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Morphometric and mersitic variation of the Ikan Puyu (*Anabas testudineus*) (BLOCH, 1792) in Malaysia

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The Ikan Puyu (*Anabas testudineus*) is one of the important indigenous fish species in Malaysia and famous for its delicious taste and flavour. However, this fish population has declined due to the overfishing, pesticides usage in agricultural runoff and deforestation. A morphometric study was conducted to retain the fish data for further conservation purposes. Approximately 75 fish sample were randomly collected from five regions of Malaysia (Pahang, Perak, Terengganu, Sabah and Sarawak). Prior to measurement, the fish were anesthetizing using benzocaine (ethyl-p-amino-benzoate) and then measured by ruler and being photographed. Fish appearance was macroscopically observed and morphometric features analyzed by conventional and truss network measurement. Data was subjected to One Way Analysis of Variance and discriminant function analyses (DFA). Results showed significant differences ($p<0.05$) in 14 features in *A. testudineus* for conventional measurement. There are significant differences ($p<0.05$) in anal fin ray for meristic counting and six landmarks for truss network measurement. Both conventional and truss measurements showed four DFA which DF1 with 63.7% and 55.4%, DF2 with 25.9% and 29.7%, DF3 with 7.6% and 12.5%, and DF4 with 2.7% and 2.4%, respectively, among group variability. DF plotting presented showed that the conventional measurement were significantly different among regions. There was a notable overlapping for the sample at region 1 and 2 based on truss network measurement finding suggested that there was intermixing among the populations. There were significantly different in morphometric features among five regions based on the statistical analyses due to the separated geographical sites and different environment. In conclusion, morphometric data of the *A. testudineus* would be beneficial as guidelines among communities in further conservation measure and fisheries management.

Keywords: ***Anabas testudineus*, ikan puyu, meristic, morphometric, truss network**

INTRODUCTION

The Ikan puyu or Climbing perch (*Anabas testudineus*) (Bloch, 1792) is one of the important indigenous fish species in Malaysia and famous for its delicious taste and flavour. This fish is believed to have medical properties for human health and has essential mineral needed for hemoglobin synthesis (Paul et al. 2017, Mohd-Khairi et al. 2018). Its unique characteristic as an omnivorous feeder, able to stand live without water and high resistance to diseases, makes this species a popular aquaculture fish in Malaysia (Nguang & Ha, 2015, Rahman et al. 2015, Hossen et al. 2017, Simi et al.2017). *A. testudineus* can be found in canal, larger rivers, swamps and estuaries which widely distributed throughout the Southeast Asian Countries (Tay et al. 2006, Dipesh et al. 2010, Hossen et al. 2017, Ahmadi, 2018). Although *A. testudineus* is a much sought-after fish among locals, this species is still recorded as data deficient under Red list status (Pal and Chaudhry, 2010, Hossain et al. 2015). In recent years, population of *A. testudineus* has declined rapidly due to ecological degradation, overfishing, and pesticides runoffs produced from agricultural runoff and management failure (Department of Fisheries, 2002, Hossain et al. 2015). It is important to conduct continuous morphometric study on *A. testudineus* as an alternative in conservational strategy.

Fish morphology information and recruitment patterns are considered as a reliable source before measures to any appropriate management and conservation are taken. Fish species identification is the initial step in conservation efforts as information like precise measurement and the fin rays' elements counting are needed as primary data. Morphometric feature is resistant and remain constant after generations (Mohhadasi et al. 2013, Mahfuj et al. 2019).

There are different methods to characterize the morphological features including conventional linear measurement and truss network system which has been employed in many fisheries research for instance investigation of life history and morphological trend across habitats (Rawat et al. 2017). Conventional method is linear measurement for shape indicator like length, area and ratio in fish. Truss network system measure a series of distances between landmarks that form two-dimensional outline to extract the fish body information (Mohaddasi et al. 2013, Solomon et al. 2015, Sotola et al. 2019).

There is no to limited documentation for the morphometric variance of *A. testudineus* due to the limited wild stock and scarcity in research on morphometric. This study aims to assess morphometric patterns of *A. testudineus* in Malaysia for conservation purposes and fisheries management.

