Distribution of the exotic parasite, *Lernaea cyprinacea* (Copepoda, Lernaeidae) in Argentina

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Abstract

Lernaea cyprinacea Linnaeus is an ectoparasitic copepod that infects the gills and skin of various freshwater fishes and amphibians tadpoles. It is capable of infecting fish at high levels, with high pathogenicity and mortality, mainly during the summer season. Besides the effects of penetration, haemorrhages and ulcerations are also caused. The objective of this study was to investigate the presence of *L. cyprinacea* in natural bodies of water of Argentina in the provinces of Buenos Aires, Mendoza and Río Negro; the finding of the parasite in Choele-Choel, in the province of Río Negro, is the most austral distribution of its presence. In our study, infections with *L. cyprinacea* showed a low host specific and they were adapted to a large variety of habitats.

Introduction

Lernaea cyprinacea Linnaeus (Copepoda, Lernaeidae), the anchor worm, is an ectoparasitic copepod that generally infects the gills and skin of various freshwater fishes (Stoskopf, 1993), amphibian tadpoles (Martins and Souza, 1996; Alcalde and Batistoni, 2005) and the larval stage of axolotl (Carnevia *et al*., 2003). These parasites are distributed naturally in Africa, central Asia, the southern region of Siberia, and have also been reported in Europe, Japan and Israel (Schäperclaus, 1991; Thatcher *et al*., 1998). The genus *L. cyprinacea* has been introduced into North and South America by the importation of tropical fishes (Figueira and Ceccarelli, 1991).

The species of *Lernaea* has nine stages in the life cycle, including three free-living naupliar stages, five copepodid stages, and one adult stage (Grabda, 1963; Hoffman, 1999). After male and female adults mate on the fish host, copulation occurs during the fourth copepodid stage. When the copulation was realized the males presumably dying while the females metamorphose and insert the anterior region of the body into the host tissue and then produce eggs (Hoffman, 1999; Nagasawa *et al*., 2007).

The adult females of the genus *Lernaea* exhibit an extreme modification of the cephalothorax. The mouth parts of the adult female are severely reduced, the body is elongate and vermiform, and the head is modified into four horn-shaped appendages, which are somewhat long and slender; the two outer or posterior ones are bifurcated and the anterior one is simple (Scott and Scott, 1913). The horns

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are used for attachment to the host and are buried beneath the epidermis. The abdomen is short and is often bent dorsally. The genital pore is located at or near the posterior extremity and the egg sacs project well beyond the body (Thatcher and Willians, 1998).

Only the adult female lernaeids are parasitic, whereas the males and immature forms of both sexes are free living (Schäperclaus, 1991).

L. cyprinacea is capable of massive attack, with high pathogenicity and mortality, mainly during the summer season. Besides the effects of penetration, haemorrhages and ulcerations are also caused (Carnevia *et al.*, 2003). The most dangerous consequence of an intensive attack results in blood loss, intense lymphocytopenia, neutrophilia, increase of monocytes (Silva-Souza *et al.*, 2000), and secondary infections (Schäperclaus, 1991). The disease is accompanied by marked emaciation and loss of weight (Stoskopf, 1993; Pavanelli, 1998). The index of mortality is high (Mancini *et al.*, 2008a). The risk of parasite pathogenicity depends on the affected organ, parasitism intensity, environmental conditions and concomitant infections, among other factors (Alvarez Pellitero, 1998).

The objective of this study was to investigate the distribution of *L. cyprinacea* in Argentina, indicating its distribution by province, taking into account that reports of lernaeid parasites infecting freshwater fish in our country are scarce.

Material and methods

The fish were collected in three Argentinean provinces: Buenos Aires, Mendoza and Rio Negro.

A total of 200 *Astyanax bimaculatus* and 50 *Odontesthes bonariensis* adult specimens were collected from the Luján river and its flooding lagoons (Buenos Aires) in March-April 2004.

