E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2017; 5(1): 233-240

www.fisheriesjournal.com
Received: 05-11-2016
Accepted: 06-12-2016

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Morphological differentiation among cultured and wild *Clarias macrocephalus, C. macrocephalus* x *C. gariepinus* hybrids, and their parental species in the Mekong delta, Viet Nam

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Abstract
Morphological differences among two cultured and three wild bighead catfish (*Clarias macrocephalus*) populations, and introduced North African catfish (*Clarias gariepinus*) and hybrids (*C. macrocephalus* x *C. gariepinus*) collected in the Mekong Delta, Viet Nam, were assessed using five meristic and 21 morphometric measurements. Wild and cultured *C. macrocephalus* differed significantly at 19 of 21 morphometric traits ($P<0.01$), indicating high their phenotypic plasticity between natural and captive conditions. *C. macrocephalus* differed from *C. gariepinus* and hybrids in the number of gill rakers on the first branchial arch and for all morphometric traits ($P<0.01$). Principle component analyses revealed that occipital process size, head size, the distance from dorsal to caudal fins (DDCF), and fin lengths were particularly important to within-, between-species variation and to hybrid identification. Hybrids exhibited intermediate phenotypes relative to the two parental species in most of meristic and morphometric parameters, except DDCF comparable to *C. macrocephalus* and the shape of the occipital process similar to *C. gariepinus*. Discriminant analysis correctly classified 97.5 and 99.0% individuals as cultured and wild *C. macrocephalus*, 80.0% as *C. gariepinus*, and their hybrids with 95.7% confidence. Misclassifications were found between cultured and wild bighead catfish, and between *C. gariepinus* and hybrids. Results from this study can be used to distinguish F1 hybrid and cultured bighead catfish from wild individuals.

Keywords: Morphology, bighead catfish, *Clarias*, hybrid identification, hybridization

1. Introduction
Morphological analysis using meristic and morphometric characters has been routinely used to detecting inter-specific hybrids in fishes [1-2]. Traditionally, individuals would be considered hybrids when their meristic and morphometric measurements which were calculated using hybrid indices were intermediate to values of two parental species (Hubbs and Kuronuma 1942; cited by Campton 1987). However, hybrids can express differently among traits, leading to violation of the intermediate assumption. More advanced, multivariate statistical approaches such as principal component analysis and discriminant function analysis [3-4] have been incorporated in analyzing morphological data to identify hybrids, which have reduced limitations of the hybrid index in morphological classification [1].
Morphological methods can also be used to identify stocks and population structure within fish species [5-6]. Morphological characteristics vary among populations due to effects of environment factors [7-8], diets, developmental stages [6, 9-10], and the genetic origin of populations [9, 11]. Morphological differences between wild and farmed fish populations have been reported for several marine species (e.g., sea bream [12]; Atlantic cod [13]). Consequently, little information is available for freshwater fish (e.g., snakehead *Channa striata* Bloch, 1793 [14]). Cultured and wild *C. striata* differed significantly in all morphometric measurements with the largest divergence in the head shape. With the same body length, cultured individuals have shorter and smaller head compared to wild fish [14]. Changes in morphometric measurements can occur rapidly in several generations [13, 15]. The morphological divergence between cultured and wild fish can have an important explanation for identification of captive individuals that have escaped to the wild [16].

Bighead catfish (*Clarias macrocephalus* Gunther, 1864) is an economically important species for aquaculture in Southeast Asian countries. In nature, its populations have declined dramatically and rapidly [17]. In addition, the gene pool of bighead catfish can be exposed to genetic risks of introgression from the introduced North African catfish *Clarias gariepinus* Burchell, 1822 [18-19] and from hatchery-bred individuals, as observed in other farmed fish [20-21]. Catfish hybrids (*C. macrocephalus* x *C. gariepinus*) have also been cultured commercially in Vietnam [22] and Thailand [18, 23]. Because of the widespread farming, captive bighead catfish and hybrid individuals can escape into the wild [19]. Identifying these individuals become critical for aquaculture and fisheries purposes. However, few studies have documented these effects in *Clarias* species.

Although molecular approaches have been employed to address *Clarias* hybrid and stock identification [24], morphological analyses can be used because of its simplicity. A previous study found some morphological differences among *Clarias* species but could not separate hybrids from parental species based on morphometric measurements, maybe because of small sample sizes (15 for hybrid, and 24 – 35 individuals for each of three *Clarias* species).

In this study, our objectives were to quantify morphological differences between cultured and wild bighead catfish populations in the Vietnamese Mekong Delta, and between catfish hybrids and their parental species. Documentation of morphological differences can be particularly important to differentiate hybrids and bighead catfish that are believed to widely escape captive conditions and mix with wild individuals.

