

Short Communication

## First Report of Chytridiomycosis in a Dying Toad (Anura: Bufonidae) from Cuba: A New Conservation Challenge for the Island

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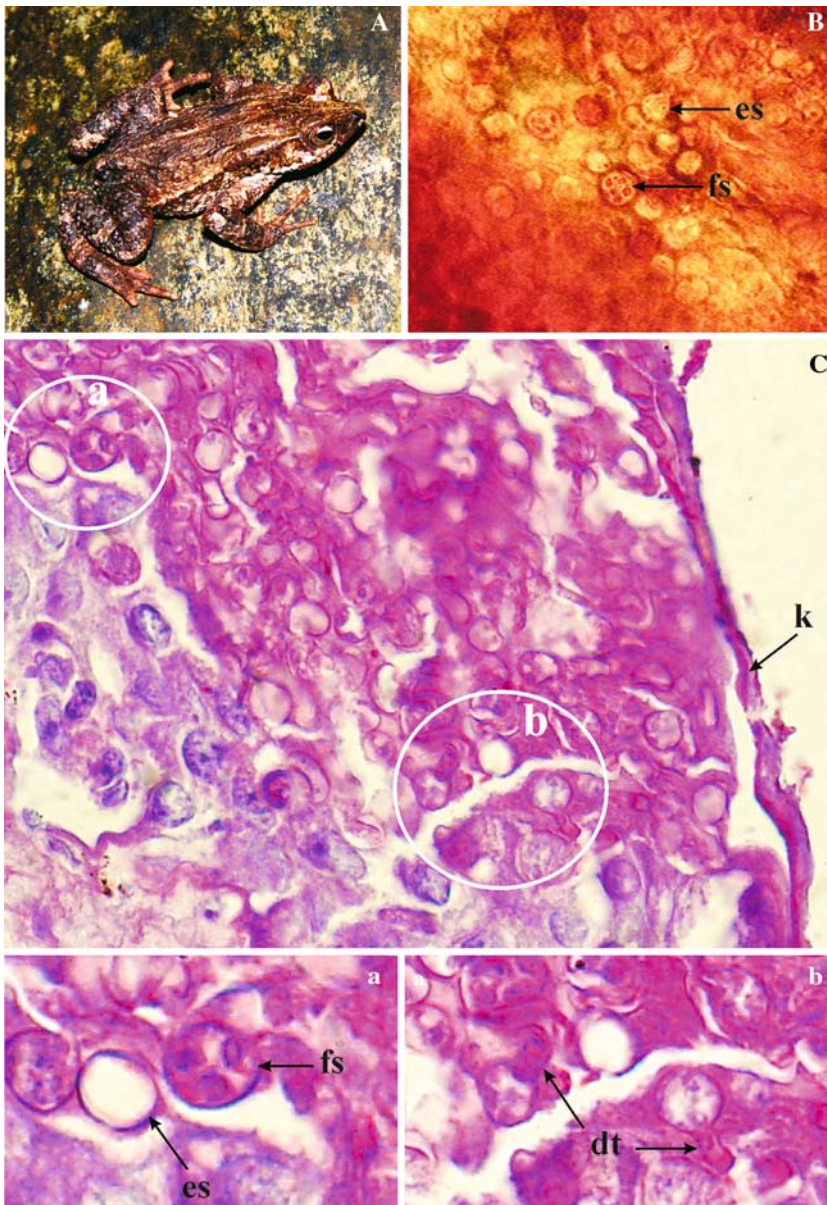
**Abstract:** Infection by the chytrid fungus *Batrachochytrium dendrobatidis* was detected in a male *Bufo longinasus dunni*, a small toad endemic to central Cuba. The individual was severely infected and in decaying condition. Sections of the skin were examined through standard histological procedures. Several stages of the fungus were confirmed and are illustrated in this contribution. Implications for amphibian conservation in Cuba are discussed.

**Keywords:** *Batrachochytrium dendrobatidis*, *Bufo longinasus*, Cuba, amphibian conservation

Amphibians represent one of the most important groups of terrestrial vertebrates in Cuba, encompassing 60 known species and the highest level of endemism (95%) among local tetrapods. Cuban amphibians have been considered to be threatened mostly by habitat loss, especially given the limited distribution of the majority of species. Long-term knowledge about the natural history and population dynamics of Cuban taxa is nearly nonexistent, imposing serious limitations for understanding local patterns of demographic fluctuations or population decline over time. This information is likewise crucial for designing and implementing management plans for amphibian conservation in the country. No studies have yet addressed the impacts of global climatic changes upon the Cuban frog fauna.

