

Molecular Laboratory (J. Wood, Boulder, Colorado, USA) for PCR (polymerase chain reaction) assay. Three of the five samples were *Bd* positive. The voucher specimens are deposited in the Saint Joseph's College zoology collection.

Annual larval surveys at the study site indicate that *A. jeffersonianum* and *A. opacum* (Marbled Salamander) populations have declined over the past ten years (R. Brodman, unpubl. data). Disease could be a cause of decline; however, we have not found dead or moribund larvae in previous years. To our knowledge, this is the first published case of *Bd* on larval salamanders. It should be noted that we have not determined the direct cause of mortality of these larval salamanders, nor the cause of the physical lesions, and it is possible that *Bd* and/or the associated physical lesions could be the cause of mortality.

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LITERATURE CITED

- BERGER L., R. SPEARE, P. DASZAK, D. E. GREEN, A. A. CUNNINGHAM, C. L. GOGGIN, R. SLOCOMBE, M. RAGANI, A. HYATT, K. McDONALD, H. HINES, K. R. LIPS, G. MARANTELLI, AND H. PARKES. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. *Proc. Natl. Acad. Sci.* 95:903–906.
- BOSCH, J., I. MARTINEZ-SOLANO, AND M. GARCIA-PARIS. 2001. Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of central Spain. *Biol. Conserv.* 97:331–337.
- BRODMAN, R. 1999. Food and space dependent effects during the interactions of two species of larval salamanders. *J. Freshwat. Ecol.* 14:431–437.
- . 2004. Intraguild predation on congeners affects size, aggression and survival among *Ambystoma* salamander larvae. *J. Herpetol.* 38:21–26.
- , AND J. M. JASKULA. 2002. Microhabitat use and activity of five species of salamander larvae. *Herpetologica* 58:346–354.
- CRUMMER, M. R., D. E. GREEN, AND E. M. O'NEILL. 2005. Aquatic chytrid pathogen detected in terrestrial plethodontid salamander. *Herpetol. Rev.* 36:248–249.
- DAVIDSON E. W., M. PARRIS, J. P. COLLINS, J. E. LONGCORE, A. P. PESSIER, AND J. BRUNNER. 2003. Pathogenicity and transmission of chytridiomycosis in tiger salamanders (*Ambystoma tigrinum*). *Copeia* 2003:601–607.
- DASZAK, P. A. A. CUNNINGHAM, AND A. D. HYATT. 2003. Infectious disease and amphibian population declines. *Diversity and Distribution* 9:141–150.
- FRIAS-ALVAREZ, P., V. T. VREDENBURG, M. FAMILIAR-LOPEZ, J. E. LONGCORE, E. GONZALEZ-BERNAL, G. SANTOS-BARRERA, L. ZAMBRANO, AND G. PARRA-OLEA. 2008. Chytridiomycosis survey in wild and captive Mexican amphibians. *EcoHealth* 5:18–26.
- HARRISON, R. G. 1969. Harrison stages and description of the normal development of the spotted salamander, *Amblystoma punctatum* (Linn.). In S. Wilens (ed.), *Organization and Development of the Embryo*, pp. 44–66. Yale University Press, New Haven, Connecticut.
- LIPS, K. R., F. BREM, R. BRENES, J. D. REEVE, R. A. ALFORD, J. VOYLES, C. CAREY, L. LIVO, A. P. PESSIER, AND J. P. COLLINS. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proc. Natl. Acad. Sci.* 103:3165–3170.
- OUELLET, M., I. MIKAELIAN, B. D. PAULI, J. RODRIGUE, AND D. M. GREEN. 2005. Historical evidence of widespread chytrid infection in North American amphibian populations. *Conserv. Biol.* 19:1431–1440.
- PADGETT-FLOHR, G. E., AND J. E. LONGCORE. 2005. *Ambystoma californiense* (California Tiger Salamander) fungal infection. *Herpetol. Rev.* 36:50–51.
- PASMANS, F., P. ZWART, AND A. D. HYATT. 2004. Chytridiomycosis in the Central American bolitoglossine salamander (*Bolitoglossa dofleini*). *Vet. Rec.* 154:153.
- SPEARE, R., AND L. BERGER. 2000. Global distribution of chytridiomycosis in amphibians. <http://www.jcu.edu.au/school/phtm/PHTM/frogs/chyglob.htm> (accessed 30 April 2008).

