Pasmans, F., F. Mutschmann, T. Halliday, and F. Zwart. 2006. Amphibian declines: the urgent need for amphibian research in Europe. Vet. J. 171:18–19.

Patrelle, C., M. B. Hjernquist, A. Laurila, F. Söderman, and J. Merilä. 2012. Sex differences in age structure, growth rate and body size of common frogs *Rana temporaria* in the subarctic. Pol. Biol. DOI 10.1007/s00300-012-1190-7.

Pounds J. A., M. R. Bustamante, L. A. Coloma, J. A. Consuega, M. P. L. Fogden, P. N. Foster, E. La Marca, K. L. Masters, A. Merino-Viteri, R. Puschendorf, S. R. Ron, G. A. Sanchez-Azofeifa, C. J. Still, and B. E. Young. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. Nature 439:161–167.

Reeves, M. K. 2008. *Batrachochytrium dendrobatidis* in wood frogs (*Rana sylvatica*) from three national wildlife refuges in Alaska, USA. Herpetol. Rev. 39:68–70.

———, and D. E. Green. 2006. *Rana sylvatica* (wood frog): chytridiomycosis. Herpetol. Rev. 37:450–450.

Ron, S. R. 2005. Predicting the distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* in the New World. Biotropica 37:209–221.

Scalera, R., M. J. Adams, and S. K. Galvan. 2008. Occurence of *Batrachochytrium dendrobatidis* in amphibian populations in Denmark. Herpetol. Rev. 39:199–200.

Schloegel, L., J. M. Hero, L. Berger, R. Speare, K. R. McDonald, and P. Daszak. 2006. The decline of the sharp-snouted day frog (*Taudactylus acutirostris*): the first documented case of extinction by infection in a free-ranging wildlife species? EcoHealth 3:35–40.

Schock, D. M., G. R. Ruthig, J. P. Collins, S. J. Kutz, S. Carrière, R. J. Gau, A. M. Veitch, N. C. Larter, D. P. Tate, G. Guthrie, D. G. Allaire, and R. A. Popko. 2010. Amphibian chytrid fungus and ranaviruses in the Northwest Territories, Canada. Dis. Aquat. Org. 92:231–40.

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

Skerratt, L. F., L. Berger, H. B. Hines, K. R. McDonald, D. Mendez, and R. Speare. 2008. Survey protocol for detecting chytridiomycosis in all Australian frog populations. Dis. Aquat. Org. 80:85–94.

Slough, B. G. 2009. Amphibian chytrid fungus in western toads (*Anaxyrus boreas*) in British Columbia and Yukon, Canada. Herpetol. Rev. 40:319–320.

ter Schure, A. F. H., P. Larsson, J. Merilä, and I. Jönsson. 2002. Latitudinal fractionation of polybrominated diphenyl ethers and polychlorinated biphenyls in frogs (*Rana temporaria*). Environ. Sci. Technol. 36:5057–5061.

Voyles, J., S. Young, L. Berger, C. Campbell, W. F. Voyles, A. Dinudom, D. Cook, R. Webb, R. A. Alford, L. F. Skerratt, and R. Speare. 2009. Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. Science 326:582–585.

Voyles, J., E. B. Rosenblum, L. Berger. 2011. Interactions between *Batrachochytrium dendrobatidis* and its amphibian hosts, a review of pathogenesis and immunity. Microb. Infect. 13:25–32.

Wells, K. 1977. The social behaviour of anuran amphibians. Anim. Behav. 25:666–693.

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Ranavirus Infection in Native Amphibians at La Selva Biological Station, Costa Rica: First Report of Ranavirus in Central America

Ranaviruses are a globally widespread group of iridoviruses capable of causing mass mortality events in amphibian populations (Gray et al. 2009; Green et al. 2002), and are acknowledged to be a significant threat to amphibian populations in many parts of the world. Central America hosts a diverse, unique, and highly threatened amphibian fauna (Whitfield et al., *in press*), yet there have been only modest efforts to detect and characterize ranaviruses in the region. No information exists on impacts of ranaviruses to amphibian hosts or populations in the Neotropics—a significant gap in knowledge that may inhibit effective conservation planning.