2. Materials and Methods

2.1 Fish collection

Fish were collected from the Mekong Delta, Viet Nam, in 2015. Bighead catfish adults were sampled from 2 grow-out ponds (60.9-327.7g) in Can Tho and Long An provinces and from 3 wild populations (31.1-82.9g) in Ca Mau, Can Tho and Long An provinces (Table 1, Fig 1). Wild populations in Ca Mau and Long An were sampled in canals, swamp sites in conservation areas, where catfish farming has not been applied. In Can Tho province, which is the center of catfish farming in the Mekong Delta, wild fish were caught in rice fields and small canals. The three wild populations were approximately 120 km apart. Main environmental differences between wild and culture conditions included fish densities, feed types and abundance, and room for movement. Hatchery-bred fish were cultured in small ponds (approximately 200 – 300 m^2) at densities of 30-50 individuals/m^2. Fish were fed twice daily with home-made feed (5-10% biomass) commonly used for other fish such as striped catfish. The feed contained 19-25% crude protein from a mixture of rice bran, broken rice, trash fish, soybean, and fish meal [26].

Thirty adult samples (204-615g) of the introduced North African catfish *Clarias gariepinus* (herein, NA catfish) were bought from a hatchery located in Can Tho province. The owner has maintained NA catfish biomass about 0.5 ton since 1990 through artificial propagation for producing commercial catfish hybrid seed. F1 Catfish hybrids (*C. macrocephalus x C. gariepinus*, hereafter hybrids) have been widely cultured popularly in the Mekong Delta, Vietnam, especially in Can Tho. Ninety-one hybrid samples (2-3 months-old, weight ranged 64-178g) were collected in three different farms (30 or 31 samples/farm) located in Can Tho (Fig 1). Fish were transported live to a laboratory at Can Tho University for morphological analyses.

2.2 Morphological measurements

Fish were individually anesthetized before measurements were taken. Meristic and morphometric traits were measured based on established methods described by Khoa and Huong (1993) [27] and Teugels (1986, cited by [25]). Five meristic traits were analyzed: the number of dorsal, pectoral, ventral, and anal fin rays, and a number of gill rakers on the first branchial arch. Morphometric traits were measured using digital calipers (0.1 mm resolution). In addition to total body weight (Wt), body characteristics consisted of 13 measurements (Fig 2) including total length (TL), standard length (SL), body depth at anus (BDA), caudal peduncle depth (CPD), distance between dorsal and caudal fins (DDCF), pre-dorsal distance (PDD), pre-anal distance (PAD), pre-ventral distance (PVD), pre-pectoral distance (PPD), dorsal fin length (DFL), anal fin length (AFL), ventral fin length (VFL), pectoral fin length (PFL). Ten head characters (Fig 2) were recorded: head length (HL), head width (HW), inter-orbital distance (IOD), eye diameter (ED), occipital process length (OPL), occipital process width (OPW), occipital fontanelle length (OFL), occipital fontanelle width (OFW), frontal fontanelle length (FFL), and frontal fontanelle width (FFW).

2.3 Statistical analyses

The range, mode, and frequency of modal values of meristic traits of each parental species and hybrids were calculated (Table 2). Morphometric measurements were reported as ratios to standard length (for seven body traits, three fins (Table 3), and head length) or to head length (for nine head measurements in Table 4). The ratio of occipital process length (OPL) to its width (OPW) was also calculated (21 ratios). However, these ratios correlated with body sizes (P<0.05, data not shown), which varied among fish groups. To remove fish size effects when comparing among populations and species, morphometric measurements were adjusted for body size differences by using the model described by Elliott *et al*. (1995) [28].

$$M_{adj} = M*(L_s/L_o)^b$$

where M_{adj}: adjusted measurement by fish length; M: original measurement; L_o: standard length of each individual, L_s: the overall mean of standard length for all individuals from all fish groups in each analysis; and parameter b estimated for each trait from the original data by the equation $M = a*(L_o)^b$. All adjusted morphometric measurements did not correlate with fish standard length (all P values > 0.05), indicating that effects of size differences among fish groups were removed. Then, adjusted morphometric measurements of bighead catfish were compared for significant differences (at a significant level of 0.05) between wild and cultured populations and among geographic locations (Ca Mau, Can Tho, and Long An) using General linear models. If wild and cultured populations differed significantly in at least two measurements, they were then treated as two separate groups to compare with NA catfish and the hybrids. Means were tested for significant differences using Duncan's multiple range tests. Statistical analyses were performed with SPSS v.20. Principal component analysis (PCA) was conducted to examine divergences in adjusted morphometric measurements which were log-transformed, among four groups of fish using the prcomp () function in R (R Core Team, 2014). In SPSS program, a stepwise discriminant analysis using Mahalanobis distance was conducted to identify important variables that contribute to population/species differentiation and to classify each individual into possible groups based on prior probabilities calculated from group sample sizes.

