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MORPHOLOGICAL DIFFERENCES IN FIVE STRAINS OF GENETICALLY IMPROVED NILE TILAPIA (*OREOCHROMIS NILOTICUS*) USING GEOMETRIC MORPHOMETRICS

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ABSTRACT

The determination of fish stock structure is important in developing optimal strategies for efficient management of aquaculture species. Morphometric analysis provides a robust, non-expensive, and statistically powerful means of stock delineation. In the Philippines, five strains of genetically improved Nile tilapia (*Oreochromis niloticus*) have been developed. This study sought to use geometric morphometrics to delineate among the five tilapia strains. Specimens were collected in June to December 2014 from various institutions in the Philippines. Images of 263 individuals were taken at four months old, and 17 landmarks were digitized. Multivariate analysis of variance (MANOVA) revealed significant shape differences between strains. The Canonical Variate Analysis (CVA) plot showed the SEAFDEC strain to be most unique in shape whereas close similarity was observed among specimens of GIFT Philippines, GIFT Malaysia and GET-EXCEL. Discriminant groupings by CVA reflect the historical relationships among the strains. Morphological traits such as the tip of the snout, insertion of the pelvic fin, ventral base of the caudal fin, and the anterior end of the dorsal fin can be used to differentiate one strain from another. Sexual dimorphism in shape was also evident. These results indicate the utility of geometric morphometrics in delineating strains of economically important fish species.

Key words: aquaculture, fish strains, genetic improvement, shape variation, stock delineation

INTRODUCTION

The tilapia is a group of cichlid fishes, which includes three economically important genera namely, *Tilapia*, *Oreochromis*, and *Sarotherodon.* It is an important commodity, ranking ninth in global aquaculture production and third in the Philippine aquaculture production (Fitzsimmons, 2000; Boyd, 2004; Fitzsimmons *et al.*, 2011). China, Egypt, Indonesia, Philippines, and Thailand are the principal producing countries of tilapia. The world tilapia production had been growing increasingly in recent years with 5.3 million metric tons in 2014 (FishstatJ, 2016). The tilapias are a great source of protein in protein-deficient inland communities (Mjoun *et al.*, 2010). Most importantly, its ability to grow fast, its large size and ease of culture are characteristics that make tilapia a desirable food fish.

In 1950, *Oreochromis mossambicus* was introduced in the country. It became a popular market fish but improper management of ponds resulted to small-sized fish (Guerrero, 1985). Introduction of another tilapia species, *O. niloticus*, in 1972 led to the expansion of tilapia industry in

the country which led to a higher demand for tilapia juveniles (Aypa, 1995; Guerrero and Guerrero, 2004). However, the greater demand for juveniles, lack of broodstock development programs, use of few broodstock in hatcheries, and introgression with *O. mossambicus* eventually led to poor tilapia production (Lal and Foscarini, 1990; Aypa, 1995).

Genetic improvement programs were initiated in the Philippines in the 1980s to improve production (Uraiwan, 1990; Eknath *et al.*, 1993; Mair *et al.*, 1997). This led to the development of five tilapia strains, namely: (1) Genetically Improved Farmed Tilapia (GIFT), which is the product of the first selective breeding program for tropical fish (Eknath *et al.*, 1993; Eknath *et al.*, 2007; World Fish Center, 2010); (2) Freshwater Aquaculture Center-selected tilapia (FaST), a product of the combination of four *O. niloticus* strains known as Taiwan, Thailand, Israel and Singapore (Bolivar and Newkirk, 2002); (3) GET-EXCEL, a cross between GIFT and FaST stock (Tayamen, 2004); (4) GIFT Malaysia, established based on the sixth generation of GIFT from the Philippines (Ponzoni *et al.*, 2005); and (5) SEAFDEC whose founding population was the Chitralada strain (Basiao and Doyle, 1999). These genetically improved tilapia strains are dispersed through national fishery agencies, foundations, research institutions and universities (Basiao and Doyle, 1999).

A fish stock is composed of individuals that are part of the same reproductive process which are contained with no immigration or emigration of individuals from one stock to another (Garcia, 2005). Determination of fish stock structure is important because it helps in the development of an optimal strategy for efficient management of fish (Coyle, 1998). Morphometric analysis is a good alternative or complement to biochemical or genetic methods of stock identification because it is cheaper and more robust than molecular techniques. External morphology, such as body shape and skin pigmentation, has been important in commercial fish farming because these can influence consumer preference (Colihueque *et al.*, 2014).

