UNIVERSIDADE ESTADUAL DE CAMPINAS
INSTITUTO DE BIOLOGIA

TAMILIE CARVALHO

DESCONEXÃO DE *HABITAT* E DINÂMICA DO QUITRÍDIO EM ANFÍBIOS DA MATA ATLÂNTICA

HABITAT SPLIT AND CHYTRID DYNAMICS IN AMPHIBIANS FROM THE ATLANTIC FOREST

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Tese apresentada ao Instituto de Biologia da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Ecologia

Thesis presented to the Institute of Biology of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor, in the area of Ecology

Orientador: Prof. Dr. Luís Felipe Toledo Ramos Pereira

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RESUMO

Os anfíbios são o grupo de vertebrados mais ameaçados em todo o mundo. Dentre as principais ameaças, se destacam a perda e fragmentação do *habitat*, doenças infecciosas emergentes e mudanças climáticas - todas resultantes diretas ou indiretamente do crescimento exponencial da população humana. Além disso, essas ameaças podem agir em sinergia, determinando consequências particularmente graves. Na Mata Atlântica, a fragmentação do *habitat* pode ser especialmente impactante para os anfíbios quando perturba a complementação da paisagem entre os diferentes *habitat* necessários para o ciclo de vida de anfíbios bifásicos (reprodutores aquáticos). Esses animais realizam migrações reprodutivas e são forçados a migrar através de ambientes perturbados quando os criadouros aquáticos e os ambientes terrestres estão desconectados. Por outro lado, os anfíbios com desenvolvimento direto completam sua ontogenia inteiramente em ambientes terrestres (reprodutores terrestres) e não devem ser afetados pela desconexão do *habitat*. Criamos um modelo baseado em indivíduo para acessar os efeitos da desconexão do *habitat* em anfíbios com diferentes histórias de vida. Nossos resultados indicam que os anfíbios reprodutores aquáticos tendem a ser mais impactados negativamente pela desconexão do *habitat* do que os anfíbios reprodutores terrestres, o que pode resultar em mudanças significativas na estrutura da comunidade. Em adição aos impactos antropogênicos na paisagem, a Mata Atlântica é um hotspot do fungo *Batrachochytrium dendrobatidis* (Bd), o patógeno causador da quitridiomicose. Embora o fungo Bd esteja coexistindo com anfíbios brasileiros por um longo período, surtos de quitridiomicose foram responsáveis por dezenas de declínios e extinções populacionais de anfíbios na Mata Atlântica. Para acessar os possíveis mecanismos responsáveis pelos surtos de quitridiomicose na Mata Atlântica, em primeiro lugar nós investigamos os efeitos das coinfecções por linhagens distintas de Bd e seu genótipo recombinante. Realizamos um experimento com infecções simples e mistas com cinco genótipos de Bd isolados na Mata Atlântica, e descobrimos que as coinfecções podem alterar drasticamente o potencial de transmissão e a virulência do patógeno. Em segundo lugar, combinamos dados históricos de Bd e clima da Mata Atlântica para acessar como as mudanças climáticas podem alterar a dinâmica da doença. Nossos achados indicam que anomalias da temperatura podem favorecer a emergência de surtos de Bd, entretanto o efeito do clima é dependente da história de vida do hospedeiro. O presente trabalho elucida os efeitos da desconexão do *habitat* para os anfíbios e fornece informações inéditas sobre a dinâmica do fungo quitrídio na Mata Atlântica.

ABSTRACT

Amphibians are the most endangered vertebrate group in the world. Habitat loss and fragmentation, emerging infectious diseases and climate change stand out as the main threats- all resulting directly or indirectly from the exponential growth of the human population. In addition, these threats can act in synergy, with particularly serious consequences. In the Atlantic Forest, habitat fragmentation can be especially impactful for amphibians when it disturbs the landscape complementation between different habitats necessary for the life cycle of biphasic amphibians (aquatic-breeding amphibians). These animals perform reproductive migrations and are forced to migrate through disturbed environments when aquatic and terrestrial environments are disconnected. On the other hand, direct-developing amphibians complete their ontogeny entirely in terrestrial environments (terrestrial-breeding amphibians) and should not be affected by habitat split. We created an individual-based model to assess the effects of habitat split on amphibians with different life histories. Our results indicate that aquatic-breeding amphibians tend to be more negatively impacted by habitat split than terrestrial breeding amphibians, which can result in significant changes in community structure. In addition to the anthropogenic impacts on the landscape, the Atlantic Forest is a hotspot of the fungus *Batrachochytrium dendrobatidis* (Bd), the pathogen that causes chytridiomycosis. Although Bd fungus has been coexisting with Brazilian amphibians for a long time, chytridiomycosis outbreaks were responsible for dozens of amphibian population declines and extinctions in the Atlantic Forest. To assess the possible mechanisms responsible for chytridiomycosis outbreaks in the Atlantic Forest, we first investigated the effects of coinfections by distinct Bd strains and their recombinant genotype. We conducted an experiment with single and mixed infections using five Bd genotypes isolated from the Atlantic Forest, and we found that coinfections can dramatically change the transmission potential and pathogen virulence. Second, we combined historical data of Bd and climate in the Atlantic Forest to assess how climate change can alter disease dynamics. Our findings indicate that temperature anomalies may favor the emergence of Bd outbreaks, however the effect of climate is dependent of the host life-history traits. The present work elucidates the effects of habitat split on amphibians and provides unprecedented information on the dynamics of the chytrid fungus in the Atlantic Forest.

SUMÁRIO

INTRODUÇÃO

Grande parte da perda de biodiversidade contemporânea está relacionada à incapacidade dos táxons de superar a força evolutiva imposta por atividades humanas (Palumbi 2001; Bull & Maron 2016). Por meio de uma combinação de diferentes atividades impactantes (e.g. mudanças climáticas, fragmentação de *habitat*, superpopulação, poluição química, espécies invasoras, superexploração de recursos naturais, movimentação facilitada de patógenos), criamos as condições para uma grave crise de extinção (Barnosky *et al.* 2011; Dirzo *et al.* 2014; Ceballos *et al.* 2015; Johnson *et al.* 2017). Os anfíbios são protagonistas da atual crise de biodiversidade, com 41% das espécies ameaçadas de extinção, um número muito superior ao observado em qualquer outra classe animal (Collins & Storfer 2003, Stuart *et al.* 2004; Chanson *et al.* 2008; Hoffmann *et al.* 2010; IUCN 2021). Os cientistas começaram a se preocupar com o declínio generalizado de populações de anfíbios quando se reuniram em 1989 no Primeiro Congresso Mundial de Herpetologia (Wake 1991, 1998). Após uma fase inicial de debate sobre a existência e quantificação do fenômeno (Pechmann *et al.* 1991), três décadas de pesquisa foram dedicadas a identificar as causas dos declínios de anfíbios. Dentre as principais, se destacam a perda e fragmentação de *habitat*, doenças infecciosas emergentes e mudanças climáticas (Stuart *et al.* 2004; Beebee & Griffiths 2005; Wake & Vredenburg 2008; Catenazzi 2015; Fisher & Garner 2020) - todas ameaças resultantes direta ou indiretamente do crescimento exponencial da população humana (Beebee & Griffiths 2005; Wake & Vredenburg 2008). Além disso, essas ameaças podem agir em sinergia, determinando consequências particularmente graves (Blaustein & Kiesecker 2002; Hof *et al.* 2011; Belasen *et al.* 2019).

A Mata Atlântica se destaca por ser um *hotspot* para a conservação da biodiversidade do mundo (Myers 2003), em especial de anfíbios (Haddad *et al.* 2013). O bioma abriga sozinho 660 espécies desses animais (Luís Felipe Toledo, comunicação pessoal), correspondendo à cerca de 8% da diversidade global (Frost 2021). Entretanto, a Mata Atlântica também abriga 92% das espécies nacionais ameaçadas (DOU 2014) e quase todos os registros de declínios e extinções populacionais de anfíbios do Brasil (Heyer *et al.* 1988; Weygoldt 1989; Eterovick *et al.* 2005; Carvalho *et al.* 2017). Assim como em outras regiões do mundo, a perda e fragmentação de *habitat*, a presença de doenças infecciosas emergentes e as mudanças climáticas representam ameaças potenciais à diversidade dos anfíbios brasileiros (Becker *et al.* 2010; Carvalho *et al.* 2017; Rebouças *et al.* 2021).

De acordo com a Avaliação Global de Anfíbios, a perda e fragmentação do *habitat* são uma grande ameaça para 63% de todas as espécies de anfíbios (Chanson *et al.* 2008). Na Mata

Atlântica, o fenômeno reduziu a vegetação original em mais de 70% (Rezende *et al.* 2018) e grande parte dos fragmentos que restaram, em uma paisagem típica do bioma, são considerados "fragmentos secos", ou seja, não se sobrepõem às zonas ribeirinhas (Becker *et al.* 2010). Esse processo de fragmentação é particularmente comum em *hotspots* de biodiversidade mundiais, onde os assentamentos humanos geralmente se concentram nos vales (locais com maior disponibilidade de água para agricultura, pecuária, indústria e consumo humano), enquanto os remanescentes florestais estão concentrados em encostas íngremes e topos de morros (Silva *et al.* 2007; Mittermeier *et al.* 2011). Este cenário cria uma desconexão induzida pelo homem entre os *habitat* usados por diferentes estágios da história de vida de uma espécie ["Habitat Split": Becker *et al.* (2007); Figura 1], o que pode impactar profundamente as migrações sazonais reprodutivas dos anfíbios (Todd *et al.* 2009).

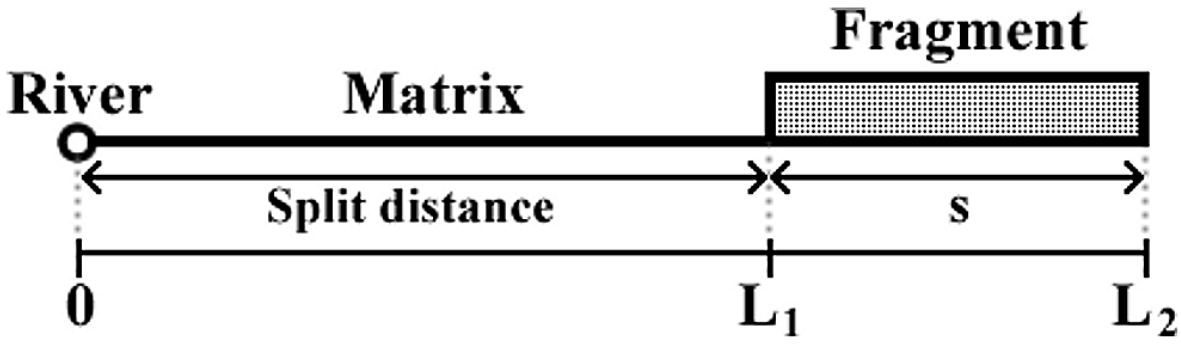

Figure 1. Modelo conceitual da paisagem com desconexão de *habitat*. Fonte: Fonseca *et al.* (2013).

A maioria das espécies de anfíbios tem um ciclo de vida bifásico, com uma fase larval aquática e uma fase terrestre pós-metamórfica [anfíbios reprodutores aquáticos; (Werner & Gilliam 1984)]. Durante o período reprodutivo, os anfíbios reprodutores aquáticos adultos deixam os ambientes terrestres em busca de criadouros, como riachos ou lagoas. Após a reprodução, os adultos e jovens migram para ambientes terrestres. Entretanto, a desconexão de *habitat* força os anuros pós-metamórficos a cruzarem *habitat* inóspitos para completar seu ciclo de vida (Becker *et al.* 2007, 2010), prejudicando as migrações sazonais de centenas de espécies reprodutoras aquáticas. Entretanto, as espécies de anfíbios que completam sua ontogenia inteiramente em ambientes terrestres por meio de (i) desenvolvimento direto dentro do ovo, (ii) desenvolvimento larval endotrófico em câmaras terrestres, ou (iii) aquelas que se reproduzem em cavidades de água de plantas terrestres (fitotelma) (anfíbios reprodutores terrestres), não requerem corpos d'água para completar seu ciclo de vida. Como resultado, em paisagens

fragmentadas os anfíbios reprodutores aquáticos tendem a ser mais impactados negativamente pela desconexão do *habitat* do que os anfíbios reprodutores terrestres, resultando em mudanças significativas na estrutura das comunidades (Becker *et al.* 2007; Fonseca *et al.* 2008). Assim, embora os efeitos da fragmentação de *habitat* nas comunidades de anfíbios sejam complexos, com algumas espécies se beneficiando do aumento de *habitat* abertos ou paisagens mais heterogêneas (Catenazzi 2015), para a maioria das espécies de anfíbios a fragmentação do *habitat* torna-se prejudicial quando perturba a complementação da paisagem, uma vez que os indivíduos devem se deslocar entre diferentes fragmentos da paisagem em busca de recursos críticos (Pope *et al.* 2000; Becker *et al.* 2007).

Recentemente, um modelo teórico foi desenvolvido para prever o impacto da distância dividida (*split distance*; Figura1) - a distância entre os *habitat* terrestres e aquáticos - na demografia da população de anfíbios e na persistência de populações de fragmentos isolados (Fonseca *et al.* 2013). Os autores evidenciaram que existe uma distância crítica máxima entre os *habitat* terrestres e os aquáticos para a persistência da população, e que populações de fragmentos acima dessa distância crítica deverão ser extintas (Fonseca *et al.* 2013). Entretanto, faltam estudos que integrem dados empíricos e modelos demográficos sazonais para prever o impacto da desconexão do *habitat* em anfíbios com histórias de vida contrastantes em paisagens fragmentadas.

Assim como a perda e fragmentação de *habitat*, doenças infecciosas emergentes representam uma grande ameaça à diversidade global de anfíbios (Scheele *et al.* 2019; Fisher & Garner 2020). Dentre as principais doenças que podem acometer os anfíbios, a quitridiomicose se destaca e tem sido alvo de um número crescente de pesquisas nas últimas décadas ao redor do mundo (Fisher *et al.* 2009; Voyles *et al.* 2011; James *et al.* 2015; O'Hanlon *et al.* 2018; Scheele *et al.* 2019; Fisher & Garner 2020). A quitridiomicose é causada pelos fungos patogênicos *Batrachochytrium dendrobatidis* (Bd), descoberto há pouco mais de 20 anos (Berger *et al.* 1998; Longcore *et al.* 1999), e *Batrachochytrium salamandrivorans* (Bsal), descoberto em 2010 (Martel *et al.* 2013). Até o momento, a doença foi associada ao declínio de mais de 500 espécies de anfíbios, incluindo 90 extinções (Scheele *et al.* 2019) (Figura 2).

A mortalidade dos anfíbios devido a quitridiomicose geralmente ocorre após eventos epizoóticos dos patógenos recém-introduzidos em populações que não tiveram contato prévio com o fungo, o que suporta a hipótese da disseminação do novo patógeno (Lips et al. 2008). Entretanto, epizootias de quitridiomicose, possivelmente devido a fatores exógenos, ocorreram em regiões onde o fungo já estava presente, apoiando a hipótese do patógeno endêmico (Goka et al. 2009, Vredenburg et al. 2013, Carvalho et al. 2017, Toledo 2017). Este parece ser o caso

da Mata Atlântica brasileira, onde a quitridiomicose possivelmente causou dezenas de declínios e extinções populacionais de anfíbios (Carvalho *et al.* 2017) quase um século após a detecção do patógeno Bd no bioma (Rodriguez *et al.* 2014).

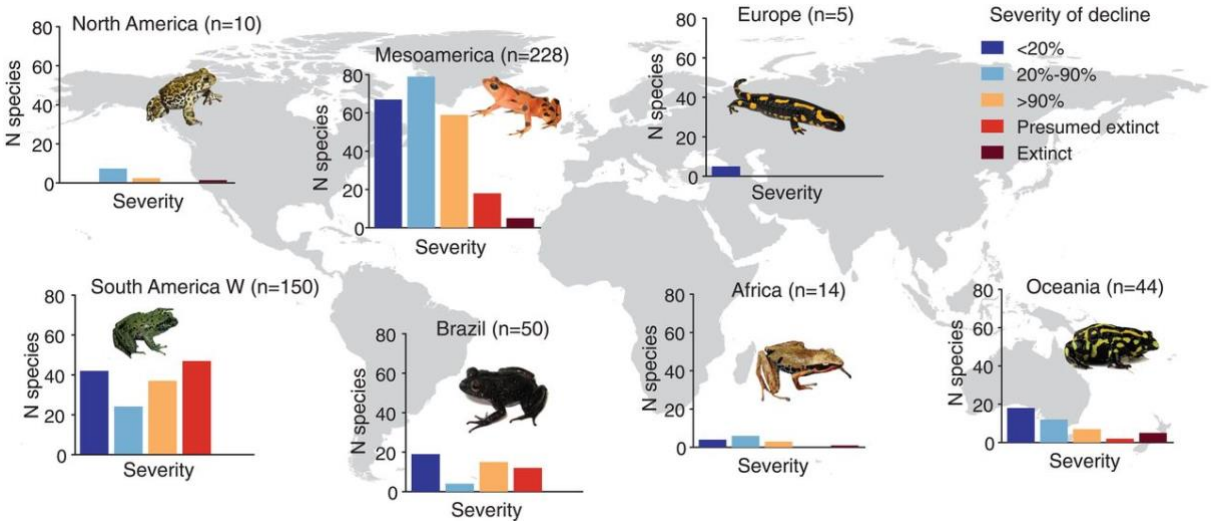

Figura 2. Distribuição mundial dos declínios e extinções populacionais de anfíbios decorrentes da quitridiomicose no mundo. Fonte: Scheele *et al.* (2019)

O fungo Bd é um patógeno cosmopolita (Bower *et al.* 2017) e generalista (Olson & Ronnenberg *et al.* 2014, Scheele *et al.* 2019), capaz de infectar centenas de espécies hospedeiras. O Bd infecta a pele dos anfíbios causando hiperplasia e hiperceratose epidérmica, o que interrompe funções fisiológicas essenciais, como equilíbrio osmótico, transporte de eletrólitos e respiração (Voyles *et al.* 2011). Por sua vez, o Bsal possui registros de ocorrência apenas na Europa e Ásia (Martel *et al.* 2013, 2014; Bower *et al.* 2017; Lötters *et al.* 2018) e ainda não está claro até que ponto o patógeno é capaz de infectar uma ampla gama de hospedeiros anfíbios (Martel *et al.* 2013). Entretanto, o Bsal é extremamente letal e sua patogenicidade envolve lesões erosivas multifocais e ulcerações profundas na pele de salamandras (Martel *et al.* 2013, 2014).

Tanto o Bd quanto o Bsal, apresentam em seus ciclos de vida um zoósporo de vida livre nadante em sua fase infectante que se transformam em zoosporângios sésseis quando colonizam o hospedeiro (Longcore *et al.* 1999; Martel *et al.* 2013; James *et al.* 2015) (Figura 3). Após um período de mobilidade (~24 horas), o zoósporo de Bd se liga a superfície da pele do anfíbio, retrai o flagelo e transfere seu conteúdo para o tecido do hospedeiro através de um tubo germinativo que penetra a membrana celular do hospedeiro (Berger *et al.* 2005; Greenspan *et al.* 2012). Em seguida, ocorre a maturação do zoosporângio e formação de pelo menos uma papila de descarga para liberação dos zoósporos recém-formados, completando o ciclo quatro

ou cinco dias após a penetração do zoósporo (Berger *et al.* 2005; Greenspan *et al.* 2012). Desse modo, os zoósporos são responsáveis pela transmissão da doença, podendo nadar e infectar novos hospedeiros ou reinfectar o próprio hospedeiro aumentando a carga do patógeno, uma situação possivelmente comum em ambientais com pouca disponibilidade de água para a dispersão dos zoósporos (Ruggeri *et al.* 2018) (Figure 3). Os mecanismos envolvidos na penetração e infecção pelo Bsal ainda não são conhecidos.

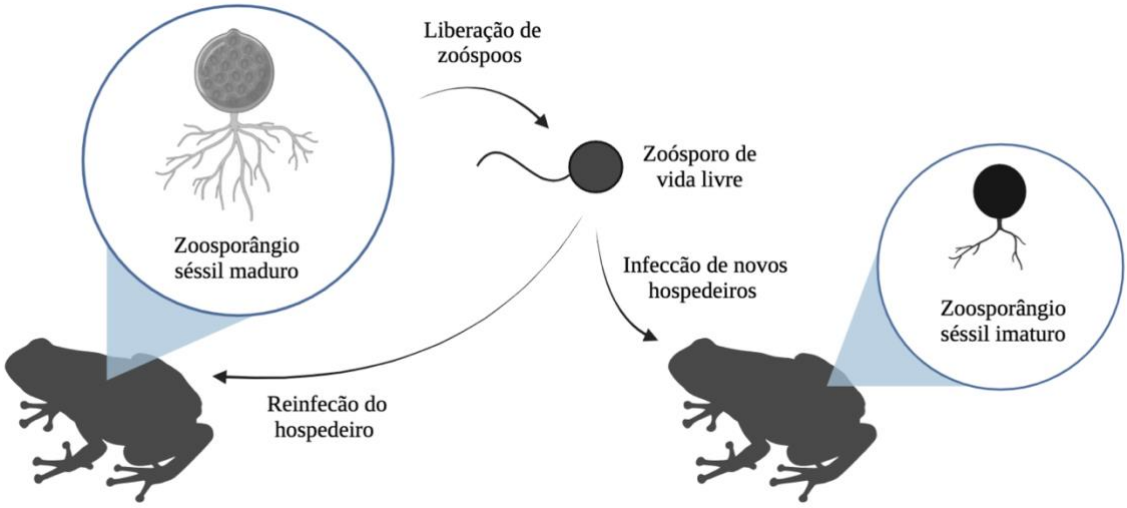

Figure 3. Esquema simplificado do ciclo de vida do Bd. Criado em BioRender.com

O fungo Bd é sensível à temperatura e sua patogenicidade é dependente dessa variável (Voyles *et al.* 2017). Estudos demostram que o Bd tem um crescimento termal ótimo "in vitro" entre 17° C e 23° C, mas pode crescer bem entre 2° C e 28° C e tolerar variações extremas de temperatura (Piotrowski *et al.* 2004; Woodhams *et al.* 2008; Voyles *et al.* 2017). Entretanto, o padrão de crescimento de diferentes genótipos do fungo em cultura pode variar (Voyles *et al.* 2011), assim como um mesmo genótipo pode apresentar profundas divergências de crescimento quando em interação com seu hospedeiro (Cohen *et al.* 2017; Sonn *et al.* 2017). Ao que sabemos, o Bsal tem um crescimento termal ótimo entre 17° C e 23° C e não resiste a temperaturas iguais ou superiores a 25° C, o que poderia explicar sua distribuição geográfica mais restrita (Martel et al. 2013).

Além da temperatura, a virulência (amplamente definida como dano induzido por patógenos) do Bd e Bsal em hospedeiros infectados frequentemente é contexto dependente de diversos fatores (Fisher & Garner 2020) (Figura 4) e pode variar de leve ou nenhum sintoma à morte (Firura 4). Fatores abióticos (e.g. temperatura, precipitação, sazonalidade e elevação), bióticos (e.g. características da história de vida do hospedeiro, tolerância da espécie, presença

de reservatórios, diversidade da comunidade, microbioma da pele do hospedeiro, coinfecções e diversidade da comunidade) e fatores genéticos intrínsecos dos fungos (e.g. proteases e fatores imunossupressores) podem influenciar e tornar complexo o desfecho da doença (Fisher & Garner 2020).

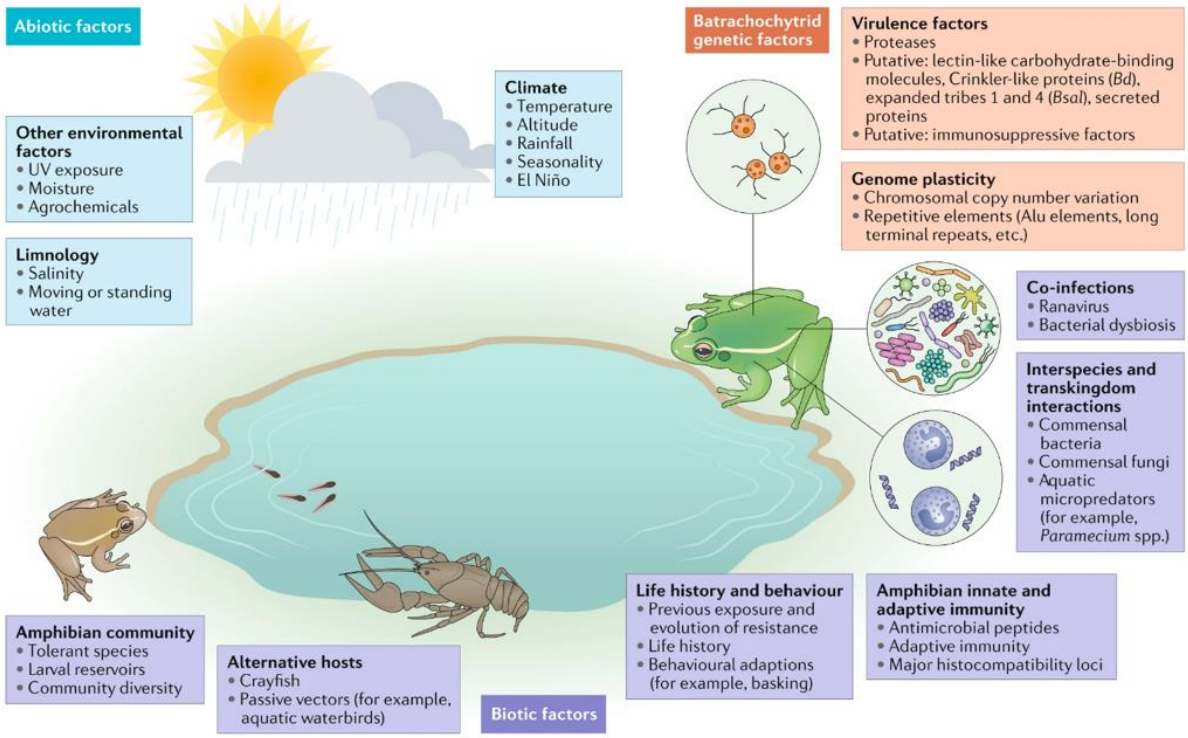

Figura 4. Fatores que influenciam a virulência dos fungos *Batrachochytrium dendrobatidis* e *Batrachochytrium salamandrivorans.* Fonte: Fisher *et al.* (2020).

Existem atualmente cinco linhagens de Bd identificadas (O'Hanlon *et al.* 2018; Byrne *et al.* 2019) (Figura 5), que potencialmente se originaram na Ásia e, posteriormente, se espalharam por todo o mundo - provavelmente através do comércio de anfíbios (Schloegel *et al.* 2012; O'Hanlon *et al.* 2018; Byrne *et al.* 2019). Essas diásporas emergentes de Bd incluíram a linhagem panzoótica global virulenta e infecciosa (Bd-GPL), a última a emergir da Ásia e aquela associada a declínios e extinções populacionais em todo o mundo (Farrer *et al.* 2011; Lips 2016; Scheele *et al.* 2019). A disseminação da linhagem Bd-GPL pelo mundo levou à coocorrência dessa linhagem panzoótica com linhagens pré-existentes que ocorrem em um estágio de infecção enzoótica em algumas regiões, o que causou a incidência de infecções mistas na população hospedeira e eventual troca genética entre linhagens coinfectantes (Schloegel *et al.* 2012; Jenkinson *et al.* 2016; O'Hanlon *et al.* 2018; Byrne *et al.* 2019). Por exemplo, na Mata Atlântica, uma invasão precoce por Bd já em 1894 (Rodriguez *et al.* 2014), presumivelmente pela linhagem enzoótica putativa Bd-Asia-2 / Brasil (Jenkinson *et al.* 2016),

foi seguida por introduções de Bd-GPL e pelo surgimento de três genótipos Bd recombinantes (Schloegel *et al.* 2012; Jenkinson *et al.* 2016), tornando um dos lugares mais diversos de anfíbios do mundo um *hotspot* do patógeno Bd (Figura 5). De forma alarmante, um dos genótipos recombinantes (CLFT 024-02) foi determinado como mais virulento do que suas linhagens parentais ao infectar anfíbios nativos da Mata Atlântica (Greenspan *et al.* 2018).

