Herpetological Review, 2009, 40(2), 175–176.

Batrachochytrium dendrobatidis in Argentina: First Record in *Leptodactylus gracilis* and Another Record in *Leptodactylus ocellatus*

ROMINA GHIRARDI
Instituto Nacional de Limnología (INALI-CONICET-UNL). José Maciá 1933 (3016) Santo Tomé, Santa Fe, Argentina

Instituto de Botánica "Carlos Spegazzini," Calle 53 Nº 477, (1900) La Plata Buenos Aires, Argentina
e-mail: romighirardi@yahoo.com.ar

JULIÁN N. LESCANO
Centro de Zoología Aplicada
Facultad de Ciencias Exactas, Físicas y Naturales
Universidad Nacional de Córdoba, Rondeau 798, (5000) Córdoba, Argentina
e-mail: lescanojul@gmail.com

MARÍA S. LONGO
and
GERARDO ROBLEDO
Laboratorio de Micología IMBIV-UNC-CONICET. C.C. 495 (5000) Córdoba, Argentina
e-mail: longosil@yahoo.com.ar; glrobledo@yahoo.com

MÓNICA M. STECIOW
Instituto de Botánica "Carlos Spegazzini," Calle 53 Nº 477, (1900) La Plata Buenos Aires, Argentina
e-mail: msteciow@museo.fcnym.unlp.edu.ar

and
MARÍA G. PEROTTI
Laboratorio de Fotobiología, INIBIOMA (UNCo-CONICET) Quintral 1250, (8400) San Carlos de Bariloche, Río Negro, Argentina
e-mail: gabyperotti@gmail.com

In the last few decades, rapid declines and extinctions of populations have been reported in more than 400 amphibian species all over the world. A pathogen that has received attention relative to these losses is the chytridiomycete *Batrachochytrium dendrobatidis* (*Bd*). Ron (2005) identified potentially suitable regions for *Bd* establishment in the New World. In South America, Ron predicted suitable regions included the Brazilian Atlantic forest, the temperate forest in Chile and western Argentina (south to 30ºS), northeastern Argentina, Uruguay, and Paraguay. Thus far in Argentina, *Bd* has been reported in four anuran species at three geographic locations: 1) Barrionuevo and Mangione (2006) found *Bd* in *Telmatobius pisanoi* and *T. atacamensis* (Anura: Leptodactylidae) in mountainous areas of Northern Argentina; 2) Arellano et al. (2006) and Herrera et al. (2005) reported *Bd* in adult specimens of *Leptodactylus ocellatus* (Anura: Leptodactilidae) in Buenos Aires province; and 3) in North Patagonia, Fox et al. (2006) found *Bd* in *Atelognathus patagonicus* (Anura: Leptodactilidae). We present *Bd* detections in central Argentina: a new occurrence in the Striped Thin-toed Frog (*Leptodactylus gracilis*) and another occurrence in the Spotted Thin-toed Frog (*Leptodactylus ocellatus*).

Dead specimens of the two leptodactilids were found during field surveys and were deposited in the collection of Centro de Zoologia Aplicada (Universidad Nacional de Córdoba, Argentina). A specimen of *Leptodactylus ocellatus* (CZA a-00011) was collected on May 2005 (31.3967ºS, 64.5936ºW) and a specimen of

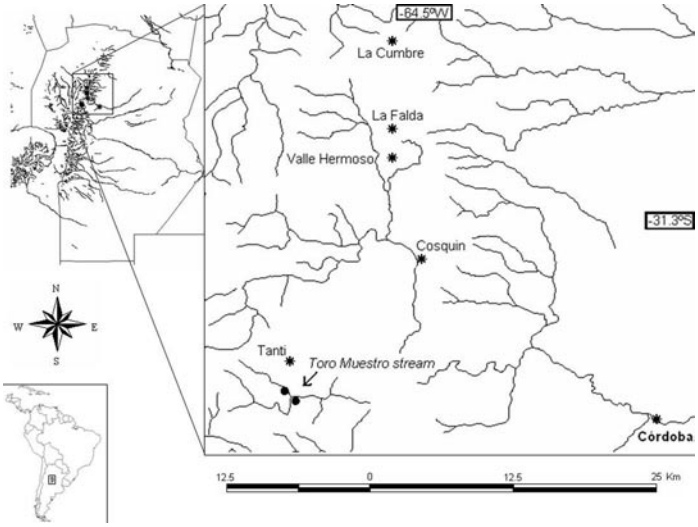

FIG. 1. Location of dead *Leptodactylus gracilis* and *L. ocellatus* specimens infected with the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, in central Argentina (black dots). Stars represent nearby localities.

