

## Parasite community of *Patagonotothen cornucola* (Pisces: Nototheniidae) from intertidal rocky pools from Strait of Magellan

*Comunidades de parásitos de Patagonotothen cornucola* (Pisces: Nototheniidae) de pozas rocosas intermareales del estrecho de Magallanes

Gabriela Muñoz<sup>1</sup>✉

### Abstract

Nototheniids are abundant fish in the Austral Ocean, therefore they establish many interactions with other species, and thus they have become important components in the ecosystem. However, the relationships between these fish and their parasites are poorly understood. The parasite community of the intertidal notothen *Patagonotothen cornucola* has been analyzed in this study considering two sampling sites at the Strait of Magellan and the sex of the fish. The fish samples were collected in January 2017 at Rinconada Bulnes (RB), and San Isidro (SI). A total of 98 fish individuals, with total body length between 4.2 and 13.4 cm, were obtained. Of these fish, 15 parasite species were recorded. Four species were the most prevalent or abundant, three digeneans (*Caudotestis kerguelensis*, *Whitegonimus ozoufae* and *Genolinea cf. bowersi*) and one acantocephalan (*Corynosoma beaglense*). The similarity of parasite composition based on the abundance of parasites showed significant differences among sites because the fish from SI had only five parasite species and two of them with higher abundances than RB. There were no differences in parasite composition between male and female fish. Therefore, the sampling site was important for composition and abundance of parasites, indicating ecological and environmental differences between sites that affect parasite transmission. This is the first study on the parasite community of a species of *Patagonotothen* in the Patagonia area, showing a wide diversity of parasite species in this little fish.

### Key words:

*Caudotestis kerguelensis*, *Whitegonimus ozoufae*, *Corynosoma beaglense*, Southern Chile.

### Resumen

Nototénidos son peces abundantes del océano Austral, que tienen múltiples interacciones con otras especies, siendo importantes componentes de su ecosistema. Sin embargo, la relación de estos peces y sus parásitos es poco conocida. En este estudio se analiza las comunidades de parásitos de *Patagonotothen cornucola* considerando dos sitios de muestreo en el estrecho de Magallanes, y el sexo de este hospedero. Las muestras de peces fueron recolectadas en enero de 2017 en Rinconada Bulnes (RB) y San Isidro (SI), obteniendo 98 peces, cuyas longitudes corporales fueron de 4,2 a 13,4 cm. Del total de peces, 15 especies de parásitos fueron reconocidas, cuatro de ellas fueron prevalentes o abundantes, 3 digeneos (*Caudotestis kerguelensis*, *Whitegonimus ozoufae* and *Genolinea cf. bowersi*) y un acantocéfalo (*Corynosoma beaglense*). La similitud de la composición de parásitos, basada en la abundancia de éstos, mostró diferencias significativas entre sitios, debido a que solo cinco especies de parásitos fueron encontrados en los peces de SI, y dos de ellas tenían mayor abundancia en comparación a RB. No hubo diferencias entre peces machos

<sup>1</sup> Centro de Investigación Costar-R, Universidad de Valparaíso, Avenida Borgoño 16344, Viña del Mar, Chile.  
✉ gabriela.munoz@uv.cl

y hembras en su composición parasitaria. Por lo tanto, el sitio de muestreo fue un factor importante para la composición parasitaria lo que significa que existen diferencias ecológicas y ambientales en los sitios que afectarían la transmisión de parásitos. Este es el primer estudio que entrega antecedentes de las comunidades parasitarias en una especie de *Patagonotothen* en el área Patagónica, en el cual se demostró una amplia diversidad a pesar del pequeño tamaño del pez.

### Palabras clave:

*Caudotestis kerguelensis*, *Whitegonimus ozoufae*, *Corynosoma beaglense*, sur de Chile.

