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ORIGINAL ARTICLE

# Morphological study of selected mudskipper species (Family: Oxudercidae) and development of key pictorial

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**Abstract**
Mudskippers (Oxudercidae) are euryhaline fish found in mudflats, sandy beaches, and mangrove swamps. Owing to the high abundances of mudskipper species in Peninsular Malaysia, the identification process is found challenging. The purpose of this study was to identify selected species of mudskipper from selected mangroves in Terengganu, east coast of Peninsular Malaysia according to morphometrical variations and development of key pictorial of mudskipper. A total of 63 mudskippers were collected using a fish net in Marang (n= 30) and Setiu (n= 33). Quantitative data on the morphometric characteristics of each individual were identified namely *Periophthalmus gracilis*, *P. variabilis* and *P. argentilineatus*. One-way analysis of variance showed significant differences ($P<0.05$) in 10 out of 16 morphometric characteristics among the three species. In the casewise statistics analysis, 92.1% of the mudskippers were correctly classified into their original groups on average. The canonical variate analysis (CVA) scatter plot showed the segregation of three identified mudskipper species into three distinct groups. In the cluster analysis, UPGMA dendrogram indicated the segregation of the three species into two distinct clades, and *P. gracilis* and *P. variabilis* were included in the sister group, whereas *P. argentilineatus* was in a separate clade. The development of the key pictorial of mudskippers showed that 10 species from five genera were successfully recognized and distinguished by comparing their body parts as key indications. This study will be helpful to researchers acquiring information for identifying mudskippers especially from the genus *Periophthalmus*.

**Keywords:** Morphometric, Discriminant function analysis, Canonical variate analysis, Key pictorial development.

## INTRODUCTION

Mudskipper (Oxudercidae) has regularly been a topic within Malaysian society with the common question being, is it a fish or an amphibian (Hui et al. 2019). The 10 genera of mudskippers in the world have 34 species. In Peninsular Malaysia, eight species from six genera were recorded, namely, *Periophthalmus, Periophthamodon, Baleophthalmus, Pseudapocryptus, Scartelaos* and *Oxuderces* (Khaironizam et al. 2003). The habitats of mudskipper are mangroves and intertidal mudflat ecosystems (Polgar 2009). They are widespread on tidal mudflats throughout tropical Africa, Australia and Asia and found in the muddy soft bottom shores of intertidal zones, estuarine ecosystems and mangrove swamps in the Indo-Pacific (Khaironizam et al. 2003). Mudskippers are usually out of the water to feed, mate, and avoid predators. They have specialized skin that retains enough water and enables them to remain on the ground for long periods. High ammonia tolerance helps fish to survive within the intertidal zone with a high concentration of $NH_3$ or $NH_4^+$ (You et al. 2018).

In Malaysia, mudskippers reside in mangrove ecosystems, where 3.7% of the total mangrove coverage on a global scale compensates for the entire land sector. In fact, mangrove forests in Malaysia comprise approximately 0.58 million hectares. Approximately 58.6% of these forests are in Sabah, 24.4% are in Sarawak, and the remaining 17% are found in Peninsular Malaysia (Kanniah et al. 2015). Over the recent decades, every mangrove area has undergone a total reduction as a result of anthropogenic activities. The remaining mangrove areas lost their pristine quality and showed ecosystem changes because of the harvesting of aquatic animals and utilization of wood. In other words, human activities have imposed crucial

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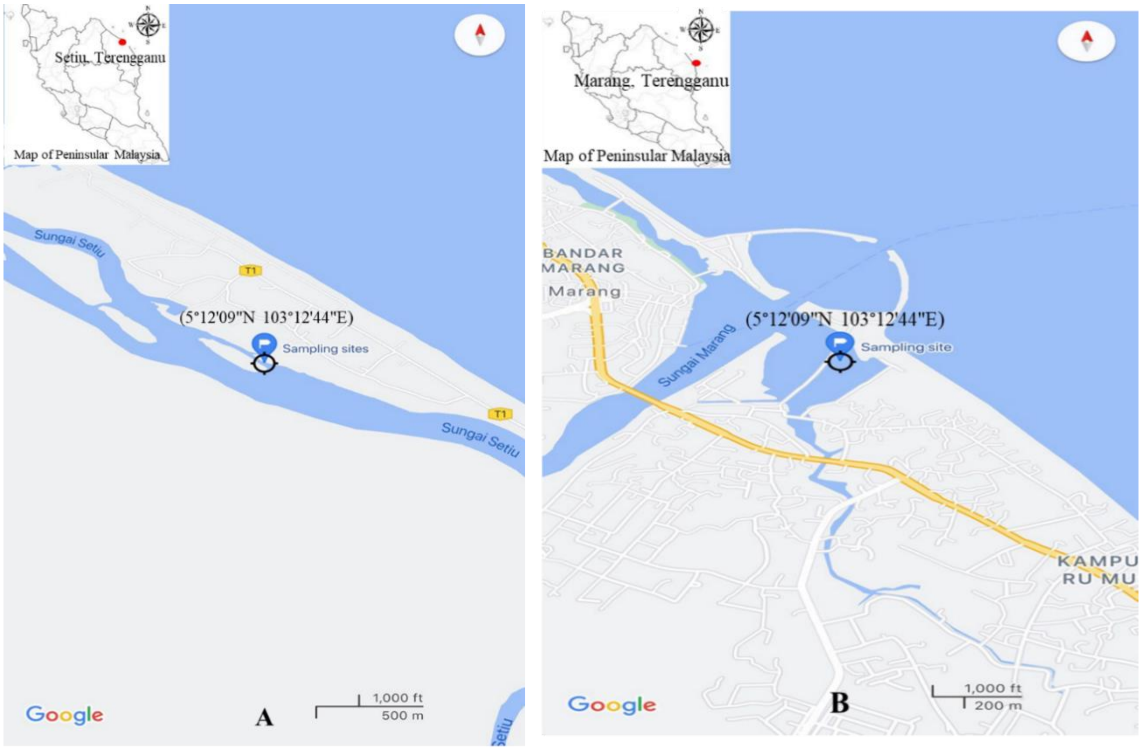


**Fig.1.** Map of sampling site in (A) Setiu, Terengganu and (B) Marang, Terengganu.

impacts on mangrove ecosystems, including overharvesting, overfishing, conversion to other uses, sedimentation, pollution and alteration of flow regimes (Hamdan et al. 2012). Therefore, not only mudskipper populations are affected by habitat disturbances, but also other aquatic organisms are under threat (Polgar & Lim 2011).