Leptodactylus gracilis (CZA a-00010) was collected on March 2006 (31.3855ºS, 64.6057ºW), on the banks of the Toro Muerto stream, in the locality of Villa Flor Serrana, in a mountainous area of Córdoba province, Central Argentina, at 800 m elevation. The stream had a mean depth of 70 cm and flowed intermittently over granitic rock. The physiognomy of the surrounding vegetation was that of serrano secondary forest and thorny shrublands, typical of the Chacoan region (Cabrera 1976). Annual rainfall is 950 mm and is mainly concentrated in spring and summer. Mean annual temperature is 18.9°C, ranging from peak values in summer that may exceed 38°C to winter frosts (Capitanelli 1979).

The specimens were fixed in the field in 10% neutral formaldehyde solution and then were transferred to 70% ethanol. In the laboratory, abdominal and hind limb ventral skin patches (~5 x 10 mm) were excised from the anurans, stored in 70% ethanol and dehydrated to embed in paraffin. Then, tissues were sectioned at 5-μm thickness with a Reitcher microtome for histology, and stained with hematoxylin & eosin. With a stereomicroscope, we looked for spores and sporangia in the corneous epithelium of the tissue samples following Berger et al. (1999) and Pessier et al. (1999).

We identified *Bd* in the two specimens analyzed. Zoosporangia at different developmental stages, empty or containing rounded basophilic zoospores, were identified in the stratum corneum. With those results, we increase the geographic distribution of *Bd* in *Leptodactylus ocellatus*, and report the first record of infection in *L. gracilis*. These two species are widespread in a broad region of Argentina, supporting the need to conduct further studies of *Bd* to investigate the status of these populations and the risk to sympatric species. The first case of *Bd* in Argentina was detected in 2002 in a dead specimen (Herrera et al. 2005), and until now eleven specimens (include our two) belonging to five different species have been found infected (Arellano et al. 2006, Barrionuevo and Mangione 2006, Fox et al. 2006, Herrera et al. 2005). Our finding expands the known distribution of *Bd* to the wilderness area of the Chacoan phytogeographic region of Argentina, Chaco Serrano district (Cabrera 1976) (Fig. 1). Although Ron's (2005) prediction map of potentially suitable regions for *Bd* establishment

has constituted a helpful tool for orientating explorations for *Bd* infections detection, these prediction have been made on the bases of only 44 points over the New World, and none of these points was either from Argentina or neighboring countries, resulting in a low precision. Further information is needed about the prevalence and habitat requirements of *Bd* infecting anurans inhabiting Argentina for modeling an actualized and more precise *Bd* distribution map. An increase in the number of amphibian species monitored will help us to estimate the health of wild populations. The collection of environmental and biological data will provide us with valuable tools to predict new scenarios and implement adequate and specific conservation policies in order to manage disease outbreaks in Argentina.

Acknowledgments.—This work was partially founded by a grant (to GR) from Neotropical Grassland Conservancy and a fellowship (RG and JNL) from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Protocols for maintenance and use of specimens followed the human and ethical rules in accordance with Animal Care guidelines. We thank Javier A. López for critically reading the manuscript.

LITERATURE CITED

ARELLANO, M. L., M. AGOSTINI, R. HERRERA, M. STECIOW, E. LAVILLA, AND D. BARRASO. 2006. Nuevo hallazgo de quitridiomicosis en el noreste de la provincia de Buenos Aires. VII Congreso Argentino de Herpetología. Corrientes. November, 2006. Libro de Resúmenes del VII Congreso Argentino de Herpetología: p 124.

BARRIONUEVO, S., AND S. MANGIONE. 2006. Chytridiomycosis in two species of *Telmatobius* (Anura: Leptodactylidae) from Argentina. Dis. Aquat. Org. 73:171–174.

BERGER, L., R. SPEARE, AND A. KENT. 1999. Diagnosis of chytridiomycosis in amphibians by histologic examination. Available at: www.jcu.edu.au/school/phtm/PHTM/frogs/histo/chhisto.htm. November 1999.

