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# Jumping into a trap: high prevalence of chytrid fungus in the preferred microhabitats of a bromeliad-specialist frog

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**ABSTRACT:** The chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) has been identified as a major threat to several amphibian populations in tropical forests. Amphibians that inhabit the phytotelmata (water tanks) of bromeliads may be especially at risk of *Bd* infection since the humid, environmentally buffered microhabitat that they prefer might also be favorable for *Bd* persistence on the host. To test this hypothesis, we sampled adults and tadpoles of the bromeligenous anuran *Phyllodytes edelmoi* (endemic to the northern Brazilian Atlantic Forest) from the bromeliad *Portea leptantha* for *Bd*, using qPCR. We also analyzed 8 bromeliad characteristics: water tank temperature and pH, canopy closure, tank diameter, number of leaves, bromeliad maximum column depth to store water, bromeliad relative volume, and season. Adult frogs preferentially selected bromeliads with a smaller diameter, more leaves and a relatively higher volume of water. We found that *Bd* was more prevalent in frogs inhabiting bromeliad phytotelmata with smaller diameters, suggesting that the behavioral preferences of *P. edelmoi* may be driving *Bd* infection patterns. Therefore, species such as *P. edelmoi* will be trapped by their own natural history traits.

**KEY WORDS:** Pathogen · *Batrachochytrium dendrobatidis* · *Phyllodytes* · Atlantic Forest · Bromeligenous

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## INTRODUCTION

The pathogenic chytrid fungus *Batrachochytrium dendrobatidis* (hereafter *Bd*) has now been detected in over 500 amphibian species worldwide (Olson et al. 2013, James et al. 2015). Despite its documented role in amphibian population declines (e.g. Lips et al. 2008, Stuart et al. 2008, Wake & Vredenburg 2008), the spatial and temporal dynamics of enzootic *Bd* are poorly understood (Lips et al. 2008, Briggs et al.

2010). The Brazilian Atlantic Forest (AF) is characterized by high levels of amphibian species richness and endemism (e.g. Toledo & Batista 2012, Haddad et al. 2013). AF amphibians are also functionally diverse, being characterized by diverse reproductive modes, and morphological and behavioral adaptations (Haddad et al. 2013). Likewise, *Bd* in the AF has a high genetic diversity (Schloegel et al. 2012, Rosenblum et al. 2013, Jenkinson et al. 2016) and has been detected in more than 130 amphibian species (James et

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al. 2015, Valencia-Aguilar et al. 2015). Despite an association between *Bd* and amphibians of the AF dating back at least 100 yr (Rodriguez et al. 2014), the role of the chytrid in amphibian population declines in this region is uncertain (Eterovick et al. 2005, Lips et al. 2005). Climatic variation, *Bd* strain, amphibian species/population susceptibility, microbiota assemblages on amphibian skin, and presence/density of non-amphibian carriers could all shape how the disease interacts with amphibians at any given site (Fisher et al. 2009, Voyles et al. 2011, 2012, Schmeller et al. 2014).

Given that *Bd* has been detected in a wide range of species, this pathogen could be considered a generalist pathogen of anurans in the AF (Valencia-Aguilar et al. 2015). However, this interpretation is probably too simplistic given the patchy distribution of *Bd* within the environment. For example, frogs that inhabit natural closed canopies, and pond breeders may be at greater risk for chytrid infections in the AF (Becker & Zamudio 2011, Gründler et al. 2012). Such increased susceptibility may also be a consequence of natural history characteristics or behavioral traits, such as microhabitat choice (Silva et al. 2012), which may allow *Bd* to thrive in specialized niches within otherwise suboptimal environments (Becker & Zamudio 2011). Conversely, some behavioral traits may decrease susceptibility to *Bd*. For example, leopard frogs that inhabit ponds with water temperatures that exceed 30°C have significantly lower rates of *Bd* infection (Forrest & Schlaepfer 2011). Thus, to better understand *Bd*–amphibian host–pathogen dynamics within a habitat, it is necessary to identify the underlying factors that influence the fine-scale spatial interactions between pathogen and hosts.

