Testing spatial and environmental factors to explain body shape variation in the widespread Central American Blackbelt cichlid *Vieja maculicauda* (Teleostei: Cichlidae)

Correspondence:
Diego A. Ardón
diegoardon1991@gmail.com

Diego A. Ardón[1], Caleb D. McMahan[2], Ernesto Velázquez-Velázquez[1] and Wilfredo A. Matamoros[1]

Body-shape variability may respond to the interaction between history and environment, particularly in species whose range includes known biogeographical barriers. Central America has traditionally been regarded to have functioned as a complete land-bridge connecting two continents since the Plio-Pleistocene and as an incomplete one since much earlier. This history has helped shape species diversification and distributions. *Vieja maculicauda* is a widely distributed cichlid, found throughout most of the Atlantic slope of Central America, across three of the four geological blocks that make up this region and whose borders are known dispersal barriers. We asked whether there is an effect of geologic blocks on body shape of *V. maculicauda* as determined by geometric morphometric assessment of 151 individuals from across its range. We asked how much variance in body shape is explained by environmental variables. We used a CVA and a MANCOVA to determine whether a relationship between body shape and geologic blocks exists. For testing body shape correlation against geographic and environmental variables, we used a Mantel test. Our results suggest that body shape in *V. maculicauda* can be segregated by geologic blocks, while the correlation results showed no strong correlation between our sets of variables.

Keywords: Allometry, Biogeographic barriers, CVA, Geological blocks, Mantel Test.

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1 Maestría en Ciencias en Biodiversidad y Conservación de Ecosistemas Tropicales, Instituto de Ciencias Biológicas, Universidad de Ciencias y Artes de Chiapas, Libramiento Norte # 1150, Col. Lajas Maciel, Tuxtla Gutiérrez, Chiapas, C.P. 29039, México. (DAA) diegoardon1991@gmail.com (corresponding author), (EVV) er_velazquez@yahoo.com, (WAM) wilmatamoros@yahoo.com.

2 Field Museum of Natural History, 1400 S. Lake Shore Drive, Chicago, IL 60605 USA. cmcmahan@fieldmuseum.org.

La variabilidad en forma de cuerpo puede responder a la interacción entre historia y medio ambiente, particularmente en especies de amplia distribución cuyo rango incluya barreras biogeográficas conocidas. Tradicionalmente se ha considerado que Centroamérica ha funcionado como un puente terrestre conectando dos masas continentales desde alrededor del Plio-Pleistoceno y como un puente incompleto desde mucho antes. Esta historia ha ayudado a moldear la diversificación y distribución de especies. *Vieja maculicauda* es un cíclido de amplia distribución, encontrado a través de la mayor parte de la vertiente Atlántica de Centroamérica, en tres de los cuatro bloques que componen la región y cuyas fronteras son bien conocidas como barreras de dispersión. Nos preguntamos si hay un efecto del bloque geológico en la forma de cuerpo de *V. maculicauda*, determinado a través de una evaluación de morfometría geométrica de 151 especímenes a lo largo de su distribución. También nos preguntamos cuánta varianza en la forma de cuerpo es explicada por variables ambientales. Utilizamos un AVC y un MANCOVA para determinar si existe una relación entre forma de cuerpo y bloque geológico. Para probar las correlaciones de la forma de cuerpo con variables geográficas y medioambientales utilizamos una prueba de Mantel. Nuestros resultados sugieren que la forma de cuerpo en *Vieja maculicauda* puede ser segregada por bloque geológico, mientras que los resultados de la correlación no mostraron una correlación fuerte entre nuestros sets de variables.

Palabras clave: Alometría, AVC, Barreras biogeográficas, Bloques geológicos, Prueba de Mantel.

INTRODUCTION

Shape variability in organisms is a complex phenomenon and may depend on genetic factors, environmental influence, such as whether the habitat is lentic or lotic and the interaction of these dynamics (Clark, 1976; McMahan *et al*., 2017b; Gómez-González *et al*., 2018). Furthermore, the phenotypic variability of a species across its distributional range may also be driven by a combination of regional and local evolutionary processes. In other words, body shape variability may respond to the interaction between history and environment, particularly in widespread species and those whose range includes known biogeographical barriers (Cardini, Elton, 2009; Cardini *et al*., 2010; Díaz-Murillo *et al*., 2017; Kaya *et al*., 2018; Mendoza *et al*., 2019).

Biogeographically, Central America has functioned as a complete land-bridge connecting the landmasses of two continents since around the Plio-Pleistocene (Mann *et al*., 2007; Bagley, Johnson, 2014; O'Dea *et al*., 2016) and although some authors propose an earlier final closure (Bacon *et al*., 2015; Montes *et al*., 2015), it can be assumed it at least functioned as an incomplete land-bridge for millions of years before that (Gutiérrez-García, Vázquez-Domínguez, 2013; McMahan *et al*., 2013). The formation of the land-bridge involved a series of tectonic events over millions of years with the formation of several barriers between land masses which have helped shape diversification patterns of taxa and the current distribution of species (Hulsey, López-Fernández, 2011; Bagley, Johnson, 2014; Mendoza *et al*., 2019). These barriers have been proposed in many cases as explanatory proxies for the unique composition of freshwater fish species, which is largely characterized by a relative lack of ostariophysan fishes and dominated by the families Poeciliidae and Cichlidae (Myers, 1966; Matamoros *et al*., 2015). Of the latter family, *Vieja maculicauda* (Regan, 1905), is the only northern Middle American cichlid which has dispersed and contributed to lower Middle American assemblages and has therefore been suggested as an important model for gaining a better understanding of the interconnectivity of Central American freshwaters (McMahan *et al*., 2017a).

