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Geographic variation in composition of metazoan parasite infracommunities in *Galaxias maculatus* Jenyns 1842 (Osmeriformes: Galaxiidae) in southern Chile (38-47° S)

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Abstract

Galaxias maculatus is an abundant freshwater fish species in Chilean continental waters where it plays important ecological functions, yet few parasitological records of this species exist in Chile and all of them cover a very limited geographic range. The objective of this study was to assess large scale geographic variation in composition of parasite infracommunities of *Galaxias maculatus*. Specifically, parasite infracommunities of this species were compared among 11 locations across 9 degrees of latitude and 3 ecosystem types (lake, river and estuary). Most taxa found had been previously reported in Chile and Argentina. However, this is the first report for *Tylodelphys* sp. in this host in Chile. Furthermore, the cranial parasite *Tylodelphys* sp. had the highest overall prevalence and abundance compared to other parasite species. Despite the fact that the abundance of *Tylodelphys* sp. was not significantly correlated with Fulton's condition factor of fish, infected fish seem to have a better body condition compared to uninfected ones. The most important source of variation in composition of infracommunities was the sampling location. Furthermore, fish from lakes have a different composition of parasite infracommunities mainly due to higher abundances of *Tylodelphys* sp.

Keywords: Geographic distribution, Estuary, River, Lake, Parasites

Background

Puye *Galaxias maculatus* Jenyns 1842 (Osmeriformes: Galaxiidae) is one of the most widely distributed freshwater fish species in the Southern Hemisphere [1]. In Chile, *Galaxias maculatus* is widespread and often abundant in rivers of central Chile and Patagonia, where it plays an important ecological role [2, 3]. There are several parasitological records for this species in Argentina, Australia and New Zealand [4–7], but just five in Chile [8–12] and these covered very limited geographic range (one to three sampling locations in each study). The aim of this study was to assess geographic variation in composition of infracommunities of metazoan parasites of *G. maculatus* in Chile (excluding Myxozoa). This was done by comparison of parasite communities among 11 locations across 9 degrees of latitude and 3 ecosystem types (lake, river and estuary, Fig. 1).

Methods

One-hundred and sixty-six individuals of *G. maculatus* were collected during 2013 using a beach seine (5 m long, 1.5 m high and 10 mm stretched mesh size). The beach seine was hauled over a distance of 10–20 m in

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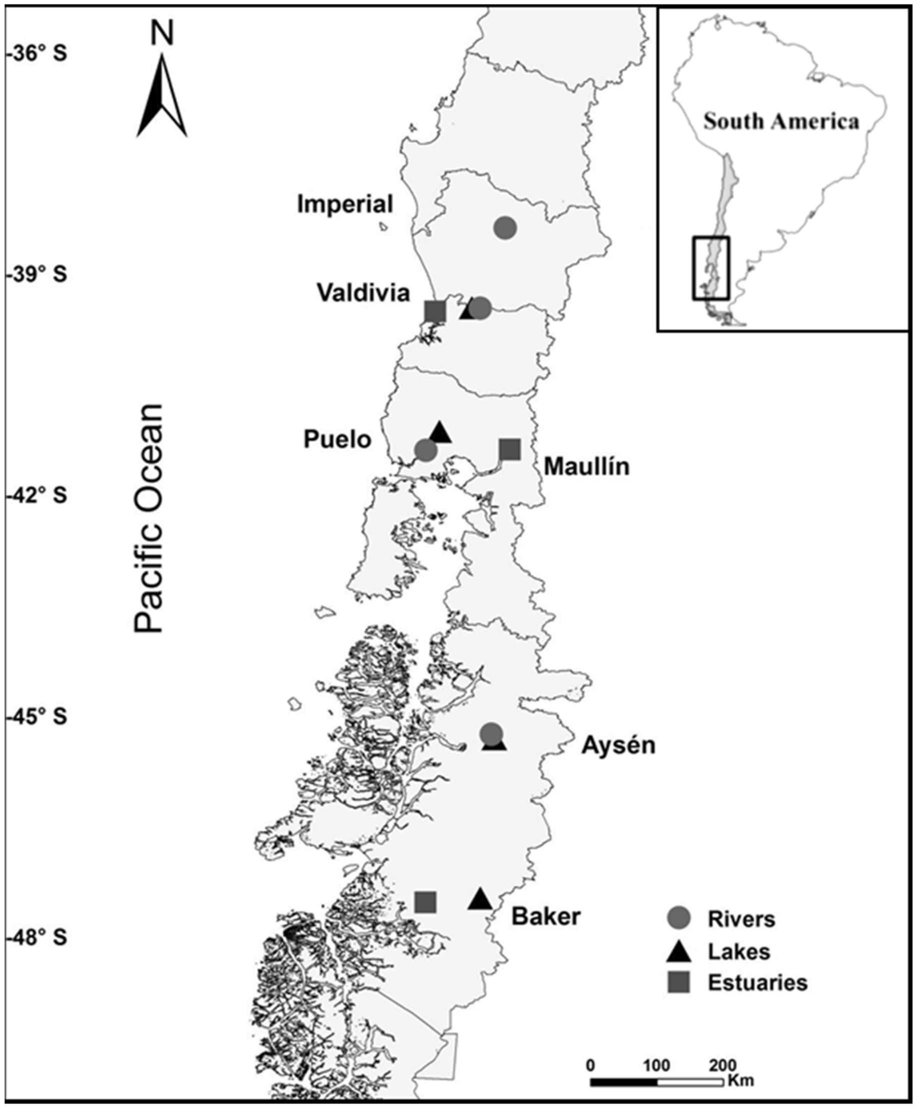