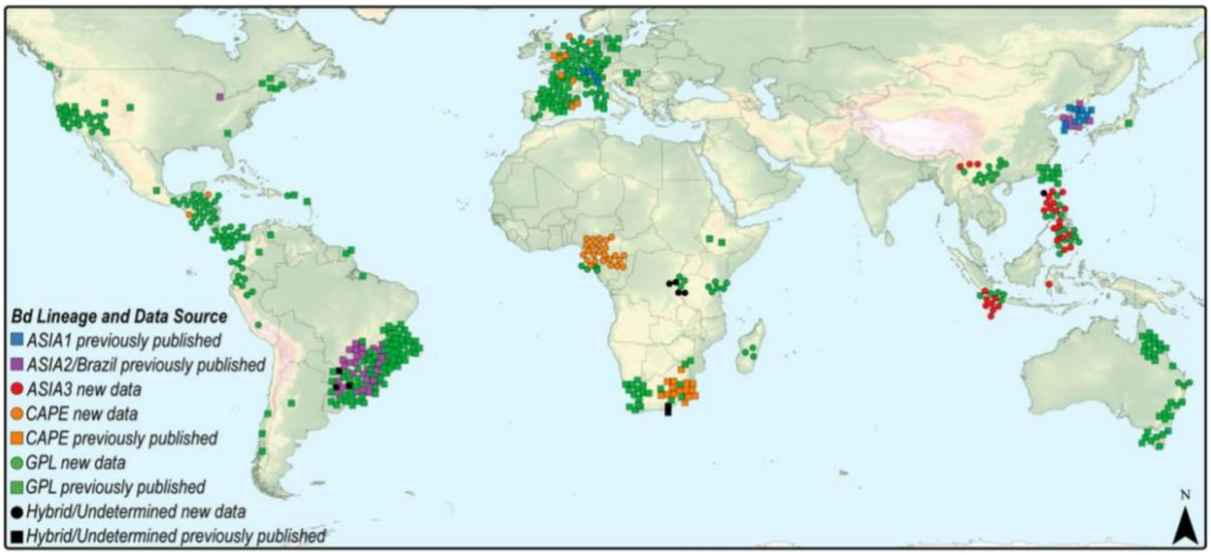

Figura 5. Distribuição das linhagens de Bd conhecidas. Fonte: Byrne *et al.* (2019).

Além da emergência de genótipos recombinantes altamente virulentos, as coinfecções podem alterar a dinâmica dos patógenos dentro do hospedeiro ao adicionar pressões seletivas sobre os patógenos coinfectantes (Alizon *et al.* 2013), cujas interações de competição, facilitação ou neutras, juntamente com os fatores do hospedeiro, influenciam o desfecho da doença (Seppälä *et al.* 2009, 2012). Nesse contexto, a "virulência geral" do hospedeiro coinfectado pode superar ou estar abaixo da virulência dos patógenos mais e menos virulentos, respectivamente, ou assumir algum nível intermediário (Alizon *et al.* 2013). Além disso, esta variação na virulência associada a coinfecções pode afetar diretamente os potenciais de transmissão dos patógenos e a frequência dos genótipos na população (Mideo *et al.* 2008). Finalmente, a competição intraespecífica entre os patógenos dentro do hospedeiro pode selecionar genótipos mais agressivos e virulentos (de Roode *et al.* 2005; Zhan & McDonald 2013). Assim, em um mundo globalizado, em que a movimentação de genótipos é facilitada pelo homem, a coinfecção por genótipos do patógeno Bd previamente isolados podem desempenhar fortes consequências para a ecologia de doenças e evolução da virulência do fungo quitrídio (Hamilton 1972; Nowak & May 1994; Mideo *et al.* 2008; Fisher *et al.* 2012; Alizon *et al.* 2013).

Recentemente, Jenkinson et al. (2018) realizaram um experimento de coinfecção com genótipos de Bd previamente isolados da Mata Atlântica. No estudo, o genótipo Bd-GPL foi competitivamente superior ao genótipo Bd-Asia-2 / Brasil dentro do hospedeiro, o que sugere uma possível exclusão da linhagem enzoótica por Bd-GPL à medida que se espalhou por todo o bioma (Jenkinson *et al.* 2018). No entanto, este foi o único estudo que investigou os efeitos de coinfecções por linhagens distintas de Bd em termos de competição intraespecífica e desfecho da doença, permanecendo escasso o nosso entendimento sobre o efeito de infecções mistas por Bd que incluem variação de linhagem e genótipos recombinantes.

Em adição aos efeitos possivelmente catastróficos das infecções mistas por Bd e a emergência de genótipos de Bd recombinantes, o efeito sinergético entre mudanças climáticas e a quitridiomicose pode agravar ainda mais a crise da biodiversidade dos anfíbios. Como as condições climáticas frequentemente são fatores-chave no desfecho de doenças (Harvell *et al.* 2002; Altizer *et al.* 2013), um número crescente de surtos de quitridiomicose e declínios de anfíbios concomitantes com eventos climáticos anômalos foram reportados nas últimas décadas (Rohr & Raffel 2010; Cohen *et al.* 2019). Esse cenário levou muitos pesquisadores a sugerirem uma relação causal entre as mudanças climáticas e o surgimento de doenças (Pounds *et al.* 2006; Bosch *et al.* 2007; Rohr & Raffel 2010; Cohen *et al.* 2019). No entanto, poucos estudos esclareceram os mecanismos subjacentes dos efeitos do ambiente na interação entre patógeno e hospedeiro (Rohr & Raffel 2010; Cohen *et al.* 2017).

Pounds *et al.* (2006) foram os primeiros a sugerirem uma relação entre quitridiomicose e mudanças climáticas. Os autores propõem que os surtos de quitridiomicose nas montanhas de Monteverde, na Costa Rica, que levou dezenas de espécies de *Atelopus* sp. ao declínio, foram causados pelo aquecimento das temperaturas oceânicas. Especificamente, o aumento da temperatura favoreceu o aumento da cobertura de nuvens nas montanhas, o que convergiu em temperaturas ótimas para o crescimento do fungo [hipótese da temperatura ótima para o quitrídio: Pounds *et al.* (2006)]. Entretanto, o estudo recebeu fortes críticas por Rohr *et al.* (2008), que argumentaram que os achados de Pounds *et al.* (2006) eram casuais devido à falta de controle temporal nas análises estatísticas. Na sequencia, Rohr & Raffel (2010) propuseram que as perdas de anfíbios do gênero *Atelopus* em Monteverde foram causadas por eventos climáticos globais do *El Niño* que aumentaram a variabilidade da temperatura, o que pode ter reduzido as defesas dos anfíbios contra o patógeno Bd. Finalmente, uma perspectiva mais mecanicista para elucidar os efeitos do clima antrópico na dinâmica da doença em hospedeiros ectotérmicos foi sugerida por Cohen et al. (2017).

A hipótese de incompatibilidade térmica (“thermal mismatch”) propõe que o efeito das anomalias climáticas da temperatura na dinâmica da doença depende dos traços da história de vida do hospedeiro (Cohen *et al.* 2017). Especificamente, a hipótese sugere que os hospedeiros ectotérmicos adaptados a climas mais frios correm maior risco de doenças em climas mais quentes e vice-versa. A hipótese prevê que hospedeiros e patógenos são adaptados localmente, e que patógenos, sendo menores que seus hospedeiros, têm tolerâncias térmicas mais amplas e se adaptam ou se aclimatam mais rapidamente que seus hospedeiros (Baas-Becking 1934; Rohr *et al.* 2018). Assim, os patógenos podem manter um alto desempenho em uma faixa mais ampla de temperaturas, e surtos de doenças são esperados quando o desempenho do hospedeiro é relativamente baixo, enquanto o patógeno permanece alto. A hipótese de incompatibilidade térmica foi testada pela primeira vez usando o sistema patógeno Bd e hospedeiro anfíbio (Cohen *et al.* 2017), e mais tarde foi apoiada por vários experimentos e análises de campo em escala global de diversos sistemas patógenos hospedeiros (Cohen *et al.* 2017, 2019, 2020). No entanto, estudos em escalas menores de comunidades com alta diversidade ainda são necessários para melhorar e mostrar a consistência da teoria.

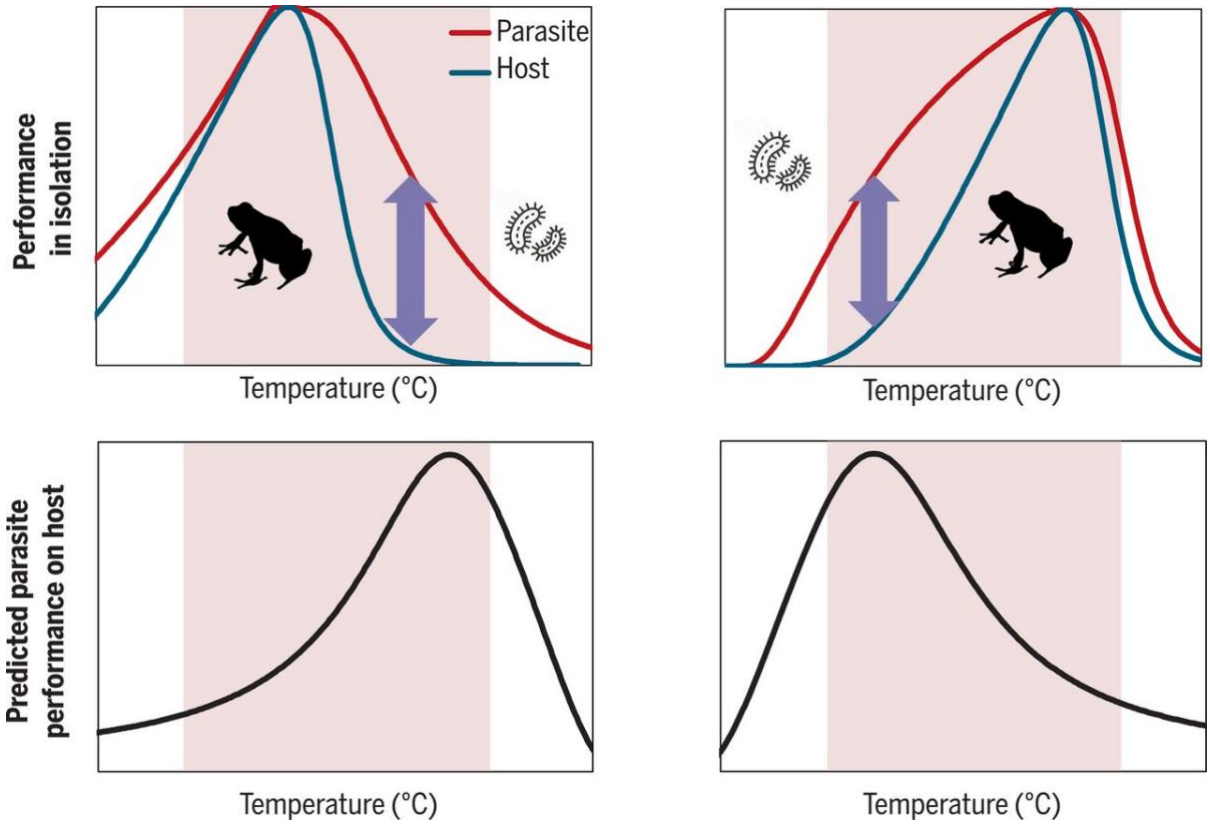

Figura 6. Previsão do desempenho térmico do hospedeiro e do parasita isoladamente adaptados a climas frios (superior esquerda) e quentes (superior direita) versus padrões de interações parasita-hospedeiro em clima frio (inferior esquerda) e quente (inferior direita). Fonte: Cohen *et.al* (2020).

Dado o contexto, o presente estudo teve como objetivos:

1. Criar um modelo baseado em indivíduos (MBI) para quantificar os efeitos da desconexão do *habitat* em anfíbios reprodutores aquáticos e terrestres e integrar com dados empíricos;
2. Compreender como a coinfecção por genótipos de Bd de duas linhagens distintas e seu genótipo recombinante presentes na Mata Atlântica afetam a virulência e o potencial de transmissão dos genótipos;
3. Testar a hipótese da incompatibilidade térmica na prevalência de Bd em hospedeiros da Mata Atlântica.

CAPÍTULO 1. Linking deforestation, breeding migrations, and population persistence in tropical amphibians using empirical field data and individual-based models

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Author contributions statement

T.C. executed model experiments, analyzed the data, and wrote the manuscript. C.G.B. conceived the idea and coordinated statistical analyses and writing. T.W. developed the

mathematical models. L.A.A., L.O.M.G. and C.F.B.H. contributed with field work. L.F.T participated in interpreting the data and helped with writing. All authors critically revised the manuscript, provided editorial advice and approved it for publication.

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ABSTRACT

Most aquatic-breeding amphibians have a biphasic life cycle, with an aquatic larval phase and a post-metamorphic terrestrial phase. To complete their life cycle, many aquatic-breeding amphibian species migrate between aquatic and terrestrial environments. However, discontinuity between terrestrial and aquatic habitats (habitat split) caused by several anthropogenic practices forces several species to migrate through disturbed environments, ultimately leading to population declines. Conversely, terrestrial-breeding amphibians complete their ontogeny entirely in terrestrial environments and should not be affected by the habitat split. Therefore, amphibians with different life histories should vary in their responses to habitat change. We analyzed field data and built seasonal Individual-Based Models (IBMs) to test and model the impact of habitat split on amphibian species with different life histories. We applied our IBM framework to a landscape of Brazil's Atlantic forest and to a series of hypothetical landscapes with varying levels of habitat split. Empirical field data and IBM results point to proportionally lower population size and occupancy of aquatic-breeding amphibians in areas with high habitat split. These findings are exacerbated under scenarios of larger split distances and low amphibian dispersal capacity. Interestingly, intermediate and high split distances combined with high dispersal rates predicted a reduction in population sizes in aquatic-breeding species. Dispersal ability and habitat split did not predict population size and occupancy of terrestrial-breeding

amphibians according to our IBMs, matching observed field patterns. Therefore, our IBM approach could facilitate conservation efforts for frogs of the Atlantic forest.

KEYWORDS

Dispersal, habitat split, life history, amphibian decline, fragmentation, Amphibia, Anura

INTRODUCTION

The ability of animals to disperse is a key factor for population persistence (Dingle & Drake, 2007). The basic driving forces for seasonal migrations are ecological and biogeographical, such as spatiotemporal distribution of resources, habitat quality, predation, and competition (Alerstam, Hedenström, & Åkesson, 2003). For instance, arctic terns fly an impressive 80,000 kilometers annually between the Arctic and Antarctic in search of more suitable habitats, maximizing their survival and reproductive success (Egevang et al., 2010). Seasonal migrations are also associated with movement and allocation of resources. Salmon spawning transfers high amounts of biomass each year from oceanic to terrestrial ecosystems, which contributes to food web stability (Gresh, Lichatowich, & Schoonmaker, 2000). Such movements affect communities and trophic structure, ultimately conditioning ecosystem functioning (Bauer & Hoye, 2014). Thus, seasonal migrations play a key role in ontogenetic development and animal population viability, but accelerated anthropogenic habitat change has been increasingly disrupting seasonal migrations in vertebrates (Robinson, Wilson, & Crick, 2001; Wilcove & Wikelski, 2008; Robinson et al., 2009; Lennox et al., 2016).

Most amphibian species have a biphasic life cycle, with an aquatic larval phase and a post-metamorphic terrestrial phase [hereafter aquatic-breeding amphibians;

(Werner & Gilliam, 1984)]. During the reproductive period, adult aquatic-breeding amphibians leave terrestrial environments in search of breeding sites such as streams or ponds. After reproduction, adults and juveniles of the year migrate to overwintering terrestrial environments. However, anthropogenic habitat disturbances can deeply impair seasonal migrations in amphibians (Todd et al., 2009) when aquatic breeding sites and remnants of natural terrestrial vegetation are disconnected through agriculture, livestock or any other man-induced interference. This discontinuity between required habitats by different amphibian life stages (termed habitat split) forces several species to migrate through disturbed environments to overcome man-made disruption to landscape complementation (Pope, Fahrig, & Merriam, 2000), ultimately leading to population declines (Becker et al., 2007, 2010). Conversely, species that complete their ontogeny entirely in terrestrial environments either through (i) direct development within the egg, (ii) endotrophic larval development in terrestrial chambers, or (iii) those that reproduce in water cavities of terrestrial plants (phytotelma) (hereafter terrestrial-breeding amphibians) do not require water bodies to complete their life cycle. As a result, aquatic-breeding amphibians tend to be more negatively impacted by habitat split than terrestrial-breeding amphibians, leading to significant shifts in community structure (Becker et al., 2007; Fonseca et al., 2008).

Habitat fragmentation may have negative or positive effects on abundance and species richness of ecological communities (Fahrig et al., 2019). For amphibians, habitat fragmentation becomes detrimental when it disrupts landscape complementation (Pope, Fahrig, & Merriam, 2000) and exacerbates habitat split (Becker et al., 2007). Fragmented landscapes of topographically complex tropical regions often suffer with high habitat split. In such places, human settlements are generally concentrated in the valleys where water is available for agriculture, livestock, industry, and human

consumption. In this scenario, forest remnants are concentrated on steep slopes and hilltops (Silva et al., 2007). A large fraction of forest fragments of Brazil's Atlantic forest does not overlap with riparian zones (Ribeiro et al., 2009; Becker et al., 2010). This scenario impairs seasonal migrations of hundreds of species of aquatic-breeding frogs, forcing post-metamorphic anurans to cross inhospitable habitats in order to complete their life cycle (Becker et al., 2007, 2010). A theoretical model has recently been developed to predict the impact of split distance – the distance between the terrestrial and aquatic habitats – on population demographics (Fonseca et al., 2013), but lacking are studies integrating empirical field data and seasonal demographic models to forecast the impact of habitat split on amphibians with contrasting life histories and across fragmented landscapes.

Here, we employed an Individual-Based Model (IBM) framework to quantify the effects of habitat split on both aquatic-breeding and terrestrial-breeding amphibians. We applied our model framework to (i) a fragmented landscape in Brazil's Atlantic forest and (ii) a series of hypothetical landscapes with varying levels of habitat split. We parameterized our model framework with available natural history information and validated it with results from our field data. We hypothesized that aquatic-breeding amphibians would be more negatively impacted by split distance and dispersal probability than terrestrial-breeding amphibians. Specifically, we predicted that our models would mirror results from empirical field data showing reduced abundance (and diversity) of forest-associated, aquatic-breeding amphibian species in a landscape with high levels of habitat split. Conversely, we predicted to find stable occupancy rates and population sizes of terrestrial-breeding amphibians in fragments of natural vegetation, independent of their dispersal or levels of habitat split. Combined, our results integrating field and modeling data emphasize that amphibian life history, dispersal, and

habitat split, when combined, are good proxies to estimate population persistence in the wild.

METHODS

Focal landscape, field sampling, and amphibian communities

The focal Atlantic forest landscape (hereafter focal landscape) encompasses a severely disturbed region (hereafter fragmented forest) and an adjacent area of pristine forest (hereafter continuous forest) located in the municipality of São Luiz do Paraitinga, state of São Paulo, Brazil (23º 13' S, 45º 18' W; Fig. 1a). The fragmented forest is dominated by a matrix of pastures and other non-natural vegetation classes that cover the most accessible, irrigated, flat, and fertile areas for agriculture. Forested areas cover ~12 % of this section of the landscape, primarily in small, natural forest fragments. The majority of forest fragments are also disconnected from permanent water bodies such as streams. Most forest fragments are located primarily at higher-elevation (mean elevation of forest fragments 844 m [± 8.0 s.e.]; mean elevation of non-natural matrix vegetation 816 m [± 7.7 s.e.]: Becker et al., 2010). A protected continuous forest (Parque Estadual da Serra do Mar - Núcleo Santa Virginia) covers the eastern portion of the focal landscape (Viana, Tabanez, & Batista, 1997; Morellato & Haddad, 2000; Fig. 1). Riparian zones and associated aquatic habitats, such as streams, ponds, and swamps, are the main breeding sites for aquatic-breeding amphibians. Natural forest understory and leaf-litter are the main breeding sites for terrestrial-breeding amphibians; thus, these species can breed in any remnant of natural forest with suitable microclimate conditions (Haddad et al., 2013).

Data on forest-associated leaf-litter frogs in the focal landscape were obtained with pitfall traps between November 2004 to February 2006 (Anjos, 2008; Giasson,

2008; Becker et al., 2010). Because of the limited ability of pitfall traps to sample treefrogs (Hylidae, Centrolenidae and Hemiphractidae) we did not include treefrogs in our analyses; we focused only on leaf-litter amphibian species. We binary-classified each species developmental type following the 39 reproductive modes of Neotropical anurans classified by Haddad et al. (2013). Leaf-litter amphibian species with reproductive modes that complete their ontogeny entirely in terrestrial environments either through direct development within the egg, endotrophic larval development in terrestrial chambers or in phytotelma were classified as terrestrial-breeding amphibians (*Tb*). Species that require an aquatic habitat to complete their development (*e.g.*, streams, ponds, marshes) were classified as aquatic-breeding amphibians (*Ab*). The historical fragmentation of Brazil's Atlantic forest facilitated range size expansion of several open-habitat specialist amphibians. For the purpose of this study we only focus on Atlantic forest endemic amphibians that evolved in natural forest environments.

Focal and hypothetical landscapes

We used a land-cover classification based on data from the year 2002 (5-m resolution SPOT image) and hydrographic maps (IBGE 1:50.000) to characterize land cover (natural *vs.* non-natural), habitat connectivity, and discontinuity between natural forest and riparian habitats (habitat split) in the focal landscape. We generated an ASCII raster grid with 50 x 50 m pixels categorized as: 1 – riparian habitat in disturbed vegetation (matrix), 2 – riparian habitat in natural forest, 3 – disturbed vegetation, and 4 – natural forest. In addition to the focal landscape, we designed four hypothetical landscapes in a gradient of split distance using ArcGis® 10.6.1 software, intellectual property of ESRI (Fig. 2a). Specifically, the hypothetical landscapes were designed with split distance gradients of 0 pixel (0P, no habitat split), 1 pixel (1P, hereafter low split distance), 2

pixels (2P, intermediate split distance), and 3 pixels (3P, high split distance) (Fig. 2b). In order to exclude habitat loss effect, we standardized the width of riparian zone to two pixels and forest fragments to eight pixels on these landscapes.

Individual-Based Model and parameterization

We designed and implemented an Individual-Based Model (IBM) to simulate the movement and reproduction of a population of frogs with different developmental modes over time in the focal and hypothetical landscapes. Although we were unable to implement species-specific IBMs for the species we sampled in the field due to limited life history information, we ran IBMs independently for both life-histories: aquatic-breeding (*Ab*) and terrestrial-breeding (*Tb*), based on general information on development and breeding habitats obtained by members of our team (Becker, 2007; Anjos, 2008; Giasson, 2008; Haddad et al., 2013). The IBM was implemented in Java, with a Perl wrapper to simplify the running of replicate simulations. Habitat was modeled using an ASCII raster grid imported from ArcGIS 10.5.1. Values were assigned to cells to designate one of four different habitat types described above. In the focal landscape, half of the grid was deemed to be 'continuous' habitat and the other half 'fragmented' habitat (see Fig. 1a). In the IBM, the different habitat types were associated with the same carrying capacities and different density-independent deaths, numbers of offspring during the breeding season, and probabilities of moving away from that cell during two seasons (breeding/non-breeding). Individuals had the characteristics age, sex, and x and y position in the habitat grid.

The IBM was initialized by uniformly seeding the habitat with five individual amphibians per cell, with sex ratio assigned with 50/50 probability and with age drawn uniformly from zero to the maximum defined longevity of three years. The simulation

was run for 30 years, with each year being divided into 12 time steps (months) in loop. To maximize ecological realism, a breeding season was defined to run from time step 4 to 7 (Haddad et al., 2013), with time steps outside this window being the non-breeding season. For each time step, individuals aged, dispersed, underwent density-dependent and -independent deaths, or died when they reached maximum longevity.

For aquatic-breeding amphibians (*Ab*), dispersal probability and direction depended on the season. A friction matrix was implemented in the IBM to induce dispersal of individuals towards aquatic habitat pixels during time steps comprising the breeding months (time steps 4 to 7), and towards natural forest pixels during time steps comprising the non-breeding season (time steps 8 to 3). Because the focal terrestrial-breeding amphibians (*Tb*) do not rely on breeding migrations, show limited dispersal capabilities (Becker, 2007), and require natural forest vegetation in order to complete their life cycle (Haddad et al., 2013), a friction matrix was implemented for the IBM to favor dispersal of *Tb* individuals towards forest pixels during the 12 time steps.

Dispersal was allowed to any of the eight surrounding cells, except to pixels at the edge of the matrix (*i.e.,* the simulation had reflecting boundaries). If females were in suitable breeding habitat during the breeding season, they were of breeding age, and males were also present in the same cell, then breeding occurred. The number of offspring per female was drawn from a Poisson distribution with a mean dependent on the habitat type. Sex of offspring was assigned with 50/50 probability. For each time step, the number of *Ab* and *Tb* individuals in each cell was counted. The IBM allowed us to keep track of the number of individuals over time in both fragmented and continuous sections of the grid.

For each landscape, we ran models independently for both *Ab* and *Tb* with five dispersal probabilities (20, 40, 60, 80, and 100 %); *i.e.*, probability of monthly dispersal

from its pixel to a neighboring one. Each model consisted of 30-year simulations performed 100 times, where we computed average total amphibian population size (abundance per pixel) and occupancy (presence at each pixel) at the end of year 30. Results were recorded separately for both fragmented and continuous sections in our focal landscape. We applied the same IBM approach described above to our four hypothetical landscapes with varying split distances (Fig. 2a, b). We parametrized models for *Ab* and *Tb* according to model parameters described in Table S1.

Statistical analysis

We ran IBMs and plotted population size and occupancy for *Ab* and *Tb* frogs across the focal and hypothetical landscapes. We used a likelihood ratio test to compare *Ab* and *Tb* IBM occupancy rates in the focal landscape between forest fragments disconnected vs. connected to aquatic habitats. Data on total population size obtained from the IBMs were also used in downstream Generalized Linear Models (GLMs) with normal distribution and identity link. We performed similar GLMs using pixel occupancy obtained during IBMs. We ran a full factorial GLM testing whether the explanatory variables habitat disturbance (*i.e.*, fragmented *vs.* continuous), dispersal (*i.e.*, 0.2, 0.4, 0.6, 0.8, and 1 probabilities), and life history (*Ab vs. Tb*), including their one-level interactions, predicted population persistence (*i.e.*, total population size or pixel occupancy) in the focal landscape. We performed four independent GLMs for hypothetical landscapes varying in split distance. In these models we tested whether split distance, dispersal, and life history (and their one-level interactions) predicted the population size and occupancy across the landscape. All statistical analyses were conducted in R statistical software.

RESULTS

The three independent field studies recorded a total of 25 leaf-litter amphibian species in the focal landscape, 18 of which were endemic to the Atlantic forest (Haddad et al. 2013) (Table S2). The proportion of Ab species among the Atlantic forest endemic amphibians recorded in the continuous and fragmented forest was 67% and 44%, respectively (Fig. 3).