Geometric morphometrics is widely used in determining shape variation. Instead of using linear measurements, as in traditional morphometrics, data are recorded in the form of coordinates of landmark points (Adams *et al.*, 2004). Application of advanced image processing techniques has significantly enhanced stock identification and discrimination in fishes (Cadrin *et al.*, 2005). Stock identification using geometric morphometrics was recently applied on *Sebastes* spp. (Valentin *et al.*, 2014), *Gasterosteus aculeatus* (Pistore *et al.*, 2016), and *Mugil curema* scales (Ibáñez *et al.*, 2017). In *O. niloticus*, geometric morphometrics has been applied to study the effect of management (Lorenz *et al.*, 2014), to examine bone growth patterns (Fujimura and Okada, 2008), to analyze effects of temperature and salinity (Ndiwa *et al.*, 2016), and to corroborate phylogenetic relationships (Clabaut *et al.*, 2007).

This study sought to discriminate among the five strains of genetically improved *O. niloticus* using geometric morphometrics. The nature of shape variation is also characterized.

MATERIALS AND METHODS

The specimens used for this study were obtained from different institutions that maintain these. The GET-EXCEL strain was obtained from the National Fisheries Technology Training Center of the Bureau of Fisheries and Aquaculture Resource, Central Luzon State University. FaST strain was obtained from the Freshwater Aquaculture Center of the Central Luzon State University. GIFT Philippines was maintained by the GIFT Foundation of the Philippines. The GIFT Malaysia strain was from the World Fish Center in Penang, Malaysia, a stock of which is maintained by the Bureau of Fisheries and Aquatic Resources. SEAFDEC strain was from the Binangonan Freshwater Station of the Southeast Asian Fisheries Development Center. At least 50 four-month old individuals of tilapia from each of the five genetically improved strains were used in this study.

Tilapia specimens were placed in ice in order to immobilize them as adopted from Iwama and Ackerman (1994). Each specimen was then placed on a platform with white paper as background, and was assigned a code for identification. A ruler was placed next to the specimen to serve as a size standard. The body posture and fins were then teased into its natural position. Using a Nikon D60 camera with an 18-55mm lens, a photograph of each of the specimen was taken. Standard length (SL) and weight (Wt) were taken. The sex was determined by dissection and examination for the presence of either the ovaries or the testes. From the digital image, 17 landmarks (Fig. 1) from the left side of the fish were digitized using tpsDig2 (Rohlf, 2006). Landmarks were modified from those of Velasco *et al*. (1996) and Lorenz *et al*. (2014). Representative specimens are shown in Fig. 2.