Geometric morphometric studies have been used to understand body shape variability in cichlids, particularly in the African Great Lakes (Kerschbaumer, Sturmbauer, 2011). In Middle America, geometric morphometric studies on cichlids are scarcer and limited to a few taxa and locations (*e.g*., *Herichthys minckleyi* (Kornfield & Taylor, 1983) Trapani, 2003; Mejía *et al*., 2015; *Amphilophus* spp. Klingenberg *et al*., 2003; Parsons *et al*., 2003; Franchini *et al*., 2014). A study on morphometric differences among populations of *Vieja melanurus* (Günther, 1862) (McMahan *et al*., 2017b), the sister species of *V*. *maculicauda*, and an analysis of *Vieja* Fernández-Yépez, 1969 species in Mexico (Soria-Barreto *et al*., 2011), are the most taxonomically similar publications to the current study. Although studying intraspecific variation across geographic regions holds potential for allowing understanding of how body shape evolves in space and time (Cardini *et al*., 2010), such studies in Middle America remain scarce, which is understandable given that few Middle American cichlid species have such widespread distributions.

Vieja maculicauda is a heroine cichlid native to Middle America with one of the largest distributions in the region, covering most of the Atlantic slope of Central America from the Central River in Belize to the Chagres River in Panama (McMahan *et al*., 2017a). Whereas *V. maculicauda* is found in the middle and lower reaches of rivers throughout its distribution, the species is especially common in coastal systems (*e.g*., estuaries and coastal lagoons). A unique feature of the range of *V. maculicauda* is that the species is found across three of the four geological blocks which make up Central America (Mann *et al*., 2007; McMahan *et al*., 2017a) and whose borders are known to work as dispersal barriers in other freshwater fish species (Perdices *et al*., 2002, 2005; Hulsey, López-Fernández, 2011; Alda *et al*., 2013; Matamoros *et al*., 2015) as well as in other taxa (Gutiérrez-García, Vásquez-Domínguez, 2012; Phillips *et al*., 2016; Mendoza *et al*., 2019). The Polochic-Motagua fault is the region where the Maya and Chortis Blocks meet and acts as the southernmost limit of many freshwater groups, while the San Juan River marks the southern limit for several species in the Honduran province and the northern limit for Costa Rican species, as well as phylogeographical breaks for at least two studied species (Fig. 1; Hulsey, López-Fernández, 2011; Bagley, Johnson, 2014).

In a phylogeographic study based on the mitochondrial cytochrome-b gene, McMahan *et al.* (2017a) reported very little genetic divergence between two geographic clades of *V*. *maculicauda* from Belize through Panama, with clade structure found to be related to the Motagua Fault. The aim of this research was to study patterns of body shape variation across populations of *V. maculicauda* and explore factors that might explain the observed variability. We tried to understand how much of the variance in body shape of *V. maculicauda* along its distribution could be explained by the geographic location of the specimens and environmental factors obtained from a database frequently used in this type of test (Fick, Hijmans, 2017).

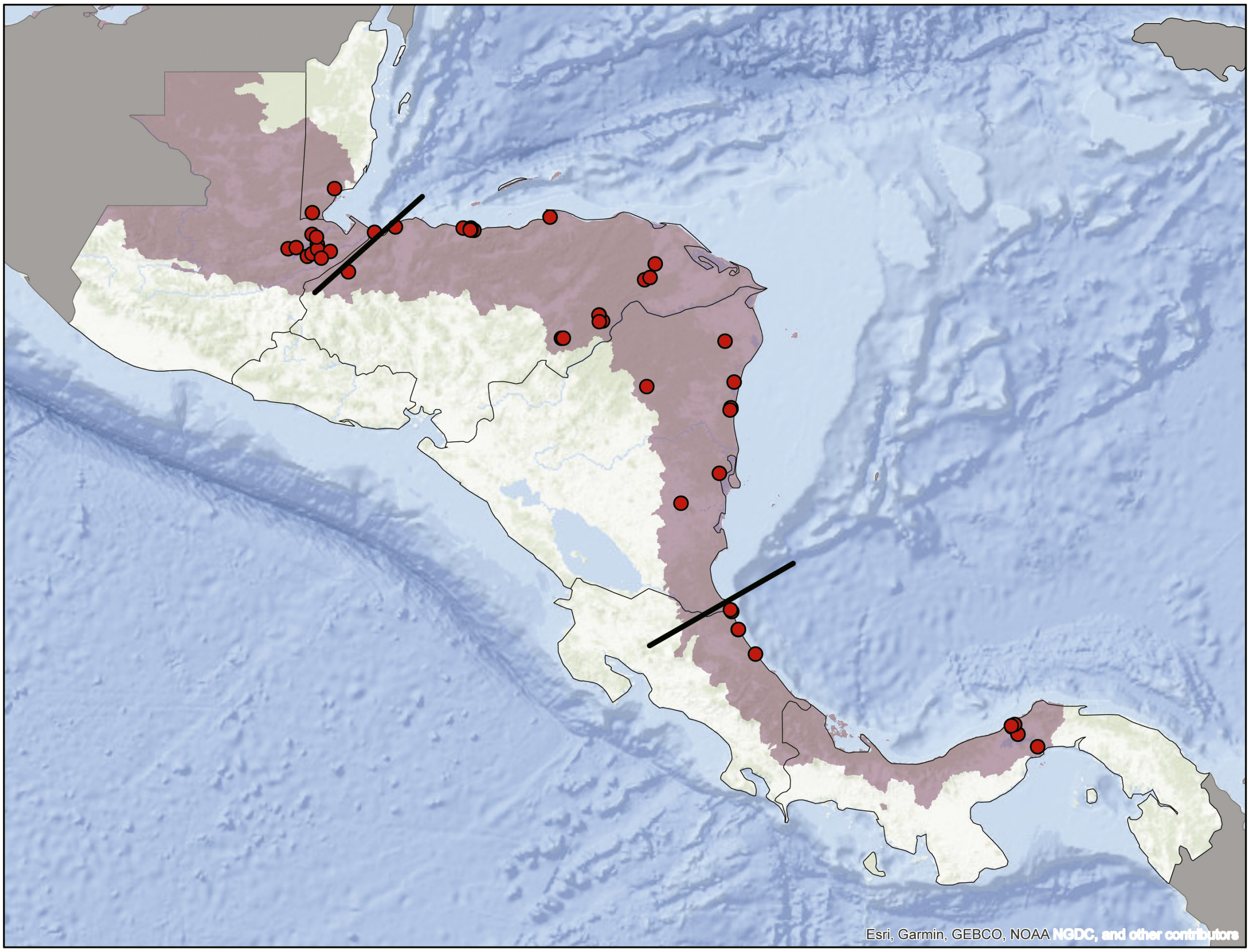

FIGURE 1 | Points representing geographic location for the lots of *Vieja maculicauda* used in the current study. Straight black lines represent the approximate location of geological block divisions. Purple shading represents a modified version of IUCN redlist data for the distribution of this species (Lyons, 2019).

