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Frogs in plants:
Ecology and conservation of a bromeliad-dwelling amphibian from Brazil

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Frogs in plants:

Ecology and conservation of a bromeliad-dwelling amphibian from Brazil

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Supervised by:

Professor Richard A. Griffiths

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1932–2015

For appreciating my interest in natural science and who was a great admirer of Archimedes of Syracuse work and his famous quote:

‘Give me a place to stand and a lever long enough, and I shall move the world’

Author's declaration

Chapters of this thesis were written by the author Izabela Menezes Barata, with comments from both supervisors, Professor Richard A. Griffiths and Professor Martin S. Ridout. This research was risk and ethically assessed and approved by the University of Kent committee. All legal permits required by the Brazilian government were obtained to conduct fieldwork. The dataset analysed during this study are available from the author on request. Detailed collaborations are given as follow:

Chapter 1: written by Izabela M. Barata and reviewed by Richard A. Griffiths. Figures produced by Izabela M. Barata.

Chapter 2: Izabela M. Barata and Eliene P. Silva conducted the field survey; Izabela M. Barata did data analyses which had inputs from Richard A. Griffiths. Izabela M. Barata wrote the manuscript for publication with comments from Richard A. Griffiths and Eliene P. Silva. All authors revised the manuscript and agreed with submission. Figures were produced by Izabela M. Barata.

Chapter 3: Izabela M. Barata conducted the field expeditions and collected the data. Izabela M. Barata ran data analyses, assisted by Vivian M. Uhlig and Lara G. Cortes. Data analysis was reviewed by Richard A. Griffiths. Izabela M. Barata wrote the manuscript for publication, and Richard A. Griffiths, Vivian M. Uhlig and Lara G. Cortes revised the manuscript. All maps were produced by Izabela M. Barata.

Chapter 4: Izabela M. Barata planned data collection and sampling design and conducted fieldwork. Izabela M. Barata did data analyses which had inputs from Martin S. Ridout. Izabela M. Barata wrote the manuscript for publication with comments from Richard A. Griffiths and Martin S. Ridout. All authors revised the manuscript and agreed with submission. Figures were produced by Izabela M. Barata.

Chapter 5: Izabela M. Barata conducted the fieldwork and collected data. Izabela M. Barata ran data analyses which was reviewed and had inputs from Richard A. Griffiths and Martin S. Ridout. Izabela M. Barata wrote the manuscript and had comments from Richard A. Griffiths and Martin S. Ridout. Figures were produced by Izabela M. Barata.

Chapter 6: Izabela M. Barata conducted the fieldwork and collected data. Izabela M. Barata ran data analyses which was reviewed and had inputs from Richard A. Griffiths and Martin S. Ridout. Izabela M. Barata wrote the manuscript and Richard A. Griffiths and Martin S. Ridout revised the manuscript. Figures were produced by Izabela M. Barata.

Chapter 7: written by Izabela M. Barata and reviewed by Richard A. Griffiths.

In name of co-authors I hereby declare that there are no competing interests.

Abstract

This thesis investigates the population ecology and population dynamics of a species of bromeligenous frog, *Crossodactylodes itambe*, endemic to a highland area in the Atlantic Rainforest of Brazil, within the Espinhaço Mountain Range. Bromeligenous frogs spend their entire life cycle inside bromeliads and dispersal of individuals is still unknown. There are 99 species of bromeligenous frogs, all restricted to the Neotropics, and most species are threatened. *Crossodactylodes* is comprised of five small-sized bromeligenous frogs and there is little information on their ecology and natural history of the whole genus. *Crossodactylodes itambe* occurs at the Itambé summit, above 1700 m in elevation, with an estimated area of occurrence of < 0.5 km^2. Individuals are known to occupy a single species of bromeliad, which is also endemic to just two localities, including the Itambé summit.

Species abundance was influenced by specific features of habitat structure, such as size of plant and presence of water, which were considered more important than local climate. Number of adults in a single plant was usually limited to one individual and the structure of the bromeliad was considered extremely important for species persistence. Distribution of plants at the Espinhaço Range was influenced by topography and specific climatic conditions, such as temperature seasonality and annual precipitation. Given the strong dependence of the frogs on the plant, using bromeliads as a surrogate for modelling frog distribution can be extended to many bromeligenous species that lack distributional data. Despite extensive survey effort, *Crossodactylodes itambe* cannot be found anywhere else within the southern limits of the Espinhaço Range, and the species is indeed naturally rare.

