Latin American Journal of Aquatic Research, 51(1): 47-66, 2023
DOI: 10.3856/vol51-issue1-fulltext-2785

Research Article

Spatiotemporal analysis of the genetic and morphological variation of *Iphigenia brasiliensis* (Mollusca: Bivalvia) from the southwest tropical Atlantic

Tainara Ravaglia Ferreira Gonçalves[1], Edson Pereira Silva[1]
Alan Bonner[1] & Michelle Rezende Duarte[1]
[1]Laboratório de Genética Marinha e Evolução, Departamento de Biologia Marinha
Universidade Federal Fluminense, Niterói, RJ, Brasil
Corresponding author: Michelle Rezende Duarte (michellerezendeduarte@yahoo.com.br)

ABSTRACT. *Iphigenia brasiliensis* is a bivalve mollusk exploited as a shellfish and subsistence resource on the Brazilian coast, mainly in the north and northeast regions. Genetic (allozyme electrophoresis revealed eight gene loci) and morphological variations (multivariate morphometry of valves used 13 linear measurements of traditional and 19 Fourier coefficients of geometric morphometry) were studied, considering the geographic (445 km of the southwest tropical Atlantic), environmental (mangroves and channels of communication with the sea of three estuaries in the state of Rio de Janeiro), and temporal dimensions (over two years). *I. brasiliensis* showed high levels of genetic variation (the average number of alleles per locus ranged from 2.8 to 3.4, and the average heterozygosity ranged from 0.441 to 0.675). Bayesian analysis of population partitioning showed that the highest LnP(D) value was achieved for K = 3. These results indicated mixed ancestry, possibly oscillations in the dispersion dynamics among the different sampling groups, and temporal oscillations in the population sizes due to the anthropogenic influence on the studied estuaries. The results of morphological variation, inferred by the PERMANOVA from the Fourier analysis, indicated that a similar influence might occur in valves (also, the discriminant analysis showed that different groups could be consistently identified). In this sense, the studied populations may be organized in a dynamic of metapopulations. Finally, these are the first data on morphological and genetic variation of the species in the latitudinal, environmental, and temporal dimensions studied simultaneously, thus providing relevant information for the exploration, management, and conservation of this commercially important species.

Keywords: *Iphigenia brasiliensis*; genetic variation; morphological variation; bivalves; allozymes; morfometry; Rio de Janeiro

INTRODUCTION

The multiplicity of factors regulating genetic and morphological variation patterns has imposed important challenges for studies interested in these phenomena (Clark 2000, Auld et al. 2010). Genetic variation is influenced by stochastic factors, such as genetic drift, inbreeding, and temporal fluctuation in population sizes, and deterministic, such as natural selection and migratory regimes (Grant 2001, Stapley et al. 2017, Fenderson et al. 2020). All these factors are subject to spatial, different environments, and temporal changes related to different natural cycles (Barton & Charlesworth 1984). Even more labile is the morphological variation. In addition to being directly linked to the patterns of gene variation (Cheverud 1984), it is subject to the different patterns of genotype reaction (Gupta & Lewontin 1982, Sultan 2017), phenotypic plasticity (West-Eberhard 1989, Scheiner 1993, Bonamour et al. 2019), epigenetic variation (Jablonka & Lamb 1989, Jablonka 2012), and environmental noises, which can cause changes in the normal patterns

Corresponding editor: José Gallardo

of development due to environmental or genetic disorders (Markow 1995, Kight & Swaddle 2011).

Regarding the morphological variation, traditional and geometric morphometric techniques have allowed describing the form patterns of organisms within and between populations, as well as correlate of them with variations of a latitudinal, temporal, and environmental nature (Bitner-Mathé et al. 1995, Van Buskirk 2009, Telesca et al. 2018). Traditional morphometry started in the late 19th century and was consolidated in the 20th century with the development of multivariate analysis techniques, which allowed studying the variation in the form of organisms through correlations between distance measurements between biological structures. However, traditional morphometry still had limitations due to its inability to capture the complete form of organisms (Rohlf & Marcus 1993) and circumscribe the problem of allometric growth (Adams et al. 2004). The development of geometric morphometry from the 90s could solve many of these limitations by applying techniques such as contour analysis. In this case, the body form is represented as a whole and allows the direct comparison of the form detected in all directions, compensating for random measurement errors (Rohlf & Marcus 1993, Roth & Mercer 2000, Adams et al. 2004, Zelditch et al. 2004).

Patterns of genetic variation in natural populations have been described using the allozyme electrophoresis technique (Bernstein et al. 1973, Carson & Johnson 1975, Rick et al. 1979, Taggart et al. 1981, Fins & Seeb 1986, Hamrick et al. 1992, Sugama et al. 1999, Uthicke & Purcell 2004) since the publication of the seminal studies by Hubby & Lewontin (1966) and Lewontin & Hubby (1966). Allozymes have been a good descriptor of genetic variation (Arunkumar et al. 2018, Pazoto et al. 2018, Jormalainen et al. 2019) despite some technical (e.g. type of material that can be used, the record of phenotypes, and underestimation of the total genetic variation) and theoretical limitations (e.g. discussion about locus neutrality), mainly when the studies concern the influence of latitudinal, temporal, and environmental effects on genetic variation (Kuzishchin et al. 2018, Montagna et al. 2018, Bonner et al. 2019, Lauterjung et al. 2019, Rossi et al. 2019). Allozymes have also been a good tool for investigating the relationships between genetic and morphological variation (Ryman et al. 1984, Zink 1988, Cruz et al. 2011, Vetrova et al. 2016, Duarte et al. 2018).

Bivalve mollusks are among the models that have been used to study the correlation between genetic and morphological variation and different factors like localities, environments, and time (Laudien et al. 2003, Kong et al. 2007, Zieritz et al. 2010, Inoue et al. 2013, Begum et al. 2018, Bonner et al. 2019). Bivalve mollusks have a wide geographic distribution, occupy a relatively wide range of environmental conditions, and are relatively abundant (Simpson & Harnik 2009, Lorion et al. 2010), besides being animals of commercial importance for coastal communities (Krause et al. 2019, Olivier et al. 2020). The southwest tropical region of the Atlantic Ocean, especially on the Brazilian coast, is home to great biodiversity of bivalves (Soares et al. 2011), such as the species of bivalve mollusks *Anomalocardia flexuosa*, *Crassostrea rhizophorae*, and *Iphigenia brasiliensis* (Nascimento et al. 1980, Maia et al. 2018, Teixeira & Campos 2019). *Anomalocardia brasiliana* is an exclusively Brazilian species that has been studied since the 1970s in different approaches (Narchi 1972b, Mouëza et al. 1999, Arruda et al. 2009, Silva-Cavalcanti & Costa 2011, Corte et al. 2017), similar to the species *Crassostrea rhizophorae* (Martino & Cruz 2004, Freitas et al. 2006, Leal et al. 2008, Kanhai et al. 2014). However, *I. brasiliensis* is poorly studied despite its commercial relevance (Narchi 1972a, Ceuta et al. 2010, Bonner et al. 2019, Costa et al. 2019).

I. brasiliensis occurs in Florida (USA), Suriname, parts of the Caribbean Sea, and Brazil between the states of Pará (00°07'04"S, 49°23'17"W) and Santa Catarina (29°19'46"S, 49°42'39"W) (Rios 1994, Scarabino et al. 2015). It is found in estuaries and mangroves (Narchi 1972a), that is, water bodies in which seawater is diluted with freshwater derived from terrestrial drainage, presenting a horizontal gradient of salinity (McLusky 1993). These regions have a high variation in physical (temperature and changes in seas) and chemical aspects (salinity, pH, and dissolved oxygen), which can vary in time and space (McLusky 1993, Chapman & Wang 2001). Thus, this species is subject to a range of environmental variables that fluctuate in time and space, as has been demonstrated that is a common condition in estuaries (Hiroki 1971, Adams et al. 2016, Kefford et al. 2016, Gomes et al. 2021). Considering the wide range of spatiotemporal variation of the ecological niche occupied by this species and its high exploration as a shellfish resource on the Brazilian coast, this study aimed to describe its genetic and morphological variation in the southwest tropical Atlantic in the geographic, environmental, and temporal dimensions. Correspondences between genetic and morphological variation patterns were analyzed in the three sampled dimensions. The hypothesis postulated by this article is that the three dimensions analyzed (geographic, environmental, and temporal)

are relevant for the patterns of genetic and morphological variation of the organisms. The obtained results provide important information for understanding the action of the factors that regulate the patterns of genetic and morphological variation of natural populations and developing strategies for the rational exploration, management, and conservation of this species.

MATERIALS AND METHODS

Study area

Four hundred twenty-nine individuals samples of the species *Iphigenia brasiliensis* were obtained from estuaries in three locations along 445 km on the coast of the state of Rio de Janeiro, Brazil, in the southwest tropical Atlantic (Fig. 1, Table 1) as follows: Cemitério Beach, where the Ostras River empties (Rio das Ostras, 22°32'02"S, 41°56'08"W); Itaipu Beach, with a communication channel with the Itaipu Lagoon (Niterói, 22°57'58"S, 43°02'41"W); and Jabaquara Beach, where the Perequê-Açú River empties (Paraty, 23°12'41"S, 44°42'55"W). Individuals from two different environments (mangrove and channel) were collected for each of the three estuaries. Each of these two different environments from the three estuaries had collections performed in two different years (Rio das Ostras in 2015/2017, Niterói in 2016/2018, and Paraty in 2017/2018), totaling 12 different sampling groups. Samples were obtained by digging the substrate to about 20 cm in depth and transported alive or on dry ice to the laboratory. Tissue and the soft part from each specimen were taken and maintained at -20º until the electrophoresis work was carried out. The valves of each individual were enumerated, and the information related to the site, environment, and collection date was recorded. All intact left valves were separated for measurements and photographs related to morphometric analysis.

Genetic variation

The genetic variation was sampled by the allozyme electrophoresis method in 12.5% starch gels (Harris & Hopkinson 1976) based on six enzyme systems (alpha esterase, leucine aminopeptidase, malate dehydrogenase, malic enzyme, phosphoglyconate dehydrogenase, and glucose-6-phosphate isomerase) interpreted as eight gene loci: *α-Est-1, α-Est-2, Lap-2, Mdh, Me-1, Me-2, Pgd,* and *Pgi*. The buffer systems used were lithium tris-hydroxide pH 8.0 (TLiOH) for the enzymes *α-Est, Lap,* and *Mdh,* and tris-citrate pH 8.0 (TC 8.0) for the enzymes *Me, Pgd,* and *Pgi* (Selander et al. 1971). The development of each enzyme system followed the procedures described by Harris & Hopkinson (1976) and Richardson et al. (1986).

The genetic variation was measured based on the average number of heterozygotes observed and expected per locus, the number of polymorphic loci, and the number of alleles per locus, estimated using the software BIOSYS-2 (Swofford & Selander 1997). The Hardy-Weinberg equilibrium tests were performed for all locations, environments, and times using the software GENEPOP 3.3 (Rousset 2008), and the significance levels p were corrected using the Bonferroni method (Haynes 2013). The degree of genetic structure among sampling groups (two times, three locations, and two environments) was estimated by F-statistics (Weir & Cockerham 1984) using the software Fstat 2.9.3.2 (Goudet 2001). A Bayesian analysis was carried out to infer the most likely number of populations or genetic groups in the data set using the software STRUCTURE 2.3.3 (Pritchard et al. 2000). The genetic identities between the 12 different sampling groups were calculated by the method of Nei (1972) and used to build a dendrogram based on the unweighted pair group method with arithmetic mean (UPGMA) algorithm using the software BIOSYS-2 (Swofford & Selander 1997).

