

# Infection by the chytrid fungus *Batrachochytrium dendrobatidis* in the yellow belly frog (*Elachistocleis bicolor*) from Argentina

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Infection by the chytrid fungus *Batrachochytrium dendrobatidis* was detected in a dead adult of the yellow belly frog (*Elachistocleis bicolor*) in Misiones province, Argentina. The analysis of the skin through standard histological procedures revealed the presence of chytrid sporangia in different stages. This is the first report of *B. dendrobatidis* occurring in hot and humid lowlands in northeastern Argentinian Atlantic forest, and the first case of infection for the frog genus *Elachistocleis*. The importance of this finding is discussed in light of the particular conservation importance of the eco-region involved, and the coinciding emergence of locally introduced American bullfrogs.

*Key words:* Anura, amphibians, disease, Microhylidae, Misiones

Chytridiomycosis is considered an emerging disease (Daszak et al., 2003) linked to mortalities of wild amphibian populations in many areas including Europe, South America, Central America, North America, New Zealand, Australia and Africa (Bishop, 2000; Bosch et al., 2001; Bradley et al., 2002; Weldon & du Preez, 2004; Barrionuevo & Mangione, 2006; Lips et al., 2006). This disease is caused by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*, Longcore et al., 1999; Phylum Chytridiomycota, Order Rhizophydiales; Letcher et al., 2006). At present, *B. dendrobatidis* has been reported in eight South American countries, including Argentina, Bolivia, Brazil, Colombia, Ecuador, Peru, Uruguay and Venezuela (Ron & Merino, 2000; Bonaccorso et al., 2003; Mazzoni et al., 2003; Carnaval et al., 2005; Herrera et al., 2005; Lampo et al., 2006; Seimon et al., 2005; Barrionuevo et al., 2008; Ruiz & Rueda-Almonacid, 2008; Velásquez et al., 2008). In Argentina,

infections by *Bd* have been recorded in five anuran species: *Atelognathus patagonicus*, *Leptodactylus gracilis*, *L. ocellatus*, *Telmatobius atacamensis* and *T. pisanoi* (Herrera et al., 2005; Barrionuevo & Mangione, 2006; Fox et al., 2006; Ghirardi et al., 2009), taxa associated with high altitudes and/or latitudes or with low temperatures.

In this contribution we report the presence of the fungus on an individual of *Elachistocleis bicolor* from Misiones Province (Argentina). *Elachistocleis bicolor* (Guerín-Méneville, 1838), is a small, ovoid-shaped microhylid, widely distributed in Uruguay, northeastern Argentina, southern Brazil and Paraguay, and southwestern Bolivia (IUCN 2008), and is considered "Least Concern" by Lavilla et al. (2000) and IUCN (2008). The province of Misiones includes all the Atlantic forest remnants in Argentina (Giraudó, 2005). Although it covers only 1.1 % of the total area of the country, it is one of the richest in biodiversity in Argentina, and one of the most threatened on a global scale (Giraudó et al., 2005).

On 26 November 2005 one individual of the yellow belly frog was found dead (without signs of have been killed by vehicle traffic) on the margin of a temporal pond on National Route N° 12, on the road to Posadas (27°26'373"S, 56°01'43"W, 122 m a.s.l.), Misiones province, Argentina (Figure 1). The specimen was fixed in 10% formalin and then maintained in 70% ethanol. The ventral pelvic and ventral hind limb skin was dehydrated, paraffin-embedded and cut (Microm GmbH HM 400 R) into five-micron-thick sections for histological analysis. Skin cuts were stained with haematoxylin and eosin and were examined with a light microscope (Olympus BX 40 Optical Co. Ltd, Tokyo, Japan) We followed the guidelines described in Berger et al. (1999) to diagnose infection produced by *Bd*. The voucher specimen is housed in the herpetological collection of the Museo de La Plata, Buenos Aires, Argentina (MLP A. 4631).

Histological examination of the epidermis of the yellow belly frog showed evidence of fungal infection. Spherical and ovoid zoosporangia, empty or containing zoospores, were identified in the keratinized cell layer of the epidermis (Fig. 2). Many of the zoosporangia, ranging from 5 to 10 µm in diameter, showed the discharge tube that releases zoospores. The infected areas included mild to moderate hyperkeratosis and areas of focal erosion adjacent to the infection. These observations agree with *Bd* infection as described by Berger et al. (1999). The stage of the infection was identified as "light", containing clusters of 4–6 spherical sporangia (Herrera et al., 2005).