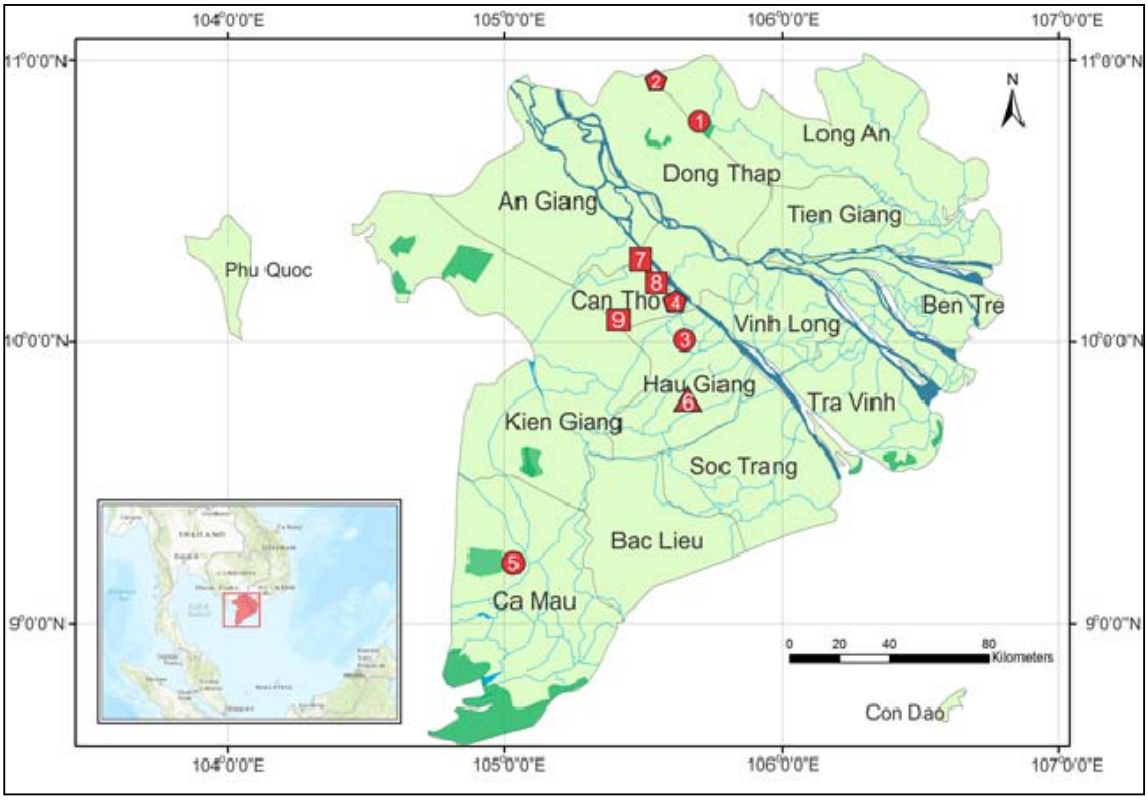

Fig 1: Map of the Vietnamese Mekong Delta showing sampling locations for wild (populations 1, 3, and 5) and cultured (populations 2 and 4) *C. macrocephalus* populations, cultured *C. gariepinus* (population 6), and three cultured *Cm*Cg* hybrids (populations 7, 8 and 9)