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Geographic Distribution of *Batrachochytrium dendrobatidis* in Puerto Rico

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Amphibian declines and extinctions have been reported across the world and infection by a pathogenic chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), is one of the factors associated with this biodiversity loss (Berger et al. 1998; Stuart et al. 2004; Lips et al. 2006). *Bd* was first diagnosed in the West Indies in 2000, from histological studies of skin from declining populations of *Eleutherodactylus coqui* and *E. portoricensis* at El Yunque, Puerto Rico (Burrowes et al. 2004). Histological studies on seven different species of *Eleutherodactylus* collected between 1961 and 1978, revealed that 1976 was the earliest record of chytridiomycosis on the island, and this record corresponded to the last known individual of *E. karlschmidti* collected from El Yunque (Burrowes et al. 2004).

The purpose of this paper is to provide a geographical and taxonomic overview of the current distribution of *Bd* in Puerto Rico. This type of information, with corresponding sampling dates, represents a baseline for further research on the pathogen's origin, potential points of introduction, movement trends, and ecological requirements. In addition, it allows us to identify amphibian species that may be at greater risk from infection or extinction, and to develop appropriate management strategies.

Methods.—Frogs sampled in the wild for *Bd* were captured in individual plastic bags worn as gloves and inverted to prevent cross contamination. All native Puerto Rican amphibians, with the exception of *Bufo lemur*, were sampled. Individuals were examined for physical condition and sampled for *Bd* by swabbing their ventrum, feet and toes as described by Kriger et al. (2006), or toe-clipped following a modification of Twitty (1966) that allowed us to take tissue from different limbs of the frog. A fresh pair of powder-free nitrile gloves was worn every time a different frog

