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ORIGINAL PAPER

Parasites of *Urophycis brasiliensis* (Gadiformes: Phycidae) as indicators of marine ecoregions in coastal areas of the South American Atlantic

Aldenice N. Pereira · Camila Pantoja · José L. Luque · Juan T. Timi

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Abstract The potential value of parasites as ecosystem markers was tested by analyzing the metazoan assemblages of *Urophycis brasiliensis* caught in four locations distributed in three ecoregions of the Warm Temperate Southwestern Atlantic. A total of 5,001 metazoan parasites belonging to 33 species were found. The identified parasites varied across locations in terms of presence, prevalence, and abundance, and their multivariate analyses resulted in clear similarity patterns. No differences were observed between two locations of the same ecoregion, whereas an evident separation of samples was observed across ecoregions in support of the existing hypotheses regarding the ecoregional division of the southwestern Atlantic. We proposed that parasite assemblages, which are composed of several metazoan phyla, are potentially useful as ecosystem indicators. This suggestion is derived from the combined evidence of the evolutionary history and biogeography of multiple lineages, which is expected to be more efficient in capturing recurrent patterns in overall biodiversity than individual lineages. Furthermore, as many parasites have complex life cycles, their distribution patterns are dependent not only on environmental conditions but also on the distribution and population density of all hosts involved in their life cycles, adding further sources of distributional variability that act synergistically to define robust geographical patterns. The selection of long-lived parasites and their comparative analysis provided evidence supporting the existence of three different stocks in the four sampled areas. The best parasite tags were those with low specificity in fish hosts, constituting promising biological tags for the stock discrimination of other fish species in the region.

Keywords Biological tags · Parasites · *Urophycis brasiliensis*

A. N. Pereira · C. Pantoja · J. L. Luque
Curso de Pós-Graduação em Ciências Veterinárias and Departamento de Parasitologia Animal, Universidade Federal Rural do Rio de Janeiro, Caixa Postal 74.540, CEP 23851-970, Seropédica, RJ, Brasil

J. T. Timi (✉)
Laboratorio de Parasitología, Instituto de Investigaciones Marinas y Costeras (IIMyC), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata - Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Funes 3350, (7600) Mar del Plata, Argentina
e-mail: jtimi@mdp.edu.ar

Introduction

Among the applied aspects of ichthyoparasitology, the use of parasites as biological tags has been established as a valuable methodology for stock identification and the determination of connectivity between populations of marine fish (Cadrin et al. 2005; Timi and MacKenzie 2014). Parasite tags are applied to discriminate stocks of individual valuable host species across various locations of their distributional range. These types of studies have been successfully applied to several host species in the southwestern Atlantic, mainly to fishes from northern and central Argentine waters (Cantatore and Timi 2014), whose populations display significant differences in composition as a consequence of the contrasting oceanographic characteristics between these regions and their effect on parasite distribution (Timi 2007). However, the potential use of parasite tags remains unexplored in most regions of the vast South American Atlantic waters, particularly along the northern coast.

Indeed, with the exception of comparisons of several fish species between the regions of Rio de Janeiro, Brazil and northern Argentine waters (Timi et al. 2005, 2010; Braicovich et al. 2012), only one study using parasites to discriminate fish stocks of *Micropogonias furnieri* from five locations along the Brazilian coast, located between Rio de Janeiro and Rio Grande do Sul near the boundary with Uruguay, has been published (Luque et al. 2010). This region is part of the large ecosystem defined as the Brazilian Shelves by Miloslavich et al. (2011); it is a very large region that is

hydrologically and topographically complex with contrasting dominant ecosystems of unique features, including mangroves, coral reefs, dunes, sand banks, sandy beaches, rocky shores, lagoons, estuaries, and salt marshes. This multiplicity of environments offers an excellent opportunity to use parasites not only as biological indicators of stock structure for an enormous variety of fish species but also as markers of the regions or waters they inhabit; consequently, they can also be used as ecosystem indicators (Cantatore and Timi 2014).

The distribution patterns and endemisms of southwestern Atlantic biota have been utilized to describe a series of biogeographical units (realms, provinces, and ecosystems) with different degrees of geographic resolution (Spalding et al. 2007; Miloslavich et al. 2011; Briggs and Bowen 2012). Specifically, the coastal and shelf region between Rio de Janeiro and the northern Argentine Sea shows a continuous distribution of warm temperate fauna and has historically been characterized as the Argentinean Province. It was recently renamed as the Temperate Western South Atlantic Province (Menni et al. 2010) or the Warm Temperate Southwestern Atlantic (Spalding et al. 2007). Its limits with neighboring provinces, as well as its subdivision in districts, are variably defined according to the group of organisms studied. For example, the region of Cabo Frio (22° 54′ S) has traditionally been proposed as the limit of this province with the northern Brazilian Province and western Atlantic Tropics based on the distribution of different zoological groups, such as mollusks, decapod crustaceans, cartilaginous fishes, etc. (Briggs 1995; Boschi 2000; Spalding et al. 2007; Menni et al. 2010). However, based on evidence obtained from mangrove gastropods and reef fishes, Floeter and Soares-Gomes (1999), Floeter and Gasparini (2000), and Floeter et al. (2001, 2008) concluded that this limit should be extended further south to Santa Catarina State. Similarly, the boundary between the two main districts of the Argentine Province (or Temperate Western South Atlantic Province), namely the southern Brazilian and Bonaerensean, has been variably located near the mouth of the Rio de la Plata, at 34° S or fluctuating between 30° S and 32° S, in front of the State of Rio Grande do Sul (Balech and Ehrlich 2008; Menni et al. 2010).

