Morphological characterization of the progenies of pure and reciprocal crosses of *Pangasianodon hypophthalmus* (Sauvage, 1878) and *Clarias gariepinus* (Burchell, 1822)

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Twenty-five traditional and thirty-four geometric morphometric comparisons were carried out on pure and reciprocal crosses of *Pangasianodon hypophthalmus* (Sauvage, 1878) and *Clarias gariepinus* (Burchell, 1822). Thirty fish samples each of the *C. gariepinus* (CH), *P. hypophthalmus* (PH), Pangapinus (♀PH × ♂CG) and the two distinct morphotypes of the Clariothalmus (♀CG × ♂PH) (Clarias-like and Panga-like) between the ages of four and six months were used for this study. Phenotypically, the Clarias-like Clariothalmus and the Pangapinus progenies were indistinguishable from their maternal parents while the Panga-like Clariothalmus was a phenotypic intermediary of the putative parents but looks more closely to the paternal parent. Hence, both univariate proportion and multivariate analysis of the collected data successfully separated the different fishes into three multivariate spaces. The analysis of the dendrogram with complete linkage and Euclidean distance further showed the close relationship of the isolated Panga-like Clariothalmus progenies to the paternal parent, however, Clarias-like Clariothalmus and the Pangapinus were completely intermingled with their maternal parents. The most important index of discrimination of these fishes into different multivariate spaces was the fin characteristic which showed 100% exclusive ranges for the individual groups in many cases.

The need for genetic improvement and aquaculture diversification has been the justification for hybridization in fishes. It is one of the biotechnological breeding tools used to develop new genetic stocks of fish for aquaculture/fisheries industries and a viable alternative to selective breeding when there is little additive genetic variation in the desired traits of pure stocks to be exploited1. However, successful genetic improvement through hybridization and other biotechnology tools requires proper identification and classification of species2. Hybrid identification is a problem linked to natural resources management because fertile hybrids could interbreed with pure species leading to genetic introgression. Despite the reliability of genetic approach, morphological characterization is still widely exploited due to its rapidity and the ease of field applicability3,4.

Traditional methods for morphological identification are based on principal component analysis and discriminant function analysis of linear measurements5. Discriminant function analysis of morphological data can be used to separate two or more groups of fish individuals into multivariate spaces. However, many authors have elucidated the fact that the result from the traditional morphological measurement is sometimes contradictory.
**Figure 1.** Some traditional morphometric measurement for (A) *Clarias gariepinus* (B) *Pangasianodon hypophthalmus*. 1 = Total length; 2 = Pre-dorsal distance; 3 = head length; 4 = pre-pelvic distance; 5 = pelvic height; 6 = body height; 7 = pre-pectoral distance; 8 = pre-anal distance; 9 = standard length; 10 = Anal fin length; 11 = Dorsal fin length; 12 = Caudal fin height; 13 = Caudal peduncle depth; 14 = Caudal fin length.

**Figure 2.** Morphological landmarks points for (A) typical pure *Clarias gariepinus/Clarias-like Clariothalmus* (B) Panga-like *Clariothalmus* (C) typical *Pangapinus/Pangasianodon hypophthalmus*. Landmark points are; Snout¹, origin of dorsal fin², posterior end of the dorsal fin³, dorsal attachment of the caudal fin to the tail⁴, ventral attachment of the caudal fin to the tail⁵, posterior end of the anal fin⁶, origin of the anal fin⁷, origin of the pelvic fin⁸, origin of the pectoral fin⁹ and the posterior point of the eye¹⁰.
Quantitative morphometric techniques are the most rigorous morphometric technique ever developed9–11. It is capable of processing morphometric data from digital images with landmark points quickly and with high precision9,12,13. The integration of geometric morphometric techniques with other analytic tools such as biochemical, geographical, molecular and morphological parameters could better describe phylogenetic relationships among fishes and shed light to many ambiguous taxonomic ranks14–16.

Recently, we produced novel hybrid progenies from intergeneric crosses of Asian catfish Pangasianodon hypophthalmus and Clarias gariepinus. (Landmark points are as indicated in Fig. 2). Table 1. Morphometric distances measured between landmarks points of pure and reciprocal Pangasianodon hypophthalmus and Clarias gariepinus. (Landmark points are as indicated in Fig. 2).

