

Spatial distribution of *Batrachochytrium dendrobatidis* in South American caecilians

Carolina Lambertini^{1,*}, C. Guilherme Becker², Cecilia Bardier^{1,3},
Domingos da Silva Leite⁴, Luís Felipe Toledo¹

¹Laboratório de História Natural de Anfíbios Brasileiros (LaHNAB), Departamento de Biologia Animal, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, São Paulo 13083-862, Brazil

²Departamento de Zoologia, Universidade Estadual Paulista, Rio Claro, São Paulo 13506-900, Brazil

³Laboratorio de Sistemática e Historia Natural de Vertebrados, Instituto de Ecología y Ciencias ambientales, Universidad de la República, Montevideo 11400, Uruguay

⁴Departamento de Genética, Evolução e Bioagentes, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, São Paulo 13083-862, Brazil

ABSTRACT: The amphibian-killing fungus *Batrachochytrium dendrobatidis* (*Bd*) is linked to population declines in anurans and salamanders globally. To date, however, few studies have attempted to screen *Bd* in live caecilians; *Bd*-positive caecilians have only been reported in Africa and French Guiana. Here, we performed a retrospective survey of museum preserved specimens to (1) describe spatial patterns of *Bd* infection in Gymnophiona across South America and (2) test whether areas of low climatic suitability for *Bd* in anurans predict *Bd* spatial epidemiology in caecilians. We used quantitative PCR to detect *Bd* in preserved caecilians collected over a 109 yr period, and performed autologistic regressions to test the effect of bioclimatic metrics of temperature and precipitation, vegetation density, and elevation on the likelihood of *Bd* occurrence. We detected an overall *Bd* prevalence of 12.4%, with positive samples spanning the Uruguayan savanna, Brazil's Atlantic Forest, and the Amazon basin. Our autologistic models detected a strong effect of macroclimate, a weaker effect of vegetation density, and no effect of elevation on the likelihood of *Bd* occurrence. Although most of our *Bd*-positive records overlapped with reported areas of high climatic suitability for the fungus in the Neotropics, many of our new *Bd*-positive samples extend far into areas of poor suitability for *Bd* in anurans. Our results highlight an important gap in the study of amphibian chytridiomycosis: the potential negative impact of *Bd* on Neotropical caecilians and the hypothetical role of caecilians as *Bd* reservoirs.

KEY WORDS: Chytrid infection dynamics · Gymnophiona · Life history · Environmental variables · Museum specimens

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INTRODUCTION

Amphibian fungal pathogens of the genus *Batrachochytrium* are linked to global population declines in anurans and salamanders through the infectious disease chytridiomycosis (Berger et al. 1998, Lips et al. 2008, Martel et al. 2013). The amphibian-killing fungus *Batrachochytrium dendrobatidis* (*Bd*) has been acknowledged as one of the most destructive patho-

gens to wildlife (Fisher & Garner 2007, Skerratt et al. 2007), causing population declines in a large fraction of infected species. *Bd* is a host-generalist pathogen among amphibians (Valencia-Aguilar et al. 2015), infecting anurans and salamanders from tropical and temperate regions (Lips et al. 2006, 2008, Vredenburg et al. 2010, Cheng et al. 2011). Reports of *Bd* infection in caecilians (Gymnophiona), however, are rare in the literature. To our knowledge, only 4

reports of *Bd* from wild-caught caecilians are available to date (Doherty-Bone et al. 2013, Gower et al. 2013, Hydeman et al. 2013, Rendle et al. 2015).