Populations were maintained over all stipulated time steps in all IBMs. For the focal landscape we found lower occupancy of aquatic-breeding frogs in fragments disconnected from aquatic habitats than in fragments connected to aquatic habitats (Likelihood ration test: Chi-square = 166.733, $P < 0.0001$). Conversely, terrestrial-breeding amphibians persisted in all forest fragments. Detailed outputs of the models are reported in tables S3 and S4.

The GLM applied to the IBM results from the focal landscape showed that habitat disturbance, life history, and dispersal independently and interactively influenced population size ($F_{(1992;7)} = 4941.88$; $r^2 = 0.945$; $P = < 0.0001$; Table S5). Dispersal positively predicted population size of *Ab* amphibian populations, whereas disturbance was a negative predictor (Fig. 1b, c). Furthermore, the interaction between all explanatory variables, including life history, was a predictor of population size. IBM results obtained from the hypothetical landscapes indicated that population size of *Ab* amphibians increased with dispersal rates and decreased with split distance (Fig. 2c). Life history and the interaction among explanatory variables were also predictors of population size in the hypothetical landscapes ($F_{(3992;7)} = 4737.60$; $r^2 = 0.892$; $P = < 0.0001$; Table S4). Both focal and hypothetical IBM models predicted constant population sizes for *Tb* amphibians, independent of dispersal (Figs. 1b, c and 2c). We found consistent patterns when using occupancy data as a response variable for IBMs

employed on focal ($F_{(1992;7)}$ = 3699.708 r^2 = 0.928; P = < 0.0001) and hypothetical landscapes ($F_{(3992;7)}$ = 4154.046; r^2= 0.879; P = < 0.0001; Table S6).

DISCUSSION

The population modeling results from the focal landscape are in agreement with empirical field data (Anjos, 2008; Giasson, 2008; Becker et al., 2010) and with results from a previous study (Becker et al., 2007), all of them pointing to proportionally lower occupancy rates, population size, and diversity of *Ab* than *Tb* amphibians in areas with habitat split. The IBM results indicate that the larger the split distance, the lower the population size in our hypothetical landscapes, and these findings are exacerbated under scenarios of low amphibian dispersal probability. Interestingly, high dispersal rates led to a reduction in population sizes under intermediate and high split distances for *Ab* frogs. As predicted, dispersal and habitat split were not associated with population size and occupancy of *Tb* amphibians. Thus, our results emphasize that habitat split, amphibian life history, and dispersal are good proxies for amphibian population persistence.

The IBM results for the focal landscape indicated that the total population size, of both amphibian life-histories, was lower in the fragmented forest when compared to the continuous forest. Several mechanisms of habitat loss and fragmentation, including disruption of breeding migrations, shifts in food resources, and shifts in microclimates, may contribute independently or synergistically to a reduction in amphibian population size under natural conditions (Vallan, 2000; Lehtinen, Ramanamanjato, & Raveloarison, 2003; Cushman, 2006; Becker et al., 2007, 2010; Olson et al., 2007; Todd et al., 2009; Hayes et al., 2010; Schneider-Maunoury et al., 2016).

Amphibians with different reproductive modes vary in their responses to habitat change (Gascon et al., 1999; Tocher, Gascon, & Meyer, 2001; Bell & Donnelly, 2006; Urbina-Cardona, Olivares-Pérez, & Reynoso, 2006; Becker et al., 2007; Werner et al., 2007) and aquatic-breeding species often need landscape complementation, relying on the integrity and connectivity between terrestrial and aquatic habitats to complete their biphasic life cycles (Werner & Gilliam, 1984; Pope, Fahrig, & Merriam, 2000; Becker et al., 2010). Adults and newly metamorphosed *Ab* individuals in habitat split fragments are forced to cross disturbed environments during reproductive migrations or searching for refuge areas (Becker et al., 2010). During this process, animals face multiple risks associated with environmental conditions within the inhospitable matrix. Dehydration, predation, parasites, pathogens, high UV-B radiation, agrochemicals, and other pollutants in the matrix may reduce fitness or lead to death, negatively affecting population persistence (Hayes et al., 2002; Daszak et al., 2004; Denoel, Dzukic, & Kalezic, 2005; Mazerolle & Desrochers, 2005; Relyea, 2005; Bancroft, Baker, & Blaustein, 2008; Cosentino, Schooley, & Phillips, 2011). On the other hand, *Tb* amphibians reproduce in the interior of fragments and often avoid crossing open environments (Gascon et al., 1999; Pardini et al., 2009; Dixo & Metzger, 2010; Ferreira, Beard, & Crump, 2016). Deforestation in the focal landscape generated fragments with and without habitat split, which allowed us to validate our model and corroborate previous findings pointing to disproportionate negative effects of habitat split on *Ab* amphibians (Becker et al., 2007, 2010).

In addition, IBM model focused on hypothetical landscapes on a gradient of split distances are consistent with the results from both the IBM focused on the focal landscape and from empirical field data. Those results reinforce our hypothesis that *Ab* amphibians are negatively affected by habitat split, while no effect is expected for *Tb*

amphibians. Specifically, our results corroborate previous studies that show that split distance is a key landscape metric to explain species richness and abundance of amphibians in forest fragments (Fonseca et al., 2013; Lion, Garda, & Fonseca, 2014). In fact, amphibian richness in ponds is negatively associated with distance to forested areas (*e.g.*, Loman, 1988; Laan & Verboom, 1990; Lehtinen, Galatowitsch, & Tester, 1999; Silva et al., 2012). Larger split distances could lead to population declines of *Ab* amphibians through three mechanisms: (i) higher split distances involve greater exposure to non-natural habitats and consequently higher probability of mortality (*e.g.*, through increased dehydration, predation, parasitism and exposure to UV-B radiation); (ii) in fragmented habitats, amphibian dispersal ability may be three to four times lower when compared to continuous habitats (Rothermel & Semlitsch, 2002), which may affect seasonal breeding migrations; and (iii) traveling longer distances increases the cost of dispersal (Fahrig, 2003; Kokko & López-Sepulcre, 2006; Schtickzelle, Mennechez, & Baguette, 2006; Bonte et al., 2012), which can reduce clutch and egg size, decreasing reproductive success (Kinnison et al., 2001; Crossin et al., 2004).

Inbreeding is another mechanism by which deforestation affects amphibian populations (Andersen, Fog, & Damgaard, 2004; Allentoft & O'Brien, 2010). Habitat fragmentation can lower the genetic diversity of populations by reducing the effective size of the remaining populations and thus decreasing connectivity (Frankham, 1996; Cushman, 2006; Johansson, Primmer, & Merilä, 2007). After fragmentation, small population sizes may lead to genetic drift, higher risks of inbreeding, lower evolutionary potential, and, consequently, higher risk of extinction (Avise, 1987; Young, Boyle, & Brown, 1996; Saccheri et al., 1998; Reed & Frankham, 2003; Allentoft & O'Brien, 2010). For example, Dixo et al. (2009) found a positive correlation between fragment size and genetic variation of the remaining amphibian populations in the Atlantic forest.

In the IBM we did not measure any parameter of population genetics, such as pairwise Fst, allelic richness, inbreeding, and genetic drift. Future work integrating field surveys of amphibian population genetics with IBM models may advance our ability to predict population persistence in amphibian populations influenced by deforestation and habitat connectivity.

Beside the risks imposed by habitat disturbance, breeding migrations through non-natural habitats are key for *Ab* frogs to complete their life cycle. Many *Ab* amphibian populations often forage or overwinter in natural terrestrial habitats 300-1000 m away from aquatic breeding sites (Semlitsch & Bodie, 2003; Schabetsberger et al., 2004; Crawford & Semlitsch, 2007; Sinsch et al., 2012), turning dispersal ability a determining attribute for *Ab* amphibian populations persistence (Gulve, 1994; Trenham, Koenig, & Bradley Shaffer, 2001; Becker, 2007; Werner et al., 2009; Campbell et al., 2010; Pittman, Osbourn, & Semlitsch, 2014). Although anthropogenic environmental changes profoundly impact the ability of animals to disperse through the landscape (Rothermel & Semlitsch, 2002; Fahrig, 2007), greater rates of dispersal can decrease the negative effects of deforestation on population persistence in fragmented landscapes with high levels of habitat split, as demonstrated here and in previous studies (Fonseca et al., 2013). One likely explanation is that increased dispersal ability may favor migration success by increasing the animal's chance of reaching aquatic breeding sites, reproducing and returning to remnants of natural terrestrial vegetation to forage and overwinter.

Interestingly, high dispersal rates led to a reduction in population sizes under intermediate and high split distances for *Ab* amphibians. Models that attempt to predict how mobility will influence species resilience to anthropogenic habitat change are conflicting. Some authors argue that high mobility is advantageous if highly mobile

species are able to recolonize areas following local extinction events (Hanski & Thomas, 1994; Tscharntke et al., 2002; Grimm et al., 2004). Others argue that more vagile species are subject to higher mortality rates in the inhospitable matrix and are thus more susceptible to local extinction (Casagrandi & Gatto, 1999; Fahrig, 2001; Flather & Bevers, 2002). Still, other authors argue that species with intermediate mobility will be most resilient to landscape change (Casagrandi & Gatto, 1999; Kean & Barlow, 2004). Indeed, predicting optimal movement in newly modified landscapes is difficult because previous ideal movements were selected prior to anthropogenic changes and may no longer be ideal (Fahrig, 2007). Thus, a possible explanation for our results is that more mobile Ab amphibians endemic to the Atlantic forest, which have evolved in a continuous forest habitats, may be maintain this behavior and cross long stretches of non-natural habitats, penetrating and moving through the inhospitable matrix and increasing population mortality rates (Fahrig, 2007).

In conclusion, although our modeling framework could not incorporate variation in species-specific life history traits such as longevity, clutch size and stress tolerance, our general IBM closely matched recent trends in tropical amphibian populations in the wild, including the focal landscape, with developmental mode and dispersal ability being important predictors of persistence in fragmented landscapes. Individual-Based Models that incorporate seasonal migrations and multiple required habitats for the ontogenetic development of vertebrates could enhance our ability to forecast population sizes in varying scenarios of anthropogenic interference, landscape configuration, and spatial connectivity among multiple required habitats. These models could be also applied to entire ecosystems in an attempt to target habitat restoration efforts and mitigation programs in understudied landscapes where habitat split likely poses persistent threats to the local fauna.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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Figure 1. Focal Atlantic forest landscape of São Luiz do Paraitinga, state of São Paulo (a). Green represents natural landcover and white represents non-natural habitats. Lines represent riparian zones. Average population size (log-transformed) in different probabilities of amphibian dispersal for aquatic-breeding (blue line) and terrestrial-breeding frogs (red line) in continuous forest (b) and fragmented forest (c).

Figure 2. Depiction of a hypothetical landscape (a). Four representations of the same area under different degrees of habitat split, where 0P = lack of habitat split, 1P = low split distance, 2P = intermediate split distance, and 3P = high split distance; black represents the water body, green represents natural landcover, and white represents non-natural habitats (b). Population size (log-transformed) in different probabilities of dispersal for aquatic-breeding (blue line) or terrestrial-breeding frogs (red line) (c).

Figure 3. Proportion of aquatic-breeding (blue bar) and terrestrial-breeding species (red bar) endemic to Brazil's Atlantic forest recorded in the focal landscape, both for fragmented and continuous forest.

Figure 1

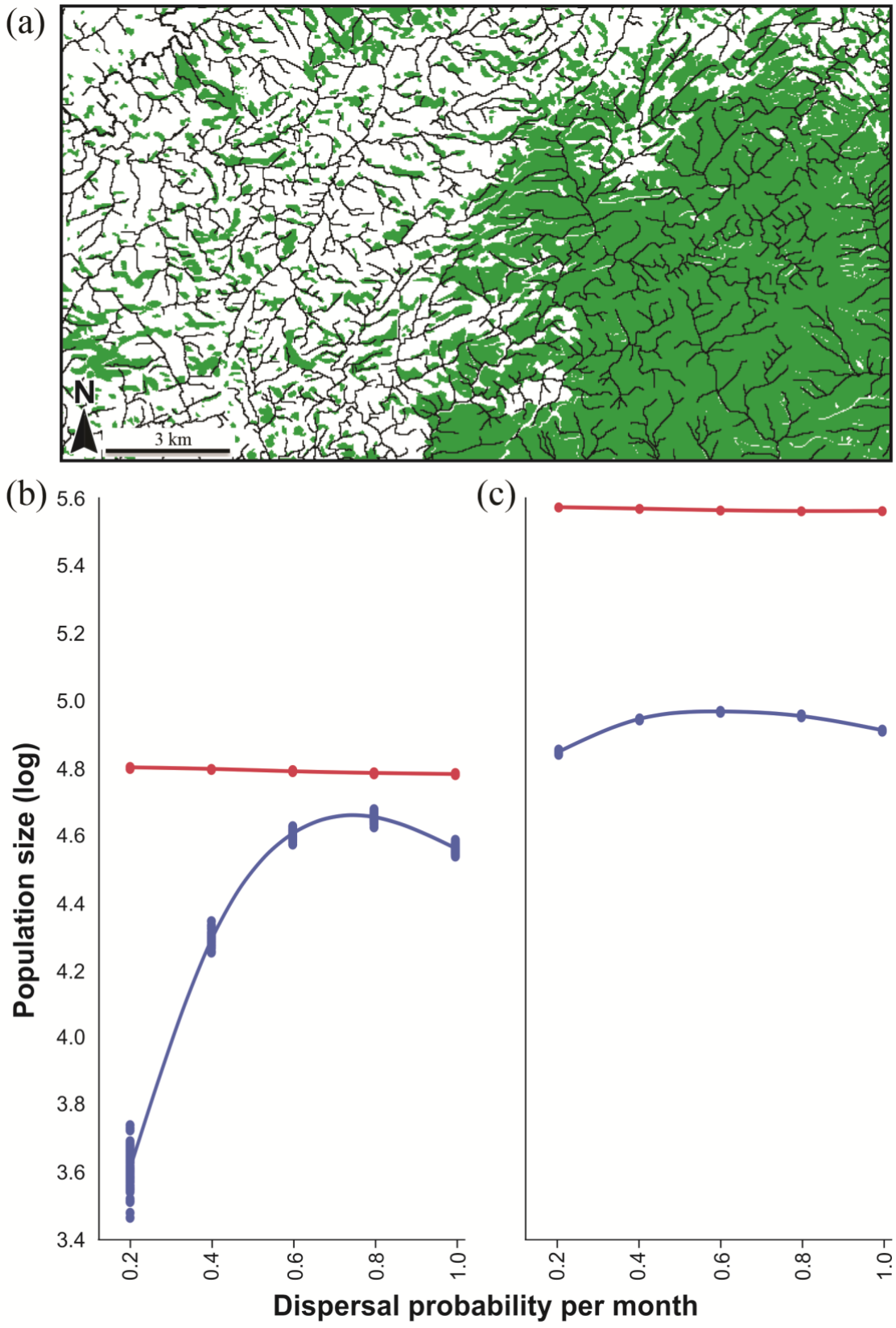

(a)
N
3 km
(b)
(c)
Population size (log)
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
0.2
0.4
0.6
0.8
1.0
Dispersal probability per month

Figure 2

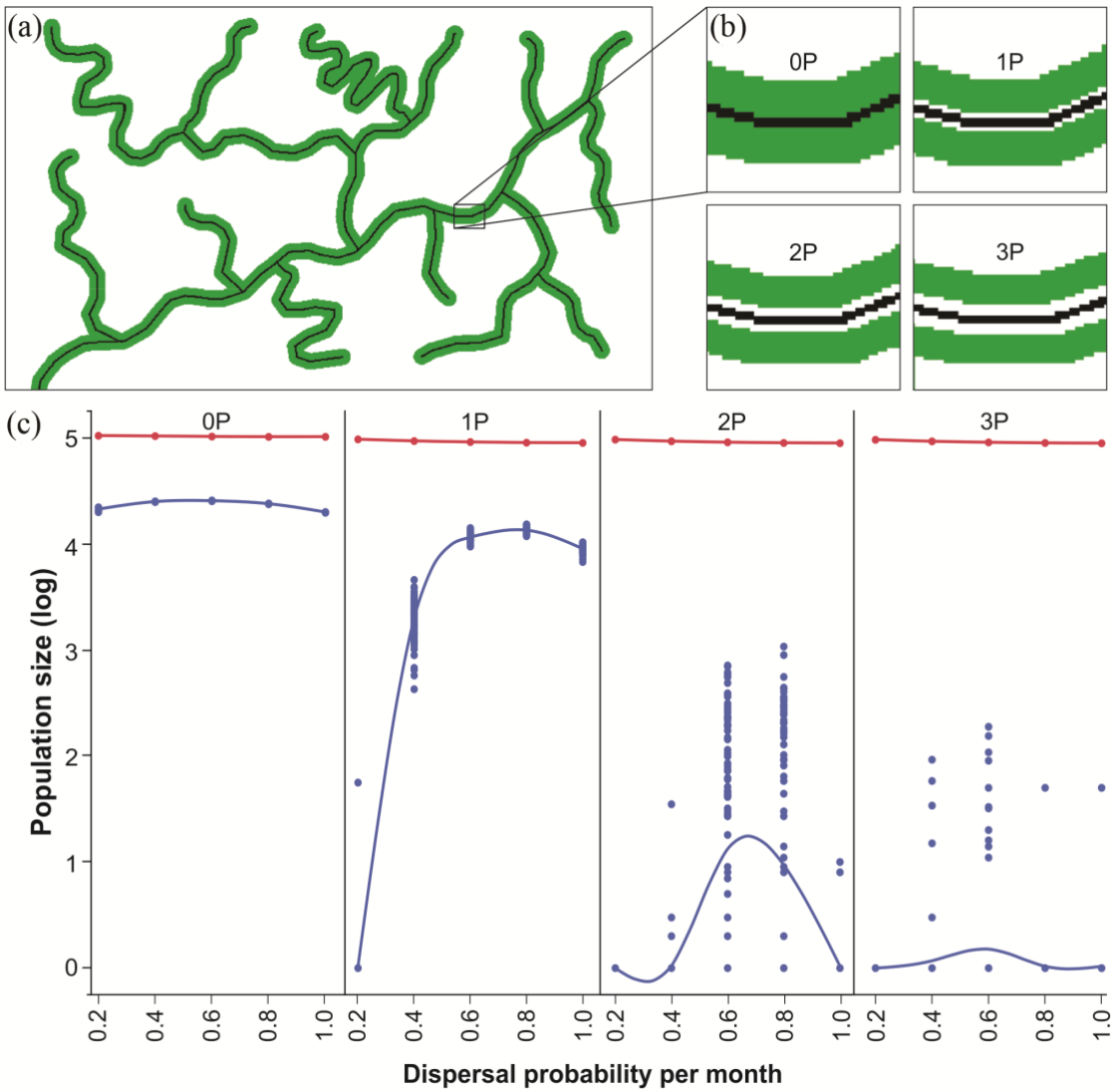
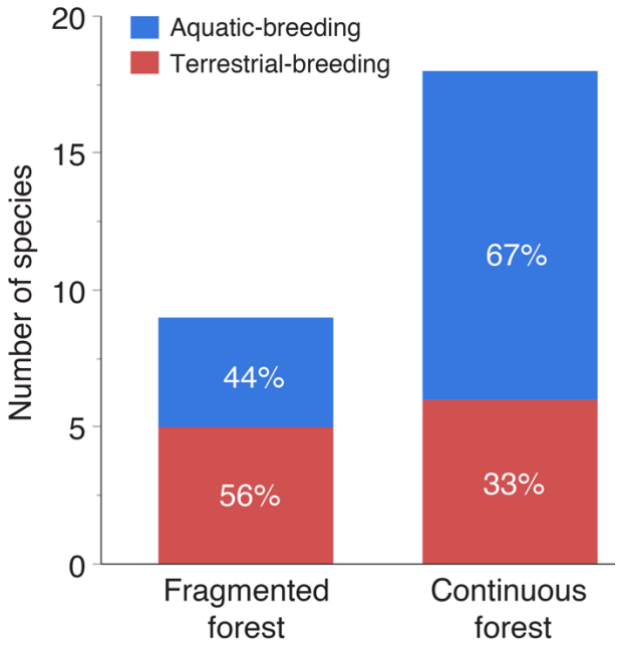

(a)
(b)
0P
1P
2P
3P
(c)
0P
1P
2P
3P
Population size (log)
0
1
2
3
4
5
0.2
0.4
0.6
0.8
1.0
Dispersal probability per month

Figure 3


Aquatic-breeding
Terrestrial-breeding
Number of species
20
15
10
5
0
44%
56%
67%
33%
Fragmented forest
Continuous forest

Electronic Supplemental Material

Linking deforestation, breeding migrations, and population persistence in tropical amphibians using empirical field data and individual-based models

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Table S1. Life-history parameters used in the Individual-Based Model (IBM) for aquatic and terrestrial breeding life-histories.

Parameters	*Ab*	*Tb*
Years	30	30
Time step (months)	12	12
Start breeding in the month	4	4
End breeding in the month	7	7
Dispersal probability per month	0 - 1	0 - 1
Breeding age (years)	2	2
Initial individuals per cell	5	5
Maximum longevity (months)	36	36
Use Friction 1 in the month	2	-
Use Friction 2 in the month	7	1-12
Carrying capacity for all cell types	10	10
Density independent death (%) per time step for different cell types		
Cell type 1 - riparian habitat in disturbed matrix	0.05	0.05
Cell type 2 - riparian habitat in natural forest	0.03	0.03
Cell type 3 - disturbed vegetation	0.08	0.08
Cell type 4 - natural forest	0.03	0.03
Offspring per female for different cell types		
Cell type 1 - riparian habitat in disturbed matrix	2	0
Cell type 2 - riparian habitat in natural forest	2	2
Cell type 3 - disturbed vegetation	0	0
Cell type 4 - natural forest	0	2
Friction time 1		
Cell type 1 - riparian habitat in disturbed matrix	0.01	0.99
Cell type 2 - riparian habitat in natural forest	0.01	0.01
Cell type 3 - disturbed vegetation	0.99	0.99
Cell type 4 - natural forest	0.99	0.01
Friction time 2		
Cell type 1 - riparian habitat in disturbed matrix	0.99	0.99
Cell type 2 - riparian habitat in natural forest	0.99	0.01
Cell type 3 - disturbed vegetation	0.01	0.99
Cell type 4 - natural forest	0.01	0.01

Table S2. List of leaf-litter anuran species recorded in continuous and fragmented forests of the focal Atlantic forest landscape in the state of São Paulo, Brazil, according to Becker *et al.* (2007), Giasson *et al.* (2008), and Anjos *et al.* (2008).

Species	Breeding mode	Atlantic forest endemic	Natural habitat
Continuous Forest			
Chiasmocleis atlantica	Aquatic	Yes	Forest
Hylodes asper	Aquatic	Yes	Forest
Hylodes phyllodes	Aquatic	Yes	Forest
Hylodes sp. (aff. *sazimai*)	Aquatic	Yes	Forest
Leptodactylus latrans	Aquatic	No	Grassland
Leptodactylus furnarius	Aquatic	No	Grassland
Leptodactylus fuscus	Aquatic	No	Grassland
Phantasmarana bocainensis	Aquatic	Yes	Forest
Paratelmatobius poecilogaster	Aquatic	Yes	Forest
Physalaemus cuvieri	Aquatic	No	Grassland
Physalaemus olfersii	Aquatic	Yes	Forest
Proceratophrys appendiculata	Aquatic	Yes	Forest
Proceratophrys boiei	Aquatic	Yes	Forest
Proceratophrys melanopogon	Aquatic	Yes	Forest
Rhinella icterica	Aquatic	Yes	Forest
Rhinella ornata	Aquatic	Yes	Forest
Adenomera marmorata	Terrestrial	Yes	Forest
Brachycephalus pitanga	Terrestrial	Yes	Forest
Haddadus binotatus	Terrestrial	Yes	Forest
Ischnocnema cf. *randorum*	Terrestrial	Yes	Forest
Ischnocnema parva	Terrestrial	Yes	Forest
Ischnocnema sp. (aff. *henseli*)	Terrestrial	Yes	Forest
Fragmented Forest			
Chiasmocleis atlantica	Aquatic	Yes	Forest
Elachistocleis cesarii	Aquatic	No	Grassland
Leptodactylus fuscus	Aquatic	No	Grassland
Leptodactylus labyrinthicus	Aquatic	No	Grassland
Leptodactylus mystacinus	Aquatic	No	Grassland
Physalaemus cuvieri	Aquatic	No	Grassland
Proceratophrys boiei	Aquatic	Yes	Forest
Rhinella icterica	Aquatic	Yes	Forest
Rhinella ornata	Aquatic	Yes	Forest
Adenomera marmorata	Terrestrial	Yes	Forest
Brachycephalus pitanga	Terrestrial	Yes	Forest
Haddadus binotatus	Terrestrial	Yes	Forest
Ischnocnema parva	Terrestrial	Yes	Forest
Ischnocnema sp. (aff. *henseli*)	Terrestrial	Yes	Forest

Table S3. Population size at different probabilities of dispersal (PD) for aquatic-breeding (*Ab*) or terrestrial-breeding species (*Tb*) in fragmented forest (FF), continuous forest (CF), landscape without split habitat (0P), landscape with one pixel of habitat split (1P), landscape with two pixels of habitat split (2P), and landscape with three pixels of habitat split (3P). Values presented as mean ± standard deviation (range).

	PD	FF	CF	0P	1P	2P	3P
Ab	**0.2**	4,187.08 ± 85.73 (4,101.35 – 4,272.81)	70,204.04 ± 110.26 (70,093.78 – 70,314.30)	21,190.69 ± 91.30 (21,099.39 – 21,281.99)	0.55 ± 1.08 (-0.53 – 1.63)	0	0
	0.4	19,356.14 ± 141.29 (19,214.85 – 19,497.43	88,154.83 ± 74.20 (88,080.63 – 88,229.03)	24,949.84 ± 24.51 (24,925.33 – 24,974.35)	2,138.79 ± 148.15 (1,990.64 – 2,286.94)	0.37 ± 0.67 (-0.30 – 1.04)	1.97 ± 2.20 (-0.23 – 4.17)
	0.6	40,186.28 ± 217.71 (39,968.57 – 40,403.99)	92,554.24 ± 65.72 (92,488.52 – 92,619.96)	25,416.85 ± 25.53 (25,391.32 – 25,442.38)	11,545.29 ± 186.48 (11,358.81 – 11,731.77)	106.58 ± 34.05 (72.53 – 140.63)	7.05 ± 5.50 (1.55 – 12.55)
	0.8	45,033.27 ± 184.35 (44,848.92 – 45,217.62)	89,775.61 ± 54.70 (89,720.91 – 89,830.31)	23,833.51 ± 23.99 (23,809.52 – 23,857.50)	13,446.51 ± 155.09 (13,291.42 – 13,601.60)	108.39 ± 37.08 (71.31 – 145.47)	0.49 ± 0.96 (-0.47 – 1.45)
	1.0	36,675.09 ± 162.20 (36,512.89 – 36,837.29)	81,501.92 ± 52.64 (81,449.28 – 81,554.55)	19,875.40 ± 25.34 (19,850.06 – 19,900.74)	8,984.08 ± 129.60 (8,854.48 – 9,113.68)	0.16 ± 0.22 (-0.06 – 0.38)	0
Tb	**0.2**	63,227.48 ± 37.98 (63,189.49 – 63,265.47)	374,591.62 ± 63.16 (374,528.46 – 374,654.78)	104,480.62 ± 108.69 (104,371.93 – 104,589.31)	96,545.73 ± 76.13 (96,469.60 – 96,621.86)	95,949.21 ± 61.48 (95,887.73 – 96,010.69)	95,826.17 ± 62.81 (95,763.36 – 95,888.98)
	0.4	62,542.27 ± 32.50 (62,509.77 – 62,574.77)	370,895.56 ± 64.43 (370,831.13 – 370,959.99)	103,559.85 ± 109.32 (103,450.53 – 103,669.17)	93,097.23 ± 78.54 (93,018.69 – 93,175.77)	92,585.24 ± 64.14 (92,521.10 – 92,649.38)	92,363.27 ± 56.17 (92,307.10 – 92,419.44)
	0.6	61,556.58 ± 27.05 (61,529.53 – 61,583.63)	366,807.12 ± 63.96 (366,743.16 – 366,871.08)	102,541.26 ± 104.12 (102,437.14 – 102,645.38)	91,041.54 ± 78.18 (90,963.36 – 91,119.72)	90,532.04 ± 61.49 (90,470.55 – 90,593.53)	90,363.96 ± 52.78 (90,311.18 – 90,416.74)
	0.8	60,853.83 ± 33.79 (60,820.04 – 60,887.62)	364,854.59 ± 62.68 (364,791.91 – 364,917.27)	101,971.89 ± 105.86 (101,866.03 – 102,077.75)	89,851.50 ± 77.94 (89,773.56 – 89,929.44)	89,382.48 ± 60.94 (89,321.54 – 89,443.42)	89,178.32 ± 60.20 (89,118.12 – 89,238.52)
	1.0	60,393.96 ± 32.75 (60,361.20 – 60,426.72)	365,114.25 ± 65.96 (365,048.29 – 365,180.21)	102,014.66 ± 109.05 (101,905.61 – 102,123.71)	89,374.98 ± 70.60 (89,304.38 – 89,445.58)	88,916.20 ± 59.95 (88,856.25 – 88,976.15)	88,730.95 ± 54.65 (88,676.30 – 88,785.60)

Table S4. Number of occupied pixels at different probabilities of dispersal (PD) for aquatic-breeding species (*Ab*) or terrestrial-breeding species (*Tb*) in fragmented forest (FF), continuous forest (CF), landscape without split habitat (0P), landscape with one pixel of habitat split (1P), landscape with two pixels of habitat split (2P), and landscape with three pixels of habitat split (3P). Values presented as mean ± standard deviation (range).

