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The Chytrid Fungus *Batrachochytrium dendrobatidis* Infecting the Creole Frog, *Leptodactylus latrans*, in a New Region of West Argentina

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The emerging infectious disease chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), can trigger rapid amphibian population declines and extirpations (Vredenburg et al. 2010). In some cases, *Bd* may persist in "reservoir hosts" which do not experience disease symptoms when infected, thus maintaining the pathogen within the ecosystem in the absence or with low density of other disease-susceptible hosts (Brannelly et al. 2017). The disease and host community dynamics of disease-susceptible and –reservoir hosts are not well understood.

There are reports of *Bd* in the Creole Frog, *Leptodactylus latrans* (Leptodactylidae) throughout its broad distribution in South America (Dall'Acqua Coutinho et al. 2015). *Leptodactylus latrans* occurs in Argentina, Venezuela, Bolivia, Brazil, Colombia, French Guiana, Guyana, Paraguay, Suriname, Trinidad and Tobago and Uruguay (Fig. 1). This species uses a wide variety of habitats, from wet grasslands to highly modified agroecosystems, occurring in habitats with tropical to temperate climates and from sea level to around 1400 m elevation (Sanabria et al. 2005). In temperate wetlands, its life expectancy is five years, with early maturation (López et al. 2017). Males and females in amplexus deposit eggs in foam nests, tadpoles are aquatic and form aggregations of several developmental stages, and remain gregarious until completing metamorphosis (Rodrigues et al. 2011).

The first case of chytridiomycosis in Argentina was reported by Herrera et al. (2005) in a dead specimen of *L. latrans*. Since then, new records of *Bd* had been reported in the country in a growing number of native species (Barrionuevo and Mangione 2006; Arellano et al. 2009; Ghirardi 2012; Lescano et al. 2013; Agostini and Burrowes 2015; Ghirardi et al. 2017a) and in the introduced American Bullfrog, *Lithobates catesbeianus* (Ghirardi et al. 2011; 2017b).

Since the report of Herrera et al. (2005), there have been additional cases of *L. latrans* individuals infected by *Bd* in Argentina (Ghirardi et al. 2009; Ghirardi 2012; Agostini and Burrowes 2015). However, according to our knowledge, there is only one study reporting *L. latrans* survival to *Bd* infection (Arellano et al. 2017), which evaluated tadpole disease susceptibility. Herein, we report *Bd* infecting wild *L. latrans* in a novel region from Argentina.

We analyzed *Bd* infection in six specimens of *L. latrans* deposited in the herpetological collection of Instituto y Museo de Ciencias Naturales from Universidad Nacional de San Juan (Table 1). The individuals were juvenile and adult frogs collected during a research project (Sanabria et al. 2005) in Argentinean Andean foothills (Departamento Zonda, San Juan Province; 31.550°S, 68.733°W; 650 m elev.; Fig. 1) during spring and summer 2002 (from September to March). *Leptodactylus latrans* were hand-captured on diurnal and nocturnal transects. The frogs were sacrificed in situ by immersion in a buffered anesthetic solution of benzocaine (Heyer et al. 1994), fixed in 10% formaldehyde, and preserved in 70% ethanol. When collected, frogs showed no evident signs of chytridiomycosis to the naked eye. No unusual sloughing of the skin was observed in any individual, nor were any dead individuals recorded during the course of the field work (Sanabria, pers. comm.).

We excised skin patches of abdominal and ventral hind limbs, and applied standard histological techniques (Ghirardi et al. 2011) using light microscopy. Briefly, we cut two samples per individual (~0.5 cm^2 each), dehydrated them, encased them in paraffin and sectioned them at 5 µm. We stained the sections with hematoxylin and eosin and examined them at 40x and 100x magnification following Berger et al. (1999) and Pessier et al. (1999).