The morphological characteristics of fish, including their structural features, should be studied for the convenient and better identification of fish species (Keat-Chuan Ng et al. 2017; Abbasi et al. 2022; Mouludi-Saleh et al. 200). Morphological variation is one of the most important typical forms of biological studies that can be used for many aspects including resource management, evolution, behaviour, ecology and phenotype plasticity (Mouludi-Saleh et al. 2019; Seçer et al. 2020; Abbasi et al. 20221). Furthermore, morphological variation in fish populations is closely related to the complicated aspects of hydrology and evolutionary history (Haas et al. 2015). Moreover, the ability of a fish species to colonize a new habitat can be accurately predicted and detected according to its external morphology alone (Azzurro et al. 2014). Thus, to survive in aquatic environments, fish species have developed various morphological structures with a wide range of biological and physical characteristics (Haas et al. 2015).

Based on the above-mentioned background, this study aimed to identify mudskipper species based on the morphometric variability from selected mangroves in Terengganu, Malaysia and to develop a key pictorial for the identification of selected mudskipper species from Peninsular Malaysia. The findings will help a useful understanding of the morphological variability of the mudskipper species in the identification process as it aids the segregation of the species. Besides, the key pictorial will be applicable for the researchers to identify mudskipper species particularly mudskippers from Peninsular Malaysia.

## MATERIALS AND METHOD

**Sampling sites:** This research was conducted in mangrove areas in Terengganu, located on the east coast of Peninsular Malaysia. Setiu (Fig. 1A) and Marang (Fig. 1B) were selected as the sites for mudskipper sampling with different descriptions

**Table 1.** Sampling sites description.

| Sampling sites | Location | Description |
|---|---|---|
| Setiu | (5°37′18″N, 102°47′36″E) - Mangrove forest - Low tide level | This site was located at the riverbank or brackish water swamp, which was filled with Rhizophora, Avicennia, and Nipa palm trees. The soil condition was muddy and soft. |
| Marang | (5°12′09″N, 103°12′44″E) - Open area and mangrove forest - Low tide level | This site was located at seawater swamp. This area was near shorelines, jetties and a few houses. There were many Rhizophora and Avicennia plants found around the sites. The area had low bulk density cover. |

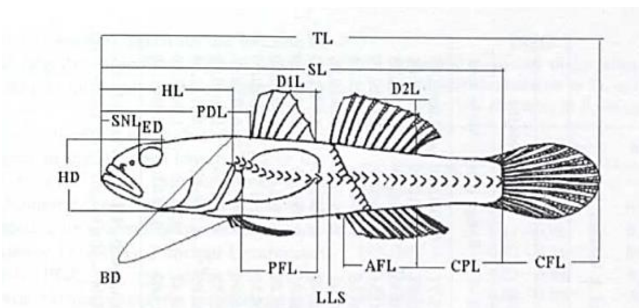


**Fig.2.** Morphometric measurements of mudskippers (Daud et al. 2005).

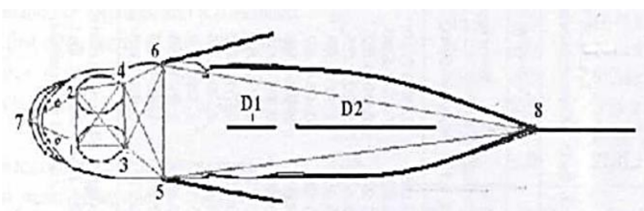


**Fig.3.** Morphometric measurements of mudskipper at upper view (Daud et al. 2005).

(Table 1). Sample collection was performed for 7 days in both sampling sites from February to July 2021. The tide tables were checked before sampling (Lee et al. 2005).

**Sampling:** Mudskippers were caught manually with the fish net in both sites. The sampling session started in the morning during low tide and when the mudflats were fully revealed. The sampling session ended when the tide began to rise with an estimated time of 4-5 h in each sampling session. The reason was that catching mudskippers was difficult when mudflats were covered with water. Captured mudskippers were placed in a polystyrene box for subsequent identification and morphometric measurements.

**Sample keeping:** The mudskippers were cleaned thoroughly with clean water for removal of mud or dirt and fixed immediately after collection. Some specimens were fixed in 10% formalin and some of them were fixed in -20℃ freezer to (Immaculate & Jamila 2018).

**Identification sample:** Each species was identified using Fishbase and WoRMS (Taniwel et al. 2020). Early identification processes were based on body parts, such as the colour of the first dorsal fin, head shape and spots on the body, and sorting was performed according to potential genus and species. All the mudskippers were then placed separately in labeled containers according to potential groups.

**Collection of morphometric data:** Sixteen morphometric characteristics were measured quantitatively in millimetres (mm) including eye diameter (ED), head diameter (HD), total length (TL), standard length (SL), head length (HL), head width (HW), body depth (BD) snout length (SNL), predorsal length (PDL), first dorsal fin length (FDFL), second dorsal fin length (SDFL), pelvic fin length (PFL1), pectoral fin length (PFL2), anal fin length (AFL), caudal fin length (CFL) and caudal peduncle fin length (CPL). A digital calliper was used (Figs. 2, 3). The measurement of morphometric characteristics was performed under a high lighting intensity, which enabled us to clearly observe all the parts of a mudskipper's body.