Detecting declines in amphibian populations is challenging and surveys should be species-specific and designed to meet specific monitoring goals. A sampling design was suggested for *Crossodactylodes itambe*, which can detect large to moderate population changes with 80% statistical power. The first analysis of population trends for a bromeligenous frog was provided. Although population changes were detected at all elevational ranges covering the current species distribution, a decline was only significant at lower elevations, where bromeliads are smaller and occur at lower density. While colonization of bromeliads by frogs was driven by habitat characteristics, local extinction was explained by seasonal variation in local weather conditions. Colonization rates were negatively affected by a stochastic fire event, which decreased dramatically in burnt plants.

This thesis provides valuable information on the drivers of distribution and abundance of this threatened frog species. The same methodological approaches could be broadly applied to many Data Deficient bromeligenous frogs, for which little information is available. This research also demonstrates how the frog-bromeliad system can be a useful small-scale model for investigating key demographic parameters, such as extinction and colonization, which might be unfeasible on a larger scale or in patchy habitats. Considering the population dynamics of this bromeligenous frog, in the short-term, habitat conservation should be a priority action when compared to climate change mitigation.

Keywords: Amphibians, Atlantic Rainforest, Bromeliads, Ecology, Endemism, Espinhaço Mountain, Long-term monitoring, Population dynamics, Rare species, Species conservation.

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Chapter 1 Introduction

1.1 Amphibian species richness in Brazil

The Neotropical region shelters great levels of biodiversity, with Brazil and Colombia encompassing the largest number of amphibian species in the world (Wake and Vredenburg 2008). The Brazilian species list comprises 1080 living amphibians of the nearly 7899 known species in the world (Segalla *et al.* 2016; Frost 2018). The number of known amphibian species changes constantly and is rapidly rising: since 1985 the species list has increased by 48% (Frost *et al.* 2006), but is still far from being complete. Global estimates of amphibian species richness range from over 9000 (Giam *et al.* 2012) to approximately 15000 (Scheffers *et al.* 2012). In Brazil, almost half of amphibian species were described in the past 40 years (Pimenta *et al.* 2005). Considering just the last five years, the Brazilian amphibian list increased by more than 15%, surpassing recent predictions of species richness (Pimm *et al.* 2010). The vast majority of species in Brazil are anurans (1039 species), followed by caecilians, with 36 species, and salamanders, with five species (Segalla *et al.* 2016).

Due to its great geographical extent, amphibian species in Brazil are distributed among several different biomes and many of them are restricted to single locations. One of the most diverse biomes in the country is the Atlantic Rainforest, which is mainly represented by tropical and subtropical moist forested habitats, located in coastal areas of Brazil. Considered a highly threatened biodiversity hotspot (Myers *et al.* 2000), between 11–16% of its original vegetation is left and more than

80% of these fragments are < 50 ha (Ribeiro *et al.* 2009). Not surprisingly, the biome shelters a considerable number of threatened species (Hoffmann *et al.* 2010) and also a high concentration of small-range species (Pimm *et al.* 2014). Amphibian richness in the Atlantic Rainforest is remarkable, with more than 500 species – of which 88% are considered endemic (Haddad *et al.* 2013).

Equally diverse and threatened, the Cerrado biome occupies the centre of Brazil and shelters a great biodiversity with high levels of threat (Myers *et al.* 2000). The Cerrado is a savanna-like mosaic of several vegetation types determined primarily by soil conditions, such as open grasslands, woodlands, dry forest and rocky ecosystems (Eiten 1978). It is the largest biome in the country, occupying 2 million km^2 (Klink and Machado 2005), and it is possibly the most threatened tropical savanna in the world (Silva and Bates 2002). Between 2002 and 2010, 40–60% of the Cerrado had been converted to anthropogenic land uses (Beuchle *et al.* 2015; Sano *et al.* 2010), less than 20% remains undisturbed (Strassburg *et al.* 2017) and only about 3% of its area is under legal protection (MMA/IBAMA 2015). With over 200 anuran species and half of them considered to be endemic (Valdujo *et al.* 2012), the Cerrado also shelters high levels of amphibian richness and endemism.

