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ARTICLE

Parasite community of a temporal notothen fish from intertidal rocky pools in south-central Chile: Is it similar to other fish from the same habitat?

Comunidad de parásitos de un pez nototénido temporal del intermareal rocoso del centro-sur de Chile: ¿es similar a la de otros peces del mismo hábitat?

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Resumen.- Los peces nototénidos (Nototheniidae) han sido registrados en las costas de Sudamérica, desde los 35°S hasta el extremo austral. Poco se sabe de su biología y de relaciones interespecíficas, incluyendo sus parásitos. En este estudio se analizó la parasitofauna de una especie de *Notothenia* juvenil, recolectada del intermareal rocoso de Lebu, centro-sur de Chile (36°S), y se comparó con las comunidades de parásitos de otras 9 especies de peces del mismo hábitat. *Notothenia* sp. mostró la mayor diversidad de parásitos en comparación a otras especies de peces (21 *vs* 1-9 taxa parasitarios). La composición parasitaria fue diferente entre las especies de peces analizadas, de 0 a 33% de similitud. *Notothenia* sp. presentó la mayor semejanza solo con *Calliclinus geniguttatus* (27%). Los parásitos con mayor abundancia y prevalencia en *Notothenia* sp. fueron el copépodo *Caligus* cf. *cheilodactyli*, larvas de Tetraphyllidea, nematodos anisákidos, y varias especies de acantocéfalos, siendo estos parásitos típicos de peces de las zonas submareales y demersales. Se concluye que los ejemplares de *Notothenia* sp. estaban parasitados cuando alcanzaron la zona intermareal. Solo unas pocas especies parásitas generalistas pudieron ser adquiridas por *Notothenia* sp. durante su corta estadía en este ambiente, tales como el copépodo *Holobomolochus chilensis* y el digeneo *Lecithaster macrocotyle*. Muchos peces nototénidos son de aguas antárticas y subantárticas, siendo posible que *Notothenia* sp. provenga de otros hábitats, submareal y demersal, pero también del extremo austral Sudamericano. En este estudio, *Notothenia* sp. no pudo ser identificada a nivel específico debido a las diferencias entre resultados morfológicos y genéticos.

Palabra clave: Comunidades de parásitos, similitud parasitaria, *Notothenia,* pozas intermareales, costa chilena

Abstract.- Notothen fishes (Nototheniidae) have been recorded on the coasts of South America, from 35°S to southern. Little is known about the biology of intertidal notothen fishes or their relationships with other species, including parasites. Here, we analyzed the parasitic fauna of a species of *Notothenia* collected from the rocky intertidal of Lebu, south-central Chile (36°S) and compared the parasite community of this host with other 9 fish species commonly found in intertidal rocky pools. Among the tested species, *Notothenia* sp. had the highest parasite richness (21 parasitic taxa *vs.* 1 to 9 parasitic taxa). The composition of parasite species was different among fishes, with similarity indices between 0 and 33%. The parasite composition of *Notothenia* sp. was most similar to *Calliclinus geniguttatus* (27%). The most abundant and prevalent parasites found in *Notothenia* sp. were the copepod *Caligus* cf. *cheilodactyli*, Tetraphyllidea larvae, anisakid nematodes, and several acanthocephalan species, which are typical of other fish from the subtidal and bento-demersal zones. Therefore, we conclude that the juveniles of *Notothenia* sp. were a few months old and had their parasites when reaching the intertidal zone, acquired some generalist parasites during their stay in this habitat, such as *Holobomolochus chilensis* and *Lecithaster macrocotyle*. Most notothen fishes are from Antarctic and Sub-Antarctic waters, therefore, it is possible that this fish came from another habitat but also from the austral region of South America. *Notothenia* sp. could not be specifically identified because of different results between morphological and genetic analyses.

Key words: Parasite communities, parasitic similarity, *Notothenia,* intertidal pools, Chilean coast

[redacted]

Introduction

The great species diversity in intertidal zones has attracted scientific interest for many years, which has been focused on establishing and describing the interspecific relationships that are formed there. In Chile, several studies have dealt with the relations of competition or coexistence among species (*e.g.,* Shinen & Navarrete 2010, Aguilera *et al.* 2013), between predators and prey (*e.g.*, Quijada & Cáceres 2000, Berrios & Vargas 2004) and relationships between parasites and hosts (*e.g.*, Muñoz & Cortés 2009, Soto *et al.* 2016). All these studies agree that intertidal systems are diverse and highly complex in their organization.

The intertidal zone has environmental characteristics that favor the recruitment of juvenile stages of many invertebrate and vertebrate species, which use this area as a temporary refuge, settlement, and for a definitive life. Thus, resident fish species, mostly benthic organisms, live permanently in the intertidal zone. This group is represented by the families Blenniidae, Clinidae, Trypteriigidae, and Labrisomidae (Quijada & Cáceres 2000, Muñoz & Delorme 2011). On the other hand, temporal fish live in that zone for a short period and can be associated with a certain ontogenetic stage (juveniles or adults), including Clupeidae, Kyphosidae, and Nototheniidae species (Pequeño & Lamilla 1995). Both resident and temporal fish groups are present along the Chilean coast, although the abundances of the species vary considerably among geographical areas (Navarrete *et al.* 2014).

Although resident and temporal fish share the habitat (same rocky pools), and food (several mollusks and crustaceans), and often have comparable body sizes, the composition of their parasite communities can differ because of different dwell times in the intertidal zone and arrival at different development stages (Muñoz & Delorme 2011). Temporal juvenile fish have presented different parasitic compositions even when they come from nearby localities (Muñoz *et al.* 2001, 2002), which indicates that their parasite communities are highly variable; whereas the resident fish have a composition of parasite communities that are less variable among nearby localities (Flores & George-Nascimento 2009, Muñoz-Muga & Muñoz 2010) and over time, although there are certain seasonal differences (Díaz & George-Nascimento 2002, Muñoz & Delorme 2011).

