
The distribution of *Batrachochytrium dendrobatidis* across the southern Appalachian states, USA

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This study documents the occurrence of *Batrachochytrium dendrobatidis* (Bd) associated with the wood frog (*Lithobates sylvatica*) from the Cumberland Gap National Historical Park. This is the first report of Bd from the Park and subsequently from natural amphibian populations in the state of Kentucky. Based on the results of the current survey, a review of the literature, and records downloaded from the database maintained by BD-Maps.net, the following conclusions can be tentatively drawn: (a) the proportion of populations harboring Bd across southern Appalachian states appears to be relatively low, (b) mortality associated with Bd appears to be rare across the region, and (c) the highlands of the southern Appalachian mountains are notably under-sampled and understudied despite their conceivably high potential to contribute to our understanding of the factors predisposing populations to chytridiomycosis.

Key words – Chytridiomycosis – chytrid distribution – Cumberland Gap National Historical Park – frogs – salamanders

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Introduction

Batrachochytrium dendrobatidis (Bd) is a chytrid fungus capable of colonizing the epidermis of amphibians and can lead to a disease condition commonly referred to as chytridiomycosis (Berger et al. 1998). In some instances infected populations may experience high mortality rates. The origin, ecological affinities, mode of transmission, and the set of conditions by which the fungus becomes pathogenic are still rather poorly understood. However, some evidence suggests that other factors (e.g., anthropogenic effects, climate

change, and pollution) may weaken amphibians thus predisposing them to the progression to chytridiomycosis (Pounds & Crump 1994, Alford & Richards 1999). Given the uncertainty of the conditions contributing to an apparent global decline in amphibians, Bd has recently been the subject of a substantial research effort worldwide. One fundamental aspect in elucidating the ecology of Bd is to develop a better understanding of its distributional patterns. Furthermore, it is imperative to identify those host populations that develop chytridiomycosis and document

their associated historical, anthropogenic, biotic, and abiotic environmental variables. The objectives of the study reported herein were to (1) test for the presence of Bd in the Cumberland Gap area where the fungus had not yet been documented to occur and (2) determine the extent of previous sampling efforts as well as the documented occurrences of Bd across eight southern Appalachian states.

The Chytridiomycota represent a basal lineage of fungi that are characterized by producing flagella. Members of this group are common and sometimes abundant components of aquatic ecosystems where they play an important role in nutrient cycling through the decomposition of plant and animal detritus. However, in the case of Bd, this species is capable of living in association with the epidermis of amphibians where it can persist as either a parasite or a pathogen. Susceptibility to Bd varies among individuals within a species, and among populations, and in many instances the disease condition, chytridomycosis, does not develop (Davidson et al. 2003). As such, some individuals or populations can function as reservoirs for the chytrid. In addition, it has been reported that at least some reptiles can function as reservoirs and or vectors for Bd. For example, the presence of Bd in two lizard and three snake species were positively correlated with Bd occurrence among co-occurring anurans (Kilburn et al. 2011). Furthermore, simply the presence of Bd does not implicate the fungus as the cause of an amphibian decline or mortality event; as such, caution must be exercised in such an assessment (Daszak et al. 2005). The current investigation was initiated after the discovery of several dead wood frogs (*Lithobates sylvatica*) in a wetland within the Cumberland Gap National Historical Park, Kentucky, USA.

Methods

Field Sampling

During the 2009 field season four study areas were established in Tennessee and in 2010 an additional study area was established in the Cumberland Gap National Historical Park, Kentucky (Table 1). All specimens were captured using a scoop net, visually inspected for marks or lesions, and measured to

determine the total body length. Each individual was handled with a clean pair of disposable latex gloves in order to reduce the potential for cross contamination.

Samples for the isolation of DNA were collected in the manner described by Pisces Molecular. In short, a sterile cotton swab was run across the dorsum, ventrum, left side, right side and the foot webbing five times each for a total of 30 strokes. The swab was immediately placed in a labeled 2.0 ml screw-capped microcentrifuge tube containing 70% ethanol. The samples were then shipped to Pisces Molecular LLC for PCR analyses.

Sampling Distribution Map

Eight southern states (Alabama, Georgia, Kentucky, North Carolina, South Carolina, Tennessee, Virginia, and West Virginia) that intercept the Appalachian Mountain chain were selected as the area of interest. The results generated from the current study, a review of the literature, and database records downloaded from BD-Maps.net were used to construct a map elucidating the extent of Bd sampling among these southern Appalachian states. The GPS coordinates along with the host species sampled were recorded from these sources and divided into three classes (a) Bd not detected, (b) Bd detected – no mortality reported, and (c) Bd detected – mortality reported. A map was then generated using the freely available DIVA-GIS software package.

