



## Parasitological evidence of stocks of *Paralichthys isosceles* (Pleuronectiformes: Paralichthyidae) at small and large geographical scales in South American Atlantic coasts

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### ABSTRACT

Three stocks of the flounder *Paralichthys isosceles* Jordan, 1861 were identified in the southwestern Atlantic by using their parasites as biological markers. Comparisons (permutational multivariate analyses of variance and canonical analysis of principal coordinates) of 78 flounders caught simultaneously at two close localities in Brazil (Cabo Frio and Niteroi) and previously published data of 51 fish from Argentina (Necochea) allowed testing the value of parasite tags as discriminators at both small and large spatial scales. A total of 5925 metazoan parasites belonging to 17 species were found in Brazilian samples. Parasite assemblages varied across localities in terms of species richness and presence and abundance of individual species. Multivariate analyses of presence and abundance of long-lived parasites resulted in clear dissimilarity patterns across all samples. Significant differences were also observed when the entire parasite assemblages were compared between both Brazilian samples. The best discriminator species at both spatial scales were represented by unspecific parasites, broadly distributed among fish species in the region. These indicator species apparently display recurrent spatial patterns across host species, being therefore expected to serve as suitable tags for population studies of other resources. Parasites constitute valuable tools to be included in further holistic stock identification studies, which may eventually allow proactive mitigation and conservation strategies for many short scale artisanal fisheries in the southwestern Atlantic coasts, which are facing severe risks of overexploitation and collapse.

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### 1. Introduction

The present status of marine fisheries, characterized by marked decreases in many regional captures as a result of overexploitation (Food and Agriculture Organization of the United Nations, 2012), has evidenced the need of precise assessments of the biological status of most fisheries around the world ocean. The success of such assessments relies on detailed knowledge on stock composition, an elusive goal in many fisheries (Costello et al., 2012) that requires different approaches and has prompted the combination of a broad spectrum of complementary techniques, including the use of par-

asite tags in recent times (Cadriin et al., 2005; Timi and MacKenzie, 2015).

The increase in the knowledge of the life cycles and ecology of marine fish parasites has improved their efficiency as biological indicators; their use has also been supported by their reliability, proved in holistic studies when incorporated in multidisciplinary sampling designs. Consequently, parasites are increasingly used worldwide as biological tags for fish stock discrimination, especially of commercially valuable species; indeed, a progressively higher number of publications on parasite tags per decade and the spread of the methodology over the world ocean have been recently documented (Timi and MacKenzie, 2015).

Recent analyses of Brazilian marine fisheries catch data have shown evidences of overexploitation (Ministério do Meio Ambiente, 2006) and indications that the marine biodiversity is being eroded (Freire and Pauly, 2010). Brazil has the longest coastline in South America as well an extensive continental shelf, represented by a multiplicity of ecosystems (Miloslavich et al.,

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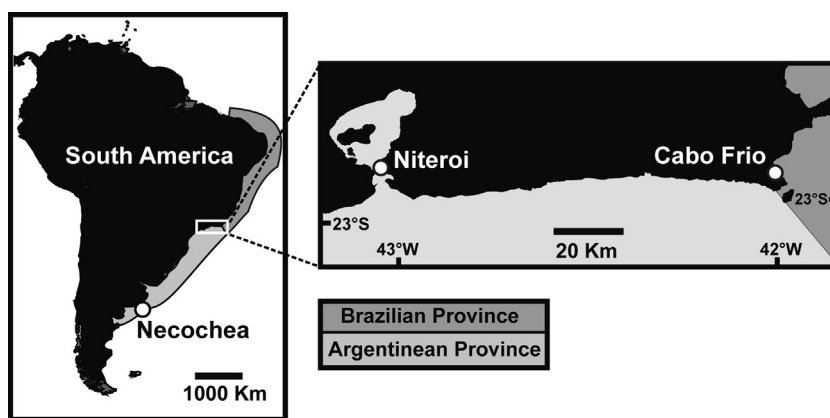


Fig. 1. Sampling localities of *Paralichthys isosceles* in the south west Atlantic.

2011), where fishing is an important economic and widely distributed activity along five different regions defined by their bathymetry, oceanographic structure, fauna, flora and fishery (Freire, 2003). This variety of environments along Brazilian coasts offers outstanding opportunities to use parasites as biological indicators of stock structure for diverse fish species (Cantatore and Timi, 2015). Nevertheless, the use of parasite tags remains unexplored in most regions of the Brazilian sea, despite the need to focus future actions on enhancing fisheries management to improve the regional ocean health (Elfes et al., 2014). In fact, at present only two studies have been carried out between Brazilian localities for the discrimination of stocks of *Micropogonias furnieri* (Luque et al., 2010) and *Urophycis brasiliensis* (Pereira et al., 2014), both comparing fish samples between distant regions with successful results derived from sampling designs covering different biogeographical provinces and ecoregions (Pereira et al., 2014). Similarly, some previous comparisons of parasite communities between fish samples from the regions of Rio de Janeiro, Brazil and northern Argentine waters are also based on large-scale spatial studies (Timi et al., 2005, 2010; Braicovich et al., 2012).

