Distinguishing Hybrids of Golden Thailand and Malaysian Strains of Climbing Perch, *Anabas testudineus* (Bloch, 1792), Using Multivariate Analyses of Morpho-Meristic Traits

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E-ISSN: 2073-3720
https://doi.org/10.33997/j.afs.2023.36.3.006

Abstract

The climbing perch, *Anabas testudineus* (Bloch, 1792), is a commercially important freshwater fish in Southeast Asia. To meet demand, establishing stock development breeding programmes is essential. However, there is a lack of scientific literature on parent-hybrids differences. This study aims to use morphometric characteristics to identify important predictors and determine their heritability to address the knowledge gap. Two strains of *A. testudineus* and their corresponding hybrid groups, represented by T1–T4 (T1: golden Thai (♀) × golden Thai (♂), T2: golden Thai (♀) × Malaysian (♂), T3: Malaysian (♀) × Malaysian (♂) and T4: Malaysian (♀) × golden Thai (♂)) were used for the experiment. Thirty individuals of each strain were randomly selected from their respective tanks for morphometric characteristics assessment. Twenty-five morphometric measurements were taken, and adjustments were made for body size effects. These measurements were then subjected to multivariate analysis with a 5 % selection intensity for genetic advancement, focusing on body depth and base of anal fin length. The morphometric characters, including postorbital length, lowest body depth, the base of anal fin length, total length, standard length, pre-pectoral fin length, the base of pectoral fin length, and base of pelvic fin length based on the canonical coefficient, were identified as distinguishing factors between hybrids and the parental population. The study showed that body length heritability was greater than 60 %, indicating a significant additive genetic effect that surpasses the impact of the environmental effect and thus could be used as a potential characteristic for selective breeding to improve the desired trait.

Keywords: heritability, multivariate analysis, phenotyping, progeny, characteristics

Introduction

The climbing perch, *Anabas testudineus* (Bloch, 1792), is a native freshwater fish species that is commercially traded in several Southeast Asian countries, including Vietnam, Thailand, Cambodia, Indonesia, the Philippines, India and Malaysia (Slamat et al., 2019). Because of its hardness, it has been successfully domesticated and cultivated in various culture facilities (Sarma et al., 2010). However, there is a need to increase its production due to challenges such as unabated overfishing, pollution, and the risk of extinction of indigenous species caused by wetland conversion (Paliwal and Bhandarkar, 2014). Therefore, there is a need to increase production through stock development via breeding programmes with enhanced variants of similar species. Furthermore, since a fast growth rate is acknowledged as one of the most desirable qualities in aquaculture, stock improvement through breeding programmes is crucial (Gjedrem and Baranski, 2010; Gjedrem and Robinson, 2014). Among the strains is the golden Thai anabas, a native to Thailand, is an exotic variety primarily traded as an ornamental fish and has been identified as a potential aquaculture candidate with merits that include superior meat quality and fast growth rates (Kohinoor and Zaher, 2006). While the native Malaysian anabas strain, though small, is valued for its flavour and robustness (Paul et al., 2017). Meanwhile, hybrids crossing between these strains, which combine desirable genotypes and phenotypes from both parents, are often indistinguishable from the parental strains (Park et al., 2003; Nwachi et al., 2020). Therefore, it is necessary to differentiate hybrids from parental strains and assess the inheritance of traits in subsequent generations.

Morphometric measurements play an important role in providing a quantitative description of organisms. Morphometrics is a statistical methodology used to differentiate between changes in phenotypic features and genetic differences in fish. It is a simple and straightforward approach to fish identification (Turan, 1999; 2006; Nwachi and Ebguchunam, 2021). Traits generated from external phenotypes of organisms can provide valuable information regarding the similarity or dissimilarity among taxa. Morphological features provide better indicators for assessing heritability compared to individual parameters, allowing the selection of optimal breeding pairs (parents) through simple phenotypic testing (Akdemir and Sánchez, 2016). Quantitatively analysing organisms, including comparing their shape, colour, scales, and morphology, enables the distinction between fish populations (Muchlisin, 2013). However, distinguishing between parents, offspring, and hybrids can be challenging since they may appear similar in shape, colour and scales to the untrained eye.

This study aimed to develop a reliable method for distinguishing between hybrid and pure strains of *A. testudineus* for ease of identification and domestication-conservation strategies. However, there is little or no information regarding the differentiating traits inherited by the hybrids from their parental species. Discriminant functional analysis (DFA) was utilised to identify and analyse crucial predictors in the differentiation process to address this gap.

Materials and Methods

Ethical approval

All fish used were handled in line with the Institutional Animal Care and Use Committee (IACUC) UPM ethical approval for animal experimental protocol with approval number R034/2019.

