

AMPHIBIAN DISEASES

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Northern Leopard Frogs (*Rana pipiens*) Infected with *Batrachochytrium dendrobatidis* in the Amphibian Trade

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Batrachochytrium dendrobatidis (*Bd*) is an aquatic fungus that infects amphibians with the disease chytridiomycosis, a disease that has been implicated as one of the causes of worldwide amphibian decline (Berger et al. 1998; Bosch et al. 2001; Rachowicz et al. 2006; Skerratt et al. 2007; Stuart et al. 2004). Although the manner in which *Bd* is spread is not fully understood, the amphibian trade has been implicated as a possible source of dissemination (Fisher and Garner 2007; Parker et al. 2002; Picco and Collins 2008; Rowley et al. 2007; Schloegel et al. 2009). Amphibian trade results in transport of large numbers of individuals and species nationally and internationally; with >7,000,000 amphibians imported into the United States between 1998 and 2002 (Schlaepher et al. 2005), and >4,000,000 amphibians imported to Hong Kong, China, in a single year via air transport alone (Rowley et al. 2007). Many US imports are subsequently exported to European and Asian markets (Schlaepher et al. 2005). The United States is one of the few countries to track import and export, but no regulations require screening for pathogens such as *Bd*. In 2008, the World Organization of Animal Health (OIE) listed *Bd* as OIE-notifiable, and developed standards for amphibian trade (http://www.oie.int/eng/normes/icode/en_sommaire.htm; accessed 5 April 2010).

Bd has been isolated from Northern Leopard Frogs, *Rana pipiens*, from two biological supply companies in Massachusetts and Minnesota, USA (Woodhams et al. 2008). This and other research has suggested that *Rana pipiens* shipped from biological-supply houses may be a carrier for *Bd* (Fischer and Garner 2007; Woodhams et al. 2008).

Charles D. Sullivan Inc. (CDS) is a major biological supply company based in Tennessee, USA, specializing in amphibians for educational and research purposes. Charles D. Sullivan Inc. ships its products, both nationally and internationally, to over 100 institutions (as claimed on their web site: <http://www.researchamphibians.com>; accessed 26 April 2010). There have been hundreds of primary research articles based on specimens provided by CDS. To determine if frogs shipped from CDS had *Bd* we tested shipments of *R. pipiens* that were ordered for research purposes.

Shipments of 14 and 25 adult *R. pipiens* were shipped overnight from CDS to Central Washington University, Ellensburg, Washington, USA, on 18 October 2006 and 12 July 2007, respectively. Both shipments were immediately unpacked and transferred into clean and unused plastic containers, with each individual housed separately. Frogs were held at 10 or 15°C and the container cleaned twice weekly. Powder-free latex gloves were worn while handling frogs, with a new pair used for each frog. To determine infection status small pieces of shed skin were collected opportunistically from the enclosures of each of the frogs within 30 days of arrival and examined using differential interference contrast microscopy for the presence of *Bd* zoospores and zoospores. Samples were examined for no more than 10 min. If no *Bd* structures were found within 10 min, the sample was considered negative for the pathogen.

Bd was found in the shed skin of all *R. pipiens* (N = 39) sampled. Upon arrival at the laboratory, several frogs exhibited skin redness on the ventral surface of the abdomen and thighs, a common symptom in infected individuals (Pessier et al. 1999). These signs resolved a few days after the frogs were transferred into their enclosures. However, many frogs displayed signs of *Bd* infection intermittently throughout the 66- or 109-day experiments (Table 1), including redness on the ventral surface, lethargy, and excessive skin sloughing. There was no consistent pattern in the appearance and disappearance of the signs of *Bd* infection throughout the experiments. Six (15%) *R. pipiens* died during the experiments (Table 1).

These results indicate amphibians shipped from CDS may be infected with *Bd*. Given that the frogs were shipped overnight and assessed for *Bd* shortly after arrival, we consider it unlikely that they were infected during transport. The high infection rate more likely reflects holding conditions before shipping or natural infection rates. The relatively low mortality rates and high rates of infection in our experiments are consistent with previous research (Padgett-Flohr 2008; Woodhams et al. 2008) and suggest that some populations of *R. pipiens* may not be susceptible to mortality from chytridiomycosis and/or some strains of *Bd* are

TABLE 1. Number of *Rana pipiens* received in Ellensburg, Washington, USA, from a laboratory supplier (Charles D. Sullivan Inc., Tennessee, USA) testing positive for *Batrachochytrium dendrobatidis*, the length of the laboratory experiments for which the frogs were used, average survival time of each cohort, and the number of mortalities during each experiment.

Date sampled	No. Frogs Received from Supplier	No. Infected Frogs	Length of Experiment (days)	Average Survival Time with Infection (days)	Mortalities During Experiment
13 October 2006	14	14	109	103	6
14 July 2007	25	25	66	66	0

less virulent.

