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Short Communication

Body shape variation between farms of tilapia (*Oreochromis* sp.) in Colombian Andes using landmark-based geometric morphometrics

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ABSTRACT. Tilapia is the most economically important fish in the aquaculture of different countries from the Americas. This species exhibits morphological plasticity under different conditions. In this study, we used landmark-based geometric morphometric to describe the shape variation of two red and one Nile tilapia farmed populations in Colombian Andes. We recorded significant morphological differences between all studied farms ($P < 0.001$, in the multivariate analysis of variance). In this way, individuals from the Nile tilapia farms were more elongated and had a more ventral position of the posterior extreme of the orbit and the insertion of pectoral fin than red tilapias. Moreover, the Nile group showed a shorter space between the mouth profile and the posterior extreme of the orbit, compared to red groups. On the other hand, individuals from farm red 2 were deep bodied and had a smaller head compared to tilapias from farm red 1. Our results provide evidence that tilapias from different farms in Colombian Andes display differences in body shape, and can be applied to selective breeding programs after establishing the contribution of genetic and environmental effects on tilapia shape as well as the preferences of consumers for the body shape of the species.

Keywords: tilapia; anatomic landmarks; morphology; variation; aquaculture

Tilapia (*Oreochromis* spp.) is one of the most important fish in aquaculture worldwide (FAO, 2016). Likewise, this species is the most economically important fish in the freshwater production of different countries from the Americas (CONAPESCA, 2013; ACEB, 2014; FAO, 2014). In Colombia, tilapia accounted for 62.5% of national fish farming production. Furthermore, Colombia is the second largest exporter of fresh tilapia fillet to the United States (US). In 2015, US imported 5,329 ton of this product from Colombia, valued at US$44,119,211 (NMFS, 2016).

Variation in the shape of the body has been extensively studied in fish and particularly in Cichlids (Clabaut *et al.*, 2007; Kassam *et al.*, 2007; Kerschbaumer & Sturmbauer, 2011). An essential element of these studies is their focus on natural populations in the context of evolutionary biology. By contrast in aquaculture, the differences in morphology between farmed populations are of great concern for selective breeding because the shape is a commercially important trait that contributes to the market value of the product (Colihueque & Araneda, 2014; de Oliveira *et al*., 2016).

One way to describe the shape quantitatively is by geometric morphometrics (GM) instead of traditional methods based on linear measurements between reference points. Landmark-based GM involves summarizing shape regarding a constellation of discrete anatomical loci, each described by Cartesian coordinates (Webster & Sheets, 2010). Key advantages of GM include: a) emphasis on the complete retention of geometric information throughout the research process, b) much higher statistical power to detect shape differences with sufficient sample sizes, c) localization

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of the spatial morphological variation, and d) visualization of the shape differences directly as illustrations (Zelditch *et al*., 2004; Slice, 2007; Klingenberg, 2013).

The morphological plasticity of tilapia has been characterized previously by GM. For example, Lorenz *et al*. (2014) determined that after an eradication attempt with rotenone, tilapia were deeper in body and head shape than pre-management individuals. Similarly, Ndiwa *et al*. (2016) found variations in the head, caudal peduncle and anal fin base of Nile tilapia from extreme environmental conditions compared to populations experiencing less extreme conditions. These authors also registered morphological differences between populations with similar genetic background. Also, Firmat *et al*. (2012) described that invasive populations of Mozambique tilapia exhibited a more elongated body shape, a shorter caudal peduncle and a more expanded anterior region relative to native populations. Concerning shape changes during growth, Fujimura & Okada (2008) assessed the developmental trajectory that leads to the adult lower jaw shape in Nile tilapia and concluded that differences in adult shapes might be due to differences arising early in development. Differences in shape between lines, farms or rearing conditions have been found previously using GM on aquaculture species such as: European sea bass (*Dicentrarchus labrax*), seabream (*Sparus aurata*), brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) (Costa *et al*., 2010; Vehanen & Huusko, 2011; Pulcini *et al*., 2013; Fragkoulis *et al*., 2016). Therefore, we hypothesized that a phenotypic variable species as tilapia would show differences in shape between separate rearing sites.

In this study, morphological variation between 265 fishes from one Nile (*Oreochromis niloticus*, n = 87) and two red farms (mainly *Oreochromis mossambicus* × *Oreochromis aureus*, red 1 n = 89, red 2 n = 89) was analyzed using landmark-based GM (Fig. 1).

