Marine Biology (2022) 169:103
https://doi.org/10.1007/s00227-022-04088-x

ORIGINAL PAPER

Contrasting morphological and genetic patterns suggest cryptic speciation and phenotype–environment covariation within three benthic marine hydrozoans

Amanda Ferreira Cunha[1,2] · David Carmelet-Rescan[3] · Antonio Carlos Marques[1] · Mary Morgan-Richards[3]

Received: 1 October 2021 / Accepted: 4 July 2022 / Published online: 28 July 2022

Abstract

Connectivity among populations of widespread marine species is expected to be correlated with their dispersal potential but the evolution of reproductive barriers may account for variations in spatial genetic patterns. Marine benthic hydroid species are traditionally considered widespread, with long-distance rafting presumably increasing their dispersal potential. In this study, we investigated the relationship between genetic, morphological and environmental variability within three benthic marine hydroid species to evaluate current patterns of genetic variation and assess the existence of cryptic speciation. Although a long-lived planktonic stage is absent in all the lineages sampled and they have an overlapping geographical ranges, we observed contrasting patterns of genetic and phenotypic divergence: *Orthopyxis sargassicola* showed little genetic variation, while *O. caliculata* and *O. crenata* each contained high genetic differentiation, primarily suggesting limited dispersal potential. Significant covariation was observed between phenotypic and environmental data in all lineages, but different environmental variables were responsible for explaining morphological variation in each case. Genetic and morphological patterns within *O. caliculata* and *O. crenata* are suggestive of cryptic speciation, while phenotypic variation in *O. sargassicola* may be plastic. Thus, morphological and genetic patterns may potentially vary among related marine lineages with shared life history traits and habitat.

Keywords Genetic structure · Cryptic speciation · Morphometrics · Hydrozoa · Dispersal · Phenotypic plasticity

Introduction

In marine environments, patterns of population genetic structure are often found to correlate with the dispersal potential of the species, suggesting that gene flow can be maintained over large spatial scales (Palumbi 1994; Levin 2006). Most clades of marine invertebrates have life cycles that include at least one planktonic stage, which is frequently the larva (see Cowen and Sponaugle 2009). As a result, the duration of their pelagic stage has commonly been considered a proxy of their dispersal potential and used to infer patterns of population connectivity (e.g. Grantham et al. 2003). However, the link between duration of the pelagic stage and dispersal potential is not pervasive among marine species (e.g. González-Wevar et al. 2018), and the influence of physical and biological processes such as ocean currents, eddies, larval behaviour and habitat specificity result in a nonlinear relationship between the pelagic stage dispersal potential and realised population connectivity (Cowen and Sponaugle 2009; Kelly and Palumbi 2010). Similarly, gene flow constitutes just one of the many processes that influence spatial patterns of population structure (Hart and Marko 2010; Marko and Hart 2011). Historical processes and the evolution of reproductive barriers may also account for contrasting results concerning the connectivity of marine

Responsible Editor: Oscar Puebla.

✉ Amanda Ferreira Cunha
amanfcunha@gmail.com

[1] Departamento de Zoologia, Instituto de Biociências, Universidade de São Paulo, Rua Do Matão, Travessa 14, 101, São Paulo 05508-090, Brazil

[2] Departamento de Biologia Animal, Centro de Ciências Biológicas e da Saúde, Universidade Federal de Viçosa, Avenida P.H. Rolfs, s/n, 36.570-900, Minas Gerais, Brazil

[3] Wildlife and Ecology Group, School of Natural Sciences, Massey University, Private Bag 11-222, Palmerston North 4410, New Zealand

invertebrate populations (e.g. Palumbi 1994; Marko 2004; Arranz et al. 2021).

Marine hydrozoans are known for their diversity of life cycles, which is generally meroplanktonic with alternation between a benthic sessile polyp stage and a free-swimming planktonic medusa stage, the latter reproducing sexually to form a planula larva that will settle and develop into a new polyp (Millard 1975; Cornelius 1990). Variations of this basic life cycle are common, and mainly involve the loss of the polyp phase, resulting in holoplanktonic species with presumably ample dispersal, as well as the reduction or complete loss of the medusa stage, resulting in meroplanktonic and benthic species with presumably limited dispersal (Cornelius 1992a; Gibbons et al. 2010). Studies have corroborated the connection between life cycle and dispersal potential in hydrozoans (Gibbons et al. 2010; Rodriguez et al. 2017), and consistently show high genetic differentiation among allopatric populations of benthic and meroplanktonic species (e.g. Schuchert 2014; Cunha et al. 2016, 2017). However, several exceptions to this pattern have also been observed, including holoplanktonic species with high genetic structure over regional scales (e.g. Pontin and Cruickshank 2012), as well as widespread benthic species with low genetic differentiation among populations (e.g. Moura et al. 2019, see also Marques 2011 for a discussion based on the bioinvasive approach).

Among hydrozoans without a medusa stage, many species have been traditionally considered nearly cosmopolitan in their distribution (Cornelius 1981, 1992b). Because most benthic hydroids are substrate generalist, and often occur on floating material (e.g. Calder et al. 2014), their wide distributions have been explained by long-distance rafting, which would significantly increase the dispersal potential of benthic species (Cornelius 1992a; Thiel and Haye 2006; Marques 2011). In fact, several recent studies suggest a central role for rafting in range expansion of hydroid species with benthic life cycles (e.g. Postaire et al. 2017a, b; Boissin et al. 2018; Moura et al. 2019). This implies that, even though lacking a long-lived planktonic stage, benthic hydroids may achieve long-distance dispersal through passive carriers, which could directly influence spatial patterns of genetic structure.

The genus *Orthopyxis* L. Agassiz 1862 comprises a group of hydroids that typically liberate a reduced medusa, though it can be facultatively retained in some species (Cornelius 1982). They are distributed widely but mainly reported from shallow waters (Millard 1975; Vervoort and Watson 2003; Calder 2013; Cunha et al. 2015), occurring in habitats with varying conditions of temperature and salinity, as well as attached to a great variety of substrates, such as algae, barnacles, mussels and other hydroids (e.g.Ralph 1957; Oliveira et al. 2016). Morphological variation within species of *Orthopyxis* (Cunha et al. 2015, 2016) is generally assumed to be plastic, and associations between phenotypic variation and temperature, depth and substrate type suggest that this variation may be adaptive (e.g. Naumov 1969; Hughes et al. 1991; Gili and Hughes 1995). However, few investigations have been conducted to assess the magnitude of phenotypic plasticity in hydroids (e.g. Van Winkle et al. 2000; Griffith and Newberry 2008).

In this study, we investigated the relationships between genetic, morphological and environmental variability in benthic marine hydroids. By contrasting genetic data in three putatively widespread species of the genus *Orthopyxis*, we aimed to investigate current patterns of genetic variation and detect less inclusive lineages that might be evidence of cryptic speciation within currently recognised species. We also documented hydrothecal shape variation with the aim of determining whether morphological variation is concordant with genetic lineages, and sought evidence of covariation between phenotypic and environmental variation. Previous studies of hydroids led us to predict size would be influenced by water temperature (Ralph and Thomson 1968) and that perisarc thickness would covary with current velocity (Boero 1984; Gili and Hughes 1995). By sampling closely related species with overlapping geographical ranges and shared life history traits, we hoped to shed light on the relative contributions of cryptic speciation and phenotype–environment covariation on the morphological variation and spatial genetic patterns of these benthic marine invertebrates.

Materials and methods

Specimens and study site

Morphological and genetic data were obtained from putative populations of the species, *Orthopyxis caliculata* (Hincks, 1853), *Orthopyxis crenata* (Hartlaub, 1901) and *Orthopyxis sargassicola* (Nutting, 1915). These species are recognised by the absence of cusps in the hydrotheca of *O. caliculata*, and the presence of annulations in the gonotheca of *O. sargassicola*, while the gonotheca is smooth in *O. crenata*. The species *O. caliculata* and *O. crenata* as currently recognised have broad distributions, with records in the north and south Atlantic and Pacific Oceans (e.g. Cornelius 1995; Vervoort and Watson 2003; Cunha et al. 2015; Oliveira et al. 2016), as well as the Indian Ocean (Millard 1975) and Mediterranean Sea (e.g. Peña Cantero and García Carrascosa 2002). Records of *O. sargassicola* are restricted to the Atlantic, ranging from the coast of Florida in the USA to the south of Brazil (e.g. Calder 2013; Oliveira et al. 2016). In this study, sympatric populations of the three species were sampled on the southern coast of Brazil (Fig. 1, Table S1 in Online Resource 1). Additional populations were sampled on the coast of northern Brazil (*O. crenata*) and New Zealand (*O.*

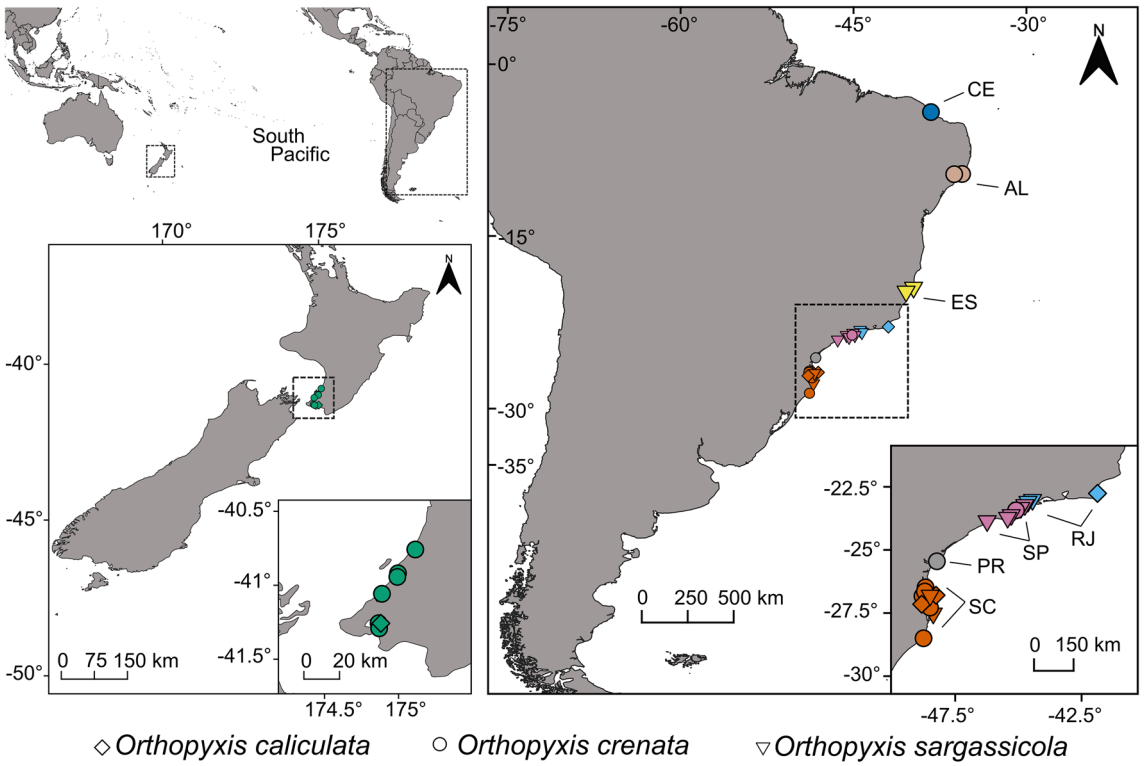


Fig. 1 Sampling sites on the coast of Brazil and New Zealand of populations of three hydroid species of the genus *Orthopyxis* analysed in this study. Different colours indicate different populations in accordance with Fig. 4. Population's acronyms (states in Brazil): CE—Ceará, AL—Alagoas, ES—Espírito Santo, RJ—Rio de Janeiro, SP—São Paulo, PR—Paraná, SC—Santa Catarina

caliculata, O. crenata; Fig. 1, Table S1). All specimens were sampled during low tide, and were predominantly found on brown algae (e.g. *Sargassum* sp., *Carpophyllum* sp.).

