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Pathogenic Fungus Batrachochytrium Dendrobatidis in Marbled Water Frog Telmatobius Marmoratus: First Record From Lake Titicaca, Bolivia

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NOTE

Pathogenic fungus *Batrachochytrium dendrobatidis* in marbled water frog *Telmatobius marmoratus*: first record from Lake Titicaca, Bolivia

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ABSTRACT: The pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*) has been associated with amphibian declines worldwide but has not been well-studied among Critically Endangered amphibian species in Bolivia. We sampled free-living marbled water frogs *Telmatobius marmoratus* (Anura: Leptodactylidae) from Isla del Sol, Bolivia, for *Bd* using skin swabs and quantitative polymerase chain reactions. We detected *Bd* on 44% of *T. marmoratus* sampled. This is the first record of *Bd* in amphibians from waters associated with Lake Titicaca, Bolivia. These results further confirm the presence of *Bd* in Bolivia and substantiate the potential threat of this pathogen to the Critically Endangered, sympatric Titicaca water frog *T. culeus* and other Andean amphibians.

KEY WORDS: Chytridiomycosis · *Telmatobius marmoratus* · *Telmatobius culeus* · Lake Titicaca · Bolivia · Andes

INTRODUCTION

Recent widespread extinctions and declines of amphibian populations have increased awareness of diseases in amphibians around the world (Voyles et al. 2009). The pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*) has received much attention as a major contributor to global amphibian declines (Ron & Merino 2000, Berger et al. 2004, Skerratt et al. 2007). The disease chytridiomycosis is caused by *Bd* and can have significant negative impacts on amphibian populations in areas of high species richness and endemism such as in South and Central America (Lips et al. 2005, Catenazzi et al. 2011), particularly at montane sites (Lips 1999). South American amphibians have undergone dramatic declines (Stuart et al. 2004, 2008, Stuart 2008), and *Bd* has been identified as a cause in a number of these (Young et al. 2001). Despite well-documented declines throughout South America, relatively little work has been published on *Bd* infections in Bolivia. However, De la Riva & Burrowes (2011) recently documented *Bd* in Bolivia at 7 of 8 study sites along a transect located between the Chilean border and the Cordillera Oriental to the east, and Barrionuevo et al. (2008) reported *Bd* in Bolivia through chytridiomycosis-related oral abnormalities in the riverine tadpoles of *Rhinella quechua.* Although *Bd* has been recorded in Bolivia, its presence in waters of Lake Titicaca has yet to be formally documented.

One particularly threatened anuran group in South America is the highland genus *Telmatobius* (Angulo 2008, De la Riva et al. 2010). Declines attributed to *Bd* have been reported for at least 8 species of this genus in Argentina, Ecuador, and Peru (Merino-Viteri et al. 2005, Seimon et al. 2005, 2007, Barrionuevo & Mangione 2006, Barrionuevo & Ponssa 2008, Catenazzi et al. 2011). Additionally, there are *Bd*-related con-

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servation concerns for the Critically Endangered Titicaca water frog *T. culeus* and the Vulnerable marbled water frog *T. marmoratus* (IUCN 2012). Apparent declines in populations of the endemic *T. culeus* have prompted conservation efforts and widespread monitoring (Reading et al. 2011). *T. marmoratus* (Duméril & Bibron 1841) is a riparian, semi-aquatic frog in Andean Puna of southeastern Peru and western Bolivia (De la Riva et al. 2010) that has been shown to carry *Bd* in captive animals within the food trade (Catenazzi et al. 2010). As these species may occur sympatrically within Lake Titicaca waters and associated tributaries, understanding the disease dynamics of both species is warranted. Highly aquatic *T. culeus* occur in deeper lacustrine habitats and may be difficult to encounter. However, *T. marmoratus* are more readily captured near shore or in small tributaries, making them an accessible study species. In light of conservation concerns for *Telmatobius* species and other Andean endemics, and the documented presence of *Bd* within the region, the objectives of our study were to determine (1) whether *Bd* was detectable in an insular population of *T. marmoratus* living within Lake Titicaca (Isla del Sol, Bolivia); and if so, (2) whether individual frogs exhibited clinical symptoms of chytridiomycosis.

MATERIALS AND METHODS

Our study took place on Isla del Sol, a small island (~10 km × 4 km) located in Lake Titicaca, Bolivia (Fig. 1). The elevation of Isla del Sol ranges from lake level at 3830 m up to its highest point at 4070 m. Island vegetation consists of terraced agricultural lands and dry scrub with limited precipitation of ~800 mm annually (Erickson 2000). Mean monthly air temperatures range from 5 to 10°C (Delclaux et al. 2007), with maxima occasionally exceeding 15°C. We searched for *Telmatobius marmoratus* on 17 and 18 March 2010 between 20:00 and 00:00 h in a small first-order tributary feeding directly into Lake Titicaca. This stream, known locally as the Fuente del Inca, is located on the southeast side of the island (16.0388° S, 69.1448° W) and flows along a well-known archeological feature known as the Inca Staircase (Fig. 1). The permanent stream is less than 1 m wide with a maximum depth of 0.1–0.3 m. Water clarity was high and the primary substrates were gravel and cobble, with small areas of sand and silt accumulation in pools and along stream edges. There was little aquatic vegetation and only slight amounts of organic debris, with low levels of suspended and dissolved organic matter. During our surveys, both water and air temperatures were 13°C, and relative humidity was 69%.

