Pertanika J. Trop. Agric. Sc. 41 (3): 1059 - 1070 (2018)

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Intraspecific Morphological Variation of Crossbanded Barb, *Puntioplites Bulu* (Bleeker, 1851) From Selected River in Peninsular Malaysia Based On Truss Network Analysis

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ABSTRACT

The study on intraspecific variation of wild crossbanded barb, *Puntioplites bulu* stock in Peninsular Malaysia was investigated based on truss network analysis constructed from the fish body. A total of 90 samples were collected from three different populations, namely Kelantan River, Perak River and Pahang River (n= 30/population). The 22 truss characters were standardised by an allometric formula and analysed by multi and uni-variate analysis. The results showed significant differences ($p< 0.05$) between mean of the three populations. The loadings of the first and second discriminant function accounted for 81.3% and 18.7% respectively in terms of group variability, and they explained 100% of the total among group variability. The results showed significant variation of *P. bulu* in morphology based on truss network caught from three different populations. The morphological differences were located mainly on the head region, body depth and median region. These findings indicate the presence of morphometric variations between three populations of *P. bulu* in Peninsular Malaysia based on their locations.

Keywords: Morphology, *Puntioplites bulu,* stock identification, truss network, variation

INTRODUCTION

Stock identification is essential in fish stock assessment and sequentially for effective fisheries management (Turan, 2004). Accuracy of stock has remained the core challenge for fisheries scientists, as

ARTICLE INFO

Article history:
Received: 02 August 2017
Accepted: 27 April 2018
Published: 29 August 2018

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ISSN: 1511-3701
e-ISSN: 2231-8542

it is not possible to directly map how far the larvae scatter. Thus, stock structure has been studied using a variety of techniques, mostly on genetic and phenotypic variations. The study on phenotypic variation between stocks can deliver as an indirect basis or initial step for stock structure. Although it does not provide direct evidence based on genetic variation, this method may be applicable for short term environmentally induced variation study (Begg, Friedland, & Pearce, 1999).

Morphometric characteristics is a quantitative description that have been effectively used for taxonomic inferences, based on a set of traditional measurements (Hubbs & Lagler, 1947). It can be defined as a technique for describing size and shape variations and has been commonly used in fisheries biology for measuring discreteness and relationship among taxonomic categories (Strauss & Bookstein, 1982). Although the traditional measurement has been criticised, it is still considered a useful tool for fish identification. Regardless, a new approach in morphometric measurement called a truss network analysis is being used, especially for stock differentiation since this system covers the entire fish in a uniform network, and theoretically would increase the probability in extracting morphometric differences within and between species or populations (Abdurahman et al., 2016; Hossain, Nahiduzzaman, Debasish, Khanam, & Alam, 2010; Muchlisin, 2013; Turan, 2004; Turan, Erguden, Gurlek, Basusta, & Turan, 2004;). This method is considered revolutionary to overcome weakness of traditional morphometric measurements, which were limited to certain body structures, such as fin and not being able to enumerate body shape (Mojekwu & Anumudu, 2015).

Crossbanded barb, *Puntioplites bulu*, or locally known as “Tenggalan”, is a cyprinid fish of the genus *Puntioplites* which are naturally found throughout Southeast Asia including Indonesia (Kalimantan), Malaysia (Perak, Pahang, Kelantan, Johor, and Sarawak), Brunei, and peninsular Thailand (Ambak, Isa, Zakaria, & Ghaffar, 2010). This omnivorous species feeds mainly on submerged aquatic plants, algae, and benthic organisms often occurring in mid water benthic level that can normally be found in large lowland rivers and lakes including streams and coastal rivers (Allen, 2011). *Puntioplites bulu* is commercially important and sought after by anglers and fishermen (Ambak et al., 2010) for its high price of RM 30–50/kg live weight. It also has a good taste. Nevertheless, in recent years, this species is at risk from fishing pressure and habitat degradation, resulting from intensive development activities (Allen, 2011). In Malaysia, total landing production of *P. bulu* recorded a falling trend; shrinking from 93.68 tonnes in 2005 to only 43.44 tonnes in 2015 (Department of Fisheries Malaysia [DOF], 2005; 2015).

In order to prevent the decline in *P. bulu* landing in Malaysia, a study on the stock structure of this species is essential but it is yet to be done. Some studies examined the distribution status of *P. bulu* (Zarul et al., 2012) and phenotypic variation of two different *Puntioplites* species in Peninsular Malaysia (Zakaria-Ismail, 1988). Therefore, in this study, examination of *P. bulu* stock structure in Peninsular Malaysia was carried out from phenotypic aspects based on truss network analysis, in order to determine its relationship between morphological variations and geographical with origins of individuals from different populations.

