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A lizard acting as carrier of the amphibian-killing chytrid *Batrachochytrium dendrobatidis* in southern Brazil

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Abstract. Fungal infections are causing widespread population declines and extinctions in all vertebrate classes. Among them, an important fungal disease chytridiomycosis, caused by the pathogenic chytrid *Batrachochytrium dendrobatidis* (*Bd*). With an aquatic infectious phase, *Bd* does not survive desiccation for long, but may be transported by non-amphibian carriers. Such mechanism is key to understand amphibian-chytrid dynamics and may contribute to local amphibian conservation action plans. Therefore, we surveyed *Bd* in reptiles from two different Brazilian rainforests, looking for possible *Bd* carriers. We sampled 35 individuals belonging to 11 squamate families, five from the Atlantic Forest and 30 from the Amazon. We detected *Bd* in one adult lizard, *Placosoma glabellum*. This lizard feeds, shelters, and breeds in the leaf-litter, and moves between Atlantic Forest streams. Hence, it may be carrying *Bd* from stream to stream, and also spreading the pathogen to direct-developing amphibians, which have no contact with water bodies and are more susceptible to chytridiomycosis than aquatic species. This is the first record of a non-amphibian chytrid carrier in South America. We suggest that additional field and museum samplings will contribute to understand whether *Bd* can actually infect reptiles, and how reptile carriers can affect chytrid dynamics in the wild.

Keywords. Amphibia, Reptilia, Squamata, Amazon, Atlantic Forest, Chytridiomycosis, Infection dynamics, Pathogen vector.

Infectious diseases, caused by a diversity of pathogenic agents, have become one of the most important threats to wildlife (Daszak et al., 2000; Daszak et al., 2013). For example, fungal infections are responsible for worldwide vertebrate population declines (Blehert et al., 2009; Turner et al., 2011; Fisher et al., 2012; Lorch et al., 2016), especially jeopardizing amphibians (Berger et al., 1998; Lips et al., 2006). Chytridiomycosis is an amphibian infectious disease caused by the waterborne fungus *Batrachochytrium dendrobatidis* (hereafter *Bd*) (Longcore et al., 1999). *Bd* infects epidermal tissues of amphibian hosts, affecting essential physiological processes as osmoregulation (Berger et al., 2005; Van Rooij et al., 2015), which may cause sub-lethal effects (e.g., Bovo et al., 2016, Salla et al., 2015), or death of infected hosts through asystolic cardiac arrest, as a consequence of reductions on blood plasma sodium and potassium concentrations (Voyles, 2009). It has also been noticed that *Bd* produces toxic factors that inhibits lymphocyte proliferation on hosts (Fites et al., 2013).

Chytridiomycosis is associated with massive losses of amphibian populations all over the globe (Olson and

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Ronnenberg, 2014; James et al., 2015; Carvalho et al., 2017). This pathogen has a biphasic life-cycle, with flagellated free-swimming zoospores, often discharged into water bodies as streams and ponds, and sessile zoosporangia with intracellular development (Rosenblum et al., 2010; Greenspan et al., 2012). *Bd* does not survive desiccation due to its aquatic life-cycle (Johnson et al., 2003), but may be carried by non-amphibian species (McMahon et al., 2013), which might contribute to pathogen transmission (Kilburn et al., 2011). Past studies have shown that non-amphibian organisms such as algae (Johnson and Speare, 2003), nematodes (Shapard et al., 2012), crayfishes (Brannelly et al., 2015), reptiles (Kilburn et al., 2011), and waterfowls (Garmyn et al., 2012) also transport *Bd* zoospores. In this sense, besides the studies with amphibian hosts it is essential to increase knowledge about other organisms that may be acting as carriers of this pathogen.

Thus, since there are no *Bd* records for reptiles in South America, here in order to find additional *Bd* potential carriers we swab-sampled reptiles from Brazil's Atlantic Forest and Amazon, both tropical rainforests, but different in terms of *Bd* occurrence and infection intensity (Carvalho et al., 2017; Lambertini et al., 2017). We swabbed reptiles collected from the states of Paraná, São Paulo and Espírito Santo in Brazil's Atlantic Forest, and from the state of Pará in the Brazilian Amazon. We sampled a total of 35 individuals of 11 Squamata families, five from the Atlantic Forest and 30 from the Amazon (Table 1).

We swabbed live individuals throughout their body, ventral and dorsal surfaces, limbs (for lizards), and head (as in Hyatt et al., 2007) to test for the presence of *Bd* zoospores. We then extracted DNA from each swab using Prep-Man Ultra® (Life Technologies) and proceeded with TaqMan® qPCR assay for *Bd* detection and quantification (Boyle et al., 2004; Lambertini et al., 2013). We considered positive results greater than or equal to one zoospore genomic equivalent (GE) (Kriger et al., 2007).

We detected one *Bd* positive individual, *Placosoma glabellum* (Peters, 1870) (currently housed at Museu de Zoologia "prof. Adão J. Cardoso" Universidade Estadual de Campinas: ZUEC-REP 3735), with a load of 3.14 *Bd* zoospore GE. To confirm the positive result, we rinsed this individual in running water for 10 minutes (following the protocols of Rodriguez et al., 2014 and Becker et al., 2016), swabbed the lizard again and run an additional qPCR experiment. In the second run we detected a load of 1.67 zoospore GE.