The knowledge of parasites in marine fishes from Brazil is concentrated in coasts of the State of Rio de Janeiro (see e.g., Luque et al., 2004). In this region, Cabo Frio (22°54'S) is considered as the limit between two zoogeographical provinces, the Argentinean and Brazilian Provinces, on the basis of the distribution of several zoological groups (Briggs, 1995; Boschi, 2000; Spalding et al., 2007; Menni et al., 2010). This is an oceanographically complex region due to the presence of different water masses and currents as well as of a strong upwelling regime (Machado et al., 2005). Upwelling events are common particularly during spring and summer, constituting the most important oceanographic feature of the area between Cabo Frio and Guanabara Bay (Machado et al., 2005; Coelho-Souza et al., 2012). Shoreline irregularities also define the embayment formation of Arraial do Cabo, north of Cabo Frio, making it an area with different intensities of upwelled water that harbours high diversity. Consequently, on a small spatial scale there are environments with tropical and subtropical features north and south of Cabo Frio, respectively (Coelho-Souza et al., 2012).

Here, we analysed the use of parasite tags at a small geographical scale, by comparing the parasite assemblages of the flounder *Paralichthys isosceles* Jordan, 1861, whose parasite fauna is relatively well known in the region (Luque and Poulin, 2004; Felizardo et al., 2009a,b, 2010, 2011; Knoff et al., 2012). *P. isosceles* is endemic of the Argentinean Zoogeographical Province, where inhabits waters at depths between 50 and 180 m (Díaz de Astarloa, 2002), being most abundant at 41–70 m depth (Díaz de Astarloa and Fabr e, 2003).

Flounders were caught in waters off two close localities in the State of Rio de Janeiro, Brazil, namely Cabo Fr o and Niteroi, distant

about 110 km each other. The contrasting physical conditions dominating both regions are expected to affect the distribution patterns of parasites of those fishes living in the region. The value of parasite tags at this small geographical scale is also analysed relative to a larger scale, comparing these samples with *P. isosceles* caught in Argentine waters, where this hosts has been also studied from a parasitological perspective (Timi et al., 2011; Alarcos and Timi, 2012).

## 2. Materials and methods

### 2.1. Fish samples and parasite inventories

Fish samples were taken from catches made by commercial trawlers operating at two close localities in the coasts of Rio de Janeiro State, Brazil (Fig. 1); namely the ports of Cabo Frio (22°52'S, 42°01'W; 38 specimens; total length (mean + standard deviation): 32.4 ± 2.4 cm; date: June 26th, 2012) and Niteroi (22°53'S, 43°06'W; 40 specimens; total length (mean + standard deviation): 31.3 ± 1.4 cm; date: June 27th, 2012). Fish were either kept fresh or deep frozen at −18 °C until examination. After thawing, specimens were measured for total length (cm). Body surface, gills, branchial and body cavities, viscera (stomach, intestine, liver, gall bladder, spleen, heart, gonads and mesenteries), swim bladder, kidneys and musculature were examined with the aid of a stereoscopic microscope. A sample from a previous study (Alarcos and Timi, 2012), carried out in waters off Necochea, Argentina (38°52'S, 58°10'W, Fig. 1; 51 specimens; total length (mean + standard deviation): 28.0 ± 2.1 cm; date: May 22th, 2009) was included in the analyses for comparative purposes.

The total length of fish was compared between samples by means of a one-way ANOVA and a posteriori Tukey tests for unequal samples (Zar, 2004).

The prevalence and mean abundance were calculated for each parasite species in each sample following Bush et al. (1997). The total number of parasites, regardless of their species (total abundance), was also calculated for each sample.

### 2.2. Comparisons of parasite assemblage structure

As both Brazilian samples were caught almost simultaneously, it was expected that their levels of similarity were not affected by short-term temporal variability of parasite loads. Consequently, all parasites were included in the comparative analyses of assemblage structure. The differences in community structure between both samples were tested using a one-way permutational multivariate analysis of the variance (PERMANOVA, Anderson et al., 2008) on the presence–absence and abundance values, using the

**Table 1**

Parasites of *Paralichthys isosceles* in three localities of the Southwestern Atlantic, with data on its development stage, prevalence (*P*) and mean abundance + standard deviation (MA + SD).