MATERIAL AND METHODS

A total of 151 specimens of *V. maculicauda* were examined from museum collections from sites throughout the species' distribution (Fig. 1). Forty-three came from the Maya Block, 78 from the Chortis Block and 30 from the Chorotega Block. Males and females not distinguished during analysis and most specimens came from lowlands near the river mouths. We used those specimens that were best preserved (*e.g.*, least bent), and photographed them on their left side with a Canon DSLR camera. Next, 17 landmarks (Fig. 2; Tab. **S1**) were digitized using the program tpsDIG (Rohlf, 2010), and 37 semi-landmarks were added using makeFan8 (Fig. 2; Sheets, 2010) to account for shape variation in the contours of the body and head of all individuals. Landmark and semilandmark data were scaled and aligned using bending energy, followed by a General Procrustes Analysis to eliminate the effects of position, scale and rotation in the dataset (Zelditch *et al*., 2012). These procedures were performed with the use of the R 4.0.0 (R Development Core Team, 2020) package geomorph 3.0.6 (Adams *et al*., 2018).

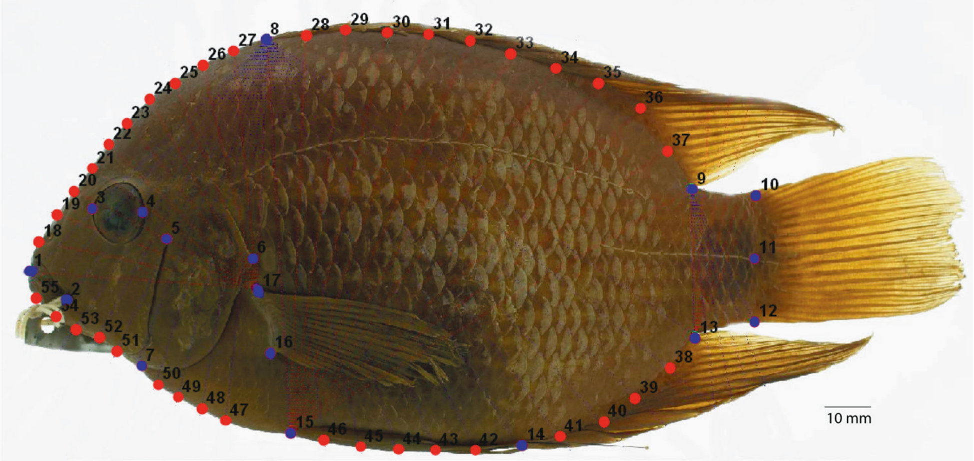

FIGURE 2 | Landmarks (in blue) and semilandmarks (in red) as placed on each specimen. Landmark positions are described on Tab. **S1**.

Institution abbreviation: Field Museum of Natural History, Chicago (FMNH); Louisiana State University Museum of Natural Science, Louisiana (LSUMZ); Royal Ontario Museum, Toronto (ROM); Universidad de Costa Rica, Escuela de Biología, Museo de Zoologia, San Jose (UCR); University of Michigan Museum of Zoology, Michigan (UMMZ); and Museum of Ichthyology, University of Southern Mississippi (USM). In the material examined section the number of specimens used from each lot is shown in parentheses.

Adjustment for allometry and multivariate analysis of covariance. Studies of the life history of *V. maculicauda* have not been published, therefore the size at maturity for this species is unknown to us. To reduce the effect of ontogeny in our sample, from a larger sample with a wider size range, we selected only specimens that were above 75 mm in standard length. Given that our sample sizes ranged from 75–251 mm SL, an assessment of allometry was carried out prior to comparing shape differences among geographic units. A multivariate regression was performed where the logarithm of centroid size of all landmarks and aligned semilandmarks were the independent variables and the Procrustes coordinates of these same landmarks were the dependent variables. Residuals from this regression were used as the set of shape variables for all subsequent analyses to remove allometric effects (Sidlauskas *et al*., 2011). To assess whether body shape in each of the three blocks was significantly different from each other, we used multivariate analysis of covariance (MANCOVA), with shape as the dependent variable, the geologic block membership as the independent variable and size as the covariate variable. Multivariate analysis of covariance was conducted using randomization of null model residuals with 1000 permutations through the function procD.lm of the geomorph package (Adams *et al*., 2018).

Geologic block and body shape. To explore the data in morphospace, a preliminary principal components analysis (PCA) was conducted on the residuals from the size regression to ascertain whether the prediction of body shape differing according to geologic block had a basis before using the more restrictive canonical variate analysis (CVA). The CVA procedure maximizes differences among defined groups and tests the validity of a prior assignment (Zelditch *et al*., 2012). In this case, group assignment was based on the Central American geologic blocks (Fig. 1). The CVA was performed in the R package Morpho 2.8 (Schlager, 2017), with each specimen assigned a classifier according to which of the three geologic blocks it belonged (*i.e*., Maya, Chortis or Chorotega block). Typical probability was calculated using the chi-square distribution of Mahalanobis distances with 10,000 permutations.

Environmental variables as explanation for variation in body shape. For the final objective of quantifying the relationship between shape, geographic and climatological variability, we used a Mantel test (Mantel, 1967). Mantel tests require distance matrices of equal size to test correlation. The morphometric dataset consisted of a triangular matrix derived from the residuals of the size regression shape variables and was first tested against a geographic matrix built from the latitude and longitude of each specimen's source locality. A resulting r value near one indicates that specimens that are geographically close will have similar shapes, while lower values of r indicate low correlation between shape and geographic location of the basin.