Morphological variation

The form of individuals by the traditional morphometry was described by 13 linear measurements between anatomical structures present in the left valves of the species *I. brasiliensis* was taken with a Mitutoyo digital caliper with an accuracy of 0.01 mm. All measurements are shown in Figure 2. The effect of allometry related to linear measurements was treated with the Burnaby allometric method (Burnaby 1966) by the software PAST 3.24 (Hammer et al. 2001). The elliptical Fourier analysis (EFA) method, which allows analyzing the form of the object from the Fourier elliptic coefficients, was used for the geometric morphometry. The object contour is decomposed into a series of harmonic ellipses (harmonically related trigonometric curves) described by four Fourier coefficients (FCs). In this sense, FCs numerically describe each harmonic ellipse's size, form, and orientation and can be used for multivariate analysis of the object form (Crampton 1995, Wishkerman & Hamilton 2018). The photographs for EFA were taken with a Canon EOS Rebel XTi digital camera. All photographic conditions, such as distance from the camera and zoom, were kept constant for all photos. The object contour was defined by transferring the photos to the program tpsDig 2.16

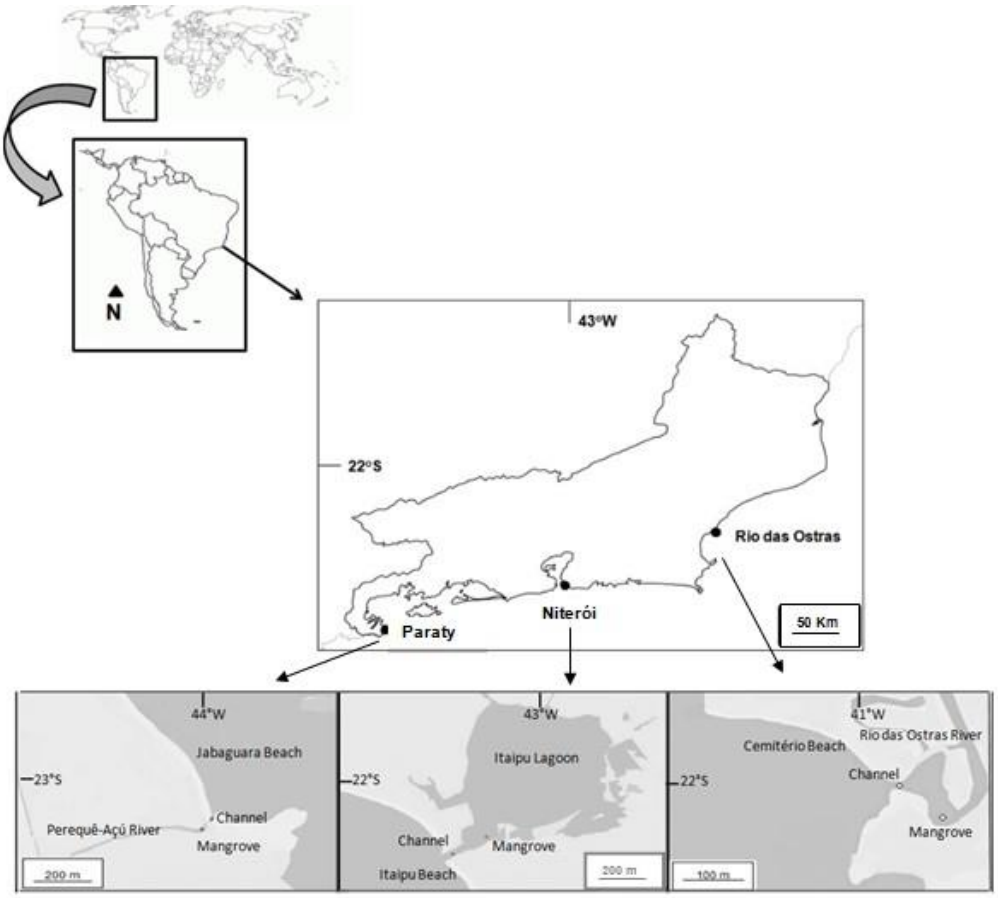

Figure 1. Location of collection points along the southeastern coast of Brazil in the southwest tropical Atlantic (adapted from Bonner et al. 2019).

Table 1. The number of valves used for the analysis of traditional morphometry (TM), geometric morphometry (GM), and the number of individuals used in the genetic analysis (GN) of each location by year and environment.

Location	Year	Environment	TM	GM	GN
Rio das Ostras	2015	Manguezal	30	30	32
		Canal	30	28	32
	2017	Manguezal	17	17	17
		Canal	25	25	28
Niterói	2016	Manguezal	30	30	35
		Canal	30	30	38
	2018	Manguezal	16	16	17
		Canal	25	25	29
Paraty	2017	Manguezal	30	30	36
		Canal	30	30	60
	2018	Manguezal	30	30	54
		Canal	30	30	51
Overall			323	321	429

(Rohlf 2015), in which 38 points were marked along the contour of the valves (Fig. 3). The coordinates were compiled into a single file using the program tpsUtil 1.76 (Rolf 2015). The software PAST 3.24 was used to obtain FCs (Hammer et al. 2001).

The morphological variation of the form of valves obtained by traditional (13 linear measurements) and geometric morphometry (19 FCs) was submitted to: 1) permutational multivariate analysis of variance (PERMANOVA) to verify a potential difference in the form of valves between the 12 different sampling groups. The data used were the Euclidean distances, and the tested hypothesis was that the three dimensions analyzed (geographic, environmental, and temporal) were relevant in determining the morphological variation pattern observed. The probabilities associated with PERMANOVA were corrected through Bonferroni correction to avoid errors derived from multiple comparisons (Haynes 2013), 2) principal component analysis (PCA), an exploratory analysis that transforms multiple correlated variables into indepen-

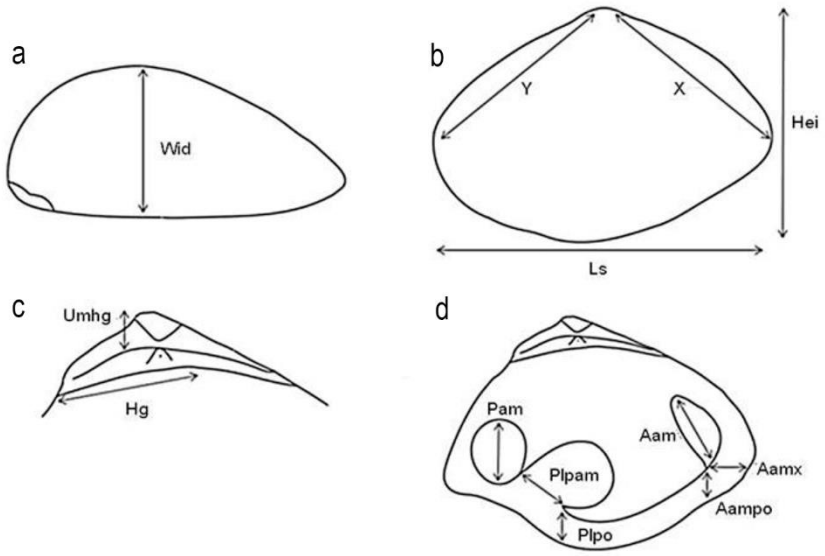

Figure 2. Description of the 13 linear measures used to describe the form of *Iphigenia brasiliensis* valves. a) Represents the measurement of the valve width (Wid; b) represents the length measurements from the umbo to the posterior ventral end (X); length from the umbo to the anterior ventral end (Y); valve length (Ls); valve height (Hei); c) represents the distance measurements between umbo and hinge (Umhg); hinge length (Hg); d) represents the length measurements of the anterior adductor muscle (Aam); distance from the anterior adductor muscle to the anterior margin (Aamx); distance from the anterior adductor muscle to the ventral end of the valve (Aampo); length of the posterior adductor muscle (Pam); distance between the paleal line and the posterior adductor muscle (Plpam); distance between the paleal line and the ventral end of the contour of the valve (Plpo).

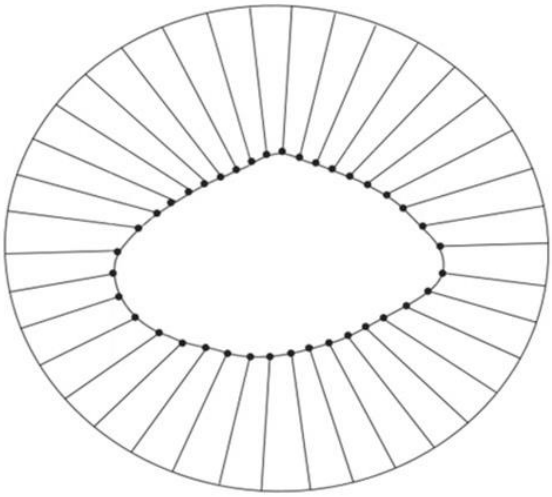

Figure 3. Representative image of the marking of the 38 points defined along the contour of a left valve of the species *Iphigenia brasiliensis*.

dent variables to verify possible patterns in the data. Here PCA was used to search for tendencies among the 12 different sampling groups, and 3) discriminant analysis (DA) to measure the chance of correctly classifying an anonymous valve in one of the sampling groups, therefore, indicating how much the form of the valve is correlated with the dimensions (geographic, environmental, temporal) studied.

All multivariate analyses (PERMANOVA, PCA, DA) were performed using PAST 3.24 (Hammer et al. 2001). Moreover, using the same software, dendrograms were built using the UPGMA algorithm (Gronau & Moran 2007) by Euclidean distances between the centroid values of the different sampling groups.

RESULTS

Allozyme electrophoresis

The six enzyme systems analyzed were interpreted as eight genetic loci. The average number of alleles per locus ranged from 2.8 to 3.4, and the average heterozygosity ranged from 0.441 (for the Rio das Ostras sample unit in the mangrove environment in 2015 - ROM 2015) to 0.675 (for the Niterói sample unit in the mangrove environment in 2018 - NITM 2018) (Table 2). Only the *α-Est-2* locus in the Paraty sampling group from the mangrove environment in 2018 (PAM 2018) was monomorphic. Most loci showed no signifi-

Table 2. Average sample size, the average number of alleles per locus, and average heterozygosity observed and expected from the *Iphigenia brasiliensis* different sampling groups of the southwest tropical Atlantic (standard deviations in parentheses). ROC: Rio das Ostras Channel, ROM: Rio das Ostras mangrove, NITC: Niterói Channel, NITM: Niterói mangrove, PAC: Paraty Channel, PAM: Paraty mangrove, 2015, 2016, 2017, and 2018 years of collection.

Sampling groups	Mean sample size	Nº alleles per locus	Polymorphism (%)	Mean heterozygosity	
				Observed	Expected
ROC 2015	21.30 ± 3.90	2.80 ± 0.30	100.00	0.553 ± 0.100	0.565 ± 0.072
ROC 2017	22.40 ± 1.30	3.40 ± 0.30	100.00	0.602 ± 0.570	0.635 ± 0.025
ROM 2015	19.50 ± 3.20	3.00 ± 0.20	100.00	0.441 ± 0.038	0.547 ± 0.025
ROM 2017	13.30 ± 0.80	3.30 ± 0.30	100.00	0.659 ± 0.107	0.623 ± 0.021
NITC 2016	32.60 ± 1.70	3.30 ± 0.30	100.00	0.569 ± 0.510	0.630 ± 0.023
NITC 2018	10.10 ± 2.30	2.80 ± 0.30	100.00	0.493 ± 0.069	0.549 ± 0.022
NITM 2016	23.40 ± 3.30	3.30 ± 0.20	100.00	0.547 ± 0.049	0.609 ± 0.027
NITM 2018	7.80 ± 1.80	2.80 ± 0.30	100.00	0.675 ± 0.116	0.590 ± 0.098
PAC 2017	45.00 ± 3.90	3.50 ± 0.30	100.00	0.562 ± 0.064	0.627 ± 0.039
PAC 2018	29.00 ± 3.60	3.00 ± 0.30	100.00	0.482 ± 0.097	0.482 ± 0.082
PAM 2017	28.50 ± 2.20	3.10 ± 0.10	100.00	0.624 ± 0.085	0.623 ± 0.021
PAM 2018	28.60 ± 4.20	3.00 ± 0.30	87.50	0.487 ± 0.099	0.541 ± 0.080
Average	23.40	3.10	98.90	0.557	0.585

Table 3. Hardy-Weinberg equilibrium probabilities (P) (Ho: joining gametes at random). Legend with the abbreviations of the different sampling groups is shown in Table 2. *Significant values after Bonferroni correction (α = 0.0005).

