

less virulent.

More investigation is needed to determine the extent of *Bd* in the amphibian trade. However, these results shed light on one possible mode of dissemination for *Bd*, and add to the evidence that *R. pipiens* shipped from biological supply houses may act as carriers for the pathogen. We recognize the importance of the amphibian trade for research and education, but our data emphasize the need to handle amphibians, and amphibian wastes, aseptically regardless of their origin. Further, in accordance with the OIE, we suggest systematic screening of imported and exported amphibians given the potentially devastating effects of *Bd* on amphibian persistence around the world.

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***Batrachochytrium dendrobatidis* in Argentina: First Record of Infection in *Hypsiboas cordobae* and *Odontophrynus occidentalis* Tadpoles, in San Luis Province**

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An estimated 43.2% of global amphibians have experienced some reduction in population size (Stuart et al. 2004), with losses in several locations explained by the emerging infectious disease chytridiomycosis caused by the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) (Berger et al. 1998; Daszak et al. 1999; Longcore et al. 1999). In Argentina, *Bd* infection was first reported in 2002, in the Spotted Thin-toed Frog, *Leptodactylus ocellatus* (Herrera et al. 2005). Since then, there have been additional detections in *Atelognathus patagonicus*, *Elachistocleis bicolor*, *Leptodactylus gracilis*, *Telmatobius atacamensis*, and *Telmatobius pisanoi* (Arellano et al. 2009; Barrionuevo and Mangione 2006; Fox et al. 2006; Ghirardi et al. 2009) in several locations in the country. Here we report the first occurrence of *Bd* in Argentinean tadpoles of two native anuran species, *Hypsiboas cordobae* and *Odontophrynus occidentalis*, in Luján, San Luis province, Argentina.

On 12 October 2009, 10 live larvae of *Hypsiboas cordobae* and *Odontophrynus occidentalis* were collected at random from a stream during a field survey in the Strict Nature Reserve

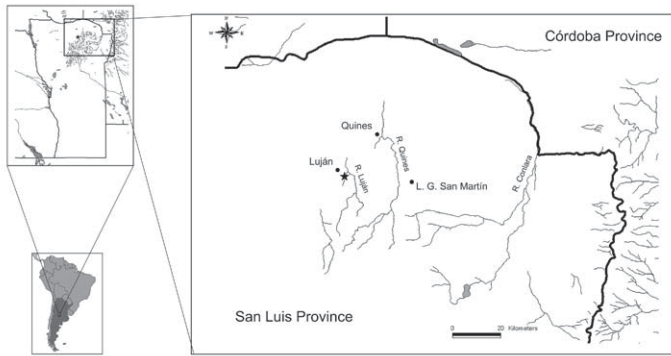


FIG. 1. Location (star) of *Batrachochytrium dendrobatidis* in San Luis province, Argentina.

“Quebarada de las Higuieritas” (32.403°S, 65.927°W) at 800 m elevation (Anderson et al. 1970) (Fig. 1). The stream had a mean depth of 15 cm and flowed intermittently. This protected area is located in the northeastern part of the San Luis province and has climatic and geomorphological characteristics of the Chaco Serrano district of the Chacoan phytogeographic region (Cabrera 1976). Annual rainfall is 500–600 mm and is concentrated in late spring and summer. Vegetation is generally woody with less representation of drought-tolerant species that occur in neighboring regions. There are also abundant areas of grassland, frequently represented in elevated areas (Anderson et al. 1970). *Hypsiboas cordobae* is an endemic mountain frog of Córdoba and San Luis, with a conservation status of Data Deficient (IUCN 2009). *Odontophrynus occidentalis* is broadly distributed in central and south-central Argentina, with an altitudinal range of 50–1500 m, and is categorized as Least Concern (IUCN 2009). Four other anuran species have been recorded at the site. During the field survey there was no evidence of anuran mortality and larvae seemed healthy.