Brood fish management and selection

A total of 100 brood stocks were procured, comprising 50 pieces from each of two distinct strains of climbing perch *A. testudineus* (Malaysian strain and the Golden Thai strain). The Golden Thai variant was obtained from a private hatchery, Three Ocean Fish Pond & Trading Sdn. Bhd., Rawang located in Selangor (3° 19' 16.68" N, 101° 34' 36.12" E), while the Malaysian strain was obtained from a government Aquaculture Pausat Development Centre in Bukit Tinggi Pahang, Bentong (3° 0' 33.462" N, 101° 26' 12.3144" E) Malaysia. Only 24 gravid fishes (16 females and 8 males) were selected as parental stocks for the breeding experiment for the two distinct strains. The size of parent stock varies from 20 g to 50 g for males and 40 g to 100 g for females. The breeding initiation was conducted at the Wet Laboratory, Department of Aquaculture, Universiti Putra Malaysia, using 100 L glass aquaria for each mating pair. The parent stocks were randomly selected and divided into four experimental groups: T1–T4. Golden Thai strain female (GT ♀) × Golden Thai strain male (GT ♂) is T1; Golden Thai strain female (GT ♀) × Malaysia strain male (M ♂) is T2; Malaysian strain female (M ♀) × Malaysian strain male (M ♂) is T3 and Malaysian strain female (M ♀) × Golden Thai strain male (GT ♂) is T4. Each experimental group (T1–T4) were designed in duplicates and nested at a ratio of 2 female:1 male. The T2 and T4 were hybrid and reciprocal crosses between strains, while pure strains (T1 and T3) were produced by mating among the same strain (Zworykin, 2012; Piwpong et al., 2016). The fish were injected with Ovatide hormone (Hemmopharma Ltd., India) intramuscularly at 0.6 mL kg^{-1} for females, while males received only half the dose (Chaturvedi et al., 2015). Brooders were removed 10 h later after successful spawning. The progeny (200) of each cross (T1–T4) were reared to a pre-grow-out weight of 3 g–5 g for 30 days, and randomly selected samples (180 fries) were then subjected to a 56-days growth performance study. On the termination of the study, 30 fish were randomly selected from each group for the morpho-meristic study.

Morphometric measurements

A total of 120 fish (30 from each group) were used for the morphological analysis. Fish were sedated with 100 mg L^{-1} clove oil using methods described by Kroon (2015) and placed onto a pile of wipes on a flat surface. A total of 15 morphometric measurements and 10 meristic measurements were made using a vernier calliper for each group by following Hossen et al. (2017) (Fig. 1).

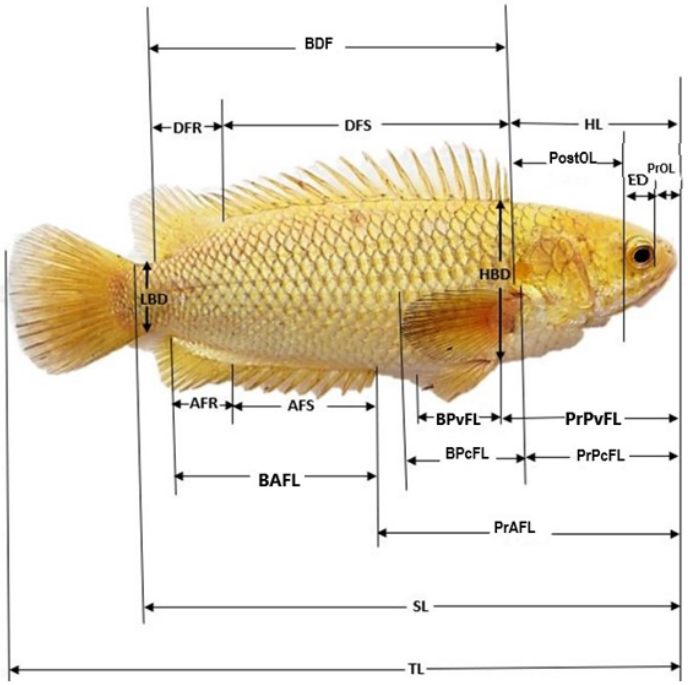
A maximum of 50 % of the water was exchanged every 10 days, and fish were fed at 3 % biomass twice a day (8 am and 5 pm). Prior to morphometric measurements, the fish were sexed by visually observing the secondary sexual characteristics. The females were identified by their slightly distended abdomen, while the males had a reddish hue and production of whitish-milky milt upon applying pressure to the abdominal cavity, as described by Akter et al. (2014). The allometric method proposed by Elliot et al. (1995) was used to eliminate size-dependent differences.

$$M_{adj} = M\,(L_s / L_0)^{b} \quad (1)$$

where M is the original measurement, M_{adj} is the adjusted size measurement, L_0 is the standard fish length, L_s is the overall standard length mean for all fish from all samples in each analysis, and b was estimated from the observed data for each character as the regression slope of log M on log L_0 using all fish from any group.

Discriminant analysis

Prior to statistical analysis, the normality of the data distribution was evaluated using the Shapiro-Wilks test, and the homogeneity of variances were



Key Symbol	Description
Morphometric	
TL	Total length
SL	Standard length
HL	Head length
PrOL	Pre-orbital distance
PostOL	Post orbital length
ED	Eye diameter
HBD	Highest body depth
LBD	Lowest body depth
BDF	Base of dorsal fin length
BPcFL	Base of pectoral fin length
BPvFL	Base of pelvic fin length
BAFL	Base of anal fin length
PrAFL	Pre anal fin length
PrPcFL	Pre pectoral fin length
PrPvFL	Pre pelvic fin length
Meristic	
DFR	Dorsal fin rays
DFS	Dorsal fin spines
PcFR	Pectoral fin rays
PvFR	Pelvic fin rays
AFR	Anal fin rays
AFS	Anal fin spines
CFR	Caudal fin ray
SoLL	Scales on lateral line
SBLL	Scales before lateral line
SALL	Scales after lateral line

Fig. 1. Morphological measurements used to compare hybrids with their parents (Malaysian and Golden Thai strains of *Anabas testudineus*).

examined using Levene's test. Since the results showed that the size of the fish did not significantly affect any of the morphometric characters, the female and male data were pooled. A multivariate analysis of variance was conducted to identify variations between the fish groups. For each trait, a separate univariate ANOVA was conducted to ascertain the impact of the genetic group. However, the Bonferroni correction of significance was applied to reduce false positive results (type 1 error) that may arise from running multiple tests on a single data set in ANOVA.