Between January and April 2005 a massive mortality of fishes was observed in the dam El Nihuil in the province of Mendoza. The Departamento General de Irrigación (D.G.I) of the government of Mendoza thus sent specimens of *Percichthys trucha*, *Oncorhynchus mykiss* and *Odontesthes bonariensis* to our laboratory.

In both cases, the fishes were collected using a nylon gill net and transported alive in plastic bags. The dead fishes were refrigerated until their observation and processing.

In January-February 2005, sport fishers captured specimens of *Rhamdia quelem* in the lagoon Del Burro, origin of the Salado river, *Cyprinus carpio* in the La Plata river, in the locality Punta Lara (both in the province of Buenos Aires), and *Cheirodon interruptus* in the Negro river, near the island Choele-Choel (province of Río Negro); the specimens were sent to our laboratory.

Specimens of *Carassius auratus*, *Corydoras paleatus*, *Hypostomus plecostomus* and parasitized cichlids were also sent to our laboratory from pet shops of La Plata, province of Buenos Aires.

Fishes were examined in the laboratory to determine the presence of parasitic copepods. Parasites were counted and copepods were dissected from the host tissue with needles and preserved in 70% alcohol.

The parasitological parameter used was the mean intensity. This parameter is the average number of parasites in a positive host. The results were analyzed in a semiquantitative form, considering them as follows: + if the host had 1-3 parasites, ++ if the host had 4-7 parasites, and +++ if the host had 8 or more parasites.

Results

In the areas studied, *L. cyprinacea* infected nine species (Table 1), including both wild and pet fish.

The infected fish showed severe physical damage in the area of high concentration of adult parasites, where they had caused petechial haemorrhages and ulcers at the site of penetration. The parasites were found on various parts of the host's body surface and appeared as small worm-like protrusions. *L. cyprinacea* seems to show a preference for a particular body area for attachment; however, the most heavily infected locations were found behind the gills, skin and the base of the pectoral fins. The lesions found were similar in all fish species. Microscopic observation revealed hyperplasia of the epithelium, abundant infiltration of inflammatory cells (lymphocytes and macrophages) and fibrosis in the site of attachment, not only in the epidermis but also in the dermis and muscular tissue.

Discussion

Reports of species of lernaeids in South America are scarce, and it should be remembered that there are literally thousands of native freshwater fishes on this continent that have never been examined for parasites (Thatcher, 2000).

Table 1. Register of fish parasitized by *L. cyprinacea* Linnaeus in Buenos Aires, Mendoza and Río Negro provinces. Average intensity: (+)= ≤ 3, (++)= 4 – 7, (+++)= ≥ 8.

Locality	Provinces	Geographical localization	Hosts	Intensity of parasitism
Luján river and its lagoons of flood, Tigre	Buenos Aires	34º26′ S 58º32′ W	*Astyanax bimaculatus* *Odontesthes bonariensis*	++
La Plata river, Punta Lara	Buenos Aires	34º49′S 57º59′W	*Cyprinus carpio*	+
Del Burro Lagoon, basin of the Salado river	Buenos Aires	35º41′S 57º55′W	*Rhamdia quelem*	+
La Plata	Buenos Aires	34º55′S 57º57′W	*Carassius auratus* *Corydoras paleatus* *Hypostomus plecostomus*	+
Atuel river, El Nihuil reservoir	Mendoza	35º05′S 68º45′W	*Percichthys trucha* *Odontesthes bonariensis* *Oncorhynchus mykiss*	+++
Negro river, Choele-Choel island	Río Negro	39º22′S 65º43′W	*Cheirodon interruptus*	+

Reports of lernaeid parasites infecting freshwater fish in Argentina are also very scarce.

According to Thatcher (2000), *Areotrachelus truchae* was found in *Percichthys trucha* (Brian, 1902), *Taurocheros salminisii* in *Salminus brevidens* and *S. maxillosus* (Brian, 1924), *Lernaea argentinensis* in *Pseudoplatystoma coruscan* and *P. fasciatum* (Paggi, 1972) and *Taurocheros tarangophilus* in *Hoplias malabaricus* (Paggi, 1976).