The larvae were fixed in 10% neutral buffered formalin in the field and then transferred to 70% ethanol. All oral structures of tadpoles were examined visually in the laboratory under a stereoscopic microscope. Samples of the keratinized mouthparts were taken, dehydrated, paraffin-embedded and cut (Leica, RM

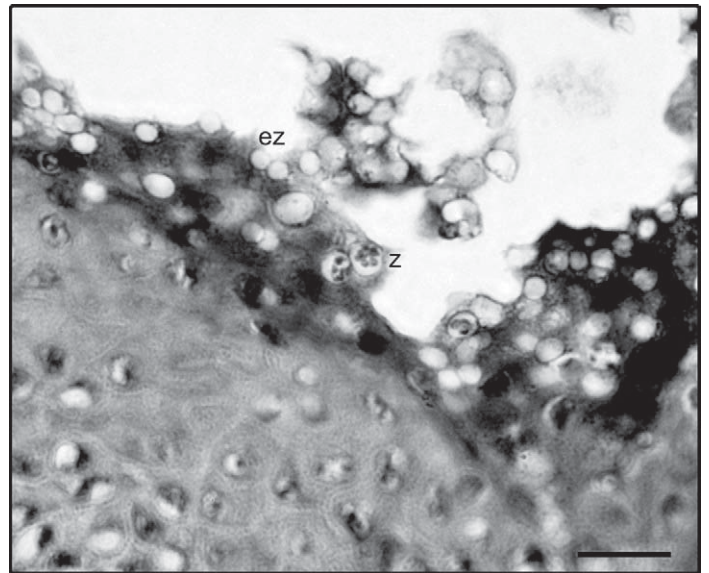


FIG. 3. Histological section of infected mouthparts of larval *Odontophrynus occidentalis* from San Luis province, Argentina, showing empty zoosporangia (ez) and containing zoospores (z). Scale bar: 30 μ m. Photo by M. L. Arellano.

2125 RT) into 5 micron-thick sections for histological analysis. Then, tissues were stained with haematoxylin and eosin and examined with a light microscope (Olympus BX 40 Optical Co. Ltd., Tokyo, Japan). For *Bd* identification we followed the procedures described by Berger et al. (1999). Voucher specimens were deposited in the herpetological collection of the Universidad Nacional de San Luis, San Luis, Argentina (CH-UNSL 0420).

We identified anomalies in the keratinized mouthparts in five of 10 individuals analyzed. The anomalies included mainly lack of pigmentation in upper and lower jaw sheaths, erosion of their cutting edge and absence of serrations, and occasional loss of some labial teeth (Fig. 2). With regard to the histological analyses, seven of 10 larvae were positive for *Bd*. Spherical and ovoid zoosporangia, empty or containing zoospores, were identified in the keratinized mouthparts. The sporangia ranged between 5 and 12 μ m in diameter (Fig. 3).

