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*Original Contribution*

# Chytridiomycosis Survey in Wild and Captive Mexican Amphibians

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**Abstract:** Mexico, a rich country in terms of amphibian diversity, hosts about 375 described species. Population declines have been documented for several species where it is evident that their habitat is being destroyed or modified. However, other species which inhabit pristine areas are declining as well. It has been suggested that the chytrid fungus *Batrachochytrium dendrobatidis (B.d.)* may be one of the causes of the enigmatic declines in Mexico. We surveyed a total of 45 localities, in 12 states across Mexico, examining a total of 360 specimens representing 14 genera and 30 species. We also examined 91 specimens of *Ambystoma mexicanum* from a captive population in Mexico City as well as one *Pachymedusa dacnicolor* obtained in a pet shop. We used a two-tiered technique to detect the pathogen. For wild-caught specimens, we utilized light microscopy to identify presence of *B.d.* sporangia in amphibian skin. Then, to verify the infection, we used a quantitative real-time PCR assay on collected skin sections which is specific for *B.d.* For captive animals, we used a nonlethal version of the real-time PCR technique. We found evidence of *B.d.* infection in 111 animals comprising 14 species in 13 localities. A large percentage (84%) of *Ambystoma mexicanum* from the colony were infected with *B.d*. The two most highly infected individuals were the endangered *Ambystoma mexicanum*, from a captive colony, and *Pachymedusa dacnicolor*, purchased at a pet shop.

**Keywords:** Mexico, *Batrachochytrium dendrobatidis*, amphibian declines, salamanders

## Introduction

Mexico, a country with extremely rich biodiversity and complex topology harbors the fourth largest amphibian fauna in the world (Ochoa-Ochoa and Flores-Villela, 2006). Currently, there are 375 described species, but the steady pace of annual species descriptions indicates that the actual number of amphibian species is greatly underestimated (Flores-Villela and Canseco-Márquez, 2004). Amphibian populations are declining worldwide and the Mexican species are no exception. In several well studied localities where salamanders and frogs were seen or collected by the hundreds in the 1970s and 1980s, it is now difficult to find even a single amphibian (Parra-Olea et al., 1999). Recent field surveys

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document that their habitat is being destroyed, modified, and fragmented, thereby seriously diminishing the diversity and number of amphibians (Parra-Olea et al., 1999). Some declines, however, have occurred in seemingly pristine areas; this pattern has been especially noticeable in tropical montane forests above 1200 m (e.g., Cerro San Felipe, Oaxaca, Mexico; Parra-Olea et al., 1999; Lips et al., 2004).

The Global Amphibian Assessment (GAA) estimated the conservation status of a total of 363 amphibian species from Mexico and report that 71 are Critically Endangered, 85 Endangered, 42 Vulnerable, 22 Near Threatened, 95 Least Concern, and 48 Data Deficient (IUCN, 2007). Habitat loss and water pollution are the most common threat, while over-collecting (e.g., for food), exotic species, and urban development are also significant factors thought to be associated with declines in Mexico. Other factors such as climate change, increased UV-B radiation, chemical contamination, and emerging infectious disease are thought to also be important, but have not properly been evaluated in Mexico. One well-known species that exemplifies the amphibian crisis in Mexico is the endangered Mexican axolotl *(Ambystoma mexicanum)*. This species, once relatively widespread and common throughout the Valle de México region, is now critically endangered and abundance has decreased dramatically (CITES, 2005; Zambrano et al., 2007). The species is clearly suffering from habitat loss, introduced predators, pollution, and illegal collection for food and medicinal uses. The reproductive biology of this species has been intensely studied and has resulted in the establishment of successful captive colonies (Armstrong et al., 1989). To help reverse the decline of this species, there is a proposal to use captive populations as a source to reintroduce the Mexican axolotl to Lake Xochimilco, the type locality and one of the only two localities where this species is found. However, recovery efforts are hampered by lack of clinical information on the captive animals as well as whether threats at Lake Xochimilco have been mitigated. Clearly, before reintroduction can occur, captive animals must be free of dangerous disease (Young et al., 2007).