## INTRODUCTION

*Patagonotothen* Balushkin, 1976 is a genus of fish distributed in the Austral Ocean along the southern coast of America on both the Atlantic and the Pacific coasts (DeWitt *et al.* 1990). Although these fish are abundant in this area, there is little knowledge about their interactions with other species. The symbiotic relationships between fish and parasites are barely documented through taxonomic records of parasites. *Patagonotothen* fish might be hosts for several parasites, such as digeneans, copepods, acantocephalans and nematodes. Most of the parasites known in *Patagonotothen* fish are digeneans, counting at least eight species (Bray & Zdzitowiecki, 2000; Jezewski *et al.* 2014), however, there are few reports in other parasitic groups (Longshaw, 1997; Laskowski & Zdzitowiecki, 2004; Muñoz & George-Nascimento, 2007; Cantatore *et al.* 2011). Apart from these parasitological records, there are no studies on the parasite communities in any species of this fish genus. A numeric analysis of the parasite community is necessary because it provides substantial information about parasite composition and variations according to ecological variables of the parasites and the fish.

*Patagonotothen cornucola* (Richardson, 1844) is a fish that reaches 13 cm in total length in the intertidal zone; it inhabits fjords and channels in southern Chile (Hüne & Vega, 2016) and is frequently found in rocky substrates (Moreno & Jara, 1984). This fish preys primarily on small crustaceans, however the diet has

had different composition between sites along Patagonia (Moreno & Jara, 1984; Hüne & Vega, 2016). There are only two parasite records in *P. cornucola*, the copepod *PhrEXOcephalus* sp. found on the fish eye (Cañete *et al.* 2013) and the nematode *Ascarophis carvajali* Muñoz & George-Nascimento, 2007 found in the intestine (Muñoz & George-Nascimento, 2007). Therefore, the objective of this study is to gain insights about the parasite community of *P. cornucola* considering the sampling sites and sex of the fish as factors.

## MATERIAL AND METHODS

From 15<sup>th</sup> to 20<sup>th</sup> of January 2017, 98 fish specimens of *P. cornucola* were collected in two intertidal sites located approximately 50 km south of Punta Arenas City (53°10'7"S; 70°54'40"W), from Rinconada Bulnes (53°35'56"S; 70°54'46"W) up to Bahía Mansa (53°44'53"S; 70°54'50"W), hereafter Rinconada Bulnes (RB), and at midway to El Faro San Isidro (53°36'21"S; 70°56'48"W), hereafter San Isidro (SI) (Fig. 1). There was major sampling effort in RB (2 days) than SI (one day), therefore cumulative parasites species curves (Jackknife 1 and 2) were applied for fish samples to determine if sample sizes were representative for the parasite diversity.

The fish were captured from rocky intertidal pools during low tides using hand nets and with 0.5% clove oil anesthetic solution (AQUI-S, Bayer®). The fish were killed with an overdose of anesthetic solution (2% clove oil) and kept in the solution for 3 to 5 minutes. Subsequently, the fish specimens were placed in individual plastic bags, labelled, and transferred to the laboratory where they were frozen at -20°C for further analysis.

The identification of the fish *P. cornucola* was confirmed based on the description provided by Lloris and Rucabado (1991). The sex of each fish was also recorded based on the color of the gonads (orange for mature female, and white-pink for males, colorless to white for juveniles). Unfortunately, the sex of fish was not recorded at the beginning of the study because it was not a variable to be considered at that time. Therefore, the fish sex was recorded only for a third of the entire sample.