Parasite species	Stage	Locality		Niteroi		Necochea <sup>a</sup>	
		Cabo Frio <i>P</i> (%)	MA ± SD	<i>P</i> (%)	MA ± SD	<i>P</i> (%)	MA ± SD
<b>Digenea</b>							
<i>Prosorhynchus</i> sp.	Adult	5.3	0.1 ± 0.4	0.0	–	0.0	–
<i>Lecithochirium microstomum</i>	Adult	36.8	0.8 ± 1.7	28.8	1.1 ± 1.8	0.0	–
<i>Derogenes varicus</i>	Adult	0.0	–	0.0	–	11.8	0.2 ± 0.7
<i>Aponurus laguncula</i>	Adult	5.3	0.1 ± 0.4	0.0	–	0.0	–
Hemiuridae gen. sp.	Adult	2.6	0.05 ± 0.3	0.0	–	0.0	–
Unidentified didymozoid	Larva	21.0	0.4 ± 0.8	34.6	0.05 ± 0.2	0.0	–
<b>Cestoda</b>							
<i>Grillotia carvajalregorum</i>	Plerocercus	94.7	59.3 ± 104.5	59.6	30.8 ± 63.4	100	332.4 ± 314.1
Tentaculariidae gen. sp.	Plerocercoid	13.2	0.4 ± 1.1	32.7	1.3 ± 2.1	58.8	6.1 ± 9.0
<i>Scolex</i> sp.	Plerocercoid	7.9	3.9 ± 18.8	0.0	–	7.8	6.5 ± 24.6
Unidentified larva	Plerocercoid	13.2	0.6 ± 1.9	3.8	0.05 ± 0.29	0.0	–
<b>Nematoda</b>							
<i>Cucullanus</i> sp.	Adult	55.3	1.9 ± 3.5	38.5	1.7 ± 2.3	0.0	–
<i>Hysterothylacium aduncum</i>	Larva III	0.0	–	0.0	–	11.8	0.1 ± 0.3
<i>Hysterothylacium</i> sp.	Larva III	100	16.5 ± 18.2	73.1	28.1 ± 27.8	37.2	0.6 ± 0.9
<i>Raphidascaris</i> sp.	Larva III	0.0	–	44.2	2.7 ± 3.0	0.0	–
<i>Contracaecum</i> sp.	Larva III	2.6	0.03 ± 0.2	0.0	–	51.0	1.4 ± 1.9
<i>Pseudoterranova cattani</i>	Larva III	0.0	–	0.0	–	2.0	0.01 ± 0.1
<i>Anisakis</i> sp.	Larva III	2.6	0.03 ± 0.2	11.5	0.2 ± 0.6	0.0	–
<i>Dichelyne pleuronectidis</i>	Adult	0.0	–	0.0	–	11.8	0.2 ± 0.5
<i>Procammallanus halitrophus</i>	Adult	18.4	0.3 ± 0.6	3.8	0.05 ± 0.2	0.0	–
<b>Acanthocephala</b>							
<i>Corynosoma australe</i>	Juvenile	7.9	0.1 ± 0.4	0.0	–	92.2	14.7 ± 20.5
<i>Bolbosoma turbinella</i>	Juvenile	36.8	0.5 ± 0.8	25.0	0.6 ± 1.0	0.0	–

<sup>a</sup> Data from Alarcos and Timi (2012).

Jaccard and Bray–Curtis indexes as the similarity measurements, respectively. Host length was introduced as a covariable (ANCOVA model) because parasites, especially long-lived ones, tend to accumulate as hosts grow, thus producing ontogenetic changes in the structure of parasite communities; and therefore fish length must be taken into account as a potential confounding variable in the interpretation of spatial patterns and stock structure when samples are not homogeneous in terms of fish size (Cantatore and Timi, 2015). The structures of parasite infracommunities between samples (1 × 2 factorial design, ‘locality’ as fixed factor) were compared, and the main effects were tested after 9999 permutations. Following Anderson et al. (2008), a permutation of residuals under a reduced model was used as the method of permutation. A sequential sum of squares (Type I SS) was applied because host length was introduced as a covariable and the samples were unbalanced (different numbers of fish examined by sample). Similarity percentages (SIMPER, Clarke, 1993), based on Bray–Curtis index, were calculated between samples to determine which species contributed largely to the average dissimilarity between parasite assemblages.

The potential use of parasites as biological tags for the discrimination of host stocks between the three sampling regions was evaluated in a similar way, now including samples from Necochea and retaining only long-lived parasites since the most important criterion for an effective parasite marker is its long residence time in the fish to avoid the effect of short-term temporal variability on parasite prevalence and abundance (Lester, 1990; Lester and MacKenzie, 2009). These parasites were the larval didymozoid, the metacestodes *Grillotia carvajalregorum*, Tentaculariidae gen. sp. and unidentified plerocerci, the larval anisakids *Contracaecum* sp., *Hysterothylacium* sp., *Hysterothylacium aduncum*, *Pseudoterranova cattani*, *Raphidascaris* sp. and *Anisakis* sp. and the juvenile acanthocephalans *Corynosoma australe* and *Bolbosoma turbinella*. PERMANOVA analyses (1 × 3 factorial design, ‘locality’ as fixed factor) were performed as in the previous section. When differences

were detected by PERMANOVA, pair-wise comparisons were used to determine which samples differed.

