Nota

Cuad. herpetol. 28 (1): 29-31 (*2014*)

Batrachochytrium dendrobatidis infecting anurans in a protected area from Santa Fe Province, Argentina

Romina Ghirardi[1], Michael G. Levy[2], Javier A. López[1,3], Mónica M. Steciow[4], Ma. Gabriela Perotti[5]

[1] Instituto Nacional de Limnología (INALI; CONICET-UNL), Ciudad Universitaria, Pje. El Pozo. 3000 Santa Fe, Santa Fe, Argentina.

[2] Department of Population Health and Pathobiology, North Carolina State University, College of Veterinary Medicine, 27607, North Carolina. United States of America.

[3] Departamento de Ciencias Naturales, Facultad de Humanidades y Ciencias, Universidad Nacional del Litoral, Ciudad Universitaria, Pje. El Pozo, 3000 Santa Fe, Santa Fe, Argentina.

[4] Instituto de Botánica Spegazzini, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, La Plata, Argentina.

[5] Laboratorio de Fotobiología, INIBIOMA (UNCOMA-CONICET), Quintral 1250, 8400, San Carlos de Bariloche, Río Negro, Argentina.

The emerging infectious diseases have been proposed as responsible of global decline of amphibians' worldwide (Bosch, 2003; Smith *et al.*, 2009). The etiologic agents of these diseases are diverse: viruses, bacteria, protists, worms, moulds and fungus (Bosch, 2003). Among these agents, the chytrid fungus *Batrachochytrium dendrobatidis -Bd-* (Fungi: Chytridiomycetes: Rhyzophydiales) (Longcore *et al.*, 1999; Letcher *et al.*, 2006) has been highlighted as suspected driver of global amphibian declines (Collins and Storfer, 2003; Daszak *et al.*, 2003).

The first case of chytrid fungus infection in anurans from Argentina was detected in a specimen of *Leptodactylus latrans* found dead in 2002 at the Pampas plains (Buenos Aires Province) (Herrera *et al.*, 2005). Since then, new records have been registered in the country in a growing number of species (Ghirardi, 2012 and cites therein), including in protected areas from different Argentinean regions (Fox *et al.*, 2006; Gutierrez *et al.*, 2010; Ghirardi, 2012 and cites therein; Lescano *et al.*, 2013). Our goal was to asses the presence of *Bd* in amphibians inhabiting the recently created National Park 'Islas de Santa Fe' (PNISF) in alluvial valley of Paraná River (Santa Fe Province, Argentina).

There is a seasonal variation in amphibians' assemblage of Paraná River wetlands, been winter the season with less specimens and species actives (López *et al.,* 2011), but low temperatures of this season are more favourable for the development of the chytrid fungus (Piotrowski *et al.*, 2004). Thus, in 2009 we conducted a winter sampling (August) in Campo Rico island (32°16' 34,86"S; 60°41' 15,35"O, elevation: 9 m a.s.l), the biggest island of PNISF (Fig. 1). We collected 28 adult anurans belonging to six species (five *Dendropsophus nanus*, ten *Hypsiboas pulchellus*, one *Leptodactylus latrans*, ten *Rhinella fernandezae*, one *R. schneideri*, and one *Scinax nasicus*). To collect DNA samples for looking *Bd* infection through molecular techniques, all specimens were gently but firmly swabbed several times on the ventral surface, hindlimbs and interdigital membrane following the techniques of Livo (2004) and Hyatt *et al.* (2007). Each swab was placed in an individually numbered plastic cryogenic vial for storage. According to the method of Boyle *et al.* (2004), samples were processed using *Bd* specific real-time Taqman (Applied Biosystems, Foster City, CA,

