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Chytridiomycosis in Wild Frogs from Southern Costa Rica

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ABSTRACT.—In 1993, the amphibian fauna of Las Tablas, Costa Rica, began to decline, and by 1998 approximately 50% of the species formerly present could no longer be found. Three years later, at the Reserva Forestal Fortuna, in western Panama, a site approximately 75 km east southeast of Las Tablas, KRL encountered a mass die-off of amphibians and a subsequent decline in abundance and species richness. The epidemiological features of the anuran population declines and die-offs at both sites were similar, suggesting a similar cause. Herein we document the presence of the fungus, *Batrachochytrium dendrobatidis*, in dead and dying wild frogs collected at Las Tablas just prior to population declines of several anuran species.

Since 1991, KRL has monitored amphibians on a private farm (8°55′N, 82°44′W) located at 1900 m elevation within the Zona Protectora Las Tablas of the Amistad Biosphere Reserve, Puntarenas Province, Costa Rica (Fig. 1). In 1993, she encountered 10 dead and dying amphibians along two adjacent 400-m transects along the headwaters of the Río Cotón, including two each of *Eleutherodactylus melanostictus*, *Atelopus chiriquiensis*, and *Hyla rivularis*, and one each of *Hyla calypsa*, *Rana vibicaria*, *Hyalinobatrachium fleischmanni*, and the salamander *Oedipina grandis* (Lips, 1998). She collected nine of these carcasses and nine additional, live, normal-appearing frogs (''controls'') from the same streams during the same month. She euthanized and preserved all specimens for future diagnostic examinations.

In 1995, RP performed microscopic diagnostic exams on three dead frogs and three control frogs, and reported a ''possible epidermal protozoal parasite'' associated with hyperkeratosis in all three dead frogs, but was unable to identify this to any known pathological agent. Approximately four years later, *Batrachochytrium dendrobatidis*, a frog-killing fungus, was described from the skin of dying captive frogs (Longcore et al., 1999). Concurrently, this same fungus was identified as the probable cause of death of 54 frogs found in Fortuna, Panama (Berger et al., 1998), an upland (1000–1400 m elevation) site located about 75 km east southeast of Las Tablas (8°42′N, 82°14′W). In both cases, infection was associated with hyperkeratosis of the epidermis, as had been seen in the dead frogs from Las Tablas. This prompted a retrospective diagnostic survey of the amphibians collected from Las Tablas in 1992–1993 to look for chytrid infection. We also surveyed additional skin samples from Fortuna specimens and from several species of amphibians collected from Las Alturas and the Jardín Botanico Wilson, in southern Costa Rica (Fig. 1). All of these sites have suffered recent losses of amphibian populations (Lips, 1999; unpubl. data), and we examined material to determine whether chytrids were present in frogs prior to population losses (Appendix 1),

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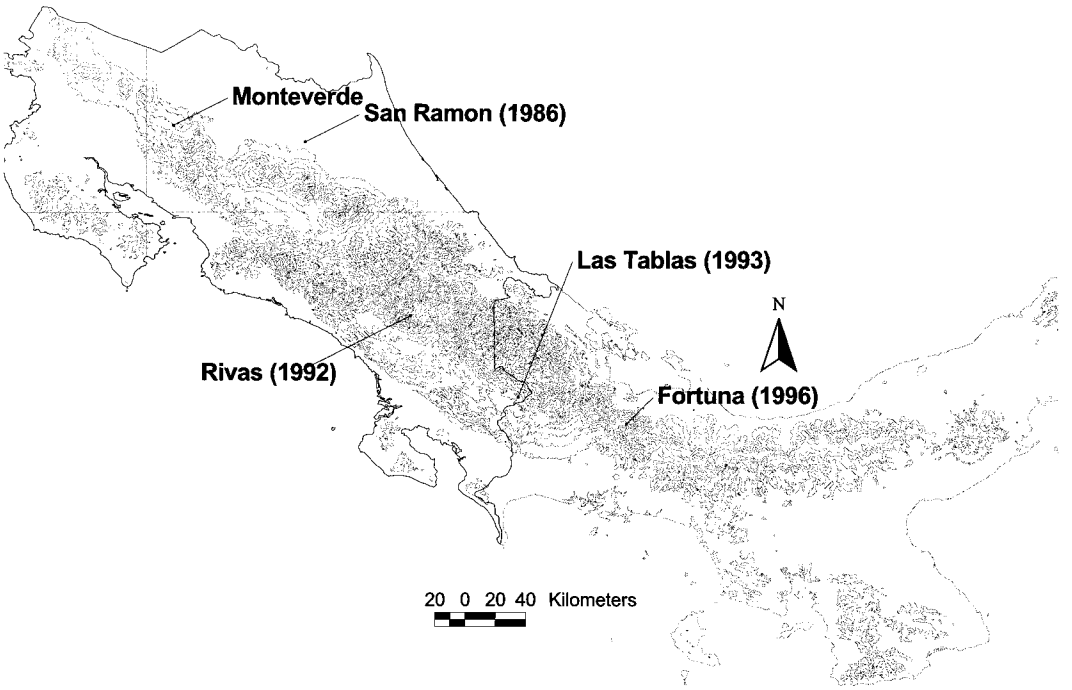

