COMPARATIVE MORPHOMETRICS OF *ATHERINELLA BRASILIENSIS* (QUOY & GAIMARD, 1824) AND *ATHERINELLA BLACKBURNI* (SCHULTZ, 1949) (ACTINOPTERYGII: ATHERINIFORMES, ATHERINOPSIDAE) IN TODOS OS SANTOS BAY, STATE OF BAHIA, NORTHEASTERN BRAZIL

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Resumo

A diferenciação morfométrica entre duas espécies de peixes aterinopsídeos ocorrentes na baía de Todos os Santos, estado da Bahia, costa nordeste do Brasil, *Atherinella brasiliensis* (Quoy & Gaimard, 1824) e *Atherinella blackburni* (Schultz, 1949), foi analisada por morfometria geométrica e técnicas de estatística multivariada. As diferenças de forma e as mudanças ontogenéticas foram examinadas usando as coordenadas de 10 marcos anatômicos obtidos em 30 espécimes de cada espécie, alinhadas pela técnica de Procrustes, como variáveis de forma. A análise de regressão das variáveis de forma no tamanho do centroide foi utilizada para examinar padrões de variação intra- e interespecífica. A análise de componentes principais das variáveis de forma mostrou que os indivíduos de *A. blackburni* são diferentes daqueles de *A. brasiliensis* quanto ao primeiro componente principal, que foi relacionado a um alongamento longitudinal global do corpo. A maior parte da alometria ontogenética ocorre ao longo do eixo ântero-posterior, sendo definida por uma tendência a um corpo mais hidrodinâmico em *A. brasiliensis*. As alterações ontogenéticas ao longo do eixo dorsoventral correspondem principalmente a um aumento na região póstero-caudal em *A. blackburni*. Estes resultados revelam diferenças significativas na forma entre *A. blackburni* e *A. brasiliensis*, que parecem estar associadas a preferências de habitat distintas (com *A. blackburni* ocorrendo em praias arenosas e *A. brasiliensis* em águas estuarinas), conforme observado em campo.

Palavras-Chave: peixes estuarinos, ecologia, morfologia, taxonomia, morfometria geométrica.

Abstract

Morphometric differentiation between two species of atherinopsid fishes occurring in Todos os Santos Bay, state of Bahia, northeastern coast of Brazil, *Atherinella brasiliensis* (Quoy & Gaimard, 1824) and *Atherinella blackburni* (Schultz, 1949), was analyzed by geometric morphometrics and multivariate statistical techniques. Shape differences and ontogenetic changes were examined using the Procrustes-aligned coordinates of 10 anatomical landmarks taken on 30 specimens of each species as shape variables.

Regression analysis of the shape variables on centroid size was used to examine patterns of intra- and interspecific variation. Principal components analysis of the shape variables showed that the individuals of *A. blackburni* are different from those of *A. brasiliensis* along the first principal component, which was related to an overall longitudinal elongation of the body. Most of the ontogenetic allometry occurs along the anteroposterior axis, being defined by a trend towards a more streamlined body in *A. brasiliensis*. Ontogenetic changes along the dorsoventral axis mostly correspond to an increase in the posterior-caudal region in *A. blackburni*. These results reveal significant differences in shape between *A. blackburni* and *A. brasiliensis,* which seem to be associated with distinct habitat preferences (with *A. blackburni* occurring on sandy beaches and *A. brasiliensis* in estuarine waters), as observed in the field.

Keywords: estuarine fishes, ecology, morphology, taxonomy, geometric morphometrics.

Introduction

The genus *Atherinella* Steindachner, 1875 belongs to the order Atheriniformes within the class Actinopterygii, encompassing a diverse group of ray-finned fishes commonly known as silversides. *Atherinella* species are primarily found in marine, estuarine, and freshwater habitats, in the temperate and tropical regions of the Americas, often forming large schools, a behavior that enhances their safety from predators and aids in foraging efficiency.