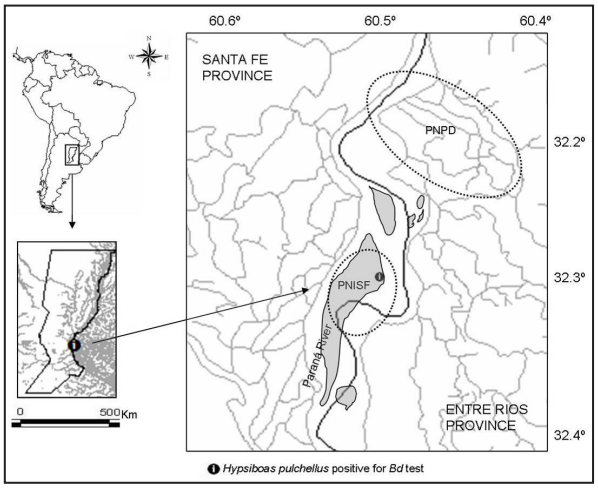

Figure 1. Geographic location of the sampled site with the *Hypsiboas pulchellus* infected by *Batrachochytrium dendrobatidis*. PNISF: Islas de Santa Fe National Park; PNPD: Pre Delta National Park.

Author for correspondence: romighirardi@yahoo.com.ar

EEUU) polymerase chain reaction in the laboratory of Population Health and Pathobiology at the North Carolina State University's School of Veterinary Medicine (USA). Appropriate negative and positive controls consisting of swabs inoculated with known numbers of zoospores were run concurrently with survey samples and the limit of detection was less than/equal to 0.5 zoospore equivalents. All swabbed individuals were released in the site of collect. To avoid possible cross-contamination of pathogens among ponds, in the field we followed sampling protocols of DAPTF Fieldwork Code of Practice and those outlined in Livo (2004).

Two out of the ten *H. pulchellus* resulted positive for *Bd* analysis. Individuals of remaining analysed species were negative for *Bd* presence. None of the specimens showed clinical signs of chytridiomycosis when collected; neither infected nor not infected ones. No unusual sloughing of the skin, no slimness and no mortality were observed.

The first case of *Bd* infecting *H. pulchellus* was described in tadpoles collected during winter and early spring (August to October) in Uruguay (Borteiro *et al.*, 2009), coinciding with our sampling season. Adults of the studied population of *H. pulchellus* have winter activity patterns (López *et al.*, 2011) and winter tadpoles with long larval period (Lajmanovich, 2000), both characteristics that confer special susceptibility to *Bd* infection (Williams and Hero, 1998; Lips *et al.*, 2003). Additionally, laboratory tests showed a particular sensibility of *H. pulchellus* to *Bd* infection when compared with other sympatric species, as for example *R. fernandezae* (Arellano M. L., pers. comm.).

Given the seasonal peaks of anuran activity (López *et al.*, 2011) it is necessary to carry out spring-summer samplings to analyze a greater number of taxa and specimens, thus assessing the spread of infection in PNISF amphibian assemblage. In addition, it is important to establish a long-term monitoring plan to evaluate whether *Bd* presence affects the dynamics of infected populations. Finally, it is essential to alert environmental authorities on the findings and issue administrators, park rangers and citizens about amphibians' conservation concern in Paraná River wetlands (see Perotti and Ghirardi, 2010).

Acknowledgements

To Dr. Fandiño, B. for allowing us join his field work trip, to Creus, E. for field assistance. Sampling was under permits of Secretaria de Estado de Medio Ambiente y Desarrollo Sustentable (Res Nº 0177 - Province of Santa Fe). The study was supported by the Student Grant Program from Neotropical Grassland Conservancy (RG) and fellowship from the Consejo Nacional de Investigaciones Científicas y Técnicas (RG and JAL).

Literature cited

Borteiro, C., Cruz, J., Kolenc, F. & Aramburu, A. 2009. Chytridiomycosis in frogs from Uruguay. *Diseases of Aquatic Organisms* 84: 159-162.

Bosch, J. 2003. Nuevas amenazas para los anfibios: enfermedades emergentes. *Munibe. Donostia- San Sebastián*. 16: 55-71.

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

Collins, J.P. & Storfer, A. 2003. Global amphibian declines: sorting the hypotheses. *Diversity and Distributions* 9: 89-98.

