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Short Communication

First molecular data for *Lernaea cyprinacea* (Copepoda: Cyclopoida) infesting *Odontesthes bonariensis*, a commercially important freshwater fish in Argentina

Primeiros dados moleculares para *Lernaea cyprinacea* (Copepoda: Cyclopoida) infestando *Odontesthes bonariensis*, um peixe de água doce comercialmente importante na Argentina

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Abstract

Parasitic copepods of the family Lernaeidae are often found infesting freshwater fishes worldwide. They cause lernaeosis, a disease that can lead to serious pathogenic effects on their fish hosts. The most common lernaeid is the *Lernaea cyprinacea*, which has been widely introduced through importation of tropical fishes, e.g. cyprinids. In South America, it is one of the most common parasites both in wild and in farmed fish in the central region of Argentina. The silverside *Odontesthes bonariensis* is the most important fish of the sport fisheries of Argentina and one of the fish most affected by lernaeosis. Six specimens of copepods were collected from 30 specimens of *O. bonariensis* collected in a Pampean shallow lake (33°25'28"S 62°53'56"W) of Córdoba (Argentina). The 28S rRNA gene of *L. cyprinacea* was amplified by means of PCR to obtain the 28S rDNA sequence. The sequence obtained of this parasite from Argentina showed high genetic similarity with those from various geographical origins. The present study provided molecular characterization of *L. cyprinacea* in South America for the first time.

Keywords: Lernaeidae, freshwater fish, *Odontesthes bonariensis*, Argentina.

Resumo

Os copépodos parasitos da família Lernaeidae são frequentemente encontrados infestando peixes de água doce em todo o mundo, causando a lernaeose, uma doença que pode levar a graves efeitos patogênicos em seus hospedeiros. O lerneídeo mais comum é a *Lernaea cyprinacea*, que tem sido amplamente introduzida por meio da importação de peixes tropicais, tais como ciprinídeos. Na América do Sul, é um dos parasitos mais comuns em peixes selvagens, bem como em peixes de cultivo na região central argentina. *Odontesthes bonariensis* é um dos peixes mais importantes para a pesca esportiva na Argentina e um dos mais afetados pela lerneose. Um total de seis espécimes desses copépodes foram coletados de espécimes de 30 *O. bonariensis* capturados em um lago de pouca profundidade nos Pampas (33°25'28"S 62°53'56"W) em Córdoba (Argentina). O gene 28S rRNA de *L. cyprinacea* foi amplificado por PCR para obter a sequência do 28S rDNA. A sequência obtida desse parasito da Argentina mostrou alta similaridade genética com aquelas de outras origens geográficas. O presente estudo forneceu, pela primeira vez, uma caracterização molecular de *L. cyprinacea* na América do Sul.

Palavras-chave: Lernaeidae, peixe de água doce, *Odontesthes bonariensis*, Argentina.

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Parasitic copepods of the family Lernaeidae (anchor worms) are often found infesting freshwater fishes worldwide. They cause lernaeosis, a disease that can lead to serious pathogenic effects on their fish hosts (LESTER & HAYWARD, 2006). Copepodites may cause disruption and necrosis on the epithelium of fish gill, and attachment of adult females usually results in hemorrhage, muscle necrosis and an intense inflammatory response, which sometimes is associated with secondary bacterial infections. (KHALIFA & POST, 1976; BERRY et al., 1991; LESTER & HAYWARD, 2006).

The most common lernaeid is the *Lernaea cyprinacea*. This parasite probably originated from Asia and it has spread to different parts of the world via movement of aquarium species (ROBINSON & AVENANT-OLDEWAGE, 1996; INNAL & AVENANT-OLDEWAGE, 2012). It has been widely introduced through fish farming and is currently found throughout North America, Europe, Asia, southern Africa and eastern Australia (HOFFMAN, 1999; LESTER & HAYWARD, 2006). In South America, *L. cyprinacea* has been introduced through importation of tropical fishes, e.g. cyprinids (FIGUEIRA & CECCARELLI, 1991).