Sampling groups	Locus							
	α-Est-1	*α-Est-2*	*Lap-2*	*Mdh*	*Me-1*	*Me-2*	*Pgd*	*Pgi*
ROC 2015	0.0198	0.0752	0.5381	0.2863	0.2153	0.1707	-	0.0040
ROC 2017	0.0874	0.0009	0.2000	0.4973	0.3540	0.1298	0.0070	0.0043
ROM 2015	0.2501	0.0020	0.2514	0.0001*	0.1905	0.2867	-	0.0310
ROM 2017	0.0466	0.1256	0.2455	0.2453	0.0096	0.6623	0.0000*	0.8740
NITC 2016	0.6765	0.0054	0.0013	0.6012	0.0020	0.0093	0.0174	0.0000*
NITC 2018	0.7203	0.8228	0.1108	1.0000	1.0000	-	0.0401	0.0476
NITM 2016	0.0750	1.0000	0.5755	0.1517	0.0072	0.5497	0.1224	0.7559
NITM 2018	0.5503	1.0000	0.1418	1.0000	1.0000	-	0.3363	1.0000
PAC 2017	0.2274	0.0000*	0.0000*	0.0148	0.2410	0.2496	0.0145	0.0068
PAC 2018	0.5339	0.0133	0.0218	0.0474	0.1728	1.0000	0.0599	0.0756
PAM 2017	0.2963	0.0000*	0.0000*	0.0194	0.2421	0.0569	0.6877	0.2319
PAM 2018	0.1406	-	0.0035	0.6762	0.0084	0.5229	0.8015	0.2098

cant deviation from what was expected by the Hardy-Weinberg equilibrium after the Bonferroni correction (Table 3). However, seven significant deviations were found, a higher number than expected at random (5% of 91 tests = 4.55). Most deviations from the Hardy-Weinberg equilibrium were towards a heterozygote deficit, except for the *α-Est-2* locus, which showed evidence of excess heterozygotes in the Paraty sampling group in the channel and mangrove environments in 2017 (PAC 2017 and PAM 2017). The inbreeding indices presented no statistically significant results when the mean of all loci was taken (Table 4).

From the three analyzed dimensions (space, time, and environment), the temporal dimension seems to have the greatest influence on the structuring of the different sampling groups of *I. brasiliensis*, as indicated by the UPGMA dendrogram (Fig. 4), which shows three groups: (1) 2015, (2) 2018, and (3) 2016 and 2017 and the Bayesian analysis of the 429 individuals which also identified three groups as the most likely number of populations (Figs. 5-6) despite the clear mixed ancestry.

Traditional morphometry

The PCA of the linear measurements of *I. brasiliensis* valves indicated form discrimination between the 12 sampling groups. The first two principal components (PC) explained 66.30% of the total variation (PC1 =

Table 4. Weir & Cockerham (1984) inbreeding indices with confidence intervals (CI) for the means by jackknife and bootstrap. Ns: not significant. *a: significant by Jackknife.

Locus	f	Θ	F
α-Est-1	-0.095^{*a}	0.019^{*a}	-0.074^{*a}
α-Est-2	-0.375^{ns}	0.156^{*a}	-0.160^{ns}
Lap-2	0.400^{ns}	0.034^{*a}	0.420^{ns}
Mdh	-0.051^{ns}	0.037^{ns}	-0.012^{ns}
Me-1	0.072^{ns}	0.041^{ns}	0.072^{ns}
Me-2	0.209^{ns}	0.028^{*a}	0.231^{*a}
Pgd	0.294^{*a}	0.013^{*a}	0.303^{*a}
Pgi	0.073^{ns}	0.122^{*a}	0.186^{*a}
Todos	0.077^{ns}	0.054^{ns}	0.127^{ns}
Jackknife (CI 95%)	[0.070; 0.078]	[0.018; 0.054]	[0.062; 0.127]
Bootstrap (CI 95%)	[-0.060; 0.207]	[0.026; 0.090]	[0.010; 0.240]
Bootstrap (CI 99%)	[-0.107; 0.247]	[0.022; 0.100]	[-0.028; 0.273]

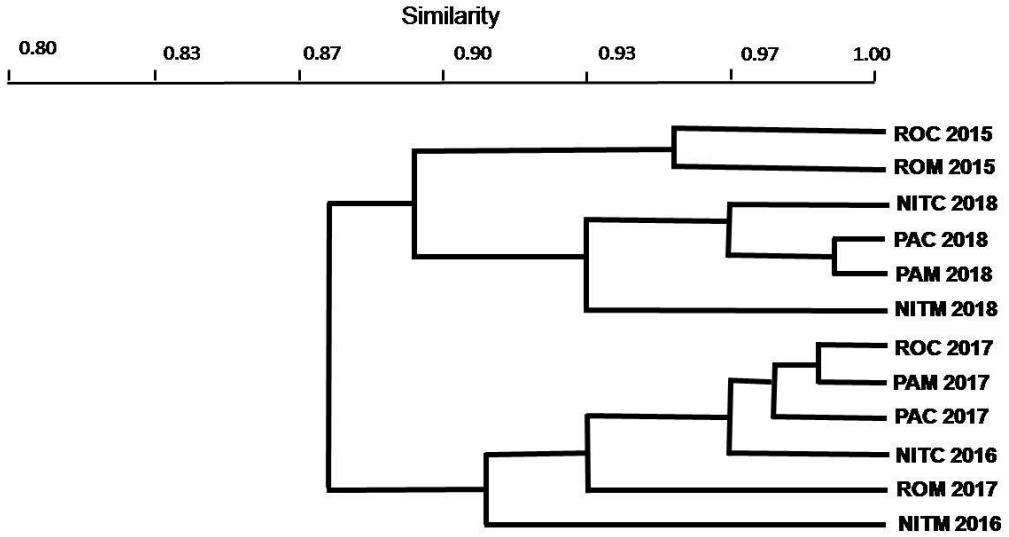

Figure 4. UPGMA dendrogram of genetic identities between different sampling groups. Legend with the abbreviations of the different sampling groups is shown in Table 2.

47.53%; PC2 = 18.80%). In addition, PC1 showed a high correlation with the length measurements of the anterior adductor muscle (Aam) and the valve height (Hei), with values of 0.96 and 0.95, respectively. In contrast, PC2 showed a high correlation with measuring the distance from the anterior adductor muscle to the anterior margin (Aamx), with a value of 0.95. The scatter plot constructed with the data provided by PCA (Fig. 7) indicates that sampling groups formed seven groupings: 1) with Rio das Ostras sampling groups in the channel and mangrove environments in 2015 (ROC 2015 and ROM 2015), Rio das Ostras in the channel environment in 2017 (ROC 2017), and Niterói in the channel environment in 2016 (NITC 2016); 2) with Paraty sampling groups in the channel and mangrove environments in 2018 (PAC 2018 and PAM 2018) and Paraty in the mangrove environment in 2017 (PAM 2017); 3) with Niterói in the channel environment in 2018 (NITC 2018); 4) with Rio das Ostras in the mangrove environment in 2017 (ROM 2017); 5) with Paraty in the channel environment in 2017 (PAC 2017); 6) with Niterói in the mangrove environment in 2016 (NITM 2016); and 7) with Niterói in the mangrove environment in 2018 (NITM 2018).

The discriminant analysis showed that the different sampling groups could be fully identified (100% discrimination) based on the variation of their 13 linear measures, except for PAC 2017, with 96.7% discrimination. The permutational multivariate analysis of variance (PERMANOVA) indicated, after Bonferroni correction, that the form of valves varied significantly

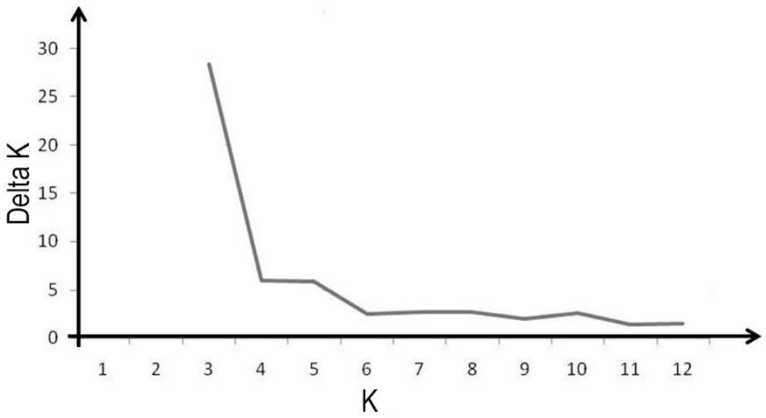

Figure 5. Delta K values relative to the number of tested populations (K) obtained in the program STRUCTURE through Bayesian analysis, where the highest Delta K value refers to the number of most likely populations.

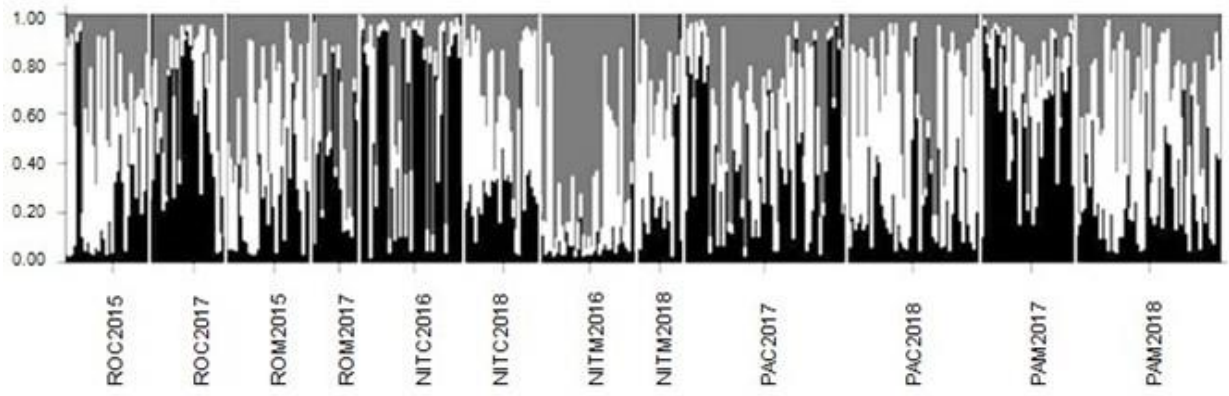

Figure 6. The graph obtained in the program STRUCTURE through Bayesian analysis shows the genetic structures between the 12 sampling groups collected from the *Iphigenia brasiliensis* populations, forming three groups. The graph shows which group the individuals in each sample unit are most present. Axis y stands for the probability of each individual to be assigned to one of the three populations (black, white and grey) defined by the STRUCTURE. Legend with the abbreviations of the different sampling groups is shown in Table 2.

between all different sampling groups in the different environments, times, and locations ($P < 0.0001$). The UPGMA dendrogram (Fig. 8) indicates the NITM 2018 isolated from the others. The influence of the temporal dimension (or the other dimensions) was not evident in the morphometric data of the linear measurements of *I. brasiliensis* valves.

Geometric morphometry

The first two PC in the analysis of the 19 FCs explained 92.7% of the total variation (PC1 = 80.8%; PC2 = 11.9%). The scatter plot in Figure 9, constructed with data provided by PCA, indicated the separation of the Niterói sampling group unit in the mangrove environment in 2016 (NITM 2016) from the other different sampling groups.

The discriminant analysis showed that the different sampling groups could be consistently identified based on the variation of the 19 FCs (Table 5), confirmed by PERMANOVA, which showed significant results for almost all different sampling groups (Table 6). The UPGMA dendrogram presented a separation of the NITM 2018 from the other different sampling groups (Fig. 10).

A signal of temporal influence on the forma of the valves can be inferred by the significant differences found among all sampling groups from 2016 in relation to the others, as well as the significant differences among Paraty sampling groups from 2018 in relation to the sampling groups of Rio das Ostras from 2015. Although it can be considered a very weak sign as it is indeed, taken together with genetic results it not at all negligible.