In relation with *L. cyprinacea*, Table 2 summarizes the previous findings in Argentina.

According to Mancini *et al.* (2008b), the wild native *O. bonariensis* and *P. trucha,* and the introduced species *C. carpio* and *O. mykiss* are among the most affected. The pet fish *Carassius auratus* was the most affected species, but *C. paleatus* and *H. plecostomus* were infected as well in the pet shops but not in wild conditions. The finding of infected *C. interruptus* is the most austral report of infection with *Lernaea* and the first record of infection in this species.

The reproduction of *L. cyprinacea* is seriously affected by water temperature (Schäperclaus, 1991; Stoskopf, 1993; Pavanelli, 1998). According to Schäperclaus (1991), the life cycle is completed in 100 days at 14°C and in 7-13 days at 28°C, but the optimum temperature lies between 23°C and 30°C. Temperature is considered of great significance; prevalence, abundance and medium intensity of *L. cyprinacea* had a higher seasonal occurrence during warm months, which coincided with blooms of Cyanobacteria and Dinoflagellates (Mancini, 2006).

The prevalence and intensity of infection was significantly reduced in April, which coincides with the decreasing water temperature. Although water temperature plays an important role, it is probably not the only factor affecting the population size of lernaeids. In the province of Mendoza there are three dams: Los Reyunos, Valle Grande and El Nihuil. The last one has a surface temperature of 24.6°C but in January 2005 a massive mortality of fish occurred, since the water temperature increased 2.8°C (Peralta *et al.*, 2006). Choele-Choel is the most austral locality with reports of infection by *L. cyprinacea* (Figure 1); this could be explained by the high temperatures that the waters of the Negro river can reach in the summer. The record of AIC (Autoridad Interjurisdiccional de Cuencas) indicates that the temperature of the Negro river in January 2005 was 23°C. The existence of the parasite and its pathogenic activity in a very austral locality demonstrates that an increase in temperature generated by contamination or climatic changes could increase significantly the geographical dissemination of *L. cyprinacea*. The relation between water temperature and infection risk is in agreement with previous findings (Berry *et al.*, 1991; Piasecki *et al.*, 2004).

Another parameter that can modify the life cycle of these copepods is water salinity. Some researchers think that the concentration of salts in lakes could determine a reduction in the life time of the Lernidae (Noga, 1996; Mancini *et al.*, 2008a). The lakes of Buenos Aires are shallow, with great variations in the

Table 2. Previous records of fish parasitized by *L. cyprinacea* Linnaeus in Argentina.

Locality	Provinces	Geographical localization	Hosts	Reference
Bahía Blanca	Buenos Aires	38°44′S 62°16′W	*Rhamdia sapo*	Vanotti and Tanzola, 2004
Napostá Grande stream, Bahía Blanca	Buenos Aires	38°48′S 62°14′W	*Oligosarcus jenynsii*	Garibotti and Guagliardo, 2004
Paraná river, Itá Ibaté	Corrientes	27°26′S 57°20′W	*Prochilodus lineatus*	Roux *et al.*, 2000
Los Sauces river, La Viña reservoir	Córdoba	31°17′S 65° 01′W	*Odontesthes bonariensis*	Mancini *et al.*, 2008b
Tanti stream	Córdoba	31°21′S 64°36′W	*Astyanax sp.* *Jenynsia sp.* *Hyla pulchella cordobae (tadpoles)*	Mancini *et al.*, 2008b Alcalde and Batistoni, 2005
Tercero river reservoir	Córdoba	32°12′S 64°28′W	*Odontesthes bonariensis*	Mancini and Grosman, 1998
Atuel river, Valle Grande reservoir	Mendoza	34°50′S 68°31′W	*Odontesthes bonariensis*	Mancini *et al.*, 2008b
Atuel river, El Nihuil reservoir	Mendoza	35°05′S 68°45′W	*Odontesthes bonariensis* *Oncorhynchus mykiss* *Percichthys trucha*	Mancini *et al.*, 2008b
Diamante river, Los Reyunos reservoir	Mendoza	34°35′S 68°40′W	*Odontesthes bonariensis*	Mancini *et al.*, 2008b