In Brazil, two species are commonly recorded: the Blackburn's silverside, *Atherinella blackburni* (Schultz, 1949), which attains a maximum size of 130 mm in length and is recorded in the western Atlantic from Belize to the state of São Paulo (southeastern Brazil), living on sandy substrate on the surf zone (Lopes and Oliveira-Silva, 2001; Mattox *et al.*, 2008; Gonzalez and Vaske Junior, 2017); and the Brazilian silverside, *Atherinella brasiliensis* (Quoy & Gaimard 1824), which attains a maximum size of 160 mm in length and occurs in the western Atlantic from Venezuela to the state of Rio Grande do Sul (southern Brazil), thriving in habitats characterized by muddy substrate, in brackish or low salinity waters of coastal lagoons, river mouths and in protected areas of the open sea (Chagas and Costa Junior, 2013; Mattox *et al.*, 2008).

Besides these differences in average size range and ecology, these species are quite similar and share several morphological features; both have a similar streamlined body shape, with dorsal and anal fins positioned posteriorly, forked caudal fin, and a distinctive silvery lateral stripe (Chernoff, 2002).

The taxonomic status of these species is still uncertain. Chernoff (1986, 2002) consider both *A. brasiliensis* and. *A. blackburni* as valid species, but other authors (Dyer, 2006; Figueiredo and Menezes, 1978; Menezes *et al.*, 2003) do not recognize the validity of *A. blackburni*. On the other hand, *A. blackburni* is recognized by a number of authors: Lopes and Oliveira-Silva (2001) recorded the presence of *A. blackburni* in the coast of Bahia State (northeastern Brazil) and Mattox *et al.* (2008) and Gonzalez and Vaske Junior (2017) found *A. blackburni* in the coast of

São Paulo State (southeastern Brazil). Using random amplified polymorphic DNA (RAPD) markers, Da Silva Cortinhas *et al.* (2010) found low genetic diversity between two subpopulations of *A. brasiliensis* in southern Brazil, but later Da Silva Cortinhas *et al.* (2016) observed very high differentiation among populations of this species in the southeastern and northeastern Brazilian coast, using both RAPD and mitochondrial (D-loop) markers Ribeiro and Molina (2013) detected marked karyotypic differentiation between *A. blackburni* and *A. brasiliensis* in the northeastern coast of Brazil.

There have been no comparative studies of the patterns of variation in the body shape of these species using quantitative methods, which contributes to the uncertainty surrounding their taxonomic status in the Brazilian coast. The aim of the present study was to analyze the differences in body shape and compare allometric relations of *A. blackburni* and *A. brasiliensis* in northeastern Brazil, using geometric morphometrics techniques, which allow the combined use of multivariate statistical methods and methods for the direct visualization of changes in biological form (Bookstein, 1991).

MATERIALS AND METHODS

Landmark-based geometric morphometric methods (Bookstein, 1991; Rohlf and Marcus, 1993; Adams *et al.*, 2004, 2013) were used to quantify and compare overall body shape in the species under study. These methods allow quantifying the shape of organisms from the coordinates of homologous landmarks, after the effects of non-shape variation (i.e. orientation, position and scale) are removed. Differences in shape among organisms can then be visually displayed as deformation grids depicting the changes in shape from one organism to another.

Cartesian coordinates were obtained from 10 anatomical landmarks defined on the external morphology (Fig. 1 and Fig. 2) of 30 specimens of *A. brasiliensis* and 30 specimens of *A. blackburni*, from images taken of the left side of each individual with a Sony DSLR-A 200K (Alpha) digital camera with a resolution of 10.2 megapixels, using the tpsDig2 v2.31 (Rohlf, 2015).