Tolerance statistics were used to measure the degree of multicollinearity among the variables in the discriminant models. These statistics helped to identify the variables that were most effective in distinguishing the four groups. Alternative methods (Discriminate analysis and cross-validation) were used to identify genetic groups and develop predictive functions. By examining the accuracy of these methods in correctly distinguishing fish between the different genetic groups, it was determined that they effectively differentiated the populations. Cross-validation using split sample validation was conducted to assess the reliability of the predictive functions. This involved removing each individual from the initial data set and then categorising the remaining data using discriminant analysis with the remaining data to assign them to their respective groups. The reliability of the functions was measured by tallying the number of correctly classified fish. The number of fish that were classified incorrectly indicated insight into the level of population mixing. All analyses were conducted using SPSS 20.0 statistical software.

Standardisation of parameters

All morphometric characters were standardised using the formula by Claytor and MacCrimmon (1987) to prevent potential biases brought about by size effects on morphometric variables.

$$ACi = \log OCi - [\beta \times (\log TLi - \log MTL) \quad (2)$$

where ACi is the adjusted logarithmic character measurements of the i^{th} specimen (i = 1, 2, 3..); OCi is the unadjusted character measurement of the i^{th} specimen (i =1, 2, 3..); β is the common within-group regression coefficient of that character against total length after the logarithmic transformation of both variables; TLi is the total length of the i^{th} specimen (i =1, 2, 3..); and MTL is the overall mean total length. NTSYS-pc 2.1 software was used to facilitate the calculations.

Computation of variance components, heritability and genetics advance

The variance components analysis was performed using SAS (Software version 9.4), employing Becker's (1992) method to effectively identify genetic variations across different groups and evaluate the influence of genetic and environmental factors on various characters. Required variance components were estimated by equating the calculated mean squares with the theoretical expectations and solutions.

Variance components

Genotypic and phenotypic variance was calculated according to Oladosu et al. (2014):

$$\sigma_g^2 = \frac{(MSG - MSE)}{r} \quad (3)$$

where MSG = the mean square of genotypes; MSE = the mean square of error and r = the number of replications.

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2 \quad (4)$$

where σ_g^2 = genotypic variance; σ_e^2 = MSE = the mean square of error.

Coefficients of variation

The phenotypic and genotypic coefficients of variation (PCV, GCV and RD), as described by Singh and Chaudhary (1985) were calculated as follows:

$$GCV = \frac{\sqrt{\sigma_g^2}}{\bar{X}} \times 100 \quad (5)$$

$$PCV = \frac{\sqrt{\sigma_p^2}}{\bar{X}} \times 100 \quad (6)$$

$$RD = \frac{PCV - GCV}{PCV} \times 100 \quad (7)$$

where σ_p^2 = phenotypic variance; σ_g^2 = genotypic variance; $\bar{X}$ = mean of the trait and RD = relative difference. The coefficients of variation were classified as low (0-10 %), moderate (10-20 %) and high (20 % and above) as inferred by Sivasubramanian and Madhavamenon (1973).

Heritability

Heritability was calculated according to Falconer (1981):

$$h_B^2(\%) = \frac{\sigma_g^2}{\sigma_p^2} \times 100 \quad (8)$$

where σ_g^2 = genotypic variance; σ_p^2 = phenotypic variance; h_B^2 = broad-sense heritability, where (0-30 %) is classified as low, (30-60 %) moderate and (≥60 %) high as given by Johnson et al. (1955).

Genetic advance

The method described by Assefa et al. (1999) was used to analyse genetic advance (GA), which was presented as a percentage of the mean with a 5% selection intensity (K). The genetic advance was categorised as low (0–10 %), moderate (10–20 %), and high (>20 %) as given by Johnson et al. (1955):

$$GA\ (\%) = K \times \frac{\sqrt{\sigma_P^2}}{\bar{X}} \times h_B^2 \times 100 \quad (9)$$

where K = selection intensity (constant 5 %, the value is 2.06); $\sqrt{\sigma_P^2}$ = phenotypic standard deviation; h_B^2 = the heritability; $\bar{X}$ = the mean of traits.

Results

Analysis

There was a significant difference among the four genetic groups on the combined morphometric characters, $F(75,275) = 4.825$, $P < 0.0001$; Wilks' Lambda = 0.082.

Further examination through separate univariate ANOVAs of each morphometric character, using the Bonferroni adjusted alpha level of 0.0125, revealed a significant genetic group effect on specific traits. These traits included the anal fin spine (AFS), pectoral fin ray (PcFR), dorsal fin ray (DFR), dorsal fin spine (DFS), the base of anal fin length (BAFL), lowest body depth (LBD), pre anal fin length (PrAFL) and eye diameter (ED) as shown in Table 1. The results showed that the hybrids exhibited morphological traits that were similar to the Malaysian anabas in terms of DRF and PcFR. The Golden Thai strain anabas shared similar PrAFL, ED and BAFL values with its hybrids. A lower value of LBD was seen for the T2 hybrid compared to other genetic groups.