MATERIALS AND METHODS

Collection of fish samples

A total of 122 samples of *A. testudineus* were randomly collected from five region of freshwater source in Malaysia including Pahang, Perak, Terengganu, Sabah and Sarawak (Table 1). The fish samples were transported to the Aquatic Laboratory of Faculty of Bioresources and Food Industry for morphometric and meristic studies.

Sample observation and measurements

Fish sample was macroscopically observed and compared in terms of morphology including body colour, appearance and body shape. For the conventional measurement, a total of 19 morphometric measurement was taken (to the nearest 0.1 cm) according to Hossen et al. (2017). The fish were anesthetizing using benzocaine (ethyl-p-amino-benzoate, 20-ppm NIKA Transmore) and photographed (high definition camera Canon IXY) during measurement. The descriptive features for conventional (Figure 1) measurement are presented in Table 2. The fish samples were measured as 13.2±1.3 cm in total length (TL) by and 44.5±14.1 g in weight.

Table 1: Fish sampling location and sample size from different region in Malaysia.

No.	Sampling site	Longitude / latitude	Sample source	Sample size
1	Terengganu	5.3296N˚, 103.1370E˚	Paddy field, culture pond	44
2	Pahang	3.9374N˚, 102.3620E˚	River	20
3	Perak	4.1116N˚, 101.2878E˚	River	20
4	Sabah	5.9804N˚, 116.0735E˚	River	15
5	Sarawak	4.3995N˚, 113.9914E˚	River	23

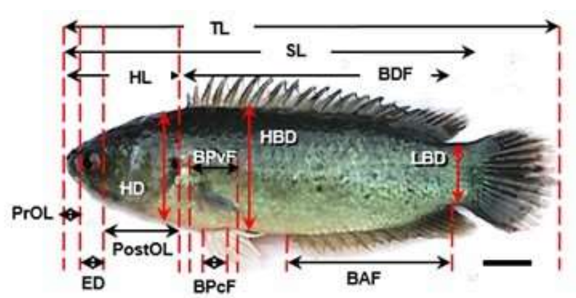

Figure 1: Conventional morphometric measurement applied on fish sample (Scale 1 cm).

A total of 11 truss network measurement were taken refer to Hossen et al. (2017). Fish was placed properly on acetate sheets using 2-cm block of expanded polystyrene before homologous landmarks on fish outline were selected using acetate sheet with a dissecting needle according to Figure 2. The descriptive features for truss network measurements are presented in Table 3. Morphometric measurements were subjected to discriminant function analysis (DFA).

A total of five meristic count includes the dorsal fins, caudal fins, anal fins, pectoral fins and pelvic fins were analyzed (Figure 3). Just after the morphometric measurement, all of the meristic counts were carried out and recorded in video for further references.

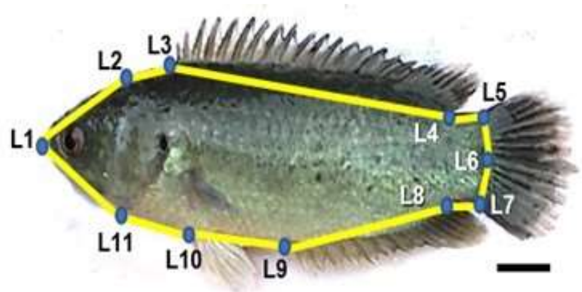

Figure 2: Location of the 11 landmarks for constructing the truss network on fish illustrate as open circles and morphometric distance measures between circles as lines (Scale 1 cm).

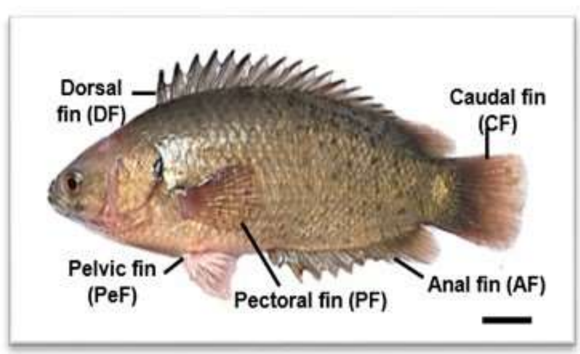

Figure 3. The meristic characters that counted for *A. testudineus* (Scale 1 cm).