Patterns of genetic variation

Nuclear and mitochondrial DNA sequence data were obtained from samples of the three species included in this study and used to infer spatial patterns of genetic variation. Part of these data was derived from a previous study with the genus *Orthopyxis* (Cunha et al. 2015), with the addition of several new population samples (Table S1). DNA was extracted from each colony (specimen) previously fixed in alcohol 90–100% using Agencourt DNAdvance extraction kit (Beckman Coulter, Beverly, USA), in accordance with the manufacture's protocol. Portions of the genes 16S (~ 610 bp) and COI (~ 650 bp), and the complete ITS (~ 700 bp including ITS1, 5.8 s and ITS2) were amplified and sequenced following protocols previously applied to the group (Cunha et al. 2015, 2017). Sequencing was carried out on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA), and both strands were sequenced for most of the samples.

Sequences were assembled and edited using Geneious v7.1 (Biomatters, Auckland, New Zealand), and aligned with MAFFT (Katoh et al. 2002). We confirmed from skim sequencing of genomic DNA from three individuals that variation in ITS exists within individuals despite concerted evolution of this multicopy marker. As a result, two sequence variants were identified in several individual ITS amplicons. The sequence variants within an individual differed by two or eight nucleotide substitutions (within samples of *O. crenata* and *O. caliculata*; Table S2 in Online Resource 2). As we obtained genomic data for individuals of only these two species, variant sites in the ITS alignments of the species *O. sargassicola* were coded as ambiguous. TCS networks (Clement et al. 2002) for the combined mitochondrial (16S + COI) and nuclear (ITS1 + ITS2) markers of each species were generated using PopART (Leigh and Bryant 2015). Summary statistics was performed with the packages *apex* v1.0.4 (Schliep et al. 2020), *pegas* v1.0–1 (Paradis 2010), and *mmod* v1.3.3 (Winter 2012) in R v4.1.0 (R Core Team 2021). For these analyses, all gaps and ambiguous sites were removed from the alignments when present (on average, 19% was removed in 16S + COI alignments and 10% in ITS1 + ITS2, see Table 1). For each species, the number of haplotypes, haplotype and nucleotide diversity, R_2 Neutrality Test (Ramos-Onsins and Rozas 2002), uncorrected p distances, as well as global (across loci) standardised fixation (Φ'_{st}, Meirmans 2006) and diversity (D, Jost 2008) indices

Table 1 Summary statistics of genetic data (mitochondrial and nuclear) of populations of *Orthopyxis*

	Species	*N*	*H*	Hd	Nd	*p* distance (mean ± sd[range])	*R2*	Φ'st (95% CI)	D_{est} (95% CI)	Length (bp)
16S + COI	*Orthopyxis caliculata*	20	13 (13)	0.9421	0.0316	0.0316 ± 0.0302 [0–0.0676]	0.1439 ns	0.60 (0.24–0.64)	0.89 (0.81–0.95)	1265/1036
	Orthopyxis crenata	28	15 (13)	0.9365	0.0706	0.0706 ± 0.0407 [0–0.1169]	0.2065 ns	0.96 (0.92–0.98)	0.89 (0.86–0.90)	1261/975
	Orthopyxis sargassicola	34	20 (18)	0.9554	0.0029	0.0029 ± 0.0015 [0–0.0068]	0.0664*	0.32 (−0.18–0.31)	0.32 (0.04–0.48)	1226/1033
ITS1 + ITS2	*Orthopyxis caliculata*	22	5 (3)	0.6234	0.0064	0.0064 ± 0.0060 [0–0.0188]	0.1534 ns	0.40 (0.20–0.45)	0.72 (0.66–0.75)	409/373
	Orthopyxis crenata	35	14 (10)	0.9076	0.0670	0.0670 ± 0.0622 [0–0.1635]	0.1595 ns	0.84 (0.75–0.84)	0.87 (0.80–0.92)	429/367
	Orthopyxis sargassicola	35	2 (1)	0.1109	0.0003	0.0003 ± 0.0008 [0–0.0024]	0.0555*	−0.02 (−0.03 to −0.02)	0.00 (−0.01–0.01)	435/407

Alignment length is given as before/after removal of gaps and ambiguous sites. Confidence intervals (95%) for global fixation and diversity indices are given in parentheses, and were corrected for upward bias as described in Winter (2012)

N number of individuals, *H* number of haplotypes/distinct sequences (number of private haplotypes), *Hd* haplotype diversity, *Nd* nucleotide diversity, *sd* standard deviation, *R2* Ramos-Onsis and Rozas (2002) neutrality test, *Φ'*st standardised global fixation index (Meirmans, 2006), *Dest* global diversity index (Jost, 2008), *ns* not significant

* $p = 0.05$

were calculated. Finally, patterns of isolation by distance were investigated by means of the Mantel test, using genetic p distance of mitochondrial data and geographic distances in km between samples, with the same packages already mentioned, as well as *ade4* v1.7–17 (Bougeard and Dray 2018), *SpatialEpi* v1.2.5 (Kim and Wakefield 2018), and *ggplot2* v3.3.5 (Wickham 2016) for graphs, in R programming language. Details of these analyses are provided in Online Resource 3.

To further investigate the presence of cryptic species, we performed phylogenetic analyses using maximum likelihood (ML) criteria on the mitochondrial (16S + COI) and nuclear (ITS1 + ITS2) datasets, as well as on the combined dataset (16S + COI + ITS1 + ITS2). For these analyses, variant sites in ITS alignments were coded as ambiguous in all species. Models of molecular evolution for each gene (16S: TIM2 + I + G; COI: GTR + I + G, ITS1: TIM2 + I + G, ITS2: TIM2ef + G) were selected based on Akaike Information Criterion (AIC) values using ModelTest-NG (Darriba et al. 2020), and ML analyses were performed on different partitions corresponding to each gene using RAxML-NG v1.1.0 (Kozlov et al. 2019), as implemented in raxmlGUI v2.0.7 (Edler et al. 2021). The analysis consisted of 20 replicate searches with taxa randomly added to the starting tree. Branch support was estimated with Bootstrap based on 1,000 replicates.

Morphometric analyses

Morphological variation among putative populations of the three species of *Orthopyxis* was evaluated with geometric morphometrics. For this, a set of two-dimensional landmarks and semi-landmarks wase used to investigate hydrothecal shape variation. Species of *Orthopyxis* frequently show variation in the shape of hydrotheca, from campanulate to cylindrical, and in the thickness of the wall, which may render a bilateral symmetry to the hydrotheca (Cunha et al. 2015, 2017). In this sense, we focused our analysis of shape variation in the hydrotheca, also ensuring that landmark determination followed the criteria of homology, repeatability and reliability, as well as providing an adequate cover of hydrothecal form (Zelditch et al. 2012). A total of 15 landmarks and semi-landmarks were used, comprising six fixed landmarks and nine sliding/semi-landmarks (Fig. 2a). Landmarks were placed on the images of specimens positioned in slides and photographed under stereo and/or compound microscope. Modelling dough was used on the edges of the cover slips to avoid pressing the hydrothecae and altering its form. Semi-landmarks were placed on the curves of the hydrothecae with the aid of a "comb", manually superimposed on the images using Inkscape (https://inkscape.org), with its teeth always perpendicular to a line between the first and third landmarks (Fig. 2a).

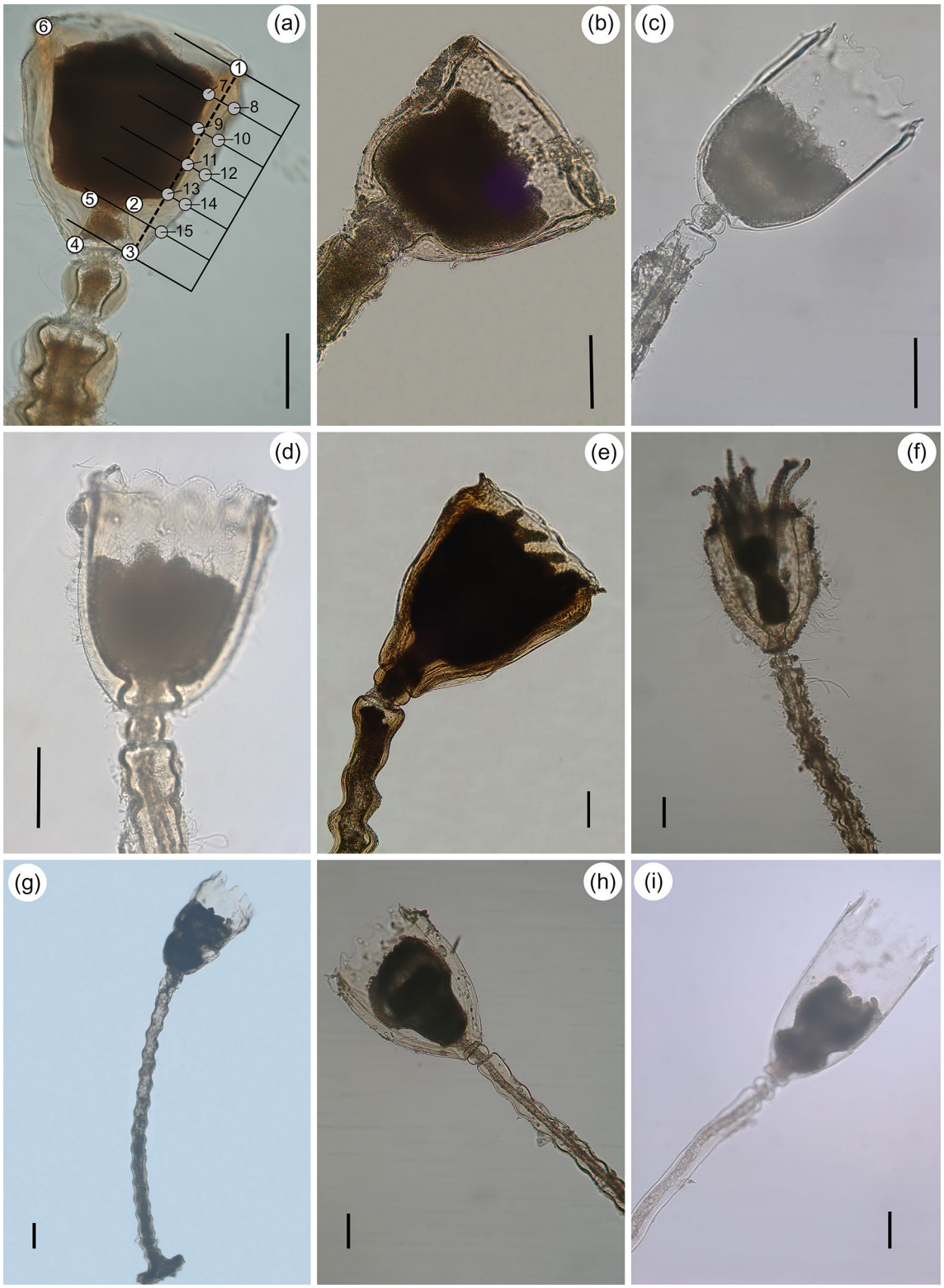


Fig. 2 Representative individuals from the lineages of *Orthopyxis* analysed in this study, with landmark configuration used in geometric morphometrics analyses. **a** *O. caliculata*-lineage-II, MZUSP 2554, specimen from Santa Catarina, Brazil; **b** *O. caliculata*-lineage-I, NIWA145063, specimen from New Zealand. Numbers in white and grey circles in (a) indicate fixed landmarks and semi-landmarks, respectively; **c** *O. crenata*-lineage-III, MZUSP 8673, specimen from Alagoas, northern Brazil; **d** *O. crenata*-lineage-II, MZUSP 2584, specimen from Paraná, southern Brazil; **e** *O. crenata*-lineage-I, NIWA145076, specimen from New Zealand; **f-i** *O. sargassicola*, **f** specimen from Espírito Santo, southern Brazil (MZUSP 2620), **g** specimen from Santa Catarina, southern Brazil (MZUSP 8691), **h-i**) specimens from São Paulo, southern Brazil, **h** MZUSP 2602, **i** MZUSP 8686. All scales 100 µm

Errors associated with manipulation of samples in microscope slides and digitalization were estimated with experimental replication, involving five photographs of the same specimen (after being removed and remounted on the slide), and by repeating five times the digitalization step on the same photograph, with a dataset comprising 4 different specimens (Online Resource 4). A Procrustes ANOVA was used to estimate the variance in shape associated with each measurement error using the package *geomorph* v4.0.0 (Adams et al. 2020) and the Intraclass Correlation Coefficient (ICC) was calculated for each term as described in Fruciano (2016) in R programming language. Repeatability values (R) were 0.99 for both manipulation and digitalization measurement errors, which were therefore considered negligible for subsequent analyses.

