Acta Herpetologica 9(1): 125-127, 2014
DOI: 10.13128/Acta_Herpetol-13516

Death in the clouds: ranavirus associated mortality in assemblage of cloud forest amphibians in Nicaragua

Tariq Stark[1,*], Carlijn Laurijssens[1], Martijn Weterings[1,2], Annemarieke Spitzen-van der Sluijs[3,4], An Martel[4], Frank Pasmans[4]

[1] *Van Hall Larenstein University of Applied Sciences, Agora 1, 8934 CJ Leeuwarden, The Netherlands. *Corresponding author.* E-mail: tariqstark@hotmail.com
[2] *Wageningen University and Research center, department of Resource Ecology, Droevendaalsesteeg 3a, 6708 PB Wageningen, The Netherlands*
[3] *Reptile, Amphibian and Fish Conservation the Netherlands, P.O. Box 1413, 6501 BK Nijmegen, The Netherlands*
[4] *Department of Pathology, Bacteriology and Avian Diseases, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium*

Submitted on 2013, 29th October; revised on 2014, 2nd April; accepted on 2014, 6th May

Abstract. Amphibian diseases are acknowledged as significant contributors to the decline and extinction of amphibian species. The main culprits currently considered are chytridiomycosis and Ranavirus. In Central America, highly endemic and geographical restricted terrestrial species may be at risk from these diseases. We collected 49 *Agalychnis callidryas* larvae, one *Lithobates forrei* and five unidentified larvae on the Nicaraguan Island Ometepe, all deceased, and skin samples were taken. The presence of Ranavirus was determined using PCR. Ranavirus was found involved in 41 of 55 tadpoles. Forty-one *Agalychnis callidryas*, one *Lithobates forrei* and another five unidentified anuran tadpoles.

Keywords. Anurans, mortality, Ranavirus, Nicaragua, Ometepe.

Amphibians are declining in all continents where they occur due to several causes like habitat destruction, habitat degradation, pollutants, climate change and diseases (Stuart et al., 2008).

Besides chytridiomycosis caused by the fungal agents *Batrachochytrium dendrobatidis* (Voyles et al., 2009) and *B. salamandrivorans* (Martel et al., 2013), Ranaviruses globally contribute to amphibian declines (Miller et al., 2011; Robert and Chinchar, 2012; Brenes, 2013). Ranaviruses belong to the family Iridoviridae and have been recorded from Asia, Central-, South- and North America and Europe (Whitfield et al., 2007; Xu et al., 2010). Although all amphibian life stages can be affected, larvae appear to be most susceptible to the disease and mortality rates are often high (Green et al., 2002; Greer et al., 2005; Kik et al., 2011; Miller et al., 2011).

Central America is one of the regions considered most struck by disease-driven amphibian declines, notably chytridiomycosis affecting mostly endemic high-elevation species with a restricted range (Duellman, 1999; Savage, 2002; Lips et al., 2008). The volcanic islands in Lake Nicaragua contain complex high-elevation amphibian assemblages in a region where Ranavirus has not been recorded before. In this study we found ranavirus to be involved in a mortality event comprising several anuran species in the crater lake of volcano Maderas on Ometepe Island.

Ometepe (11°30'N, 85°35'W) is situated in Lake Nicaragua (8,264 km^2) and is comprised out of two volcanos (McKaye, 1995; Nemitz, 2008). Our survey was conducted on the Maderas volcano, the smallest one, with cloud forests on its upper slopes.

This survey was carried out in October 2011, coinciding with the rainy period. Surveys had a strong focus on locating and sampling anurans and their breeding waters. Since breeding animals and vocalizing males were found to be most active during the hours between sunset and midnight, surveys were carried out between these.

ISSN 1827-9635 (print)
ISSN 1827-9643 (online)

