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## RESEARCH ARTICLE

**MORPHOLOGICAL VARIATION BETWEEN HATCHERY AND WILD ANABAS TESTUDINEUS, BASED ON TRUSS NETWORK ANALYSIS**

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## ARTICLE DETAILS

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## ABSTRACT

Several body measurement methods used to identify stock have recently been criticized because of inherent biases and weaknesses. As an alternative, a new system of morphometric measurement called the truss network has been increasingly used for stock identification. Therefore, the morphometric differentiations between wild and captive populations of *Anabas testudineus* were carried out throughout this study using a truss network. Truss measurements based on anchored at ten homologous landmarks with 19 distances of 120 specimens were measured. Discriminant analysis was performed to investigate distinctions and patterns of morphological variations between wild and captive populations of *Anabas testudineus*. The findings support the use of the truss network to study the morphological variation among populations as it provides interesting perspectives for the study of biodiversity patterns.

## KEYWORDS

Morphology, *Anabas testudineus*, truss network, population.

## 1. INTRODUCTION

Recently *Anabas testudineus* has been widely cultured in Asia, especially India, Bangladesh, Thailand, Vietnam, and Malaysia. The demand for this species is now on the rise, and the numbers of farms culturing them are growing. This growth is due to the decrease in the number of this species being caught in the wild. It has been well documented that many wild fish stocks have reached their maximum sustainable yield due to overexploitation, habitat degradation, and pollution (Di Franco et al., 2013). Thus, there is an urgent demand to increase the productivity of this aquaculture species recently in order to meet its increasing consumer demand. However, the current productivity of *A. testudineus* is not sufficient to meet the high demand for this species. The potentials offered by this species are not fully explored.

Currently, the seeds that are produced and used in culturing this species are based on unimproved stock (Lowe-McConnell, 1995). The *A. testudineus* cultured stocks in current use originated from the wild stocks, so the performance is similar or inferior to the wild stocks (Fischer, 2012). To date, there is no documentation on the selective breeding program or improvement program of *A. testudineus* in order to improve the quality and performance of its cultured products (Wootton, 2012). Morphometric analysis is a useful tool to describe aquaculture stocks. Phenotypic plasticity of a population can be used to distinguish a distinct stock, where phenotypic plasticity of a fish population is not directly under genetic control but is also influenced by environmental changes (Keith and Hutchings, 2012; Perez-Rodriguez et al., 2011).

Thus, genetic information alone is not enough to help in choosing the base population for selective breeding. GIFT program has also adopted both genetic and morphometric information in their stock evaluation before starting the selection program (Layman and Silliman, 2002). To date, there is still no information on the genetic and morphological variations of *A. testudineus* that can be used in aiding the base population selection. Hence, it is essential to gather as much possible information about the varying status of this species before initiating selective breeding (Lopez-Peralta and Arcila, 2002). Therefore, in order to initiate a selective breeding program for *A. testudineus*, the objectives of this study are to assess the morphological variation of climbing perch, *A. testudineus* between hatchery and wild stocks in Malaysia and selected neighboring countries (Mablouke et al., 2013).

## 2. METHODOLOGY

## 2.1 Sample Collection

The samples were collected at different sites around Peninsular Malaysia from May 2011 to October 2019, and samples from Vietnam and Thailand were collected from December 2012 to June 2019, respectively, as shown in Table 1. The wild samples were bought from fishermen and markets, but most of the samples were caught using gill nets and angling (Cordova-Tapia et al., 2015; Winemiller, 1989). For the hatchery samples, samples were bought from hatcheries listed by DOF (Department of Fisheries) directory as the main *A. testudineus* captive producers. All the collected samples were brought to the laboratory for further morphological and molecular analyses.

