

Shape and size variation of *Jenynsia lineata* (Jenyns 1842) (Cyprinodontiformes: Anablepidae) from different coastal environments

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Abstract A key question in ecological speciation is to understand the causes and consequences of phenotypic divergence among populations. In this work, we analyzed the body shape and size variation in *Jenynsia lineata* across different coastal habitats along the Atlantic coast of South America. We hypothesized that *J. lineata* presents morphological variations to inhabit contrasting environments and that these adaptations are sex specific. We analyzed 13 populations from five coastal habitats, using linear and geometric morphometry, and tested the correlation of body shape variation with environmental variables to understand which environmental factors may influence body shape and size variation. *Jenynsia lineata* showed differences in body shape and size among populations,

and these differences are specific to each sex. While females showed a variation in the caudal peduncle correlated with water current, we did not find such trait variation and correlation in males. Alternatively, individuals from marine rocky pools have a convex body curvature along the dorsal profile and larger body sizes, in both sexes. With these results, we describe the shape and size morphological variation of *J. lineata* and discuss this uncommon habitat-dependent sexual dimorphism in a Neotropical livebearer fish.

Keywords Geometric and linear morphometrics · *Jenynsia multidentata* · Local adaptation · Phenotypic plasticity · Sexual dimorphism

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Introduction

Morphological variation in populations of fish inhabiting divergent environments provides important examples of the processes related to evolution (Langerhans & DeWitt, 2004; Haas et al., 2010; Torres-Dowdal et al., 2012; Theis et al., 2014; Rowiński et al., 2015). Different environments across a species' distribution range may generate an array of distinct selection regimes, which could promote adaptive variation among populations (Townsend et al., 2008), driving plastic responses and/or genetic adaptive divergence (Crispo, 2008). In the absence of other evolutionary mechanisms and constraints, these individuals with local trait variation would have, on average, a higher relative fitness in their local environment than individuals from other habitats (Kawecki & Ebert, 2004).

A key question in the research about ecological speciation is to understand the causes and consequences of phenotypic divergence among populations. In fishes, some environmental conditions have been recognized as important causes of morphological variation and are often related to locomotion (Haas et al., 2010; Theis et al., 2014; Gaston & Lauer, 2015; Lauder, 2015) or feeding (Langerhans et al., 2004; Gomes & Monteiro, 2008; Araújo et al., 2014; Zanella et al., 2015; Ingleby et al., 2016). Another common observation is that fishes show convergence in body shape when populations occur along similar environmental gradients, allowing the elaboration of an eco-morphological paradigm for the correlation between body shape and environmental selective pressure (Langerhans et al., 2004). For example, different fish species tend to have a more compressed body shape (longer dorsal–ventral axis and shorter anterior–posterior axis) in populations of lentic water environments compared to those of lotic environments, where body shape is typically more fusiform (Haas et al., 2010; Gaston & Lauer, 2015). Such an eco-morphological paradigm has also been established for different predation pressures. In this case, populations of phylogenetically or geographically distinct species feature a pattern of variation in the body shape, according to the presence or absence of predators, that is related to the ability of predator escape (burst-swim) (Langerhans et al., 2004).

The one-sided livebearer *Jenynsia lineata* (Jenyns, 1842) is one of the most abundant brackish and

freshwater fish in the subtropical regions of South America (Garcia et al., 2004; Goyenola et al., 2011) and has recently been recognized as a senior synonym of *J. multidentata* (Jenyns, 1942) (see Amorim, 2018). This species is omnivorous (Bastos et al., 2017), with viviparous reproduction, and shows a remarkable sexual dimorphism whereby females are larger than males, while males feature a copulatory organ (gonopodium) formed by a modification of the anal fin (Betito, 2006; Lopez-Rodriguez et al., 2017). Popularly known as 'barrigudinho' (Brazil), 'overito', or 'madrecita' (Argentina and Uruguay), *J. lineata* is widely distributed and occurs in a variety of habitats, such as estuaries (Ramos & Vieira, 2001; Garcia et al., 2004; Mai et al., 2007; Bastos et al., 2017), coastal washouts (Bastos et al., 2013), lagoons (Fontoura et al., 1994), freshwater streams (Volcan et al., 2012; Corrêa et al., 2015), and marine rocky pools (Calviño & Alonso, 2016). This variability of environments, associated with its abundance, makes *J. lineata* an interesting organism to understand local adaptation to Neotropical coastal habitats.

Heterogeneous aquatic environments, such as coastal habitats, have long been in the focus of research related to adaptive divergence in fish, and body variation is often correlated with a salinity gradient characteristic for such habitats (Norris et al., 2010; Olsen et al., 2016; Dennenmoser et al., 2017). In *J. lineata*, for example, specimens are larger in brackish populations than in those from freshwater environments (Fontoura et al., 1994; Mai et al., 2005). Other Cyprinodontiformes, such as *Poecilia vivipara* (Bloch & Schneider, 1801) (Gomes & Monteiro, 2008; Araújo et al., 2014) and *Gambusia affinis* (Baird & Girard, 1853) (Langerhans et al., 2004), or even fishes from other orders, such as *Clupea harengus* (Linnaeus, 1758) (Clupeiformes) (Jørgensen et al., 2008) or *Gasterosteus aculeatus* (Linnaeus, 1758) (Gasterosteiformes) (Marchinko & Schluter, 2007; Berner et al., 2008, 2009; Baker et al., 2015; Foster et al., 2015), show a similar variation in body size and shape along salinity gradients. Different environments are an important source of divergent natural selection, and adaptation to those habitats may, under some circumstances, lead to speciation (see, e.g., Schluter, 2009; Nosil, 2012). To understand the dynamics of adaptation to different habitats, it is important to characterize morphological variation at the level of individuals and

populations as well as at ecological scales (Shukla & Bhat, 2017).

An important issue when one is interested in understanding the contribution of habitat environments to body shape variation is to consider the patterns of sex differences in body shape (Mokodongan et al., 2018). Since body shapes could be specific to each sex, species with remarkable sexual dimorphism might show males and females with distinct habitat-related body shape variation. In such cases, trade-offs between natural and sexual selection often underlie the diversification of sexes (Heinen-Kay et al., 2015), especially because evolutionary forces that have shaped the breeding success of males are fundamentally different from those acting on females (Bronson, 1985). Species with marked sexual dimorphism are the primer evidence that sexual selection is a strong force in the evolution of this intraspecific divergence (Kocher, 2004). However, habitat choice can also contribute to adaptive correlations between phenotype and environment (Porter & Akcali, 2018). In this context, *J. lineata* seems to be an adequate species to investigate inter- and intrapopulation body variation and the phenotype versus habitat correlations in Neotropical coastal environments, since this organism shows a remarkable sexual dimorphism and inhabits distinct ecosystems across its distributional range.

