4.2	95.8	100
Kudat, Sabah	24	50	50	100
Kota Kinabalu, Sabah	24	0	100	100
Bintawa, Sarawak	24	0	100	100
Pulau Bruit, Sarawak	24	16.7	83.3	100
Mukah, Sarawak	24	8.3	91.7	100
Kuantan, Pahang	24	4.2	95.8	100
Lumut, Perak	24	0	100	100
Endau, Johor	24	0	100	100
Batu Maung, Penang	24	25	75	100
Total	416			

Table 4. Principal component analysis scores of variations within the population of *Euthynnus affinis*

PC	Eigenvalue	% Variance	Cumulative %
1	9.948	82.902	82.902
2	1.141	9.511	92.414
3	0.420	3.499	95.912
4	0.149	1.245	97.157
5	0.109	0.909	98.066
6	0.093	0.773	98.839
7	0.076	0.634	99.474
8	0.027	0.228	99.702
9	0.021	0.175	99.877
10	0.007	0.057	99.935
11	0.005	0.041	99.976
12	0.003	0.024	100.000

Discriminant Function Analysis (DFA)

The discriminant function analysis has properly recognized ten functions, namely functions 1 to 10, with the variances being 47.9%, 24.8%, 11.0%, 8.3%, 3.1%, 2.9%, 1.3%, 0.5%, 0.2%, and 0.1%, respectively. Functions 1 to 4 revealed a notable association for population differentiation with values of 0.86, 0.78, 0.63, and 0.58, respectively (Table 1). Wilk's Lambda values were derived from analyzing 10 functions (designated as functions 1 through 10). The corresponding significance values for the first six functions are as follows: 0.026, 0.102, 0.254,

0.423, 0.635, and 0.755, yielded probabilities of $p = 0.000$ and 0.001 (Table 1). We discover that the distance of the pectoral fin (DP) had the highest correlation score at 0.969 among the measurement variables, followed closely by the length of the first dorsal fin (DD1) at 0.912, ventral fin's length (DV) at 0.876, anal fin's length (DA) at 0.798, Head Length (HL) at 0.711, second dorsal fin's length (DD2) at 0.686, Total Length (TL) at 0.683, Fork Length (FL) at 0.672, and Standard Length (SL) at 0.660 when setting up the DFA based on the variables' scores in function 1 (Table 2). Therefore, based on DFA, the highest percentage of correct identifications (100%) among 20 populations of *E. affinis* into their original groups was observed in Pulau Tenggol and Kukup. In contrast, the lowest percentage of correct identifications (0%) was found in the populations from Kota Kinabalu, Bintawa, Lumut, and Endau (Table 3). In this current study, the assessment of DFA demonstrated that the highest percentage of accurately classified was from Pulau Tenggol (Terengganu) and Kukup (Johor), both with 100%. In contrast, the highest percentage of misclassification was observed in the populations from Kota Kinabalu (Sabah), Bintawa (Sarawak), Lumut (Perak), and Endau (Johor), all with 100%, respectively. The canonical discriminant function analysis indicated that the mean body patterns of specimens from Malaysian Borneo and Peninsular Malaysia were morphologically identical. In contrast, the mean body patterns for populations from Mukalla City (Yemen) were segregated (Figure 3).