## Quick Response Code



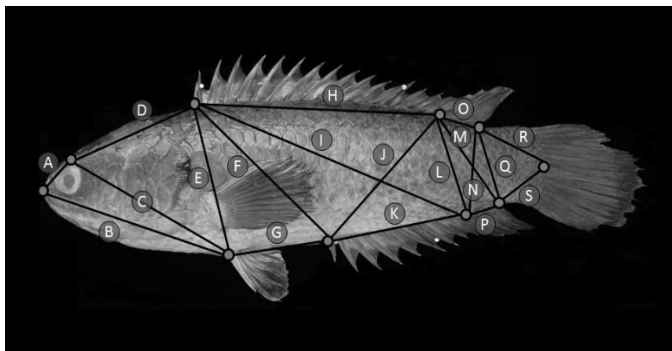
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Table 1: Sampling sites, coordinate and sample size of <i>A. testudineus</i> represent different hatcheries and wild populations			
Region	Sampling Site	Sample size (N)	Remark
Kedah	Alor Setar	9	Wild
	Kuala Muda	11	Wild
	Sungai Petani	12	Wild
	Serdang	35	Hatchery
Kelantan	Tumpat	10	Wild
	Kota Bharu	12	Wild
	Tok Bali	8	Wild
	Pasir Puteh	35	Hatchery
Johor	Segamat	9	Wild
	Tangkak	7	Wild
	ParitJawa	15	Wild
	Batu Pahat	30	Hatchery
Selangor	Tg. Karang	12	Wild
	Sungai Buloh	7	Wild
	Kuala Selangor	14	Wild
	Rawang	40	Hatchery
Thailand	Pattani	30	Hatchery
Vietnam	Can Tho	22	Hatchery

## 2.2 Sample Analyses

The morphological measurements were conducted based on the truss network anchored at ten homologous landmarks (Figure 1), resulting in 19 linear measurements (Nelson, 1979). These measurements were performed to the nearest 0.01 mm using a digital caliper.



**Figure 1:** Illustration of *A. testudineus*, showing 10 location of homologous landmark for constructing the truss network measurement based on morphological features (A; Snout to above eye, B; Snout to insertion of pelvic fin, C; Above eye to insertion of pelvic fin, D; Above eye to origin of dorsal fin, E; Origin of dorsal fin to insertion of pelvic fin, F; Origin of dorsal fin to origin of anal fin, G; Insertion of pelvic fin to origin of anal fin, H; Origin of dorsal fin to origin of dorsal soft rays, I; Origin of dorsal fin to origin of anal soft rays, J; Origin of anal fin to origin of dorsal soft rays, K; Origin of anal fin to origin of anal soft rays, L; Origin of dorsal soft rays to origin of anal soft rays, M; Origin of dorsal soft rays to end of anal soft rays, N; Origin of anal soft rays to end of dorsal soft rays, O; Origin of dorsal soft rays to end of dorsal soft rays, P; Origin of anal soft rays to end of anal soft rays, Q; End of dorsal soft rays to end of anal soft rays, R; End of dorsal soft rays to caudal fin, S; End of anal soft rays to caudal fin).

## 2.3 Data Analyses

The original measurements of the truss network were firstly standardized for size (Gerking, 2014). This transformation reduces the effect of different specimen sizes (Baker et al., 2014; Hayes, 1990). It normalizes the individuals in a sample to a single arbitrary size, common to all samples, but maintains the individual variation (Jalal, 1996). In this study, standard length (SL) was used as a common factor since it strongly correlates with other morphological characters (Baker et al., 2014; Hayes, 1990). An allometric formula used for the transformation was as follows (Dantas et al., 2015):

$$M_{adj} = M (Ls/Lo) b$$

Where,  $M_{adj}$ : Standardized character measurement;  $M$ : Observed character measurement;  $Ls$ : Overall mean of standard length of fish;  $Lo$ : Standard

length of fish;  $b$ : Slope of the regression of  $\log M$  on  $\log Lo$  for all fish. The data obtained from the truss network technique were analyzed by one-way ANOVA and multivariate analysis of DFA using Statistical Package for Social Science (SPSS) version 16.0 software for windows. The statistical methods applied in morphological analyses are univariate analysis of variance (ANOVA), and multivariate analysis of discriminant function analysis (DFA) performed using the SPSS version 16.0 program. The relative importance of discriminant variables (functions) is determined based on three measures: the relative percentage of the eigenvalue and the percent of variance existing in the discriminating values, the associated canonical correlation, and Wilk's Lambda and its corresponding chi-square.