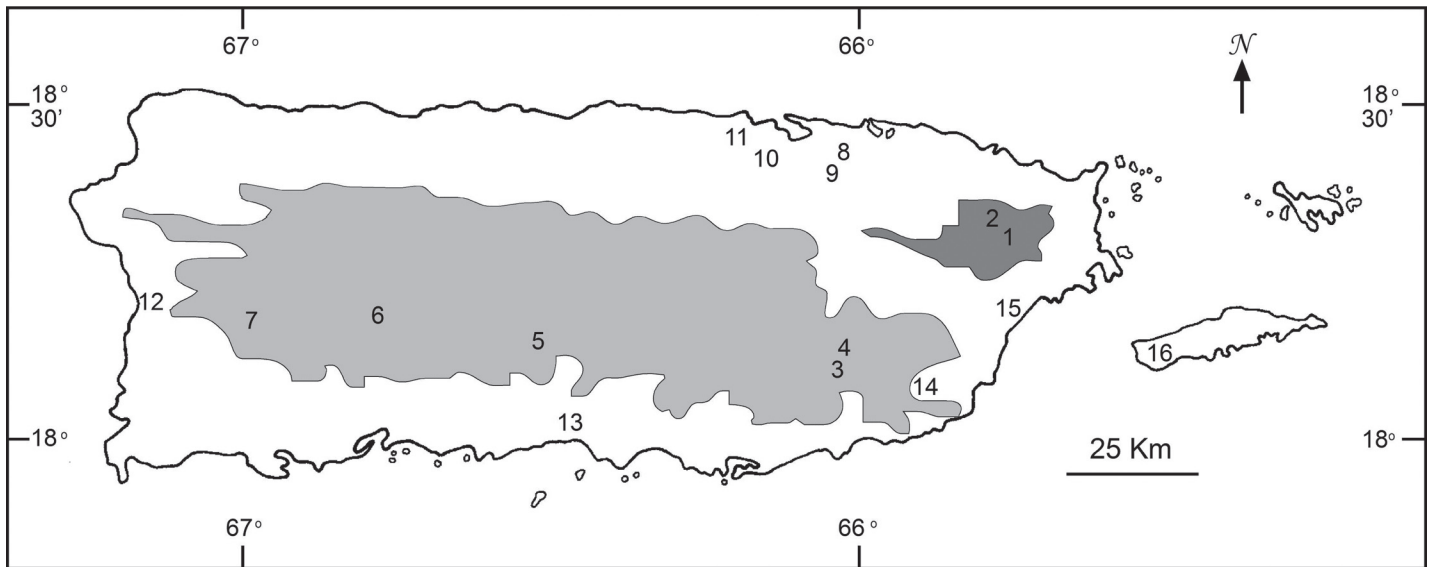


FIG. 1. Map of Puerto Rico showing locations of frogs sampled for *Batrachochytrium dendrobatidis* with the exception of Mona Island. Location numbers correspond to those described in Table 1. The light-shaded area represents the Central Cordillera and the dark-shaded area is The Sierra de Luquillo.

was manipulated. Survey sites were determined opportunistically according to other studies conducted by some of the authors. The number of frogs sampled per species and the timing in which species were sampled varied across this study (Table 1). Some species were sampled only once and *Bd* diagnosis is reported as positive or negative for that year only. Other species were sampled continuously during the dry and wet seasons in Puerto Rico, and for those, we use a dash next to the year to indicate ongoing *Bd* status until present (i.e., 2008; see Table 1).

Bd diagnostics was done by one of the following methods: histology of the skin performed by D.E. Green at the U.S.G.S. National Wildlife Health Center on wild-caught animal tissues collected in the year 2000, and on museum specimens from prior dates obtained from the University of Kansas Natural History Museum, University of Puerto Rico at Mayagüez, and the private collections of Richard Thomas and Rafael Joglar; Polymerase Chain Reaction (PCR) performed by Pisces Molecular (Boulder, Colorado, U.S.A.), following Annis et al. (2004); and Taqman quantitative PCR according to Boyle et al. (2004) at Zoological Society of London and University of Puerto Rico facilities. PCR methods were used for tissues from wild-caught animals from 2002 until present (Table 1).

Results.—A total of 2439 frogs were examined for *Bd* infection across Puerto Rico including two of its off-shore islands, Mona and Vieques. *Bd* was detected at seven of 17 locations sampled, in nine *Eleutherodactylus* species and one species of *Leptodactylus* (Table 1). *Bd* was not detected in another eight species of *Eleutherodactylus*, *Bufo marinus*, and *Rana catesbeiana*. For a few species that were examined from museum or private collections, geographic data was reported only as a region, or nature reserve. These species are reported within the closest locality sampled in our studies.

Discussion.—Previous studies suggested that *Batrachochytrium dendrobatidis* was introduced to Puerto Rico in the mid 1970s, in years characterized by cooler than average temperatures and a greater number of periods with more than five consecutive

days without rain (Burrowes et al. 2004). Considering the advanced road infrastructure on the island, the disease could have spread easily and rapidly by humans to all environments that were suitable for its growth. As an epidemic pathogen during 1976–1981, *Bd* probably drove to extinction two species, *Eleutherodactylus karlschmidti* and *E. jasperi*, that were more dependent on water than any of the other 15 congeners. *Eleutherodactylus karlschmidti* was a stream-dweller with fully webbed toes (Rivero et al. 1963), and *E. jasperi* was an obligate bromeliad dweller, considered more aquatic because it occurred within the inner axils, always flooded with water (Drewry 1986). At present the pathogen seems to be endemic above 600 m, occurring from the eastern Luquillo Mountains (El Yunque), throughout the Central Cordillera up to Maricao (Table 1 and Fig. 1). Species that are widespread in the island, such as *E. coqui*, *E. antillensis*, *E. brittoni* and *Leptodactylus albilabris*, are infected only at high elevations (Table 1). The absence of *Bd* from lower elevations in Puerto Rico may be explained by the high diurnal temperatures which are often above the thermal tolerance (17–25°C) reported for this fungus (Piotrowski et al. 2004). This suggests that high ambient temperatures may serve as a limiting factor for this disease in terrestrial, direct-developing anurans of the genus *Eleutherodactylus*, and point conservation efforts to the more vulnerable highland endemics in Puerto Rico. Nonetheless, in Costa Rica some lowland amphibians seem to cope well with low-level *Bd* infections (Pushendorf et al. 2006), and in Dominica, *Bd* has been associated with the drastic decline of *Leptodactylus fallax* at sea level (García et al. 2007). This suggests that *Bd* can infect amphibians at higher ambient temperatures, and that other factors such as behavior (Rowley and Alford 2007), reproductive mode, (Lips et al. 2003), or strain virulence (Berger et al. 2005) may influence vulnerability to chytridiomycosis among amphibian taxa.