The catastrophic effects of an emerging infectious disease to amphibian declines was first reported nine years ago (Berger et al., 1998). Microscopic and histological examination of frogs found dead in the field in both Australia and Panama revealed that the frogs were parasitized by a fungus of the phylum Chytridiomycota. The fungus was cultured from a dead frog and described as a new genus and species, *Batrachochytrium dendrobatidis (B.d.)*, in 1999 (Longcore et al., 1999). This was the first report of a member of the phylum infecting and killing living vertebrates. The fungus was not found in archived museum specimens that had been collected in Panama before population declines. In lab experiments, healthy frogs that were exposed to skin scrapings from chytrid-infected live frogs died or became moribund within 3 weeks (Berger et al., 1998).

Chytridiomycosis, the disease caused by the fungal pathogen *B.d.*, has been shown to be the direct cause for the decline of at least 43 amphibian species in Latin America (Lips et al., 2006). Most of the work by Lips and colleagues has focused on study areas in Costa Rica and Panama; however, in Mexico, *B.d.* has been positively identified using histology in five species in the states of Guerrero (Lips et al., 2004), Chiapas (Quintero-Díaz et al., 2004), and Sonora (Hale, 2001). In addition, 11 species in the states of Guerrero, Oaxaca, and Puebla (Santos-Barrera, 2004; Lips et al., 2004; Meik et al., 2005) have exhibited typical clinical signs of chytridiomycosis, but *B.d.* was not identified histologically. To date, there has been no systematic survey of the extent of *B.d.* and resulting chytridiomycosis in amphibians occurring in Mexico. The present study has two main objectives: a) survey a large geographic area in Mexico to test for the presence of *B.d.* in wild populations (45 populations) of amphibians, and b) to screen all of the individuals from a captive colony of *A. mexicanum* for presence of *B.d.* This colony is a source population for a planned reintroduction and recovery effort for the species.

## Materials and Methods

Field work was conducted in several regions, from the northern states of Baja California to the southern states of Oaxaca and Tabasco (Fig. 1), during Summer and Fall 2005, and Summer, Fall, and Winter 2006. We chose these populations to cover a large proportion of the country, a wide array of habitat types (permanent stream, ephemeral pond, etc. Table 1) and localities from lowlands to high elevation forest above 3000 m. (Fig. 1; Table 1). We included localities where amphibian declines were documented by Parra-Olea et al., (1999) and Lips et al. (2004).

Wild caught animals did not show any clinical signs of *B.d.*, however one *Ambystoma mexicanum* (captive colony) and *Pachymedusa dacnicolor* (pet shop) did show typical signs such as sloughed skin and lethargic behavior.

We visited a total of 45 localities in 12 states. Our localities included pristine areas such as Reserva de la Biosfera de la mariposa monarca, and Centro Ceremonial

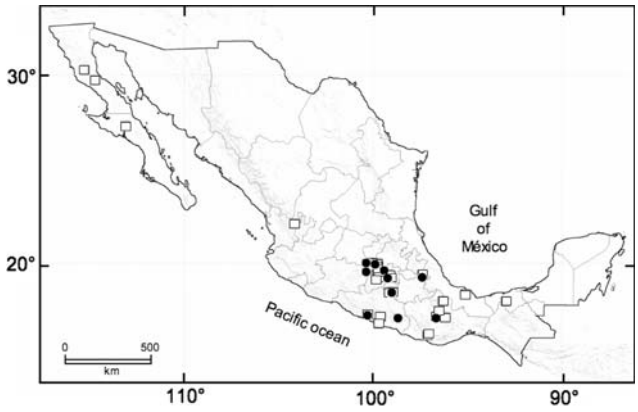


**Figure 1.** Map of localities surveyed for Chytridiomycosis. Closed circles indicate localities were *B.d.* was detected; open squares indicate localities surveyed but *B.d.* was not detected.

Otomí, as well as localities in the vicinity of big cities such as Toluca and Mexico City. Collecting techniques varied. We hand-collected terrestrial frogs and salamanders and used dip nets to capture frogs and salamanders along shorelines. We used SCUBA to collect salamanders occurring at deeper depths (5–25 m). A total of 360 wild caught amphibians were examined (94 tadpoles, 266 adults), representing 14 genera, and 30 species.