Digitalization of landmarks, as well as the assignment of sliding landmarks and Procrustes analysis were conducted with the packages *geomorph* and *RRPP* v0.6.1. (Collyer and Adams 2018) in R programming language. Hydrothecal shape variation was evaluated with Canonical Variate Analysis (CVA) using the package *Morpho* v2.8 (Schlager 2017) in R. In addition, differences in shape and size within each species were investigated with Procrustes ANOVA (*geomorph*), followed by pairwise tests (*RRPP*). Centroid size was included as a response variable in linear models investigating size variation and as a predictor in models with shape as the response variable, to consider the relative amount of shape variation attributed to size. Similarly, the geographic location of samples (different states in Brazil and New Zealand) and, for the species *O. caliculata*, the different mtDNA lineages, were included as predictors in all models to investigate patterns of both shape and size variation. Details of these analyses are provided in Online Resource 5.

Environmental data and covariation with morphological data

Variation in environmental conditions was evaluated based on marine environmental layers available from Bio-ORACLE (Assis et al. 2018). Data from the sites of occurrence of the specimens were extracted from the layers using the packages *sdmpredictors* v.0.2.8 (Bosch 2018) and *leaflet* v.2.0.3 (Cheng et al. 2019) in R programming language (R Core Team 2021). Considering that the hydroids are epibionts on benthic substrates, we extracted data from benthic layers of maximum depth, except for bathymetry, which gives the average depth of the site. The following mean annual environmental variables were considered for the analyses: (1) temperature (temp); (2) chlorophyll concentration (chlo); (3) dissolved oxygen concentration (dissox); (4) salinity; (5) concentration of silicate or ortho-silicic acid [$Si(OH)_4$]; (6) nitrate concentration; (7) phosphate concentration; (8) dissolved iron concentration; (9) currents velocity (curvel); (10) phytoplankton concentration (carbonphyto); (11) primary productivity (pp); (12) light at bottom (lightbot); (13) bathymetry.

To investigate the association between morphological and environmental data in putative populations of the three species of *Orthopyxis*, Procrustes aligned landmark coordinates and environmental data of each species was analysed with Partial Least Squares (PLS, Rohlf and Corti 2000) using the packages *geomorph* and *mixOmics* v6.16.2 (Rohart et al. 2017). Collinearity among environmental variables was investigated with the package *usdm* v1.1–18 (Naimi et al. 2014) and three variables (phosphate, carbonphyto and nitrate) were excluded from subsequent analyses based on their Variance Inflation Factor (VIF) and a maximum linear correlation with any other variable greater than 0.80. Additionally, a repeated cross-validation approach (*mixOmics*) was used to evaluate the performance of the fitted models and determine the minimum number of components necessary for an appropriate representation of the data. Variable Importance in the Projection (VIP) coefficients were computed and a cut-off of 1 was applied to determine the most important environmental variables for explaining the variation in morphology. Details of these analyses are provided in Online Resource 5.

Results

Structure and diversity within currently recognised *Orthopyxis* species

Both mitochondrial and nuclear DNA sequence data show high levels of global fixation (Φ'_{st}) and differentiation (D_{est}) indices among samples of the species *Orthopyxis caliculata* and *O. crenata* (Table 1). Additionally, *O. crenata* showed a signature of isolation by distance (IBD; Mantel test, $r = 0.69$, $P = 0.001$) while *O. caliculata* showed a marginally significant pattern of IBD ($r = 0.30$, $P = 0.042$; Fig. 3), which does not hold with the removal of the most distant population (New Zealand; $r = -0.10$, $P = 0.723$). Our sample of *O. caliculata* was found to consist of two distinct genetic lineages: the specimen from New Zealand (H12) and five specimens from Brazil (H11 and H13) had distinct mtDNA haplotypes (~6.5% divergent) and distinct ITS sequences (hereafter referred to as *O. caliculata*-lineage-I, while other samples from Brazil are referred to as *O. caliculata*-lineage-II). These two lineages are sympatric at Santa Catarina where only one sample did not show concordance of its mitochondrial lineage with its nuclear lineage (Bob33—H4/A3, Fig. 4a-b, Table S1). Similarly, our sample of *Orthopyxis crenata* consisted of four mitochondrial lineages concordant with three ITS lineages and location (Fig. 4c-d). One lineage was sampled in New Zealand (*O. crenata*-lineage-I),

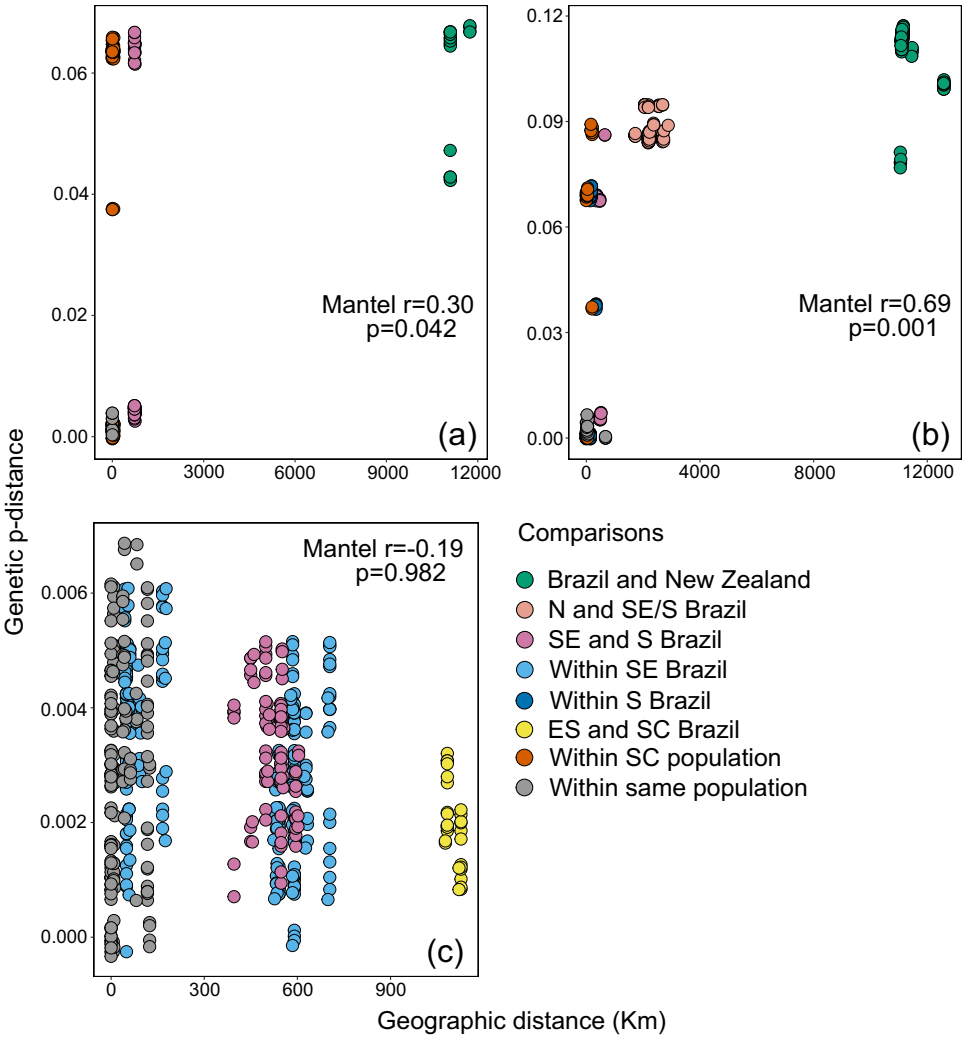


Fig. 3 Lack of isolation by distance in two hydroid species is revealed by the relationship between pairwise genetic distance (p distance, 16S + COI dataset) and geographic distance (km) between populations of the species *Orthopyxis caliculata* (**a**), *Orthopyxis crenata* (**b**) and *Orthopyxis sargassicola* (**c**). Results of the Mantel test are given for each species. N = populations from northern Brazil (Alagoas and Ceará); SE = populations from southeastern Brazil (Rio de Janeiro in a, São Paulo in b and Espírito Santo, Rio de Janeiro, and São Paulo in c); S = populations from south Brazil (Paraná and Santa Catarina in b, Santa Catarina in a and c); ES = population from Espírito Santo, Brazil; SC = population from Santa Catarina, Brazil

one in southern Brazil (Rio de Janeiro, São Paulo, Paraná, Santa Catarina; *O. crenata*-lineage-II) and one lineage in northern Brazil (Alagoas, Ceará; *O. crenata*-lineage-III). These lineages differed in mtDNA by 10.8% (New Zealand compared to Brazil) and 8.7% (northern Brazil compared to southern Brazil) and in nuclear DNA (ITS) by 15.2 and 8.3%, respectively (Fig. 3b). Interestingly, the individuals of *O. crenata*-lineage-II with very similar ITS sequences have mitochondrial haplotypes that differ by up to 8.9% (Fig. 4c).

In contrast, our population samples of *Orthopyxis sargassicola* revealed low genetic distances between haplotypes, with low levels of fixation (Φ'_{st}) and differentiation (D_{est}) indices, although many private haplotypes were observed (Table 1, Fig. 4e-f). Accordingly, no significant correlation between genetic and geographic distances were detected, and populations of this species do not show a pattern of isolation by distance (Mantel test, $r = -0.19$, $P = 0.982$) (Fig. 3c). Only two ITS sequences were detected within our sampling ($N = 35$, over ~ 1200 km of coast) that differed by a single nucleotide (Fig. 4f). We should note, however, that sampling of this species was restricted to southern Brazil, thus covering a smaller geographical area than the samplings of the other two *Orthopyxis* species. If we focus within their overlapping geographical range in southern Brazil, mtDNA divergence reaches a maximum of 0.68% for *O. sargassicola,* 6.7% for *O. caliculata* and 8.9% for *O. crenata*.

