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First parasitological study of the African clawed frog (*Xenopus laevis*, Amphibia) in Chile

Primeiro estudo parasitológico em rã com garras Africano (*Xenopus laevis*, Anfibia) no Chile

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Abstract

Introduced species can arrive into new territories with parasites; however, these species are expected to face lower parasite richness than in their original regions. Both introduced hosts and parasites can affect native fauna. Since their release into the wild in Chile following laboratory use, *Xenopus laevis* Daudin, 1802 has widely spread throughout central Chile. The only pathogen described on the host is the fungus *Batrachochytrium dendrobatidis* Longcore, Pessier, Nichols, 1999; thus, this is the first parasitological study of this species in Chile. In 10 localities in central Chile, 179 specimens of *X. laevis* were captured and examined for parasites in the gastrointestinal tube, cavities, lungs, liver, and skin. Only nine specimens of the genus *Contracaecum* Railliet, Henry, 1912 were found in six specimens of *X. laevis* from a private dam in La Patagua. It is likely that these parasites originated from species of native birds. This is the first record of *Contracaecum* sp. in Chilean amphibians.

Keywords: *Contracaecum*, nematode, invasive, anisakid.

Resumo

Espécies exóticas podem se introduzir em um novo território com seus parasitas, porém nesses casos, a riqueza parasitária seria menor. Contudo, hospedeiros exóticos e seus parasitas associados podem afetar a fauna nativa. Depois de ser dispensado do uso em laboratórios e solto em ambientes naturais, *Xenopus laevis* Daudin, 1802 tem se espalhado massivamente no Chile central. O único patógeno descrito para este anuro é o fungo *Batrachochytrium dendrobatidis* Longcore, Pessier, Nichols, 1999. O presente estudo constitui a primeira pesquisa parasitológica realizada nesta espécie de rã introduzida no Chile. Em 10 localidades do Chile central, foram capturados 179 espécimes de *X. laevis* que foram examinadas em busca de parasitos dentro tubo digestivo, cavidades corporais, pulmões, fígado e pele. Nove espécimes do gênero *Contracaecum* Railliet, Henry, 1912 foram encontrados em seis espécimes de *X. laevis* de uma barragem em La Patagua. É provável que a origem destes parasitas sejam espécies de aves nativas. Este é o primeiro relato de *Contracaecum* sp. em anuros do Chile.

Palavras-chave: *Contracaecum*, nematoide, invasivo, anisaquídeos.

Introduction

Invasive species constitute some of the most important causes of biodiversity loss (WILCOVE et al., 1998; WILCOVE & MASTER, 2005). In this context, introduced (non-native) free-living species usually arrive to a new territory with lower parasite richness when compared to what is observed in their original locality (TORCHIN et al., 2003). This can be due to the fact that some parasites are not represented in the population taken from the native geographic range; other parasites are taken with the hosts, but they do not arrive to the new territory, and those parasites that do arrive to the new territory may not survive and reproduce therein (MACLEOD et al., 2010). This suggests that there is an advantage for those invasive species that release from their parasites. However, some parasites can be transported to a

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new territory and naturalize in such a way that the introduced host may act as source of introduced parasites for native free-living species (SMITH & CARPENTER, 2006; LYMBERY et al., 2014), or they can also catch native parasites (KELLY et al., 2009a; JOHNSON & THIELTGES, 2010; MASTITSKY & VERES, 2010), affecting the dynamic of both native hosts and parasites (KELLY et al., 2009b; LYMBERY et al., 2014). Thus, the study of parasites of invasive species fosters an understanding of the underlying processes that positively or negatively affect these species.

