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First Record of *Batrachochytrium dendrobatidis* in *Physalaemus fernandezae* (Anura: Leiuperidae) for Buenos Aires Province, Argentina

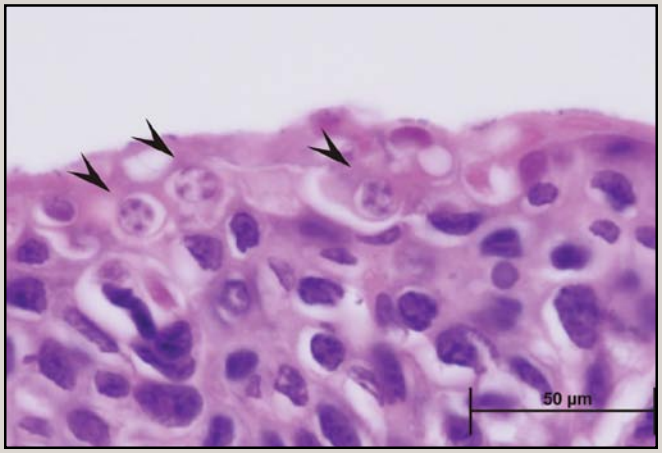

FIG. 1. Histologic section of ventral epidermis of an adult specimen of *Physalaemus fernandezae* from Punta Lara Natural Reserve, Buenos Aires province, Argentina. Arrows indicate the presence of zoosporangia of *Batrachochytrium dendrobatidis* with zoospores.

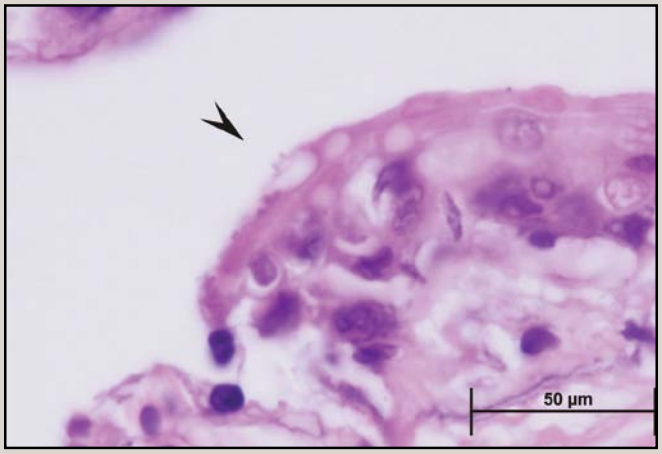

FIG. 2. Histologic section of ventral epidermis of an adult specimen of *Physalaemus fernandezae* from Punta Lara Natural Reserve, Buenos Aires province, Argentina. The arrow indicates an empty zoosporangium, with discharge tube.

In Argentina, *Batrachochytrium dendrobatidis* (*Bd*) is known from Buenos Aires, Córdoba, Misiones, Neuquén, San Luis, Salta, and Tucumán provinces (Arellano et al. 2009; Barrionuevo and Mangione 2006; Fox et al. 2006; Ghirardi et al. 2009; Gutierrez et al. 2010; Herrera et al. 2005). We provide the first record of *Bd* infection in a population of the pond-breeding anuran *Physalaemus fernandezae*, from Punta Lara Natural Reserve (34.8033˚S, 58.0099˚W), Ensenada, Buenos Aires province, Argentina.

Punta Lara Natural Reserve is located on the western bank of Río de La Plata. It has a warm-temperate climate with a mean annual temperature of 16˚C (-4˚C minimum; 42˚C maximum), and has a few days with frost, mostly in June and July. Annual precipitation is slightly over 1000 mm (SMN 2011).

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Physalaemus fernandezae is distributed in Buenos Aires and Entre Ríos provinces, Argentina, and some localities from southern Uruguay (Barrio 1964). It reproduces mainly in marshy grasslands with two reproductive events per year (Barrio, *op. cit.*). The dominant breeding season takes places between May and August (colder temperature months), but may extend until December, in many cases overlapping reproductive events with others sympatric anurans: e.g., *Hypsiboas pulchellus*, *Odontophrynus americanus*, and *Scinax squalirostris*. The second reproductive event involves fewer animals and takes place approximately between February to March, coinciding with reproduction activities of *Dendrosophus nanus*, *D. sanborni*, *Hypsiboas pulchellus*, *Pseudopaludicola falcipes*, *Pseudis minuta*, *Scinax squalirostris*, *S. berthae*, and *S. granulatus*. Currently, *P. fernandezae* has an IUCN conservation status of Minor Concern (IUCN 2011).

Seven adult specimens of *P. fernandezae* were collected at Punta Lara Natural Reserve in September 2007, preserved in 10% formalin and deposited in the herpetological collection of Museo de La Plata (MLP-A 5385–5391). A skin sample (length: 5 mm; width: 2 mm) was taken from the ventral zone of selected specimens, immersed in paraffin, thin-sectioned every 5 µm with a microtome (Leica, RM 2125 RT), mounted onto a microscopic slide and stained with haematoxilin and eosin, after Drury and Wallington (1980). Histological slides were analyzed for *Bd* following procedures described in Berger et al. (1999), with a binocular microscope (Olympus Optical Co. Ltd., Tokyo, Japan; model BX 50). Diagnostic images were taken with an Olympus DP 71 digital camera mounted to the scope.