Phylogenetic patterns confirmed the monophyly of the morphologically identified *O. caliculata* and *O. sargassicola* specimens (Figs. S1–S3 in Online Resource 6). Although analysis of the mitochondrial sequences inferred monophyly of the specimens identified as *O. crenata* (Fig. S1), this relationship was not supported by the ITS DNA sequences. The nuclear dataset recovered the lineage from New Zealand as sister to a clade comprising both *O. sargassicola* and the remaining samples of *O. crenata*, although with weak support (bootstrap 48%, Fig. S2). The concatenation of all genes did not markedly contribute to improved resolution among major lineages (Fig. S3).

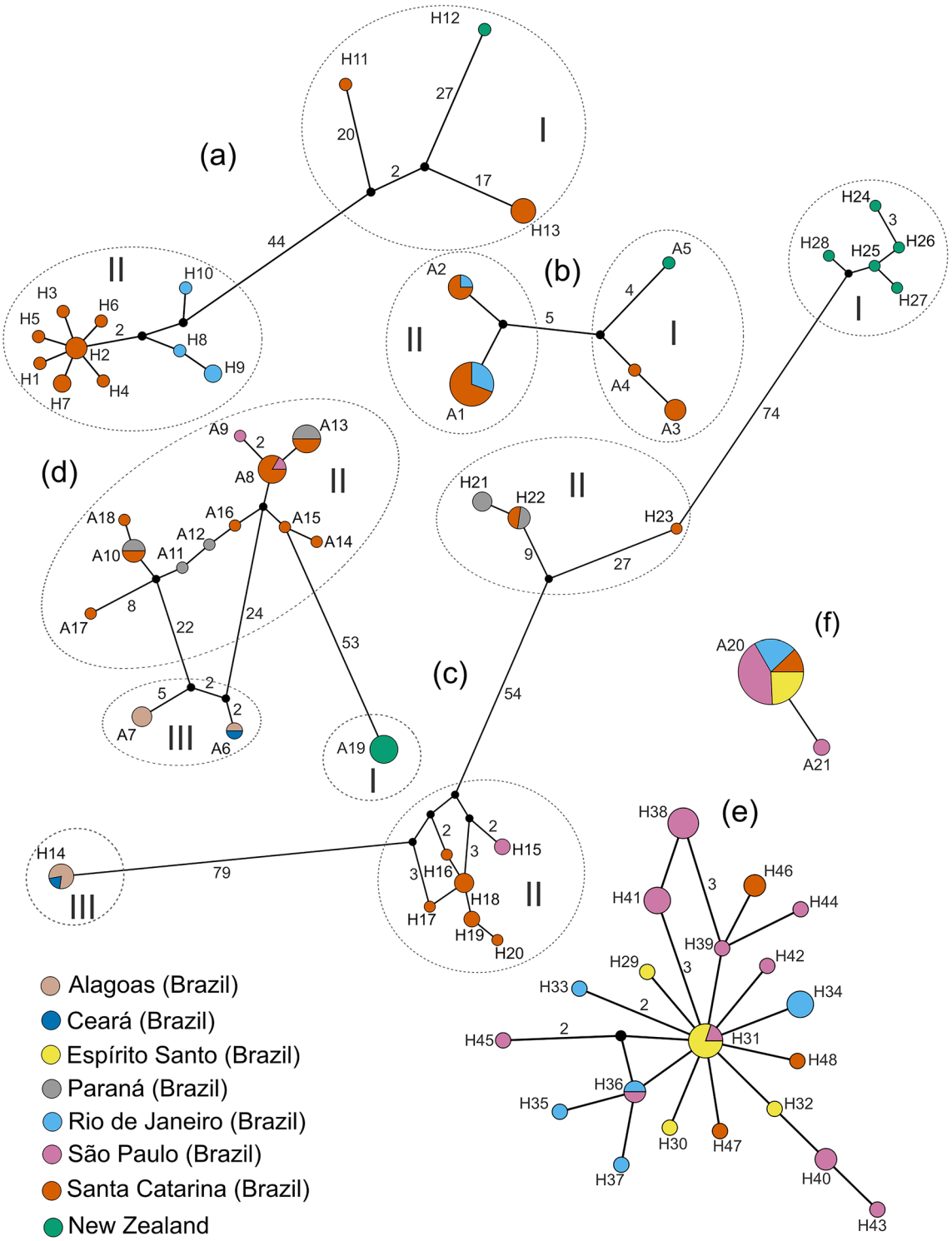


Fig. 4 Genetic variation within three hydroid species based on their concatenated mitochondrial (16S + COI) and nuclear (ITS1 + ITS2) DNA sequences is illustrated with TCS haplotype networks. a–b, *Orthopyxis caliculata*, **a** mitochondrial, **b** nuclear; **c-d** *Orthopyxis crenata*, **c** mitochondrial, **d** nuclear; **e–f** *Orthopyxis sargassicola*, **e** mitochondrial, **f** nuclear. Colours correspond to collecting locations. Solid circle sizes are proportional to the number of individuals with each haplotype, and distances between circles correspond to the number of mutations between haplotypes (if not indicate, distance equals 1). Cryptic lineages are indicated with dashed circles and roman numerals (see results for further details)

Morphological variation

Geometric morphometric analysis indicated that size and shape variation is better explained by the different genetic lineages than sampling location in *O. caliculata*. Individuals from the population of Santa Catarina in Brazil, corresponding to *O. caliculata*-lineage-I (haplotypes H11 and ITS sequences A4, Fig. 4a-b), were smaller when compared to remaining individuals ($F = 5.121$, $P = 0.008$; Fig. 5a, Table 2). Similarly, shape is also different among genetic lineages ($F = 3.305$, $P = 0.001$; Fig. 5b, Table 2). The CVA with haplotypes as the grouping variable had an overall classification accuracy of 84.42% and showed that specimens of *O. caliculata*-lineage-II (haplotype group H1-10; ITS A1-2) have a thicker hydrothecal wall (Fig. 5b, compare with Fig. 2a-b).

Considering the species *O. crenata*, geometric morphometric data showed that specimens from Brazil (*O. crenata*-lineage-II and III) are smaller than specimens from New Zealand (*O. crenata*-lineage-I; $F = 64.712$, $P = 0.001$; Fig. 5c, Table 2), and that shape variation can be explained by geographic origins (which are concordant with genetic lineages). The shape of the hydrotheca in our population sample of *O. crenata*-lineage-III (from northern Brazil) differed significantly from the *O. crenata*-lineage-I and II (sampled from southern Brazil and New Zealand; $F = 6.951$, $P = 0.001$; Table 2). The CVA using the same grouping has an overall classification accuracy of 95.45% and shows that the shape of the hydrothecae vary from more campanulate in the populations of New Zealand and southern Brazil to more cylindrical in populations from northern Brazil (Fig. 5d, also see Fig. 2c-e).

Although no differences in size were observed among population samples of *O. sargassicola* ($F = 1.676$, $P = 0.158$; Fig. 5e, Table 2), they show differences in shape (CVA overall classification accuracy = 77.42%), with populations from Espírito Santo (ES) having a thicker perisarc and more campanulate hydrothecae when compared to populations of Rio de Janeiro and Santa Catarina (SC), which have thinner perisarc and more cylindrical hydrothecae ($F = 3.5252$, $P = 0.001$; Fig. 5f, Table 2). The wide morphological variation among the population samples of São Paulo probably explains the lack of significant differences when compared to populations of ES and SC in pairwise post hoc tests (Fig. 5f, compare with Fig. 2f-i).

Association between environmental and morphological data

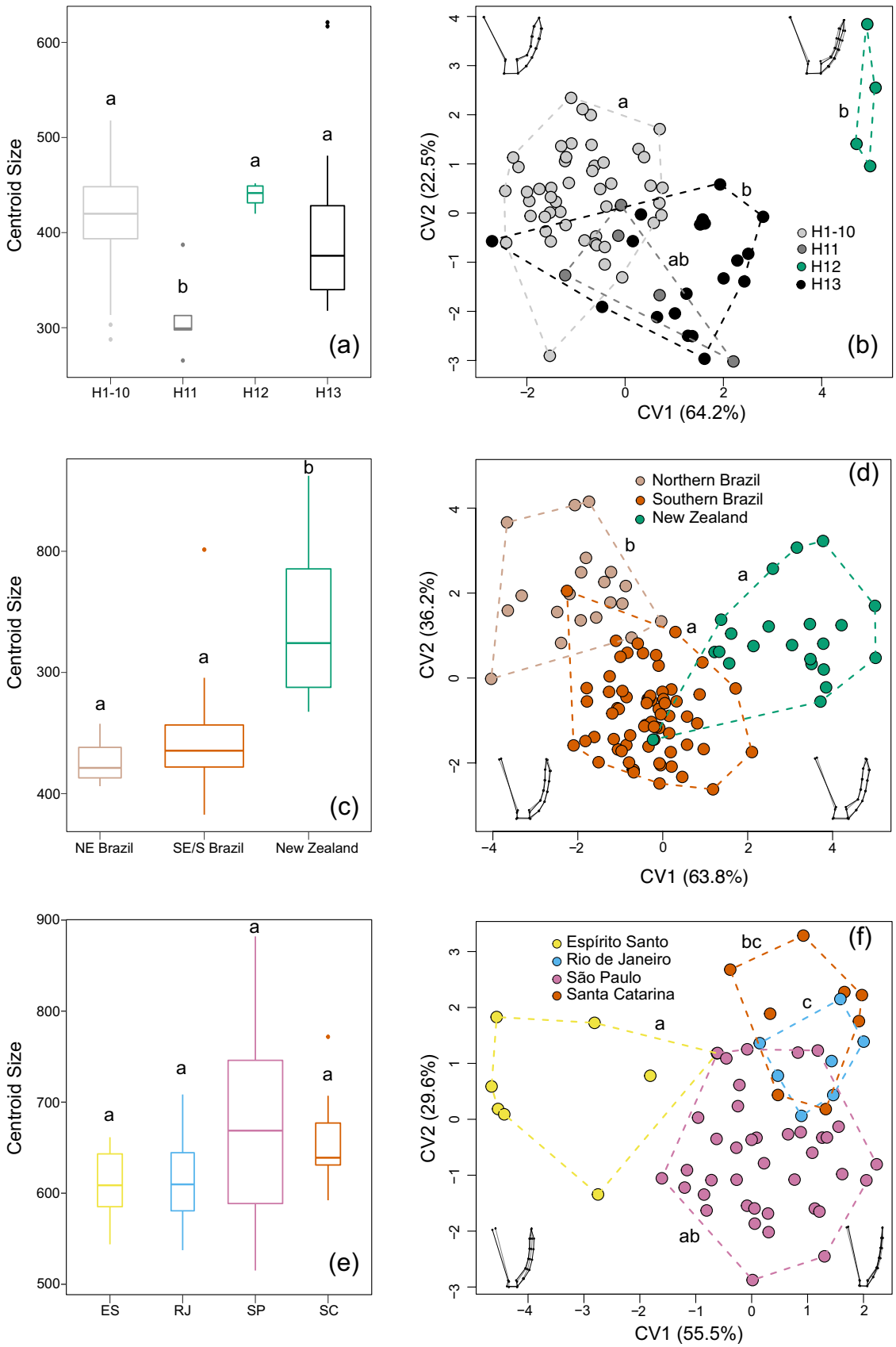
Our sampling of *Orthopyxis* covered more than 2000 kms of the Brazilian coast and included sites in New Zealand, therefore considerable variation in environmental conditions was detected among sampling locations (Fig. 1). Partial least squares analysis revealed significant covariation between morphology and environmental variables (*O. caliculata*: r-PLS = 0.518, $P = 0.001$; *O. crenata*: r-PLS = 0.502, $P = 0.001$; *O. sargassicola*: r-PLS = 0.528, $P = 0.001$), but Variable Importance in the Projection (VIP) scores suggest that those covariations are carried by different environmental variables in each species (Fig. 6). For instance, while salinity showed an important contribution to the observed shape differences in all species, bathymetry was more important for *O. caliculata*, temperature for *O. crenata*, and current velocity for *O. sargassicola*. In fact, locations within São Paulo showed clear environmental differences, which correlated with the differences observed in perisarc thickness of population samples of *O. sargassicola* (Figs. 5f, 6c).