The aim of this study is to test the potential value of parasites as ecosystem markers (Cantatore and Timi 2014) by analyzing the metazoan assemblages of the Brazilian codling *Urophycis brasiliensis* (Kaup, 1858), a common inhabitant of the coastal waters of the southwestern Atlantic between the regions of Rio de Janeiro, Brazil and the northern Argentine Sea (Cousseau and Perrotta 2004). The parasite fauna of this species is relatively well known in the extremes of its distribution (Szidat 1961; Ivanov et al. 1997; Suriano and Labriola 1999; Martorelli et al. 2000; Alves et al. 2003, 2004; Lanfranchi et al. 2004; Menoret and Ivanov 2009); however, only Alves et al. (2004) have focused on the quantitative aspects of parasite assemblages. Conversely, information on parasites of *U. brasiliensis* in the central region of its distribution is scarce (Pereira et al. 1996). The data were analyzed following the geographical frame proposed by Spalding et al. (2007), which divides the southwestern Atlantic coasts in three biogeographical provinces (Tropical Southwestern Atlantic, Warm Temperate Southwestern Atlantic, and Magellanic), which in turn are subdivided in ecoregions (Fig. 1).

U. brasiliensis is exploited by artisanal and industrial fisheries along its broad distributional range, particularly in Uruguay (Acuña et al. 2000, 2007; Acuña and Verocai 2001); however, no specific assessment of stocks, a prerequisite for control and management plans, has been performed for this resource to date. In the zoogeographical context of parasite distribution, we also analyzed the potential use of parasites as tags for the stock discrimination of *U. brasiliensis*, focusing on a subset of long-lived parasite species to provide information that could be applied to the implementation of management strategies for *U. brasiliensis* fisheries.

Materials and methods

Fish samples and parasite inventories

Fish samples were taken from catches made by commercial trawlers operating at four locations in the southwestern Atlantic, three from Brazil and one from Argentina (Table 1). Following Spalding et al. (2007), samples were assigned to three ecoregions belonging to the Warm Temperate Southwestern Atlantic (Table 1). A total of 222 specimens of *U. brasiliensis* were collected and examined for parasites following standard procedures. 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), gas bladder, kidneys, and musculature were examined with the aid of a stereoscopic microscope.

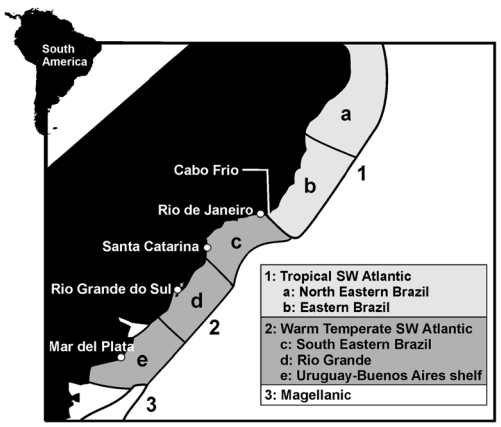

Fig. 1 Study area, sampling localities, and biogeographical provinces and ecoregions adapted from Spalding et al. (2007)

Table 1 Composition of samples of *Urophycis brasiliensis* caught in four regions of the southwestern Atlantic

State/province	Locality	Coordinates	Ecoregion[a]	Season/year	Number	Total length (cm)±SD
Rio de Janeiro (RJ)	Cabo Frio	22° 52′ S, 42° 01′ W	Southeastern Brazil	Autumn, 2012	67	26.6±2.8
Santa Catarina (SC)	Florianopolis	27° 35′ S, 48° 33′ W	Southeastern Brazil	Autumn, 2012	40	34.9±3.1
Rio Grande do Sul (RS)	Rio Grande	32° 02′ S, 52° 06′ W	Rio Grande	Summer, 2012	53	45.0±2.3
Buenos Aires (BA)	Mar del Plata	38° 00′ S, 57° 30′ W	Uruguay-Buenos Aires Shelf	Winter, 2012	62	36.6±2.5

[a] According to Spalding et al. (2007)

Fish length was compared between samples using a one-way ANOVA (Zar 1999). The prevalence and mean abundance were calculated for each parasite species in each sample following Bush et al. (1997). Unparasitized fish were excluded from subsequent analyses at the infracommunity level.

Parasites as biological indicators of ecoregions

Given the variability of the samples in relation to the season of capture and particularly fish length, we expected that a considerable proportion of the observed differences in parasite abundance would be explained by these factors. Consequently, most analyses were conducted using presence/absence data to diminish the effect of season and host length on parasite loads, allowing the location effects to be more evident. However, given that samples from Rio de Janeiro (RJ) and Santa Catarina (SC) were caught almost simultaneously, we consequently expected that their levels of similarity were not affected by the seasonality of parasite loads. 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 abundance values for all the identified parasite species, introducing host length as a covariable (ANCOVA model). The structures of parasite infracommunities between samples (1×2 factorial design, "location" as fixed factor) were compared, and the main effects were tested after 9,999 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). The Jaccard index was used as the similarity measure; this index uses binary presence/absence data (Magurran 1988) to compare species composition between samples. These procedures were repeated using the Bray-Curtis index as a similarity measure. As the Bray-Curtis coefficient is undefined when two samples contain no individuals (e.g., uninfected fish) (Clarke et al. 2006), only fish harboring at least one parasite species were included in the analyses.

Nonmetric multidimensional scaling (MDS) (Clarke and Gorley 2006) was performed to visualize the geographic patterns in the composition of parasite assemblages across the four locations. MDS was conducted using the Jaccard similarity index (presence/absence data) at the level of both the infracommunities and component communities (sensu Bush et al. 1997) in each location. In all cases, the fit of the MDS ordinations was quantified by a value of stress. To determine a possible differential effect of multivariate dispersion of infracommunities between groups, differences in dispersion were calculated using the routine PERMDISP. Dispersions were measured as distances to the centroids, and each term in the analysis was tested using 9,999 permutations (Anderson et al. 2008).

As a more quantitative approach to the analysis of parasite distribution, MDS was also conducted at the component community level using a Bray-Curtis similarity index on prevalence data. We expected a less pronounced effect of season and host length on parasite prevalence than on abundance. In addition, the Bray-Curtis index, although it includes quantitative information, emphasizes compositional dissimilarity over quantitative values in comparison with other dissimilarity and distance measures that are routinely applied in the multivariate analyses of communities (Anderson 2006; Anderson et al. 2006, 2011). A hierarchical agglomerative clustering was applied to the component communities using group-average linking, and resemblance levels were overlaid on the MDS plot (Clarke and Gorley 2006).

