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Prevalence of the Amphibian Chytrid Fungus among Zoo and Pet Store Collections in the Northeastern United States

Recent studies suggest that the amphibian trade has promoted the global transmission of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*) (Fisher and Garner 2007; Goka et al. 2009; James et al. 2009; Farrer et al. 2011), which is a pathogen implicated as a cause of global amphibian declines (Berger et al. 1999; Daszak et al. 1999; Skerratt et al. 2007; Kilpatrick et al. 2010). In particular, pet shops and zoos constantly acquire and exchange animals depending on their needs and exhibitions, making amphibian collections vulnerable to harboring and transmitting *Bd*. For instance, one study determined 28% of amphibians sold in a number of Japanese pet stores were infected with the fungus (Goka et al. 2009) and several studies reported *Bd*-positive samples among zoo amphibians (Longcore et al. 1999; Miller et al. 2008; Jones et al. 2012; Churgin et al. 2013; Goeroff et al. 2013). Despite the role that pet stores and zoos may play in propagating the disease agent, there is a paucity of studies investigating such possibilities in the United States. Thus, the purpose of this study is to enhance the current knowledge about *Bd* infection among traded amphibians in the United States by examining the prevalence of *Bd* in a variety of pet store and zoo amphibian collections.

A large number of pet stores are widely distributed throughout the United States that internationally and domestically import and sell amphibians. For example, Petco Inc. and Petsmart Inc. are the two largest pet store chains, each of which owns over 1000 stores throughout the United States (Petco: available from http://www.petco.com/ [Accessed 19 August 2013]: Petsmart: available from http://www.petsmart.com/ [Accessed 19 August 2013]). These numbers translate into the distribution of on average 40 or more pet stores per state between these two companies alone. It is likely that some pet owners have disposed of dead or alive purchased amphibians into wild habitats, making it possible that alien *Bd* strains carried by pet amphibians (e.g., Goka et al. 2009) can be transmitted to native amphibian populations. Zoos also may serve as a pathway for bringing *Bd* into amphibian collections and possibly into the local amphibian community, as they receive amphibians from a variety of sources. However, many zoos and aquariums have an Association of Zoos and Aquariums (AZA) accreditation, which sets standard procedures for the acquisition and husbandry of animals which should counter the risk of spreading disease (Poole and Grow 2012). These standards should decrease the likelihood of both presence and transmission of *Bd*.

In November 2012, we collected swab samples of 20 adults of four amphibian species from five different pet stores throughout Pennsylvania (three stores) and Massachusetts (two stores). The pet stores from which we sampled animals included several privately owned and recognized chain stores. From pet stores, we sampled 14 *Bombina orientalis* (Oriental Fire-belied Toads, native to the northeastern Asia), two *Lithobates catesbeianus* (American Bullfrogs, native to the eastern USA), three *Hyla cinerea* (Green Treefrogs, native to the southeastern USA), and one *Ceratophrys ornata* (Bell's Horned Frog, native to South America). Additionally, 30 adult amphibians of nine species were tested for *Bd* from a zoo located in Pennsylvania with AZA accreditation in September 2012. From this zoo we sampled three *L. catesbeianus*, three *Pyxicephalus adspersus* (African Bullfrogs, native to the southern Africa), three *Phyllobates terribilis* (Golden Poison Frogs, native to South America), four *Dendrobates tinctorius* (Dyeing Poison Frogs, native to South

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TABLE 1. Prevalence of *Bd* infection for each species in zoo and pet store amphibian collections.