FIG. 1. Map of Costa Rica and Panama indicating sites mentioned in text. Sites with dates indicate documented cases of chytrid infection of amphibians (Berger et al., 1998; Puschendorf, 2003; this study).

which might provide information on the epidemiology of this disease.

MATERIALS AND METHODS

DEG destructively sampled 10 formalin-fixed carcasses (four dead, seven controls) collected from Las Tablas in 1993 and examined 29 2 × 2 mm skin swatches sampled by KRL from museum specimens (Appendix 1). Because of limited sample sizes, we could only determine presence or absence of chytrid; therefore, we did not examine all specimens, but deposited some dead frogs as vouchers in the CRE collection of J. M. Savage, now housed in the LACM. Tissues were decalcified, processed routinely into paraffin blocks, sectioned and stained with hematoxylin and eosin. RP's original paraffin blocks made from tissues of dead frogs collected in 1993 were reaccessed and embedded tissues were reexamined where possible.

RESULTS

The dead amphibians collected from Las Tablas in 1993 appeared well nourished and showed no external gross abnormalities, although four frogs had epidermal chytrid infections. The fungal infection was most extensive in the skin from chin to vent and in the digital and tarso-metatarsal skin of the hind limbs. Additionally, one apparently healthy *Atelopus chiriquiensis* collected as a control had a minimal infection characterized by widely scattered minute clusters of chytridial sporangia on the skin of the thorax, abdomen, and digits. In histologic sections of eight hind-limb digits of this frog, only two minute clusters of chytrids were found. In all cases, only a few scattered chytrids were found in the epidermis of the head and dorsal body. Cultures for viruses, bacteria, and fungi could not be done on amphibians captured in 1993 because all animals were fixed in formalin and preserved in ethanol.

Histological examinations of skin snips from 29 additional frogs and toads of 14 species collected from Las Tablas (1990–1991), and the Jardín Botanico Wilson (1974–1987) in Costa Rica, and Fortuna, Panama (1995) were consistently negative for epidermal chytrid fungi (Appendix 1).

DISCUSSION

We document the association of epidermal chytridiomycosis with a previously unexplained die-off and subsequent population decline in several taxa of wild amphibians from Las Tablas, Costa Rica (Lips, 1998). Although we were unable to identify chytrids in 18 animals collected from Las Tablas three years prior to die-offs and from seven animals collected from Fortuna one year prior to that die-off, we acknowledge that this level of effort would only be able to detect chytrid if it had a very high prevalence in the population at that time (R. Alford, pers. comm.). These retrospective examinations were restricted to the pelvic patch where chytrid infection is heaviest and thus most likely to be found, but it is possible that some of these animals had infections elsewhere. One of the seven clinically normal Las Tablas frogs had a minimal infection of epidermal chytridiomycosis com-

pared to the three dead frogs, and we believe that this individual was incubating the fungus and would have eventually died.