Discussion

The three species of *Orthopyxis* included in this study share common life history traits, are traditionally considered widespread and co-occur in the southwestern Atlantic Ocean (Cornelius 1982; Cunha et al. 2015; Oliveira et al. 2016). However, our analyses revealed they have important differences in genetic and morphological patterns. While the species *O. sargassicola* showed low genetic diversity and no geographical structure nor isolation by distance, the specimens identified as *O. caliculata* and *O. crenata* showed high genetic differentiation suggesting the occurrence of cryptic speciation within these nominal species. Within *O. caliculata* this divergence was not restricted to allopatric populations separated by large geographic distances, but was also observed among sympatric population samples in Brazil. We detected contrasting genetic patterns among these three taxa despite all sharing an absence of a long-lived medusa stage. This observation suggests that their presumed limited dispersal is not the sole factor determining the levels of population connectivity within these hydroid lineages.

Spatial patterns of genetic variation

All three species of *Orthopyxis* studied here are known to release a reduced medusa (medusoid) during their life cycle (Hirohito 1969; Cornelius 1982, 1995; Migotto 1996), but there are reports of this medusoid being facultatively retained in some species (Cornelius 1982, 1995; Llobet et al. 1991). The reduced medusa does not have manubrium, mouth, or tentacles, and it is liberated with fully developed gonads (Russel 1953; Hirohito 1969; Migotto 1996). Although medusoids have been reported to live in the plankton for up to a few days, spawning occurs concomitantly or shortly after release (Russel 1953; Cornelius 1982; Migotto 1996), presumably constraining long-distance dispersal. In addition, planula larvae liberated directly from


Centroid Size
600
500
400
300
a
b
a
a
H1-10
H11
H12
H13
(a)
CV2 (22.5%)
CV1 (64.2%)
a
b
ab
H1-10
H11
H12
H13
(b)
Centroid Size
800
300
400
a
a
b
NE Brazil
SE/S Brazil
New Zealand
(c)
CV2 (36.2%)
CV1 (63.8%)
Northern Brazil
Southern Brazil
New Zealand
b
a
a
(d)
Centroid Size
900
800
700
600
500
a
a
a
a
ES
RJ
SP
SC
(e)
CV2 (29.6%)
CV1 (55.5%)
Espírito Santo
Rio de Janeiro
São Paulo
Santa Catarina
a
bc
c
ab
(f)

◂**Fig. 5** Shape variation of hydrotheca as detected by geometric morphometric analysis within three hydroid species is associated with either genetic cluster (haplotype groups) or centroid size and collection location (see Table 2). Boxplots (**a**, **c**, **e**) and Canonical Variate Analysis (**b**, **d**, **f**) plots, showing morphometric patterns of populations of *Orthopyxis*. **a**, **b** *Orthopyxis caliculata* (haplotype groups as in Fig. 4a); **c**, **d** *Orthopyxis crenata* (in d, Northern Brazil = populations from Alagoas and Ceará, Southern Brazil = populations from São Paulo, Paraná, and Santa Catarina); **e**, **f** *Orthopyxis sargassicola*. Lower case letters indicate significant differences of post hoc pairwise tests. Numbers in parentheses indicate percentages of variation explained by each canonical variate. Boxplots are shown with widths proportional to the square roots of the number of observations in each group. Shape predictions are based on minimum and maximum values of canonical variate 1

hydroid colonies also have limited dispersal, usually settling close to the mother colony, pending on the availability of suitable substrate (Sommer 1992; Gili and Hughes 1995). The absence of a long-lived planktonic stage in all taxa investigated in this study is consistent with the patterns of high genetic differentiation over small spatial scales and with increasing geographical distance observed (Cunha et al. 2016; Postaire et al. 2017a, b; Boissin et al. 2018). Our sample of *O. caliculata* consisted of two distinct genetic lineages that, despite sympatry off the coast of Brazil (Santa Catarina), showed more than 6% divergence (mitochondrial DNA sequence) among samples collected less than 10 km apart (H11-13), and concordance of nuclear and mtDNA markers. Similarly, our samples of *O. crenata* revealed genetic divergences (mtDNA) of 6–8% within Santa Catarina (10 km to over 200 km apart), and more than 8% divergence when comparing samples from northern Brazil (Alagoas and Ceará).

The absence of a long-lived planktonic stage in these hydroids, however, is not easily reconciled with the lack of genetic differentiation along southern Brazil within both *O. sargassicola* and *O. caliculata*-lineage-II. Populations of the species *O. sargassicola* occurring in sympatry with lineages of *O. crenata* and *O. caliculata* (in Santa Catarina and Rio de Janeiro) showed low genetic differentiation (< 1% divergence) over 1200 km and no signature of isolation by distance suggesting high gene flow or recent expansion over this coastline. In addition, *O. caliculata*-lineage-II (H1-10) also has low (1%) divergence among haplotypes sampled from populations over 700 km (in Santa Catarina and Rio de Janeiro), suggesting high dispersal ability.

Dispersal of *Orthopyxis* is probably facilitated by rafting on macroalgae, since biofouling and transport of colonies on ship hulls is unlikely given their low frequency on hard substrata (Millard 1975; Hirohito 1995; Vervoort and Watson 2003; Cunha et al. 2015). For instance, all records of these species in South America have been reported either on algae or epizoic on other hydroids (Oliveira et al. 2016). Additionally, accounts of hydroids occurring on ship hulls in Brazil or collected in harbours and marinas within the range of our sampling areas do not include records of species of *Orthopyxis* (Marques et al. 2013; Rocha et al. 2013). Conversely, *O. crenata*, *O. caliculata* and *O. sargassicola* are commonly reported in epiphytic associations, growing on a wide diversity of algae and seagrasses (Oliveira and Marques 2007, 2011; Cunha and Jacobucci 2010). It is also important to consider that rafting could be responsible for long-distance migrations and range expansions that would otherwise be interpreted as human-mediated introductions (see Marques 2011). In fact, our samples of the three species of *Orthopyxis* were predominantly found on benthic macroalgae, mainly of the genus *Sargassum* (Table S1). Several macroalgae, including *Sargassum*, are positively buoyant and known to float for extensive distances after their detachment from the benthos, driven by oceanic circulation and wind (Thiel and Gutow 2005a; Macaya et al. 2016). Moreover, dispersal by rafting on macroalgae, including trans-oceanic dispersal, is considered one of the main drivers of current patterns of distribution and population structure for several species of hydroids (Cornelius 1981, 1992b; Postaire et al. 2017a, b; Boissin et al. 2018; Moura et al. 2019), and could explain the observed patterns of low genetic differentiation of the species *O. sargassicola* and *O. caliculata*-lineage-II. The southern coast of Brazil is influenced by the southbound Brazil Current (BC) (Castro and Miranda 1998; Silveira et al. 2000), which could contribute to connectivity among the populations analysed in this study. However, to fully understand their current patterns of connectivity, it will be important to investigate additional Atlantic populations of these species (for instance, there is only one previous record of *O. sargassicola* in northern Brazil; see Oliveira et al. 2016).

Our analyses suggest that cryptic lineages exist within many currently recognised species of *Orthopyxis* and, therefore, inferences about dispersal based on species distributions need to be made with care. It is important to consider that *O. caliculata* and *O. crenata* are known from southern Chile (Strait of Magellan and Lennox Island), while *O. caliculata* has also been reported for southern Argentina (Puerto San Julián), Uruguay (Oliveira et al. 2016 and references therein), and western Africa (records of *O. integra* by Millard (1975) and Gili et al. (1989) most likely belong to *O. caliculata*; see Cunha et al. 2015). African records of *O. crenata* are from the Indian Ocean (Millard and Bouillon 1973; Millard 1975). Interestingly, the possibility of trans-Atlantic dispersal via rafting through the South Equatorial Current (SEC) has been recently suggested for several species of Plumularioidea hydroids (Moura et al. 2019). The SEC has also been suggested as a potential route for the recent reports of floating masses of *Sargassum* in coastal areas in Brazil and western Africa (Smetacek and Zingone 2013; Sissini et al. 2017), and could be a potential source

Table 2 Results of Procrustes analysis of variance (ANOVA) for three species of hydroids (*Orthopyxis*). Intraspecific geometric morphometric variation in hydroids were associated with either genetic cluster (haplotype groups) or centroid size and collection location in a species-specific manner

Species	Procrustes ANOVA				
Orthopyxis caliculata	**Size**	**Df**	**MS**	***F***	***p***
	Haplotypes	3	18,292.7	5.121	0.008
	Location	1	166.3	0.047	0.797
	Residuals	72	3571.9		
	Total	76			
	Shape	**Df**	**MS**	***F***	***p***
	Centroid Size	1	0.014	2.256	0.059
	Haplotypes	3	0.021	3.305	0.001
	Location	1	0.003	0.555	0.730
	Residuals	71	0.006		
	Total	76			
Othopyxis crenata	**Size**	**Df**	**MS**	***F***	***p***
	Region	2	363,614	64.712	0.001
	Location	3	2320	0.412	0.657
	Residuals	104	5619		
	Total	109			
	Shape	**Df**	**MS**	***F***	***p***
	Centroid Size	1	0.038	7.102	0.001
	Region	2	0.037	6.951	0.001
	Location	3	0.005	0.890	0.564
	Residuals	103	0.005		
	Total	109			
Orthopyxis sargassicola	**Size**	**Df**	**MS**	***F***	***p***
	Location	3	10,959.2	1.676	0.158
	Residuals	58	6538.8		
	Total	61			
	Shape	**Df**	**MS**	***F***	***p***
	Centroid Size	1	0.103	27.064	0.001
	Location	3	0.013	3.516	0.001
	Residuals	57	0.004		
	Total	61			

Df degrees of freedom, *MS* mean squares

of dispersal for species of the genus *Orthopyxis*. Ensuring all cryptic lineages within these taxa are incorporated into sampling design will be necessary for further investigating their spatial genetic patterns and dispersal routes.