Daszak, P., Cunningham, A. & Hyatt, A. 2003. Infectious disease and amphibian population declines. *Diversity and Distributions* 9: 141-150.

Fox, S., Greer, A., Torres-Cervantes, R. & Collins, P. 2006. First case of ranavirus associated morbidity and mortality in natural populations of the South American frog *Atelognathus patagonicus*. *Diseases of Aquatic Organisms* 72: 87-92.

Ghirardi, R. 2012. Estudio de quitridiomicosis por *Batrachochytrium dendrobatidis* en anfibios anuros del Litoral, Cuyo y Patagonia Argentina. Tesis Doctoral. Facultad de Ciencias Naturales y Museo. Universidad Nacional de La Plata. La Plata.

Gutierrez, R.F., Arellano, M.L., Moreno, L.E. & Natale, G.S. 2010. *Batrachochytrium dendrobatidis* in Argentina: first record of infection in *Hypsiboas cordobae* and *Odontophrynus occidentalis* tadpoles, in San Luis province. *Herpetological Review* 41: 323-324.

Herrera, R., Steciow, M. & Natale, G. 2005. Chytrid fungus parasitizing the wild amphibian *Leptodactylus ocellatus* (Anura: Leptodactylidae) in Argentina. *Diseases of Aquatic Organisms* 64: 247-252.

Hyatt, A.D., Boyle, D.G., Olsen, V., Boyle, D.B., Berger, L., Obendorf, D., Dalton, A., Kriger, K.M., Hero, J.M., Hines, H., Phillott, A.D., Campbell, R., Marantelli, G., Gleason, F.H. & Collins, A. 2007. Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms* 73: 175-192.

Lajmanovich, R.C. 2000. Interpretación ecológica de una comunidad larvaria de anfibios anuros. *Interciencia* 25: 71-79.

Lescano, J.N., Longo, M.S. & Robledo, G. 2013. Chytridiomycosis in endemic amphibians of the mountain tops of the Córdoba and San Luis ranges, Argentina. *Diseases of Aquatic Organisms* 102: 249-254.

Letcher, P., Powel, M., Churchill, P. & Chambers, J. 2006. Ultrastructural and molecular phylogenetic delineation of a new order, the Rhizophydiales (Chytridiomycota). *Mycological Research* 110: 898-915.

Lips, K., Reeve, J.D. & Witters, L. 2003. Ecological traits predicting amphibian population declines in Central America. *Conservation Biology* 17: 1078-1088.

Livo, L.J. 2004. Methods for obtaining *Batrachochytrium dendrobatidis* (*Bd*) samples for PCR testing: 64-68. *In*: Rogers, K.B. (ed.) Boreal Toad Research Report 2003. Colorado Division of Wildlife. Colorado. USA.

Longcore, J.E., Pessier, A.P. & Nichols, D.K. 1999. *Batrachochytrium dendrobatidis* gen. et sp. nov. a chytrid pathogenic to amphibians. *Mycologia* 91: 219-227.

López, J.A., Scarabotti, P.A. & Ghirardi, R. 2011. Seasonal patterns of abundance and recruitment in an amphibian assemblage from the Paraná River floodplain. *Interciencia* 36: 538-544.

Perotti, M.G. & Ghirardi, R. 2010. Como desinfectar el material de campo cuando se trabaja en humedales. Disposición 000774. Administración de Parques Nacionales, Buenos Aires.

Piotrowski, J.S, Annis, S.L. & Longcore, J.E. 2004. Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. *Mycologia* 96: 9-15.

Smith, K.G., Acevedo-Whitehouse, K. & Pedersen, A.B. 2009. The role of infectious diseases in biological conservation. *Animal Conservation* 12: 1-12.

Williams, S.E. & Hero, J.M. 1998. Rainforest frogs of the Australian wet tropics: Guild classification and the ecological similarity of declining species. *Proceedings of the Royal Society of London B* 265: 597-602.

Recibida: 03 Diciembre 2012
Revisada: 25 Enero 2013
Aceptada: 22 Abril 2013
Editor Asociado: M. Vaira