In the MDS performed at the component community level, a fifth sample was included (sample code RJ2004) for comparative purposes. The data were obtained from a previous study by Alves et al. (2004) on 75 specimens of *U. brasiliensis* caught in a broad area of the region of the State of Rio de Janeiro, Brazil (21–23° S, 41–45° W) during a period of 1 year. The re-examination of preserved materials in the authors' collection confirmed that the materials identified as *Raphidascaris* sp. by Alves et al. (2004) were actually members of *Hysterothylacium* sp. These authors also discriminated two species of larval *Nybelinia* sp.; as it was not possible to identify the plerocercoids found in the present study as belonging to one of these species, this group of parasites was excluded from the analyses.

Parasites as biological tags for stock discrimination

The potential use of parasites as biological tags for the discrimination of host stocks between sampling regions was evaluated on values of parasite abundance. 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 abundance (Lester 1990; Lester and MacKenzie 2009). Following these guidelines, only long-lived parasites were retained for the analyses. These parasites were the metacestodes *Grillotia carvajalregorum*, *Callitetrarhynchus gracilis*, and Tentacullariidae gen. sp.; the larval anisakids *Contracaecum* sp., *Hysterothylacium* sp., and *Terranova* sp.; and the juvenile acanthocephalans *Corynosoma australe*, *Corynosoma cetaceum*, and *Bolbosoma turbinella*. However, long-lived parasites tend to accumulate as hosts grow, thus producing ontogenetic changes in the structure of parasite communities; fish length should be taken into account as a potential confounding variable in the interpretation of spatial patterns and stock structure (Cantatore and Timi 2014). Therefore, for this set of analyses, fish length was introduced as a covariable to mitigate its effect on parasite abundance and achieve consistent conclusions. The selection of fish parasitized only by long-lived parasites reduced the sample size from the Brazilian locations, particularly from RJ and SC; consequently, these samples were combined as SB (Southeastern Brazil, following Spalding et al. 2007), given that no differences between the regions were observed in previous analyses.

PERMANOVA analyses (1×3 factorial design, "location" as fixed factor) were performed as in the previous section. When differences were detected by PERMANOVA, pairwise 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 9,999 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 overparameterization 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 9,999 permutations. Multivariate analyses were implemented in PERMANOVA+ for PRIMER package (Anderson et al. 2008).

Results

General results

Mean host body lengths were significantly different between samples ($F_{3,\ 218}$=471.66; P<0.01), with all pairs of samples differing significantly in fish length (all P<0.01). A high proportion of hosts were parasitized in all samples (Table 2), although eight individuals of *U. brasiliensis* were uninfected, five in the sample from RJ, one in SC, and two in Rio Grande do Sul (RS); the remaining individuals were infected by at least 1 of 33 parasite species (Table 3). A total of 5,001 metazoan parasites were recorded in the entire sample, with an uneven distribution across locations based on both abundance and composition (Table 3). The highest parasite loads and total species richness were observed in Buenos Aires (BA), and the species richness values were the same as those for RS; BA also displayed the richest infracommunities (Table 2).

Only two species, namely *Hysterothylacium* sp. and the plerocercoids of Tentacullariidae, both in the larval stage, were present in all locations; the highest number of shared species

Table 2 Descriptors of parasite assemblages of *Urophycis brasiliensis* caught in the four regions of the southwestern Atlantic

Locality	Total prevalence	Total mean abundance	Total species richness	Mean infracommunity species richness	Number of "exclusive" species	Multivariate dispersion[a]
Cabo Frio	92.5	7.6	15	2.6	2	57.55
Florianopolis	97.5	8.3	12	2.6	1	53.52
Rio Grande	96.2	19.3	19	3.2	2	59.53
Mar del Plata	100	50.7	19	8.3	7	41.87

[a] Measured as the averaged distance from an individual unit to the group centroid, defined in the principal coordinate space of the Jaccard dissimilarity

Table 3 Parasites of *Urophycis brasiliensis* in four localities of the southwestern Atlantic, with data on its development stage, prevalence (*P*) and mean abundance (MA), and new host records denoted with asterisks