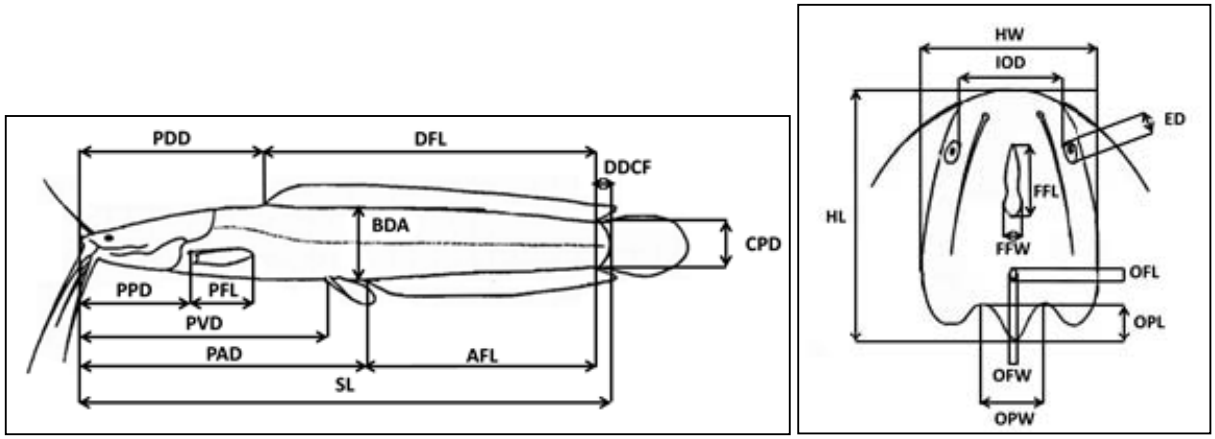

Fig 2: Morphometric measurements on the body and the head of *Clarias* catfish (Teugels *et al*., 1998)

Note: BDA: body depth at anus; CPD: caudal peduncle depth; DDCF: distance between dorsal and caudal fins; PDD: pre-dorsal distance; PAD: pre-anal distance; PVD: pre-ventral distance; PPD: pre-pectoral distance; DFL: dorsal fin length; AFL: anal fin length; PFL: pectoral fin length. HW: head width; OPL: occipital process length; OPW: occipital process width; IOD: inter-orbital distance; ED: eye diameter; FFL: frontal fontanelle length; FFW: frontal fontanelle width; OFL: occipital fontanelle length; and OFW: occipital fontanelle width

Table 1: Sampling locations, sample size, and range of fish sizes of *C. macrocephalus* (*Cm*), *C. gariepinus* and *Cm*Cg* hybrids

No.	Fish groups	Location (province)	Latitude	Longtitude	Sample size	Weight of fish (g)	Total length (cm)
1.	Wild *C. macrocephalus*	Lang Sen (Long An)	10°46'31.2"N	105°42'38.2"E	31	42.8-172.0	17.4-26.5
2.	Cultured *C. macrocephalus*	Tan Hung (Long An)	10°55'27.9"N	105°33'16.5"E	41	60.9-166.0	19.5-26.3
3.	Wild *C. macrocephalus*	Phong Dien (Can Tho)	10°00'37.4"N	105°38'41.8"E	31	31.1-120.8	14.6-23.4
4.	Cultured *C. macrocephalus*	O Mon (Can Tho)	10°08'53.5"N	105°36'04.2"E	40	83.2-327.7	21.7-33.7
5.	Wild *C. macrocephalus*	U-Minh Ha (Ca Mau)	9°19'28.9"N	104°53'20.9"E	30	102.9-182.9	22.9-28.7
6.	*C. gariepinus*	Phung Hiep (Hau Giang)	9°46'17.9"N	105°39'22.7"E	30	204-615	29.7-44.6
7.	*Cm*Cg* hybrids	Thot Not (Can Tho)	10°17'42.5"N	105°30'28.6"E	30	70.1-147.8	21.3-26.7
8.	*Cm*Cg* hybrids	Binh Thuy (Can Tho)	10°04'23.7"N	105°45'11.4"E	30	63.7-178.2	19.7-26.9
9.	*Cm*Cg* hybrids	Phong Dien (Can Tho)	9°59'21.4"N	104°59'18.9"E	31	42.2-133.5	17.7-26.5

Table 2: Range, mode, and modal frequency of meristic parameters of *C. macrocephalus* (*Cm*), *C. gariepinus* and *Cm*Cg* hybrids

Trait		Wild *C. macrocephalus*	Cultured *C. macrocephalus*	*C. gariepinus*	Hybrid
Gill rakers	Range	25-31	25-30	54-67	28 - 31
	Mode (frequency, %)	30 (20)	29 (40)	60 (40)	30 (50)
Dorsal fin ray	Range	60-65	64-69	60-78	61 - 74
	Mode (frequency, %)	65 (40)	67 (40)	69 (40)	68 (40)
Pectoral fin ray	Range	I,8-9	I,8-9		I, 8 - 10
	Mode (frequency, %)	I,9 (90)	I,9 (50)		I, 9 (70)
Annal fin ray	Range	46-50	46-54	44-60	46 - 55
	Mode (frequency, %)	46 (50)	47 (30)		46 (30)
Ventral fin ray	Range	6	6		5 - 7
	Mode (frequency, %)	6 (100)	6 (100)		6 (60)

Table 3: Body morphometric ratios and length ratios (mean ± SD) of head and fins relative to standard length (%) of (wild and cultured) *C. macrocephalus* (*Cm*), *C. gariepinus* (*Cg*) and *Cm*Cg* hybrids