Table 2 provides an overview of the data for the four sub-groups, presenting various descriptive measures to aid in understanding their characteristics. The table includes eigenvalues, cumulative variance, canonical correlation, Wilks' Lambda, and Chi-square values.

Wilks' Lambda (λ) values for the groups were 0.082, 0.286, and 0.563, respectively, and the Chi-square test ($\chi 2$) statistics showed values of 261.763, 130.737, and 59.984 ($P \leq 0.001$). These results confirmed the significance of the three discriminant functions obtained, thereby validating the discriminant analysis. Functions 1, 2, and 3 account for 59.00 %, 22.80 %, and 18.20 % of the total variation, respectively, as explained. Table 3 displays the total variation explained by the canonical variables and standardised canonical coefficients. Each variable's corresponding value represents its contribution to the overall variation within the genetic group.

Therefore, among the three genetic group variables, function 1 showed the most easily distinguished capability, with TL, SL, and PrPcFL, playing prominent roles in the linear contribution of characteristics. Additionally, in function 2, the four genetic group variables that were most easily distinguished were

Table 1. Effect of genetic group on morpho-meristic characters of *Anabas testudineus*.

Morpho-meristic characters	T1	T2	T3	T4
Total length (TL)	131.85 ± 4.44	132.06 ± 1.72	131.13 ± 1.76	130.73 ± 1.80
Standard length (SL)	103.96 ± 8.81	105.80 ± 7.67	104.30 ± 11.20	110.62 ± 9.69
Head length (HL)	33.89 ± 1.87	33.06 ± 1.59	34.45 ± 1.33	33.01 ± 1.31
Pre orbital length (PrOL)	7.42 ± 0.75	6.78 ± 0.95	6.60 ± 0.920	6.80 ± 1.02
Eye diameter (ED)	6.65 ± 0.64^{ab}	6.53 ± 0.32^{b}	6.98 ± 0.58^{a}	6.85 ± 0.49^{ab}
Post orbital length (PostOL)	21.52 ± 1.46	21.43 ± 0.85	22.49 ± 0.93	21.51 ± 0.67
Highest body depth (HBD)	33.55 ± 2.14	32.34 ± 3.58	35.06 ± 2.55	32.17 ± 2.01
Lowest body depth (LBD)	14.32 ± 1.05^{a}	13.22 ± 1.66^{b}	14.32 ± 0.94^{a}	14.89 ± 0.85^{a}
Pre anal fin length (PrAFL)	61.06 ± 5.34^{b}	62.53 ± 4.94^{ab}	65.94± 6.81^{a}	63.76 ± 3.71^{ab}
Pre pelvic fin length (PrPvFL)	39.98 ± 2.73	39.17 ± 1.88	40.48 ± 2.56	39.49 ± 2.24
Base of pectoral fin length (BPcFL)	34.94 ± 2.35	34.04 ± 1.64	35.57 ± 2.14	34.45 ± 1.67
Base of dorsal fin length (BDF)	66.29 ± 3.95	65.84 ± 1.83	64.25 ± 2.14	64.85 ± 2.56
Base of pelvic fin length (BPvFL)	18.79 ± 2.65	17.84 ± 1.35	18.89 ± 1.26	18.02 ± 1.27
Base of anal fin length (BAFL)	39.18 ± 2.83^{a}	38.23 ± 2.68^{a}	35.54 ± 2.22^{b}	38.06 ± 2.04^{a}
Pre pectoral fin length (PrPcFL)	24.48 ± 1.50	23.61 ± 1.11	23.18 ± 1.41	22.22 ± 1.18
Dorsal fin spine (DFS)	18.36 ± 0.55^{a}	18.43 ± 1.25^{a}	17.80 ± 0.66^{b}	17.00 ± 0.64^{c}
Dorsal fin ray (DFR)	9.6 ± 0.81^{a}	9.00 ± 0.83^{b}	9.07 ± 0.64^{b}	9.00 ± 0.74^{b}
Pectoral fin rays (PcFR)	16.23 ± 1.07^{a}	15.47 ± 0.97^{b}	14.97 ± 0.49^{bc}	14.90 ± 0.55^{c}
Pelvic fin ray (PvFR)	5.00 ± 0.37	4.97 ± 0.18	5.00 ± 0.00	4.90 ± 0.31
Anal fin spine (AFS)	9.93 ± 0.45^{a}	10.03 ± 0.56^{a}	9.50 ± 0.73^{b}	9.23 ± 0.57^{b}
Anal fin ray (AFR)	10.50 ± 0.90	9.47±0.63	9.40 ± 0.77	9.43 ± 0.77
Caudal fin ray (CFR)	14.73 ± 1.11	14.70 ± 1.47	14.97 ± 0.62	15.03 ± 0.56
Scale on lateral line (SoLL)	30.17 ± 2.09	30.07 ± 1.34	30.77 ± 1.70	29.23 ± 2.40
Scale after lateral line (SALL)	4.17 ± 0.38	3.93 ± 0.64	3.97 ± 0.18	3.93 ± 0.25
Scale before lateral line (SBLL)	10.17 ± 0.79	10.27 ± 0.79	10.33 ± 0.48	10.30 ± 0.47

Data are means of three replicates (n = 3) ± standard error. Where, T1 GT(♀) × (♂), T2 GT(♀) × M(♂), T3 M(♀) × (♂) and T4 M(♀) × GT(♂)
T1–T4 = groups, GT = golden Thai strain, M = Malaysian strain, (♀) = female, (♂) = male.