In this study, we investigated the body shape and size variation in *J. lineata* across different coastal habitats along the Atlantic coast of South America. For this purpose, we analyzed, through linear and geometric morphometrics approaches, 13 populations of *J. lineata* from five different coastal ecosystems. Geometric morphometrics is based on landmark coordinates, permitting the exploration and visualization of large high-dimensional data sets (Mitteroecker & Gunz, 2009). Linear measurements, or traditional morphometrics, involve summarizing morphology in terms of length measurements, ratios, or angles (Webster & Sheets, 2010). In general, the geometric morphometrics approach provides better insights into the underlying functional relationships than linear traditional measurements (Sidlauskas et al., 2011; Fabre et al., 2014). However, the combination of linear measurements and geometric morphometrics should be used in harmony to yield the most complete understanding of morphology (Ginter et al., 2012). Here, we used geometric morphometrics to evaluate

the variation in body shape and size (through centroid size, a composite size measure based on all landmarks (Mitteroecker et al., 2013)), with the aim to investigate the phenotypic variation in *J. lineata* among distinct habitats. We used linear measurements to improve the size analyses and to obtain information regarding the size of specific structures of the body (such as fins and body lengths), not available from geometric morphometrics data. We then related body shape with environmental variables to understand which environmental factors could influence body shape variation. Beyond, we investigated how each sex responds morphologically to the habitat variation. With this study, we aimed to determine how a fish species with remarkable sexual dimorphism could be phenotypically adapted to distinct environments. Specifically, we wanted to understand whether *J. lineata* presents phenotypic variation between environments, what varies in body size and shape, which environmental factors could be associated with such variation, and if males and females present the same responses. Our hypothesis was that *J. lineata* should present morphological adaptation to inhabit contrasting environments, such as marine versus freshwater or lotic versus lentic habitats, and that adaptation is sex specific: due to the morphological size and shape distinction between sexes, males and females would show distinct morphological adaptation, even considering the same habitat.

Materials and methods

Study area

We analyzed shape and body variation of *J. lineata* from five different environments: coastal washout, estuary, freshwater stream, lagoon, and marine rocky pool (Fig. 1a, Table 1). The coastal washouts, estuaries, and lagoon populations were in the coastal plain of Rio Grande do Sul, Brazil. This large plain (~ 620 km long) is characterized by a rectilinear coastline, extensive dune fields, and numerous lagoons (including the Patos-Mirim system) and others water bodies, which may temporarily be connected through small channels (Castelao & Moller-Jr, 2006).

The coastal washouts, locally known as ‘*sangradouros*’, are freshwater streams (creeks) that cross the dune belt toward the beach. They play an important

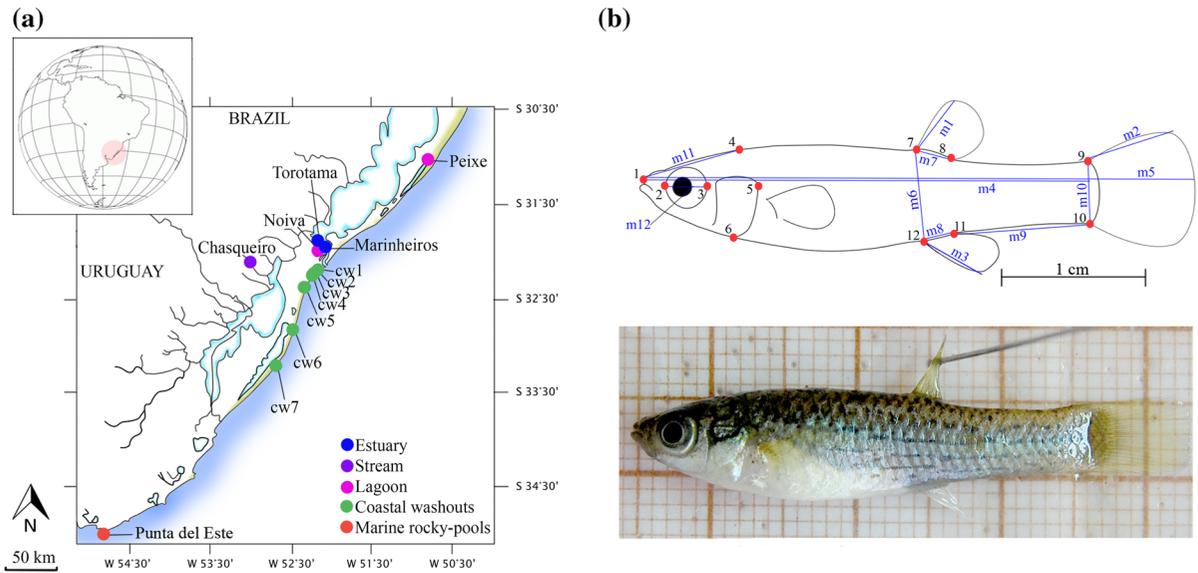


Fig. 1 Sampling sites (a) and landmark positions and linear measurements of *Jenynsia lineata* in left lateral view (b). Landmarks (red dots): 1 snout anterior margin upper jaw; 2 eye anterior most margin; 3 eye posterior most margin; 4 supraoccipital process posterior margin; 5 dorsal margin of gill opening; 6 ventral margin of gill opening; 7 dorsal fin origin; 8 dorsal fin base posterior margin; 9 caudal fin base dorsal margin;

10 caudal fin base ventral margin; 11 anal fin base posterior margin; 12 anal fin origin. Linear measurements (blue lines): m1 dorsal fin length; m2 caudal fin length; m3 anal fin length; m4 standard length; m5 total length; m6 body depth; m7 dorsal fin width; m8 anal fin width; m9 ventral width of caudal peduncle; m10 caudal peduncle depth; m11 head length; m12 eye diameter

role in the drainage of pluvial water from swamps located behind the frontal dune systems (Figueiredo & Calliari, 2006). These washouts have specific spatial and temporal dynamics with varying levels of salinity (Gandara-Martins et al., 2014). In this study, we sampled fish from seven coastal washouts in the supralittoral region (near/between dunes), a zone under the effect of sea spray that can be inundated by ocean water during extreme events (high tides or storms) (Gianuca, 1998). The distance between the sampled washouts varied from approximately 2 km (between the coastal washouts 1, 2, 3, and 4) to more than 48 km (between the coastal washouts 5, 6, and 7) (the distance between washouts 4 and 5 was approximately 20 km) (see Fig. 1a). Note that these distances are not strict, as the coastal washouts change their course depending on the season (Figueiredo & Calliari, 2006). The substrate in the washouts was dominated by fine sand, and submerge and emergent macrophytes were observed in these water bodies.

Located at coastal margins, estuarine habitats are exposed to both fresh- and salt water, depending on the tides, land drainage, wind, and local morphology, thereby showing a great variation in salinity (Scanes

et al., 2017). In this study, we sampled two estuarine populations along the coast of the Torotama and Marinheiros Islands, located in the Patos Lagoon. On Torotama Island, sampling was performed along the island coast, an open beach region without vegetation and with sandy substrate. On the Marinheiros Island, we sampled along a small channel (approximately 100 m long) near to the island coast, a region characterized by muddy substrate with vegetation (including trees) along the channel borders.

On Marinheiros Island, we also sampled a second population of *J. lineata* from a shallow lagoon, known as Noiva Lagoon. This lagoon is in the middle of the island, behind sand dunes, and is constituted by freshwater, sandy to muddy substrate, and the typical vegetation found in shallow lakes. This environment can vary in size according to climatic conditions, with low water levels during the dry season (summer) and temporal connections with adjacent small and shallow lakes during the rainy season (winter) (Quintela et al., 2009, 2018). We also sampled in a second lagoon, called Peixes Lagoon, with shallow water depths (average of 30 cm, except on the channels) (Loebmann & Vieira, 2005); in this lagoon, the northern part (limnetic region) was sampled.