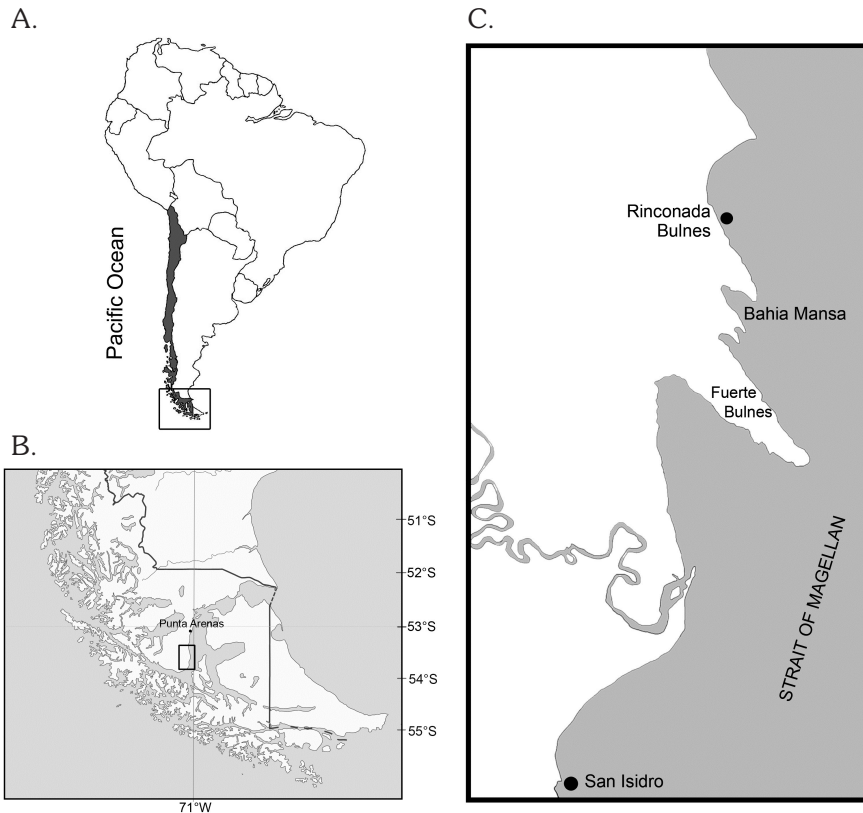


Fig. 1. Sites at the Strait of Magellan, Southern Chile (A), from 50 km from Punta Arenas city (B), *P. cornucola* were collected at the two sampling sites, indicated with the name and black circles (C).

Fish were placed under a stereoscopic microscope, observing the skin, fins, and gills to detect ectoparasites. Subsequently, the fish were dissected for the collection of their endoparasites from the body cavity, digestive tract, and musculature. Thereafter, each organ was sieved using a 250  $\mu\text{m}$  mesh. Both ecto- and endoparasites were stored, labeled, and preserved in 70% ethanol in Eppendorf tubes.

The parasites were identified at the lowest taxonomic level possible, considering bibliographic references such as Szidat and Graefe (1967), Kazachenko (1995), Zdzitowiecki (1997), Boxshall and Bravo (2000), Rocka (2004), Muñoz and George-Nascimento (2007), Jezewski *et al.* (2009), Cantatore *et al.* (2011), Jezewski *et al.* (2014), and Laskowski and Zdzitowiecki (2017). Prevalence and average abundance (*sensu* Bush *et al.* 1997) were calculated for each parasite species per sample (each site and sex). Two descriptors of

parasite infracommunity were also considered: total abundance (number of all the parasites) and parasite richness (number of species) per fish sample.

The abundance of parasites per species as well as the infracommunity abundance did not fit with normal distribution. Therefore, all the statistical analyses applied were non-parametric. The total abundance and richness of the parasite infracommunity was also compared (separately) between sites and sex with Mann-Whitney tests. The parasite prevalence of parasite species common in the sampling sites were compared with 2 x 2 contingency tables, using Fisher exact probability. Also, the fish body length was also compared between sampling sites and sexes with a Mann-Whitney test (Zar, 1996). Abundance and species richness of the parasite infracommunity were correlated with fish body length by the Spearman correlation analysis (Zar, 1996).

The similarity of parasite composition between fish samples was calculated using the Jaccard index based on the presence-absence of the parasite species found in each sample; it was the number of species shared between two samples divided by the total number of species in the two samples expressed in percentage (Chao *et al.* 2006). This index was calculated between host samples of different sites and sexes.

The similarity of parasite communities, using the parasite abundance of each species, was obtained between fish sample through the index of similarity of Bray-Curtis and analyzed with the multivariate non-parametric test (ANOSIM). The indices obtained for samples were contrasted with 9999 aleatory matrices from the original data. The results of this analysis were represented with the coefficient of dissimilarity (R) between the samples, ranging from R=0 (similarity) to R>0 (dissimilarity) with the respective statistical probability (Clarke, 1993). All the analyses were performed with the

software PAST 3.13 (<https://folk.uio.no/ohammer/past/>). The significance level for all of the analyses was  $P < 0.05$ .