Morphological and environmental variation

Our sampling of *Orthopyxis* encompassed considerable environmental variation among locations. Annual mean water conditions vary even on local scales, such as within Santa Catarina and within São Paulo locations (Fig. 6). In all hydroid lineages, significant covariation was observed between phenotypic and environmental data, suggesting

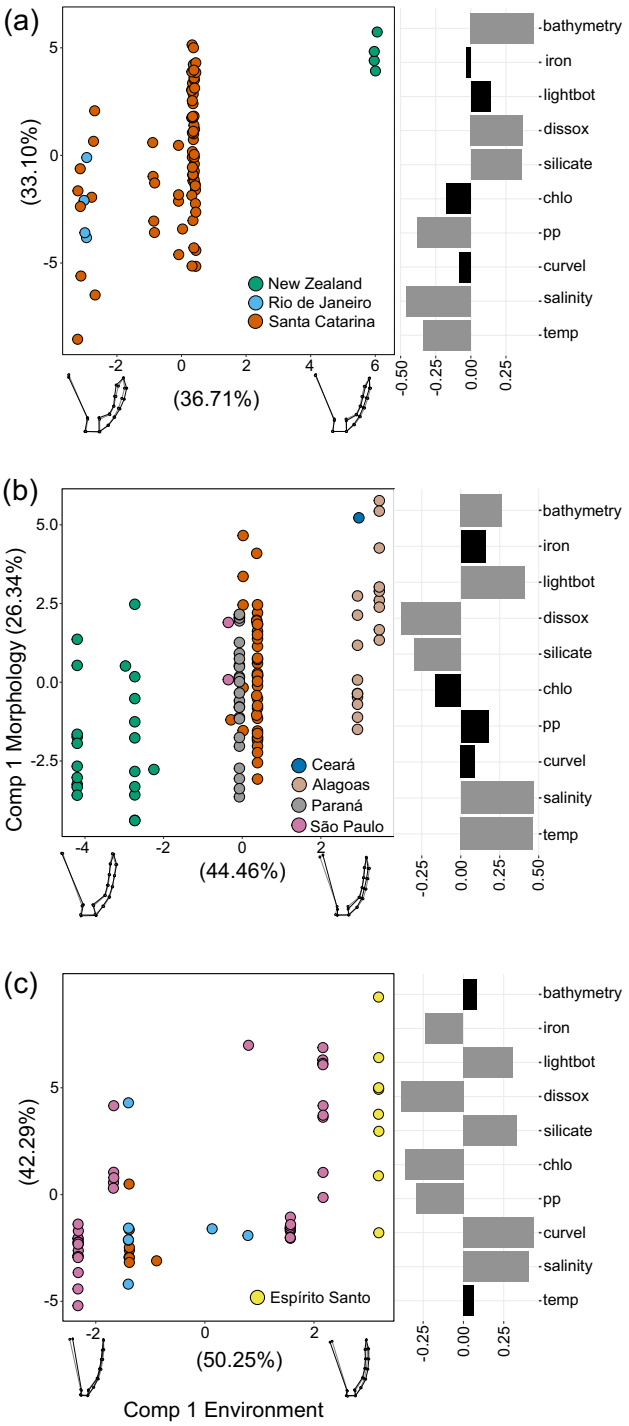


Fig. 6 Associations between local environmental conditions and the shape of hydrotheca within three species of *Orthopyxis*, according to the results from Partial Least Squares analysis. **a** *Orthopyxis caliculata*; **b** *Orthopyxis crenata*; **c** *Orthopyxis sargassicola*. Shape predictions are based on minimum and maximum values of component 1 of the environmental dataset. Horizontal bars represent the Variable Importance for the Projection (VIP), with values higher than 1 shown in grey. Abbreviations of variables: temp = temperature, chlo = chlorophyll, dissox = dissolved oxygen, curvel = currents velocity, carbonphyto = phytoplankton, pp = primary productivity, lightbot = light at bottom

that phenotypic differences were at least partially explained by variation in environmental conditions. As expected, water temperature was one of the most important variables responsible for the covariation patterns shown by lineages within *O. crenata* and could explain the larger size of New Zealand specimens, which occur in colder water when compared to Brazil. Temperature has been shown to influence energy allocation to either growth or reproduction in hydroids (Ralph and Thomson 1968), with colonies found in cooler temperatures usually being taller and having longer hydrothecae in several campanulariid species (e.g. Ralph 1956; Ralph and Thomson 1968; Naumov 1969). However, this pattern is not entirely consistent among benthic hydroids, with colony size generally decreasing with depth probably as a result of the interaction between lower temperatures, reduced growth rates and less availability of food (Fernandez et al. 2020). Nevertheless, these evidences attest to the importance of temperature as an environmental input triggering morphological variation among hydroid populations.

Unexpectedly, size variation was not observed among samples from Brazil and New Zealand of *O. caliculata*. Instead, covariation patterns in *O. caliculata* were related to differences in perisarc thickness and best explained by variations in bathymetry and salinity (Fig. 6a). Similarly, samples of *O. crenata* from northern and southern Brazil were not differentiated by size differences but variations in shape of the hydrothecal wall (campanulate or cylindrical, Fig. 6b). In fact, both *O. caliculata* and *O. crenata* showed differences in shape and size among genetic lineages, further suggesting that we might be looking at interspecific, not intraspecific variation. Despite the significant associations between environmental variables and morphometric variation we did not detect convergent shapes among sympatric lineages, and the signal of isolation by distance within *O. crenata* is compatible with morphological divergence due to drift. Importantly, the possibility that lineages within currently defined species might represent cryptic reproductively isolated units means that future analyses need to focus on variation within these genetic lineages. Common garden experiments will also contribute to revealing the underlying processes of phenotypic divergence.

We expected high current velocity locations would be associated with hydroids with relatively thick perisarc in all three species, but only in *O. sargassicola* was there an association of morphology and mean water current velocity. The perisarc is a chitin-protein exoskeleton that in most leptothecate hydroids covers the whole colony, including the hydranth (=hydrothecae), and is involved in the fixation to substrates, support, flexibility of the colony and ultimately, protection (Mendoza-Becerril et al. 2016). The thickness of the perisarc in the hydrocaulus and branches has been shown to directly affect feeding efficiency in regard to flow velocity (Harvell and Labarbera 1985; Gili and Hughes 1995), as well as to vary with hydrodynamics (Boero 1984; Gili and Hughes 1995). In particular, the perisarc of the hydrotheca is considered to be involved in the protection of the hydranth and could offer a protection against abrasion of the alga or dislodgment in epiphytic species (Hughes 1992; Gili and Hughes 1995). Considering the low genetic differentiation among populations of *O. sargassicola* observed in this study, it is possible that morphological variation in this species is a result of adaptive plasticity. Although additional sampling is required to evaluate this hypothesis, rafting as a potential source of dispersal could expose migrating individuals to considerable environmental variation, and contribute to connectivity over large spatial scales (> 1000 km; Thiel and Gutow 2005a, b). Considering that temporal and spatial environmental variation with gene flow may favour the occurrence of phenotypic plasticity (Crispo 2008; Sultan and Spencer 2002), rafting could directly influence the occurrence of phenotypic plasticity in hydroids. Indeed, strategies involving higher dispersal rates in marine species have been shown to be associated with higher phenotypic plasticity (Hollander 2008).

Cryptic speciation in *Orthopyxis caliculata* and *Orthopyxis crenata*

The presence of high genetic diversity and geographical structure with concordance of mitochondrial and nuclear markers among allopatric populations within *O. caliculata* and *O. crenata* is suggestive of cryptic speciation. In addition, the two distinct lineages of *O. caliculata* are sympatric in Brazil (in the state of Santa Catarina) which reinforces the existence of cryptic speciation within these southwestern Atlantic hydroids. However, within our sampling of *O. caliculata* a single specimen revealed that hybridisation was possible between the two genetic lineages as one individual carried the mtDNA haplotype of *O. caliculata*-lineage-II but an ITS sequence from *O. caliculata*-lineage-I (Bob33—H4/A3, Table S1). Despite this evidence of gene flow, the two lineages may be differentiated based on the thickness of the hydrothecal wall. Cryptic speciation is common in hydrozoans, particularly in presumed widespread species (e.g. Schuchert 2014; Cunha et al. 2017; Postaire et al. 2017a, b; Boissin et al. 2018; Moura et al. 2018; Miglietta et al. 2019; Vaga et al. 2020). However, defining whether these cryptic lineages deserve the species status is not an easy task, and it may not be possible to find morphological diagnostic characters for recognising each lineage, or determining whether there is a deficit of intermediates (see Schuchert 2014; Mallet 1995).

In a previous analysis including more conserved nuclear markers (18S and 28S, Cunha et al. 2017), the species *O. crenata* was recovered as monophyletic, although showing

a marked subdivision among a clade with specimens from Brazil and another clade with specimens from Argentina and New Zealand. Our analysis, using a linked but more variable nuclear marker (ITS) failed to recover *O. crenata* as monophyletic, although monophyly was recovered with mitochondrial sequences. Considering both the genetic divergence and the concordance of nuclear and mitochondrial data, we identified three genetic lineages within *O. crenata* (New Zealand, northern Brazil, and southern Brazil). These lineages were shown to vary in size (New Zealand *vs* Brazil) and shape of the hydrothecae (southern *vs* northern Brazil; Fig. 5c-d). Neither of the two putative species in Brazil correspond to the holotype (type locality of *O. crenata* is French Pass, New Zealand; Hartlaub 1901; Vervoort and Watson 2003). Similarly, there are at least two potential cryptic species within *O. caliculata* (Lineage-I in New Zealand and Brazil; Lineage-II in Brazil), but the study of sequences from the type locality (Pegwell Bay, England; Hincks 1853) is necessary to elucidate affinities between the lineages observed herein. Lineages within *O. caliculata* varied in size and thickness of the hydrothecal wall (Fig. 5a-b). Interestingly, variations in perisarc thickness are traditionally considered within the range of intraspecific variations of *O. crenata* and *O. caliculata*, as well as the presence of a more cylindrical or campanulate hydrotheca in *O. crenata* (Ralph 1957; Vervoort and Watson 2003). On the other hand, *O. sargassicola* provided evidence of intraspecific variation in perisarc thickness and hydrothecal shape. Intraspecific variations in campanulariid hydroids have been consistently shown to parallel interspecific variations (Cunha et al. 2016, 2020), and this might also be the case for the nominal species *O. caliculata* and *O. crenata* analysed in this study. Although we provide evidence for morphological differences between cryptic lineages, they need to be combined with additional molecular data and/or the study of their reproductive and defensive structures (nematocysts) to be confirmed as diagnostic.

Following several recent studies (e.g. Postaire et al. 2017a, b; Boissin et al. 2018; Moura et al. 2019), we found patterns of high genetic differentiation over not only large but also local spatial scales among benthic species of hydroids, with additional evidence of morphological divergence. These uncovered cryptic patterns suggest biodiversity has not yet been fully resolved/documented in the genus *Orthopyxis*, and this might still be the case for several other benthic hydroid species. However, these patterns were not pervasive among all species studied, and low genetic divergence with wide morphological and environmental variation suggests a role for phenotypic plasticity in *O. sargassicola*. Moreover, our study highlights the potential of variation in spatial genetic patterns among related marine lineages that share similar life history traits and habitat.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00227-022-04088-x.

Acknowledgements We would like to thank all colleagues from the Marine Evolution Lab and Molecular Evolution Lab (IB-USP) for their constant support and valuable suggestions during the development of this study. We are also very grateful to our colleagues from the Phoenix Group (Massey University) for all their assistance and support for this study. Our special thanks to J. García-Rodríguez, G.C. Manzoni and students, T.P. Miranda, K. Paresque, S. Trewick, R. D'Archino, D. Aguirre and S. Thompson for their assistance with field work. Finally, we would like to thank the editor and two anonymous reviewers for their valuable suggestions on previous version of this manuscript. This study was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—finance code 001, National Council for Scientific and Technological Development (CNPq) (Grant #316095/2021-4) and São Paulo Research Foundation (FAPESP) (Grants #2011/50242-5, #2017/00641-7 and #2018/24781-5).

Author contributions AFC, ACM and MM-R: conceived the ideas; AFC: collected the data; AFC and DC-R: analysed the data; AFC and MM-R: led the writing with contributions from ACM and DC-R.

Funding Funding for this research was provided by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—finance code 001, National Council for Scientific and Technological Development (CNPq) (grant #316095/2021–4) and São Paulo Research Foundation (FAPESP) (Grants #2011/50242–5, #2017/00641–7 and #2018/24781–5).

Availability of data and material All data supporting the findings of this study are available within the manuscript and its Supplementary Information. DNA sequence data generated for this study were deposited in GenBank (accession numbers in Online Resource 1, Table S1).

Declarations

Conflict of interests The authors have no conflict of interest to declare.