	PD	FF	CF	0P	1P	2P	3P
Ab	**0.2**	1,863.40 ± 36.61 (1,826.78 – 1,900.01)	25,493.24 ± 37.52 (25,455.72 – 25,530.76)	6,129.53 ± 24.18 (6,105.58 – 6,153.47)	0.33 ± 0.64 (-0.31 – 0.97)	0	0
	0.4	8,654.34 ± 63.35 8,590.98 – 8,717.69)	32,004.92 ± 20.58 (31,984.34 – 32,025.50)	7,654.24 ± 7.77 (7,646.46 – 7,662.02)	883.87 ± 58.94 (824.92 – 942.81)	0.26 ± 0.45 (-0.19 – 0.71)	1.34 ± 1.38 (-0.04 – 2.72)
	0.6	17,440.29 ± 91.32 (17,348.97 – 17,531.61)	33,348.38 ± 20.21 (33,328.17 – 33,368.59)	8,042.84 ± 8.76 (8,034.07 – 8,051.60)	4,359.37 ± 71.26 (4,288.10 – 4,430.63)	54.39 ± 15.54 (38.84 – 69.93)	4.25 ± 2.95 (1.29 – 7.20)
	0.8	20,751.94 ± 81.40 (20,670.53 – 20,833.35)	34,481.80 ± 17.38 (34,464.41 – 34,499.19)	8,422.85 ± 8.54 (8,414.30 – 8,431.40)	5,619.32 ± 63.51 (5,555.80 – 5,682.83)	59.24 ±19.36 (39.87 – 78.60)	0.40 ± 0.78 (-0.38 – 1.18)
	1.0	18,996.09 ± 80.46 (18,926.22 – 19,065.96)	34,698.27 ± 21.33 (34,676.94 – 34,719.61)	8,398.08 ± 8.92 (8,389.15 – 8,407.00)	4,530.82 ± 64,70 (4,466.11 – 4,595.52)	0.16 ± 0.22 (-0.06 – 0.38)	0
Tb	**0.2**	10,835.97 ± 5.24 (10,830.72 – 10,841.22)	60,504.75 ± 2.09 (60,502.65 – 60,506.85)	15,160.25 ± 14.59 (15,145.65 – 15,174.85)	13,456.01 ± 10,00 (13,446.00 – 13,466.02)	13,377.82 ± 7.90 (13,369.91 – 13,385.73)	13,348.11 ± 7.18 (13,340.92 – 13,355.30)
	0.4	10,967.72 ± 4.68 (10,963.04 – 10,972.40)	60,521.57 ± 2.12 (60,519.45 – 60,523.69)	15,172.63 ± 14.99 (15,157.63 – 15,187.63)	13,463.33 ± 10,00 (13,453.33 – 13,473.33)	13,383.47 ± 7.63 (13,375.84 – 13,391.10)	13,358.28 ± 7.83 (13,350.44 – 13,366.12)
	0.6	10,980.30 ± 4.18 (10,976.12 – 10,984.48)	60,501.80 ± 2.21 (60,499.59 – 60,504.01)	15,169.12 ± 14.94 (15,154.17 – 15,184.07)	13,451.66 ± 10,23 (13,441.30 – 13,462.02)	13,375.06 ± 7.85 (13,367.20 – 13,382.92)	13,348.47 ± 7.73 (13,340.73 – 13,356.21)
	0.8	10,975.42 ± 4.01 (10,971.40 – 10,979.44)	60,489.94 ± 2.40 (60,487.54 – 60,492.34)	15,162.85 ± 14.65 (15,148.20 – 15,177.50)	13,441.41 ± 10,23 (13,431.65 – 13,451.65)	13,365.36 ± 7.72 (13,357.63 – 13,373.09)	13,336.15 ± 7.26 (13,328.88 – 13,343.42)
	1.0	10,965.45 ± 3.42 (10,962.02 – 10,968.88)	60,487.44 ± 2.59 (60,484.84 – 60,490.04)	15,159.63 ± 14.89 (15,144.74 – 15,174.52)	13,433.58 ± 9,97 (13,423.61 – 13,443.55)	13,357.11 ± 7.90 (13,349.21 – 13,365.01)	13,330.60 ± 7.39 (13,323.21 – 13,337.99)

Table S5. Generalized linear models (GLMs) testing the independent and interactive effects of life history (*Ab vs. Tb*), dispersal probability (0.2, 0.4, 0.6, 0.8, 1), habitat disturbance (continuous *vs.* fragmented; only for focal Atlantic forest landscape), and split distance (0, 1, 2, 3 pixels; only for hypothetical landscape) predicting total population size.

		Estimate	**Std Error**	**t Ratio**	**Prob > |t|**
Focal landscape	*Intercept*	4.7320784	0.005879	804.97	< 0.0001*
	Life history	-0.272163	0.002507	-108.6	< 0.0001*
	Habitat disturbance	-0.3392991	0.002507	135.36	< 0.0001*
	Life-history * Habitat disturbance	-0.048722	0.002507	-19.44	< 0.0001*
	Dispersal	0.2901758	0.008862	32.74	< 0.0001*
	Life history * Dispersal	0.3104478	0.008862	35.03	< 0.0001*
	Habitat disturbance * Dispersal	-0.263384	0.008862	-29.72	< 0.0001*
	Life history * Habitat disturbance * Dispersal	-0.268961	0.008862	-30.35	< 0.0001*
Hypotetical landscape	*Intercept*	4.3100478	0.028987	148.69	< 0.0001*
	Life history	-1.495236	0.010729	-139.4	< 0.0001*
	Split distance	-0.786831	0.009596	-82.00	< 0.0001*
	Life history * Split distance	-0.770897	0.009596	-80.34	< 0.0001*
	Dispersal	0.5824457	0.037931	15.36	< 0.0001*
	Life history * Dispersal	0.6166254	0.037931	16.26	< 0.0001*
	Split distance * Dispersal	-0.192563	0.033927	-5.68	< 0.0001*
	Life history * Split distance * Dispersal	-0.184409	0.033927	-5.44	< 0.0001*

Table S6. Generalized linear models (GLMs) testing the independent and interactive effects of life history (*Ab* vs. *Tb*), dispersal probability (0.2, 0.4, 0.6, 0.8, 1), habitat disturbance (continuous vs. fragmented; only for focal landscape), and split distance (0, 1, 2, 3 pixels; only for hypothetical landscape) predicting pixel occupancy.

		Estimate	**Std Error**	**t Ratio**	**Prob > |t|**
Focal landscape	*Intercept*	9.5100281	0.012313	772.37	< 0.0001*
	Habitat disturbance	-0.7123922	0.00525	135.69	< 0.0001*
	Life history	-0.179094	0.00525	-34.11	< 0.0001*
	Habitat disturbance * Life history	-0.142519	0.00525	-27.15	< 0.0001*
	Dispersal	0.7772991	0.018562	41.88	< 0.0001*
	Habitat disturbance * Dispersal	-0.605401	0.018562	-32.61	< 0.0001*
	Life history * Dispersal	0.7714572	0.018562	41.56	< 0.0001*
	Habitat disturbance * Life history * Dispersal	-0.599012	0.018562	-32.27	< 0.0001*
Hypotetical landscape	*Intercept*	8.4474386	0.059585	141.77	< 0.0001*
	Life history	-2.718736	0.022053	-123.3	< 0.0001*
	Split distance	-1.621865	0.019725	-82.22	< 0.0001*
	Life history * Split distance	-1.582847	0.019725	-80.25	< 0.0001*
	Dispersal	1.3339055	0.07797	17.11	< 0.0001*
	Life history * Dispersal	1.3357023	0.07797	17.13	< 0.0001*
	Split distance * Dispersal	-0.47817	0.069738	-6.86	< 0.0001*
	Life history * Split distance * Dispersal	-0.477659	0.069738	-6.85	< 0.0001*

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Giasson, L. (2008). Atividade sazonal e uso do ambiente por anfíbio da Mata Atlântica no alto da serra do mar. PhD.

CAPÍTULO 2. Panzootic chytrid genotypes drive divergent infection dynamics in frogs with multi-lineage infections

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ABSTRACT

Globalization can facilitate the spread of pathogens and the emergence of genetically diverse infections from previously isolated pathogens. Coinfections are predicted to alter disease dynamics and favor more virulent pathogens. Numerous theoretical studies have examined the links between coinfections and virulence. However, empirical studies examining genetically diverse infections remain relatively limited. In the Brazilian Atlantic forest, globalization has led to the cooccurrence of an enzootic lineage (*Bd*-Asia-2/Brazil) and a panzootic lineage (*Bd*-GPL) of the fungus *Batrachochytrium dendrobatidis* (*Bd*). Host coinfection by both lineages allowed the emergence of a recombinant genotype, making the Atlantic forest a hotspot of the *Bd* pathogen. In addition, most amphibian declines occurred in the zone where all genotypes cooccur, suggesting that coinfections can lead to more severe short-term infections or to select more virulent pathogens. We examined how coinfection with different *Bd* genotypes from two distinct lineages, including a recombinant genotype, affects competitive interactions within hosts, and how these interactions alter pathogen virulence and transmission potential. Our results indicate that coinfection may have divergent results in pathogen load and transmission potential. At the population-level, coinfection could lead to 1) competitive suppression that helps to reduce the transmission potential of more virulent pathogens or 2) facilitative interactions that increase the transmission potential of less virulent pathogens. Furthermore, we found a link between competitiveness and virulence, which helps to explain the global epidemiological pattern of the panzootic lineage of *Bd*. Regardless of the particular genotypic combinations, coinfection was generally a lose-lose scenario for the host.

KEYWORDS

Coinfection, virulence evolution, *Batrachochytrium dendrobatidis*, disease, Amphibia, Anura

INTRODUCTION

Globalization creates numerous opportunities for spreading pathogens from one location to another (Daszak et al. 2001; Anderson et al. 2004). This process can expose hosts to completely novel pathogen species or genotypes, and can also lead to the emergence of new pathogen genotypes by means of recombination between allochthonous and autochthonous genotypes (Brasier 2001; Stukenbrock et al. 2012). These pathogens also compete for limited host resources, and constantly evolves to escape immune responses (Alizon et al 2013). Such intense competition ultimately determines which pathogen genotype(s) will successfully transmit to new hosts (de Roode et al. 2005; Alizon et al 2013).

Genetically diverse infections are common in the wild and predicted to favor more virulent pathogens; even if more aggressive pathogens kill their hosts and reduce their own fitness, less virulent pathogens suffer a disproportionate disadvantage (linked to reduced transmission) and may be eliminated by natural selection (May & Nowak 1995, Read & Taylor 2001). For instance, a virulent genotype can increase its frequency in the population if it maximizes the number of secondary infections between hosts (Alizon et al 2013). This process could be facilitated when multiple pathogen genotypes coinfect the host, because competitively superior genotypes generally exploit host resources faster, and thus, limits the reproduction and transmission of prudent pathogens (Read & Taylor 2001; Alizon et al 2013). This hypothesis offers the intriguing possibility that reducing genetically mixed infections could present novel

targets for disease intervention by reducing competition between genotypes within-hosts and subsequently, reducing selection for increased virulence. (Bell et al. 2006; Galvani 2003). Yet, quantifying how coinfection shapes the evolution of virulence remains challenging. Numerous theoretical studies have examined links between genetically-diverse coinfections and virulence (Hamilton 1972; Bremermann & Pickering 1983; Frank 1996). On the other hand, empirical studies examining genetically diverse coinfections are scattered in the literature.

Moreover, few studies considered how globalization-driven introductions of pathogen genotypes may affect coinfections in wild populations (Brasier 2001; Stukenbrock et al. 2012). For example, an introduced genotype may influence the genetic diversity of the pathogen population and thus the genetic relatedness of coinfecting genotypes. For instance, in Brazil's Atlantic forest, a hotspot of amphibian biodiversity (Toledo et al. 2021), globalization has led to the co-occurrence of enzootic (*Bd*-Asia-2/Brazil) and panzootic (*Bd*-GPL) lineages and to the origin of novel recombinant genotypes of the amphibian pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*; Jenkinson et al. 2016; O'Hanlon et al. 2018; Fisher & Garner 2020). *Bd* is a cutaneous fungus that disrupts key physiological functions of amphibian skin (Voyles et al. 2011), leading to the potentially fatal disease chytridiomycosis (Scheele et al. 2019). *Bd* genotypes from the enzootic and panzootic lineages vary in phenotypic traits, competitive ability, and virulence (Lambertini et al. 2016; Becker et al. 2017; Jenkinson et al. 2018; Greenspan et al. 2018).

In addition, recombinant genotypes (*Bd*-recombinant), such as the one resulting from the recombination between *Bd*-Asia-2/Brazil and *Bd*-GPL, was more virulent relative to their parental lineages in an experimental trial (Greenspan et al. 2018), at least in single-genotype infections. The co-occurrence of enzootic, panzootic and novel

recombinant genotypes of *Bd* in Brazil's Atlantic forest frogs suggests that coinfections among these genotypes are likely to occur (O'Hanlon et al. 2018; Byrne et al. 2019).

Notably, in Brazil, most of amphibian declines have occurred in 'hybrid zones'; locations where *Bd*-Asia-2/Brazil, *Bd*-GPL, and *Bd*-recombinant co-occur (Carvalho et al. 2017; Jenkinson et al. 2016). This observation suggest that coinfections may either lead to more severe infections or select for more virulent lineages. Therefore, it is pivotal to better understand whether coinfection shapes competitive interactions among these genotypes and alters virulence and *Bd* transmission.

Thus, we hereby tested how coinfection with different *Bd* genotypes from two distinct lineages, including a recombinant one — and with varied introduction histories — affects competitive interactions within hosts. Then, we asked whether these interactions altered pathogen virulence (host survival and lifespan) and transmission potential (using *Bd* load as a proxy for future transmission potential). Finally, we compared our results to epidemiological patterns to better understand whether global patterns of *Bd* genotype prevalence and epizootic severity reflect the outcomes of these competitive interactions.

METHODS

We used five *Bd* genotypes that vary in their degree of relatedness, competitive ability (using early establishment as a metric), virulence, and transmission potential (total *Bd* load) to examine how coinfection affects infection outcomes. We analyzed it for each genotype alone or when in mixed-infections with one or two other genotypes. We used genotypes from the most recently derived lineage of *Bd*, the Global Panzootic Lineage (*Bd*-GPL; Fig. 1), hereafter, 'P1' or 'P2' as the reference genotypes against which we competed *Bd* enzootic ('E1' and 'E2') and *Bd* recombinant ('R') genotypes. We use P1

and P2 as reference genotypes in an effort to capture the arrival of these genotypes in Brazil, where coinfections with the local enzootic lineages led to the emergence of the recombinant genotype.

Pathogens and amphibian host

Pure cultures of *Bd* isolates were isolated from tadpoles of captive bullfrogs (P2 and E2) or wild anurans (P1, E1 and R) (Table S1). Isolation, culturing and genotyping procedures to identify *Bd* genotypes followed Jenkinson et al. (2016). Prior to the experiment, isolates were maintained at 4 ºC, passaged every four months, and the number of passages ranged from 4 to 20 (Table S1). Although virulence attenuation can occur in Bd isolates serially passaged for long periods (Langhammer et al. 2013; Refsnider et al. 2015), we do not believe that the passages of our isolates have affected our results. There is no clear evidence of a decrease in pathogen virulence over time in culture (Voyles et al. 2014), and virulence attenuation was detected in isolates passed at least 30 times.

We used the terrestrial frog species *Eleutherodactylus johnstonei* (Anura; Eleutherodactylidae), which is a direct-developing species (without larval stage, embryos hatch as froglets), is native to the Lesser Antilles (Schwartz 1967) and was introduced in the city of São Paulo, Brazil, at least 25 years ago (Toledo & Measey 2018), from where they were collected. *Eleutherodactylus johnstonei* is known to tolerate *Bd* infections and is considered a potential reservoir species in Dominica and Montserrat (Hudson et al. 2019). However, the population of *E. johnstonei* has remained restricted to a small urban habitat and free of *Bd* in Brazil. Two recent studies, after testing 100 frogs in total, did not detect *Bd* at this location (Forti et al. 2017; Mesquita et al. 2017). Besides, this host population exhibited high levels of

susceptibility to *Bd* in previous experimental inoculations (Mesquita et al. 2017), therefore being a good model-species for our experiments.

At the collection site, we selected adults with about the same size (mean = 21.12 ± sd 2.66 mm, n = 95), and handled each adult frog with a new pair of disposable gloves to avoid potential *Bd* cross-contamination, and gently placed them into individual containers. We then transported the frogs to the laboratory in refrigerated coolers. To confirm that all hosts were *Bd*-free prior experimental inoculations, we swabbed the skin of all the frogs upon arrival at the laboratory following a standard protocol (Hyatt et al. 2007). We stored swabs at -20 ºC until processing.

After swabbing, we housed the frogs individually in plastic enclosures (22 x 15 x 8 cm), with a layer of sphagnum moss that was previously autoclaved and moistened with distilled water. Frogs were housed in temperature-controlled rooms at 20 ºC on a 12-hour day-night cycle. We monitored frogs twice a day throughout the experiment and fed them calcium-enriched crickets *ad-libitum* twice a week.

Infection assay

Experimental treatments included single-genotype exposures and mixed genotype exposures with either two or three genotypes with all possible inter-lineage combinations. In total, the experiment included 17 *Bd* exposed treatments and one control group for a total of 297 hosts of the invasive species *E. johnstonei*. (Table S2).

To prepare *Bd* solutions for the challenge assay, we transferred liquid cultures of each genotype to individual agar plates containing 1% tryptone and allowed them to grow for eight days at 11 ºC under dark conditions. We then collected zoospores of each *Bd* genotype by flooding culture plates with 2 ml of distilled water for 45 minutes to induce zoospore release and then gently scraping the surface to maximize collection.

From each zoospore suspension, we prepared standard inoculation solutions (concentration $2.98x10^6$ zoospores per ml) by collecting a 1 ml subsample and quantifying the zoospore density using a hemocytometer.

For pathogen exposure, individual hosts were placed in Petri dishes with 1.5 ml of zoospore suspension containing a fixed dose (2.98×10^6 zoospores / ml), and were exposed for 45 min at 20 ºC. Frogs from the control group were exposed to 1.5 ml of distilled water. Individuals from coinfection treatments (those exposed to two or three genotypes) were exposed to a zoospore suspension containing equal proportions of each *Bd* genotype suspension (final volume of 1.5 ml). To quantify *Bd* infection following exposure, we collected skin swabs on day 21 post exposure and after death or at the end of the experiment (day 76) following a standard protocol (Hyatt et al. 2007). Both collection days span multiple *Bd* life cycles (Berger et al. 2005).

Molecular analyses

We leveraged two genotyping assays to detect the presence or absence of *Bd*-Asia-2/Brazil, *Bd*-GPL, and *Bd* recombinant (Schloegel et al. 2012) in a mixed sample. Assay *Bd*mt_26360 targets a mitochondrial SNP and distinguishes between *Bd*-Asia-2/Brazil (allele A, 6-FAM) and *Bd*-GPL or *Bd*-recombinant (allele G, VIC) (Jenkinson et al. 2018). If only allele G was detected in a sample by the mtDNA assay, then we performed a second genotyping reaction using assay SC9_621917 (Rodriguez unpublished) to target a SNP in the diploid/aneuploid nuclear genome to determine whether the recombinant genotype (allele C, 6-FAM) was present.

We used the skin swab DNA extractions as input for qPCRs in 20 ml volumes that comprised 5 ml of the sample template, 10 ml of TaqMan Fast Advanced 2X Master Mix (Applied Biosystems, Inc.), 4 ml of nuclease-free water, and 1 ml of the

SNP assay mix (Thermo Fisher) at 20X concentration (18 mM forward primer, 18 mM reverse primer, 4 mM 6-FAM probe, and 4 mM VIC probe). On a QuantStudio 3 (Applied Biosystems, Inc.), cycling conditions consisted of 95 °C for 20 s, then 95 °C for 1 s and 60 °C for 20 s (data collection step) for 50 cycles. We analyzed amplification curves using the Standard Curve application that is part of the Thermo Fisher Connect cloud-based software.

Statistical analyses

To clarify that our infection methodology was consistent across treatments, we first quantified the proportion of hosts that became infected using Generalized Linear Models (GLMs) with binomial errors. Then, we tested for differences in the competitive ability (using early establishment of *Bd* as a metric – 21 days post-inoculation), virulence (lifespan and survival), and transmission potential (using total *Bd* load – after death or on day 76) of each genotype when alone and when in competition with either one or two other genotypes using a combination of generalized linear models (GLMs), planned *a priori* orthogonal contrasts, and survival analyses.

For GLMs we chose the most appropriate distribution for each model using the R package *fitdistrplus*. For virulence and transmission potential, we included only the animals that were infected (and excluded animals that were exposed but uninfected; n = 3: E1+R = 1, P2+R = 1, P2+E2+R = 1). To understand how coinfections affected key traits of the reference genotypes, we used planned *a priori* orthogonal contrasts (Gotelli & Ellison 2004, Bolker 2008, Crawley 2012).

We computed survival curves using the Kaplan-Meier method, which was implemented using the *survfit* function from the package *survival* (Therneau 2021), and compared survival curves by conducting log rank tests using the function *survdiff* (also

from the package survival). We conducted *post-hoc* comparisons of survival curves using the function *pairwise_survdiff* from the package *survminer* (Kassambara et al. 2019), which computes adjusted *p*-values to correct for false discovery rate (we used the method 'BH', Benjamini and Hochberg 1995). We examined the effect of coinfections by comparing survival curves of *Bd* genotypes from the panzootic lineage in single-genotype infections (P1 or P2) with their respective mixed infection treatments, which included the *Bd* genotypes from the enzootic lineage (E1 and E2) and the recombinant *Bd* variant (R). All statistical analyses were conducted in R statistical software version 1.4.11 (R Core Team 2020). The data and full code for all statistical tests are available at dryad (https://insertpostpublication).

RESULTS

The proportion of hosts that became infected did not differ across *Bd* genotypes or coinfections (Fig. S1A; Binomial GLM, all *p*-values > 0.05). Also, no individual in the control group died or were infected during the experiment. These results serve as a quality control metric and clarify that the infection methodology was consistent across treatments. We found links between virulence and competitive ability, but the direction of this relationship varied across particular *Bd* genotypes and their introduction history (*i.e.*, enzootic *vs*. panzootic). The most competitive genotype (P1) was also the most virulent whereas the enzootic and recombinant genotype were less competitive than the panzootic genotypes but were of intermediate virulence.

The panzootic *Bd* genotype (P1), which was the quickest to establish and outgrow all other genotypes in single-genotype infections (comparing the *Bd* load on day 21 of P1 to all other genotypes: $p < 0.001$; Fig. 2*a*), suffered competitive suppression; in all mixed infections involving P1, its early establishment was

suppressed relative to the single infections (GLM single/mixed: $p < 0.001$; Fig. 2*b*). For P1, the degree of competitive suppression was decreased in triple infections, potentially due to competitive interactions between the E2 and R genotypes (Fig. 2 *a-c*; *3 a-c*).

Competitive suppression also reduced the virulence and transmission potential of P1. In single-genotype infections, P1 was more virulent than in mixed-genotype infections in terms of both mean lifespan (GLM single/mixed: $p = 0.052$; Fig. 2*d* vs. 2*e*) and survival (Log-rank test: $X^2 = 12.50$, df = 5, $p = 0.03$; Fig. 2 *g-i*). In other words, in most cases, host survival rates slightly increased when coinfected. This trend was evident in the two-genotype infection with the recombinant genotype, although this trend was not statistically significant (median survival time P1: 31.5 days; P1+R: 48.5 days; adjusted-$p = 0.09$).

These longer lifespans of *E. johnstonei*, however, did not always lead to an overall increase in transmission potential. In all coinfections involving P1, transmission potential (total *Bd* load) was lower than in single-genotype infections (GLM single/mixed: $p = 0.025$; Fig. 4 *a,b*). Thus, even though coinfected hosts lived longer, coinfections with the enzootic lineages still suppressed transmission potential for the most virulent genotype (P1) relative to single-genotype infections.

In contrast, P2, which was the slowest to establish and replicate in single infections and therefore, had a low competitive ability (Fig. 2*a*), facilitated the early establishment of the E1 genotype ($p = 0.049$; Figs. 2*a*, 2*c*, and 3*a*). The trend was the same for the R genotype, though this effect was not statistically significant ($p = 0.271$; Figs. 2*a* and 2*c* and *3b-c*). In mixed infections with P2, it is likely that facilitation occurred for two reasons. First, the total *Bd* load (transmission potential) was higher in mixed infections relative to single-genotype infections (GLM single/mixed: $p < 0.001$; Fig. 4). For E1 in particular, coinfections with P2 lead to drastic increases in total *Bd*

loads with levels reaching those similar to P1 single-genotype infections (Fig. 4 *a*-*b* vs. *c*). Second, the proportion of P2 in these mixed infections was lower relative to either the E1 or E2 genotypes (Fig. 3 *a*, *c*). In triple infections, however, this facilitation was hindered by the recombinant genotype (R) (Fig. 2 *a*-*c* and Fig. 3).

Importantly, despite the increase in pathogen loads (Fig. 4), coinfections involving P2 did not substantially increase virulence (Figs. 2). For instance, mixed infections with P2 had no effect on mean lifespan (GLM single/mixed: $p = 0.113$; Fig. 2*f*) but slightly decreased host survival rates (Log-rank test: $X^2 = 13.30$, df = 5, $p = 0.02$; Fig. 2 *g*-*i*).

DISCUSSION

Coinfections by multiple pathogen genotypes are common in human (reviewed in Balmer & Tanner 2011), plant (Hood 2003; Susi et al. 2015), and wildlife (Rauch et al. 2005; Pickering et al. 2000) diseases. Theory predicts that coinfections can fundamentally alter processes within- and between-hosts with important implications for epidemiological dynamics and virulence evolution (reviewed by Alizon et al. 2013). Based on our results we suggest that coinfection can have divergent outcomes on pathogen load and transmission potential over the course of infection — depending on the particular combination of pathogen genotypes and their introduction history. Importantly, for the most virulent genotype (P1), these within-host interactions among pathogen genotypes reflect both global (O'Hanlon et al. 2018) and regional patterns of *Bd* outbreaks (Carvalho et al 2017).