We also examined 91 specimens of *Ambystoma mexicanum* from a captive population in Mexico City as well as one *Pachymedusa dacnicolor* obtained in a pet shop in the state of Michoacan. For captive animals, we sampled each one by rubbing a sterile synthetic swab (MW 100–100, Medical Wire & Equipment, Corsham, Wiltshire, UK) in a stroke-like fashion 10 times over the following locations: (1) the ventral surface from the inguinal or throat area to the vent, (2) the undersides of the thighs, and (3) the salamander's sides from the groin or vent area to the armpit. Swabs were then air dried and stored at −20°C. All salamanders were handled with unused non-powdered latex gloves to prevent disease transmission between animals.

We used Longcore's method of wet preparation of whole skin to detect *B.d.* [J.E. Longcore, personal communication]. In the case of wild-caught amphibians, if sporangia were identified visually, then we collected tissue and used a real-time PCR assay (Boyle et al., 2004) to verify *B.d.* presence. The assay also allows quantification of infection load (Boyle et al., 2004). All of the captive animals were analyzed with real-time PCR.

### Wet Preparations

Field-collected animals were euthanized in a solution of MS-222 and specimens were kept damp and cool until examination. Amphibian adult tissues were dissected using a dissecting microscope at 20× or 40× magnification using sterile needle-nosed forceps. A small section of skin (up to 5 mm × 5 mm) was removed from between foot digits (webbing) or elsewhere on the ventral surface of the animal. In two cases, shed skin was used (*Ambystoma mexicanum* and *Pachymedusa dacnicolor*). All samples were placed on a microscope slide in a drop of sterile distilled water and covered with a cover slip. Jaw sheaths of tadpoles were removed with a sterile scalpel and needle-nosed forceps. The tissue was placed on a microscope slide in a drop of sterile distilled water and covered with a cover slip. Digital photographs were taken when empty sporangia, sporangia containing zoospores or internal septa within sporangia of *B.d.* were visualized.

### PCR

We tested 119 samples for *B. dendrobatidis* using the real-time PCR assay described by Boyle et al. (2004). This assay uses species-specific primers ITS1–3 Chytr and 5.8S Chytr and the probe ChytrMGB2 to amplify ITS-1 and 5.8S region. For wild-caught animals, tissue samples consisted of pieces of tissue taken from the inguinal region, toes, or interdigital webbing from the specimens which were identified as positive by wet preparations. For captive animals, we used sterile synthetic swabs *(Ambystoma mexicanum)* and shed skin tissue (one *Ambystoma mexicanum*, and *Pachymedusa dacnicolor*).

DNA was extracted using the PrepMan Ultra protocol for DNA extraction. DNA standards (provided by A.D. Hyatt) were diluted to give 100, 10, 1 genome equivalents for use in Taqman assay (Boyle et al., 2004). Results from the assay are presented quantitatively as the number of genomic equivalents or zoospore equivalents recovered from tissue or from the synthetic swab from each specimen.

## Results

We examined a total of 360 specimens using light microscopy and 119 samples with real-time PCR (Fig. 2). We found evidence of *B.d.* infection in 111 animals comprising 14 species: *Agalychnis moreletti*, *Ambystoma altamirani*, *A. granulosum*, *A. mexicanum*, *A. rivulare*, *A. velasci*, *Hyla euphorbiaceae*, *H. eximia*, *Exerodonta melanomma*, *Pachymedusa dacnicolor*, *Rana megapoda, R. montezumae*, *R. neovolcanica, R. spectabilis* (Table 1).