Bromeliads, a plant family (Bromeliaceae) mostly distributed in the Neotropics (Lehtinen 2004, Horres et al. 2007, Schulte et al. 2009), are a potential model system for studying fine-scale interactions between *Bd* and amphibians. Specifically, bromeliads provide a good system for studying *Bd* spatial patterns and effects of host habitat selection on *Bd* infection. Bromeliads are discrete units in the landscape, which makes it fairly straightforward to quantify spatial patterns and patterns of microhabitat selection. Indeed, bromeliad phytotelmata ('plants that hold water') have been specifically identified as good sites for hosting anurans that carry *Bd* (McCracken et al. 2009, Rodriguez et al. 2014). Bromeliad morphological traits are generally chosen by anurans based on diverse ecological requirements such as parental care, food availability, predator avoidance, or to re-

duce competition (e.g. Brown et al. 2008, Poelman et al. 2013, Ryan & Barry 2011). Amphibians have differing degrees of association with bromeliads (e.g. Jared et al. 2005, Romero et al. 2010). Peixoto (1995) functionally split anurans that use bromeliads into bromelicolous, when using bromeliads sporadically as shelter, and bromeligenous/bromeligens, when they complete their entire life cycle within bromeliads (e.g. Lehtinen 2004, Pederassi et al. 2012, Sabagh et al. 2012). Critically, their ability to hold water makes them excellent (sometimes unique) refuges during adverse drier environmental conditions when microhabitats are less hospitable for both fungi (Holmes et al. 2014) and amphibians (Kitching 2000, Lehtinen 2004).

Here, we used bromeliads as a model system to investigate anuran–*Bd* interactions at a fine spatial scale. Specifically, we explored habitat associations between *Bd* and the bromeligenous species *Phyllostictes edelmoi* Peixoto, Caramaschi & Freire, 2003 in a rocky outcrop habitat. Although the conservation status (IUCN Red List category = Data Deficient, DD) and the systematics of *P. edelmoi* still need revision (Freire & Peixoto 2004, Wiens et al. 2005, 2010, Jowers et al. 2008, Pyron & Wiens 2011), it is well documented that this genus spawns in bromeliads (reproductive mode 6, sensu Haddad & Prado 2005). Specifically, we evaluated (1) whether *P. edelmoi* is associated with larger phytotelmata due to their greater capacity to buffer adverse environmental conditions; (2) whether *Bd* infection in *P. edelmoi* is associated with certain bromeliad characteristics; and (3) whether phytotelmata characteristics are associated with the presence of both *P. edelmoi* and *Bd*.

## MATERIALS AND METHODS

Estação Ecológica de Murici (ESEC de Murici) is located in the Murici, Flexeiras, and Messias municipalities, state of Alagoas, northeastern Brazil (see Fig. S1 in the Supplement at [www.int-res.com/articles/suppl/d121p223\\_supp.pdf](http://www.int-res.com/articles/suppl/d121p223_supp.pdf)). Samples were taken in 2 rocky outcrops (between 500 and 600 m a.s.l.) in an open area inside the Mata da Bananeira forest fragment (35.8667° S, 9.2000° W and 35.8583° S, 9.21305° W; Datum WGS84). The climate is subtropical (Köppen's climate classification) with a hot and humid rainy season between April and August and a dry period between November and January (Alvares et al. 2013). This forest fragment has an area of 26.28 km<sup>2</sup> and is located at the north biogeographic region of the AF defined as the Pernambuco interior

(semi-deciduous) eco-region (Vasconcelos et al. 2014). Deforestation has been a major threat in this area (Ribeiro et al. 2009) with remaining forests representing about 11.5% (56 400 km<sup>2</sup>) of the original forest (Ribeiro et al. 2009).

We focused our sampling on the tank bromeliad species *Portea leptantha* Harms (Bromeliaceae; Fig. S2B in the Supplement), a medium- to large-sized bromeliad that can be epiphytic or terrestrial, and occurs in rocky patches in northeastern Brazil (Gomes & Alves 2010). We recorded the presence of the endemic hyloid bromeligenous species *Phyllo-dytes edelmoi* (Fig. S2C,D), known from the states of Alagoas and Pernambuco (from sea level up to 650 m a.s.l.). This species can be distinguished from other species in the *Phyllo-dytes luteolus* group based on coloration (Faivovich et al. 2005). It is a medium-sized species (snout-vent length 23.5 to 28.8 mm), with adults characterized by a deep golden yellow color and a conspicuous pearl white spot on the dorsum on the tadpole (Peixoto et al. 2003).