All three farms are land-based aquaculture systems. Red 1 is geographically separated from red 2 by 5.45 km, red 1 from the Nile by 120.65 km and, finally, red 2 from the Nile by 116.5 km. The Nile farm is a raceway system of export-oriented production of fillets. This intensive farm uses a high water flow rate and high stocking densities. Red farms are semi-intensive productions oriented to the national market. In the three farms, the water demand is calculated from a mass oxygen balance, and no supplemental oxygen is used. The farms were chosen according to the following criteria: a) their relative high contribution to tilapia production in Antioquia region (Colombia), b) they had their breeders and hatcheries, and c) they had their processing facilities. Although the broodstocks have been kept in these farms for more than five generations, specific sources and pedigree information was not registered by the farmers. In the three farms, fry sex reversal was accomplished by oral administration of 17α-methyltestosterone.

General methods followed those of Kavembe *et al*. (2016), and the handling procedures followed the section seven of the Aquatic Animal Health Code about the welfare of farmed fish (OIE, 2016). Images of the left side of fish with a scale included were taken after harvesting and before stunning at each one of the processing facilities, using an 18-megapixel EOS 7D digital camera with a 50 mm 1:2.5 lens (Canon USA, Inc.) mounted on a tripod stand. The coordinates of 11 landmarks (Fig. 2) were digitalized in the same order on each image after setting the scale factor using TPSDIG2 v2.30 (Rohlf, 2015). For shape analysis, the data set containing the x-y coordinates was then imported into MORPHOJ v1.06d (Klingenberg, 2011).

In order to translate, rotate and uniformly scale the specimens relative to each other so as to minimize a total sum of squares, a full Procrustes fit and a projection of the data to the tangent space (Dryden & Mardia, 1998) was conducted. Next, an inspection for outliers of the new dataset was performed. A regression analysis with Procrustes coordinates as the dependent variable and log-transformed centroid size as the independent variable with a permutation test against the null hypothesis of independence including 10,000 randomization rounds (Klingenberg, 2016) was carried out to examine the statistical association between size and shape. A canonical variate analysis (CVA) of the covariance matrix of the shape coordinates (Mitteroecker & Bookstein, 2011) was used to assess body shape differences between farmed populations of tilapia.

Shape changes were visualized using a wireframe graph as well as a transformation grid superimposed with their warped outline drawing for each canonical variate. To test the significance of the shape differences between farmed populations, a multivariate analysis of variance (MANOVA) was performed using PAST v3.15 (Hammer *et al*., 2001). A random permutation of individuals testing the significance of each pair-wise Mahalanobis distance among groups with cross-validation was performed to assess the accuracy of the morphometric classification, using the module PAD of the CLIC package (Dujardin, 2008).

The regression analysis with the group centered scores of Procrustes coordinates as the dependent variable, and log-transformed centroid size as an independent variable (Fig. 3) showed: a) superimposition of the values of the majority of the three groups of tilapia examined, b) a high range of values of the regression score for a small range of log-transformed