The Sub-Antarctic region, according to oceanographic characteristics, extents from central-south of Chile and Argentina (38-40°S), ending in the waters located south of Cape Horn in the Drake Strake (55-56°S). In this region, Nototheniidae is a predominant fish family (Norman 1937), which includes benthic or pelagic species that live in cold waters at the southern hemisphere (Eastman 1991). Most of notothen records are from 45°S to south (Reyes & Hüne 2012). There are two species found in rocky intertidal pools at the central-southern coast of Chile, in northern distribution limit; *Notothenia microlepidota* in Mehuín (39°22'S) (Small 1976) and *Notothenia* cf. *angustata* close to Concepción City [Maule 37°0'S; 73°14'W, and Isla Santa María 37°S; 73°30'W (Muñoz *et al.* 2001), as well as a record of a *Notothenia* sp. at Burca 36°32'S; 72°55'W (Quijada & Cáceres 2000)]. These species have been found during the austral summer as juveniles, with body lengths between 8 and 13 cm (Small 1976, Quijada & Cáceres 2000, Muñoz *et al.* 2001).

Little is known about intertidal notothen fishes; there is only some dietary information (Pequeño 1976, Quijada & Cáceres 2000, Muñoz *et al.* 2001) and one parasitological record for *N.* cf. *angustata* (Muñoz *et al.* 2001). According to the few reports addressing notothen species from central-south of Chile described above, these species are unlikely to be recruited from the intertidal zone. Consequently, it is worth asking how much of the parasitic fauna of a notothen species is brought from another habitat and if parasitic species can be acquired during their intertidal stay. The objectives of this study were: 1) to identify a notothen species obtained from Lebu, a close locality from Concepción, central-southern Chile; 2) to describe the parasite community of that fish species; and 3) to compare this with those parasites of other intertidal fish species from the central-southern zone of Chile.

Materials and methods

Fish collection and identification

Between March and May 2014-2015, 388 fish were collected in the area of Lebu, from 36°45'3.971"S-73°11'2.738"W to 36°47'4.265"S-73°12'21.532"W, located approximately 145 km south of Concepción City (36°49'29.485"S -73°3'41.965"W), south-central Chile.

The fish were captured in rocky intertidal pools during low tides, using hand nets and with 1% benzocaine anesthetic solution (BZ-20®, Veterquímica, Chile). All of the captured specimens were placed in individual plastic bags, labelled, and transferred to the laboratory, where they were frozen (at -20 °C) for further analysis.

At the time of fish dissection, each specimen was identified according to Williams (1990), Stepien (1993) and Reyes & Hüne (2012). In the case of Nototheniidae, the taxonomic identification of species has been controversial, and with few taxonomic keys available and updated (*e.g.*, Fischer & Hureau 1985). Therefore, prior to the dissection of the specimens, a meristic analysis was made to compare it with other notothen species, based on the information obtained from several bibliographic sources (*e.g.*, Pequeño 1976, 1984; Fischer & Hureau 1985, Reyes & Hüne 2012, McMillan *et al.* 2014) and the website <www.fishbase.com> (Froese & Pauly 2017). The distribution of the hypural bones was also observed according to the descriptions given by Fischer & Hureau (1985). The total length and the wet weight were recorded for each fish specimen.

The meristic comparisons between Nototheniidae species were carried out with cluster analysis, using the simple union similarity index, and the distance between the fish species by the Euclidean method (De Vienne *et al.* 2011) carried out in the STATISTICA 7.0 program. Several species had a few meristic measures. Therefore, 2 clusters were carried out. The first analysis considered 7 measurements (spines and dorsal fin rays, anal fin rays, pectoral and pelvic fin rays, number of upper and lower lateral line scales) in 10 notothen species. The second analysis considered 3 meristic variables (spines and dorsal fin rays, anal fin rays) of 30 notothen species. In both analyses we include a juvenile fish determined as *Notothenia* cf. *angustata* by Muñoz *et al.* (2001) that was from an area close to the present study. The maximum and minimum values of each meristic measurement were considered as separate variables. Thus, the first working matrix contained 13 variables in 10 species, and the second 6 variables in 30 species.

Genomic DNA was extracted from single specimens of the notothen fish to determine their taxonomic identity. Tissue samples (muscles) were stored in 96% ethanol and DNA was extracted by E.Z.N.A.® Tissue DNA kit (Omega Bio-Tek, Norcross, GA, USA). The barcode region of the cytochrome oxidase I (COI) was the gene chosen to obtain an approximation at the genus or species level of the fish. The primers employed for PCR amplification were LCO (GGT CAA CAA ATC ATA AAG ATA TTG G) and HCO (TAA ACT TCA GGG TGA CCA AAA AAT CA) for the COI gene (Folmer *et al*. 1994).

Each PCR assay had a final volume of 25 µl and included 0.125 µl of Taq polymerase, 2.5 µl of 10 × PCR buffer, 0.5 µl of dNTPs (10 mM), 4 µl of $MgCl_2$ (25 mM), 1 µl of primers (according to the gene analysed), 1.5 µl of BSA (BioLabs) (19 mg mL^{-1}), 3 µl of concentrated DNA (10 to 200 ng of DNA) and 11.375 µl of water. The thermocycling conditions for amplification were: an initial denaturation step at 95 °C (5 min), followed by 40 cycles at 95 °C (45 s), 50 °C (45 s) and 72 °C (1 min), and a final extension step at 72 °C (10 min) (Folmer *et al.* 1994).

