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First Report of *Batrachochytrium dendrobatidis* in the Warty Toad, *Rhinella spinulosa* (Wiegmann, 1834), from the Argentinean Andean Foothills

The chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) has been highlighted as one of the suspected drivers of amphibian declines world-wide (Schloegel et al. 2010). Herrera et al. (2005) detected the first case of chytridiomycosis in Argentina in a dead specimen of the native species *Leptodactylus latrans*. Since then, new records of *Bd* have been reported in a growing number of native species (Ghirardi 2012 and cites therein; Lescano et al. 2013; Agostini and Burrowes 2015).

The bufonid genus *Rhinella* includes 91 species broadly distributed around the world. The Warty Toad, *R. spinulosa,* inhabits the Andean slopes of Argentina, Chile (scrubland and grassland of the Altiplano region in the North to the southern *Nothofagus* forest region), Bolivia and Perú (Frost 2016) in a wide altitudinal range from sea level to 5100 m elev. (IUCN 2016). In Argentina, *R. spinulosa* occurs from the northwest section of the country (~22ºS, 67ºW) to the southwest of the Paragonian region (~46ºS, 69ºW), in the 900–4000 m elevational range (Cei 1980) (Fig. 1). There are reports of *Bd* in three species of this genus in ~~the country~~: *R. achalensis* and *R. arenarum* (Lescano et al. 2013), and *R. fernandezae* (Agostini and Burowes 2015). Herein we report the presence of *Bd* in the Warty Toad *Rhinella spinulosa* (*sensu lato*) from populations in the Argentinean Andean foothills.

During October and November 2009, and February 2010, we sampled 13 populations of *Rhinella spinulosa* in Argentina for *Bd* presence (Table 1). We carried out the study in the Andean foothills spanning a latitudinal range of ~1000 km and an altitudinal range of ~2600 m elev., in the Provinces of Jujuy, Salta, Catamarca, La Rioja, and San Juan (Fig. 1, Table 1). The region turns from partial to extremely arid from north to south. Annual precipitation ranges from 200 to 400 mm, which falls almost exclusively between November and February (summer). Summers are long and hot, with average high temperatures between 21°C and 35°C; afternoon thunderstorms are common, as well as heat waves that can bring temperatures as high as 36°C to 40°C for a few days. In winter, temperature lows range between 5°C to 19°C.

For fieldwork we followed the sampling protocols of DAPTF (1998) and Livo (2004) to avoid possible cross-contamination of pathogens among ponds. To collect samples we gently but firmly swabbed individuals (Livo 2004; Hyatt et al. 2007) and placed each swab in a numbered plastic cryogenic vial for storage. At populations 1 to 12, we collected pooled samples, where a swab of one or more individuals was combined for a single laboratory test (Table 1). At population 13, we swabbed and tested individual toads independently. We performed laboratory detection and quantification of *Bd* 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, California, USA) quantitative polymerase chain reaction (qPCR), according to the method of Boyle et al. (2004). We analyzed the cycle threshold of positive results (Ghirardi et al. 2014).

We found *Bd* presence in 5 of 22 (23%) swabs, at 5 of 13 (38%) populations across 3 of the 5 sampled Provinces (Table 1). Four of 15 (27%) swabs tested for *Bd* presence in *R. spinulosa* populations 1 to 12 were *Bd*-positive, including one pooled sample of 31 toads. However, it should be noted that if *Bd* prevalence were low, our small sample size may have precluded detection (Skerratt et al. 2008). One specimen f~~orm~~ Iruya Iturbe (population 1) was found dead and it was positive for *Bd* (Table 1, Fig. 1). In Huerta de Huachi (population 13), 1 of 7 (14%) independently swabbed individuals was positive for *Bd* infection (Ct 40.16) (Table 1).