Parasite species	Stage	Rio de Janeiro		Santa Catarina		Rio Grande do Sul		Mar del Plata	
		P	MA±SD	*P*	MA±SD	*P*	MA±SD	*P*	MA±SD
Monogenea									
Diclidophoroides maccallumi	Adult	0.0	–	0.0	–	11.5	0.2±0.6	24.2	0.4±0.8
Pseudempleurosoma sp.	Adult	13.4	0.3±0.8	10.0	0.2±0.7	25.0	1.5±3.2	0.0	–
Digenea									
Prosorhynchus australis	Adult	0.0	–	0.0	–	11.5	0.3±0.9	48.4	2.9±6.1
Bucephalus urophyci	Adult	0.0	–	0.0	–	0.0	–	19.4	0.4±1.3
*Ellytrophalloides oatesi**	Adult	0.0	–	0.0	–	0.0	–	1.6	0.02±0.1
*Lecithocladium cristatum**	Adult	0.0	–	0.0	–	13.5	0.2±0.7	12.9	0.2±0.7
*Lecithochirium microstomum**	Adult	6.0	0.1±0.5	10.0	0.2±0.5	19.2	0.6±1.5	0.0	–
Derogenes varicus	Adult	0.0	–	0.0	–	11.5	0.2±0.6	50.0	1.2±1.8
Parahemiurus merus	Adult	9.0	0.3±1.7	0.0	–	0.0	–	0.0	–
Ectenurus virgula	Adult	3.0	0.1±0.4	0.0	–	0.0	–	0.0	–
Aponurus laguncula	Adult	3.8	1.7±4.9	0.0	–	11.5	0.4±1.2	0.0	–
Pseudolepidapedon brasiliensis	Adult	26.9	0.7±1.5	12.5	0.3±1.2	0.0	–	0.0	–
Stephanostomum sp.	Adult	7.5	0.1±0.4	0.0	–	15.4	0.3±0.8	74.2	6.8±11.5
*Cainocreadium oscitans**	Adult	7.5	0.2±1.0	2.5	0.1±0.3	0.0	–	0.0	–
Cestoda									
Grillotia carvajalregorum	Plerocercus	0.0	–	0.0	–	0.0	–	85.5	14.5±15.7
*Callitetrarhynchus gracilis**	Plerocercus	0.0	–	0.0	–	0.0	–	14.5	0.4±1.4
Tentaculariidae gen. sp.	Plerocercoid	25.4	0.5±1.1	15.0	0.2±0.5	13.5	0.2±0.5	46.8	1.3±2.2
Scolex sp. 1	Plerocercoid	0.0	–	0.0	–	9.6	0.2±0.7	25.8	0.4±0.8
Scolex sp. 2	Plerocercoid	0.0	–	0.0	–	11.5	0.2±0.8	51.6	2.8±9.5
Nematoda									
Cucullanus bonaerensis	Adult	0.0	–	0.0	–	0.0	–	85.5	4.6±3.5
Cucullanus cirratus	Adult	55.2	1.8±3.3	67.5	4.1±5.4	65.4	9.0±13.0	0.0	–
Ascarophis marina	Adult	0.0	–	0.0	–	0.0	–	67.7	4.3±6.3
*Hysterothylacium aduncum**	Adult	0.0	–	0.0	–	0.0	–	22.6	0.3±0.7
Hysterothylacium sp.	Larva III	6.0	0.1±0.2	12.5	0.2±0.4	30.8	4.6±14.0	33.9	0.4±0.7
Contracaecum sp.	Larva III	3.0	0.05±0.3	0.0	–	15.4	0.2±0.6	37.1	1.5±5.3
Terranova sp.*	Larva III	0.0	–	0.0	–	5.8	0.1±0.2	0.0	–
Procamallanus halitrophus	Adult	28.4	1.0±2.9	52.5	1.9±3.3	25.0	0.7±1.6	0.0	–
*Capillaria gracilis**	Adult	0.0	–	20.0	0.3±0.4	0.0	–	0.0	–
Acanthocephala									
Corynosoma australe	Juvenile	0.0	–	10.0	0.2±0.6	15.4	0.3±1.0	83.9	6.6±6.2
*Corynosoma cetaceum**	Juvenile	0.0	–	0.0	–	13.5	0.2±0.6	43.6	1.6±2.5
*Bolbosoma turbinella**	Juvenile	28.4	0.5±1.0	35.0	0.7±1.2	0.0	–	0.0	–
Copepoda									
Acanthochondria triangularis	Adult	3.0	0.04±0.3	7.5	0.1±0.4	0.0	–	0.0	–
Isopoda									
Gnathia sp.*	Praniza	0.0	–	0.0	–	3.4	0.04±0.2	0.0	–

was observed for BA and RS with 12 parasites, although RJ also shared a high proportion of species with the other two Brazilian locations (ten and nine with SC and RS, respectively). Many long-lived species (those not affected by short-term or seasonal variations) occurred with a high prevalence in several locations while being absent in others (i.e., *G. carvajalregorum* in BA and *B. turbinella* in RJ and SC; both were absent in RS).

Table 4 One-factor PERMANOVA results of infracommunity data of parasites of *Urophycis brasiliensis* in two samples from the ecoregion Southeastern Brazil, based on Jaccard and Bray-Curtis dissimilarity measures with host length as covariable. *P* values obtained after 9,999 permutations

Dissimilarity measure	Source	*d.f.*	SS	MS	Pseudo *F*	*P* (perm)
Jaccard	Host length	1	16,133	16,133	4.8947	<0.001
	Locality	1	5,815.5	5,815.5	1.7643	>0.05
	Host length×locality	1	3,877.3	3,877.3	1.1763	>0.05
	Residual	97	3.1972 e^5	3,296.1		
	Total	100	3.4555 e^5			
Bray-Curtis	Host length	1	22,617	22,617	6.6655	<0.0001
	Locality	1	4,627	4,627	1.3636	>0.05
	Host length×locality	1	3,641.6	3,641.6	1.0732	>0.05
	Residual	97	3.2914 e^5	3,393.2		
	Total	100	3.6002 e^5			

Parasites as biological indicators of ecoregions

The results of PERMANOVA analyses on both presence/absence and abundance data (Table 4) showed a strong effect of host length on the response variables, although no interaction was observed between the host length and location. After taking into account the variations among samples due to host length, no significant variability was detected among the parasite assemblages.

The multidimensional scaling of infracommunities based on the presence/absence data was calculated after omitting three fish considered as outliers (two from RJ, harboring only *Cainocreadium oscitans*, and one from SC, with monospecific infection by *Acanthochondria triangularis*). The MDS revealed an apparent pattern of separation between several of the locations (Fig. 2), and the stress level (0.14) indicated that community structure was substantially different from random. The fish from BA were clearly separated from the rest and showed a more homogeneous distribution in the bidimensional space. The fish from RS occupied an intermediate (central) position in the biplot, whereas those from SC and RJ were distributed toward the right side and were indistinguishable from each other. All the samples from the Brazilian locations showed a broad dispersion, reflecting a higher heterogeneity in the composition of samples.