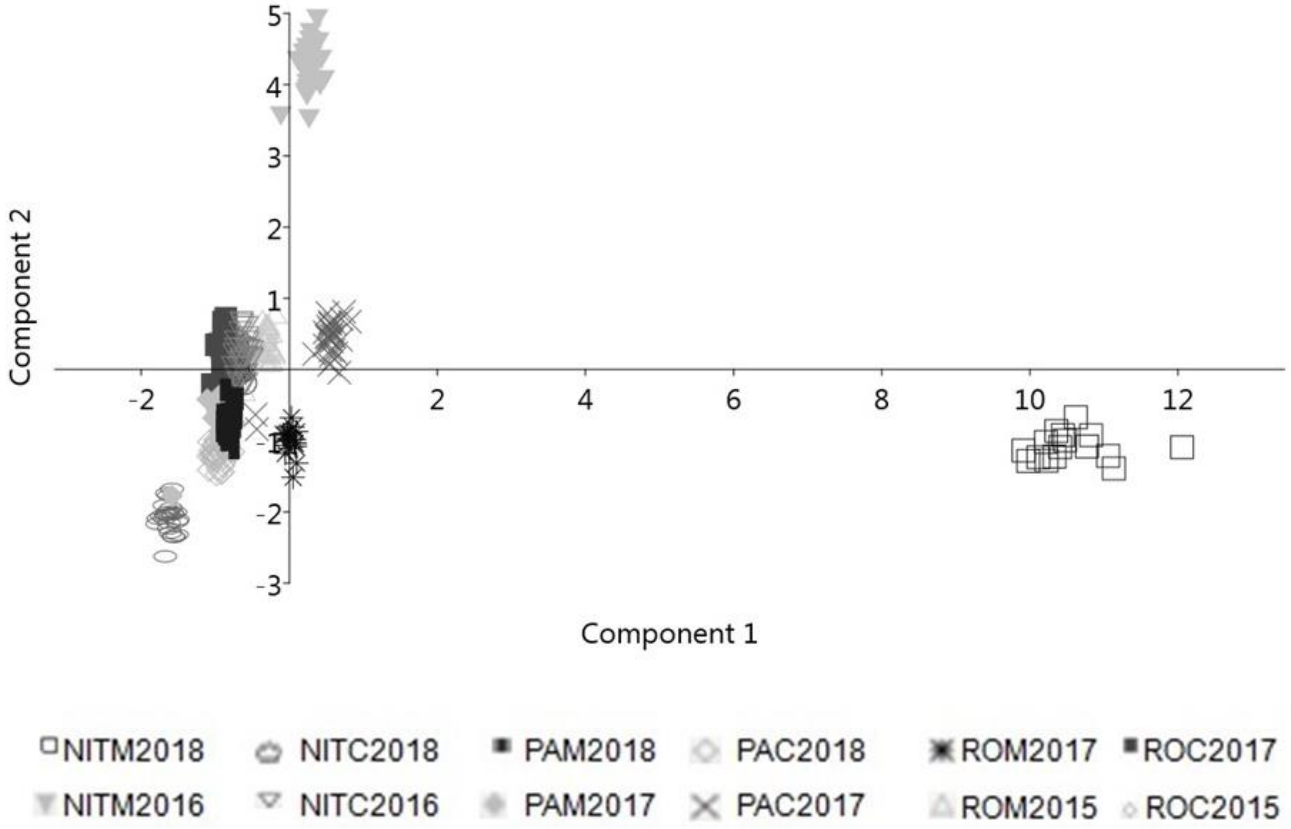

Figure 7. Scatter plot of the 12 different sampling groups of *Iphigenia brasiliensis* as a function of the two principal components of the 13 linear measurements. Legend with the abbreviations of the different sampling groups is shown in Table 2.

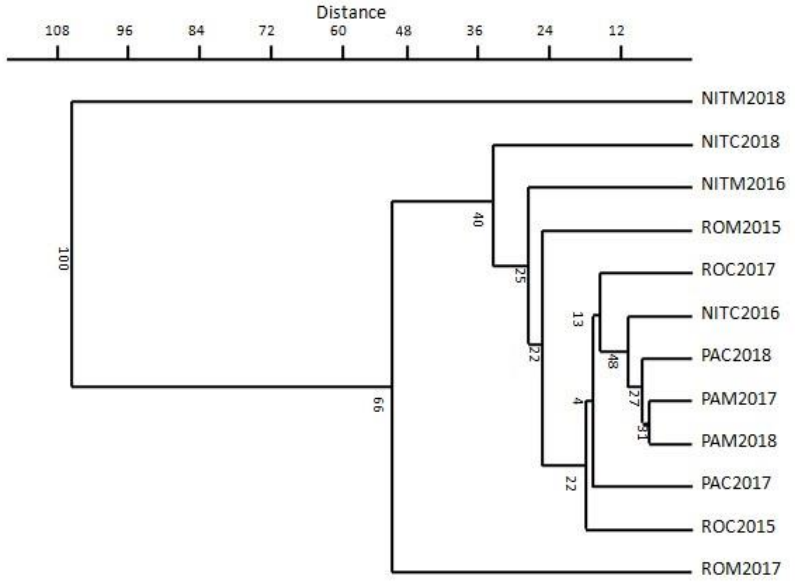

Figure 8. UPGMA dendrogram generated from the Euclidean distances of the 12 different sampling groups of *Iphigenia brasiliensis* analyzed through 13 linear measurements. The values in the branches indicate the support associated with each of these groupings. Legend with the abbreviations of the different sampling groups is shown in Table 2.

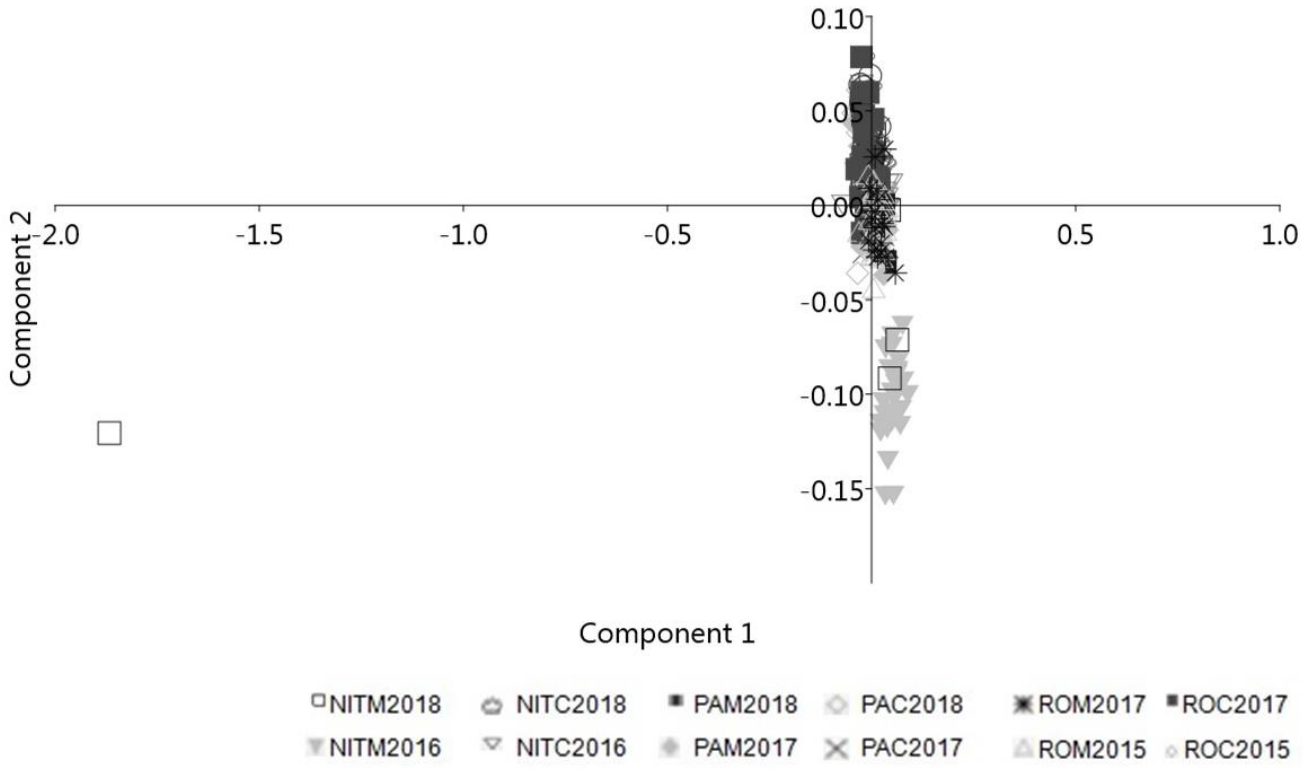

Figure 9. Scatter plot of the 12 different sampling groups of *Iphigenia brasiliensis* as a function of the two principal components of the 19 Fourier coefficients. Legend with the abbreviations of the different sampling groups is in Table 2.

Table 5. Percentage of *Iphigenia brasiliensis* individuals correctly classified in their sample unit from the discriminant analysis of the 19 Fourier coefficients. Legend with the abbreviations of the different sampling groups is shown in Table 2.

Different sampling groups	ROC 2015	ROC 2017	ROM 2015	ROM 2017	NITC 2016	NITC 2018	NITM 2016	NITM 2018	PAC 2017	PAC 2018	PAM 2017	PAM 2018
ROC2015	92.86	3.57	0	0	0	0	0	3.57	0	0	0	0
ROC2017	12.00	80.00	0	0	0	0	0	0.	4.00	4.00	0	0
ROM2015	3.33	0	86.67	6.67	0	0	0	0	3.33	0	0	0
ROM2017	0	0	5.88	94.12	0	0	0	0	0	0	0	0
NITC2016	0	0	0	0	86.67	6.67	0	3.33	0	3.33	0	0
NITC2018	4.00	0	4.00	0	0	88.00	0	0	0	0	4.00	0
NITM2016	0	0	0	0	0	0	100.00	0	0	0	0	0
NITM2018	0	0	0	0	6.25	0	12.50	81.25	0	0	0	0
PAC2017	6.67	0	0	0	0	0	0	0	80.00	6.67	3.33	3.33
PAC2018	0	3.33	0	0	0	3.33	0	0	10.00	66.67	3.33	13.33
PAM2017	0	0	3.33	0	0	0	0	0	3.33	10.00	76.67	6.67
PAM2018	0	0	0	0	0	0	0	0	10.00	0	13.33	76.67

DISCUSSION

Genetic variation

The genetic variation observed for *Iphigenia brasiliensis* in the different sampling groups was high but within what was found for *I. brasiliensis* by Bonner et al. (2019) and other bivalve species belonging to the family Donacidae, such as the species *Donax vittatus* (H_o between 0.389 to 0.460, Fernández-Pérez et al. 2019) and *Donax trunculus* (H_o between 0.207 to 0.768, Marie et al. 2016). Regarding the Hardy-Weinberg equilibrium, seven loci violated the null hypothesis of the random union of gametes, six of them towards a heterozygote deficit. Heterozygote deficiency is com-

Table 6. Permutational multivariate analysis of variance (PERMANOVA) among the 12 different sampling groups of *Iphigenia brasiliensis* using the 19 Fourier coefficients. Legend with the abbreviations of the different sampling groups is shown in Table 2. *Significant values after Bonferroni correction.