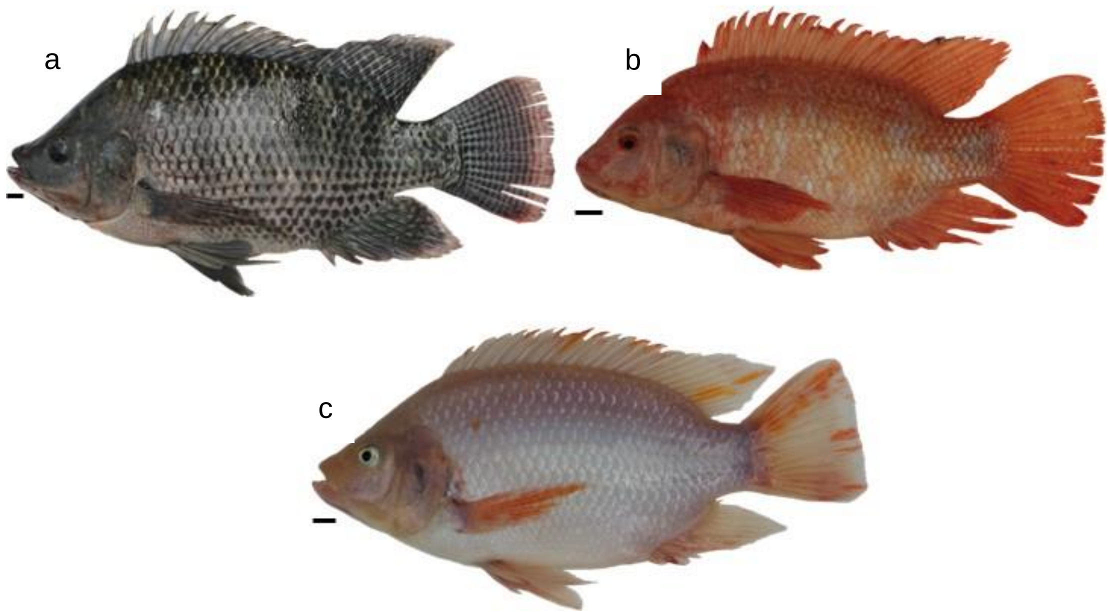

Figure 1. Representative images for each group of tilapia examined. a) Nile, b) red 1, and, c) red 2. Scale bar = 1 cm.

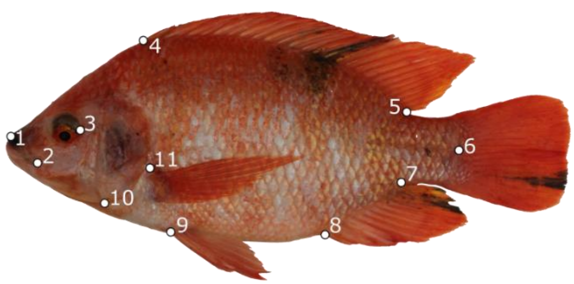

Figure 2. The position of landmarks used in the present study. 1) the intersection between the upper lip and body outline in the nasal-palatine anterior area, 2) most posterior corner of the maxilla when the mouth is closed, 3) the posterior extreme of the orbit, 4) anterior insertion of the first dorsal spine, 5) posterior insertion of the last dorsal ray, 6) last pore of the lateral line, 7) posterior insertion of the last anal ray, 8) anterior insertion of the first anal spine, 9) anterior insertion of the first pelvic spine, 10) upper insertion of pectoral fin, and 11) most ventral corner of interoperculum-suboperculum joint. Image from farm red 2.

centroid sizes for each group of tilapia, c) only 2.28% of shape variation in the tilapias studied covaried with size (*P*-value < 0.0001 at 10,000 permutations), and d) in the present study, a small range of sizes were examined (no fingerlings or juveniles were measured and the range of values of centroid size in Figure 3 was small).

Therefore, only a short section of the growth trajectory was covered by our data. Consequently, no further size correction was applied. The canonical variate analysis displayed a distinct separation between the three tilapia farms. In this analysis, the first and second axes accounted for 70.7 and 29.3% of the total shape variation respectively (Fig. 4). Also, the CVA indicated significant differences among farms of tilapia (*P*-value < 0.0001 in all cases). The Nile group in this defined morphospace was entirely separated by the first CV from the red groups, while red 1 and red 2 were mainly separated along the second CV.

As illustrated by the wireframe graphs and transformation grids, individuals from the Nile farm were more elongated and had a more ventral position of the posterior extreme of the orbit and the insertion of pectoral fin than red tilapias. Moreover, the Nile group showed a shorter space between the mouth profile (defined by the landmarks one and two) and the posterior extreme of the orbit, compared to red groups. On the other hand, individuals from farm red 2 were deep bodied and had a smaller head compared to tilapias from farm red 1. Finally, the MANOVA detected significant differences in shape variables among farms of tilapia (Wilk's lambda: 0.03775, *F*: 56,45; *P* < 0.001) and the cross-validated classification, correctly reassigned 92 and 100% of the individuals from the farms red 1, red 2 and Nile, respectively.

We found that individuals from different farms of tilapia from Antioquia showed significant differences in body shape. Prior research has identified that both environmental and genetic factors influence the body shape of fishes. For example, Crichigno *et al*. (2012) achieved plastic induction of body shape of *Odontesthes*