Histological examination revealed presence of *Bd* in all six studied specimens. The stratum corneum presented zoosporangia at different developmental stages, spherical and ovoid, empty or containing zoospores. Many of the zoosporangia

TABLE 1. Museum specimens of *Leptodactylus latrans* collected in 2002 in the Argentinean Andean foothills examined using histology techniques for *Batrachochytrium dendrobatidis* (*Bd*) infection. Female (F); Male (M); Juvenile with no identified sex (*). All individuals tested positive for *Bd*. Specimen identification (ID) numbers: Instituto y Museo de Ciencias Naturales, Universidad Nacional de San Juan, Argentina.

Specimen ID	Collection date	Snout–vent length (cm)	Sex
IMCN UNSJ 5100	October 2002	9.2	F
IMCN UNSJ 5101	October 2002	9.5	F
IMCN UNSJ 5102	October 2002	5.6	*
IMCN UNSJ 5103	October 2002	10.5	M
Nº V	February 2002	8	*
Nº VIII	February 2002	9.8	F

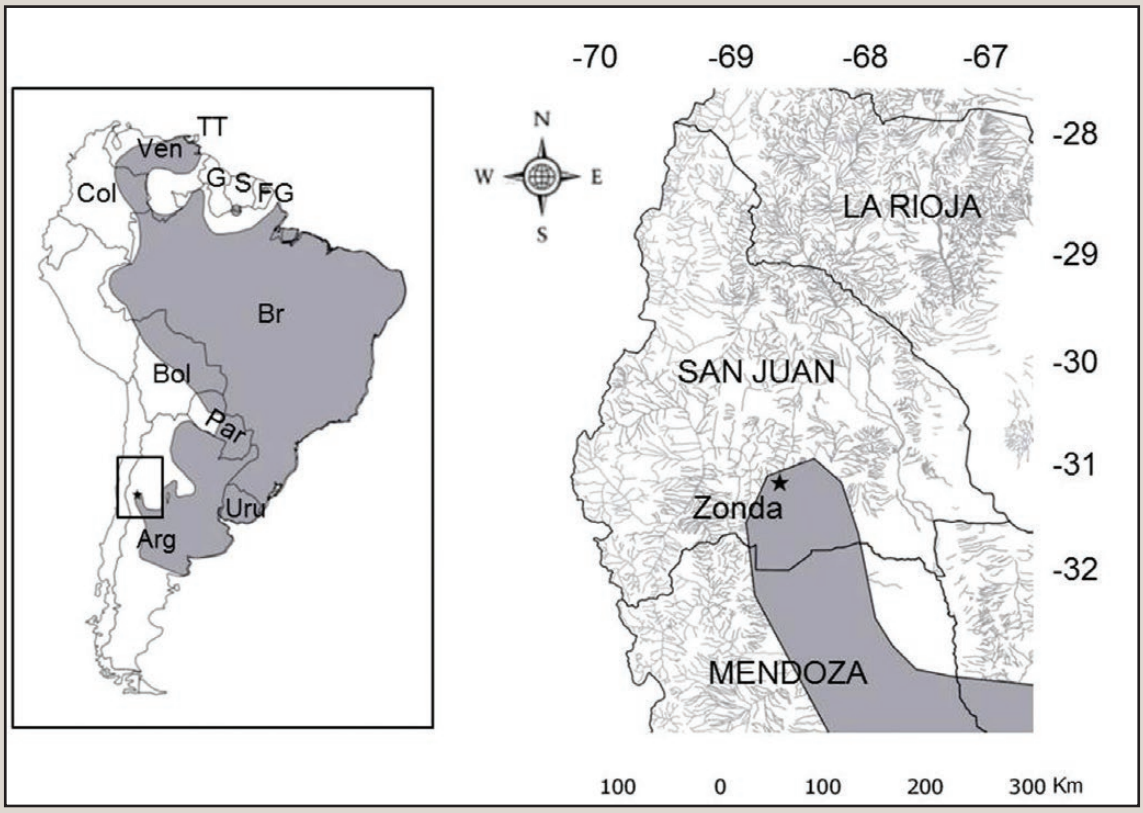

FIG. 1. Map of west-central Argentina showing the collection site of *Leptodactylus latrans* specimens (black star) tested for *Batrachochytrium dendrobatidis* infection. Latitude (°S) and longitude (°W) are indicated. Inset map (left) shows the distribution of *L. latrans* in South America. Arg: Argentina; Uru: Uruguay; Par: Paraguay; Bol: Bolivia; Br: Brasil; Col: Colombia; Ven: Venezuela; TT: Trinidad and Tobago; G: Guyana; S: Suriname; FG: French Guiana.