MATERIALS AND METHODS

All samples (n = 90) with 30 samples for every population were collected from three different sites (Kelantan River, Perak River and Pahang River) (Figure 1) between January 2016 and January 2017. Total landing of *P. bulu* from these three states (Perak, Pahang, Kelantan) was the highest in Peninsular Malaysia with 14.35 tonnes, 5.35 tonnes, 4.71 tonnes respectively (DOF, 2015). In this context, the evaluation of stock structure between these three important rivers would be important for management purposes of this species.

The sampling locations and certain biological aspects of the samples are presented in Table 1. The collected samples were systematically identified based on literature findings (Ambak et al., 2010). The main morphological feature that distinguishes this species from the others in the genus of *Puntioplites* is the patches of darkened scale which form oblique cross bands (Ambak et al., 2010). All fish were preserved with ice before transported to the research laboratory for further analysis.

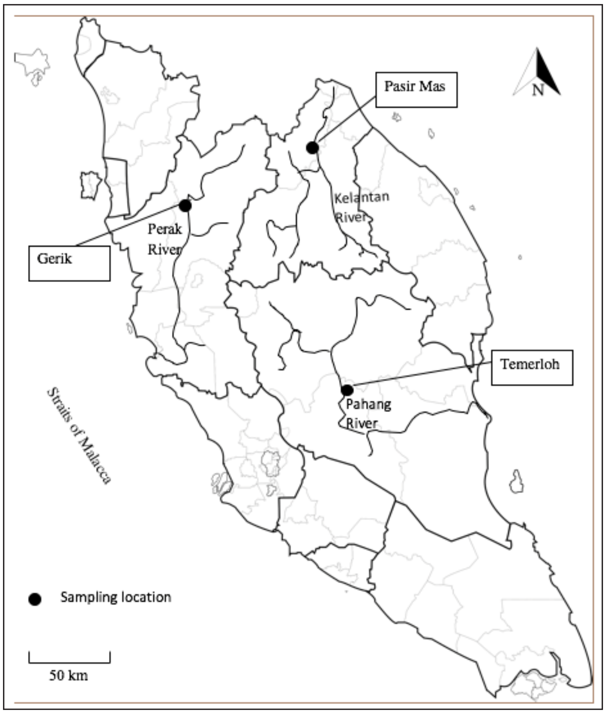


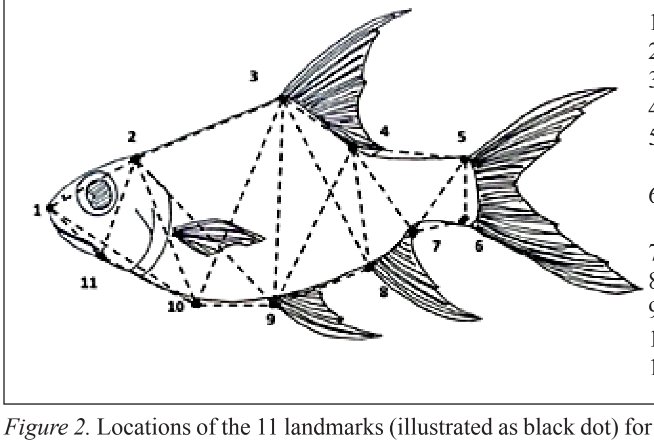
Figure 1. The map of the sampling sites of *P. bulu* in Kelantan River, Perak River and Pahang River

Table 1
Location, sampling sites and sample size of P. bulu *sample*

Sampling sites	Locations	Sample size	SL (cm) (min-max)	Mean SL (cm)	BW(g) (min-max)	Mean BW (g)
Kelantan River	6°02’N, 102°08’E (Pasir Mas Area)	30	12.3-24.5	16.17±3.1	59.9-172	144.7 ± 34.1
Perak River	5°24’N, 101°09’E (Gerik Area)	30	12.1-20.5	14.86±1.9	55-196.8	101.1 ± 36.1
Pahang River	3°31'N, 102°25'E (Temerloh Area)	30	9.7-14.5	11.53±3.1	30.4-104.9	46.9 ± 16.5

Note: SL: standard length (cm), BW: body weight (g) of samples

In the laboratory, body weight of the fish was recorded with a digital balance to the nearest 0.01g and internal gonad inspection for sex determination was done after obtaining the truss network analysis measurement. Sex of the samples were determined based on the gonad maturation stages as well as gonad external morphology appearance, such as colour, shape, size and degree of visualisation of the gonads (De Souza et al., 2011; Soetingya, Suryobroto, Kamal, & Boediono, 2017). Prior to analysis, the fish were defrosted and placed on a polystyrene board. The fish were then located on their left side on acetate sheets, with the body posture and fins placed into a natural position (Elliot, Haskard, & Koslow, 1995; Turan, 1999). Dissecting needles were used by piercing the acetate sheet in order to mark the 11 truss homologous landmark resulted in 22 liner measurements. The 11 landmarks produced for *P. bulu* are illustrated in Figure 2. All measurements were performed using a Mitutoyo digital calliper to the nearest 0.01 mm. All measurements were done four times in order to get the accuracy of the measurements.