| SN | Landmark points | Character description |
|----|----------------|----------------------|
| 1  | 1–2            | Snout to the origin of dorsal fin. |
| 2  | 2–3            | Dorsal fin length. |
| 3  | 3–4            | Posterior end of the dorsal fin to the dorsal attachment of the caudal fin to the tail. |
| 4  | 4–5            | Dorsal attachment of the caudal fin to the tail to ventral attachment of the caudal fin. |
| 5  | 5–6            | Ventral attachment of the caudal fin to the tail to the posterior end of the anal fin. |
| 6  | 6–7            | Anal fin length. |
| 7  | 7–8            | Origin of the anal fin to the origin of the pelvic fin. |
| 8  | 8–9            | Origin of the pelvic fin to the origin of the pectoral fin. |
| 9  | 9–10           | Origin of the pectoral fin to the posterior point of the eye. |
| 10 | 1–10           | Snout to the posterior point of the eye. |
| 11 | 2–10           | Origin of the dorsal fin to the posterior point of the eye. |
| 12 | 3–10           | Posterior end of the dorsal fin to the posterior point of the eye. |
| 13 | 4–10           | Dorsal attachment of the caudal fin to the tail to the posterior point of the eye. |
| 14 | 8–10           | Origin of the pelvic fin to the posterior point of the eye. |
| 15 | 7–10           | Origin of the anal fin to the posterior point of the eye. |
| 16 | 6–10           | Posterior end of the anal fin to the posterior point of the eye. |
| 17 | 5–10           | Ventral attachment of the caudal fin to the tail to the posterior point of the eye. |
| 18 | 2–9            | Origin of the dorsal fin to the origin of the pectoral fin. |
| 19 | 3–9            | Posterior end of the dorsal fin to the origin of the pectoral fin. |
| 20 | 4–9            | Dorsal attachment of the caudal fin to the tail to the origin of the pectoral fin. |
| 21 | 7–9            | Origin of the anal fin to the origin of the pectoral fin. |
| 22 | 6–9            | Posterior end of the anal fin to the origin of the pectoral fin. |
| 23 | 5–9            | Ventral attachment of the caudal fin to the tail to the origin of the pectoral fin. |
| 24 | 2–8            | Origin of the dorsal fin to the origin of the pelvic fin. |
| 25 | 2–7            | Origin of the dorsal fin to the origin of the anal fin. |
| 26 | 2–6            | Origin of the dorsal fin to the posterior end of the anal fin. |
| 27 | 2–5            | Origin of the dorsal fin to the ventral attachment of the caudal fin to the tail. |
| 28 | 3–8            | Posterior end of the dorsal fin to the origin of the pelvic fin. |
| 29 | 3–7            | Posterior end of the dorsal fin to the origin of the anal fin. |
| 30 | 3–6            | Posterior end of the dorsal fin to the posterior end of the anal fin. |
| 31 | 3–5            | Posterior end of the dorsal fin to the ventral attachment of the caudal fin to the tail. |
| 32 | 4–8            | Dorsal attachment of the caudal fin to the tail to the origin of the pelvic fin. |
| 33 | 4–7            | Dorsal attachment of the caudal fin to the tail to the origin of the anal fin. |
| 34 | 4–6            | Dorsal attachment of the caudal fin to the tail to the posterior end of the anal fin. |

and ambiguous4–8. Geometric morphometric on the other hand is a landmark-based technique and considered the most rigorous morphometric technique ever9–11. It is capable of processing morphometric data from digital images with landmark points quickly and with high precision9,12,13. The integration of geometric morphometric data with other analytic tools such as biochemical, geographical, molecular and morphological parameters could better describe phylogenetic relationships among fishes and shed light to many ambiguous taxonomic ranks14–16.

Recently, we produced novel hybrid progenies from intergeneric crosses of Asian catfish Pangasianodon hypophthalmus (S.) and African catfish Clarias gariepinus (B.)17,18. Beyond the production of a novel aquaculture candidate, we also anticipated providing solutions to some of the breeding problems associated with the production of the pure crosses by the hybridization between the two species. For instance, the killing of male Clarias gariepinus to obtain testis is perfectly complimented and could be avoided due to the ease of sperm stripping from the male Pangasianodon hypophthalmus. Also, the early maturity (9months) and high fecundity of the female Clarias gariepinus eliminate seasonal production characteristics associated with Pangasianodon hypophthalmus brood fish due to the difficulty in obtaining gravid female (late maturity period of about 3 years). More so, early report of the hybrid 9P. hypophthalmus × Clarias gariepinus has demonstrated superior growth performance and heterosis over both pure parents17. In view of the performance characteristics of the hybrids and popularity of the pure crosses, it is important to urgently provide a quick and rapid identification tools for the hybrid since data on genetic discrimination is not available. In this study, morphological data were collected using traditional and geometric measurement hence, overcoming the drawbacks inherent in the use of traditional multivariate techniques alone. It is believed that the combination of both approaches would allow for accurate characterization of the novel hybrids between the African and Asian catfishes.
different species were divided separately into two portions. One portion was used for the production of pure prog-

ers of \( P. \ hypophthalmus \) and \( C. \ gariepinus \) expressed as percentages of standard length. Numbers in each cell are

measures in means (%) ± standard error. Mean in the same row with different superscript differ significantly

(\( P < 0.05 \)). Keys: DFL = Dorsal fin length; DFH = Dorsal fin height; DBDAF = Distances between dorsal fin end

and adipose fin origin; PDD = Pre dorsal distance; PFL = Pelvic fin length; PFH = Pelvic fin height; PPD = Pre

pelvic distance; PeFL = Pectoral fin length; PeFH = Pectoral fin height; AFL = Anal fin length; AFH = Anal fin

height; CFL = Caudal fin length; CFH = Caudal fin height; CPD = Caudal peduncle depth; BH = Body height,