Fig. 1 The study area indicating fish sampling locations and ecosystems (rivers, lakes and estuaries)

the following river basins in southern Chile (Fig. 1: Imperial (river, 38°44′52.42″S, 73°24′27.19"O), Valdivia (lake, river and estuary, 39°51′55.33″S, 73°21′21.89"O), Maullín (lake and river, 41°12′38.98″S, 73°2′3.86"O), Puelo (estuary, 41°39′08.75″S, 72°18′16.78″W), Aysén (lake and river, 45°24′17.87″S, 72°47′41.62″W) and Baker (lake and estuary, 47°47′25.62″S, 73°29′57.42″W). Sample sizes ranged from 6 to 20 host fish per sampling location. Small sample size in some locations was due to low host abundances (catch per unit effort). Each fish specimen was measured (total length, TL; in mm) and weighed (total weight, W; in g), then preserved in 70% ethanol and stored individually. Subsequently, each specimen was thoroughly scraped on the body surface, fins and gills to look for ectoparasites. For endoparasites, we examined eyes, brain, heart, intestine, stomach, liver as well as the cranial and body cavity under a stereomicroscope. Finally, longitudinal cuts were made in the musculature to verify presence of metacercariae.

The variation in TL of *G. maculatus* among locations and ecosystem types was assessed using general linear models. Subsequently, the size effect was analyzed using partial eta-squared statistic. These analyses were performed in SAS/STAT/PROC GLM software, Version 9.3 (2011). A posteriori tests were run using GT-2 because of unequal sample sizes. The variation in the composition of parasite infracommunities was analysed using permutational multivariate analysis of variance (PERMANOVA) [13] based on fourth-root transformed data and Bray Curtis + 1 similarity index. In this analysis, the ecosystem type, sampling locations (nested within ecosystem type) were considered as factors, with total body length as covariate. A Principal Coordinates Analysis (PCoA) was used to visually display the distribution of parasite infracommunities among sampling locations and ecosystems using the vectors whose Spearman correlation coefficients were higher than 0.5. Further insight on this ordination was carried out with a One-way ANOVA on the scores of the first PCoA axis with the type of ecosystem as a factor. Furthermore, distance-based test for homogeneity of multivariate dispersions (PERMDISP) was used to compare composition of parasite infracommunities among ecosystems. These analyses were performed in PRIMER statistical program Version 7 [14]. In addition, a Spearman correlation coefficient was used to assess the association between the distances of sampling locations (in km) with the similarity in composition of parasite infracommunities. These analyses were performed in XLSTAT® software. Finally, the eventual effect of *Tylodelphys* sp. on Fulton's condition factor of fishes (a quantitative indicator of host's well-being) was assessed in two ways: by examining the statistical significance of the Spearman correlation coefficient between the abundance of parasites and the condition factor, and by performing a Mann-Whitney test to compare condition factor of fishes with and without *Tylodelphys* sp.