Locations/ Populations	BDA	CPD	DDCF	PDD	PAD	PVD	PPD	HL	DFL	AFL	PFL
Wild *Cm*	18.6±1.5	5.90±0.63	2.40±0.67	31.1±1.5	51.5±2.6	43.2±1.9	20.6±2.3	27.1±1.0	69.3±2.5	48.9±2.3	15.3±1.5
Cultured *Cm*	16.4±1.3	5.75±0.58	1.78±0.55	30.1±1.7	51.2±2.4	42.8±2.2	17.8±0.9	26.0±1.5	68.3±2.6	46.5±2.7	13.2±1.2
Hybrid	16.0±1.1	6.48±0.50	2.52±0.52	34.0±1.5	55.7±2.2	46.6±2.5	21.1±1.4	29.1±1.4	63.9±2.2	44.1±2.3	13.3±1.1
Cg	14.4±0.8	7.88±0.57	5.02±0.95	35.2±1.8	60.2±2.5	49.2±2.0	23.8±1.3	30.5±1.8	65.4±2.6	42.9±2.3	13.6±0.8
P value*	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: (*) P values show significant differences in means of adjusted morphometric measurements (see statistical analyses for more detail)
BDA: body depth at anus; CPD: caudal peduncle depth; DDCF:distance between dorsal and caudal fins; PDD: pre-dorsal distance; PAD: pre-anal distance; PVD: pre-ventral distance; PPD: pre-pectoral distance; DFL: dorsal fin length; AFL: anal fin length; PFL: pectoral fin length.

Table 4: Head morphometric ratios and fontanelle characters relative to head length (%) of (wild and cultured) *C. macrocephalus* (*Cm*), *C. gariepinus* (*Cg*) and *Cm*Cg* hybrids

Locations & Populations	HW/HL	OPL/HL	OPW/HL	IOW/HL	ED/HL	FFL/HL	FFW/HL	OFL/HL	OFW/HL	OPL/OPW
Wild *Cm*	72.8±3.1	12.0±2.9	45.2±2.6	43.9±2.7	8.01±1.11	17.4±2.2	8.82±1.66	9.86±1.66	6.91±1.37	26.4±6.81
Cultured *Cm*	66.4±4.3	14.1±2.6	42.7±3.5	38.1±1.7	8.89±1.06	15.5±3.4	7.56±1.51	8.18±1.93	5.60±1.50	33.0±6.01
Hybrid	64.8±3.7	14.5±1.9	25.3±2.0	39.0±1.8	7.14±0.97	19.3±2.6	8.49±1.02	9.25±1.31	5.76±1.07	57.7±8.6
Cg	59.3±2.4	15.3±1.5	20.5±1.9	43.4±2.1	6.92±0.87	21.2±4.6	7.13±1.06	8.05±2.11	4.95±1.38	75.2±9.37
P value*	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: (*) P values as noted Table 3.
HW: head width; HL: head length; OPL: occipital process length; OPW: occipital process width; IOD: inter-orbital distance; ED: eye diameter; FFL: frontal fontanelle length; FFW: frontal fontanelle width; OFL: occipital fontanelle length; and OFW: occipital fontanelle width.

Table 5: The number and the percentage (in parentheses) of correct assignment into their original groups of wild and cultured *C. macrocephalus* (*Cm*), *C. gariepinus* (*Cg*) and *Cm*Cg* hybrids.

Original group	Predicted Group Membership				Total
	1	2	3	4	
1. Wild *Cm*	95(99.0)	1(1.0)	0(0)	0(0)	96(100.0)
2. Cultured *Cm*	2(2.5)	78(97.5)	0(0)	0(0)	80(100.0)
3. *Cg*	0(0)	0(0)	24(80.0)	6(20.0)	30(100.0)
4. Hybrid	0(0)	0(0)	4(4.3)	89(95.7)	93(100.0)

3. Results

3.1 Meristic parameters

The largest difference in meristic traits between the two *Clarias* species was gill raker number on the first branchial arch (25 – 31 in bighead catfish and 54 – 67 in NA catfish), whereas hybrids were similar to maternal species (bighead catfish) (Table 2). Other meristic traits overlapped between the two species and hybrids. These traits showed different levels of variation (modal frequencies) within (for bighead wild and cultured individuals) and among species, and hybrids.

3.2 Differences in morphometric measurements between wild and cultured bighead catfish

Within bighead catfish species, wild and cultured populations differed in 19 of 21 adjusted morphometric parameters (data not shown), except pre-anal distance (PAD) and dorsal fin length (DFL). Wild and cultured bighead catfish populations showed the greatest differences in body depth, head size, fin length, and six morphometric measurements on fish's head. The magnitude of differences between wild and cultured fish depended on sampling location ($P_{population\ X\ location}<0.01$).