Table 2. Summary of canonical discriminant functions for four genetic groups of *Anabas testudineus*.

Function	Eigen values	Cumulative variance	Canonical correlation	Wilks' lambda	Chi square
1	2.504	59.00	0.845	0.082***	261.763***
2	0.968	81.80	0.701	0.286***	130.737***
3	0.775	100.00	0.661	0.563***	59.984***

Table 3. Standardised canonical discriminant function coefficients for four genetic groups of *Anabas testudineus*.

Morpho-meristic characters	Function		
	1	2	3
Total length (TL)	-0.870	0.329	-3.169
Standard length (SL)	0.696	-0.433	0.869
Head length (HL)	-0.405	-0.006	0.334
Pre orbital distance (PrOL)	0.287	-0.398	0.201
Eye diameter (ED)	-0.238	-0.005	0.011
Post orbital length (PostOL)	-0.418	0.692	-0.384
Highest body depth (HBD)	0.525	0.057	0.387
Lowest body depth (LBD)	-0.110	-0.600	0.680
Pre anal fin length (PrAFL)	-0.308	0.090	0.245
Pre pelvic fin length (PrPvFL)	-0.246	0.090	0.083
Base of pectoral fin length (BPcFL)	-0.081	0.779	0.504
Base of dorsal fin length (BDF)	0.334	-0.275	-0.007
Base of pelvic fin length (BPvFL)	0.081	0.036	0.712
Base of anal fin length (BAFL)	-0.333	-1.049	-0.080
Pre pectoral fin length (PrPcFL)	0.857	0.287	0.262
Dorsal fin spine (DFS)	0.562	0.301	-0.199
Dorsal fin ray (DFR)	0.465	0.111	0.210

Table 3. Continued.

Morpho-meristic characters	Function		
	1	2	3
Pectoral fin ray (PcFR)	0.375	-0.212	0.023
Pelvic fin ray (PvFR)	-0.230	0.311	-0.063
Anal fin spine (AFS)	0.191	-0.082	-0.261
Anal fin ray (AFR)	0.292	-0.205	0.452
Caudal fin ray (CFR)	0.039	0.110	0.116
Scale on lateral line (SoLL)	0.343	0.384	0.006
Scale above lateral line (SALL)	0.200	0.076	-0.130
Scale below lateral line (SBLL)	-0.153	-0.341	0.036
Total variance	59.000	22.800	18.200

PostOL, LBD, BPcFL, and BAFL. Function 3 showed only four characters with loading larger than 0.60, namely TL, SL, LBD, and BPvF.

Table 4 shows the canonical correlation coefficients for the *A. testudineus* genetic groups. Among the variables examined, DFS (0.402*), BAFL (0.442*), and LBD (0.338*) showed a strong correlation with the first, second, and third functions, respectively. The effectiveness of the discriminant analysis in classifying fish based on their genetic groups is demonstrated in Table 5. Function 1 correctly classified 103 out of 120 fish, based on the original group assignment and cross-validated classification of predicted group members of *A. testudineus* and its hybrids. Cross-validation using split-sample method resulted in an overall success rate of 65.00 % was achieved. Specifically, 73.30 % of T1, 50.00 % of T2, 63.30 % of T3, and 73.30 % of T4 were correctly assigned to their respective genetic group. The connections between the four genetic groups were confirmed, and overlap was observed between the groups as demonstrated in the two-dimensional plot of function 1 and 2 characters (Fig. 2).

Table 4. Canonical correlation coefficients of discriminant functional analysis loading of characters of *Anabas testudineus*.

Morpho-meristic characters	Function		
	1	2	3
Total length (TL)	-0.115	-0.162*	-0.074
Standard length (SL)	-0.146	-0.174*	-0.048
Head length (HL)	-0.092*	-0.017	0.088
Pre orbital distance (PrOL)	0.076	-0.257*	0.087
Eye diameter (ED)	-0.187	0.027	0.191*
Post orbital length (PostOL)	-0.151*	0.026	0.070
Highest body depth (HBD)	-0.065	0.104	0.153*
Lowest body depth (LBD)	-0.212	-0.233	0.338*
Pre anal fin length (PrAFL)	-0.212*	0.025	0.010
Pre pelvic fin length (PrPvFL)	-0.116*	-0.075	0.069
Base of pectoral fin length (BPcFL)	-0.114*	-0.036	0.113
Base of dorsal fin length (BDF)	-0.056	-0.223*	-0.088
Base of pelvic fin length (BPvFL)	-0.025	-0.004	0.175*
Base of anal fin length (BAFL)	-0.013	-0.442*	-0.118
Pre pectoral fin length (PrPcFL)	0.129*	-0.091	-0.017
Dorsal fin spine (DFS)	0.402*	0.187	-0.283
Dorsal fin ray (DFR)	0.172	-0.087	0.203*
Pectoral fin ray (PcFR)	0.401*	-0.201	0.063
Pelvic fin ray (PvFR)	0.076	0.105*	0.035
Anal fin spine (AFS)	0.313*	0.040	-0.304
Anal fin ray (AFR)	0.315*	-0.231	0.299
Caudal fin ray (CFR)	-0.080*	0.010	0.079
Scale on lateral line (SoLL)	0.081	0.260*	0.046
Scale above lateral line (SALL)	0.125	-0.057	0.152*
Scale below lateral line (SBLL)	-0.052*	0.051	-0.014

Table 5. Group classification of predicted membership of *Anabas testudineus*.

