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Parasites communities in the clingfish *Gobiesox marmoratus* from central Chile

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Abstract

This study investigated the factors (i.e., season, locality, sampling year, total length and maturity stage of the hosts) that might influence the structure of parasite populations and communities in the clingfish *Gobiesox marmoratus*. The parasite community was described and analyzed using numerical descriptors, such as prevalence, intensity and species richness, between factors previously mentioned. A total of 260 clingfish were collected from 2 localities of central Chile, four seasons and during 3 year cycles (from July 2006 to July 2009). In the whole clingfish sample, 668 parasites were found, which belonged to 14 parasite taxa; 9 of them were new records in *G. marmoratus*. Parasite infracommunity richness ranged 0–3 species, although 1 trematode species, *Helicometrina nimia*, represented 80% of all parasites collected and was the most abundant and prevalent parasite species. The average of parasite abundance and intensity (± SD) was 2.5 ± 8.2 and 7.5 ± 12.7, respectively. Generalized linear model showed that parasite communities were influenced by season, locality, sampling year, and maturity stage when considering the abundance and intensity of parasites. For the parasite richness, only the locality and maturity of fish was determinant for explaining the differences. The populations and communities of the parasite variations were variable due to differences in fish body length because prevalence, abundance and intensity of parasites significantly correlated with the fish body length. Concordantly, maturity fish were longer than immature fish. Thus, clingfish from El Tabo were longest and mature, which harbored higher parasite richness than those fish from Las Cruces.

Keywords

Parasite communities, parasite populations, intertidal zone, Gobiesocidae, clingfish, Chile

Introduction

Clingfish, which belong to the Gobiesocidae family, are characterized for having a sucking ventral disc that is used to attach to rocks. This group of fish is composed of approximately 120 known species which lives in different habitats, showing worldwide distribution (McEachran 2003). Little is known about the biology of these fish, especially with respect to their parasites.

Several parasitological studies have focused on certain parasite species (Montú 1980; Oliva and Zegers 1988; Dojiri 1989; Korniychuk and Gaevskaya 1999; Belofastova and Korniychuk 2000; Muñoz and George-Nascimento 2002; Salgado-Maldonado 2006), and a few reports have addressed the parasite communities in clingfish (*Sicyases sanguineus* Müller et Troschel, 1843; *Gobiesox mamoratus* (Jenyns 1842) from South America only (Pardo-Gandarillas *et al*. 2004; Iannacone and Alvariño 2011; Muñoz and Zamora 2011).

Four clingfish species have been recorded in Chile: *Sicyases hildebranchi* Schultz, 1944 (= *Sicyases brevirostris* (Guichenot, 1848), which has been found only in an insular system (Juan Fernández Archipelago), and *S. sanguineus*, *G. marmoratus*, and *Tomicodon chilensis* Brisout de Barneville, 1846, which are distributed widely along the continental coast of Chile (Pequeño 1989). Although clingfish have been frequently recorded in the intertidal coastal zone of Chile, they are not very abundant because they represent between 3% and 14% of the total abundance of the fish assemblage of central Chile (Muñoz and Ojeda 1997, 1998; Quijada and Cáceres 2000; Berrios and Vargas 2004; Muñoz and Cortés 2009). Consequently, biological studies have been based on small sample sizes, which may affect the statistical significance of the analyzed data.

In Chile, the trophic habits of 3 clingfish have been reported (Cancino and Castilla 1988; Muñoz and Ojeda 1997, 1998; Quijada and Cáceres 2000; Berrios and Vargas 2004;

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Pardo-Gandarillas *et al.* 2004; Muñoz and Zamora 2011), and a few of these studies have included parasite community analyses (Pardo-Gandarillas *et al.* 2004; Muñoz and Zamora 2011). In particular, *G. marmoratus* is a small carnivorous fish that reaches up to 15 cm in length and harbors at least 10 eumetazoan parasites and 5 myxosporideans (Pardo-Gandarillas *et al.* 2004). Moreover the parasite communities of this clingfish do not significantly vary with the body size of the fish or its locality along the Chilean coast (Pardo-Gandarillas *et al.* 2004), although for other sympatric intertidal fish species, some studies have shown that the parasite community changes according to season (e.g., Muñoz and Delorme 2011) and the locality (e.g., Flores and George-Nascimento 2009, Díaz and Muñoz 2010), both of which may sometimes be linked to body length. Therefore, the aim of this study was to determine whether parasite communities vary in the clingfish species *G. marmoratus*, from central Chile, depending on the season, locality, sampling year, and total length of the hosts.