There is a previous report of *Bd* in *Rhinella spinulosa* from the Andes highlands of northern Chile (Solís et al. 2015) but this is the first report of *Bd* infection in *R. spinulosa* populations from

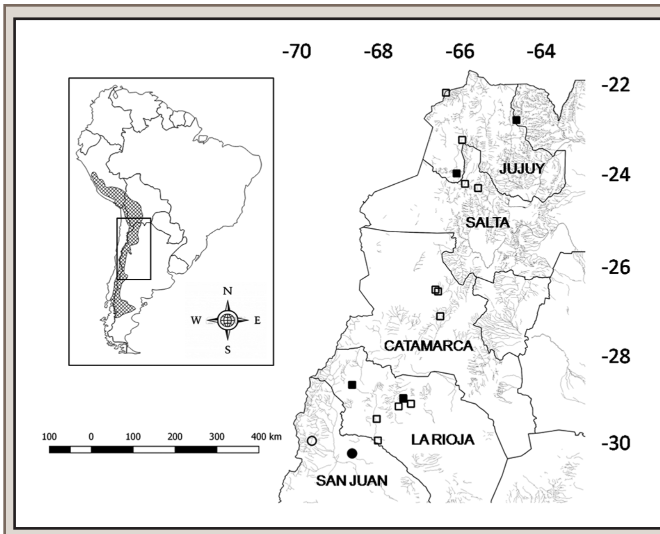

Fig. 1. *Rhinella spinulosa* populations tested for *Batrachochytrium dendrobatidis* (*Bd*) in five Provinces of Argentina. Squares: *R. spinulosa spinulosa*. Circles: *R. spinulosa papillosa*. Solid symbols: *Bd*-positive. Open symbols: *Bd*-negative. Shaded area of inset map is the distribution of the species (IUCN 2016). Latitude (°S) and longitude (°W) are indicated for sampled area.

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Table 1. Locations of *Rhinella spinulosa* samples tested for *Batrachochytrium dendrobatidis* (*Bd*) in Argentina. ID: population identification number. NBd: number of swabs testing *Bd*-positive; I: number of individual swabbed; S: number of swabs analyzed; d: dead individual sampled; Oct: fieldwork conducted in October 2009; Feb: fieldwork conducted in February 2010; the rest of fieldwork conducted in November 2009.

ID	Province	Site	Latitude °S	Longitude °W	Elevation (m)	NBd	I/S
1 Oct	Jujuy	Iruya Iturbe	-22.282811	-66.391081	3312	1	1/1d
2	Jujuy	RN40	-23.282811	-66.391080	3888	0	4/1
3	Jujuy	Puesto Sey	-23.282833	-66.391081	3681	1	1/1
4	Salta	RN 151	-24.316194	-66.050302	~3800	0	2/1
5	Salta	San Antonio de los Cobres River	-24.224369	-66.330236	~4100	0	1/1
6	Catamarca	Laguna Blanca	-26.534669	-66.908606	~3200	0	2/1
7	Catamarca	Villa Vil	-22.086444	-66.821353	2100	0	5/1
8	Catamarca	El Chorro	-26.495761	-66.964125	3200	0	2/1
						0	2/1
9	La Rioja	Río Aschavil	-28.824881	-67.654908	2200	0	9/1
						0	3/1
10	La Rioja	Río El Marco	-28.836903	-67.651344	2116	1	4/1
11	La Rioja	Araya	-29.001453	-67.754492	~4000	0	1/1
12	La Rioja	Arroyo El Peñón	-28.538914	-68.752383	3050	1	31/1
						0	3/1
13 Feb	San Juan	Huerta de Huachi	-30.011694	-68.753222	1497	1	7/7

Argentina. *Rhinella spinulosa* is categorized as Least Concern at national and international levels; it has a presumed large population and is unlikely to be declining (Vaira et al. 2012; IUCN 2016). Although the effects of *Bd* on *R. spinulosa* are uncertain at this time, *Bd*-mediated population declines have not been indicated (Bielby et al. 2008; Ghirardi et al. 2014), and it is possible that the species complex may serve a role as a reservoir host (Catenazzi et al. 2013; Solis et al. 2015). It is worth noting that the taxonomic status of *R. spinulosa* is being examined and there are two subspecies proposed by Cei (1980), corresponding to the two groups sampled in our study. Neither subspecies is included in a threat category at the national level (Vaira et al. 2012; not evaluated at subspecies level by IUCN), and populations of both subspecies included *Bd*-infected toads in our analysis: *R. spinulosa spinulosa* (populations 1 to 12; Table 1) and *R. spinulosa papillosa* (population 13; Table 1).