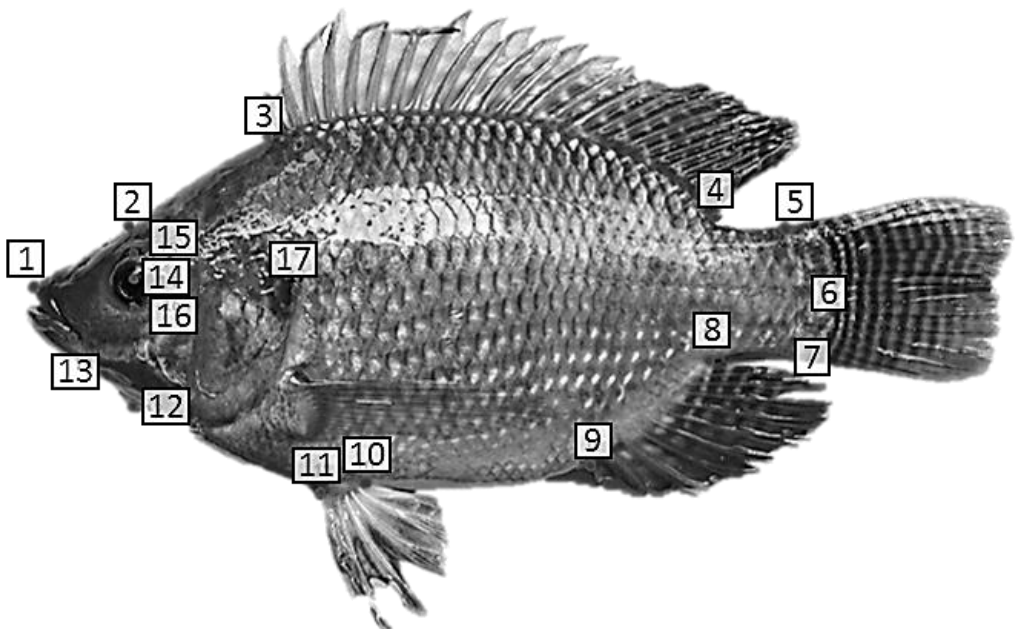

Fig. 1. Locations of the 17 landmarks used for the shape analysis of *Oreochromis niloticus.* (1) snout tip, (2) edge of the head directly above the eye, (3) anterior base of the dorsal fin, (4) posterior base of the dorsal fin, (5) dorsal base of the caudal fin, (6) base of the caudal fin at the level of the lateral line, (7) ventral base of the caudal fin, (8) posterior end of the anal fin base, (9) anterior end of the anal fin base, (10) posterior insertion of the pelvic fin, (11) anterior insertion of the pelvic fin, (12) edge of the head directly below the eye, (13) corner of the mouth, (14) center of the eye, (15) top of the eye, (16) bottom of the eye, and (17) the most posterior edge of the operculum.

Digital images were assigned to their respective strain and sex. General Procrustes Analysis (GPA) was performed, superimposing landmarks into a common coordinate system, while removing variation due to size, location, and orientation (Addis *et al.*, 2010). Canonical Variate Analysis (CVA) was performed to discriminate among strains based on shape variables. GPA and CVA were conducted using programs from the Integrated Morphometrics Package (IMP; Sheets, 2003).

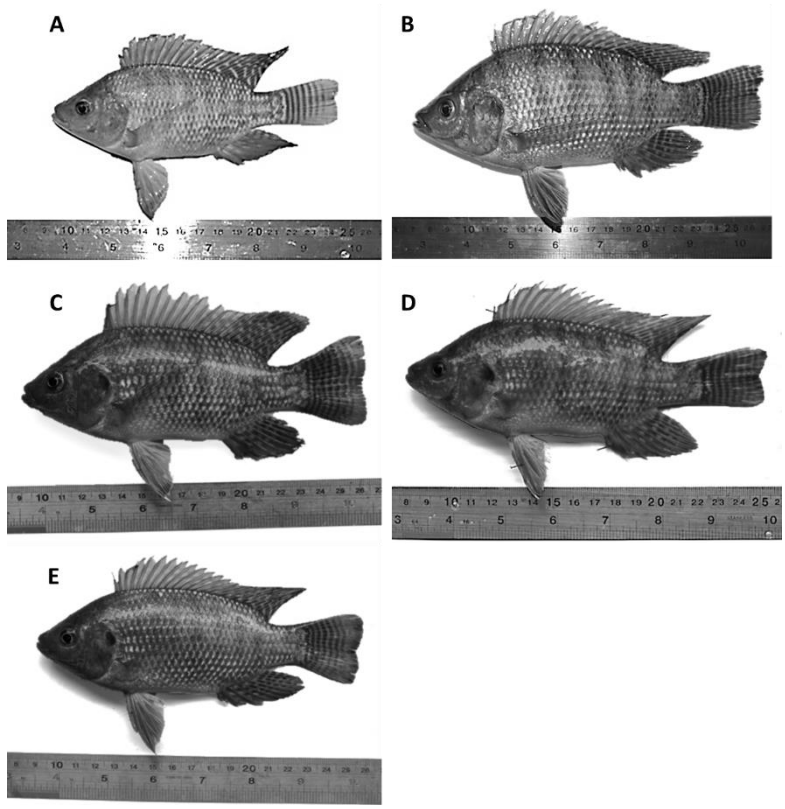

Fig. 2. Representative *O. niloticus* specimens per strain. a.) GET-EXCEL, b.) FaST, c.) GIFT Philippines, d.) GIFT Malaysia, e.) SEAFDEC

RESULTS AND DISCUSSION

Variations in SL and Wt are summarized in Tables 1 and 2. Differences in average SL and Wt were significant. The FaST strain was superior in those parameters. GIFT Malaysia was the smallest but were not significantly different from GET-EXCEL and SEAFDEC. In all strains, differences in SL and Wt between sexes were not significant (Table 2). Males were observed to be longer and heavier in GET-EXCEL, FaST, GIFT Malaysia. The opposite was observed for the GIFT Philippines and SEAFDEC strains.