Our second Mantel test used the same morphometric distance matrix as before, but tests of correlation were based on a climatic variable matrix generated from Worldclim data (Fick, Hijmans, 2017) and extracted for each specimen through the geographic location of each respective source locality. Prior to running the Mantel test the climatic variables where subjected to a Pearson correlation test and all variables with correlation values above 0.75 were eliminated, reducing the matrix to the following eight variables: Annual Mean Temperature (BIO1), Isothermality (BIO3), Max Temperature of Warmest Month (BIO5), Mean Temperature of Wettest Quarter (BIO8), Mean Temperature of Driest Quarter (BIO9), Mean Temperature of Coldest Quarter (BIO11), Precipitation Seasonality (BIO15), Precipitation of Wettest Quarter (BIO16) and Precipitation of Warmest Quarter (BIO18). The multicollinearity fix for Worldclim data is a common procedure in this kind of study (McMahan *et al*., 2017a; Hallas *et al*., 2021). The Mantel test was carried out using the mantel function of the package vegan using 999 iterations of permutational significance testing (Oksanen *et al*., 2017).

RESULTS

The multivariate regression of shape on size showed that body size explained 10.1% (p=0.001) of shape variation in our data. The MANCOVA analysis which also tested whether body shape was significantly different between the populations of each geologic block showed that this was the case, with block membership explaining 13.8% of the variation (p=0.001), and the interaction of size and block membership explaining 2.2% (p=0.015) (Tab. 1).

Geologic block and body shape. The first two principal components on the residuals from the allometric regression were graphed and coded by the geologic block on which each specimen was collected. A pattern in body shape is shown, particularly in

TABLE 1 | Results for multivariate regression of body shape on size and group membership.

	R^2	Pr(>F)
Size	0.101	0.001
Group membership	0.138	0.001
Size: group	0.0216	0.015
Residuals	0.739	

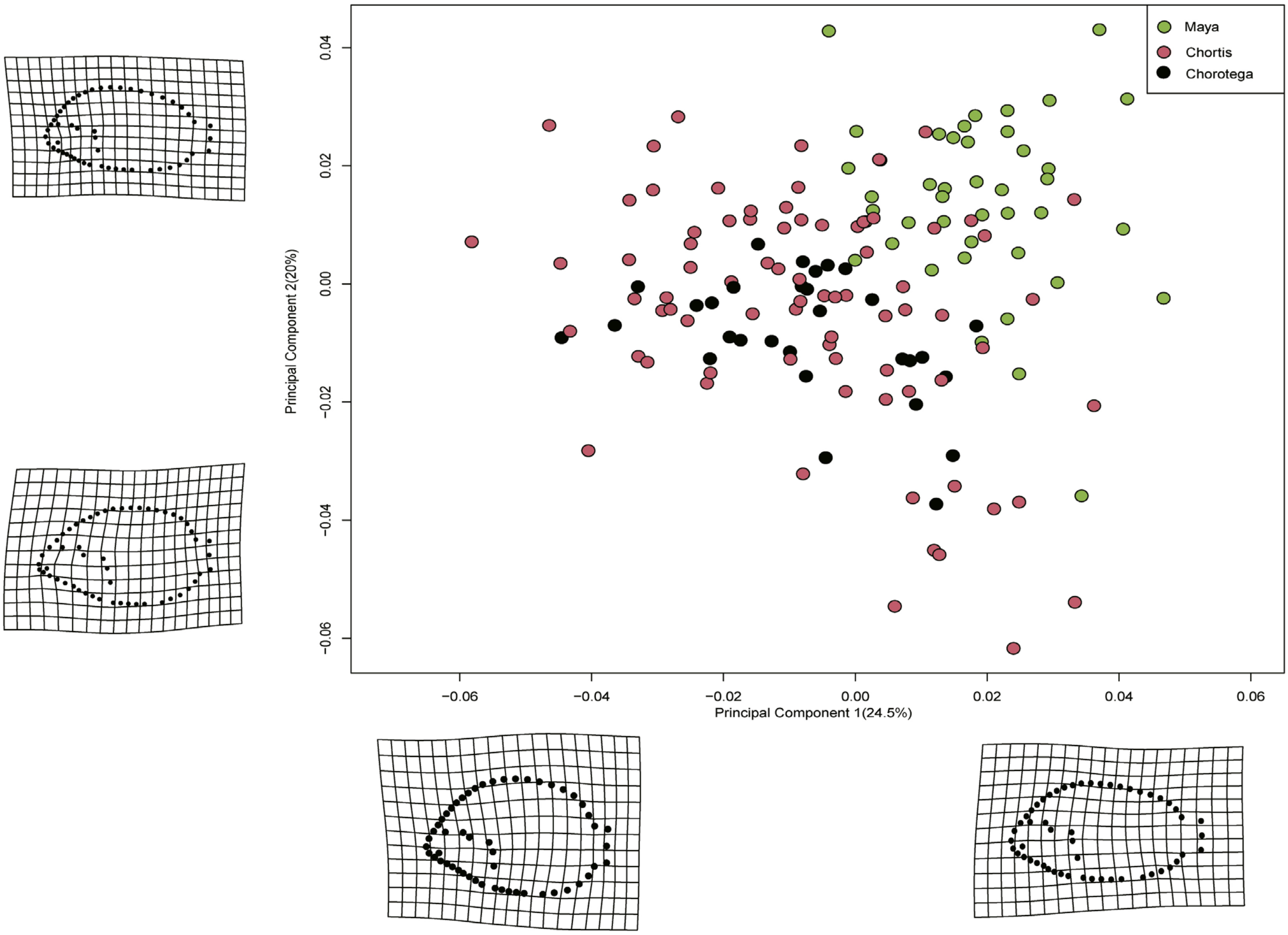

FIGURE 3 | Principal component analysis of size-corrected shape and deformation grids along each axis.

a tendency for individuals of the Maya Block to group towards the positive ends of both PC1 and PC2 (Fig. 3). In body shape, these positive ends represent a longitudinally larger body with a mid-body compression in positive PC1 and changes in the mouth shape and position of internal landmarks representing the opercle, preopercle and pectoral fin insertions in PC2. Individuals from Chorotega Block are completely embedded in those of Chortis Block, and no discernible difference can be made out from these two principal component axes.