Although the study is based on one specimen, the presence of *B. dendrobatidis* in *E. bicolor* from Misiones (Argentina) is relevant for several reasons. It represents the southernmost record of *Bd* in the Paranean forest ecoregion (*sensu* Burkart et al., 1999). The Atlantic forest, considered one of the five biodiversity hotspots originally reported for South America (Myers et al., 2000; Conservation International, 2005), is currently reduced to 7–8% of its original size (Galindo Leal & Gusmão Câmara, 2005), and not only houses the highest diversity of am-



**Fig. 1.** Report site (indicated by star) of the yellow belly frog infected by *Bd* in Misiones province, northeastern Argentina. The triangles represent four *Lithobates catesbeianus* (American bullfrog) localities (Pereyra et al., 2006).

phibians in Argentina and Paraguay, but also is one of the richest batrachological areas in Brazil (Brusquetti & Lavilla, 2006; Cochran, 1955; Cei, 1980). The Atlantic forest is also characterized by its high level of endemic species (Placi & Di Bitetti, 2005). While only about 3% and 10% of the original cover exist in Brazil and Paraguay, respectively, Argentina still maintains 50% of the original forest (Holz & Placci, 2005; Giraud et al., 2005). Previously, *Bd* was reported from several localities from Jaqueira (Pernambuco, Brazil; Carnaval et al., 2005, 2006; Toledo et al., 2006) at a straight-line distance of about 2900 km to Misiones.

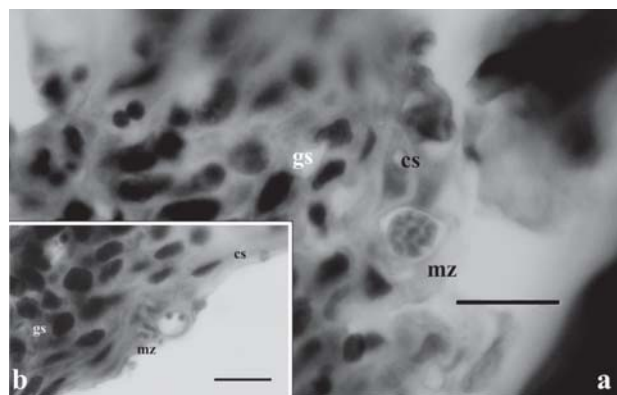
Our study area has a humid-subtropical climate (*sensu* Köppen, 1936), where the mean minimum temperature is 21.8 °C for the hottest month (January, local summer) and 11.1 °C for the coldest (June, local winter; statistics for the period 1981–1991; Servicio Meteorológico Nacional, 2008). Our finding constitutes the first record of *Bd* in a lowland and subtropical area of Argentina during spring. Previous records of *Bd* in the country were associated with colder temperatures, either due to winter records (Buenos Aires Province, 11 m a.s.l., 4 July 2002; Herrera et al., 2005), mid or high altitudes (Salta Province, 3885 m a.s.l., Tucumán Province, 2352 m a.s.l., Barrionuevo & Magione, 2006; Córdoba Province 800 m a.s.l., Ghirardi et al., 2009), or high latitudes (Neuquén Province, Laguna Blanca, 39°S, 70°W, 1276 m a.s.l., Fox et al., 2006). It is interesting to note that reports of *Bd* around the world are scarce in lowlands (Mazzoni et al., 2003; Carnaval et al.,

2006; Puschendorf et al., 2006) and mainly occur in farmed animals and/or under low temperatures.

Interestingly, the presence of *Bd* in the Paranean forest of Misiones coincides with the finding in the same ecoregion of feral populations of the American bullfrog *Lithobates catesbeianus* (Pereyra et al., 2006), which can act as an efficient pathogen carrier (Kats & Ferrer, 2003; Garner et al., 2006). *Lithobates catesbeianus* is considered resistant to the chytrid fungus (Daszak et al., 2004; Hanselmann et al., 2004), and infected specimens have been recorded in three South American countries, including Brazil (Garner et al., 2006), Uruguay (Mazzoni et al., 2003) and Venezuela (Hanselmann et al., 2004). Aside from Misiones, populations of bullfrogs in Argentina have also been recorded in San Juan (Sanabria et al., 2005), Córdoba (Akmentins et al., 2009), Buenos Aires (Barraso et al., 2009) and Salta (Akmentins & Cardozo, 2009), with *Bd* reports in the latter three provinces (Barrionuevo & Mangione, 2006; Ghirardi et al., 2009; Herrera et al., 2005). Specific studies of *Bd* infection in *L. catesbeianus* are necessary to confirm if this anuran species is acting as a carrier of the disease in Misiones province.

Up to now, no other *Bd* infections have been recorded in Misiones province. At the pond where the specimen was collected, a further 16 anuran species (adults and/or tadpoles) have been recorded (Diego Baldo, pers. comm.). Studies on species susceptibility to *Bd* are needed to quantify its potential to affect amphibian populations in Argentina, considering that this fungal disease has been suggested to cause death in whole frog communities from Australian and Central American Forests (Berger et al., 1998; Lips, 1999).

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**Fig. 2.** Histological section of ventral skin of the yellow belly frog. a) Mature zoosporangium (mz) containing zoospores in the stratum corneum. b) Mature zoosporangium almost empty; cs: stratum corneum; gs: stratum granulosum. Scale bar (a, b) = 18 μm.

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