Two field trips of 7 d each (dry and rainy season) were conducted from 20 to 27 September 2013 (rainy season), and 14 to 21 March 2014 (dry season). We sampled for anuran presence or absence in both seasons (Keating & Cherry 2004). Floor bromeliad patches of 4878 and 7690 m<sup>2</sup> were sampled at open forest rock outcrops (Fig. S2A). Floor bromeliads with phytotelmata were divided into 2 groups. One group consisted of plants occupied by frogs; frogs were collected and swabbed for *Bd* from a random sample of 19 plants. A second group contained a random sample of 37 plants without frogs. We considered each *P. leptantha* bromeliad as a functional sampling unit due to its water reservoir characteristics. In each path, bromeliads were visited only once and were sampled at least 8 m apart from each other (based on geolocation accuracy) to improve independent replication. In total, 56 bromeliads were sampled across 2 sites in both seasons (Fig. S3 in the Supplement).

We first conducted a visual encounter survey for the presence of *P. edelmoi* in bromeliads during their nocturnal activity with a total of 24 h of person effort (between 19:00 and 22:00 h); bromeliads containing frogs were marked as occupied bromeliads. The next day, we counted a second set of bromeliads, using another observer to draw numbers from a random table to select which bromeliad to sample, and marked these as unoccupied. Afterwards, we measured environmental data from both previously occupied and unoccupied samples (between 10:00 and 17:00 h) and post-checked for anuran absence in bromeliads that were marked as unoccupied (night

survey of the same bromeliads). Specifically, we recorded 7 bromeliad morphological characteristics: tank diameter (tank size along the second leaf of the bromeliad), size of the largest leaf, relative volume of water at bromeliad center calculated as  $V = 1/3 \times b \times h$  where  $V$  is the volume of a conic solid (l) with a distance base ' $b$ ' and the height ' $h$ ' (the perpendicular distance from the base to the apex), maximum column capacity available to store water measured from the inner bottom to the opening of the phytotelma (using a 5 m measuring rod in the center of the bromeliad), number of leaves, pH inside bromeliads' water with a digital pH meter (Model PH-107), water microhabitat temperature in the center of the bromeliad ( $T_m$ ) measured with an infrared thermometer Benetech GM 300 (°C). Relative humidity (RH) and ambient air temperature ( $T_a$ ) were measured with a Digital Thermohygrometer HC-520. Canopy closure was measured with a Nikon Coolpix 950 automatic mode photos on a tripod at 1.45 m above the ground with an angle lens >30° (Paletto & Tosi 2009). The camera LCD was always facing north. Seasons were defined by the total amount of precipitation (mm) of each month of survey. Bromeliad spatial distributions were taken with a Garmin GPS eTrex. Finally, we swabbed adults of *P. edelmoi* (see Table S1 in the Supplement) following the protocol proposed by Hyatt et al. (2007). In total, frogs were swabbed 30 times across specific body parts: 5 times on each side of the upper thighs/flanks, 5 times on the digits of each fore foot, and 5 times on the digits of each hind foot. Entire tadpoles were stored in 99% ethanol and swabs from adults were stored dry; both samples were stored in cryotubes. Prior to molecular analyses, tadpoles' mouthparts were extracted and used as samples (Lambertini et al. 2013). To avoid cross-contamination, each animal was allocated into a separate plastic bag after collection, and handled with a fresh pair of sterile gloves.

### Molecular analyses

To quantify the presence and infection intensity of *Bd* in each sample, we extracted DNA from swabs using PrepMan ULTRA® (Life Technologies), and then quantified infection intensities using a Taqman® qPCR Assay (Life Technologies), in singlicates, with standards of 0.1, 1, 10, 100, and 1000 zoospore genomic equivalents (GE) (Kriger et al. 2006, Lambertini et al. 2013) using primers designed for the ITS-1 region of *Bd* strain CLFT 023 (from Monte Verde, Camanducaia, Minas Gerais State, Brazil).

Table 1. Microclimatic conditions and phytotelm characteristics of bromeliads *Portea leptantha* both occupied and unoccupied by *Phyllodytes edelmoi* during dry and wet seasons. Diameter: bromeliad diameter; length: length of the largest bromeliad leaf; leaves: number of bromeliad leaves; column: maximum column size available to store water; volume: relative bromeliad volume;  $T_m$ : microhabitat temperature;  $T_a$ : ambient air temperature; RH: ambient relative humidity; canopy closure: proportion of sky obscured by vegetation at a single point. All data are presented as mean  $\pm$  SD. (\*) indicates significance level ( $p < 0.05$ ) for Mann-Whitney test ( $W$ ) or Welch's  $t$ -test ( $t$ )