Statistical analysis

The external morphometric diversities were ascribed to the differences of body features but not to the relation of fish sizes. In this study, significant linear correlations were observed among all of the measured features and the total length (TL) of the fish sample. These size-dependent variations from all morphometric measurement must be removed. Thus, prior to analysis, the size effects from the data set were eliminated by standardized the morphometric parameter using allometric formula (Elliot et al. 1995): $M_{adj} = M (L_s / L_o)^b$, where the M_{adj} refer as size-adjusted measurement, M refer as original measurement, L_s refer as the TL of fish sample, and L_o mean of the whole of TL of fish sample. Factor b was estimated for each feature from the data set as the slope of the regression of log M and log L_o using the entire fish group.

One-way ANOVA test and DFA using SPSS statistical software with significant at *p*-value was used to analyze data obtained from conventional, truss networks measurement and meristic count. Biometric analysis using morphometric characteristics was done to identify and relate different fish species and populations.

RESULTS

The greenish-grey colour is associated with black spot on body of fish collected from Terengganu, Pahang and Perak (Figure 4 a, 4b and 4c) while fish from Sabah and Sarawak were black in color (Figure 4d). Body depth was less deep with both head and anterior parts showed subcylindrical, hinder part compressed, snout blunt, mouth small, and oblique that extended to anterior part of orbit.

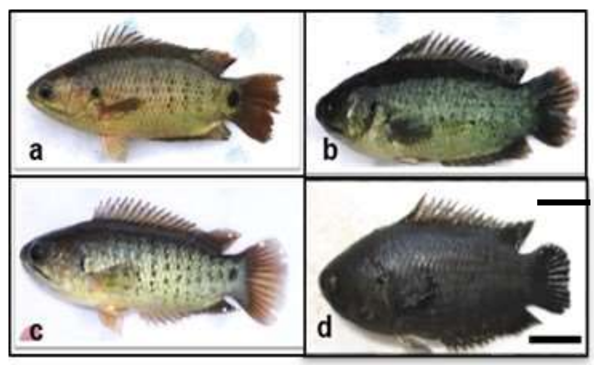

Figure 4. Different colour of fish sample from the (a) Terengganu, (b) Pahang, (c) Perak, (d) Sabah states in Malaysia (Scale, 2 cm).

Orbit of pectoral was just before or behind the opercle as well as dorsal origin while origin of ventral was a little behind and below origin of pectorals and reached a little behind anus. Body depth was less than the head length in standard length.

Significant different ($p<0.05$) in TL, HL, BD, PrOL, ED, PoSTOL HBD, HDF, HPvF, HAF, BPcF, BPvF, UJL and LJL of *A. testudineus* were illustrated in Table 2 for conventional measurement.

Table 2: The F value and *p*-value of conventional measurement of an ANOVA-test for differences among the morphometric features.

Morphometric feature	F value	*p*-value
Total length (TL)	5.214	0.001*
Standard length (SL)	2.423	0.052
Head length (HL)	14.261	0.000*
Body depth (BD)	2.821	0.028*
Pre-orbital length (PrOL)	5.106	0.001*
Eye diameter (ED)	15.605	0.000*
Post-orbital length (PoSTOL)	5.099	0.001*
High Body Depth (HBD)	4.906	0.001*
Lower Body Depth (LBD)	2.175	0.076
Height of Dorsal Fin (HDF)	49.739	0.000*
Height of Pectoral Fin (HPcF)	1.716	0.151
Height of Pectoral Fin (HPvF)	12.834	0.000*
Height of Anal Fin (HAF)	10.139	0.000*
Base length of Dorsal Fin (BDF)	1.002	0.409
Base length of Pectoral Fin (BPcF)	7.708	0.000*
Base length of Pelvic Fin (BPvF)	10.952	0.000*
Base length of Anal Fin (BAF)	2.071	0.089
Upper Jaw Length (UJL)	10.690	0.000*
Lower Jaw Length (LJL)	8.211	0.000*

Significant difference (p<0.05) in landmarks L1, L2, L3, L5, L8 and L10 of *A. testudineus* for truss measurement were shown in Table 3. There were significant differences (p<0.05) of types of fin rays of dorsal, caudal, anal, pelvic and pectoral were counted individually in meristic count (Table 4).