BW = Body width.

| Parameter | \( \varphi_G \times \varphi_G \) | \( \varphi_G \times \varphi_H \) | Clarias-like | Panga-like | \( \varphi_H \times \varphi_G \) | \( \varphi_H \times \varphi_H \) | P-Value |
|-----------|-----------------|-----------------|-------------|-----------|-----------------|-----------------|---------|
| HW        | 67.47 ± 1.75\textsuperscript{a} | 67.77 ± 0.94\textsuperscript{a} | 62.47 ± 1.63\textsuperscript{a} | 73.56 ± 1.76\textsuperscript{a} | 72.96 ± 0.79\textsuperscript{a} | 0.001 |
| ED        | 9.52 ± 0.27\textsuperscript{a} | 8.03 ± 0.19\textsuperscript{a} | 12.80 ± 0.25\textsuperscript{a} | 14.48 ± 0.51\textsuperscript{a} | 18.86 ± 0.24\textsuperscript{a} | 0.001 |
| UMBL      | 101.50 ± 2.80\textsuperscript{a} | 109.34 ± 2.18\textsuperscript{a} | 83.82 ± 2.22\textsuperscript{a} | 55.43 ± 3.47\textsuperscript{a} | 57.58 ± 1.11\textsuperscript{a} | 0.001 |
| LMBL      | 96.76 ± 2.47\textsuperscript{a} | 95.85 ± 1.60\textsuperscript{a} | 64.49 ± 2.34\textsuperscript{a} | 29.67 ± 2.42\textsuperscript{a} | 29.02 ± 0.62\textsuperscript{a} | 0.001 |
| MW        | 39.73 ± 1.11\textsuperscript{a} | 38.68 ± 0.38\textsuperscript{a} | 37.13 ± 0.99\textsuperscript{a} | 45.79 ± 1.35\textsuperscript{a} | 47.90 ± 1.05\textsuperscript{a} | 0.001 |
| POL       | 23.75 ± 0.67\textsuperscript{a} | 25.12 ± 0.33\textsuperscript{a} | 24.73 ± 0.27\textsuperscript{a} | 29.39 ± 0.81\textsuperscript{a} | 28.63 ± 0.50\textsuperscript{a} | 0.001 |

Materials and Methods

Progenies of \( C. \ gariepinus \) (CH), \( P. \ hypophthalmus \) (PH), and the reciprocal crosses Pangapinus (\( \varphi_P \times \varphi_G \)) and Clariothalmus (\( \varphi_G \times \varphi_H \)) were obtained from similar breeding history using the method described by Okomoda et al. \textsuperscript{17,18}. In brief, six sexually mature \( P. \ hypophthalmus \) and \( C. \ gariepinus \) (between 1–2.5 kg) were injected with Ovaprim® hormone at a dosage of 0.5 ml/kg. After stripping of the females, the pooled eggs of the different species were divided separately into two portions. One portion was used for the production of pure progenies (\( \varphi_G \times \varphi_G \) and \( \varphi_P \times \varphi_H \)); while the other portion was used for the reciprocal crosses Clariothalmus (\( \varphi_G \times \varphi_H \)) and Pangapinus (\( \varphi_P \times \varphi_G \)).

The progenies obtained were cultured for at least four months (4–6 months) at the School of Fisheries and Aquaculture Sciences hatchery of the Universiti Malaysia Terengganu, Malaysia before morphological analysis was done. Thirty\textsuperscript{19} fish samples each of the progenies of the pure \( C. \ gariepinus \), \( P. \ hypophthalmus \), Pangapinus (Panga-like) and the two observed morphotypes of the Clariothalmus (Clarias-like and Panga-like) were used for morphological characterization in this study. The experimental protocols for this study were approved by the Universiti Malaysia Terengganu committee on research. All methods used in this study involving the care and use of animals were in accordance with international, national, and institutional guidelines. Twenty-five conventional traditional morphometric data were collected from each fish (some of which are described in Fig. 1).

These includes total length (TL), standard length (SL), dorsal fin length (DFL), dorsal fin height (DFH), distances between dorsal fin end and adipose fin origin (DBDAF), predorsal distance (PDD), pelvic fin length (PFL), pelvic
identified namely Snout1, origin of dorsal fin 2, posterior end of the dorsal fin 3, dorsal attachment of the caudal fin to the tail 4, ventral attachment of the caudal fin to the tail 5, posterior end of the anal fin 6, origin of the anal fin 7, origin of the pelvic fin 8, origin of the pectoral fin 9 and the posterior point of the eye 10. Thirty-six distances between the different landmark points were recorded as shown in Table 1. Values from the landmark distance measured were expressed as percentages of standard length.

fin height (PFH), pre pelvic distance (PPD), pectoral fin length (PeFL), pectoral fin height (PeFH), anal fin length (AFL), anal fin height (AFH), caudal fin length (CFL), caudal fin height (CFH), caudal peduncle depth (CPD), body height (BH), body width (BW), head length (HL), head width (HW), eye diameter (ED), upper maxillary barbel length (UMBL), lower maxillary barbel length (LMBL), mouth width (MW) and pre-orbital length (POL).