For the most virulent and competitive genotype (P1), competitive suppression during coinfection reduced both virulent effects on host survival and transmission potential (total *Bd* load). The decline in transmission potential was likely driven through

competitive suppression such that even though coinfected hosts lived longer (which could lead to higher shedding rates), overall pathogen production was reduced relative to hosts infected with a single pathogen genotype. For less virulent genotypes, however, facilitative interactions among pathogen genotypes had little effect on virulence, but did increase transmission potential. Thus, while highly virulent genotypes may be subject to competitive suppression during coinfections, less competitive and virulent genotypes may facilitate other genotypes leading to increased transmission potential.

For P1, the observed link between competitiveness and virulence is consistent with theory (van Baalen & Sabelis 1995, Read & Taylor 2001) and empirical studies, for example, in the rodent malaria system (deRoode et al. 2005), bacteria (Ben-Ami et al. 2008, Inglis et al. 2009, Kinnula et al. 2017), baculoviruses (Hodgson et al. 2004), and may potentially underlie epidemiological dynamics in powdery mildew (Susi et al. 2015). For *Bd*, the link between competitiveness and virulence in the genotype P1 might help explain the global epidemiological patterns of this panzootic pathogen. Specifically, *Bd*-GPL is both globally distributed and associated with mass mortalities of amphibian hosts (James et al. 2015; Lips et al. 2016). Additionally, our results are in agreement with a previous study (e.g. Jenkinson et al. 2018) that demonstrated that *Bd*-GPL was more competitive in the first four weeks post-inoculation, a critical phase for successful pathogen colonization.

The consistency of virulence patterns in *Bd*-GPL from both field observations and empirical studies spanning multiple host species is somewhat surprising. Variation within *Bd*-GPL in phenotypic traits (e.g., zoosporangia and zoospore size) have been observed in both laboratory (Becker et al. 2017; Jenkinson et al. 2018) and field studies (Lambertini et al. 2016), and has been linked to pathogen incidence in natural populations (Lambertini et al. 2016) and virulence in exposure experiments (Fisher et

al. 2009; Farrer et al. 2011). Additionally, over multiple generations, competitive suppression could select for P1 to invest greater resources into phenotypically-plastic transmission stages such that coinfections could actually lead to higher relative transmission (van Baalen & Sabelis 1995). Within this context, pronounced phenotypic variation in P1 (and other *Bd*-GPL genotypes) may therefore help explain its ability to successfully outcompete other genotypes, establish a successful infection within-individual hosts and spread through the population, regardless of the high level of virulence.

The less virulent P2 genotype was isolated from a bullfrog farm, which high density and low diversity of hosts can favor the emergence of highly virulent genotypes by increasing transmission potential (Frank 1996). However, the low production of infectious stages and virulence observed in P2 may, in contrast, indicate that conditions associated with bullfrog farms (e.g. high host density and low diversity) may also select for less virulent genotypes due to a constant availability of hosts resources. In addition, bullfrog conditions may also allow pathogens to reallocate resources to suppress the host immune system. Thus, the evolution of pathogen virulence under bullfrog farm conditions is complex and difficult to predict, as suggested by Ribeiro et al. (2019) in a study where distinct *Bd* genotypes with different degrees of virulence were isolated from these types of farms.

Unlike previous studies, the pathogen genotypes examined here span a range of relatedness and introduction histories (i.e., enzootic vs. panzootic). Theory predicts that the competitive outcomes among coinfecting pathogens depends on their genetic relatedness (Hamilton 1972, Frank 1996, Brown et al. 2002; Buckling & Brockhurst 2008). Distantly related pathogens are predicted to compete more strongly than closely related (and cooperating) pathogens (Hamilton 1972; Buckling & Brockhurst 2008).

However, we found that both facilitation and strong competition occurred among the most distantly related genotypes, P2+E1 vs. P1+E1, respectively. Fully testing current theoretical predictions in this system would require a larger and logistically challenging experiment with both intra- and inter-lineage combinations that was beyond the scope of this current study. In the meantime, our results underscore an important point that is often overlooked by current theory: genotype by genotype interactions and introduction history may play stronger roles than relatedness *per se*.

We corroborate previous studies and help to explain why pathogens in general, and *Bd* in particular, can be highly virulent to their hosts despite their reliance on hosts resources for their own growth and fitness. An important effort for future studies is to understand the extent to which greater phenotypic plasticity in *Bd*-GPL may enable it to maintain fitness in novel environments and reduce the costs of virulence that are predicted to limit transmission. Identifying the particular genes or pathways that enable such plasticity may offer a novel target for managing outbreaks in *Bd* and other fungal pathogens.

Mounting evidence indicate that pathogen infections often consist of multiple distinct genotypes (reviewed by Read & Taylor 2001). In the amphibian chytrid, which is implicated in decimating amphibian populations around the globe (Scheele et al. 2019), coinfections with multiple strains may be common and likely to arise largely from the amphibian world trade (Byrne et al. 2019). We found that coinfections can have important implications for disease dynamics and virulence evolution and warrant further monitoring in wild populations (and the pet trade if such a goal was possible). Regardless of the particular genotypic combinations, coinfection was generally a lose-lose scenario for the host. In the case of the most virulent and widely distributed

genotype (P1), this effect was strong enough that it is reflected in global epidemiological patterns.

Understanding how these within-host dynamics play out over multiple host and pathogen generations and in the wild carries important implications for the evolutionary trajectory of *Bd* and any strategies aimed at virulence management. This underscores the need for ongoing *Bd* monitoring efforts to begin tracking coinfection.

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Figure 1. An overview of the phylogeny, geographical distribution of *Batrachochytrium dendrobatidis* (*Bd*), and experimental design for single- and mixed-genotype infections. (*A*) Phylogenetic tree of *Bd* genotypes used in the challenge assay. (*B*) Map showing the spatial distribution of locations where lineages have been isolated from the Brazilian Atlantic forest (Table S3). On the map, each symbol represents the location where the genotypes used in this challenge assay were isolated from. White triangle: P1, black triangle: P2, diamond: E2, star: E1 and R. (*C*) Overview of infection assay. In order to prepare the *Bd* solutions, we flooded the *Bd* cultures with distilled water, quantified *Bd* zoospore density using a hemocytometer and adjusted all solutions to the same concentration (see Methods for additional experimental details).

Figure 2. Competitive interactions, virulence on mean lifespan and host survival varies across hosts infected with single- and mixed-genotypes of *Batrachochytrium dendrobatidis* (*Bd*). Grey bars highlight single-genotype infections. (*A-C)* Competitiveness of each genotype was measured as the average *Bd* load during the first time point of the assay (day 21). (*D-F*) The most virulent genotype (P1) had a competitive advantage in mixed-genotype infections, while the least virulent genotype (P2) tended to have a facilitative effect on other genotypes. (*G* and *I*) The presence of multiple genotypes reduced the virulence of the most virulent genotype (P1). (*G* and *I*) Coinfections with the least virulent genotype (P2), however, had little effect on survival or median survival time. For survival curves, p-values are from Log-rank tests.

Figure 3. Competitive outcomes in mixed-genotype infections of *Batrachochytrium dendrobatidis* (*Bd*). (*A-C*) Competitive outcomes for the mixed-genotype infections

were based on the proportion of the total pathogen load that was either the most and least virulent genotype (P1 and P2, respectively).

Figure 4. Transmission potential of *Batrachochytrium dendrobatidis* (*Bd*) in both single- and mixed-genotype infections. Grey bars highlight single-genotype infections. (*A* and *B*) Competitive suppression could either help reduce the transmission potential of more virulent genotypes (P1) or (*A* and *C*) increase the transmission potential of less virulent genotypes (P2), likely through facilitation.

Figure 1

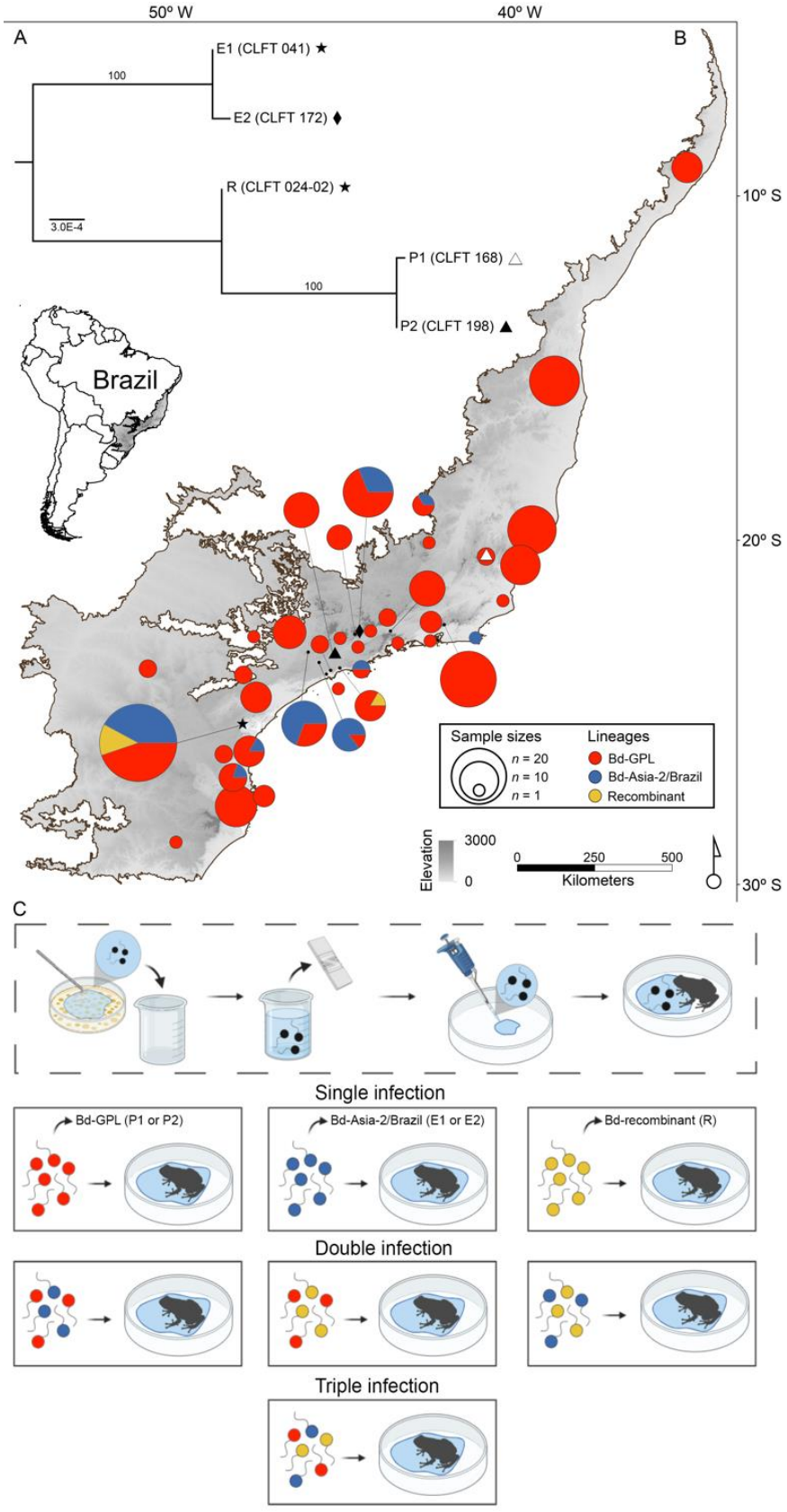

50° W
40° W
A
B
E1 (CLFT 041) ★
100
E2 (CLFT 172) ♦
R (CLFT 024-02) ★
3.0E-4
P1 (CLFT 168) △
100
P2 (CLFT 198) ▲
Brazil
10° S
20° S
30° S
Sample sizes
n = 20
n = 10
n = 1
Lineages
Bd-GPL
Bd-Asia-2/Brazil
Recombinant
Elevation
3000
0
0
250
500
Kilometers
C
Single infection
Bd-GPL (P1 or P2)
Bd-Asia-2/Brazil (E1 or E2)
Bd-recombinant (R)
Double infection
Triple infection

Figure 2

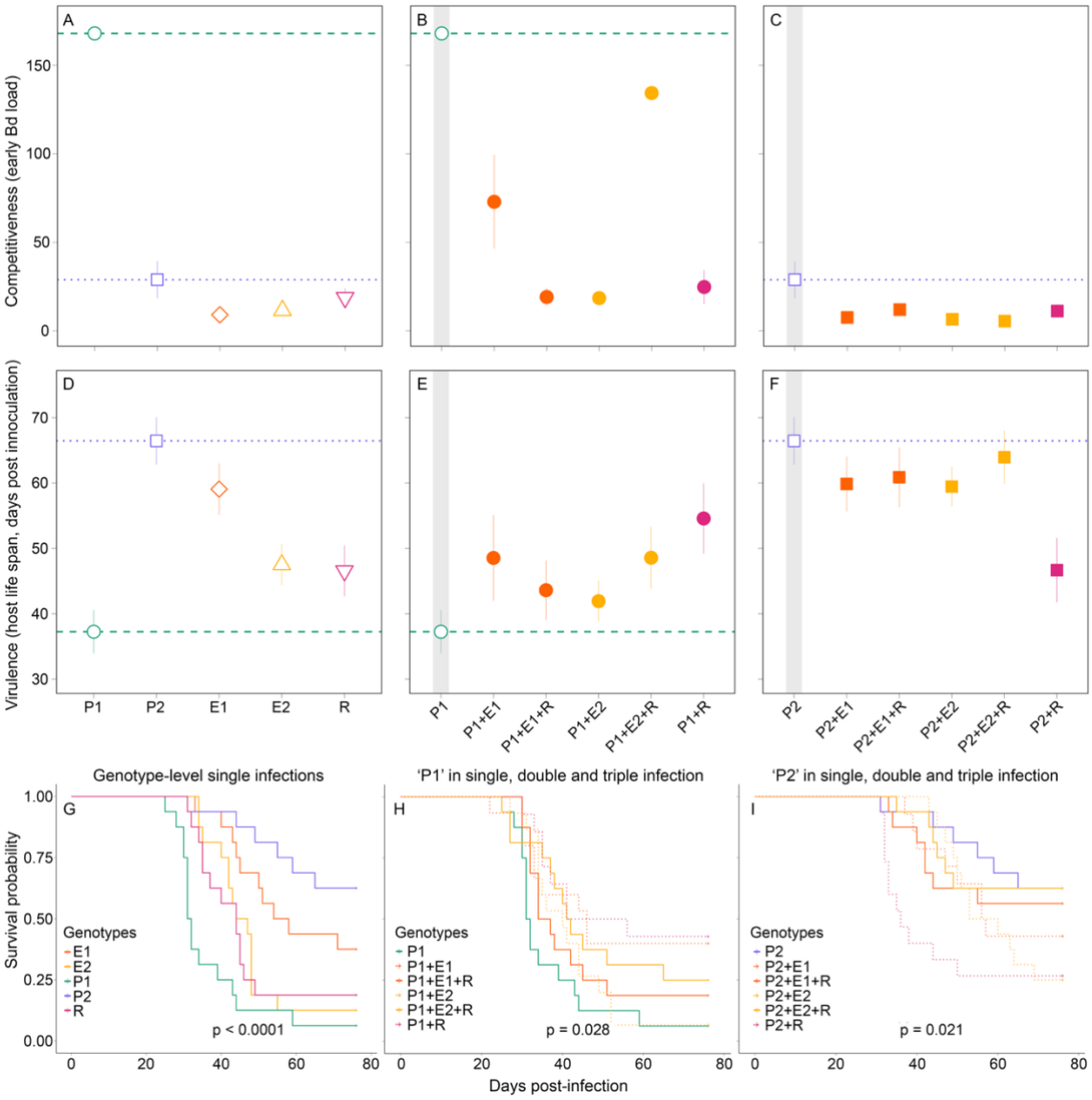

A
B
C
D
E
F
G
H
I
Competitiveness (early Bd load)
Virulence (host life span, days post innoculation)
P1
P2
E1
E2
R
P1+E1
P1+E1+R
P1+E2
P1+E2+R
P1+R
P2+E1
P2+E1+R
P2+E2
P2+E2+R
P2+R
Genotype-level single infections
'P1' in single, double and triple infection
'P2' in single, double and triple infection
Survival probability
Genotypes
p < 0.0001
p = 0.028
p = 0.021
Days post-infection

Figure 3

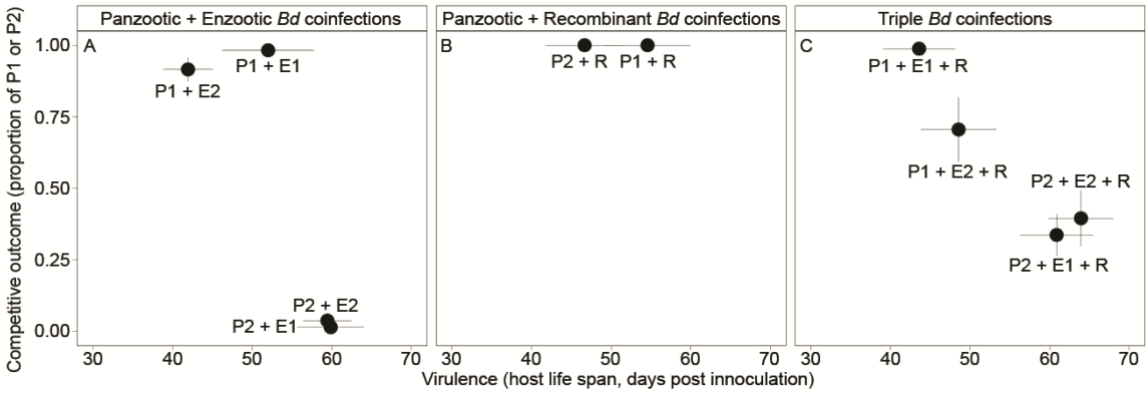
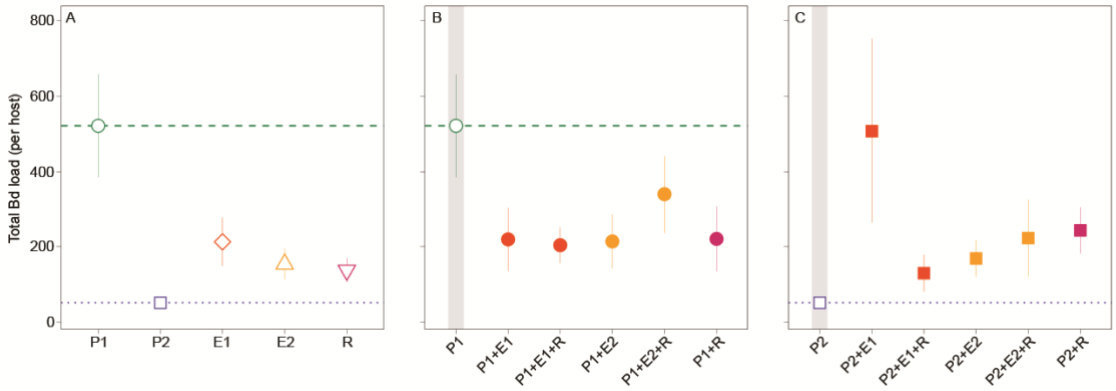

Panzootic + Enzootic *Bd* coinfections
Panzootic + Recombinant *Bd* coinfections
Triple *Bd* coinfections
A
B
C
P1 + E1
P1 + E2
P2 + E2
P2 + E1
P2 + R
P1 + R
P1 + E1 + R
P1 + E2 + R
P2 + E2 + R
P2 + E1 + R
Competitive outcome (proportion of P1 or P2)
1.00
0.75
0.50
0.25
0.00
30
40
50
60
70
Virulence (host life span, days post innoculation)

Figure 4


A
B
C
Total Bd load (per host)
800
600
400
200
0
P1
P2
E1
E2
R
P1
P1+E1
P1+E1+R
P1+E2
P1+E2+R
P1+R
P2
P2+E1
P2+E1+R
P2+E2
P2+E2+R
P2+R

Electronic Supplemental Material

Panzootic chytrid genotypes drive divergent infection dynamics in frogs with multi-lineage infections

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Table S1. *Batrachochytrium dendrobatidis* (*Bd*) genotypes used in the challenge assay. Isolate/genotype name, designation (panzootic 'P', enzootic 'E' and recombinant 'R'), chytrid lineage, locality (municipality, state), host species, year of isolation, and number of passages for each isolate used are given.

Isolate	Designation	Lineage	Locality	Host species	Year	Passage
CLFT 168	P1	Bd-GPL	Alto Caparaó, MG	*Phantasmarana apuana*	2015	12
CLFT 198	P2	Bd-GPL	Santa Isabel, SP	*Aquarana catesbeiana*	2016	9
CLFT 041	E1	Bd-Asia-2/ Brazil	Morretes, PR	*Bokermannohyla hylax*	2013	18
CLFT 172	E2	Bd-Asia-2/ Brazil	Pindamonhangaba, SP	*Aquarana catesbeiana*	2016	9
CLFT 024-02	R	Recombinant	Morretes, PR	*Hylodes cardosoi*	2011	24

Table S2. Treatment groups of the infection assay with five different genotypes of the fungus *Batrachochytrium dendrobatidis* (*Bd*), which included single-genotype and mixed genotype exposures with either two or three genotypes in all possible combinations, and a control group. Designations (panzootic 'P', enzootic 'E' and recombinant 'R'), number of hosts per treatment, and Bd lineage.

Treatment Groups	n	Lineages
P1	16	Bd-GPL
P2	16	Bd-GPL
E1	16	Bd-Asia-2/Brazil
E2	16	Bd-Asia-2/Brazil
R	16	Recombinant
P1 + E1	15	Bd-GPL + Bd-Asia-2/Brazil
P1 + E2	15	Bd-GPL + Bd-Asia-2/Brazil
P1 + R	14	Bd-GPL + Recombinant
P2 + E1	14	Bd-GPL + Bd-Asia-2/Brazil
P2 + E2	16	Bd-GPL + Bd-Asia-2/Brazil
P2 + R	15	Bd-GPL + Recombinant
E2 + R	16	Bd-Asia-2/Brazil + Recombinant
E1 + R	16	Bd-Asia-2/Brazil + Recombinant
P1 + E1 + R	16	Bd-GPL + Bd-Asia-2/Brazil + Recombinant
P1 + E2 + R	16	Bd-GPL + Bd-Asia-2/Brazil + Recombinant
P2 + E1 + R	16	Bd-GPL + Bd-Asia-2/Brazil + Recombinant
P2 + E2 + R	16	Bd-GPL + Bd-Asia-2/Brazil + Recombinant
Control	32	Control

Table S3. *Batrachochytrium dendrobatidis* (*Bd*) genotypes isolated from the Brazilian Atlantic Forest. Isolate name or museum record of the host, host species, host stage, collector name, isolation year, chytrid lineage or genotype, municipality, state, latitude (Lat), longitude (Lon), and reference of each isolate are given.

Isolate/Museum record	Host species	Stage	Collector	Isolation year	Lineage	Municipality	State	Lat	Lon	Reference
CLFT 001/10	*Hylodes japi*	Tadpole	CA Vieira	2010	Bd-Asia-2/ Brazil	Jundiai	SP	-23.25	-46.95	CLFT
CLFT 021	Unidentified	Tadpole	CA Vieira	2010	Bd-GPL	Cabreuva	SP	-23.31	-47.10	CLFT
CLFT 023	*Boana* sp.	Tadpole	CA Vieira	2011	Bd-GPL	Camanducaia	MG	-22.87	-46.03	CLFT
CLFT 024-01	*Hylodes cardosoi*	Tadpole	CA Vieira	2011	Bd-GPL	Morretes	PR	-25.36	-48.88	CLFT
CLFT 024-02	*Hylodes cardosoi*	Tadpole	CA Vieira	2011	Recombinant	Morretes	PR	-25.36	-48.88	CLFT
CLFT 026	*Boana faber*	Tadpole	C Lambertini	2011	Bd-GPL	Iporanga	SP	-24.58	-48.60	CLFT
CLFT 029-00	*Boana* cf. *albopunctata*	Tadpole	C Lambertini	2011	Bd-GPL	Jundiai	SP	-23.25	-46.95	CLFT
CLFT 029-01	*Scinax hiemalis*	Tadpole	C Lambertini	2011	Bd-GPL	Jundiai	SP	-23.25	-46.95	CLFT
CLFT 030	*Hylodes phyllodes*	Tadpole	C Lambertini	2012	Bd-GPL	Bertioga	SP	-23.71	-46.03	CLFT
CLFT 031	*Hylodes phyllodes*	Tadpole	C Lambertini	2012	Bd-GPL	Bertioga	SP	-23.71	-46.03	CLFT
CLFT 032	*Hylodes phyllodes*	Tadpole	C Lambertini	2012	Bd-GPL	Bertioga	SP	-23.71	-46.03	CLFT
CLFT 033	*Hylodes phyllodes*	Tadpole	C Lambertini	2012	Bd-GPL	Bertioga	SP	-23.71	-46.03	CLFT
CLFT 034	*Hylodes phyllodes*	Tadpole	TS Jenkinson	2013	Bd-GPL	Bertioga	SP	-23.71	-46.03	CLFT

CLFT 035	*Boana faber*	Tadpole	KR Zamudio	2013	Bd-GPL	Iporanga	SP	-24.58	-48.60	CLFT
CLFT 036	*Boana faber*	Tadpole	D Rodriguez	2013	Bd-GPL	Iporanga	SP	-24.58	-48.60	CLFT
CLFT 037	*Boana faber*	Tadpole	KR Zamudio	2013	Bd-GPL	Iporanga	SP	-24.58	-48.60	CLFT
CLFT 038	*Bokermannohyla hylax*	Tadpole	TS Jenkinson	2013	Recombinant	Morretes	PR	-25.36	-48.88	CLFT
CLFT 039	*Bokermannohyla hylax*	Tadpole	TS Jenkinson	2013	Recombinant	Morretes	PR	-25.36	-48.88	CLFT
CLFT 040	*Bokermannohyla hylax*	Tadpole	LF Toledo	2013	Bd-Asia-2/ Brazil	Morretes	PR	-25.36	-48.88	CLFT
CLFT 041	*Bokermannohyla hylax*	Tadpole	D Rodriguez	2013	Bd-Asia-2/ Brazil	Morretes	PR	-25.36	-48.88	CLFT
CLFT 042	*Boana faber*	Tadpole	C Betancourt	2013	Bd-GPL	Iporanga	SP	-24.58	-48.60	CLFT
CLFT 043	*Bokermannohyla hylax*	Tadpole	TS Jenkinson	2013	Bd-GPL	Morretes	PR	-25.36	-48.88	CLFT
CLFT 044	*Hylodes cardosoi*	Tadpole	C Betancourt	2013	Bd-Asia-2/ Brazil	Morretes	PR	-25.36	-48.88	CLFT
CLFT 045	*Hylodes cardosoi*	Tadpole	TS Jenkinson	2013	Bd-GPL	Morretes	PR	-25.36	-48.88	CLFT
CLFT 046	*Bokermannohyla hylax*	Tadpole	C Betancourt	2013	Bd-GPL	Morretes	PR	-25.36	-48.88	CLFT
CLFT 047	*Bokermannohyla hylax*	Tadpole	C Betancourt	2013	Bd-GPL	Morretes	PR	-25.36	-48.88	CLFT