**Size adjusted measurement:** To eliminate sizedependent variation from morphometric data, an allometric method (Elliot et al. 1995) was used in order to avoid biases due to the size of the morphometric variables. All of them were standardized using the following formula: $M_{adj} = M(L_s/L_o)^b$, where $M$ is the original measurement, $M_{adj}$ is the adjusted size of the measurement, $L_o$ is the standard length of the fish, $Ls$ is the mean of standard length for all fish for all samples, and $b$ is the slope of the regression of $\log M$ on $\log L_o$ of all samples. The results of the allometric method were evaluated through the correlation between the transformed variables and standard length of the samples.

**Analysis of variance (ANOVA):** Data on each morphometric characteristic in mudskipper populations were analysed using one-way analysis of variance (ANOVA) performed on Statistical Package for the Social Science (SPSS), and significant differences in morphological parameters among individuals in different locations were determined (Ethin et al. 2019). Only morphometric characters with significant variation ($P<0.05$) were used to obtain a stable result from multivariate analysis (DFA, CVA and UPGMA).

**Discriminant function analysis (DFA) and canonical variate analysis (CVA):** Discriminant function analysis (DFA) was run using SPSS and based on the 16 morphometric characteristics for the extraction of significant functions among the morphometric variables Eigenvalues, percentage of variance and Wilks' lambda tests were used. Then, the highest characteristic loadings from the extracted functions were identified as important variables in population differentiation (Mousavi-Sabet & Anvarifar 2013). These significant functions were further analysed for the examination of the patterns of morphometric discrimination among the populations of mudskippers through canonical variate analysis. Afterwards, the percentage of correctly and incorrectly classified mudskippers were identified (Colihueque et al. 2017).

**Cluster analysis:** In addition to discriminant analysis, morphometric distances among the individuals from the three groups of mudskippers were determined through cluster analysis by using the Euclidean distance algorithm on Minitab (Veasey et al. 2001). The unweighted pair group method with arithmetic mean (UPGMA) dendrogram was constructed to show the clustering algorithm among the *Periophthalmus* species (Cruz et al. 2014).

**Key pictorial identification development:** The taxonomic key (dichotomous key) was developed by distinguishing characteristics for genus and species keys and adjusting and utilising characteristics from original mudskipper species descriptions and existing keys (Craig & Bonner 2019). The specific categories following the keys and descriptions obtained from morphometric and meristic characters and colour patterns of the mudskippers were identified by comparing the morphological features of mudskipper specimens. The picture of each species was collected for the reference material of each key.

## RESULTS

**Morphometric measurement:** A total of 63 of mudskippers (family Oxudercidae) were collected from mangroves in Terengganu Setiu [n = 33] and Marang [n = 30]. Detailed observations based on morphological characteristics showed that they were classified into one genus of *Periophthalmus* and further identified as *P. gracilis* (n= 30; Fig. 4), *P. variabilis* (n= 28; Fig. 5) and *P. argentilineatus* (n= 5; Fig. 6). The descriptive statistics of morphometric characteristics for three selected species were carried out and were reported as range and mean and standard deviation. *Periophthalmus variabilis* had the largest mudskippers with lengths ranging from 63.38mm to 66.16mm with a mean value of 64.87mm. By contrast, *P. gracilis* comprised the smallest mudskippers, which had lengths ranging from 58.59mm to 64.22mm with a mean value of 60.46mm. **Species identification:**

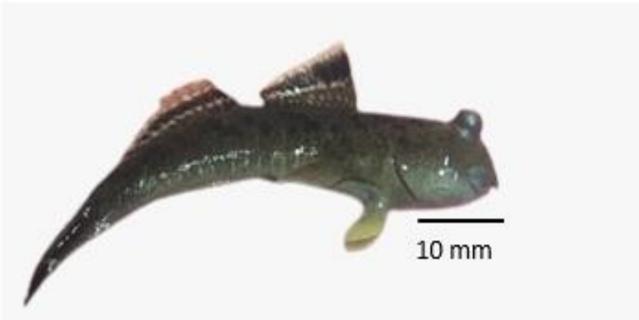


**Fig.4.** Sample of *Periophthalmus gracilis*.


**Fig.5.** Sample of *Periophthalmus variabilis*.


**Fig.6.** Sample of *Periophthalmus argentilineatus*.

Based on the 14 out of 16 morphometric characteristics data (*P*<0.05) among three species of genus *Periophthalmus* were observed, the data were further used in DFA, canonical variate analysis (CVA) and cluster analysis by using UPGMA method. Thus, the contributions of variables (morphometric characteristics) to principal components or function were identified for the identification of morphometric measurement mainly influences species differentiations (Mousavi-Sabet & Anvarifar 2013).

Based on the result, the DFA successfully brings out two significant functions, Function 1 and Function 2 which explained 94.2% and 5.8% of the variance respectively (Table 4). Function 1 had higher character loadings, which had higher variations in characteristics in the identification of *Periophthalmus* species (Table 5). The characteristic loadings on Function 1 were total length, standard length, eye diameter, head length, predorsal length, first dorsal fin length, second dorsal fin length, anal fin length and in Function 2 consisted of Body depth, head diameter, head width, snout length, pectoral fin length, and caudal fin length.

How a function effectively separates mudskippers into three species groups can be determined through Wilk's lambda statistic (Table 6). The tests of Function 1 through Function 2 (Wilk's lambda = 0.032) had a probability of 0.000 and Function 2 (Wilk's lambda = 0.515) had a probability of 0.001. Both had a significance level of *P*<0.05. The small value of Wilk's lambda for Function 1 confirmed that the characters had greater discriminatory ability among the three groups (Gonzalez-Martinez et al. 2021). The highest character loadings observed in Function 1 and Function 2 were total length (0.640) and head width (1.091), respectively, as shown in Table 7. This result indicated that both characters are the greatest degree morphological characters to be used for species identification among the *Periophthalmus* samples in this study.

