

A Global Assessment of Parasite Diversity in Galaxiid Fishes

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Abstract: Free-living species often receive greater conservation attention than the parasites they support, with parasite conservation often being hindered by a lack of parasite biodiversity knowledge. This study aimed to determine the current state of knowledge regarding parasites of the Southern Hemisphere freshwater fish family Galaxiidae, in order to identify knowledge gaps to focus future research attention. Specifically, we assessed how galaxiid–parasite knowledge differs among geographic regions in relation to research effort (i.e., number of studies or fish individuals examined, extent of tissue examination, taxonomic resolution), in addition to ecological traits known to influence parasite richness. To date, ~50% of galaxiid species have been examined for parasites, though the majority of studies have focused on single parasite taxa rather than assessing the full diversity of macro- and microparasites. The highest number of parasites were observed from Argentinean galaxiids, and studies in all geographic regions were biased towards the highly abundant and most widely distributed galaxiid species, *Galaxias maculatus*. Parasite diversity generally increased with the number of studies and individual fish examined, however studies which examined parasites from all body tissues could overcome the effects of low study effort. In order to promote further understanding of galaxiid–parasite biodiversity, we provide a series of recommendations, including the use of molecular techniques to verify parasite identity, and highlight the future roles both fish biologists and parasitologists can play.

Keywords: Galaxiidae; *Aplochiton*; *Brachygalaxias*; *Galaxias*; *Galaxiella*; *Lovettia*; *Neochanna*; *Paragalaxias*; infection



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1. Introduction

Parasites represent an often neglected, yet numerically and functionally important, component of global biodiversity [1]. Given the predominantly negative attention parasites receive, it is unsurprising that free-living species (hosts) have received greater biodiversity conservation attention as opposed to the affiliated parasites they support (e.g., [2]). However, as parasites are dependent on their hosts for survival, host population declines and extinctions likely result in parasite co-extinctions, especially for specialist parasites adapted to single host species [3–5]. Parasite conservation efforts may be further hindered by considerable disparities in our knowledge of parasite diversity amongst geographic regions, ecosystem types, host, and parasite groups [6–8].

Understanding which factors drive observed differences in parasite diversity has long been of interest in parasitology. At the geographic region scale, parasite diversity is strongly linked to the number of potential host species, which in turn is associated to a region's size (e.g., [9,10]), with larger geographical regions supporting a greater number of both host and parasite species. At the host species scale, parasite diversity is often linked to a series of host-specific ecological traits (e.g., body size, geographical range, diet), phylogenetic history (e.g., evolutionary age or distinctness), and environmental factors

(e.g., latitude [11,12]). In particular, host body size, geographical range size, and population density have been shown to be consistent drivers of parasite species richness across a wide range of host groups [13], with a greater variety of parasites likely to be encountered for those host species which occupy large geographical ranges and/or are large-bodied [14,15].

Research effort also plays a major role in the number of parasites documented in any host species [16], with fish parasites, for instance, often displaying aggregated distributions, both across the geographic range of their hosts and amongst individuals from a single locality [17]. There may also be mismatches between regions of high parasitology research effort and those with high freshwater fish diversity [18]. Furthermore, the taxonomic expertise of parasitology researchers may not encompass all macro- and microparasite groups encountered [19], with such problems even greater for non-parasitologists who may mistake parasites as ingested food items (Paterson pers. obs.) or morphological features of the host [20,21]. The use of molecular sequencing, which has become the standard practice for recently described parasite taxa [22,23], may partially assist in addressing limitations in morphological taxonomic expertise. Molecular sequencing has also revealed the presence of multiple genetically distinct cryptic parasite species from previously morphologically described single species [24].

The current study forms part of a special issue on Galaxiidae fish, a family of diadromous and landlocked freshwater fishes of Gondwanan origin that occur only in the cool temperate waters of the Southern Hemisphere (Oceania, South America, Africa [25,26]), and focuses on the diversity of parasites from the seven known galaxiid genera (*Aplochiton*, *Brachygalaxias*, *Galaxias* [Ga], *Galaxiella* [Gx], *Lovettia*, *Neochanna*, *Paragalaxias*). With the notable exception of *Galaxias maculatus*, and to a lesser extent *Aplochiton zebra*, *Galaxias platei* (Argentina, Chile and Islas Malvinas/Falkland Islands) and *Galaxias brevipinnis* (Australia and New Zealand), the majority of galaxiid species have restricted geographic distributions (e.g., *Galaxias globiceps*—two Chilean localities [27]). Whilst natural speciation and geomorphological processes have shaped galaxiid distribution patterns [28,29], the impacts of anthropogenic stressors (e.g., habitat fragmentation, interactions with exotic fish [30–32]) have reduced the geographic extent of many galaxiid species, which are now considered vulnerable or critically endangered [2]. However, previous studies (e.g., [33,34]) suggest that there are many galaxiid species and geographic regions for which a basic understanding of host–parasite interactions is lacking.

The primary objective of this study was to determine the current state of knowledge of parasite diversity in fishes of the family Galaxiidae and, as a consequence, identify knowledge gaps to focus future research attention. Specifically, we aimed to identify which galaxiid species were most understudied at the geographical region level. To do this, we assessed the study effort in terms of number of studies, locations, and fish examined, the extent of tissue examination, and taxonomic resolution of the described parasite assemblages. Furthermore, we evaluated whether ecological traits known to influence parasite richness were important drivers of observed differences in parasite assemblages among galaxiid species.