Table 1 Environmental parameters (mean ± SD) and number of females and males analyzed for each population

Environment	Population	Females	Males	pH	OD (mg/ml)	Sal (ppm)	Temp (°C)	Depth (cm)	Micro (mg/s)	Super (m/s)	Substrate
Coastal washouts (CW)	CW-1	55	12	8.0 ± 0.1	8.9 ± 1.9	0.3 ± 0.0	22.9 ± 0.8	100.0 ± 3.8	0.9 ± 0.2	0.1 ± 0.0	Sandy
	CW-2	25	10	8.0 ± 0.0	8.4 ± 1.2	0.3 ± 0.0	23.4 ± 0.1	8.9 ± 5.5	6.2 ± 0.8	0.3 ± 0.0	Sandy
	CW-3	15	11	8.1 ± 0.0	9.6 ± 1.7	0.2 ± 0.0	22.2 ± 0.0	21.0 ± 7.0	5.6 ± 0.1	0.4 ± 0.0	Sandy
	CW-4	24	7	8.0 ± 0.1	10.1 ± 2.6	0.2 ± 0.0	22.8 ± 0.1	29.0 ± 9.2	5.8 ± 1.3	0.2 ± 0.0	Sandy
	CW-5	21	14	7.3 ± 0.1	10.6 ± 2.6	0.1 ± 0.0	19.9 ± 0.0	53.0 ± 8.6	4.5 ± 0.7	0.3 ± 0.0	Sandy
	CW-6	11	10	7.4 ± 0.1	11.2 ± 3.1	0.1 ± 0.0	18.7 ± 0.7	-	2.6 ± 0.5	~ 0	Sandy
	CW-7	39	16	-	-	-	-	-	-	-	-
Estuary	Torotama Island	22	19	7.0 ± 0.1	11.0 ± 0.4	0.0 ± 0.0	16.3 ± 0.1	36.0 ± 3.8	3.5 ± 0.9	0.2 ± 0.0	Sandy
	Marinheiros Island	20	11	6.5 ± 0.1	5.2 ± 0.6	0.0 ± 0.0	18.4 ± 0.1	32.0 ± 1.9	0.5 ± 0.4	~ 0	Moody
Lagoon	Noiva Lagoon	46	17	7.9 ± 0.3	10.0 ± 0.3	0.0 ± 0.0	18.9 ± 0.7	84.0 ± 7.2	1.2 ± 0.9	0.1 ± 0.0	Moody
	Peixe Lagoon	36	36	8.0 ± 0.3	10.4 ± 0.7	0.1 ± 0.0	21.3 ± 0.1	33.0 ± 2.6	1.3 ± 0.1	0.1 ± 0.0	Moody
Stream	Chasqueiro Stream	17	14	6.1 ± 0.3	10.4 ± 0.9	0.0 ± 0.0	18.6 ± 0.2	120 ± 23.5	1.4 ± 0.6	0.1 ± 0.0	Moody
	Punta del Este	26	20	8.8 ± 0.1	9.9 ± 0.4	21.3 ± 0.6	19.6 ± 1.1	31.0 ± 16.5	1.1 ± 0.5	~ 0	Rocky

Environmental variables for CW-7 population were not available for analyses, and also depth for CW-6

OD oxygen dissolved, Sal salinity, Temp water temperature, Depth depth of water column, Micro microhabitat water current, Super superficial water current

A freshwater stream population was sampled in the Sul-Riograndense shield, South Brazil. The sampled stream is called Chasqueiro and belongs to the Mirim Lagoon hydrographic system. It is located in the city Arroio Grande, where it is used as freshwater reservoir. Our sampling was performed upstream of the reservoir, a region that has an average width of about 8 m and a depth of about 30 cm, with a strong water current (lotic environment) (Corrêa et al., 2015).

Additionally, a marine rocky pool population was sampled in Uruguay, near the port of Punta del Este (Maldonado, Uruguay). This population was described as the first record of *J. lineata* in a truly marine environment (Calviño & Alonso, 2016). At that location, *J. lineata* inhabits shallow rocky pools that have no connection to freshwater.

Sampling and data acquisition

Thirteen populations of *J. lineata* ($N = 554$, females = 357, males = 197) were sampled between January 2016 and February 2017, using a beam trawl and hand nets (see Table 1 for details). In the laboratory, the individuals were anesthetized by immersion in clove oil solution and digital images were taken in a standardized way. Specifically, photographs of the lateral left side of each living organism were taken using a digital camera (Nikon® D90 or P600) mounted at approximately 50 cm. The specimens were positioned on a graph paper, their fins were extended, and the dorsal and anal fins were pinned.

Digital images were converted into .tps files, using the tpsUtil 1.64 software (Rohlf, 2013). For each specimen, 12 landmarks and 12 linear measurements (Fig. 1b) were recorded using the software tpsDIG2 2.30 (Rohlf, 2015). Linear measurements were recorded between some landmarks (LM1–LM4: estimation to head size; LM7–LM8: dorsal fin width; LM11–LM12: dorsal fin width; LM10–LM11: ventral width of caudal peduncle; LM9–LM10: caudal peduncle depth) and between landmarks and the extremities of the anal, caudal, and dorsal fins (LM12, LM9, and LM7, respectively). To avoid bias related to the acquisition of landmarks and linear measurements, the same person performed all processes (GXP). We used only adult specimens (standard length > 19 mm, according to Garcia et al., 2004) and avoided pregnant females (identified visually).

Environmental parameters were recorded at each sampling location. Dissolved oxygen, pH, salinity, and water temperature were measured using a multi-parameter water quality checker (Horiba®, model U50). Surface water current and microhabitat current were determined according to Theis et al. (2014), with the following modifications: surface water current was estimated by measuring the time a 500 ml plastic bottle filled with 250 ml of water travelled 10 m. Microhabitat current was estimated considering the level of the water current. To this end, we used lollipops (Florestal® Flopito Baby, strawberry flavor, individually wrapped) to measure the relative rate of dissolution. Prior to the measurement, each lollipop was precisely weighted. For the measurements, four lollipops were mounted underwater for 6 min (using a fixed line), while one was exposed, for the same period, to water taken from the same location, but left in a beaker filled with 500 ml. This control treatment was performed to determine the baseline dissolution rate for each site, without water current. After recovery, the lollipops were dried at ambient temperature for at least 24 h and then weighted to calculate the mass lost relative to the baseline for each site. The water column depth of each environment was determined using a measuring tape. Substrate type was classified as sandy (type 1), muddy (type 2), or rocky (type 3).

All parameters were determined five times at each sampling site, except for microhabitat current, which was measured four times. The different measurements were taken at slightly different sites, about 10 m away from the previous one. For habitat characterization, the mean across all measurements was used. These environmental variables were measured during the spring season of 2017 for 12 populations (note that it was not possible to obtain environmental information from the coastal washout 7, Fig. 1). Although habitat parameters might seasonably vary to some extent, the relative differences between sampling habitats are likely to be consistent.

Data analysis

Geometric morphometry

Due to a pronounced sexual dimorphism in this species, females and males were analyzed separately. Geometric morphometric analyses were performed

with shape coordinates obtained from generalized procrustes analyses (GPA) (Rohlf & Slice, 1990), which minimizes the differences in translation, scaling, and rotation between landmarks (Zelditch et al., 2012). We also obtained the centroid size values from GPA, characterized as the square root of the sum of the squares of the distance of each landmark from the centroid (mean of all coordinates) of the configuration (Bookstein, 1991). Normality distribution and homogeneity of variances of centroid size values were checked by Shapiro–Wilk and Levene’s tests, respectively, and the size variation among populations was analyzed through analysis of variance (ANOVA), followed by Tukey’s *post hoc* test with Bonferroni correction. A Welch *F* test was performed in the cases that homogeneity of variances was rejected. Possible allometric effects, caused by different ontogenetic stages among specimens, were removed by regressing Procrustes coordinates (shape variable) into centroid size (size variable). Multivariate analyses were performed with the covariance matrix calculated from the resulting regression residuals (Stange et al., 2016). Principal components analysis (PCA) was used to identify the axes of maximal shape variance among all specimens and the patterns associated with this variance as well as to identify grouping of variance among the specimens. Multivariate analysis of variance (MANOVA), followed by pairwise comparisons, was performed to analyze shape statistical differences using the scores of informative principal components (based on a broken-stick distribution) as dependent variable and habitat as independent variable. The MANOVA was followed by Wilks’ λ test to identify the proportion of the variance that is explained by the independent variable (population). We used the canonical variates analysis (CVA) to describe the differences among groups (habitats) and to form mathematical functions, which were used to assign specimens to groups through jackknife cross-validation analyses (Zelditch et al., 2012).