**Correlation among identified *Periophthalmus* spp.:** Differences among the three mudskippers species are shown through canonical variate analysis plot (Fig. 7), where the tested variables were values of Function 1 and Function 2 derived from DFA. In addition, the canonical variate analysis plot revealed no overlaps occurs among three groups of *Periophthalmus* species, where the conspecific populations overlapped significantly more than heterospecific populations especially for *P. gracilis* and *P. variabilis*. At the Function 1 axis, it was primarily a contrast between *P. variabilis* and

**Table 3.** The ANOVA result for morphometric measurements of *Periophthalmus* species in mangrove areas of Setiu and Marang, Terengganu.

| Morphometric characteristics | Between species | |
|---|---|---|
| | F value | P value |
| TL | 200.2 | 0.000* |
| SL | 5.342 | 0.007* |
| BD | 6.658 | 0.002* |
| ED | 25.860 | 0.000* |
| HL | 35.952 | 0.000* |
| HD | 4.789 | 0.012* |
| HW | 10.30 | 0.000* |
| SNL | 5.077 | 0.009* |
| PDL | 33.017 | 0.000* |
| FDFL | 8.547 | 0.001* |
| SDFL | 25.144 | 0.000* |
| PFL1 | 1.112 | 0.335 |
| PFL2 | 34.495 | 0.000* |
| CFL | 0.945 | 0.394 |
| CPL | 19.350 | 0.000* |
| AFL | 23.794 | 0.000* |

**Table 4.** Eigenvalues for discriminant function analysis (DFA) of three mudskippers species from selected mangrove in Terengganu.

| Function | Eigenvalue | Variance (%) | Cumulative (%) | Canonical Correlation |
|---|---|---|---|---|
| 1 | 15.232* | 94.2 | 94.2 | 0.969 |
| 2 | 0.940* | 5.8 | 100.0 | 0.696 |

**Note:** (*) most significant value $P<0.05$.

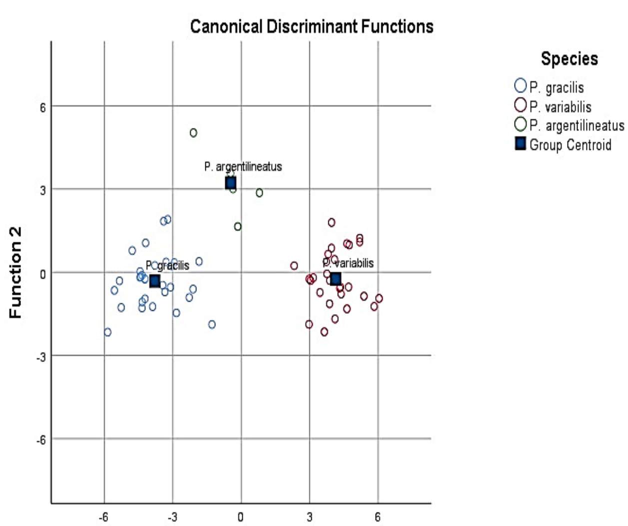


**Fig.7.** Plots of canonical variate analysis (CVA) discriminating Function 1 and Function 2 for three *Periophthalmus* species identification.

*P. gracilis* (Fig. 7), as these correspond to differences in their morphological relative, such as eye diameter, caudal peduncle and anal fin length.

The Function 2 axis showed *P. argentilineatus* were shortly apart from the other two species and was associated with some key difference in head width. This corresponded to *P. argentilineatus* which has a wider head than *P.variabilis* and *P. gracilis* according to the mean on head width (HW) in Table 2. In casewise statistics, the CVA plot analysis among identified *Periophthalmus* species were further explained in Table 8, and 100% of the samples were correctly classified into their original groups. Then, a breakdown of successful identification according to species showed 100% success rates for *P. gracilis*, *P. variabilis* and

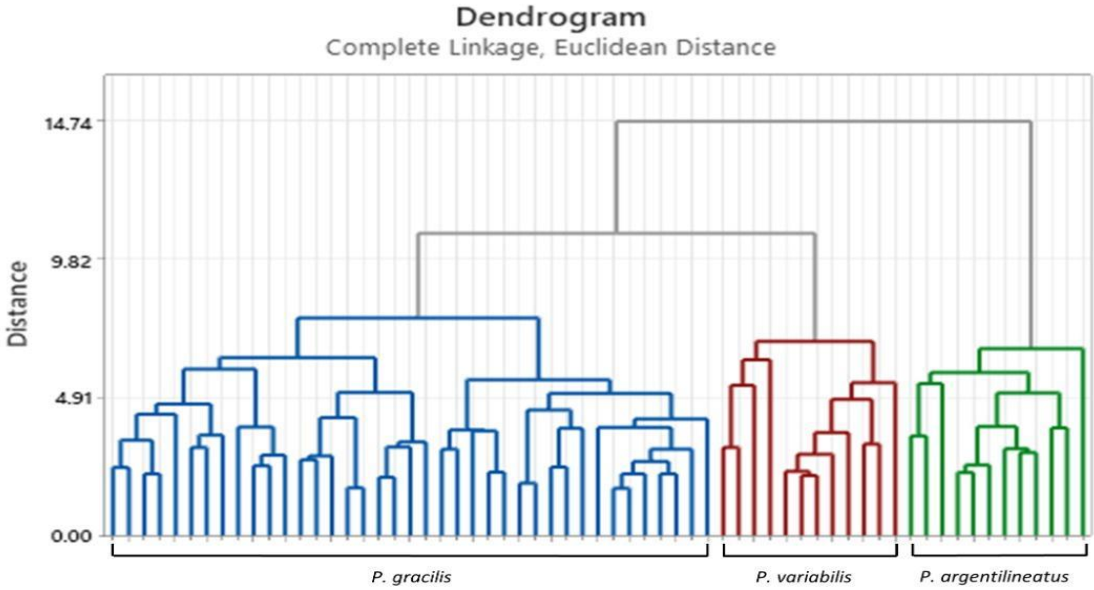


**Fig.8.** UPGMA cluster analysis of *Periophthalmus* species from selected mangrove in Terengganu based on morphometric data.

