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Research Note

First Survey of Nematode Parasites in Introduced American Bullfrogs (*Lithobates catesbeianus*) in Argentina

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ABSTRACT: We provide the first report of nematode parasites from the introduced American bullfrog (*Lithobates catesbeianus*) in San Juan Province, Argentina. Sixteen individuals (7 adult females, 6 adult males, and 3 juveniles) of *L. catesbeianus* were studied. Thirteen (81.3%) of the frogs harbored 2 species of nematodes: larvae of *Contracaecum* sp. (Nematoda, Anisakidae) in the serosal surface of the stomach wall and at the gastroduodenal junction, and adults of *Falcaustra sanjuanensis* (Nematoda, Kathlaniidae) in the large intestine. Previous studies of introduced populations of American bullfrogs in Argentina have focused on population structure, trophic ecology, and their impacts on native amphibian and other vertebrate populations. More studies of their parasites need to be conducted in South America in general, and in Argentina specifically, in order to assess the extent to which bullfrogs either introduce new parasites or are infected by native parasites and to determine the potential impacts of nonnative parasites on native frog species.

KEY WORDS: American bullfrog, *Lithobates catesbeianus*, introduced species, nematode parasites, San Juan, Argentina.

The introduction of nonnative species by humans is a global biological concern. The reasons for such introductions are varied, and they include biological control, human consumption, and the pet trade. Impacts of nonnative species include extinction or extirpation of native species, biotic homogenization, disruption of food webs, and changes in the primary productivity of the ecosystem. Moreover, such invasive species may act as vectors of disease (Kraus, 2009).

In recent years, the role of parasites in animal invasions has been extensively studied (Torchin et al., 2003; Prenter et al., 2004; Dunn, 2009; Dunn et al., 2012). In a comparative study of parasites of exotic species in their native and introduced range, Torchin et al. (2003) found that hosts from introduced populations had half the number of parasitic species and a lower proportion of infected individuals than those from native populations. Prenter et al. (2004) emphasized the important role parasites can play in biological invasions and established that parasites can indirectly effect interspecific interactions such as competition and predation (see also Hatcher et al., 2006). Understanding these complex interactions requires first documenting the parasites present in introduced species following their successful establishment in new regions.

The American bullfrog, *Lithobates catesbeianus* (Shaw, 1802), is considered one of the most harmful invasive species in the world (Ficetola et al., 2007). This species was first reported in Argentina from San Juan Province in 2005 (Sanabria et al., 2005). Subsequently, bullfrogs have been found in 6 other provinces in the country (Akmentis and Cardozo, 2010; Sanabria et al., 2011). Here we report 2 nematode species found parasitizing a population of American bullfrogs in San Juan Province.

Bullfrogs were collected from the Río Castaño, 130 km west of the city of San Juan, Calingasta Department, San Juan Province, Argentina (31°25′15″S, 69°44′17″W; 1331 m). We collected 7 adult females, 6 adult males, and 3 juveniles of *L. catesbeianus* in February 2010 ($n = 3$) and in December 2012 ($n = 13$). The frogs were euthanized immediately after capture, fixed in 10% formalin, and preserved in 70% ethanol. We thoroughly examined each frog for nematodes via a stereomicroscopic examination of the external surface, mouth, body cavity, lungs, stomach, small intestine, colon, urinary bladder, liver, kidneys, mesentery, and leg musculature. Host sex was determined by observation of reproductive organs. Nematode parasites were fixed in 10% formalin,

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Table 1. Prevalence (as %), mean intensity ±1 SD, and range for nematode parasites from *Lithobates catesbeianus* from San Juan province, Argentina.

	Prevalence	Mean intensity	Range
Contracaecum sp.	43.7	1.14 ± 0.37	1–2
Falcaustra sanjuanensis	43.7	4.28 ± 5.02	1–15

preserved in 70% ethanol, and cleared with lactophenol for light microscopic examination. We calculated the prevalence (percentage of sample infected) and mean intensity (mean ± SE number of parasites divided by the number of infected hosts) for each nematode species. Samples of nematodes species were deposited in the Helminthological Collection of the Museo de La Plata, La Plata, Argentina (*Falcaustra sanjuanensis*, MLP-He 6705, 5 males and 5 females) and in the Helminthological Collection of the Centro de Ecología Aplicada del Litoral, Corrientes, Argentina (*Contracaecum* sp., CECOAL 13111101, 8 specimens; *F. sanjuanensis*, CECOAL 13111102, 5 males and 15 females).