1. anterior tip of snout at upper jaw
2. posterior end of neurocranium
3. origin of dorsal fin base
4. end of dorsal fin
5. anterior attachment of dorsal membrane from caudal fin
6. anterior attachment of ventral membrane from caudal fin
7. end of anal fin
8. origin of anal fin
9. origin of pelvic fin
10. insertion of pectoral fin and
11. posterior-most point of maxillary

Figure 2. Locations of the 11 landmarks (illustrated as black dot) for constructing the truss network on *P. bulu* and distance measured between the dots as line

Prior to analysis, each measurement value was standardised according to the following formula to reduce the allometric effects on these value (Komiya, Fujita & Watanabe, 2011).

> Yi = log (Mi) – b {log (SLi) – mean (log(SL)}
>
> Where Yi and Mi are the adjusted and original values for characters in individual i (i=1, …..N), SLi is the standard length and b is the slope regression coefficient of the logarithm M on the logarithm of SL using all fish in all groups.

The transformation will reduce the size effect on different specimen sizes as variation should be attributable to body shape differences and not associated to size of fish (Turan, 2004). Standard length (SL) was used as a common factor since it correlates strongly with other morphometric characters (Abdurahman et al., 2016). The size adjustment efficiency was assessed by testing the significance of the correlation and incomplete removal of size effects can be determined by a significant correlation between standard length and transformed variables (Turan, Oral, Ozturk, & Duzgunes, 2006).

All transformed data was evaluated using one-way ANOVA and multivariate analysis of discriminant function analysis (DFA) using Statistical Package for Social Science (SPSS) version 16.0 software for windows. One-way ANOVA was used to classify any significant variables among the truss measurement and a post-hoc test was carried out when significant results ($p < 0.05$) were obtained from the ANOVA result in order to investigate the groups that were significant to each other. The eigenvalues, cumulative percentage, percentage of total variances and canonical correlation were generated in this study. The DFA combines a selection of body shape measures into a linear mode to produce a mathematical function to be used to classify individuals into group. The group separation was is in a scatterplot of a function 1 versus function 2 (Figure 3).

RESULTS AND DISCUSSION

The descriptive data of SL and BW including mean values and standard deviation for each sample are presented in Table 1. None of the 22 transformed truss measurement showed a significant correlation with the standard of the fish indicating that the allometric formula has successfully removed the size effect from the data. Univariate statistics results in Table 2 showed that out of 22 truss measurements, 17 measurements were significantly different ($p < 0.05$) among three different populations except for measurements (1-2, 1-11, 9-10, 4-7, 6-5). The morphometric characters did not differ significantly ($p > 0.05$) between both sexes. Therefore, data for both sexes were pooled for subsequent analysis. According to Kocovsky, Adam & Bronte (2009), recommended ratio of the sample number relative to the landmark positions must be at least 3-3.5 times in order to avoid false conclusions on variations among groups. In this analysis, 22 characters were used and the number of fish examined (N) relative to the number of truss elements (P), N:P ratio was 4.09 for all 22 truss measurements. Summary of the relevant statistics of the DFA for *P. bulu* from three populations are shown in Table 3.

The eigenvalue designates the percentage of variance explained, with a large eigenvalue is related to a strong

Table 2
Univariate statistics testing differences between samples from all truss measurements

Variables	Characters	F	Significance
V1	1-2	2.985	0.056
V2	2-10	19.049	0.000*
V3	11-10	7.362	0.001*
V4	1-11	1.468	0.236
V5	2-11	10.823	0.000*
V6	1-10	7.373	0.001*
V7	2-3	3.776	0.027*
V8	3-9	9.258	0.000*
V9	9-10	0.492	0.613
V10	2-9	5.425	0.006*
V11	3-10	4.549	0.013*
V12	3-4	8.380	0.000*
V13	4-8	7.917	0.001*
V14	8-9	6.956	0.002*
V15	4-9	6.654	0.002*
V16	3-8	7.197	0.001*
V17	4-5	4.672	0.012*
V18	5-7	4.710	0.011*
V19	7-8	5.570	0.005*
V20	4-7	2.997	0.055
V21	6-7	9.130	0.000*
V22	6-5	0.038	0.963

*Indicate significance level at $p<0.05$. Characters are defined in Figure 2.