The PCR products were visualised on 1% agarose gel via electrophoresis and purified using the E.Z.N.A.® Gel Extraction kit (Omega Bio-Tek, Norcross, GA, USA). The PCR products for each specimen were sequenced using an automated capillary electrophoresis sequencer (ABI 3730XL, Macrogen, Inc., Seoul, Korea)[1]. The sequences were blasted to other data available in GenBank, using the IgBLAST 1.8.0 tool program (National Center for Biotechnology Information, Bethesda, MD)[2].

Parasitological analysis

Ectoparasites were sought under a stereoscopic microscope observing the skin, fins and gills of the fish. Subsequently, fish were dissected for the collection of endoparasites from the coelomic cavity, digestive tract and musculature. For detailed observation under a magnifying glass, each organ was sieved using a 250 µm mesh. Both ecto- and endoparasites were stored, labeled, and preserved in 70% ethanol in Eppendorf tubes.

When possible, parasites were identified at the lowest taxonomic level, considering bibliographic references indicated in Soto *et al.* (2016) and adding others, such as Petrochenco (1971), Zdzitowiecki (1997), Amin *et al.* (2011), and Laskowski & Zdzitowiecki (2017). Abundance and prevalence (*sensu* Bush *et al.* 1997) were calculated for each species of parasite in each host sample. We also calculate the total prevalence as the percentage of a fish sample parasitized with any parasite species.

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[1] <http://www.macrogen.com>

[2] <https://www.ncbi.nlm.nih.gov/>

Parasite infracommunity abundance and richness were calculated for each host and averaged by fish species sample. Then, these descriptors were compared among fish species using the Kruskal-Wallis test. The total prevalence of parasite community per fish species was also calculated and then compared between fish species through contingency tables. Both the total prevalence and the average abundance and infracommunity parasite richness were correlated with the average body weight of the fish, using Pearson's correlation analysis. A significance level of 0.05 was used for all statistical analyzes.

The similarity of parasite composition among fish species was calculated using the Jaccard similarity coefficient (Brower & Zar 1977) based on presence-absence of parasite taxa in the fish. Parasite taxa that were present in one fish specimen were not considered for this analysis. Subsequently, to test whether groups of fish were distinguished according to their parasitic fauna, a Nonmetric-Multidimensional Scaling (NMDS) analysis was carried out together with an Analysis of Similarities (ANOSIM) (Clarke & Warwick 1991, Chapman & Underwood 1999), using Past 3.2 software (Øyvind Hammer, Natural History Museum, University of Oslo)[3].

Results

Taxonomic identification of a notothen fish

The distribution of the hypural bones of notothen specimens is in accordance to the genus *Notothenia* (Fischer & Hureau 1985). The morphological comparison, when considering the matrix of 13 variables and 10 species (Fig. 1A), shows that all of the meristic ranges of *Notothenia* sp. were similar to *Notothenia* cf. *angustata* (juveniles in Muñoz *et al.* 2001) and *N. angustata* (adults in Fischer & Hureau 1985). The analysis with the matrix of 6 variables and 30 species also showed that specimens of the present study were within a small group composed of *N. microlepidota*, *Notothenia* cf. *angustata*, *N. angustata*, and *Paranotothenia magellanica* (Fig. 1B, Table 1). This group was distant from all of the other notothen species considered in this analysis.

Table 1. Meristic morphology of the notothenioid species most similar to the fish species collected for the present study (*Notothenia* sp.). *N. coriiceps* is also included due to its similitude in the sequence of the COI gene to *Notothenia* sp. / Morfología merística de las especies nototénidas más parecidas a la especie recolectada en el presente estudio (*Notothenia* sp.). Se incluye también a *N. coriiceps* por ser cercana en la secuencia genética del gen COI a *Notothenia* sp.

Scientific name	Max. TL (cm)	Dorsal spines	Dorsal soft rays	Anal soft rays	Pectoral rays	Pelvic spines	Pelvic rays	Caudal rays	Scales lateral lines (UP; LO)*
Notothenia sp. †1	16	4-6	27-30	22-25	17-18	1	5	14	52-56; 10-16
Notothenia cf. *angustata*†2	13	3-6	28	23-25	18-19		6	15-16	50-56; 14-16
Notothenia angustata / *Paranotothenia angustata*	41	3-6	28-36	23-25	17-19	0-1	5-6	15-16	50-56; 12-16
Notothenia magellanica / *Paranotothenia magellanica* / *Notothenia macrocephala*	38	3-6	28-31	22-26	16-18				
Notothenia microlepidota	70	6-7	27-28	23-24					
Notothenia microlepidota†3	11	5-6	27-31	23-25	17-19				57; 12
Notothenia coriiceps	62	4-6	35-38	26-30	16-19				34-49; 6-17

*UP: upper lateral lines, LO: lower lateral line.
†Juveniles, data from: [1]this study, [2]Muñoz *et al.* (2001), [3]Pequeño (1976)