Speare (1991) reported an iridovirus, likely a ranavirus, from *Rhinella marina* in Costa Rica. Picco and Collins (2007) tested 41 amphibians from Monteverde, Costa Rica and 43 amphibians from Las Cruces, Costa Rica but did not detect ranavirus at either site. Lips et al. (2006) assayed 38 amphibians during a mass mortality event at El Copé, Panama and detected no ranavirus. To our knowledge, there are only two reports of infection by ranaviruses anywhere in the American tropics: Galli et al.(2006) reported ranavirus infections in cultivated, non-native American Bullfrogs, *Lithobates catesbeianus*, in Brazil; and Zupanovic et al. (1998) reported infection by "Guatopo Virus"—a novel strain of ranavirus similar to frog virus 3 (FV3)—from wild frogs in Venezuela. Here, we provide the first robust evidence for the presence of ranavirus in amphibians in Central America.

We conducted this study at La Selva Biological Station, a 16 km^2 private biological reserve in the northeastern lowlands of Sarapiquí, Heredia Province, Costa Rica (10.433°N, 83.983°W) with a very well-known herpetofauna (Guyer and Donnelly 2005; Whitfield et al. 2007). We collected toe clip tissue samples from amphibians between July 2006 and May 2008 in association with a mark-recapture study using 19 study plots within primary forest. Our sampling for this study was focused on forest-floor amphibians, the majority of which are direct-developing species with no free-living tadpole stage. Toe clips were stored in 70% ethanol until we performed laboratory analyses.

We haphazardly selected 104 of these toe clip samples for molecular detection of ranavirus using quantitative real-time PCR (qPCR). We extracted nucleic acids from toe clips with Qiagen DNeasy spin columns, and used the extract to perform qPCR following the standard ranavirus protocol (Kerby and Storfer 2009). We used 13µL reactions containing 2µL of extracted DNA template, 900-nmol forward primer, 900-nmol reverse primer, 250

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TABLE 1. Results of qPCR assays for ranavirus at La Selva Biological Station in Costa Rica.

Taxon	Number positive / number tested
Bufonidae	
Rhaebo haematiticus	0 / 2
Hylidae	
Scinax eleaochroa	0 / 3
Eleutherodactylidae	
Diasporus diastema	0 / 9
Strabomantidae	
Pristimantis cerasinus	0 / 1
Prisimantis ridens	0 / 3
Craugastoridae	
Craugastor bransfordii	4 / 28
Craugastor crassidigitus	0 / 1
Craugastor fitzingeri	0 / 7
Craugastor megacephalus	0 / 8
Craugastor mimus	0 / 34
Craugastor noblei	0 / 1
Craugastor talamancae	0 / 5
Total amphibians sampled	4 / 104

nmol probe, and 2X Taqman Fast Universal Master Mix (Applied Biosystems, Foster City, California). We ran plates for one cycle of 95°C (20 sec) and 50 cycles of 95°C (3 sec) and 60°C (20 sec) on a StepOnePlus qPCR machine (Applied Biosystems, Foster City, California) in a molecular core facility at the University of South Dakota. Each plate included a negative control and a standard curve of $1 \times 10^3 - 1 \times 10^7$ copies of the complete major capsid protein sequence of FV3 (Mao et al. 1997), 3900bp in length, derived from a bacterial plasmid (pCRII) obtained from V. G. Chinchar. All samples were run in triplicate and samples with two or more positive wells were considered positive. Samples with only one positive well were rerun and designated as positive if further wells were positive and negative if further wells were negative.

We used DNA from one positive sample to sequence a 407-bp portion of the MCP gene with primers MCP4 and 5 (Mao et al. 1997) in 20µL reactions of Qiagen (Valencia, California) multiplex master mix following the manufacturer's instructions (no Q additive and annealing temperatures of 54.5° and 50.5°C). We cleaned PCR products by polyethylene glycol (Rosenthal et al. 1993), cycle sequenced with Applied Biosystems (Carlsbad, California) BigDye v3.1, and sephadex cleaned (Zoon 1987) prior to sequencing on an Applied Biosystems 3500 instrument.

Of the 104 individuals from 12 species and 5 families, four individuals tested positive for ranavirus, all from a single species: *Craugastor bransfordii*. All four individuals were determined to be adults on the basis of body size. Infected animals were detected in April 2007, May 2007, September 2007, and April 2008. Viral loads for infected animals averaged 5.16×10^6 viral copies (range 1.10×10^5 to 1.70×10^7). These are seemingly high concentrations—for comparison Brunner and Collins (2009) found ~10^5 plaque forming units (each of which almost certainly represents many copies of the viral genome) per nanogram of DNA, and Kerby and Storfer (2009) found ~10^6 viral genomes in *Ambystoma tigrinum* larvae that died of ranavirus infection—but we did not notice apparent symptoms of ranaviral disease in any of these infected individuals. The sequence produced in this study was identical to basepairs 205 to 611 of seven FV3 strains from Genbank.

