

## Research Article

## The invasive parasitic copepod *Lernaea cyprinacea*: updated host-list and distribution, molecular identification and infection rates in Patagonia

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### Abstract

Although competition and predation are mechanisms more studied in biological invasions, ecologists and conservation managers have become aware of the threat imposed by the co-introduction of parasites. Parasites can be co-introduced into new areas with a non-native host, and become co-invaders when they spill over to native hosts. The copepod *Lernaea cyprinacea* was presumably co-introduced to Patagonia along with the common carp, *Cyprinus carpio*. Our aims were to compose an updated list of the hosts of *L. cyprinacea* and its distribution in South America, to confirm the specific identity of Patagonian specimens using molecular tools, and to enlarge the list of hosts and localities in Patagonia, by comparing infection rates between non-native and native fishes to evaluate spillover processes. Between the years 2011 and 2017, native and non-native fish species were collected in freshwater environments from Northern Patagonia in basins where common carp and non-native fishes belonging to the Brazilian biogeographic sub-region, like Characiforms, Siluriforms, and Cyprinodontiforms, have been introduced. The sampled fishes were examined under a stereoscopic microscope to search for *L. cyprinacea*. At present, in South America, records of *L. cyprinacea* parasitizing native and non-native hosts exist for Colombia, Uruguay, Brazil, Chile and Argentina, with a total of 43 host fish species. Complete sequence data from the 18S region corroborate the identity of the Patagonian parasite based on morphological characteristics. A total of 754 fish belonging to 13 species were collected and 388 copepods were recovered. *Lernaea cyprinacea* was registered in almost every surveyed freshwater system in Patagonia, even in places where the original host, *C. carpio*, was absent. The copepod was found parasitizing two native and four non-native fish species, and in general, native fishes showed higher infection values. The present study enlarges the list of localities in Patagonia. It also provides molecular confirmation of the specific identity. In summary, our results show how an alien parasite may switch host and disperse beyond the actual distribution range of the original host. This study provides an overview of the process of colonization of *L. cyprinacea* in Patagonia, which represents a threat to native species due to its pathogenicity.

**Key words:** invasive parasites, host-switching, spillover, freshwater fishes, *Cyprinus carpio*

### Introduction

The introduction of non-native species is considered to be a major cause of biodiversity loss, mainly driven by competition and predation. However, ecologists and conservation managers have become increasingly aware of

the threat imposed by the co-introduction of parasites along with non-native hosts (Kelly et al. 2009). Introductions can lead to novel host-parasite relationships changing community structures (Poulin 2017), as parasites can be co-introduced into new areas with a non-native host, and become a co-invasive parasite when it switches to native hosts (LyMBERY et al. 2014). Non-native parasites can affect native host populations, depending on their ability to parasitize native hosts, even if the native fish are not phylogenetically close to the original hosts (Lebarbenchon et al. 2009; Telfer and Bown 2012). On the other hand, native parasites may interact with exotic hosts, leading to a process known as spillback, if alien hosts amplify transmission with the subsequent increase in infection of native hosts (Kelly et al. 2009; Poulin 2017), or it can reduce transmission resulting in a decrease in infection of natives hosts (Paterson et al. 2011; Poulin et al. 2011).

The cosmopolitan copepod *Lernaea cyprinacea* Linnaeus, 1758, known as anchor worm, has been introduced to almost every continent along with cyprinids, and has been recorded in hundreds of freshwater fishes (Piasecki et al. 2004; Poulin and Morand 2004; Avenant-Oldewage 2012; Sayyadzadeh et al. 2016). At present, *L. cyprinacea* is found throughout North and South America, Europe, Asia, Southern Africa, and Australia (Avenant-Oldewage 2012). The life cycle comprises of three free-swimming nauplius instars followed by five copepodid stages (Kearn 2004). The cyclopoid males and females are able to swim freely and, after mating, the males die while females attach permanently to the host, and then undergo metamorphosis (Avenant-Oldewage 2012).

In South America, three species of the genus *Lernaea* have been recorded parasitizing native and introduced fishes. *Lernaea argentinensis* Paggi, 1972 and *Lernaea devastatrix* Boxshall, Montú and Schwarzbald, 1997 are native (Boxshall et al. 1997). Meanwhile, *L. cyprinacea* was introduced in the beginning of the 20<sup>th</sup> century along with imported cyprinids (Piasecki et al. 2004). There are records of the presence of this parasite in native and introduced fishes in Colombia, Brazil, Uruguay, Chile and Argentina (Gabrielli and Orsi 2000; Carnevia and Speranza 2003; Piazza et al. 2006; Alvarez-León 2007; Habit et al. 2015). In Argentina, in particular, *L. cyprinacea* is widely distributed, with formal records in several provinces since 1993 (Gilbert et al. 1993; Mancini et al. 2008; Plaul et al. 2010; Biolé et al. 2012; Waicheim et al. 2017; Soares et al. 2018).