TABLE 2 | Cross validation results for group assignment after CVA.

Specimens (%)	Chorotega	Chortis	Maya
Chorotega	30 (100%)	0 (0%)	0 (0%)
Chortis	0 (0%)	78 (100%)	0 (0%)
Maya	0 (0%)	0 (0%)	43 (100%)

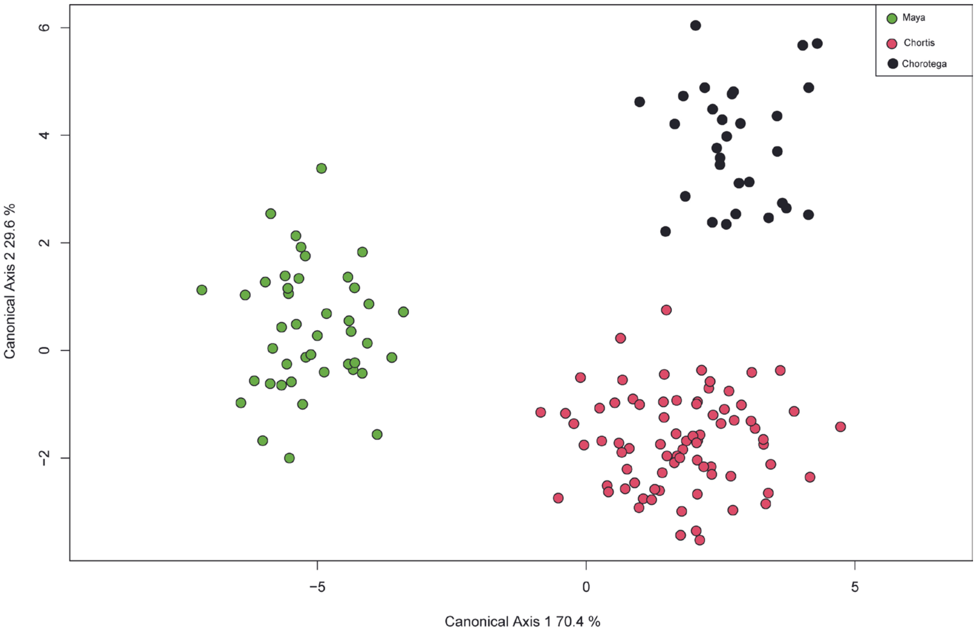

FIGURE 4 | Canonical variate analysis and shape changes along both axes. Shape change has been magnified by two for increased visualization.

The CVA explained 70.4% of variance on its first canonical variate (cv1) and 29.6% on its second canonical variate (cv2, Fig. 4). The cross-validation results indicated an overall classification accuracy of 100% (Tab. 2). Individuals of *V. maculicauda* above 75 mm in standard length can be completely segregated among geologic blocks based on their geometric morphometric measurements.

Geographic environmental covariation with body shape. The results for the Mantel correlation between body shape and geographic location of the basin of origin of the specimen gave an insignificant r value (Mantel r = 0.02708, p = 0.320), and the correlation between body shape differences and differences in environmental data extracted from Worldclim gave a significant result (Mantel r = 0.111, p = 0.004, Tab. 3), but the r value is still low, and the significance is likely an effect of the treatment of the data, which was not merged to a single point per basin. The low r values indicate that high levels of variation in body shape occur between geographically close sites, similarly shaped individuals can be found in distant localities, and that differences in climatological conditions are also not a good explanation for body shape. Local site ecological factors related to habitat type may be better at explaining the variation (McMahan *et al.*, 2017a,b; Gómez-González *et al.*, 2018), but our current dataset is not appropriate for testing this hypothesis.

TABLE 3 | Mantel correlation results.

Matrix 1 (n)	Matrix 2	Mantel r	% explained	p-value
Distance between size-corrected shapes (151)	Geographic distance from latitude and longitude coordinates	0.02708	0.073%	0.320
Distance between size-corrected shapes (151)	Ecological distance from climatic variables extracted from WorldClim	0.111	1.23%	0.004

Material examined. Belize: FMNH 82071 (n=1, 157 mm SL), FMNH 97729 (n=13, 102–173 mm SL), UMMZ 195944 (n=1, 155 mm SL). Guatemala: FMNH 127884 (n=3, 82–94 mm SL), FMNH 127885 (n=2, 77–85 mm SL), FMNH 127886 (n= 2, 79–80 mm SL), FMNH 127887 (n=1, 121 mm SL), FMNH 127888 (n=1, 93 mm SL), FMNH 127889 (n=4, 76–96 mm SL), FMNH 127890 (n=3, 77–87 mm SL), FMNH 127892 (n=7, 78–134 mm SL), FMNH 129603 (n=3, 87–111 mm SL), FMNH 130871 (n=2, 179–232 mm SL), FMNH 134419 (n=1, 144 mm SL), FMNH 5537 (n=2, 176–192 mm SL), LSUMZ 15630 (n=1, 152 mm SL). Honduras: FMNH 84990 (n=2), USM 31767 (n=1, 103 mm SL), USM 35975 (n=2, 96–97 mm SL), USM 43284 (n=1, 143 mm SL), USM 43313 (n=2, 100–115 mm SL), USM 31901 (n=4, 100–165 mm SL), USM 43317 (n=4, 75–85 mm SL), USM 43359 (n=4, 76–86 mm SL), USM 42383 (n=4, 82–85 mm SL), USM 44187 (n=1, 80 mm SL), USM 44213 (n=1, 96 mm SL), USM 44937 (n=3, 75–92 mm SL), LSUMZ 14423 (n=5, 75–91 mm SL), LSUMZ 14455 (n=3, 83–100 mm SL), LSUMZ 14491 (n=3, 94–99 mm SL), LSUMZ 16002 (n=1, 104 mm SL), LSUMZ 16003 (n=2, 110–143 mm SL), LSUMZ 16028 (n=3, 163–191 mm SL), LSUMZ 17181 (n=1, 77 mm SL), LSUMZ 17185 (n=1, 79 mm SL), LSUMZ 17283 (n=1, 161 mm SL), LSUMZ 17291 (n=4, 80–195 mm SL), LSUMZ 17292 (n=3, 80–82 mm SL), LSUMZ 17300 (n=1, 197 mm SL). Nicaragua: UCR 457 (n=2, 90–104 mm SL), UMMZ 188312 (n=2, 167–194 mm SL), UMMZ 196949 (n=2, 190–224 mm SL), LSUMZ 15303 (n=5, 80–222 mm SL), LSUMZ 15317 (n=2, 78–83 mm SL), LSUMZ 15340 (n=1, 75 mm SL), LSUMZ 15351 (n=2, 109 mm SL), LSUMZ 15425 (n=1, 77 mm SL). Costa Rica: ROM 84144 (n=2, 125–196 mm SL), ROM 87729 (n=1, 86 mm SL), UCR 1067 (n=3, 108-139 mm SL), UCR 1083 (n=1, 251 mm SL), UCR 2858 (n=1, 103 mm SL), UCR 991 (n=2, 124–134 mm SL), UCR 605 (n=1, 127 mm SL), UMMZ 180667 (n=3, 88–149 mm SL). Panama: FMNH 29115 (2, 152–154 mm SL), FMNH 29117 (1, 194 mm SL), FMNH 29118 (1, 164 mm SL), FMNH 29119 (6, 78–93 mm SL), FMNH 29152 (1, 96 mm SL), FMNH 36951 (1, 157 mm SL), FMNH 36952 (2, 78–104 mm SL), FMNH 52275 (1, 85 mm SL), FMNH 8091 (1, 92 mm SL).