Table 3: The F value and *p*-value of truss dimensions of an ANOVA-test for differences among the landmarks.

Landmark	F value	*p*-value
L1 (anterior tip of snout at upper jaw)	11.050	0.000*
L2 (posterior aspect of neurocranium)	9.780	0.000*
L3 (origin of DF)	11.222	0.000*
L4 (insertion of DF)	0.703	0.591
L5 (anterior attachment of dorsal membrane from CF)	7.427	0.000*
L6 (posterior end of vertebrae column)	2.135	0.81
L7 (anterior attachment of ventral membrane from CF)	0.163	0.956
L8 (insertion of AF)	14.295	0.000*
L9 (origin of AF)	2.565	0.042
L10 (insertion of PeF)	4.264	0.003*
L11 (insertion of PF)	2.037	0.094

Table 4: The F value and *p*-value of meristic count of an ANOVA-test for differences among the types of fin rays.

Types of fin	F value	*p*-value
Dorsal fin	2.428	0.056
Caudal fin	0.907	0.465
Anal fin	2.942	0.026*
Pectoral fin	1.052	0.387
Pelvic fin	-	-

Discriminant function analysis provided four discriminant functions (DF1 to DF4) for the conventional and truss network measurements (Table 5) and showed 100% of the total among groups variability. All samples showed differ in morphometric and separated from each other in the discriminant space (Figure 5 and Figure 6). There was a notable overlapping for the sample at region 1 and 2 based on truss network measurement. The finding suggested that there was intermixing among the populations.

Table 5: The discriminant functions for the conventional and truss network measurements.

Discriminant function	Conventional (% in variance)	Truss network (% in variance)
DF1	63.7	55.4
DF2	25.9	29.7
DF3	7.6	12.5
DF4	2.7	2.4

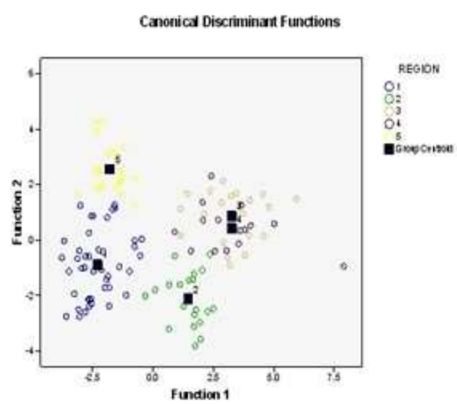

Figure 5: Sample centroids of discriminant function scores based on conventional measurement (Region 1 = Terengganu, 2 = Pahang, 3 = Perak, 4 = Sabah and 5 = Sarawak).

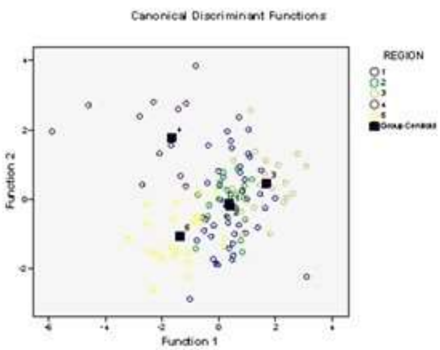

Figure 6: **Sample centroids of discriminant function scores based on truss network measurement (Region 1 = Terengganu, 2 = Pahang, 3 = Perak, 4 = Sabah and 5 = Sarawak).**

Pooled within-group correlations among the discriminant variables and DF in showed in 19 conventional measurements: HDF and PrOL contributed to DF1, ED, HAF, LJL and UJL contributed to DF2, BPcF, TL, BPvF and HPcF contributed to DF3, and the other eight dominantly contributed to DF4 (Table 6). For 11 truss measurements: L3 and L8 contributed to DF1, L1, L5, L6 and L9 contributed to DF2, L2, L10 and L11 contributed to DF3 and the other two landmarks contributed to DF4 (Table 6).