2. Materials and Methods

2.1. Data Collection

Studies investigating parasite taxa in fishes of the family Galaxiidae were obtained from literature databases (Web of Science, Google Scholar), the Google search engine, and fish-parasite species checklists (e.g., [34]) for 53 galaxiid species recognized in FishBase (pre-March 2020, [35]). The following search terms in English and Spanish were used to obtain studies: (“*Aplochiton*” OR “*Brachygalaxias*” OR “*Galaxias*” OR “*Galaxiella*” OR “*Lovettia*” OR “*Neochanna*” OR “*Paragalaxias*” OR “galaxiid”) AND (“parasit*” OR “infect*” OR “disease*”), in addition to individual searches for each galaxiid species in each geographic region. To evaluate the full extent of study efforts related to galaxiid parasite research, our database also included records obtained from technical reports (e.g., [36]) and unpublished theses (e.g., [37–39]). All possible efforts were made to obtain original publications through

direct contact with authors and inter-library requests, however parasite records were included from secondary data sources in instances where original publications were not available (e.g., [40,41]).

Our database includes both macro- (i.e., acanthocephalans, cestodes, copepods, molluscs, monogeneans, nematodes, trematodes, leeches) and microparasite species (e.g., myxozoans, ciliates). However, we acknowledge that there is likely a research attention bias towards macroparasite species [8,42], in addition to standard preservation techniques (e.g., freezing; formalin or ethanol fixing) being more suited for macroparasites.

We considered Argentina (AR), Australia (AU; including Lord Howe Island), Chile (CL), Islas Malvinas/Falkland Islands (M/F), New Caledonia (NC), New Zealand (NZ; including Auckland, Campbell, Chatham, and Stewart Island/s), and South Africa (SA) as separate geographic regions. From each study, we obtained the study location (waterbody name) and the number of individual fish sampled to quantify the study effort for each galaxiid species. Examination effort (full body, focal tissues, single species, or incidental) for each study was classified based on whether parasite assemblages were assessed from all body tissues, examination of focal tissues (e.g., alimentary tract [43], brain [44]), a single target parasite species (e.g., *Acanthocephalus galaxii* [45], *Philureter trigoniopsis* [46]), or were incidentally observed from a non-parasitology study (e.g., diet analysis [47]). We also determined the level of taxonomic resolution for each reported parasite taxa (species to phylum), in addition to whether the taxa were verified by molecular sequencing in addition to morphological identification.

To reflect changes in both valid taxonomy and taxonomic description level for each recorded parasite, the following parasite taxa were standardized between studies: *Coitocaecum anaspidis* (e.g., [48]) to *Coitocaecum parvum* [49]; *Stephanostomum* sp. [50] to *Acanthostomoides apophalliformis* [51,52]; *Diphyllobothium* (e.g., [53,54]) to *Dibothriocephalus* [55,56], *Nippotaenia* sp. [57,58] to *Ailinella mirabilis* (ex *Ga. maculatus* [59]) and *Galaxitaenia toloi* (ex *Ga. platei* [60]), *Echinocasmus* sp. [57] to *Stephanoprora uruguayense* [61], and *Diplostomum minutum* [62] to *Diplostomum* sp. [57]. We also standardized “nematode sp.” and “nematodes” to unidentified nematode (e.g., [63,64]); “adult cestodes” and “cestodes” to unidentified cestode (e.g., [65,66]); “encysted larval trematodes” to unidentified trematode (e.g., [67]). Furthermore, “cyst parasites”, “unidentified cysts”, or “species not specified” were standardized to unidentified parasite (e.g., [68,69]) to account for multiple parasite families known to encyst in intermediate or paratenic fish hosts. A full checklist of all parasite taxa described from each galaxiid species is provided in Table S1. In terms of galaxiid hosts, *Galaxias scribei* [70] and *Galaxias o’connori* [71] were standardized to *Ga. maculatus* and *Ga. olidus*, respectively, to reflect current taxonomy. We also recognize that Argentinean *Aplochiton taeniatus* populations studied by Ortubay et al. [57] are now considered to be *A. zebra* [72]. Records from unknown *Galaxias* sp. (e.g., [73]) and hybrids (e.g., *Galaxias depressiceps* x *Galaxias* sp. [68]) were excluded from the database.

2.2. Predictors of Parasite Diversity

We obtained information on the dominant traits recognized to influence parasite diversity in a host species (see [13]). For each fish species, host length (maximum standard length, mm) was obtained from FishBase [35], whereas the geographical range size was estimated for each geographic region as the distance (km) between the minimum and maximum reported latitude for each fish species (where 1° of latitude equals 111.19 km [26,35,74–78]). Population density was not consistently available across galaxiid species and was, therefore, not included in this study.