DISCUSSION

The present study has found that specimens of *Vieja maculicauda* present different shapes that are related to its geographic position among the three geologic blocks that make up its distribution across Central America. Although a size effect in shape in the analyzed sample remains, most of the variation of individuals as they keep growing is due to their differing locations. The exploratory PCA hints at this geographic pattern in body shape in *Vieja maculicauda*. The MANCOVA effectively confirms that body shape differs significantly between geologic blocks. Although separation between these same blocks has been documented for other species at the genetic level (Gutiérrez-García, Vásquez-

Domínguez, 2012; McMahan *et al*., 2017a; Mendoza *et al*., 2019), this is the first time that it has been found in body shape.

The CVA and MANCOVA results suggest that there exists a signature in body shape that correlates with geologic blocks throughout Central America. Our results coincide with the previous molecular study on *V. maculicauda*, which reported subtle differences among populations in relation to the geological blocks of Central America (McMahan *et al*., 2017a), with populations west and north of the Motagua Fault (those in Guatemala and Belize) presenting the most genetic differentiation with respect to populations from the two geologic blocks east of the Motagua Fault. Our results add support to the idea that the Motagua Fault could be working as a biogeographic barrier for gene flow, which in turn is also reflected in body shape. There also exists some difference between populations in the Chortis Block and those in the Chorotega Block although this is minimal.

Correlating body shape with explanatory variables was one of the main aims of this study. We hypothesized that geographic distance between basins might be associated with shape differences, with specimens from geographically close basins sharing greater morphometric similarities than specimens from basins located farther apart. However, the Mantel test between body shape and geographic distance of the matrixes resulted in a very low and insignificant correlation (Mantel r = 0.02708, p-value = 0.320), suggesting that body shape of *V. maculicauda* is variable but that the level of variation is consistent throughout the distribution of the species. These results contrast with body shape variability in sister species *V. melanurus* (McMahan *et al*., 2017b), where two distinct clusters in morphospace were associated with different aquatic habitats and river basins in which the species occurs.

In addition to correlation between body shape variation and geographic distances, we also sought to explain body shape variation in *V. maculicauda* in relation to climatological variables. Overall, the resulting Mantel correlation between body shape and environmental variables suggests a better but still weak relationship (Mantel r = 0.111, p-value = 0.004). There is an available phylogenetic assessment in this species carried out with the mitochondrial gene *cyt b* (McMahan *et al*., 2017a), but its results show low variability across the species range, and it is possible that finer markers (*e.g*., SNPs from genomic data) need to be used to find significant correlations between body shape and genetic factors.

Our study thus concludes that the geographical and available climatological variables have a low to no effect on body shape and that they are not suitable explanations for the variation in body shape across the distribution of this fish. The hypothesized high explanatory power of geography was rejected and evidence from these results suggests that the variables most related to body shape variation in *V. maculicauda* remain to be identified. One possibility is that our classifiers are not suitable for explaining more of the observed variability in body shape, which might be better explained by more localized ecological variation and processes including river morphology factors such as mean annual run-off or other river characteristics (Haas *et al*., 2015), or aspects of trophic ecology (Rüber, Adams, 2001; Franssen *et al*., 2013) as suggested for other fishes. A second possibility is simply a combination of phylogenetic constraint on body shape in this species and habitat conservatism, with *V. maculicauda* occurring in relatively similar types of habitats throughout its large distribution. Future work should be aimed at carrying out a drainage specific study where *V. maculicauda* can be captured along different locality points in the river continuum and where quantitative ecological variables can be directly collected. Analyses of body shape and correlation tests with these variables and empirical environmental data have the potential to possess a higher explanatory power than the ones used in this study.