Argentinean Patagonia has a depauperate fish fauna composed by 36 species, 19 of which are native, including some endemic and emblematic species (Liotta 2005; Aigo et al. 2008; Pérez 2008; Pérez et al. 2015). The introduced fish species in Patagonia are the salmonids, the common carp *Cyprinus carpio* Linnaeus, 1758, and several small fish species from the Brazilian biogeographic sub-region including *Astyanax eigenmanniorum* (Cope, 1894), *Astyanax pampa* Casciotta, Almirón and Azpelicueta, 2005,

*Cheirodon interruptus* (Jenyns, 1842), *Oligosarcus jenynsii* (Günther, 1864), *Corydoras paleatus* (Jenyns, 1842), *Jenynsia alternimaculata* (Fowler, 1940), *Jenynsia multidentata* (Jenyns, 1842), *Cnesterodon decemmaculatus* (Jenyns, 1842), and *Crenicichla lacustris* (Castelnau, 1855) (Almirón et al. 1997; Liotta 2005; Alvear et al. 2007; Pascual et al. 2007; Aigo et al. 2008; Crichigno et al. 2016). The salmonids were introduced as embryos into Patagonia at the beginning of the 20<sup>th</sup> century from the Northern Hemisphere (Macchi and Vigliano 2014). The common carp is one of the most widely distributed freshwater fish in the world; it came from Eurasia and was introduced into Argentina during the 19<sup>th</sup> century, but since the 1970s it has spread rapidly in many provinces (Rosso 2007; Cousseau et al. 2010; Maiztegui et al. 2016). At present, common carp is distributed in the northern and central provinces of the country, reaching the Negro River basin in the north of Patagonia (López Cazorla and Sidorkewicj 2002; Alvear et al. 2007; Waicheim et al. 2014; Crichigno et al. 2016). The Brazilian fish species were accidentally introduced into Patagonia due to movement by the aquarium trade and fishing activities, occurring in the Colorado and Negro rivers and in Valcheta Stream (Almirón et al. 1997; Liotta 2005; Alvear et al. 2007; Pascual et al. 2007; Aigo et al. 2008; Pérez et al. 2015; Crichigno et al. 2016).

The most important rivers of Northern Patagonia, in terms of water volume, are the Colorado and Negro rivers. The Negro River emerges from the confluence of the Neuquén and Limay rivers. Both the Colorado and Negro river basins drain an area that extends from the Andes to the Atlantic Ocean and each one is more than 1,000 km long. Several dams have been constructed in both basins. Colorado River has Casa de Piedra Reservoir; Limay River has five reservoirs, of which Ramos Mexia and Arroyito are located in the lower reaches of the river. Neuquén River has a series of dams forming Los Barreales, Mari Menuco, El Chañar, Pellegrini, and Ingeniero Ballester reservoirs. The headwaters of Valcheta Stream basin are included in a protected area that harbors two endemic species: the characiform *Gymnocharacinus bergii* Steindachner, 1903 and the anuran *Pleurodema somuncurensis* (Ceí, 1969) (Pérez et al. 2015). At present, the only genetic confirmation of *L. cyprinacea* in South America are that of Soares et al. (2018) for specimens parasitizing *Odontesthes bonariensis* (Valenciennes, 1835) from Córdoba Province, Argentina. The aim of the present work comprises three main objectives: (1) to compose an updated list of the hosts of *L. cyprinacea* and the distribution of this parasite in South America, (2) to confirm the specific identity of Patagonian specimens using molecular tools, and (3) to enlarge the list of hosts and localities in Patagonia comparing infection rates between native and non-native hosts to evaluate spillover processes.

## Materials and methods

### *Updated list of hosts and distribution*

The review and update of the hosts and geographical distribution range of the anchor worm was made from national and international scientific journals. In the case of Argentina, available technical reports, theses, and congress summaries were also included. Due to the existence of other species of the genus *Lernaea* in South America (Paggi 1972; Boxshall et al. 1997), records of “*Lernaea* sp.” were not included, with the exception of the cases where the author suggests *L. cyprinacea* as the specific identity, and in the previous records from Colorado and Negro river basins (Table 1).

### *Sample collections in Northern Patagonia*

Between the years 2011 to 2017, during spring and summer seasons, specimens of five native fish species were collected: *Galaxias maculatus* (Jenyns, 1842), *Odontesthes hatcheri* (Eigenmann, 1909), *G. bergii*, *Mugil liza* Valenciennes, 1836 and *Percichthys trucha* (Valenciennes, 1833). Also, eight introduced fish species were collected: *C. carpio*, *C. interruptus*, *C. paleatus*, *Salmo trutta* Linnaeus, 1758, *Oncorhynchus mykiss* (Walbaum, 1792), *O. bonariensis*, *C. decemmaculatus*, *J. multidentata* (Table 2). Samples were taken from basins where common carp, non-native Brazilian fishes, and Salmonids were introduced. Fishes were captured using hand nets, gill nets and seine nets, from freshwater systems situated in the Colorado, Neuquén, Limay and Negro rivers, and in Valcheta Stream (Figure 1, Supplementary material Table S1). Fishes were put on ice and immediately transported to the laboratory. Teguments, gills, and opercular and buccal cavities, were examined for *Lernaea* infection using a stereoscopic microscope. Copepods were counted and carefully removed from the fish tissues using needles and blades, and preserved in 96% alcohol. Prevalence and mean intensity were calculated (Bush et al. 1997). Due to the small sample sizes of some fish species, localities were grouped by basin for the comparisons of infection rates between hosts. Further divisions were made in Neuquén and Limay rivers, where sites were separated into two groups: upstream and downstream to the first dam that prevents the upstream migration of *C. carpio*. Kruskal-Wallis and Multiple Comparison *a posteriori* tests were performed to compare: 1) *L. cyprinacea* abundance among fish species in Neuquén, Limay and Negro Rivers, and 2) *L. cyprinacea* abundance in *P. trucha* among the three rivers.