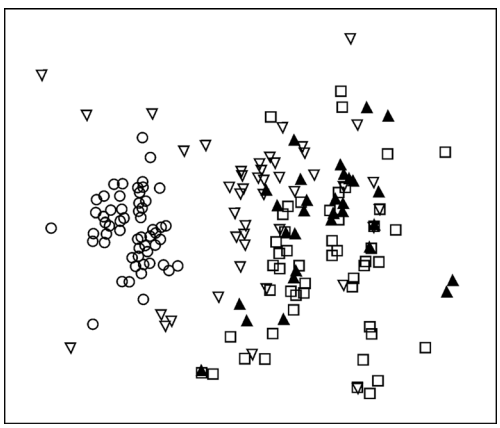

Fig. 2 Nonmetric two-dimensional ordination plot using Jaccard similarity-based presence/absence data of metazoan parasite species across 211 individual *Urophycis brasiliensis* from four samples from the southwest Atlantic: Cabo Frio, Rio de Janeiro, Brazil (*white squares*); Florianopolis, Santa Catarina, Brazil (*black triangles*); Rio Grande, Rio Grande do Sul, Brazil (*white inverted triangles*); and Mar del Plata, Buenos Aires, Argentina (*circles*)

The overall differences in multivariate dispersions (Table 3) of parasite infracommunities in terms of their deviations from centroids were significant ($F_{3,\ 210}$=36.97, *P* (perm)<0.01). The pairwise comparisons showed that the sample from BA was significantly less dispersed than its Brazilian counterparts (all *P* (perm)<0.01), which in turn did not differ from each other (all *P* (perm)>0.01).

The MDS and cluster analyses based on the Jaccard index as a resemblance measure and applied to the component communities revealed an apparent pattern of separation between samples following a latitudinal pattern (Fig. 3a), and the stress level (0.0) indicated a community composition substantially different from random. The fish from RJ were clearly associated with those from SC and showed the highest values of similarity; both samples clustered together at lower similarity values with those from RJ2004. However, a high level of compositional similarity was evident for samples from RS and BA. Similarly, in the MDS on prevalence (Fig. 3b) with the same stress level (0.0), the samples from RJ and SC were the most closely related, with the difference that fish RS were more similar to RJ and SC than to BA. RJ2004 again showed a low level of similarity to the other Brazilian samples.

Parasites as biological tags for stock discrimination

After selecting hosts with one or more long-lived parasite species, 64 unparasitized fish were excluded from the analyses, including 27 from RJ, 17 from SC, and 20 from RS. The results of PERMANOVA analyses on both presence/absence

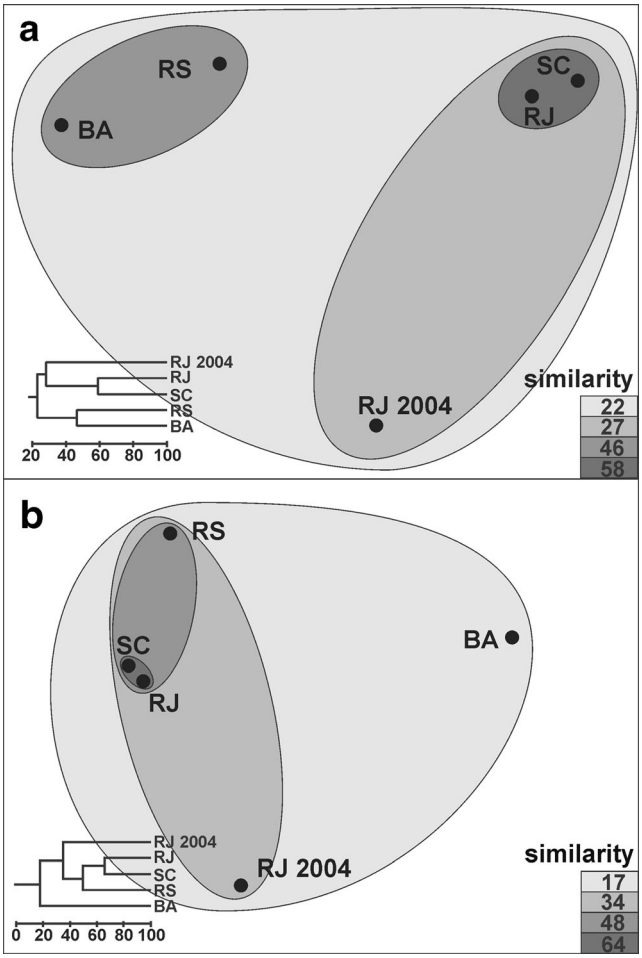

Fig. 3 Nonmetric two-dimensional ordination plot and cluster analyses of parasite component communities of *Urophycis brasiliensis* from four regions in the southwest Atlantic. **a** Using Jaccard (presence/absence) similarity and **b** using Bray-Curtis similarity based on untransformed parasite prevalence. Results of a hierarchical agglomerative clustering are shown as dendrograms and overlaid on the MDS plot with similarity levels represented by a gray scale. *BA* Buenos Aires, *RJ* Rio de Janeiro, *RS* Rio Grande, *SC* Santa Catarina

and abundance data (Table 5) 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 (perm)<0.01).

A proportion of these differences can be attributed to differences in the multivariate dispersions of parasite infracommunities in terms of their deviations from centroids because the PERMDISP results were significant for both the Jaccard and Bray-Curtis indices ($F_{2,\ 147}$=23.05 and $F_{2,\ 147}$= 28.85; both P (perm)<0.01). Indeed, the pairwise test showed significant differences in the multivariate dispersions between all pairs of samples for the Bray-Curtis index (all P (perm)< 0.01) but only for those involving fish from BA for the Jaccard index (both P (perm)<0.01), with fish from both northern locations displaying similar values of average deviations from centroids (P (perm)>0.05).