CABRERA, A. L. 1976. Regiones fitogeográficas de la Republica Argentina. Enciclopedia Argentina de Agricultura y Jardinería. ACME 2:1–85.

CAPITANELLI, R. G. 1979. Clima. *In* J. B. VÁZQUEZ, R. A. MIATELLO, AND M. A. ROQUÉ (eds.), Geografía Física de la Provincia de Córdoba, pp. 45–138. Editorial Bold. Buenos Aires, Argentina.

FOX, S., J. YOSHIOKA, M. E. CUELLO, AND C. ÚBEDA. 2006. First case of ranavirus-associated morbidity and mortality in natural populations of the South American frog *Atelognathus patagonicus*. Dis. Aquat. Org. 72:87–92.

HERRERA, R., M. STENCIOW, AND G. NATALE. 2005. Chytrid fungus parazitizing the wild amphibian *Leptodactylus ocellatus* (Anura: Leptidactylidae) in Argentina. Dis. Aquat. Org. 64:247–252.

PESSIER, A. P., D. K. NICHOLS, J. C. LONGCORE, AND M. S. FULLER. 1999. Cutaneous chytridiomycosis in poison dart frogs (*Dendrobates* spp.) and White's tree frogs (*Litoria caerulea*). J. Vet. Diagn. Invest. 11:194–199.

RON, S. R. 2005. Predicting the distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* in the New World. Biotropica 37(2):209–221.

Herpetological Review, 2009, 40(2), 176–179.

Detection of *Batrachochytrium dendrobatidis* in Amphibians from the Great Smoky Mountains of North Carolina and Tennessee, USA

MATTHEW W. H. CHATFIELD
University of Michigan
Department of Ecology and Evolutionary Biology and Museum of Zoology
1109 Geddes Avenue, Ann Arbor, Michigan 48109-1079, USA
e-mail: mattchat@umich.edu

and
BETSIE B. ROTHERMEL*
CHAD S. BROOKS
JILLIAN B. KAY
Austin Peay State University, The Center of Excellence for Field Biology
P.O. Box 4718, Clarksville, Tennessee 37044, USA

**Present address:*
Archbold Biological Station, P.O. Box 2057
Lake Placid, Florida 33862, USA

Amphibians in the southern Appalachian Mountains of the United States comprise a diverse and globally significant biological resource (Dodd 2004). There are ample reasons to be concerned about the welfare of amphibian populations in the Southern Appalachians, given expected changes in environmental conditions with climate change (Bernardo and Spotila 2006; Corser 2001; Dodd 1997) and reports of population declines (Corser 2001; Highton 2005). Furthermore, the pathogenic fungus, *Batrachochytrium dendrobatidis* (hereafter called *Bd*), has been detected in many sites throughout the southeastern U.S. (e.g., Daszak et al. 2005; Rothermel et al. 2008). Although Rothermel et al. (2008) detected *Bd* infection in 10 species within the families Ranidae, Hylidae, and Salamandridae, none of the 143 amphibians collected in Great Smoky Mountains National Park in 1999–2001 were infected. Importantly, this sample included only two individuals in the family Plethodontidae. Other studies have reported *Bd* infections in wild-caught plethodontid salamanders of two terrestrial species (*Plethodon neomexicanus*, Cummer et al. 2005; *P. cinereus*, Lauer et al. 2007) and three stream-associated species (*Eurycea cirrigera*, Byrne et al. 2008; *E. bislineata* and *Desmognathus fuscus*, Grant et al. 2008). Researchers have also collected morbid terrestrial plethodontid species (*Oedipina* spp. and *Bolitoglossa* spp.) during mass mortality events in Mexico and Panama (Lips et al. 2003; Parra-Olea et al. 2005). Therefore, there is an urgent need for more information regarding *Bd* occurrence and susceptibility within the range of the many endemic species of salamanders in the Southern Appalachians.

Three methods have been used to detect the presence of *Bd* in amphibians: histological examination, conventional polymerase chain reaction (PCR), and quantitative (real-time) PCR (qPCR). The relative benefits of histological examination versus the more sensitive molecular methods have been described previously (Smith 2007). Since the publication of Boyle et al. (2004), qPCR has become widely adopted in studies seeking to detect or quantify the presence of *Bd* in amphibians. A recent study has supported the increased sensitivity of qPCR over conventional PCR in detecting *Bd* (Kriger et al. 2006), but there are few empirical tests