Linear morphometry

Standardized relative measures (called RM) were used for linear morphometric analyses, which were obtained by dividing each measure by the individual standard length, according to Shukla & Bhat (2017). A total of 525 specimens (335 females and 190 males) were used for the linear morphometry analyses

because it was not possible to obtain measures for all sampled specimens (some specimens presented damaged fins, making its measurements impossible). Normality distribution and homogeneity of variances for relative distances were checked by Shapiro–Wilk and Levene’s tests, respectively. The variation among populations for each RM was analyzed through analysis of variance (ANOVA), followed by Tukey’s *post hoc* test with Bonferroni correction. A Welch F test was performed in the cases that homogeneity of variances was rejected. Factorial analyses were performed to describe the interdependent relationship between the linear measures. To this end, Bartlett’s test of sphericity and the Kaiser–Meyer–Olkin (KMO) criterion were used to check the assumptions of non-correlation among variables and the adequacy of the data matrix, respectively, with factors with eigenvalues > 1 being selected.

General linear models

To understand the relationships of shape variation and environmental variables, we developed predictive models using the scores of informative principal components as response variables and selected environmental variables as predictive variables. First, we checked the correlation of these variables individually with the informative principal components scores, separately for each sex. Variables with Pearson’s correlation coefficient > 0.2 were selected to develop the models. When predictive variables correlated with each other ($r > 0.7$), we selected the one with higher correlation with the response variable. Linear regression analyses were performed independently for each informative PC (as response variable), using the selected environmental variables as predictors. The models were developed based on a stepwise regression modeling method, where the environmental variables that best explained the response variables (shape variation) were selected as the best model for each shape variable, using the adjusted R^2 , P value, and the AIC (Akaike information criterion) values as criteria for the selection of predictive models (Shukla & Bhat, 2017). The assumptions of linearity, normality, and homogeneity of variances were checked through plots of residuals versus fitted values, normal Q–Q, and scale-location (squared root of standardized residuals versus fitted values), respectively. When some of these assumptions were not achieved, logarithmical

transformations were performed with the predictive variables to fit assumptions, and subsequently, the predictive models were developed. Extreme values were cut off based on Cook’s D plot.

Analyses were performed in R environment (R Core Team, 2013), using the Geomorph (Adams & Otárola-Castillo, 2013; Adams et al., 2017) and MASS (Venables & Ripley, 2002) packages for geometric morphometric analyses, the Hmisc (Harrell, 2014) and Psych (Revelle, 2017) packages for predictive models, and nFactors (Raiche, 2010) and REdaS (Hatzinger et al., 2014) for factorial analyses. Graphs were edited using the software Inkscape v0.92. Differences were considered significant at $P < 0.01$.

Results

Geometric morphometric analyses

The results of the MANOVA indicated that mean body shape was distinct among the habitats, both in females ($F_{4,352} = 26.498$, Wilks’ $\lambda = 0.45805$, $P < 0.001$) and in males ($F_{4,192} = 9.232$, Wilks’ $\lambda = 0.49896$, $P < 0.001$). The difference was significant among all habitats ($P < 0.001$), except for males from stream and coastal washouts habitats ($P = 0.1603$). The jackknife cross-validation analyses indicated an overall classification accuracy of 82.1% for females and 72.1% for males, with correct classification among habitats varying from 42 to 96% (Table 2).

For females, the broken-stick model indicated PC1, PC2, and PC3 as the informative principal components, which together accounted for 58.7% of the total variation. These PC axes were mainly related to the caudal peduncle length, curvature of the body, as well as the head size (Table 3). Specimens from lagoon environments were distributed mainly in the negative end of the PC1 (which explained 28.3% of the total variation), having shorter and wider caudal peduncles when compared with specimens from the stream and coastal washouts, which have longer and narrower caudal peduncles. The PC2 (explaining 17.8% of the total variation) distinguished two groups based on body curvature. Specimens from marine rocky pools showed a convex body curvature along the dorsal profile, with a more ventral position of the mouth and the caudal peduncle, as compared to specimens from all other populations (Fig. 2).

Table 2 Classification results (%) from CVA jackknife cross-validation

Habitat	Estuary	Stream	Lagoon	CW	Marine	Correct classification
Females						
Estuary	47.6	0.0	16.7	35.7	0.0	47.6
Stream	5.9	52.9	0.0	41.2	0.0	52.9
Lagoon	6.1	0.0	79.3	12.2	2.4	79.3
CW	2.6	1.6	4.2	91.6	0.0	91.6
Marine	0.00	0.0	0.0	3.8	96.1	96.1
Males						
Estuary	60.0	0.0	20.0	16.7	3.3	60.0
Stream	7.1	42.9	7.1	42.9	0.0	42.9
Lagoon	3.8	0.0	71.7	24.5	0.0	71.7
CW	3.7	2.5	12.5	78.7	2.5	78.7
Marine	5.0	0.0	0.0	10.0	85.0	85.0

Table 3 Explained variation of the informative principal components of the shape attributes related with the higher loadings landmarks

Principal component	Explained variation (%)	Landmarks with higher loadings	Fish shape attributes
Females			
PC1	28.31	9, 10, 11, 12 (<i>x</i> -axis)	Caudal peduncle length
PC2	17.77	9 (<i>y</i> -axis), 4 (<i>x</i> -axis), 12, 11 (<i>y</i> -axis)	Body curvature
PC3	12.59	6, 4, 1, 5 (<i>x</i> -axis)	Head size
Males			
PC1	22.18	12, 9, 6 (<i>x</i> -axis), 1 (<i>y</i> -axis)	Caudal peduncle and head length, and mouth position
PC2	19.39	4, 1, 5, 2 (<i>x</i> -axis)	Head size
PC3	13.91	10, 8, 7, 4 (<i>x</i> -axis)	Caudal peduncle length and head size
PC4	10.42	12, 7, 8 (<i>x</i> -axis), 10 (<i>y</i> -axis)	Caudal peduncle length

For males, we identified the first four PC axes as informative components, accounting for 65.9% of the total variation (Table 3). In contrast to females, it was not possible to clearly distinguish groups along the males' PC axes. However, males from marine rocky pools also occupied a distinct position in the morphospace indicating the same body curvature as females from the same environment, as observed in both PC1 (22.2% of total variation) and PC2 (19.4% of total variation) (Fig. 2).