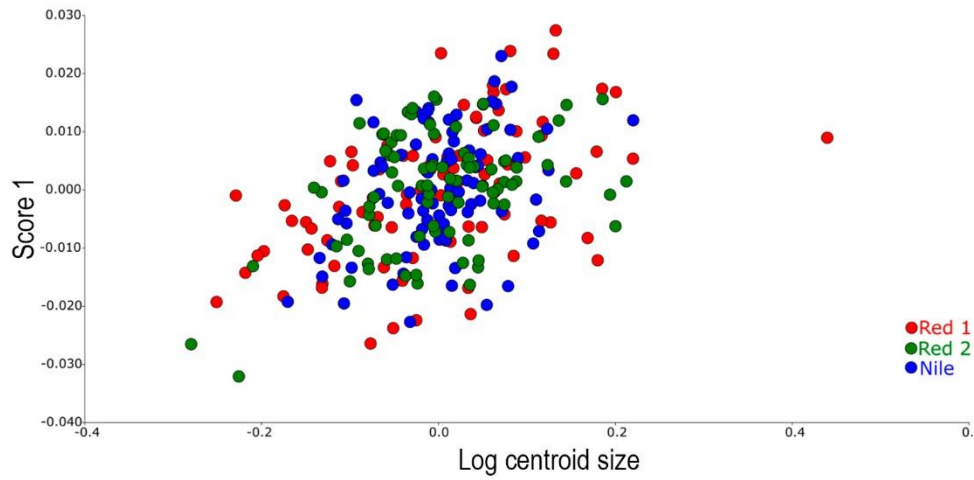

Figure 3. Group-centered scores of the regression between Procrustes coordinates and log-transformed centroid size.

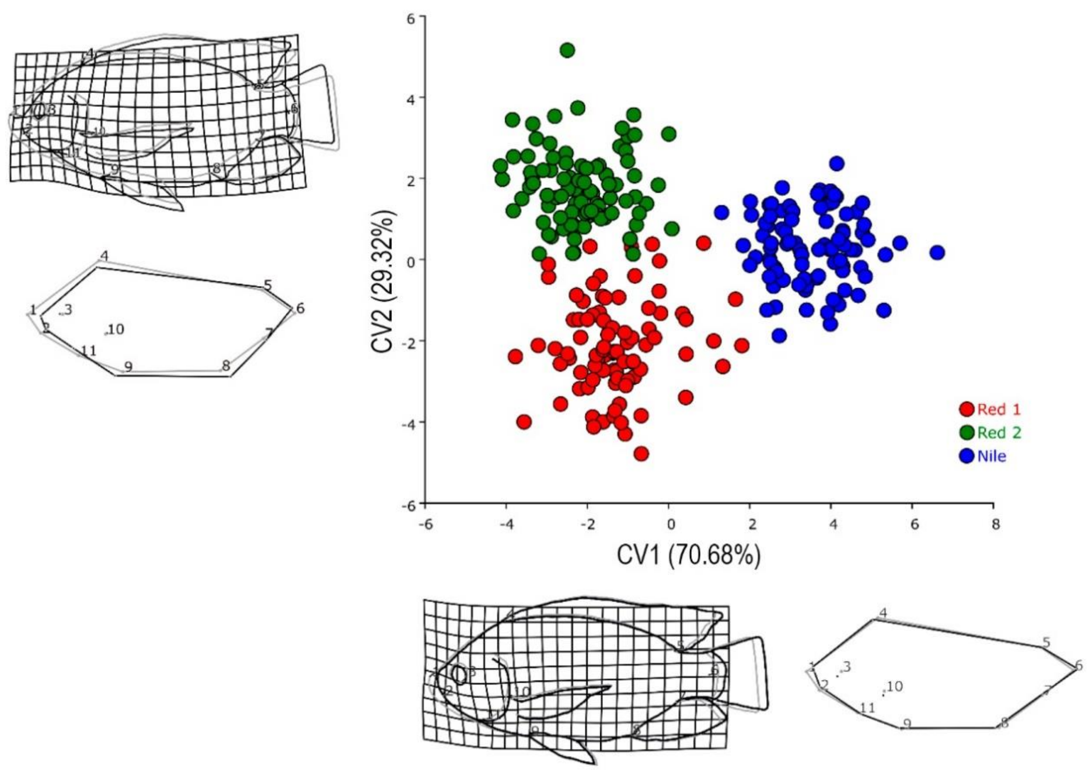

Figure 4. Canonical variate analysis (CVA) of the covariance matrix of the shape coordinates. The axis represents the canonical variates 1 and 2 (CV1 and CV2) with their respective graphs for visualizations of shape changes.