The first study screening for *Bd* in caecilians reported 53 *Bd*-positive caecilians out of 85 tested individuals from Cameroon (Doherty-Bone et al. 2013). In the same year, Gower et al. (2013) reported the first case of lethal chytridiomycosis in caecilians from Cameroon and Tanzania. They reported that wild-caught specimens of *Geotrypetes seraphini* that tested positive for *Bd* died in captivity with signs of chytridiomycosis. *Bd* infections were also confirmed in an endemic caecilian (*Schistometopum thomense*) from an island of the archipelago of São Tomé and Príncipe (Hydeman et al. 2013). The only record of *Bd* infecting caecilians outside Africa was recently published for a wild-caught specimen from French Guiana (Rendle et al. 2015). Despite the observed high *Bd* prevalence in African caecilians, sampling efforts to detect *Bd* in Gymnophiona have been made in other regions but without any *Bd*-positive samples from specimens collected in the wild in peninsular Malaysia (n = 2; Savage et al. 2011), Colombia (n = 1; Vásquez-Ochoa et al. 2012), West Africa (n = 6; Penner et al. 2013), and the Seychelles archipelago (n = 78; Labisko et al. 2015). The small sample sizes of these published reports reflect the difficulty and/or lack of effort in working with this generally inconspicuous taxon (e.g. Gower & Wilkinson 2005); thus, it cannot be ruled out that Gymnophiona are infected with *Bd* as extensively as anurans are. Furthermore, conspicuous die-offs due to chytridiomycosis, such as those observed in anurans, are relatively unlikely to be observed in caecilians (Gower et al. 2013) due to their fossorial or fully aquatic life styles (Wells 2007, Vitt & Caldwell 2014). Caecilians are broadly distributed in the tropics (Taylor 1968, Frost 2016), often co-occurring with anuran populations heavily impacted by chytridiomycosis (Seimon et al. 2007, Gower et al. 2012, Bataille et al. 2013, James et al. 2015) and inhabiting microhabitats within *Bd*'s optimal growth conditions of temperature and humidity (Piotrowski et al. 2004). Determining whether caecilians are affected by chytridiomycosis as much as anurans and salamanders are, and whether caecilians are serving as pathogen reservoirs, is relevant for amphibian conservation (Gower & Wilkinson 2005).

Several environmental factors influence *Bd* infection in anurans. Infection prevalence and zoospore loads are often positively correlated with elevation (Brem & Lips 2008, Gründler et al. 2012, Catenazzi

et al. 2013), vegetation density (Puschendorf et al. 2009, Becker & Zamudio 2011, Becker et al. 2016), and precipitation (Becker & Zamudio 2011), and negatively correlated with temperature (Becker & Zamudio 2011, Ruggeri et al. 2015, Becker et al. 2016). Because most caecilians are fossorial, they are likely exposed to lower microclimatic fluctuations dictated by land cover, insolation, and humidity. Therefore, microclimatic optima/averages for caecilians might fall within the optimal growth conditions of *Bd*, allowing *Bd* to persist in areas where it would otherwise not endure year-round.

Here, we performed a retrospective survey of museum preserved specimens to (1) describe spatial patterns of *Bd* infection in Gymnophiona in South America and (2) test whether areas of low climatic suitability for *Bd* in anurans predict spatial epidemiology of *Bd* in caecilians. We used quantitative PCR to detect *Bd* in preserved caecilians collected over a 109 yr period, and performed autologistic regressions to test the effect of bioclimatic metrics of temperature and precipitation, vegetation density, and elevation on the likelihood of *Bd* occurrence. Our results provide novel information on *Bd* spatial epidemiology and suggest that caecilians could be potentially threatened or serve as *Bd* reservoirs in regions where anurans are not infected during most of the year.