Variable	Occupied				Unoccupied			
	Dry season	Rainy season	Test	p	Dry season	Rainy season	Test	p
Diameter (cm)	15.24 $\pm$ 4.26	17.3 $\pm$ 3.15	$t = -1.13$	0.28	18.3 $\pm$ 3.05	17.7 $\pm$ 4.24	$t = -0.50$	0.62
Length (cm)	64.5 $\pm$ 24.7	78.1 $\pm$ 14.64	$t = -1.31$	0.22	77.3 $\pm$ 14.9	67.6 $\pm$ 17.2	$t = -1.92$	0.06
Leaves (number)	15 $\pm$ 2.38	16.1 $\pm$ 1.87	$W = 28$	0.34	14.1 $\pm$ 2.6	14.8 $\pm$ 3.05	$W = 187$	0.55
Column depth (cm)	27.6 $\pm$ 6.9	30.4 $\pm$ 3.76	$t = -0.99$	0.35	26.8 $\pm$ 5.2	31.3 $\pm$ 4.63	$t = 2.92$	<b>0.005*</b>
Volume (ml)	170.2 $\pm$ 130.5	209.9 $\pm$ 95.9	$t = -0.69$	0.50	140.9 $\pm$ 77.0	167.0 $\pm$ 94.7	$t = 0.96$	0.34
pH	5 $\pm$ 0.67	4.3 $\pm$ 0.72	$W = 14$	<b>0.03*</b>	5.25 $\pm$ 0.46	4.25 $\pm$ 0.52	$W = 39$	<b>0.001*</b>
$T_m$ ( $^{\circ}$ C)	27.5 $\pm$ 3.25	26.4 $\pm$ 4.57	$W = 29$	0.42	27.8 $\pm$ 3.01	24.1 $\pm$ 3.06	$t = -3.83$	<b>0.001*</b>
$T_a$ ( $^{\circ}$ C)	30.53 $\pm$ 2.38	29.3 $\pm$ 4.93	$t = 0.7$	0.49	31.57 $\pm$ 2.94	28.2 $\pm$ 5.52	$t = -2.38$	<b>0.02*</b>
RH (%)	66.29 $\pm$ 6.47	68.8 $\pm$ 14.58	$t = -0.50$	0.62	64.24 $\pm$ 8.30	66.2 $\pm$ 15.39	$t = 0.51$	0.61
Canopy closure (%)	29.02 $\pm$ 22.2	5.9 $\pm$ 13.03	$W = 13$	<b>0.01*</b>	20.6 $\pm$ 20.44	9.81 $\pm$ 12.49	$W = 155$	0.15

We considered a sample as positive ( $Bd^+$ ) when the infection load was  $\geq 1$  GE (Kriger et al. 2006).

### Data analyses

Images of the canopy were converted to an 8 bit format with a binary (black/white pixels) format, and the percentage of pixels that were black were counted with IMAGEJ analysis software (Schneider et al. 2012). Spatial autocorrelation for each variable was assessed by Moran's  $I$  test using 'spdep' with the 'sp.correlogram' function in R. Pearson's correlations were performed between normally distributed variables (see Tables S5 & S6 in the Supplement) that characterize bromeliads and microclimatic conditions. Consequently, variables without high correlation coefficients ( $r < 0.7$ ) were selected. Variables were analyzed with a logistic regression generalized linear model (GLM) (R Package 'ape' and 'vegan') with a 'backward and forward stepwise' procedure to suggest which variables were associated with  $Bd$  presence, *P. edelmoi* adult occupation, and tadpole presence. Significant likelihood-ratio explained the variation when the dependent variable was present (Peng et al. 2002). Significance in predictor variables was evaluated with likelihood-ratio chi-squared tests (R Package 'car') based on a predictive model compared to a null model. Model fit was evaluated with a goodness-of-fit test based on deviance likelihood-ratio. In the same way, a multiple logistic regression (GLM) with a step- by-step procedure was conducted to test which variables were most influential in predicting the presence of  $Bd$ . We started the backward

and forward stepwise selection with 5 variables and later aggregated variables for all possible combinations. All analyses were performed in the R software package (R Development Core Team 2014).