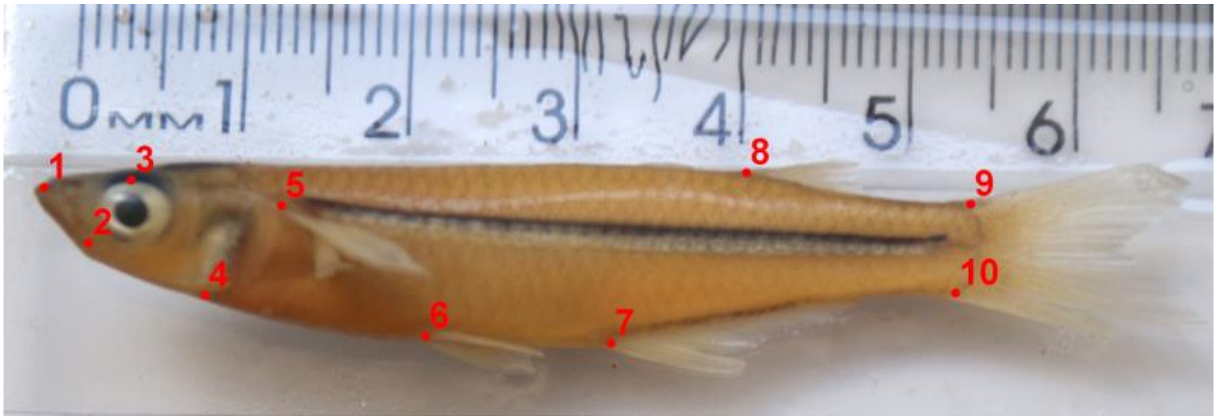

Figure 1 – Anatomical landmarks (numbered) for *Atherinella brasiliensis*

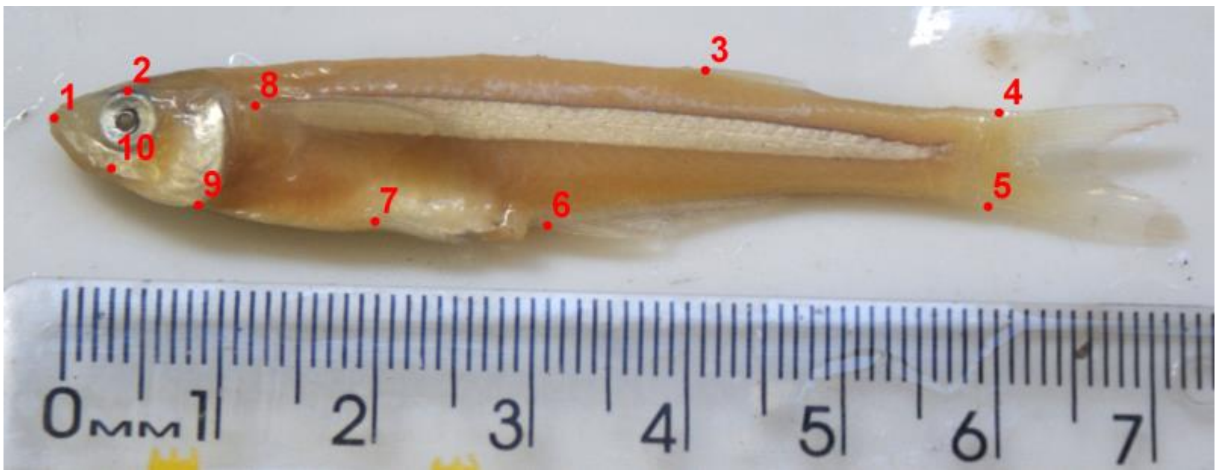

Figure 2 – Anatomical landmarks (numbered) for *Atherinella blackburni*

The specimens analyzed come from six localities around the Todos os Santos Bay, state of Bahia, northeastern Brazil, and are housed at the Fish Division, Museum of Zoology (Department of Biological Sciences at the State University of Feira de Santana). Encompassing an area of more than 1,200 km^2, the Todos os Santos Bay it is the largest bay in Brazil, and contains about 90 islands, Itaparica Island near its entrance being the largest of them (Cirano and Camargo, 2007). The oceanographic characteristics of the Todos os Santos Bay show a clear difference between the coastal waters, bathed by the Atlantic Ocean, and the inland waters of the bay, with large variations in temperature (which can be 3°C) and salinity (which can reach more than 4 ppm) between the inner and coastal parts (Cirano and Lessa, 2007). All specimens of *A. blackburni* were collected at Ponta da Ilha Beach, located in the southeastern of Itaparica Island. This beach is not inland within the Todos os Santos Bay, but rather at its entrance. Thus, it is directly bathed by the Atlantic Ocean. Ponta da Ilha Beach has no rocks, being exclusively a sandy-bottomed beach. The specimens of *A. brasilensis*, in turn, were collected on muddy-bottomed beaches inland within the Todos os Santos Bay, or in locations on Itaparica Island where mangroves existed or still exist.