The clawed frog, *Xenopus laevis* Daudin, 1802, is native to Africa and has been introduced to Europe, Asia, North America, and South America, for both scientific use and pet trade (MEASEY et al., 2012). In Chile, this frog was introduced into the wild in 1973, when an unknown number of individuals was dumped into the Caren Lagoon, which is close to Santiago's international airport (JAKSIC, 1998). The first naturalized population (stage III, after COLAUTTI & MACISAAC, 2004) was recorded at the beginning of the 1980s (VELOSO & NAVARRO, 1988). *Xenopus laevis* spread from this lagoon on its own to other lagoons, ponds, dams, and watercourses around Santiago, although it has also translocated from Caren Lagoon to other bodies of water (LOBOS & JAKSIC, 2005). The spread rate of this frog in Chile was estimated to be between 3.1 and 5.4 km/year, reaching an invaded area of about 21,200 km^2 in the last decade; this area accounts for four of the fifteen administrative regions (LOBOS & JAKSIC, 2005) and the African clawed frog is expected to invade further north and south in Chile (LOBOS et al., 2013).

At present, the only important pathogen reported in *X. laevis* in Chile is *Batrachochytrium dendrobatidis* Longcore, Pessier, Nichols, 1999, which is the etiological agent underlying chytridiomycosis – a disease that has resulted in the population decline and extinction of several anuran species worldwide (SOLÍS et al., 2010) and is considered a notifiable disease by OIE (2010). Studies on the helminth parasites of *X. laevis* have not been performed in Chile; however, many helminth species have been described in feral populations in North America (KUPERMAN et al., 2004) and in wild populations in Africa (PRITCHARD, 1964; MACNAE et al., 1973; WADE, 1982; TINSLEY, 1996). Thus, in order to describe the antecedents involved in the process *X. laevis* invasion in Chile, in this study, we aimed to analyze the gastrointestinal and external parasite community of this species of frog in this territory.

Materials and Methods

From 1997-2014, 179 adult *X. laevis* were caught from 10 localities in central Chile: El Tabo (Córdova stream; coordinates: 33°26'0.05"S, 71°38'44.61"W; n = 9 individuals), El Yali National Reserve (Los Molles dam: 33°48'3.60"S, 71°41'49.21"W; n = 2), Tejas Verdes (Maipo river: 33°37'42.25"S, 71°36'30.80"W; n = 3), Batuco (Batuco lagoon: 33°12'0.01"S, 70°50'0.01"W; n = 43), Ibacache (Ibacache stream: 33°27'5.18"S, 71°19'59.41"W; n = 24), La Pintana (Universidad de Chile: 33°34'23.88"S, 70°37'57.64"W; n = 5), Las Chilcas (Chilcas stream: 32°52'17.24"S, 70°50'43.19"W; n = 8), Alhue (Carén stream: 34° 3'57.71"S, 71°15'5.94"W; n = 3), Palmilla (Tinguiririca river: 34°35'49.14"S, 71°21'20.29"W; n = 3), and La Patagua (private dam: 34°42'59.26"S, 71°23'5.94"W; n = 79).

Frogs of La Patagua were actively caught from a dam with use of an *ad hoc* mesh; in this dam, the frogs were apparent in high densities. In the other localities, the frogs were captured using simple funnel traps (buckets with tight-fitting lids, modified by the lateral insertion of open cones) and baited with liver. After they were euthanized in benzocaine immersion (see CHARBONNEAU et al., 2010), the frogs were preserved in 70% ethanol and examined in the laboratory under stereomicroscope. They were assessed for helminths through the gastrointestinal tube, cavities, lungs, liver, and skin. Nematodes were preserved in 70% ethanol and cleared with lactophenol for light microscope examination. For the prevalence of infection, refer to Margolis et al. (1982). The Comité de Ética of the Facultad de Ciencias Veterinarias – Universidad de Concepción approved and certified the study (certificate number CBE 23-12). Specimens of nematode parasites were deposited into the Helminthological Collection of Centro de Ecología Aplicada del Litoral (CECOAL 16110301; there were a total of 7 specimens).

Results and Discussion

Only nine specimens of nematodes were found in six frogs (prevalence: 3.4%) from La Patagua. They were found encapsulated in the intestinal serosa and were subsequently identified as larvae of the genus *Contracaecum* Railliet and Henry, 1912. Genus identification was based on morphological attributes (HARTWICH, 2009): the presence of a posterior ventricular appendix, an anterior intestinal caecum, the excretory pore at the base of the ventral labia, and a rounded tail (Figure 1). Measurements (μm; mean ± standard deviation) of eight larvae (unless otherwise stated) were: total length 2,100 ± 380, width: 116.66 ± 12.74, cecum length: 173.75 ± 69.7, esophagus length: 269 ± 54, distance excretory pore – anterior end: 15 ± 42 (two individuals), distance nerve ring - anterior end: 47 (one individual), tail length: 76.25 ± 11.08 (four individuals). Given the small number of specimens and the fact that they were larvae, it was not possible to identify the species. No external parasites were found.