Materials and Methods

Two hundred and sixty *G. marmoratus* specimens were collected in the rocky intertidal pools formed during low tide on the central coast of Chile (33°S, 71°W), including Las Cruces (33°13´S) and El Tabo (33°27´S), which are ~3 km apart. The fish were seasonally sampled between August 2006 and July 2009, over 3 years, which were classified as follows: year 1 (August 2006 – July 2007), year 2 (August 2007 – July 2008) and year 3 (August 2008 – July 2009). The fish were captured by hand, and sometimes, an anesthetic solution was used (BZ-20®). Most of the fish (~80%) were frozen until dissection; the other were dissected when fresh. Sample sizes of the clingfish collected in each locality, season, year and maturity stage are shown in Table I.

The fish specimens were identified as previously described by Chirichigno (1974) and then necropsied to collect the parasites. Before the parasitological analysis, the total fish lengths (TL, in cm) were measured, and the sexes were determined by external observation of the gonads, according to the maturity stage of the specimen. Basically, mature females have long, pink-orange ovaries when mature, while mature males have compact, long, white testicles. Those specimens with small and translucent gonads were classified as immature. The fish were externally and internally examined for ecto- and endoparasites, respectively. The parasites collected were fixed in 5% formalin. For taxonomical identification, the platyhelminthes were stained with hematoxylin, while the crustaceans and nematodes were cleared with lactophenol.

Table I. Sample size of clingfish *Gobiesox marmoratus* collected in each year, season, locality and maturity stage

Factors	Groups	n
Year	Year 1	82
	Year 2	113
	Year 3	65
Season	Summer	71
	Autumn	53
	Winter	75
	Spring	61
Locality	El Tabo	188
	Las Cruces	72
Maturity	Mature	53
	Immature	207

The prevalence and average of intensity and abundance of parasites was determined to each parasite species in the whole sample (n = 260). The abundance, intensity and richness were determined in each individual host (Bush *et al.* 1997). Generalized Linear Models, GLM (using Poisson distribution, log-link, and likelihood Type I error) were applied to infracommunity raw data (abundance, intensity and richness of parasites) in order to know the influence of 4 variables: sampling year, season, locality and maturity stage of the fish. Also, correspondence analyses were done between prevalence, abundance and intensity of parasites species and host groups, according to the 4 variables, to know the parasites which were more associated to some host groups. For this latter analysis, only the parasite species shared among the fish groups were considered.

The fish TL was transformed to $\log_{10}$ to achieve normality and homogeneity of the variances using the Kolmogorov-Smirnov and Levine's tests, respectively, in order to apply parametric statistic (Zar 1996). Then, the fish TL were compared between seasons, localities, sampling years and maturity stages, using main effects ANOVA to analyze the first order (non-interactive) effects of multiple factors.

The prevalence, intensity and richness of parasites were correlated with the fish TL using Spearman correlations to determine the relation between these variables. To correlate the parasitic prevalence and the clingfish TL, the whole sample of fish (n = 260) was classified by their body size into 10 range classes, and the prevalence was calculated in each class of TL. The prevalence of parasites was compared between the groups of each variable using contingency tables by considering the number of both parasitized and unparasitized hosts (Zar 1996).

Results

Parasite community description

Of the clingfish sample (n = 260), 34% were parasitized by at least one parasite species. In total, 668 parasites and 14 parasite taxa were found (Table II). Most of the parasites were adults: an undetermined leech species (Piscicolidae gen. sp.), 4 copepods, 4 trematodes, and 2 nematodes. Some larval parasites were also found: 2 undetermined cestodes (Tetraphyllidea gen. sp. and Pseudophyllidea gen. sp.) and 1 acanthocephalan species (*Corynosoma* sp.). Nine of these parasite taxa were recorded for the first time in *G. marmoratus*: *A. sicyasis*, *L. zbigniewi*, *H. chilensis*, 2 *Proctoeces* spp., Tetraphyllidea and Pseudophyllidea cestode larvae, *Similascarophis* sp. and *Pseudodelphis chilensis* (Table II).

Most parasitized clingfish (67.4%) harbored only one parasite species, and 4 parasite taxa was the maximum richness reached by a clingfish, found in only one specimen. The trematode *Helicometrina nimia* Linton, 1920 was the most abundant parasite; 538 individuals of this species were collected, which represents 80% of all parasites found (Table II).

