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# Batrachochytrium dendrobatidis Detected in Amphibians from National Forests in Eastern Texas, USA

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# *Batrachochytrium dendrobatidis* Detected in Amphibians from National Forests in Eastern Texas, USA

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The amphibian disease chytridiomycosis, caused by the pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*, Longcore et al. 1999), is well known as a major threat to amphibians resulting in mass die-offs and population declines throughout the world (Berger et al. 1998; Blaustein and Keisecker 2002; Daszak et al. 2003; McCallum 2005; Rachowicz et al. 2006). *Batrachochytrium dendrobatidis* has been detected on amphibians from sites across North America (Ouellet et al. 2005; Woodhams et al. 2008) and appears to be most prevalent in the western and the northeastern United States (Longcore et al. 2007; Schlaepfer et al. 2007). Whereas infected anurans also have been found throughout the southeastern US (Green and Dodd 2007), there have been no reports of *Bd* from amphibians in eastern Texas, a broad area encompassing 10,000,000 ha. We sampled amphibians for the presence of *Bd* in four National Forests in eastern Texas (approximately 31°N latitude).

Amphibians were sampled for *Bd* from 9 January to 27 May 2009 in the Angelina, Davy Crockett, and Sabine National Forests, and the Stephen F. Austin Experimental Forest (Fig. 1). The Stephen

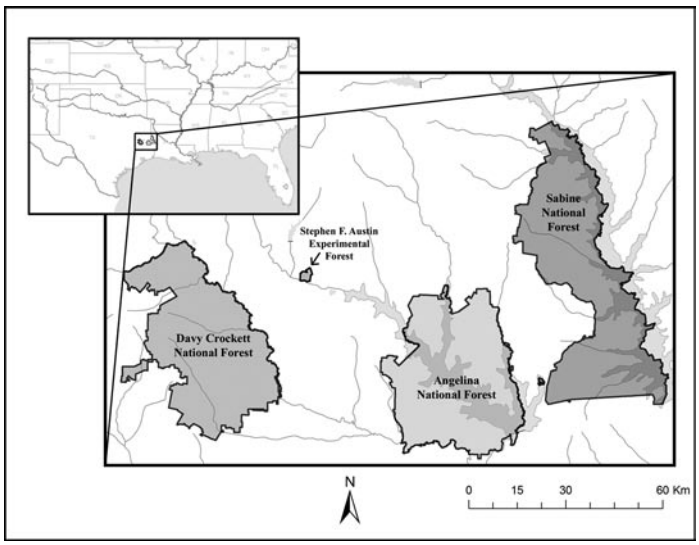


Fig. 1. Locations of the Angelina, Davy Crockett, Sabine National Forests, and the Stephen F. Austin Experimental Forest where six of 18 amphibian species tested positive for the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*.

TABLE 1. Amphibian species tested for the presence of *Batrachochytrium dendrobatidis* (*Bd*) within Angelina, Davy Crockett, and Sabine National Forests, and the Stephen F. Austin Experimental Forest, Texas. Bold font indicates that *Bd* was detected in the species.

| | | No. animals infected/examined | No. animals infected/examined | No. animals infected/examined | No. animals infected/examined |
|---|---|---|---|---|---|
| Family | Species | Angelina National Forest | Davy Crockett National Forest | Sabine National Forest | Stephen F. Austin Experimental Forest |
| Ambystomatidae | *Ambystoma maculatum* | 0/5 | - | - | - |
| | *Ambystoma opacum* | 0/10 | 0/2 | 0/5 | 0/10 |
| | *Ambystoma talpoideum* | 0/2 | - | - | - |
| Plethodontidae | ***Eurycea quadradigitata*** | 0/8 | - | 0/8 | **3/10** |
| Salamandridae | *Notophthalmus viridescens* | 0/1 | - | - | 0/2 |
| Bufonidae | ***Anaxyrus woodhousii*** | **1/7** | 0/10 | **-** | 0/1 |
| | *Incilius nebulifer* | 0/3 | 0/8 | 0/1 | - |
| Hylidae | ***Acris crepitans*** | **1/10** | **1/10** | **-** | **-** |
| | *Hyla cinerea* | 0/10 | 0/6 | **-** | 0/10 |
| | *Hyla versicolor* | 0/8 | 0/10 | **-** | 0/8 |
| | ***Pseudacris crucifer*** | 0/10 | **1/10** | **-** | 0/10 |
| | ***Pseudacris fouquettei*** | **3/5** | **-** | **-** | **2/10** |
| Microhylidae | *Gastrophryne carolinensis* | 0/5 | - | - | 0/2 |
| Ranidae | ***Lithobates catesbeianus*** | 0/1 | 0/6 | - | **1/1** |
| | *Lithobates clamitans* | 0/2 | 0/4 | - | 0/2 |
| | *Lithobates palustris* | - | - | 0/1 | 0/4 |
| | *Lithobates sphenocephalus* | 0/8 | 0/7 | - | 0/10 |
| Scaphiopodidae | *Scaphiopus hurteri* | - | 0/1 | - | - |
| | **TOTAL** | **5/95 (5.3%)** | **2/76 (2.6%)** | **0/15 (0.0%)** | **6/80 (7.5%)** |