Given the various processes that make the arrival and establishment of parasites to new territories difficult (MACLEOD et al., 2010), the reduced parasite richness of an invasive host species in the colonized territory, when compared to its original geographic range, is expected. Thus, our results were in line with our expectations, particularly as a greater richness of gastrointestinal and cavity helminths have been recorded in *X. laevis* in South Africa. For instance, some of the helminth species reported in African clawed frogs include *Cephalochalamys namaquensis* Cohn, 1906 (cestode); *Protopolystoma xenopodis* Price, 1943; *Gyrdicotylus gallieni* Vercammen-Grandjean, 1960 (monogenean); *Oligolecithus jonkershoekensis* Pritchard, 1964; and *Progonimodiscus doyeri* Ortlepp, 1926 (digenean) (PRITCHARD, 1964; THEUNISSEN et al., 2014). For a further review, see Tinsley (1996). In addition, in this study, the prevalence of *Contracaecum* sp. was low, and the parasites were present in only one geographical location, La Patagua. This lack of helminths in most clawed frogs means that there is a lack of enemies (parasitic helminths), enhancing the survival,

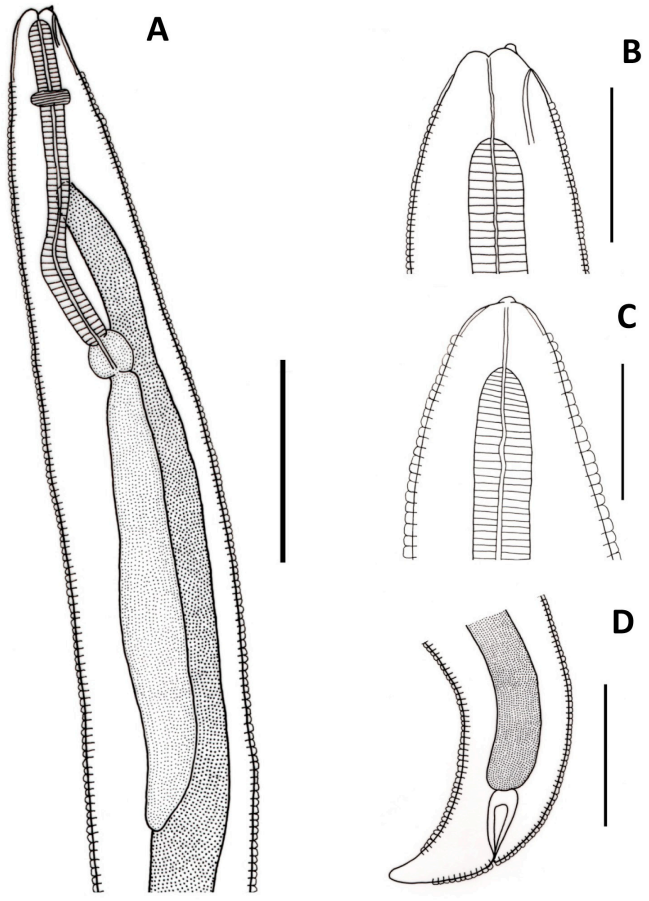

Figure 1. *Contracaecum* sp. larvae from the intestinal serosa of *Xenopus laevis* from La Patagua, Chile: (A) Anterior end; (B) Lateral view of the cephalic portion; (C) Dorsal view of the cephalic portion; (D) Lateral view of the posterior end. Scale bars: A, D: 100 µm; B, C: 50 µm.

which may favor the process of invasion by *X. laevis*. More studies are necessary to confirm this hypothesis. The parasitic richness found in our study is also lower than what was previously found in other territories invaded by this frog, including California, where at least seven species of helminths have been reported in the same anatomical parts of the frogs investigated in this study. These helminths included the following: *C. namaquensis*, *G. gallieni*, *P. xenopodis*, *Clinostomum* sp., *Contracaecum* sp., *Eustrongylides* sp., and *Acanthocephalus* sp. (LAFFERTY & PAGE, 1997; KUPERMAN et al., 2004). Further studies are necessary to test the possible reasons underlying this difference.

