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First Record of *Batrachochytrium dendrobatidis* in Paraná, Brazil

Batrachochytrium dendrobatidis (*Bd*) has been reported in seven of twenty-seven federative states of Brazil, including Minas Gerais, Mato Grosso do Sul, Pernambuco, Rio de Janeiro, Rio Grande do Sul, Santa Catarina, and São Paulo (Toledo et al. 2006). Despite its apparent widespread occurrence in Brazil, there are many distributional gaps in our knowledge that are yet to be filled. Understanding the distribution of *Bd* is urgently needed for the development and implementation of amphibian conservation action plans (Verdade et al. 2012). Herein, we provide the first report *Bd* in the state of Paraná, Brazil.

Samples were collected on 26 March 2011 at the municipality of Morretes, within the Atlantic Forest (Fig. 1). Nine tadpoles of two species [*Hylodes cardosoi* (Hylodidae; N = 8) and *Hypsiboas faber* (Hylidae; N = 1)] were collected at the Estrada da Graciosa (PR-410, 25.351297°S, 48.882148°W, 470 m elev.) and examined in the laboratory for *Bd*. Two methods of chytrid diagnosis were applied: cytology (direct observation under the microscope without stains); and isolation of fungus strains in cultures (Longcore et al. 1999).

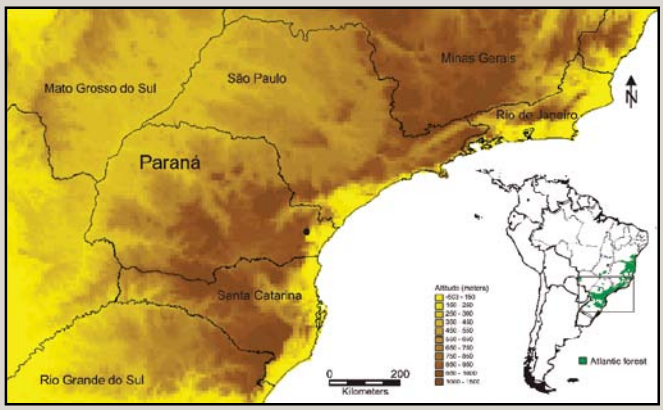

FIG. 1. Location of *Batrachochytrium dendrobatidis* sampling in the city of Morretes, state of Paraná, southern Brazil. SP = São Paulo, PR = Paraná, SC = Santa Catarina.

We detected *Bd* in 7 of 9 individuals we examined: 6 of 8 *Hylodes cardosoi* and 1 of 1 *Hypsiboas faber* were *Bd*-positive. Diagnosis was confirmed by the lack of keratin in mouthparts of the infected tadpoles, and the presence of *Bd* zoosporangia in the still keratinized regions of the mouth; some zoosporangia presented a medium septum (Berger et al. 2000) (Fig. 2). Following microscopic analysis, we isolated the *Bd* fungus (strain CLFT 024) in solid growth medium cultures of 1% tryptone agar (Fig. 2).

Our detection of *Bd* from Morretes fills a 200 km knowledge gap in the distribution of *Bd* in Brazil. The site is approximately 100 km north of São Bento do Sul, Santa Catarina, and 100 km

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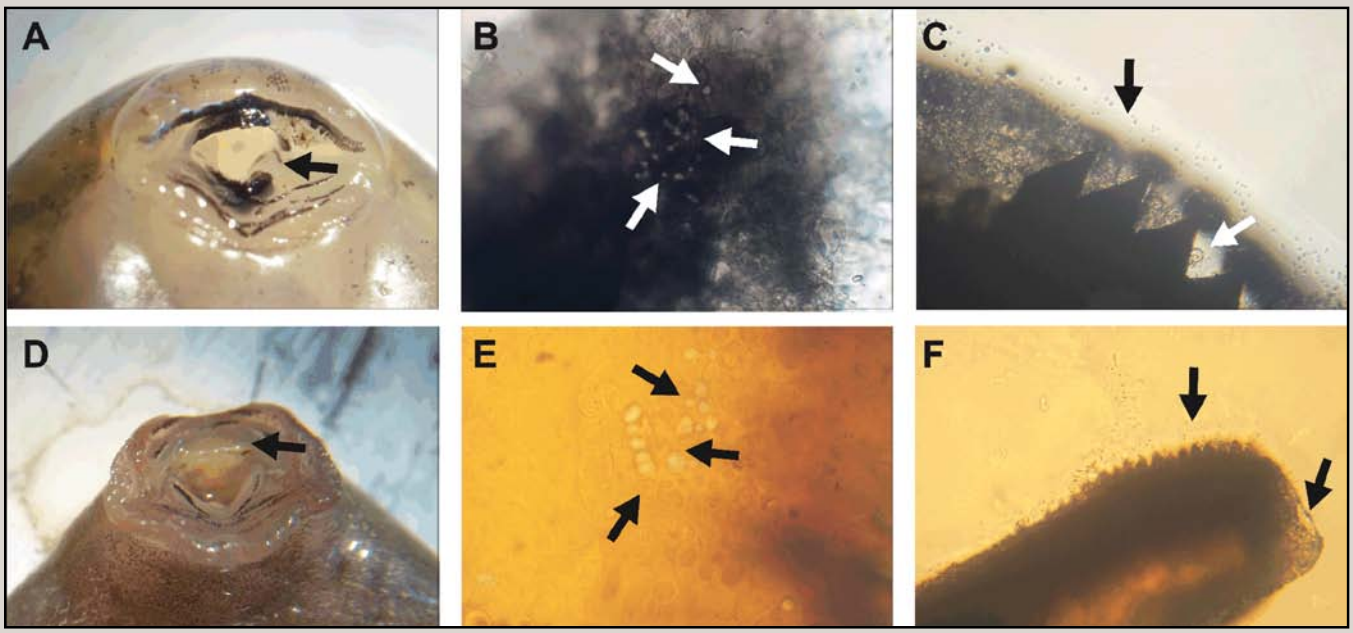