Parasite populations related to factors

The parasite infrapopulations did not differ significantly between variables (years, seasons, localities and maturity stages) when the parasite prevalence (X^2<10.76, P>0.064), abundance and intensity (X^2<5.68, P>0.459) were analyzed. The inertia percentage of the dimensional axes that represents the percent of variance explained by each dimension, varied from 16.2 to 23.8 for prevalence and 13.1 to 20.0 for intensity meaning that there was a little correspondence between parasite species and factors, in other words, several parasite species had similar prevalences between years, seasons, localities and maturity stages (Fig. 1 A-B). Distribution of parasites and factors, in the correspondence analyses, were similar for abundance and intensity of parasites (plots not shown). The trematode *Helicometrina nimia* was the only parasite associated with some factor; it was more prevalent in second sampling year than the other years (X^2 = 7.54; f.d. = 2; P = 0.023), it was also more abundant (z = 2.32, P = 0.02) and prevalent (X^2 = 11.26; f.d. = 1; P = 0.009) in El Tabo than in Las Cruces (Fig. 1A), and in mature than immature fish (abundance: z = 4.66, P<0.001; prevalence: X^2 = 30.5; f.d. = 1; P<0.001, Fig. 1B). The prevalence (n = 10, rs: 0.90, P<0.05), abundance (n = 260, rs = 0.54, P<0.05) and intensity (n = 46, rs = 0.59, P<0.05) of *H. nimia* significantly correlated with the clingfish TL.

Parasite communities related to factors

The GLM showed that abundance and intensity of the parasite infracommunity was affected by the 4 variables considered: sampling year, season, locality and maturity stage of the fish (Table IIIA). Maturity stage had the major effect (highest values of Chi-square) on the abundance and intensity of parasites, whereas, locality mostly influenced on parasite richness (Table IIIA). The correspondence analysis between factors and parasitological descriptors showed a similar results for abun-

Table II. Number of parasite individuals (No. par.), prevalence (P), mean intensity (X INT) and its (standard deviations, SD) of each parasite species found in the clingfish *Gobiesox marmoratus* of central Chile

Parasite Taxa	TOTAL (n = 260)			
	No. Par.	X INT	SD	P(%)
HIRUDINEA				
Piscicolidae gen sp.	5	1.00	0	1.92
COPEPODA				
Acanthochondria sicyases	6	1.50	0.58	1.54
Lepeophtheirus zbigniewi	3	1.00	0.00	1.15
Holobomolochus chilensis	2	1.00	0.00	0.77
Trifur c.f. tortuosus	7	1.40	0.89	1.92
TREMATODA				
Helicometrina nimia	538	11.70	14.84	17.69
Proctoeces lintoni	16	3.20	2.05	1.92
Proctoeces sp.	40	2.50	2.76	6.15
Lecisthaster sp.	40	1.29	0.64	11.92
CESTODA				
Tetraphyllidea (larvae)	5	1.67	1.15	1.15
Pseudophyllidea (larvae)	2	1.00	0	0.77
NEMATODA				
Similascarophis sp.	1	1.00	–	0.38
Pseudodelphis chilensis	1	1.00	–	0.38
ACANTHOCEPHALA				
Corynosoma sp.	2	1.00	0	0.77

dance, intensity and parasite richness; i.e., two groups of fish specimens, according to combined factors, were detectable in the plot dimensions of the correspondence analyses, one characterized by maturity stage-season-year (dashed line) and other determined by locality-season-maturity stage (intermittent line) (Fig. 2), meaning that juveniles were associated with Las Cruces during winter and spring, while adults were associated with El Tabo during autumn and winter.

Influence of clingfish TL in parasite communities

The clingfish TL ranged from 2.3 cm to 14.2 cm. The unparasitized clingfish TL ranged from 2.3 to 10.8 cm, while in the parasitized fish TL ranged between 3.0 and 14.2 cm. The total prevalence (rs = 0.94, n = 10, P < 0.001), abundance (n = 260; rs = 0.53; $P < 0.05$), intensity (n = 87, rs = 0.63, $P < 0.05$) and richness of parasites were strongly correlated with the fish TL.