**Table 1.** List of Species and Localities Surveyed for Chytridiomycosis[a]

| Species | Mexican state | Geographical coordinates N | W | Elevation (m) | Season, year collected | Breeding habitat | IUCN Red List Category | Wet preparation organisms No. examined | infected/No. (prevalence %) | PCR organisms No. infected/No. examined (prevalence %) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Tadpoles | Adults | Tadpoles | Adults |
| ***Ambystoma mexicanum*** | Distrito Federal | Captive | Captive | — | — | — | CR | | **1**/1 (100) | | **76**/90 (84.4) |
| ***Pachymedusa dacnicolor*** | Michoacán | Pet shop | Pet shop | — | — | — | LC | | **1**/1 (100) | | **1**/1 (100) |
| Captive total | | | | | | | | | **2**/2 (100) | | **77**/91 (84.6) |
| Wild | | | | | | | | | | | |
| ***Agalychnis moreletii*** | Guerrero | 17°17′28″ | 100°16′52″ | 939 | Summer 2006 | PS | CR | | **1**/2 (50) | | |
| ***Ambystoma altamirani*** | México | 19°35′ | 99°25′ | 2800 | Fall 2005 | PS | EN | | **2**/2 (100) | | **1**/2 (50) |
| ***Ambystoma granulosum*** | México | 20°00′60″ | 99°56′74″ | 2840 | Fall 2005 | EP | CR | | **1**/1 (100) | | **1**/1 (100) |
| *Ambystoma granulosum* | México | 20°00′38″ | 99°44′58″ | 2634 | Fall 2005 | PP | | | 0/15 | | |
| ***Ambystoma granulosum*** | Michoacán | 20°0′2″ | 100°1′3″ | 2300 | Fall 2005 | PP | | | **4**/4 (100) | | **4**/4 (100) |
| *Ambystoma granulosum* | Michoacán | 19°45′51″ | 100°17′50″ | 2428 | Fall 2005 | PP | | | 0/4 | | |
| *Ambystoma mexicanum* | Distrito Federal | 19°17′25″ | 99°06′14″ | 2230 | Winter 2006 | PP | CR | | 0/1 | | |
| *Ambystoma mexicanum* | Distrito Federal | 19°25′ | 99°11′ | 2230 | Winter 2006 | PP | | | 0/1 | | |
| *Ambystoma mexicanum* | Distrito Federal | 19°17′ | 99°05′ | 2230 | Winter 2006 | PP | | | 0/1 | | |
| ***Ambystoma rivulare*** | Michoacán | 19°40′07″ | 100°16′41″ | 3236 | Fall 2005 | PS | DD | | **9**/17 (52.9) | | **8**/8 (100) |
| *Ambystoma taylori* | Puebla | 19°25′ | 97°24′ | 2320 | Fall 2005 | PP | CR | | 0/33 | | |
| ***Ambystoma velasci*** | Puebla | 19°22′ | 97°21′ | 2350 | Fall 2005 | PP | LC | | **5**/9 (55.5) | | **5**/5 (100) |
| *Ambystoma velasci* | Puebla | 19°22′ | 97°23′ | 2350 | Fall 2005 | PP | | | 0/5 | | |
| *Bufo marinus* | Guerrero | 17°10′12″ | 99°35′15″ | 151 | Fall 2005 | PS | LC | | 0/5 | | |
| *Bufo marinus* | Guerrero | 16°49′11″ | 99°42′42″ | 12 | Fall 2005 | PP | | | 0/5 | | |
| *Bufo marinus* | Oaxaca | 18°00′34″ | 96°19′20″ | 29 | Fall 2005 | PS | | | 0/4 | | |
| *Bufo marinus* | Oaxaca | 18°00′29″ | 96°16′20″ | 51 | Fall 2005 | PS | | | 0/8 | | |
| *Bufo marinus* | Veracruz | 18°22′24″ | 95°07′44″ | 338 | Fall 2005 | PP | | | 0/6 | | |
| *Bufo valliceps* | Veracruz | 18°22′24″ | 95°07′44″ | 338 | Fall 2005 | PP | LC | | 0/1 | | |
| *Eleutherodactylus sp.* | Oaxaca | 17°08′30″ | 96°10′32″ | 2302 | Summer 2005 | T | Nd | | 0/1 | | |
| *Eleutherodactylus sp.* | Oaxaca | 17°28′38″ | 96°30′44″ | 2924 | Summer 2005 | T | | | 0/1 | | |
| *Eleutherodactylus sp.* | Oaxaca | 17°28′49″ | 96°30′26″ | 2959 | Summer 2005 | T | | | 0/2 | | |
| *Eleutherodactylus sp.* | Oaxaca | 17°11′44″ | 96°38′16″ | 2899 | Summer 2005 | T | | | 0/3 | | |
| ***Exerodonta melanomma*** | Guerrero | 17°07′38″ | 98°39′07″ | 1600 | Summer 2005 | ES | VU | | **2**/5 (40) | | |
| *Hyalinobatrachium fleischmanni* | Guerrero | 17°17′28″ | 100°16′52″ | 939 | Summer 2006 | PS | LC | | 0/2 | | |