Sampling groups	ROC 2015	ROC 2017	ROM 2015	ROM 2017	NITC 2016	NITC 2018	NITM 2016	NITM 2018	PAC 2017	PAC 2018	PAM 2017	PAM 2018
ROC2015	-	1.0000	0.0066*	0.0066*	0.0066*	1.0000	0.0066*	1.0000	0.0066*	0.0528	0.0066*	0.0066*
ROC2017	1.0000	-	0.0066*	0.0066*	0.0462*	0.8250	0.0066*	1.0000	0.9372	1.0000	0.0132*	0.0396*
ROM2015	0.0066*	0.0066*	-	1.0000	0.0066*	0.0066*	0.0066*	1.0000	0.0066*	0.0066*	0.0132*	0.0066*
ROM2017	0.0066*	0.0066*	1.0000	-	0.7986	0.0066*	0.0066*	1.0000	0.0066*	0.0066*	0.1848	0.033*
NITC2016	0.0066*	0.0462*	0.0066*	0.7986	-	0.1122	0.0066*	1.0000	1.0000	0.1650	1.0000	1.0000
NITC2018	1.0000	0.8250	0.0066*	0.0066*	0.1122	-	0.0066*	1.0000	0.0198*	0.0792	0.0066*	0.0396*
NITM2016	0.0066*	0.0066*	0.0066*	0.0066*	0.0066*	0.0066*	-	0.0066*	0.0066*	0.0066*	0.0066*	0.0066*
NITM2018	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0066*	-	1.0000	1.0000	1.0000	1.0000
PAC2017	0.0066*	0.9372	0.0066*	0.0066*	1.0000	0.0198*	0.0066*	1.0000	-	1.0000	1.0000	1.0000
PAC2018	0.0528	1.0000	0.0066*	0.0066*	0.1650	0.0792	0.0066*	1.0000	1.0000	-	0.7524	0.9042
PAM2017	0.0066*	0.0132*	0.0132*	0.1848	1.0000	0.0066*	0.0066*	1.0000	1.0000	0.7524	-	1.0000
PAM2018	0.0066*	0.0396*	0.0066*	0.0330*	1.0000	0.0396*	0.0066*	1.0000	1.0000	0.9042	1.0000	-

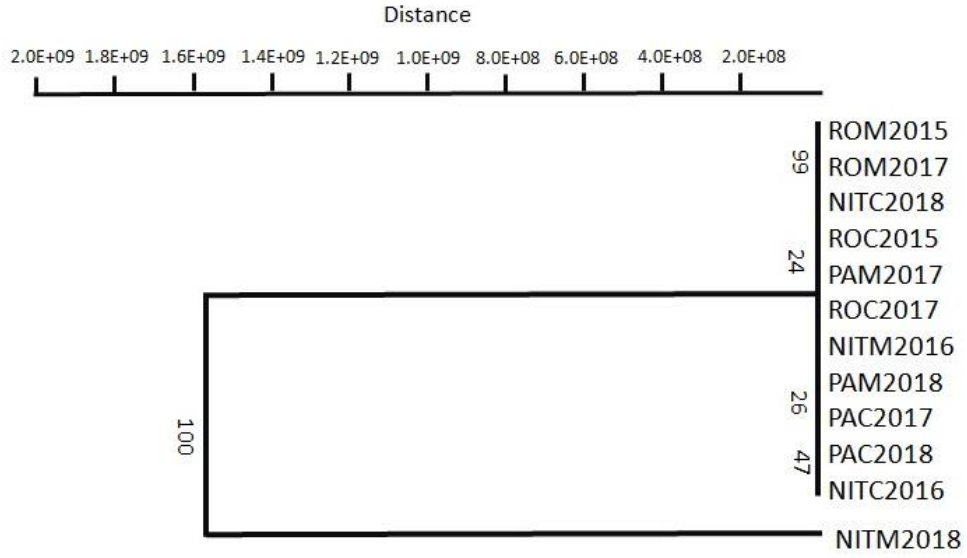

Figure 10. UPGMA dendrogram generated from the Euclidean distances of the 12 different sampling groups of *Iphigenia brasiliensis* analyzed through 19 Fourier coefficients. The values in the branches indicate the support associated with each of these groupings. Legend with the abbreviations of the different sampling groups is shown in Table 2.

monly reported in studies with bivalves (Laudien et al. 2003, Zhao et al. 2009, Rajaei et al. 2014). Its explanation is related to inbreeding (Bierne et al. 1998), natural selection (Bonner et al. 2019), null alleles (Zhao et al. 2009), and the Wahlund effect (Hare et al. 1996). In the present study, the Wahlund effect and inbreeding do not seem to be good explanations, as they would produce effects in all loci (Zhao et al. 2009), which was not observed. Also, the results of inbreeding indices (f) were not statistically significant. However, the null alleles and natural selection hypotheses cannot be ruled out due to the sample design used in this study.

Excesses of heterozygotes are not commonly observed in bivalves, but they have been previously observed by Bonner et al. (2019) for the same loci (*α-Est-2*) in individuals also collected in Paraty in the channel and mangrove environments. The phenomenon has also been observed for other bivalves, such as *Mactra corallina* (Chetoui et al. 2012) and *Solen marginatus* (Hmida et al. 2012). The causes associated with the excess of heterozygotes in the described cases were the action of natural selection against homozygosis (Hmida et al. 2012) and a high rate of migration and dispersion of planktonic larvae (Chetoui et al. 2012). In the present

study, the observed excesses could not have their cause defined. However, explanations such as the action of natural selection under the loci in variable environments cannot be ruled out, as discussed by Bonner et al. (2019), or variations in migration, dispersion, and recruitment of larvae.

It is expected to find geographic structuring among populations of estuarine organisms because migration may not occur in the dispersing process from one estuary to another due to the large open sea segments between different estuaries (Calazans et al. 2017) and wide variations in temperature and salinity to which the larvae are exposed in their migratory path (Braby & Somero 2006). Moreover, immigrant larvae also encounter barriers that influence their settlement and survival in different estuarine habitats (Christensen & Pyne 2020) due to the different salinity levels and substrates, for example (Peterson 2003).

The channel has the highest salinity levels (due to its proximity to the ocean) and sandy substrate among the studied estuarine habitats, different from the mangroves, where the substrate is muddy, and the salinity levels are lower than in the channel. Species present in these habitats, such as *I. brasiliensis*, are always subject to environmental and temporal variations in temperature, salinity, and nutrients, among others, which can influence the patterns of genetic variation (Sgrò & Hoffmann 2004, Lee & Boulding 2009). However, the results did not indicate a statistically significant influence of the geographic and environmental dimensions studied in the genetic variation, although signs of a low temporal influence were observed.

Spatiotemporal analyses carried out with other bivalve species have already indicated the influence of the temporal dimension on the population structure (Arnaud & Laval 2004, Lee & Boulding 2009). The explanation for this behavior is based on the different patterns of recruitment that individuals from populations of different geographic regions may present over the years (Lee & Boulding 2009) or variations in reproductive success between cohorts (Vera et al. 2016). The influence of changes in gene flow patterns (Wade & McCauley 1988) between the different sampling groups worked on in this study cannot be neglected and may be the result of oscillations in the extinction and recolonization events over the years, which characterizes a metapopulation structure.

The dynamics of metapopulations have already been described for bivalves such as *Cerastoderma edule* (Genelt-Yanovskiy et al. 2018) and *Mytilus galloprovincialis* (Gardner & Westfall 2012). Metapopulations can be defined as a network formed by populations structured in clusters under the strong influence of local migration dynamics, with extinction and recolonization as the main processes (Hanski 1997, Baguette et al. 2017). Oscillations in the extinction and recolonization processes can be determined by geographic, environmental, and temporal factors that affect the dispersion, recruitment, and reproduction of organisms over time (Figueroa-Fábrega et al. 2018). According to Hanski (1997), a metapopulation must satisfy four conditions: 1) the habitat considered adequate must occur in discrete patches and be occupied by local populations that reproduce; 2) even the largest population must be at significant risk of extinction; 3) habitat patches must not be isolated to the point of preventing recolonization; and 4) local populations cannot have fully synchronized dynamics.

In estuaries, natural barriers are formed by several factors, such as salinity gradients and different substrates along their length. Natural barriers or habitat fragmentation caused, for instance, by anthropogenic activities, can determine habitat fragmentation and the organization of populations in patches (Collingham & Huntley 2000). Anthropogenic actions such as organic pollution and urbanization can also cause habitat fragmentation (Quintino et al. 2009, Stocken et al. 2019), influencing the connectivity between populations of estuarine organisms (Peacock & Smith 1997, Thrush et al. 2008). All these conditions are common in the estuaries where *I. brasiliensis* was studied, thus satisfying conditions 1 and 3. However, the results did not indicate connectivity limitations between different sampling groups.

Impacts on commercially important bivalve population sizes due to overexploitation have been described in the literature for commercially exploited bivalve species (Blaber et al. 2000, Coleman & Williams 2002, Bhattacharya & Sarkar 2003), as is the case of *I. brasiliensis* (Teixeira & Campos 2019). The commercial exploitation of bivalve species is seasonal and can be very intense at certain times of the year due to the time of maturation and growth (Katsanevakis et al. 2008, Hausmann & Meredith-Williams 2017). Also, the estuary of the Itaipu Lagoon has records of strong anthropogenic influence on its environmental conditions (Cerda et al. 2013, Laut et al. 2016), which may determine an environmental fragmentation with consequent reduction of population sizes. Thus, satisfying the two conditions necessary to postulate a metapopulation structure.

The dynamics between subpopulations of a metapopulation vary over time due to several factors, such as natural barriers, environmental changes, and anthropogenic influences, which can affect migration

between them or determine extinction events of local subpopulations (Figueroa-Fábrega et al. 2018). Thus, *I. brasiliensis* is under the most diverse environmental conditions in the estuaries, and the dynamics between different sampling groups can change over time, subject to condition 4.

In summary, the results indicated clear patterns of mixed ancestry with evidence of temporal influence in structuring patterns of the genetic variation of *I. brasiliensis* which may indicate fluctuations in the dynamics between sampling groups reflecting the action of natural barriers, such as temperature and salinity gradients, which interfere with the dispersion and colonization processes (Hanski 1997) and temporal fluctuations in population sizes under the strong anthropogenic influence, determining a regime of extinction and recolonization over time. In this sense, signs of temporal influence in the structuring of *I. brasiliensis* indicate a possible organization of the species in the studied locations, environments, and times in metapopulations.

Morphological variation

Traditional morphometry showed significant differences between all sampling groups, but it was unable to define which of the analyzed dimensions defined these differences. Morphological variations in bivalves are generally associated with natural selection, geographic isolation, genetic drift, or phenotypic plasticity (Fassatoui et al. 2014). The most plausible explanation is phenotypic plasticity, which occurs through the expression of several phenotypes from the interaction of a single genotype with local environmental conditions (Thompson 1991, Agrawal 2001, Pimpinelli & Piacentini 2020). Thus, the different environmental variables, latitudinal gradients, and temporal variation may have influenced the form of valves. In contrast, the results of geometric morphometry evidenced a signal of the temporal influence on the form of *I. brasiliensis* valves.

Geometric morphometry detects subtle variations in form, which are often not detectable by traditional morphometry (Zelditch et al. 2004, Mitteroecker & Gunz 2009, Adams & Otárola-Castillo 2013). Willsie et al. (2020) sought to determine whether morphometric analyses could be used to distinguish the species *Fusconaia flava* and *Pleurobema sintoxia* reliably, which are similar species regarding shell shape and color, resulting in many identification errors and observed that traditional morphometry was less useful in detecting differences between the two species than geometric morphometry. Similarly, Morán et al. (2018) identified variations regarding the form of valves of the bivalve *Ameghinomya antiqua* between the Holocene and the present using geometric morphometry. In this case, the study of the form of valves of this species was used for paleoenvironmental reconstructions. The authors indicated that the differences found could be associated with differentiation in environments over time, such as wave actions, tidal influences, temperature variations, and substrate on the sea surface, which have important effects on the form and size of the shells of this species.

Variations in shells may be related to phenotypic plasticity, genetic variability, or their combination. The signs of temporal influence in the form of *I. brasiliensis* valves, indicated by the geometric morphometry, coincide with what was found in the genetic variation. Associations between genetic and morphological variation have been previously reported for bivalves (Zhao et al. 2009, Zieritz et al. 2010, Rajaei et al. 2014) but not simultaneously related to temporal influences (Paolucci et al. 2014). Analyses of geometric morphometry and genetic variation conducted by Rajaei et al. (2014) found significant differences between two populations of the species *Pinctada radiata* from different locations, probably due to the stressful conditions of the environments. Also, significant associations were found between genetic and morphological variations between populations of the species *Coelomactra antiquata* (Kong et al. 2007) and *Cyclina sinensis* (Zhao et al. 2009), showing high differences between the populations collected from different locations along the coast of China, indicating the effect of possible physical barriers on gene flow.

In the present study, the evidence is the simultaneity of the temporal effect on the genetic and morphological variation. These effects can be explained by seasonal influences of environments, which can act on genotypes and generate a diversity of phenotypes over time (Ghalambor et al. 2007). Moreover, temporal influences on migration rates between different sampling groups can cause phenotypic variations. In this case, according to the model proposed by Sultan & Spencer (2002), moderate to high migration rates between subpopulations of a metapopulation can promote phenotypic plasticity, contributing to the difference in morphological patterns between subpopulations over time. These possibilities are plausible explanations for the results obtained here, considering the heterogeneity of estuarine environments in which the species *I. brasiliensis* inhabits and the lack of structure between different sampling groups.

In short, the results of this study indicated a pattern of mixed ancestry and signs that genetic variation may fluctuate over time. These results are reinforced by the evidence that a similar influence may occur on the morphological variation of valves inferred by geometric morphometry. The fact that the genetic results show only a small temporal influence may be related to the short period analyzed; that is, the influence of the temporal dimension may be stronger than it was possible to infer from the results. Thus, prolonged temporal accompaniment studies would be required for the species *I. brasiliensis* at these same locations to better understand the dynamics in action. However, the data shown in this study are the first on morphological and genetic variation of the species in the latitudinal, environmental, and temporal dimensions, simultaneously representing relevant information for the exploration, management, and conservation of this commercially important species.