Zoo (Z) or Pet store (P)	Species	No. infected / No. swabbed
P	*Bombina orientalis*	0/14
P	*Lithobates catesbeianus*	0/2
P	*Hyla cinerea*	1/3
P	*Ceratophrys ornata*	0/1
		Total (P): 1/20 = 5%
Z	*Lithobates catesbeianus*	3/3
Z	*Pyxicephalus adspersus*	0/3
Z	*Phyllobates terribilis*	0/3
Z	*Dendrobates tinctorius*	0/4
Z	*Rhinella marina*	0/1
Z	*Ambystoma mexicanum*	0/1
Z	*Trachycephalus resinifictrix*	0/6
Z	*Phyllomedusa bicolor*	0/2
Z	*Polypedates otilophus*	0/7
		Total (Z): 3/30 = 10%
		Total infected: 4/50=8%

America), one *Rhinella marina* (Cane Toad, native to Central and South America), one *Ambystoma mexicanum* (Axolotl, native to Mexico), six *Trachycephalus resinifictrix* (Boatman Frogs, native to South America), two *Phyllomedusa bicolor* (Waxy-Monkey Tree Frogs, native to South America), and seven *Polypedates otilophus* (File-eared Tree Frogs, native to the southeastern Asia). In total, 50 adult individuals of 13 amphibian species were analyzed.

Each individual was swabbed five times on the ventral side of each limb and abdominal surface using a cotton-tipped swab. In order to avoid cross contamination, nitrile gloves, together with cotton swabs, were replaced between each specimen. Skin swabs were stored dry in a 2.0-mL screw cap vial and kept in a -20°C freezer until DNA extraction. DNA was extracted from swabs using PrepMan Ultra (Life Technologies, Grand Island, New York, United States) and 1:10 diluted DNA samples for the subsequent PCR assay were prepared. The PCR primers and cycle were determined based on Annis et al. (2004), consisting of an initial denaturation (94°C for 5 min), followed by 30 cycles of replication and final extension (72°C for 10 min). Each cycle consisted of denaturation (30°C for 45 s), annealing (55°C for 30 s), and extension (72°C for 1 min). PCR products were ran on ethidium bromide stained agarose gels using electrophoresis and visualized under UV light. These products included both a positive control from a known *Bd* source and a negative control containing sterile distilled water. Conventional PCR combined with agarose gel electrophoresis was used instead of real-time PCR, as a recent study recommended this technique for studies that require only prevalence data (Garland et al. 2011). PCR and gel electrophoresis were repeated three times for all positive samples to prevent any false negative or positive results.

Four of 50 (8%) amphibians tested positive for the presence of *Bd* (Table 1). Three *L. catesbeianus* from the zoo were *Bd* positive, and one *H. cinerea* from a national chain pet store in Pennsylvania was *Bd* positive. These samples consistently showed positive results in the three replicates of the PCR assays.

Whereas only 8% of individuals and 2 of 13 (15%) species were infected with *Bd*, this could have profound effects on *Bd* transmission given the scale of the amphibian trade. The infection that we found in *H. cinerea* sold at the chain pet store poses a potentially devastating risk, because the species is native to the central and southeastern United States and is a commonly sold pet amphibian in the United States and around the world. For instance, between 1998 and 2002 at least 87,536 Kg of *H. cinerea* were exported from the United States as pets (Schlaepfer et al. 2005). Given the high *Bd* infection rate detected among traded amphibians (Goka et al. 2009; Schloegel et al. 2009), the large vendors and breeding centers responsible for rearing and trading these amphibians, as well as the pet stores themselves, likely have lax regulations and protocols in place to prevent the spread of *Bd*. Additionally, some state regulators have not passed specific laws protecting harvested or imported amphibians, particularly concerning amphibians infected with *Bd* (Wilson 2005). Thus, our results predict that the number and distribution of infected pet amphibians in the United States could be substantial.