2.3. Statistical Analysis

All statistical analyses were conducted in R version 3.6.1 [79]. Islas Malvinas/Falkland Islands were excluded from all analyses due to small sample sizes but are included in figures for illustrative purposes. Continuous variables were centered on the mean and scaled by two standard deviations prior to analysis [80]. In all instances, parasite taxa

refer to the lowest taxonomic level reported for each documented parasite. The combined effects of geographic region, study effort, host length, and geographic range size on the number of parasite taxa reported in each galaxiid species were tested using a generalized linear model (glm) fitted with a quasipoisson distribution to account for over-dispersed count data. Study effort was modelled in two separate analyses as (i) the number of studies per galaxiid species where parasites were reported or (ii) the number of individual fish examined for parasites per galaxiid species, with the latter representing a reduced dataset, since the number of fish examined was not reported in all data sources (e.g., [34,81,82]). Post hoc pairwise comparisons for interactions between categorical (i.e., geographic region) and continuous (i.e., study effort, host length, geographic range size) variables were made by calculating estimated marginal means using `emmeans::lsmeans`, whereas estimated marginal means of linear trends were calculated for interactions between two continuous variables (`emmeans::emtrends` [83]). Differences in the number of study locations per galaxiid species between geographic regions were tested using a generalized linear model fitted with a quasipoisson distribution to account for overdispersion, with significant differences between geographic regions tested with estimated marginal means. Separate glms fitted with a binomial distribution were used to test whether (i) the proportion of parasite taxa described to species level or (ii) the portion of macroparasites out of the total number of parasite taxa described for each galaxiid species differed amongst geographic regions.

3. Results

Parasite taxa were reported from half of all 53 galaxiid species (Argentina (3/3), Australia (12/24), Chile (5/8), Islas Malvinas/Falkland Islands (2/3), and New Zealand (13/22 species; Table 1)). No parasite records were found for the single galaxiid species of South Africa (*Galaxias zebratus*) or New Caledonia (*Galaxias neocaledonicus*), the four Australian *Paragalaxias* species, nor for galaxiid species occurring on Australian or New Zealand offshore islands. The most widely distributed galaxiid species, *Ga. maculatus*, was also the most commonly studied species in terms of each geographic region, the total number of studies (92/143 studies) and the number of individuals examined (28,931/37,730 individuals; Table 1).

The number of parasite taxa recognized from galaxiid species was influenced by two-way interactions between the number of studies, geographic region, and latitudinal range size (all $p < 0.02$; Table 2). Whilst a greater number of parasite taxa were documented from Argentinean galaxiids overall (Figure 1a), pairwise comparisons of slope estimates demonstrated that the number of parasites reported from Argentinean galaxiids showed the least variation from the least to the most studied galaxiid species (n studies/n parasite taxa: *A. zebra* 3/18; *Ga. maculatus* 48/37) compared to all other geographic regions (Tables A1 and A2). In contrast, an increasing number of studies had the strongest influence on the number of parasite taxa reported from Chilean galaxiids, ranging from a single *A. taeniatus* study reporting one parasite to 23 parasite taxa recognized from 14 *Ga. maculatus* studies. An increasing number of studies had intermediate effects on the number of parasite taxa reported from Australian and New Zealand galaxiids, which did not differ from one another. Whilst an interaction between latitudinal range size and geographic region on the number of parasite taxa reported was also detected (Table 2, Figure 1b), post hoc comparisons suggest this relationship is weak (Tables A1 and A2). The positive effect of latitudinal range size on the number of parasite taxa reported was shown to decrease with increasing study effort (n studies), with latitudinal range size of galaxiids having little influence on the number of parasite taxa reported when the study effort approached 15 studies per fish host species (Figure 2). Standard length did not influence the number of parasites reported across all studies.

Table 1. Cont.

Geographic Region	Galaxiidae Species	N Studies	N Fish ^a	N Locations	Max. Standard Length (cm)	Latitudinal Range Size (km)	N Taxa (Macro/Micro)	Taxonomic Resolution						N Molecular ^e	Examination ^f			
								Species	Genus	Family	Order	Class	Phylum		Full Body	Focal Tissue	Single Species	Incidental
New Zealand	<i>Galaxias anomalus</i>	4	743	6	6.1	111.7	7 (7/0)	6	1	0	0	0	0	0	1	0	3	0
	<i>Galaxias argenteus</i>	2	114	2	34.0	173.4	4 (4/0)	3	0	0	0	1	0	0	0	1	0	1
	<i>Galaxias brevipinnis</i>	12	36	10	28.0	1319.9	13 (11/2)	7	4	0	1	1	0	0	0	4	4	0 ^d
	<i>Galaxias cobitinis</i>	-	-	-	7.0	142.3	-	-	-	-	-	-	-	-	-	-	-	-
	<i>Galaxias depressiceps</i>	1	51	4	7.3	129.5	2 (2/0)	2	0	0	0	0	0	0	0	1	0	0
	<i>Galaxias divergens</i>	2	unknown	2	6.0	542.4	4 (4/0)	3	1	0	0	0	0	0	0	0	1	0 ^d
	<i>Galaxias eldonii</i>	-	-	-	7.5	47.8	-	-	-	-	-	-	-	-	-	-	-	-
	<i>Galaxias fuscatus</i>	1	unknown	1	21.5	1423.9	1 (1/0)	1	0	0	0	0	0	0	0	0	0	0 ^d
	<i>Galaxias gollumoides</i>	1	7	1	17.2	249.1	2 (2/0)	2	0	0	0	0	0	0	0	1	0	0
	<i>Galaxias gracilis</i>	-	-	-	6.2	80.3	-	-	-	-	-	-	-	-	-	-	-	-
	<i>Galaxias macronasus</i>	-	-	-	7.0	99.3	-	-	-	-	-	-	-	-	-	-	-	-
	<i>Galaxias maculatus</i>	20	989	11	19.0	1424.5	18 (17/1)	9	4	0	3	2	0	1	1	4	12	1 ^d
	<i>Galaxias paucispondylus</i>	-	-	-	8.8	468.7	-	-	-	-	-	-	-	-	-	-	-	-
	<i>Galaxias postvectis</i>	1	unknown	1	26.0	1229.1	1 (1/0)	1	0	0	0	0	0	0	0	0	0	0 ^d
	<i>Galaxias prognathus</i>	-	-	-	8.0	213.5	-	-	-	-	-	-	-	-	-	-	-	-
	<i>Galaxias pullus</i>	-	-	-	13.1	50	-	-	-	-	-	-	-	-	-	-	-	-
	<i>Galaxias vulgaris</i>	4	52	4	8.9	374.7	3 (2/0) ^c	1	0	0	0	0	1	0	1	1	1	0 ^d
	<i>Neochanna apoda</i>	4	312	8	9.6	450.8	8 (6/1) ^c	3	1	2	0	0	1	1	0	1	1	0
	<i>Neochanna burrosius</i>	1	1091	4	13.3	218.9	4 (0/2) ^c	1	1	0	0	0	0	0	0	1	0	0
	<i>Neochanna diversus</i>	1	unknown	1	12.2	434.9	1 (1/0)	0	0	0	0	0	1	0	0	0	0	0 ^d
<i>Neochanna heletos</i>	-	-	-	10.5	28.4	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Neochanna rekohtua</i>	-	-	-	6.6	4.7	-	-	-	-	-	-	-	-	-	-	-	-	
South Africa	<i>Galaxias zebraus</i>	-	-	-	6.8	444.8	-	-	-	-	-	-	-	-	-	-	-	-