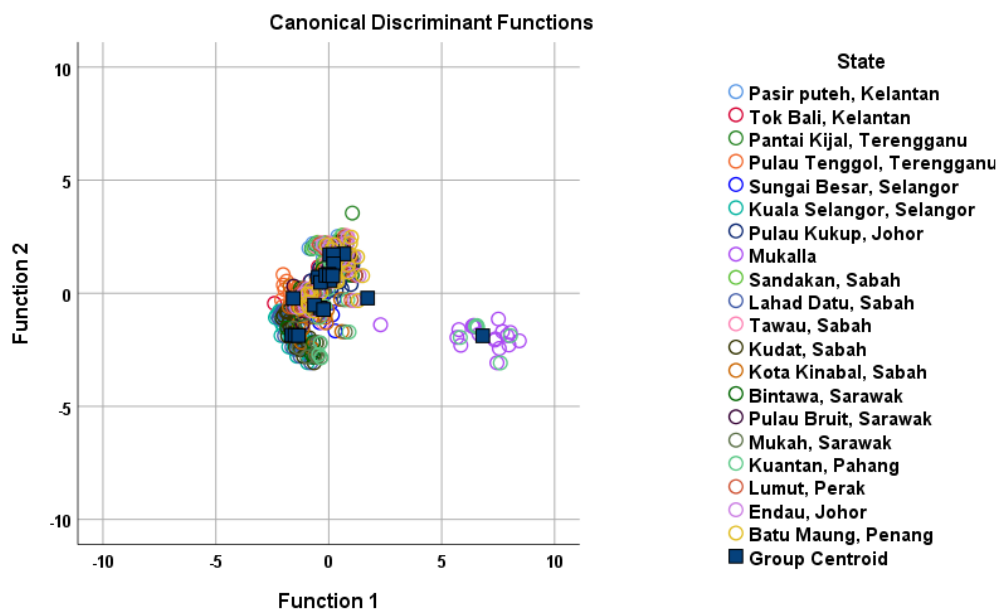


Figure 3. Canonical discriminant analysis based on morphometric measurements of 20 populations of *Euthynnus affinis*

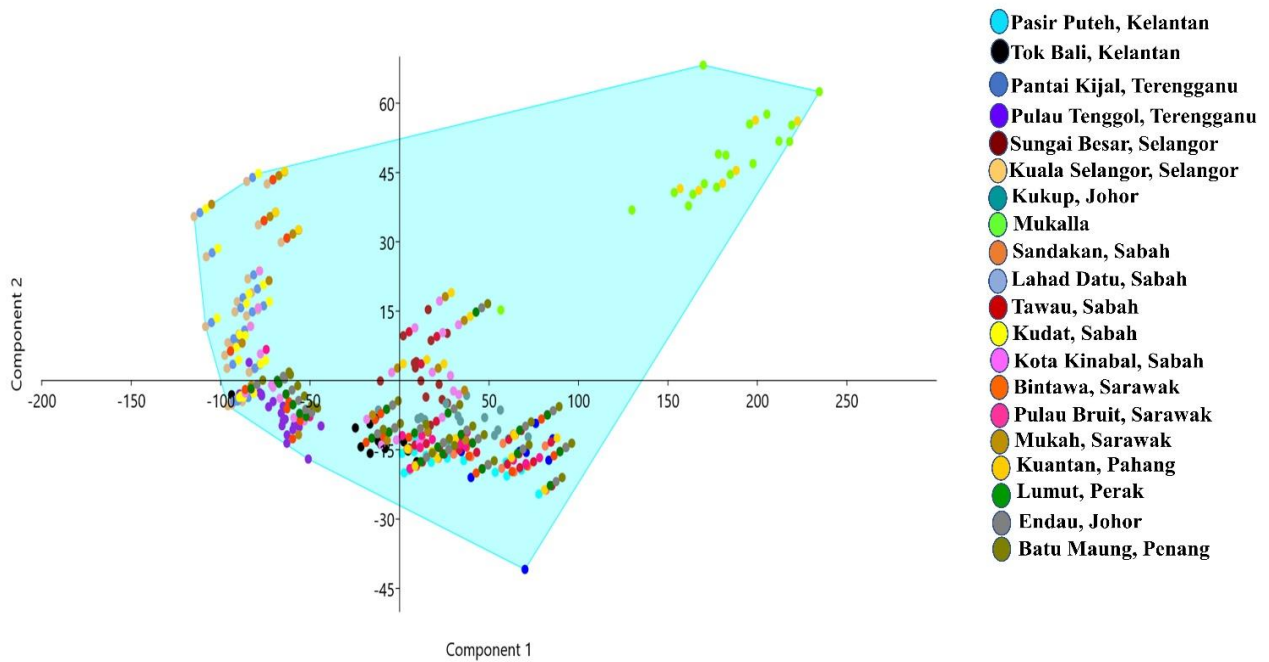


Figure 4. The principal components analysis of *Euthynnus affinis* shows PC1= = 82.902% and PC2= = 9.511%, accounting for 92.414% of the total variation in 416 samples