The fungus *Batrachochytrium dendrobatidis* is considered the most important pathogen associated with amphibian declines and extinctions worldwide (Berger et al., 1998; Lips, 1999; Longcore et al., 1999; Ron and Merino-Viteri, 2000; Bosch et al., 2001; Green and Sherman, 2001; Hopkins and Channing, 2003; Lane et al., 2003; La Marca et al., 2005; Puschendorf et al., 2006). Although it has been the focus of attention of Cuban herpetologists for a few years, no reports of infection for the island existed to date. In the West Indies, chytridiomycosis has been nonetheless detected in Puerto Rico (Burrowes et al., 2004), where a synergistic interaction between droughts and the pathological effect of the chytrid fungus is suggested as the most probable cause of local population declines and extinctions.

On the morning of October 22, 2006 (approximately 10:00 a.m.), a male of *Bufo longinasus dunni* (Fig. 1A) was found on a rock next to a small stream in Topes de Col-



**Figure 1.** **A:** Infected individual of *Bufo longinasus durni*, photographed in its natural habitat prior to collection. **B:** *B. dendrobatidis* in the toad skin, directly observed with Congo Red stain. **C:** Histological skin sample stained with PAS. Encircled are two sections, better magnified below, showing: **(a)** three sporangia in different stages of development (note the mature zoospores in the signaled sporangium); **(b)** two sporangia with discharge tubes; the left one is releasing a zoospore. es, empty sporangium; fs, sporangium with zoospores; dt, sporangia with discharge tubes; k, keratin layer (stratum corneum). Photos by Luis M. Díaz (A, B), and Agustín Chong (C). All photographs were adjusted with the software CorelDraw 11.633 (© 2002 Corel Corporation).

lantes (21° 55' N, 80° 01' W; approximately 780 m a.s.l), Macizo de Guamuhaya, central Cuba. Although the animal seemed normal at first glance, an unusual lethargic behavior was detected after observing the individual for several minutes. A close examination of the toad confirmed that the hind limbs were unusually swollen and that the skin was shedding off the hands, forelimb insertion, throat, feet, thighs, and the pelvic patch. The toad was not able to move normally and was definitely in a decaying condition. The animal died while being handled and was preserved in ethanol 70%. All symptoms were immediately tied to a suspicion of chytridiomycosis. The specimen was deposited in the herpetological collection of the Museo Nacional de Historia Natural de Cuba (MNHNCu 1000).

Pieces of the shed skin were initially examined in the laboratory under a clinical Olympus BX51 microscope (Tokyo, Japan) after application of Congo Red stain procedure proposed by Briggs and Burgin (2004). The observations revealed the existence of advanced chytridiomycosis, with abundant and mostly clustered sporangia in different stages of development (Fig. 1B) as described by Berger et al. (2005). In a second and most precise approach, a piece of the pelvic patch skin measuring 8 × 2 × 1 mm (l × w × d) was processed with standard histological protocols using a tissue processor Sakura Tissue Tek II and a microtome Sakura Accu-Cut SRM (Tokyo, Japan). Skin cuts were stained (separately) with hematoxylin and eosine (H&E), periodic acid-Schiff (PAS), and Grocott's silver-methenamine. All

procedures enabled visualization of typical stages of *Batrachochytrium dendrobatidis*, which were better observed with PAS (Fig. 1C). Skin hyperkeratosis and hyperplasia, observed in histological samples, were the probable cause of the swollen appearance of the hind limbs and of the excessive shedding, as previously reported (Berger and Speare, 1998; Berger et al., 2000, 2005).

To date, chytridiomycosis was not known to occur in Cuba. To our knowledge, this was not only the first report for the island, but also the first time that a Cuban amphibian was found in a decaying condition, possibly tied to an advanced stage of the disease. We were unable to detect other individuals with the same symptoms during our short visit to the locality, but now it is clear that, at the very least, this toad population is confronting a serious threat. *Bufo longinasus dunni* is endemic to the mountains of central Cuba. The species was considered endangered (EN) by Hedges and Díaz (2004), following the categories and criteria of IUCN. The four local subspecies of *Bufo longinasus* (*B. l. longinasus*, *B. l. dunni*, *B. l. cajalbanensis*, and *B. l. ramsdeni*) are closely associated with water, especially the males, and all have somewhat restricted spatial distributions in Cuba's mountain ranges (Schwartz and Henderson, 1991). A preliminary captive breeding protocol for *Bufo longinasus longinasus* was described by Díaz and Cádiz (2006). With some modifications, it may be successful for *B. l. dunni* as an emergency solution for this taxon, if need be. Ex situ conservation programs may be the only option to avoid extinction for many species (Mendelson et al., 2006). Other populations of *B. longinasus* may be under threat, and the fungus itself might have a broader distribution throughout the country than previously suspected, possibly affecting other species of frogs.