There were no significant differences in the fish TL between year cycles, although the clingfish TL varied between seasons, locality and maturity stages (Table IV). Clingfish collected in the autumn were smaller (average ± standard deviation: 5.49 ± 2.33 cm) than those collected in other seasons (between 5.75 ± 1.78 cm in winter and 6.55 ± 2.67 cm in summer) (Scheffé's test, P<0.05). Besides the LT variation among factors, there were no significant differences in prevalence,

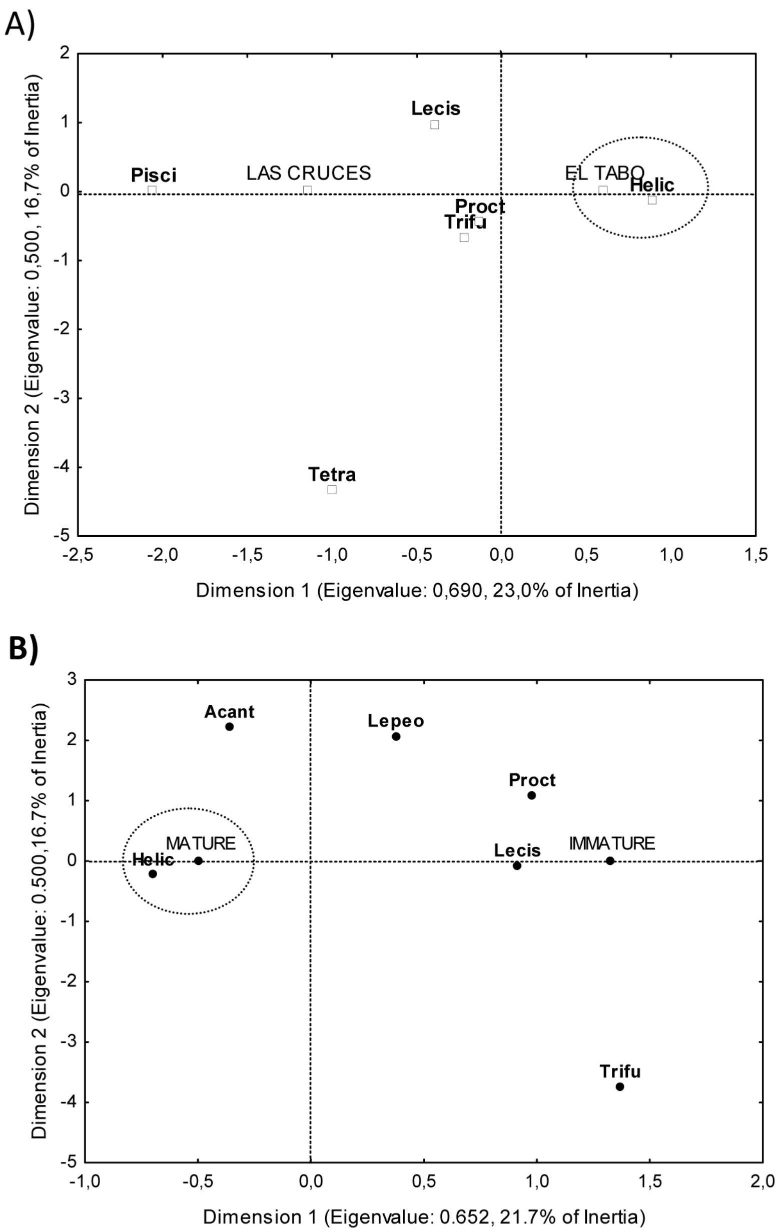

Fig. 1. Correspondence analysis between prevalence of the parasite species found in *Gobiesox marmoratus* and A) locality (El Tabo, Las Cruces), and B) maturity stage of the fish (mature and immature). Parasite species were abbreviated using the 5 first letters of the scientific name

Table III. GLM results (f.d.= freedom degree, X^2 = Chi-square, P = likelihood Type I) showing the main factors that influenced the parasitological descriptors of the clingfish; A) considering only the 4 factors, and B) considering the clingfish TL (cm) as a covariate. Bold probabilities indicate significant differences among the groups