[3]<https://folk.uio.no/ohammer/past/>

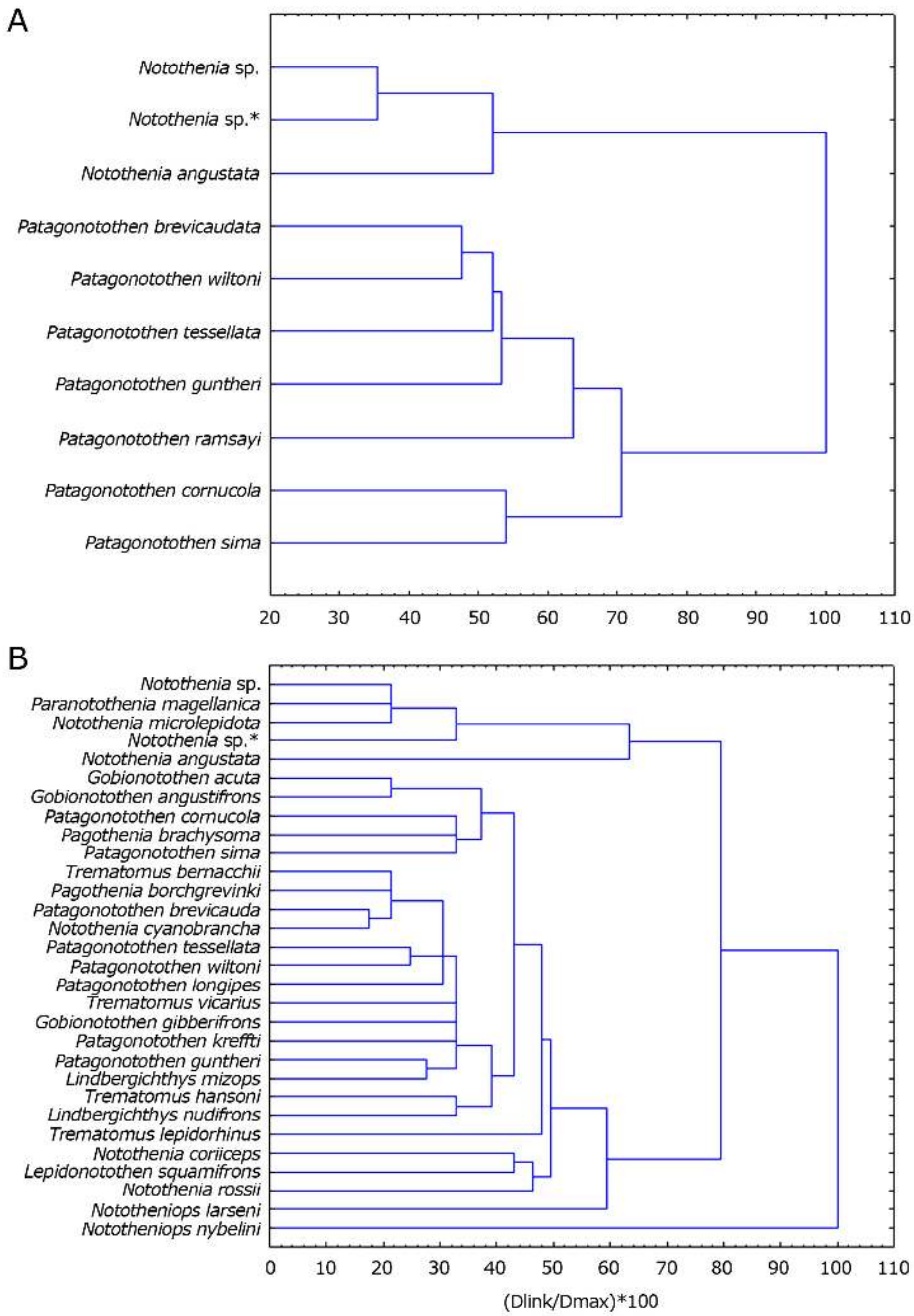

Figure 1. Conglomerate analysis according to the percentage of similarity based on meristic morphological variables of notothenid species. A) 13 variables in 10 fish species; B) 6 variables in 30 fish species. *specimens considered in Muñoz *et al.* (2001) identified as similar to *N. angustata* / Análisis de conglomerados según porcentaje de similitud en base a la morfología de variables merísticas de especies nototénidas. A) 13 variables en 10 especies de peces; B) 6 variables en 30 especies de peces. *especímenes considerados en Muñoz *et al.* (2001) identificados como similares a *N. angustata*

The genetic results show that the three specimens of *Notothenia* sp. (from the present study) was closer to *Notothenia coriiceps,* with 94% of similarity and 89-98% of alignment, considering sequences of 14 specimens available in GenBank. *Notothenia* sp. was also closer with *Paranotothenia magellanica*, with 94% of similarity sequences but 88% alignment of one sequence available.

Composition of parasite community in intertidal fish

Thirteen fish species were caught in the intertidal rocky zone of Lebu, with different sample sizes (Table 2); *Notothenia* sp. and *Bovichthus chilensis* were the most abundant. Ten fish species, with at least 5 specimens each, were considered for all statistical analyses.

Individual body sizes varied between 3.8 to 19.5 cm (total length); the smallest average was for clingfish *G. marmoratus*, and the largest was for *S. sanguineus* (Fig. 2A). Total weight varied from 0.7 to 113 g, the smallest average was for *B. chilensis* and the largest was for *S. sanguineus* (Fig. 2B). *Notothenia* sp. averaged 9.2 cm in length, being one of the largest in the fish assembly. However, its average body weight was 12.7 g, being within the medium range and similar to several other fish species (Fig. 2B).

Thirty parasitic taxa were found in the whole fish assemblage, most of the parasites identified at least to the family level (Table 3). Twelve fish species had parasites. There were clear differences in the richness and abundance of parasites between fish species. Most of the fish had between one and 6 parasite taxa. However, *Notothenia* sp. harbored the greatest richness, with 21 taxa, followed by *C. geniguttatus* with 9 taxa (Table 3). Total prevalence and the average abundance of parasites

Table 2. Intertidal fish species and their sample sizes (n) obtained from Lebu, south-central Chile. Parasitological data, such as the component community richness, total prevalence, and average abundance of the infracommunity (± standard deviation, DE) are shown for each fish species. Different superscript letters (in prevalence and abundance) indicate significant differences among fish species / Especies de peces intermareales, con sus respectivos tamaños muestrales (n), obtenidos en la zona de Lebu, centro-sur de Chile. Para cada una de ellas se muestran algunos datos parasitológicos, tales como la riqueza de la comunidad componente, la prevalencia total y la abundancia infracomunitaria promedio (± desviación estándar, DE). Letras distintas en superíndices (en prevalencia y en abundancia promedio de parásitos) indican diferencias significativas entre especies de peces