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REFERENCES

- **Adams DC, Collyer ML, Kaliontzopoulou A.** Geomorph: Software for geometric morphometric analyses. R package version 3.0.6; 2018. Available from: https://cran.r-project.org/package=geomorph
- **Alda F, Reina RG, Doadrio I, Bermingham E.** Phylogeny and biogeography of the *Poecilia sphenops* species complex (Actinopterygii, Poeciliidae) in Central America. Mol Phylogenet Evol. 2013; 66(3):1011–26. http://dx.doi.org/10.1016/j.ympev.2012.12.012
- **Bacon CD, Silvestro D, Jaramillo C, Smith BT, Chakrabarty P, Antonelli A.** Biological evidence supports an early and complex emergence of the Isthmus of Panama. Proc Natl Acad Sci USA. 2015; 112(19):6110–15. https://doi.org/10.1073/pnas.1423853112
- **Bagley JC, Johnson JB.** Phylogeography and biogeography of the lower Central American Neotropics: diversification between two continents and between two seas. Biol Rev. 2014; 89(4):767–90. https://doi.org/10.1111/brv.12076
- **Cardini A, Elton S.** Geographical and taxonomic influences on cranial variation in red colobus monkeys (Primates, Colobinae): Introducing a new approach to 'Morph' Monkeys. Global Ecol Biogeogr. 2009; 18(2):248–63. https://doi.org/10.1111/j.1466-8238.2008.00432.x
- **Cardini A, Diniz-Filho JAF, Polly PD, Elton S.** Biogeographic analysis using geometric morphometrics: Clines in skull size and shape in a widespread African Arboreal Monkey. In: Elewa AMT, editor. Morphometrics for Nonmorphometricians. Lecture notes in Earth Sciences. Berlin: Springer; 2010. p.191–217.
- **Clark WC.** The environment and the genotype in polymorphism. Zool J Linn Soc. 1976; 58(3):255–62. https://doi.org/10.1111/j.1096-3642.1976.tb00831.x
- **Díaz-Murillo BP, Ruiz-Campos G, Piller KR, McMahan CD, García-De-León FJ, Camarena-Rosales F.** Assessing population-level morphometric variation of the Mountain Mullet *Agonostomus monticola* (Teleostei: Mugilidae) across its Middle American distribution. Neotrop Ichthyol. 2017; 15(4):e170036. https://doi.org/10.1590/1982-0224-20170036

- **Fick SE, Hijmans RJ.** WorldClim 2: new 1km spatial resolution climate surfaces for global land areas. Int J Climatol. 2017; 37(12):4302–15.
- **Franchini P, Fruciano C, Spreitzer ML, Jones JC, Elmer KR, Henning F *et al*.** Genomic architecture of ecologically divergent body shape in a pair of sympatric crater lake cichlid fishes. Mol Ecol. 2014; 23(7):1828–45. https://doi.org/10.1111/mec.12590
- **Franssen NR, Harris J, Clark SR, Schaefer JF, Stewart LK.** Shared and unique morphological responses of stream fishes to anthropogenic habitat alteration. Proc R Soc Lond B Biol Sci. 2013; 280(1752):20122715. https://doi.org/10.1098/rspb.2012.2715
- **Gómez-González AE, Álvarez F, Matamoros WA, Velázquez-Velázquez E, Schmitter-Soto JJ, González-Díaz A *et al*.** Redescription of *Vieja hartwegi* (Taylor & Miller 1980) (Teleostei: Cichlidae) from the Grijalva River basin, Mexico and Guatemala, with description of a rheophilic morph. Zootaxa. 2018; 4375(3):371–91. https://doi.org/10.11646/zootaxa.4375.3.5
- **Gutiérrez-García TA, Vázquez-Domínguez E.** Biogeographically dynamic genetic structure bridging two continents in the monotypic Central American rodent *Ototylomys phyllotis*. Biol J Linn Soc Lond. 2012; 107(3):593–610. https://doi.org/10.1111/j.1095-8312.2012.01966.x
- **Gutiérrez-García TA, Vázquez-Domínguez E.** Consensus between genes and stones in the biogeographic and evolutionary history of Central America. Quaternary Res. 2013; 79(3):311–24. https://doi.org/10.1016/j.yqres.2012.12.007
- **Haas TC, Heins DC, Blum MJ.** Predictors of body shape among populations of a stream fish (*Cyprinella venusta*, Cypriniformes: Cyprinidae). Biol J Linn Soc Lond. 2015; 115(4):842–58. https://doi.org/10.1111/bij.12539
- **Hulsey CD, López-Fernández H.** Nuclear Central America. In: Albert JS, Reis RE, editors. Historical biogeography of Neotropical freshwater fishes. Berkeley: University of California Press; 2011. p.279–91.
- **Kaya A, Gharakhloo MM, Coşkun Y.** Geographic variation in the skull morphology of *Ellobius lutescens* Thomas, 1897 (Mammalia: Rodentia) by geometric morphometric analyses. Vertebr Zool. 2018; 68(2):157–64. Available from: https://www.senckenberg.de/wp-content/uploads/2019/07/04_vertebrate_zoology_68_2_kaya_et_al_157-164.pdf
- **Kerschbaumer M, Sturmbauer C.** The utility of geometric morphometrics to elucidate pathways of cichlid fish evolution. Int J Evol Biol. 2011; 2011(290245):1–08. https://doi.org/10.4061/2011/290245
- **Klingenberg CP, Barluenga M, Meyer A.** Body shape variation in cichlid fishes of the *Amphilophus citrinellus* species complex. Biol J Linn Soc. 2003; 80(3):397–408. https://doi.org/10.1046/j.1095-8312.2003.00246.x
- **Lyons TJ.** *Vieja maculicauda*. The IUCN Red List of threatened species 2019: e.T192924A2179918. https://dx.doi.org/10.2305/IUCN.UK.2019-2.RLTS.T192924A2179918.en. Accessed on 18 March 2022.
- **Mann P, Rogers RD, Gahagan L.** Overview of plate tectonic history and its unresolved tectonic problems. In: Bundschuh J, Alvarado GE, editors. Central America: Geology resources hazards. Leiden: Taylor & Francis; 2007. p.205–41
- **Mantel N.** The detection of disease clustering and a generalized regression approach. Cancer Res. 1967; 27(2):209–20. Available from: https://cancerres.aacrjournals.org/content/canres/27/2_Part_1/209.full.pdf
- **Matamoros WA, McMahan CD, Chakrabarty P, Albert JS, Schaefer JF.** Derivation of the freshwater fish fauna of Central America revisited: Myers's hypothesis in the twenty-first century. Cladistics. 2015; 31(2):177–88. https://doi.org/10.1111/cla.12081
- **McMahan CD, Davis MP, Domínguez-Domínguez O, García-de-León FJ, Doadrio I, Piller KR.** From the mountains to the sea: phylogeography and cryptic diversity within the mountain mullet, *Agonostomus monticola* (Teleostei: Mugilidae). J Biogegr. 2013; 40(5):894–904. https://doi.org/10.1111/jbi.12036