Table 3
Summary of canonical discriminant for P. bulu *from three populations*

Function	Eigen-value	Variance (%)	Cumulative (%)	Canonical Correlation
1	1.514	81.3	81.3	0.776
2	0.394	18.7	100.00	0.508

First two canonical discriminant functions were used in the analysis.

function. The canonical correlation summarises the degree of relatedness between the groups (populations), where larger value indicates greater degree of association connection and 1.0 considered as their utmost value. A total of 17 significant variables explain 100% of total variability with the first function described 81.3% of discriminating power, while second function with 18.7% respectively (Table 4).

Table 4
Standardised canonical discriminant function coefficients

Variables	Characters	Function 1	Function 2
V2	2-10	-0.509*	0.360
V21	6-7	0.367*	0.127
V13	4-8	-0.329*	0.227
V6	1-10	-0.323*	0.178
V14	8-9	0.320*	-0.117
V3	1-11	-0.318*	0.216
V16	3-8	-0.185	0.571*
V15	4-9	-0.161	0.571*
V8	3-9	-0.267	0.549*
V10	2-9	-0.149	0.511*
V11	3-10	-0.100	0.506*
V18	5-7	0.131	0.486*
V19	7-8	-0.193	0.452*
V7	2-3	0.104	0.449*
V5	2-11	-0.347	0.436*
V12	3-4	-0.295	0.417*
V17	4-5	0.227	0.291*

Pooled within-groups correlations between discriminating variables and standardised canonical discriminant functions. Characters are defined in Figure 2.
* denotes the largest correlation between each variable and any discriminant function.

The truss distances with loadings on first factor (DF1) were 1-2, 6-7, 4-8, 1-10, 8-9, 1-11 while second factor (DF2) were loaded by characters 3-8, 4-9, 3-9, 2-9, 3-10, 5-7, 7-8, 2-3, 2-11, 3-4, 4-5. The characters which contributed to Function 1, were strongly correlated to head region whilst characters which contributed to Function 2, were strongly correlated to median region of the body implying that these characters are the most important in the description of population characteristics. The discrimination of *P. bulu* from three different populations based on truss network measurements was clearly illustrated in scatter plot as shown in Figure 3. The results showed that Function 1 successfully discriminated the individuals into three independent groups.

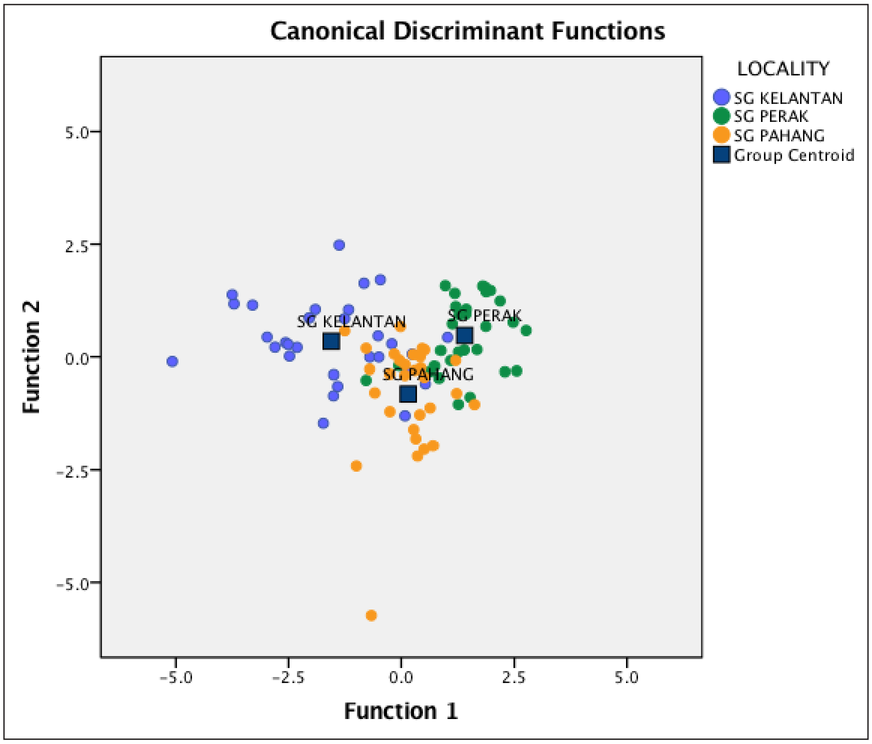


Figure 3. Discriminant scatter plot of *P. bulu* from Kelantan River, Perak River and Pahang River

