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Distribution Limits of *Batrachochytrium dendrobatidis*: A Case Study in the Rocky Mountains, USA

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ABSTRACT: Knowledge of the environmental constraints on a pathogen is critical to predicting its dynamics and effects on populations. *Batrachochytrium dendrobatidis* (*Bd*), an aquatic fungus that has been linked with widespread amphibian declines, is ubiquitous in the Rocky Mountains. As part of assessing the distribution limits of *Bd* in our study area, we sampled the water column and sediments for *Bd* zoospores in 30 high-elevation water bodies that lacked amphibians. All water bodies were in areas where *Bd* has been documented from neighboring, lower-elevation areas. We targeted areas lacking amphibians because existence of *Bd* independent of amphibians would have both ecologic and management implications. We did not detect *Bd*, which supports the hypothesis that it does not live independently of amphibians. However, assuming a detection sensitivity of 59.5% (based on sampling of water where amphibians tested positive for *Bd*), we only had 95% confidence of detecting *Bd* if it was in ≥16% of our sites. Further investigation into potential abiotic reservoirs is needed, but our results provide a strategic step in determining the distributional and environmental limitations of *Bd* in our study region.

Key words: Amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, chytridiomycosis, pathogen reservoir, water sampling.

The decline of many amphibians worldwide has been attributed to chytridiomycosis (Berger et al., 1998; Lips et al., 2006), an infectious disease caused by the aquatic fungus *Batrachochytrium dendrobatidis* (*Bd*; Longcore et al., 1999). Despite the proliferation of *Bd*-related surveys and research, much about its ecology is still unknown, including whether it parasitizes nonamphibian hosts or uses other mechanisms to persist in the environment. The fungus has been detected from environmental samples, but sampling efforts have been focused on sites occupied by amphibians (Lips et al., 2006; Kirshtein et al., 2007; Walker et al., 2007; Cossel and Lindquist, 2009). Pathogens with abiotic or biotic reservoirs can maintain a higher force of infection and increase extinction risk for hosts (McCallum, 2005; Mitchell et al., 2008), making it important to determine if the distribution of *Bd* is dependent upon amphibians.

Most flagellated fungi (phylum Chytridiomycota) are plant parasites or soil saprobes (James et al., 2006). Although *Bd* may be capable of functioning as a saprobe, it is the only chytridiomycete known to parasitize vertebrates (Longcore et al., 1999). Recent evidence of possible recombination suggests that *Bd* may be able to produce desiccation-resistant sporangia (Morgan et al., 2007). The ability to survive as a saprobe and the production of a resting stage would increase the potential for *Bd* to occupy a wide range of habitats independent of amphibians.

Batrachochytrium dendrobatidis is found on >35% of amphibians annually in the Rocky Mountains and seems to be enzootic (Fig. 1; Young et al., 2007; Muths et al., 2008; E.M., B.R.H., and P.S.C., unpubl.). Because the ubiquity of host-associated *Bd* in the region has been established, we instead asked: Where is it *not* present? We addressed this question by using a recently developed method to sample water bodies in areas naturally