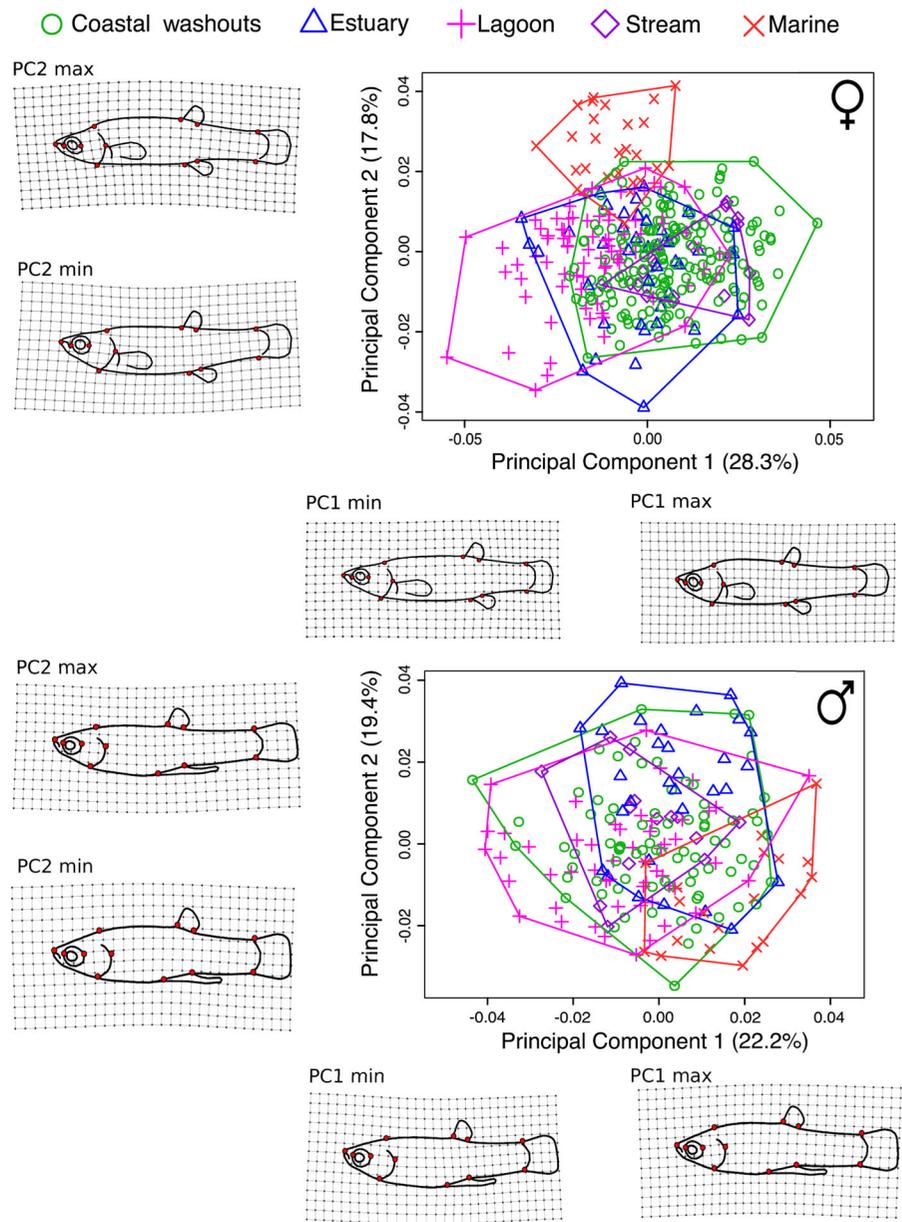
The CVA, for both females and males, evidenced the distinction among the specimens from marine rocky pools and the others along CV1, which accounted for 56.7 and 47.4% of the total variation, respectively (Fig. 3). This distinction was also clear when the Procrustes distance was observed (Table 4). This difference regarding the marine rocky pools was

also observed for body size, where an ANOVA indicated that centroid size was different among the populations, irrespective of sex (Table 5). Mean centroid size of marine rocky pools specimens was larger than the mean centroid size from specimens from other populations (Fig. 4). However, regarding centroid size, pairwise comparisons showed that most populations were statistically different ($P < 0.001$), i.e., mean centroid size was significantly different within habitats.

Linear measurement analyses

Total length (RM5) was different among the populations for both sexes (Fig. 5). For females, body depth (RM6), caudal peduncle length (RM9), and depth (RM10) also differed among populations, whereas

Fig. 2 Principal components analysis of body shape in females and males, showing PC1 versus PC2. For each PC, the shape alteration of extreme PC values in relation to the mean shape through fishes' warped drawings on grid of deformation is represented



males differed with respect only to caudal fin length (RM2) (Table 5). For males, the specimens from marine rocky pools were larger in terms of total length.

In females, factorial analysis indicated four factors with eigenvalues > 1, accounting for 59% of the total variation. The higher loadings for the factor 1 were those associated with the caudal peduncle (RM9 and RM10) and with the body depth (RM6), whereas the total length (RM5) and caudal fin length (RM2) loadings were higher for factor 2. The remaining

factors were mainly related to eye diameter (RM12) and dorsal fin length (RM1). In males, we identified five factors with eigenvalues > 1, with 67% of the total variation. The first factor, which accounted for 21% of the total variation, was mainly related to body length (RM5) and caudal fin length (RM2). Factors 2 and 3 (12% of total variation each) were associated with gonopodium size (RM7) and body depth (RM6). The remaining factors were associated with caudal

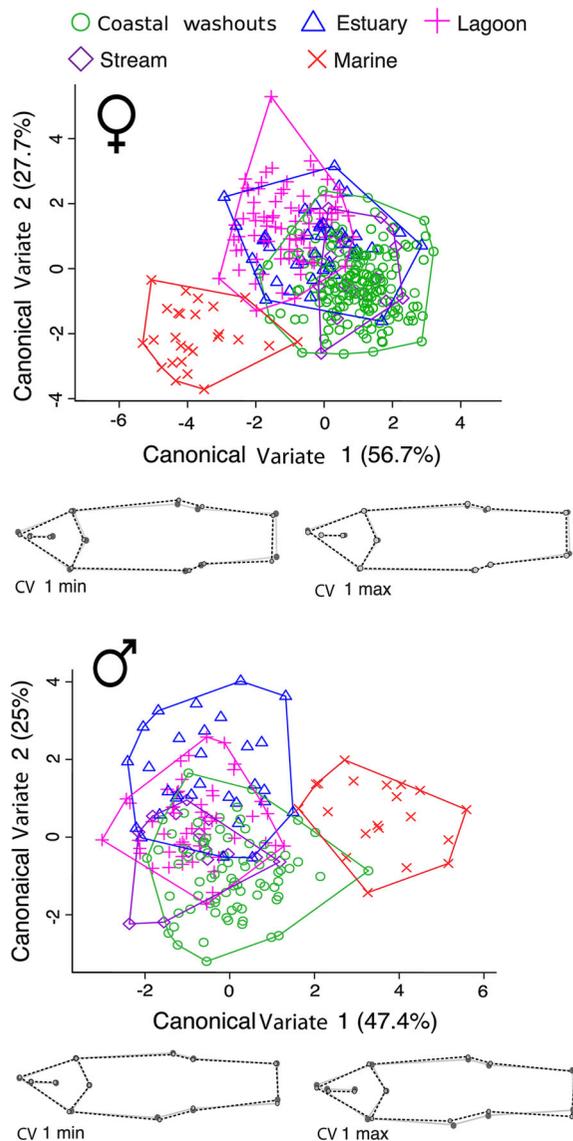


Fig. 3 Canonical analysis of variance among habitats for body shape in females and males, showing CV1 versus CV2. Maximum and minimum body shape alteration estimates are shown for CV1 from each sex through warped drawings

peduncle (RM9 and RM10) and eye diameter (RM12) (Table 6, Fig. 6).