This study provides strong evidence of infection by ranavirus in wild native amphibians in the lowlands of Costa Rica. Prevalence of infection among all species at La Selva was relatively low (3.8%; 95% CI 1.1–9.5%), although prevalence in the one species in which we detected infections, *Craugastor bransfordii,* was considerably higher (14.2%, 95% CI: 4.0–32.6%). We noted no obvious symptoms of ranaviral disease in these frogs, but have no data available to determine whether infections have any adverse effect on individuals or populations. Our genetic characterization of this FV3 strain is currently insufficient to assess whether this represents a strain native to Central America or a strain recently introduced from other areas.

This study represents the first report of ranavirus infection in amphibians from Central America, and one of very few reports from the hyper-diverse Neotropical region. Other studies from Central America have failed to detect ranaviruses (Lips et al. 2006; Picco and Collins 2007; but see Speare et al. 1991), but were based on small sample sizes (each <100 samples) and used traditional PCR for pathogen detection, which is less sensitive than the qPCR used in this study. We suspect that the absence of reports of ranaviruses from tropical regions is likely a result of limited sampling rather than a true absence of these pathogens. We expect that further studies of ranavirus in tropical regions are very likely to increase the known distribution of these pathogens. It will be important to determine how the ranavirus we detected is related to other ranaviruses, whether this strain is endemic or introduced, and whether it presents a risk to the neotropical amphibian assemblages already decimated by chytridiomycosis-associated declines.

LITERATURE CITED

BRUNNER, J. L., AND J. P. COLLINS. 2009. Testing assumptions of the trade-off theory of the evolution of parasite virulence. Evol. Ecol. Res. 11:1169–1188.

GALLI, L., A. PEREIRA, A. MARQUEZ, AND R. MAZZONI. 2006. Ranavirus detection by PCR in cultured tadpoles (*Rana catesbeiana* Shaw, 1802) from South America. Aquaculture 257:78–82.

GRAY, M. J., D. L. MILLER, AND J. T. HOVERMAN. 2009. Ecology and pathology of amphibian ranaviruses. Dis. Aquat. Org. 87:243–266.

GREEN, D. E., K. A. CONVERSE, AND A. K. SCHRADER. 2002. Epizootiology of sixty-four amphibian morbidity and mortality events in the USA, 1996–2001. Ann. New York Acad. Sci. 969:323–339.

GUYER, C., AND M. A. DONNELLY. 2005. Amphibians and Reptiles of La Selva, Costa Rica, and the Caribbean Slope. University of California Press, Berkeley, California. 300 pp.

KERBY, J. L., AND A. STORFER. 2009. Combined effects of atrazine and chlorpyrifos on susceptibility of the tiger salamander to *Ambystoma tigrinum* Virus. Ecohealth 6:91–98.

LIPS, K. R., F. BREM, R. BRENES, J. D. REEVE, R. A. ALFORD, J. VOYLES, C. CAREY, L. LIVO, A. P. PESSIER, AND J. P. COLLINS. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proc. Nat. Acad. Sci. USA 103:3165–3170.

MAO, J., R. P. HEDRICK, AND V. G. CHINCHAR. 1997. Molecular characterization, sequence analysis, and taxonomic position of newly isolated fish Iridoviruses. Virology 229:212–220.

PICCO, A. M., AND J. P. COLLINS. 2007. Fungal and viral pathogen occurrence in Costa Rican amphibians. J. Herpetol. 41:746–749.

ROSENTHAL, A., O. COUTELLE, AND M. CRAXTON. 1993. Large-scale production of DNA sequencing templates by microtitre format PCR. Nucleic Acids Res. 21(1):173–174.

Speare, R., W. J. Freeland, and S. J. Bolton. 1991. A possible iridovirus in erythrocytes of *Bufo marinus* in Costa Rica. J. Wildl. Dis. 27:457–462.

Whitfield, S. M., K. E. Bell, T. Philippi, M. Sasa, F. Bolanos, G. Chaves, J. M. Savage, and M. A. Donnelly. 2007. Amphibian and reptile declines over 35 years at La Selva, Costa Rica. Proc. Nat. Acad. Sci. USA 104:8352–8356.

———, K. R. Lips, and M. A. Donnelly. In press. Decline and conservation of amphibians in Central America. *In* H. H. Heatwole, C. Barrio-Amoros, and J. W. Wilkenson (eds.), Status of Conservation and Declines of Amphibians: Western Hemisphere. Surrey Beatty and Sons, Pty. Ltd., Sydney.