Table 6: Pooled within-groups correlations among the discriminating variables and standardized canonical discriminant functions based on conventional measurement (Variables ordered by absolute size of correlation within function).

Feature	DF1	DF2	DF3	DF4
HDF	-0.493*	0.262	0.204	0.003
PrOL	0.157*	0.077	-0.090	0.083
ED	0.174	0.354*	-0.136	-0.277
HAF	0.078	-0.339*	-0.167	-0.025
LJL	0.115	-0.280*	-0.019	-0.073
UJL	0.154	-0.270*	-0.214	-0.125
LBD	0.047	0.150*	0.075	0.024
BPcF	-0.038	0.092	-0.553*	0.170
TL	0.083	-0.048	0.419*	-0.001
BPvF	0.141	0.257	-0.320*	-0.207
HPcF	-0.023	0.010	-0.262*	-0.131
HL	0.071	0.387	-0.208	-0.0436*
SL	0.045	0.019	0.169	0.428*
HPvF	0.164	0.273	-0.247	0.396*
HD	0.000	0.137	-0.120	0.384*
PostOL	0.122	0.138	-0.040	-0.359*
BDF	0.019	0.023	0.070	0.322*
HBD	-0.119	0.089	-0.217	0.308*
BAF	0.051	0.114	0.142	0.165*

* Largest absolute correlation between each variable and any discriminant function.

Table 7: Pooled within-groups correlations among the discriminating variables and standardized canonical discriminant functions based on truss network measurement (Variables ordered by absolute size of correlation within function).

Feature	DF1	DF2	DF3	DF4
L3	0.507*	-0.040	0.427	0.171
L8	0.567*	0.055	0.510	-0.099
L1	0.210	0.628*	0.422	0.023
L5	0.086	0.591*	0.094	-0.222
L6	0.039	0.315*	-0.006	0.238
L9	-0.033	0.342*	0.018	0.324
L2	-0.367	-0.264	0.623*	0.226
L10	0.233	0.279	0.119*	0.585*
L11	0.042	0.032	0.475*	0.165
L4	-0.050	0.160	0.025	-0.239*
L7	0.013	0.017	0.068	-0.261*

* Largest absolute correlation between each variable and any discriminant function.

DISCUSSION

Fish are delicate to environmental changes and adjust promptly by shifting their morphometric to adapt the surrounding environment (Kipanyula and Maina, 2016, Wang and Guo, 2019). This study is preliminary research on the morphometric and meristic features of *A. testudineus* in Malaysia. Results indicated a distinct division of species in *A. testudineus* by both conventional and truss measurements. Among the 19 morphometric, proximately 14 of morphometric was significantly different ($p<0.05$) among the fish samples. The phenotypic differ of *A. testudineus* among the region is considered cause by the similar river resources but separate geographical site, environmental deviation such as from cultured pond or paddy field and genetic disparity (Ha et al. 2011, Abaad et al. 2016, Tams et al. 2018, Ha et al. 2017, Ahammad et al. 2018, Mahfuj et al. 2019). The morphometric variation in *A. testudineus* occurred might due to environmentally induced morphological differences. Morphometric and meristic results showed that head and anal part in fish were the major features that distinguished fish species with similar observation on *Pangasius* sp. and *Tor* sp. obtained from Pahang River, Malaysia (Muchlisin, 2013). The distinguished features could be related to differences in food availability and swimming behaviour (Muchlisin, 2013, Oufiero and Whitlow, 2016, Tams et al. 2018). Changes in ecosystem pursued the fish hunting adaptation in which changes their body morphology to increase forage ability and survival in the ecosystems (Abaad et al. 2016, Oufiero and Whitlow, 2016, Kelley et al. 2017). The rapid morphological evolution mostly concentrated on the head part to enable prey hunting ability of fish in a complex ecosystem with differ effects of temperature, salinity, dissolved oxygen and water flow (Brraich and Akhter, 2015, Kelley et al., 2017). Variation in water flow is assumed to affect the body shape and swimming behavior of the fishes (Langerhans 2008, Kelley et al. 2017).