Table 4. Distance between landmark points expressed as percentages of the standard length of pure and reciprocal crosses of *Pangasianodon hypophthalmus* and *Clarias gariepinus*. Numbers in each cell are means values in percentages (%) ± standard error. Mean in the same row with different superscript differ significantly (*P < 0.05*). (landmark distance is as stated in Table 1).
To ensure that variations in this study were only attributed to body shape differences, and not to the relative sizes of the fish, size effects from the data set were eliminated, by standardizing the morphometric parameters (from traditional and trust network measurement) using the allometric formula given by Elliott et al.20:

\[ M_{\text{adj}} = M \left( \frac{L_s}{L_o} \right)^b \]

where \( M \) is the original measurement, \( M_{\text{adj}} \) is the size-adjusted measurement, \( L_o \) is the TL of the fish, and \( L_s \) is the overall mean of the TL for all fish from all samples. Parameter \( b \) was estimated for each character from the observed data as the slope of the regression of \( \log M \) on \( \log L_o \), using all fish in all groups.

The data collected were subjected to Principal component analysis (PCA) using PAST free software to obtain sample centroids graph and then determine the morphological character that contributes most to the separation of the fishes into distinct groups. Dendrograms with complete linkage and Euclidean distances of the fishes were also determined.

**Result and Discussion**

Despite the ease of phenotypic discrimination between two species involved in interspecific or intergeneric crosses, the use of morphological characterization could pose a number of challenges because of inappropriate techniques and the occurrence of growth allometry16,21. Hence, discrimination depending solely on size influenced morphometric traits becomes difficult or largely inaccurate. It is, however, important to eliminate size effect or growth-related shape changes and then elucidate shape differences among the different fish groups. The morphometric parameters expressed as a proportion of standard/head length revealed three distinct multivariate spaces for the five fish groups in this study. The Clarias-like Clariothalmus and the Pangapinus progenies

| Variable | PC1 | PC2 | PC3 | PC4 |
|----------|-----|-----|-----|-----|
| TL       | 0.18| -0.01| 0.43| -0.10|
| SL       | 0.10| -0.08| 0.39| 0.40|
| DFL      | 0.27| 0.05| 0.07| -0.01|
| DFH      | 0.27| 0.04| 0.003| 0.01|
| DBDAF    | -0.12| -0.21| -0.51| 0.10|
| PDD      | -0.14| -0.17| 0.47| 0.11|
| PFL      | -0.23| -0.06| 0.22| -0.13|
| PFH      | -0.18| -0.04| 0.02| -0.31|
| PPD      | 0.17| -0.31| 0.07| 0.10|
| PFL      | 0.05| -0.36| -0.07| -0.40|
| PFH      | -0.23| -0.08| 0.01| 0.01|
| AFL      | 0.26| 0.06| -0.00| -0.04|
| AFH      | 0.26| 0.05| 0.03| -0.04|
| CFL      | -0.21| -0.20| 0.07| 0.01|
| CFH      | -0.25| 0.13| 0.004| -0.08|
| CPD      | -0.16| -0.38| 0.07| -0.10|
| BH       | -0.23| -0.19| 0.04| -0.09|
| BW       | 0.17| -0.27| -0.17| -0.18|
| HL       | 0.14| -0.31| -0.17| 0.42|
| HW       | 0.15| -0.33| 0.07| -0.09|
| ED       | -0.26| -0.01| -0.08| 0.09|
| UMBL     | 0.25| 0.004| -0.13| -0.07|
| LMBL     | 0.26| -0.03| -0.11| -0.04|
| MW       | 0.15| -0.17| 0.12| -0.45|
| POL      | -0.02| -0.37| -0.004| 0.26|
| Eigenvalue | 13.43 | 2.74 | 1.99 | 1.13 |
| % of variance | 53.71 | 10.97 | 7.96 | 4.52 |
| Cumulative % variance | 53.71 | 64.68 | 72.64 | 77.16 |

Table 5. Principal component analysis of transformed morphometric data of the progenies of pure and reciprocal crosses of *Pangasianodon hypophthalmus* and *Clarias gariepinus* (n = 30). Numbers are component loading. Keys: TL = Total length; SL = Standard length; DFL = Dorsal fin length; DFH = Dorsal fin height; DBDAF = Distances between dorsal fin end and adipose fin origin; PDD = Pre dorsal distance; PFL = Pelvic fin length; PFH = Pelvic fin height; PPD = Pre pelvic distance; PeFL = Pectoral fin length; PeFH = Pectoral fin height; AFL = Anal fin length; AFH = Anal fin height; CFL = Caudal fin length; CFH = Caudal fin height; CPD = Caudal peduncle depth; BH = Body height, BW = Body width; HL = Head length; HW = Head width; ED = Eye diameter; UMBL = Upper maxillary barbel length; LMBL = Lower maxillary barbel length; MW = Mouth width; POL = Preorbital length.
were indistinguishable phenotypically to their maternal parents and had similar morphological and geometric proportions (Tables 2–4). The Panga-like Clariothalimus on the other hand had intermediate/shared features of both parents, however, looks more like the paternal parent. This is justified by the discriminant function analysis of morphometric which clustered the Panga-like Clariothalimus between the parental clusters with slight or no overlapping (Tables 5 and 6; Figs 3 and 4) indicating possible intermediate inheritance for the studied characters.