CLFT 048	*Hylodes meridionalis*	Tadpole	C Betancourt	2013	Bd-GPL	Rancho Queimado	SC	-27.67	-49.09	CLFT
CLFT 049	*Hylodes meridionalis*	Tadpole	TS Jenkinson	2013	Bd-GPL	Rancho Queimado	SC	-27.67	-49.09	CLFT
CLFT 050	*Hylodes meridionalis*	Tadpole	C Betancourt	2013	Bd-GPL	Rancho Queimado	SC	-27.67	-49.09	CLFT
CLFT 051	*Hylodes meridionalis*	Tadpole	TS Jenkinson	2013	Bd-GPL	Rancho Queimado	SC	-27.67	-49.09	CLFT
CLFT 052	*Hylodes meridionalis*	Tadpole	C Betancourt	2013	Bd-GPL	Rancho Queimado	SC	-27.67	-49.09	CLFT
CLFT 053	*Hylodes meridionalis*	Tadpole	KR Zamudio	2013	Bd-GPL	Rancho Queimado	SC	-27.67	-49.09	CLFT
CLFT 054	*Hylodes meridionalis*	Tadpole	D Rodriguez	2013	Bd-GPL	Rancho Queimado	SC	-27.67	-49.09	CLFT
CLFT 055	*Hylodes meridionalis*	Tadpole	TY James	2013	Bd-GPL	Rancho Queimado	SC	-27.67	-49.09	CLFT
CLFT 056	*Hylodes meridionalis*	Tadpole	TS Jenkinson	2013	Bd-GPL	Rancho Queimado	SC	-27.67	-49.09	CLFT
CLFT 057	*Hylodes meridionalis*	Tadpole	C Betancourt	2013	Bd-GPL	Rancho Queimado	SC	-27.67	-49.09	CLFT
CLFT 058	*Hylodes meridionalis*	Tadpole	TS Jenkinson	2013	Bd-GPL	Rancho Queimado	SC	-27.67	-49.09	CLFT

CLFT 060	*Hylodes meridionalis*	Tadpole	TS Jenkinson	2013	Bd-GPL	Pomerode	SC	-26.77	-49.19	CLFT
CLFT 061	*Hylodes meridionalis*	Tadpole	C Betancourt	2013	Bd-Asia-2/ Brazil	Pomerode	SC	-26.77	-49.19	CLFT
CLFT 062	*Hylodes meridionalis*	Tadpole	C Betancourt	2013	Bd-GPL	Pomerode	SC	-26.77	-49.19	CLFT
CLFT 063	*Hylodes meridionalis*	Tadpole	C Betancourt	2013	Bd-GPL	Pomerode	SC	-26.77	-49.19	CLFT
CLFT 064	*Hylodes meridionalis*	Tadpole	C Betancourt	2013	Bd-GPL	Pomerode	SC	-26.77	-49.19	CLFT
CLFT 065	*Hylodes japi*	Tadpole	C Betancourt	2013	Bd-Asia-2/ Brazil	Jundiai	SP	-23.25	-46.95	CLFT
CLFT 066	*Hylodes japi*	Tadpole	J Longcore	2013	Bd-Asia-2/ Brazil	Jundiai	SP	-23.25	-46.95	CLFT
CLFT 067	*Hylodes japi*	Tadpole	C Betancourt	2013	Bd-Asia-2/ Brazil	Jundiai	SP	-23.25	-46.95	CLFT
CLFT 068	*Hylodes japi*	Tadpole	C Betancourt	2013	Bd-Asia-2/ Brazil	Jundiai	SP	-23.25	-46.95	CLFT
CLFT 070	*Hylodes japi*	Tadpole	J Longcore	2013	Bd-Asia-2/ Brazil	Jundiai	SP	-23.25	-46.95	CLFT
CLFT 071	*Hylodes japi*	Tadpole	C Betancourt	2013	Bd-Asia-2/ Brazil	Jundiai	SP	-23.25	-46.95	CLFT

CLFT 073	*Aplastodiscus* sp.	Tadpole	C Betancourt	2013	Bd-GPL	Teresopolis	RJ	-22.46	-43.00	CLFT
CLFT 074	Unidentified	Tadpole	C Betancourt	2013	Bd-GPL	Teresopolis	RJ	-22.46	-43.00	CLFT
CLFT 075	Unidentified	Tadpole	TY James	2013	Bd-GPL	Teresopolis	RJ	-22.46	-43.00	CLFT
CLFT 076	*Bokermannohyla* sp.	Tadpole	C Betancourt	2013	Bd-GPL	Teresopolis	RJ	-22.46	-43.00	CLFT
CLFT 077	*Bokermannohyla* sp.	Tadpole	C Betancourt	2013	Bd-GPL	Teresopolis	RJ	-22.46	-43.00	CLFT
CLFT 078	*Bokermannohyla* sp.	Tadpole	TY James	2013	Bd-GPL	Teresopolis	RJ	-22.46	-43.00	CLFT
CLFT 079	*Bokermannohyla* sp.	Tadpole	TY James	2013	Bd-GPL	Teresopolis	RJ	-22.46	-43.00	CLFT
CLFT 080	*Bokermannohyla* sp.	Tadpole	C Betancourt	2013	Bd-GPL	Teresopolis	RJ	-22.46	-43.00	CLFT
CLFT 081	Unidentified	Tadpole	C Betancourt	2013	Bd-GPL	Teresopolis	RJ	-22.46	-43.00	CLFT
CLFT 082	*Bokermannohyla* sp.	Tadpole	C Betancourt	2013	Bd-GPL	Teresopolis	RJ	-22.46	-43.00	CLFT
CLFT 083	*Scinax hayii*	Tadpole	C Betancourt	2013	Bd-GPL	Teresopolis	RJ	-22.46	-43.00	CLFT
CLFT 084	*Bokermannohyla* sp.	Tadpole	C Betancourt	2013	Bd-GPL	Teresopolis	RJ	-22.46	-43.00	CLFT
CLFT 085	Unidentified	Tadpole	C Betancourt	2013	Bd-GPL	Teresopolis	RJ	-22.46	-43.00	CLFT
CLFT 086	Unidentified	Tadpole	C Betancourt	2013	Bd-GPL	Teresopolis	RJ	-22.46	-43.00	CLFT
CLFT 087	*Scinax hayii*	Tadpole	C Betancourt	2013	Bd-GPL	Teresopolis	RJ	-22.46	-43.00	CLFT
CLFT 088	*Scinax hayii*	Tadpole	C Betancourt & TS Jenkison	2013	Bd-GPL	Teresopolis	RJ	-22.46	-43.00	CLFT
CLFT 089	*Aplastodiscus sibilatus*	Tadpole	LFM Lima	2013	Bd-GPL	Maceio	AL	-9.23	-35.86	CLFT

CLFT 090	*Aplastodiscus sibilatus*	Tadpole	LFM Lima	2013	Bd-GPL	Maceio	AL	-9.23	-35.86	CLFT
CLFT 091	*Aplastodiscus sibilatus*	Tadpole	LFM Lima	2013	Bd-GPL	Maceio	AL	-9.23	-35.86	CLFT
CLFT 092	*Aplastodiscus sibilatus*	Tadpole	ABCR Lima	2013	Bd-GPL	Maceio	AL	-9.23	-35.86	CLFT
CLFT 093	*Aplastodiscus sibilatus*	Tadpole	ABCR Lima	2013	Bd-GPL	Maceio	AL	-9.23	-35.86	CLFT
CLFT 094	*Aplastodiscus sibilatus*	Tadpole	C Lambertini	2013	Bd-GPL	Maceio	AL	-9.23	-35.86	CLFT
CLFT 095	*Aplastodiscus* sp.	Tadpole	TS Jenkinson	2014	Bd-GPL	Camacan	BA	-15.38	-39.57	CLFT
CLFT 096	*Aplastodiscus* sp.	Tadpole	C Lambertini	2014	Bd-GPL	Camacan	BA	-15.38	-39.57	CLFT
CLFT 097	*Aplastodiscus* sp.	Tadpole	AV Aguilar	2014	Bd-GPL	Camacan	BA	-15.38	-39.57	CLFT
CLFT 098	*Aplastodiscus* sp.	Tadpole	C Lambertini	2014	Bd-GPL	Camacan	BA	-15.38	-39.57	CLFT
CLFT 099	*Aplastodiscus* sp.	Tadpole	TS Jenkinson	2014	Bd-GPL	Camacan	BA	-15.38	-39.57	CLFT
CLFT 100	*Bokermannohyla* sp.	Tadpole	C Lambertini	2014	Bd-GPL	Camacan	BA	-15.38	-39.57	CLFT
CLFT 101	*Aplastodiscus* sp.	Tadpole	TS Jenkinson	2014	Bd-GPL	Camacan	BA	-15.38	-39.57	CLFT
CLFT 102	*Bokermannohyla* sp.	Tadpole	TS Jenkinson	2014	Bd-GPL	Camacan	BA	-15.38	-39.57	CLFT
CLFT 103	*Bokermannohyla* sp.	Tadpole	AV Aguilar	2014	Bd-GPL	Camacan	BA	-15.38	-39.57	CLFT
CLFT 104	*Bokermannohyla* sp.	Tadpole	AV Aguilar	2014	Bd-GPL	Camacan	BA	-15.38	-39.57	CLFT

CLFT 105	*Bokermannohyla* sp.	Tadpole	TS Jenkinson	2014	Bd-GPL	Camacan	BA	-15.38	-39.57	CLFT
CLFT 106	*Bokermannohyla* sp.	Tadpole	TS Jenkinson	2014	Bd-GPL	Camacan	BA	-15.38	-39.57	CLFT
CLFT 107	*Bokermannohyla* sp.	Tadpole	C Lambertini	2014	Bd-GPL	Camacan	BA	-15.38	-39.57	CLFT
CLFT 108	*Bokermannohyla* sp.	Tadpole	TS Jenkinson	2014	Bd-GPL	Camacan	BA	-15.38	-39.57	CLFT
CLFT 109	*Bokermannohyla* sp.	Tadpole	TS Jenkinson	2014	Bd-GPL	Camacan	BA	-15.38	-39.57	CLFT
CLFT 110	*Bokermannohyla* sp.	Tadpole	AV Aguilar	2014	Bd-GPL	Camacan	BA	-15.38	-39.57	CLFT
CLFT 111	*Bokermannohyla* sp.	Tadpole	TS Jenkinson	2014	Bd-GPL	Santa Teresa	ES	-19.83	-40.59	CLFT
CLFT 112	*Aplastodiscus* sp.	Tadpole	AV Aguilar	2014	Bd-GPL	Santa Teresa	ES	-19.83	-40.59	CLFT
CLFT 113	Unidentified	Tadpole	TS Jenkinson	2014	Bd-GPL	Santa Teresa	ES	-19.83	-40.59	CLFT
CLFT 114	*Bokermannohyla* sp.	Tadpole	AV Aguilar	2014	Bd-GPL	Santa Teresa	ES	-19.83	-40.59	CLFT
CLFT 115	*Bokermannohyla* sp.	Tadpole	AV Aguilar	2014	Bd-GPL	Santa Teresa	ES	-19.83	-40.59	CLFT
CLFT 116	*Bokermannohyla* sp.	Tadpole	TS Jenkinson	2014	Bd-GPL	Santa Teresa	ES	-19.83	-40.59	CLFT
CLFT 117	*Bokermannohyla* sp.	Tadpole	C Lambertini	2014	Bd-GPL	Santa Teresa	ES	-19.83	-40.59	CLFT
CLFT 118	*Bokermannohyla* sp.	Tadpole	AV Aguilar	2014	Bd-GPL	Santa Teresa	ES	-19.83	-40.59	CLFT
CLFT 119	*Bokermannohyla* sp.	Tadpole	AV Aguilar	2014	Bd-GPL	Santa Teresa	ES	-19.83	-40.59	CLFT
CLFT 120	*Bokermannohyla* sp.	Tadpole	AV Aguilar	2014	Bd-GPL	Santa Teresa	ES	-19.83	-40.59	CLFT
CLFT 121	*Bokermannohyla* sp.	Tadpole	AV Aguilar	2014	Bd-GPL	Santa Teresa	ES	-19.83	-40.59	CLFT
CLFT 122	*Bokermannohyla* sp.	Tadpole	TS Jenkinson	2014	Bd-GPL	Santa Teresa	ES	-19.83	-40.59	CLFT

CLFT 123	*Bokermannohyla* sp.	Tadpole	C Lambertini	2014	Bd-GPL	Santa Teresa	ES	-19.83	-40.59	CLFT
CLFT 124	*Bokermannohyla* sp.	Tadpole	AV Aguilar	2014	Bd-GPL	Santa Teresa	ES	-19.83	-40.59	CLFT
CLFT 125	Unidentified	Tadpole	C Lambertini	2014	Bd-GPL	Santa Teresa	ES	-19.83	-40.59	CLFT
CLFT 126	*Phyllomedusa* sp.	Tadpole	AV Aguilar	2014	Bd-GPL	Vargem Alta	ES	-20.66	-41.00	CLFT
CLFT 127	*Dendropsophus minutus*	Tadpole	TY James	2014	Bd-GPL	Vargem Alta	ES	-20.66	-41.00	CLFT
CLFT 128	*Aplastodiscus* sp.	Tadpole	TY James	2014	Bd-GPL	Vargem Alta	ES	-20.66	-41.00	CLFT
CLFT 129	*Aplastodiscus* sp.	Tadpole	AV Aguilar	2014	Bd-GPL	Vargem Alta	ES	-20.66	-41.00	CLFT
CLFT 130	*Scinax fuscovarius*	Tadpole	AV Aguilar	2014	Bd-GPL	Vargem Alta	ES	-20.66	-41.00	CLFT
CLFT 131	*Aquarana catesbeiana*	Tadpole	TS Jenkinson	2014	Bd-GPL	Vargem Alta	ES	-20.66	-41.00	CLFT
CLFT 132	*Dendropsophus minutus*	Tadpole	AV Aguilar	2014	Bd-GPL	Vargem Alta	ES	-20.66	-41.00	CLFT
CLFT 133	*Phyllomedusa* sp.	Tadpole	AV Aguilar	2014	Bd-GPL	Vargem Alta	ES	-20.66	-41.00	CLFT
CLFT 134	*Phyllomedusa* sp.	Tadpole	TS Jenkinson	2014	Bd-GPL	Vargem Alta	ES	-20.66	-41.00	CLFT
CLFT 135	*Scinax fuscovarius*	Tadpole	KR Zamudio	2014	Bd-GPL	Vargem Alta	ES	-20.66	-41.00	CLFT
CLFT 136	*Bokermannohyla hylax*	Tadpole	TS Jenkinson	2014	Bd-Asia-2/ Brazil	Morretes	PR	-25.36	-48.88	CLFT
CLFT 137	*Hylodes cardosoi*	Tadpole	TS Jenkinson	2014	Bd-GPL	Morretes	PR	-25.36	-48.88	CLFT

CLFT 138	*Hylodes cardosoi*	Tadpole	C Lambertini	2014	Bd-GPL	Morretes	PR	-25.36	-48.88	CLFT
CLFT 139	*Hylodes cardosoi*	Tadpole	TS Jenkinson	2014	Bd-Asia-2/ Brazil	Morretes	PR	-25.36	-48.88	CLFT
CLFT 141	*Hylodes cardosoi*	Tadpole	LFM Lima	2014	Bd-Asia-2/ Brazil	Morretes	PR	-25.36	-48.88	CLFT
CLFT 142	*Crossodactylus caramaschii*	Tadpole	PP Morao	2014	Bd-Asia-2/ Brazil	Morretes	PR	-25.36	-48.88	CLFT
CLFT 143	*Hylodes cardosoi*	Tadpole	TS Jenkinson	2014	Bd-Asia-2/ Brazil	Morretes	PR	-25.36	-48.88	CLFT
CLFT 144	*Hylodes cardosoi*	Tadpole	TS Jenkinson	2014	Bd-Asia-2/ Brazil	Morretes	PR	-25.36	-48.88	CLFT
CLFT 145	*Hylodes cardosoi*	Tadpole	PP Morao	2014	Bd-Asia-2/ Brazil	Morretes	PR	-25.36	-48.88	CLFT
CLFT 146	*Hylodes cardosoi*	Tadpole	TS Jenkinson	2014	Bd-Asia-2/ Brazil	Morretes	PR	-25.36	-48.88	CLFT
CLFT 148	*Hylodes cardosoi*	Tadpole	TS Jenkinson	2014	Bd-Asia-2/ Brazil	Morretes	PR	-25.36	-48.88	CLFT
CLFT 149	*Hylodes cardosoi*	Tadpole	TS Jenkinson	2014	Bd-Asia-2/ Brazil	Morretes	PR	-25.36	-48.88	CLFT
CLFT 150	*Hylodes cardosoi*	Tadpole	PP Morao	2014	Bd-Asia-2/ Brazil	Morretes	PR	-25.36	-48.88	CLFT

CLFT 151	*Hylodes cardosoi*	Tadpole	PP Morao	2014	Bd-Asia-2/ Brazil	Morretes	PR	-25.36	-48.88	CLFT
CLFT 152	*Crossodactylus caramaschii*	Tadpole	TS Jenkinson	2014	Bd-GPL	Morretes	PR	-25.36	-48.88	CLFT
CLFT 153	*Hylodes cardosoi*	Tadpole	TS Jenkinson	2014	Bd-Asia-2/ Brazil	Morretes	PR	-25.36	-48.88	CLFT
CLFT 154	*Hylodes cardosoi*	Tadpole	TS Jenkinson	2014	Bd-GPL	Morretes	PR	-25.36	-48.88	CLFT
CLFT 156	*Hylodes cardosoi*	Tadpole	TY James	2015	Bd-GPL	Morretes	PR	-25.36	-48.88	CLFT
CLFT 157	*Hylodes cardosoi*	Tadpole	TS Jenkinson	2015	Bd-GPL	Morretes	PR	-25.36	-48.88	CLFT
CLFT 158	*Hylodes cardosoi*	Tadpole	TY James	2015	Bd-GPL	Morretes	PR	-25.36	-48.88	CLFT
CLFT 159	*Hylodes cardosoi*	Tadpole	T Carvalho	2015	Bd-GPL	Morretes	PR	-25.57	-48.90	CLFT
CLFT 160	*Hylodes cardosoi*	Tadpole	TS Jenkinson	2015	Recombinant	Morretes	PR	-25.57	-48.90	CLFT
CLFT 161	*Hylodes cardosoi*	Tadpole	TY James	2015	Bd-GPL	Morretes	PR	-25.57	-48.90	CLFT
CLFT 162	*Hylodes cardosoi*	Tadpole	TS Jenkinson	2015	Bd-GPL	Morretes	PR	-25.57	-48.90	CLFT
CLFT 163	*Hylodes cardosoi*	Tadpole	TS Jenkinson	2015	Bd-GPL	Morretes	PR	-25.57	-48.90	CLFT
CLFT 164	*Hylodes cardosoi*	Tadpole	TY James	2015	Bd-GPL	Morretes	PR	-25.57	-48.90	CLFT
CLFT 165	*Hylodes cardosoi*	Tadpole	TS Jenkinson	2015	Recombinant	Morretes	PR	-25.36	-48.88	CLFT
CLFT 166	Unidentified	Tadpole	PP Morao	2015	Bd-GPL	Campos do Jordao	SP	-22.78	-45.62	CLFT
CLFT 167	Unidentified	Tadpole	TS Jenkinson	2015	Bd-GPL	Campos do Jordao	SP	-22.78	-45.62	CLFT

CLFT 168	*Phantasmarana apuana*	Tadpole	C Lambertini	2015	Bd-GPL	Alto Caparao	MG	-20.42	-41.85	CLFT
CLFT 169	*Phantasmarana apuana*	Tadpole	LFM de Lima	2015	Bd-GPL	Alto Caparao	MG	-20.42	-41.85	CLFT
CLFT 170	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-Asia-2/ Brazil	Pindamonhangaba	SP	-22.85	-45.49	CLFT
CLFT 171	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-Asia-2/ Brazil	Pindamonhangaba	SP	-22.85	-45.49	CLFT
CLFT 172	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-Asia-2/ Brazil	Pindamonhangaba	SP	-22.85	-45.49	CLFT
CLFT 173	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Pindamonhangaba	SP	-22.85	-45.49	CLFT
CLFT 174	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Pindamonhangaba	SP	-22.85	-45.49	CLFT
CLFT 175	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-Asia-2/ Brazil	Pindamonhangaba	SP	-22.85	-45.49	CLFT
CLFT 176	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Pindamonhangaba	SP	-22.85	-45.49	CLFT
CLFT 177	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Pindamonhangaba	SP	-22.85	-45.49	CLFT
CLFT 178	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Pindamonhangaba	SP	-22.85	-45.49	CLFT

CLFT 179	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Pindamonhangaba	SP	-22.85	-45.49	CLFT
CLFT 180	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Pindamonhangaba	SP	-22.85	-45.49	CLFT
CLFT 181	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Pindamonhangaba	SP	-22.85	-45.49	CLFT
CLFT 182	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Pindamonhangaba	SP	-22.85	-45.49	CLFT
CLFT 183	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-Asia-2/ Brazil	Pindamonhangaba	SP	-22.85	-45.49	CLFT
CLFT 184	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Santa Barbara D'Oeste	SP	-22.76	-47.41	CLFT
CLFT 185	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Santa Barbara D'Oeste	SP	-22.76	-47.41	CLFT
CLFT 186	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Santa Barbara D'Oeste	SP	-22.76	-47.41	CLFT
CLFT 187	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Santa Barbara D'Oeste	SP	-22.76	-47.41	CLFT
CLFT 188	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Santa Barbara D'Oeste	SP	-22.76	-47.41	CLFT
CLFT 189	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Santa Barbara D'Oeste	SP	-22.76	-47.41	CLFT

CLFT 190	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Santa Barbara D'Oeste	SP	-22.76	-47.41	CLFT
CLFT 191	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Santa Isabel	SP	-23.32	-46.23	CLFT
CLFT 192	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Santa Isabel	SP	-23.32	-46.23	CLFT
CLFT 193	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Santa Isabel	SP	-23.32	-46.23	CLFT
CLFT 194	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Santa Isabel	SP	-23.32	-46.23	CLFT
CLFT 195	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Santa Isabel	SP	-23.32	-46.23	CLFT
CLFT 196	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Santa Isabel	SP	-23.32	-46.23	CLFT
CLFT 197	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Santa Isabel	SP	-23.32	-46.23	CLFT
CLFT 198	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Santa Isabel	SP	-23.32	-46.23	CLFT
CLFT 199	Unidentified	Tadpole	LP Ribeiro & T Carvalho	2016	Bd-GPL	Jundiai	SP	-23.25	-46.95	CLFT
CLFT 201	Unidentified	Tadpole	C Lambertini	2016	Bd-GPL	Capitolio	MG	-20.65	-46.26	CLFT

CLFT 202	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-Asia-2/ Brazil	Sao Paulo	SP	-23.55	-46.63	CLFT
CLFT 203	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-Asia-2/ Brazil	Sao Paulo	SP	-23.55	-46.63	CLFT
CLFT 204	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-Asia-2/ Brazil	Sao Paulo	SP	-23.55	-46.63	CLFT
CLFT 205	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-Asia-2/ Brazil	Sao Paulo	SP	-23.55	-46.63	CLFT
CLFT 206	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-GPL	Sao Paulo	SP	-23.55	-46.63	CLFT
CLFT 207	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-Asia-2/ Brazil	Sao Paulo	SP	-23.55	-46.63	CLFT
CLFT 209	*Aquarana catesbeiana*	Tadpole	LP Ribeiro	2016	Bd-Asia-2/ Brazil	Sao Paulo	SP	-23.55	-46.63	CLFT
JEL 648	*Hylodes japi*	Tadpole	J Longcore	2010	Bd-Asia-2/ Brazil	Jundiai	SP	-23.25	-46.95	Schloegel et al 2012
JEL 649	*Hylodes japi*	Tadpole	J Longcore	2010	Bd-Asia-2/ Brazil	Jundiai	SP	-23.25	-46.95	Schloegel et al 2012
LMS 902	*Aquarana catesbeiana*	Adult	LM Schloegel	2008	Bd-GPL	Pindamonhangaba	SP	-22.85	-45.49	Schloegel et al 2012
LMS 925	*Aquarana catesbeiana*	Adult	LM Schloegel	2008	Bd-GPL	Pindamonhangaba	SP	-22.85	-45.49	Schloegel et al 2012

LMS 931	*Aquarana catesbeiana*	Adult	LM Schloegel	2009	Bd-GPL	Tremembe	SP	-23.00	-45.51	Schloegel et al 2012
MNRJ 1352	*Cycloramphus brasiliensis*	Adult	D Rodriguez	1926	Bd-GPL	Petropolis	RJ	-22.50	-43.18	Rodriguez et al 2014
MNRJ 1484	*Hylodes asper*	Adult	D Rodriguez	1928	Bd-GPL	Petropolis	RJ	-22.50	-43.18	Rodriguez et al 2014
MNRJ 1516	*Hylodes asper*	Adult	D Rodriguez	1928	Bd-GPL	Sao Jose do Barreiro	SP	-22.64	-44.56	Rodriguez et al 2014
MNRJ 189	*Cycloramphus semipalmatus*	Adult	D Rodriguez	1922	Bd-GPL	Cubatao	SP	-23.89	-46.42	Rodriguez et al 2014
MNRJ 2142	*Fritziana fissilis*	Adult	D Rodriguez	1951	Bd-GPL	Sao Jose do Barreiro	SP	-22.64	-44.56	Rodriguez et al 2014
MNRJ 34713	*Melanophryniscus moreirae*	Adult	D Rodriguez	1949	Bd-GPL	Petropolis	RJ	-22.50	-43.18	Rodriguez et al 2014
MNRJ 43610	*Ololygon ariadne*	Adult	D Rodriguez	1951	Bd-GPL	Sao Jose do Barreiro	SP	-22.64	-44.56	Rodriguez et al 2014
MNRJ 531	*Cycloramphus fuliginosus*	Adult	D Rodriguez	1923	Bd-GPL	Campos dos Goytacazes	RJ	-21.74	-41.33	Rodriguez et al 2014
MNRJ 546e	*Hylodes perplicatus*	Adult	D Rodriguez	1915	Bd-GPL	Joinville	SC	-26.30	-48.84	Rodriguez et al 2014
MNRJ 546g	*Hylodes perplicatus*	Adult	D Rodriguez	1915	Bd-GPL	Joinville	SC	-26.30	-48.84	Rodriguez et al 2014

MNRJ 5549	*Melanophryniscus moreirae*	Adult	D Rodriguez	1902	Bd-GPL	Itatiaia	RJ	-22.35	-44.63	Rodriguez et al 2014
MNRJ 5592	*Hylodes perplicatus*	Adult	D Rodriguez	1916	Bd-Asia-2/ Brazil	Joinville	SC	-26.30	-48.84	Rodriguez et al 2014
MNRJ 5594	*Hylodes perplicatus*	Adult	D Rodriguez	1916	Bd-GPL	Joinville	SC	-26.30	-48.84	Rodriguez et al 2014
MNRJ 5607	*Hylodes perplicatus*	Adult	D Rodriguez	1916	Bd-GPL	Joinville	SC	-26.30	-48.84	Rodriguez et al 2014
MNRJ 5613	*Hylodes perplicatus*	Adult	D Rodriguez	1916	Bd-GPL	Joinville	SC	-26.30	-48.84	Rodriguez et al 2014
MNRJ 840	*Scinax perpusillus*	Adult	D Rodriguez	1924	Bd-GPL	Angra dos Reis	RJ	-23.01	-44.31	Rodriguez et al 2014
MNRJ/ALMN 1390	*Crossodactylus gaudichaudii*	Adult	D Rodriguez	1927	Bd-GPL	Santo Andre	SP	-23.78	-46.30	Rodriguez et al 2014
MNRJ/ALMN 1999	*Crossodactylus gaudichaudii*	Adult	D Rodriguez	1929	Bd-GPL	Rio De janeiro	RJ	-22.90	-43.20	Rodriguez et al 2014
MNRJ/ALMN 2065	*Crossodactylus dispar*	Adult	D Rodriguez	1930	Bd-GPL	Sao Jose do Barreiro	SP	-22.64	-44.56	Rodriguez et al 2014
MNRJ/ALMN 2071	*Crossodactylus dispar*	Adult	D Rodriguez	1930	Bd-GPL	Sao Jose do Barreiro	SP	-22.64	-44.56	Rodriguez et al 2014
MNRJ/ALMN 480	*Crossodactylus gaudichaudii*	Adult	D Rodriguez	1923	Bd-Asia-2/ Brazil	Cabo Frio	RJ	-22.86	-42.04	Rodriguez et al 2014

