Vol. 95: 259–261, 2011
doi: 10.3354/dao02369

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published July 12

NOTE

New records of *Batrachochytrium dendrobatidis* in Chilean frogs

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ABSTRACT: We used molecular techniques to examine 11 species of frogs in 6 localities in southern Chile to ascertain the incidence of the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*). We detected the fungus in 2 localities (Coñaripe and Raúl Marín Balmaceda) in 3 species: *Batrachyla leptopu*s, *Pleurodema thaul* and *Rhinoderma darwinii*. Our findings expand the list of *Bd* hosts to include *B. leptopus* and *P. thaul* and extend the spatial distribution in Chile to include the southernmost *Bd* record at Raúl Marín Balmaceda.

KEY WORDS: *Batrachochytrium dendrobatidis* · Chytridiomycosis · Amphibia · Anura · Chile

INTRODUCTION

Because of multiple variables, such as habitat loss, environmental pollution, introduction of non-native species, climate change and infectious diseases, amphibians are more often threatened with extinction than are birds and mammals (IUCN 2010). Chytridiomycosis is caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*) (Longcore et al. 1999), which occurs on every continent (Rachowicz et al. 2005) and has been linked to amphibian declines (Stuart et al. 2004, Pounds et al. 2006)

Within Chilean fauna, amphibians have the highest percentage rate of threatened species (36.2%) and the highest level of endemism (69%). Chile ranks 11th worldwide in terms of endemism rates and 13th in terms of the percentage of Endangered or Extinct species (i.e. the percentage of the total number of amphibian species in the respective countries) (Stuart et al. 2008). Most amphibian species inhabit the rainy temperate forest in southern Chile, and this area accounts for the high level of endemism. However, the temperate forest is rapidly being destroyed by plantations of introduced species (Neira et al. 2002), causing biodiversity to suffer and many species to be threatened (Debinski & Holt 2000, Watson et al. 2004).

Almost one-third of Chilean amphibian species are described as evolutionarily distinct and globally endangered (EDGE; www.edgeofexistence.org/species/complete_search.php). Among the ecosystems of Chile, the temperate forest is considered a hotspot of biodiversity and conservation priority (Conservation International; www.biodiversityhotspots.org/xp/hotspots/chilean_forests/ Pages/default.aspx). The Chilean temperate forest, however, also includes a region that is 1 of 11 in the New World thought to be optimal for the presence of *Bd*: the ecological conditions of this region coincide with the environmental conditions suitable for the fungus (Ron 2005, Rödder et al. 2010).

In Chile. *Bd* has been detected recently in the introduced frog species *Xenopus laevis* (Solis et al. 2010) and in the native species *Rhinoderma darwinii* (Bourke et al. 2010). In the present study, we obtained additional information on the distribution of *Bd* in Chile and on the species infected.

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MATERIALS AND METHODS

During the southern hemisphere summer between December 2009 and February 2010 we manually captured frogs from 6 populations from the temperate forest of Chile (Fig. 1). Frogs were swabbed for PCR detection of *Bd* and then released at the site of capture. We wiped a sterile cotton swab along the skin of captured frogs for ~30 s, focusing on forelimbs, hindlimbs and the pelvic region. DNA was extracted from swabs with DNeasy Blood & Tissue Kits (Qiagen), following the extraction protocol of the manufacturer. Isolated DNA was preserved in Eppendorf microcentrifuge tubes containing 98% ethanol.

DNA of *Batrachochytrium dendrobatidis* was detected with a modified real-time (rt) TaqMan PCR assay (Boyle et al. 2004). In this assay the TaqMan probe was not conjugated with a minor groove binder but included locked nucleic acid (LNA, N+) bases to elevate the melting temperature (5′-6FAM-CGA GTC+ G+AA+ C+A+A+ AAT-BBQ-3′). All rtPCR reactions were performed in a final volume of 20 µl on a LightCycler 480 (Roche Applied Science). The reaction mixtures contained 0.25 µM of each primer (ITS1-3 Chytr and 5.8S Chytr; Boyle et al. 2004), 62.5 µM of each dNTP, 1.5 mM $MgCl_2$, 6% BSA, 2 µl of 10× reaction buffer (BD, Solis BioDyne), 1 unit *Taq* DNA polymerase (AmpliTaq, ABI) and 1 µl DNA. The cycling profile consisted of an initial denaturation step at 96°C for 5 min, followed by 50 cycles of 10 s at 96°C and 1 min at 60°C. We included negative controls and a positive control for each amplification. rtPCR reactions were performed twice for each sample.