### *Molecular characterization*

Molecular identification was performed by the extraction of Genomic DNA from eight individual copepods of two different hosts and from three localities (Table 2). We used the E.Z.N.A.® Insect DNA Kit – Omega Bio-Tek, and following the manufacturers recommendations amplified a fragment

**Table 1.** Recorded wild hosts parasitized with the copepod *Lernaea cyprinacea* in South America, indicating host origin (a: alien, n: native) and source of data.

Country	Host Species	Origin	Reference
Colombia	Cypriniformes		
	<i>Carassius auratus</i> (Linnaeus, 1758)	a	Álvarez-León (2007)
	<i>Cyprinus carpio</i> Linnaeus, 1758	a	Álvarez-León (2007)
Uruguay	Perciformes		
	<i>Trichogaster microlepis</i> (Günther, 1861)	n	Álvarez-León (2007)
	Cypriniformes		
Brasil	<i>Carassius auratus</i> (Linnaeus, 1758)	a	Carnevia and Speranza (2003)
	Amphibia		
Brasil	Caudata		
	<i>Ambystoma mexicanum</i> (Shaw and Nodder, 1798)	n	Carnevia and Speranza (2003)
	Cypriniformes		
	<i>Cyprinus carpio</i> Linnaeus, 1758	a	Gabrielli and Orsi (2000)
	Characiformes		
	<i>Astyanax bimaculatus</i> (Linnaeus, 1758)	n	Gabrielli and Orsi (2000); Magalhães (2006)
	<i>Brycon cephalus</i> (Günther, 1869)	n	Gabrielli and Orsi (2000)
	<i>Colossoma macropomum</i> (Cuvier, 1816)	n	Luque et al. (2013)
	<i>Hoplias malabaricus</i> (Bloch, 1794)	n	Luque et al. (2013)
	<i>Leporinus elongatus</i> Valenciennes, 1850	n	Gabrielli and Orsi (2000)
	<i>Leporinus friderici</i> (Bloch, 1794)	n	Gabrielli and Orsi (2000)
	<i>Leporinus macrocephalus</i> Garavello and Britski, 1988	n	Gabrielli and Orsi (2000)
	<i>Piaractus mesopotamicus</i> (Holmberg, 1887)	n	Gabrielli and Orsi (2000)
	<i>Prochilodus brevis</i> Steindachner, 1875	n	Luque et al. (2013)
	<i>Prochilodus lineatus</i> (Valenciennes, 1837)	n	Gabrielli and Orsi (2000)
	<i>Schizodon intermedius</i> Garavello and Britski, 1990	n	Gabrielli and Orsi (2000)
	<i>Schizodon nasutus</i> Kner, 1858	n	Gabrielli and Orsi (2000)
	<i>Serrasalmus spilopleura</i> Kner, 1858	n	Gabrielli and Orsi (2000)
	<i>Steindachnerina insculpta</i> (Fernández-Yépez, 1948)	n	Acosta et al. (2013)
	Siluriformes		
	<i>Clarias gariepinus</i> (Burchell, 1822)	a	Gabrielli and Orsi (2000)
	<i>Franciscodoras marmoratus</i> (Lütken, 1874)	n	Luque et al. (2013)
	<i>Iheringichthys labrosus</i> (Lütken, 1874)	n	Gabrielli and Orsi (2000)
	<i>Pimelodus blochii</i> Valenciennes, 1840	n	Luque et al. (2013)
	<i>Pseudoplatystoma corruscans</i> (Spix and Agassiz, 1829)	n	Gabrielli and Orsi (2000)
	<i>Rhamdia quelen</i> Quoy and Gaimard, 1824	n	Luque et al. (2013)
	Cyprinodontiformes		
	<i>Poecilia sphenops</i> Valenciennes, 1846	n	Piazza et al. (2006)
	<i>Xiphophorus maculatus</i> (Günther, 1866)	a	Piazza et al. (2006)
	Perciformes		
<i>Oreochromis niloticus</i> (Linnaeus, 1758)	a	Gabrielli and Orsi (2000)	
<i>Pachyurus squamipennis</i> Agassiz, 1831	n	Luque et al. (2013)	
Chile	Characiformes		
<i>Cheirodon galusdae</i> Eigenmann, 1928	n	Habit et al. (2015)	
Perciformes			
<i>Percichthys trucha</i> (Valenciennes, 1833)	n	Habit et al. (2015)	
Argentina	Cypriniformes		
<i>Cyprinus carpio</i> Linnaeus, 1758	a	Mancini et al. (2008); Plaul et al. (2010); Biolé et al. (2012)	
<i>Carassius auratus</i> (Linnaeus, 1758)	a	Mancini et al. (2008); Plaul et al. (2010); Biolé et al. (2012)	
Characiformes			
<i>Astyanax bimaculatus</i> (Linnaeus, 1758)		Plaul et al. (2010)	
<i>Astyanax eigenmanniorum</i> (Cope, 1894)	n	Mancini et al. (2008); Biolé et al. (2012)	
<i>Astyanax hermosus</i> Miquelarena, Protogino and López, 2005	n	Ramallo and Terán (2014)	
<i>Astyanax</i> sp.		Mancini et al. (2008)	
<i>Bryconamericus iheringii</i> (Boulenger, 1887)	n	Mancini et al. (2008); Biolé et al. (2012)	