Northern populations of *R. spinulosa spinulosa* in Argentina co-occur with ~~frog~~ populations of the genus *Telmatobius*, some species of which are threatened at national and international levels (IUCN 2016; Vaira et al. 2012). In particular, *T. ceiorum*, *T. laticeps*, and *T. pisanoi* have had drastic population declines (Barrionuevo and Ponssa 2008), and there was a report of *Bd* infections in *T. atacamensis* and *T. pisanoi* in the northwest region of Argentina (Barrionuevo and Mangione 2006), near some of the populations of *R. spinulosa* evaluated here (populations 3, 5, 6, 7, and 8). Further research is warranted to assess whether *Bd*-infected *R. spinulosa* may play a role as a vector of *Bd* to other native species with threatened status (Solis et al. 2015).

More than a decade after the first red list of herpetofauna proposed by the Herpetology Association of Argentina (Asociación Herpetológica Argentina) (Lavilla et al. 2000), a group of specialists reevaluated the conservation status of amphibians from Argentina in 2012 (Giraudo et al. 2012; Vaira et al. 2012). The threat of 175 amphibian species was analyzed following compilation of systematic (Taxon) and geographic (Province level) information (Vaira et al. 2012). Our results add new information about *Bd* infections in a previously unassessed species and from new geographic regions (Provinces), which can inform adaptive management of species extinction risk categorizations at regional, national and international levels.

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Report of an Oomycete Infection in a Western Lesser Siren (*Siren intermedia nettingi*) from Southeastern Oklahoma, USA

Infections from oomycetes (water molds) are among the top pathogens responsible for global amphibian declines (Blaustein et al. 1994; Blaustein and Kiesecker 2002; Densmore and Green 2007). In North America, several salamanders of all aquatic life stages have been reported to be infected with oomycetes, including the Spotted Salamander (*Ambystoma maculatum*), Marbled Salamander (*Ambystoma opacum*), Oklahoma Salamander (*Eurycea tynerensis*), Mudpuppy (*Necturus maculosus*), and Greater Siren (*Siren lacertina*) (Bishop 1926; Goin 1961; Petranka 1989; Connior et al. 2014; Urban et al. 2015). In a species account, Leja (2005) did not mention any reports of oomycetes in the Western Lesser Siren *S. intermedia*. Here, I document an oomycete infection in a *S. i. nettingi* from southeastern Oklahoma, USA.

During October and November 2016, five juvenile *S. i. nettingi* (mean ± 1SD SVL = 108 ± 32.5; range 72–143 mm) were collected with a dipnet from a private lake in Idabel, McCurtain Co., Oklahoma (33.864773°W, 94.859133°N). Specimens were kept in lake water and taken to the laboratory where they were placed in a 20-L aquarium. On immediate examination, one of the *S. i. nettingi* (72 mm SVL) was noted as having what appeared to be unknown white-to-light brown fluffy or cotton wool-like patches/lesions on the body (Figs. 1A–C). This specimen was euthanized with a dilute chloretone solution and the growth was sampled by removing a portion with fine sterilized forceps, wet mounted on a microscopic slide with tap water and cover slip, and examined by light microscopy. Photomicrographs were obtained using a Swift Model 10LB-S digital video light microscope and a Motic K400 stereomicroscope fitted with a Canon conversion lens (Martin Microscope LA-DC58E). The voucher specimen of *S. i. nettingi* was deposited in the Arkansas State University Museum of Zoology (ASUMZ), Herpetological Collection as ASUMZ 33608.

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Without culturing and DNA sequencing, it was not possible to determine with certainty which water mold genus was present in this case. Microscopic examination revealed aseptate, multinucleate and unbranched or branched hyphae (Fig. 1D) with zoospore (Fig. 1E) similar to species in the water mold (Oomycetes) order Saprolegniales (Webster and Weber 2007). Pathogenic oomycetes belonging to this order include the genera *Achlya*, *Aphanomycetes*, and *Saprolegnia*. The clinical signs of saprolegniasis in larval amphibians include the external appearance of colonies that appear like those in the present case.

Frye and Gillespie (1989) reported salamanders may experience anorexia, lethargy, weight loss, vomiting, and respiratory distress, with death resulting in severe cases, presumably from osmoregulatory impairment. On necropsy, the salamander in the present case did not contain any visible fat bodies, and no food was present in the gastrointestinal