## RESULTS

There was a great difference of the fish specimens caught at each site, 12 in SI and 86 in RB. Fifteen parasite species were found in the entire fish sample, five parasite species at SI and 15 at RB (Table 1). The estimators of the maximum parasite species for both sites were seven and 19 species respectively (by Jackknife 2). The 90% of the total parasite richness in the accumulative curve was reached at 4 specimens in SI and 36 specimens in RB.

Several parasite species were present in few host individuals, and four species were the most prevalent, three digeneans [*Caudotestis kerguelensis* (Prudhoe & Bray, 1973), *Whitegonimus ozoufae* Jezewski, Zdzitowiecki & Laskowski, 2009 and *Genolinea cf. bowersi* (Leiper

Table 1. Parasite taxa found in *Patagonotothen cornucola*. Infection site in the fish body, number of parasite individuals (n), mean abundance and standard deviation (ABU  $\pm$  SD), and the percentage of prevalence (PREV) of each parasite species for two sampling sites at Strait of Magellan.

PARASITIC TAXA	Infection site	SAN ISIDRO (n=12)				RINCONADA BULNES (n=86)			
		n	ABU	SD	PREV	n	ABU	SD	PREV
ECTOPARASITES									
<i>Caligus rogercresseyi</i>	BS					2	0.02	0.15	1.2
<i>Acanthochondria lilianae</i>	Gi					4	0.05	0.21	3.5
<i>Haemobaphes</i> sp.	Gi	3	0.25	0.62	16.7	4	0.05	0.26	1.2
ENDOPARASITES									
<i>Caudotestis kerguelensis</i>	In					171	1.99	3.69	43.0
<i>Whitegonimus ozoufae</i>	In	20	1.66	5.78	8.3	46	0.53	1.63	16.3
<i>Genolinea cf. bowersi</i>	St					47	0.55	1.06	27.9
<i>Lecithaster macrocotyle</i>	In	1	0.08	0.288	8.3	8	0.09	0.33	8.1
<i>Corynosoma beaglense</i>	BC	13	1.08	2.46	25.0	40	0.46	1.24	20.9
<i>Corynosoma australe</i>	BC					1	0.01	0.11	1.2
<i>Dichelyne cf. fraseri</i>	In					15	0.17	0.89	5.8
<i>Ascarophis carvajali</i>	In	65	5.41	11.91	41.7	10	0.12	0.42	9.3
<i>Pseudodelphis</i> sp.	In					9	0.10	0.48	5.8
<i>Anisakis</i> sp.	BC					1	0.01	0.11	1.2
<i>Pseudoterranova</i> sp.	BC					2	0.02	0.15	2.3
<i>Contracaecum</i> sp.	In					1	0.01	0.11	1.2

Abbreviations: BS: body surface, Gi: gills, In: intestine, St: stomach, BC: body cavity.

& Atkinson, 1914)] and one acantocephalan (*Corynosoma beaglense* Laskowski, Jeżewski & Zdzitowiecki, 2008) (Table 1).

There were significant differences in the prevalence of two parasite species present in both sites; *Haemobaphes* sp. (Fisher exact  $P=0.038$ ) and *Ascarophis carvajali* (Fisher exact  $P=0.008$ ) were more prevalent in SI than in RB. From the 15 parasite species recorded in *P. cornucola*, only a third part was shared between hosts from different sampling sites (Table 2A). Comparison of parasite composition based on the abundance of each parasite species showed significant differences between sampling sites (ANOSIM,  $R=0.288$ ,  $P=0.005$ ) (Table 1). Four parasite species constituted the difference, namely *Whitegonimus ozoufae* and *Ascarophis carvajali* which were more abundant in SI than RB, whereas *Caudotestis kerguelensis* and *Genolinea cf. bowersi* were absent at SI but abundant at RB (Tables 1, 2A).

Table 2. Similitude indices for parasite communities in host fish *P. cornucola* for sampling sites (A) and sex (B). Values over the diagonal line correspond to the Jaccard index, and values below the line correspond to the Bray-Curtis index. Asterisks indicate significant difference between the fish groups according to ANOSIM test.