More investigation is needed to determine the extent of *Bd* in the amphibian trade. However, these results shed light on one possible mode of dissemination for *Bd*, and add to the evidence that *R. pipiens* shipped from biological supply houses may act as carriers for the pathogen. We recognize the importance of the amphibian trade for research and education, but our data emphasize the need to handle amphibians, and amphibian wastes, aseptically regardless of their origin. Further, in accordance with the OIE, we suggest systematic screening of imported and exported amphibians given the potentially devastating effects of *Bd* on amphibian persistence around the world.

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LITERATURE CITED

- BERGER, L., R. SPEARE, P. DASZAK, D. E. GREEN, A. A. CUNNINGHAM, C. L. GOGGIN, R. SLOCOMBE, M. A. RAGAN, A. D. HYATT, K. R. McDONALD, H. B. HINES, K. R. LIPS, G. MARANTELLI, AND H. PARKES. 1998. Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proc. Natl. Acad. Sci., USA*, 95:9031–9036.
- BOSCH, J., I. MARTÍNEZ-SOLANO, AND M. GARCÍA-PARÍS. 2001. Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of central Spain. *Biol. Conserv.* 97:331–337.
- 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. *Fung. Biol. Rev.* 21:2–9.
- PADGETT-FLOHR, G. E. 2008. Pathogenicity of *Batrachochytrium dendrobatidis* in two threatened California amphibians: *Rana draytonii* and *Ambystoma californiense*. *Herpetol. Conserv. Biol.* 3:182–191.
- PARKER, J. M., I. MIKAELIAN, N. HAHN, AND H. E. DIGGS. 2002. Clinical diagnosis and treatment of epidermal chytridiomycosis in African clawed frogs (*Xenopus tropicalis*). *Comp. Med.* 52:265–268.
- PESSIER, A. P., D. K. NICHOLS, J. E. LONGCORE, AND M. S. FULLER. 1999. Cutaneous chytridiomycosis in poison dart frogs (*Dendrobates* spp.) and White's tree frogs (*Litoria caerulea*). *J. Vet. Diagn. Investig.* 11:194–199.
- PICCO, A. M., AND J. P. COLLINS. 2008. Amphibian commerce as a likely source of pathogen pollution. *Conserv. Biol.* 22:1582–1589.
- RACHOWICZ, L. J., R. A. KNAPP, J. A. T. MORGAN, M. J. STICE, V. T. VREDENBURG, J. M. PARKER, AND C. J. BRIGGS. 2006. Emerging infectious disease as a proximate cause of amphibian mass mortality. *Ecology* 87:1671–1683.
- ROWLEY, J. J. L., S. K. F. CHAN, W. S. TANG, R. SPEARE, L. F., SKERRATT, R. A., ALFORD, K. S. CHEUNG, C. Y. HO, AND R. CAMPBELL. 2007. Survey for the amphibian chytrid *Batrachochytrium dendrobatidis* in Hong Kong in native amphibians and in the international amphibian trade. *Dis. Aquat. Org.* 78:87–95.
- SCHLAEPFER, M. A., C. HOOVER, AND C. K. DODD. 2005. Challenges in evaluating the impact of the trade in amphibians and reptiles on wild populations. *Bioscience* 55:256–264.
- SCHLOEGEL, L. M., 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.
- STUART, S. N., J. S. CHANSON, N. A. COX, B. E. YOUN, A. S. L. RODRIGUES, D. L. FISCHMAN, AND R. W. WALLER. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306:1783–1786.
- WOODHAMS, D. C., A. D. HYATT, D. G. BOYLE, AND L. A. ROLLINS-SMITH. 2008. The northern leopard frog *Rana pipiens* is a widespread reservoir species harboring *Batrachochytrium dendrobatidis* in North America. *Herpetol. Rev.* 39:66–68.

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***Batrachochytrium dendrobatidis* in Argentina: First Record of Infection in *Hypsiboas cordobae* and *Odontophrynus occidentalis* Tadpoles, in San Luis Province**

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An estimated 43.2% of global amphibians have experienced some reduction in population size (Stuart et al. 2004), with losses in several locations explained by the emerging infectious disease chytridiomycosis caused by the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) (Berger et al. 1998; Daszak et al. 1999; Longcore et al. 1999). In Argentina, *Bd* infection was first reported in 2002, in the Spotted Thin-toed Frog, *Leptodactylus ocellatus* (Herrera et al. 2005). Since then, there have been additional detections in *Atelognathus patagonicus*, *Elachistocleis bicolor*, *Leptodactylus gracilis*, *Telmatobius atacamensis*, and *Telmatobius pisanoi* (Arellano et al. 2009; Barrionuevo and Mangione 2006; Fox et al. 2006; Ghirardi et al. 2009) in several locations in the country. Here we report the first occurrence of *Bd* in Argentinean tadpoles of two native anuran species, *Hypsiboas cordobae* and *Odontophrynus occidentalis*, in Luján, San Luis province, Argentina.

On 12 October 2009, 10 live larvae of *Hypsiboas cordobae* and *Odontophrynus occidentalis* were collected at random from a stream during a field survey in the Strict Nature Reserve