*hatcher*y by manipulation of incubation temperature and diet. In the same fashion, Staszny *et al.* (2013) reared under identical environmental conditions or different diets two inbred lines of *Danio rerio.* These authors found that genetic and environmental factors markedly determined the shape of scales. Divanach & Koumoundouros (2014) concluded that developmental temperature significantly affected the position of the bases of some head bones and fins of *Sparus aurata* juveniles. Environmental factors such as water velocity, depth, rearing density, diet, farming method (*i.e*., pond or cage) and temperature as well as genetics would affect the shape of fishes under culture conditions (Pakkasmaa & Piironen, 2001; Kause *et al*., 2003; Ramler *et al*., 2014). In this way, individuals from the same gene pool but reared in different condi-

Table 1. Water quality of the tilapia farms. Data are shown as mean ± SD; different letters indicate significant ($P < 0.05$) differences among the farms.

Farm	Dissolved oxygen (mg L^{-1})		Oxygen saturation (%)		Temperature (°C)	pH
	Inlet	Outlet	Inlet	Outlet		
Nile	7.16 ± 1.0	5.59 ± 1.49	93.61 ± 10.17	70.75 ± 18.50	24.88 ± 1.35b	7.39 ± 0.60
Red 1	7.5 ± 1.78	6.02 ± 2.12	94.83 ± 25.59	78.41 ± 28.79	27.21 ± 4.46a	7.54 ± 0.43
Red 2	5.81 ± 1.42	4.8 ± 1.95	72.71 ± 16.77	60.43 ± 24.16	26.84 ± 1.12ab	7.71 ± 0.71

Table 2. Water quality of the tilapia farms. Data are shown as mean ± SD; different letters indicate significant ($P < 0.05$) differences among the farms.

Farm	Alkalinity (mg L^{-1})	Phosphate (mg L^{-1})	Ammoniacal nitrogen (mg L^{-1})	Nitrate (mg L^{-1})	Total solids (mg L^{-1})	Dissolved solids (mg L^{-1})
Nile	14.88 ± 9.17a	0.08 ± 0.04	0.70 ± 0.84	1.50 ± 0.58a	91.02 ± 46.38	55.13 ± 27.07a
Red 1	54.38 ± 20.76a	0.10 ± 0	1.14 ± 1.92	3.05 ± 1.32ab	187.63 ± 59.81	132.88 ± 35.38b
Red 2	101.63 ± 9.23b	0.10 ± 0	1.17 ± 2.02	4.10 ± 1.82b	213.63 ± 13.46	145.01 ± 22.80b

tions show differences in body shape (Costa *et al.*, 2010; Vehanen & Huusko, 2011; Fragkoulis *et al.*, 2016). Recently, Montoya-López (*unpubl. data*) characterized the genetic diversity and population structure of the broodstocks from the three farms analyzed in this study using short tandem repeats. This author found that broodstocks from both red farms belonged to a single genetic cluster.

In contrast, the Nile broodstocks formed a separate cluster. However, an important difference between the two red farms was the presence and number of private alleles, particularly in the farm red 2. The Tables 1 and 2 show the water quality of the three tilapia farms evaluated from Betancur *et al*. (2016). Dissolved oxygen, oxygen saturation, pH, phosphate, ammoniacal nitrogen, and total solids, show no significant difference between farms. Conversely, the temperature was significantly lower in the Nile farm than in red 1, alkalinity and nitrate was significantly lower in the Nile than in red 2 and dissolved solids were significantly higher in red 1 and red 2 than in the Nile. However, the contribution of both genetic and environmental effects on tilapia shape remains to be experimentally determined.

We found that Nile individuals were more elongated and had a more ventral position of the posterior extreme of the orbit while individuals from the two red farms were separated by differences in body depth and head shape. These findings are similar to previous studies in tilapia by GM, which identified changes in body depth and head shape as the main variable characteristics (Firmat *et al*., 2012; Lorenz *et al*., 2014). In like manner, Clabaut *et al*. (2007) compared specimens from 45 species of Lake Tanganyika cichlids and concluded that the most important differences in body shape between species were related to body length as well as the proportion of sizes of head and caudal peduncle.

Our results provide clear evidence that tilapias from different farms in Colombia display differences in body shape. This fact can be applied to selective breeding programs after establishing the preferences of consumers for the body shape of tilapia because consumer perceptions and public attitudes toward specific characteristics of shape in this species remain unclear in Colombia. Therefore, future work should include experiments such as progeny tests to clarify the influence of genetics, environment and their interaction in the body shape of this species.

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