It is evident that the range of responses of amphibians to infection by *Bd* varies among taxa, environmental conditions, climatic regimes and *Bd* strains (Berger et al. 2005, Retallick and

TABLE 1. Geographical distribution of *Batrachochytrium dendrobatidis* in Puerto Rico. Species names are followed by year of first diagnosis and sample size; a dash after a year indicates persistence until present. An asterisk denotes species for which exact localities were not available and, thus, have been reported within closest locality in their geographical range. The number in the first column corresponds to localities in the map (Fig. 1).

Map No.	Location	Coordinates	Elevation (m)	<i>Bd</i> positive (date, No. detected/No. sampled)	<i>Bd</i> negative (date, No. sampled)	Method of Detection
1	El Yunque (Palo Colorado Forest)	18.3006°N 65.785389°W	671	<i>E. coqui</i> (1978, 2/9; 2000-, 430/784) <i>E. portoricensis</i> (2000-, 8/15) <i>E. karlschmidti</i> (1976, 2/2)* <i>E. wightmanae</i> (2005, 1/1)	<i>E. eneidae</i> (1961-1965, 8) <i>E. hedricki</i> (2006, 1) <i>E. locustus</i> (1961-1965, 8) <i>Leptodactylus albilabris</i> (2003-, 5)	Histology PCR qPCR
2	El Yunque (Elfin Forest)	18.301867°N 65.794583°W	850–907	<i>E. coqui</i> (2003-, 154/532) <i>E. portoricensis</i> (2003-, 204/375) <i>E. unicolor</i> (2007-, 3/6)	<i>E. gryllus</i> (2005-, 5)	Histology PCR qPCR
3	Patillas (Casas de la Selva)	18.079483°N 66.034972°W	623	<i>E. coqui</i> (2004-, 19/95) <i>E. brittoni</i> (2004-, 3/25) <i>E. wightmanae</i> (2004-, 53/147) <i>Leptodactylus albilabris</i> (2004-, 4/6)		PCR qPCR
4	Carite	18.0916°N 66.034233°W	686	<i>E. coqui</i> (2005-, 6/6) <i>E. locustus</i> (2005-, 17/18) <i>E. portoricensis</i> (2005-, 1/1) <i>E. richmondi</i> (2005-, 6/11)	<i>E. jasperi</i> (1976, 3)* <i>E. brittoni</i> (2005, 5) <i>Leptodactylus albilabris</i> (2005, 1)	Histology PCR qPCR
5	Toro Negro	18.172367°N 66.358022°W	851–1178	<i>E. coqui</i> (2005-, 20/39) <i>E. antillensis</i> (2005-, 20/20) <i>E. brittoni</i> (2005-, 3/3) <i>E. wightmanae</i> (2005-, 10/12) <i>Leptodactylus albilabris</i> (2005-, 6/6)	<i>Bufo marinus</i> (2005, 1)	PCR qPCR
6	Adjuntas	18.173231°N 66.772531°W	733	<i>E. coqui</i> (2006, 4/10) <i>E. brittoni</i> (2006, 3/5)		qPCR
7	Maricao	18.14455°N 66.9801°W	800	<i>E. coqui</i> (2005-, 14/48) <i>Leptodactylus albilabris</i> (2005-, 4/10)	<i>E. wightmanae</i> (2005-, 5)	PCR qPCR
8	San Juan (UPR)	18.406644°N 66.041414°W	17		<i>E. coqui</i> (2005-, 107) <i>E. cochranae</i> (2007-, 5) <i>Leptodactylus albilabris</i> (2005-, 11)	PCR qPCR
9	San Juan (Bosque San Patricio)	18.411578°N 66.094417°W	64		<i>E. coqui</i> (2005-, 6)	PCR
10	Bayamón	18.369414°N 66.18205°W	45		<i>E. coqui</i> (2005-, 10)	PCR qPCR
11	Toa Baja	18.431847°N 66.200644 °W	10		<i>E. juanariveroi</i> (2006, 5) <i>E. coqui</i> (2006, 5)	qPCR
12	Mayagüez	18.210536°N 67.138144°W	32		<i>E. coqui</i> (2004-, 6) <i>Leptodactylus albilabris</i> (2004-, 4)	qPCR
13	Juana Díaz	18.135083°N 66.358022°W	215		<i>E. coqui</i> (2005-, 7) <i>Leptodactylus albilabris</i> (2006-, 8)	PCR qPCR
14	San Lorenzo	18.081281°N 65.989544°W	407		<i>E. cooki</i> (2004-, 10)	PCR qPCR
15	Humacao	18.166667°N 65.766667°W	3		<i>Bufo marinus</i> (2000, 4) <i>Rana catesbeiana</i> (2000, 3)	Histology
16	Vieques	18.116892°N 65.560786°W	2.5		<i>E. antillensis</i> (2006, 10) <i>Leptodactylus albilabris</i> (2006, 2)	PCR
17	Mona Island	18.088508°N 67.935914°W	10		<i>E. monensis</i> (2006, 8)	qPCR