MNRJ/ALMN 87	*Crossodactylus gaudichaudii*	Adult	D Rodriguez	1920	Bd-GPL	Teresopolis	RJ	-22.46	-43.00	Rodriguez et al 2014
MZUSP 109133	*Crossodactylus gaudichaudii*	Adult	D Rodriguez	1968	Bd-GPL	Sao Jose do Barreiro	SP	-22.64	-44.56	Rodriguez et al 2014
MZUSP 109513	*Crossodactylus bokermanni*	Adult	D Rodriguez	1971	Bd-GPL	Catas Altas	MG	-20.05	-43.45	Rodriguez et al 2014
MZUSP 109645	*Crossodactylus caramaschii*	Adult	D Rodriguez	1958	Bd-GPL	Florianopolis	SC	-27.49	-48.40	Rodriguez et al 2014
MZUSP 109650	*Crossodactylus caramaschii*	Adult	D Rodriguez	1959	Bd-GPL	Florianopolis	SC	-27.49	-48.40	Rodriguez et al 2014
MZUSP 109659	*Crossodactylus caramaschii*	Adult	D Rodriguez	1959	Bd-GPL	Florianopolis	SC	-27.49	-48.40	Rodriguez et al 2014
MZUSP 110332	*Ololygon ariadne*	Adult	D Rodriguez	1968	Bd-GPL	Sao Jose do Barreiro	SP	-22.64	-44.56	Rodriguez et al 2014
MZUSP 110336	*Ololygon ariadne*	Adult	D Rodriguez	1967	Bd-GPL	Sao Jose do Barreiro	SP	-22.64	-44.56	Rodriguez et al 2014
MZUSP 110631	*Crossodactylus bokermanni*	Adult	D Rodriguez	1964	Bd-GPL	Santana do Riacho	MG	-18.96	-43.71	Rodriguez et al 2014
MZUSP 112587	*Hylodes asper*	Adult	D Rodriguez	1965	Bd-GPL	Marumbi	PR	-23.70	-51.63	Rodriguez et al 2014
MZUSP 112588	*Hylodes asper*	Adult	D Rodriguez	1965	Bd-GPL	Marumbi	PR	-23.70	-51.63	Rodriguez et al 2014

MZUSP 118	*Boana pardalis*	Adult	D Rodriguez	1942	Bd-GPL	Itatiaia	RJ	-22.35	-44.63	Rodriguez et al 2014
MZUSP 130376	*Hylodes perplicatus*	Adult	D Rodriguez	2000	Bd-GPL	Monte Alegre dos Campos	RS	-28.78	-50.79	Rodriguez et al 2014
MZUSP 136242	*Fritziana fissilis*	Adult	D Rodriguez	2006	Recombinant or Coinfection	Bertioga	SP	-23.71	-46.03	Rodriguez et al 2014
MZUSP 209	*Bokermannohyla luctuosa*	Adult	D Rodriguez	1897	Bd-GPL	Piquete	SP	-22.61	-45.17	Rodriguez et al 2014
MZUSP 240	*Boana faber*	Adult	D Rodriguez	1907	Bd-GPL	Santo Andre	SP	-23.78	-46.30	Rodriguez et al 2014
MZUSP 296	*Scinax hayii*	Adult	D Rodriguez	1902	Bd-GPL	Campos do Jordao	SP	-22.73	-45.59	Rodriguez et al 2014
MZUSP 53055	*Vitreorana eurygnatha*	Adult	D Rodriguez	1976	Bd-GPL	Itapeva	SP	-23.96	-48.90	Rodriguez et al 2014
MZUSP 53058	*Vitreorana eurygnatha*	Adult	D Rodriguez	1976	Bd-GPL	Itapeva	SP	-23.96	-48.90	Rodriguez et al 2014
MZUSP 53330	*Megaelosia goeldii*	Adult	D Rodriguez	1977	Bd-GPL	Teresopolis	RJ	-22.46	-43.00	Rodriguez et al 2014
MZUSP 56836	*Crossodactylus bokermanni*	Adult	D Rodriguez	1979	Bd-Asia-2/ Brazil	Santana do Riacho	MG	-18.96	-43.71	Rodriguez et al 2014
MZUSP 58677	*Hylodes heyeri*	Adult	D Rodriguez	1972	Bd-GPL	Iporanga	SP	-24.48	-48.65	Rodriguez et al 2014

MZUSP 58744	*Cycloramphus boraceiensis*	Adult	D Rodriguez	1982	Bd-Asia-2/ Brazil	Sao Sebastiao	SP	-23.76	-45.41	Rodriguez et al 2014
MZUSP 60698	*Hylodes perplicatus*	Adult	D Rodriguez	1982	Bd-GPL	Sao Sebastiao	SP	-23.76	-45.41	Rodriguez et al 2014
MZUSP 64712	*Phyllomedusa distincta*	Adult	D Rodriguez	1949	Bd-GPL	Sao Bento do Sul	SC	-26.24	-49.38	Rodriguez et al 2014
MZUSP 64714	*Phyllomedusa distincta*	Adult	D Rodriguez	1949	Bd-GPL	Sao Bento do Sul	SC	-26.24	-49.38	Rodriguez et al 2014
MZUSP 75694	*Aplastodiscus perviridis*	Adult	D Rodriguez	1970	Bd-GPL	Botucatu	SP	-22.89	-48.45	Rodriguez et al 2014
MZUSP 76436	*Fritziana goeldii*	Adult	D Rodriguez	1964	Bd-GPL	Teresopolis	RJ	-22.46	-43.00	Rodriguez et al 2014
MZUSP 76695	*Proceratophrys boiei*	Adult	D Rodriguez	1964	Bd-GPL	Teresopolis	RJ	-22.46	-43.00	Rodriguez et al 2014
MZUSP 77615	*Crossodactylus bokermanni*	Adult	D Rodriguez	1973	Bd-GPL	Santana do Riacho	MG	-18.96	-43.71	Rodriguez et al 2014
MZUSP 86	*Boana prasina*	Adult	D Rodriguez	1905	Bd-GPL	Campos do Jordao	SP	-22.73	-45.59	Rodriguez et al 2014

* CLFT: Coleção Luís Felipe Toledo, MZUSP: Museu de Zoologia da Universidade de São Paulo, MNRJ: Museu Nacional do Rio de Janeiro, AL: State of Alagoas, BA: State of Bahia, ES: State of Espirito Santo, MG: State of Minas Gerais, PR: State of Parana, RJ: State of Rio de Janeiro, SC: State of Santa Catarina, SP: State of Sao Paulo.

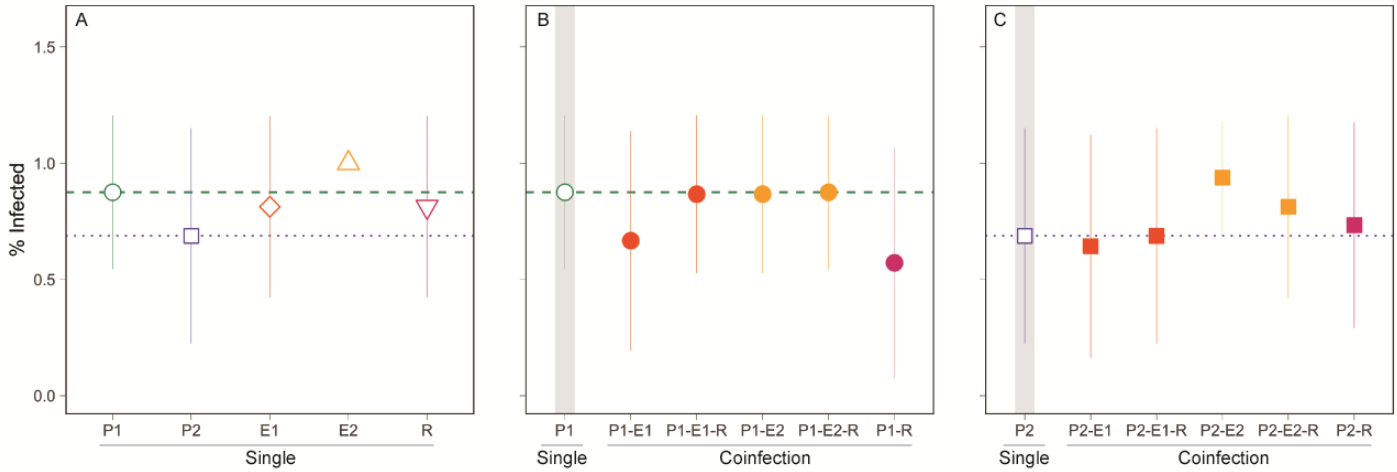

Figure S1. The presence of multiple genotypes did not affect the proportion of hosts infected (Binomial GLM, all *p*-values > 0.05). Grey bars highlight single-genotype infections. These results serve as a quality control metric and clarify that our infection methodology was consistent across treatments.

Appendix S1. *Batrachochytrium dendrobatidis (Bd*) genotype sequences in Fasta format.

>CLFT024
CGCATGGCAGAGGCAAGATGGCAGCGCGAATGTCARCAGCTTGTTCAAAAA
TTAGAATACGAGGTAGATGGTTTAAGCCTGCTAAATTTACCACAACAAAAA
TCTAATATGCATGATATAGTTGAACAGGTCAGATGAGTTGCAGCGTCTTTTC
GATCAGCAAGTGCTCAAGACACGGGAATTAGAGAGRGAGCTGGCTGACACC
CGACTGCTTTTGCACCAGACTCAATCTGGTTAGTGCAGTGTTTACTTTTTAAC
TATTTGTCGTTGAATTTCATTCGAATTAACTTATTTATCTGATGCGTGGAGGG
TATTACCACGAAGTTCGGCTTCACGGATAGTACGAGCCAAATTGTCACCTGG
AAGAGCCTAGTAGAGATCATTGATTACAACATTCAACAATGAAGGGTTAGT
ATGCGCGATTTACTACGACTGAGCTGATGCATCATGTAATAGGATCAGCAA
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CAPÍTULO 3. Thermal mismatch explains *Batrachochytrium dendrobatidis* outbreaks in the Atlantic rainforest

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Abstract

Global climate change is increasing the frequency of unpredictable weather conditions, which has triggered the emergence of disease outbreaks in wildlife around the world. Host-pathogen dynamics can be especially affected by climate in systems involving ectothermic hosts, given their dependence to temperature for basic physiological processes. However, few studies have shown the effect of climatic anomalies in the distribution of diseases. The recently emerged thermal mismatch hypothesis proposes that ectothermic hosts susceptibility to a pathogen increases as temperatures depart from its thermal optima. It also suggests that hosts adapted to cooler and warmer climates should be at greatest risk of infection under warm and cool conditions, respectively. Here, we tested the effect of thermal mismatch on the historical (50 years) prevalence of the *Batrachochytrium dendrobatidis* (Bd) pathogen along an elevational gradient in amphibian hosts from the Brazilian Atlantic Forest. Bd outbreaks have caused dozens of amphibian declines in the Atlantic Forest, even after a long period of coexistence with amphibian hosts. We expect higher Bd prevalence in lowland warm-adapted hosts during cooler periods and vice versa. In addition, because most of the declines have occurred at high elevations, and because high elevation amphibians generally experience less thermal variation, we expect a greater effect of the thermal mismatch in cool-adapted amphibians. Our findings are consistent with the thermal mismatch hypothesis, and corroborate that there are context-dependent effects of temperature on Bd prevalence in amphibian populations. However, we found a stronger effect of thermal mismatch on lowland warm-adapted amphibians. Our study indicates that Bd outbreaks in the Atlantic Forest were possibly triggered by changes in climatic conditions and highlight the need of considering species specific elevation breadth when evaluating the environmental context of pathogen infections.

Keywords

Climate anomaly, amphibians, host-pathogen dynamics, chytrid fungus

Introduction

Anthropogenic climate change and emerging infectious diseases are synergistically causing unprecedented threats to biodiversity and human health (Daszak et al. 2000; Pecl et al. 2017). Human actions, besides influencing mean global temperature and precipitation, are also increasing the variability of these factors (Easterling 2000; Schär et al. 2004; Cai et al. 2014), leading to a less predictable climate. Increased climatic anomalies may be one of the main causes of disease outbreaks worldwide (Rohr & Raffel 2010; Cohen et al. 2019a), due to the strong link between climate and host-pathogen dynamics (Harvell et al. 2002; Altizer et al. 2013). However, although the relationship between climatic anomalies and disease outbreaks has been suggested, few studies have addressed the underlying mechanisms via which these anomalies affect host-pathogen interaction (Rohr & Raffel 2010; Cohen et al. 2017).

Host-pathogen dynamics can be especially affected by climate in systems involving ectothermic hosts. Ectotherms have a limited capacity to produce metabolic heat and generally depend on external sources of heat to maintain their body temperature (Seebacher et al. 2015). Thus, fluctuations in temperature can push these animals closer to their thermal limits, negatively affecting their natural behavior and physiological processes (Huey & Stevenson 1979; Dell et al. 2011; Amarasekare & Sifuentes 2012). In addition, disease dynamics that are influence by differences in optimum thermal performance between ectothermic hosts and their pathogens may be altered as thermal regimes shift (Cohen et al. 2017). Thus, understanding ectothermic

hosts-pathogen dynamics provides an ideal system to assess the impact of climate change on disease outbreaks.

In terms of the mechanism via which anthropic climate change could influence disease dynamics in ectothermic hosts, Cohen et al. (2017) proposed the thermal mismatch hypothesis, which states that host susceptibility to a pathogen increases as temperatures depart from its thermal optima. For instance, the hypothesis predicts that ectothermic hosts adapted to cooler temperatures can experience the greatest susceptibility to pathogen infection at warmer climates and *vice-versa* (Cohen et al. 2017). The thermal mismatch hypothesis is based on the assumptions that hosts and pathogens are locally adapted, that cool- or warm-adapted hosts and parasites occur near their thermal limits of performance, and that pathogens, being smaller than their hosts, have broader thermal tolerances and adapt or acclimate faster than their hosts (Raffel et al. 2006; Cohen et al. 2017; Rohr et al. 2018). Thus, considering that a pathogen can maintain high performance over a wider range of temperatures compared to the host, disease outbreaks are expected to occur at a temperate range that reduces host performance while that of the pathogen remains relatively high. The thermal mismatch hypothesis was first proposed and supported using amphibians and their skin pathogenic fungus *Batrachochytrium dendrobatidis* (Bd) as a study system (Cohen et al. 2017), and it was later corroborated by multiple experiments and field studies at global scale using diverse host-pathogen systems (Cohen et al. 2019a, 2019b, 2020; Sauer et al. 2020). However, assessments of the thermal mismatch hypothesis using historical data of host-pathogen dynamics in megadiverse communities are still lacking, and may provide a critical system to further expand the predictive ability of this hypothesis.

Chytridiomycosis, the emergent infectious disease caused by Bd, has been associated with amphibian population declines and extinctions worldwide (Scheele et al. 2019; Fisher & Garner 2020). Bd has been found infecting the keratinized mouthparts of tadpoles and skin of post-metamorphic individuals of over 500 amphibian species worldwide (Lips 2016). Amphibian mass-mortalities have been observed during epizootic events following the introduction of Bd [the novel spreading pathogen hypothesis (Lips et al. 2006; Lips et al. 2008)], and in areas where the fungus was already present [endemic pathogen hypothesis (Goka et al. 2009; Vredenburg et al. 2013; Carvalho et al. 2017; Toledo 2017)]. In both scenarios, most amphibian declines occurred in pristine forests at high elevations (Lips et al. 2008; Carvalho et al. 2017; Scheele et al. 2019), possibly due to a greater sensitivity to thermal mismatches in amphibians at these localities (Cohen et al. 2019b), where temperatures are generally constant (Brattstrom 1968; Navas 1996) and highly endemic hosts adapted to mountain tops have narrow thermal breadths (Rohr & Raffel 2010). Although Cohen et al. (2019b) demonstrated an effect of elevation on the contribution of thermal mismatch to Bd occurrence, whether the thermal mismatch hypothesis is applicable to explain Bd susceptibility of lowland species still requires further investigation.

Here, we tested the effect of thermal mismatch (by assessing the interaction between elevation and temperature anomaly) on Bd prevalence along an elevation gradient, for which we used a 50-year time series dataset of Bd incidence in amphibians from the Brazilian Atlantic Forest, one of the most biodiverse regions in the world (Toledo et al. 2021). In this Neotropical region, chytridiomycosis outbreaks were detected at least a century after the first Bd detection (Rodriguez et al. 2014) and affected several amphibian populations across the entire Atlantic Forest (Carvalho et al.

2017). Most of the declines occurred at high elevation sites (Carvalho et al. 2017), where the prevalence of Bd is usually higher when compared to lowlands (Gründler et al. 2012). In this study, considering the predictions of the thermal mismatch hypothesis, we expect to determine 1) a higher prevalence of Bd in lowland (warm-adapted) host populations during cooler periods, and 2) the opposite pattern for highland (cool-adapted) host populations – higher Bd prevalence during warmer periods, after accounting for other environmental factors such as rainfall metrics. In addition, because most of the declines have occurred at high elevations, and because high elevation amphibians generally experience less thermal variation, we expect to find a greater effect of thermal mismatch on cool-adapted amphibians at high elevation sites (Cohen et al. 2017).

Methods

Study site and Bd prevalence data

The Atlantic Forest can be divided into two divergent bioclimatic regions, the Northern and Southern portions (NAF and SAF, respectively), with the Rio Doce as the biogeographic boundary between them (Oliveira-Filho & Fontes 2000; Thomé et al. 2014; Carnaval et al. 2014). Relative to the NAF region, the SAF region, which extends from 19 to 32 degrees of latitude, presents a lower mean annual temperature, a greater volume of precipitation and higher variation in elevation, ranging from sea level to about 3,000 m (Grimm 2003; Carlucci et al. 2021; Lambertini et al. 2021; Lins-e-Silva et al 2021). In terms of Bd, different pathogen dynamics have been observed between these two regions (Ruthsatz et al. 2020; Lambertini et al. 2021). Though, these regions differ in the amount of sampling effort invested to detect Bd in the temporal and spatial scale, being higher for the SAF region. In an extensive sampling of museum specimens

from the Atlantic Forest that comprised a time frame of 85 years (1930-2015), Carvalho et al. (2017) realized that the majority of amphibian samples had been collected in the SAF, allowing to conduct powerful "natural experiments (Körner 2007), such as an assessment of the thermal mismatch hypothesis to predict the pattern of Bd outbreaks along temperature gradients. To avoid mixing distinct methods of Bd detection, we compiled data of 17,616 tadpoles visually inspected for Bd infection in the southern Atlantic Forest between 1963 and 2013 from a single study (Carvalho et al. 2017), using ArcGis v.10.8 (ESRI, 2019).

Environmental data

We extracted data of historic monthly mean temperatures and total rainfall from the Hadley Climate Research Unit (Harris et al. 2014), and of elevation from the Bioclim raster (Fick & Hijmans 2017). To extract the data, we specified the georeferenced location where each individual was collected in the field, and used the function *extract* from the *raster* package (Hijmans 2014). Monthly climate data were available with a resolution of 2.5 minutes (~ 21 km^2), and elevation with 30 seconds (1 km^2). Using the monthly historical data of climate, we calculated two metrics: an annual mean and a 50-year period mean for temperature and rainfall (average of the monthly mean temperature and rainfall of the month of collection and previous eleven or 599 months, respectively). Lastly, we subtract the 50-year means from the annual means to calculate temperature and rainfall annual anomalies. All analyzes were performed in the R statistical software v. 4.0.2 (R Core Team, 2020).

Statistical analyses

To assess the thermal mismatch hypothesis across elevations, we determined the effect of environmental anomaly (for rainfall and temperature) and elevation, and their interaction on the pattern of Bd prevalence using Generalized Linear Mixed Effect Models (GLMM). We fit the GLMMs using a binomial error distribution (link logit) and a covariance structure AR(1), to account for temporal autocorrelations across years and seasons, using the *glmmTMB* function and package (Brooks et al. 2017). Thermal mismatch was included in the model as an interaction term between elevation and annual temperature anomaly of each location where amphibians were collected. Elevation is a strong proxy for long-term climate adaptation (Sternberg & Thomas 2014), while annual temperature anomaly indicates if temperatures are raising or lowering based on the past five decades. As we expect that lowland warm-adapted host populations would have a higher Bd prevalence under cool periods, and vice versa, the estimate of the coefficient of bidirectional thermal mismatch interaction is hypothesized to be positive. To control for latitude and rainfall anomaly effects, which have been shown to influence Bd prevalence (Kriger et al. 2007; Becker & Zamudio 2011; Ruggeri et al. 2018; Moura-Campos et al 2021), we have included latitude in absolute values as a random effect in the model, while all other variables, including rainfall anomaly, were included as fixed effects. In addition, latitude accounted for low spatial autocorrelation (Moran's I test, observed = 0.053, expected = -0.002, standard deviation = 0.021, $P = 0.009$), because all latitude and longitude combinations were unique location in our data.

We fit the models using an automated model selection approach to test the relative importance of each environmental variable and the interaction between elevation and temperature anomaly using the *dredge* function of the *MuMin* package (Bartoń K, 2020). This function generates models using all combination of variables,

and rank the models based on corrected Akaike information Criterion (AICc). The variance inflation factors (VIF) of the final model was tested to be inferior to 4, using the *multicollinearity* function of the *performance* package (Lüdecke et al. 2020). Finally, to test our hypothesis that amphibians from higher elevations are more susceptible to Bd following thermal mismatches, we conducted a Flinger–Killeen test for homogeneity of variances (*fligner.test* function) to assess how the variance of mean temperature changed with elevation.

The fit of the best model was assessed visually and statistically using a simulation-based approach in the package *DHARMa* (Hartig, F 2020). First, we used the function *simulateResiduals* to create scaled residuals simulated from the best model (adjusted for 1,000 simulations). Then, we performed a Kolmogorov-Smirnov (KS), dispersion and outliers tests to detect overall deviations from the expected distribution using the function *testResiduals*. Lastly, we test whether the expected number of zeros based on our fitted model differs from the number of observed zeros, using the *testZeroInflation* function. The model was robust in all tests ($P > 0.05$), except for the KS test, although visually it does not show a strong deviation (Figure S1). In addition, on the package *DHARMa* documentation, it is unlikely to have a model, containing a large sample size, with a perfect fit (Hartig, F 2020). Lastly, to visually inspect our result, we generated partial residual plots using the *visreg* function and package (Breheny & Burchett, 2017). This function isolates the effects of certain factors while controlling for all other factors in the statistical model that are not being displayed in the figure.

Results

The automated model selection ranked the models with thermal mismatch terms as the most parsimonious model, with no other submodels presenting a $\Delta AIC \leq 2$ (Table S1). In addition, rainfall anomaly was excluded from the model with the highest Akaike weight (Table S1), which intrinsic variation was potentially account for by the factor 'seasons' (Table S2).

The binomial mixed-effects model results showed that elevation ($\beta = 0.0023$; $P < 0.01$) and temperature anomaly ($\beta = -0.5015$; $P < 0.01$) were both predictors of Bd prevalence (Table 1). Furthermore, the effect of temperature anomaly on Bd prevalence depended on elevation, as shown by the significant interaction between temperature anomaly and elevation ($\beta = 0.0006$; $P < 0.01$; Table 1; Figure 1A). In other words, Bd prevalence increased in lowland warm-adapted hosts during cooler years, while Bd prevalence increased during warmer years in highland cool-adapted hosts. In addition, the effect of thermal mismatch was higher in lowland warm-adapted hosts relative to that of highland cool-adapted hosts (Figure 1A). Lastly, a Flinger–Killeen test for homogeneity of variances determined that host species at lower elevations in our dataset experienced greater temperature variability ($\chi^2 = 125.58$, $P < 0.0001$).

Discussion

Species interactions can be sensitive to environmental conditions. In recent decades, an increasing number of disease outbreaks triggered by anomalous climatic events have been reported worldwide (Rohr & Raffel 2010; Cohen et al. 2019a). Within this context, our findings show that chytridiomycosis outbreaks over a 50-year time series in the southern Brazilian Atlantic forest (SAF) were possibly triggered by changes in climatic conditions. In particular, our findings are consistent with the

thermal mismatch hypothesis, and corroborate that there are context-dependent effects of temperature on Bd prevalence in amphibian populations (Cohen et al. 2017). Specifically, highland cool-adapted hosts showed higher Bd prevalence in the warmest years, while lowland warm-adapted showed hosts higher Bd prevalence in the coolest years. In addition, our findings indicate a stronger effect of thermal mismatch in lowland warm-adapted amphibians than in highland cool-adapted amphibian hosts.

The evidence that climatic anomalies could also influence the severity of chytridiomycosis in hyper diverse amphibian populations in the SAF show that global climate change can favor Bd outbreaks even in regions where the pathogen have coexisted with amphibians for a long time (Toledo 2017). Besides such environmental effects, chytridiomycosis outbreaks could also be related to the emergence of highly virulent recombinant variants (Jenkinson et al. 2016; Greenspan et al. 2018), a sudden increase in virulence in local genotype/s through genetic recombinations, mutations or hybridizations (Fisher et al. 2009; Rosenblum et al. 2013; Phillips & Puschendorf 2013), pathogen phenotypic plasticity (Lambertini et al. 2016), or even to the arrival of a novel genotype of the Global Panzootic Lineage (Bd-GPL) (O'Hanlon et al. 2018), which is the most virulent clade and associated with declines globally (Lips et al. 2008; James et al. 2015). However, although we cannot rule these factors out, they are unlikely to have emerged in several locations within the SAF in a short period of time, which makes the regional climate effect the most parsimonious explanation for Bd outbreaks in area.

Our findings indicate that the effect of climatic anomalies on Bd dynamics varies along the elevation gradient. Specifically, based on our results, we expect warmer years to negatively impact amphibian populations at higher elevations, and cooler years to impact amphibian populations at lower elevations (Figure 1A). Although many

studies have found higher Bd prevalence positively associated with elevation (Brem & Lips 2008; Gründler et al. 2012), others described that elevation was negatively associated (Catenazzi et al. 2011), had no effect on Bd incidence (Kriger & Hero 2008; Lambertini et al. 2016; Zornosa-Torres et al. 2021), or was confounded with elevational shifts in land cover (Becker & Zamudio 2011). Thus, we suggest that incorporating the thermal mismatch approach may be more suitable when evaluating temporal changes in Bd prevalence along elevation gradients.

Our study is the first to provide evidence that supports the thermal mismatch hypothesis over a 50-year time series in a megadiverse amphibian community. Contrary to our predictions, however, our results indicate that lowland warm-adapted amphibians from the SAF may be more sensitive to climatic anomalies despite experiencing greater variations in average temperatures (Figure 1A). A possible explanation for this would be that cooler years could negatively affect several physiological processes by reducing the metabolic rate (Huey & Stevenson 1979; Angilletta 2009; Dell et al. 2011) and suppress the immune system of warm-adapted amphibians (Maniero & Carey 1997; Carey et al. 1999), possibly amplifying the thermal mismatch effect. Future studies should use a temperature gradient to assess the interaction between Bd and immune system in amphibian species inhabiting lowlands.