The infection prevalence was 81.3% ($n = 13$) for the 16 frogs examined. In these amphibians we only found 2 species of nematode parasites: coiled larvae of the genus *Contracaecum* Raillet and Henry 1912 (Nematoda: Anisakidae) in cysts attached to the serosal surface of the stomach wall and at the gastroduodenal junction, and adults of *F. sanjuanensis* González, Sanabria and Quiroga, 2013 (Nematoda: Kathlaniidae) in the large intestine. Prevalence, mean intensity, and range are given in Table 1. The following criteria allowed identification of these larvae to the genus level: an esophagus provided with a ventriculus, the presence of a posterior ventricular appendix and an anterior intestinal cecum, the situation of the excretory pore at the level of the base of the lip, and the presence of a tooth-like structure on the labium ventrolateral to the oral opening.

Recent studies concerning introduced anuran amphibian species and their helminth parasites have focused on the cane toad (*Rhinella marina*) in both Australia (Kelehear and Jones, 2010; Pizzatto and Shine, 2011; Pizzatto et al., 2012, 2013; Kelehear et al., 2013) and in Hawaii (Barton and Pichelin, 1999), as well as on *Eleutherodactylus coqui* (Marr et al., 2008, 2010) in Hawaii. The helminth parasites of *L. catesbeianus* have been reported in many studies over its native and introduced range. In these studies, realized principally in the northern hemisphere, the digeneans (specifically lung flukes) were determined to be the most prevalent parasites in bullfrogs (Goldberg et al., 1998; Novak and Goater, 2013). It is interesting to note that other helminths, such as cestodes and trematodes, were not found in the specimens examined in our study. A recent review (Mata-López et al., 2010) listed species of monogenean, digenean, cestode, acanthocephalan, and nematode parasitic worms from 6 countries (Canada, Cuba, Japan, Korea, United Kingdom, and the United States). However, in South America, the only study of the helminth parasites of the American bullfrog refers to specimens bred on a farm in Ubatuba, São Paulo, Brazil, that were destined for human consumption (Antonucci et al., 2012). In that study, 1 nematode species (*Longibucca catesbeianae* Souza Junior, Artigas and Martins, 1993) was reported from *L. catesbeianus*.

To our knowledge, and despite the numerous records of American bullfrog populations in Argentina, ours is the first study of their helminth parasites in the country. One of 2 nematode species we found, *Contracaecum* sp., has been reported from *L. catesbeianus* in the Northern Hemisphere (Goldberg and Bursey, 2002). Species of *Contracaecum* do not achieve sexual maturity in frogs. They require aquatic invertebrates as intermediate hosts to complete their life cycle, with the definitive host being piscivorous birds and aquatic mammals (Anderson, 2000). Our study also includes the first record of *F. sanjuanensis* from the bullfrog. Species of *Falcaustra* parasitize fishes, amphibians, and reptiles, but their life cycle is poorly known. Larval stages have been reported from freshwater fishes and from the tissues of a freshwater snail, so these organisms are likely paratenic hosts of these nematodes (Anderson, 2000). Adult *Falcaustra* spp. have been reported principally from the digestive tracts of turtles and amphibians around the world (Anderson, 2000), but the modes of infection and transmission are unknown. *Falcaustra sanjuanensis* was described from the native frog, *Odontophrynus* cf. *barrioi*, which was collected in Quebrada de Las Flores, San Juan Province, Argentina (González, Sanabria, et al., 2013). This raises a question as to whether the infection occurred first in the native species and subsequently infected bullfrogs, or whether the bullfrogs brought *F. sanjuanensis* to San Juan. The type locality of *F. sanjuanensis* is 145 km east of Calingasta, where the bullfrogs were collected for our study and which is an isolated population. Moreover, the helminths of *L. catesbeianus* have not been well characterized in the Neotropics. Given these facts and the limited data in hand, we cannot address the source of *F. sanjuanensis* infection.

Studies of the consequences of the introduction of the American bullfrog into Argentina are still in their infancy (<10 years old) and most address the population structure and trophic ecology of these amphibians and their potential impact on populations of native amphibians and other vertebrates. To our knowledge, there are only 2 other studies of the parasites of anurans in San Juan province, and these involved the native species *Rhinella arenarum* (Hensel, 1867) and *O*. cf. *barrioi* (see González, Sanabria et al., 2013; González, Quiroga et al. 2013). Therefore, the study presented herein extends our knowledge of the nematode parasites in this province. As such, it would be interesting to compare (e.g., in terms of species richness) the parasites of American bullfrogs from San Juan province with parasites of populations of this host from other areas of Argentina, especially because they occur in a variety of habitats (e.g., Atlantic forests, Precordillera, Pampean grasslands, and Chacoan xeric forest). With parasite data from such diverse habitats, we would be able to address questions concerning habitat or site-specific rates of prevalence or infection as well as the prevalence and direction of host switching.

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