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First Record of *Batrachochytrium dendrobatidis* in *Pleurodema somuncurense*, a Critically Endangered Species from Argentina

The Valcheta Frog, *Pleurodema somuncurense* (Cei 1969), is an endemic species from the Somuncura Plateau (northern Patagonia, Argentina) with a high degree of habitat specialization and a very small distributional range. It is an almost wholly aquatic frog that inhabits permanent thermal springs and the warm headwaters of the Valcheta Stream, a watercourse located at the edge of the plateau. The Valcheta Frog is one of most endangered species of Argentina (Vaira et al. 2012) and one of the three amphibians in this country listed as Critically Endangered by the IUCN Red List (IUCN 2016) due to a restricted range and a combination of threats including exotic predatory fish species (*Oncorhynchus mykiss* and *Salvelinus fontinalis*), habitat fragmentation, and livestock encroachment (Velasco et al. 2016). The potential threat of disease to *P. somuncurense*, namely the emerging fungal disease chytridiomycosis caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*), is unknown. However, the pathogen was recently reported by Arellano et al. (2015) in *Atelognathus reverberii*, another endemic species from Somuncura Plateau that inhabits high altitude lagoons. *Bd* is widely distributed throughout Argentina, infecting several species (Herrera et al. 2005; Barrionuevo and Mangione 2006; Fox et al. 2006; Arellano et al. 2009; Ghirardi et al. 2009, 2014; Gutierrez et al. 2010; Delgado et al. 2012; Lescano et al. 2013). However, there are few *Bd*-related mortality records, with the exception of a case in *Atelognathus patagonicus* (Ghirardi et al. 2014).

In February 2015 during a population study of the Valcheta Frog, we conducted visual encounter surveys covering the whole range of the species, which encompasses two pairs of stream branches that comprise the headwaters of Valcheta Stream (Fig. 1; Velasco et al. 2016). These branches are locally identified as the cold and warm branches, with temperatures ranging from 20.5 to 22.5°C and from 22 to 26°C, respectively (Ortubay et al. 1997). During surveys, we hand-captured 15 adult frogs (using latex rubber examination gloves) from the western cold branch (N = 7), eastern cold branch (N = 4), and western warm branch (N = 4). Individuals were gently but firmly swabbed 10 times using sterile fine-tipped rayon swabs with plastic shafts, on the ventral surface, hind limbs and interdigital membrane following the techniques of Hyatt et al. (2007).

We also found two dead individuals at the eastern warm branch (Fig. 1). These individuals were fixed in formalin, and then we took samples of shed skin from hind limbs. These samples were observed with a light microscope at 400x magnification to search for the characteristic *Bd* zoosporangia.

The swab samples were preserved in absolute ethanol. DNA extractions were performed using 40 µl of PrepMan Ultra (Applied Biosystems) using procedures as Boyle et al. (2004). The extracts were diluted to 1:10 with PCR grade water and stored at -20°C until they were analyzed in duplicate quantitative PCR following the methods of Kerby et al. (2013).

PCR analyses were performed on a Quant Studio 3 Real-Time PCR System (Applied Biosystems). Samples were run twice in duplicate for a total of four reactions per sample, and showed two individuals to be consistently *Bd*-positive and another two

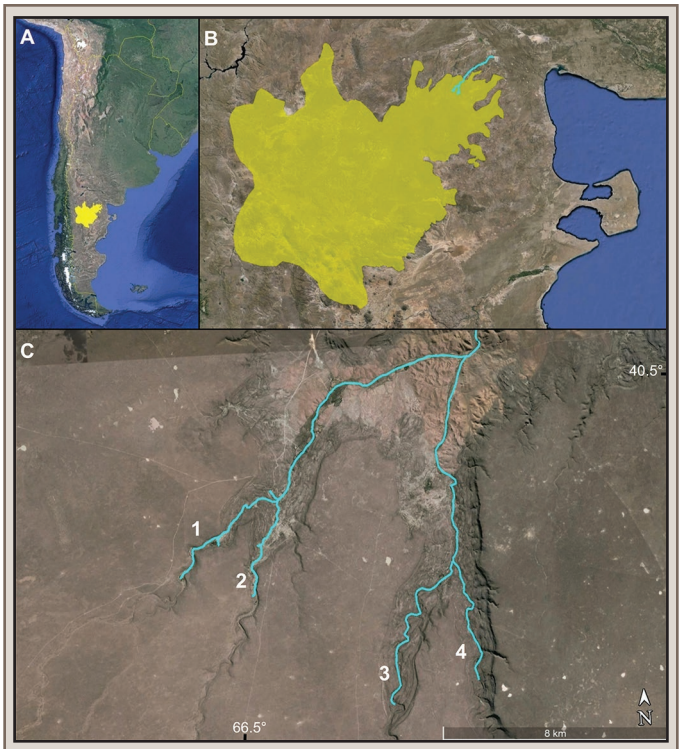

Fig. 1. Map of Argentina showing location of Somuncura Plateau (A), the Valcheta Stream (B), and the branches that form the headwaters of Valcheta Stream (C): 1) Western cold branch; 2) Eastern cold branch; 3) Western warm branch; and 4) Eastern warm branch.