At the same standard length, wild bighead catfish had greater body depth, longer fins (except DFL), and larger head size compared to cultured individuals ($P<0.01$, Table 3). In addition, the shape of the occipital process was also different ($p<0.001$; Table 4), being wider and shorter in wild individuals compared with cultured ones. Moreover, frontal and occipital fontanelle of wild catfish were significantly larger in size compared to the cultured fish ($P<0.01$; Table 4).

Under the main effect of sampling locations, bighead catfish collected in different provinces were not different in body

depth, head length, and PAD and PDD but they varied significantly in the other traits (data not shown). Principal component analysis (PCA) using 21 log-transformed morphometric measurements indicated a significant divergence between wild and cultured populations (Fig 3) but no significant divergence between geographic locations. Because of large differences between wild and cultured bighead catfish, they were treated as two groups to be compared with NA catfish and their hybrids.

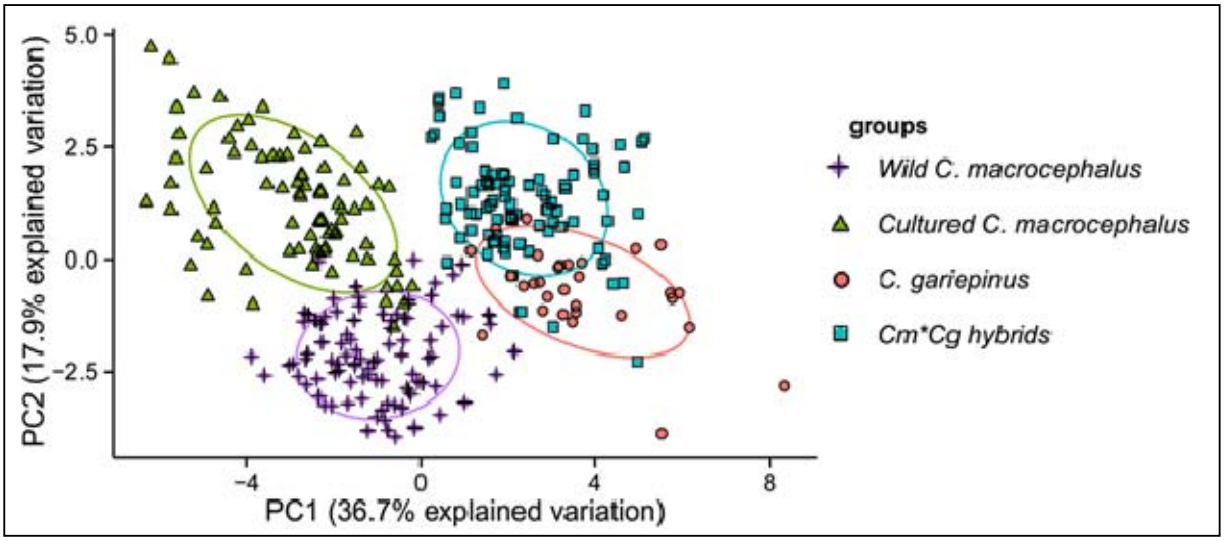

Fig 3: Two-dimension plot of principle component (PC) analyses using 21 log-transformed morphometric measurements adjusted for body size among four groups of fish including *C. macrocephalus (Cm), C. gariepinus (Cg)* and *Cm*Cg* hybrids. The ellipse circles indicate 68% probability for each fish group.

3.3 Differences in morphometric measurements between North African catfish, bighead catfish and their hybrids

All adjusted morphometric measurements were significantly different among four groups of fish (all P values <0.001). In which, NA catfish differed 19 of 21 traits from wild (except the head width, HW, and occipital fontanelle width, OFW) or cultured (except body depth, BD, and eye diameter, ED) bighead catfish. The largest magnitude of differences between the two species was found in the width and length of the occipital process (OPW and OPL), head length (HL), the frontal fontanelle length (FFL), and distances of pre-dorsal (PDD), pre-anal (PAD) and pre-ventral fins (PVD). Most adjusted morphometric values (18 measurements, 85.7%) of the hybrids fell in ranges of both parental species, in which the distance between dorsal and caudal fins (DDCF) was close to maternal species, while the shape of the occipital process (occipital process width and its length - width ratios, OPL/OPW) were similar to paternal species.