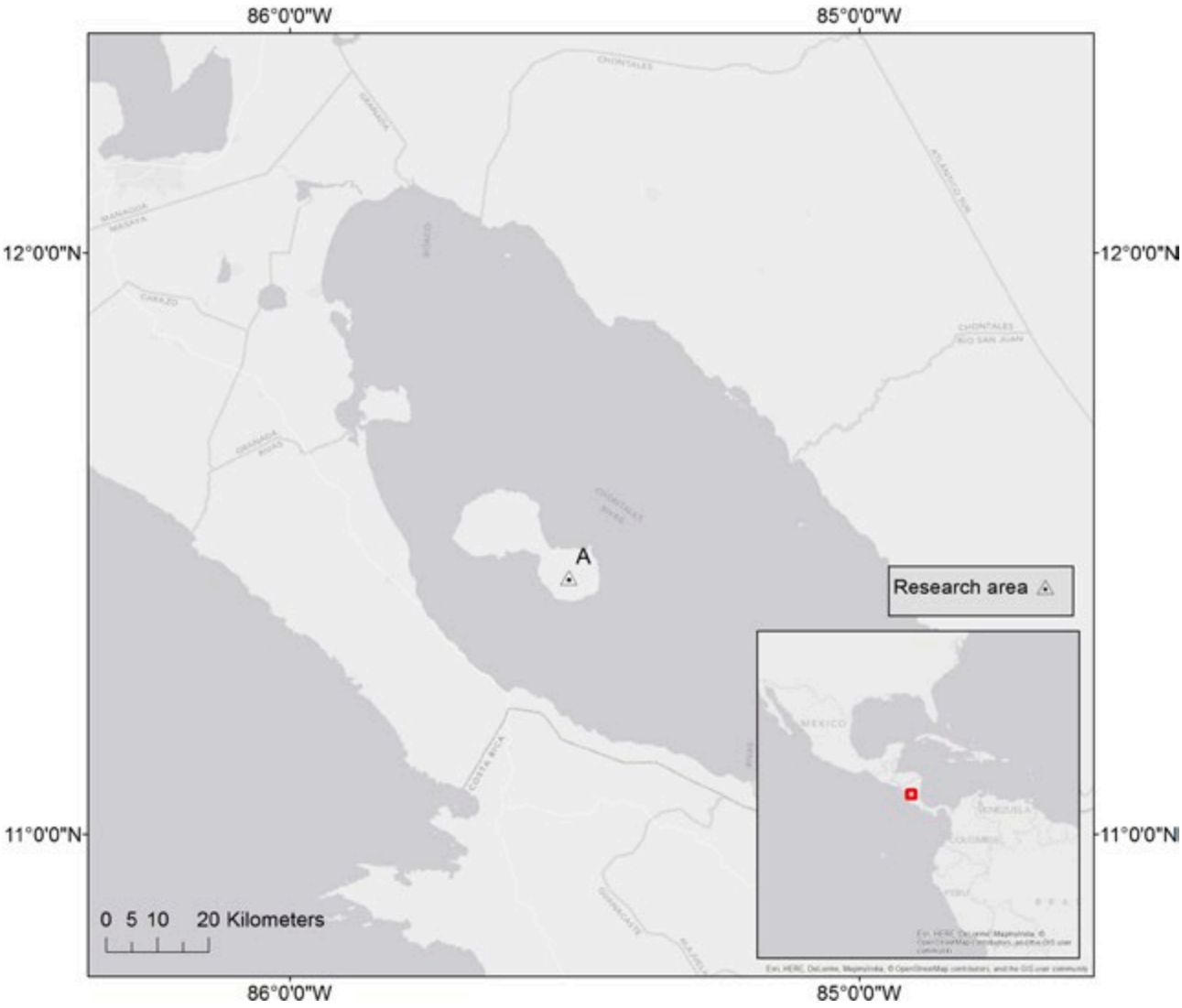

Fig. 1. Ometepe Island. The study area on Volcano Maderas, one of the two volcanoes that comprises the island, is marked with A.

On 21 October 2011 55 tadpoles of two anuran species (*Agalychnis callidryas* and *Lithobates forrei*), were found dead in the cloud forest of Maderas at an elevation of 1,203 meters (Nicaragua, 11°26'N, 85°30'E). From 54 of these tadpoles, skin swabs were collected and processed for the detection of Bd using qPCR (Boyle et al., 2004) and for the presence of ranavirus DNA using the PCR described by Mao et al. (1997). We tested for these two pathogens because these two are known to cause amphibian declines in Central America. Thirty-five out of 49 dead larvae of *Agalychnis callidryas* (in various stages of development), one dead *Lithobates forrei* tadpole and five dead unidentified tadpoles (due to post mortem decay), tested positive for Ranavirus. None of these larvae tested positive for Bd.

Ranavirus was associated in this case with a mortality event in the larvae of two anuran species on Ometepe. However, only PCR was performed because it was not possible to collect dead animals to perform a histological examination. The latter is needed to confirm an etiological diagnosis (Greer et al., 2005).

During an outbreak, Ranavirus has been known to kill almost 100% of larvae and post metamorphs (Green et al., 2002). Ranavirus may thus have a big impact on geographically restricted and small populations of amphibians on Maderas like the micro-endemic *Bolitoglossa insularis* (status vulnerable IUCN, 2013), *Craugastor laevissimus* (status endangered IUCN, 2013). As the current study only encompassed a limited time frame, we cannot exclude an effect on other species

within the entire community of amphibians in this cloud forest.

If we consider the most likely scenario that the outbreak is due to a recent introduction of Ranavirus on Ometepe, the route of viral entry is hypothetical. Few tourists venture up Maderas due to the very poor accessibility of the volcano (personal observation). The infrastructure of both the lowland on Ometepe and the accessibility of Maderas are, however, being improved since 2011 (personal observation). With these changes it might be easier to reach the higher ranges of the volcano, which can lead to more people in the cloud forest acting as vectors for Ranavirus. Continuous monitoring of the amphibian assemblages at this site is highly recommended.

