Western Toads, warrants further investigation.

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Chytridiomycosis-Associated Mortality in a *Rana palustris* Collected in Great Smoky Mountains National Park, Tennessee, USA

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Batrachochytrium dendrobatidis (*Bd*) has been associated with global amphibian population declines and occasionally with species extinctions (Daszak et al. 1999). Further, recent evidence suggests that surviving amphibian communities may persist with sublethal infections (Rettalick et al. 2004). Although *Bd* has been detected in amphibians in Great Smoky Mountains National Park (GSMNP; Chatfield et al. 2009), USA, mortality associated with chytridiomycosis has not been reported.

The Southern Appalachian Mountain Range, where GSMNP occurs, is known for its amphibian species richness (Dodd 2004). Within the GSMNP, Cades Cove, Tennessee, is one of the most highly visited sites by tourists, and thus an area where anthropogenic stressors (e.g., introduction of chemical agents or novel pathogens) may impact native species. Although amphibian mortality was observed in Cades Cove from 1999 to 2001, these mortality events were attributed to pathogens other than *Bd* (Dodd 2004; Rothermel et al. 2008). On 5 April 2009, a recently deceased adult male *Rana palustris* was found in a pond in Cades Cove (35.606667°N, 83.816944°W) while conducting field surveillance for *Ranavirus*. The animal was collected and shipped overnight on ice to the University of Georgia, Veterinary Diagnostic and Investigational Laboratory for diagnostic evaluation.

Gross examination revealed only mild (ca. 48 h under refrigeration post field collection) postmortem autolysis. Grossly there was diffuse erythema (reddening) on the ventromedial legs and random petechia (pin-point hemorrhages), ecchymosis (red blotches), and irregular patches of discoloration elsewhere on the ventral body (Fig. 1A). Vascular congestion was noted in the kidneys but other organs appeared normal. Sections of all organs were collected and fixed in 10% phosphate-buffered formalin, embedded in paraffin, cut at 4 μm sections, stained with hematoxylin and eosin, and examined microscopically. Additionally, sections of skin lesions and toes were collected for qPCR and conventional PCR for *Bd*, and sections of skin lesions, liver and kidney were collected for