Because PERMANOVA is sensitive to differences in multivariate dispersion between groups (sensu homogeneity of variances, which can inflate Type 1 error even when centroids have identical locations), the same models were tested for differences in dispersion using the routine PERMDISP (Anderson et al., 2008). Dispersions were measured as distance to the centroid, and each term in the analysis was tested using 9999 permutations.

Differences between infracommunities among samples were examined in more detail using canonical analysis of principal coordinates (CAP) (Anderson and Willis, 2003; Anderson et al., 2008). The potential for over-parameterization was prevented by choosing the number of PCO axes (*m*) that maximized a leave-one-out allocation success to groups (Anderson and Robinson, 2003). CAP analyses were based on abundance data using a Bray–Curtis dissimilarity coefficient and repeated on the qualitative composition of samples using Jaccard coefficients. To test for significant differences between infracommunities among the samples, a permutation “trace” test (sum of squared canonical eigenvalues) was applied; the *P* was obtained after 9999 permutations.

Multivariate analyses were implemented in PERMANOVA+ for PRIMER package (Anderson et al., 2008).

### 3. Results

Mean host body lengths were significantly different between samples ( $F_{2,126} = 61.01$ ;  $P < 0.01$ ), with all pairs of samples differing significantly in fish length (both comparisons including samples from Necochea  $P < 0.01$ ; comparison between Niteroi and Cabo Frío  $0.01 < P < 0.05$ ).

For the Brazilian samples, all hosts were parasitized by at least 1 of 17 parasite species (Table 1). A total of 5925 metazoan parasites were recorded in both samples, with fish from Cabo Frío harboring a higher mean total abundance and species richness than those

**Table 2**  
Descriptors of parasite assemblages of *Paralichthys isosceles* caught in three regions of the Southwestern Atlantic.

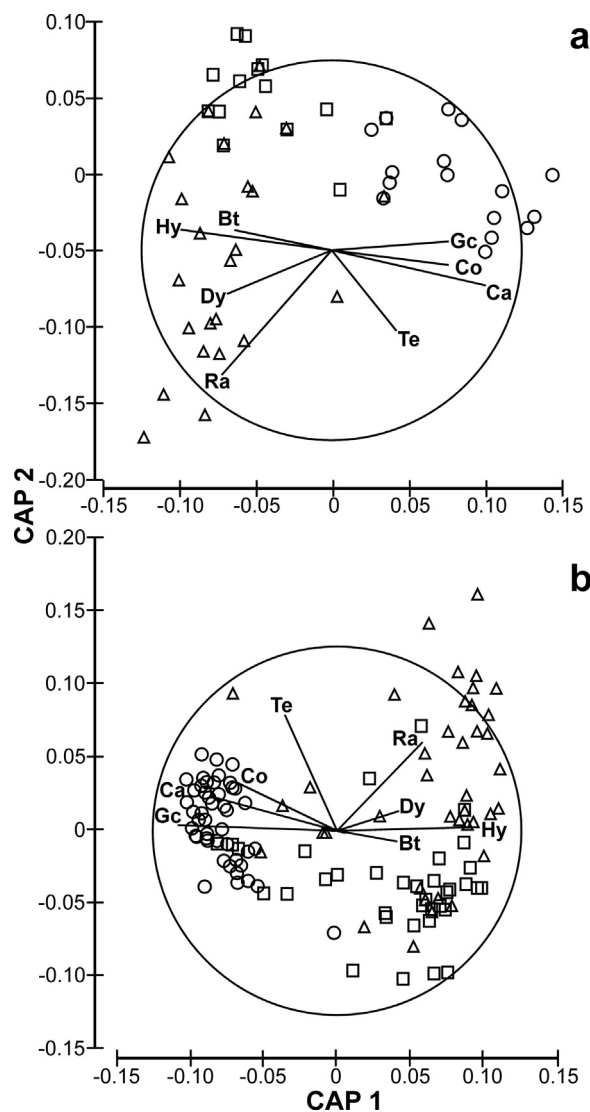
Locality	Total prevalence	Total mean abundance	Total species richness	Mean infracommunity species richness (range)
Cabo Frio	100	84.9	16	4.2 (2–7)
Niteroi	100	67.5	11	4.6 (1–9)
Necochea	100	362.0	11	3.8 (1–6)

from Niteroi, although mean infracommunity species richness was slightly higher in Niteroi (Table 2). Five species were found parasitizing only host from Cabo Frio, although at low prevalence, whereas *Raphidascaris* sp. was only found in Niteroi at a high prevalence (44.2%) (Table 1). An uneven distribution across localities was observed also for most parasites shared by both samples, in terms of both prevalence and abundance (Table 1), especially for those displaying the highest parasite loads, namely *G. carvajalregorum* and *Hysterothylacium* sp.