Predictive models

There was a strong correlation between microhabitat current and superficial water current ($r > 0.7$). For this reason, only microhabitat water current was used to develop the models. In females, multivariate linear

regression with stepwise selection indicated microhabitat water current and substrate as the main predictors for the PC1 scores ($F_{2,309} = 40.99$, $P < 0.001$, $R^2 = 0.205$), but only microhabitat water current was statistically significant at $P < 0.01$. Substrate and pH fit the model with the PC2 scores ($F_{2,309} = 59.61$, $P < 0.001$, $R^2 = 0.274$). For males, the model indicated a correlation between the PC1 scores and salinity and water temperature ($F_{2,179} = 15.08$, $P < 0.001$, $R^2 = 0.135$), with salinity being the main predictor. Substrate and water temperature best explained PC2 (lower AIC value and higher R^2 adjusted). The remaining informative PC scores did not show any significant correlation with the available environmental variables (Table 7).

Discussion

Fishes often exhibit phenotypic divergence in response to habitat differences (Bruckerhoff & Magoulick, 2017). Variation in the water current has been identified as one of the most important factors associated with morphological adaptation in fishes (see, e.g., Gomes & Monteiro, 2008; Berner et al., 2009; Haas et al., 2010; Foster et al., 2015). However, only relatively few studies have looked at differences in body shape related to water current between males and females. Here, we examined phenotypic divergence in body shape in *J. lineata* inhabiting different coastal habitats. We found that females and males showed distinct shape variations with respect to the analyzed environmental variables. In females, shape variation primarily involved the caudal peduncle, whereas males did not show any variation in this trait. Females from environments with lower microhabitat current showed wider and shorter caudal peduncles compared with specimens living in habitats with higher microhabitat current, which is concordant to what has been observed in other species (Haas et al., 2010; Theis et al., 2014; Gaston & Lauer, 2015; Lauder, 2015). Surprisingly, water current was not correlated with shape variation in males of *J. lineata*.

This habitat-related sexual dimorphism means that the environmental conditions might exert different selection pressures in variation of body shape and size among sexes. This pattern of sex differences in body shape and size is critical for our understanding of the role of natural selection (e.g., resource availability,

Table 4 Procrustes distances from least square means generated in CVA

	Estuary	Stream	Lagoon	CW	Marine
Females					
Estuary	0	0.01536845	0.01419506	0.01249562	0.03260140
Stream		0	0.02669847	0.01426437	0.03968148
Lagoon			0	0.02182658	0.03020874
CW				0	0.03403277
Marine					0
Males					
Estuary	0	0.01963892	0.02252585	0.01919011	0.03234595
Stream		0	0.01853569	0.01195373	0.03219391
Lagoon			0	0.01461908	0.03052316
CW				0	0.02594600
Marine					0

Table 5 Centroid size and linear measurements differences among populations, through analysis of variance (ANOVA)

	DF	Sum sq.	Mean sq.	F value	P value
Females					
RM5: total length					
Population	12	0.13486	0.011239	53.13	< 0.001
Residual	322	0.06811	0.000212		
RM6: body depth					
Population	12	0.07129	0.005941	60.76	< 0.001
Residual	322	0.03148	0.000098		
RM9: ventral width of caudal peduncle					
Population	12	0.06780	0.005650	25.21	< 0.001
Residual	322	0.07215	0.000224		
RM10: caudal peduncle depth					
Population	12	0.02201	0.0018345	42.88	< 0.001
Residual	322	0.01378	0.0000428		
Centroid size					
Population	12	25.92	2.1597	48.85	< 0.001
Residual	322	14.23	0.044		
Males					
RM2: caudal fin length					
Population	12	0.06373	0.005311	24.3	< 0.001
Residual	177	0.03868	0.000219		
RM5: total length					
Population	12	0.06155	0.005129	24.32	< 0.001
Residual	177	0.03734	0.000211		
Centroid size					
Population	12	18.444	1.5370	51.65	< 0.001
Residual	177	5.267	0.0298		

It is presented just those measurement which were statistically important (i.e., with P value < 0.01 and with the population sum of squares higher/equal than residuals sum of squares)

presence of predators, water current) in sexual dimorphism evolution, especially in species with remarkable morphological sex differences, such as *J. lineata*.

Regarding sexual dimorphism, two main hypotheses have been proposed as drivers of the evolution of this characteristic: sexual selection and intraspecific niche

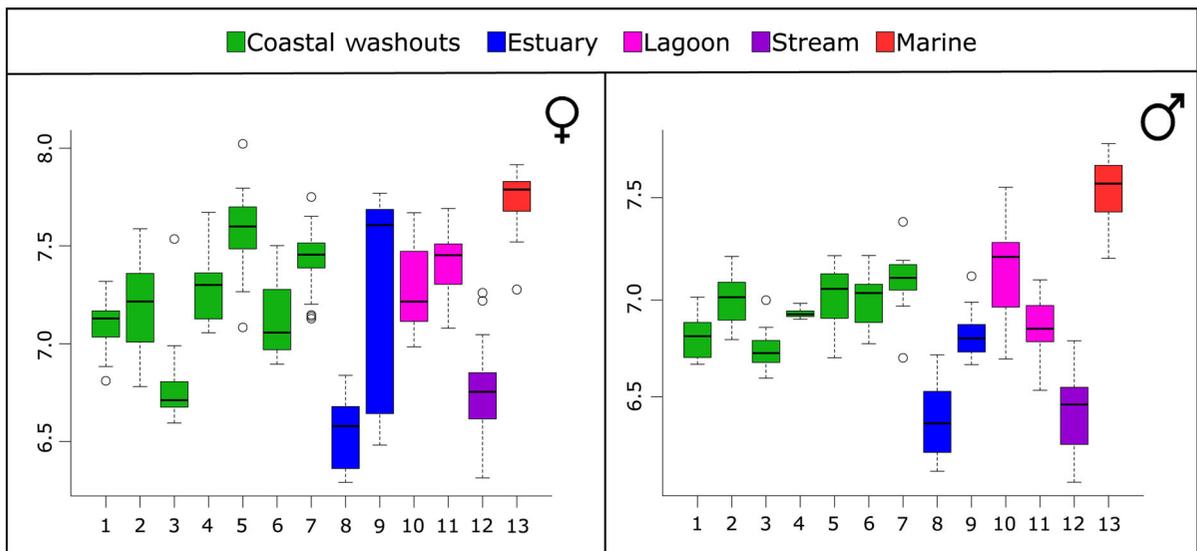


Fig. 4 Centroid size boxplot for females and males from each population (numbered from 1 to 13 in the same order as in Table 1)

divergence with adaptation of each sex to different ecological niches within the same environments (Shine, 1986). Both hypotheses are not mutually exclusive. Hence, it is possible that females and males of *J. lineata* occupy distinct niches, where females are more exposed to water current than males, occupying different places in the microhabitat. Other *Jenynsia* species (*J. alternimaculata* (Fowler, 1940) and *J. maculate* (Regan, 1906)) have been observed using different microhabitats in northwestern Argentina when recorded with underwater videos and visualizations; larger individuals used the central portion of the creek, while smaller individuals and males occupied the margins (Felipe Alonso, pers. obs. unpublished data). Indeed, it is well established that sexual dimorphism in size is extremely important in driving sexual segregation, at least in ungulates (see Ruckstuhl, 2007). This sexual segregation occurs when females and males of a species have a differential use of space (Peterson & Weckerly, 2017). This behavioral phenomenon is widespread in the animal kingdom, but poorly documented in aquatic environments (Wearmouth & Sims, 2008).