Zoon, R. A. 1987. Safety with 32P- and 35S-labeled compounds. Methods in Enzymology 152:25–29.

Zupanovic, Z., C. Musso, G. Lopez, C. L. Louriero, A. D. Hyatt, S. Hengstberger, and A. J. Robinson. 1998. Isolation and characterization of iridoviruses from the giant toad *Bufo marinus* in Venezuela. Dis. Aquat. Org. 33:1–9.

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Batrachochytrium dendrobatidis and Ranavirus in Anurans Inhabiting Decorative Koi Ponds near Minneapolis, Minnesota, USA

Evidence of widespread chytrid infection in North American amphibian populations has been documented (Longcore et al. 2007; Ouelett et al. 2005; Woodhams et al. 2008) and *Batrachochytrium dendrobatidis* (*Bd*) has been reported from several locations in Minnesota (Rodriguez et al. 2009; Sadinski et al. 2010; Woodhams et al. 2008). Ranaviruses also have been linked to mass amphibian die-offs in North America (Daszak et al. 1999; Gray et al. 2009; Greer et al. 2005; Jancovich 2005), including Minnesota (Green et al. 2002; Vandenlangenberg et al. 2003). The geographic distribution of these pathogens in Minnesota is still unknown, however, and additional observations will aid assessment of the threat to local amphibian populations.

We report the presence of ranavirus and *Bd* in anurans inhabiting four decorative koi ponds in Burnsville, Minnesota, a suburb of Minneapolis (Fig. 1). The ponds were closely observed for indications of pathogenic activity following the discovery of dead Wood Frogs (*Lithobates sylvaticus*) floating on the surface during the last week of August 2011. Selected specimens were tested for *Bd* and ranavirus.

These four rubber-lined ponds were typical of those constructed by koi- and water-gardening enthusiasts. Their combined surface area was approximately 30 m^2 and their maximum depth was 1.05 m. Koi and goldfish were held in the ponds throughout the year. Total fish biomass was roughly 3.3 kg. The largest fish measured approximately 0.35 m, total length. Water was circulated through three of the four ponds by a pump connected to a simple filtering system employing fiberglass batting as filter material. A similar system was used to filter water in the fourth pond. Water lost via evaporation was replaced by precipitation and partial refilling from a tap connected to a chlorinated city water line.

The ponds were situated within a 260 m^2 area separated from a 970 ha park reserve by a short (100–150 m) strip of undeveloped land. The distance to the nearest permanent body of water was approximately 112 m.

From 25 August to 4 September 2011, three dead Wood Frogs were recovered from the ponds. All three specimens were fresh and exhibited significant reddening of the ventral surface. No signs of traumatic injury were observed and disease was determined to be a likely cause of death.

We visited the ponds daily from 7 September to 24 October 2011, following the initial frog deaths. During each visit, the ponds and immediate surroundings were visually surveyed for amphibians. Observations of dead and dying individuals were recorded and dead specimens were collected for examination, condition permitting. Anurans exhibiting unusual behavior were photographed.

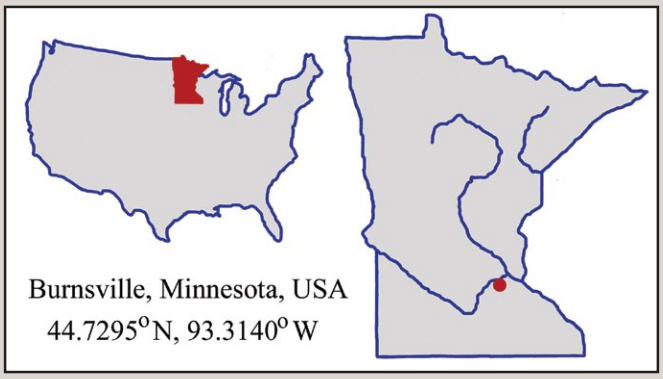

Fig. 1. Location of koi ponds in Minnesota, USA where frogs were found infected with *Batrachochytrium dendrobatidis* and ranavirus.

Our daily visits produced 202 sightings of six species (Table 1). Two dead Wood Frogs, one dead Green Frog (*Lithobates clamitans*), and two dead American Toads (*Anaxyrus americanus*, formerly *Bufo americanus*) were recovered from 7 September to 24 October 2011. Most of the dead specimens were retrieved from the ponds. The lone exception was an American Toad found within 0.25 m of a pond. Detailed examination of the Green Frog and one of the toads was precluded by advanced decomposition. The other toad was recovered in very good condition and transported

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