The CAP analysis based on binary data showed significant differences among the samples (tr=1.28; P=0.0001). The selected orthonormal PCO axes (m=6) described 97.46 % of the variation in the data cloud, with a high percentage of correct allocations (88 %). The two first canonical axes resulting from the CAP analysis clearly separated samples from BA, but those from Brazilian locations were not clearly distinguishable (Fig. 4a). However, 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 (δ_1=0.92 and δ_2=0.66). By superimposing vectors corresponding to the Spearman correlations of individual species with the CAP axes (restricted to

Table 5 One-factor PERMANOVA results of infracommunity data of long-lived parasites of *Urophycis brasiliensis* in three samples based on Jaccard and Bray-Curtis dissimilarity measures with host length as covariable. P values obtained after 9,999 permutations

Dissimilarity measure	Source	*d.f.*	SS	MS	Pseudo F	P (perm)
Jaccard	Host length	1	45,805	45,805	18.361	<0.0001
	Locality	2	1.1884 e^5	59,422	23.820	<0.0001
	Host length×locality	2	36,799	1,839.9	0.7375	>0.05
	Residual	144	3.5223 e^5	2,494.7		
	Total	149	5.2756 e^5			
Bray-Curtis	Host length	1	39,266	39,266	14.439	<0.0001
	Locality	2	1.3639 e^5	68,193	25.077	<0.0001
	Host length×locality	2	4,672.6	2,336.3	0.8591	>0.05
	Residual	144	3.9159 e^5	2,719.4		
	Total	149	5.7192 e^5			

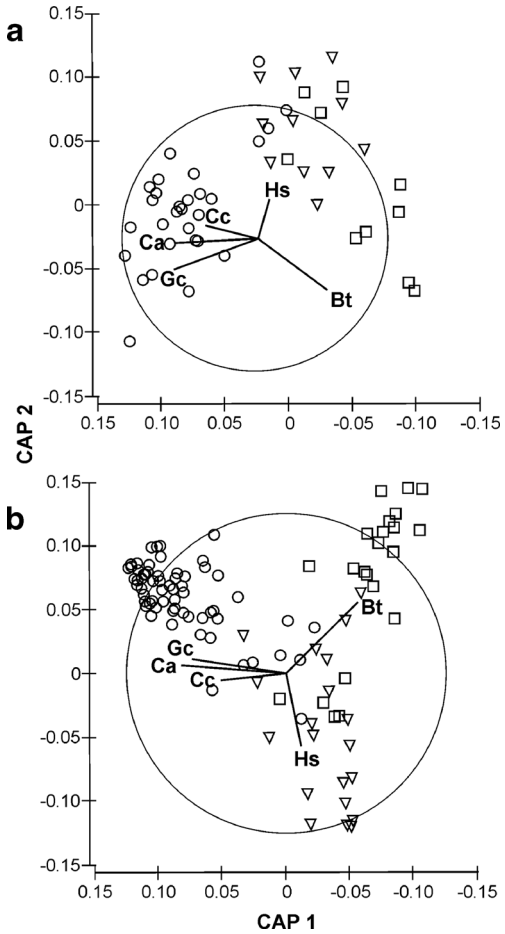

Fig. 4 Canonical analysis of principal coordinates (CAP) biplot based on **a** presence/absence data and Jaccard dissimilarities and **b** parasite untransformed abundance and Bray-Curtis dissimilarities of parasites in samples of *Urophycis brasiliensis* from three ecoregions in the southwest Atlantic according to Spalding et al. (2007): Southeastern Atlantic=Rio de Janeiro+Santa Catarina, Brazil (*squares*); Rio Grande, Rio Grande do Sul, Brazil (*triangles*); and Mar del Plata Buenos Aires, 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*; *Cc*, *Corynosoma cetaceum*; *Gc*, *Grillotia carvajalregorum*; *Hs*, *Hysterothylacium* sp.

those species with lengths >0.30), we observed that *C. australe*, *C. cetaceum*, and *G. carvajalregorum* were indicators of samples from BA, whereas the abundances of *Hysterothylacium* sp. and *B. turbinella* were mainly associated with fish from RS and SB, respectively. Similar results were obtained after CAP analysis based on the abundance data, which also showed significant differences among the samples (tr=1.42; P=0.0001). The selected orthonormal PCO axes (m=10) described 97.28 % of the variation in the data cloud, with the same percentage of correct allocations (88 %). The two first canonical axes resulting from CAP analysis clearly separated the three samples (Fig. 4b) with high values of canonical correlations (δ_1=0.93 and δ_2=0.75). 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).

Table 6 Discriminant analysis classification showing the numbers and percentages of *Urophycis brasiliensis* classified in each locality (number of correctly classified fish in each sample in bold) using Jaccard and Bray-Curtis dissimilarity measures (rows correspond to group memberships)

	Jaccard				Bray-Curtis			
	SB	RS	MP	%	SB	RS	MP	%
Southeastern Brazil	**50**	7	0	87.7	**50**	7	0	87.7
Rio Grande	2	**29**	0	93.5	3	**26**	2	83.9
Mar del Plata	2	7	**53**	87.7	2	4	**56**	90.3

SB Southeastern Brazil (Cabo Frio + Florianopolis), *RS* Rio Grande, *MP* Mar del Plata, *%* percentage of correctly classified fish per locality

The cross-validation of the results showed that in both sets of analyses, high percentages of correctly allocated fish occurred in all locations (Table 6). The same percentage of correctly classified hosts was observed for SB with both indices; however, fishes from RS showed higher percentages when discriminant analysis was based on assemblage composition. By contrast, a high proportion of fish from BA was allocated to this location when the analysis was based on abundance.

Discussion

The parasite fauna of specimens of *U. brasiliensis* examined in the present study was represented by 33 metazoan species; 11 of these species were new host records, which when added to previous records, resulted in a richness of 40 species of parasites in the entire distributional range of *U. brasiliensis*. This value may be even larger given that some parasites were identified at the species complex level, i.e., *Scolex* sp. or *Contracaecum* sp. (Chambers et al. 2000; Mattiucci and Nascetti 2008). It is notable that most of the previously cited species that were not found in the present study were recorded in the region of Rio de Janeiro (Alves et al. 2004), whereas almost all the species known in *U. brasiliensis* from Argentine waters were recorded. This could indicate that the predictability of the composition of parasite assemblages is greater at higher latitudes, which is in agreement with the observed results when comparing multivariate dispersions across samples.