MATERIALS AND METHODS

Species sampling

We sampled 193 museum-preserved specimens of Gymnophiona: 160 from Brazil (Caeciliidae, Siphonopidae, and Typhlonectidae) and 33 from Uruguay (Typhlonectidae; Table 1). We screened specimens from 3 out of 4 South American caecilian families housed at the following herpetological collections: Museu Paraense Emílio Goeldi (MPEG), Museu de Zoologia prof. Adão José Cardoso, Universidade Estadual de Campinas (ZUEC), Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ), Coleção de Anfíbios Célio F. B. Haddad, Universidade Estadual Paulista (CFBH), and Colección de Vertebrados de la Universidad de La Republica, Montevideo (ZVCB) (see Table S1 in the Supplement at www.int-res.com/articles/suppl/d124p109_supp.pdf). For standardization purposes, we did not include in our analyses published *Bd* data from wild-caught caecilians from French Guiana, Guyana, and Colombia (Vásquez-Ochoa et al. 2012, Gower et al. 2013, Rendle et al. 2015). We gathered GPS coordinates in

Table 1. Families, species, environment, and country where individual caecilians were captured, and proportion of animals infected by *Batrachochytrium dendrobatidis* (as the number of positives/total number screened). All species were collected in Brazil, apart from *C. indistinctum* (collected in Uruguay)

| Family | Habit | Habitat | Proportion infected |
|---|-----------|--------------------------|---------------------|
| Caeciliidae | | | |
| <i>Caecilia gracilis</i> | Fossorial | Rainforest | 0/1 |
| Siphonopidae | | | |
| <i>Luetkenotyphlus brasiliensis</i> | Fossorial | Rainforest | 3/9 |
| <i>Siphonops annulatus</i> | Fossorial | Rainforest | 1/12 |
| <i>Siphonops cf. annulatus</i> | Fossorial | Rainforest | 1/1 |
| <i>Siphonops paulensis</i> | Fossorial | Rainforest | 2/8 |
| <i>Siphonops cf. paulensis</i> | Fossorial | Rainforest, Grassland | 0/15 |
| <i>Siphonops</i> sp. (aff. <i>paulensis</i>) | Fossorial | Grassland | 0/2 |
| <i>Siphonops hardyi</i> | Fossorial | Rainforest | 0/2 |
| <i>Siphonops</i> sp. | Fossorial | Rainforest, Grassland | 1/7 |
| Typhlonectidae | | | |
| <i>Atretochoana eiselti</i> | Aquatic | Rainforest | 0/4 |
| <i>Chthonerpeton braestrupi</i> | Aquatic | Rainforest | 0/6 |
| <i>Chthonerpeton indistinctum</i> | Aquatic | Grassland | 4/33 |
| <i>Chthonerpeton indistinctum</i> | Aquatic | Rainforest | 0/1 |
| <i>Chthonerpeton noctinetes</i> | Aquatic | Rainforest | 0/8 |
| <i>Chthonerpeton</i> sp. | Aquatic | Rainforest | 0/1 |
| <i>Chthonerpeton tremembe</i> | Aquatic | Grassland | 0/4 |
| <i>Chthonerpeton viviparum</i> | Aquatic | Rainforest | 0/8 |
| <i>Potamotyphlus kaupii</i> | Aquatic | Rainforest | 9/39 |
| <i>Typhlonectes compressicauda</i> | Aquatic | Rainforest | 3/32 |
| Total | | | 24/193 |

decimal degrees for each sampled specimen based on museum data. We used the geographic centroid of municipalities as an approximation when precise geographic coordinates were not available. We did not consider records of *Bd* from captive and live specimens for methodological consistency (e.g. Raphael & Pramuk 2007, Churgin et al. 2013).

***Bd* detection**

Retrospective sampling of museum specimens has been widely used to determine historical dynamics of *Bd* across space and time (Weldon et al. 2004, Ouellet et al. 2005, Soto-Azat et al. 2010, Cheng et al. 2011, Rodriguez et al. 2014, Courtois et al. 2015, Talley et al. 2015, Becker et al. 2016). We swabbed individual specimens on the head, anal disc, and dorsal and ventral surfaces with a single swab per individual, following Rendle et al. (2015), and stored each sample in a 1.5 ml dry sterile tube. We extracted DNA from each swab using 50 µl PrepMan ULTRA® and

proceeded with molecular detection with the TaqMan® qPCR assay (Life Technologies), using strain CLFT 023 as a quantitative standard for the reactions diluted from 10^3 to 10^{-1} zoospore genomic equivalents (GE; Boyle et al. 2004, Lambertini et al. 2013). We considered *Bd*-positive samples with $GE \geq 1$ (Kriger et al. 2007).