**Table 1.** (continued).

Country	Host Species	Origin	Reference
	<i>Cheirodon interruptus</i> (Jenyns, 1842)	n	Plaul et al. (2010) ; Biolé et al. (2012)
	<i>Cyphocharax voga</i> (Hensel, 1870)	n	Mancini et al. (2008)
	<i>Hoplias malabaricus</i> (Bloch, 1794)	n	Biolé et al. (2012)
	<i>Oligosarcus jenynsii</i> (Günther, 1864)	n	Mancini et al. (2008); Plaul et al. (2010); Biolé et al. (2012)
	<i>Prochilodus lineatus</i> (Valenciennes, 1837)	n	Roux et al. (2000)
	Siluriformes		
	<i>Rhamdia quelen</i> (Quoy and Gaimard, 1824)	n	Mancini et al. (2008); Plaul et al. (2010); Biolé et al. (2012)
	<i>Corydoras paleatus</i> (Jenyns, 1842)	n	Plaul et al. (2010)
	<i>Hypostomus plecostomus</i> (Linnaeus, 1758)	a	Plaul et al. (2010)
	<i>Rhamdia sapo</i> (Quoy and Gaimard, 1824)	n	Vanotti and Tanzola (2005)
	<i>Parapimelodus valenciennis</i> (Lütken, 1874)	n	Biolé et al. (2012)
	Salmoniformes		
	<i>Oncorhynchus mykiss</i> (Walbaum, 1792)	a	Gilbert et al. (1993); Mancini et al. (2008); Plaul et al. (2010)
	Atheriniformes		
	<i>Odontesthes bonariensis</i> (Valenciennes, 1835)	n	Mancini and Grosman (1998); Mancini et al. (2008); Plaul et al. (2010); Biolé et al. (2012)
	<i>Odontesthes hatcheri</i> (Eigenmann, 1909)	n	Colavincenzo (1996)
	Cyprinodontiformes		
	<i>Jenynsia multidentata</i> (Jenyns, 1842)	n	Montes (2012)
	<i>Jenynsia</i> sp.	n	Mancini et al. (2008)
	<i>Poecilia</i> sp.	n	Mancini et al. (2008)
	Perciformes		
	<i>Percichthys trucha</i> (Valenciennes, 1833)	n	Gilbert et al. (1993); Ortubay et al. (1994); Mancini et al. (2008); Plaul et al. (2010); Waicheim et al. (2017)
	Amphibia		
	Anura		
	<i>Hypsiboas cordobae</i> (Barrio, 1965)	n	Alcalde and Batistoni (2005)
	<i>Lithobates catesbeianus</i> (Shaw, 1802)	a	Salinas et al. (2016)

of the 18S region with the following primers: 18SF (AAGGTGTGMCCT ATCAACT) and 18SR (TACTTCTCTAAACGCTC) (Song et al. 2008). The PCR reactions were prepared with a final volume of 25 µl, containing 1x buffer, 1.5 mM MgCl<sub>2</sub>, 0.4 µM of each primer, 0.4 µM of the dNTPs mix and 1.25 U of recombinant Taq DNA Polymerase (ThermoFisher) with the following thermo-cycling conditions: 94 °C for 5 min, following 30 cycles of 94 °C for 30 s, 54 °C for 30 s and 72 °C for 1 min and a final extension at 72 °C for 5 min (Song et al. 2008). The PCR products were run in 2% agarose gels, and the amplified products were purified using the Exo-SAP chemistry and sent to Macrogen (Korea) for Sanger sequencing.

Six out of eight sequences were readable, and then aligned using ClustalX incorporated in Mega7. All sequences were checked and corrected by eye before and after the alignment. We estimated the evolutionary divergence between sequences as the numbers of base differences per site between sequences. The analysis involved 6 individuals and none of the sequences had gaps or missing values, summing up a total of 795 positions in the final dataset. Using this alignment (accession numbers: MH198049 to MH198054) a Blast search was performed, and the first 6 sequences were added. Those corresponding to *L. cyprinacea* were added to the analyses (accession numbers: KP235363.1, KM281816.1, DQ107554.1, DQ107555.1, DQ107556.1 and DQ107557.1) to investigate the genetic differences and evaluate the genetic identity of them.

**Table 2.** Localities, fish species, fish origin (a: alien, n: native), number of fish examined (n), host length (mean  $\pm$  SE), and infection rates of *Lernaea cyprinacea* (the highest values by locality are underlying). Individuals with DNA extraction (\*).