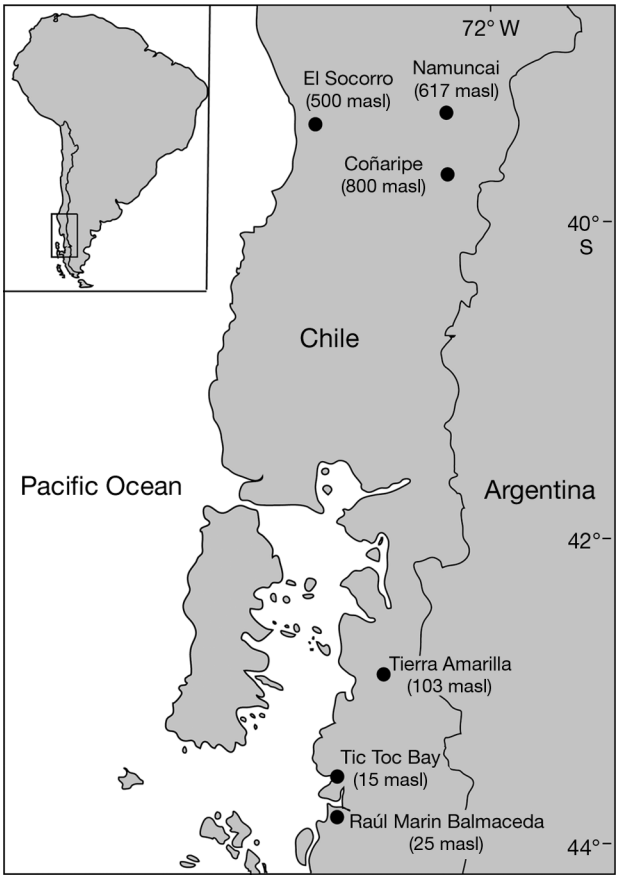

Fig. 1. Chilean frog populations analyzed for the presence of *B. dendrobatidis*: Raúl Marín Balmaceda (43° 47′ S, 72° 57′ W); Tic Toc Bay (43° 36′ S, 72° 53′ W); Tierra Amarilla (43° 00′ S, 72° 29′ W); El Socorro (39° 12′ S, 73° 01′ W); Namuncai (39° 11′ S, 71° 51′ W); Coñaripe (39° 32′ S, 71° 55′ W). masl: m above sea level. Inset shows location of study area in southern Chile

RESULTS

Of 79 amphibians tested, 9 (11.4%) were infected with *Bd*: *Batrachyla leptopus*, n = 2 out of 11 (2/11, 18.2%); *Pleurodema thaul*, n = 4/14 (28.6%); *Rhinoderma darwinii*, n = 3/6 (50%). No infection was detected in *Alsodes igneus* (n = 2), *Batrachlya antartica* (n = 1), *B. taeniata* (n = 2), *Eupsophus calcaratus* (n = 37), *E. vertebralis* (n = 3), *E. migueli* (n = 1), *E. roseus* (n = 1) or *E. emiliopugini* (n = 3), but, except for *E. calcaratus*, sample sizes were small. We detected chytrid infections only at Coñaripe (n = 4/14, 28.6%) and Raúl Marín Balmaceda (n = 5/14, 35.7%).

DISCUSSION

Our findings expand the list of amphibian species susceptible to *Bd* and the distribution of infected species in Chile, including the most southern record of *Bd* at Raúl Marín Balmaceda. *Bd* was detected in *Batrachyla leptopus* and *Pleurodema thaul* for the first time during our survey, which adds to concerns about the threat of *Bd* to Chile's unique and endangered fauna.

The incidence of chytridiomycosis may vary seasonally from low to massive infections within a population, with infection rates usually being higher during cooler seasons (Woodhams & Alford 2005, Woodhams et al. 2008). The low prevalence of infections in frogs and the high proportion of uninfected populations in the temperate forest of Chile may be a short-term phenomenon because we collected our samples exclusively during the southern hemisphere summer (January to February).

We have insufficient data to estimate the effect of *Bd* on amphibian diversity in Chile. Additional individuals, populations and species must be examined, and infected populations must be monitored to correlate the infection rate to population dynamics. Analysis of

preserved collection material may provide information about the historical patterns of *Bd* occurrence in Chile.

Acknowledgements. We thank D. Guzman, E. Gonzales, L. Castro and M. Nonque (Pumalín), P. Camoglio and R. Calcagni (Namuncai) and G. Rebolledo (Universidad Católica de Temuco) for their help and land permits, and Thomas Uzzell (Philadelphia) for reviewing the English. F. Gómez and M. Sierra assisted in the field work. Scientific permits were provided by Servicio Agrícola Ganadero (SAG), Chile. This research was funded by Chester Zoo/North of England Zoological Society in collaboration with Leipzig Zoo, the Zoologische Gesellschaft für Arten- und Populationsschutz (ZGAP), Reptilia, and the Deutsche Forschungsgemeinschaft (grants PL 213/6-1 GR 1467/11-1). J.B. acknowledges a grant from DAAD-CONICYT.

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Editorial responsibility: Alex Hyatt, Geelong, Victoria, Australia

Submitted: November 11, 2010; Accepted: April 27, 2011
Proofs received from author(s): June 29, 2011