

Short note

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Pathogenic fungus in feral populations of the invasive North American bullfrog in Argentina

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Nearly a third (32.4%) of the world's amphibian species are either threatened with extinction or already extinct (1). The chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) (Chytridiomycetes: Ryzophydiales) (2) has been recognized as responsible for mass mortalities or population extinctions of numerous anuran species in different continents (e.g., 3,4,5 and citations therein). There is evidence that legal and illegal trade in amphibians as pets, food and for bio-control is one of the reasons for the spread of *Bd* (6,7,8,9).

The North American bullfrog, *Lithobates catesbeianus* (Shaw, 1802) (Anura: Ranidae), has been introduced accidentally or intentionally for aquaculture into numerous countries (10). *L. catesbeianus* is a highly invasive species that disperses rapidly within an ecosystem (11). The oldest report of specimens of *L. catesbeianus* parasitized by *Bd* dates from 1928, and corresponds to individuals from the State of California, USA (12). Bullfrogs can be asymptotically infected with *Bd* (13), and infected specimens can readily spread the disease into new geographic regions (14).

Initial unsuccessful attempts to introduce bullfrogs into Argentina for farming date from 1935. A subsequent successful introduction occurred during the 1980s (10,15). Escaped individuals became established and the species spread rapidly (16). Nevertheless assessments of the presence of *Bd* in feral bullfrogs from Argentina are lacking (17,18).

Of 179 amphibian species known in Argentina, 29.14% are considered threatened (19). The potential impact of *Bd* dispersed by invasive bullfrogs to the Argentine amphibians is large. The aim of this study was to assess the presence of *Bd* in feral populations of *L. catesbeianus* established in the western highlands of Argentina.

We sampled bullfrogs from three feral populations in permanent water bodies in the Argentinean western highlands [phytogeographical region of Monte Province, Chacoan Domain; (20)] (Fig. 1 and Table 1). Sampling took place in a rainy month with high activity of *Lithobates catesbeianus* (February 2008, February 2010 and February 2011). To capture bullfrogs, we used visual transects and sampling effort standardized by search time (the first two hours after sunset). Amphibians were caught by hand and handled using latex rubber examination gloves. Following HYATT *et al.* (21) and LIVO (22), we gently but firmly swabbed 10 times on the ventral surface, hind limbs and interdigital membrane of each specimen collected and placed each swab in an individually numbered plastic cryogenic vial, frozen at -18°C for storage. We followed field sampling protocols of DAPTF Fieldwork Code of Practice and those outlined in LIVO (22) to avoid possible cross-contamination of pathogens among ponds.

Laboratory detection and quantification of infection with *Bd* were performed at the Laboratory of Vector Borne Disease Diagnostics from North Carolina State University's School of Veterinary Medicine, using *Bd*-specific real-time Taqman (Applied Biosystems, Foster City, CA) quantitative polymerase chain reaction (qPCR), according to the method of BOYLE *et al.* (23) with PrepMan Ultra Kit (Applied