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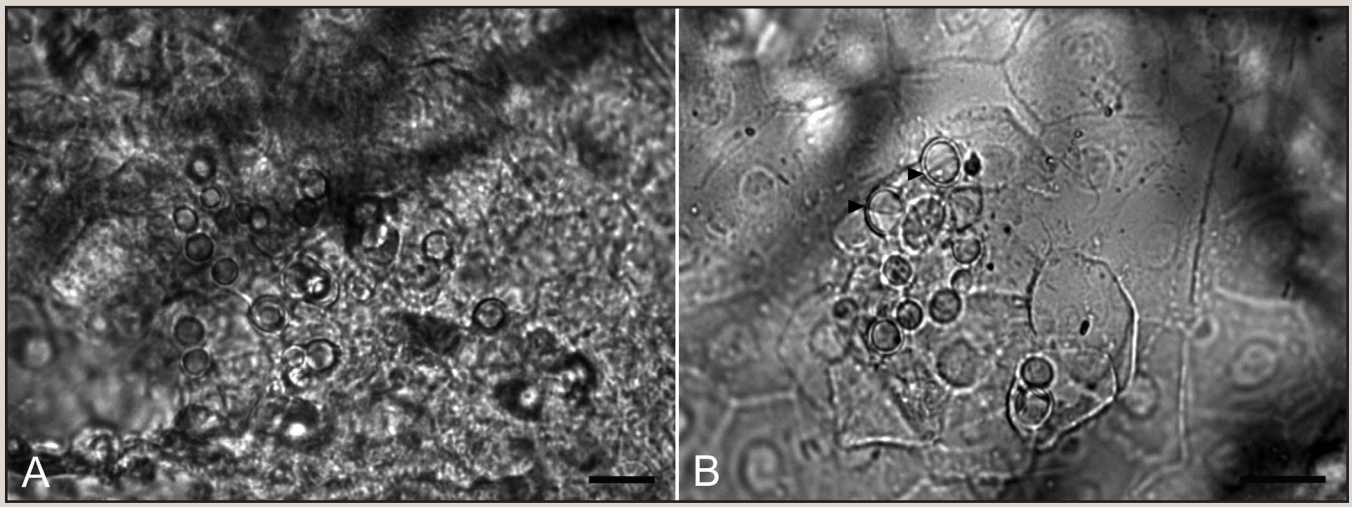

FIG. 2. Unstained wet shedding skin from *Pleurodema somuncurense* infected with *Batrachochytrium dendrobatidis* (A: 400x; B: 600x) in Argentina. Arrows (B) indicate zoosporangia with septum. Scale bars = 20 µm.

borderline *Bd*-positive (samples with mean load > 1 ZE but not all replicates amplifying), all from the eastern cold branch (Fig. 1). Loads among the four positive individuals ranged from 1.2–29.3 zoospore equivalents (ZE) averaged across technical replicates. Moreover, one of both skin samples taken from dead individuals was *Bd* infected (Fig. 2), and although this infection load was not quantified by qPCR, qualitatively the density of zoosporangia observed was higher than in other tissues already analyzed (Arellano, unpubl. data).

Although we have not found many mortality events in the field, a few individuals have been found dead (two adults during this study, and two larvae) in different field surveys. Even though few individuals were tested in this study, *Bd* prevalence (26.6%) is not insignificant compared with *Bd* prevalence in previous works (Vredenburg et al. 2013; Petersen et al. 2016). These values of prevalence and the existence of other threats, including exotic predators, trampling by livestock, and human-related disturbs on aquatic habitats (Velasco et al. 2016) might reinforce the species' threat.

We have no *Bd* information on the other two amphibian species sharing the stream (*Rhinella arenarum* and *Odonthophrynus occidentalis*), but the fungus was previously detected in *Atelognathus reverberii* (Arellano et al. 2015), an IUCN vulnerable species inhabiting volcanic lagoons scattered over the plateau.

The presence of *Bd* zoosporangia in skin samples of the dead individual coming from the eastern warm branch, which reach temperatures of up to 26°C, suggests the occurrence of a *Bd* strain adapted to higher temperatures (*Bd* does not grow well above 25°C: Piotrowsky et al. 2004). Since the two pairs of branches differ by almost 4°C, and knowing that temperature may influences patterns of *Bd* infection (Kinney et al. 2011; Olson et al. 2013; Fernández-Beaskoetxea et al. 2015), future field-studies should assess the prevalence of the *Bd* infection in subpopulations of Valcheta Frog inhabiting at different environmental conditions along the stream. Considering the critically endangered status of the species, detailed monitoring of the stability of the population is warranted in order to elucidate the long-term consequences of the presence of this exotic disease.

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Detection of High Prevalence of *Batrachochytrium dendrobatidis* in Amphibians from Southern Oklahoma, USA

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Oklahoma is home to 54 species of amphibians (31 species of frogs, 23 species of salamanders; Sievert and Sievert 2011), a group of vertebrates shown to be highly susceptible to infectious pathogens, such as the fungus *Batrachochytrium dendrobatidis* (*Bd*; Vredenburg et al. 2010; Cheng et al. 2011). *Bd* has been documented in all states bordering Oklahoma, but little is known about *Bd* within Oklahoma (Young et al. 2007; Rothermel et al. 2008; Steiner and Lehtinen 2008; Gaertner et al. 2009a,b; Rimer and Briggler 2010; Lannoo et al. 2011). Previous studies sampled for *Bd* in four isolated sites spread out over four counties, with *Bd* detected in three of these sites (Steiner and Lehtinen 2008; Lannoo et al. 2011; *Bd*-Maps 2015). Recent research on historical museum specimens indicated that *Bd* has been present in Oklahoma since at least 1926, but little is known about current prevalence rates (Watters et al. 2016). Our study addresses the paucity of data for *Bd* infection in Oklahoma amphibians, where there is a great need to increase sampling efforts so that conservation actions can be implemented to mitigate potential negative effects of the pathogen on native species.

From March–May 2015, we conducted six sampling trips to southern Oklahoma to collect amphibians and sample for *Bd*; research during this time coincided with the breeding season of amphibians and avoided the seasonal drop in detection of and infection by *Bd* due to high temperatures during the summer