Genetic group (*Anabas testudineus*)	T1	T2	T3	T4
Golden Thai strain	90.0	6.70	0.00	3.30
Malaysian strain × Golden Thai strain	3.30	76.70	10.00	10.00
Malaysian strain	3.30	10.00	83.40	3.30
Golden Thai strain × Malaysian strain	0.00	0.00	6.70	93.30
Error level	0.11	0.30	0.20	0.07
Priors	0.25	0.25	0.25	0.25

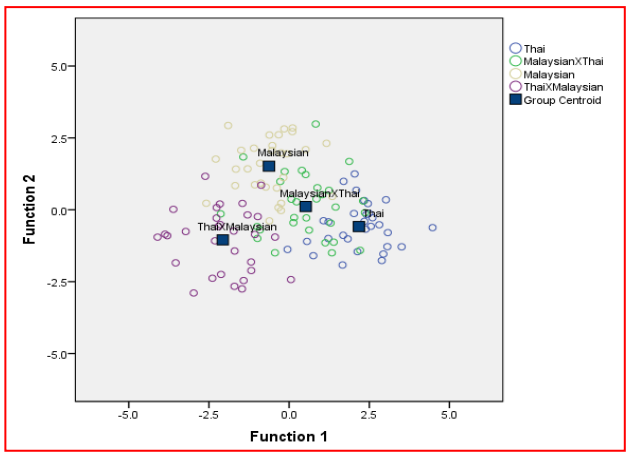


Fig. 2. Scatterplot of canonical representation of the four genetic groups of *Anabas testudineus*. Golden Thai strain female × Golden Thai strain male is T1; Golden Thai strain female × Malaysia strain male is T2; Malaysian strain female × Malaysian strain male is T3 and Malaysian strain female × Golden Thai strain male is T4.

Morphological appearance of Anabas testudineus

Based on colour pattern comparison, live specimens (Fig. 3a) of Malaysian strain were olive green with a characteristic diamond-shaped mark on its operculum and caudal peduncle. While hybrid T2 (Fig. 3c1, 3c2) was light brownish with scattered black spots across its body and a black diamond shape on its operculum and caudal peduncle. Hybrid T4 (Fig. 3d2) were darker brownish-green with a broader body shape than its golden Thai parents, with dark spots all over its body and a diffused diamond mark on its peduncle. The hybrid T4 had some progeny displaying lighter body colouration (Fig. 3d1) devoid of spots but with diffused diamond marks at the tail region. None of the hybrids exhibited their parental colour.

The study revealed morphological variations among the hybrid anabas and their parents based on the morphological characters. The morphological characters measured in the present study were similar (Table 6), however, significant components of variance ($P < 0.05$) were seen in some body characteristics. The results show that the trait TL/SL (length) was high but not significantly different within the groups when compared to LBD (body depth), which was significantly different with higher values recorded for hybrid (T4) 14.89 cm when compared to hybrid T2 which measured 13.25 cm.

Table 6 shows a broad sense heritability values for 25 measured morpho-meristic characteristics. Among these characteristics, the estimated body length and height values indicated a significant predicted genetic effect on these traits. In particular, groups T1 and T4, displayed a high heritability rate (>60 %) and a moderate genetic advance (10–20 %). The parental stock demonstrated intermediate values between the two groups.

Discussion

The present study establishes a relationship between body size and shape in fish, which can lead to morphological changes (Wimberger, 1992). It is important to note that various environmental factors can cause morphological modifications, potentially resulting in the expression of genetic differences and even genetic impoverishment (Teimori et al., 2012). Previous research by Svanbäck and Eklöv (2006) supports the idea that interactions between environmental factors and genetic plasticity contribute to morphological changes, but identifying the causes of morphological differences between populations can be challenging (Cadrin, 2000). Significant variability was observed among the four genetic groups, indicating intermixing among individuals. Additionally, migration could be a significant factor contributing to the observed differences (Sibinamol et al., 2020). Although the hybrid fish displayed similar morphological characteristics to their golden Thai parent, they differed in qualitative traits such as colour. The Thai stock might have originated from Thailand, or there could have been mixing during the spawning process