A)		Intensity		Abundance		Richness	
Factors	f.d.	X^2	P	X^2	P	X^2	P
Year	2	44.29	**<0.001**	18.24	**<0.001**	0.48	0.783
Season	3	23.55	**<0.001**	80.48	**<0.001**	2.10	0.550
Locality	1	42.54	**<0.001**	149.30	**<0.001**	19.94	**<0.001**
Maturity	1	68.81	**<0.001**	283.38	**<0.001**	19.05	**<0.001**
B)		Intensity		Abundance		Richness	
Factors	f.d.	X^2	P	X^2	P	X^2	P
Fish TL	1	636.63	**<0.001**	1592.38	**<0.001**	102.29	**<0.001**
Year	2	24.20	**<0.001**	38.54	**<0.001**	10.64	**0.005**
Season	3	32.60	**<0.001**	75.21	**<0.001**	7.39	0.060
Locality	1	<0.01	0.970	4.98	**0.025**	8.32	**0.003**
Maturity	1	8.04	**0.004**	16.41	**<0.001**	0.01	0.924

intensity and richness of parasite infracommunities between sampling years (Table IV).

The clingfish TL correlated with locality and maturity stage, because clingfish from El Tabo (6.4 ± 2.4 cm of TL) were significantly larger than the clingfish from Las Cruces (5.2 ± 1.9 cm) (Table II), and most clingfish from Las Cruces were sexually immature or juveniles (Fig. 2). Immature clingfish (5.6 ± 2.17 cm), which were mostly juveniles, were smaller than mature fish (7.87 ± 2.21 cm) (Table IV) and also exhibited significant differences in the intensity, richness (Table III-A) and prevalence of parasites ($X^2 = 18.78$, $P < 0.001$). Thus, clingfish from EL Tabo had more parasites (average intensity: 8.37 ± 13.46, average richness: 0.58 ± 0.85, and total prevalence: 38.8%) than the clingfish from Las Cruces (average intensity: 3.56 ± 7.09, average richness: 0.25 ± 0.49, and total prevalence: 22.2%) (Table II). This is corroborated with another GLM analysis which considered the clingfish TL as covariate (Table III-B), all the parasitological descriptors were significantly influenced by the clingfish TL (highest values of Chi-square) and the effects of the 4 factors were greatly reduced.

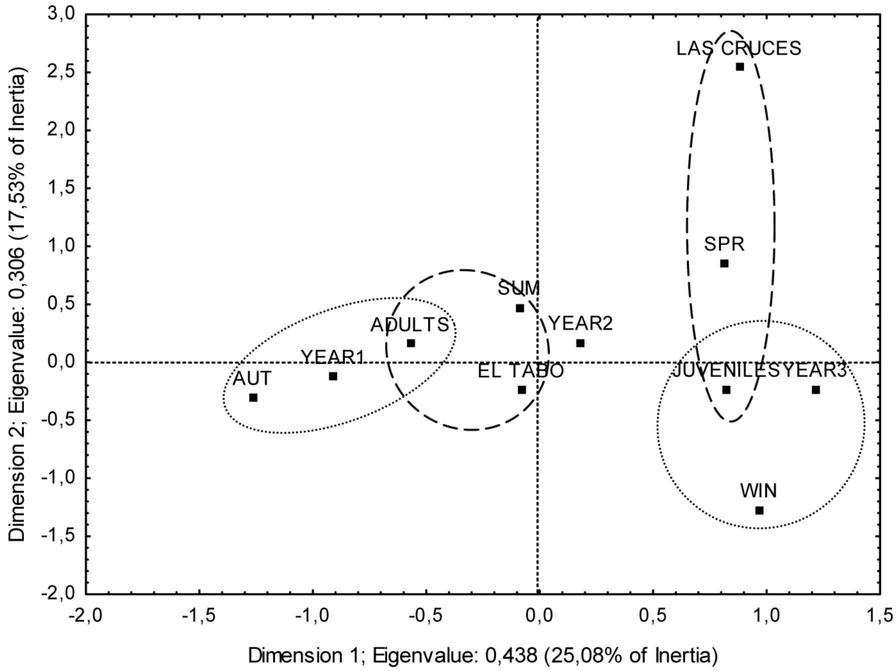

Fig. 2. Multiple correspondence analysis between the intensity of parasites of *Gobiesox marmoratus* and 4 factors: sampling year (1, 2, 3), season (autum, winter, spring and summer determined by the 3 first letters), locality (El Tabo, Las Cruces), and maturity stage of the fish

Table IV. Results of the main-effects ANOVA comparing the body length of the clingfish *Gobiesox marmoratus* between factors

	Clingfish TL			
Factors	**DF**	**MS**	**F**	***P***
Year	2	0.055	2.55	0.080
Seasons	3	0.087	3.97	**0.008**
Localities	1	0.195	8.92	**0.003**
Maturity	1	0.988	45.06	**<0.001**
Error	252	0.022		