The results of PERMANOVA analyses on both presence/absence and abundance data of all parasites of *P. isosceles* from Cabo Frio and Niteroi (Table 3) showed no effect of host length on the response variables and no interaction between the host length and locality. Significant variability was detected between the localities in both analyses. These differences cannot be attributed to differences in the multivariate dispersions of parasite infracommunities in terms of their deviations from centroids, because the PERMDISP results were not significant for both the Jaccard and Bray–Curtis indices ( $F_{1,76} = 0.01$  and  $F_{1,76} = 1.76$ , respectively; both  $P(\text{perm}) > 0.05$ ). SIMPER results showed that the main contributors to dissimilarity between both samples were *G. carvajalregorum* and *Hysterothylacium* sp., contributing to 49.2 and 39.5% of dissimilarity, respectively, followed in importance by *Raphidascaris* sp. with a contribution of only 3.7%.

For the large scale analysis, we selected 11 long-lived parasite species, including the sample from Necochea. The results of PERMANOVA on both presence/absence and abundance data (Table 4) showed a strong effect of host length on the response variables and therefore on the parasite community structure. The interaction of host length with the samples showed that the nature of the relationship between the covariate and the multivariate response did not differ within different levels of the factor. Furthermore, taking into account the variations among samples due to host length, significant variability was detected among the parasite assemblages. The pairwise tests showed that there were significant differences in all pairs of samples (all  $P(\text{perm}) < 0.01$ ). However, both samples from Brazil were more similar each other in composition and abundance than when compared with those from Necochea (Table 5).

A proportion of these differences can be attributed to differences in the multivariate dispersions of parasite infracommunities in terms of their deviations from centroids (Table 2), because the PERMDISP results were significant for both the Jaccard and Bray–Curtis indices ( $F_{2,126} = 8.53$  and  $F_{2,126} = 19.96$ ; both  $P(\text{perm}) < 0.001$ ). Indeed, the pairwise test showed significant differences in the multivariate dispersions for those comparisons involving Niteroi (both  $P(\text{perm}) < 0.01$ ), but not between Cabo Frio and Necochea ( $P(\text{perm}) < 0.01$ ) for the Jaccard index, whereas for those calculations based on the Bray–Curtis index, significant differences were observed for comparisons involving Necochea (both  $P(\text{perm}) < 0.01$ ). SIMPER results showed that the main contributors to dissimilarity between Brazilian and Argentine samples were the same two species than between both Brazilian samples, namely *G. carvajalregorum* and *Hysterothylacium* sp., contributing to 82.8% and 7.2% of dissimilarity, respectively, when comparing Cabo Frio and Necochea, and to 79.1% and 10.5%, respectively, when comparing Niteroi and Necochea. These species were followed in



**Fig. 2.** Canonical analysis of principal co-ordinates (CAP) biplot based on (a) presence/absence data and Jaccard dissimilarities and (b) untransformed abundance and Bray–Curtis dissimilarities of parasites in samples of *Paralichthys isosceles* from three localities in the south west Atlantic. Cabo Frio, Brazil (squares); Niteroi, Brazil (triangles) and Necochea, Argentina (circles). Vector overlay are Spearman correlations of parasite species with the CAP axes (restricted to those having  $r > 0.3$ ): Bt: *Bolbosoma turbinella*; Ca: *Corynosoma australe*; Co: *Contracecum* sp.; Dy: Unidentified didymozoid larvae; Gc: *Grillotia carvajalregorum*; Hy: *Hysterothylacium* sp.; Ra: *Raphidascaris* sp.; Te: *Tentaculairiidae* gen. sp.

importance by *C. australe*, with a contribution of 6.1% when comparing Necochea and Cabo Frio and 5.3% in the case of Niteroi.

The CAP analysis based on binary data showed significant differences among the samples ( $\text{tr} = 1.31$ ;  $P = 0.0001$ ). The selected orthonormal PCO axes ( $m = 6$ ) described 96.51% of the variation in the data cloud, with a high percentage of correct allocations (88.4%). The two first canonical axes resulting from the CAP analysis clearly separated the samples in 3 groups, with fish from Necochea separated along the CAP 1 and situated at the right of the CAP biplot, while both Brazilian samples were separated along the CAP 2 (Fig. 2a). The strength of the association between the multivariate data cloud and the hypothesis of group differences was indicated by the large size of their canonical correlations ( $\delta_1 = 0.92$  and  $\delta_2 = 0.69$ ). By superimposing vectors corresponding to the Spearman correlations of individual parasite species with the CAP axes (restricted to those species with lengths  $> 0.30$ ), it was observed that *C. australe*, *G. carvajalregorum*, *Contracecum* sp. and

**Table 3**

One-factor PERMANOVA results of infracommunity data of parasites of *Paralichthys isosceles* in two samples from the coastal region of Rio de Janeiro State, Brazil, based on Jaccard and Bray–Curtis dissimilarity measures with host length as covariable. *P*-values obtained after 9999 permutations.

