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Short communication

The amphibian chytrid fungus along an altitudinal transect before the first reported declines in Costa Rica

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ABSTRACT

Amphibian populations have declined and disappeared in protected and apparently undisturbed areas around the world, especially in montane areas of the tropics. The amphibian chytrid fungus, *Batrachochytrium dendrobatidis* has been implicated in many of these declines. In Costa Rica most declines occurred in the highlands. We examined an amphibian collection made in Braulio Carrillo National Park in 1986 for the amphibian chytrid fungus *B. dendrobatidis*, prior to the well documented amphibian declines in Monteverde, along an altitudinal transect from 100 to 2600 m elev. Skin from the pelvic patch of 202 specimens corresponding to 30 species was examined histologically to determine whether the disease was present in the highlands of the park before amphibian populations declined. For comparison, in 2002 we collected and examined 18 specimens of seven species of *Eleutherodactylus* and *Craugastor* from two other lowland Caribbean sites. The chytrid fungus was present in almost all altitudes in 1986, including lower areas. The pathogen was also found in both species that later declined and in species that did not do so. We detected chytrid fungus on amphibians collected at almost all altitudes in 1986, including those sampled at 280 m. *B. dendrobatidis* was abundant in frogs collected in the 2002 survey, and seems to be endemic in most of Costa Rica. More retrospective museum surveys are needed in order to determine whether it can be found in the area before 1986.

1. Introduction

The decline and extinction of many amphibian populations in protected areas around the world is an alarming phenomenon with several possible causes (Alford and Richards, 1999; Stuart et al., 2004; Beebee and Griffiths, 2005). In Central America, this phenomenon occurred at elevations above 500 m (Young et al., 2001).

Costa Rican declines have been characterized by a general reduction of abundance in almost all amphibian species at mid to high elevations, and by the extirpation of endemics that used to inhabit the higher parts of the mountains (Pounds et al., 1997; Lips, 1998; Bolaños, 2002). In the Neotropics, one of the best indicators of amphibian population decline is the case of the harlequin frogs of the genus *Atelopus* (Lips et al., 2003a; La Marca et al., 2005). Diurnal and brightly colored these animals are severally threatened, and their sudden disappearances is strongly linked to a general amphibian decline in the area were they occurred (La Marca et al., 2005).

What follows is an account of individual *Atelopus* populations reported in the literature and unpublished reports by other researchers of where these disappeared throughout

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Costa Rica's mountains starting on the northwest and going down to the southeast.

The most northern population of any *Atelopus* species existed at Volcán Cacao, in the province of Guanacaste (Savage, 2002). *Atelopus varius* was last seen here in 1990 (Flowers, pers. comm.). Approximately 95 km southeast, harlequin frog populations suffered total extirpation in apparent synchrony with the one of golden toad (*Bufo periglenes*), in the cloud forest of Monteverde (Pounds and Crump, 1994; Pounds et al., 1997). A few kilometers further south, in the San Ramón reserve amphibians suffered strong declines as well, with the apparent loss of many species, including *A. varius* populations which were last seen in 1986, and were absent in 1990 (Bolaños and Ehmcke, 1996; Bolaños and Chaves, unpublished). *Bufo holdridgei*, which was endemic to Cerro Chompipe (Central highlands), and *Atelopus senex*, also found at this site, may now be extinct (Savage, 2002). In Tapantí National Park, *Atelopus* was last seen in 1986 (Wake, pers. comm.), and in Las Tablas (southwestern highlands) *Hyla calypsa* and *Bufo fastidiosus* disappeared at the same time as *Atelopus chiriquiensis* (Lips, 1998). Significant amphibian declines, including the loss of harlequin frogs have also occurred at Fortuna, in the Panamanian Talamanca region, an area close to the Costa Rican border (Lips, 1999). Twenty-three species of Costa Rican anurans have been affected; 10 of these have not been seen for more than a decade, including *A. senex* and *A. chiriquiensis* (Bolaños, 2002). Most of Costa Rica's mountains seem to have been affected by this phenomenon.