Puschendorf (2003) documented the oldest record of infection by *B. dendrobatidis* in Costa Rican museum specimens of *Atelopus varius* collected in San Ramón, Sarapiquí in 1986 and an infected *A. varius* from Rivas, San Isidro de General in 1992 (Fig. 1). We report the first record of chytrid infection from dead Costa Rican frogs from a protected area known to have experienced subsequent population crashes in multiple species of wild amphibians. We hypothesize that chytrid infection is the proximate cause of amphibian population declines at Las Tablas and encourage further examinations of preserved museum material from sites with documented amphibian declines (e.g., Young et al., 2001). We emphasize the usefulness of museum collections for retrospective investigations of parasite load and prevalence of infection in wild populations.

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APPENDIX 1. Material examined and diagnostic findings by D. E. Green (DEG) and R. Papendick (RP). Site abbreviations are as follows: Las Tablas (LT) and Jardín Botanico Wilson (JBW) Costa Rica, and Fortuna, Panama (Fort). All specimens collected in 1993 were destroyed during examination.

Species	Site and year	Diagnostic findings
I. LAS TABLAS DIE-OFF		
(A) Dead amphibians:		
Atelopus chiriquiensis USC 12517	LT 1993	RP: Possible epidermal parasite DEG: Moderate chytrid infection
Eleutherodactylus melanostictus USC 12528	LT 1993	DEG: Moderate chytrid infection
E. melanostictus USC 12516	LT 1993	RP: Possible epidermal parasite DEG: Heavy chytrid infection
Oedipina grandis USC 12518	LT 1993	RP: degeneration of skin, no epidermal parasites DEG: Not examined
(B) ''Controls''		
Atelopus chiriquiensis USC 12519	LT 1993	RP: No epidermal parasites DEG: Slight chytrid infection
A. chiriquiensis USC 12520	LT 1993	RP: No epidermal parasites DEG: No chytrids
A. chiriquiensis USC 12525	LT 1993	RP: No epidermal parasites DEG: No chytrids
Hyla rivularis USC 12522	LT 1993	No chytrids
H. rivularis USC 12523	LT 1993	No chytrids
H. rivularis USC 12526	LT 1993	No chytrids
H. rivularis USC 12527	LT 1993	No chytrids
II. Historic resurvey		
Atelopus varius LACM 149590	JBW 1974	No chytrids
A. varius LACM 149593	JBW 1987	No chytrids
A. varius LACM 149595	JBW 1987	No chytrids
A. varius LACM 149592	JBW 1987	No chytrids
A. chiriquiensis LACM 148838	LT 1990	No chytrids
A. chiriquiensis LACM 148841	LT 1990	No chytrids
A. chiriquiensis LACM 148834	LT 1990	No chytrids
A. chiriquiensis LACM 148835	LT 1990	No chytrids
Bufo fastidiosus LACM 149573	LT 1990	No chytrids
B. fastidiosus LACM 149575	LT 1990	No chytrids
Caecilia volcani	Fort 1995	No chytrids
Hyalinobatrachium fleischmanni LACM 149605	Fort 1995	No chytrids
Centrolene prosoblepon USC 12575	Fort 1995	No chytrids
Eleutherodactylus melanostictus LACM 148792	LT 1990	No chytrids
E. melanostictus LACM 148794	LT 1990	No chytrids
Hyla calypsa LACM 146204	LT 1990	No chytrids
H. calypsa LACM 146203	LT 1990	No chytrids
H. calypsa LACM 146200	LT 1990	No chytrids
H. calypsa LACM 146078	LT 1991	No chytrids
H. colymba LACM 149572	Fort 1995	No chytrids
H. lancasteri LACM 149606	Fort 1995	No chytrids
H. rivularis LACM 149599	LT 1990	No chytrids
H. rivularis LACM 149598	LT 1990	No chytrids
H. rivularis LACM 149597	LT 1990	No chytrids
H. rivularis LACM 149596	LT 1990	No chytrids
H. uranochroa LACM 149603	Fort 1995	No chytrids
Rana vibicaria LACM 148829	LT 1990	No chytrids
R. warzewitschii CRE 5231	LT 1990	No chytrids
Smilisca phaeota LACM 149604	Fort 1995	No chytrids