Changes of body shape too are observed in differences of body depth among *A. testudineus* in this study. Changes in body depth could affect the overall fusiform shape of the fish and therefore, it may change the hydrodynamic power and swimming ability of the fishes (Liao, 2007, Kelley et al.2017). The changes in shape and size of body including scales are most probably linked to the ecological conditions of the studied habitats in Terengganu, Pahang, Sarawak, and Sabah that suggested eco-morphological variation (Sfakianakis et al. 2011, Brraich and Akhter, 2015). Wider and bigger anal and dorsal fins in the population probably caused by the high-water flow in the natural habitat (Langerhan and Reznick, 2010, Kelley et al. 2017)

For the meristic counting, anal fin is regarded as the only fin ray that has significant differences (p<0.05) among the region. In contrast, Nakamura (2002) found distinctive meristic count in Japanese charr (*Salvelinus leucomaenis*) among the river systems associated with Naka River (Ashinagasawa, Akasawa, Ushirosawa and Moot-akashiraswa streams). These phenotypic distinct suggest a direct relationship between the extent of phenotypic divergence and geographical separation is a limiting factor to migration among stock. The morphometric distinct that occurs among these species is due to geographical isolation and may come from the different ancestors (Abaad et al. 2016, Kelley et al. 2017, Tams et al. 2018).

In this study, there are no significant differences found in meristic character of dorsal fin, pectoral fin and pelvic fin. These suggest that the environmental variation have no influence on these meristic characters. Similar kind observation was made in meristic character of the population od *Clupea harengus* sampled from different environmental condition in Baltic Sea (Jorgensen et al. 2008) and population of *Arius jella* collected from different estuaries of Sri Lanka (Gunawickroma, 2007). These results suggest that variation arising in meristic character may take long time to evolve. It also may be the reason for morphometric handling error or abnormal growth of fishes.

Truss network or landmark measurement is a great device to identify fish stocks and detect minutest changes in morphology. It is cheaper cost compared to molecular techniques (Ethin et al. 2019). In this study, among 11 landmarks, six landmarks were significantly different among the fish samples and region (p<0.05). This indicated that the variable important in discrimination of morphological variation in *A. testudineus*. Comparable to Mahfuj et al. (2019), there were nine features (8 morphometric and 28 truss network measurements) significantly difference in the freshwater garfish in South-Estern Bangladesh. With the similar measurement methods, Ahammad et al. (2017) revealed all features (10 morphometric and 22 truss) were significantly different in the wild Bhagna (*Labeo ariza*) among the three river at Bangladesh. In this study, DFA showed there were differ in morphometric and separated from each other in the discriminant space but there were overlapping for region 1 and 2 (Terengganu and Pahang) under truss network measurement. This result proposed that there was merging among the populations. For further study, genetic analysis could be carried out for detail confirmation of the *A. testudineus* (Ha et al., 2011). In this study, DFA plotting showed clear isolation in conventional and truss network measurements. Similar clear DFA plotting also showed for the morphological in *Cyclocheilichthys apogon* from three rivers in North-Eastern Thailand (Kenthao and Jearranaiprepame, 2018) and cultured and wild African catfish (*Clarias garieinus*) (Solomon et al., 2015). More information could be obtained if cluster analysis is applied on the data set. A dendrogram is suggested to use for analyzing further clustering pattern of the fish populations.

CONCLUSION

Conventional and truss measurements and meristic feature resulted significant differences among all treatments. Related information and research of stock structure are important for environment re-establishment, stable exploitation, fisheries management and conservation. The baseline information from this study would be beneficial as guidelines among communities in fish conservation and fisheries management. More research for instance genetic studies are necessary to determine the environmental effects on the morphometric parameters in *A. testudineus*.

CONFLICT OF INTEREST

The authors declared that the present study has no conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

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