ACKNOWLEDGMENTS

The authors would like to thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for scholarships for MRD (Post-doctorate), TRFG (MSc) and AB (MSc).

REFERENCES

Adams, D.C. & Otárola-Castillo, E. 2013. Geomorph: an R package for the collection and analysis of geometric morphometric shape data. Methods in Ecology and Evolution, 4: 393-399. doi: 10.1111/2041-210X.12035

Adams, J.B., Bate, G.C. & Riddin, T. 2016. Ecology and biodiversity of estuaries. South African Journal of Botany, 107: 1-4. doi: 10.1016/j.sajb.2016.08.019

Adams, D.C., Rohlf, F.J. & Slice, D.E. 2004. Geometric morphometrics: ten years of progress following the 'revolution'. Italian Journal of Zoology, 71: 5-16. doi: 10.1080/11250000409356545

Agrawal, A.A. 2001. Phenotypic plasticity in the interactions and evolution of species. Science, 294: 321-326. doi: 10.1126/science.1060701

Arnaud, J-F. & Laval, G. 2004. Stability of genetic structure and effective population size inferred from temporal changes of microsatellite DNA polymorphisms in the land snail *Helix aspersa* (Gastropoda: Helicidae). Biological Journal of the Linnean Society, 82: 89-102. doi: 10.1111/j.1095-8312.2004.00320.x

Arruda, C.C.B., Beasley, C.R., Vallinoto, M., Marques-Silva, N.D.S. & Tagliaro, C.H. 2009. Significant genetic differentiation among populations of *Anomalocardia brasiliana* (Gmelin, 1791): a bivalve with planktonic larval dispersion. Genetics and Molecular Biology, 32: 423-430. doi: 10.1590/S1415-47572009000200033

Arunkumar, A.N., Warrier, R.R., Shanthi, A. & Joshi, G. 2018. Allozyme variations to measure genetic diversity in clonal accessions of Indian sandalwood (*Santalum album*). International Journal of Forestry and Horticulture, 4: 1-8.

Auld, J.R., Agrawal, A.A. & Relyea, R.A. 2010. Re-evaluating the costs and limits of adaptive phenotypic plasticity. Proceedings of the Royal Society B: Biological Sciences, 277: 503-511. doi: 10.1098/rspb.2009.1355

Baguette, M., Michniewicz, R. & Stevens, V.M. 2017. From genes to metapopulations. Nature Ecology and Evolution, 1: 0130. doi: 10.1038/s41559-017-0130

Barton, N.H. & Charlesworth, B. 1984. Genetic revolutions, founder effects, and speciation. Annual Review of Ecology and Systematics, 15: 133-164. doi: 10.1146/annurev.es.15.110184.001025

Begum, S., Brey, T. & Held, C. 2018. Lack of association between genetical and morphological variations for bivalve *Arctica islandica* from six different sites of the NE Atlantic Ocean. Estuarine, Coastal and Shelf Science, 212: 34-39. doi: 10.1016/j.ecss.2018.06.014

Bernstein, S.C., Throckmorton, L.H. & Hubby, J.L. 1973. Still more genetic variability in natural populations. Proceedings of the National Academy of Sciences, 70: 3928-3931. doi: 10.1073/pnas.70.12.3928

Bhattacharya, A. & Sarkar, S.K. 2003. Impact of overexploitation of shellfish: northeastern coast of India. AMBIO: A Journal of the Human Environment, 32: 70-75. doi: 10.1579/0044-7447-32.1.70

Bierne, N., Launey, S., Naciri-Graven, Y. & Bonhomme, F. 1998. Early effect of inbreeding as revealed by microsatellite analyses on *Ostrea edulis* larvae. Genetics, 148: 1893-1906.

Bitner-Mathé, B.C., Peixoto, A.A. & Klaczko, L.B. 1995. Morphological variation in a natural population of *Drosophila mediopunctata*: altitudinal cline, temporal changes and influence of chromosome inversions. Heredity, 75: 54-61. doi: 10.1038/hdy.1995.103

Blaber, S.J.M., Cyrus, D.P., Albaret, J-J., Ching, C.V., Day, J.W., Elliott, M., et al. 2000. Effects of fishing on the structure and functioning of estuarine and nearshore ecosystems. ICES Journal of Marine Science, 57: 590-602. doi: 10.1006/jmsc.2000.0723

Bonamour, S., Chevin, L.M., Charmantier, A. & Teplitsky, C. 2019. Phenotypic plasticity in response

to climate change: the importance of cue variation. Philosophical Transactions of the Royal Society B: Biological Sciences, 374: 20180178. doi: 10.1098/rstb.2018.0178

Bonner, A., Ferreira, M.S.N., Duarte, M.R. & Silva, E.P. 2019. Genetic variation and asymmetry in populations of *Iphigenia brasiliensis* (Lamarck, 1818) from different localities and environments. Journal of Natural History, 53: 73-88. doi: 10.1080/00222933.2019.1576934

Braby, C.E. & Somero, G.N. 2006. Following the heart: temperature and salinity effects on heart rate in native and invasive species of blue mussels (genus *Mytilus*). Journal of Experimental Biology, 209: 2554-2566. doi: 10.1242/jeb.02259

Burnaby, T.P. 1966. Growth-invariant discriminant functions and generalized distances. Biometrics, 22: 96-110. doi: 10.2307/2528217

Calazans, S.H., Walters, L.J., Fernandes, F.C., Ferreira, C.E.L. & Hoffman, E.A. 2017. Genetic structure provides insights into the geographic origins and temporal change in the invasive charru mussel (Sururu) in the southeastern United States. Plos One, 12: e0180619. doi: 10.1371/journal.pone.0180619

Carson, H.L. & Johnson, W.E. 1975. Genetic variation in Hawaiian *Drosophila*. I. Chromosome and allozyme polymorphism in *D. setosimentum* and *D. ochrobasis* from the island of Hawaii. Evolution, 29: 11-23. doi: 10.2307/2407138

Cerda, M., Nunes-Barboza, C.D., Scali-Carvalho, C.N., Andrade-Jandre, K. & Marques, A.N. 2013. Nutrient budgets in the Piratininga-Itaipu lagoon system (southeastern Brazil): effects of sea-exchange management. Latin American Journal of Aquatic Research, 41: 226-238. doi: 10.3856/vol41-issue2-fulltext-3

Ceuta, L.O., Boehs, G. & Santos, J.J.B. 2010. Hermaphroditism among dioecious *Tagelus plebeius* (Lightfoot, 1786) (Mollusca, Psammobiidae) and *Iphigenia brasiliana* (Lamarck, 1818) (Mollusca, Donacidae) on the Cachoeira River Estuary, Ilhéus, Bahia, Brazil. Brazilian Journal of Biology, 70: 125-127. doi: 10.1590/S1519-69842010000100017

Chapman, P.M. & Wang, F. 2001. Assessing sediment contamination in estuaries. Environmental Toxicology and Chemistry, 20: 3-22. doi: 10.1002/etc.5620200102

Chetoui, I., El Cafsi, M. & Boussaid, M. 2012. Allozymic and morphological variation in three populations of surf clam *Mactra corallina* (Bivalvia: Mactridae) from Tunisian sandy beaches. Cahiers de Biologie Marine, 53: 409-417.

Cheverud, J.M. 1984. Quantitative genetics and developmental constraints on evolution by selection. Journal of Theoretical Biology, 110: 155-171. doi: 10.1016/S0022-5193(84)80050-8

Christensen, A.B. & Pyne, M.I. 2020. The effect of a permanent saltwater barrier on *Rangia cuneata*, an indicator species of brackish conditions. Estuarine, Coastal and Shelf Science, 243: 106800. doi: 10.1016/j.ecss.2020.106800

Clark, A.G. 2000. Limits to prediction of phenotypes from knowledge of genotypes. In: Clegg, M.T., Hecht, M.K. & Macintyre, R.J. (Eds.). Evolutionary Biology. Springer, Boston, pp. 205-224.

Coleman, F.C. & Williams, S.L. 2002. Overexploiting marine ecosystem engineers: potential consequences for biodiversity. Trends in Ecology and Evolution, 17: 40-44. doi: 10.1016/S0169-5347(01)02330-8

Collingham, Y.C. & Huntley, B. 2000. Impacts of habitat fragmentation and patch size upon migration rates. Ecological Applications, 10: 131-144. doi: 10.1890/1051-0761(2000)010%5B0131:IOHFAP%5D2.0.CO;2

Corte, G.N., Coleman, R.A. & Amaral, A.C.Z. 2017. Environmental influence on population dynamics of the bivalve *Anomalocardia brasiliana*. Estuarine, Coastal and Shelf Science, 187: 241-248. doi: 10.1016/j.ecss.2017.01.016

Costa, V.C., Amorim, F.A.C., Babos, D.V. & Pereira-Filho, E.R. 2019. Direct determination of Ca, K, Mg, Na, P, S, Fe and Zn in bivalve mollusks by wavelength dispersive X-ray fluorescence (WDXRF) and laser-induced breakdown spectroscopy (LIBS). Food Chemistry, 273: 91-98. doi: 10.1016/j.foodchem.2018.02.016

Crampton, J.S. 1995. Elliptic Fourier shape analysis of fossil bivalves: some practical considerations. Lethaia, 28: 179-186. doi: 10.1111/j.1502-3931.1995.tb01611.x

Cruz, D.T., Selbach-Schnadelbach, A., Lambert, S.M., Ribeiro, P.L. & Borba, E.L. 2011. Genetic and morphological variability in *Cattleya elongata* Barb. Rodr. (Orchidaceae), endemic to the campo rupestre vegetation in northeastern Brazil. Plant Systematics and Evolution, 294: 87-98. doi: 10.1007/s00606-011-0444-0

Duarte, M.R., Otegui, A.C.P., Fernandes, F.C. & Silva, E.P. 2018. High levels of genetic and morphological variability in invasive *Limnoperna fortunei* (Dunker, 1857) populations in South America. Malacologia, 62: 171-186. doi: 10.4002/040.062.0106

Fassatoui, C., Jenhani, A.B.R. & Romdhane, M.S. 2014. Geographic pattern of shell morphology in the

endemic freshwater mussel *Unio ravoisieri* (Bivalvia: Unionidae) from northern Tunisia. Journal of Molluscan Studies, 81: 152-160. doi: 10.1093/mollus/eyu069

Fenderson, L.E., Kovach, A.I. & Llamas, B. 2020. Spatio-temporal landscape genetics: investigating ecology and evolution through space and time. Molecular Ecology, 29: 218-246. doi: 10.1111/mec.15315

Fernández-Pérez, J., Nantón, A., Arias-Pérez, A., Insua, A. & Méndez, J. 2019. Fifteen novel microsatellite loci, developed using next-generation sequencing, reveal the lack of genetic structure in *Donax vittatus* from Iberian Peninsula. Estuarine, Coastal and Shelf Science, 217: 218-225. doi: 10.1016/j.ecss.2018.11.011

Figueroa-Fábrega, L., Bravo-Samaha, J., Silva-Haun, R. & Padilla, T. 2018. Dinámica metapoblacional, ocupación espacial y migración en bivalvos de importancia comercial: el caso de la almeja *Tawera gayi* (Bivalvia: Veneridae) en el Mar Interior de Chiloé. Anales del Instituto de la Patagonia, 46: 7-21. doi: 10.4067/S0718-686X2018000100007

Fins, L. & Seeb, L.W. 1986. Genetic variation in allozymes of western larch. Canadian Journal of Forest Research, 16: 1013-1018. doi: 10.1139/x86-177

Freitas, L.E.L.D., Feitosa, C.V. & Araújo, M.E.D. 2006. Mangrove oyster (*Crassostrea rhizophorae*) (Guilding, 1928) farming areas as artificial reefs for fish: a case study in the state of Ceará, Brazil. Brazilian Journal of Oceanography, 54: 31-39. doi: 10.1590/S1679-87592006000100003

Gardner, J.P.A. & Westfall, K.M. 2012. Geographic distribution and molecular identification of a metapopulation of blue mussels (genus *Mytilus*) in northeastern New Zealand. Journal of Molluscan Studies, 78: 66-73. doi: 10.1093/mollus/eyr037

Genelt-Yanovskiy, E., Nazarova, S., Tarasov, O., Mikhailova, N. & Strelkov, P. 2018. Phylogeography of the temperate marine bivalve *Cerastoderma edule* (Linnaeus, 1758) (Bivalvia: Cardiidae) in the Subarctic: unique diversity and strong population structuring at different spatial scales. Journal of Zoological Systematics and Evolutionary Research, 57: 67-79. doi: 10.1111/jzs.12231

Ghalambor, C.K., McKay, J.K., Carroll, S.P. & Reznick, D.N. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Functional Ecology, 21: 394-407. doi: 10.1111/j.1365-2435.2007.01283.x

Gomes, V.J.C., Asp, N.E., Siegle, E., Gomes, J.D., Silva, A.M.M., Ogston, A.S. & Nittrouer, C.A. 2021. Suspended-sediment distribution patterns in tide-dominated estuaries on the eastern Amazon coast: geomorphic controls of turbidity-maxima formation. Water, 13: 1568. doi: 10.3390/w13111568

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). [http://www2.unil.ch/popgen/softwares/fstat.htm]. Reviewed: February 26, 2021.