Collection localities, geographic coordinates, and number of specimens sampled are listed in Table 1 and shown on the map of Figure 3.

Table 1 – Locality, geographic coordinates, and number of specimens of the six samples of *Atherinella brasiliensis* and *A. blackburni* included in this study.

Species	Locality	Longitude	Latitude	Number of specimens
A. brasiliensis	Acupe	38°45' W	12°39' S	2
	Cabuçu	38°46' W	12°46' S	4
	Cacha-Pregos	38°47' W	13°7' S	1
	Itapema	38°45' W	12°41' S	8
	Maragogipe	38°55' W	12°46' S	15
A. blackburni	Ponta da Ilha	38°46' W	13°7' S	30

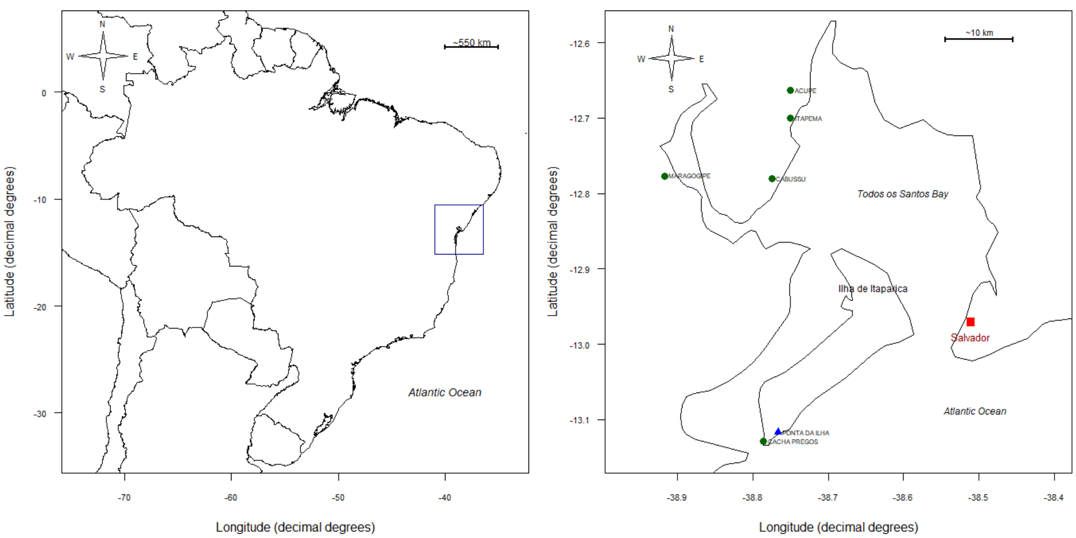

Figure 3 – Map of the six samples of *A. brasiliensis* and *A. blackburni* analyzed in this study (full circle = *A. brasiliensis*, full triangle = *A. blackburni*).

The geometric size of each specimen was estimated by the logarithm of centroid size, which is the square root of the sum of the squared distances from each landmark to the location on the individual that minimizes that sum (Bookstein, 1991). For each specimen, the coordinates were aligned by the generalized Procrustes method of least squares (Rohlf and Slice, 1990) and were treated as shape variables in all subsequent statistical analyses. Allometric shape variation (caused by size differences) was tested by linear regression analysis of the Procrustes-aligned coordinates on log-transformed centroid size (Sokal and Rohlf, 1995). The statistical significance of the regression model was tested by analysis of variance (ANOVA) from 10,000 random permutations of the original data set. Principal components analysis of the shape variables

(Morrison, 2005) was used to assess overall patterns of body form variation in the two species. The shape changes described by each principal axis were visualized using thin-plate spline (TPS) deformation grids, produced for means of each species and compared with a consensus configuration representing the average across all specimens, to facilitate biological interpretation of the observed shape differences.