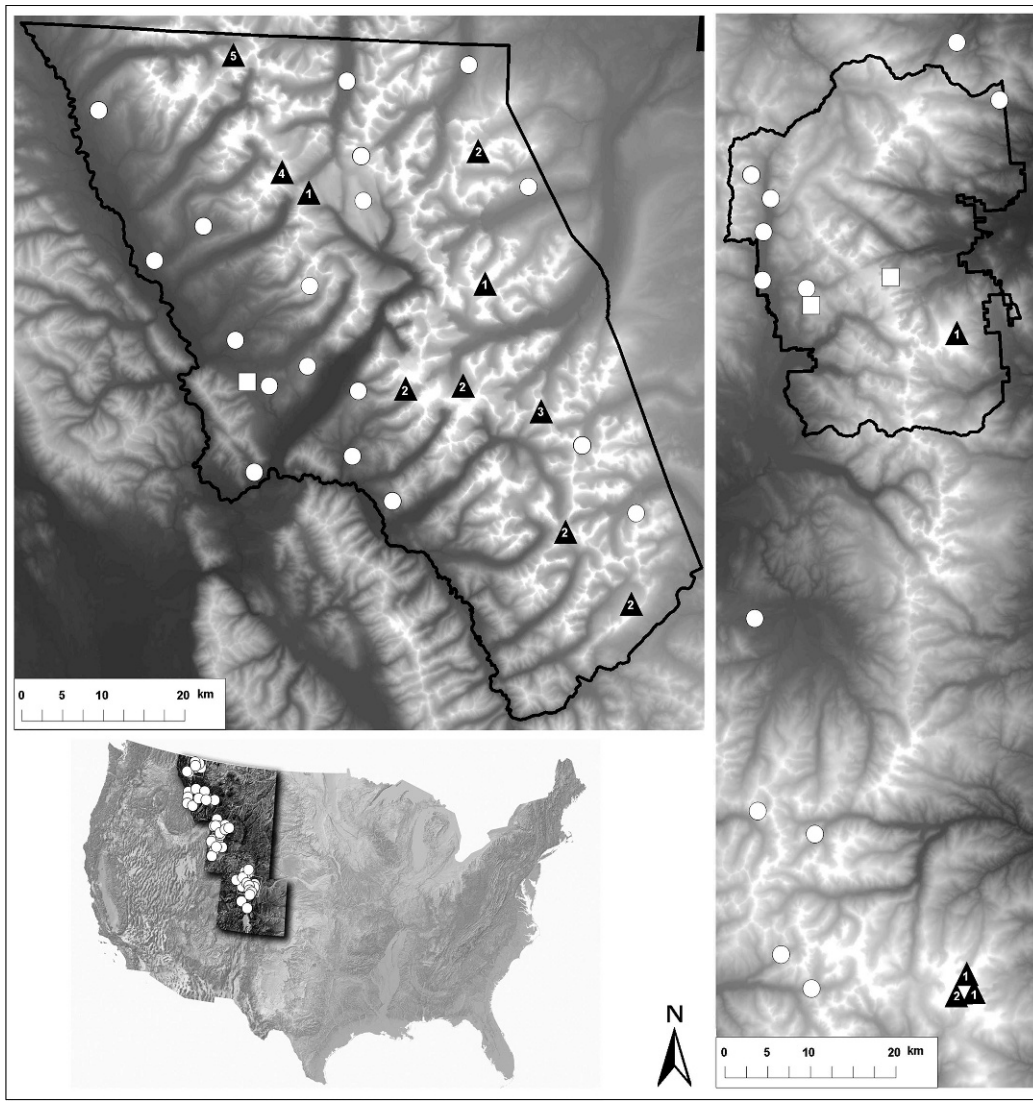

FIGURE 1. Elevation map of the areas sampled in Glacier National Park (left panel; map range: 894–3,192 m), Montana, and Colorado (right panel; map range: 2,194–4,351 m). Rocky Mountain National Park is outlined in Colorado. High-elevation areas are highlighted in white. Black triangles designate groups of water bodies that we sampled for *Batrachochytrium dendrobatidis* (*Bd*); inset numerals indicate the number of sites. White circles on maps of the study areas and the conterminous United States are a subset of the locations where *Bd* has been detected from amphibians in our study region (Muths et al., 2008; B.R.H. and P.S.C., unpubl. data). Each circle represents all amphibians sampled within a 1.5-km radius. White squares indicate where *Bd* was detected by sampling water in 2005 (Kirshtein et al., 2007).

lacking amphibians (Kirshtein et al., 2007).

We collected triplicate water samples from 25 high-elevation water bodies in Glacier National Park, Montana (48°46′52″N, 113°53′05″W), and five water bodies in northern Colorado (40°22′37″N, 105°31′38″W), including one in Rocky Mountain National Park (Fig. 1 and Table 1). Many of the sites in Glacier National Park were in high-elevation areas where the growing season is too short to sustain amphibians; the others were in cirques that are mostly shielded from the sun. The five sites in Colorado

TABLE 1. Mean (range) of values for water bodies sampled in Glacier National Park, Montana, and Colorado. Volume sampled is the average filtrate per filter. We disturbed the sediment prior to collecting the third sample from each water body, which greatly reduced the volume filtered. Specific conductance was not measured in Colorado.

Study area	Volume sampled (l)	Water-body size (ha)	Specific conductance ($\mu S\ cm^{-1}$)	Temperature (C)	Elevation (m)
Glacier National Park ($n=25$)	0.73 (0.06–1.00)	0.76 (0.01–10)	53 (<5–140)	13 (4–24)	2,120 (1,931–2,318)
Colorado ($n=5$)	0.60 (0.28–1.00)	12.24 (0.01–28)	NA	10 (6–15)	3,830 (3,766–4,017)

were above local elevation limits for amphibians (~3,660 m; Hammerson, 1999; E.M., pers. obs.). One site in Colorado contained stocked trout. All samples were collected in July and August 2007, when amphibian larvae (if present) would have been detected easily. There is an extensive survey history in both areas (Corn et al., 1997, 2005), and we have no reason to suspect that amphibians have occupied the sampled areas recently.