Similarly, the analysis of the dendrogram with complete linkage and Euclidean distance showed the closeness of Panga-like Clariothalimus to their paternal parent but uniquely separated (Figs 5 and 6). However, Clarias-like Clariothalimus and the Pangapinus were intermingled with their maternal parents. This is a pointer to differentiation in genetic inheritance in the hybrid progenies of the reciprocal cross.

In interspecific hybridization between Yellow flounder *Limanda ferruginea* (S.) and Winter flounder *Pseudopleuronectes americanus* (W.), morphometric assessed by body proportions indicated that hybrids display a morphology intermediate between the maternal and paternal species92. Bhowmick *et al.*93 and Jana94 had also earlier reported that the ♀ *Gibelio calla* (H.) × ♂ *Labeo rohita* (H.) hybrid exhibited intermediate morphometric traits of the parents. However, this hybrid tends more towards the paternal parent (*L. rohita*) with regard to body proportions and fin ray counts. The coloration of the body of the hybrid was reported similar to *G. calla* while

| Variable          | PC1  | PC2  | PC3  | PC4  | PC5  |
|-------------------|------|------|------|------|------|
| Total Length      | -0.16| -0.17| 0.10 | -0.09| -0.04|
| Standard length   | -0.14| -0.23| 0.15 | -0.10| 0.18 |
| 1–2               | 0.14 | -0.24| 0.12 | 0.24 | -0.15|
| 2–3               | -0.22| -0.002| -0.01| -0.02| -0.07|
| 3–4               | -0.20| -0.04| -0.03| 0.18 | -0.21|
| 4–5               | 0.15 | -0.05| 0.10 | -0.26| 0.32 |
| 5–6               | -0.21| 0.05 | -0.06| 0.05 | -0.02|
| 6–7               | -0.21| 0.04 | -0.01| -0.16| -0.02|
| 7–8               | 0.13 | -0.20| 0.14 | 0.18 | -0.34|
| 8–9               | -0.13| -0.20| 0.06 | 0.17 | 0.47 |
| 9–10              | -0.18| 0.01 | -0.03| 0.10 | -0.07|
| 1–10              | 0.16 | 0.09 | -0.04| 0.001| 0.13 |
| 2–10              | 0.11 | -0.29| 0.15 | 0.23 | -0.19|
| 3–10              | -0.22| 0.03 | -0.03| -0.01| -0.01|
| 4–10              | -0.18| -0.18| 0.11 | -0.09| 0.06 |
| 5–10              | -0.19| -0.12| -0.01| 0.21 | 0.27 |
| 6–10              | -0.12| -0.29| 0.09 | 0.27 | 0.13 |
| 7–10              | -0.22| -0.03| 0.01 | -0.03| 0.03 |
| 8–9               | -0.19| -0.18| 0.12 | -0.05| 0.01 |
| 9–10              | 0.16 | -0.26| 0.08 | 0.12 | -0.08|
| 1–9               | -0.20| -0.05| -0.16| -0.08| -0.04|
| 2–9               | -0.19| -0.18| 0.12 | -0.05| 0.01 |
| 3–9               | -0.21| -0.003| -0.15| -0.15| 0.07 |
| 4–9               | 0.14 | 0.16 | 0.33 | 0.04 | 0.12 |
| 5–9               | -0.22| -0.01| -0.03| -0.04| -0.10|
| 6–9               | -0.21| -0.14| -0.09| -0.07| -0.21|
| 7–9               | 0.01 | -0.33| -0.28| -0.05| -0.01|
| 8–9               | -0.20| -0.11| -0.12| -0.11| 0.02 |
| 9–9               | -0.09| 0.10 | 0.43 | 0.13 | 0.06 |
| 2–8               | 0.10 | -0.26| -0.18| 0.03 | 0.27 |
| 2–7               | 0.09 | -0.31| -0.24| -0.05| 0.07 |
| 3–6               | 0.20 | -0.003| -0.15| -0.15| 0.07 |
| 2–5               | 0.14 | 0.16 | 0.33 | 0.04 | 0.12 |
| 1–3               | -0.22| -0.01| -0.03| -0.04| -0.10|
| 3–8               | -0.09| -0.14| -0.09| -0.07| -0.21|
| 3–6               | -0.21| 0.03 | -0.04| 0.08 | -0.15|
| 3–5               | -0.21| 0.02 | -0.04| 0.08 | -0.14|
| 4–8               | 0.01 | -0.24| 0.25 | -0.35| -0.29|
| 4–7               | 0.03 | -0.17| 0.24 | -0.54| -0.03|
| 4–6               | 0.20 | -0.11| 0.10 | -0.10| -0.04|
| Eigenvalue % of variance | 20.39| 5.66 | 3.93 | 1.71 | 1.07 |
| Cumulative % variance | 56.63| 15.73| 10.94| 4.77 | 2.97 |