*P. argentilineatus.* This relative segregation was correlated with CVA, which allowed the visual examination of the plotted Function1 and Function 2 scores for each sample.

**Clustering of *Periophthalmus* species:** The taxonomic relationships were calculated using the Euclidean distance between the species. Actual distances were not plotted, and the distance to numbers between 0 to 14.74 (Fig. 8) was plotted. Based on the UPGMA result, three main branches with different colours showed the three selected *Periophthalmus* species, which were clustered accordingly. In the first branch, *P. gracilis* was clustered as the nearest taxon to *P. variabilis.* Moreover, these two species were indicated as sisters to *P. argentilineatus* as the branch showed high divergence between them.

**Key Pictorial of Mudskipper species in Peninsular Malaysia**

1a. Blue spots appear on the dorsal fin (Fig. 9a) ....…...............................Genus *Baleophthalmus*, 2

1b. No blue spots appear on the dorsal fin (Fig. 9b) …………………………………………...……….3

2a Base of the eyes are blue (Fig. 10a) ……………………..*Baleophthalmus pectinirostris*

2b Base of the eyes are greyish (Fig. 10b) …..………………….......... *Baleophthalmus boddarti*

3a Caudal fin elongate and spade (Fig. 11a) ………………….……….*Pseudapocryptes elongatus*

3b Caudal fin elongate and rounded (Fig. 11b) ……….……………..…………..……………..……..4

4a Present of a longitudinal stripe along the body (Fig. 12a) ....……...... *Periophthalmodon schlosseri*

4b Absent of a longitudinal stripe along the body (Fig. 12b).…...… ………… ………….………....5

5a Black spots appear on the second dorsal fin (Fig. 13a) …………...........……*Scartelaos histophorus*

5b Transparent background with medial dark brown stripe on second dorsal fin (Fig. 13b)

6a Have rounded posterior margin of first dorsal fin (Fig. 14a)…..………..…*Periophthalmus walailake*

6b Have straight posterior margin of first dorsal fin (Fig.14b).……………............…………..............7

7a Pelvic fins are fused by a basal membrane (Fig. 15a)………………....*Periophthalmus chrysospilos*

7b Pelvic fins are completely separated (Fig. 15b). 8

8a First Spine on the first dorsal fin is elongate (Fig. 16a).……………………*Periophthalmus variabilis*

8b No elongated first spine present on first dorsal fin (Fig. 16b)…………………...…………..……9

9a The membranes of the caudal fin are transparent (Fig. 17a)………………....*Periophthalmus gracilis*

9b Membranes of caudal fin are dusky to brownish speckles (Fig. 17b)...*Periophthalmus argentilineatus*

**Table 5.** Correlations between the measured morphometric variables and standardized canonical discriminant functions of three mudskipper from selected mangrove in Terengganu.

| Morphometric characters | Function 1 | Function 2 |
|---|---|---|
| TL | 0.661* | 0.145 |
| SL | 0.108* | 0.041 |
| BD | 0.084 | 0.348* |
| ED | 0.237* | 0.090 |
| HL | 0.280* | 0.061 |
| HD | 0.091 | 0.190* |
| HW | 0 .019 | 0.599* |
| SNL | 0.082 | 0.265* |
| PDL | 0.263* | -2.26 |
| FDFL | 0.137* | 0.032 |
| SDFL | 0.234* | 0.080 |
| PFL2 | 0.253 | 0.428* |
| CPL | 0.198 | 0.222* |
| AFL | -0.226* | 0.113 |

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

**Table 6.** Wilks' lambda for discriminant function analysis (DFA) of three mudskippers species from selected mangroves in Terengganu.

| Test of Function | Wilks' Lambda | Chi-square | df | Sig. |
|---|---|---|---|---|
| 1 through 2 | 0.032 | 184.572 | 28 | 0.000 |
| 2 | 0.515 | 35.467 | 13 | 0.001 |

**Table 7.** Standardized canonical discriminant function coefficient of three mudskipper species from selected mangrove in Terengganu.

| Character | Function | |
|---|---|---|
| | 1 | 2 |
| TL | 0.640* | 0.197 |
| SL | 0.099 | 0.055 |
| BD | -0.228 | 0.448 |
| ED | 0.143 | -0.073 |
| HL | 0.204 | -0.245 |
| HD | 0.245 | -0.785 |
| HW | -0.324 | 1.091* |
| SNL | -0.256 | 0.281 |
| PDL | 0.503 | -0.379 |
| FDFL | -0.126 | -0.007 |
| SDFL | 0.546 | -0.435 |
| PFL2 | 0.149 | -0.414 |
| CPL | 0.602 | 0.072 |
| AFL | 0.441 | 0.100 |

Note: (*) Highest diagnostic character loadings in each function.

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**Table 2.** Descriptive data (range and mean ± standard deviation) on the morphometric characteristics of *Periophthalmus* spp. in the mangrove areas of Setiu and Marang, Terengganu.