We sampled by filtering water in situ with a new 0.22-μm Sterivex™ filter and sterile 60-ml syringe at each of three shoreline points. Sampling materials were transported in sealed bags and handled with new gloves. Samples were collected from gently sloped areas in each water body because these would be more likely to collect organic material that could support saprobes. We stirred the sediment with a new chopstick before the third sample because we suspected that *Bd* may be more likely to be detected on sediment or other material than the clear water typical of these sites. Disturbing the sediment typically reduced the volume of filtrate by ≥50%. Sampling was terminated after 1 l or when the filters were nearly clogged (Table 1). We flushed excess dissolved organic carbon from the filters with 50 ml of phosphate-buffered saline (Kirshtein et al., 2007) and preserved filter contents with 0.9 ml of cell lysis solution.

After collecting water samples, we measured water temperature and conductivity (Glacier National Park only) using electronic meters. Size of water bodies was estimated visually for small unmapped sites or from a wetland or lake coverage using a geographic information system. Elevation of each water body was taken from a 7.5-minute topographic map.

We used the SYBR® quantitative polymerase chain reaction (qPCR) assay for detection of *Bd* from the filter media (Kirshtein et al., 2007). All samples were run in duplicate. We checked for PCR inhibition by measuring recovery of 100 copies of a plasmid-containing insert of a 146 base-pair (bp) *Bd* ITS-1 amplicon that was added to a third qPCR reaction for each extract, which also provided a positive control for every sample. Based on the recovery rate of zoospores from spiked pond water (Kirshtein et al., 2007) and the presence of organic matter and other PCR inhibitors, five to 10 zoospores/l would have been required for detection in our samples.

Detection efficiency of *Bd* with the sampling technique we used is uncertain because little is known about the temporal and spatial heterogeneity of the fungus in the environment. We used program FreeCalc2 (Cameron and Baldock, 1998) to estimate a detection probability for the water bodies. In 37 sampling events either reported by Kirshtein et al. (2007) or from other lentic sites in our region where amphibians have tested positive for *Bd* (C.W.A., unpubl.), 22 have resulted in at least one *Bd*-positive filter, and there was no relationship between detection of *Bd* and filtrate volume per filter or per site.

The conservative assumption that *Bd* was present during all sampling events yields a 59.5% detection rate. This value is similar to that from a study in Spain (62%) that used a similar method, where more turbid water bodies (with lower filtrate samples) yielded greater detections of *Bd* (Walker et al., 2007). Using a detection sensitivity of 59.5%, we had 95% confidence of detecting it in at least one water body if its prevalence was ≥16%.

We did not detect *Bd*. Our results support the hypothesis that the fungus does not exist independently of amphibians. Based on our data, however, we can only conclude that it is rare in water bodies lacking amphibians in the two areas we sampled. Several alternative explanations for not finding *Bd* are possible.

The sites we sampled may have been inhospitable to *Bd* regardless of amphibian absence. Muths et al. (2008) found reduced prevalence of *Bd* on amphibians at higher elevations in the Rocky Mountains, including sites near those in this study, and hypothesized that low maximum temperatures limit the distribution of the fungus. We would have preferred to sample lower-elevation wetlands that were warmer and more productive, but it is unlikely they are never used by amphibians. In work with a similar objective as ours, Rowley et al. (2007) sampled terrestrial retreat sites used by *Bd*-positive stream frogs (*Litoria* spp.) and did not detect *Bd*. The fungus can survive ≥12 wk on moist sand (Johnson and Speare, 2005), has been found on several strictly terrestrial species (Beard and O'Neill, 2005; Cummer et al., 2005; Kriger and Hero, 2007), and it does not require an amphibian host in the laboratory (Longcore et al., 1999); whether this means *Bd* can survive terrestrially or in other environments independently of amphibians remains unknown.

We may have simply failed to detect *Bd*, which may be distributed patchily in water bodies or which may be present in quantities below our detection limits. Kirshtein et al. (2007) did not detect *Bd* at all sample points in water bodies where it was found. Using a similar sampling technique, Walker et al. (2007) also did not detect *Bd* from some water bodies where chytridiomycosis was confirmed on resident amphibians. However, the opposite pattern has been found in the US Appalachian Mountains: *Bd* was detected from stream water samples even though extensive sampling of amphibians yielded no detections (S. Walls and W. Barichivich, US Geological Survey, pers. comm.). These results indicate that sampling of the environment is complementary to sampling amphibians and may provide unique information.

Our data suggest that *Bd* is rare in Rocky Mountain water bodies that lack amphibians. This result is not necessarily unexpected, but the distribution of *Bd* in the environment has only been evaluated in a few locations and has important implications for conservation, because whether or not *Bd* exists independently of amphibians is one of the most critical gaps in our knowledge of the epidemiology of chytridiomycosis (McCallum, 2005; Mitchell et al., 2008).

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