A) Sampling sites		RB	SI
Rinconada Bulnes (RB)		--	0.30
San Isidro (SI)		0.0046*	--
B) Host sex		Males	Females
Males		--	0.53
Females		0.27	--

In the entire sample of hosts used for comparison between sexes, 14 parasite species were found. A half of them were shared between males and females (Table 3). However, there was no significant difference in the composition of parasites between fish sexes (ANOSIM,  $R=0.052$ ,  $P=0.272$ ) (Tables 2B, 3), and no

Table 3. Parasite taxa found in males and females of *Patagonotothen cornucola*. Number of parasite individuals (n), mean abundance and standard deviation (ABU ± SD), and the percentage of prevalence (PREV) of each parasite species.

PARASITIC TAXA	n	FEMALES (n=17)			n	MALES (n=9)		
		ABU	SD	PREV		ABU	SD	PREV
<b>ECTOPARASITES</b>								
<i>Caligus rogercresseyi</i>	1	0.06	0.24	5.9				
<i>Acanthochondria liliana</i>					2	0.22	0.44	22.2
<i>Haemobaphes</i> sp.	1	0.06	0.24	5.9	1	0.11	0.33	11.1
<b>ENDOPARASITES</b>								
<i>Caudotestis kerguelensis</i>	2	0.12	0.33	11.8	28	3.11	6.15	33.3
<i>Whitegonimus ozoufae</i>	16	0.94	2.05	29.4	3	0.33	1.00	11.1
<i>Genolinea cf. bowersi</i>	9	0.53	1.01	29.4	5	0.56	0.88	22.2
<i>Lecithaster macrocotyle</i>	3	0.18	0.53	11.8	2	0.22	0.44	22.2
<i>Corynosoma beaglense</i>	7	0.41	1.28	11.8	6	0.67	2.00	11.1
<i>Corynosoma australe</i>					1	0.11	0.33	11.1
<i>Dichelyne cf. fraseri</i>	5	0.29	0.99	11.8				
<i>Ascarophis carvajali</i>	5	0.29	0.47	29.4				
<i>Pseudodelphis</i> sp.	4	0.24	0.75	11.8	1	0.11	0.33	11.1
<i>Pseudoterranova</i> sp.	1	0.06	0.24	5.9	1	0.11	0.33	11.1
<i>Contracaecum</i> sp.					1	0.11	0.33	11.1



difference was found in the prevalence of parasite species between male and female fish (Fisher exact  $P > 0.302$ ).

The total abundance of parasites was not different between sampling sites ( $U=386$ ,  $P=0.158$ ) neither between sexes ( $U=75$ ,  $P=0.935$ ). The species richness was not different between sampling sites ( $U=363.5$ ,  $P=0.098$ ), neither between sexes ( $U=73.5$ ,  $P=0.871$ ).

The total length of the fish ranged 4.2 to 13.4 cm, and it was similar between SI and RB ( $U=457$ ,  $P=0.522$ ). There was no difference in the fish length of males (4.8-11.5 cm) and females (4.9-13.4 cm) (Mann Whitney test:  $U=66$ ,  $P=0.571$ ). Considering the largest fish group (RB,  $n=86$ ), there was no significant correlation between parasite abundance and fish total length ( $r_s=-0.121$ ,  $P > 0.20$ ) neither for parasite richness and fish total length ( $r_s=0.107$ ,  $P > 0.20$ ). Only the richness and abundance of parasites were positively correlated ( $r_s=0.783$ ,  $P < 0.001$ ).

## DISCUSSION

The fish sample size analyzed in this study was different between sites due to the sampling effort, thus the results about differences of the parasite communities between samples must be cautiously interpreted. Small fish sample size can affect the parasitological descriptors, however the minimum host sample size is variable for parasites and host species. In this study, the estimators of parasite richness differed little with the observed richness in each sampling site, meanings that representative parasite richness was found at each site. However, the difference of the total parasite richness, five species in SI and 15 species in RB, was determined by the fish sample size, *i.e.*, the more host specimens the more parasite species. Nevertheless, the greater abundance of some parasites at SI (*W. ozoufae* and *A. carvajali*) than RB confirmed the differences in parasite community.