Miera 2007; Morgan et al. 2007). Puerto Rico is not an exception to these observations. Species extinctions, population extirpations from certain geographical areas, and population fluctuations in species that seem to resist the disease, have occurred on the island. Our data (Table 1) provides a valuable research tool to compare future changes in *Bd* spread, test for evolution of genetic strains, and study environment-specific variation in prevalence of this disease in amphibian communities.

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LITERATURE CITED

- ANNIS, S. L., F. P. DASTOOR, H. ZIEL, P. DASZAK, AND J. LONGCORE. 2004. J. Wildl. Dis. 40:420–428.
- BERGER, L., G. MARANTELLI, L. F. SKERRATT, AND R. SPEARE. 2005. Virulence of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, varies with the strain. Dis. Aq. Org. 68(1):47–50.
- , R. SPEARE, P. DASZAK, D. E. GREEN, A. A. CUNNINGHAM, C. L. GOGGIN, R. SLOCOMBE, M. A. RAGAN, A. D. HYATT, K. R. McDONALD, H. B. HINES, K. R. LIPS, G. MARANTELLI, AND H. PARKES. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rainforests of Australia and Central America. Proc. Nat. Acad. Sci. 95:9031–9036.
- BOYLE, D. G., D. B. BOYLE, V. OLSEN, J. A. T. MORGAN, AND A. D. HYATT. 2004. Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Dis. Aq. Org. 60:141–148.
- BURROWES, P. A., R. L. JOGLAR, AND D. E. GREEN. 2004. Potential causes for amphibian declines in Puerto Rico. Herpetologica 60:141–154.
- DREWRY, G. E. 1986. Golden Coqui recovery survey and brief description of five other Puerto Rican *Eleutherodactylus* species. Report prepared for the Caribbean Field Office of the U.S. Fish and Wildlife Service.
- GARCÍA, G., A. A. CUNNINGHAM, D. L. HORTON, T. W. J. GARNER, A. HYATT, S. HENGSTBERGER, J. LOPEZ, A. OGRODOWCZYK, C. FENTON, AND J. E. FA. 2007. Mountain chickens *Leptodactylus fallax* and sympatric amphibians appear to be disease free on Montserrat. Oryx 41(3):398–401.
- KRIGER, K. M., H. B. HINES, A. D. HYATT, D. G. BOYLE, AND J. M. HERO. 2006. Techniques for detecting chytridiomycosis in wild frogs: comparing histology with real-time Taqman PCR. Dis. Aq. Org. 71:141–148.
- LIPS, K. R., F. BREM, R. BRENES, J. D. REEVE, R. A. ALFORD, J. VOYLES, C. CAREY, L. LIVO, A. P. PESSIER, AND J. P. COLLINS. 2006. Emerging infectious disease and the loss of biodiversity in a neotropical amphibian community. Proc. Nat. Acad. Sci. 103(9):3165–3170.
- , J. REEVE, AND L. WITTERS. 2003. Ecological traits predicting amphibian population declines in Central America. Cons. Biol. 17(4):1078–1088.
- MORGAN, J. A. T., V. T. VREDENBURG, L. J. RACHOWICZ, R. A. KNAPP, M. J. STICE, T. TUNSTALL, R. E. BINGHAM, J. M. PARKER, J. E. LONGCORE, C. MORITZ, C. J. BRIGGS AND J. W. TAYLOR. 2007. Population genetics of the frog-killing fungus *Batrachochytrium dendrobatidis*. Proc. Nat. Acad. Sci. 104:13845–13850.
- PIOTROWSKI, J. S., S. L. ANNIS, AND J. E. LONGCORE. 2004. Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. Mycologia 96:9–15.