The instances of infection in the three *L. catesbeianus* were not surprising, as this species is a globally well-known vector of *Bd* (Garner et al. 2006; Schloegel et al. 2010). Some zoos acquire their amphibians from a variety of sources, including many of the centers responsible for breeding amphibians for the pet trade. The zoo staff revealed that the *L. catesbeianus* individuals examined in our study were obtained from a commercial bullfrog farm (L. Brennan, pers. comm.). Acquiring amphibians from such sources comes with the risk of bringing *Bd* into the zoo collection. Despite no clinical signs of *Bd* infection shown by the *L. catesbeianus*, however, the zoo managed their amphibian collection properly and prevented *Bd* from spreading from those *L. catesbeianus*, which all were kept in the same container. The AZA Amphibian Husbandry Resource Guide details preventative and treatment procedures against *Bd* (Poole and Grow 2012). Although we examined only one zoo, our case exemplifies a quality husbandry standard followed by an AZA accredited institution. Meanwhile, it is important to note that if zoos maintain collections that contain *Bd* as detected in our study, they run the risk of counteracting any sorts of conservation breeding efforts (De Paula et al. 2012).

A study like the present one is important as it disseminates critical information about *Bd* prevalence among zoo and pet store amphibians. Our results suggest that stricter protocols and routine *Bd* screens are warranted to protect traded and captive amphibians from disease infection and transmission. A similar type of protocol was implemented when *Salmonella* infection in humans became prominent due to lenient regulations protecting reptiles in the pet trade, which dramatically decreased the number of reported *Salmonella* cases associated with handling reptiles (de Jong et al. 2005). Continued monitoring on the prevalence of *Bd* among traded amphibians at a larger scale is essential to promote amphibian conservation.

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Literature Cited

Annis, S. L., F. P. Dastoor, H. Ziel, P. Daszak, and J. E. Longcore. 2004. A DNA-based assay identifies *Batrachochytrium dendrobatidis* in amphibians. J. Wildl. Dis. 40:420–428.

Berger, L., R. Speare, and A. Hyatt. 1999. Chytrid fungi and amphibian declines: overview, implications and future directions. *In* A. Campbell (ed.), Declines and Disappearances of Australian Frogs, pp. 23–33. Environment Australia, Commonwealth of Australia.

Churgin, S. M., B. L. Raphael, J. B. Pramuk, J. G. Trupkiewicz, and G. West. 2013. *Batrachocytrium dendrobatidis* in aquatic caecilians (*Typhlonectes natans*): a series of cases from two institutions. J. Zoo Wildl. Med. 44:1002–1009.

Daszak, P., L. Berger, A. A. Cunningham, A. D. Hyatt, D. E. Green, and R. Speare. 1999. Emerging infectious diseases and amphibian population declines. Emerg. Infect. Dis. 5:735–748.

de Jong, B., Y. Andersson, and K. Ekdahl. 2005. Effect of regulation and education on reptile-associated salmonellosis. Emerg. Infect. Dis. 11:398–403.

De Paula, C. D., E. C. Pacífico-Assis, and J. L. Catão-Dias. 2012. *Batrachochytrium dendrobatidis* in amphibians confiscated from illegal wildlife trade and used in an ex situ breeding program in Brazil. Dis. Aquat. Org. 98:171–175.

Farrer, R. A., L. A. Weinert, J. Bielby, T. W. J. Garner, F. Balloux, F. Clare, J. Bosch, A. A. Cunningham, C. Weldon, L. H. Du Preez, L. Anderson, S. L. K. Pond, R. Shahar-Golan, D. A. Henk, and M. C. Fisher. 2011. Multiple emergences of genetically diverse amphibian-infecting chytrids include a globalized hypervirulent recombinant lineage. Proc. Nat. Acad. Sci. 108:18732–18736.

Fisher, M. C., and T. W. J. Garner. 2007. The relationship between the emergence of *Batrachochytrium dendrobatidis*, the international trade in amphibians and introduced amphibian species. Fungal Biol. Rev. 21:2–9.

Garland, S., J. Wood, and L. F. Skerratt. 2011. Comparison of sensitivity between real-time detection of a TaqMan assay for *Batrachochytrium dendrobatidis* and conventional detection. Dis. Aquat. Org. 94:101–105.