Fish Family	Fish species	Fish species abbreviation*	Parasitological descriptor			
			n	Richness	Prevalence	Abundance ± DE
Nototheniidae	*Notothenia* sp.	Noto	95	21	90.5^{a}	8.62 ± 6.56^{a}
Labrisomidae	*Calliclinus geniguttatus*	Cgen	38	9	36.8^{b}	1.24 ± 2.38^{c}
	Auchenionchus variolosus	Avar	3	0	0.0	0.00 ± 0.00
Bovichthidae	*Bovichthus chilensis*	Bchi	103	6	12.6^{b}	0.26 ± 0.84^{c}
Gobiesocidae	*Sicyases sanguineus*	Ssan	19	4	78.9^{a}	$2.63 \pm 2.75^{a,\ c}$
	Gobiesox marmoratus	Gmar	29	1	10.3^{b}	$1.00 \pm 0.83^{c,}$
Eleginopsidae	*Eleginops maclovinus*		2	5	100.0	3.00 ± 1.40
Clinidae	*Myxodes cristatus*	Mcri	7	3	$42.9^{a,\ b}$	0.43 ± 0.53^{c}
	Myxodes viridis	Mvir	17	2	11.8^{b}	0.18 ± 0.53^{c}
Gobiidae	*Ophiogobius jenynsi*	Ojen	5	1	40.0^{b}	0.40 ± 0.55^{c}
Blenniidae	*Scartichthys viridis*	Svir	42	1	2.4^{b}	$0.02 \pm 0.15^{c,\ d}$
	Hypsoblennius sordidus	Hsor	27	3	11.1^{b}	0.15 ± 0.46^{c}
Aplodactylidae	*Aplodactylus punctatus*		1	0	0.0	0.00 ± 0.00

*Abbreviated names are used in other results (Table 4 and Fig. 4)

Table 3. Prevalence (P, in %) and abundance (ABU) of parasite species found in 10 fish species collected in the rocky intertidal of Lebu, south-central Chile / Prevalencia (P, in %) y abundancia (ABU) de las especies de parásitos encontradas en 10 especies de peces recolectados en el intermareal rocoso de Lebu, centro-sur de Chile

Parasitic taxa	MS	*Notothenia* sp. (n 95)		*C. geniguttatus* (n 38)		*B. chilensis* (n 103)		*S. sanguineus* (n 19)		*H. sordidus* (n 27)		*M. cristatus* (n 7)		*M. viridis* (n 17)		*O. jenynsi* (n 5)		*S. viridis* (n 42)		*G. marmoratus* (n 29)	
		P	ABU	P	ABU	P	ABU	P	ABU	P	ABU	P	ABU	P	ABU	P	ABU	P	ABU	P	ABU
HIRUDINEA																					
Platybdellinae gen. sp.	A	1.1	0.01																		
COPEPODA																					
Acanthochondria sicyases	A							5.3	0.11												
Caligus cf. *cheilodactyli*	A§	51.5	1.37							3.7	0.04										
Holobomolochus chilensis	A	1.1	0.01	5.2	0.11																
Pennelidae gen sp.	A											14.3	0.14	5.9	0.06						
MONOGENEA																					
Microcotyle sp.	A			13.1	0.29							14.3	0.14								
Neobenedenia melleni	A			2.6	0.03																
DIGENEA																					
Helicometrina labrisomi	A			5.3	0.26																
Hemipera cribbi	A							5.3	0.05	3.7	0.07										
Lecithaster macrocotyle	A	80	4.66	18.4	0.37	97	0.02	31.5	0.89			14.3	0.14					2.4	0.024	10.3	1.00
Proctoeces sicyases	A							68.4	1.53												
Prosorhynchoides carvajali	I									3.7	0.04										
Lecithochirium sp.	I	10.5	0.14	2.6	0.08																
Opecoelidae gen. sp.	A	1.1	0.06																		
CESTODA																					
Tetraphyllidea gen. sp. (trilocular bothridia)	L§	12.6	0.02																		
Grillotidae gen. sp.	L§	6.3	0.11	2.6	0.03																
Nybelinia sp.	L§	8.4	0.084	2.6	0.03											20	0.2				
NEMATODA																					
Ascarophis sp.	A	1.1	0.01																		
Hysterothylacium sp.	L§	3.2	0.053	2.6	0.03	1	0.01														
Anisakis sp.	L§	8.4	0.178																		
Pseudoterranova sp.	L§	1.1	0.01																		
ACANTHOCEPHALA																					
Spiracanthus bovichthys	A					8.7	0.20														
Profilicollis bullocki	L	1.1	0.03			1	0.01														
Rhadinorhynchus sp. 1	A*§	1.1	0.01																		
Rhadinorhynchus sp. 2	A*§	9.5	0.12																		
Aspersentis sp.	A§	3.2	0.03											5.9	0.12						
Hypoechinorhynchus magellanicus	A§	50.5	1.42																		
Corynosoma australe	L	1.1	0.02																		
Corynosoma beaglense	L	3.2	0.05			1	0.01														
Polymorphidae gen. sp.	I	4.2	0.06			1	0.01														

MS: Maturity stage of parasites: L= larva: A= adult, I= immature. *: Sp. 1 with many and strong spines on anterior trunk and two lateral set of spines, proboscis long 14 rows with 23-24 hooks, including 1-2 basal hooks slightly smaller than anterior hooks; Sp. 2: with spines on the tenth anterior portion of trunk, dense distribution of spines on ventral than dorsal side, proboscis long with 24-25 hooks, including 11-12 basal hooks which are smaller than anterior hooks. §: Species not common in intertidal resident fish