Localities	Fish Species	Origin	n	Total Length (cm)	Prevalence (%)	Mean Intensity
Colorado River	<i>Cyprinus carpio</i>	a	3	27.0 $\pm$ 7.8	33.0	1.0
	<i>Odontesthes bonariensis</i>	a	5	25.9 $\pm$ 10.5	–	–
	<i>Odontesthes hatcheri</i>	n	3	18.2 $\pm$ 5.5	–	–
	<i>Oncorhynchus mykiss</i>	a	2	18.5 $\pm$ 0.7	50.0	1.0
	<i>Jenynsia multidentata</i>	a	44	3.3 $\pm$ 1.6	2.0	1.0
Neuquén River (Upstream Ing. Ballester Dam)	<i>Cheirodon interruptus</i>	a	38	4.1 $\pm$ 0.9	5.3	1.0
	<i>Corydoras paleatus</i>	a	12	1.9 $\pm$ 2.4	–	–
	<i>Oncorhynchus mykiss</i>	a	5	37.6 $\pm$ 1.3	–	–
	<i>Odontesthes hatcheri</i>	n	30	29.6 $\pm$ 2.9	26.7	1.3
	<i>Cnesterodon decemmaculatus</i>	a	19	2.4 $\pm$ 1.1	–	–
	<i>Jenynsia multidentata</i>	a	65	4.0 $\pm$ 1.4	16.9	1.5
	<i>Percichthys trucha</i> *	n	39	35.0 $\pm$ 7.9	<u>43.6</u>	<u>3.5</u>
Neuquén River (Downstream Ing. Ballester Dam)	<i>Cyprinus carpio</i>	a	31	34.2 $\pm$ 13.9	–	–
	<i>Oncorhynchus mykiss</i>	a	5	36.3 $\pm$ 6.7	–	–
	<i>Odontesthes hatcheri</i>	n	20	27.6 $\pm$ 6.7	5.0	1.0
	<i>Cnesterodon decemmaculatus</i>	a	7	3.0 $\pm$ 0.4	–	–
	<i>Jenynsia multidentata</i>	a	11	2.0 $\pm$ 1.9	9.1	2.0
	<i>Percichthys trucha</i>	n	9	29.1 $\pm$ 10.9	<u>66.7</u>	<u>4.0</u>
Limay River (Upstream Arroyito Dam)	<i>Odontesthes hatcheri</i>	n	10	39.1 $\pm$ 2.2	–	–
	<i>Oncorhynchus mykiss</i>	a	8	49.4 $\pm$ 1.7	–	–
	<i>Salmo trutta</i>	a	2	59.0 $\pm$ 2.1	–	–
	<i>Percichthys trucha</i>	n	9	39.2 $\pm$ 6.8	–	–
Limay River (Downstream Arroyito Dam)	<i>Cyprinus carpio</i>	a	24	29.0 $\pm$ 19.8	4.2	1.0
	<i>Galaxias maculatus</i>	n	12	3.4 $\pm$ 2.3	–	–
	<i>Oncorhynchus mykiss</i>	a	18	44.4 $\pm$ 4.2	11.1	4.5
	<i>Odontesthes hatcheri</i> *	n	37	29.7 $\pm$ 10.5	10.8	5.0
	<i>Jenynsia multidentata</i>	a	11	2.9 $\pm$ 1.4	–	–
	<i>Percichthys trucha</i> *	n	29	30.6 $\pm$ 9.7	<u>31</u>	<u>14.7</u>
Negro River	<i>Cyprinus carpio</i>	a	111	20.7 $\pm$ 13.0	11.7	2.0
	<i>Cheirodon interruptus</i>	a	12	4.3 $\pm$ 0.9	25.0	1.0
	<i>Corydoras paleatus</i>	a	2	1.8 $\pm$ 1.0	–	–
	<i>Odontesthes hatcheri</i> *	n	22	20.1 $\pm$ 10.2	22.8	1.6
	<i>Jenynsia multidentata</i>	a	14	4.1 $\pm$ 2.1	–	–
	<i>Mugil cephalus</i>	n	16	52.2 $\pm$ 3.4	–	–
	<i>Percichthys trucha</i> *	n	24	20.3 $\pm$ 8.2	<u>58.3</u>	<u>5.1</u>
Valcheta Stream	<i>Cheirodon interruptus</i>	a	15	5.0 $\pm$ 0.5	–	–
	<i>Cnesterodon decemmaculatus</i>	a	15	3.5 $\pm$ 0.3	–	–
	<i>Gymnocharacinus bergii</i>	n	15	3.7 $\pm$ 0.4	–	–