ACKNOWLEDGEMENTS

Specimens were collected under permit Number 011-102010/DGPN issued by The Ministry of the Environment and Natural Resources Nicaragua (Marena). We also thanks the Universidad Nacional Autónoma de Nicaragua-Leon, León, Nicaragua, for helping obtaining collecting permits through Javier Sunyer. We are very grateful to Fundacion Colibolca and José M Zolotoff-Pallais (Momabacho) for their guidance, assistance and use of the field station. We are also grateful for Rachael Antwis for supplying us with various field equipment. Ignas Dummer we wish to thank for creating the map of the research area depicted in this paper. Jordi Janssen is thanked for proof reading this paper.

REFERENCES

Boyle, D.G., Boyle, D.B., Olsen, V., Morgan, J.A.T., Hyatt, A.D. (2004): Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. Dis. Aquat. Organ. **60**: 141-148.

Brenes, R.M. (2013): Mechanisms Contributing to the Emergence of Ranavirus in Ectothermic Vertebrate Communities. Doctoral dissertation. University of Tennessee.

Duellman, W.E. (1999): Patterns of distribution of amphibians: a global perspective. John Hopkins University Press, Baltimore, Maryland.

Green, D.E., Converse, K.A., Schrader, A.K. (2002): Epizootiology of sixty-four amphibian morbidity events in the USA, 1996-2001. Ann. Ny. Acad. Sci. **969:** 323-339.

Greer, A.L., Berrill, M., Wilson, P.J. (2005): Five amphibian mortality events associated with ranavirus infection in south central Ontario Canada, Dis Aquat Organ. **67:** 9-14.

IUCN (2013). IUCN Red List of Threatened Species. Version 2013.2. <www.iucnredlist.org>. Downloaded on 20 May 2014.

Kik, M., Martel, A., Spitzen-van der Sluijs, A., Pasmans, F., Wohlseind, P., Grönea, A., Rijksa, J.M. (2012): Ranavirus-associated mass mortality in wild amphibians, The Netherlands, 2010: A first report. Vet. J. **190:** 284-286.

Lips, K.R., Diffendorfer, J. Mendelson, J.R., Sears, M.W. (2008): Riding the wave: Reconciling the roles of disease and climate change in amphibian declines. PLoS Biol. **6**: 72.

Martel, A., Spitzen-van der Sluijs, A., Blooi, M., Bert, W., Ducatelle, R., Fisher, M.C., Woeltjes, A., Bosman, W., Chiers, K., Bossuyt, F., Pasmans, F. (2013): *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. PNAS **110:** 15325-15329.

Miller, D., Gray, M., Storfer, A. (2011): Ecopathology of Ranaviruses Infecting Amphibians. Viruses **3**: 2351-2373.

McKaye, K.R., Ryan, J.D., Stauffer, J.R., Perez, L.J.L., Vega, G.I., van den Berghe, E.P. (1995): African Tilapia in Lake Nicaragua. Ecosystem in transition. **45:** 406-411.

Nemitz, D. (2008): An assessment of sampling detectability for global biodiversity monitoring: results from sampling grids in different climatic regions, Unpublished Master thesis. Faculty of Biology, Georg-August-Universität Göttingen.

Robert, J., Chinchar, V.G. (2012): Ranaviruses: An emerging threat to ectothermic vertebrates. Report of the First International Symposium on Ranaviruses, Minneapolis MN July 8, 2011. Dev. Comp. Immunol. **2:** 259-261.

Savage, J.M. (2002): The amphibians and reptiles of Costa Rica: a herpetofauna between two continents, between two seas. University of Chicago Press, Chicago.

Stuart, S.N., Hoffmann, M., Chanson, J.S., Cox, N.A., Berridge, R.J., Ramani, P., Young, B.E. (eds.) (2008): Threatened Amphibians of the World. Lynx Edicions, Barcelona, Spain.

Voyles, J., Young, S., Berger, L., Campbell, C., Voyles, W.F., Dinudom, A., Cook, D., Webb, R., Alford, R.A., Skerrat, L.F., Speare, R. (2009): Pathogenesis of Chytridiomycosis, a Cause of Catastrophic Amphibian Declines. Science **326:** 582-585.

Whitfield, S.M., Geerdes, E., Chacon, I., Ballesteros, E., Jimenez, R., Donnelly, M.A., Kerby, J.L. (2013): Infection and co-infection by the amphibian chytrid fungus and ranavirus in wild Costa Rican frogs. Dis. Aquat. Organ. **104:** 173-178.

Xu, K., Zhu, D., Wei, Y., Schloegel, L.M., Chen, X., Wang, X. (2010): Broad distribution of ranavirus in free ranging *Rana dybowskii* in Heilongjiang, China. Ecohealth **7:** 18-23.