- PUSCHENDORF, R., F. BOLAÑOS, AND G. CHAVES. 2006. The amphibian chytrid fungus along an altitudinal transect before the first reported declines in Costa Rica. Biol. Cons. 2006:136–142.
- RETALLICK, R. W. R., AND V. MIERA. 2007. Strain differences in the amphibian chytrid *Batrachochytrium dendrobatidis* and non-permanent, sub-lethal effects of infection. Dis. Aq. Org. 75:201–207.
- RIVERO, J. A., J. MALDONADO, AND H. MAYORGA. 1963. On the habits and food of *Eleutherodactylus karlschmidti* Grant. Carib. J. Sci. 3(1):25–27.
- ROWLEY, J. L., AND R. A. ALFORD. 2007. Behaviour of Australian rainforest stream frogs may affect the transmission of chytridiomycosis. Dis. Aq. Org. 77:1–9.
- STUART, S. N., J. S. CHANSON, N. A. COX, B. E. YOUNG, A. S. L. RODRIGUEZ, D. L. FISCHMAN, AND R. W. WALLER. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–1786.
- TWITTY, V. C. 1966. Of Scientists and Salamanders. Freeman, San Francisco, California.

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Batrachochytrium dendrobatidis in Amphibian Populations in Italy

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The pathogen responsible for the disease chytridiomycosis is the fungus *Batrachochytrium dendrobatidis* (*Bd*) (Berger et al. 1998), which has been detected in numerous localities around the world (Skerratt et al. 2007). In Italy, it has been detected in *Bombina pachypus*, *Rana latastei*, and *Rana catesbeiana* populations in the north (Stagni et al. 2004; Garner et al. 2006; T. Garner, pers. comm. 2008) and in a *Rana kl. esculentalis/Rana lessonae* population in Umbria (Simoncelli et al. 2005; Di Rosa et al. 2007). *Bd* has also been detected on Sardinia (the second largest Italian island) in endemic populations of *Euproctus platycephalus* (Bovero et al. 2008). Here, we report surveys for the pathogen in 13 species of amphibian at 15 locations in Italy in 2007.

We hand captured individual amphibians and sampled them for *Bd* by rubbing a cotton-tipped swab over the body of each individual. Frogs were held separately prior to swabbing and technicians wore a new pair of gloves for each individual handled. As the frog was restrained, the swab was firmly rubbed back and forth 25 times over the drink patch and was also rubbed over the mouth and webbing between each toe. The swab was