We suggest that a synergistic effect between climatic anomalies and chytridiomycosis may have triggered several declines in the SAF. Despite the higher effect of thermal mismatch on lowland warm-adapted amphibians, most declines (at least those recorded) have occurred at higher elevations (Carvalho et al. 2017). This scenario suggests that in addition to the thermal mismatches, other mechanisms may be affecting disease dynamic. For example, pathogen load is expected to be a determining factor in amphibian mortality (Vredenburg et al. 2010; Ribeiro et al. 2019), and it can

be negatively affected by the dilution effect in amphibian communities (Becker et al. 2014). As amphibian diversity generally decreases with elevation (Stevens 1992), infection loads at low elevations may not be sufficient to trigger amphibians mass mortalities.

We hereby provide support for the thermal mismatch hypothesis and highlight the need of considering species specific elevational breadth when evaluating the environmental context of pathogen infections. In addition, this study highlights the inconsistent pattern of Bd prevalence along elevation gradients. A striking finding that warrants further research is the observation that lowland warm-adapted amphibians may be relatively more sensitive to the effect of thermal mismatch between host and pathogen, contrary to previous findings (Cohen et al. 2019b). Understanding the mechanisms that influence host-pathogen dynamics in wildlife can guide efforts to prevent future vertebrate extinctions in a climatically unpredictable world.

Acknowledgements

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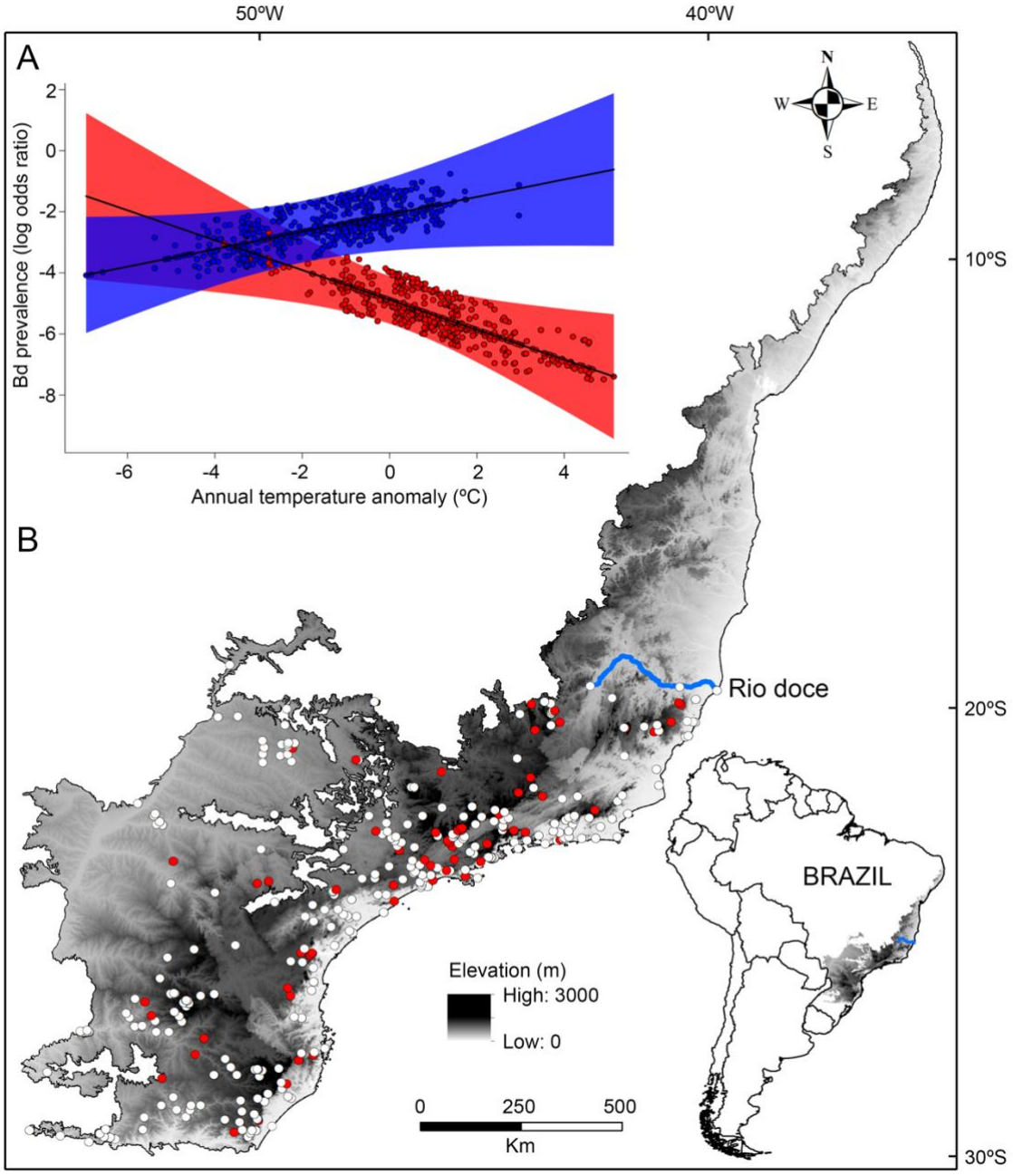
Table 1. Parameter estimates of Generalized Linear Mixed Models with temporal autocorrelations (AR1: year and seasons) assessing the effects environment variables on *Batrachochytrium dendrobatidis* prevalence, and variance inflation factors. Parameter estimates are on the logit scale, and can be back-transformed as $e^x/(1+e^x)$.

Predictors	Estimate	Std. Error	*P*	VIF
Intercept	-7.2826	1.3604	< 0.01	
Elevation	0.0023	0.0006	< 0.01	1.18
Annual temperature anomaly	-0.5015	0.1925	< 0.01	2.28
Elevation:Annual temperature anomaly	0.0006	0.0001	< 0.01	2.04

Latitude: Variance = 12.336, Times series Year: Variance = 4.045, Corr(ar1) = 0.91, Times series Seasons: Variance = 0.635, Corr(ar1) = 0.13, n = 17,616.

Figure 1. A) Estimated relationship between thermal mismatch (significant interaction term: elevation + annual temperature anomaly) and disease prevalence. Partial residual plot of *Batrachochytrium dendrobatidis* (Bd) prevalence are from binomial mixed-effects model. Red represent lowland warm-adapted hosts (10^{th} percentile elevation centred on 20 m) and blue highland cool-adapted hosts (90^{th} percentile elevation centred on 1250 m). Points represent individual hosts inspected for Bd, and shading shows 95 % confidence interval. B) Geographical distribution of Bd-infected (red dots) and Bd-undetected tadpoles (white dots) collected between 1963 and 2013 in the southern Atlantic forest.

Figure 1


50°W
40°W
A
Bd prevalence (log odds ratio)
2
0
-2
-4
-6
-8
-6
-4
-2
0
2
4
Annual temperature anomaly (°C)
N
W
E
S
B
10°S
20°S
30°S
Rio doce
BRAZIL
Elevation (m)
High: 3000
Low: 0
0
250
500
Km

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Electronic Supplemental Material

Thermal mismatch explains *Batrachochytrium dendrobatidis* outbreaks in the Atlantic rainforest

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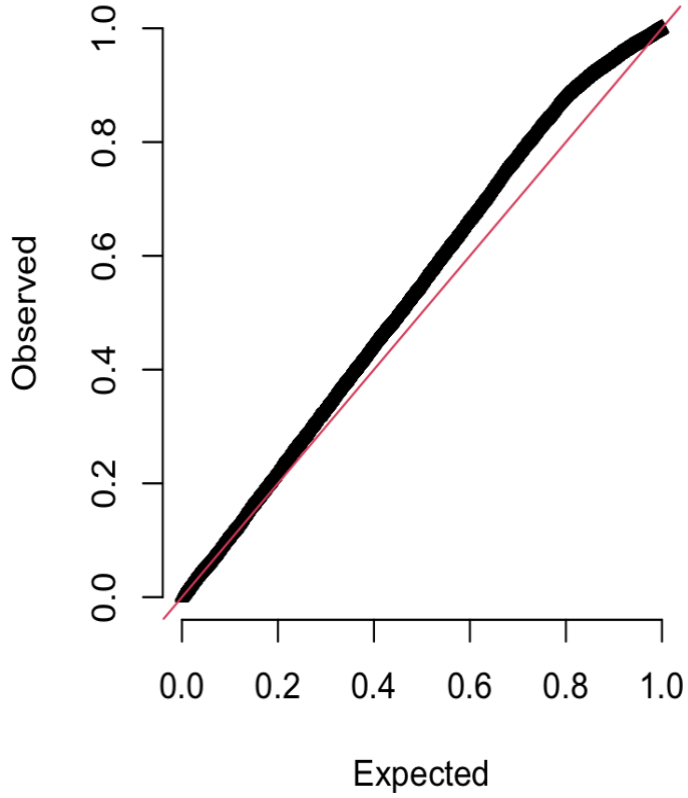

Figure S1. Residual diagnostic plot of the Generalized Linear Mixed Effect Models testing the effects of environment variables on *Batrachochytrium dendrobatidis* prevalence. Red line represents expected distribution and black dots represent observed distribution. Kolmogorov-Smirnov test: $P = 0$.

Table S1. Results of automated model selection. Shown are standardized regression coefficients for numeric predictors.

Model Rank	Intercept	Elevation	Annual temperature anomaly	Annual rainfall anomaly	Annual temperature anomaly: Elevation	df	Log-likelihood	AICc	delta	Akaike weights
1	-7.283	0.0023	-0.5015		0.0006	9	-5456.195	10930.4	0.00	0.506
2	-7.304	0.0023	-0.4872	-0.0034	0.0006	10	-5455.275	10930.6	0.16	0.466
3	-7.159	0.0019		-0.0036		8	-5461.080	10938.2	7.77	0.010
4	-7.161	0.0019				7	-5462.156	10938.3	7.92	0.010
5	-7.124	0.0018	-0.0288	-0.0036		9	-5461.055	10940.1	9.72	0.004
6	-7.112	0.0018	-0.0411			8	-5462.105	10940.2	9.82	0.004
7	-6.017		-0.2557			7	-5466.484	10947.0	16.57	0
8	-6.012		-0.2463	-0.0035		8	-5465.494	10947.0	16.60	0
9	-5.905			-0.0039		7	-5468.126	10950.3	19.86	0
10	-5.902					6	-5469.365	10950.7	20.33	0

Table S2. Parameter estimates of Generalized Linear Mixed Models with temporal autocorrelation (AR1: year) assessing the effects environment variables on *Batrachochytrium dendrobatidis* prevalence, and variance inflation factors. Parameter estimates are on the logit scale, and can be back-transformed as $e^x/(1+e^x)$.

Predictors	Estimate	Std. Error	*P*	VIF
Intercept	-7.4844	0.9767	<0.0001	
Elevation	0.0027	0.0007	<0.0001	1.16
Annual temperature anomaly	-0.4546	0.1943	<0.0001	3.57
Annual rainfall anomaly	-0.0117	0.0023	<0.0001	1.02
Elevation:Annual temperature anomaly	0.0008	0.0002	<0.0001	3.36

Latitude: Variance = 13.438, Times series Year: Variance = 3.346, Corr(ar1) = 0.82, n = 17,616

CONCLUSÕES

Ações antrópicas e doenças infecciosas emergentes representam ameaças sem precedentes à biodiversidade, particularmente para os anfíbios, o grupo de vertebrado mais ameaçado em todo o mundo (IUCN 2021). Quase a totalidade das espécies do Brasil ameaçadas e dos declínios ou extinções populacionais de anfíbios se concentram na Mata Atlântica (Heyer *et al.* 1988; Weygoldt 1989; Eterovick *et al.* 2005; DOU 2014 Carvalho *et al.* 2017), bioma que se destaca por ser um hotspot para a conservação da biodiversidade do mundo (Myers 2003), em especial de anfíbios (Haddad *et al.* 2013).

No presente trabalho, elucidamos como a desconexão de *habitat*, a quitridiomicose e as mudanças climáticas podem ameaçar a diversidade de anfíbios da Mata Atlântica.

Um modelo baseado em indivíduo demostrou que os anfíbios reprodutores aquáticos (que realizam migrações reprodutivas entre fragmentos florestais e corpos d'água) são mais afetadas pela desconexão de *habitat* do que os anfíbios reprodutores terrestres. Ainda, descobrimos que o grau de desconexão pode ser um fator importante para a manutenção de populações de anfíbios e encontramos suporte para nosso modelo analisando dados empíricos de uma paisagem fragmentada da Mata Atlântica.

Acessamos o efeito de coinfecções por diferentes genótipos de Bd na dinâmica da quitridiomicose através de um experimento laboratorial que contemplava infecções simples e mistas com duas linhagens do patógeno Bd e um genótipo recombinante. Nossos resultados indicam que em parte as coinfecções podem ser benéficas a curto prazo para os hospedeiros, que tiveram cargas dos patógenos mais virulentos reduzida e apresentaram maior tempo de vida em infecções mistas quando comparada às infecções simples, possivelmente devido a competições interespecíficas entre os genótipos coinfectantes. Entretanto, genótipos mais virulentos superam genótipos menos virulentos quando em coinfecção, o que a longo prazo permite a dominância de genótipos mais virulentos na população. Ainda, encontramos interações facilitativas entre genótipos de Bd menos virulentos, o que acarretou em cargas maiores dos patógenos quando em coinfecção. No geral, o resultado da doença foi contexto dependente dos genótipos de Bd em infecções simples e mistas.

Mostramos fortes evidencias de que as mudanças climáticas podem ter desempenhado um papel importante para os surtos de quitridiomicose na Mata Atlântica. Testamos a hipótese da incompatibilidade térmica em anfíbios amostrados para Bd ao longo de uma série temporal de 50 anos. Nossos achados suportam a hipótese da incompatibilidade térmica, evidenciando que anfíbios adaptados ao frio de regiões elevadas apresentaram maior prevalência do patógeno Bd em anos mais quentes, enquanto anfíbios de baixa elevação adaptados ao calor apresentaram maior prevalência do Bd em anos mais frios. Assim, mesmo em regiões onde o fungo Bd coexiste com anfíbios por um longo período, as anomalias climáticas podem alterar a dinâmica Bd-anfíbio, o que pode facilitar a emergência de surtos de quitridiomicose.

Com base em nossos resultados, sugerimos que futuros trabalhos abordem (i) possíveis efeitos sinergéticos entre a divisão do *habitat* e a quitridiomicose em comunidades de anfíbios, (ii) a ocorrência de seleção de parentesco e como esse mecanismo pode determinar as relações entre os genótipos de Bd dentro dos hospedeiros e (iii) possíveis diferenças fisiológicas entre populações de anfíbios adaptados ao calor e ao frio da Mata Atlântica, no âmbito de evidenciar quais mecanismos podem estar atuando para que as espécies de baixa elevação sofram um maior impacto das incompatibilidades térmicas.

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ANEXOS

1. Licença de coleta e manutenção de animais em cativeiro

Ministério do Meio Ambiente –MMA
Instituto Chico Mendes de Conservação da Biodiversidade –ICMBio
Sistema de Autorização e Informação em Biodiversidade –SISBIO

Autorização para atividades com finalidade científica

Número: 71780-1	Data da Emissão: 03/09/2019 10:20:00	Data da Revalidação*: 03/09/2020
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: Tamilie Carvalho	CPF: 367.720.108-09
Título do Projeto: Efeito da competição intraespecífica na evolução da virulência do fungo Batrachochytrium dendrobatidis	
Nome da Instituição: UNIVERSIDADE ESTADUAL DE CAMPINAS	CNPJ: 46.068.425/0001-33

Cronograma de atividades

#	Descrição da atividade	Início (mês/ano)	Fim (mês/ano)
1	Coleta e manutenção em cativeiro	11/2019	09/2020

Equipe

#	Nome	Função	CPF	Nacionalidade
1	Luisa de Pontes Ribeiro	Coleta	384.418.958-05	Brasileira
2	Janaina de Andrade Serrano	Coleta	409.749.308-65	Brasileira

Observações e ressalvas

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5	As atividades de campo exercidas por pessoa natural ou jurídica estrangeira, em todo o território nacional, que impliquem o deslocamento de recursos humanos e materiais, tendo por objeto coletar dados, materiais, espécimes biológicos e minerais, peças integrantes da cultura nativa e cultura popular, presente e passada, obtidos por meio de recursos e técnicas que se destinem ao estudo, à difusão ou à pesquisa, estão sujeitas a autorização do Ministério de Ciência e Tecnologia.
6	O titular de licença ou autorização e os membros da sua equipe deverão optar por métodos de coleta e instrumentos de captura direcionados, sempre que possível, ao grupo taxonômico de interesse, evitando a morte ou dano significativo a outros grupos; e empregar esforço de coleta ou captura que não comprometa a viabilidade de populações do grupo taxonômico de interesse em condição in situ.
7	Este documento não dispensa o cumprimento da legislação que dispõe sobre acesso a componente do patrimônio genético existente no território nacional, na plataforma continental e na zona econômica exclusiva, ou ao conhecimento tradicional associado ao patrimônio genético, para fins de pesquisa científica, bioprospecção e desenvolvimento tecnológico. Veja maiores informações em www.mma.gov.br/cgen.

Este documento foi expedido com base na Instrução Normativa nº 03/2014. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Sisbio/ICMBio na Internet (www.icmbio.gov.br/sisbio).

Código de autenticação: 0717800120190903 Página 1/3

Ministério do Meio Ambiente –MMA
Instituto Chico Mendes de Conservação da Biodiversidade –ICMBio
Sistema de Autorização e Informação em Biodiversidade –SISBIO

Autorização para atividades com finalidade científica

Número: 71780-1	Data da Emissão: 03/09/2019 10:20:00	Data da Revalidação*: 03/09/2020
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: Tamilie Carvalho	CPF: 367.720.108-09
Título do Projeto: Efeito da competição intraespecífica na evolução da virulência do fungo Batrachochytrium dendrobatidis	
Nome da Instituição: UNIVERSIDADE ESTADUAL DE CAMPINAS	CNPJ: 46.068.425/0001-33

Locais onde as atividades de campo serão executadas

#	Descrição do local	Município-UF	Bioma	Caverna?	Tipo
1	Rua Miranda Guerra, Jardim Cordeiro	São Paulo-SP	Mata Atlântica	Não	Fora de UC Federal

Atividades X Táxons

#	Atividade	Táxon	Qtde.
1	Captura de animais silvestres in situ	Eleutherodactylus johnstonei	–
2	Manutenção temporária (até 24 meses) de vertebrados silvestres em cativeiro	Eleutherodactylus johnstonei	–
3	Coleta/transporte de espécimes da fauna silvestre in situ	Eleutherodactylus johnstonei	336

Materiais e Métodos

#	Tipo de Método (Grupo taxonômico)	Materiais
1	Amostras biológicas (Anfíbios)	Outras amostras biológicas(Animal inteiro)
2	Método de captura/coleta (Anfíbios)	Captura manual

Destino do material biológico coletado

#	Nome local destino	Tipo destino
1	UNIVERSIDADE ESTADUAL DE CAMPINAS	Laboratório

Ministério do Meio Ambiente –MMA
Instituto Chico Mendes de Conservação da Biodiversidade –ICMBio
Sistema de Autorização e Informação em Biodiversidade –SISBIO

Autorização para atividades com finalidade científica

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Nome da Instituição: UNIVERSIDADE ESTADUAL DE CAMPINAS	CNPJ: 46.068.425/0001-33

Registro de coleta imprevista de material biológico

De acordo com a Instrução Normativa nº03/2014, a coleta imprevista de material biológico ou de substrato não contemplado na autorização ou na licença permanente deverá ser anotada na mesma, em campo específico, por ocasião da coleta, devendo esta coleta imprevista ser comunicada por meio do relatório de atividades. O transporte do material biológico ou do substrato deverá ser acompanhado da autorização ou da licença permanente com a devida anotação. O material biológico coletado de forma imprevista, deverá ser destinado à instituição científica e, depositado, preferencialmente, em coleção biológica científica registrada no Cadastro Nacional de Coleções Biológicas (CCBIO).

Táxon*	Qtde.	Tipo de Amostra	Qtde.	Data

* Identificar o espécime do nível taxonômico possível.

2. Cadastro no Conselho de Gestão do Patrimônio Genético – SISGen

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Cadastro de Acesso Nº A8246D0

Tipo de Usuário:	**INDEPENDENTE**
Responsável pelo cadastro:	**36772010809**
Objeto do Acesso:	**Patrimônio Genético**
O acesso foi realizado antes de 17/11/2015 ou obteve autorização de acesso antes de 17/11/2015?	**Não, sem solicitação de autorização em tramitação**
Finalidade do Acesso:	**Pesquisa**
Estas atividades são baseadas em acesso realizado anteriormente?:	**Não**
Este cadastro está vinculado a cadastro anterior de remessa?	**Não**

Patrimônio Genético

Título da Atividade:	**Coinfecção de anfíbios pelo fungo Batrachochytrium dendrobatidis**
Título da Atividade em inglês:	**Coinfection of amphibians by the fungus Batrachochytrium dendrobatidis**
Resumo da atividade (incluindo objetivos e resultados esperados ou obtidos, conforme o caso)	**Investigamos a relação entre a biodiversidade do patógeno Batrachochytrium dendrobatidis e o declínio de anfíbios, realizando um experimento de coinfecção com diferentes genótipos do fungo Batrachochytrium dendrobatidis. Encontramos que uma maior diversidade de patógenos pode ser benéfica para os anfíbios.**
Resumo não sigiloso da Atividade em Inglês	**We investigated the relationship between the biodiversity of the pathogen Batrachochytrium dendrobatidis and the decline of amphibians, carrying out a co-infection experiment with different genotypes of the fungus. We found that a greater diversity of pathogens can be beneficial to amphibians.**
Palavra(s)-chave:	**Anfíbios, doenças, Batrachochytrium dendrobatidis, epidemiologia**
Palavra(s)-chave em inglês:	**Amphibians, diseases, Batrachochytrium dendrobatidis, epidemiology**
Período das Atividades:	**01/01/2018** **01/03/2020**

Equipe

Nome Completo	Documento	Instituição	Nacionalidade
Tamilie Carvalho	367.720.108-09	UNICAMP	Brasil

Sobre o Componente do Patrimônio Genético Acessado

O acesso ao patrimônio genético será realizado em área indispensável à segurança nacional ou águas jurisdicionais brasileiras, plataforma continental e zona econômica exclusiva:	**Não**

Tipo de Componente:	**Fauna**
Nome Científico:	**Eleutherodactylus johnstonei**
Reino:	**Animalia**
Filo/Divisão:	**Chordata**
Classe:	**AMPHIBIA**
Ordem:	**ANURA**
Família:	**Eleutherodactylidae**
Trata-se de variedade tradicional local ou crioula ou raça localmente adaptada ou crioula?	**Não**

Sobre a Procedência Do Patrimônio Genético

Procedência da amostra:	**In situ**
Tipo de fonte ex situ:	**Outras coleções ex situ**
UF:	**SP**
Município:	**São Paulo**
Latitude:	**23º 38" 11.11' S**
Longitude:	**46º 41" 0.04' W**
Bioma:	**Mata Atlântica**
Data da coleta	**10/04/2018**

Data do Cadastro:	**17/05/2021 16:07:43**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situacão cadastral conforme consulta ao SisGen em **16:16** de **17/05/2021**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - SISGEN

3. Autorização da Comissão de Ética no Uso de Animais da Universidade Estadual de Campinas

UNICAMP

CEUA/UNICAMP

CERTIFICADO

Certificamos que a proposta intitulada **Efeito da competição e virulência por coinfecção de linhagens do fungo Batrachochytrium dendrobatidis**, registrada com o nº **5398-1/2019**, sob a responsabilidade de **Prof. Dr. Luís Felipe Toledo e Tamilie Carvalho**, que envolve a produção, manutenção ou utilização de animais pertencentes ao filo *Chordata*, subfilo *Vertebrata* (exceto o homem) para fins de pesquisa científica (ou ensino), encontra-se de acordo com os preceitos da **LEI Nº 11.794, DE 8 DE OUTUBRO DE 2008**, que estabelece procedimentos para o uso científico de animais, do **DECRETO Nº 6.899, DE 15 DE JULHO DE 2009**, e com as normas editadas pelo **Conselho Nacional de Controle da Experimentação Animal (CONCEA), tendo sido aprovada pela Comissão de Ética no Uso de Animais da Universidade Estadual de Campinas - CEUA/UNICAMP**, em reunião de **10/10/2019**.

Finalidade:	**( ) Ensino (X) Pesquisa Científica**
Vigência do projeto:	15/11/2019 a 15/03/2020
Vigência da autorização para manipulação animal:	10/10/2019 a 15/03/2020
Espécie / linhagem/ raça:	Anfíbio** / Eleutherodactylus johnstonei
No. de animais:	16
Idade/Peso:	1.00 Anos / 20.00 Gramas
Sexo:	8 Machos 8 Fêmeas
Espécie / linhagem/ raça:	Anfíbio** / Eleutherodactylus johnstonei
No. de animais:	16
Idade/Peso:	1.00 Anos / 20.00 Gramas
Sexo:	8 Machos 8 Fêmeas
Espécie / linhagem/ raça:	Anfíbio** / Eleutherodactylus johnstonei
No. de animais:	16
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Sexo:	8 Machos 8 Fêmeas
Espécie / linhagem/ raça:	Anfíbio** / Eleutherodactylus johnstonei
No. de animais:	16
Idade/Peso:	1.00 Anos / 20.00 Gramas
Sexo:	8 Machos 8 Fêmeas

Espécio / linhagem/ raça:	Anfíbio** / Eleutherodactylus johnstonei
No. de animais:	16
Idade/Peso:	1.00 Anos / 20.00 Gramas
Sexo:	8 Machos 8 Fêmeas
Espécie / linhagem/ raça:	Anfíbio** / Eleutherodactylus johnstonei
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No. de animais:	16
Idade/Peso:	1.00 Anos / 20.00 Gramas
Sexo:	8 Machos 8 Fêmeas
Espécie / linhagem/ raça:	Anfíbio** / Eleutherodactylus johnstonei
No. de animais:	16
Idade/Peso:	1.00 Anos / 20.00 Gramas
Sexo:	8 Machos 8 Fêmeas
Espécie / linhagem/ raça:	Anfíbio** / Eleutherodactylus johnstonei
No. de animais:	32
Idade/Peso:	1.00 Anos / 20.00 Gramas
Sexo:	16 Machos 16 Fêmeas
Origem:	Animais invasores que serão coletados nos jardins de residências da cidade de São Paulo
Biotério onde serão mantidos os animais:	LaDiVert, DBA/IB/UNICAMP

A aprovação pela CEUA/UNICAMP não dispensa autorização a junto ao **IBAMA, SISBIO** ou **CIBio** e é **restrita** a protocolos desenvolvidos em biotérios e laboratórios da Universidade Estadual de Campinas.

Campinas, **18 de outubro de 2019**.

Cinthia Baú Betim Cazarin
Prof. Dr. Wagner José Fávaro
Presidente

Rosangela dos Santos
Secretária Executiva

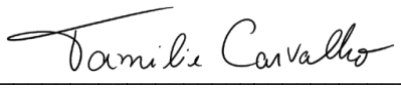
IMPORTANTE: Pedimos atenção ao prazo para envio do relatório final de atividades referente a este protocolo: até 30 dias após o encerramento de sua vigência. O formulário encontra-se disponível na página da CEUA/UNICAMP, área do pesquisador responsável. A não apresentação de relatório no prazo estabelecido impedirá que novos protocolos sejam submetidos.

4. Declaração referente aos direitos autorais

Declaração

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Dissertação/Tese de Mestrado/Doutorado, intitulada **Desconexão de habitat e a dinâmica do quitrídio em anfíbios da Mata Atlântica**, não infringem os dispositivos da Lei n.° 9.610/98, nem o direito autoral de qualquer editora.

Campinas, 20 de setembro de 2021

Assinatura : 
Nome do(a) autor(a): **Tamilie Carvalho**
RG n.° 45.715.056-7

Assinatura : 
Nome do(a) orientador(a): **Luís Felipe Toledo Ramos Pereira**
RG n.° 28465361-5