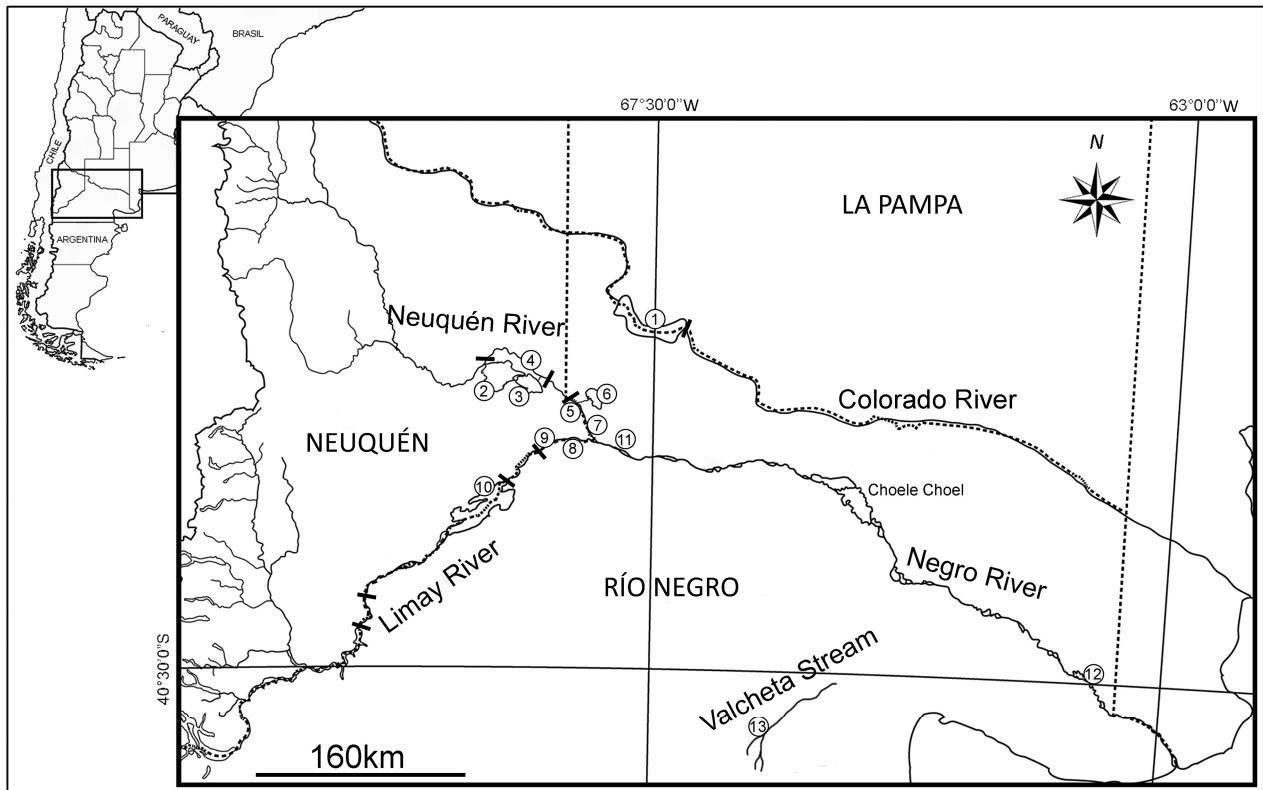
## Results

### Updated host-list and distribution

At present, in South America, there are records of *L. cyprinacea* parasitizing native and non-native hosts in Colombia, Uruguay, Brazil, Chile and Argentina (Table 1). A total of 3 amphibians and 43 fish taxa identified at species level, belonging to 7 orders, were found to be parasitized by *L. cyprinacea*. Characiformes was the most parasitized Order.

### Molecular analyses

In total, six 18S sequences obtained in this study and six 18S (rDNA sequences) gathered from GenBank ([www.ncbi.nlm.nih.gov/genbank/](http://www.ncbi.nlm.nih.gov/genbank/)), were employed in our analyses. After alignment, the resulting 18S rDNA data



**Figure 1.** Localities of fish collections in Northern Patagonia. Colorado River: 1. Casa de Piedra Reservoir; Neuquén River: 2. Los Barreales Reservoir, 3. Mari Menuco Reservoir, 4. El Chañar Reservoir, 5. Downstream Ingeniero Ballester Dam, 6. Pellegrini Reservoir, 7. Centenario; Limay River: 8. China Muerta, 9. Downstream Arroyito Dam, 10. Ramos Mexia Reservoir, Negro River: 11. Allen, 12. Guardia Mitre and Valcheta Stream: 13. La Horqueta (for details see Supplementary material Table S1). Black bars show the location of dams across the rivers.

were 795 bp in length, as some sequences had to be pruned to allow comparisons. The evolutionary divergence (numbers of base differences per site) between these two groups (the NCBI sequences from Japan, Iran and China and our specimens) was 0.32%. Each group shows an evolutionary divergence within sequences of 0.04% for the NCBI and 0.15% for our sequences. The overall mean distance for all the sequences as a group was 0.22%. The G+C content was 50.7% for both the new and the GenBank retrieved sequences. The sequences analyzed as one group showed five variable non-synonymous substitution sites.

#### *Hosts and localities in Northern Patagonia*

A total of 754 fish belonging to 13 species were collected and 388 copepods were recovered. The copepod *L. cyprinacea* was registered in almost every surveyed freshwater system, except for two environments (upstream Arroyito Dam in Limay River and in Valcheta Stream) (Table 2). The copepod was found parasitizing two native (*O. hatcheri* and *P. trucha*) and four non-native (*C. carpio*, *C. interruptus*, *O. mykiss* and *J. multidentata*) fish species.

#### *Comparisons of infection rates*

Native fishes generally had high infection rates, with *P. trucha* showing the highest infection rates, reaching up to 66.7% prevalence in Neuquén River