### RESULTS

Microclimatic conditions and phytotelm characteristics for a total of 56 *Portea leptantha* bromeliads showed that microhabitat characteristics were similar within the same season, both in those occupied and not occupied by *Phyllodytes edelmoi* (Table 1). However, bromeliads occupied by frogs during the dry season (Table 1) were associated with significantly higher pH ( $5 \pm 0.67$  SD) in phytotelmata than that of bromeliads in the rainy season (Mann-Whitney test,  $W = 14$ ,  $p = 0.02$ ). Similarly, occupied and unoccupied bromeliads showed little variation in the different seasons (Table S2 in the Supplement), and there were similar numbers of bromeliads occupied by *P. edelmoi* detected by time effort survey (dry season  $n = 7$ ; rainy season  $n = 12$ ).

Binary GLM analysis for each *P. edelmoi* stage (adult males and tadpoles) in bromeliads took into account 8 uncorrelated variables (see Tables S5 & S6). In the Moran's  $I$  analysis, variables associated with bromeliad samples showed no significant autocorrelation, except canopy closure and relative humidity ( $p < 0.05$ ; Tables S3 & S4, Fig. S3 in the Supplement); but these 2 variables were dismissed by stepwise Akaike's information criterion (AIC) selection. Variables that influenced *P. edelmoi* adult selection and tadpole presence in bromeliads included diameter,

Table 2. Logistic regressions on the presence of *Phyllodytes edelmoi* adult males (full model AIC = 43.48) and tadpoles (full model AIC = 64.47) in bromeliads *Portea leptantha* as predicted by bromeliad habitat characteristics selected by stepwise procedure using Akaike's information criterion (AIC).  $\Delta$ AIC is the change of AIC by subtracting the difference of each variable from the full model. The likelihood-ratio (LR) goodness of fit test was for adult selection ( $\chi^2 = 4.8081$ , df = 1, p = 0.028), and tadpole presence ( $\chi^2 = 6.6542$ , df = 1, p = 0.009). Bromeliad variables as defined in Table 1. Adult (n = 50); tadpole (n = 56). (\*) indicates significance level (p < 0.05) for likelihood-ratio chi-squared test statistic

Bromeliad variable	$\Delta$ AIC	LR $\chi^2$ -statistic	df	Pr(>  $\chi^2$  )
<b>Adult male</b>				
Diameter	7.05	9.0379	1	<b>0.026*</b>
Leaves	2.81	4.8081	1	<b>0.028*</b>
Volume	3.02	4.9908	1	<b>0.025*</b>
<b>Tadpole</b>				
Diameter	4.27	6.2638	1	<b>0.012*</b>
Leaves	4.65	6.6542	1	<b>0.009*</b>
Volume	0.25	2.2349	1	0.135

number of leaves, and relative bromeliad volume of water (Table 2). Patterns of tadpole presence and adult selection of bromeliads are shown in Fig. 1.

Both adults (n = 22) and tadpoles (n = 17) of *P. edelmoi* were infected. In general, all bromeliads with positive adult amphibians also showed positive tadpole samples (n = 3), and all tadpoles in the same bromeliad were also positive. Likewise, bromeliads in which adults tested negative contained tadpoles that were also negative (n = 4). *P. edelmoi* adults exhibited an average GE load of 40.6 ( $\pm 82.7$  SD), and prevalence was 31.8% (7/22). Similarly, *P. edelmoi* tadpoles showed a high prevalence of 41.1% (7/17). Eight predictor variables were used for a binary logistic regression (GLM) (microhabitat temperature, pH, diameter, volume, number of leaves, column total capacity, canopy closure, and season) for *Bd* presence. Overall, variables associations with *Bd* were selected by stepwise AIC (Table 3). Although canopy closure was spatially autocorrelated (p = 0.003), it was not selected by AIC for the model. *Bd* presence was associated negatively with phytotelm diameter, and positively with maximum column capacity for storage of water (Table 3). Nevertheless, only phytotelm diameter was significant according to the model (p = 0.039). Variables in the model were plotted for presence of *Bd* from anurans in bromeliads (Fig. 2). The likelihood-ratio (LR) goodness-of-fit test was significant (p < 0.05), indicating that the model fit well for our *Bd* presence data (*P. edelmoi* infected with *Bd*:  $\chi^2 = 8.6888$ , df = 1, p = 0.003).