Statistical analyses

We described patterns of *Bd* infection in Caecilians from Brazil and Uruguay (proportion of *Bd*-infected individuals \pm binomial 95% CI). We classified species based on their predominant life history (aquatic or fossorial) and reported the proportion (\pm binomial 95% CI) of infected individuals for each life-history category and ecoregion. We also described spatiotemporal patterns of *Bd* infections from 1905 to 2014; 28 specimens lacking information for their collection year were excluded from these calculations.

Furthermore, we conducted multi-model inference using autologistic regressions to test for the effect of bioclimatic variables, vegetation density, and elevation on the likelihood of *Bd* infection while accounting for the effects of spatial autocorrelation (Rangel et al. 2010). For each sampling loca-

tion, we extracted 19 bioclimatic variables of temperature and precipitation averaged over a period of 50 yr (Hijmans et al. 2005), vegetation density (FAO 2010), and elevation, using Arc Map v.10.1 (ESRI). We used a model averaging procedure, including *Bd* as the response variable (presence vs. absence) and the aforementioned environmental factors as explanatory variables. Our model averaging ranked all possible models based on Akaike's information criterion (AIC) and averaged beta coefficients of variables present in 90% of models within $\Delta AIC < 2$. We reported the strength and the direction that each environmental variable influenced *Bd*. We used SAM v4.0 to perform spatial statistics (Rangel et al. 2010).

RESULTS

Our qPCR reactions detected *Bd* in 24 out of 193 screened specimens (12.4%, 95% CI = 0.08–0.17; Fig. 1). Infected individuals belonged to the families



Fig. 1. *Batrachochytrium dendrobatidis* sampling in museum-preserved caecilians from Brazil and Uruguay (positive samples: red circles; negative samples: white circles), and wild-caught caecilians (positive sample: red cross; negative samples: white crosses; Vásquez-Ochoa et al. 2012, Gower et al. 2013, Rendle et al. 2015, present study). *Siphonops paulensis* is a fossorial terrestrial species found in eastern Brazil, and *Chthonerpeton indistinctum* is an aquatic species that is the only caecilian species found in Uruguay. Photographs: Daniel Loebmann. Service layer source: US National Park Service

Siphonopidae ($n = 8$ individuals) and Typhlonectidae ($n = 16$) distributed across the Uruguayan savanna (proportion of infected individuals = 12.1%, 95% CI = 0.03–0.28, $n = 33$ total individuals sampled), the Amazon Basin (16%, 95% CI = 0.08–0.26, $n = 75$), and Brazil's Atlantic Forest (13.5%, 95% CI = 0.06–0.24, $n = 59$). We did not detect *Bd* in individuals of Caeciliidae or in samples from the Brazilian Cerrado, Caatinga, and Pantanal, but our sampling sizes in these ecoregions were small ($n = 2, 22,$ and 2 , respectively). We detected a proportion of infected individuals of 11.7% in aquatic species (95% CI = 0.06–0.18, $n = 136$) and 14% in terrestrial (typically fossorial) species (95% CI = 0.06–0.25, $n = 57$).

Although our sampling spanned 109 yr, most of our *Bd*-positive specimens ($n = 16$) were collected after 1994. Only 5 samples before this period tested positive for *Bd*, and were collected from the wild between 1965 and 1994, and 3 positive samples had no collecting date. However, 21 samples known to have been collected prior to 1971 were screened, and they all tested negative for *Bd*.