Notes: Geographic regions—Australia (including Lord Howe Island), New Zealand (including Auckland, Campbell, Chatham, and Stewart Island/s). ^a Number of fish examined from studies reporting study effort, ^b Tritt [37] reported no parasite taxa from examined fish, ^c unidentified parasites, ^d examination effort unknown or partially known among studies, ^e number of parasite taxa identified by molecular methods, ^f extent of tissues examined.

Table 2. Influence of study effort (number of studies or fish), geographical region, fish length (standard), and latitudinal range size (km) on the number of parasite taxa reported from galaxiid fish. Statistically significant differences in parameters ($\alpha = 0.05$) are in bold.

	Df	Deviance	Residual Df	Residual Deviance	F	p
NULL			32	251.20		
N studies	1	134.32	31	116.88	423.59	<0.001
Region	3	20.95	28	95.93	22.03	<0.001
Fish length	1	1.44	27	94.49	4.54	0.050
Range size	1	21.79	26	72.70	68.73	<0.001
N studies × Region	3	49.95	23	22.75	52.51	<0.001
N studies × Fish length	1	0.47	22	22.28	1.47	0.245
N studies × Range size	1	9.49	21	12.79	29.93	<0.001
Region × Fish length	3	2.61	18	10.18	2.74	0.080
Region × Range size	2	3.81	16	6.37	6.01	0.012
Fish length × Range size	1	1.39	15	4.98	4.39	0.054
NULL			24	172.76	NA	NA
N fish	1	67.68	23	105.07	40.21	<0.001
Region	3	40.13	20	64.94	7.95	0.012
Fish length	1	3.79	19	61.15	2.25	0.177
Range size	1	20.98	18	40.17	12.46	0.010
N fish × Region	3	18.44	15	21.73	3.65	0.072
N fish × Fish length	1	0.56	14	21.17	0.33	0.582
N fish × Range size	1	3.11	13	18.06	1.85	0.216
Region × Fish length	3	0.67	10	17.39	0.13	0.938
Region × Range size	2	3.79	8	13.60	1.13	0.376
Fish length × Range size	1	1.91	7	11.69	1.14	0.322