**Table 1.** continued

| Species | Mexican state | Geographical coordinates N | W | Elevation (m) | Season, year collected | Breeding habitat | IUCN Red List Category | Wet preparation organisms No. examined | infected/No. (prevalence %) Tadpoles | Adults | PCR organisms No. examined | infected/No. (prevalence %) Tadpoles | Adults |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Hyla arenicolor* | Jalisco | 22°06′35″ | 104°08′22″ | 2370 | Winter 2006 | EP | LC | | | 0/1 | | | |
| ***Hyla euphorbiacea*** | Oaxaca | 17°11′39″ | 96°37′53″ | 2924 | Summer 2005 | EP | NT | | | **1**/6 (16.6) | | | |
| *Hyla eximia* | México | 20°00′60″ | 99°56′74″ | 2840 | Summer 2005 | EP | LC | 0/9 | | | | | |
| ***Hyla eximia*** | México | 19°50′18″ | 99°51′42″ | 2604 | Summer 2005 | PP | | 0/11 | | **1**/1 (100) | | | **1**/1 (100) |
| *Hyla eximia* | México | 19°50′57″ | 99°51′01″ | 2525 | Summer 2005 | PP | | 0/4 | | | | | |
| *Hyla eximia* | México | 20°02′10″ | 100°01′20″ | 2575 | Summer 2005 | PP | | 0/2 | | | | | |
| *Hyla eximia* | México | 20°02′22″ | 100°01′35″ | 2543 | Summer 2005 | PP | | 0/6 | | 0/2 | | | |
| *Hyla eximia* | México | 20°00′42″ | 99°56′56″ | 2824 | Summer 2005 | PP | | 0/1 | | | | | |
| *Hyla eximia* | México | 20°00′38″ | 99°56′56″ | 2865 | Summer 2005 | PP | | 0/1 | | | | | |
| *Hyla eximia* | México | 20°00′36″ | 99°56′56″ | 2863 | Summer 2005 | PP | | 0/1 | | | | | |
| *Hyla plicata* | Michoacán | 19°40′07″ | 100°16′41″ | 3236 | Fall 2005 | PS | LC | 0/9 | | | | | |
| *Leptodactylus melanonotus* | Guerrero | 16°49′11″ | 99°42′42″ | 12 | Fall 2005 | PP | LC | | | 0/3 | | | |
| *Leptodactylus melanonotus* | Veracruz | 18°22′24″ | 95°07′44″ | 338 | Fall 2005 | PP | | | | 0/6 | | | |
| *Leptodactylus melanonotus* | Tabasco | 17°59′29″ | 92°58′21″ | 11 | Winter 2006 | EP | | | | 0/5 | | | |
| *Plectrohyla pentheter* | Oaxaca | 16°11′38″ | 97°06′48″ | 1514 | Summer 2006 | ES | EN | | | 0/1 | | | |
| *Pseudacris cadaverina* | Baja California | 29°43′36″ | 114°42′48″ | 534 | Winter 2006 | EP | LC | | | 0/3 | | | |
| *Pseudacris regilla* | Baja California | 30°15′46″ | 115°15′33″ | 530 | Winter 2006 | PS | LC | | | 0/18 | | | |
| *Pseudacris regilla* | Baja California Sur | 27°17′53″ | 113°06′08″ | 103 | Winter 2006 | EP | | | | 0/20 | | | |
| *Ptychohyla leonhardschultzei* | Oaxaca | 16°11′38″ | 97°06′48″ | 1514 | Summer 2006 | ES | EN | | | 0/3 | | | |
| *Ptychohyla leonhardschultzei* | Oaxaca | 16°11′47″ | 97°07′26″ | 1641 | Summer 2006 | ES | | | | 0/1 | | | |
| *Rana megapoda* | México | 19°34′58″ | 99°45′38″ | 2536 | Summer 2005 | PP | VU | 0/8 | | 0/1 | | | |
| *Rana megapoda* | México | 20°02′10″ | 100°01′20″ | 2575 | Summer 2005 | PP | | 0/3 | | | | | |
| *Rana megapoda* | México | 20°02′22″ | 100°01′35″ | 2543 | Summer 2005 | PP | | 0/3 | | | | | |
| ***Rana megapoda*** | México | 20°00′36″ | 99°56′56″ | 2863 | Summer 2005 | PP | | **1**/12 (8.3) | | 0/3 | | | **1**/1 (100) |
| *Rana megapoda* | México | 20°00′42″ | 99°56′56″ | 2824 | Summer 2005 | PP | | 0/1 | | | | | |
| ***Rana montezumae*** | Distrito Federal | 19°18′54″ | 99°11′24″ | 2337 | Fall 2005 | PP | LC | **1**/1 (100) | | 0/1 | **1**/1 (100) | | |
| ***Rana neovolacanica*** | México | 20°00′60″ | 99°56′74″ | 2841 | Fall 2005 | PP | NT | | | **2**/2 (100) | | | **2**/2 (100) |
| *Rana neovolcanica* | México | 20°00′36″ | 99°56′56″ | 2863 | Fall 2005 | PP | | | | 0/1 | | | |
| ***Rana neovolcanica*** | Michoacán | 19°40′07″ | 100°16′41″ | 3236 | Fall 2005 | PP | | | | **3**/6 (50) | | | **3**/3 (100) |