The present study revealed evidence of significant morphometric heterogeneity among samples from three different rivers in Peninsular Malaysia. Findings generated from DFA showed that truss measurements that represent body depth and head region were the most discriminate characters among all the samples. A visual examination of the plots through F1 and F2 scores indicated that samples were grouped into three major areas with a certain overlap among them. Both higher mean values functions were recorded for *P. bulu* from the Perak River, in (V2-head region) and (V16-median region of the body). The main differences between these populations were observed in the head region area. The variation among the stocks could be due to environmental response, geographic position, ecological change and uncommon hydrological conditions (Khan, Miyan, & Khan, 2012; Mir, Sarkar, Dwiyedi, Gusain, & Jena, 2013; Siddik, Hanif, Chaklader, Nahar, & Fotedar, 2016). Decreasing food availability and heavy pollution load may lead to smaller body depth and head length (Khan et al., 2012). The Kelantan River population showed a comparatively smaller body depth which may be related to water quality and uncommon hydrological conditions in that area as argued by Radhi, Roshaliney and Zarul (2017). Mir et al. (2013) noted similar observation in Roho lobeo, *Labeo rohita* from Ganga basin India, where the uncommon hydrological conditions such as differences in alkalinity, temperatures, current pattern and turbidity contribute to phenotypic plasticity. Komiya et al. (2011) studied the morphological differences of Japanese gudgeon, *Sarcocheilihthys* between two different habitats in Lake Biwa Japan, and found significant morphometric divergence where fish inhabiting pebbly zones had e streamlined body with a short round head compared with fish inhabit rocky zone found to have deep compressed body with a long head. According to Muchlisin (2013), the morphometric system generally showed that the head and caudal region were major characters in distinguishing the group and same similar findings have been observed in Ariid catfishes, *Plicofollis* spp. (Abdurahman et al., 2016) in Peninsular Malaysia as well as Pangasiids catfishes, *Pangasius* spp and Mahseers*, Tor* spp. in the Pahang River (Akhbar, Jalal, Nasuha, Faizul, & Ambak, 2015; Akhbar et al., 2016).

The variation among *P. bulu* from three different populations based on the truss network system suggests a direct relationship between the level of morphometric divergence and geographic separation. The detected variation may signify reproductive isolation among local *P. bulu* populations. It is quite difficult to explain the reasons for morphological variations between populations but it is expected that these differences may be genetically associated or might be related by phenotypic plasticity in response to different environmental factors (Murta, Pinto, & Abaunza, 2008). Fish are very subtle to environmental changes and demonstrate greater variances in morphology both within and between populations compared with other vertebrates for their phenotypic plasticity. This allows them to adapt to environmental changes, which modifies their behaviour and physiology, leading to changes in their morphology, reproduction, and survival that reduce effects of environmental changes (Hossain et al., 2010; Wimberger, 1992). Consequently, effects of some environmental factors, such as temperature, food availability, and migration distance may affect and determine the phenotypical discreteness of *P. bulu*. Phenotypic variation within the same species in different populations may have advantages in stock structure analysis, particularly when time is limited for significant genetic differentiation to accumulate among populations. This method has also been successfully used in stock identification and to differentiate stock of horse mackerel, *Trachurus trachurus* (Murta et al., 2008), Abu mullet, *Liza abu* from three different populations (Turan & Erguden, 2004), and anchovy, *Engraulis encrasicolus* in the Mediterranean seas (Turan et al., 2004). Thus, a morphological analysis could be the initial step in exploring large population size of species. The morphometric variations between stocks are predictable as they are geographically separate and may originate from different ancestors. The truss network system can effectively be used to study separations of stock within a species as reported in other freshwater and marine environments (Mir et al., 2013).

CONCLUSION

These present findings reveal the potential power of the truss network method for identification of *P. bulu* stock in Peninsular Malaysia, suggesting a need for separate management approach to sustain the stock for future use. Environmental factors may be contribute to morphometric variations of *P. bulu* between the three populations. However, the results from this finding can be further confirmed based on biochemical and molecular procedures to support the morphometric data.

ACKNOWLEDGEMENT

The authors are thankful to Department of Agriculture, Universiti Putra Malaysia and Department of Science and Biotechnology, Universiti Selangor for providing necessary facilities to undertake this study.

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