Table 3. Logistic regressions for the presence of *Batrachochytrium dendrobatidis* in *Phyllodytes edelmoi* as predicted by bromeliad habitat characteristics (full model AIC = 18.3) selected by stepwise procedure using Akaike's information criterion (AIC).  $\Delta$ AIC is the change of AIC by subtracting the difference of each variable from the full model. Bromeliad variables as defined in Table 1 (n = 20). (\*) indicates significance level (p < 0.05) for likelihood-ratio chi-squared statistic test

Bromeliad variable	$\Delta$ AIC	LR $\chi^2$ -statistic	df	Pr(>  $\chi^2$  )
Diameter	10.03	12.0247	1	<b>0.0005*</b>
Column depth	6.69	8.6888	1	<b>0.003*</b>

## DISCUSSION

*Phyllodytes edelmoi* individuals were *Bd*<sup>+</sup> in the Mata Bananeira, ESEC Murici; the chytrid fungus showed high prevalence on this bromeligenous species, but low infection intensity. Likewise, non-bromeliad anurans have also shown high prevalence and low infection intensity in this region (Valencia-Aguilar et al. 2015). In fact, high prevalence patterns have been reported for the south AF including frogs in phytotelm microhabitats (Rodríguez et al. 2014). Specifically, anurans inhabiting phytotelmata had a high *Bd* prevalence of 26.8% (95% CI 21.9–32.4) (Rodríguez et al. 2014). Therefore, the prevalence reported in our study fits well within the parameters of prevalence in the southern AF. No clinical effects due to chytridiomycosis were observed in *P. edelmoi*. In fact, asymptomatic chytridiomycosis had been commonly reported for anurans in the AF (Toledo et al. 2006, Rodríguez et al. 2014). We found relatively fewer *Bd*-infected individuals (tadpoles and adults) during the dry season. These open patches of bromeliads are exposed to high ambient air temperatures (see Table 1), and we observed some amphibians inside bromeliad water tanks that were beyond the upper 30°C thermal tolerance reported for *Bd* in laboratory (Piotrowski et al. 2004). Thus, it is plausible that *Bd* infection can be regulated by a 'hot bath' (Forrest & Schlaepfer 2011).

In a region such as the north AF, which is characterized by high temperatures and less precipitation than the rest of the AF (Alvares et al. 2013, Vasconcelos et al. 2014), *Bd* might experience limited zoospore growth on its hosts (Becker & Zamudio 2011). Alternatively, different *Bd* strains (Rosenblum et al. 2013) may have different climatic tolerances (Stevenson et al. 2013), and hence, the observed trends could be influenced by strain type (which is unknown for