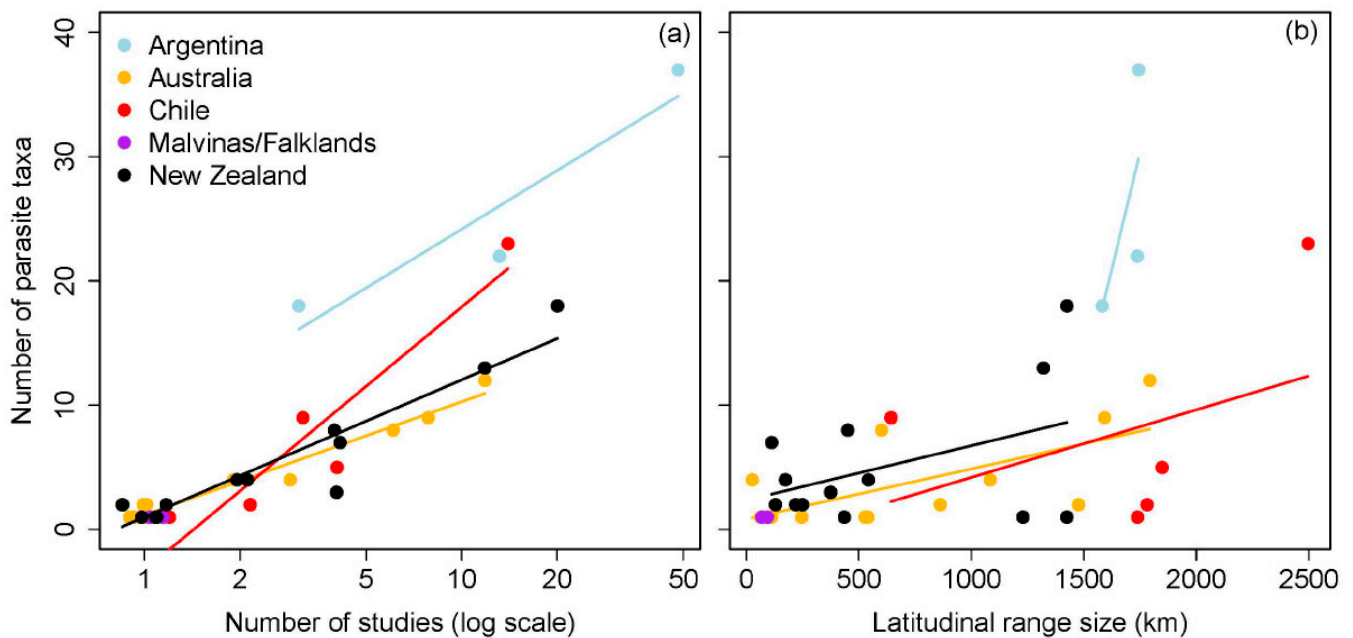


Figure 1. Influence of the (a) number of studies (log scale) and (b) latitudinal range size (km) on the number of parasite taxa reported for each galaxiid species per geographic region.

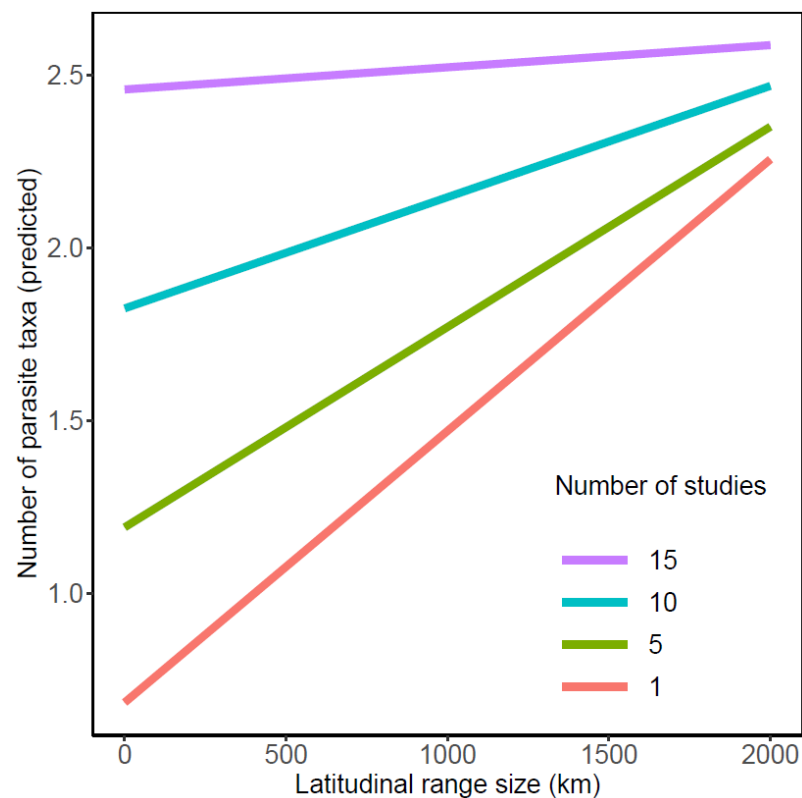


Figure 2. Influence of latitudinal range size (km) and the number of studies on the predicted number of parasite taxa reported in galaxiid fishes.

The number of fish examined was reported from 66.4% of studies ($n = 40$ Argentina, 18 Australia, 16 Chile, 2 Malvinas/Falklands, 19 New Zealand). The number of parasite taxa was positively correlated with both the number of fish examined and latitudinal range size (Table 2, Figure 3) and also differed between geographic regions, with significantly more parasite taxa reported in Argentina than other geographic regions (Tukey HSD post hoc test: all $p < 0.05$). Standard length did not influence the number of parasite taxa per host species among studies where the number of fish examined was reported.

The number of locations from which parasites were studied differed among geographic regions (GLM_{COUNTRY}: $F_{3,29} = 6.49$, $p = 0.002$; Table 1), with a greater number of localities per galaxiid species studied in Argentina compared to New Zealand and Australia (pairwise comparisons, $p < 0.001$; Table A3), where parasites were frequently assessed from one or two locations only. Thirty or more individual fish were examined from all study locations for three galaxiid species, *Galaxiella toourtkoourt* (Australia, n locations = 4), *A. zebra* (Malvinas/Falklands, $n = 1$), *Neochanna burrowsius* (New Zealand, $n = 4$), with parasite assemblages observed from fewer than 30 individual fish per location for most other galaxiid species (Table 1).

Parasites from Argentinean and Chilean galaxiids were most commonly recorded from single species studies, whereas parasites from Australian and New Zealand galaxiids were observed from both studies focusing on single parasite species and examinations of focal tissues (Table 1). *Aplochiton zebra* from Argentina was the only galaxiid species for which all studies ($n = 3$) in a single geographic region involved full examinations of all body tissues. Incidental observations of parasites observed during non-parasitology studies contributed a total of seven studies across all galaxiid species. We also note that no parasite taxon was detected from the single parasitological study assessing Australian *Galaxiella nigrostriata* ($n = 779$ fish [37]), however this study focused on the detection of a single parasite species.