PCA analysis showed that the first 5 components explained 71.2% total variation in morphometric measurements. In which, the first and second components (PC1 and PC2) explained 36.7% and 17.9% of the variation, respectively. Two parental species and the hybrid were differentiated from each other on PC1 where OPW, OPL/OPW, FFL, HL, and pre-fin distances (PDD, PAD, and PVD) were main contribution variables. With large differences among four groups, the discriminant analysis could classify individuals into their original groups with correct assignments of 99% for wild bighead catfish (N=96), 97.5% for cultured ones (N=80), 80% for NA catfish (N=30), and 95.7% for hybrids (N=93). Mismatch assignments were found between wild and cultured bighead catfish (1% and 2.5%, respectively) and between NA catfish and hybrids (20% pure NA catfish were misclassified as hybrids and 4.3% hybrids were misclassified as pure NA catfish) (Table 5). Cross-validation analysis gave the same result of group assignments.

4. Discussion

The present study revealed morphological differentiation among two *Clarias* species, bighead- and North African catfish, and their hybrid, and morphological divergence between wild and cultured bighead populations but not between sampling locations. Important morphological characteristics contributing to between and within catfish species differentiation included the number of gill rakers on the first branchial arch (for between species), head characters, the distance from dorsal to caudal fins, pre-fin distances, and fin lengths. The hybrid exhibited intermediate values between the two parental species in most of morphometric (85.7%) and meristic parameters.

Meristic traits that are usually highly heritable [29] are commonly used for species classification. Among five meristic parameters counted in this study, the number of gill rakers on the first branchial arch differs between two catfish species, which is almost double in NA catfish compared to bighead catfish. This trait is also used to identify other *Clarias* species, for example, *C. bachatrus* with around 20 [25], and *C. anguillaris* with 14 to 40 gill-rakers [30]. Gill rakers and other meristic parameters of the catfish hybrid were similar to and thus difficult to distinguish from bighead catfish, as well as between wild and cultured bighead catfish populations. Small or no variations in meristic parameters among populations were also reported in several fishes such as brook lamprey *Lethenteron reissneri* [31] *Tilapia zilli* [32]. 2015). However, other studies found that some meristic traits such as lower gill arch rakers were significantly differentiated among populations of European whitefish (*Coregonus lavaretus* L.) that symmetrically live in Lake Femund, Norway [33]. Therefore, depending on fish species, meristic parameters can be useful indicators for population differentiation.

The shape of the occipital process has been used as a key character for the classification of species in the genus *Clarias* [25, 27]. It was found in this study that the occipital process was

rounded and short in bighead catfish, compared to M'- shaped in NA catfish, whereas, that of hybrids was more similar to NA catfish than bighead catfish. In addition, Liem (2008) [34] reported that the shape of the occipital process (especially the width) reciprocal hybrids was more likely inherited from NA catfish (Fig 4). The width of the occipital process was also different between bighead catfish populations. It was longer and narrower in cultured bighead catfish than wild individuals and that its shape also varied between sampling locations. Cultured catfish in the present study had longer but similar width occipital processes (14.2±2.9% HL and 42.7±3.5% HL, respectively) than catfish in Malaysia (10.2±0.92% HL and 42.9±1.9% HL, respectively) [34]. The data indicated that the shape of the occipital process could vary depending on living (natural or captive) environments.

Wild and cultured bighead catfish also differed significantly in body depth, head size, and DDCF. Cultured catfish had deeper body height with shorter fins, and smaller sizes of head, frontal fontanelle, and occipital fontanelle compared to wild catfish. In NA catfish, head measurements could be used to differentiate among populations [35]. Similar results were reported in other freshwater fish (e.g., *Tilapia zilli* [32]) and marine species such as Atlantic cod Gadus morhua [13], gilthead seabream *Sparus aurata* [16]. In Atlantic cod, for instance, farmed cod had relatively smaller head and jaw, shorter fins (dorsal, anal, ventral and pectoral) but larger body depth than those of wild cod.

Differences in morphology between fish species can be explained by genetic makeup. In addition, within a species, fish can exhibit high plasticity in morphometric characters to different diets, physical environments (i.e., turbidity, water temperature and current), and fish densities [10, 13, 35]. Environmental factors in natural and captive conditions could contribute to the morphological divergence between wild and cultured bighead catfish in the present study. In nature, fish have to compete for space, feeding, survival, and finding mates. Compared to cultured fish, wild fish need longer fins for instantaneous movement and rapid swimming for feeding or escaping predators [36]. The natural diet of wild bighead catfish depends on the availability and accessibility of favorite food. In contrast, cultured catfish live in small ponds with high stocking densities, periodic feeding rate, and available food. Hence, cultured fish may require less locomotion to obtain adequate food than wild individuals. In addition, crowding in high densities may also cause shorter fins in some individuals due to fin nipping behavior, as reported in salmonids [37, 38]. Environmental factors could vary between sampling locations in nature and in culture conditions, resulting in the observed significant interaction effects of populations and sampling locations on morphometric measurements of bighead catfish.