RB is a place with rocks and stones of different sizes forming several rocky pools, contrasting with SI that is characterized by long and flat rocks with few spaces for pools. That explained why was easier to collect more fish at RB than SI. The sampling sites of this study are

located at 10 km distant (on a straight line) one another. Although this distance is not long, it had a significant influence in the parasite communities of the fish. The processes that explain differences of the parasite community along the space are related to parasite transmissions which can be affected by the capacity of displacements of hosts which this limits the dispersion of parasites (Thieltges *et al.* 2009), fish interactions with other species (prey, sympatric hosts), and environmental characteristics (Poulin, 2003). Although Poulin (2003) indicated that the dissimilarity between parasites is not necessarily related to the vagility of the host, the notothenid fish have some particularities in their swimming performances because they lack swim bladder, and the fish from intertidal habitats have low static buoyancy that combined with low metabolic rate (as occur in *P. cornucola*) results in a less active lifestyle (Fernández *et al.* 2012). Thus, the distance of 10 km might be significant for little fish, and the specific site they used to live in might have differences in some aspects (physical conditions and composition of other species) that affect their parasite community.

In other studies, the difference in parasite communities of fish has been correlated to difference of fish body length (*e.g.*, Muñoz-Muga & Muñoz, 2010; Muñoz, 2014) which is not the case for *P. cornucola* because the fish samples have similar body lengths. Therefore, differences in parasite composition between sites might be due to other ecological characteristics, for example invertebrate composition. Most endoparasites use invertebrates as intermediate hosts. In fact, Hüne and Vega (2016) found differences in the diet of *P. cornucola* collected from different localities, which is one of the primary reasons behind different parasite compositions (*e.g.* Muñoz & Zamora, 2011).

There is no information about the intermediate hosts for the parasite species found in *P. cornucola*. However, the endoparasite species that were present in different abundances in SI have indirect life cycles; this implies that they need one or two invertebrate hosts before reaching the fish. *Ascarophis* nematodes usually used decapod crustaceans as intermediate hosts (Leiva *et al.* 2015) that go into the fish through predation. Therefore, the high prevalence and abundance of *A. carvajali* can

be possible if there are more decapod crustaceans or crustaceans more infected with the nematode in SI than the other sites. A similar explanation might apply for the digeneans one present in high abundance (*Whitegonimus ozoufae*) in fish from SI and the other digenean (*Caudotestis kerguelensis* and *Genolinea cf bowersi*) were absent in the fish from this site. Digeneans primarily use mollusks and crustaceans as primary and secondary intermediate hosts, respectively (Olsen, 1974). Therefore, if one of the intermediate hosts needed by the parasites is abundant or might not have a significant influence in the life cycle dynamic of the parasites. Predation and environmental conditions regulate emergence and mobility of parasitic larval stages, which also affect the infection rate to the final host (Prinz *et al.* 2011).

Sex did not have an effect on the parasite community of *P. cornucola*. This fish did not show difference in body length, and a previous study has shown that there are no differences in diet between sexes (Hüne & Vega, 2016). Usually the sex of the hosts has little or no effect on the parasite communities (e.g. González & Acuña, 2000; González *et al.* 2001) that happen when male and female hosts have ecological similarities (similar diet, habitats and behavior) which does not affect to the parasites.

It is important to highlight the presence of the copepod *Caligus rogercresseyi* Boxshall and Bravo 2000 on wild fish from the most austral zone of Chile, because this parasite has a great impact in salmon culture. This copepod is distributed from Puerto Montt (41°S) to the austral zone (53°S), at the Pacific and Atlantic coasts (Bravo *et al.* 2008). *Caligus rogercresseyi* is usually a parasite of wild fish (Carvajal *et al.* 1998), such as *Eleginops maclovinus* (Valenciennes, 1830), *Odontesthes regia* (Humboldt, 1833) and *Paralichthys microps* (Günther, 1881). Thus, in the present study, *P. cornucola* is added as a new host for *C. rogercresseyi*.

In conclusion, this study revealed that the sampling site was important for parasites. This is likely due to differences in the habitat conditions and/or free-living species composition which might serve as hosts for parasites and might have several effects on parasite transmission that finally affect the parasite community structure in *P. cornucola*.

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