**Table 1.** continued

| Species | Mexican state | Geographical coordinates N | W | Elevation (m) | Season, year collected | Breeding habitat | IUCN Red List Category | Wet preparation organisms No. infected/No. examined (prevalence %) Tadpoles | Adults | PCR organisms No. infected/No. examined (prevalence %) Tadpoles | Adults |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Rana sp.* | México | 19°18′08″ | 99°23′18″ | 2991 | Summer 2005 | PP | Nd | 0/9 | | | |
| *Rana sp.* | México | 19°11′55″ | 99°50′24″ | 3191 | Summer 2005 | PP | | 0/2 | | | |
| *Rana sp.* | México | 19°43′35″ | 99°50′09″ | 2531 | Summer 2005 | PP | | 0/10 | | | |
| *Rana sp.* | México | 19°50′18″ | 99°51′42″ | 2604 | Summer 2005 | PP | | 0/1 | | | |
| ***Rana spectabilis*** | Morelos | 18°30′07″ | 99°00′07″ | 1097 | Fall 2005 | ES | LC | | **1**/2 (50) | | **1**/1 (100) |
| *Rana vaillanti* | Veracruz | 18°22′24″ | 95°07′44″ | 338 | Fall 2005 | PP | LC | | 0/36 | | |
| *Rana vaillanti* | Tabasco | 17°59′29″ | 92°58′21″ | 11 | Winter 2006 | EP | | | 0/1 | | |
| *Rana zweifeli* | Morelos | 18°30′06″ | 99°00′05″ | 1094 | Fall 2005 | ES | LC | | 0/1 | | |
| *Smilisca baudinii* | Guerrero | 16°49′11″ | 99°42′42″ | 12 | Fall 2005 | PP | LC | | 0/3 | | |
| *Smilisca baudinii* | Veracruz | 18°22′24″ | 95°07′44″ | 338 | Winter 2006 | PP | | | 0/1 | | |
| Wildlife total | | | | | | | | **2**/94 (2.1) | **32**/266 (12) | **1**/1 (100) | **27**/28 (96.4) |
| Captive total | | | | | | | | | | | **77**/91 (84.6) |

[a]Boldface names and numbers indicate samples positive for *B.d.* Breeding habitat: PS, permanent stream; ES, ephemeral stream; EP, ephemeral pond; PP, permanent pond; T, terrestrial. IUCN Red List Category: CR, Critically Endangered; DD, Data Deficient; EN, Endangered; LC, Least Concern; NT, Near Threatened; VU, Vulnerable; Nd, No data.