Results

Field Survey

Twenty-five specimens representing eleven species (eight anuran and three caudates) were obtained and swabbed from the Tennessee sites and all of these specimens tested negative for Bd (Table 2). Seventeen wood frog specimens were obtained from the Kentucky site. PCR analysis indicated that 33% of these samples were positive for Bd. Interestingly, when these specimens were divided into small (<5cm) and large (>5cm) size classes, none of the specimens from the small size class were positive for Bd, but 66% of those from the large size class were positive for Bd.

Table 1 General information pertaining to the study areas sampled.

Site	Coordinates	Elevation (m)	State	County
S-1	36.2835 -83.8333	457	TN	Union
S-2	36.2469 -84.0993	314	TN	Campbell
S-3	36.4413 -83.5314	441	TN	Claiborne
S-4	36.5801 -83.6661	430	TN	Claiborne
CUGA-1	36.6022 -83.6922	349	KY	Bell

Distribution

Collectively the information gathered from the current survey, the review of the literature, and the records obtained from the BD-Maps database resulted in the identification of 150 sites within the eight states that have been surveyed for the presence of Bd (Fig. 1). Seventy-three percent of the sampled localities tested negative for Bd, whereas 24% tested positive with no reports of mortality and the remaining 3% tested positive with reports of associated mortality. Overall, ten anuran and ten caudate species have been reported to harbor Bd from these eight states (Table 3). Mortality associated with populations harboring Bd in this region has been reported from only four species: wood frog, eastern newt (*Notophthalmus viridescens*), chorus frog (*Pseudacris* sp.), and an unidentified species of *Lithobates*; however, it is important to note that this does not necessarily implicate Bd as the causative agent.

Discussion

The current report of Bd associated with wood frogs from the Cumberland Gap National Historical Park is a new distributional record for this chytrid and to the best knowledge of the authors represents the first report from a natural amphibian population for Kentucky. Although mortality has been associated with this population, an attempt to determine if Bd was the causative agent was not undertaken as part of the current project.

The Appalachian Mountains particularly the highlands of Kentucky, North Carolina, Tennessee, and Virginia are seemingly under-sampled and relatively poorly known with respect to Bd. Other studies, particularly those conducted in tropical regions (Puschendorf et al. 2006) and in the western United States (Muths et al. 2003) seem to suggest an increased potential for the development of chytridiomycosis in association with cool microenvironmental conditions. It is

Table 2 Summary of species collected during the 2009 and 2010 field surveys. Note “-” indicates negative for Bd, “+” indicates positive for Bd, and a blank space indicates that the species was not present from the site.

Species	Common Name	TN	KY
Anurans			
<i>Anaxyrus americanus</i>	American Toad	-	
<i>Anaxyrus fowleri</i>	Fowler's Toad	-	
<i>Hyla versicolor</i>	Gray Tree Frog	-	
<i>Lithobates catesbeiana</i>	American Bullfrog	-	
<i>Lithobates clamitans</i>	Green Frog	-	
<i>Lithobates palustris</i>	Pickerel Frog	-	
<i>Lithobates sylvatica</i>	Wood Frog	-	+
<i>Scaphiopus holbrookii</i>	Eastern Spadefoot Toad	-	
Caudates			
<i>Eurycea bislineata</i>	Northern Two-Lined Salamander	-	
<i>Eurycea longicauda</i>	Long-Tailed Salamander	-	
<i>Eurycea lucifuga</i>	Spotted-Tail Salamander	-	