This is the first report of *Bd* in a reptile from South America. This pathogen has already been detected in lizards and snakes from Panama, also by qPCR detection and without histological analysis, which would confirm whether these reptiles are infected with *Bd* or only act as carriers (Kilburn et al., 2011). Anyway, despite the absence of histological analyses, it is clear that reptiles, including arboreal snakes and semiaquatic or terrestrial lizards, carry *Bd*, contributing to zoospore dispersion

Table 1. Sampled species for *Batrachochytrium dendrobatidis* presence. Number of sampled individuals is in parentheses. Species in bold was positive for *Bd*. Taxonomy follows Costa and Bérnils (2018).

Species	Municipality, State	Biome
Squamata: "Lizards"		
Dactyloidae		
Norops sp.(1)	Belém, Pará	Amazon
Gekkonidae		
Hemidactylus mabouia (1)	Belém, Pará	Amazon
Gymnophthalmidae		
Leposoma scincoides (1)	Santa Teresa, Espírito Santo	Atlantic Forest
***Placosoma glabellum* (2)**	Morretes, Paraná	Atlantic Forest
Phyllodactylidae		
Thecadactylus rapicauda (2)	Belém, Pará	Amazon
Polychrotidae		
Polychrus sp.(1)	Belém, Pará	Amazon
Mabuyidae		
Copeoglossum nigropunctatum (1)	Belém, Pará	Amazon
Sphaerodactylidae		
Chatogekko amazonicus (1)	Belém, Pará	Amazon
Coleodactylus sp. (1)	Belém, Pará	Amazon
Gonatodes sp.(1)	Belém, Pará	Amazon
Lepidoblepharis sp. (2)	Belém, Pará	Amazon
Tropiduridae		
Plica umbra (3)	Belém, Pará	Amazon
Uranoscodon sp. (3)	Belém, Pará	Amazon
Squamata: "Serpentes"		
Boidae		
Boa constrictor (1)	Belém, Pará	Amazon
Dipsadidae		
Dipsas catesbyi (1)	Belém, Pará	Amazon
Erythrolamprus sp. (1)	Belém, Pará	Amazon
Helicops angulatus (2)	Acará, Pará	Amazon
Imantodes cenchoa (2)	Belém, Pará	Amazon
Imantodes lentiferus (1)	Acará, Pará	Amazon
Leptodeira annulata (1)	Belém, Pará	Amazon
Oxyrhopus clathratus (1)	Peruíbe, São Paulo	Atlantic Forest
Thamnodynastes sp. (1)	Dores do Rio Preto, Espírito Santo	Atlantic Forest
Typhlopidae		
Amerotyphlops reticulatus (4)	Belém, Pará	Amazon

across different habitats (Kilburn et al., 2011; present study).

The *Bd*-positive lizard was collected on 9 January 2013, at the Estrada da Graciosa road (PR-410), in the municipality of Morretes, state of Paraná. This site is within the same area where three divergent *Bd* lineages were isolated: *Bd*-Brazil/Asia-2, *Bd*-GPL and a hybrid between them (Schloegel et al., 2012; Jenkinson et al., 2016; O'Hanlon et al., 2018). Besides that, this individual lizard was collected on top of a rock inside a stream, syntopic to an endemic rheophilic frog species, *Cycloramphus rhyakonastes* (Anura: Cycloramphidae) (Nunes-de-Almeida et al., 2016). This is the only known population of this species (Silvano, 2004), and given that *Bd* infected individuals were already recorded [with a prevalence of 55% (10 out of 18 sampled), L.F.T., unpublished data], continuous exchange of *Bd* in this area can facilitate hybridization of the pathogen (Jenkinson et al., 2016), producing some strains that could potentially be hypervirulent (Greenspan et al., 2018), posing a real short-term threat to the endemic *C. rhyakonastes*. Furthermore, *Placosoma glabellum* is a terrestrial small-size lizard with distribution restricted to south and southeast of Brazil (Uetz, 2017). Studies have shown that *Bd* can survive up to seven weeks out of amphibian hosts, specifically in lake water (Johnson and Speare, 2003). Also, it can survive up to three hours on dry bird feathers (Johnson and Speare, 2005). This ability to survive may lead to a greater range of *Bd* zoospore dissemination by other organisms. In this specific case, the *Bd*-positive lizard species could carry *Bd* zoospores from the stream into the forest, potentially transmitting this pathogen to other water bodies, or to direct-developing anurans that lives in leaf-litter inside forested areas, and can be less tolerant to chytridiomycosis than indirect-developing aquatic anurans (Mesquita et al., 2017).

Given that reptiles are more vagile than amphibians, and less restricted to water bodies, they may play an important role in *Bd* dissemination in the wild. In this sense, we highlight the need for more studies attempting to understand how reptiles and other non-amphibian species may contribute to *Bd* transmission dynamics in wildlife.

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