Results

Total length of 166 *G. maculatus* specimens showed medium size effect and significant variations among sampling locations (overall mean ± S.D. = 3.62 ± 0.56 cm, $F_{10, 155} = 15.04$, $R^2 = 0.49$, $P < 0.0001$, size effect partial eta-squared 90% confidence limits = 0.37–0.54). Specifically, the mean TL of specimens of the small sample from Puelo Estuary ($n = 6$) differed from other locations. Furthermore, fish collected from the Baker River basin (Lbak) were the smallest (Table 1). TL size effect showed significant though small variations among ecosystems ($F_{2, 163} = 4.13$, $P = 0.018$, $R^2 = 0.049$, size effect partial eta-squared 90% confidence limits = 0.00–0.10). More specifically, fish from rivers were larger than those from lakes (GT-2 Test, $P < 0.05$).

Sixty-four percent of the specimens harbored at least one parasite. A total of 12 metazoan taxa of parasites was recorded. Most taxa had low prevalence and abundance, except for a diplostomulum type larva found free (unencysted) in the meningeal space (overall prevalence = 49%). This parasite accounted for 89% of all 1214

Table 1 Number of fish examined, total length (mm), weight (g) standard deviation, and number of parasites per sampling location and taxon. Total number of taxa per location (last row) and total number of parasites per location as well as the range of locations in which each taxon occur (right column)

Sampling location	Ebak	Epue	Eval	Lays	Lbak	Lmau	Lval	Rays	Rimp	Rmau	Rval		
N fishes examined	15	6	15	15	15	6	22	20	20	19	13		
Total length (mm)	37	44	32	37	27	36	36	32	40	46	32		
Standard deviation (mm)	1,1	1,5	3,9	2,1	3,8	2,4	4,2	3,5	5,7	8,9	2,4		
Weight (g)	0,27	0,59	0,32	0,53	0,18	0,56	0,49	0,32	0,56	0,55	0,33		
Standard deviation (g)	0,17	0,14	0,11	0,13	0,11	0,14	0,13	0,10	0,18	0,09	0,07		
Parasite taxa (si; S)[a]												N parasites	N locations
Argulus sp. (sk, f; A)	0	0	1	0	2	0	0	0	10	24	0	37	4
Dactylogyridae gen. sp. (g; A)	0	0	0	0	0	3	7	6	1	1	0	18	5
Camallanus corderoi (i; L)	0	2	0	0	0	1	0	0	0	0	0	3	2
Cystidicoloides sp. (s; A)	0	1	0	0	0	0	1	0	1	1	0	4	4
Contracaecum sp. (m, bc; L)	2	1	0	0	1	5	0	0	20	3	0	32	6
Tylodelphys sp. (cc; L)	7	140	36	9	472	148	68	5	151	0	48	1084	10
Posthodiplostomum sp. (i, s; L)	0	0	0	0	0	1	0	0	1	0	0	2	2
Achanthostomoides apophalliformis (bc; L)	0	0	0	0	1	0	1	0	0	0	0	2	2
Metacercaria *A. apophalliformis* (l; M)	0	2	0	0	0	6	2	0	0	0	0	10	3
Allocreadium pichi (i; A)	0	0	0	0	0	9	0	0	0	0	0	9	1
Larva Cyclophyllidea (i; L)	1	0	0	0	0	1	0	0	3	0	0	5	3
Acanthocephalus sp. (i; A)	4	0	0	4	0	0	0	0	0	0	0	8	2
Total	14	146	37	13	476	174	79	11	187	29	48	1214	
N taxa	3	5	2	2	4	8	5	2	7	4	1		

[a] *si* site of infection, *i* intestine, *cc* cranial cavity, *g* gills, *m* mesenteries, *f* fins, *bc* body cavity, *l* liver, *s* stomach, *sk* skin
S Stage, *A* Adult, *L* Larva, *M* Metacercaria

Table 2 Summary of PERMANOVA of the composition of metazoan parasite infracommunities in 166 *G. maculatus* according to their total length (TL), type of ecosystem (Ec) and sampling location (Lo)