Table 6. Principal component analysis of transformed data from landmark point of the progenies of pure and reciprocal crosses of *Pangasianodon hypophthalmus* and *Clarias gariepinus* (n = 30). Numbers are component loading.
Figure 3. Principal component analysis of transformed morphometric data obtained from pure and reciprocal crosses of *Pangasianodon hypophthalmus* and pure *Clarias gariepinus* \((n = 30)\). Cross = pure *C. gariepinus*; dot = Clarias-like Clariothalmus; circle = Panga-like Clariothalmus; multiplication = Pangapinus; square = *P. hypophthalmus*.

| Parameter | Pure *C. gariepinus* vs Pure *P. hypophthalmus* |
|-----------|-----------------------------------------------|
|           | Pure ♂*CG* × ♂*CG* | Pure ♂*PH* × ♂*PH* |
| DFL       | 57.35–67.22 (100%) | 5.49–7.65 (100%) |
| DFH       | 4.39–9.00 (100%)  | 14.47–20.47 (100%) |
| PFL       | 1.82–3.26 (100%)  | 3.42–4.92 (100%)  |
| PFH       | – (0.0%)          | 11.69–12.19 (13.33%) |
| PeFL      | 5.09–5.39 (20.0%) | 3.75–3.85 (3.33%)  |
| PeFH      | 10.23–12.96 (76.67%) | 14.01–16.96 (83.33%) |
| AFL       | 37.96–46.06 (100%) | 28.48–33.52 (100%) |
| AHF       | 3.29–6.45 (100%)  | 11.10–14.10 (100%) |
| CFL       | 6.63–9.00 (100%)  | 9.43–11.73 (96.67%) |
| CFH       | 13.89–20.37 (96.67%) | 22.58–30.25 (96.67%) |

Table 7. Comparison between Pure *Pangasianodon hypophthalmus* and *Clarias gariepinus* for the exclusive ranges of fin measurement expressed as a percentage of standard length \((n = 30)\). Minimum-maximum exclusive range observed (percentage). Keys: DFL = Dorsal fin length; DFH = Dorsal fin height; PFL = Pelvic fin length; PFH = Pelvic fin height; PeFL = Pectoral fin length; PeFH = Pectoral fin height; AFL = Anal fin length; AHF = Anal fin height; CFL = Caudal fin length; CFH = Caudal fin height.
the shape of the mouth was more like those of *L. rohita*. Also, the hybrid ♀ *C. gariepinus* × ♂ *Clarias batrachus* (L.) was phenotypically similar to *C. batrachus*. The reciprocal hybrid between *Pangasius djambal* (B.) and *P. hypophthalmus* showed intermediate phenotypic characteristics but had a strong similarity with the latter than the former. Legendre *et al.* and Akinwande *et al.*, had earlier concluded that the display of intermediate phenotypic characteristics in the ♀ *C. gariepinus* × ♂ *Heterobranchus longifilis* (V.) hybrid was an indication of true hybridization. Hence, it is thought that they resulted from the fusion of the genetic material of both parents. They further hypothesized that phenotypical inheritance in true hybrid has paternal dominance. In the study, the Panga-like Clariothalmus progeny had an adipose fin and a forked tail just like the paternal parent. However, it has an elongated dorsal fin just like those in the maternal parent (but shorter than), hence may be the only true hybrid of the reciprocal crosses. According to Chevassus, fertilization of a fish egg by a heterospecific sperm may lead to the production of haploid, gynogenesis/androgenesis, hybrid diploid, hybrid triploid, hybrid tetraploid, a combination of some or all the mentioned or death. Progenies of many distance hybridization are often composed of different phenotypic characters which is suggestive of significant differences in genetic composition and inheritance pattern. Therefore, the largely indistinguishable morphology of Purebred *C. gariepinus* and *P. hypophthalmus* with the Clarias-like Clariothalmus and Pangapinus progenies respectively may be a pointer to the

Figure 4. Principal component analysis of transformed morphometric data obtained from pure and reciprocal crosses of *Pangasianodon hypophthalmus* and pure *Clarias gariepinus* (*n* = 30). Cross = pure *C. gariepinus*; dot = Clarias-like Clariothalmus; circle = Panga-like Clariothalmus; multiplication = Pangapinus; square = *P. hypophthalmus*. 
presence of gynogenetic individuals. Environmental, geographical, and genetic adaptation has been implicated as possible causes of phenotypic variations between strains in many previous studies\cite{19,30}, observations of the present study, however, is largely linked to differences in species and pattern of genetic inheritance in the different morphotypes of the reciprocal hybrids.