Our spatial regression models indicated a significant effect of macroclimate on the likelihood of *Bd* occurrence (Table 2). Our autologistic model averaging showed a negative effect of maximum temperature of the warmest month and precipitation in the wettest quarter, and a positive effect of annual precipitation on *Bd* infection likelihood; the full set of significant variables is provided in Table 2. Vegetation density had a weak negative effect on *Bd* occurrence, and elevation was not a significant variable in our models (Table 2, Table S2).

DISCUSSION

Seasonal variations in temperature and precipitation strongly mediate *Bd* infections by changing optimal physiological conditions of hosts and pathogen (Piotrowski et al. 2004, Becker & Zamudio 2011, Ruggeri et al. 2015). These environmental constraints are revealed in several environmental niche models, indicating that much of South America is unsuitable for *Bd* during at least part of the year

Table 2. Model averaging results ranking significant environmental variables explaining *Batrachochytrium dendrobatidis* occurrence in South American caecilians. Rank importance corresponds to the number of models in which each variable was present (also see Table S2 in the Supplement). CI: confidence interval. Bio 3: isothermality, Bio 5: temperature of warmest month, Bio 9: mean temperature of driest quarter, Bio 10: mean temperature of warmest quarter, Bio 12: annual precipitation, Bio 13: precipitation of wettest month, Bio 14: precipitation of driest month, Bio 15: precipitation seasonality, Bio 16: precipitation of wettest quarter, Bio 17: precipitation of driest quarter, Bio 18: precipitation of warmest quarter, Bio 19: precipitation of coldest quarter

| Variable | Rank importance | Beta coefficient | 95 % CI |
|--------------------|-----------------|------------------|---------|
| Bio 5 | 43 | -0.038 | 0.002 |
| Bio 12 | 32 | 0.003 | 0.001 |
| Bio 16 | 22 | -0.009 | 0.004 |
| Bio 3 | 12 | 0.076 | 0.032 |
| Bio 13 | 11 | 0.039 | 0.011 |
| Bio 17 | 10 | 0.002 | 0.008 |
| Bio 15 | 9 | -0.029 | 0.006 |
| Bio 14 | 9 | -0.027 | 0.023 |
| Bio 18 | 7 | 0.003 | 0.001 |
| Bio 19 | 7 | 0.002 | 0.001 |
| Vegetation density | 6 | -0.011 | 0.003 |
| Bio 10 | 4 | -0.034 | 0.003 |
| Bio 9 | 3 | -0.011 | 0.006 |

(Rödger et al. 2009, Liu et al. 2012, James et al. 2015, Becker et al. 2016). Although most of the *Bd*-positive records overlapped with reported areas of high climatic suitability for the fungus in the Neotropics (Rödger et al. 2009, Liu et al. 2012, James et al. 2015, Becker et al. 2016), several *Bd*-positive records extend far into areas of poor suitability for *Bd* in anurans (e.g. central Amazon: see Becker et al. 2016). Our data points to widespread *Bd* infections in Neotropical caecilians, and that this taxon may serve as an environmental reservoir, perhaps because hosts are able to avoid harsh seasonal extremes where *Bd* would otherwise not persist year-round. These results, combined with a recent report of lethal chytridiomycosis in wild-caught caecilians (Gower et al. 2013), indicate that Gymnophiona are potentially experiencing silent population declines in the wild due to *Bd*.

Spatial regressions are also consistent with the observed associations between macroclimate and *Bd* infection in anuran species (Becker & Zamudio 2011, James et al. 2015, Becker et al. 2016). Specifically, we detected a positive effect of precipitation and a negative effect of temperature variables on the likelihood of *Bd* infection in caecilian hosts. Vegetation