These striking population declines in Costa Rica have been linked to climate change (Pounds and Crump, 1994; Pounds et al., 1997; Pounds et al., 1999), and to outbreaks of the chytrid fungus *Batrachochytrium dendrobatidis* (Lips et al., 2003b), a recently discovered species that grows on the epidermis of amphibians (Berger et al., 1998; Pessier et al., 1999; Longcore et al., 1999). It is reported to be non lethal for amphibian larvae (Berger et al., 1999; Longcore et al., 1999). However, Blaustein et al. (2005) found that *Bufo boreas* tadpoles exposed to this disease experienced increased mortality and behavior that indicate an effected by this pathogen. In culture the fungus grows well at temperatures below 23 °C but dies at temperatures greater than 29 °C (Longcore et al., 1999). This pathogen occurs on at least five continents (Berger et al., 1998; Ron and Merino, 2000; Lips et al., 2004; Bosch et al., 2001; Green and Sherman, 2001; Puschendorf, 2003; Bonaccorso et al., 2004; Weldon et al., 2004) and initial studies of it's population genetics suggest that it is a clonally reproducing organism with little genetic variation (Morehouse et al., 2003).

Woodhams et al. (2003) show that elevating the body temperature of infected animals can clear the disease from their skin. Temperature appears to be an important factor that may limit the effect of chytridiomycosis.

Puschendorf (2003) examined skin samples of most of the specimens of *A. varius* in the Museum of Zoology of the University of Costa Rica, and found an infected specimen that had been collected during a survey in 1986 in Braulio Carillo National park, on the Atlantic slope of the Cordillera Volcánica Central. Because well-documented declines occurred 75 km west of this site in Monteverde (Pounds and Crump, 1994) two years later, archived frogs collected during the 1986 survey provided an opportunity to check if *B. dendrobatidis* occurred in this area prior to disappearance of the golden toad (*B. periglenes*) in 1989. Declines occurred in this area in the higher portion of the transect, around Cerro Chompipe and Volcán Barva, although no exact date exists for this, since no systematic studies were conducted here until 1990, when no frogs were found. (Bolaños and Chaves, unpublished data). Herpetological research has been carried out in the area of the lower part of the 1986 transect since the late 1940s (Guyer and Donnelly, 2005). In an intense study between 1982 and 1983 nearly all species known historically to this site were present (Clark, 1988); no dramatic changes have occurred since then (Bolaños and Chaves, unpublished data), as they have in higher elevations.

In 2002, we collected and histologically examined specimens from Reserva Escalera de Mono and Guayacán, two lowland sites to see if the chytrid fungus could be found in amphibian populations more recently.

Our objective was to determine to what extent and in what species *B. dendrobatidis* was present in 1986, when amphibian populations were still robust. In an attempt to learn about the evolution of *B. dendrobatidis* in Costa Rican amphibians, we compare infection status of host species in 1986 with altitude (a surrogate for temperature), reproductive mode and current population status.

2. Methods

2.1. *Study regions and collection of specimens*

2.1.1. *The La Selva–Volcán Barva expedition of 1986*

Braulio Carrillo National Park and La Selva Biological Station encompasses a 46,000 ha reserve (Clark, 1990) on the Atlantic slope of Cordillera Volcánica Central in Costa Rica. Four different life zones are included in the transect: tropical wet forest, premontane rain forest, lower montane rain forest and mountain rain forest (Holdridge, 1982; Hartshorn and Peralta, 1988; Lieberman et al., 1996).

In March and April 1986, a team of biologists including botanists, mammalogists, entomologists, invertebrate zoologists and herpetologists participated in a month long survey from La Selva (30–137 m elev.) to Volcán Barva (2906 m elev.) during which museum specimens were collected (Timm et al., 1989). No dead or dying animals were encountered during the survey and there was no reason to believe that any of the animals collected were infected.

The team of herpetologists worked out of seven camps, situated roughly at 300 m elev., 700 m elev., 1000 m elev., 1500 m elev., 1800 m elev., 2050 m elev. and 2600 m elev. Thirty-five species of anurans were collected. Elevations at these sites were determined using topographic maps.

The herpetological specimens were divided between three different museums; we examined all the anuran specimens available from the Museum of Vertebrate Zoology, University of California at Berkeley (MVZ) and the Museo de Zoología, Universidad de Costa Rica (UCR).

2.1.2. *The reserva forestal Escalera de Mono (EARTH) and Guayacán surveys, 2002*

Guayacán (10°02′N, 83°32′W, 650 m elev.) is one of the most herpetologically diverse sites in Costa Rica (Savage, 2002). Reserva Forestal Escalera de Mono at EARTH University