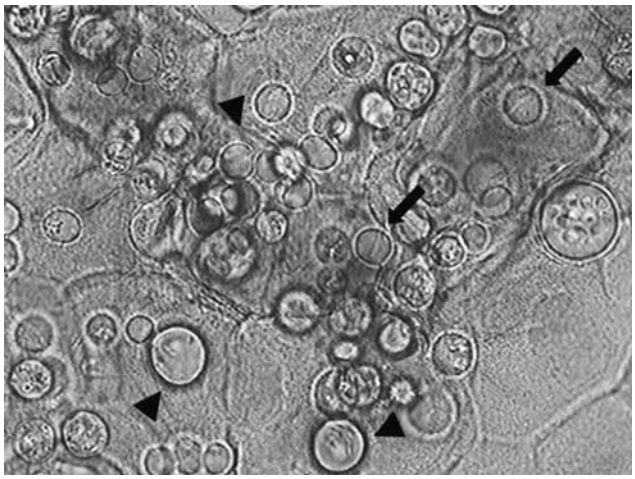

**Figure 2.** *Batrachochytrium dendrobatidis* infection in *Ambystoma mexicanum* viewed with 100× objective lens. Arrows indicate thalli with septae; arrowheads indicate empty sporangia.

*B.d.* was detected at 13 localities from the following seven states: Distrito Federal, Estado de México, Guerrero, Michoacán, Morelos, Oaxaca, and Puebla, all between 939–3236 m elevation (Fig. 1; Table 1). Out of the 36 positives from the wet preparation method, only 31 specimens had tissues available for the PCR assay. No tissue was available for *Agalychnis moreleti*, *Hyla euphorbiacea*, and *Exerodonta melanomma*. The PCR assay resulted in *B.d.* detection in 30 of the 31 specimens categorized as infected from the wet preparation.

We also examined 90 specimens of *Ambystoma mexicanum* from a captive colony and 76 resulted in the amplification of *B.d.* DNA (i.e., positive for *B.d.*). Real-time PCR results varied from zero genomic or zoospore equivalents (i.e., no evidence for infection) to 1726.29 zoospore equivalents.

The zoospore equivalents reported are not directly comparable among our samples because different amounts of tissues were used for the extraction (e.g., we tested toes and skin from wild-caught specimens, and synthetic swabs for captive animals). The two most highly infected samples were *A. mexicanum* and *Pachymedusa dacnicolor* collected using our synthetic swab technique. Both samples were obtained from captive populations.

## Discussion

Using a combination of techniques, we confirmed the presence of *B.d.* in a growing number of amphibians endemic to Mexico, and our results suggest that it is widely distributed along the Transmexican Volcanic Belt (TVB) in high elevation forests (Fig. 1). We found chytridiomycosis in relatively disturbed areas as well as in pristine forests such as the Reserva de la Biosfera Mariposa Monarca in the state of Michoacán and the Centro Ceremonial otomí in the state of Mexico. According to Lips et al. (2003) declines due to chytrid epizootics are most common at higher elevations in the tropics. One hypothesis suggests that cooler temperatures allow for optimal growth for the fungus (17°–25°C; Piotrowski et al., 2004; Pounds et al., 2006). Our findings concur with Lips et al. (2003) in that infected samples were found at higher elevations from 939 m to 3200 m in elevation. In addition, *B.d.* has also been reported for a number of species in localities in the lowlands (lower than 100 m) such as in Hawaii (Beard and O'Neill, 2005), Puerto Rico (Burrowes et al., 2004 ), Honduras (Puschendorf et al., 2006), and Brazil (Carnaval et al., 2006). None of our lowland samples were infected, indicating that *B.d.* is not likely to occur in tropical rainforest (Veracruz) or lowland desert (Baja California). However, there is an unpublished report of a frog found dead in the Peninsula de Yucatán and diagnosed with *B.d.* [J. Voyles, personal communication.]. If verified, this record would greatly expand the range of *B.d.* in Mexico.

We found the presence of *B.d.* in historical sites where amphibian population declines had been reported by Parra-Olea et al. (1999) and Lips et al. (2004). Lips et al. (2004) reported *Agalychnis moreleti* from the state of Guerrero (their region 1) as locally extinct, but Duran-Fuentes et al. [unpublished data] surveyed this site and found two specimens of this species. We swabbed those specimens and one of them was infected with *B.d.* We also found another species in the same region, *Exerodonta melanomma*, to be infected as well. Other areas where enigmatic declines have occurred include Sierra de Juárez in the state of Oaxaca. In this study, we found very few specimens of *Eleutherodactylus sp.* (four specimens) and *Hyla euphorbiacea* (six specimens) in our survey. We found one of the *H. euphorbiacea* to be infected. Thus our data confirm the presence of *B.d.* in historical decline sites and suggest *B.d* as a factor for declines of some species in these areas.