Source	d.f.	SS	MS	Pseudo-F	P (perm)	Perms
Total length	1	5384	5384,1	1134	0,340	999
Ecosystem	2	4759	2379,5	0,282	0,956	998
Lo(Ec)	8	61,025	7628,2	13,648	0,001	998
TLx Ec2		540	269,8	0,483	0,822	999
TL x Lo(Ec)	8	4407	550,9	0,986	0,469	997
Residuals	144	80,485	558,9			
Total	165	1566E+ 05				

parasitic individuals collected, and was the most prevalent and widespread parasite across locations (Table 1). This parasite was determined as *Tylodelphys* sp., in the absence of molecular confirmation. However, it is morphometrically similar to *Tylodelphys barilochensis* Quaggiotto and Valverde 1992 (Digenea: Diplostomatidae) (14), based on its body length and width (in microns) (body length: average ± S.D. = 456.3 ± 126.3, maximum body width: 127.3 ± 15.0, $n = 10$). There was no significant correlation between the Fulton's condition factor and the abundance of *Tylodelphys* sp. ($r_s = -0.196$, $P = 0.081$). However, condition factor of non-infected fish was lower than that of fishes infected with *Tylodelphys* sp. (Mann-Whitney U one-tailed test, U = 2871, $P = 0.032$).

Multivariate dispersion of parasite infracommunities did not differ among ecosystems (Pseudo-F $_{2,\ 163} = 2.09$, P (perm) = 0.188). The largest part of the variation was associated with sampling locations (GT-2 test, $P < 0.05$, Table 2). Two first axes of the PCoA analysis of the composition of parasite infracommunities accounted for 81.5% of the variation (Fig. 2). The infracommunities of the Maullín River separated from all other locations, mostly due to the absence of *Tylodelphys* sp. in this basin. Two other parasite taxa were relatively prevalent: the branchiuran *Argulus* sp. (mostly found in Maullín River), and the larval nematode *Contracaecum* sp. The one-way ANOVA on the scores of the first PCoA axis with the type of ecosystem as a factor revealed significant differences in composition of infracommunities in fish coming from lakes ($F_{7,\ 158} = 9.438$, $P < 0.0001$) due to higher abundance of *Tylodelphys* sp. in lakes than in rivers or estuaries (Table 2). The number of taxa per sampling location ranged from 1 to 8, and was not correlated with the number of fish examined (Spearman