**Figure 5.** Dendrogram with complete linkage and Euclidean distance for the morphometric parameter of pure and reciprocal crosses of *Pangasianodon hypophthalmus* and pure *Clarias gariepinus* (*n = 30*). Cross = pure *C. gariepinus*; dot = Clarias-like Clariothalmus; circle = Panga-like Clariothalmus; multiplication = Pangapinus; square = *Phyophthalmus*.

**Figure 6.** Dendrogram with complete linkage and Euclidean distance for data collected from Trust network on pure and reciprocal crosses of *Pangasianodon hypophthalmus* and pure *Clarias gariepinus* (*n = 30*). Cross = pure *C. gariepinus*; dot = Clarias-like Clariothalmus; circle = Panga-like Clariothalmus; multiplication = Pangapinus; square = *Phyophthalmus*.
| Parameter | Exclusive range with \( \text{CG} \times \text{CG} \) | Exclusive range with \( \text{PH} \times \text{CG} \) | Exclusive range with \( \text{PH} \times \text{PH} \) |
|-----------|------------------------|------------------------|------------------------|
| DFL       | 57.34–59.66 (13.3%)    | −(0.0%)                | 5.49–7.65 (100%)       |
| DFH       | 8.06–9.00 (66.7%)      | −(0.0%)                | 14.47–20.47 (100%)    |
| PFL       | 1.82–2.53 (60%)        | 3.32–3.45 (13.3%)      | 3.42–4.92 (100%)      |
| PFH       | 11.22–11.68 (10.0%)    | 7.41–8.50 (30.0%)      | 11.25–12.19 (50.0%)   |
| PeFL      | 3.85–3.98 (10.0%)      | 5.43–5.84 (16.67%)     | 3.75–3.85 (6.67%)     |
| PeFH      | 10.23–10.26 (3.33%)    | 13.97–14.46 (3.33%)    | 14.47–16.95 (66.67%)  |
| AFL       | 37.96–39.78 (10.0%)    | −(0.0%)                | 28.48–33.52 (100.0%)  |
| AFH       | 6.02–6.45 (3.33%)      | −(0.0%)                | 11.45–14.10 (100%)    |
| CFL       | 6.63–9.00 (56.67%)     | 9.09–9.89 (36.67%)     | 10.00–11.73 (90.0%)   |
| CFH       | 18.28–21.32 (46.67%)   | 12.69–13.77 (16.67%)   | 21.13–30.25 (100%)    |

Table 8. Exclusive ranges and percentages of fin measurement expressed as a percentage of standard length for Clarias-like Clariothalmus in comparison with the pure parents \( n = 30 \). Minimum-maximum recorded range (percentage). Keys: DFL = Dorsal fin length; DFH = Dorsal fin height; PFL = Pelvic fin length; PFH = Pelvic fin height; PeFL = Pectoral fin length; PeFH = Pectoral fin height; AFL = Anal fin length; AFH = Anal fin height; CFL = Caudal fin length; CFH = Caudal fin height.

| Parameter | Exclusive range with \( \text{CG} \times \text{CG} \) | Exclusive range with \( \text{PH} \times \text{CG} \) | Exclusive range with \( \text{PH} \times \text{PH} \) |
|-----------|------------------------|------------------------|------------------------|
| DFL       | 57.35–67.22 (100%)     | 32.58–44.22 (100%)     | 5.49–7.65 (100%)       |
| DFH       | 4.40–9.00 (100%)       | 10.07–13.97 (100%)     | 14.47–20.47 (100%)    |
| PFL       | 1.82–1.98 (13.3%)      | 3.29–4.31 (56.67%)     | 4.32–4.92 (63.3%)     |
| PFH       | 8.52–8.55 (6.67%)      | 11.81–12.84 (10.0%)    | 7.53–8.59 (3.33%)     |
| PeFL      | 3.85–4.06 (13.33%)     | 5.41–5.59 (20.0%)      | 3.75–4.03 (13.33%)    |
| PeFH      | 10.23–13.98 (70.0%)    | 14.05–17.58 (63.3%)    | 12.80–12.90 (20.0%)   |
| AFL       | 37.96–46.01 (100%)     | 32.87–39.46 (100%)     | 33.60–39.46 (86.67%)  |
| AFH       | 3.29–5.69 (96.67%)     | 6.76–15.38 (90.0%)     | 6.08–11.22 (90.0%)    |
| CFL       | 6.62–7.35 (43.33%)     | 9.38–11.03 (86.67%)    | 7.38–7.60 (66.7%)     |
| CFH       | 13.89–18.81 (66.67%)   | 21.67–22.29 (16.67%)   | 18.82–21.11 (83.33%)  |