For the shape variation, four distinct canonical variates were observed, indicating that the five strains can be dichotomized based on shape characters. GIFT Philippines and GIFT Malaysia showed the greatest overlap among the strains and SEAFDEC, FaST and EXCEL clustered separately (Fig. 3). Shape deformation along CV1 shows variation in the position of the ventral base of the caudal fin as well as expansion of the anterior and posterior ends. Shape deformation along CV2 also shows variation in the ventral base of the caudal fin as well as ventral compression. CV1 and CV2 contributed to 75% of the total variation. Differences between strains along CV1 and CV2 were significant as shown by one-way ANOVA (Table 1). CV1 and CV2 effectively differentiates SEAFDEC from the rest of the strains. GIFT Malaysia was significantly different from FaST based on CV1. CV2 delineates among three distinct subgroups, namely, SEAFDEC strain, the subgroup of GIFT Philippines and FaST, and the subgroup of GIFT Malaysia and GET-EXCEL.GET-EXCEL showed the highest percentage of correct classification (Table 3).

Table 1. Descriptive statistics of specimens of *O. niloticus*, comparing among strains.

Measure	Strains					F value (P value)
	GET-EXCEL (n=53)	**FaST (n=53)**	**GIFT PHILIPPINES (n=55)**	**GIFT MALAYSIA (n=51)**	**SEAFDEC (n=51)**	
SL	129.35ab±13.18	138.96c±20.22	135.49bc±21.98	125.10a±13.43	130.32ab±15.19	5.106 (0.00)
	(107.76-171.49)	(101.26-186.61)	(90.10-183.02)	(91.54-154.82)	(96.19-156.15)	
Wt	48.92ab±4.76	53.88c±8.63	51.79b±8.09	48.07a±5.74	49.19ab±6.43	6.259 (0.00)
	(38.77-64.82)	(36.95-74.59)	(36.00-71.51)	(33.64-62.01)	(33.83-63.14)	
CV1	0.0003ab±0.0038	0.0015b±0.0029	0.0007ab±0.0027	-0.0001a±0.002	-0.0025±0.0026	13.693 (0.00)
	(-0.0057-0.0074)	(-0.0034-0.0076)	(-0.0072-0.0052)	(-0.0065-0.0039)	(-0.0075-0.0018)	
CV2	0.0006b±0.0026	-0.0015a±0.0019	-0.0022a±0.0018	0.0002b±0.0017	0.0031±0.0024	49.335 (0.00)
	(-0.0054-0.0062)	(-0.0054-0.0059)	(-0.0064-0.0016)	(-0.0032-0.0038)	(-0.0018-0.0070)	
CV3	-0.0003ab±0.0023	0.0003ab±0.0032	0.0005b±0.0022	0.0006b±0.0028	-0.0010a±0.0024	3.52 (0.08)
	(-0.0054-0.0054)	(-0.0060-0.0076)	(-0.0057-0.0043)	(-0.0068-0.0066)	(-0.0075-0.0026)	

*Values are mean ± standard deviation and range (in parentheses). Means followed by the same letter are not significantly different at α=0.05 using Tukey's Post hoc analysis. n, sample size; Wt, weight (g); SL, standard length (mm); CV1, canonical variate 1; CV2, canonical variate 2; CV3, canonical variate 3.

Among the different strains, greatest overlap was observed between GIFT Philippines and GIFT Malaysia. This reflects the origin of GIFT Malaysia as the sixth generation of selection from GIFT Philippines (Ponzoni *et al.*, 2005). FaST was grouped separately because it wasn't based on the GIFT Philippines strain but rather the initial selection was based on four strains of *O. niloticus* which included Taiwan, Thailand, Israel and Singapore strains (Lester *et al.*, 1988). The GET-EXCEL strain overlapped with the cluster of GIFT Philippines and also with GIFT Malaysia and FaST because it was based on the eighth generation of GIFT Philippines strain, 13th generation of FaST, Egypt strain and Kenya strain (Tayamen, 2004). The SEAFDEC selected strain (Basiao and Doyle, 1999) showed a very distinct separate clustering from the rest of the four strains. The founding population of the SEAFDEC strain was the Chitralada strain from the National Institute of Fisheries (NIFI) in Thailand. The Chitralada strain which originally came from Egypt was a gift from the Emperor of Japan to the King of Thailand. High percent of correct assignment support significant differences of body shape among strains.