On the other hand, mating preferences could play a role in the sexual dimorphism observed in *J. lineata*, since organisms exhibiting genitalia that cannot be retracted are particularly susceptible to pre-mating sexual selection and natural selection (Langerhans, 2010). Analyzing the effects of male genital size on

attracting mates in different predation regimes, Langerhans et al. (2005) found that females from other livebearer fish species exhibited mating preference for the large-gonopodium males. However, relatively large gonopodia seem to incur in a cost of reduced burst-swimming speed because of increased hydrodynamic drag (however, see Booksmythe et al., 2016). In this case, gonopodium size seems to reflect an evolutionary trade-off between pre-mating sexual selection, favoring a larger gonopodium, and natural selection pressures related to predation avoidance, favoring a smaller gonopodium (Langerhans et al., 2005). It is possible that this kind of trade-off could be also occurring in *J. lineata*. Besides, this species features coercive mating, whereby males approach females from behind and try to thrust their copulatory organ, the role of females is not a passive one; when observed together with males, females showed avoidance and aggression, which leads us to infer that struggling may represent a way by which the female assesses the skill and endurance of males (Bisazza et al., 2000). The trade-off between sexual selection and selection of swimming performance could promote a specific adaptive body shape in males of *J. lineata*, for which occupying microhabitats with less water current than females (as observed for others *Jenynsia* species) should be better due to the drag caused by the prominent copulatory organ. Another explanation for such caudal peduncle differentiation

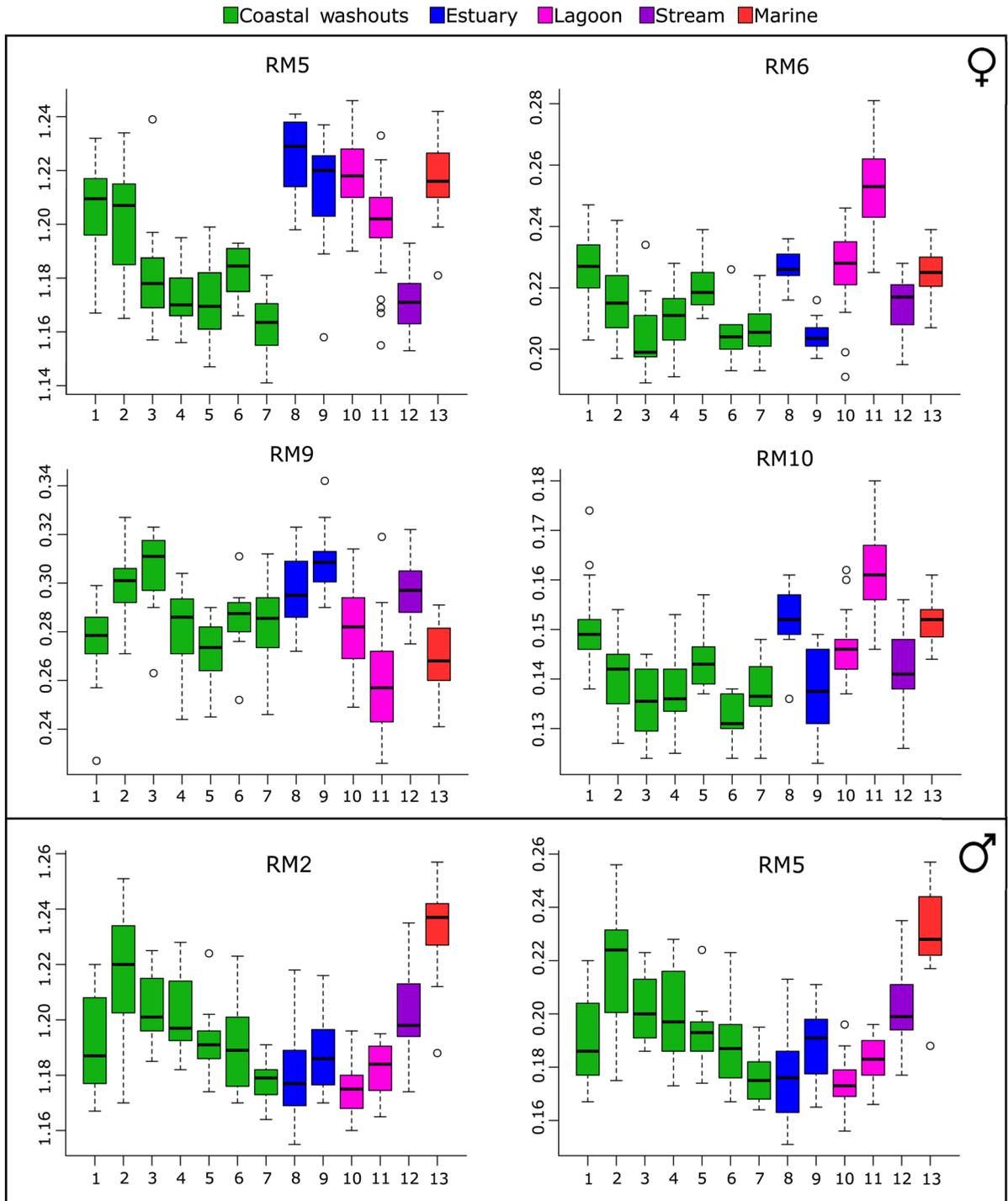


Fig. 5 Boxplots with linear measures statistically different among the 13 populations (numbered from 1 to 13 in the same order as in Table 1) for females and males

Table 6 Loadings and proportion of total variation of factor analysis from linear relative measures (RM)

Females					Males					
Measure	Factor 1	Factor 2	Factor 3	Factor 4	Measure	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
RM6	0.84				RM2	0.95				
RM9	-0.63				RM5	0.98				
RM10	0.86				RM6		-0.59			
RM2		0.90			RM7		0.94			
RM5		0.96			RM8			0.97		
RM11			0.52		RM9				0.98	
RM12			0.89		RM10				-0.51	
RM1				0.68	RM12					0.84
RM3				0.47	RM1					
RM7				0.49	RM3					
RM8					RM11					
% variation	20	18	12	9	% variation	21	12	12	12	10