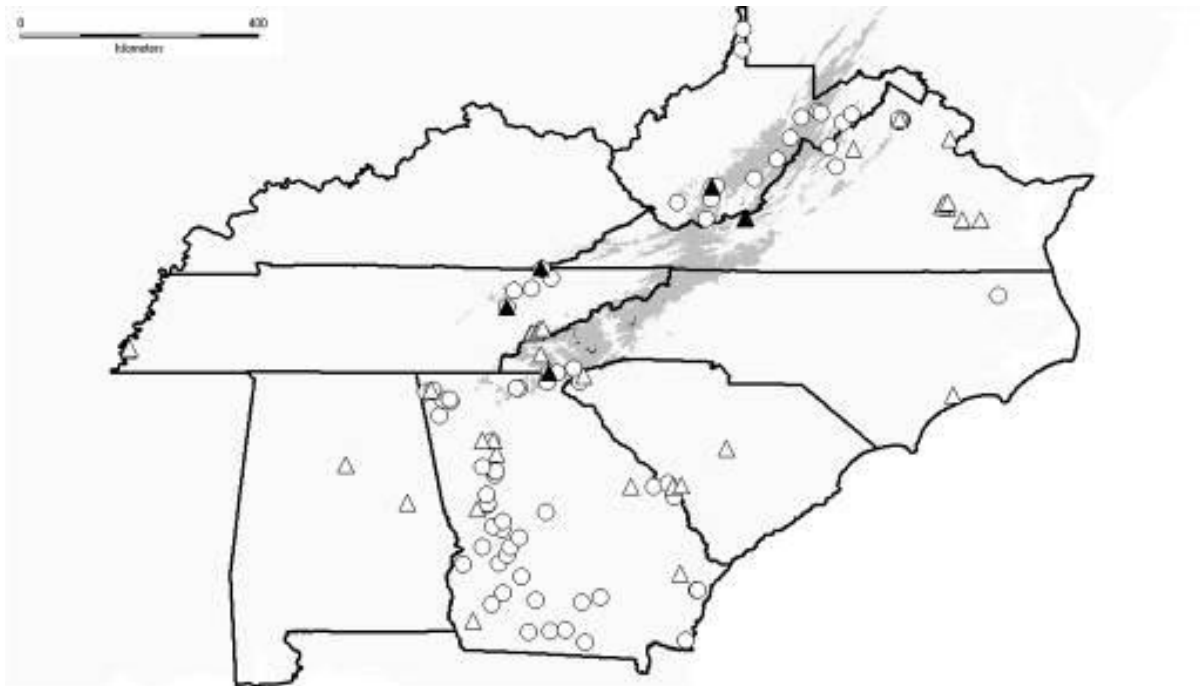


Fig.1 – The distribution of *Batrachochytrium dendrobatidis* surveys across the southern Appalachian states (USA) based on a review of the literature, BD-Maps.net, and the current survey. Open circles indicate a negative test for Bd, open triangles indicate positive result for Bd and no mortality reported, and shaded triangles indicates positive result for Bd and mortality reported.

Table 3 Summary of the species reported to host *Batrachochytrium dendrobatidis* in the southern Appalachian states (USA) based on literature, BD-Maps.net, and the current survey. Note “+” indicates a positive test of occurrence and “M” indicates a positive test of Bd and reports of associated mortality.

Species	Common Name	AL	GA	KY	NC	SC	TN	VA	WV
Anurans									
<i>Acris crepitans</i>	Northern Cricket Frog							+	
<i>Anaxyrus</i> sp.	Toad						+		
<i>Lithobates catesbeiana</i>	American Bullfrog		+		+	+		+	
<i>Lithobates clamitans</i>	Green Frog	+	+					+	
<i>Lithobates heckscheri</i>	River Frog		+						
<i>Lithobates palustris</i>	Pickerel Frog		+			+	+		
<i>Lithobates</i> sp.			M						
<i>Lithobates sphenoccephala</i>	Southern Leopard Frog		+		+	+	+	+	
<i>Lithobates sylvatica</i>	Wood Frog			M			+		
<i>Pseudacris</i> sp.	Chorus Frogs						M		
Caudates									
<i>Desmognathus conanti</i>	Spotty Dusky Salamander		+						
<i>Desmognathus monticola</i>	Seal Salamander							+	
<i>Desmognathus quadramaculatus</i>	Black-Bellied Salamander								+
<i>Eurycea bislineata</i>	Two-Lined Salamander							+	
<i>Eurycea cirrigera</i>	Southern Two-Lined Salamander	+							
<i>Eurycea guttolineata</i>	Three-Lined Salamander		+						
<i>Eurycea</i> sp.	Lungless Salamander	+							
<i>Notophthalmus viridescens</i>	Eastern Newt	+	+		+		+	M	
<i>Plethodon cinereus</i>	Red Back Salamander							+	
<i>Pseudotriton ruber</i>	Red Salamander					+			

interesting to note that the five localities in the southern Appalachian states that have reported mortality events are located within or near mountainous areas (Fig. 1). As such, the upland areas of the central and southern Appalachians would seem to represent an ideal opportunity to advance our understanding with respect to the ecology of Bd. It would be particularly interesting to undertake an ecological survey that critically evaluates the occurrence of Bd and associated biotic and abiotic environmental parameters along a series of elevational transects.

Based on the available data, it appears that Bd occurrence in association with amphibians across this region is relatively low and accompanying mortality events are rare. However, this may simply represent an artifact of sampling efforts to date. For example, the highlands of West Virginia appear to be fairly well sampled (Fig. 1), but these localities are the result of a single study that only considered salamanders (Bartkus 2009). As such, a more widespread sampling effort across the region that encompasses several species of amphibians and reptiles needs to be undertaken in order to gain a clearer picture with respect to the ecology and distribution of Bd.

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