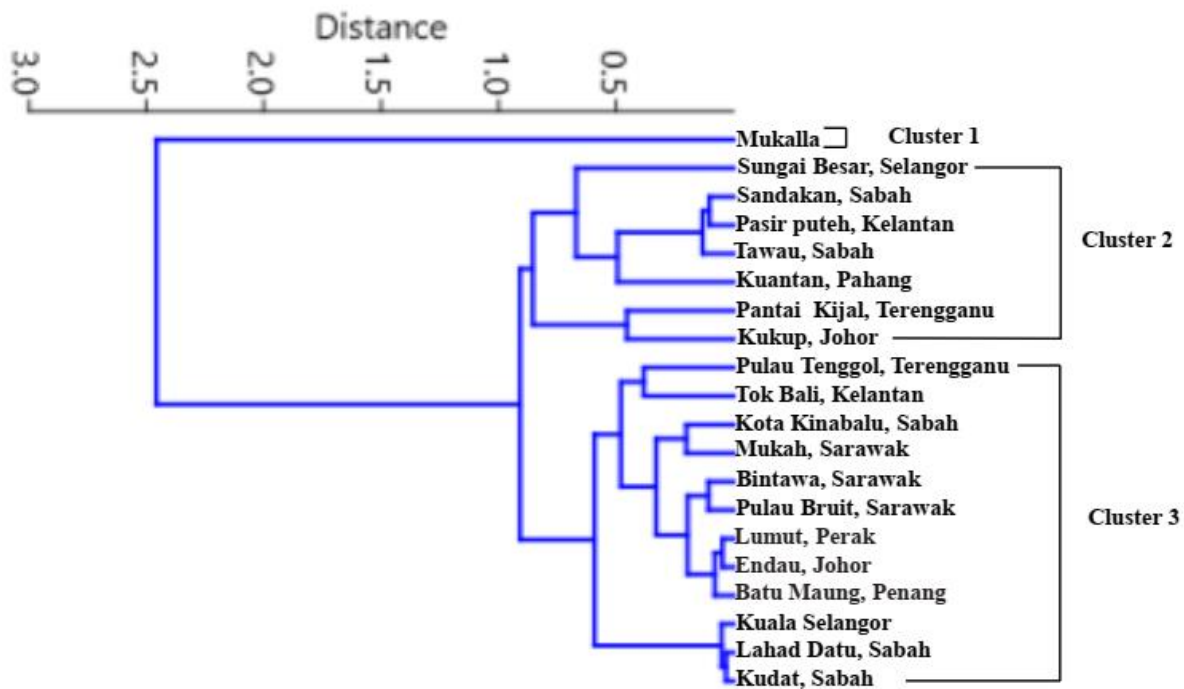


Figure 5. A dendrogram was generated from cluster analysis based on Mahalanobis distances among 20 populations of *Euthynnus affinis* using morphometric measurements

Principal Component Analysis (PCA)

The analysis of PCA performed on 416 individuals revealed 12 components that were instrumental in identifying variations in body shape. The first component exhibited the highest variance, accounting for 82.902% and possessing an eigenvalue of 9.948, which attests to its significant influence (an eigenvalue more than 0.3 is

regarded as significant). However, when considering the first four Principal Components (PCs), the breakdown of variance was as follows: 82.902% for PC1, 9.511% for PC2, 3.499% for PC3, and 1.245% for PC4, summing up to an overall variance of 97.157% that elucidates the variations in body shape (Table 4). Our findings indicate that the subtle morphological deviation among *E. affinis*

individuals was primarily influenced by the overlaying structure observed in the graph of PC1 vs. PC2, where a remarkably notable eigenvalue was significant in discerning *E. affinis* based on shared body shape characteristics (Figure 4). In summary, Figure 4 illustrates that the populations exhibited significant overlap, with no clear difference, signifying a high degree of morphological homogeneity across Peninsular Malaysia and Malaysian Borneo populations. Notably, the population from Mukalla City (Yemen) stood out as a distinct and separate group or cluster. In general, the results obtained from PCA confirmed the DFA results. Hierarchical cluster analysis based on the Mahalanobis distances demonstrated that 20 populations of *E. affinis* were grouped into three separate clusters (Figure 5). The first group comprises populations exclusively from Mukalla City. The second group encompasses populations from Sungai Besar (Selangor), Sandakan (Sabah), Pasir Puteh (Kelantan), Tawau (Sabah), Kuantan (Pahang), Pantai Kijal (Terengganu), and Kukup (Johor). The third and final group includes populations from Pulau Tenggol (Terengganu), Tok Bali (Kelantan), Kota Kinabalu (Sabah), Mukah (Sarawak), Bintawa (Sarawak), Pulau Buit (Sarawak), Lumut (Perak), Endau (Johor), Batu Maung (Penang), Kuala Selangor, Lahad Datu (Sabah), and Kudat (Sabah).