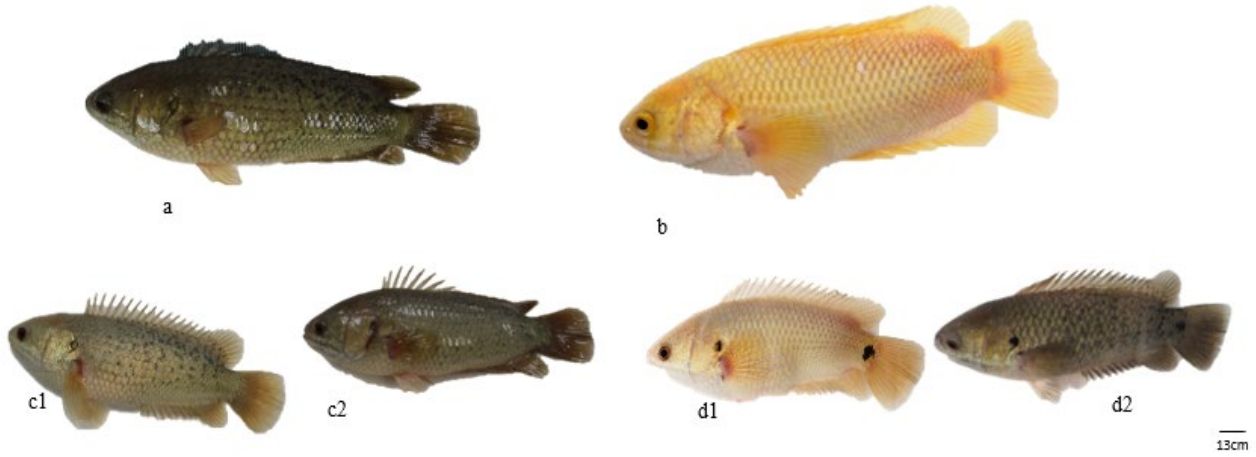


Fig. 3. Morphological appearance Malaysian strain (a) and Golden Thai strain (b) *Anabas testudineus* and their Hybrid T2 (c1 and c2) and T4 (d1 and d2). Where, T2 GT(♀) × M(♂) and T4 M(♀) × GT(♂) (T1–T4 =groups, GT = golden Thai strain, M = Malaysian strain, (♀) = female, (♂) = male).

Table 6. Genetic variability, heritability, and genetic advancement of Malaysian and golden Thai parent and their hybrids.

Morpho-meristic characters	GV	PV	PCV %	GCV %	RD	h^2_B %	GA
Total length (TL)	38.57	73.02	6.50	4.72	27.32	52.82	7.07
Standard length (SL)	32.10	52.27	6.81	5.34	21.63	61.41	8.62
Head length (HL)	1.71	5.10	6.73	3.89	42.10	33.53	4.65
Pre orbital distance (PrOL)	0.02	0.39	9.08	2.21	75.64	5.93	1.11
Eye diameter (ED)	-0.01	0.10	4.76	0.00	100.00	-6.45	-0.63
Post orbital length (PostOL)	0.73	2.01	6.53	3.93	39.74	36.32	4.88
Highest body depth (HBD)	7.04	10.31	9.64	7.96	17.37	68.27	13.56
Lowest body depth (LBD)	1.50	2.15	10.33	8.63	16.45	69.81	14.86
Pre anal fin length (PrAFL)	-2.99	15.87	6.29	0.00	100.00	-18.87	-2.45
Pre pelvic fin length (PrPvFL)	4.32	7.17	7.71	5.99	22.38	60.25	9.57
Base of pectoral fin length (BPcFL)	0.62	2.53	6.81	3.38	50.40	24.61	3.45
Base of dorsal fin length (BDF)	12.14	19.72	6.80	5.34	21.54	61.57	8.62
Base of pelvic fin length (BPvFL)	0.73	1.94	7.58	4.65	38.66	37.63	5.88
Base of anal fin length (BAFL)	6.18	9.92	8.33	6.58	21.08	62.29	10.69
Pre pectoral fin length (PrPcFL)	3.25	8.78	7.45	4.53	39.16	37.02	5.68
Dorsal fin spine (DFS)	0.01	0.13	1.99	0.00	1.00	44.74	1.83
Dorsal fin ray (DFR)	0.00	0.11	3.67	0.00	1.00	-23.53	-1.78
Pectoral fin ray (PcFR)	0.00	0.20	2.93	0.00	0.00	-47.54	-2.87
Pelvic fin ray (PvFR)	0.00	0.01	1.65	0.00	1.00	-50.00	-1.69
Anal fin spine (AFS)	0.00	0.08	2.92	0.00	1.00	-12.50	-0.75
Anal fin ray (AFR)	0.00	0.18	4.37	0.00	1.00	-38.63	-3.47
Caudal fin ray (CFR)	0.00	0.25	3.35	0.00	1.00	-47.98	-3.31
Scale on lateral line (SoLL)	0.00	1.76	4.41	0.00	1.00	-45.39	-4.13
Scale above lateral line (SALL)	0.00	0.05	5.81	0.00	1.00	-49.94	-5.97
Scale below lateral line (SBLL)	0.00	0.18	4.15	0.00	1.00	-13.05	-1.12

GV = genotypic variance, PV = phenotypic variance, PCV = phenotypic coefficient of variance, GCV = genotypic coefficient of variance, RD = relative difference, h2B = broad sense heritability, GA= genetic advance.

for the Malaysian strain. Further studies are needed to determine the genetic stocks in Malaysia and validate the current findings using other available tools.

Discriminant function analysis (DFA) is used to identify key factors for distinguishing between hybrid anabas and their parental species. The hybrid T2 GT (♀) × M (♂) group closely resembled the paternal parent, while the T4 M (♀) × GT (♂) group resembled the maternal parent. However, both hybrids exhibited intermediate features, consistent with findings from previous studies (Okomoda et al., 2018). The analysis revealed that eight traits (TL, SL, PFL, PostOL, PPF, BAF, LBD, and BPvF) were the primary components for

differentiating between body and fin characteristics. These components (1 through 3) were crucial in distinguishing traits with significant variation, demonstrating their discriminative power. Variables with a loading of 0.60 or higher are generally considered to make substantial contributions as discriminative variables. Moreover, freshwater fish are known to exhibit morphological differences. However, the extent to which changes influenced these differences in body size or environmental factors remains unclear in many taxa (Bell and Jacquemin, 2017). In this study, factors such as food and temperature were standardised for all specimens.