The canonical correlation is a measure of association that summarizes the degree of relatedness between the groups (population) and the discriminant function. The larger the value, the higher the degree of association and 1.0 as being the maximum value. While Wilk's Lambda is an inverse measure of the discriminating power in the original variables, hence, the larger the value, the less discriminating the function. The standardized discriminant function coefficient describes the relative contribution of its associated variables to the functions; the sign indicates a positive or negative contribution. The larger the magnitude of the coefficient (disregarding sign), the greater the variables' contribution to the function, while the opposite is true for the lowest coefficients.

## 3. RESULTS AND DISCUSSION

One way ANOVA suggested that all 19 truss measurements in this study were significantly correlated, with  $p < 0.05$ . Therefore, all 19 truss network measurements derived from 10 homologous landmarks were used in the DFA multivariate analysis. A total of seven functions was extracted, where 100% of variance was used to discriminate between the populations. The degrees of variation calculated for all seven functions extracted were supported by a high value of canonical correlations (McLusky, 2013). The canonical correlations for all the seven functions are 0.996, 0.929, 0.889, 0.815, 0.307, 0.273 and 0.224, respectively (Table 2). The value of canonical correlations suggested that all functions described in this analysis are highly discriminating. Thus, these functions have a high possibility to discriminate the wild and hatchery populations of *A. testudineus* in this study.

The Wilk's lambda values calculated for all the seven functions were 0.000, 0.008, 0.056, 0.267, 0.796, 0.879 and 0.950, respectively (Table 2). The values calculated were closed to zero, which indicates the significance of these discriminant functions. Wilk's lambda analysis suggested that the variables described by the functions were different. This suggestion was supported by the observed high significant difference, with  $p < 0.05$ . Therefore, wild and hatchery populations of *A. testudineus* could be discriminated based on all the variables described in the functions.

The functions at group centroids (Table 3) and scatter plot of discriminant function (Figure 2) were produced in order to discriminate the wild and hatchery populations of *A. testudineus*. The hatchery populations showed higher group centroid values for function 1 until function 4, compared to the wild population. The wild populations showed higher group centroid values for function 5 until function 7, compared to hatchery populations. These values showed that these populations were highly separated from each other, which means this function could be used to discriminate wild and hatchery populations of *A. testudineus*.

Scatter plots produced showed high levels of differentiation between wild and hatchery populations. This is shown in the scatter plot by the relative distance of each point on the plot. In the discriminant function scatter plot, hatchery and wild populations were plotted far from each other. This indicates that the functions described were able to discriminate between wild and hatchery populations of *A. testudineus* in this study.

Important variables were selected based on the standardized discriminant function coefficient values (Table 4). The higher the coefficient value, the greater the discriminating ability of the variables in describing the

functions. From all 19 variables used in this study, the highest coefficient value was observed for variables B, C, A, K, L, S, and D, with the values of 0.356, -0.336, 0.531, -0.407, 0.658, 0.571 and 0.718, respectively. The variable B represents the measurement from snout to insertion of the pelvic fin, variable C represents above the eye to insertion of the pelvic fin, variable A represents snout to above the eye, variable K represents the origin of the anal fin to the origin of anal soft rays, variable L represents origin of dorsal soft rays to origin of anal soft rays, variable S represents the end of anal soft rays to the caudal fin, and variable D represents above the eye to the origin of the dorsal fin.

Stock identification is fundamental to fisheries and hatchery management. Fish stock is referring to a group of fish large enough to be self-reproduce and the members of the group having similar life history (Deka and Gupta, 2013). Fish stocks are identified based on differences between characteristics belonging to different groups (Dantas et al., 2013).