Dissimilarity measure	Source	d.f.	SS	MS	Pseudo F	<i>P</i> (perm)
Jaccard	Host length	1	3359.5	3359.5	1.4511	>0.05
	Locality	1	10261	10261	4.4320	<0.01
	Host length × locality	1	1863.8	1863.8	0.8051	>0.05
	Residual	74	17132 e <sup>5</sup>	2315.1		
	Total	77	1.868 e <sup>5</sup>			
Bray–Curtis	Host length	1	2341.6	2341.6	1.3373	>0.05
	Locality	1	12086	12086	6.920	<0.0001
	Host length × locality	1	1278.5	1278.5	0.7301	>0.05
	Residual	74	1.2958 e <sup>5</sup>	1751.1		
	Total	77	1.4529 e <sup>5</sup>			

**Table 4**

One-factor PERMANOVA results of infracommunity data of long-lived parasites in three samples of *Paralichthys isosceles* from the southwestern Atlantic based on Jaccard and Bray–Curtis dissimilarity measures with host length as covariable. *P*-values obtained after 9999 permutations.

Dissimilarity measure	Source	d.f.	SS	MS	Pseudo F	<i>P</i> (perm)
Jaccard	Host length	1	43071	43071	35.456	<0.0001
	Locality	2	65696	32848	27.04	<0.0001
	Host length × locality	2	3279.8	1639.9	1.3499	>0.05
	Residual	123	1.494 e <sup>5</sup>	1214.8		
	Total	128	2.6147 e <sup>5</sup>			
Bray–Curtis	Host length	1	64538	64538	35.117	<0.0001
	Locality	2	61763	30882	16.804	<0.0001
	Host length × locality	2	4629.6	2314.8	1.2596	>0.05
	Residual	123	2.2605 e <sup>5</sup>	1837.8		
	Total	128	3.5698 e <sup>5</sup>			

**Table 5**

Average similarity within (main diagonals) and between samples of *Paralichthys isosceles* in three localities of the South West Atlantic, based on Jaccard and Bray–Curtis similarity measures.

Localities	Jaccard			Bray–Curtis		
	Cabo Frio	Niteroi	Necochea	Cabo Frio	Niteroi	Necochea
Cabo Frio	59.73			36.95		
Niteroi	44.53	45.85		33.32	35.20	
Necochea	31.47	24.27	58.86	22.67	13.80	58.30

Tentaculariidae gen. sp. were indicators of samples from Necochea, whereas *Hysterothylacium* sp., *B. turbinella*, the unidentified didymozoids and *Raphidascaris* sp. were associated with fish from Brazil, with the latter two species associated to hosts from Niteroi. Similar results were obtained after CAP analysis based on the abundance data, which also showed significant differences among the samples ( $\text{tr} = 1.05$ ;  $P = 0.0001$ ). The selected orthonormal PCO axes ( $m = 8$ ) described 99.82% of the variation in the data cloud, with 77.5% of correct allocations. The two first canonical axes resulting from CAP analysis also clearly separated the three samples (Fig. 2b) with high values of canonical correlations ( $\delta_1 = 0.86$  and  $\delta_2 = 0.55$ ). The same set of indicator species was identified after superimposing vectors corresponding to the Spearman correlations of individual species with the CAP axes (restricted to those species having lengths >0.30).

The cross validation of the results showed that in both sets of analyses, high percentages of correctly allocated fish occurred in all localities (Table 6), especially for samples from Necochea, being also higher for analyses based on the Jaccard than on the Bray–Curtis indexes.

#### 4. Discussion

The parasite fauna of specimens of *P. isosceles* examined in the present study in the Brazilian coasts was represented by 17 metazoan species. This value may be even larger given that some parasites were identified at the genus or family level, although

more than one species have been previously recorded in this host for these taxa. For example Felizardo et al. (2010) recorded two species of larval tentaculariid trypanorhynch, *Nybelinia lingualis* and *Heteronybelinia nipponica* in *P. isosceles* from the littoral of the State of Rio de Janeiro; similarly Felizardo et al. (2010) described two species of juvenile didymozoid trematodes, *Torticaecum* sp. and *Neotorticaecum* sp., in the same region. More recently, Knoff et al. (2012) erected *Hysterothylacium deardorffoverstreeterorum* based on a genetic and morphological characterization of larvae parasitizing *P. isosceles* from the region of Rio de Janeiro. However, phylogenetic analyses based on mtDNA of larvae obtained from *Trichiurus lepturus* and *Pseudopercis numida* caught in the same region demonstrated the presence of different clades under *H. deardorffoverstreeterorum*, suggesting either polymorphism or the presence of 2 distinct species (Borges et al., 2012; Pantoja et al., 2015). In the present study these parasites were considered as Tentaculariidae gen. sp., unidentified Didymozoid and *Hysterothylacium* sp., respectively, with no differentiation at a lower taxonomic level. This was due to the need of avoiding a possible effect of temporal and spatial variability in samples composition, thus fish were caught at the same day and locality and consequently, most specimens were frozen until examination. The state of preservation of frozen parasites did not allow a higher taxonomic resolution for these species. Nevertheless, if different species of these parasites were detected, it is not expected that the result of comparisons at community level will be affected since both groups were not

**Table 6**  
Discriminant analysis classification showing the numbers and percentages of *Paralichthys isosceles* classified in each locality (number of correctly classified fish in each sample in bold) using Jaccard and Bray–Curtis dissimilarity measures of long-lived parasite assemblages (rows correspond to group memberships).