Efforts must be made to detect new cases of chytridiomycosis in Cuba, especially in montane species linked to aquatic habitats, which includes not only riparian frogs (as many in the genus *Eleutherodactylus*; e.g., *E. turquinensis*), but also bromeliad dwelling anurans (e.g., *E. melacara*), cloud forests ground dwellers (e.g., *E. albipes*), and tadpoles. Altitudinal distribution and ecological specialization, such as restriction to stream or bromeliad habitats, were considered common denominators in the decline of Puerto Rican anurans (Joglar and Burrowes, 1996; Joglar, 1998). Likewise, it is necessary to intensify studies on the health and fluctuations of local populations through close monitoring efforts. It will be important to access also whether climate is tied to local outbreaks of the disease (Bosch et al., 2006; Pounds et al., 2006). The dry and colder season, in

which ground dweller and semiarboreal anurans tend to search for humidity along the edges of streams, might be a sensible moment for infection in many species that can eventually overcrowd in those still damp microhabitats.

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## REFERENCES

- Berger L, Speare R (1998) Chytridiomycosis—a new disease of amphibians. *ANZCCART News* 11:1–3
- Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, et al. (1998) Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Sciences USA* 95:9031–9036
- Berger L, Speare R, Kent A (2000) Diagnosis of chytridiomycosis in amphibians by histologic examination. *Zoos' Print Journal* 14:1–4
- Berger L, Hyatt AD, Speare R, Longcore JE (2005) Life cycle stages of the amphibian chytrid *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms* 68:51–63
- Bosch J, Matínez-Solano I, García-París M (2001) Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of central Spain. *Biological Conservation* 97:9031–9036
- Bosch J, Carrascal LM, Durán L, Walker S, Fisher MC (2006) Climate change and outbreaks of amphibian chytridiomycosis in a montane area of central Spain; is there a link?. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 274:1–8
- Briggs C, Burgin S (2004) Congo Red, an effective stain for revealing the chytrid fungus, *Batrachochytrium dendrobatidis*, in epidermal skin scrapings from frogs. *Mycologist* 18:98–103

- Burrowes P, Joglar R, Green DE (2004) Potential causes for amphibian declines in Puerto Rico. *Herpetologica* 60:141–154
- Díaz LM, Cádiz A (2006) Pflege und Vermehrung von *Bufo longinasus* Stejneger, 1905: Ein Beitrag zur Erhaltung dieser Art. *Aquaristik Fachmagazin and Aquarium heute* 38:18–21
- Green DE, Sherman CK (2001) Diagnostic histological findings in Yosemite toads (*Bufo canorus*) from a die-off in the 1970s. *Biological Conservation* 120:92–103
- Hedges SB, Díaz LM (2004) IUCN Red List of Threatened Species. Available: <http://www.redlist.org>
- Hopkins S, Channing A (2003) Chytrid fungus in northern and western cape frog populations, South Africa. *Herpetological Review* 34:334–336
- Joglar R (1998) *Los Coquíes de Puerto Rico: Su Historia Natural y Conservación*. San Juan, Puerto Rico: Editorial de la Universidad de Puerto Rico
- Joglar R, Burrowes P (1996) Declining amphibian populations in Puerto Rico. In: Powell R, Henderson RW (editors), *Contributions to Herpetology*, Vol 12. Contributions to West Indian Herpetology: A Tribute to Albert Schwartz Ithaca, NY: Society for the Study of Amphibians and Reptiles, pp 371–380
- Lane EP, Weldon C, Bingham J (2003) Histological evidence of chytridiomycete fungal infection in a free-ranging amphibian, *Afrana fruscigula* (Anura: Ranidae) in South Africa. *Journal of the South African Veterinary Association* 74:20–21
- La Marca E, Lips K, Lotters S, Puschendorf R, Ibañez R, Jueda-Almonacid J, et al. (2005) Catastrophic population declines and extinction in neotropical harlequin frog (Bufonidae: *Atelopus*). *Biotropica* 37:190–201
- Lips KR (1999) Mass mortality and population declines of anurans at an upland site in western Panama. *Conservation Biology* 13:117–125
- Longcore JE, Pessier AP, Nichols DK (1999) *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia* 91:219–227
- Mendelson JR, Lips KR, Gagliardo RW, Rabb GB, Collins JP, Diffendorfer JE, et al. (2006) Confronting amphibian declines and extinctions. *Science* 313:48
- Pounds JA, Bustamante MR, Coloma LA, Consuegra JA, Fogden MPL, Foster PN, et al. (2006) Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* 439:161–167 (DOI:10.1038/nature04246)
- Puschendorf R, Castañeda F, McCranie JR (2006) Chytridiomycosis in wild frogs from Pico Bonito National Park, Honduras. *Diseases of Aquatic Organisms* 3:178–181
- Ron S, Merino-Viteri A (2000) Amphibian declines in Ecuador: overview and first report of chytridiomycosis from South America. *Froglog* 42:2–3
- Schwartz A, Henderson R (1991) *Amphibians and Reptiles of the West Indies: Descriptions, Distributions, and Natural History*. Gainesville, FL: University of Florida Press, pp 720