Discussion

Discriminant Function Analysis (DFA)

In identifying *E. affinis*, function 1 in DFA is the best attribute with a variance value of 47.9% (Table 1), and Wilk's lambda score is 0.026. When loading into the analysis, distance of pectoral fin (DP) is revealed to have a crucial role in recognizing *E. affinis* with the greatest contribution values/score, followed by distance of the first dorsal fin (DD1), ventral fin's distance (DV), anal fin's distance (DA), head length (HL), second dorsal fin's distance (DD2), Total Length (TL), Fork Length (FL), and Standard Length (SL) in function 1 (Table 2). Our results confirm the study by Aminan et al. (2020) and Gain et al. (2017), in which they highlighted that function 1 in DFA was able to distinguish intrapopulation in samples of *Rasbora* spp. and *Cirrhinus cirrhosus*. Furthermore, a similar study in Tunisian waters revealed that fork length, standard length, and pectoral fin are important characteristics in differentiating between bluefin tuna populations *Auxis rochei* (Allaya et al. 2016). Likewise, a study conducted by Karakulak et al. (2016) demonstrated that there is a clear relationship between the length of the head and the length of the pectoral fin, the first predorsal, and the height of the second dorsal fin between *Thunnus thynnus* and *E. alletteratus*. Furthermore, Griffiths et al. (2017) divulged a highly significant relationship between fork length-total length and length-weight among four Scombridae fish species (*E. affinis*, *T. tonggol*, *R. kanagurta*, and *C. elegans*). Body characteristic variations were influenced by exterior factors such as food and water quality availability and dependent and independent elements (Kumar et al. 2019; Sawalman and Madduppa 2020). Differentiation in the shape of head pattern is also

attributable to using different environmental niches, food, and prey species (Cronin-Fine et al. 2013).

The assessment of DFA carried out in this study demonstrated that the group member of *E. affinis* was accurately recognized, with a maximum percentage (100%) of classifications for the populations from Pulau Tenggol (Terengganu) and Kukup (Johor), followed by populations from Mukalla City (94.4%), Sungai Besar (Selangor) (92.9%), Tok Bali (Kelantan) (71.4%), and Pantai Kijal (Terengganu) (62.5%). On the other hand, the high percentage of misclassified (100%) was in the populations from Kota Kinabalu (Sabah), Bintawa (Sarawak), Lumut (Perak), and Endau (Johor), followed by populations (95.5%) in the populations from Lahad Datu and Tawau (Sabah), Kuantan (Pahang), and Mukah (Sarawak) (91.7%), Pulau Buit (Sarawak) (83.3%), Kuala Selangor (Selangor) (62.5%), Pasir Puteh (Kelantan) (61.1%), Sandakan (Sabah), and Batu Maung (Penang) (75%), and Kudat (Sabah) (50%) (Table 3). The results from our study were comparable to Ganjali et al. (2021), who reported that the overall correctly classified topmouth gudgeon (*Pseudorasbora parva*) from Iranian waters was 74.5%. The data on predicted group membership was derived from the study of components that exhibited a significant level of similarity (Kočičová and Mišanková 2014; Aminan et al. 2020). Furthermore, canonical discriminant assessment asserted that morphological similarities prevailed between Peninsular Malaysia and Malaysian Borneo populations. The population from Mukalla City is clearly separate from the population caught in Malaysian waters (Figure 3). Generally, DFA has exhibited significant variabilities between 20 *E. affinis* populations sampled. Our findings were analogous to those of Hajje et al. (2013) and Pérez-Quinonez et al. (2017), who reported the presence of morphologically segregated groups of *E. alletteratus* and *Opisthonema*, respectively using DFA. According to some theories, environmental and genetic variations could have caused the gap across populations (see Verma et al. 2014; Madduppa et al. 2017). Generally, various causes influence morphological variances; environmental influences, genetic factors, and habitat diversity are only a few examples (Tzeng 2004). Because of the genetic effects on the ontogenies of individuals, selection, and environment, geographic separation possibly occurs in the evolution of varied physical features between fish populations (Sajina et al. 2011). The primary factor contributing to the divergence of fish populations in distinct aquatic habitats is geographical segregation, which frequently results in reduced gene flow between these populations. Following Konan et al. (2010), geographical obstacles foster genetic and morphological variations across populations, facilitating differentiation. As Mahfuj et al. (2022) specified, the variation in phenotypic features observed among stocks can be attributed to genetic variations arising from population migration, natural selection, mutation, and environmental factors. These factors, in turn, are influenced by the geographical location. Based on the characteristics of the Arabian Sea, it exhibits various features that can lead to differences in the fish body shape, including high productivity, food availability, salinity, biodiversity, a wide