	Jaccard				Bray–Curtis			
	Cabo Frio	Niteroi	Necochea	%	Cabo Frio	Niteroi	Necochea	%
Cabo Frio	<b>35</b>	2	1	92.1	<b>26</b>	6	6	68.4
Niteroi	10	<b>29</b>	1	72.5	11	<b>25</b>	4	62.5
Necochea	1	0	<b>50</b>	98.0	2	0	<b>49</b>	96.1

#: Percentage of correctly classified fish per locality.

important contributors to the observed dissimilarity. Furthermore, as both parasites showed different values of prevalence and mean abundance between localities, a higher taxonomic identification will not necessarily lead to an under-interpretation of biological differences between them (Bray and Cribb, 2015).

At short special scale and at the component community level, both Brazilian samples differed in terms of species richness and parasite total abundance, although those species found only in Cabo Frio were observed at low abundance. *Grillotia carvajalregorum* and *Hysterothylacium* sp. dominated largely the assemblages in terms of abundance, accounting for 88.4% of all parasites recorded (58.8% and 29.6%, respectively), the former contributing therefore to the higher total abundance registered for flounders from Cabo Frio, where it was twice more abundant than in Niteroi. Conversely, *Hysterothylacium* sp. in fish from Niteroi doubled those from Cabo Frio in abundance. Not surprisingly, these two species were the main contributors to dissimilarity between both samples. The third species in order of importance as responsible of the observed dissimilarity, namely *Raphidascaris* sp., made a moderate contribution to the multivariate differences between assemblages, due to its low values of abundance. However, their importance as discriminator relies in its absence from Cabo Frio, whereas it reached a prevalence over 44% in Niteroi. In fact, most incorrectly allocated hosts in both CAP analyses were represented by fish from Niteroi classified as belonging to Cabo Frio; this misclassification was as a consequence of the absence of *Raphidascaris* sp. in most of them (all ten hosts in CAP analysis based on presence-absence and nine out of eleven in analyses on abundance, data not shown). *Raphidascaris* sp. constitutes, therefore, a promissory marker at small spatial scales in this region, where these larvae are broadly distributed in several fish species (Luque and Poulin, 2004).

The structure of parasite assemblages in both zones, largely influenced by these species in terms of abundance and composition, was different enough to consider that different stocks of *P. isosceles* inhabit both localities. The observed differences could be a consequence of the tropical and subtropical characteristics of waters north and south of Cabo Frio, respectively (Coelho-Souza et al., 2012). Indeed the north region is characterized by oligotrophic conditions due to the influence of tropical waters, rocky bottoms and a mostly narrow continental shelf, whereas in waters south of Cabo Frio primary production is mainly driven by seasonal upwelling of nutrient-rich, cold subtropical waters pumped by alongshore winds and by cyclonic vortexes originating from the Brazil Current (Bakun and Parrish, 1990; Vasconcellos et al., 2011). Although these large scale environmental features and processes surely determine at a large extent the structure of parasite assemblages, it is possible that small scale characteristics of the coastal systems also play a role in such structure and composition. In example, the absence of *Raphidascaris* sp. in flounders from Cabo Frio is not a consequence of the main oceanographic conditions in this region, because larvae of this species have been reported in other fish species at relative high loads in northern localities (Luque et al., 2010). Indeed, the heterogeneity of the Brazilian continental shelf, represented by contrasting dominant ecosystems of unique features, includ-

ing coral reefs, dunes, sand banks, sandy beaches, rocky shores, lagoons, mangroves, salt marshes, and estuaries (Miloslavich et al., 2011), provide a multiplicity of environments where fish parasite communities are expected to display significant differences at small geographical scales, offering excellent possibilities for using parasite tags for the enormous variety of fish species that this region harbors.