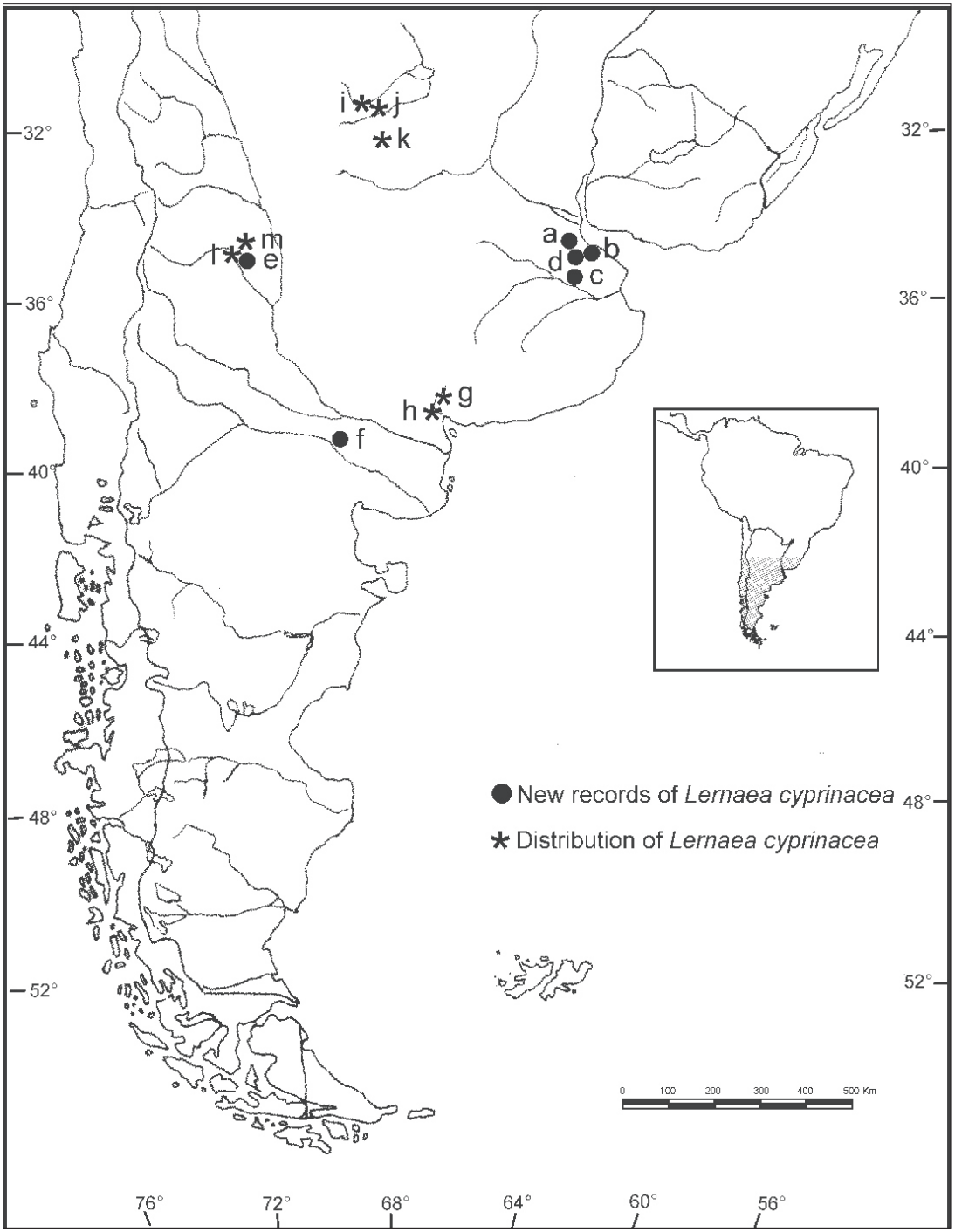

Figure 1. Distribution of *L. cyprinacea* Linnaeus in Argentina. **a.** Luján river and its lagoons of flood, Tigre, **b.** La Plata river, Punta Lara, **c.** Del Burro Lagoon, basin of the Salado river, **d.** La Plata, **e.-l.** Atuel river, El Nihuil reservoir, **f.** Negro river, Choele-Choel island, **g.** Bahía Blanca, **h.** Napostá Grande stream, Bahía Blanca, **i.** Los Sauces river, La Viña reservoir, **j.** Tanti stream, **k.** Tercero river reservoir, **m.** Atuel river, Valle Grande reservoir.

salinity and circulation of water, are naturally eutrophic but have been in ambient stress during the last few years (Quirós, 2005). These lakes have a great concentration of soluble halures, Na^+ and HCO_3^- (Ringuelet *et al.*, 1972). The dam El Nihuil and the river Negro are mesoeutrophic and are highly impacted by irrigation agriculture. Their waters are rich

in sulphates, with Ca^{+2} as the predominant cation and Na^{+} in lower concentrations. In January and February 2005, Cl^{-} was the predominant anion. However, no differences were observed in the mean parasitic intensity in the Negro river, except for an increase in the parasitic charge in the fish captured in the dam El Nihuil. In this case, during January and February 2005, the anion concentration was greater, the water temperature increased 2.8°C, the O_2 pressure was lower and the planktonic community and surface macrophytas were reduced. These extreme conditions facilitate the proliferation of the parasites. The situation was complicated because the fishes were probably immunosuppressed as a consequence of the environmental stress.

L. cyprinacea is one of the most common parasites in wild and aquaculture fishes of Argentina's central region and produces serious economic losses to the fish industry (Mancini *et al.*, 2008b). In our observations, copepod infections were low host specific and adapted to a large variety of habitats offered by different hosts and different sites on these geographical locations.