Discussion

All factors considering in this study, i.e., sampling year, season, locality and fish maturity stage, were important for the structure of parasite communities of the clingfish *G. marmoratus*. However, most of the effects on the parasitological descriptors were determined by the clingfish TL. In other studies, the fish TL has proven to be one of the primary variables that affects the burden and prevalence of parasite infection. In many host species, some parasitological descriptors, such as the abundance, richness, diversity and prevalence, have correlated with an increase in the host body size (e.g., Lo *et al.* 1998; Muñoz and Delorme 2011; the current study). Although the fish body length is a measurable variable and is recognized as the "habitat size" for parasites, the fish body size actually reflects several intrinsic variables of the host that should be important for parasites (see Thomas *et al.* 2002). However, it is difficult to know which specific variables, related to the size of the fish, actually affect the parasites. For example, energetic budgets, oxygen consumption and hormonal actions are physiological changes related to the age and body size of the organism (Wilmer *et al.* 2006). In addition, these physiological characteristics are associated with animal behavior, such as distribution, displacement, diet and habitat preferences (Restif *et al.* 2001), which are factors that can influence parasite transmission and infection of the hosts (e.g., Møller and Rózsa 2005; Hoye *et al.* 2012).

Las Cruces and El Tabo differ in some physical characteristics (beach slope, kind of rocks), which may influence the parasite transmissions (Kaltz and Shykoff 1998; Restif *et al.* 2001), but also the clingfish could have different population dynamics, specifically related to body size (smaller fish in Las Cruces and larger fish in El Tabo) and trophic habits. Small clingfish (≤5.0 cm) belong to a trophic guild that prey mainly on amphipods, while larger clingfish (>5.1 cm) belong another guild that prey mainly on decapods crustaceans (Muñoz and Ojeda 1998). Therefore, considering that prey items transmit most of the endoparasite species, the differences in the parasite infrapopulations may be due to diet, which is associated to fish TL; thus fish from El Tabo prey on certain items that can transmit more and different parasites than those from Las Cruces.

Pardo-Gandarillas *et al.* (2004) recorded 10 eumetazoan parasites in this clingfish from 3 geographical locations, of which 4 parasite species were found in hosts from El Tabo (one of the location used in the present study). However, their results contrast with the 14 parasite taxa found in this study that is considered to be a diverse parasite-fauna for a relatively small fish. It is likely that the small sample size examined by those authors (n = 25) did not allow for the identification of additional parasites, especially when the parasitic prevalence was low. The parasite communities of *G. marmoratus* were composed of generalist and infrequent parasite species, as all the parasites recorded for this fish have been already found in other intertidal fish species (e.g., Muñoz and Delorme 2011). Only *Proctoeces* spp. was found exclusively in the other gobiesocid species, *S. sanguineus*, in Chile (Muñoz and Zamora 2011) and Perú (Iannacone and Alvariño 2011), whereas *H. nimia* have been recorded in several other intertidal and subtidal fish (Muñoz and Delorme 2011, González *et al.* 2013), and this species was the most abundant parasite species in clingfish in this study.

Therefore, this study not only reveals the influences of several factors on the parasite community structure of *G. marmoratus*, also provides new records of parasites species, and showed some evidence of spatial-temporal segregation of clingfish in the coast of central Chile; there was more adult clingfish in El Tabo, associated to autumn and summer, while clingfish from Las Cruces were mostly juveniles, associated to winter and spring. El Tabo and Las Cruces are relatively close to each other, but differ in some environmental conditions which can be preferred differently by clingfish; to spend the earlier period of development in Las Cruces, and when adults move to El Tabo to reproduce. This is not strange for clingfish species because *S. sanguineus*, which also live in the intertidal rocky zone of central Chile, use to spend the juvenile period in rock grieves, and when being adults move to the subtidal zone. This species also exhibit great differences in parasite communities between juveniles and adults, which is related to changes in body size, habitats and trophic habits (Muñoz and Zamora 2011). Other example is given by Henríquez *et al.* (2011) who also corroborate trough the analysis of the parasite community structure, that the fish *Eleginops maclovinus* (Valenciennes, 1830) had spatial segregation between juvenile males and females. Consequently, the spatial segregation between hosts of a certain species, even in closer habitats, may have a great impact on populations and communities of parasites, as was observed in the present study.

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