Effects of polygenic and non-genetic factors on morphology contribute to difficulties in morphologically classifying hybrids from their parental species [1, 2, 39]. In this study, catfish hybrids exhibited different levels of variation among meristic and morphometric measurements. PCA and discriminant function analyses based on morphometric measurements adjusted for body size differences could identify hybrids with 95.7% of correct assignments. Incorrect classifications occurred between NA catfish and the hybrids, indicating that morphometric traits of the hybrids were more similar to parent species (i.e., NA catfish) than mother species.

Hybrid identification based on morphology only valid for F1 catfish hybrids which have been used in catfish hybrid farming in Vietnam [22] and Thailand [18-19]. Misclassification of beyond F1 hybrids or backcrossed individuals as pure bighead catfish would be common [2, 39]. To solve hybrid identification problems, more advanced, DNA-based methods [40-41] associated with Bayesian clustering approaches [42-43] have been used in studying hybridization and inter-specific genetic introgression. Previous studies employed allozyme [18-19], microsatellite and mitochondrial markers to identify F1 catfish hybrids [24].

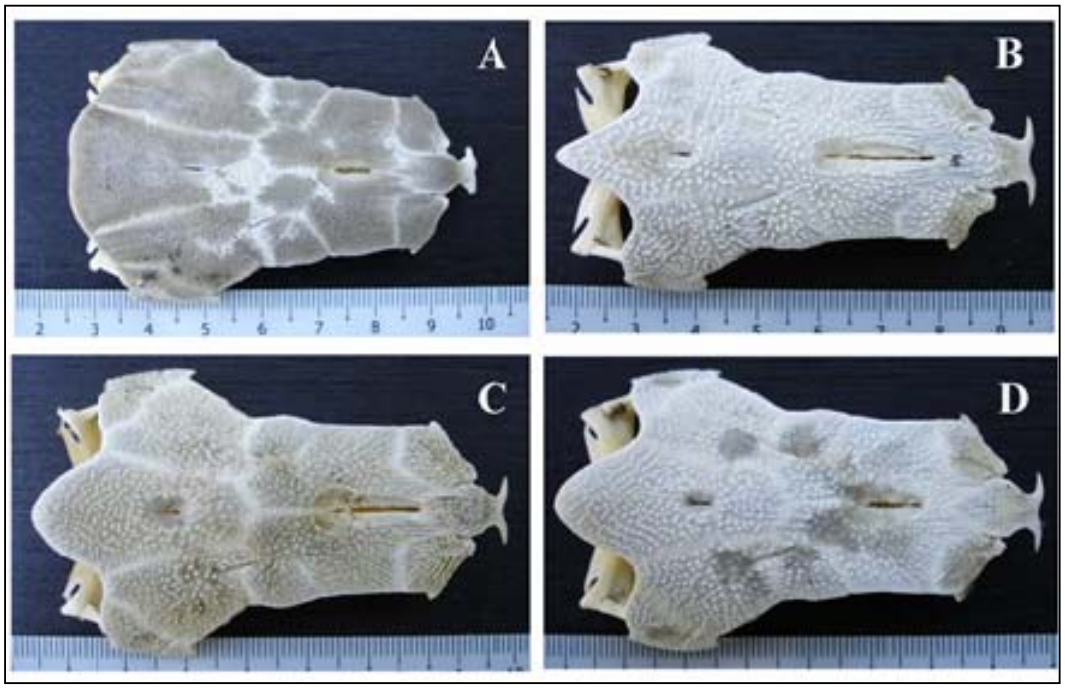

Fig 4: The shape of the head, occipital process, and frontal fontanelle of (A) *C. macrocephalus* (*Cm*), (B) *C. gariepinus* (*Cg*), (C) *Cm*Cg* hybrid, and (D) *Cg*Cm* hybrid (Taken by Liem, 2008) [34]

5. Conclusion and implications

Results of the present study indicated that high phenotypic plasticity of bighead catfish species in response to natural and captive environments and morphological differences of *Clarias* hybrids from their parental species.

In practice, the study provides a simple method to distinguish F1 hybrid and cultured bighead catfish from wild individuals. The misuse of F1 individuals as pure bighead catfish individuals in broodstock selection can lead to reducing aquaculture production [19]. In addition, identifying correctly wild bighead catfish individuals is also necessary to replenish domesticated stock, which might be reduced genetic diversity due to domestication and inappropriate broodstock management [29].

6. Acknowledgements

The authors would like to thank Drs. Barry Clough and Kim Scribner for editing and providing comments on previous drafts. This research was funded by the Viet Nam National Foundation for Science and Technology Development (NAFOSTED) under grant number: 106-NN.05-2014.86.

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