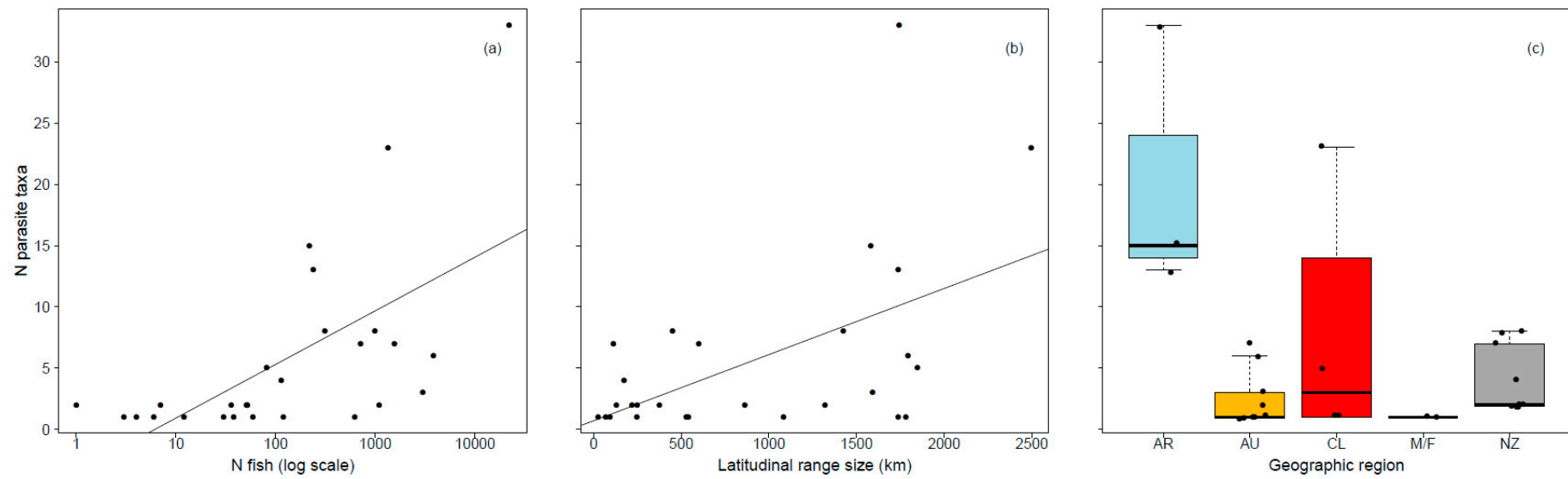


Figure 3. Influence of the (a) number of fish (log scale), (b) latitudinal range size (km), and (c) geographic region on the number of parasite taxa reported in galaxiid fishes (AR Argentina, AU Australia, CL Chile, M/F Islas Malvinas/Falkland Islands, NZ New Zealand).

Approximately 60% of parasite taxa described from Galaxiidae were described to species level using morphological taxonomy, with no observed difference in the proportion of parasites described to species-level between regions (GLM_{REGION}: $\chi^2 = 0.99$, $df = 3$, $p = 0.805$; Figure 4). Of these parasites, identification was verified with molecular sequencing for one new (host–parasite (country): *Ga. maculatus*—*Ortholinea lauquen* (AR) [84]) and three previously described species (*Galaxiella pusilla*, *Gx. toourtkoourt*—*Apatemon gracilis* (AU) [85], *Galaxias occidentalis*—*Lernaea cyprinacea* (AU) [86], *Ga. maculatus*—*H. spinigera* (NZ) [87]). Molecular tools were also used to describe a further two new parasite taxa to family level (*Ga. maculatus*, *Ga. occidentalis*—Diplostomoidea (AU) [37]) and subfamily levels (*Neochanna apoda*—Capillariinae (NZ) [88]). Furthermore, all geographic regions consistently reported more macroparasite taxa (mean 86.5%) than microparasites from studied galaxiids with no differences among regions (GLM_{REGION}: $\chi^2 = 1.09$, $df = 3$, $p = 0.779$; Table 1).

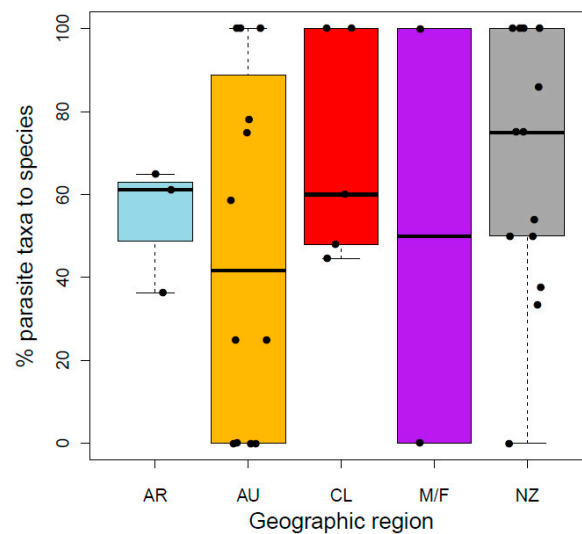


Figure 4. Percentage of parasite taxa described to species level in each galaxiid species per geographic region (AR Argentina, AU Australia, CL Chile, M/F Islas Malvinas/Falkland Islands, NZ New Zealand).