Grant, P.R. 2001. Reconstructing the evolution of birds on islands: 100 years of research. Oikos, 92: 385-403. doi: 10.1034/j.1600-0706.2001.920301.x

Gronau, I. & Moran, S. 2007. Optimal implementations of UPGMA and other common clustering algorithms. Information Processing Letters, 104: 205-210. doi: 10.1016/j.ipl.2007.07.002

Gupta, A.P. & Lewontin, R.C. 1982. A study of reaction norms in natural populations of *Drosophila pseudoobscura*. Evolution, 36: 934-948. doi: 10.2307/2408073

Hammer, Ø., Harper, D.A.T. & Ryan, P.D. 2001. PAST: Paleontological statistics software package for education and data analysis. Paleontologia Electronica, 4: 1-9.

Hamrick, J.L., Godt, M.J.W. & Sherman-Broyles, S.L. 1992. Factors influencing levels of genetic diversity in woody plant species. New Forests, 6: 95-124. doi: 10.1007/BF00120641

Hanski, I. 1997. Metapopulation dynamics: from concepts and observations to predictive models. In: Hanski, I. & Gilpin, M.E. (Eds.). Metapopulation biology: ecology, genetics, and evolution. Academic Press, San Diego, pp. 69-91.

Hare, M.P., Karl, S.A. & Avise, J.C. 1996. Anonymous nuclear DNA markers in the American oyster and their implications for the heterozygote deficiency phenomenon in marine bivalves. Molecular Biology and Evolution, 13: 334-345. doi: 10.1093/oxfordjournals.molbev.a025593

Harris, H. & Hopkinson, D.A. 1976. Handbook of enzyme electrophoresis in human genetics. North-Holland Publishing Company, Amsterdam.

Hausmann, N. & Meredith-Williams, M. 2017. Seasonal patterns of coastal exploitation on the Farasan Islands, Saudi Arabia. Journal of Island and Coastal Archaeology, 12: 360-379. doi: 10.1080/15564894.2016.1216478

Haynes, W. 2013. Bonferroni correction. In: Dubitzky, W., Wolkenhauer, O., Cho, K-H. & Yokota, H. (Eds.). Encyclopedia of systems biology. Springer, New York, pp. 154.

Hiroki, K. 1971. Fisioecologia de invertebrados marinhos: resistência à anoxia. Boletim de Zoologia e Biologia Marinha, 28: 315-341. doi: 10.11606/issn.2526-3366.bzbm.1971.121223

Hmida, L., Fassatoui, C., Ayed, D., Ayache, N. & Romdhane, M.S. 2012. Genetic characterization of the razor clam *Solen marginatus* (Mollusca: Bivalvia: Solenidae) in Tunisian coasts based on isozyme markers. Biochemical Systematics and Ecology, 40: 146-155. doi: 10.1016/j.bse.2011.10.016

Hubby, J.L. & Lewontin, R.C. 1966. A molecular approach to the study of genic heterozygosity in natural populations. I. The number of alleles at different loci in *Drosophila pseudoobscura*. Genetics, 54: 577-594. doi: 10.1093/genetics/54.2.577

Inoue, K., Hayes, D.M., Harris, J.L. & Christian, A.D. 2013. Phylogenetic and morphometric analyses reveal ecophenotypic plasticity in freshwater mussels *Obovaria jacksoniana* and *Villosa arkansasensis* (Bivalvia: Unionidae). Ecology and Evolution, 3: 2670-2683. doi: 10.1002/ece3. 649

Jablonka, E. 2012. Epigenetic variations in heredity and evolution. Clinical Pharmacology & Therapeutics, 92: 683-688. doi: 10.1038/clpt.2012.158

Jablonka, E. & Lamb, M.J. 1989. The inheritance of acquired epigenetic variations. Journal of Theoretical Biology, 139: 69-83. doi: 10.1016/S0022-5193(89) 80058-X

Jormalainen, V., Boothroyd, J.C., Dove, A. & Shuster, S.M. 2019. A comparison of genetic variation in two endemic thermal spring isopods, *Thermosphaeroma thermophilum* and *T. milleri* (Crustacea-Isopoda: Sphaeromatidae). In: Ghafor, I.M. (Ed.). Crustacea. IntechOpen, London.

Kanhai, L.D.K., Gobin, J.F., Beckles, D.M., Lauckner, B. & Mohammed, A. 2014. Metals in sediments and mangrove oysters (*Crassostrea rhizophorae*) from the Caroni Swamp, Trinidad. Environmental Monitoring and Assessment, 186: 1961-1976. doi: 10.1007/s10 661-013-3510-y

Katsanevakis, S., Lefkaditou, E., Galinou-Mitsoudi, S., Koutsoubas, D. & Zenetos, A. 2008. Molluscan species of minor commercial interest in Hellenic seas: distribution, exploitation and conservation status. Mediterranean Marine Science, 9: 77-118. doi: 10.12681/mms.145

Kefford, B.J., Buchwalter, D., Canedo-Argüelles, M., Davis, J., Duncan, R.P., Hoffmann, A. & Thompson, R. 2016. Salinized rivers: degraded systems or new habitats for salt-tolerant faunas? Biology Letters, 12: 20151072. doi: 10.1098/rsbl.2015.1072

Kight, C.R. & Swaddle, J.P. 2011. How and why environmental noise impacts animals: an integrative, mechanistic review. Ecology Letters, 14: 1052-1061. doi: 10.1111/j.1461-0248.2011.01664.x

Kong, L., Li, Q. & Qiu, Z. 2007. Genetic and morphological differentiation in the clam *Coelomactra antiquata* (Bivalvia: Veneroida) along the coast of China. Journal of Experimental Marine Biology and Ecology, 343: 110-117. doi: 10.1016/j.jembe.2006. 12.003

Krause, G., Buck, B.H. & Breckwoldt, A. 2019. Socio-economic aspects of marine bivalve production. In: Smaal, A.C., Ferreira, J.G., Grant, J., Petersen, J.K. & Strand, Ø. (Eds.). Goods and services of marine bivalves. Springer, Cham, pp. 317-334.

Kuzishchin, K.V., Gruzdeva, M.A., Pavlov, S.D., Semenova, A.V. & Pavlov, D.S. 2018. Features of spatial structure of mikizha *Parasalmo mykiss* in the Kol River, Western Kamchatka: on the problem of the population integration in the complex river system. Journal of Ichthyology, 58: 694-709. doi: 10.1134/ S0032945218050144

Laudien, J., Flint, N.S., Van der Bank, F.H. & Brey, T. 2003. Genetic and morphological variation in four populations of the surf clam *Donax serra* (Röding) from southern African sandy beaches. Biochemical Systematics and Ecology, 31: 751-772. doi: 10.1016/ S0305-1978(02)00252-1

Laut, L.L.M., Martins, M.V.A., Fontana, L.F., Silva, F.S., Mendonça-Filho, J.G., Clemente, I.M.M.M., et al. 2016. Ecological status evaluation of Itaipu Lagoon (Niterói) based on biochemical composition of organic matter. Journal of Sedimentary Environments, 1: 297-315. doi: 10.12957/jse.2016.25903

Lauterjung, M.B., Montagna, T., Bernardi, A.P., Silva, J.Z., Costa, N.C.F., Steiner, F., et al. 2019. Temporal changes in population genetics of six threatened Brazilian plant species in a fragmented landscape. Forest Ecology and Management, 435: 144-150. doi: 10.1016/j.foreco. 2018.12.058

Leal, D.A.G., Pereira, M.A., Franco, R.M.B., Branco, N. & Neto, R. 2008. First report of *Cryptosporidium* spp. oocysts in oysters (*Crassostrea rhizophorae*) and cockles (*Tivela mactroides*) in Brazil. Journal of Water and Health, 6: 527-532. doi: 10.2166/wh.2008.065

Lee, H.J. & Boulding, E.G. 2009. Spatial and temporal population genetic structure of four northeastern Pacific littorinid gastropods: the effect of mode of larval development on variation at one mitochondrial and two nuclear DNA markers. Molecular Ecology, 18: 2165-2184. doi: 10.1111/j.1365-294X.2009.041 69.x

Lewontin, R.C. & Hubby, J.L. 1966. A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. Genetics, 54: 595-609. doi: 10.1093/genetics/54.2.595

Lorion, J., Buge, B., Cruaud, C. & Samadi, S. 2010. New insights into diversity and evolution of deep-sea Mytilidae (Mollusca: Bivalvia). Molecular Phylogenetics and Evolution, 57: 71-83. doi: 10.1016/j.ympev.2010.05.027

Maia, A.M.L., Medeiros, E. & Henry-Silva, G.G. 2018. Distribution and density of the bivalve *Anomalocardia brasiliana* in the estuarine region of northeastern Brazil. Brazilian Journal of Biology, 78: 32-40. doi: 10.1590/1519-6984.02316

Marie, A.D., Lejeusne, C., Karapatsiou, E., Cuesta, J.A., Drake, P., Macpherson, E., et al. 2016. Implications for management and conservation of the population genetic structure of the wedge clam *Donax trunculus* across two biogeographic boundaries. Scientific Reports, 6: 39152. doi: 10.1038/srep39152

Markow, T.A. 1995. Evolutionary ecology and developmental instability. Annual Review of Entomology, 40: 105-120. doi: 10.1146/annurev.en.40.010195.000541

Martino, R.C. & Cruz, G.M.D. 2004. Proximate composition and fatty acid content of the mangrove oyster *Crassostrea rhizophorae* along the year seasons. Brazilian Archives of Biology and Technology, 47: 955-960. doi: 10.1590/S1516-89132004000600015

McLusky, D.S. 1993. Marine and estuarine gradients-an overview. Netherland Journal of Aquatic Ecology, 27: 489-493. doi: 10.1007/BF02334809

Mitteroecker, P. & Gunz, P. 2009. Advances in geometric morphometrics. Evolutionary Biology, 36: 235-247. doi: 10.1007/s11692-009-9055-x

Montagna, T., Lauterjung, M.B., Candido-Ribeiro, R., Silva, J.Z.D., Hoeltgebaum, M.P., Costa, N.C.F.D., et al. 2018. Spatial genetic structure, population dynamics, and spatial patterns in the distribution of *Ocotea catharinensis* from southern Brazil: implications for conservation. Canadian Journal of Forest Research, 48: 506-516. doi: 10.1139/cjfr-2017-0446

Morán, G.A., Martínez, J.J., Boretto, G.M., Gordillo, S. & Boidi, F.J. 2018. Shell morphometric variation of *Ameghinomya antiqua* (Mollusca, Bivalvia) during the late quaternary reflects environmental changes in North Patagonia, Argentina. Quaternary International, 490: 43-49. doi: 10.1016/j.quaint.2018.05.027

Mouëza, M., Gros, O. & Frenkiel, L. 1999. Embryonic, larval and postlarval development of the tropical clam, *Anomalocardia brasiliana* (Bivalvia, Veneridae). Journal of Molluscan Studies, 65: 73-88. doi: 10.1093/mollus/65.1.73

Narchi, W. 1972a. On the biology of *Iphigenia brasiliensis* Lamarck, 1818 (Bivalvia, Donacidae). Journal of Molluscan Studies, 40: 79-91. doi: 10.1093/oxfordjournals.mollus.a065213

Narchi, W. 1972b. Comparative study of the functional morphology of *Anomalocardia brasiliana* (Gmelin, 1791) and *Tivela mactroides* (Born, 1778) (Bivalvia, Veneridae). Bulletin of Marine Science, 22: 643-670.