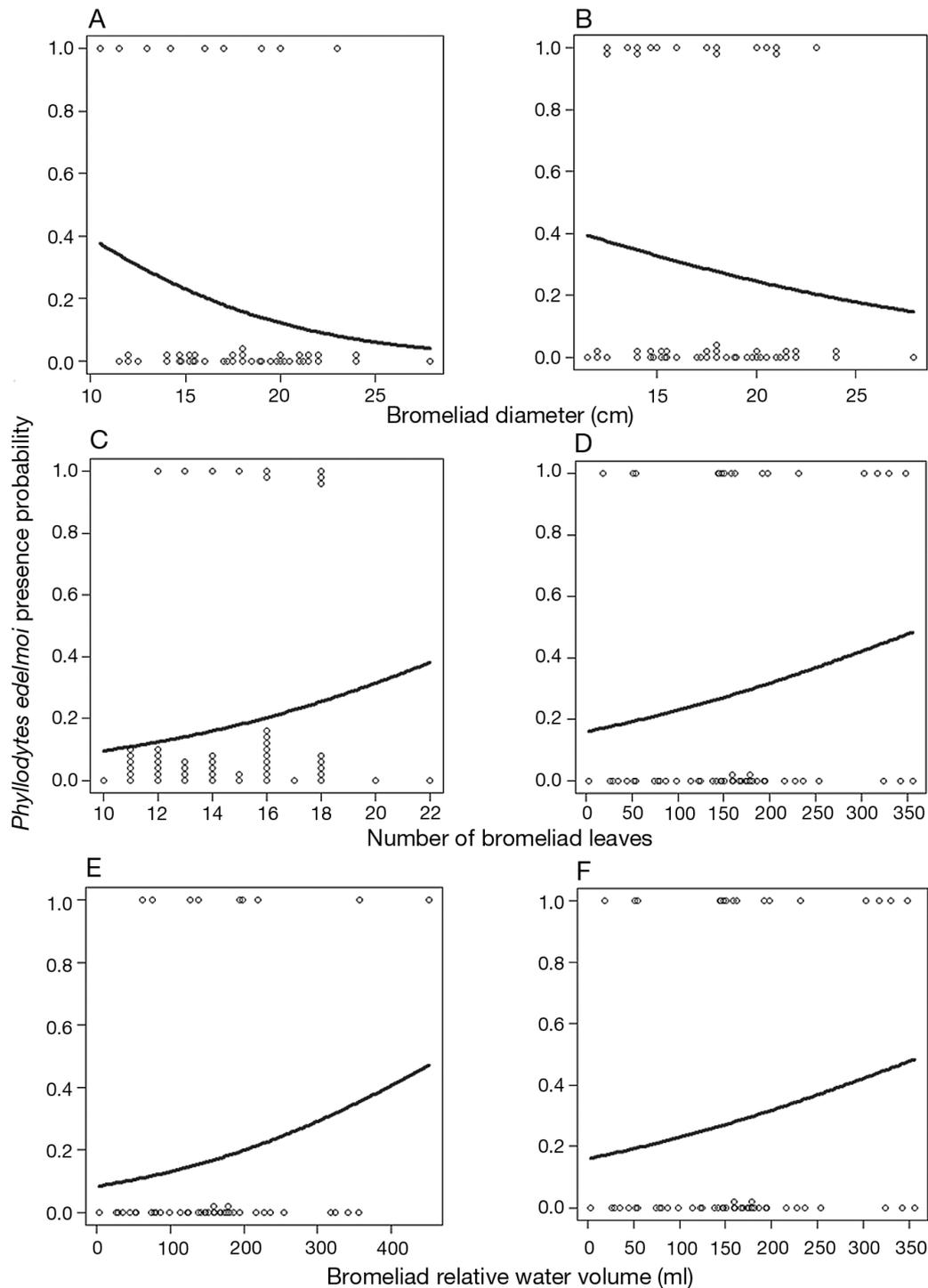


Fig. 1. Interaction of (A,B) bromeliad diameter (cm), (C,D) number of leaves, and (E,F) relative volume of water (ml) in the logit model fit to *Phyllodytes edelmoi* (A,C,E) adult males and (B,D,F) tadpole presence data in bromeliads *Portea leptantha*. Bromeliad variables as defined in Table 1

these bromeliad species), or by local adaptation that makes it possible for *Bd* to persist in these harsh climatic conditions. Finally, frog species that have tolerance for open rocky outcrops may be less susceptible to *Bd* (Becker & Zamudio 2011).

The bromeliad morphological traits of volume of water, number of leaves, size of leaves and size of bromeliad have been positively associated with the presence of anurans in the Neotropical region (e.g. Oliveira & Navas 2004, Stuckert et al. 2009, Pederassi

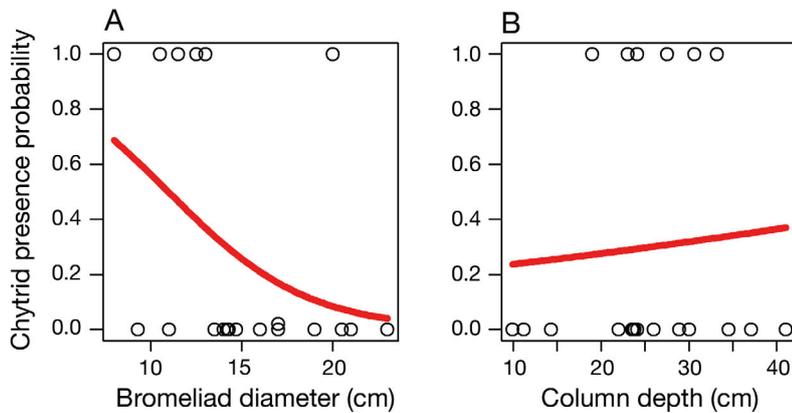


Fig. 2. Interaction of (A) bromeliad diameter (cm) and (B) bromeliad maximum column depth to store water (ml) in the logit model fit to *Phyllodytes edelmoi* *Bd* presence data in bromeliads *Portea leptantha*. Bromeliad variables as defined in Table 1