4. Discussion and Conclusions

Our study demonstrates a number of knowledge gaps in relation to the current understanding of galaxiid–parasite diversity, including the absence of parasitological studies from 26 out of 53 recognized galaxiid species. This suggests that the diversity of parasites supported by this fish family is only partially understood. However, in the global context of the >34,000 recognized fish species, previous studies (e.g., [89,90]) suggest that most of the other 579 fish families are likely to have parasites described from less than half of their recognized species. It is, however, important to note that whilst galaxiids may in fact represent one of the better-known fish families in terms of parasitological investigations, it does not necessarily imply that research efforts have been distributed evenly across member species. Our study demonstrates that *Ga. maculatus* has been extensively studied in most regions, though relatively limited parasitological knowledge is available for other galaxiid species. We also note that almost 20 years after McDowall [33] highlighted the absence of parasitological studies from Australian and New Zealand offshore islands, South Africa, New Caledonia, and Islas Malvinas/Falkland Islands, progress towards characterizing the galaxiid–parasite assemblages has only been made in the latter island group [47,91].

In general, study effort, in terms of the number of studies or the number of fish examined per galaxiid species, was a strong predictor of the number of parasite taxa reported; however, examination effort has the potential to overcome the effects of low study effort in some instances. Whilst the majority of Argentinean studies were focused on single parasite taxa, all three studies ($n = 254$ fish) investigating the parasites of *A. zebra*

consisted of full examinations of all host tissues. This resulted in a greater number of parasite taxa documented from this fish species relative to study effort in terms of either the number of studies or fish examined and the least discrepancy in the number of parasite taxa reported with regard to study effort for Argentinean galaxiids. Differences in study effort among geographic regions may result from a combination of local researcher interests and the diversity of freshwater fish assemblages in each geographic region. The eight known galaxiid species of South America, distributed across the ichthyogeographic Patagonian province and the south of the Chilean Province [92,93], occupy a region of relatively low freshwater fish diversity (29 fish species [26]). In contrast, galaxiids comprise 22 out of New Zealand's 62 freshwater fish species, with even greater freshwater fish diversity occurring in South Africa (1 galaxiid/180 freshwater fish species), and the majority of Australian states where galaxiids are distributed (e.g., New South Wales: 4/81, Queensland: 2/191, Western Australia: 5/134 [35]). Bearing in mind the recent expansion in galaxiid taxonomic resolution (e.g., [94–96]), the number of recognized galaxiid species with undescribed parasite assemblages is set to grow.

Surprisingly, fish length was not found to influence the number of parasite taxa reported in galaxiids, despite larger, long-lived hosts, with associated ontogenetic diet changes often resulting in encounters with a wide range of parasites [97–99]. The absence of such patterns may be attributed to the majority of studies focusing on *Ga. maculatus*, a relatively small (<110 mm), short-lived species (maximum age class 3+ years [100]). The close proximity of research laboratories to this highly abundant, widely distributed galaxiid, combined with an ever-increasing list of described parasite taxa, has ensured that *Ga. maculatus* has remained highly attractive to fish parasitologists (e.g., [101,102]). If body size and age do in fact influence parasite diversity within the Galaxiidae family, then a considerable number of parasite species may currently be undiscovered, especially from the large, long-lived species such as *Ga. platei* (max. age = 18 years [103]) and *Galaxias argenteus* (20 years [104]). Positive correlations between latitudinal range size and the number of parasites also suggest that widely distributed species such as the Chilean *A. zebra* and *A. taeniatus* or Australian *Ga. brevipinnis* (range size >1500 km) have the potential to support a greater diversity of parasite taxa than the New Caledonian *Ga. neocaledonicus* or New Zealand's *Neochanna rekohua* (range size <5 km), however our results display that the effects of latitudinal range size may diminish with increasing study effort.

Our study demonstrated that with the exception of recent taxonomic studies, there has been limited use of molecular techniques to confirm the identity of galaxiid parasites. For example, the trematode responsible for black spot disease in the muscles of Australian galaxiids has now been reclassified (from *Diplostomum galaxiae* to "Dip01" aligned with *Posthodiplostomum* spp., and family Strigeidae [37]). However, the majority of parasite taxa reported from galaxiid hosts rely on morphological descriptions only, of which many parasite taxa were first described in other freshwater fish in the same geographic region. Whilst galaxiids may share generalist parasite species capable of infecting fish species from other families (e.g., *Acanthocephalus tumescens* in Atherinopsidae, Galaxiidae, Diplomystidae, Percichthyidae, and Salmonidae [58]), the increasing recognition of cryptic parasite species suggests that galaxiids may be host to their own unique parasites. For instance, molecular approaches demonstrate that the trematode *Stegodexamene anguillae* and the acanthocephalan *A. galaxii*, which are common parasites of New Zealand galaxiid fishes, may consist of multiple cryptic species, each very host-specific (Hernandez-Orts unpublished, [105]).

Our study also suggests that although the current understanding of macroparasite diversity in galaxiids may be patchy, our knowledge of microparasite diversity is even more limited, with a greater proportion of species reported being macroparasite taxa rather than microparasites, from both single parasite studies and full assessments of parasite assemblages (i.e., parasite component population and community [106]). Although the majority of microparasite species have been reported from galaxiids originating from Argentina (e.g., [84,107–109]), this may be evidence of study bias towards the research interests of local parasitologists [18], rather than the absence of microparasites from other

regions. Reporting bias is also likely to occur with macroparasite taxa, though to a lesser degree, where single target parasite studies are unlikely to report the presence of other encountered parasite taxa [110].