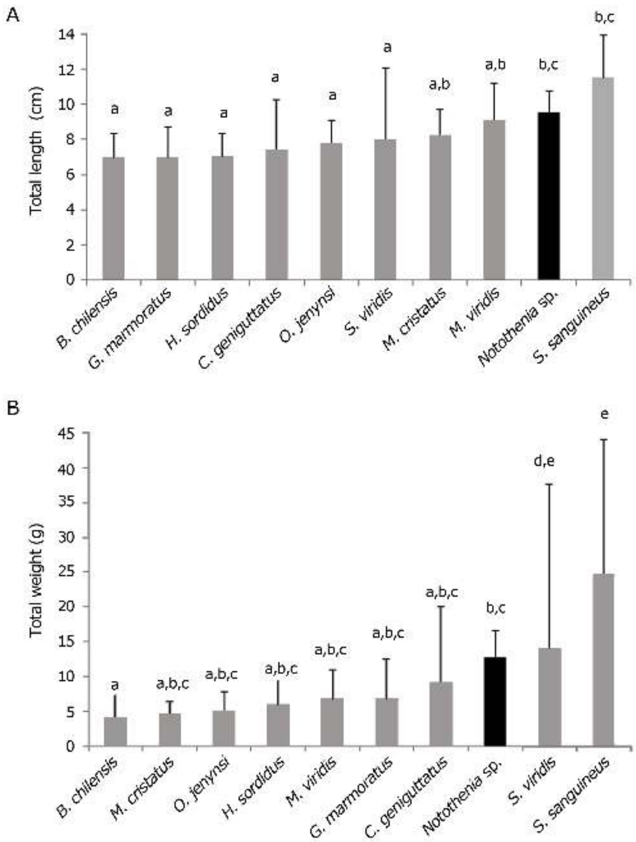

Figure 2. Averages of body sizes, A) total length and B) weight of each fish species obtained from Lebu, south-central Chile and organized from lowest to highest fish size. Different letters on the bars indicate significant differences in body sizes among species / Promedios de los tamaños corporales, A) longitud total y B) peso de cada especie de pez obtenidos de Lebu, centro-sur de Chile y organizadas de menor a mayor tamaño. Letras distintas sobre las barras indican diferencias significativas en tamaños corporales entre especies

were also highest in *Notothenia* sp. (Table 2). The parasite descriptors were not significantly related to fish body weight ($P > 0.2$) (Fig. 3). Although parasite prevalence tends to increase with fish weight, this correlation was not significant (Fig. 3).

The parasite community composition was also different between fish species; 16 (53.3%) out of the 30 parasite taxa found in total were only present in one host species; 10 taxa (33%) were in 2 host species, 3 taxa were present in 3 fish species, and only one parasite species (the digenean *Lecitasther macrocotyle*) parasitized 7 host species (Table 3). Nine parasite taxa of *Notothenia* sp. were also found in other fish species (Table 4). Consequently, the Jaccard similarity index was low, with a maximum of 33% for most pairs of host species, and high (100%) only for *G. marmoratus* and *S. viridis*, which were only parasitized by the digenean *L. macrocotyle* (Tables 3 and 4). In the NMDS diagram, few fish species were close to each other (except *G. marmoratus* and *S. viridis*) and several others were distant according to their parasite taxa (Fig. 4), that was supported by the ANOSIM results ($R= 0.63$; $P= 0.001$). However, the parasitic similarity among fishes showed in the NMDS had a stress value of 0.36 (Fig. 2; Stress= 0.06) suggesting a poor ordination fit (Clarke & Warwick 1994).

In addition to the high parasite richness of *Notothenia* sp., 2 parasite taxa stand out for their high prevalence and abundance: the copepod *Caligus* cf. *cheilodactyli* and the acanthocephalan *Hypoechinorhynchus magellanicus*, but also the presence of other parasites, such as *Rhadinorhynchus* spp., *Anisakis* sp. and several cestodes, are normally not present in intertidal resident fish, whereas *Lecithaster macrocotyle* is prevalent and abundant in several fish species (Table 3).