Ethics approval The authors declare that all necessary approvals for sampling of specimens and access to genetic heritage have been obtained (sampling permit 16802–3 SISBIO/ICMBIO—Instituto Chico Mendes de Conservação da Biodiversidade; registry code AB6239D, SISGEN—Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado).

Consent to participate Not applicable.

Consent to publish Not applicable.

References

Adams DC, Collyer ML, Kaliontzopoulou A (2020) Geomorph: software for geometric morphometric analyses. R Package Vers 3(2):1

Arranz V, Thakur V, Lavery SD (2021) Demographic history, not larval dispersal potential, explains differences in population structure of two New Zealand intertidal species. Mar Biol 168:105. https://doi.org/10.1007/s00227-021-03891-2

Assis J, Tyberghein L, Bosch S, Verbruggen H, Serrão EA, De Clerck O (2018) Bio-ORACLE v2.0: Extending marine data layers for

bioclimatic modelling. Glob Ecol Biogeogr 27:277–284. https://doi.org/10.1111/geb.12693
Boero F (1984) The ecology of marine hydroids and effects of environmental factors: a review. Mar Ecol 5:93–118. https://doi.org/10.1111/j.1439-0485.1984.tb00310.x
Boissin E, Hoareau TB, Postaire B, Gravier-Bonnet N, Bourmaud CAF (2018) Cryptic diversity, low connectivity and suspected human-mediated dispersal among 17 widespread Indo-Pacific hydroid species of the south-western Indian Ocean. J Biogeogr 45:2104–2117. https://doi.org/10.1111/jbi.13388
Bosch S (2018) sdmpredictors: species distribution modelling predictor datasets. R package version 0.2.8.
Bougeard S, Dray S (2018) Supervised multiblock analysis in R with the ade4 package. J Stat Softw 86:1–17
Calder DR (2013) Some shallow-water hydroids (Cnidaria: Hydrozoa) from the central east coast of Florida, USA. Zootaxa 3648:1–72
Calder D, Choong H, Carlton J, Chapman J, Miller J, Geller J (2014) Hydroids (Cnidaria: Hydrozoa) from Japanese tsunami marine debris washing ashore in the northwestern United States. Aquat Invasions 9:425–440. https://doi.org/10.3391/ai.2014.9.4.02
Castro BM, Miranda LB (1998) Physical oceanography of the western Atlantic continental shelf located between 4°N and 34°S coastal segment (4, W). In: Robinson AR, Brink KH (eds) The Sea. John Wiley & Sons Inc, United Kingdom, pp 209–251
Cheng J, Karambelkar B, Xie Y (2019) leaflet: create interactive web maps with the javascript "Leaflet" library. R Package Vers 2:3
Clement M, Snell Q, Walker P, Posada D, Crandall K (2002) TCS: estimating gene genealogies. In Proceedings of the 16th International Parallel and Distributed Processing Symposium. IEEE, USA, 7 pp.
Collyer ML, Adams DC (2018) RRPP: An R package for fitting linear models to high-dimensional data using residual randomization. Methods Ecol Evol 9:1772–1779. https://doi.org/10.1111/2041-210X.13029
Cornelius PFS (1981) Life cycle, dispersal and distribution among the Hydroida. Porcup Newsl 2:47–50
Cornelius PFS (1982) Hydroids and medusae of the family Campanulariidae recorded from the eastern North Atlantic, with a world synopsis of genera. Bull Br Museum Nat Hist 42:37–148
Cornelius PFS (1990) Evolution in leptolid life-cycles (Cnidaria: Hydroida). J Nat Hist 24:579–594. https://doi.org/10.1080/00222939000770391
Cornelius PFS (1992b) The Azores hydroid fauna and its origin, with discussions of rafting and medusa suppression. Arquipelago, Life Earth Sci 10:75–99
Cornelius PFS (1995) North-west European thecate hydroids and their medusae. Part 2. Sertulariidae to Campanulariidae. Synopses Br Fauna New Ser 50:1–386
Cornelius PFS. (1992a) Medusa loss in leptolid Hydrozoa (Cnidaria), hydroid rafting, and abbreviated life-cycles among their remote-island faunae: an interim review. Sci Mar 56:245–261.
Cowen RK, Sponaugle S (2009) Larval dispersal and marine population connectivity. Ann Rev Mar Sci 1:443–466. https://doi.org/10.1146/annurev.marine.010908.163757
Crispo E (2008) Modifying effects of phenotypic plasticity on interactions among natural selection, adaptation and gene flow. J Evol Biol 21:1460–1469. https://doi.org/10.1111/j.1420-9101.2008.01592.x
Cunha AF, Jacobucci GB (2010) Seasonal variation of epiphytic hydroids (Cnidaria: Hydrozoa) associated to a subtropical *Sargassum cymosum* (Phaeophyta: Fucales) bed. Zool 27:945–955. https://doi.org/10.1590/S1984-46702010000600016
Cunha AF, Genzano GN, Marques AC (2015) Reassessment of morphological diagnostic characters and species boundaries requires taxonomical changes for the genus *Orthopyxis* L Agassiz, 1862 (Campanulariidae, Hydrozoa) and some related campanulariids. PLoS ONE 10:e0117553. https://doi.org/10.1371/journal.pone.0117553
Cunha AF, Maronna MM, Marques AC (2016) Variability on microevolutionary and macroevolutionary scales: a review on patterns of morphological variation in Cnidaria Medusozoa. Org Divers Evol 16:431–442. https://doi.org/10.1007/s13127-016-0276-4
Cunha AF, Collins AG, Marques AC (2017) Phylogenetic relationships of Proboscoida Broch, 1910 (Cnidaria, Hydrozoa): Are traditional morphological diagnostic characters relevant for the delimitation of lineages at the species, genus, and family levels? Mol Phylogenet Evol 106:118–135. https://doi.org/10.1016/j.ympev.2016.09.012
Cunha AF, Collins AG, Marques AC (2020) When morphometry meets taxonomy: morphological variation and species boundaries in Proboscoida (Cnidaria: Hydrozoa). Zool J Linnean Soc 190:417–447. https://doi.org/10.1093/zoolinnean/zlz166
Darriba D, Posada D, Kozlov AM, Stamatakis A, Morel B, Flouri T (2020) ModelTest-NG: A new and scalable tool for the selection of DNA and protein evolutionary models. Mol Biol Evol 37:291–294. https://doi.org/10.1093/molbev/msz189
Edler D, Klein J, Antonelli A, Silvestro D (2021) raxmlGUI 2.0: a graphical interface and toolkit for phylogenetic analyses using RAxML. Methods Ecol Evol 12:373–377. https://doi.org/10.1111/2041-210X.13512
Fernandez MO, Collins AG, Gittenberger A, Roy K, Marques AC (2020) Traits and depth: What do hydroids tell us about morphology and life-history strategies in the deep sea? Global Ecol Biogeogr 29:908–924. https://doi.org/10.1111/geb.13074
Fruciano C (2016) Measurement error in geometric morphometrics. Dev Genes Evol 226:139–158. https://doi.org/10.1007/s00427-016-0537-4
Gibbons MJ, Janson LA, Ismail A, Samaai T (2010) Life cycle strategy, species richness and distribution in marine Hydrozoa (Cnidaria: Medusozoa). J Biogeogr 37:441–448. https://doi.org/10.1111/j.1365-2699.2009.02226.x
Gili JM, Hughes RG (1995) The ecology of marine benthic hydroids. Oceanogr Mar Biol an Annu Rev 33:351–426
Gili JM, Vervoort W, Pagès F (1989) Hydroids from the West African coast: Guinea Bissau, Namibia and South Africa. Sci 53:67–112
González-Wevar CA, Segovia NI, Rosenfeld S, Ojeda J, Hüne M, Naretto J, Saucède T, Brickle P, Morley S, Féral J-P, Spencer HG, Poulin E (2018) Unexpected absence of island endemics: long-distance dispersal in higher latitude sub-Antarctic Siphonaria (Gastropoda: Euthyneura) species. J Biogeogr 45:874–884. https://doi.org/10.1111/jbi.13174
Grantham BA, Eckert GL, Shanks AL (2003) Dispersal potential of marine invertebrates in diverse habitats. Ecol Appl 13:108–116. https://doi.org/10.1890/1051-0761(2003)013[0108:DPOMII]2.0.CO;2
Griffith KA, Newberry AT (2008) Effect of flow regime on the morphology of a colonial cnidarian. Invertebr Biol 127:259–264. https://doi.org/10.1111/j.1744-7410.2008.00127.x
Hart MW, Marko PB (2010) It's About time: divergence, demography, and the evolution of developmental modes in marine invertebrates. Integr Comp Biol 50:643–661. https://doi.org/10.1093/icb/icq068
Hartlaub C (1901) Hydroiden aus dern Stillen ocean. Ergebnisse einer Reise nach dem Pacific. Zool Jahrbücher 14:349–379
Harvell CD, Labarbera M (1985) Flexibility: a mechanism for control of local velocities in hydroid colonies. Biol Bull 168:312–320. https://doi.org/10.2307/1541243
Hincks T (1853) Further notes on British Zoophytes, with descriptions of new species. Ann Mag Nat Hist 11:178–185
Hirohito ES (1969) Some hydroids of the Amakusa Islands. Biological Laboratory, Imperial Household, Tokyo, Japan