Many galaxiid species for which parasites have not been described represent species that have either been recently described, are of conservation concern, and/or are from remote localities, thus obtaining specimens from which to evaluate parasite diversity may be logistically difficult. Ethical constraints must also be taken into consideration given that, with the exception of some ectoparasites, current practices for assessing parasite diversity in fishes usually involve lethal sampling, though the development of non-lethal methods for detecting fish endoparasites offers a promising solution [111,112]. Whilst our study suggests that incidentally reported parasites in non-parasitology studies have made only minor contributions to knowledge of parasite diversity in galaxiids, greater communication between fish biologists and parasitologists could ensure that full biological information is obtained (i.e., host taxonomy, age structure, diet, and their parasites [113]), provided that collected material is preserved in a way that is useful to parasitologists [114]. Parasitologists must also play their part by sampling all parasites from collected fishes, especially when conservation or ethical considerations are involved [18]. However, there may be some fish species for which parasite diversity must remain unknown or only partially evaluated, since gaining knowledge about parasite diversity should not come at the expense of sampling either a parasite or its host to extinction.

Access to parasite specimens will only partially address galaxiid–parasite knowledge gaps, since the number of taxonomically skilled parasitology researchers and availability of funding to support taxonomic research in a geographical region may be limited. Here, collaborations with research institutes with the necessary morphological and molecular parasite taxonomy expertise is vital and may also help to address the lack of attention directed towards microparasite species. Existing collaborations between Argentinean and Chilean researchers have aided the description of new parasites from *Ga. maculatus* [115] and *B. bullocki* [116], with such collaborations largely stemming from a strong history of joint parasitology meetings between these regions. However, additional effort is required to address the gaps in parasite taxonomy expertise, and thus we encourage measures that enable taxonomic experts based outside the geographic range of galaxiids to access samples. We support Poulin et al.'s [18] suggestion of an online database of parasite specimens, which not only documents the host species and geographic localities surveyed for parasites, but also facilitates greater international collaboration among parasitologists. Furthermore, we strongly encourage the deposition of voucher specimens into museum collections, especially from host species that have received little parasitological attention.

Improving our understanding of galaxiid–parasite associations has the potential to shift general attitudes concerning parasites in the conservation and management of galaxiid populations. However, there remains much progress to be made until parasites are no longer regarded as simply threats to galaxiid survival that need to be minimized or eliminated in management efforts [67,117,118]. The recognition that the unique and potentially co-endangered parasite assemblages supported by galaxiid hosts are themselves important in biodiversity conservation [119] will contribute to efforts to co-manage hosts and parasites and ensure the retention of parasites and the ecosystem functions they provide in conservation programs.

Supplementary Materials: The following are available online at <https://www.mdpi.com/1424-2818/13/1/27/s1>, Table S1. Checklist of parasite taxa detected in fishes of the Galaxiidae family.

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Appendix A

Table A1. Slope average estimates describing the effect of study effort (number of studies) and latitudinal range size on the number of parasite taxa reported from galaxiid fishes from each geographical region.

Base Variable	Country	Slope Estimate	SE	95% Confidence Interval (Lower, Upper)
N studies	Argentina	18.94	1.10	16.68, 21.19
	Australia	6.18	0.51	5.13, 7.22
	Chile	9.08	0.66	7.71, 10.44
	New Zealand	6.23	0.42	5.37, 7.09
Latitudinal range size	Argentina	−31.44	31.80	−96.93, 34.06
	Australia	4.57	1.63	1.22, 7.93
	Chile	3.78	3.95	−4.35, 11.92
	New Zealand	6.42	1.75	2.82, 10.02
Latitudinal Range Size	N Studies	Trend Estimate	SE	Asymptotic 95% Confidence Interval (Lower, Upper)
	1	0.0008	0.0002	0.0003, 0.001
	5	0.0006	0.0002	0.0002, 0.001
	10	0.0003	0.0003	−0.0003, 0.0001
	15	<0.0001	0.0005	−0.001, 0.001

Table A2. Tukey pairwise comparisons investigating the effect of geographic region on the number of reported parasite taxa per galaxiid species in relation to number of studies. Statistically significant estimates ($\alpha = 0.05$) are in bold.

Base Variable	Contrast	Estimate	SE	t Ratio	p
N studies	Argentina–Australia	12.76	1.21	10.56	<0.001
	Argentina–Chile	9.86	1.28	7.70	<0.001
	Argentina–New Zealand	12.70	1.17	10.83	<0.001
	Australia–Chile	−2.90	0.83	−3.47	<0.001
	Australia–New Zealand	−0.05	0.66	−0.08	1.000
	Chile–New Zealand	2.84	0.78	3.63	0.007
Latitudinal range size	Argentina–Australia	−36.01	31.84	−1.13	0.674
	Argentina–Chile	−35.22	32.04	−1.10	0.693
	Argentina–New Zealand	−37.85	31.85	−1.19	0.640
	Australia–Chile	0.79	4.27	0.19	0.998
	Australia–New Zealand	−1.84	2.39	−0.77	0.866
	Chile–New Zealand	−2.63	4.32	−0.61	0.928

Table A3. Post host comparisons between geographic regions on the total number of locations from which galaxiid–parasite associations have been studied. Statistically significant estimates ($\alpha = 0.05$) are in bold.

Contrast	Estimate	SE	z Ratio	p
Argentina–Australia	1.88	0.48	3.93	<0.001
Argentina–Chile	1.37	0.54	2.54	0.054
Argentina–New Zealand	1.96	0.48	4.10	<0.001
Australia–Chile	−0.51	0.59	−0.86	0.826
Australia–New-Zealand	0.08	0.53	0.15	0.999
Chile–New-Zealand	0.59	0.59	0.99	0.753

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