| | | Population | | | | | |
|---|---|---|---|---|---|---|---|
| | | Setiu *P. variabilis* (N=28) | | Setiu *P. argentilineatus* (N=5) | | Marang *P. gracilis* (N=30) | |
| | | Range(mm) | Mean ± sd | Range(mm) | Mean ± sd | Range(mm) | Mean ± sd |
| Morphometric characters | TL | 63.38-66.16 | 64.872±0.56 | 62.28-63.06 | 62.74±0.29 | 58.594-64.22 | 60.46±1.08 |
| | SL | 43.10-63.00 | 54.014±4.68 | 42.30-57.60 | 52.32±5.84 | 41.60-60.30 | 49.92±4.71 |
| | BD | 7.76-9.64 | 8.67±0.38 | 8.66-9.49 | 9.06±0.32 | 7.04-9.74 | 8.35±054 |
| | ED | 3.042-3.824 | 3.43±0.16 | 3.24-3.34 | 3.28±0.04 | 2.55-3.52 | 3.06±0.24 |
| | HL | 12.97-14.70 | 13.86±0.46 | 12.70-13.73 | 13.21±0.38 | 10.99-14.00 | 12.51±0.74 |
| | HD | 7.86-10.26 | 9.20±0.54 | 9.02-9.64 | 9.32±0.23 | 7.75-9.81 | 8.83±0.49 |
| | HW | 7.81-11.70 | 9.54±1.09 | 11.03-11.66 | 11.41±0.26 | 7.47 -10.72 | 9.37±0.84 |
| | SNL | 1.46-3.323 | 2.34±0.43 | 2.49-2.59 | 2.56±0.04 | 1.21-3.17 | 2.05±0.44 |
| | PDL | 17.31-19.70 | 18.25±0.57 | 13.76-17.80 | 16.61±1.62 | 14.71-18.36 | 16.54±0.86 |
| | FDFL | 9.61-11.61 | 10.63±0.59 | 9.79-10.49 | 10.19±0.32 | 7.69-12.14 | 9.69±1.11 |
| | SDFL | 10.24-12.19 | 11.34±0.59 | 10.38-11.30 | 10.87±0.39 | 9.05-11.27 | 10.24±0.62 |
| | PFL1 | 12.44-14.30 | 13.28±0.48 | 12.58-13.44 | 13.15±0.33 | 11.12-14.15 | 13.05±0.74 |
| | PFL2 | 2.34-3.64 | 2.93±0.28 | 2.77-3.50 | 3.00±0.27 | 2.17-2.96 | 2.45±0.18 |
| | CFL | 9.37 -12.15 | 10.86±0.56 | 9.96-10.74 | 10.42±0.29 | 9.26-14.18 | 10.66±0.94 |
| | CPL | 11.38-13.54 | 12.58±0.54 | 12.16-12.77 | 12.49±0.26 | 9.7-13.28 | 11.55±0.77 |
| | AFL | 7.26-10.17 | 9.26±0.60 | 8.23 – 9.50 | 8.84±0.52 | 6.73-9.75 | 8.09±0.70 |

Note: TL= Total length, SL= Standard length, BD= Body depth, ED= Eye diameter, HL= Head length, HD= Head depth HW= Head width, SNL= Snout length, PDL= Predorsal length, FDL= First dorsal fin length, SDFL= Second dorsal fin length, PFL1= Pectoral fin length, PFL = Pelvic fin length, CFL= Caudal fin length, CP = Caudal peduncle length, AFL= Anal fin lengthOut of 16 morphometric characteristics assessment, ten characters revealed as the most significant characteristics in segregating them into three different species which were the total length (TL), eye diameter (ED), head length (HL), head width (HW), predorsal length (PDL), second dorsal fin (SDFL), pelvic fin length (PFL2), snout length (SNL), caudal peduncle length (CPL) and anal fin length (AFL) with the value of $P<0.05$ (Table 3). In this finding, all statistical tests were based on alpha level of 0.05 as a significant criterion.

**Table 8.** Casewise statistics on predicting the correct group for individuals of *Periophthalmus* species from selected mangrove in Terengganu.

| | Species | Predicted group membership | | | Total |
|---|---|---|---|---|---|
| | | *P. gracilis* | *P. variabilis* | *P. argentilineatus* | |
| Count | *P. gracilis* | 30 | 0 | 0 | 30 |
| | *P. variabilis* | 0 | 28 | 0 | 28 |
| | *P. argentilineatus* | 0 | 0 | 5 | 5 |
| (%) | *P. gracilis* | 100.0 | 0 | 0 | 100 |
| | *P. variabilis* | 0 | 100.0 | 0 | 100 |
| | *P. argentilineatus* | 0 | 0 | 100.0 | 100 |

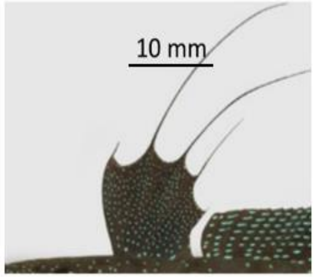

**Fig.9a** (Polgar 2014)

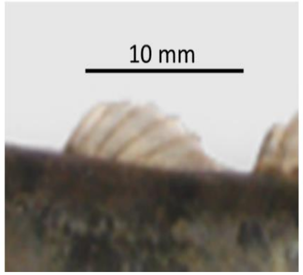

**Fig.9b** (Polgar) 2013)

**Fig.10a**.

**Fig.10b** (Pormansyah et al. 2021)

**Fig.11a** (Polgar 2013)

**Fig. 11b** (Polgar 2016)

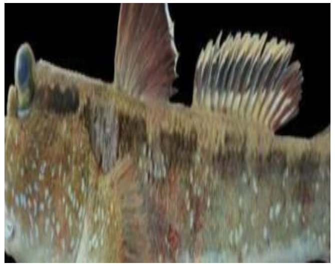
**Fig.12a** (Shetty 2017)