The parasites varied in terms of presence, prevalence, and abundance across locations, with a very low proportion (two species) shared by all the samples. The number of shared species was extended to five when considering the three ecoregions, with all the species displaying a clearly higher

prevalence in BA, particularly when compared with the northernmost locations. The remaining parasites were unevenly distributed among the samples, with seven species found only in BA. Notwithstanding, these patterns are not determined by the distribution of parasites in all cases. In fact, some species found only in the Argentine samples, such as *Diclidophoroides maccallumi* and *Ascarophis marina*, were previously recorded in *U. brasiliensis* from RJ (Alves et al. 2004). By contrast, *Ectenurus virgula*, which was not found in BA in the present study, was reported by Szidat (1961) in *U. brasiliensis* from this region. These species are short-lived parasites, and their occurrence could depend on seasonal variations that are potentially associated with seasonal changes in diet (Acuña et al. 2007) or reproductive migrations (Acuña et al. 2000). Seasonality or short-term temporal variability can also explain the absence of *Lecithocladium cristatum* in Brazilian samples and *Lecithochirium microstomum*, *Parahemiurus merus*, and *Aponurus laguncula* in BA, given that these digeneans have been recorded in other host species from their respective regions, although not in *U. brasiliensis* (Timi et al. 1999; Braicovich et al. 2009). Similar results have been observed for long-lived parasites, which are not affected by seasonality, such as *G. carvajalregorum* and *Callitetrarhynchus gracilis* in Brazilian waters in hosts other than *U. brasiliensis* (Menoret and Ivanov 2009; Braicovich et al. 2012; Fonseca et al. 2012) and *B. turbinella* in other fish species from BA (Braicovich and Timi 2010; Braicovich et al. 2012). Because all these species are transmitted trophically, the absence of such parasites in *U. brasiliensis* from regions where they have previously been reported could be explained by the differential composition of host diet, prey availability, or prey selectivity across host distribution. Although *U. brasiliensis* feeds at similar trophic levels along the southwestern Atlantic coasts, taxonomical differences in prey composition (crustaceans, fishes, and mollusks) have been observed among regions in Brazil, Uruguay, and Argentina (Acuña et al. 2007). However, although these species appear to be distributed throughout the biogeographical province, they display variability in terms of abundance and prevalence across regions (see below). In addition, a possible effect of differences in host length on diet composition across samples cannot be disregarded because this host undergoes ontogenetic changes in its feeding ecology and switches from invertebrates to primarily fish as it grows (Mora and Pintos 1980; Goldstein 1986; Acuña et al. 2007).

The differential occurrence of other groups of parasites likely appears to be a consequence of their own distribution patterns, either directly due to the effect of environmental conditions or indirectly due to the distribution of their intermediate hosts. The cucullanids found in *U. brasiliensis* represent a clear example, as *Cucullanus cirratus* was only found in the Brazilian samples and was replaced by *Cucullanus bonaerensis* in BA. *C. cirratus* is a typical parasite of gadiform fishes, including *Urophycis blennoides* (Brünnich), and it has only been reported in European waters (Berland 1970; Moravec 1994), whereas its previous identification in the sciaenid *Micropogonias undulatus* (Linnaeus, 1766) from Brazil by Vicente and Santos (1973) was considered erroneous (Lanfranchi et al. 2004). However, the presence of *C. bonaerensis* in *U. brasiliensis* from BA constituted an accidental infection in an unsuitable host (Lanfranchi et al. 2004), with its definitive host later identified as the flounder *Xystreuris rasile* (Jordan, 1891) (Alarcos and Timi 2012).

Similarly *C. cetaceum*, *Bucephalus margaritae*, *Prosorhynchus australis*, *Ellytrophalloides oatesi*, *Derogenes varicus*, and *Hysterothylacium aduncum* were only found in BA and have never been cited in Brazilian hosts (Kohn et al. 2007; Santos et al. 2008; Luque et al. 2011). These results are expected for the latter three species, which are characteristic of the cold waters of the Magellanic zoogeographical province (Cantatore and Timi 2014). Conversely, several other species have never been recorded in Argentina, namely *Procamallanus halitrophus*, *C. oscitans*, *Pseudolepidapedon brasiliensis*, and the host-specific *Capillaria gracilis*, *Pseudempleurosoma* sp., and *A. triangularis*.

Independent of the different processes that yield the geographic distribution of individual parasite species, the combination of such patterns in multivariate analyses resulted in clear similarity patterns. In fact, no differences were observed between both locations in the ecoregion of southeastern Brazil (RJ and SC), whereas the evident separation of samples was observed in the MDS plots across ecoregions, particularly for the component communities given the higher values of multivariate dispersions of infracommunities in all the Brazilian samples. These results are in agreement with a previous study aimed to discriminate stocks of the whitemouth croaker *M. furnieri* (Luque et al. 2010); the authors considered the samples of croaker from SC and RJ as a single population but different from those of RS. In the study by Luque et al. (2010), two additional samples from northern latitudes (states of Ceará and Bahia) were identified as representatives of a single different population, which was not surprising as these Brazilian coasts belong to a different biogeographical province that is separated from the Warm Temperate Southwestern Atlantic by the Cabo Frio region (Briggs 1995; Boschi 2000; Spalding et al. 2007; Menni et al. 2010). The observed separation of the sample RJ2004 is explained by the existence of this boundary between provinces. In fact, Alves et al. (2004) analyzed samples gathered over a year in a region comprising the northern and southern coastal zones of this limit (21–23° S, 41–45° W). The resulting sample was composed of a mixture of hosts from both biogeographical provinces. Despite these results, RJ2004 was more similar in species composition to the northern samples than to RS and BA.

Different results were found in the MDS analysis based on parasite prevalence, where fish from RS were more closely

related to other Brazilian samples than to BA. This result was found using a more quantitative analysis and reflects the transitional condition of the RS region, which has been considered as the boundary between the two main districts of the Temperate Western South Atlantic Province (Balech and Ehrlich 2008). In fact, the compositional similarity of RS in relation to BA is because most (12 out of 19) of its species are shared with BA; however, when prevalence is taken into account, the samples from BA exhibited a high number of exclusive species with an extremely high prevalence, as well as a higher prevalence for most species shared with RS. By contrast, the most prevalent species in RS, namely *C. cirratus*, was found at similar levels in the other Brazilian samples. Because the prevalence data were not transformed, the results of the MDS analysis were heavily influenced by the most prevalent species.