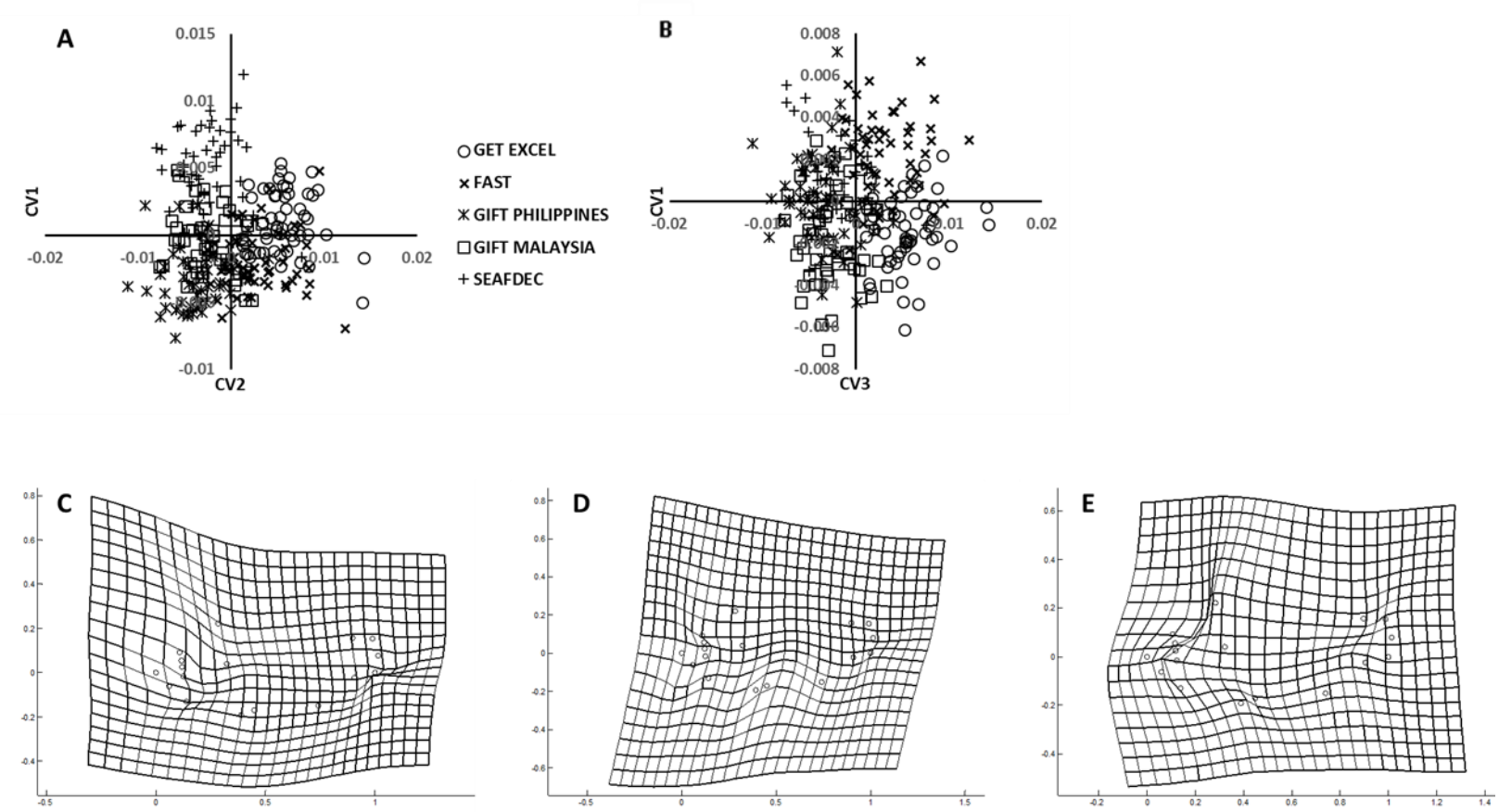

Fig. 3. Results of CVA of warp scores of both female and male samples of GET-EXCEL (n=53), FaST (n=53), GIFT Philippines (n=55), GIFT Malaysia (n=51) and SEAFDEC selected (n=51) strains of *O. niloticus*; A, CVA plot showing CV1 versus CV2; B, CVA plot showing CV1 versus CV3; C, Deformation grid showing shape change along CV1; D, Deformation grid showing shape change along CV2; E, Deformation grid showing shape change along CV3. For the deformation grids, the reference form is equivalent to mean of all configurations.

Table 2. Comparison of sexes between strains of *O. niloticus* specimens. Values are mean ± standard deviation and range (in parentheses). N, sample size; Wt, weight (g); SL, standard length (mm); CV1, canonical variate 1.