Morphological characteristic is among other methods used in stock identification. Body shape and measurement of particular morphological features of various dimensions in the body part is included as morphological characteristics.

From 19 truss measurement used in this study, it shows evidence of a highly significant morphological difference in *A. testudineus* populations. The DFA analysis (Figure 2) shows the significance level of differentiation between wild and hatchery populations. Based on the DFA analysis of wild and hatchery *A. testudineus* population in this study, these populations showed different morphological characteristics that enable them to be discriminated from each other. This evidence suggested that wild and hatchery *A. testudineus* belonged to a different stock. Even though the hatchery population may be originated from wild populations, but they have morphologically changed due to several factors affecting their changes (Pimentel, 1967).

**Table 2:** The summary of eigenvalue, canonical correlation, and Wilk's lambda attained from morphological differences between hatchery and wild populations of *A. testudineus*

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation	Wilks' Lambda	Chi-square	df	Sig.
1	134.137 <sup>a</sup>	91.6	91.6	0.996	0.000	1090.358	133	0.000
2	6.299 <sup>a</sup>	4.3	95.9	0.929	0.008	543.307	108	0.000
3	3.781 <sup>a</sup>	2.6	98.5	0.889	0.056	321.673	85	0.000
4	1.982 <sup>a</sup>	1.4	99.8	0.815	0.267	147.204	64	0.000
5	0.104 <sup>a</sup>	0.1	99.9	0.307	0.796	25.390	45	0.992
6	0.081 <sup>a</sup>	0.1	100.0	0.273	0.879	14.379	28	0.984
7	0.053 <sup>a</sup>	0.0	100.0	0.224	0.950	5.725	13	0.956

a. First seven canonical discriminant functions were used in the analysis

**Table 3:** Functions at group Centroids/ Means of canonical variances scores from morphological differences between hatchery and wild populations of *A. testudineus*

Region	F1	F2	F3	F4	F5	F6	F7
Johor	1.986	-1.147	0.110	<b>3.635</b>	0.046	-0.012	0.000
Kedah	11.792	1.973	<b>2.897</b>	-0.453	-0.011	0.004	-0.008
Kelantan	-0.799	<b>5.123</b>	-3.210	0.052	-0.002	-0.047	-0.017
Selangor	<b>13.432</b>	-2.920	-1.830	-0.877	-0.006	0.012	0.016
Wild Johor	-13.213	-0.816	0.532	-0.397	-0.528	-0.386	<b>0.384</b>
Wild Kedah	-12.775	-0.766	0.293	-0.332	-0.490	0.452	-0.375
Wild Kelantan	-13.043	-0.369	0.371	-0.538	<b>0.530</b>	0.456	0.336
Wild Selangor	-12.901	-1.125	0.544	-0.681	0.466	<b>0.479</b>	-0.341

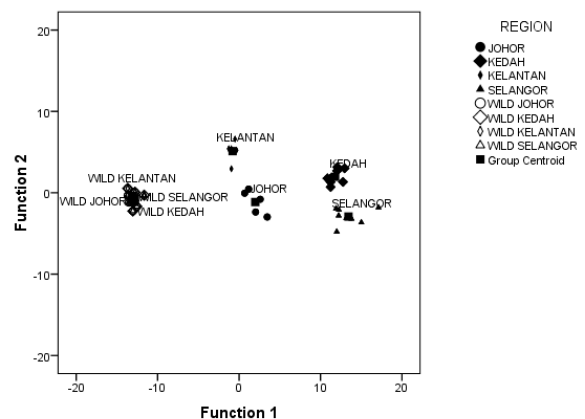
\* Unstandardised canonical discriminant functions evaluated at group means

**Table 4:** Standardised Canonical Discriminant Function Coefficients scored based on morphometric characters of hatchery and wild *A. testudineus* populations