Nascimento, I.A., Pereira, S.A. & Souza, R.C. 1980. Determination of the optimum commercial size for the mangrove oyster (*Crassostrea rhizophorae*) in Todos os Santos Bay, Brazil. Aquaculture, 20: 1-8. doi: 10.1016/0044-8486(80)90056-3

Nei, M. 1972. Genetic distance between populations. American Naturalist, 106: 283-292. doi: 10.1086/282771

Olivier, A.S., Jones, L., Vay, L.L., Christie, M., Wilson, J. & Malham, S.K. 2020. A global review of the ecosystem services provided by bivalve aquaculture. Reviews in Aquaculture, 12: 3-25.

Paolucci, E.M., Sardiña, P., Sylvester, F., Perepelizin, P.V., Zhan, A., Ghabooli, S., et al. 2014. Morphological and genetic variability in an alien invasive mussel across an environmental gradient in South America. Limnology and Oceanography, 59: 400-412. doi: 10.4319/lo.2014.59.2.0400

Pazoto, C., Ventura, C., Duarte, M. & Silva, E. 2018. Genetic variation and population homogeneity of the sea star *Coscinasterias tenuispina* (Forcipulatida: Asteroidea) on the coast of Rio de Janeiro, Brazil. Latin American Journal of Aquatic Research, 46: 355-363. doi: 10.3856/vol46-issue2-fulltext-11

Peacock, M.M. & Smith, A.T. 1997. The effect of habitat fragmentation on dispersal patterns, mating behavior, and genetic variation in a pika (*Ochotona princeps*) metapopulation. Oecologia, 112: 524-533. doi: 10.1007/s004420050341

Peterson, M.S. 2003. A conceptual view of environment-habitat-production linkages in tidal river estuaries. Reviews in Fisheries Science, 11: 291-313. doi: 10.1080/10641260390255844

Pimpinelli, S. & Piacentini, L. 2020. Environmental change and the evolution of genomes: transposable elements as translators of phenotypic plasticity into genotypic variability. Functional Ecology, 34: 428-441. doi: 10.1111/1365-2435.13497

Pritchard, J.K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics, 155: 945-959.

Quintino, V., Sangiorgio, F., Ricardo, F., Mamede, R., Pires, A., Freitas, R., et al. 2009. *In situ* experimental study of reed leaf decomposition along a full salinity gradient. Estuarine, Coastal and Shelf Science, 85: 497-506. doi: 10.1016/j.ecss.2009.09.016

Rajaei, M., Farahmand, H., Poorbagher, H., Mortazavi, M.S. & Farhadi, A. 2014. Sympatric morphological and genetic differentiation of the pearl oyster *Pinctada radiata* (Bivalvia: Pterioida) in the northern Persian Gulf. Journal of the Marine Biological Association of the United Kingdom, 95: 537-543. doi: 10.1017/S0025315414001611

Richardson, B.J., Baverstock, P.R. & Adams, M. 1986. Allozyme electrophoresis - a handbook for animal systematics and population studies. Academic Press, Sydney.

Rick, C.M., Fobes, J.F. & Tanksley, S.D. 1979. Evolution of mating systems in *Lycopersicon hirsutum* as deduced from genetic variation in electrophoretic and morphological characters. Plant Systematics and Evolution, 132: 279-298.

Rios, E.C. 1994. Seashells of Brazil. Editora da FURG, Rio Grande.

Rohlf, F.J. 2015. The tps series of software. Hystrix the Italian Journal of Mammalogy, 26: 9-12. doi: 10.4404/hystrix-26.1-11264

Rohlf, F.J. & Marcus, L.F. 1993. A revolution morphometrics. Trends in Ecology & Evolution, 8: 129-132. doi: 10.1016/0169-5347(93)90024-J

Rossi, A.R., Colangelo, P., Berline, L., Angiulli, E., Ardizzone, G., Fassatoui, C. & Sola, L. 2019. Influence of hydrodynamic connectivity on the genetic structure and gene flow of the common pandora *Pagellus erythrinus*. Hydrobiologia, 834: 103-117. doi: 10.1007/s10750-019-3914-y

Roth, V.L. & Mercer, J.M. 2000. Morphometrics in development and evolution. American Zoologist, 40: 801-810. doi: 10.1093/icb/40.5.801

Rousset, F. 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Molecular Ecology Resources, 8: 103-106. doi: 10.1111/j.1471-8286.2007.01931.x

Ryman, N., Lagercrantz, U.L.F., Andersson, L., Chakraborty, R. & Rosenberg, R. 1984. Lack of correspondence between genetic and morphologic variability patterns in Atlantic herring (*Clupea harengus*). Heredity, 53: 687-704. doi: 10.1038/hdy.1984.127

Scarabino, F., Zelaya, D.G., Orensanz, J.L., Ortega, L., Defeo, O., Schwindt, E., et al. 2015. Cold, warm, temperate and brackish: bivalve biodiversity in a complex oceanographic scenario (Uruguay, south-western Atlantic). American Malacological Bulletin, 33: 1-18. doi: 10.4003/006.033.0219

Scheiner, S.M. 1993. Genetics and evolution of phenotypic plasticity. Annual Review of Ecology and Systematics, 24: 35-68. doi: 10.1146/annurev.es.24.110193.000343

Selander, R.K., Smith, M.H., Yang, S.Y., Johnson, W.E. & Gentry, J.R. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I: Variation in the old-field mouse (*Peromyscus polionotus*). Studies in genetics VI. University of Texas, 7103: 49-90.

Sgrò, C.M. & Hoffmann, A.A. 2004. Genetic correlations, tradeoffs and environmental variation. Heredity, 93: 241-248. doi: 10.1038/sj.hdy.6800532

Silva-Cavalcanti, J.S. & Costa, M.F. 2011. Fisheries of *Anomalocardia brasiliana* in tropical estuaries. Pan-American Journal of Aquatic Sciences, 6: 86-99.

Simpson, C. & Harnik, P.G. 2009. Assessing the role of abundance in marine bivalve extinction over the post-Paleozoic. Paleobiology, 35: 631-647. doi: 10.1666/0094-8373-35.4.631

Soares, L.S.H., Lopez, J.P., Muto, E.Y. & Giannini, R. 2011. Capture fishery in northern Todos os Santos Bay, tropical southwestern Atlantic, Brazil. Brazilian Journal of Oceanography, 59: 61-74. doi: 10.1590/S1679-87592011000100005

Stapley, J., Feulner, P.G., Johnston, S.E., Santure, A.W. & Smadja, C.M. 2017. Variation in recombination frequency and distribution across eukaryotes: patterns and processes. Philosophical Transactions of the Royal Society B: Biological Sciences, 372: 20160455. doi: 10.1098/rstb.2016.0455

Stocken, T.V., Wee, A.K.S., Ryck, D.J.R., Vanschoenwinkel, B., Friess, D.A., Dahdouh-Guebas, F., et al. 2019. A general framework for propagule dispersal in mangroves. Biological Reviews, 94: 1547-1575. doi: 10.1111/brv.12514

Sugama, K., Tridjoko, T., Haryanti, H., Budi, S.M. & Cholik, F. 1999. Genetic variation and population structure in the humpback grouper, *Cromileptes altivelis*, throughout its range in Indonesian waters. Indonesian Fisheries Research Journal, 5: 32-38. doi: 10.15578/ifrj.5.1.1999.32-38

Sultan, S.E. 2017. Developmental plasticity: reconceiving the genotype. Interface Focus, 7: 20170009. doi: 10.1098/rsfs.2017.0009

Sultan, S.E. & Spencer, H.G. 2002. Metapopulation structure favors plasticity over local adaptation.

American Naturalist, 160: 271-283. doi: 10.1086/341015

Swofford, D.L. & Selander, R.B. 1997. Biosys-2, a computer program for the analysis of allelic variation in population genetics and biochemical systematics. University of Illinois, Illinois. [http://lamar.colostate.edu]. Reviewed: June 26, 2020.

Taggart, J., Ferguson, A. & Mason, F.M. 1981. Genetic variation in Irish populations of brown trout (*Salmo trutta* L.): electrophoretic analysis of allozymes. Comparative Biochemistry and Physiology - Part B: Biochemistry & Molecular Biology, 69: 393-412. doi: 10.1016/0305-0491(81)90330-8

Teixeira, S.F. & Campos, S.S. 2019. Mollusc gathering in tropical regions of Brazil. In: Diarte-Plata, G. & Escamilla-Montes, R. (Eds.). Molluscs. IntechOpen, London.

Telesca, L., Michalek, K., Sanders, T., Peck, L.S., Thyrring, J. & Harper, E.M. 2018. Blue mussel shell shape plasticity and natural environments: a quantitative approach. Scientific Reports, 8: 2865. doi: 10.1038/s41598-018-20122-9

Thompson, J.D. 1991. Phenotypic plasticity as a component of evolutionary change. Trends in Ecology & Evolution, 6: 246-249. doi: 10.1016/0169-5347(91)90070-E

Thrush, S.F., Halliday, J., Hewitt, J.E. & Lohrer, A.M. 2008. The effects of habitat loss, fragmentation, and community homogenization on resilience in estuaries. Ecological Applications, 18: 12-21. doi: 10.1890/07-0436.1

Uthicke, S. & Purcell, S. 2004. Preservation of genetic diversity in restocking of the sea cucumber *Holothuria scabra* investigated by allozyme electrophoresis. Canadian Journal of Fisheries and Aquatic Sciences, 61: 519-528. doi: 10.1139/f04-013

Van Buskirk, J.V. 2009. Natural variation in morphology of larval amphibians: phenotypic plasticity in nature? Ecological Monographs, 79: 681-705. doi: 10.1890/08-1692.1

Vera, M., Carlsson, J., Carlsson, J.E., Cross, T., Lynch, S., Kamermans, P., et al. 2016. Current genetic status, temporal stability and structure of the remnant wild European flat oyster populations: conservation and restoring implications. Marine Biology, 163: 239-252. doi: 10.1007/s00227-016-3012-x

Vetrova, V.P., Ekart, A.K., Kravchenko, N.A. & Larionova, A.Y. 2016. Relationships between the allozyme and phenotypic diversities of *Picea ajanensis* populations. Russian Journal of Genetics: Applied Research, 6: 560-568. doi: 10.1134/S2079059716050142

Wade, M.J. & McCauley, D.E. 1988. Extinction and recolonization: their effects on the genetic differentiation of local populations. Evolution, 42: 995-1005. doi: 10.2307/2408915

Weir, B.S. & Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population. Evolution, 38: 1358-1370. doi: 10.2307/2408641

West-Eberhard, M.J. 1989. Phenotypic plasticity and the origins of diversity. Annual Review of Ecology and Systematics, 20: 249-278. doi: 10.1146/annurev.es.20.110189.001341

Willsie, J.A., Morris, T.J. & Zanatta, D.T. 2020. Morphometric analyses distinguish wabash pigtoe (*Fusconaia flava*) and round pigtoe (*Pleurobema sintoxia*) mussels. Diversity, 12: 337. doi: 10.3390/d12090337

Wishkerman, A. & Hamilton, P.B. 2018. Shape outline extraction software (DiaOutline) for elliptic Fourier analysis application in morphometric studies. Applications in Plant Sciences, 6: e01204. doi: 10.1002/aps3.1204

Zelditch, M.L., Swiderski, D.L., Sheets, H.D. & Fink, W.L. 2004. Geometric morphometrics for biologists: a primer. Academic Press, London.

Zhao, Y., Li, Q., Kong, L. & Mao, Y. 2009. Genetic and morphological variation in the venus clam *Cyclina sinensis* along the coast of China. Hydrobiologia, 635: 227-235. doi: 10.1007/s10750-009-9916-4

Zieritz, A., Hoffman, J.I., Amos, W. & Aldridge, D.C. 2010. Phenotypic plasticity and genetic isolation-by-distance in the freshwater mussel *Unio pictorum* (Mollusca: Unionoida). Evolutionary Ecology, 24: 923-938. doi: 10.1007/s10682-009-9350-0

Zink, R.M. 1988. Evolution of brown towhees: allozymes, morphometrics and species limits. The Condor, 90: 72-82. doi: 10.2307/1368435

Received: June 25, 2021; Accepted: October 28, 2022