Measure	Sex		F value	P value
	GET-EXCEL			
	Male (n=26)	Female (n=27)		
SL	130.55±14.11	128.09±12.29	0.457	0.502
	(108.32-160.95)	(107.76-171.49)		
Wt	49.77±4.75	48.03±4.70	1.778	1.88
	(40.63-60.64)	(38.77-64.82)		
CV1	-0.0016±0.0062	0.0015±0.0006	324.475	0.000
	(-0.0027-(-0.0004))	(0.00020.0030)		
	FaST			
	Male (n=26)	Females (n=27)		
SL	142.31±17.64	135.73±22.30	1.359	0.249
	(101.97-164.47)	(101.26-186.61)		
Wt	55.69±8.42	52.14±8.64	2.208	0.144
	(36.95-69.66)	(35.37-39.22)		
CV1	-0.0012±0.0006	0.0011±0.0006	206.799	0.00
	(0.00-0.00)	(0.00-0.00)		
	GIFT PHILIPPINES			
	Male (n=27)	Female (n=28)		
SL	138.27±19.78	133.00±23.85	1.567	0.217
	(110.71-180.49)	(90.10-183.02)		
Wt	52.61±7.17	71.06±8.89	2.311	1.35
	(24.58-40.81)	(35.51-36.00)		
CV1	0.0015±0.0007	-0.0015±0.0010	159.624	0.00
	(0.00-0.00)	(0.00-0.00)		
	GIFT MALAYSIA			
	Male (n=27)	Female (n=25)		
SL	127.40±13.86	122.71±12.82	1.359	0.249
	(91.54-145.92)	(96.98-154.82)		
Wt	49.26±5.66	46.84±5.67	2.208	0.144
	(33.64-57.42)	(37.48-62.01)		
CV1	0.0015±0.0009	-0.0016±0.0011	121.934	0.00
	(-0.0001-0.0015)	(-0.0043-(-0.0002))		
	SEAFDEC			
	Male (n=26)	Female (n=25)		
SL	129.13±14.96	135.73±22.30	0.306	0.583
	(99.90-153.00)	(101.26-186.61)		
Wt	48.99±6.36	52.14±8.64	0.048	0.827
	(36.32-57.79)	(39.22-74.59)		
CV1	-0.0018±0.0009	0.0019±0.00059	316.261	0.00
	(-0.0034-0.0002)	(0.0009-00.3351)		

Table 3. Canonical Variate Analysis (CVA) classification for GET-EXCEL, FaST, GIFT Philippines, GIFT Malaysia and SEAFDEC populations.

A priori* assignment		***A posteriori* assignment**	
		% Correct Classification	**% Misclassification**
Males and Females combined among strains			
	GET-EXCEL	92	7
	FaST	79	20
	GIFT Philippines	74	25
	GIFT Malaysia	72	27
	SEAFDEC	82	17
Between Sexes			
GET-EXCEL			
	Female	100	0
	Male	100	0
FaST			
	Female	100	0
	Male	96	3.85
GIFT Philippines			
	Female	96	3
	Male	96	3
GIFT Malaysia			
	Female	100	0
	Male	100	0
SEAFDEC			
	Female	100	0
	Male	96	3

**A priori* assignments are based on strain and sex to which the specimen belongs while *a posteriori* assignments make use of Mahalanobis-based approach to predict the group membership of each specimen based on CVA.

Results indicate that the head region above and below the eyes can be used to differentiate among strains for both male and female samples. Additionally, the snout can be used to differentiate between strains when dealing with female samples except when differentiating between GET-EXCEL and GIFT Malaysia, further supporting the similarity of shape between these strains. It is possible that the difference observed in the snout region can be attributed to the mouth-brooding characteristic of *O. niloticus* (Tran *et al.,* 2011). The dorsal and ventral base of the caudal fin can be used to differentiate between strains when dealing with male individuals. Differences in the size of caudal fin can affect swimming performance, swimming behavior and routine activity (Plaut, 2000). A high aspect ratio caudal fin is required for steady swimming while a large caudal fin is required for unsteady swimming (Webb, 1982). Also, differences observed may be due to the differences in the growth rates of strains which was observed in Red, GIFT and Supreme strains in the study of dos Santos *et al.* (2013). SEAFDEC can be separated from the rest of the strains using all these characters, showing that this strain is distinct from the rest of the strains being studied.

CONCLUSION

Shape differences were observed among strains of *O. niloticus*. The tip of the snout, insertion of the pelvic fin, ventral base of the caudal fin, and the anterior end of the dorsal fin are traits that can best differentiate one strain from another. Geometric morphometrics is a good tool in delineating strains of important species like *O. niloticus*. This would contribute to better management of this economically important fish.

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