The TVB is a region with complex geological history, where components of the neartic and the neotropical biota meet resulting in a large diversity of plants and animals and a large number of endemic species (Ochoa-Ochoa and Flores-Villela, 2006). The total amphibian fauna in the TVB is approximately 136 species of which 80% are endemic to

the region (Flores-Villela and Gerez, 1994). We surveyed 19 species (in 26 localities) along the TVB and found evidence of chytridiomycosis in 9 of them (47%). We believe it is very important to further document the extent of the disease in the region, and answer questions such as: How many species present in the TVB are infected with *B.d.*? Has any species gone extinct? Is *B.d.* present all year round? Are all infected species declining in population size? Only when we have answered these questions can we proceed to propose a conservation management plan appropriate for the taxa in the TVB.

The broad geographic range and complex topography of Mexico have resulted in a unique and specious amphibian fauna. Among Central and North American countries, Mexico has the greatest diversity of amphibians and over 65% of the taxa are endemic to the country (Campbell, 1999; IUCN, 2007) [Flores-Villela et al., unpublished data]. Two of the genera that contribute greatly to the total number of endemic species are *Ambystoma* (95% of the species are endemic) and *Rana* (69% of the species are endemic). Our results indicate that these two groups had the highest levels of infection. Of the *Ambystoma* and *Rana* we studied, five out of six, and four out of five, respectively, were infected with *B.d.* In the Global Amphibian Assessment, Stuart et al. (2004), established that the families Ambystomatidae and Ranidae in Mexico are the most at risk. Our result offer further support for the endangered status of those taxa.

Amphibian species most at risk from extinction are those with small ranges (Stuart et al., 2004). Alarmingly, all of the species we found to be infected are Mexican endemics with highly restricted distributions (Flores-Villela and Gerez, 1994). The only exception is the positive we got from the pet trade *(Pachymedusa dacnicolor)*. Perhaps the most interesting case is the Mexican axolotl, *Ambystoma mexicanum*, which is restricted to two lakes (Xochimilco and Chalco). The species has survived in the wild despite a high level of exploitation for human consumption over perhaps several hundred years, and urban growth that has completely surrounded the only two bodies of water where it still occurs. The conditions are precarious and the survival of the species is far from secure. In Xochimilco, the populations have been steadily decreasing, and during the last five years the abundance has been reduced by 60% (Graue, 1998; Zambrano et al., 2007).

The demand for axolotls for international trade is very high, and the most common form of trade is live specimens. The interest both for international trade and in the hope for re-introduction has resulted in the establishment of breeding colonies around the world. In Mexico City, there are at least three breeding colonies, with constant specimen translocation between them to ensure genetic mixing. These colonies are established at the two larger Federal Universities and serve to provide not only live animals for domestic and international markets, but most importantly, for research and conservation. The main objective of the captive colonies is to provide source animals for re-introduction into the wild to establish more secure self-supporting wild populations.

We found that 85% of the captive *Ambystoma mexicanum* at the Instituto de Biologia, UNAM colony was infected with *B.d.* At this point, it is hard to establish the source of infection at the captive colony because some of the specimens came from Xochimilco while others originated in the other captive colony. The specimens that we captured and swabbed from the field in Xochimilco were negative, but our sample size is too small ($n = 2$) to be able to declare that *Ambystoma mexicanum* from that site are disease-free. We suspect that all of the captive colonies in Mexico might be infected and strongly recommend that animal translocation should stop immediately and that captive animals should be treated with anti-fungal treatment regimes until no positives can be detected in the captive colonies. Clearly, re-introduction of *A. mexicanum* from the colonies into the wild should not proceed until all specimens are healthy. The introduction of infected axolotls into the wild could cause further declines of the species in the wild and may also spread the disease to heterospecifics.

Given the alarming declines and disappearances of Mexican amphibians, examination of the potential decline factors is urgent. This must include systematic surveys of the presence and incidence of chytridiomycosis in wild and captive populations of amphibians. Special attention should be placed in areas such as the Transmexican Volcanic Belt where *Batrachochytrium dendrobatidis* seems to be widely dispersed.

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