In addition to *X. laevis* from California (with prevalences (P) similar to our study, varying from 0 to 4%), the genus *Contracaecum* was reported in Argentina in another introduced amphibian species: *Lithobates catesbeianus* Shaw, 1802 (P = 43.7%) (GONZÁLEZ et al., 2014); this genus was also reported in the native *Bufo marinus* Linnaeus, 1758 (ESPINOZA-JIMÉNEZ et al., 2007) from Mexico (P = 4.2%); in both cases showing higher prevalences than in our study. In Chile, this genus was found in other groups of vertebrates, including birds (P > 17.4%) and fish (P = 13.3%) (TORRES et al., 1982, 1983, 1991, 2005; TORRES & CUBILLOS, 1987; GONZÁLEZ-ACUÑA et al., 2008). However, as far as we know, this is the first record of an anisakid species in amphibians from Chile. Other taxa of nematodes found in amphibians native to Chile include *Rhabdias* sp. (P = 36%) (Rhabditida, Rhabdiasidae); *Parapharyngodon sceleratus* Travassos, 1923 (P = 20-100%); *Spauligodon maytacapaci* Vicente, Ibáñez, 1968 (P = 40-92%) (Oxyurida, Pharyngodonidae); *Aplectana artigasi* Puga, Torres, 1997 (P = 23-100%); *Aplectana chilensis* Lent, Freitas, 1948 (P = 64%); *Cosmocerca chilensis* Lent, Freitas, 1948 (unreported prevalence) (Ascaridida, Cosmocercidae); *Skrjabinelazia* sp. (P = 3.3%) (Ascaridida, Seuratidae); *Physaloptera* cf. *lutzi* Cristófaro, Guimaraes, Rodríguez, 1976 (P = 3-19%); *Physaloptera* sp. (P = 15%) (Spirurida, Physalopteridae); *Oswaldocruzia neghmei* Puga, 1981 (P = 7-100%); and *Oswaldocruzia* sp. (P = 7%) (Sotrongylida, Molineidae) (GARÍN & GONZÁLEZ-ACUÑA, 2008).

Given that *Contracaecum* display an indirect cycle in which the frogs are intermediate hosts, and where laboratory-bred clawed frogs serve as the source of the invasive population (LOBOS et al., 2014), the source of infection for those frogs with *Contracaecum* sp. was more likely the native fauna of Chile, particularly native birds (definitive hosts) than the native range of *X. laevis* (i. e., African infection that persisted through the laboratory breeding in Chile).

Birds are frequently mentioned as hosts of *Contracaecum* sp. One cormorant species, *Phalacrocorax brasilianus* (syn *olivaceus*) Gmelin, 1789 (TORRES, 1983; TORRES et al., 1991, 2005), and three gull species – *Larus dominicanus* Lichtenstein, 1823, *Larus* (syn. *Chroicocephalus*) *maculipennis* Lichtenstein, 1823, and *Larus serranus* Tschudi, 1844 (TORRES et al., 1983) – were found to be parasitized with *Contracaecum* in Chile, with *Contracaecum rudophii* Hartwich, 1964 as the one parasite species found in all cases. In addition, birds, including *L. dominicanus*, are also the main predators of *X. laevis*; there are two other bird species reported as predators of *X. laevis*: *Athene* (syn. *Speotyto*) *cunicularia* Molina, 1782 and *Nycticorax nycticorax* Linnaeus, 1758 (LOBOS & JAKSIC, 2005). While this suggests that *X. laevis* facilitates the infection of this gull by serving as an intermediate host for *Contracaecum* sp. and as prey for *L. dominicanus*, the low prevalence and abundance of this parasite in *X. laevis* may mean that this frog is of low importance in the infection of gulls by *Contracaecum* nematodes.

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