All morphometric analyses were performed with the software environment for statistical computing and graphics R v4.2.1 (R Development Core Team, 2022) using routines in the package geomorph v4.0.6 (Adams and Otarola-Castillo, 2013, Baken *et al.*, 2021).

RESULTS

The projection of the regression scores of the individuals of *A. blackburni* and *A. brasiliensis* along centroid size suggests that the change in shape in these species is allometric, that is, it shows a dependence on the shape in relation to the size (Fig. 4). Most of the ontogenetic changes in shape in both species occur along the longitudinal axis in the posterior region of the body, which can be characterized by an increase in the height of the posterior part of the body in relation to the head region, with a trend towards a more streamlined body in *A. brasiliensis*. Ontogenetic changes along the dorsoventral axis, mostly correspond to an increase in the posterior-caudal region in *A. blackburni*. In both species, the larger individuals have a relatively taller body and a relatively smaller head (Fig. 4, lower right). Analysis of variance showed statistically significant differences in shape with size ($P < 0.001$) between *A. brasiliensis* and *A. blackburni* (Tab. 2).

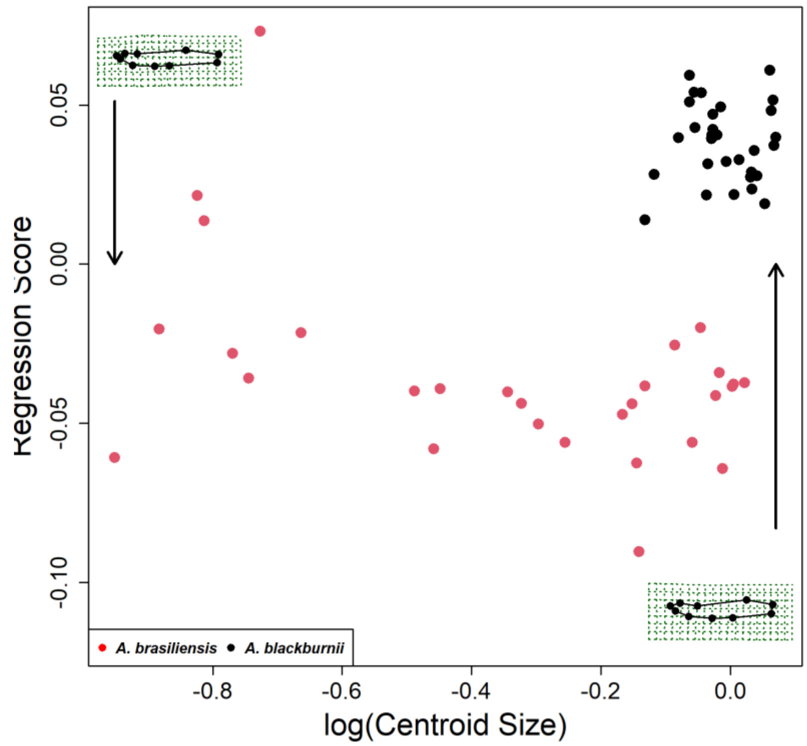

Figure 4 – Scatter diagram of *Atherinella brasiliensis* and *A. blackburni* regression scores of shape in relation to size (ontogenetic allometry). Deformation diagrams of allometric changes in *Atherinella brasiliensis* (upper) and *A. blackburni* (lower) are shown for a small (above) and a large (below) specimen.

Table 2 – Results of Procrustes regression for size covariation on body shape (allometry) of *Atherinella* species. Df, degrees of freedom; SS, sums of squares; MS, mean squares; Rsq, R-squared (coefficient of determination); F, F-values; Z, Z-values or standard score (standard deviations from the means); Pr(>|F|): probability to find F-values by chance.