Garner, T. W. J., M. W. Perkins, P. Govindarajulu, D. Seglie, S. Walker, A. A. Cunningham, and M. C. Fisher. 2006. The emerging amphibian pathogen *Batrachochytrium dendrobatidis* globally infects introduced populations of the North American bullfrog, *Rana catesbeiana*. Biol. Lett. 2:455–459.

Georoff, T. A., R. P. Moore, C. Rodriguez, A. P. Pessier, A. L. Newton, D. McAloose, and P. P. Calle. 2013. Efficacy of treatment and long-term follow-up of *Batrachochytrium dendrobatidis* PCR-positive anurans following itraconazole bath treatment. J. Zoo Wildl. Med. 44:395–403.

Goka, K., J. Yokoyama, Y. Une, T. Kuroki, K. Suzuki, M. Nakahara, A. Kobayashi, S. Inaba, T. Mizutani, and A. D. Hyatt. 2009. Amphibian chytridiomycosis in Japan: distribution, haplotypes and possible route of entry into Japan. Mol. Ecol. 18:4757–4774.

James, T. Y., A. P. Litvintseva, R. Vilgalys, J. A. T. Morgan, J. W. Taylor, M. C. Fisher, L. Berger, C. Weldon, L. du Preez, and J. E. Longcore. 2009. Rapid global expansion of the fungal disease chytridiomycosis into declining and healthy amphibian populations. PLoS Pathogens 5:e1000458.

Jones, M. E. B., D. Paddock, L. Bender, J. L. Allen, M. D. Schrenzel, and A. P. Pessier. 2012. Treatment of chytridiomycosis with reduced-dose itraconazole. Dis. Aquat. Org. 99:243–249.

Kilpatrick, A. M., C. J. Briggs, and P. Daszak. 2010. The ecology and impact of chytridiomycosis: an emerging disease of amphibians. Trends Ecol. Evol. 25:109–118.

Longcore, J. E., A. P. Pessier, and D. K. Nichols. 1999. *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. Mycol. Soc. Amer. 91:219–227.

Miller, D. L., S. Rajeev, M. Brookins, J. Cook, L. Whittington, and C. A. Baldwin. 2008. Concurrent infection with ranavirus, *Batrachochytrium dendrobatidis*, and *Aeromonas* in a captive anuran colony. J. Zoo Wildl. Med. 39:445–449.

Poole, V. A., and S. Grow (eds.). 2012. Amphibian Husbandry Resource Guide. 2.0 edition. Association of Zoos and Aquariums, Silver Spring, Maryland. 238 pp.

Schlaepfer, M. A., C. Hoover, and C. K. Dodd, Jr. 2005. Challenges in evaluating the impact of the trade in amphibians and reptiles on wild populations. BioScience 55:256–264.

Schloegel, L. M., C. M. Ferreira, T. Y. James, M. Hipolito, J. E. Longcore, A. D. Hyatt, M. Yabsley, A. M. C. R. P. F. Martins, R. Mazzoni, A. J. Davies, and P. Daszak. 2010. The North American bullfrog as a reservoir for the spread of *Batrachochytrium dendrobatidis* in Brazil. Animal Conserv. 13:53–61.

———, A. M. Picco, A. M. Kilpatrick, A. J. Davies, A. D. Hyatt, and P. Daszak. 2009. Magnitude of the US trade in amphibians and presence of *Batrachochytrium dendrobatidis* and ranavirus infection in imported North American bullfrogs (*Rana catesbeiana*). Biol. Conserv. 142:1420–1426.

Skerratt, L. F., L. Berger, R. Speare, S. Cashins, K. R. McDonald, A. D. Phillott, H. B. Hines, and N. Kenyon. 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth 4:125–134.

Wilson, A. B. 2005. Commercial trade. *In* M. J. Lannoo (ed.), Amphibian Declines: the Conservation Status of United States Species, pp. 146–148. University of California Press, Berkeley, California.