range of temperatures (Jawad 2021), geographical barriers, and discrete spawning time (Barth et al. 2017). Interestingly, prior research has indicated notable dissimilarities between Yellowfin tuna (*Thunnus albacares*) specimens procured from the Arabian Sea and those sourced from the Atlantic, Indo-Pacific regions, and the Bay of Bengal (Kunal et al. 2013; Barth et al. 2017).

Principal Component Analysis (PCA)

The first four principal components (PCs) addressed 97.157% of the variation in body shape across all variables, demonstrating high significance with eigenvalues exceeding 0.3. An eigenvalue greater than 0.3 is considered significant (Lombarte et al. 2012). The elevated scores of the first four PCs indicate substantial variations in the overall body shape among all examined populations of *E. affinis*. (Figure 4 and Table 4). This finding aligns with the outcomes achieved by Claverie and Wainwright (2014), who found that the first four PCs consequences in 75.3% of morphological variations in the reef fishes. Similar findings were also recorded in studies by Imtiaz and Naim (2018) (80% of body shape variations in the Genus *Nemipterus*) and Mounir et al. (2019) (88.96% of body shape variations in *Sardina pilchardus*). In the same way, Addis et al. (2014) revealed that the first two PCs accounted for 56.97% of the shape variations in *T. thynnus*. Particularly, we outlined that the differences in the morphological patterns of *E. affinis* prevailed in the overall body pattern. As a result, our findings are comparable to those of Jalili et al. (2015), which determined that phenotypic differences between *Alburnus filippii* depend on snout tip and body depth variations. Similarly, Mounir et al. (2019) highlighted head length, dorsal fins, and body depth as critical variables characterizing differences in *S. pilchardus* populations along the Moroccan Atlantic coast.

The PCA results generally corroborated the DFA findings, indicating that morphological homogeneity existed among Peninsular Malaysia and Malaysian Borneo populations. However, specimens in Mukalla City were clearly separated and established their cluster. The samples' overlapping distribution could be attributed to significant seasonal migration in these waters, showing that these populations have a temporal and spatial residence in these areas. Interestingly, our findings proved that the previous genetic studies on the genetic makeup of *E. affinis* from various oceanic provinces such as Philippines and Peninsular Malaysia (Pangkor Island, Penang) (Santos et al. 2010), North Indian Ocean (Kumar et al. 2012), Straits of Malacca (Masazurah et al. 2012), and Tanzania coastal waters (Johnson et al. 2016), which reported that the genetic structure of *E. affinis* was a single stock. Based on the outcomes derived from the hierarchical cluster analysis, the populations of *E. affinis* were clustered into three clusters, as illustrated in Figure 5. These differences between population differences can be ascribed to each population's distinct geographical locations and environmental constraints (Siddik et al. 2016). According to the cluster analysis, our findings are comparable to the results obtained by Siddik et al. (2016) and El Mghazli et al. (2021), who found that the populations of Gangetic

whiting (*Sillaginopsis panijus*) from the Bangladesh and Atlantic horse mackerel (*Trachurus trachurus*) from the Moroccan Atlantic coast were each clustered into two groups. Fish exhibit notable morphological diversity within and across populations, making them particularly sensitive to environmental adaptation, eventually influencing their phenotypes (Verma et al. 2014). Furthermore, these variations have been linked to marine ecology, encompassing light penetration, food availability, and water currents (Imtiaz and Naim 2018).

In conclusion, this current research is the first of its kind to employ morphometric techniques for determining and discriminating Malaysian populations of *E. affinis* [Peninsular Malaysia, Borneo (Sabah and Sarawak)], and Mukalla City, Yemen. Discriminant function analysis (DFA), principal component analysis (PCA), and cluster analysis (CA) have revealed significant differences in body form among 20 *E. affinis* populations investigated. The findings will deliver precious information for the proper governance and preservation of *E. affinis* in these regions. Further research combining morphometric and molecular techniques is needed to interpret population delimitation accurately.

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