Frogs were captured by hand using clean nitrile gloves and placed individually in new plastic bags. We measured the snout–vent length (SVL) to the nearest millimeter and mass to the nearest gram using a Pesola™ spring scale. We examined frogs for lesions, sloughing skin, and normal muscle activity (posture and righting reflex). Each frog was swabbed using a sterile fine-tipped swab (Medical Wire & Equipment, MW 100-100) according to the protocol

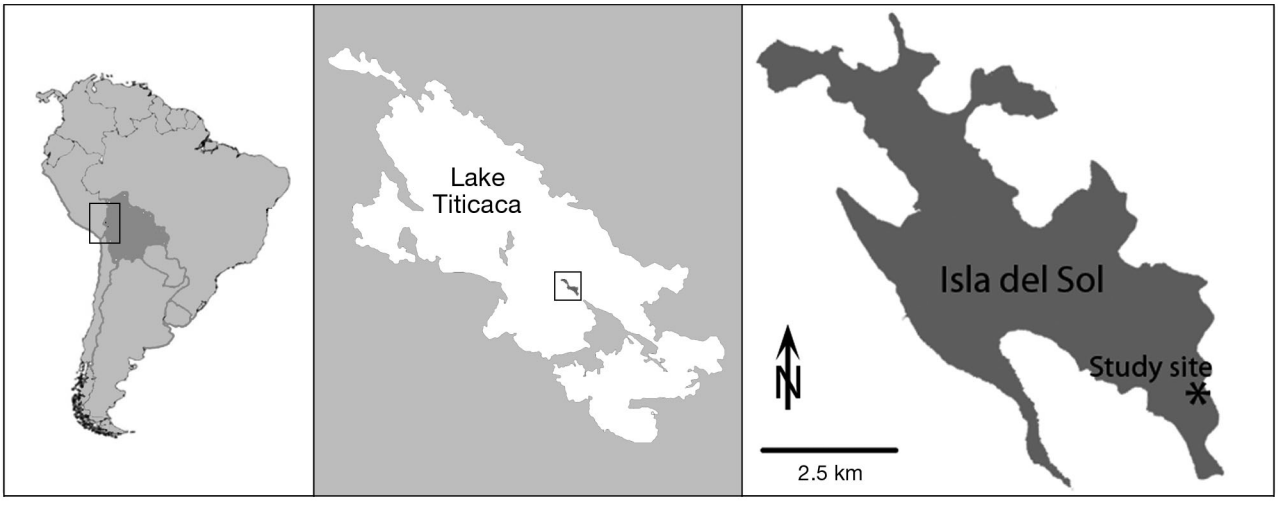

Fig. 1. Marbled water frogs *Telmatobius marmoratus* were sampled for the presence of the pathogenic fungus *Batrachochytrium dendrobatidis* in a small stream (16.0388° S, 69.1448° W) on Isla del Sol that feeds directly into Lake Titicaca, Bolivia

outlined by Hyatt et al. (2007). After data collection, frogs were released at their point of capture. Swab samples were allowed to air dry and were protected from heat extremes. Under normal field conditions, swab samples are stable (Hyatt et al. 2007, Van Sluys et al. 2008). However, as a precaution, swabs were stored at −20°C upon return to the laboratory (<72 h after sample collection).

We analyzed swabs using quantitative polymerase chain reaction (qPCR) as described by Boyle et al. (2004), with a 48-well Applied Biosystems Step One Plus™ qPCR machine. Successful PCR reactions were determined using an internal positive control (TaqMan® Exogenous Internal Positive Control; Applied Biosystems). Each plate had a negative control of water and qPCR reagents. Samples that were positive in singlicate analysis were re-analyzed in triplicate as suggested by Kriger et al. (2006), with a slight change of using standards at twice the protocol concentration.

RESULTS

We captured 11 male and 7 female *Telmatobius marmoratus* ranging in length from 41 to 75 mm SVL and ranging in mass from 6 to 44 g (Table 1). All but 2 individuals were adults; 1 male and 1 female were subadults as determined by size (Veloso et al. 1982). We detected *Bd* on 44.4% of the frogs sampled (Table 1). None of the frogs exhibited lesions or abnormal behaviors, although some limited skin sloughing was observed.