**Fig.12b** (Bray 2016)


**Fig.13a** (Bray

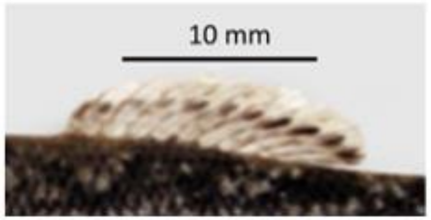

**Fig. 13b** (Polgar 2012)

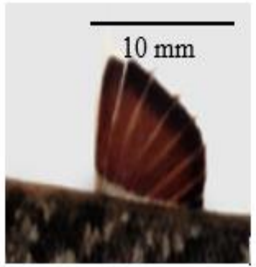

**Fig.14a** (Polgar 2012)

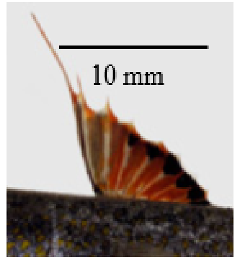

**Fig.14b** (Polgar 2012)

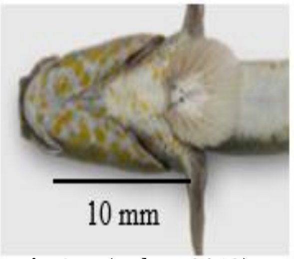

**Fig.15a** (Polgar 2012)

**Fig.15b.**

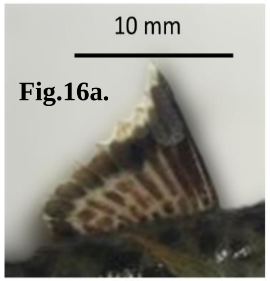

**Fig.16a.**

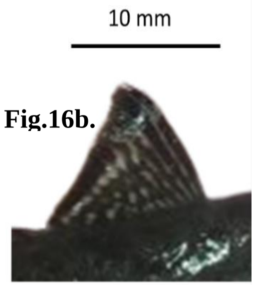

**Fig.16b.**

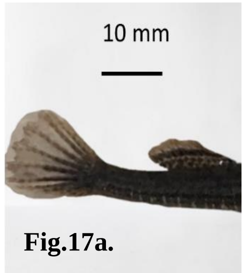

**Fig.17a.**

**Fig.17b.**

## DISCUSSION

**Morphometric measurement:** Morphological characters are widely used in fisheries biology for the estimation of discreteness and relationships between various taxa (Gonzalez-Martinez et al. 2020) and have been used in identifying stocks of fish (Daud et al. 2005; Ukenye et al. 2020). An organism's morphometric and meristic characteristics are important tools for identifying variants of identical species and involve the classification of subtle shape variations and differentiation through size (Bhakta et al. 2020). Both characteristics are considered the easiest and most authentic tools for identifying a specimen which is termed as morphological systematic (Brraich & Akhter 2015). In general, morphometric characteristics for mudskippers refer to the measured length structure, such as the fin, the length of the head, the diameter of the eye, or the ratio between measurements. Whereas the meristic character includes nearly all the countable structures, including the number of fin rays, scales, and gills (González et al. 2016).

Several examples in previous studies applying morphometric and meristic characteristics for species identification were carried out on three species on mudskippers in Ambon Island coastal waters in Indonesia namely *P. argentilineatus*, *P. kalolo* and *P. minitus* (Sangur et al. 2020). Furthermore, variation in shape of the same species of mudskippers depends on locality. For instance, the populations of *Scartelaos tenuis* from five different stations in Oman Sea and Persian Gulf are distinguishable using morphometric and meristic characteristics (Ghanbarifardi et al. 2020). The findings of multivariate morphometric investigation are similar to those of several previous studies, which delineated *Sillaginopsis panijus* stock structure based on morphometric characters of the species (Siddik et al. 2016). Moreover, Turan et al. (2011) studied nine species of Mediterranean grey mullet and successfully identified according to systematic relationships among species and comparison of morphometric and meristic characters.

**Species identification:** The identification of mudskipper samples in this study was performed using several analyses, including the DFA and CVA. In general, DFA is an effective method for distinguishing different stocks of the same species or different species of the same genus, according to stock management methods (Siddik et al. 2016). In addition, a high character variability corresponds to the important morphometric characteristics that determine species grouping (Boussou et al. 2010). Similar approach has been introduced, which applies this method for the identification of morphological variations and stock structures of the selected Tilapia fish (Samaradivakara et al. 2012), the Indian major carp, *Labeo rohita* (Mir et al. 2013) and the five fish species of subfamily Barbinae (Gupta et al. 2018).

The CVA plot helps confirm species assignment with morphological characters owing to the linear combinations of the original variables that maximally separate the mudskipper groups, *Periophthalmus* spp. A similar approach was applied to the population study of *Caranx* species (Torres & Santos 2019) and successfully applied to Midas cichlid species (Elmer et al. 2010). The DFA helps calculate the multivariate distance from an unknown specimen to the centroids for classification (McKeown et al. 2013). The discriminant analysis creates an equation, which minimises the possibility of misclassifying cases into respective groups (Riffenburgh 2012). Canonical and discriminant analyses are often used in assessing patterns of intergroup variation and identification of the biological affinity of individual specimens (McKeown et al. 2013).

The *Periophthalmus* spp. can be separated into their assigned species according to morphological and meristic measurements. However, overlaps were found on several axes. With the help of case-wise analysis which is able to indicate which cases are extreme outliers. The case-wise diagnosis of *Periophthalmus* samples helps demonstrate a low degree of intermingling among the three *Periophthalmus* populations possibly because of the huge distances of the studied area, where the samples were collected (Colihueque et al. 2017). Most studies utilize case-wise diagnosis to identify outliers among their samples (Gustiano & Pouyaud 2008). However, in this study, the data were useful in determining the potential of morphometric and meristic characters in the classification of *Periophthalmus* spp. The possible reasons for these misidentifications occurred because species had morphological traits that were nearly identical to those of other species in their early life stages although the traits slowly changed through time, as they become older, or occupy different environments (Kirsch et al. 2018).

**Clustering of *Periophthalmus* species:** In the hierarchical cluster analysis, UPGMA dendrogram was constructed using Euclidean distance and averaging the measures of dissimilarity, thus avoiding clustering dissimilarity on morphometric data among *Periophthalmus* species (Cruz et al. 2014). Euclidean distance helps compute the distance between two data of the same variables (Barrett 2005). The clustering of *Periophthalmus* species was appropriately constructed by UPGMA. This finding was similar to the study on fish from the genus *Cobitis* (family Cobitidae; Mousavi-Sabet & Anvarifar 2013) and the five fish species from the subfamily Barbinae (Gupta et al. 2018). UPGMA is proven reliable in separating a studied population according to morphological characteristics.