	Df	SS	MS	Rsq	F	Z	Pr(>F)
log(Csize)	1	0.018642	0.018642	0.06372	5.6834	2.8961	0.0014**
species	1	0.088251	0.088251	0.30165	26.9058	5.1674	1e-04***
log(Csize):species	1	0.001988	0.001988	0.00679	0.6061	-0.5780	0.7198
Residuals	56	0.183681	0.003280	0.62784			
Total	59	0.292562					

The first principal component extracted from the shape variables accounted for 49% of the total variation, whereas the second principal component explained about 15.6%. The projection of the individual scores on the morphospace defined by the first two principal components shows that the two species are almost completely discriminated along the x-axis, to an overall longitudinal elongation of the body (Fig. 5).

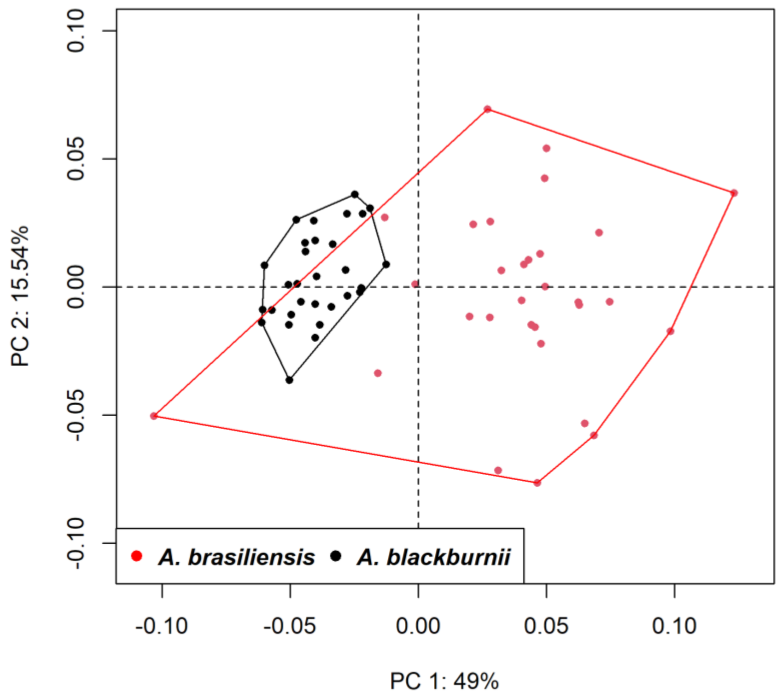

Figure 5 – Scatterplot of the individual scores of *Atherinella* species on the first two principal components

DISCUSSION

The results of the present study indicate that these two species of atherinopsid fishes, although morphologically very similar, can be clearly distinguished by their body shape and changes in shape with size during growth (ontogenetic allometry) using geometric morphometric analyses. These findings do agree with the marked differences in habitat occupation and karyotypic variation between *A. blackburni* and *A. brasiliensis* observed by other authors (Lopes and Oliveira-Silva, 2001; Mattox *et al.*, 2008; Ribeiro and Molina, 2013). The two species also differ in body coloration, with *A. blackburni* specimens having an orange tail and greenish tones on the body, while *A. brasiliensis* has a more hyaline and uniform coloration (Lopes and Oliveira-Silva, personal observation). On the other hand, the most recent revision of the South American atherinopsids (Dyer, 2006), although recognizing *Atherinella brasiliensis*, does not even mention

A. blackburni (which may not be a valid species but a variation of *A. brasiliensis*). The ecological segregation and karyoptic variation observed between the two species could suggest an incipient speciation process (Mayr, 1977), inasmuch as, although these species overlap in part of their geographic range, they show a distinct separation in terms of habitat, with *A. blackburni* occurring on exposed sandy beaches and *A. brasiliensis* being associated with muddy bottoms, mangrove areas, and estuarine environments. We suggest that the findings reported here may inform future studies on the taxonomic status of *A. blackburni*.

Additional analyzes with a larger number of population samples throughout their range will be necessary to further elucidate the patterns of body shape variation and to better evaluate the possible morphometric differences occurring between these species.

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