FIG. 2. *Batrachochytrium dendrobatidis* diagnosis by visual examination (A and D: lack of keratin in the tadpole mouthparts indicated by an arrow); cytology (B and E: optical microscope at 400× amplification, arrows indicating zoosporangia), and isolation (C and F: mTGh culture medium, with the arrows indicating zoospores and zoosporangia) methods. Top row of images A–C were taken from *Hylodes cardosoi*; bottom row images D–F were taken from *Hypsiboas faber*, as labeled.

south of Apiaí, São Paulo, which were the nearest sites last reported (Carnaval et al. 2006; Toledo et al. 2006). Also, this site is the type and only known locality of *Cycloramphus rhyakonastes* (Cycloramphidae; Heyer 1983), which is listed as endangered for the state (Mikich and Bérnils 2004). As the tadpoles of both species (*H. cardosoi* and *H. faber*) may live for one year or more in the water bodies, they may be serving as reservoirs of the fungus and infecting other species in the area. Hence, further *Bd* monitoring at this site is warranted to assess the potential threat of chytridiomycosis to these populations.

Other *Bd* distributional gaps exist in Brazil. For example, the occurrence of the fungus has not been studied between the states of Rio de Janeiro and Pernambuco, indicating a lack of sampling across the Atlantic Forest.

Three *Bd* strains have been previously isolated from Brazil; two (CLFT 001/10 and CLFT 021/01) from Serra do Japi, Jundiaí and Cabreúva, São Paulo, and one (CLFT 023/01) in Monte Verde, Camanducaia, Minas Gerais (unpublished data). We report the isolation of a fourth strain (CLFT 024) from the Brazilian Atlantic Forest. The isolation of strains is important because it provides the basis for studies of fungal molecular biology, virology, biogeography, physiology, morphology, and amphibian conservation. In particular, amphibian host-specific virulence patterns of different *Bd* strains are not well known, which could have direct relevance to amphibian conservation efforts.

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Resumo.—Reportamos aqui pela primeira vez a ocorrência do fungo quitrídio (*Batrachochytrium dendrobatidis*) no estado brasileiro do Paraná. A descoberta é preocupante, pois espécies endêmicas e ameaçadas de extinção vivem nos mesmos corpos d'água onde foi encontrado o fungo, organismo que pode ser letal para anfíbios.

LITERATURE CITED

BERGER, L., R. SPEARE, AND A. KENT. 2000. Diagnosis of chytridiomycosis in amphibians by histologic examination. Zoos' Print Journal 15(1):184–190.

CARNAVAL, A. C. O. Q., R. PUSCHENDORF, O. L. PEIXOTO, V. K. VERDADE, AND M. T. RODRIGUES. 2006. Amphibian chytrid fungus broadly distributed in the Brazilian Atlantic rain forest. EcoHealth 3:41–48.

HEYER, W. R. 1983. Variation and systematics of frogs of the genus *Cycloramphus* (Amphibia, Leptodactylidae). Arq. Zool., São Paulo 30:235–339.

LONGCORE, J. E., A. P. PESSIER, AND D. K. NICHOLS. 1999. *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. Mycologia 91:219–227.

MIKICH, S. B., AND R. S. BÉRNILS. 2004. Livro vermelho da fauna ameaçada no Estado do Paraná. http://www.pr.gov.br/iap. Accessed on 29 August 2009.

TOLEDO, L. F., F. B. BRITTO, O. G. S. ARAÚJO, L. M. O. GIASSON, AND C. F. B. HADDAD. 2006b. The occurrence of *Batrachochytrium dendrobatidis* in Brazil and inclusion of 17 new cases. S. Am. J. Herpetol. 1:185–191.

VERDADE, V. K., P. H. VALDUJO, A. C. CARNAVAL, L. SCHIESARI, L. F. TOLEDO, T. MOTT, G. V. ANDRADE, P. C. ETEROVICK, M. MENIN, B. V. S. PIMENTA, C. NOGUEIRA, C. S. LISBOA, C. D. DE PAULA, AND D. L. SILVANO. 2012. A leap further: the Brazilian Amphibian Action Plan. Alytes 29(1–4):27–42.