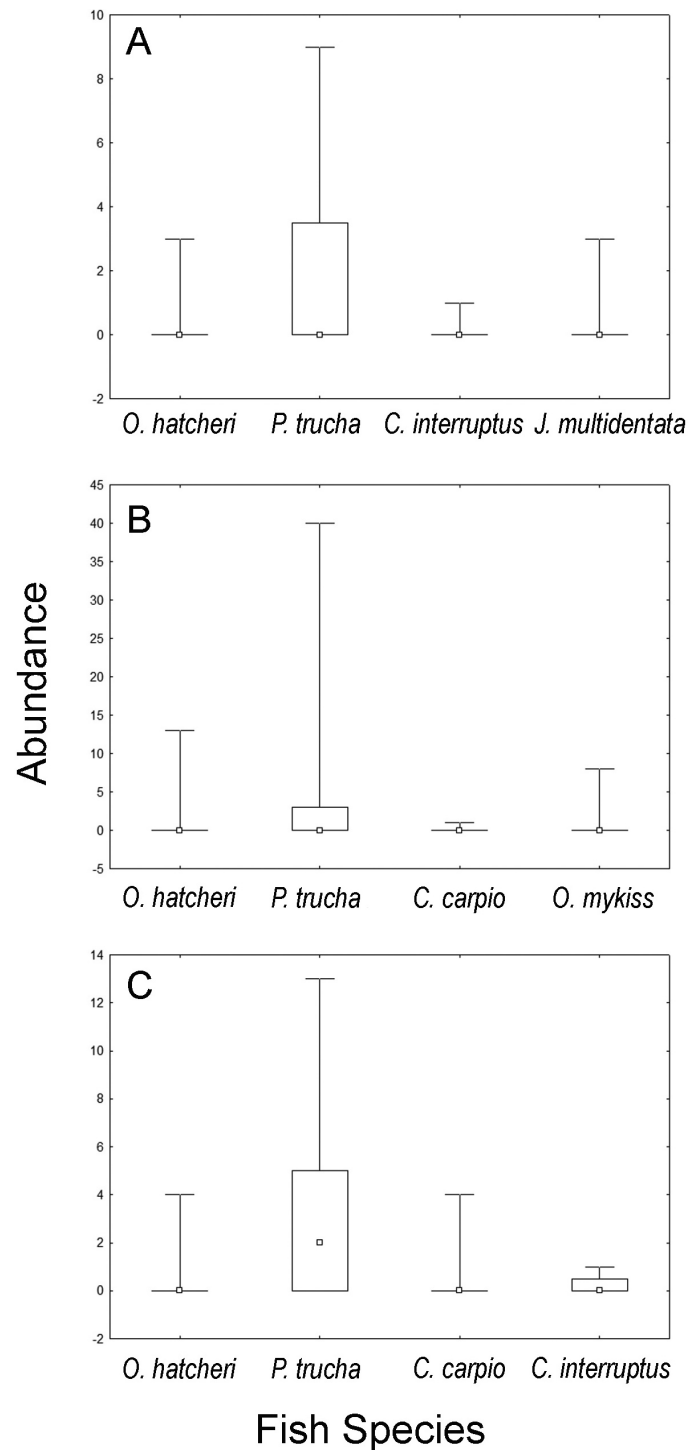


downstream Ingeniero Ballester Dam, and 14.7 of mean intensity in Limay River downstream Arroyito Dam (Table 2). The highest prevalence values for non-native species were recorded for *C. interruptus* (25.0%), *J. multidentata* (16.9%) and *O. mykiss* (11.1%). Mean intensity values were generally low for non-native fishes, mainly 1, reaching up to 4.5 in *O. mykiss* from Limay River downstream Arroyito Dam. Prevalence in *C. carpio* ranged between 0.0 and 11.7%, and the mean intensity varied between 1 and 2. In the three river basins (Neuquén, Limay and Negro), the abundance of *L. cyprinacea* differed significantly among the fish species (Neuquén River:  $H_{3,212} = 33.8$ ;  $p < 0.001$ ; Limay River:  $H_{3,108} = 9.9$ ;  $p = 0.019$ ; Negro River:  $H_{3,169} = 32.7$ ;  $p < 0.001$ ). *Percichthys trucha* showed the highest abundance values in all the basins (Figure 2). *Lernaea cyprinacea* abundance in *P. trucha*, among the 3 river basins, showed no significant differences ( $H_{2,101} = 2.8$ ;  $p = 0.246$ ).

## Discussion

The copepod *L. cyprinacea* has been spread widely throughout the world in association with the translocation of fishes, especially cyprinids (Avenant-Oldewage 2012; Smit et al. 2017). It is thought that in South America the anchor worm was absent until the beginning of the 20<sup>th</sup> century when it was accidentally co-introduced along with cyprinids (Piasecki et al. 2004). As far as we are aware, there are records of *L. cyprinacea* in Colombia, Uruguay, Brazil, Chile, and Argentina (Table 1). This invasive copepod has been registered parasitizing 43 native and non-native fish species, corresponding to 7 orders, and it has been also recorded in 3 amphibian species. In almost every country, it has been recorded parasitizing introduced cyprinids as hosts. It is worth noting that Characiformes, with 20 parasitized species, is the most infected Order in South America, whereas in South Africa the infection is almost exclusively found in Cypriniformes (Smit et al. 2017). Brazil and Argentina are the countries with the greatest number of records of *L. cyprinacea*, being found in 40 fish species. As parasitological studies are scarce in other countries of South America, we believe that this parasite has an even wider distribution in the continent than is currently recognized. Although in South America and South Africa the first records of the introduction of *L. cyprinacea* date back to the 1980s, in Africa only ten fish species were infected (Smit et al. 2017). This difference could indicate that the anchor worm was introduced in South America long before that in South Africa. In support of this, there are records in Argentina of the presence of *C. carpio* since middle of 19<sup>th</sup> century, and of *Carassius auratus* (Linnaeus, 1758) since middle of 20<sup>th</sup> century (Liotta 2005).

We provide the first molecular data on the invasive copepod *L. cyprinacea* from Patagonia, and the second in South America (Soares et al. 2018). By comparing molecular data of the 18S rDNA obtained in our study with those



**Figure 2.** Boxplot showing median abundance of *Lernaea cyprinacea* parasitizing different fish species in 3 rivers (A: Neuquén River, B: Limay River, and C: Negro River). Dots inside the boxes represent the median values, lower and upper box boundaries represent the 25 and 75% of the data, and whiskers indicate min-max values.

available in GenBank, we show that genetic differences are indicative of intraspecific variation. The levels of divergence between the individuals sequenced in our work and those obtained from the GenBank were similar, and of the same magnitude as for other Copepoda groups. For example, among the family Ergasilidae, evolutionary divergence within groups of 3 different genera (*Sinergasilus*, *Ergasilus* and *Paraergasilus*) ranged from 0.14%

to 3.65% (Song et al. 2008); thus, the evolutionary divergence of 0.3% within the *Lernaea* is similar. Although *L. cyprinacea* has a cosmopolitan distribution and it has been widely studied, molecular data is scarce in areas where it is native, as well as in new colonized regions (Welicky et al. 2017).