density, which is often positively associated with *Bd* infection in anurans (Raffel et al. 2010, Becker & Zamudio 2011, Becker et al. 2012), showed a weak negative effect on *Bd* in caecilians. This finding might be due to the high degree of fossoriality of terrestrial caecilian species, which spares them from the direct or indirect effects of habitat quality, with downstream shifts in both macro- and microclimates. Elevation, which is often positively associated with *Bd* infection in anurans (Walker et al. 2010, Piovia-Scott et al. 2011, Gründler et al. 2012) due to optimal growth conditions in highlands (Piotrowski et al. 2004), showed no effect on *Bd* in Caecilians. Although our sampling spanned 1000 m in elevation, most of our samples were collected at lower altitudes. This uneven sampling across the elevation gradient may have thus impacted our ability to detect a significant effect of elevation in our analyses. Although large-scale climate may play a role in *Bd* epizootiology of caecilians, these results indicate that infection dynamics in caecilians and anurans might be different.

Although our results suggest that caecilians could act as pathogen reservoirs in environments or periods of harsh microclimatic conditions for *Bd*, limited natural history information is available for most caecilian species (Gower & Wilkinson 2005, Vitt & Caldwell 2014). Basic information on foraging behavior, population densities, and breeding habits that would be key to quantifying transmission dynamics between Gymnophiona and Anura are typically lacking. It is known that 5 out of 10 families of caecilians are found in South America (Wilkinson et al. 2011, Frost 2016), and that these 5 families range from being obligate fossorial to completely aquatic (Haddad et al. 2013, Vitt & Caldwell 2014). We predict that fossorial and fully aquatic species are less likely to be exposed to environmental and climatic fluctuations than terrestrial anurans because they spend longer periods of time underground or underwater. Future studies of caecilian foraging behavior and habitat use may help test the link between habitat use and temperature variability. Lower temperature extremes and variability are linked to higher *Bd* growth and persistence in amphibian hosts both in the wild and in the laboratory (Pounds et al. 2006, Raffel et al. 2013, 2015). Because *Bd* is a waterborne fungus (Longcore et al. 1999, Kilpatrick et al. 2010), we also expect fully aquatic caecilians to be exposed to the pathogen not only during their early life stages. Therefore, fossorial and aquatic life styles observed in caecilians are life history traits that likely make an efficient host reservoir, espe-

cially in areas where *Bd* does not persist in anuran hosts year-round.

In areas of low predicted suitability for *Bd* such as the Amazon basin (Ron 2005, James et al. 2015, Becker et al. 2016), we detected an infection prevalence of 16%, which is surprisingly high compared to the observed ~3% in museum-preserved anurans in this region (Becker et al. 2016). In contrast, the proportion of infected caecilians in the Atlantic Forest was slightly lower than what has been observed for preserved anurans (~23%) in this ecoregion (Rodriguez et al. 2014). Nonetheless, limited sample sizes for caecilians prior to the 1970s precludes us from making any concrete spatio-temporal comparison between *Bd* in caecilians and in anurans from both the Atlantic Forest (Rodriguez et al. 2014) and the Amazon basin (Becker et al. 2016). Although most aquatic caecilians included in this study were sampled from the Amazon basin and most terrestrial caecilians from the Atlantic Forest, we did not detect a significant effect of host life style (aquatic vs. terrestrial) on the likelihood of *Bd* infection. These results further indicate that *Bd* infection dynamics in Gymnophiona might experience a lower pressure from macroclimate than in Anura.

Our results highlight an important gap in the study of amphibian chytridiomycosis: the possible impact of *Bd* on Neotropical caecilians and the hypothetical role of caecilians as *Bd* reservoirs. To date, information on the susceptibility of caecilian hosts to *Bd* infection is still lacking. Therefore, *Bd* genotypes detected in caecilians may present different adaptations to host histophysiology or microclimates, which provides a key opportunity to isolate and genotype new *Bd* isolates from live caecilians and test the virulence of these new isolates in anurans. Because *Bd* has a disproportionately higher impact in tropical amphibians, a better understanding of *Bd* infection dynamics in Gymnophiona may increase our knowledge about chytridiomycosis and advance our conservation efforts in the wild.

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