The difference in shape plays a crucial role in size variation, where biological factors such as different growth rates and selective environments contribute to size variation. Furthermore, sexual dimorphism between the sexes is an additional source of variation (Bell and Jacquemin, 2017). Therefore, the original measurements need to be converted into shape variants to account for size-related effects. This is particularly important for species with determinate growth, as size variation within or between taxa can be inherently intriguing (Cadrin and Friedland, 2005; Mojekwu and Anumudu, 2015). Deviations in shape and size may not be correlated, and although not universally applicable to all species, they can be linked, as suggested by Berglund et al. (1986), to a balance between fecundity constraints and success. Measurements are normalised for all specimens, as was done by Mohamed et al. (2019) for Pangasius hybrid to eliminate discrepancies arising from size effects.

According to Nwachi et al. (2020), body colour differences and morpho-meristic variation can be used to distinguish hybrids from their parents. Moreover, morphometric features assess metrics that reflect body shape, such as absolute sizes of body parts; consequently, Omasaki et al. (2019) propose that traits with high genotypic coefficient of variation, heritability, and genetic enhancement should be selected. For instance, vibrant colouration can be an initial method to distinguish between different anabas strains. In addition to strong heritability, genotypic coefficients of variation offer more comprehensive information than individual parameters.

Hybrid production is widely practised in aquaculture for its economic benefits. Commercial hybrid production has been achieved for species such as snakehead, *Channa* sp., catfish, *Ictalurus* sp., and tilapia, *Oreochromis* sp. (Chapman, 2000; Ligeon et al., 2004; Li et al., 2018). However, determining pure lineages and distinguishing hybrids requires appropriate techniques. In the present study, univariate and multivariate analyses effectively differentiated hybrids. As observed by Hubbs (1955), Park et al. (2003) concluded that some hybrids exhibit intermediate characteristics between their parents, particularly in the F1 generation. Examples include the interspecific cross between yellow flounder (♀ *Limanda ferruginea*) and winter flounder (♂ *Pseudopleuronectes americanus*) (Park et al., 2003), where the hybrids more closely resembled their maternal species; and the hybridisation between Catla (♀*Catla catla*) and Fimbriatus (♂ *Labeo fimbriatus*) (Basavaraju et al., 1995), was observed to exhibit a faster growth pattern similar to the paternal Catla parent. Similarly, the hybrid ♀ *Clarias gariepinus* × ♂ *Clarias batrachus* closely resembled *C. batrachus* (Mollah and Khan, 1997). Phenotypic inheritance often exhibits paternal dominance in true hybrids, as reported by Legendre et al. (1992) and Akinwande et al. (2013).

The heritability value in a selection programme increases with greater direct and correlated selection responses (Pérez-Rostro and Ibarra, 2003). Fish strain productivity is typically assessed based on one or two growth traits that enhance economic value. The allometric formula was effectively used to remove the size effect from the data by correlating total length and modified characters, as shown in Equation 8. Thus, the total length was initially omitted and not transformed since all other parameters used it as a standard. Heritability values for body height (0.68) as observed, were higher than those reported for the growth performance of eight strains of tilapia, *Oreochromis niloticus* (Linnaeus, 1758), tested in different farm environments (Eknath et al., 1993). The present study's results identified several characteristics, including TL, SL, HBD, LBD, BDF and AFS, that can be used to quantify growth trait scores based on Allan and Burnell's (2013) heritability estimates for aquatic animals. These identified characters (TL, SL, HBD, LBD, BDF, and AFS) exhibited values ranging from 44 % to 62 % across groups, with a rate greater than 30 %, indicating high heritability for the growth traits in this study. Regarding sexual maturity, the results indicate that hybrid anabas can be identified based on appearance and a score greater than 40 % for body depth (HBD). It is important to note that heritability reports sometimes focus only on expressed features, neglecting traits and body parts that are not easily noticeable but could contribute to a positive heritability report.

Some level of heritability occurs during hybridisation, although the inherited traits may not be immediately apparent but could manifest before the hybrid's lifespan ends. Careful evaluation of the parent stock and their associated traits, which may increase or change with age, is crucial. However, selecting the best pair of parents for breeding can be accomplished through simple phenotypic evaluations. This study was conducted in a single environment to eliminate common environmental effects, highlighting the need for further research to assess the effects of multiple conditions and subsequent generations.

Conclusion

This study highlights the significance of population characteristics in establishing the foundation for the sustainable management of the indigenous population. Morphological analysis is a powerful tool for identifying structures that impact natural resources and serves as a better predictor for managing populations. Morphological appearance can be used to distinguish hybrid anabas from parental species, and the hybrids showed good vigour. Furthermore, domesticating native species through hybridisation may aid domestication-conservation strategies. Additionally, this work can provide insights for future research on genetic resource biodiversity for breeding programs.

Acknowledgements

The authors are grateful to the authorities of Universiti Putra Malaysia for providing the necessary facilities to carry out this work and the financial support from the Universiti Putra School of Graduate Studies International Graduate Student Scholarship (IGSS) Fellowship.

Conflict of interest: The authors declare that they have no conflict of interest.

Author contributions: Awawu Dasuki: Conceptualisation, methodology, validation, formal analysis, investigation, data curation, writing-original draft, writing-review and editing, visualisation, project administration. Yuzine B. Esa: Conceptualisation, methodology, validation, formal analysis, resources, data curation, writing-review and editing, visualisation, supervision, project administration. Annie Christianus: Methodology, validation, resources, writing-review and editing, visualisation, supervision. Mohammad Fadhil Syukri Ismail: Methodology, validation, resources. Samuel Ijabo Ogah: Formal analysis, data curation.

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