In Patagonia, the first records of this parasite (cited as *Lernaea* sp.) date back to the 1990s, in the non-native fish *O. mykiss* and in the native *P. trucha* and *O. hatcheri* from the Colorado and Negro rivers (Gilbert et al. 1993; Ortubay et al. 1994; Colavincenzo 1996). No formal records were undertaken until 2010, when the presence of *L. cyprinacea* was reported in the non-native fish *C. interruptus* in Negro River (Plaul et al. 2010). In the present survey, from a total of 13 fish species analyzed, we found *L. cyprinacea* on 6 species of fish, 2 of which are native (*O. hatcheri*, and *P. trucha*) and 4 non-native (*C. carpio*, *O. mykiss*, *J. multidentata*, and *C. interruptus*). Although it is not clear how and when *L. cyprinacea* was first introduced in Patagonia, this parasite could have been co-introduced with common carp (*C. carpio*). It is thought that this fish could have reached the Colorado River in 1983, due to an extraordinary flood from the Salado River (López Cazorla and Sidorkewicz 2002).

*Lernaea cyprinacea* was registered in almost all surveyed basins, except in the upstream Limay River (upstream Arroyito Dam) and in Valcheta Stream. These results correspond with the spread of *C. carpio* in these basins, which has colonized new areas along the Colorado, Neuquén, Limay and Negro rivers (Crichigno et al. 2016). It is known that after their first introduction, common carp was restricted to central Argentina, but since the 1970s it has shown an exponential expansion in its distribution range (Maiztegui et al. 2016). This large expansion could be associated not only with a high dispersal capability, but also facilitated by construction of reservoirs and by stocking (Zambrano et al. 2006; Maiztegui et al. 2016). In Patagonia, dams appear to be acting as barriers for the copepod dispersion upstream. However, in the last few years, some fish species from the Brazilian sub-region have circumvented these obstacles (unpublished own data; Aigo et al. 2008). For example, in Neuquén River, *C. carpio* is restricted to downstream of the Ingeniero Ballester dam, but Brazilian fishes reached environments upstream, and they might have co-introduced the parasite into this new area. The absence of *L. cyprinacea* upstream of Arroyito Dam in Limay River could be related to the lack of carp and Brazilian fishes. Valcheta Stream, where *L. cyprinacea* is absent, is part of an endorreic basin, so it is not connected to Negro River basin, and this fact might constitute a barrier for invasions of non-native species. However, two salmonid species, *O. mykiss* and *S. fontinalis*, and three Brazilian fishes (*J. multidentata*, *C. decemmaculatus* and *C. interruptus*), were introduced to Valcheta Stream (Pérez et al. 2015). These findings could represent a major cause of concern, as Valcheta Stream harbors two endemic and emblematic vertebrates, the characid fish *G. bergii*, the

southernmost endemic characid in South America, and the leptodactylid frog *P. somuncurensis*. Periodical controls of fish parasites are needed in this particular basin.

We registered significant differences in infection intensities among host species. The highest infection rates were found on the native *P. trucha*, with similar abundance values in Neuquén, Limay and Negro rivers, even though the parasite could have colonized first the middle of the Negro River and afterward the Neuquén and Limay rivers (Plaul et al. 2010; Waicheim et al. 2017). On the other hand, low infection rates were registered in its original cyprinid host *C. carpio*. This striking result has been reported in other studies, where native species show higher infection rates than the original non-native host (Marina et al. 2008). Further, in South Africa, none of the invasive cyprinid species that were potentially responsible for the co-introduction and subsequent co-invasion of this parasite was found to be infected (Smit et al. 2017). Mean intensities higher than two parasites per host were registered only in large fish species (*P. trucha*, *O. hatcheri*, and *O. mykiss*). These differences in mean intensities might be related to the mortality induced by the parasite in small fish species. Although carp have large bodies, mean intensities were one or two copepods per host.

All the studied localities from Limay River and upstream Choele Choele in the Negro River, and in Neuquén River (except for Los Barreales Reservoir, Waicheim et al. 2017), represent novel localities for the parasite. *Cyprinus carpio* and *J. multidentata* are new hosts for the parasite in Patagonia. Due to the recent colonization of *L. cyprinacea* in Patagonia, it is expected that this parasite will spill over to more fish species and enlarge its distribution range.

## Conclusions

Not only the common carp, but other native and non-native species, are acting as dispersal agents for *L. cyprinacea*, beyond the distribution range of the original fish host. At sites such as Valcheta Stream, where introductions of ornamental fishes could occur, the invasion of this copepod may affect environments with important endemism in the future. In reservoirs situated in Limay River, upstream of Arroyito Dam, there are several salmonid farms that also could eventually be affected by invasions of this copepod. In addition, the lakes in the headwaters of north Patagonian rivers are included in protected areas of National Parks. Thus, careful surveillance and monitoring are necessary to detect parasitic introductions in these environments with low diversities of species, which makes them particularly vulnerable to biological invasions. Almost all lakes and reservoirs in Patagonia have temperatures below the thermal range of growth and preference for fishes from warmer habitats. However, climate change might generate new environmental scenarios that favor the establishment of alien fishes and their parasites.

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### Supplementary material

The following supplementary material is available for this article:

**Table S1.** Localities of fish collections in Northern Patagonia

This material is available as part of online article from:

[http://www.aquaticinvasions.net/2019/Supplements/AI\\_2019\\_Waicheim\\_et\\_al\\_Table\\_S1.xlsx](http://www.aquaticinvasions.net/2019/Supplements/AI_2019_Waicheim_et_al_Table_S1.xlsx)