The life cycle of this parasite does not include an intermediate host. It has nine stages in its life cycle, including three free-living naupliar stages, five copepodites stages and one adult stage (HOFFMAN, 1999). After male and female adults mate on the fish host and then males die, females metamorphose, insert their anterior body into the host tissue and then produce eggs (LESTER & HAYWARD, 2006; NAGASAWA et al., 2007).

The economic importance of lernaeids has increased due to numerous epizootics among the most important farmed fish species in several parts of the world, especially when fish are young (fingerlings), which may lead to death caused by only a small number of parasites (KABATA, 1985; MOLNÁR, 1987; HEMAPRASANTH et al., 2008).

Reports of lernaeids in South America are still scarce, but is worth mentioning that there are thousands of native freshwater fish on this continent that have never been examined for parasites (THATCHER, 2000). However, *L. cyprinacea* is one of the most common parasites both in wild and in farmed fish in the central region of Argentina, and it gives rise to a variety of problems in fisheries (MANCINI et al., 2008b).

The Argentinean silverside, *Odontesthes bonariensis*, is one of the most important freshwater fish for consumption and for sport fishing in the Pampas region, as well as one of the species most affected by lernaeosis (MANCINI et al., 2008a). In chronic diseases, *L. cyprinacea* produces serious consequences in *O. bonariensis,* mainly secondary bacterial infections. (MANCINI et al., 2006). This fish is the main species used for restocking because of its high adaptability and economic importance. Furthermore, it has been introduced into numerous freshwater environments in Argentina and other countries (e.g. Japan, Italia, Peru, Bolivia, Uruguay and Chile) due to the economic activities generated by pejerrey game fish and aquaculture (GROSMAN, 1995; MANCINI et al., 2016).

Thirty specimens of *O. bonariensis* were collected in a Pampean shallow lake (33°25'28"S 62°53'56"W) of Córdoba (Argentina) and analyzed for parasites. Six specimens of copepods were collected, washed in 0.75% NaCl solution and preserved in 80% ethanol. The morphological identification of the copepods follows Kabata (1979). The genomic DNA was isolated using the phenol/chloroform protocol (BILLINGS et al., 1998). The 28S rRNA gene was amplified by PCR with the designed primers 28SF (5' – ACA ACT GTG ATG CCC TTA G – 3') and 28SR (5' – TGG TCC GTG TTT CAA GAC G – 3') (SONG et al., 2008). PCR reactions (25 µL) consisted of 2.5 µl of 10× PCR buffer minus Mg, 1.5 µL of MgCl2 (50 mM), 2 µL of dNTP's (2.5 mM), 1.25 µL of each primer (10 mM), 0.2 µL of Platinum Taq DNA polymerase (5 U/µL) (Invitrogen, Carlsbad, California), 13.8 µL of H_2O, and 2.5 µL of genomic DNA. PCR cycling parameters were according to Song et al. (2008).

PCR results were visualized through electrophoresis in a 1.5% agarose gel stained with ethidium bromide. The amplified products were purified with Exo-SAP-IT Kit (GE Healthcare Life Sciences) according to the manufacturer's instructions, Sanger sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems-Perkin Elmer) in a MegaBACE sequencer (GE Healthcare Life Science), with same primers of PCR reactions. Contiguous sequences were assembled in Geneious ver.9 (KEARSE et al., 2012) and deposited in GenBank under accession number KX908211.

Specimens of *L. cyprinacea* had prevalence of 20.0%. The copepods were found attached around the fins of *O. bonariensis*, mainly on the pectorals fins (Figure 1). All parasites were morphologically similar to the description of *L. cyprinacea* (KABATA, 1979).

Sequence obtained in this study, plus those retrieved from GenBank, were aligned separately according to region using the E-INS-I algorithm of the program MAFFT (KATOH et al., 2002) implemented in Geneious, and ambiguously aligned positions were excluded. Sequences for comparison were chosen according to the following criteria: belonging to *L. cyprinacea* and with sequence length of the 28S rRNA > 661bp. The alignment was subjected to maximum likelihood (ML) under the evolution model Kimura-2-parameters (K2P) for estimation of the genetic distances (KIMURA, 1980).

