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First Case of Chytridiomycosis in an Adult Specimen of a Native Anuran from Uruguay

In Uruguay, the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) was first detected by Mazzoni et al. (2003). They reported chytridiomycosis in a captive population of *Lithobates catesbeianus* (Anura, Ranidae) which suffered a massive death of metamorphs at a commercial farm. Later, Garner et al. (2006) also confirmed the presence of *Bd* at the same farm and in a new one. Subsequently, Borteiro et al. (2009) found *Bd* by histological analysis in the oral epithelium of native wild species larvae: *Odontophrynus maisuma* (Anura, Cycloramphidae), *Physalaemus henselii* (Anura, Leiuperidae), *Hypsiboas pulchellus* and *Scinax squalirostris* (Anura, Hylidae).

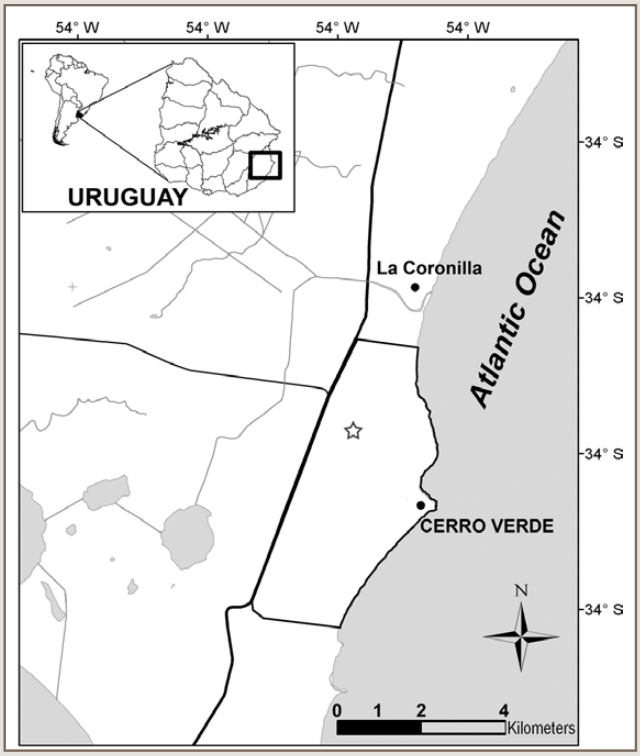

Fig. 1. Location of the Cerro Verde Protected Area, Rocha Department, Uruguay, where *Batrachochytrium dendrobatidis* was detected on *Pleurodema bibroni* (star).

We sampled native, wild amphibians for *Bd* in June 2009 at the Cerro Verde Protected Area, Rocha Department, Uruguay (33.945833°S, 53.508611°W) (Fig. 1). This area is characterized by humid subtropical climate (average temperature 16°C, annual rainfall 950 mm) and an ocean coastline with dunes (20–30 m elevation) and plains (Alonso and Bassagoda 2003). During nighttime sampling, *Pleurodema bibroni* were heard calling at a temporary pond of a coastal meadow. One frog was collected as a voucher specimen of the survey and another individual was collected under suspicion of being infected by *Bd* because it possessed some lesions on the skin (two ulcerations: on right side and dorsum, as described by Berger et al. 2005). Both specimens were taken to the laboratory and the injured frog died a few days later. Both were deposited at Vertebrate Zoology Collection, Facultad de Ciencias, Universidad de la República (ZVC-B 19776, ZVC-B 19775).

To assess the presence of *Bd* in both frogs, skin of the underside of the legs, interdigital membrane of feet, and drink patch was comprehensively swabbed (10 times ea.). Each swab was broken off into an empty Eppendorf tube, and then analyzed

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FIG. 2. Skin section of *Pleurodema bibroni* (ZVC-B 19775), collected from Cerro Verde Protected Area, Rocha Department, Uruguay, showing empty *Batrachochytridum dendrobatidis* zoosporangium (EZ) and zoosporangium containing zoospores (Z), one with a discharge tube (DT). Scale bar: 20µm.

for the presence of *Bd* DNA using qPCR (Boyle et al. 2004). *Bd* infection was also analyzed histologically. Abdominal and hind limb ventral skin patches (~5 × 10 mm) and two toe tips were excised from the anurans and routinely processed for histological examination following Berger et al. (1999).

Both analyses (qPCR and histology) were *Bd*-positive only for the apparently healthy specimen. Zoospores and zoosporangia at different stages were found at multiple sections of toe tips and hind limb of the infected individual, and some degree of hyperkeratosis at the sections from hind limbs of the specimen was found (Fig. 2).

This is the first record of *Bd* in an adult native wild-caught amphibian in Uruguay, and the first report of *Bd* infection for *Pleurodema bibroni*. This frog is considered Near Threatened by the IUCN Red List (IUCN 2006) and is Threatened at a country level for Uruguay (Maneyro and Langone 2001; Canavero et al. 2010). The pathogen was detected 100 km northeast of the nearest record in Uruguay (Borteiro et al. 2009) inside a Protected Area, which is both alarming and suggests that measures for control and prevention of human-mediated *Bd* dispersal warrant consideration. This is particularly important because populations of other native species in the area, such as *Melanophryniscus montevidensis* (Núñez et al. 2004), may be at risk (IUCN RED LIST: Vulnerable; IUCN 2006).

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Resumen.—En Uruguay la infección causada por *Batrachochytrium dendrobatidis* (*Bd*) había sido detectada en *Lithobates catesbeianus* de criaderos comerciales y en larvas silvestres de especies autóctonas, pero no había sido confirmada aún en ejemplares adultos de ninguna especie nativa. En junio de 2009 se colectaron dos machos adultos silvestres de *Pleurodema bibroni* en el Cerro Verde (33.935°S, 53.5070556°W, Rocha, Uruguay), se les tomaron muestras para análisis de ADN (qPCR) para detectar *Bd*. Uno de ellos resultó positivo y el análisis histológico de la piel del ejemplar también confirmó la infección. Por tanto se diagnostica por primera vez la infección por *Bd* en adultos silvestres de esta especie en Uruguay, que además está considerada como *Amenazada* a nivel nacional y *Near Threatened* para la IUCN.

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