Table 9. Exclusive ranges and percentages of fin measurement expressed as a percentage of standard length for Panga-like Clariothalmus in comparison with the pure parents \( n = 30 \). Minimum-maximum recorded range (percentage). Keys: DFL = Dorsal fin length; DFH = Dorsal fin height; PFL = Pelvic fin length; PFH = Pelvic fin height; PeFL = Pectoral fin length; PeFH = Pectoral fin height; AFL = Anal fin length; AFH = Anal fin height; CFL = Caudal fin length; CFH = Caudal fin height.
Table 11. Comparison between the progenies of Clariothalmus and Pangapinus for the exclusive ranges of fin measurement expressed as a percentage of standard length (n = 30). Minimum-maximum exclusive range observed (percentage). Keys: DFL = Dorsal fin length; DFH = Dorsal fin height; PFL = Pelvic fin length; PFH = Pelvic fin height; PeFL = Pectoral fin length; PeFH = Pectoral fin height; AFL = Anal fin length; AFH = Anal fin height; CFL = Caudal fin length; CFH = Caudal fin height.

| Parameter | Clarias-like 0.0% | Clarias-like 33.33% | Clarias-like 66.67% | Clarias-like 100% | Pangapinus 0.0% | Pangapinus 33.33% | Pangapinus 66.67% | Pangapinus 100% | Pangapinus 33.33% | Pangapinus 66.67% | Pangapinus 100% |
|-----------|------------------|-------------------|-------------------|-----------------|----------------|-----------------|-----------------|----------------|----------------|----------------|--------------|
| DFL       | 32.58–45.64 (100%) | 32.87–39.46 (100%) | 40.0–45.64 (100%) | 29.19–36.61 (100%) | 37.07–39.46 (33.33%) | 29.19–36.61 (76.67%) |                  |                  |                  |                  |              |
| DFH       | 10.07–13.97 (100%) | 11.27–13.97 (100%) | 40.0–45.64 (100%) | 29.19–36.61 (100%) | 37.07–39.46 (33.33%) | 29.19–36.61 (76.67%) |                  |                  |                  |                  |              |
| PFL       | 3.49–4.31 (33.33%) | 3.51–5.44 (93.33%) | 3.51–5.44 (93.33%) | 3.51–5.44 (93.33%) | 3.51–5.44 (93.33%) | 3.51–5.44 (93.33%) |                  |                  |                  |                  |              |
| PFH       | 2.61–3.45 (80.0%)  | 3.49–4.31 (33.33%) | 3.49–4.31 (33.33%) | 3.49–4.31 (33.33%) | 3.49–4.31 (33.33%) | 3.49–4.31 (33.33%) |                  |                  |                  |                  |              |
| PeFL      | 4.27–5.44 (13.33%) | 2.61–3.45 (80.0%)  | 2.61–3.45 (80.0%)  | 2.61–3.45 (80.0%)  | 2.61–3.45 (80.0%)  | 2.61–3.45 (80.0%)  |                  |                  |                  |                  |              |
| PeFH      | 2.04–3.15 (43.33)  | 2.61–3.45 (80.0%)  | 2.61–3.45 (80.0%)  | 2.61–3.45 (80.0%)  | 2.61–3.45 (80.0%)  | 2.61–3.45 (80.0%)  |                  |                  |                  |                  |              |

The morphometric variability among the three groups in this study was mainly due to the variation of characters related to fins, and body characteristic. This is because the effect of size was successfully eliminated by the allometric transformation and demonstrated by univariate proportion and multivariate analysis. However, with 100% exclusive ranges observed for the different groups for most fin characters (Tables 7–11), this is likely the easiest and most rapid index of discrimination of the three groups applicable in the field. Haddad and Willis13 stated that morphometrics of the head and body depth have been regarded as the most important characters for discrimination of Devil anglerfish Lophius vormerinus (V.), Pacific herring Clupea pallasi pallasi (V.) and Orange roughy Hoplostethus atlanticus (C.).14, Solomon et al.15, however, reported head length, body depth at anus and the eye diameter as the most influential morphometric parameters used to discriminate fish strains from cultured and wild Clarias gariepinus. However, the suitability of fin and body characteristics in the study as opposed to reports from the previous study may be due to the clear and unambiguous intergeneric phenotypic differences of the pure crosses used. Based on the results of the present study, the fin characteristic appears to be the most promising index of morphological discrimination. Despite the fact that the morphological approach alone is insufficient to investigate hybridization status of fishes, this research has provided useful assumption on the nature of the hybrid progenies and a quick/cheap identification tools for field application. However, a combination of both morphological, genetic and cytogenetic data in future studies could provide a clearer understanding of the nature of the progenies gotten.

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**Author Contributions**
The study was conceptualised and designed by K.I.C.C., H.A., A.T. and S.M.S. The study was conducted by O.V.T. as part of his Ph.D research under the supervision of the other co-authors. In addition, O.V.T. analysed the results, prepared all the figures and wrote the draft of the manuscript which was corrected and approved for submission by all the coauthors.

**Additional Information**

**Competing Interests:** The authors declare no competing interests.

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