The presence of *Bd* was confirmed for 3 of 7 (42.85%) skin samples analyzed. Different developmental stages of chytridiomycosis were clearly visible, namely: zoosporangia (isolated and grouped), empty and containing zoospores (Fig. 1), diagnostic

characteristics such as a septum and discharge tube (Fig. 2), and hyperplasic epidermis.

It is worth mentioning that the population of *P. fernandezae* used in this study has been studied by local herpetologists since 2001, but no moribund or dead specimens have been recorded to date. Moreover, the individuals on which infection was detected had been engaged in reproductive activities.

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Literature Cited

Arellano, M. L., D. P. Ferraro, M. M. Steciow, and E. O. Lavilla. 2009. Infection by the chytrid fungus *Batrachochytrium dendrobatidis* in the yellow belly frog (*Elachistocleis bicolor*) from Argentina. Herpetol. J. 19:217–220.

Barrio, A. 1964. Relaciones morfológicas, eto-ecológicas y zoogeográficas entre *Physalaemus henseli* (Peters) y *P. fernandezae* (Müller) (Anura, Leptodactylidae). Acta Zool. Lilloana 20:285–305.

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.

Drury, R. B., and E. A. Wallington. 1980. Carleton's Histological Technique. Oxford University Press. 520 pp.

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.

Ghirardi, R., J. N. Lescano, M. S. Longo, G. Robledo, M. M. Steciow, and M. G. Perotti. 2009. *Batrachochytrium dendrobatidis* in Argentina: first record in *Leptodactylus gracilis* and another record in *Leptodactylus ocellatus*. Herpetol. Rev. 40:175–176.

Gutierrez, F. R., M. L. Arellano, L. E. Moreno, and G. S. Natale. 2010. *Batrachochytrium dendrobatidis* in Argentina: First record of infection in *Hypsiboas cordobae* and *Odontophrynus occidentalis* tadpoles, in San Luis province, Argentina. Herpetol. Rev. 41:323–325.

Herrera, R., M.M. Steciow, and G.S. Natale. 2005. Chytrid fungus parasitizing the wild amphibian *Leptodactylus ocellatus* (Anura: Leptidactylidae) in Argentina. Dis. Aquat. Org. 64:247–252.

IUCN [International Union for the Conservation of Nature]. 2011. IUCN Red List of Threatened Species. Available at: www.iucnredlist.org. Accessed 06 August 2011.

SMN [Servicio Meteorológico Nacional]. 2011. Valores medios de temperatura y precipitación (1961–1990). Climatología. Productos elaborados. Available at: http://www.smn.gov.ar. Accessed 11 August 2011.

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Low Prevalence of *Batrachochytrium dendrobatidis* in Two Plethodontid Salamanders from North Carolina, USA

Although the pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*) was first isolated from anuran amphibians, subsequent research has clearly shown that it also infects many species of caudate amphibians. Opportunistic sampling surveys have shown that at least 56 species of salamanders from the families Ambystomatidae, Amphiumidae, Salamandridae, Cryptobranchidae, and Plethodontidae in the United States harbor *Bd* (reviewed in Bryne et al. 2008; Olson 2010). The effect of *Bd* on salamander demography, however, is less understood than its impact on anuran populations. Laboratory challenge experiments have shown that salamanders can be infected by *Bd* and show mortality from chytridiomycosis (Chinnadurai et al. 2009; Vazquez et al. 2009; Weinstein 2009), but more sampling and monitoring of salamanders will help elucidate potential ecological and/or climatic variables that may influence the susceptibility of salamanders in the wild. Here, we contribute information on the prevalence and distribution of *Bd* in salamander populations by testing 166 individuals from two localities in North Carolina, USA. Our results expand upon previous reports of *Bd* infection in amphibian populations in North Carolina (e.g., Bryne et al. 2009; Keitzer et al. 2011; Rothermel et al. 2008).

We opportunistically sampled two plethodontid salamander species at two collection sites on 8–12 August 2010 (Fig. 1). At Deep Gap, we collected salamanders by turning over rocks and logs that were located up to 15 m from a stream. The collecting site at Wayah Bald was a grassy area near the forest edge. We swabbed each salamander 30 times using sterile swabs (Medical wire No. 113) in the manner of Boyle et al. (2004) and Kriger et al. (2006). Each animal was handled with a clean pair of latex gloves. No animals showed any outward signs of disease. After swabbing, animals were retained for additional studies under permits issued by the North Carolina Wildlife Resources Commission and the U.S. Forest Service to LDH. Swabs were stored in 100% ethanol and transported to Cornell University, where molecular testing was performed by Kiemnec-Tyburczy.

Genomic DNA was extracted from the swabs using Prepman Ultra following the protocol of Boyle et al. (2004). The level of *Bd* zoospore load was assessed using the method of Boyle et al. (2004). Briefly, this method used Taqman quantitative PCR to determine the total number of *Bd* zoospore genome equivalents in each unknown sample, based on known standards that were run simultaneously. To maximize cost efficiency but retain individual information, PCRs were run in singlicates at 1:10

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