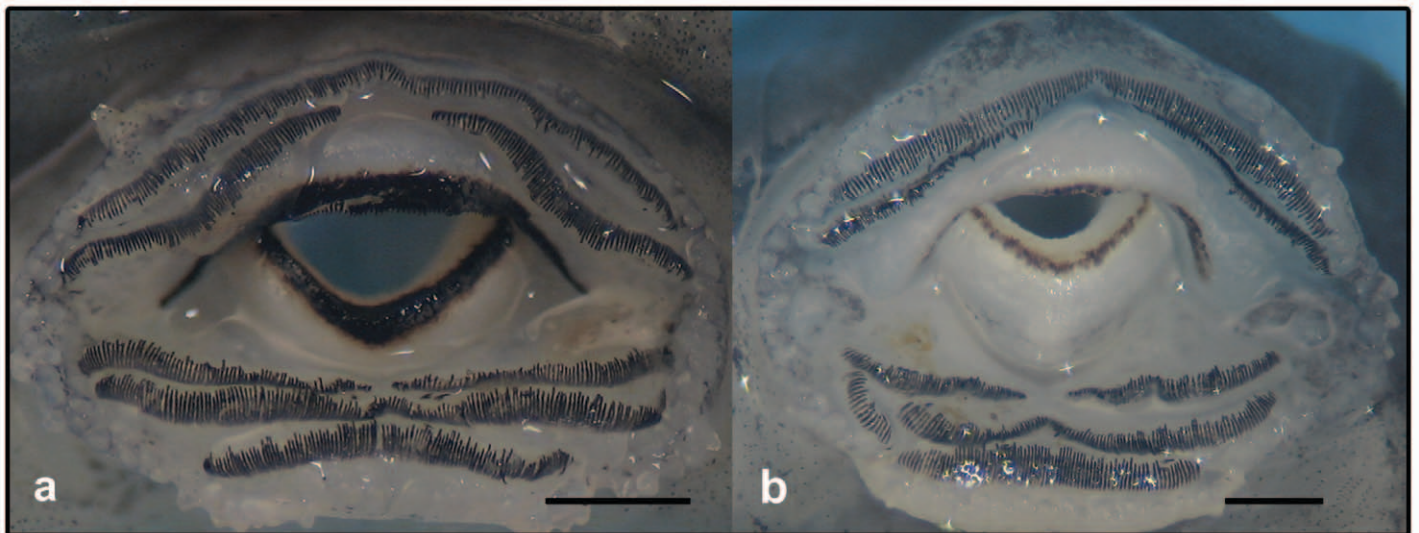


FIG. 2. Normal (a) and abnormal depigmented (b) keratinized mouthparts of larval *Hypsiboas cordobae* from San Luis province, Argentina. Scale bar: (a) 42 mm; (b) 46 mm. Photo by M. L. Arellano.

Our report of *Bd* infection extends the known distribution of the fungus to another region of the Chaco Serrano district (Cabrera et al. 1976), and to a new Argentinean province. This new locality is ca. 120 km from the locality of Ghirardi et al. (2009) in the province of Cordoba. Our discovery of *Bd* in San Luis province represents the first case of infection in tadpoles in Argentina and the presence of the fungus in two new native amphibian species. At present there are few data available on the susceptibility of Argentinean amphibians to *Bd*, and conservation efforts are needed to provide information on *Bd* distribution, ecological requirements, and pathogen-host interactions.

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Chytrid Infection of *Rana draytonii* in the Sierra Nevada, California, USA

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Amphibian chytridiomycosis, caused by the chytridiomycete fungus *Batrachochytrium dendrobatidis* (*Bd*), has been correlated with the decline and extinction of many amphibian species worldwide (Lips et al. 2006). This emergence of this disease appears to be recent, based on samples taken from the California Academy of Science Herpetological Collection, located in San Francisco, California, USA, between 1897 and 2005. *Bd* infection was not detected in any of the 223 sampled amphibians from central California collected prior to 1961 (Padgett-Flohr and Hopkins 2009). Of the 687 individuals sampled overall, including Pacific Chorus Frog (*Pseudacris regilla*), American Bullfrog (*Lithobates catesbeianus*), Foothill Yellow-legged Frog (*Rana boylei*) and California Red-legged Frog (*R. draytonii*), 13.2% were infected with *Bd*, with the most frequent infection occurring in *Pseudacris regilla* (Padgett-Flohr and Hopkins 2009). Within the southern portion of the Sierra Nevada range in central California, populations of the Sierra Nevada Yellow-legged Frog (*R. sierrae* [formerly *muscosa*]) and Southern Mountain Yellow-legged Frog (*R. muscosa*) also have been found to support *Bd* (Fellers et al. 2001; Rachowicz et al. 2006).

Little research has addressed *Bd* presence or prevalence in *Rana draytonii* in the Sierra Nevada foothills, where eight independent populations of this federally listed Threatened species occur. To aid in this regard, we sampled for *Bd* within four of the eight known Sierra Nevada *R. draytonii* populations: Big Gun Diggings, Spivey Pond, Sailor Flat, and Hughes Pond (Fig. 1). We also update the occurrence of *Bd* at Hughes Pond, where *Bd* was first detected in 2007 (www.spatial-epidemiology.net/Bd-maps/surveillance/s_country.asp; accessed March 2010). Information on disease occurrence is important to guide recovery efforts for *R. draytonii* and to manage existing populations.

Big Gun Diggings is located on the Michigan Bluff 7.5-minute topographic quadrangle, Placer County (98–1055 m elevation) and is designated as the PLACER 1 Critical Habitat (CH) Unit (USFWS 2008). The site is on a south-facing slope above the Middle Fork of the American River and includes six man-made ponds of various sizes up to 0.4 ha, which range in depth from being dry in the summer to >2.5 m deep.

Spivey Pond is located on the Sly Park 7.5-minute topographic quadrangle, El Dorado County (975 m elevation) and is identified as the ELDORADO 1 CH Unit (USFWS 2008). The site is on the North Fork Weber Creek, a tributary to the South Fork American River. Weber Creek flows from east to west through the pond, which is a 0.3-ha reservoir created by a weir on the western end of the creek.