F. Austin Experimental Forest is a disjunct unit of the Angelina National Forest, administered by the Southern Research Station, US Forest Service. The dominant habitats of these areas include secondary growth Loblolly (*Pinus taeda*), Longleaf (*P. palustris*), and Shortleaf (*P. echinata*) upland pine forests and mixed deciduous bottomland forests. East Texas experiences occasional freezing temperatures, warm winter days, and extremely hot summers (Chang et al. 1996). From 1901 to 1993, the overall mean air temperatures for January and August are 8.4°C and 27.8°C, respectively (Chang et al. 1996). In winter, cold air masses often meet warm moist air pushed up from the Gulf of Mexico resulting in frequent rain events, placing the study sites in one of the wettest regions of Texas (Bomar 1995).

We searched for amphibians near ponds, streams, moist lowland areas, and upland pine forest habitat. We captured specimens by hand. Each individual was handled with a new pair of sterile nitrile gloves. We sampled for *Bd* by rubbing a sterile cotton swab on the dorsum, ventral surfaces, and feet of each frog for approximately 30 seconds, after which the animal was released at its place of capture. The swab was then immediately placed in a sterile microcentrifuge tube containing 1 ml of 70% ethanol and later sent to Pisces Molecular Laboratory (Boulder, Colorado, USA) for PCR analyses. Global positioning system (GPS) coordinates were taken at each capture site using a Garmin® GPS unit.

Overall, we sampled a total of 266 adult amphibians of 18 different species, from 8 different families (Table 1). Of these 18 species, six had at least one individual that tested positive for *Bd*. Thirteen of the 266 individuals tested positive for an overall detection rate of 4.8%. The Stephen F. Austin Experimental Forest had the highest detection rate among the four areas sampled, with six of 80 (7.5%) individuals testing positive for *Bd* (Table 1). During sampling, no sick or dead frogs were observed at any sites.

We found similar *Bd* detection rates for anurans and caudates (4.93% and 4.76%, respectively), although the only salamanders to test positive for *Bd* were three individuals of *Eurycea qudradigitata* from the Stephen F. Austin Experimental Forest. Despite 34 Ambystomatids being sampled, none tested positive for *Bd*.

Five of the 15 (33.33%) *Pseudacris fouquettei* samples tested positive for *Bd*, which was the highest detection rate among anuran species, including species which were breeding at the same time and place where the *Bd*-positive *Pseudacris fouquettei* were found. Individuals of both *P. crucifer* and *Lithobates sphenocephalus* were collected at locations where *Bd*-positive *P. fouquettei* were found, yet no *L. sphenocephalus* and only one of 30 (3.33%) *P. crucifer* samples tested positive for *Bd*. However, small sample sizes make it difficult to assess whether there is actually a higher detection rates among *P. fouquettei* than other species.

*Batrachochytrium dendrobatidis* has been present in North American amphibian populations since at least the early 1960s (Ouellet et al. 2005). Yet, within the United States, *Bd* has been associated with amphibian die-offs predominantly in western states (e.g., Bradley et al. 2002; Briggs et al. 2005; Muths et al. 2003).

Although the fungus is present in the southeastern United States, to our knowledge no amphibian declines have been attributed to chytridiomycosis in this region. Several studies have detected higher rates of *Bd* infections during the fall or winter months (McDonald et al. 2005; Ouellet et al. 2005; Retallick et al. 2004), a phenomenon possibly explained by the fact that amphibian immune systems function less effectively at cooler temperatures (Carey 2000; Cooper et al. 1992; Maniero and Carey 1997). It is possible that the immune systems of amphibians inhabiting eastern Texas are more capable of resisting *Bd* infections because of the relatively warm winters and hot summers.

Also, it is possible that the fungus may never reach an epizootic state in eastern Texas because the warm air temperatures are less than optimal for *Bd* growth and infection of amphibians (Kriger and Hero 2006; Longcore et al. 1999; Retallick et al. 2004). Evidence for an enzootic state includes the relative low incidence of detection in PCR samples and the fact that no sick or dead frogs were encountered. Amphibians appeared to be abundant at all four of our study sites. Follow-up population and *Bd* sampling is needed to confirm what impacts *Bd* may be having on the National Forests in Texas.

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