- **McMahan CD, Ginger L, Cage M, David KT, Chakrabarty P, Johnston M *et al*.** Pleistocene to Holocene expansion of the black-belt cichlid in Central America, *Vieja maculicauda* (Teleostei: Cichlidae). PLoS ONE. 2017a; 12(5):e0178439 https://doi.org/10.1371/journal.pone.0178439
- **McMahan CD, Kutz J, Murray C, Chakrabarty P, Geheber AD, Elías D.** Objectively measuring subjectively described traits: geographic variation in body shape and caudal coloration pattern within *Vieja melanurus* (Teleostei: Cichlidae). Rev Biol Trop. 2017b; 65(2):623–31. http://dx.doi.org/10.15517/rbt.v65i2.25500
- **Mejía O, Pérez-Miranda F, León-Romero Y, Soto-Galera E, Luna E.** Morphometric variation of the *Herichthys bartoni* (Bean, 1892) species group (Teleostei: Cichlidae): how many species comprise *H. labridens* (Pellegrin, 1903). Neotrop Ichthyol. 2015; 13(1):61–76. https://doi.org/10.1590/1982-0224-20140067
- **Mendoza AM, Bolívar-García W, Vázquez-Domínguez E, Ibáñez R, Parra-Olea G.** The role of Central American barriers in shaping the evolutionary history of the northernmost glassfrog, *Hyalinobatrachium fleischmanni* (Anura: Centrolenidae). PeerJ. 2019; 7:e6115. http://doi.org/10.7717/peerj.6115
- **Montes C, Cardona A, Jaramillo C, Pardo A, Silva JC, Valencia V *et al.*** Middle Miocene closure of the Central American seaway. Science. 2015; 348(6231):226–29. http://www.doi.org/10.1126/science.aaa2815
- **Myers GS.** Derivation of the freshwater fish fauna of Central America. Copeia. 1966; 1966(4):766–73. http://dx.doi.org/10.2307/1441405
- **O’dea A, Lessios HA, Coates AG, Eytan RI, Restrepo-Moreno SA, Cione AL *et al.*** Formation of the Isthmus of Panama. Sci Adv. 2016; 2(8):e1600883. http://www.doi.org/10.1126/sciadv.1600883
- **Oksanen JF, Blanchet G, Friendly M, Kindt R, Legendre P, McGlinn D *et al*.** Vegan: Community Ecology Package. R package Version 2.4-3; 2017. Available from: https://CRAN.R-project.org/package=vegan
- **Parsons KJ, Robinson BW, Hrbek T.** Getting into shape: An empirical comparison of traditional truss-based morphometric methods with a newer geometric method applied to New World cichlids. Environ Biol Fish. 2003; 67:417–31. https://doi.org/10.1023/A:1025895317253
- **Perdices A, Doadrio I, Bermingham E.** Evolutionary history of the Synbranchid eels (Teleostei: Synbranchidae) in Central America and the Caribbean Islands inferred from their molecular phylogeny. Mol Phylogenet Evol. 2005; 37(2):460–73. https://doi.org/10.1016/j.ympev.2005.01.020
- **Perdices A, Bermingham E, Montilla A, Doadrio I.** Evolutionary history of the genus *Rhamdia* (Teleostei: Pimelodidae) in Central America. Mol Phylogenet Evol. 2002; 25(1):172–89. https://doi.org/10.1016/S1055-7903(02)00224-5
- **Phillips JG, Deitloff J, Guyer C, Huetteman S, Nicholson KE.** Biogeography and evolution of a widespread Central American lizard species complex: *Norops humilis*, (Squamata: Dactyloidae). BMC Evol Biol. 2016; 15(143):1–13. https://doi.org/10.1186/s12862-015-0391-4
- **R Development Core Team.** R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2020. Available from: https://www.R-project.org/
- **Rohlf FJ.** tpsDig: Digitize Landmarks and Outlines from Image Files, Scanner or Video, Ver 2.15. New York: Stony Brook; 2010.
- **Rüber L, Adams DC.** Evolutionary convergence of body shape and trophic morphology in cichlids from Lake Tanganyika: Morphometrics of eretmodine cichlids. J Evol Biol. 2001; 14(2):325–32. https://doi.org/10.1046/j.1420-9101.2001.00269.x
- **Schlager S.** Morpho and Rvcg - Shape analysis in R. In: Zheng G, Lin S, Székely G, editors. Statistical shape and deformation analysis: Methods, implementation and applications. Massachusetts: Academic Press; 2017. p.217–56.
- **Sheets HD.** MakeFan 1: Buffalo. Canisius College; 2010. Available from: www3.canisius.edu/~sheets/morphsoft.html

- **Sidlauskas BL, Mol JH, Vari RP.** Dealing with allometry in linear and geometric morphometrics: A taxonomic case study in the *Leporinus cylindriformis* group (Characiformes: Anostomidae) with description of a new species from Suriname. Zool J Linn Soc. 2011; 162(1):103–30. https://doi.org/10.1111/j.1096-3642.2010.00677.x
- **Soria-Barreto M, Rodiles-Hernández R, González-Díaz AA.** Morfometría de las especies de *Vieja* (Cichlidae) en ríos de la cuenca del Usumacinta, Chiapas, México. Rev Mex Biodivers. 2011; 82(2):569–79.
- **Trapani J.** Geometric morphometric analysis of body-form variability in *Cichlasoma minckleyi*, the Cuatro Cienegas cichlid. Environ Biol Fish. 2003; 68:357–69. https://doi.org/10.1023/B:EBFI.0000005763.96260.2a
- **Zelditch ML, Swiderski DL, Sheets HD.** Geometric morphometrics for biologists: A Primer. London: Academic Press; 2012.

AUTHORS’ CONTRIBUTION

Diego A. Ardón: Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing-original draft, Writing-review and editing.

Caleb D. McMahan: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Visualization, Writing-review and editing.

Ernesto Velázquez-Velázquez: Formal analysis, Methodology, Supervision.

Wilfredo A. Matamoros: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Writing-review and editing.

ETHICAL STATEMENT

The present work did not require specific collection permits since all specimens belong in museum collections.

COMPETING INTERESTS

The authors declare no competing interests.

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