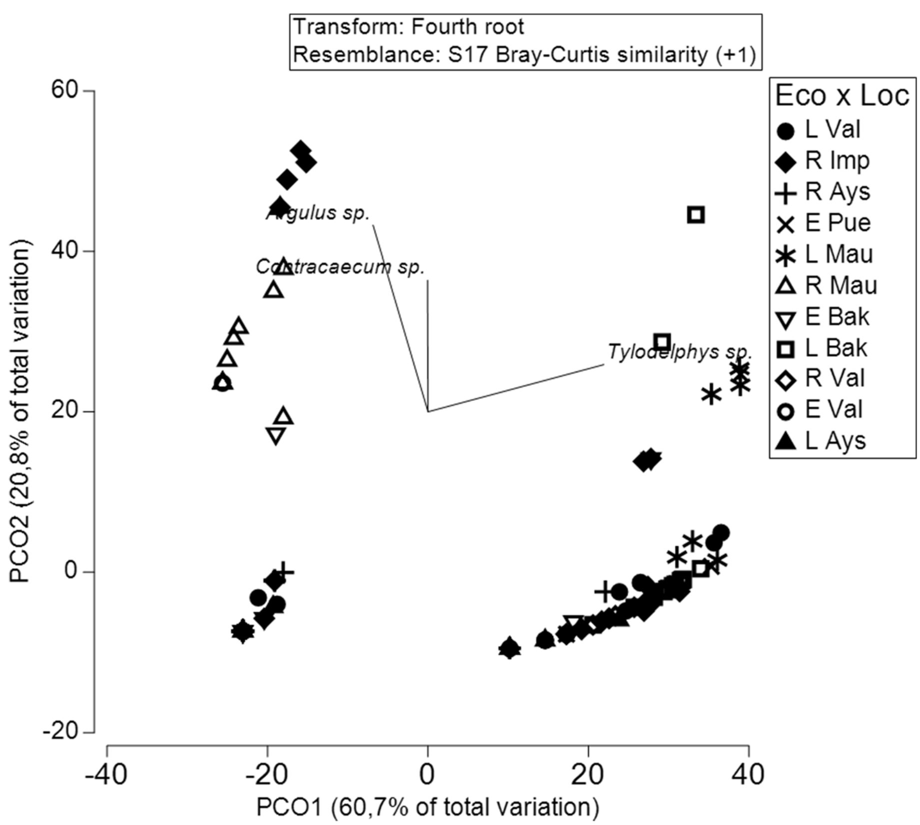

Fig. 2 Principal Coordinates Analysis (PCo) of the composition of parasite infracommunities in *G. maculatus* according to sampling locations and ecosystems. Vectors indicate species that had Spearman's rank correlations > 0.5

correlation, $r_s = 0.01$, $n = 11$, $P = 0.96$, Table 1). Finally, the distance between sampling locations was not correlated with the similarity of parasite infracommunities (Spearman correlation, $r_s = 0.007$, $n = 55$ pairs of distances between 11 sampling locations, $P = 0.55$).

Discussion

This report differs from previous ones in Chile, mainly due the broader geographical range and variety of ecosystems sampled. Previously, the only study on parasites of *G. maculatus* that considered more than two sampling locations in Chile dealt with *Stephanostomum* sp. metacercariae only [8]. Another difference of this report is that it is the first carried out at the infracommunity level. Major taxonomic differences found with previous studies was the lack of reports on *Tylodelphys* sp. for this host in Chile. In neighbouring Argentina, *Tylodelphys* spp. have been widely reported from several fish species [5, 6, 15–19]. This is also a frequent worm reported for many fish species and places elsewhere (see [20, 21]). *Austrodiplostomum mordax* Szidat and Nani, 1951 and *Tylodelphys destructor* Szidat and Nani 1951 were reported in another freshwater fish, *Basilichthys australis* Eigenmann 1927, from Lake Riñihue in the South of Chile [22, 23]. It is not surprising that fish infected with *Tylodelphys* sp. show a higher condition factor compared to uninfected fish, as previously reported for other endoparasites in freshwater fish [24]. Furthermore, this better condition is expected to be associated with strategies of transmission to definitive hosts [24].

Differences found in parasite infracommunity composition among sampled locations may be associated with the availability and vagility of intermediate and definitive hosts. For example, the absence of records of *Tylodelphys* sp. in *G. maculatus* in Chile may be due to the low abundance of its first intermediate host *Chilina dombeyana* (Bruguiére, 1789) in some sampling locations. In addition, spatial proximity of sampling locations was not a good predictor of similarity of parasite infracommunities, what has also been shown in other freshwater fish species [25]. With a mixture of larval and adult stages of parasites, there is likely a wide variety of hosts involved in parasite transmission, many of which are invertebrates that have limited dispersal ability, potentially resulting in clumped distributions. Although some results indicated that type of ecosystem was not a relevant factor to account for variations in composition of parasite infracommunities, the ANOVA on the scores of the first PCoA axis revealed that fish from lakes were different to those from rivers and estuaries, mainly due to higher abundances of *Tylodelphys* sp.. This pattern may be an effect of differential influence of autogenic and allogenic parasite species on patterns of parasite distribution and community composition [26, 27].

Abbreviations

ANOVA: Analysis of variance; Ec: Ecosystem; GLM: Procedure uses the method of least squares to fit general linear models; GT-2: Performs pairwise comparisons based on the studentized maximum modulus and Sidak's uncorrelated-t inequality; Lo: Locality; PCoA: Principal coordinates analysis; PERMANOVA: Permutational multivariate analysis of variance; PERMDISP: Test for homogeneity of multivariate dispersions; PROC: Procedure; command to run an analysis in SAS; S.D.: Standard deviation; SAS: Statistical Analysis System; online statistical software suite developed by SAS Institute for data management; TL: Total length; W: Weighed; XLSTAT: Data analysis add-in for Excel®

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Authors' contributions

MG-N analyzed and interpreted the data regarding the composition of infracommunities. RL performed the parasitological examination of the fishes, and was a major contributor to writing of the manuscript. KG sampled the fish and was a major contributor to the writing of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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