Figure 1. *Lernaea cyprinacea* specimen (arrow) attached to the base of the pectoral fin of *Odonthestes bonariensis* from a Pampean shallow lake (33°25'28"S 62°53'56"W) of Rio Cuarto, Córdoba (Argentina).

Table 1. Genetic identity, Kimura-2-parameters (K2P) and CG content (%) of sequences of the 28S rDNA from *L. cyprinacea* obtained in the present study and retrieved from GenBank database.

	KX908211*		DQ107546		DQ107547		KP235364		KM281817		KX258626		CG%
	GI	K2P	GI	K2P	GI	K2P	GI	K2P	GI	K2P	GI	K2P	
KX908211*			99.86%	0.001	99.71%	0.003	99.86%	0.001	99.14%	0.007	100%	0	52.0%
DQ107546	99.86%	0.001			99.86%	0.001	99.71%	0.003	98.99%	0.009	99.85%	0.001	51.7%
DQ107547	99.71%	0.003	99.86%	0.001			99.57%	0.004	98.85%	0.010	99.69%	0.003	51.9%
KP235364	99.86%	0.001	99.71%	0.003	99.57%	0.004			98.99%	0.009	99.85%	0.001	52.0%
KM281817	99.14%	0.007	98.99%	0.009	99.85%	0.010	98.99%	0.009			100%	0.007	52.5%
KX258626	100%	0	99.85%	0.001	99.69%	0.003	99.85%	0.001	100%	0.007			51.9%

The newly obtained sequence of the 28S rDNA for *L. cyprinacea* from Argentina deposited in GenBank (Accession No: KX908211) showed high genetic similarity with those from various geographical origins, e.g., Japan (KP235364), China (DQ107546, DQ107547) and Iran (KM281817) have 99% of genetic identity, and the isolate from Egypt (KX258626) had 100% of genetic identity (Table 1). Within all the sequences, the K2P distance varied between 0 and 0.010 (Table 1), and the percentages of cytosine and guanine ranged from 51.7% to 52.5%. Voucher specimens were deposited in the Crustacea Collection of the National Museum of Rio de Janeiro (MNRJ).

The taxonomic features used for identifying species of *Lernaea* seem to be ill-defined. The shape of the anchors is used as the most reliable characteristic for taxonomic comparison, in which the growth of the anchors is considered. This growth depends on the thickness of the tissue to which the parasite is attached (HARDING, 1950; FRYER, 1961, 1968). Conversely, this feature is in fact unreliable for distinguishing species within *Lernaea*, since it may exhibit high intraspecific variation, which creates much taxonomic confusion.

In the present study, the size of the sequence was 661 bp. The mean G + C content (Guanine + Cytosine) was 51.9%. The G+C content of a genome is frequently used in taxonomic descriptions of species and genera. According to Klenk et al. (2014), the G+C content can vary up to 3–5% between species and no more than 1% within species, if computed from genome sequences. In this study the G + C content varied no more than 0.8% (Table 1). There was small sequence divergence within the 28S rRNA gene of representative of *L. cyprinacea* (K2P distances from 0 to 0.010).

The high genetic similarities among the sequences suggest that species of *L. cyprinacea* constitute a genetically homogeneous group, independent of geographical distribution. Moreover, these high genetic similarities suggest that all representatives belong to the same species, according to the sequences of the 28S rRNA gene.

In this light, it is evident that a complete revision of the genus needs to be undertaken. Further descriptions of new species need to be made through an integrated approach that includes use of molecular data.

According to Kabata (1979) there are around 37 valid species of *Lernaea*. With the advent of molecular biology as an integrative tool for morphological identification, the number of valid species of *Lernaea* is likely to be reduced. Despite the importance of the molecular ecology and population genetics of this parasite in relation to freshwater aquaculture, these characteristics of this parasite still remain unexplored (SU et al., 2016).

The present study provided molecular characterization of *L. cyprinacea* in South America for the first time. It represents the first step towards future studies on this still-neglected parasite in Argentinean waters. Furthermore, this integrative approach has proven to be a powerful tool for shedding light on the complicated taxonomy of *Lernaea* spp.

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