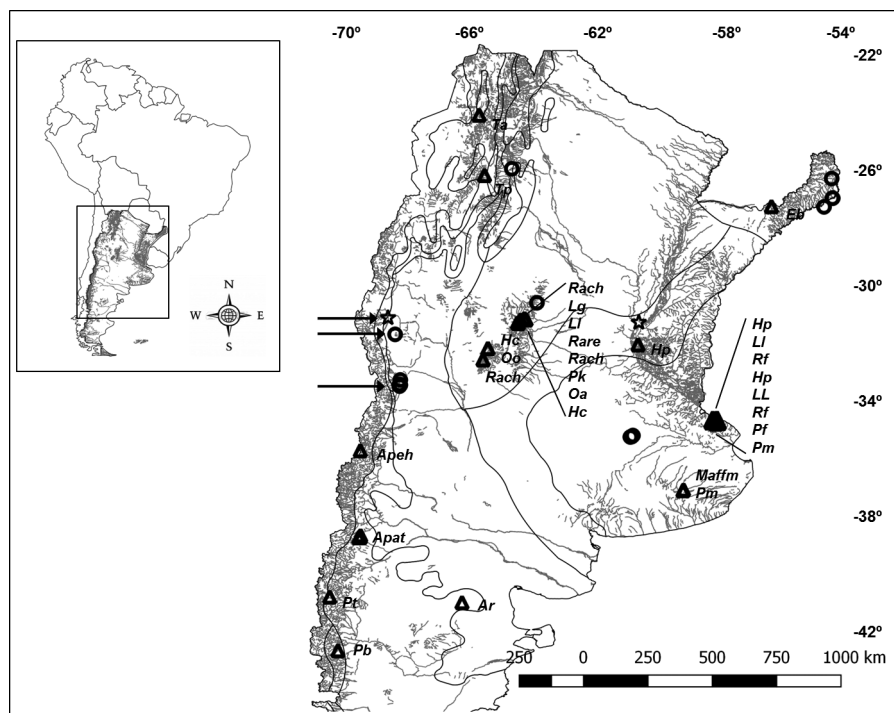


Figure 1. – Chytrid fungus and *Lithobates catesbeianus* (Shaw, 1802) (*Lc*) reports in Argentina. Arrows: sampled *Lithobates catesbeianus* populations tested for *Batrachochytrium dendrobatidis* (*Bd*) for this work. Stars: *L. catesbeianus* *Bd* positive. Circles: feral populations of *Lc* reported in literature. Triangles: native species infected with *Bd* reported in literature. Species acronyms: (*Apeh*) *Alsodes pehuenche*; (*Apat*) *Atelognathus patagonicus*; (*Ar*) *A. reverberii*; (*Eb*) *Elachistocleis bicolor*; (*Hc*) *Hypsiboas cordobae*; (*Hp*) *H. pulchellus*; (*Lg*) *Leptodactylus gracilis*; (*LI*) *L. latrans*; (*Maffm*) *Melanophryniscus aff. montevidiensis*; (*Oo*) *Odontophrynus occidentalis*; (*Oa*) *O. achalensis*; (*Pf*) *Physalaemus fernandezae*; (*Pb*) *Pleurodema bufoninum*; (*Pk*) *P. kriegi*; (*Pt*) *P. thaul*; (*Pm*) *Pseudis minutus*; (*Rach*) *Rhinella achalensis*; (*Rare*) *R. arenarum*; (*Rf*) *R. fernandezae*; (*Tp*) *Telmatobius pisanoi*; (*Ta*) *T. atacamensis*.

TABLE 1

Lithobates catesbeianus infected with *Batrachochytrium dendrobatidis* (*Bd*) in sampled localities. Elevation are in m asl. N/*Bd*+: total analyzed individuals over number of specimens infected with *Bd*. Ct: cycle threshold of positive individuals.

Locality	Coordinate	Elevation	N/ <i>Bd</i> +	Ct
Puchuzúm	31° 20'S; 69° 50'O	≈1800	82/12	38.24±3.43
Cerro Blanco	31° 55'S; 68° 70'O	650	10/0	
Capiz	≈ 33° 41'S; 69° 01'O	902	11/0	

Biosystems, California, USA). Appropriate negative and positive controls were run concurrently with survey samples, and the limit of detection was less than/equal to 0.5 zoospore equivalents. We analysed the cycle threshold of positive results.

We examined 103 individuals of which 12 tested positive for *Bd*. The cycle threshold was 38.24±3.42 (Table 1). All infected bullfrogs belonged to only one of the three sampled localities (12 *Bd*-positive from 82 individuals sampled at Puchuzúm) (Table 1 and Fig. 1). When collected, frogs showed no evident signs of chytridiomycosis to the naked eye. No unusual sloughing of the skin or sliminess was observed in any individual swabbed, nor were any dead individuals recorded during the course of this study.

The most extensive biological invasions of the North American bullfrog into South America are in Brazil and Argentina, with feral populations identified over a large extent of their territories (24,25,16). Until now, the only other work that has looked into *Bd* infection of feral bullfrogs in central Argentina did not detect chytrid (25 specimens examined histologically) (17). Our results confirmed the presence of *Bd* in feral bullfrogs from western Argentina.

Experimental data show that *L. catesbeianus* is among the species capable of being infected by *Bd* without progression of clinical signs or death (13, 26). The infected individuals analyzed in this study showed no evident signs of chytridiomycosis, supporting the findings of DASZAK *et al.* (13) and ESKEW *et al.* (26). Although the cycle threshold for positive results indicated moderate to minimal amounts of *Bd* DNA in the samples (23), bullfrogs become reservoirs and potential vectors of *Bd* to native cohabitant species.

The first detection of chytridiomycosis in native anuran species from Argentina was reported by HERRERA *et al.* (27). Since then, specimens in this country belonging to more than 20 native anuran species have been found to be infected (Fig. 1). However, to confirm that introduced bullfrogs are *Bd* vectors to Argentinean native species, the genetics of *Bd* strains should be analyzed to help discriminate among the possible origins and directions of transmission. Furthermore, the number of wild and captive bullfrog populations (and individuals) analyzed should be increased.

Humans are believed to be a major dispersal agent for *Bd* (3,28) and responsible for exotic species introduction and spread (7). Through informal interviews during fieldwork, local people told us that direct dispersion of bullfrog larvae by human translocation to new water bodies is carried out, in order to increase the bullfrog population in nearby areas, since frogs are used as food, live bait for fishing, and for ritual purposes. This activity may favour dispersion of bullfrogs infected by *Bd* to *Bd*-free locations.

This is the first study that identifies the presence of *Bd* in feral populations of *L. catesbeianus* in Argentina; this presence represents a major potential threat to native amphibian species. Sampling more specimens

from the other two locations, Capiz and Cerro Blanco, would contribute to elucidating whether these populations are *Bd* free or if our sampling effort was insufficient for detecting chytrid fungus infection. Strict regulations on the transportation and farm breeding of bullfrogs should be implemented to prevent potential spread of *Bd* by *L. catesbeianus*. Testing the prevalence of infections in bullfrogs, and monitoring their trade and releases, would further contribute to understanding the role of the commercial trade in the dispersion of pathogens in ecosystems (29).

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