Table 1. Quantitative polymerase chain reaction (qPCR) analyses were used to detect the presence of the pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*) on marbled water frogs *Telmatobius marmoratus*. Frogs were captured on 17 and 18 March 2010 in a small stream on Isla del Sol that feeds directly into waters of Lake Titicaca, Bolivia. F: female; M: male; SVL: snout–vent length; ND: not detected; (+) positive

Sex	SVL (mm)	Mass (g)	*Bd* infection status
F	42	12	ND
F	60	23	ND
F	67	30	ND
F	68	30	ND
F	68	36	+
F	71	33	+
F	75	44	ND
M	41	6	ND
M	53	17	ND
M	54	14	ND
M	54	21	+
M	55	22	+
M	58	20	+
M	60	23	ND
M	60	24	ND
M	60	24	+
M	60	21	+
M	62	26	+

DISCUSSION AND CONCLUSIONS

Bd has recently been documented as occurring in Bolivia (Barrionuevo et al. 2008, De la Riva & Burrowes 2011), and we further confirmed the presence of this pathogenic fungus. However, our work is the first to document the presence of *Bd* on free-living *Telmatobius marmoratus* within waters associated with Lake Titicaca. The presence of *Bd*-positive frogs in a stream feeding directly into Lake Titicaca raises concern over pathogen presence in the Critically Endangered Titicaca water frog *T. culeus*. *Bd* has been implicated in population declines of other *Telmatobius* species. Seimon et al. (2007) associated population declines of *T. marmoratus* in Peru with *Bd* infections. Barrionuevo & Mangione (2006) found *Bd* on individuals of *T. pisanoi* and *T. atacamensis* in Argentina, and Merino-Viteri et al. (2005) speculated that the apparent extinction of 3 *Telmatobius* species in Ecuador was due to *Bd* infection.

The level of threat that *Bd*-positive frogs might have towards sympatric *T. culeus* may be dependent on whether the pathogen is tolerated by *T. marmoratus*, allowing them to function as reservoirs. Such a scenario was documented by Reeder et al. (2012) in the Sierra Nevada range, USA, where *Bd*-tolerant, widespread, and abundant Sierran chorus frogs *Pseudacris regilla/sierra* were likely increasing pathogen persistence and increased exposure risk for the Endangered mountain yellow-legged frog species complex (*Rana muscosa/sierrae*) occurring sympatrically. None of the frogs we sampled exhibited severe signs of chytridiomycosis, suggesting the possibility that *T. marmoratus* may be tolerant of *Bd* infection. However, it is important to confirm suspected tolerance and/or resistance to *Bd* by *T. marmoratus* via laboratory challenge experiments. If *T. marmoratus* populations in Lake Titicaca waters prove to be tolerant of *Bd*, they could function as a reservoir species, increasing the exposure risk for sympatric *T. culeus*. Similar challenge experiments assessing the tolerance and/or resistance to *Bd* by *T. culeus* are also warranted, and would allow con-

servationists to weigh the potential threat of *Bd* documented in Lake Titicaca waters to these Critically Endangered endemic frogs.

Frogs in the genus *Telmatobius* are important culturally, as both *T. marmoratus* and *T. culeus* are used by indigenous people for food and traditional medicinal practices (Reading et al. 2011). These practices may have direct negative consequences on *Telmatobius* species but also raise concerns about disease spread. For example, Catenazzi et al. (2010) documented the occurrence of *Bd* on 100% of captive *T. marmoratus* sampled from a live-animal market in Cusco, Peru, and cautioned that the transport of amphibians within the region could exacerbate amphibian declines. Even greater concern is warranted if *T. marmoratus* is indeed tolerant of *Bd* and could function as a disease reservoir.

Our study population is insular and occurs ~0.9 km from the nearest mainland shore. Possible mechanisms for transporting *Bd* to Isla del Sol include migratory waterfowl (Johnson & Speare 2005, Garmyn et al. 2012), amphibians, or other aquatic vectors. Conceivably, local residents could have transported *Bd*-contaminated equipment (nets/traps) or water from surrounding mainland areas out to the island. Additionally, *Bd*-positive frogs from mainland live-animal markets may have been transported to the island, although this is doubtful due to presence of these species on the island.

Educational efforts aimed at minimizing the spread of *Bd*-positive animals may be helpful, and the cooperative efforts between the Denver Zoo and Peruvian agencies to establish captive breeding populations of the sympatric Critically Endangered *T. culeus* are timely and warranted (Reading et al. 2011). Our study was limited spatially, and future work should focus on determining the extent of *Bd* infection within the Lake Titicaca system. Although the prevalence of *Bd* we detected was fairly high (44.4%), our limited sample size precludes wide-scale inference until larger samples sizes are obtained. Repeated sampling across seasons could also help determine how our results fit within an epidemiological model (i.e. novel or established pathogen in this system). Despite the small sample size, our work further documents the presence of *Bd* within Bolivia and for the first time establishes its presence in Lake Titicaca. The confirmed presence of *Bd* makes it a plausible causative agent among several agents for previously observed amphibian declines in this region and highlights the potential threat to Andean amphibian diversity.

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