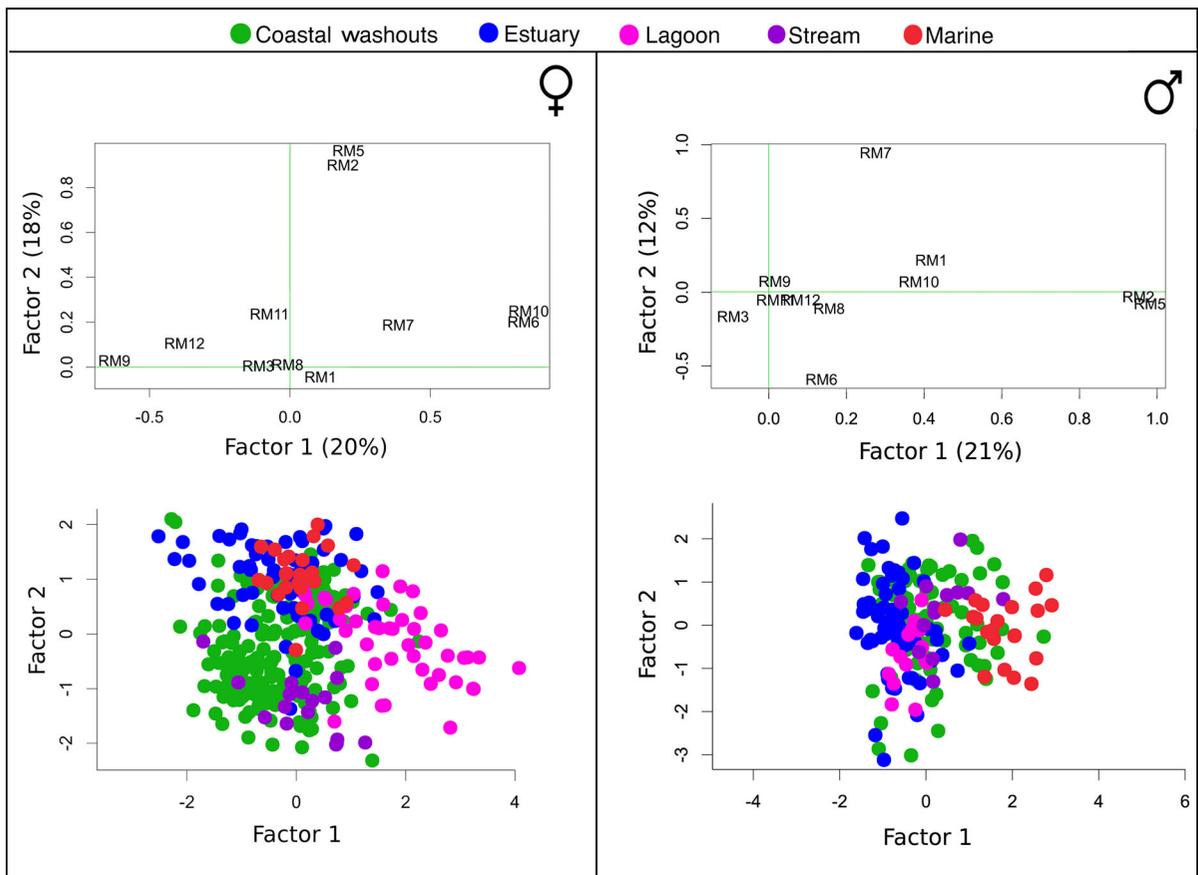


Fig. 6 Factorial analysis of linear measurements. For each sex, a plot with Factor 1 versus Factor 2 is shown, along with the contribution of the relative linear measures explaining each

factor (superior plots) and the distribution of each specimen according its Factor 1 versus Factor 2 scores (inferior plots)

Table 7 Predictive models indicating the relationship between the shape variation (from informative principal components scores—PCs) and the environmental variables through linear models

Sex	Response variables	Selected models	AIC value	F value	P value	Adjusted R ²
Females	PC1 scores	Microhabitat water current	− 2,617.7	78.39 _(1,310)	< 0.001	0.199
		Substrate	− 2,682.8	5.298 _(1,310)	0.022	0.014
		Microhab. + substrate	− 2,683.9	40.99 _(2,309)	< 0.001	0.205
	PC2 scores	Substrate	− 2,731.4	96.26 _(1,310)	< 0.001	0.234
		pH	− 2,788.5	28.85 _(1,310)	< 0.001	0.082
		pH + substrate	− 2,803.5	59.61 _(2,309)	< 0.001	0.274
Males	PC1 scores	Log(salinity)	− 1,506.7	11.44 _(1,180)	< 0.001	0.054
		Water temperature	− 1,512.2	5.647 _(1,180)	0.018	0.025
		Log(salinity) + temp.	− 1,527.4	15.08 _(2,179)	< 0.001	0.135
	PC2 scores	Water temperature	− 1,556.8	15.77 _(1,181)	< 0.001	0.075
		Substrate	− 1,544.9	28.98 _(1,181)	< 0.001	0.133
		Temp. + substrate	− 1,576.6	27.63 _(2,180)	< 0.001	0.226

between sexes could be related to a different swimming performance. However, with morphological data alone it is not possible to answer the question why females and males show distinct phenotype responses associated with the environmental variables studied herein.

We also observed a second pattern of body shape variation in *J. lineata* of both sexes from marine rocky pools. Their bodies were more curved, and both mouth and the caudal fin had a more ventral position. This characteristic might be related to the specific environmental conditions in their habitat: permanent water current in variable directions due to the action of waves, getting water in and out of the pools. Also, variations associated with the tides could play a role as drastic alterations in environmental factors can occur in these habitats (i.e., temperature, salinity, dissolved oxygen, pH). As a result, some fish species living in this kind of environment have developed a range of adaptations (including morphological ones) that allow them to tolerate this variable environment (Laming et al., 1982; Gibson, 1986). Adults of *Kelloggella disalvoi* (Randall, 2009) (a Gobiidae from the Easter Island, Pacific Ocean) show a similar pattern of body curvature as those found in *J. lineata* from Punta del Este rocky pools (Vera-Duarte et al., 2017). In *K. disalvoi*, the specific adaptation has been associated with diet: specimens with an inferior mouth feed more on bivalves, whereas specimens with an anterior mouth primarily prey upon copepods (Vera-Duarte et al., 2017). There is no data about the diet of *J.*

lineata from rocky pools. However, this species has been found to live on an omnivorous diet, with diet shifts between environments (Bastos et al., 2017). Possibly, the morphological variations in *J. lineata* from rocky pool populations might also be associated with foraging habits. However, it is also highly possible that numerous complex evolutionary processes act together to shape the local morphological differences among the studied populations.

An additional interesting characteristic of *J. lineata* from rocky pools is its body size. Specimens from Punta del Este showed the largest centroid size in both sexes, and males of that population were the largest ones of all tested. Previous works in *J. lineata* revealed variations in size according to salinity (Fontoura et al., 1994; Mai et al., 2005). This relation between size and salinity has been described for numerous fish species (Langerhans & DeWitt, 2004; Gomes & Monteiro, 2008; Jørgensen et al., 2008; Araújo et al., 2014; Baker et al., 2015; Foster et al., 2015). One explanation for this variation could be the different predation pressure regime present in marine habitats with higher salinity. Salinity changes the environmental conditions, causing alterations in the habitat structure and influencing the entire ecological community. Generally, brackish water environments contain fewer piscivorous fishes than freshwater ones, where prey needs to spend more energy to escape from predators, leading to shorter bodies (Gomes & Monteiro, 2008). We did not find any piscivorous fishes in the marine rocky pools from Punta del Este (Calviño & Alonso, 2016), and it is

therefore possible that the size difference observed in *J. lineata* could be related to the predation pressure. (However, it is also possible that other fishes inhabit these pools and were not sampled.) However, size changes could be a by-product of physiological mechanisms under other constraints with no adaptive positive value. Salinity influences growth in numerous fish species by affecting standard metabolic rate, food intake, food conversion, and/or hormonal stimulation. In fact, numerous hormones are involved in osmoregulation and growth regulation (Boeuf & Payan, 2001). Different environments may present divergent environmental conditions that may act together and interact. Therefore, experiments manipulating those variables under controlled conditions are necessary to assess the extent and contribution of those factors to morphological changes and to evaluate whether those are due to genetic adaptive responses, phenotypic plasticity, consequences of other restrictions, or random effects such as genetic drift and founder effects.

In conclusion, in different habitats, *J. lineata* shows variations in relation to body shape and size, and these variations are not the same for males and females. Water current seems to be an important environmental factor correlated to body shape variation, while the salinity degree is strongly correlated with body size. We highlight both locomotor and foraging habits as the main functions that might be related to the body shape and size variation observed in *J. lineata*. Apart from some issues that could not be resolved in this study, our findings present the morphological adaptation of *J. lineata* inhabiting contrasting environments, and this adaptation is sex specific. To test whether the observed phenotypic variation is due to phenotypic plasticity or to genetic polymorphism (allelic variation at coding loci), common garden experiments are needed. In this sense, genomic investigations could also be interesting to identify polymorphisms that could be related to the observed phenotypic variation.

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