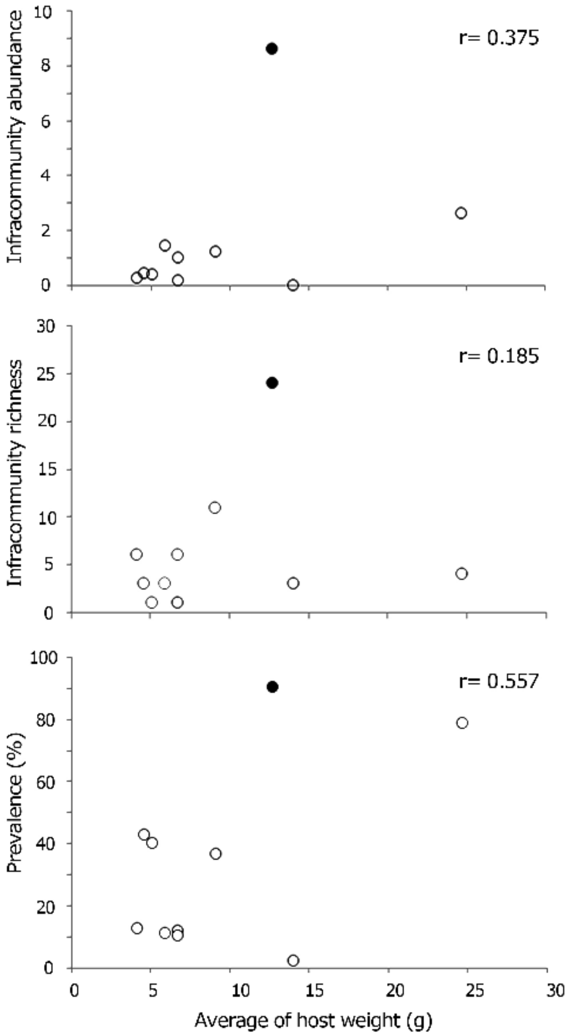

Figure 3. Mean abundance and infracommunity parasite richness and total prevalence of parasites related to host body weight of several species. The black dot on each graph indicates the position of *Notothenia* sp. r= Pearson correlation / Promedio de abundancia y riqueza infracomunitaria de parásitos y prevalencia total de parásitos en relación al peso corporal promedio de varias especies de peces. El punto negro en cada gráfico indica la posición de *Notothenia* sp. r= correlación de Pearson

Discussion

Identification of the notothen fish

The identification of *Notothenia* sp. from the intertidal of the central-south of Chile remains unclear. On the one hand, the specimens belong to the genus *Notothenia* because hypural 3 and 4 are completely fused (Fischer & Hureau 1985). On the other hand, *Notothenia* sp. has a morphological affinity with *Notothenia angustata*.

Table 4. Matrix of similarity on parasitological data, using the Jaccard index, between pairs of intertidal fish species of Lebu. Fish names are abbreviated as in Table 2 / Matriz de similitud basada en los datos parasitológicos, mediante el índice de Jaccard, entre pares de especies de peces intermareales de Lebu. Nombre de peces están abreviados como en Tabla 2

	Noto	Cgen	Bchi	Ssan	Hsor	Meri	Mvir	Ojen	Svir	Gmar
Noto	-	0.272	0.227	0.045	0.045	0	0	0.045	0	0.045
Cgen		-	0.153	0.083	0	0.2	0	0.111	0.111	0.111
Bchi			-	0.111	0	0.125	0	0	0.111	0.166
Ssan				-	0.2	0.166	0	0	0.25	0.25
Hsor					-	0	0	0	0	0
Meri						-	0.333	0	0.333	0.333
Mvir							-	0	0	0
Ojen								-	0	0
Svir									-	1
Gmar										-

However, the latter has been considered as *Paranotothenia* due to the distribution of its hypural bones (hypural 3 and 4 are partially fused). The specimens of this study were also similar to *N. microlepidota*, for which meristic data were from intertidal specimens of southern Chile (Pequeño 1976). Fischer & Hureau (1985) considered that *N. microlepidota*, *N. angustata,* and *P. magellanica* are morphologically similar and also co-occur in the same areas, and that they belong to the genus *Paranotothenia* because of the conformation of the hypural bones. Recently, a study based on both molecular and morphological aspects of notothen fish indicated that *Paranotothenia* should be considered as *Notothenia* (Sanchez *et al.* 2007) because of their high genetic similarity. Therefore, the use of the distribution of hypural bones can be a distinctive character among species and not between genera.

In our molecular analysis of *Notothenia* sp., the maximum genetic similarity (94%) was with *N. coriiceps* (which is Antartctic fish), but it was not concordant with the morphological analysis (Table 2). With the all information analyzed, it is not possible to determine with certainty the *Notothenia* species of the present study. This fish was morphologically similar to *N. microlepidota* recorded by Pequeño (1976) and *N.* cf. *angustata* used by Muñoz *et al.* (2001), all juveniles from the intertidal zone. These fishes formed one distant group from several other notothen species (Fig. 1); therefore, it is quite possible that this 'group of fish'may be one undescribed species.

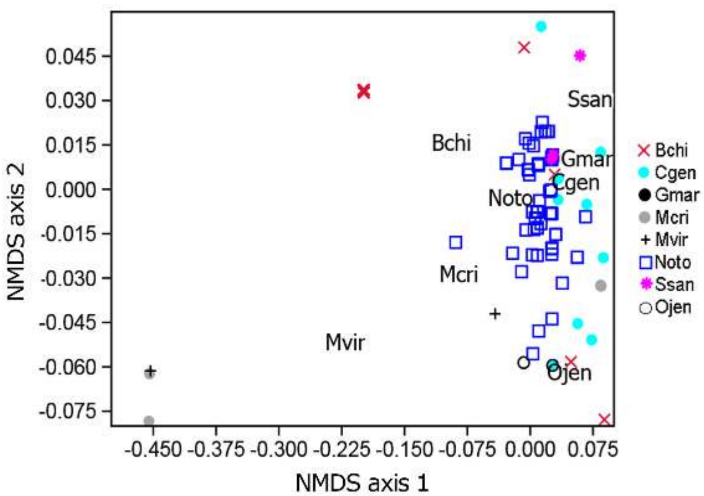

Figure 4. NMDS analysis of 10 host fish using the Jaccard similarity index based on the presence-absence of the parasite species. Fish names abbreviated as in Table 2 / Análisis NMDS en 10 peces hospederos, mediante el índice de similitud de Jaccard en base a la presencia-ausencia de las especies parásitas. Nombre de peces abreviados como en la Tabla 2

Composition of parasite community in intertidal fish

Of all fish species collected, *Notothenia* sp. had the greatest richness and abundance values at the level of parasite infracommunity. Moreover, *Notothenia* sp. hosted parasites that are not typical for intertidal fish, such as some anisakid nematodes, cestodes and acanthocephalans (Table 3). In addition to the high parasite richness, prevalence and average abundance of parasites was also high relative to other intertidal fish, which cannot be explained by the influence of body size – *Notothenia* sp. was a medium-sized fish (Fig. 2). The parasite fauna of this species was different, sharing no more than one-third of the parasite fauna with other fish species. There are three studies of parasite communities in *Notothenia* species, all of them related to *N. coriiceps* from the Antarctic zone (Szidat 1965, Palm *et al.* 1998, Zdzitowiecki & Laskowski 2004), which contrast with the parasite community of *Notothenia* sp. of this study, sharing only 4.2 to 5.2% (Jaccard index) of their parasite species. Whereas other Antarctic fish, such as *Trematomus* spp. (Zdzitowiecki & Ozouf-Costaz 2013), share between 14 and 23% of the parasite species with *Notothenia* sp. Hence, juveniles of *Notothenia* sp. are different from intertidal fish and also from other notothens.