The several hydrographic river basins mentioned in this work are not connected to each other; therefore, the extensive geographical distribution can not be directly attributed to dispersion by natural means. The human activity could have an indirect influence, by the scattering of *L. cyprinacea* when using live specimens of fish as bait in other lakes and rivers (Mancini and Larriestra, 1997; Piasecki *et al.*, 2004). In Uruguay, Carnevia and Speranza (2003) found *L. cyprinacea* in the skin of the goldfish *C. auratus*; the common escapes from aquarium fishes could spread this crustacean and generate an incontrollable epidemiological condition (Barroso de Magalhaes, 2006).

Our observations give account of the necessity to increase the knowledge on the distribution of these parasites in Argentina in order to determine affected and free zones, laying the foundations for epidemiologist control programmes to limit the effects of the introductions on native populations of fishes. The monitoring of aquatic environments by measuring the density of parasite vectors in the water is very important. The way in which water quality and eutrophication influence the prevalence and intensity of several fish parasites has been well characterized previously (Mancini et al, 2008a). The present work demonstrates the presence of *L. cyprinacea* in a localization more austral than the one previously reported, but the temperature of the water of this finding was in the necessary range for the growth of this parasite. Furthermore, it is important to point out that the findings show a lack of host specificity and a high adaptability to numerous habitats of fresh water. In laboratory conditions a higher salinity of the water is a partially effective mechanism to control the adults, but not the larvae. This treatment is very difficult in a natural habitat, and according to our observations, the seasonal increase in the salinity in lakes is not sufficient to limit the vital cycle of the parasite.

Acknowledgements

We thank to Med. Vet. P. Fernando Andrés Laube and Raúl Herrera for petshop and field sampling. We also thank to Dr. Jose

Celso de Oliveira Malta for the taxonomic characterization of the material and Dr. Pablo I. Marino for your technical assistance.

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