et al. 2012, Poelman et al. 2013). Specifically, presence of a bromeligenous hylid species of the genus *Scinax* in the AF was greater in larger bromeliads (Oliveira & Navas 2004, Pederassi et al. 2012). Similarly, our study also showed that selection was not random. Males of *P. edelmoi* preferred smaller phytotelmata. This selection may be explained by the frogs' reproductive mode (Haddad & Prado 2005) which may include parental care and territorial behavior, based on frequent observations of single males in bromeliads with conspecific tadpoles (G. Ruano-Fajardo pers. obs.). Similar behavioral inferences have been made for other bromeligenous species (e.g. *Dendropsophus bromeliaceus* and *Phyllodytes luteolus*); nevertheless, these observations require further investigation (Ferreira et al. 2012, 2015). Neotropical species of poison frogs (family Dendrobatidae), which display parental care, benefit by selecting smaller phytotelmata (Brown et al. 2008, Ryan & Barry 2011). Moreover, this preference has also been observed in other closely related species, suggesting that this is a general trait of this group of dendrobatids (Brown et al. 2008). In other words, breeding habitat selection seems to have evolved together with changes in parental care. Smaller phytotelmata appear to have lower microorganism diversity (Kitching 2001, Jocque & Field 2014); a suggestive advantage, as it means a less competitive microorganism environment for tadpoles, predation by other tadpoles or invertebrates (Brown et al. 2008). Furthermore, the choice of *P. edelmoi* individuals for a less rich organism microhabitat may also be an advantage for both tadpole fitness and *Bd* infection.

*P. edelmoi* does not choose bromeliads randomly. Our model shows that bromeliads chosen by this spe-

cies were characterized by having a smaller diameter, a relatively higher volume of water, and more leaves capable of holding water. Therefore, this suggests that choice of plants used by *P. edelmoi* is based on maximal water availability. Furthermore, the chytrid fungus in *P. edelmoi* was more frequently found in individuals inhabiting smaller diameter bromeliads. In other words, this bromeligenous anuran prefers small bromeliads—probably for physiological reasons—but *Bd* is more abundant there. This might be for several reasons: (1) if there are more frogs, there are more hosts for *Bd*. It is more likely to find more frogs in this type of

bromeliad, and consequently, more frogs will be detected as *Bd*<sup>+</sup>. (2) The presence of more frogs increases the chance for horizontal transmission. In density-dependent transmission, the contact rate between susceptible and infected individuals could be amplified by the higher number of *P. edelmoi* individuals. For instance, evidence of frog host density affecting the transmission rates of *Bd* has been observed in temperate regions and microcosm studies (e.g. Briggs et al. 2010, Venesky et al. 2014). Also, our observations suggest that vertical transmission of *Bd* may occur between parents and tadpoles and contribute to the maintenance of prevalence in the bromeligenous population. Although our sample is small, a similar transmission dynamic was suggested by Holmes et al. (2014) in a previous study based on Jamaican bromeliad anurans with parental care. (3) The lower richness of aquatic invertebrate fauna in small bromeliads (Richardson 1999, Kitching 2001) may result in less *Bd* predation. This may be advantageous for *Bd* persistence, as bromeliads may lack typical zoospore predators. Microorganisms such as daphnia, ciliates, and rotifers are effective in regulating *Bd* infection because they consume *Bd* zoospores in aquatic environments (e.g. Buck et al. 2011, Schmeller et al. 2014).

Chytrid presence in certain bromeliads in rocky outcrops could also be explained by other factors. First, Cossel & Lindquist (2009) found *Bd* inside phytotelma water of bromeliads that may infect individuals. *Bd* cannot survive for long periods without water (Johnson & Speare 2005) and consequently, it needs the water reservoirs inside the bromeliad. Therefore, the limits for *Bd* survival in open rocky outcrops are apparently restricted by desiccation, as

observed in other regions (Puschendorf et al. 2009, Holmes et al. 2014). Second, other host species (bromelicolous anurans) might carry *Bd* inside the bromeliads, changing the likelihood of encountering *Bd* or transmitting it to other individuals (Searle et al. 2011). For instance, low anuran richness found in the outcrop patches in the ESEC Murici, where *Pristimantis ramagii* (Craugastoridae) was sporadically found in bromeliads and recorded as *Bd*-positive in adjacent forested areas (Valencia-Aguilar et al. 2015), may be carrying *Bd* from other areas to the bromeliads in the outcrop patches. Consequently, bromelicolous species may be re-infected constantly and spread the zoospores to other *Bd*-susceptible species in a source–sink population dynamic.

Our observations of host–pathogen dynamics inside the bromeliad microhabitat should be further investigated. For example, whether micropredators play an important role in this system is an important question. Moreover, understanding microhabitat preferences of both host and pathogen is important for predicting and counteracting disease risk. As for many ecological studies (Kitching 2001), it appears that the tank of bromeliads may be an interesting system for the study of *Bd* dynamics in the field.

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