Variables	F1	F2	F3	F4	F5	F6	F7
B	<b>0.356</b>	-0.102	-0.074	-0.162	0.081	0.080	-0.117
C	0.351	<b>-0.336</b>	-0.094	-0.031	-0.154	0.331	-0.212
A	0.069	0.168	<b>0.531</b>	-0.304	0.495	0.016	-0.075
K	0.102	0.063	-0.039	<b>-0.407</b>	0.157	0.065	0.257
L	0.043	-0.004	0.104	-0.291	<b>0.658</b>	0.215	-0.138
H	0.274	-0.066	-0.083	-0.184	0.650	-0.019	0.034
E	0.093	-0.162	0.180	-0.175	0.530	0.112	0.096
I	0.216	-0.062	0.029	-0.297	0.509	0.116	0.079
R	0.100	0.048	-0.213	0.024	0.463	0.118	-0.222
J	0.109	-0.009	0.176	-0.307	0.437	0.194	0.163
G	0.166	-0.022	0.028	0.116	0.396	-0.50	0.293
P	0.039	0.034	0.105	0.104	-0.280	-0.175	-0.057
F	0.165	-0.198	-0.010	-0.221	0.225	0.055	-0.076
M	0.095	0.014	0.153	-0.048	0.202	0.017	-0.090
S	0.103	0.081	-0.238	0.017	0.221	<b>0.571</b>	-0.041
O	0.051	0.020	-0.070	-0.085	-0.114	-0.133	-0.014
D	0.206	0.012	0.059	0.031	-0.353	-0.053	<b>0.718</b>
N	0.098	0.010	0.121	-0.213	0.198	0.188	-0.441
Q	0.069	-0.160	-0.070	-0.009	0.251	-0.100	0.393

Wild and hatchery condition is far apart from each other. The hatchery provides almost everything to ensure the survival of rear fish, there was enough food, water quality was monitored and shelter for the fish to stay alive. In a natural environment, the conditions are uncertain and providing natural selection for the fittest (Yang et al., 2014). Fish are known to be very sensitive to environmental changes, and they will quickly adapt to the new environment. Changes in water condition, source of food, and stress will demonstrate variation between different environments. They are more susceptible to environmental induce morphological variation. Coupled with every difference between wild and hatchery conditions, the morphology between these two populations was discrete from each other, as displayed in this study.

To make the deviation clearer, in a natural environment, each population experience natural selection and evolutionary process. Natural environments influence changes in the wild population by a random process of mutation, genetic drift, and gene flow. Each of these forces contributes to the variation among the wild population (Yang et al., 2014). Furthermore, in the wild population, only the fittest will survive natural selection such as disease, environmental challenge, and predatory. However, selection in the hatchery population was done by artificial selection based on criteria decided by a human. Fishes with criteria favorable by commercial need were selected. These selections have deviated from natural selection and, in the end, exhibited in morphological differences between wild and hatchery populations.



**Figure 2:** Scatter plot of discriminant functions analysis (DFA) scored based on morphometric characters of hatchery and wild *A. testudineus* populations

Therefore, this study suggested that wild populations can be used to supplement different variations in selective breeding programs to increase variations within the hatchery population. Wild populations have been used as reservoirs in species variation, and this practice has been carried out extensively in aquaculture (Kim et al., 2011). It is also well known that there are higher variations within the wild population due to natural selection, and on this basis, the wild population contained a fitter individual compared to the hatchery population. However, hatchery populations that have gone through artificial selection have an adaptive value of commercial need. Conversely, artificial selection may also reduce the fitness of the hatchery population (Nikolsky, 1963). The selection of wild populations as a supplement to the hatchery population has been used in some selective breeding programs to improve fitness in hatchery populations, especially to increase resistance to disease (Davies et al., 2012; Pitcher, 2012).

#### 4. CONCLUSION

Morphological analysis between hatchery and wild populations showed that *A. testudineus* populations were from a different stock of populations, where, each population was not closely related to each other. Thus, the mating of individuals from different populations suggested in this study will not lead to inbreeding as they are not closely related to each other.

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