**Development of key pictorial:** Species identification is a major step in any research project and plays an important role in the behavioural study (Brraich & Akhter 2015), and traditionally, species identification approaches rely on morphological and meristic characteristics as field guides (Omer 2017). Typically, the main objectives of key pictorial identification are the accurate identification of species, species biology, and geographical distribution as well (Tolis et al. 2011). Hence, the development of key pictorial is crucially important to the determination of mudskipper species in five genera of the family Oxudercidae, which are potential bio-indicators in environmental monitoring and assessment of coastal waters (Ansari et al. 2014). Key pictorial evidence has been proven useful in the study of sciaenid fish (family Sciaenidae) from the Taiwan Strait where a new species, *Johnius taiwanese* was identified. This species has been confused and misidentified for decades as *J. macrorhynus, J. belangerii, J. macrorhynus* or *J. sina* (Chao et al. 2019). Development of *Periophthalmus* key pictorial utilised different body parts of mudskippers to help distinguish one species from another. This observation was aided by another mudskipper genus. The mudskipper was first recognised based on the presence of a blue spot on their first dorsal fin. This key characteristic is important to the discrimination between the genus *Periophthalmus* and the rest of the mudskipper genera (Pormansyah et al. 2021). The stripes on the body were used in differentiating *Periophthalmus* spp. from *P. schlosseri*. The latter was recognised to have a black longitudinal stripe that starts from the eye and runs uninterrupted along the upper part of each side of the body to the caudal peduncle area (Jaafar et al. 2006).

Next, the colour present on the second dorsal fin was identified as *Scartelaos histophorus* (Fig. 13a) having black spots on the posterior half of the second dorsal fin. Then, the transparent background with a medial dark brown stripe on the second dorsal fin was indicated to the genus *Periophthalmus* (Fig. 13b), namely, *P. walailake* (Polgar & Khaironizam 2008). The pelvic fin for *P. gracilis* was fully fused with a basal membrane, and the other two, *P. argentilineatus* and *P. gracilis*, were completely separated. But to distinguish between *P. argentilineatus* (Fig. 17b) and *P. gracilis* (Fig. 17a) the colour of the caudal fin membrane from dusky to brownish speckles and the membrane of the caudal fin are transparent, respectively (Taniwel & Leiwakabessy 2020).

In conclusion, a study on morphological characteristics of mudskipper species in selected mangrove areas in Terengganu, west coast of Peninsular Malaysia, has successfully identified them as *P. gracilis*, *P. variabilis* and *P. argentilineatus*. The successful species assignment was due to several diagnostic parameters techniques that were enough to distinguish all three mudskipper species. DFA and CVA are widespread techniques for assessing and displaying variation among groups relative to the variation within groups. From this analysis as well, the most significant difference in morphometric characteristics is reliable to identifying the *Periophthalmus* mudskipper species which were the total length and standard length. In addition, UPGMA cluster analysis also displayed a complete separation and grouping after species grouping. Through this study, a key pictorial in recognising and distinguishing mudskippers species were successfully developed guided by pictures of prominent body parts, involving 10 species from the five genera of mudskippers.

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**مقاله کامل**

# بررسی ریخت‌شناسی گونه‌های منتخب گل‌خورک (خانواده: گل‌خورک‌ها) و توسعه مصور کلیدی

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**چکیده:** گل‌خورک‌ها (Oxudercidae) ماهی‌های یوری‌هالین (تحمل شوری بالا) هستند که در زمین‌های گلی، سواحل شنی و باتلاق‌های حرا یافت می‌شوند. با توجه به فراوانی گونه‌های گل‌خورک‌ها در شبه جزیره مالزی، فرآیند شناسایی چالش برانگیز است. هدف از این مطالعه شناسایی گونه‌های منتخب گل‌خورک‌ها از جنگل‌های حرا در ترنگانو، سواحل شرقی شبه‌جزیره مالزی با توجه به تغییرات ریخت‌سنجی و توسعه مصور کلیدی گل‌خورک‌ها بود. در مجموع ۶۳ گل‌خورک با استفاده از تور ماهی‌گیری در مرنگ (۳۰ قطعه) و ستیو (۳۳ قطعه) جمع‌آوری شد. داده‌های کمی در مورد ویژگی‌های ریخت‌سنجی نمونه‌های *Periophthalmus gracilis*، *P. variabilis* و *P. argentilineatus* توصیف شد. آنالیز تجزیه واریانس یک‌طرفه نشان داد که در ۱۰ ویژگی از ۱۶ ویژگی ریختی در بین سه گونه تفاوت معنی‌داری (*P*<۰/۰۵) وجود دارد. در تجزیه و تحلیل تابع تشخیصی، ۹۲/۱ درصد از گل‌خورک‌ها به‌طور متوسط در گروه‌های اصلی خود طبقه‌بندی شدند. نمودار تحلیل همبستگی کانونی (CVA) پراکندگی تفکیک سه گونه گل‌خورک شناسایی شده را به‌صورت سه گروه مجزا نشان داد. در تجزیه و تحلیل خوشه‌ای، نمودار UPGMA نشان داد که تفکیک سه گونه به دو کلاد مجزا، و *P. gracilis* و *P. variabilis* در یک گروه خواهری قرار گرفتند، در حالی‌که *P. argentilineatus* در یک شاخه جداگانه قرار گرفت. توسعه تصاویر کلیدی گل‌خورک‌ها نشان داد که ۱۰ گونه از پنج جنس با مقایسه اعضای بدن آن‌ها به‌عنوان نشانه‌های کلیدی با موفقیت شناسایی و متمایز شدند. این مطالعه برای پژوهشگرانی که اطلاعاتی برای شناسایی گل‌خورک‌ها به‌ویژه از جنس *Periophthalmus* کسب می‌کنند، مفید خواهد بود.

**کلمات‌کلیدی:** ریخت‌سنجی، تحلیل تابع تشخیصی، تحلیل همبستگی کانونی، توسعه تصاویر کلیدی.