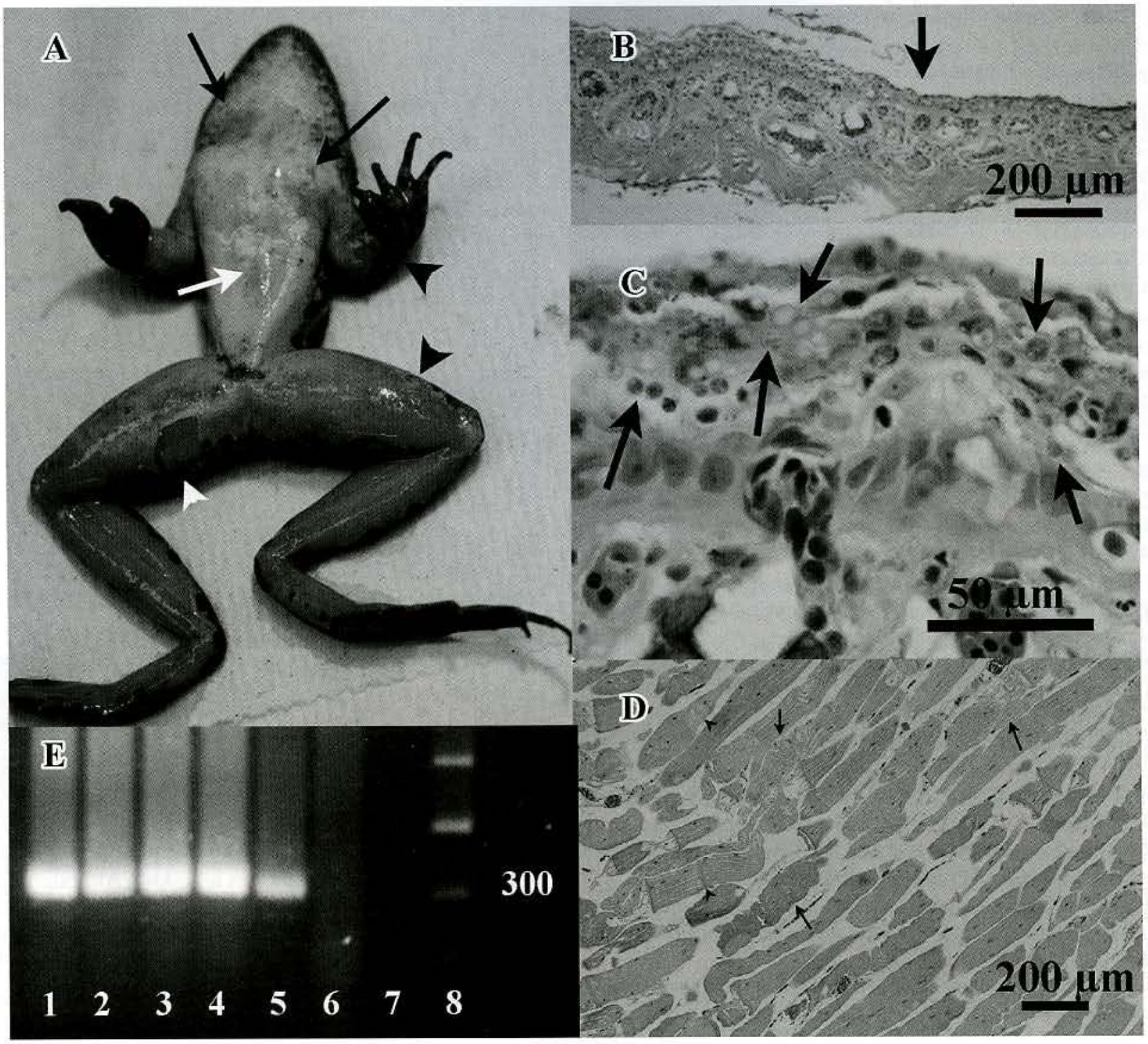

FIG. 1. A) Gross changes observed in *Rana palustris* found dead in Great Smoky Mountains National Park, Tennessee, USA. There is diffuse erythema on the ventromedial legs (black arrowheads) and random petechia, ecchymosis (white arrow), and irregular patches of discoloration (black arrows) elsewhere on the ventral body. The white arrowhead indicates an area of post-mortem tissue sampling. B) Photomicrograph of a hematoxylin and eosin (H&E) stained section of skin from the frog showing epidermal proliferation. The area to the left of the arrow is more proliferative and the area to the right is more normal. C) Closer view of the proliferative skin area in B (H&E stain), showing the organisms consistent with *Batrachochytrium dendrobatidis* zoospores (arrows). D) Photomicrograph of an H&E stained section of skeletal muscle subjacent to the skin lesions, and showing swollen fibers that had lost cross-striations and were often fragmented (arrows), and occasional contraction bands (arrowheads). E) Gel electrophoresis of the PCR products isolated from skin and toe samples collected from the frog pictured in A. The 300-bp products were consistent with *Bd*. Lanes 1 and 2 are Frog Sample 1, Lanes 3 and 4 are Frog Sample 2, Lane 5 is the Positive *Bd* Control, Lane 6 is the Negative Tissue Control, and 7 is Negative Control, Lane 8 is the molecular weight marker.

qPCR and conventional PCR for *Ranavirus*. Chytrid qPCR was performed using the method described by Boyle et al. (2004), and conventional PCR was performed following the protocol by Annis et al. (2004). *Ranavirus* qPCR was performed using the protocol by Picco et al. (2007), and conventional PCR was performed following the protocol by Mao et al. (1997).

Histologically, the epidermis was multifocally thickened (2–4 cell layers; Fig. 1B) with intracytoplasmic 3–5 μm round organisms (Fig. 1C) consistent with *Bd* organisms (thalli). This type of lesion was consistently found in the epidermis overlying the digits and in the skin lesions observed grossly. The skeletal muscles subjacent to the skin lesions had swollen fibers and patches of myofiber disarray, fragmentation, and loss of cross-striations (Fig. 1D). Conventional PCR was positive for *Bd* (Fig. 1E), but negative for *Ranavirus*. Likewise, qPCR was positive for *Bd*, but negative for *Ranavirus*.

The histological and molecular findings were consistent with chytridiomycosis caused by the amphibian pathogen *Bd*. Sensitivity to *Bd* varies by amphibian species (Blaustein et al. 2005), and may be partially attributed to differences in skin antimicrobial properties (Woodhams et al. 2004). Ultimately, chytridiomycosis is believed to interfere with osmoregulation (Voyles et al. 2007), which leads to muscular degeneration and eventually death (Miller et al. 2008). Other factors (e.g., environmental contaminants,

climate change) are hypothesized to exacerbate the effects of or make amphibians more susceptible to this pathogen (Bosch et al. 2007; Davidson et al. 2007).

We are uncertain what factors contributed to this *R. palustris* developing chytridiomycosis. Rothermel et al. (2008) found that 23 of 35 *R. palustris* collected from the southeastern United States were positive for *Bd*; however, chytridiomycosis was not found. In a Canadian survey, *Bd* prevalence in *R. palustris* was found to be low (3%; Ouellet et al. 2005). Given the findings from these studies and the lack of detection of other pathological changes in the *R. palustris* in our study, an exogenous stressor or combination of stressors (e.g., chemical exposure, habitat change, breeding) may have contributed to the development of chytridiomycosis in this case.

The finding of chytridiomycosis in this *R. palustris* verifies the need for continued surveillance and testing of amphibian populations in the GSMNP. Given the wealth of amphibian biodiversity in the GSMNP, this location serves as an ideal place to study the variation in pathogen prevalence and species susceptibility of amphibian populations. The co-occurrence of two emerging amphibian pathogens (*Ranavirus* [Dodd 2004] and *Bd*) in Cades Cove warrants continued monitoring of amphibian breeding ponds in this highly visited area of GSMNP.

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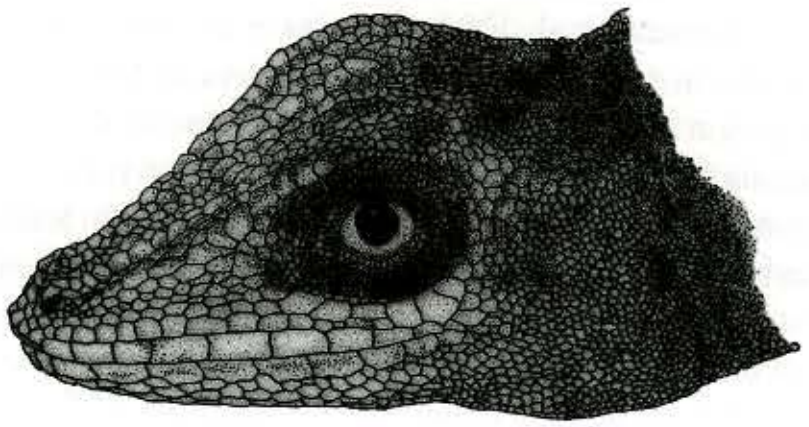

Anolis fraseri (Polychrotidae). Reserva Forestal Bosque de Yotoco, Department of Valle del Cauca, Colombia. Illustration by Fernando Vargas Salinas, based on a photograph by FVS.