

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

Romina Ghirardi¹, Michael G. Levy², Javier A. López^{1,3}, Mónica M. Steciow⁴, Ma. Gabriela Perotti⁵

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: Rhizophydiales) (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 assess 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 active (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,

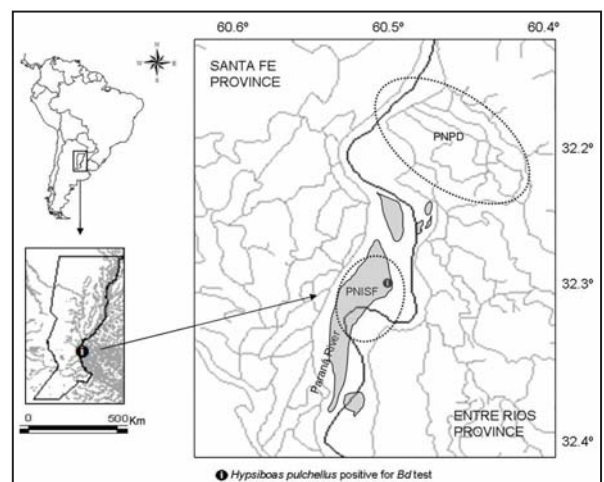


Figure 1. Geographic location of the sampled site with the *Hypsiboas pulchellus* infected by *Batrachochytrium dendrobatidis*. PNISF: Islas de Santa Fe National Park; PNPD: Pre Delta National Park.

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 (Bortei-ro *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).

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