Similar to the prevalence, the species richness of the component communities was higher in the southern samples. We observed clearer latitudinal patterns for the mean total abundance and infracommunity species richness, with both increasing southward. However, these patterns must be reviewed cautiously due to the low number of locations sampled along the latitudinal gradient and because the latitudinal gradient in diversity can be disrupted by variations in species richness due to other positional (i.e., longitude, depth) and environmental variables, particularly in shallow water systems (Gaston 2000). In fact research on the latitudinal patterns in marine parasites has yielded opposite conclusions depending on the taxonomic groups of both the hosts and parasites, as well as on the spatial scale investigated (Rohde and Heap 1998; Rohde 1999; Studer et al. 2013; Kamiya et al. 2014).

However, at the individual species level, previous studies in the southwest Atlantic have demonstrated that several parasite species display marked latitudinal gradients of prevalence or abundance, including several of the parasites found in *U. brasiliensis*, with these clines being evident when analyzed across biogeographical provinces (Timi 2003, 2007; Cantatore and Timi 2014). These parasites have a low specificity and infect all available hosts; therefore, their distribution can be traced beyond the boundaries of the endemic areas of fish hosts. For example, *G. carvajalregorum* and *C. australe* decreased in prevalence and abundance from northern Argentine waters toward the southern waters of the Patagonian Shelf (Cantatore and Timi 2014); however, their occurrence also decreased toward lower latitudes in *U. brasiliensis* and the other host species, such as *Cynoscion guatucupa*, *Pinguipes brasilianus*, *M. furnieri*, and *Trachurus lathami* (Timi et al. 2005, 2010; Luque et al. 2010; Braicovich et al. 2012), indicating that their endemic region is the northern Argentine and the Uruguayan seas. By contrast, species such as *B. turbinella*, which is present in several of these host species, displays higher loads in the northern regions, whereas *H. aduncum*, for example, showed the opposite pattern.

The variation across regions in the occurrence of the parasites with restricted distributions, driven by singular suites of oceanographic and biological features in each zone, resulted in similarity patterns that are supported by existing hypotheses about the distribution of ecoregions in the southwestern Atlantic (Spalding et al. 2007). Indeed, excluding the sample from 2004, the results of clustering the ecoregions were the same as those obtained in a biogeographical study on mollusk species with the same ecoregional perspective (Fortes and Absalão 2011), indicating the potential of parasites as suitable tools for research on marine biogeography.

Parasites are ubiquitous components of fish communities, and their assemblages in fish hosts are composed by several phyla that generally include platyhelminthes (monogeneans, trematodes, cestodes), nematodes, acanthocephalans, and crustaceans, among the metazoans. We propose here that the potential usefulness of parasite assemblages as ecosystem indicators (Cantatore and Timi 2014) is derived from the combined evidence of the evolutionary history and biogeography of multiple lineages, which is expected to be more efficient in capturing the recurrent patterns in overall biodiversity rather than individual lineages. Furthermore, as many parasites have complex life cycles including both invertebrate and vertebrate hosts, their distribution patterns are not only dependent on environmental conditions but also on the distribution and population density of all hosts involved in their life cycles (Timi 2007), adding consequently other sources of distributional variability that act synergistically to define robust geographical patterns. Similarly, the ecoregional classification of coastal systems of Spalding et al. (2007), which is mirrored by parasites of *U. brasiliensis* in the southwestern Atlantic, is based on composite studies that combined multiple divergent taxa and oceanographic drivers in the derivation of its boundaries.

The usefulness of parasites as ecoregional markers is enhanced by clines in the prevalence of broadly distributed species when this descriptor is included in the analyses. Indeed, characteristics of the regional parasite faunas are demonstrated in these more quantitative analyses, such as the dominance of a suite of long-lived larval parasites in BA, a feature common to all fish species so far investigated in the region (Cantatore and Timi 2014). Due to their persistence in hosts, these species are more preferable than transient species as biological tags for stock discrimination (Lester and MacKenzie 2009) and, consequently, were selected for the analyses in this study. Indeed, analyses restricted to long-lived parasites that incorporated abundance data provided evidence supporting the existence of three different stocks in the four sampled areas. Several of the parasite species that contributed the most to the separation of the samples agreed with those identified as markers of the northern Argentine Sea (Cantatore and Timi 2014), namely *G. carvajalregorum*, *C. australe*, and *C. cetaceum*, whereas *Hysterothylacium* sp. and *B. turbinella*

were associated with hosts from RS and SB, respectively. Due to their low specificity, these species constitute promissory biological tags for stock discrimination of other fish species along the Brazilian coast.

The identification of three stocks in the regions is a not surprising result, given the output of previous analyses and the singularity of the biogeographic forcing agents that define each ecoregion. However, more extensive studies in both time and space are desirable to accurately assess the stock composition of *U. brasiliensis* along its geographical range, particularly at lower geographical scales because variations in reproductive parameters have been observed in nearby locations of Uruguay (Acuña et al. 2000). The usefulness of parasites at these smaller scales needs to be tested, as stock assessment is the first step in the development of sustainable fisheries.

In addition, parasite tags are promissory for studies on population structure at higher spatial scales including samples from other biogeographical provinces, such as the Brazilian Province and north to Cabo Frio, where parasite communities apparently have a different structure (Alves et al. 2004). Samples should also be included from southern regions such as the Magellanic Province, where *U. brasiliensis* has been recently recorded together with a group of other species typical of more temperate northern waters (Bovcon et al. 2011). In these large-scale studies, parasites that are broadly distributed and exhibit low specificity, with geographical ranges beyond those of the individual host species and marked latitudinal gradients of abundance, will likely be helpful to identify the geographical patterns of host population structure.

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