Hirohito ES (1995) The hydroids of Sagami Bay II. Publications of the Biological Laboratory Imperial Household, Tokyo, Thecata
Hollander J (2008) Testing the grain-size model for the evolution of phenotypic plasticity. Evolution 62:1381–1389. https://doi.org/10.1111/j.1558-5646.2008.00365.x
Hughes RG (1992) Morphological adaptations of the perisarc of the intertidal hydroid *Dynamena pumilla* to reduce damage and enhance feeding efficiency. Sci 56:269–277
Hughes RG, Johnson S, Smith ID (1991) The growth patterns of some hydroids that are obligate epiphytes of seagrass leaves. Hydrobiologia 216(217):205–210. https://doi.org/10.1007/BF00026463
Jost L (2008) GST and its relatives do not measure differentiation. Mol Ecol 17:4015–4026. https://doi.org/10.1111/j.1365-294X.2008.03887.x
Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 30:3059–3066. https://doi.org/10.1093/nar/gkf436
Kelly RP, Palumbi SR (2010) Genetic Structure Among 50 Species of the Northeastern Pacific Rocky Intertidal Community. PLoS ONE 5:e8594. https://doi.org/10.1371/journal.pone.0008594
Kim A, Wakefield J (2018) SpatialEpi: methods and data for spatial epidemiology. R Package Vers 1(2):3
Kozlov AM, Darriba D, Flouri T, Morel B, Stamatakis A (2019) RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. Bioinformatics 35:4453–4455
Leigh JW, Bryant D (2015) POPART: Full-feature software for haplotype network construction. Methods Ecol Evol 6:1110–1116. https://doi.org/10.1111/2041-210X.12410
Levin LA (2006) Recent progress in understanding larval dispersal: new directions and digressions. Integr Comp Biol 46:282–297. https://doi.org/10.1093/icb/icj024
Llobet I, Gili J-M, Hughes RG (1991) Horizontal, vertical and seasonal distributions of epiphytic hydrozoa on the alga *Halimeda tuna* in the Northwestern Mediterranean Sea. Mar Biol 110:151–159
Macaya EC, López B, Tala F, Tellier F, Thiel M (2016) Float and raft: role of buoyant seaweeds in the phylogeography and genetic structure of non-buoyant associated flora. In: Hu ZM, Fraser C (eds) Seaweed Phylogeography. Springer, Netherlands, Dordrecht, pp 97–130
Mallet J (1995) A species definition for the Modern Synthesis. Trends Ecol Evol 10:294–299. https://doi.org/10.1016/0169-5347(95)90031-4
Marko PB (2004) 'What's larvae got to do with it?' Disparate patterns of post-glacial population structure in two benthic marine gastropods with identical dispersal potential. Mol Ecol 13:597–611. https://doi.org/10.1046/j.1365-294X.2004.02096.x
Marko PB, Hart MW (2011) The complex analytical landscape of gene flow inference. Trends Ecol Evol 26:448–456. https://doi.org/10.1016/j.tree.2011.05.007
Marques AC (2011) Invasives: sea of data still to come. Science 333:936. https://doi.org/10.1126/science.333.6045.936-a
Marques AC, Klôh AS, Migotto AE, Cabral A, Rigo APR, Bettim AL, Razzolini EL, Matthews-Cascon H, Bardi J, Kremer LP, Vieira LM, Bezerra LEA, Haddad MA, Oliveira Filho RR, Gutierre SMM, Miranda TP, Franklin W Jr, Rocha RM (2013) Rapid assessment survey for exotic benthic species in the São Sebastião Channel, Brazil. Lat Am J Aquat Res 41:265–285. https://doi.org/10.3856/vol41-issue2-fulltext-6
Meirmans PG (2006) Using the AMOVA framework to estimate a standardized genetic differentiation measure. Evolution 60:2399–2402. https://doi.org/10.1554/05-631.1
Mendoza-Becerril MA, Maronna MM, Pacheco MLAF, Simões MG, Leme JM, Miranda LS, Morandini AC, Marques AC (2016) An evolutionary comparative analysis of the medusozoan (Cnidaria) exoskeleton. Zool J Linn Soc 178:206–225. https://doi.org/10.1111/zoj.12415
Miglietta MP, Maggioni D, Matsumoto Y (2019) Phylogenetics and species delimitation of two hydrozoa (phylum Cnidaria): Turritopsis (McCrady, 1857) and Pennaria (Goldfuss, 1820). Mar Biodivers 49:1085–1100. https://doi.org/10.1007/s12526-018-0891-8
Migotto AE (1996) Benthic shallow-water hydroids (Cnidaria, hydrozoa) of the coast of São Sebastião, Brazil, including a checklist of Brazilian hydroids. Zool Verh 306:1–125
Millard NAH (1975) Monograph on the Hydroida of Southern Africa. Ann South African Museum 68:1–513
Millard NAH, Bouillon J (1973) Hydroids from the Seychelles (Coelenterata). Musee Royal De L'afrique Centrale Sci Zool 206:1–106
Moura CJ, Lessios H, Cortés J, Nizinski MS, Reed J, Santos RS, Collins AG (2018) Hundreds of genetic barcodes of the species-rich hydroid superfamily Plumularioidea (Cnidaria, Medusozoa) provide a guide toward more reliable taxonomy. Sci Rep 8:1–14. https://doi.org/10.1038/s41598-018-35528-8
Moura CJ, Collins AG, Santos RS, Lessios H (2019) Predominant east to west colonizations across major oceanic barriers: Insights into the phylogeographic history of the hydroid superfamily Plumularioidea, suggested by a mitochondrial DNA barcoding marker. Ecol Evol. https://doi.org/10.1002/ece3.5608
Naimi B, Na H, Groen TA, Skidmore AK, Toxopeus AG (2014) Where is positional uncertainty a problem for species distribution modelling. Ecography 37:191–203. https://doi.org/10.1111/j.1600-0587.2013.00205.x
Naumov D V (1969) Hydroids and hydromedusae of the USSR. Israel Program for Scientific Translations, Jerusalem
Oliveira OMP, Marques AC (2007) Epiphytic hydroids (Hydrozoa: Anthoathecata and Leptothecata) of the World. Check List 3:21
Oliveira OMP, Marques AC (2011) Global and local patterns in the use of macrophytes as substrata by hydroids (Hydrozoa: Anthoathecata and Leptothecata). Mar Biol Res 7:786–795. https://doi.org/10.1080/17451000.2011.578647
Oliveira OMP, Miranda TP, Araujo EM, Ayón P, Cedeño-Posso CM, Cepeda-Mercado AA, Córdova P, Cunha AF, Genzano GN, Haddad MA, Mianzan HW, Migotto AE, Miranda LS, Morandini AC, Nagata RM, Nascimento KB, Júnior MN, Palma S, Quiñones J, Rodriguez CS, Scarabino F, Schiariti A, Stampar SN, Tronolone VB, Marques AC (2016) Census of Cnidaria (Medusozoa) and Ctenophora from South American marine waters. Zootaxa 4194:1–256
Palumbi SR (1994) Genetic divergence, reproductive isolation, and marine speciation. Annu Rev Ecol Syst 25:547–572. https://doi.org/10.1146/annurev.es.25.110194.002555
Paradis E (2010) pegas: an R package for population genetics with an integrated-modular approach. Bioinformatics 26:419–420. https://doi.org/10.1093/bioinformatics/btp696
Peña Cantero AL, García Carrascosa AM (2002) The benthic hydroid fauna of the Chafarinas Islands (Alborán Sea, western Mediterranean). Zool Verh 337:1–180
Pontin DR, Cruickshank RH (2012) Molecular phylogenetics of the genus Physalia (Cnidaria: Siphonophora) in New Zealand coastal waters reveals cryptic diversity. Hydrobiologia 686:91–105. https://doi.org/10.1007/s10750-011-0994-8
Postaire B, Gélin P, Bruggemann JH, Magalon H (2017a) One species for one island? Unexpected diversity and weak connectivity in a widely distributed tropical hydrozoan. Heredity (edinb) 118:385–394. https://doi.org/10.1038/hdy.2016.126
Postaire B, Gélin P, Bruggemann JH, Pratlong M, Magalon H (2017b) Population differentiation or species formation across the Indian and the Pacific Oceans? An example from the brooding marine

hydrozoan *Macrorhynchia phoenicea*. Ecol Evol 7:8170–8186. https://doi.org/10.1002/ece3.3236
R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/. Acessed 21 September 2021
Ralph PM (1956) Variation in *Obelia geniculata* (Linnaeus, 1758) and *Silicularia bilabiata* (Coughtrey, 1875) (Hydroida, F. Campanulariidae). Trans R Soc New Zeal 84:279–296
Ralph PM (1957) New Zealand Thecate Hydroids. Part I. Campanulariidae and Campanulinidae. Trans R Soc New Zeal 84:811–854
Ralph PM, Thomson HG (1968) Seasonal changes in growth of the erect stem of *Obelia geniculata* in Wellington Harbour, New Zealand. Zool Publ from Victoria Univ Wellingt 44:1–21
Ramos-Onsins SE, Rozas J (2002) Statistical properties of new neutrality tests against population growth. Mol Biol Evol 19:2092–2100. https://doi.org/10.1093/oxfordjournals.molbev.a004034
Rocha RM, Vieira LM, Migotto AE, Amaral ACZ, Ventura CRR, Serejo CS, Pitombo FB, Santos KC, Simone LRL, Tavaros M, Lopes RM, Pinheiro U, Marques AC (2013) The need of more rigorous assessments of marine species introductions: a counter example from the Brazilian coast. Mar Pollut Bull 67:241–243. https://doi.org/10.1016/j.marpolbul.2010.12.014
Rodriguez CS, Marques AC, Mianzan HW, Tronolone VB, Migotto AE, Genzano GN (2017) Environment and life cycles influence distribution patterns of hydromedusae in austral South America. Mar Biol Res 13:659–670. https://doi.org/10.1080/17451000.2017.1280170
Rohart F, Gautier B, Singh A, Le Cao K-A (2017) mixOmics: an R package for 'omics feature selection and multiple data integration. PLoS Comput Biol 13(11):e1005752
Rohlf JF, Corti M (2000) Use of Two-Block Partial Least Squares to study covariation in shape. Syst Biol 49:740–753. https://doi.org/10.1080/106351500750049806
Russel FR (1953) The Medusae of the British Isles. University Press, Cambridge
Schlager S (2017) Morpho and Rvcg- Shape Analysis in R. In: Li S, Szekely G (eds) Zheng G. Statistical Shape and Deformation Analysis, Academic Press, pp 217–256
Schliep K, Jombart T, Kamvar ZN, Archer E, Harris R (2020) apex: phylogenetic methods for multiple gene data. R Package Vers 1:4
Schuchert P (2014) High genetic diversity in the hydroid *Plumularia setacea*: A multitude of cryptic species or extensive population subdivision? Mol Phylogenet Evol 76:1–9. https://doi.org/10.1016/j.ympev.2014.02.020
Silveira ICA, Schmidt ACK, Campos EJD, Godoi SS, Ikeda Y (2000) A Corrente do Brasil ao largo da costa leste brasileira. Rev Bras Oceanogr 48:171–183
Sissini MN, Barreto MBBB, Széchy MTM, Lucena MB, Oliveira MC, Gower J, Liu G, Bastos EO, Milstein D, Gusmão F, Martinelli-Filho JE, Alves-Lima C, Colepicolo P, Ameka G, Graft-Johnson K, Gouvea L, Torrano-Silva B, Nauer F, Nunes JMC, Barufi JB, Rörig L, Riosmena-Rodríguez R, Mello TJ, Lotufo LVC, Horta PA (2017) The floating *Sargassum* (Phaeophyceae) of the South Atlantic Ocean – likely scenarios. Phycologia 56:321–328. https://doi.org/10.2216/16-92.1
Smetacek V, Zingone A (2013) Green and golden seaweed tides on the rise. Nature 504:84–88. https://doi.org/10.1038/nature12860
Sommer C (1992) Larval biology and dispersal of *Eudendrium racemosum* (Hydrozoa, Eudendriidae). Sci 56:205–211
Sultan SE, Spencer HG (2002) Metapopulation structure favors plasticity over local adaptation. Am Nat 160:271–283. https://doi.org/10.1086/341015
Thiel M, Gutow L (2005a) The ecology of rafting in the marine environment: I. The floating substrata. In: Gibson RN, Atkinson RJA, Gordon JDM (eds) Oceanography and Marine Biology: An Annual Review. CRC Press, pp 181–264.
Thiel M, Gutow L (2005b) The ecology of rafting in the marine environment. II. The rafting organisms and community. In: Gibson RN, Atkinson RJA, Gordon JDM (eds) Oceanography and Marine Biology: an Annual Review. pp 279–418.
Thiel M, Haye P (2006) The ecology of rafting in the marine environment. III. Biogeographical and evolutionary consequences. In: Gibson RN, Atkinson RJA, Gordon JDM (eds) Oceanography and Marine Biology: an Annual Review. CRC Press, pp 323–429.
Vaga CF, Kitahara MV, Nascimento KB, Migotto AE (2020) Genetic diversity of the *Pennaria disticha* Goldfuss, 1820 (Cnidaria, Hydrozoa) complex: new insights from Brazil. Mar Biodivers. https://doi.org/10.1007/s12526-020-01102-7
Van Winkle DH, Longnecker K, Blackstone NW (2000) The effects of hermit crabs on hydractiniid hydroids. Mar Ecol 21:55–67. https://doi.org/10.1046/j.1439-0485.2000.00693.x
Vervoort W, Watson JE (2003) The Marine Fauna of New Zealand: Leptothecata (Cnidaria:Hydrozoa) (Thecate Hydroids). NIWA (National Institute of Water and Atmospheric Research), Wellington
Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York
Winter DJ (2012) mmod : an R library for the calculation of population differentiation statistics. Mol Ecol Resour 12:1158–1160. https://doi.org/10.1111/j.1755-0998.2012.03174.x
Zelditch ML, Swiderski DL, Sheets HD (2012) Geometric morphometrics for biologists: a primer. Academic Press