*Grillotia carvajalregorum*, *Hysterothylacium* sp. and *Raphidascaris* sp., identified as suitable biological tags in this study are also broadly distributed among fish species in the region (Luque and Poulin, 2004) and are therefore expected to serve as suitable indicators for population studies of other hosts. These species also met the most important criterion for an effective parasite marker in having long residence time in the host (Lester, 1990; Lester and MacKenzie, 2009), which allows comparisons between samples independently of the date or season of capture, permitting further studies that include samples from other regions and dates. Indeed, the analyses based on long-lived parasites and involving samples from Argentina showed similar results between Brazilian samples, as well as a clear differentiation from *P. isosceles* from the northern Argentine Sea. This latter result is expectable for a population living in a different ecoregion (Spalding et al., 2007), under markedly dissimilar oceanographic conditions, whose contrasting parasite assemblage structure is clearly shown by CAP analyses based on both binary data and abundances. Those species identified by SIMPER as the main contributors to dissimilarity between samples (*G. carvajalregorum*, *C. australe* and *Hysterothylacium* sp.) acted, among others, as discriminator species between both ecoregions in CAP analyses. They also displayed latitudinal patterns of prevalence and abundance similar to those observed in previously studied host species. Indeed, parasite loads of *G. carvajalregorum* and *C. australe* were higher in Argentina than in Brazil in *Cynoscion guatucupa* (Timi et al., 2005), *Pinguipes brasiliensis* (Timi et al., 2010), *Trachurus lathami* (Braicovich et al., 2012) and *U. brasiliensis* (Pereira et al., 2014), whereas the opposite pattern was observed for *Hysterothylacium* sp. in the same hosts, except for *U. brasiliensis* possibly due to the smaller size of Brazilian specimens. These recurrent patterns of suitable parasite tags across host species in the southwestern Atlantic reinforce previous studies in this region showing that parasite assemblages can be used as ecosystem indicators to characterize hosts living in different zoogeographical provinces (Cantatore and Timi, 2015) and, as suggested by Pereira et al. (2014) for studies at larger spatial scales, as indicators of ecoregions within zoogeographical provinces.

As mentioned earlier, samples in the present study were caught at three different ecoregions (Spalding et al., 2007), belonging to two zoogeographical provinces, namely the Brazilian (Cabo Frio) and Argentinean (Niteroi and Necochea) Provinces (Menni et al., 2009 and references therein), or Tropical Southwestern Atlantic and Warm Temperate Southwestern Atlantic (Spalding et al., 2007), respectively. As a consequence of this hierarchical biogeographical scheme, it should be expected a higher similarity between samples caught within the same biogeographical province. How-

ever, in this case, flounders from Niteroi (Argentine Province) were more similar to those from Cabo Frio (Brazilian Province) than to fish from Necochea, contradicting the zoogeographical hypothesis. These results can be a consequence of the large distance separating both localities situated at the extremes of the Argentine Province (about 2200 km, calculated as the shortest linear distance between localities obtained from a map, following the coastline below the 50 m isobaths, Timi et al., 2010), whereas the distance separating both Brazilian localities was about ten times shorter. A distance decay of similarity in species composition among communities is a pattern commonly reported in parasite ecology (Poulin and Morand, 1999; Poulin, 2003). This pattern can be observed as exponential decay of similarity that implies a constant proportional reduction in similarity per unit distance (Poulin, 2003), and has been reported for parasite assemblages of *P. brasiliensis* (Timi et al., 2010) in a large proportion of the study area sampled in the present work. This pattern was attributed to the existence of geographical gradients in environmental conditions, determined by the water currents characterizing circulation in the southwestern Atlantic (Timi et al., 2010). On the other hand, the higher similarity between both Brazilian localities regarding Argentine samples could be not only a consequence of their geographical closeness, but also to the transitional characteristics of Cabo Frio between tropical and subtropical waters (Coelho-Souza et al., 2012). Indeed, some zoogeographical studies consider the region of Rio de Janeiro within the Brazilian Province, extending southern their limit with the Argentine Province to Santa Catarina (Floeter et al., 2008; Briggs and Bowen, 2012).

Beyond the zoogeographical implications of the similarities and differences between both Brazilian samples, parasitological evidence suggests that these fish, caught in close localities, can be considered as belonging to different stocks on the basis of differences in the loads of unspecific parasites which, in turn, are expected to display similar spatial patterns in other hosts. This information can be useful for the implementation of policies to help supporting sustainable coastal artisanal fisheries, which are broadly distributed along Brazilian coasts, and account for approximately 54% of the total marine landings in the country (Vasconcellos et al., 2011). Due to the lack of such strategies, these small-scale fisheries face many challenges, including over-exploitation and the depletion of resources (Salas et al., 2007). Indeed, approximately 60% of the stocks in the region are probably overfished and unable to support higher yields in the future (Vasconcellos et al., 2007, 2011). Parasites can be used as ancillary tools for the recognition of population structure these fishery resources, which is a prerequisite for the implementation of policies that ensure an efficient and sustainable management of resources.

Furthermore, the inclusion of parasites in holistic approaches involving a broad spectrum of complementary techniques is increasingly recommended in stock identification studies (Begg and Waldman, 1999; Baldwin et al., 2012; Catalano et al., 2014; Cantatore and Timi, 2015). Indeed, the integration of parasites with other techniques may resolve fish stock structure over small geographic areas by increasing the number of spatial and temporal scales studied simultaneously leading to methods for successful management of marine fish species (Baldwin et al., 2012). At light of the observed capability of parasites to discriminate stocks of *P. isosceles* at different spatial scales, and due to the fact that the best indicator species were unspecific parasites which seem to display recurrent patterns across fish species in the region, we recommend that the including parasites in further stock identification studies may eventually allow proactive mitigation and conservation strategies for short scale artisanal fisheries in the southwestern Atlantic coasts.

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