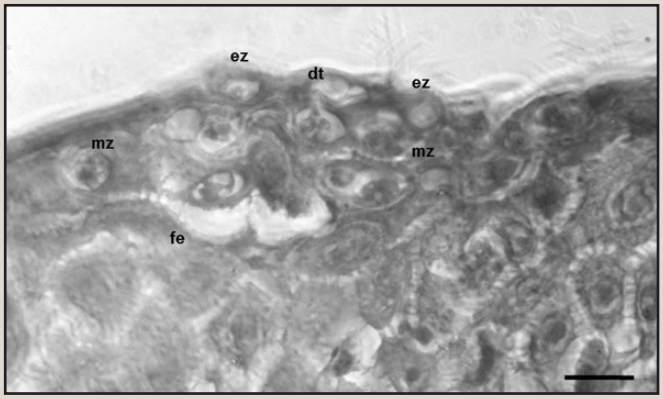

FIG. 2. *Leptodactylus latrans* skin section with hematoxylin and eosine histological technique. Zoosporangia at different developmental stages: mature zoosporangia (mz), empty zoosporangia (ez), zoosporangia with discharge tube oriented toward the skin surface (dt). Also shown is the focal erosion adjacent to the infection (fe). Scale bar: 15 µm.

had a discharge papilla oriented toward the skin surface (Fig. 2). The infected areas included mild to moderate hyperkeratosis and areas of focal erosion adjacent to the infection (Fig. 2). According to Longcore et al. (1999), *Bd* grows within skin, and is able to damage keratinized cells. In histological sections of analyzed *L. latrans*, skin affected by the fungus showed alterations in the normal epidermis architecture.

The high prevalence of *Bd* in the samples (100%) in contrast with the possibility of false negatives caused by the limited sensitivity of histological techniques (Puschendorf and Bolaños 2006), supports a high prevalence of infection in individuals of our studied population. Overall, studies in diverse amphibian assemblages have found that those species with aquatic habits (such as *L. latrans*) had a higher likelihood of *Bd* infection (e.g., Lips et al. 2003; Agostini and Burrowes 2015). This pattern may be explained by the increased probability of contact with the *Bd* aquatic zoospores, which represent the infectious stage of this pathogen (Longcore et al. 1999). Furthermore, in *L. latrans*, the gregarious behavior of tadpoles and post-metamorphic individuals (Rodrigues et al. 2011) also might explain the high *Bd* prevalence found in our results. However, Gervasi et al. (2017) found that amphibian species associated with more ephemeral aquatic environments in general tended to become more heavily infected and experienced greater mortality when they were exposed to *Bd* than those that inhabit permanent aquatic environments. *Leptodactylus latrans* inhabit a great variety of habitats, from wet grasslands to arid and highly modified agroecosystems, from ephemeral to permanent ponds, making it a suitable host for *Bd* under each of the hypotheses raised above.

Our results add a new record of *Bd* to the list of *L. latrans* infected and in a new bioregion from Argentina. Moreover, the apparently widespread infection of this chytrid fungus in populations of *L. latrans* from different climates, elevations, and bioregions (Ghirardi et al. 2009; Ghirardi 2012; Agostini and Burrowes 2015; Dall'Acqua Coutinho et al. 2015), without a single report of population declines ascribable to *Bd*, suggests that *L.*

latrans may act as a reservoir host. Although a reservoir-host explanation might be consistent with *Bd* infection dynamics in *L. latrans*, with only six samples in the current study and the few other available studies, further screening for chytridiomycosis is warranted, as well as population monitoring, especially in *L. latrans* populations and in other species that are sympatric with *L. latrans* across its broad distribution.

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