The low similarity of parasite composition between intertidal fishes has been recorded before, which is due to several parasites of intertidal fishes are highly host-specific (Muñoz & Cortes 2009). Moreover, a temporal fish that spend a short time in the intertidal zone, as *Notothenia* sp., has low chance to become infected with parasites in that habitat. Consequently, a few generalist parasites could be acquired in the intertidal, such as the copepod *Holobomolochus chilensis* which has been recorded on several fish from the central coast of Chile (*e.g.,* Muñoz & Olmos 2007, Muñoz & Delorme 2011, Muñoz & Castro 2012), and the digenean *Lecithaster macrocotyle* that is a frequent parasite in several intertidal fish of central Chile (Soto *et al.* 2016), but it has also been found in different notothen species (Zdzitowiecki 1997), therefore, it is difficult to know if this digenean was acquired in the intertidal zone of central Chile.

Addressing how much of the parasitic fauna of *Notothenia* sp. is brought from another habitat, we found that several parasite taxa of *Notothenia* sp. are common in other environments (Table 3), for example, Tetraphyllidea, *Anisakis*, *Pseudoterranova*, and *Rhadinorhynchus* spp. are frequent parasites of subtidal fish, such as *Aphos porosus*, which are normally distributed from 5 m deep, and pelagic and demersal fish, *e.g., Trachurus murphyi, Scomber japonicus, Merluccius* spp., *Genypterus* spp., among others (Muñoz & Olmos 2008), whose bathymetric distribution can exceed 50 m. The copepod *Caligus* cf. *cheilodactyli* is common in subtidal fish, such as *Cheilodactylus variegatus*, *Chromis crusma, Eleginops maclovinus, Odontesthes regia, Prolatilus jugularis*, and *Sebastes capensis*, which live at depths comprised between 5 and 20 m, but *C. cheilodactyli* has not been recorded in intertidal fish (Muñoz & Olmos 2007). Moreover, the presence of the acanthocephalans

Aspersentis sp. and *Hypoechinorhynchus magellanicus* is interesting because they are normally found in notothen fish from Antarctic and sub-Antarctic zones (Laskowski & Zdzitowiecki 2017). *Hypoechinorhynchus magellanicus* has been also recorded in the benthopelagic fish *Eleginops maclovinus* (Sepúlveda *et al.* 2004), and in some intertidal fish although with low abundance and prevalence (Muñoz *et al.* 2002, Sepúlveda *et al.* 2004). Taken together, this information indicates that at least 11 parasite species of *Notothenia* sp. (nematodes, cestodes, acanthocephalans and a copepod) were acquired from a habitat other than the intertidal. Most notothen fishes are in Antarctic or Sub-Antarctic waters, mainly associated to the Cape Horn Current (DeWitt *et al.* 1990), but is also possible that *Notothenia* sp. is come from the Antarctic or sub-Antarctic zone and arrives, for a while, to the intertidal zone of central Chile for feeding, growing and surviving. Therefore, *Notothenia* sp. is one of the few notothen species that can reach northern distribution (up to 36°S).

Within the intertidal temporal fish species, the behavior of *Notothenia* sp. is unusual. Several species arrive as a larval stage, settle, and remain for a time as juveniles, until they have grown sufficiently to migrate to the subtidal, such Kyphosidae (*e.g.*, *Girella laevifrons* and *Graus nigra*) and Bovichthidae (*Bovichthus chilensis*), whereas other adult fish migrate from other habitats to the intertidal at a certain time of the year, to reproduce, as is the case of *Aphos porosus*. However, *Notothenia* sp. juveniles show a narrow range of body length, which corresponds to 104 to 126 days-old based on daily microincrements of the otoliths, determined in fish of 8 and 11 cm in length (Dr. Plaza, pers. comm.)[4]. This means that *Notothenia* sp. reaches the intertidal at an age of approximately 3 to 4 months-old. The narrow range of body lengths (between 8 and 14 cm in the whole sample) indicates that the stay in the intertidal zone is for only a short period of time. Instead, other fish, such as clingfish larvae (*S. sanguineus* and *G. marmoratus*), remain for 1 to 3 months in the plankton and settle in the intertidal with body lengths of 15-17 mm (corresponding to a recruiting age of 51 to 97 days) to become juveniles (Contreras *et al.* 2013). Similarly, *S. viridis* reach the intertidal zone after 30-45 days of larval development with a length of approximately 4 cm (Hernandez-Miranda & Ojeda 2006). Therefore, *Notothenia* sp. was a large fish for its age, suggesting a faster grow rate compared to other fish of similar age from the intertidal zone.

In conclusion, *Notothenia* sp. has high parasite richness, especially if we consider that the specimens analyzed were very young. We suggest that the majority of its parasitic fauna was acquired in an environment different from the intertidal, possibly between the subtidal and demersal zone, but also from the Southern Chile where notothens are common. Only a few generalist parasite species could be acquired when living in the intertidal zone. This interesting fish species has not yet been well identified and little or nothing is known about its ecological aspects, except those shown in the present study.

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