Between these two Brazilian biomes lies the Espinhaço Mountain Range, a mountain chain of 1200 km extension considered to be the geographical divider of the Atlantic Rainforest, to the east, and the Cerrado, to the west. The Espinhaço Range is a quartzite massif with a unique ecosystem (defined as 'campos rupestres'), characterized by open field habitats with shrubs and herbs growing on an extensive rocky outcrop above 800 m (Alves *et al.* 2014). The Cerrado physiognomies contribute to a vast open grassland habitat with low vegetation cover, while the high level of humidity is provided by the Atlantic Rainforest moisture winds, creating a semi-permanent fog effect that enhances rainfall and mist cover.

The South Espinhaço Range is considered a priority area for biodiversity conservation (Drummond *et al.* 2005), a centre of plant diversity (Davis, Heywood and Hamilton 1995), one of the Global 200 Ecoregions in the world (Olson *et al.* 2001) and an Important Bird Area for endemic species (Mugica *et al.* 2009). Despite its biological importance, the Espinhaço Range is severely threatened by human activity (Alves *et al.* 2014; Monteiro *et al.* 2016) and climate change (Bitencourt *et al.* 2016). The unique geological condition of the Espinhaço Range contributes to a high level of species richness and endemism for several taxa, including amphibians (Leite, Eterovick and Juncá 2008). The anuran community composition within the Espinhaço Range is partially influenced by the Cerrado-Rainforest gradient (Barata, Correia and Ferreira 2016). For example, while some endemic amphibians occur only on the western slopes (under influence of the Cerrado), other species occur in localities from the eastern slopes, which is covered by the Atlantic Rainforest (Valdujo *et al.* 2012).

Nonetheless, uneven distribution of survey effort is a problem within the entire Espinhaço Range, with the number of species recorded in the northern part of the Mountain Range much lower than that recorded in the south (Leite 2012). In the southern part of the Espinhaço Range, amphibian sampling effort is unequally distributed (Barata, Correia and Ferreira 2016) and the investigation of unexplored isolated summits (especially areas above 1700 m) can lead to the discovery of new narrowly endemic species (Leite *et al.* 2008). The recent description of the narrowly endemic *Crossodactylodes itambe* (Barata *et al.* 2013) corroborates the importance of the highlands of Espinhaço Range to the diversification and conservation of the Brazilian biota.

1.2 Bromeligenous frogs and *Crossodactylodes* species

The genus *Crossodactylodes* comprises five species of frogs that are endemic to the Atlantic Rainforest and known from the type localities only: *C. pintoi* (Cochran 1938), *C. bokermanni* and *C. izecksohni* (Peixoto 1982), *C. septentrionalis* (Teixeira *et al.* 2013) and *C. itambe* (Barata *et al.* 2013). *Crossodactylodes* species have very small areas of occurrence and are also restricted to highland areas, over 650 m above sea level (a.s.l.). *Crossodactylodes bokermanni* occurs at approximately 650 m a.s.l. (Silvano and Peixoto 2004a); *C. izecksohni* and *C. pintoi*, at 675 m a.s.l. and 1200 m a.s.l., respectively (Peixoto and Carvalho-e-Silva 2004; Silva and Peixoto 2004b), *C. septentrionalis* at 930 m a.s.l. (Teixeira *et al.* 2013) and *C. itambe* is recorded at the highest elevation, from 1700 m to 2062 m a.s.l. (Barata *et al.* 2013).

These small-sized frog species (usually up to 3 cm) are strictly dependent on bromeliads where they spend their entire life cycle (Barata *et al.* 2013; Teixeira *et al.* 2013), and are also known as bromeligenous frogs (Peixoto 1995). Bromeliads are flowering terrestrial or arboreal plants native to the Neotropics and widespread in South America (Benzing 2000), capable of holding water and providing a unique microhabitat, also known as a phytotelm (Lehtinen 2004). There are currently 99 species of bromeligenous frogs (i.e., frogs that complete their reproduction without leaving the plant) and 35% are either Data Deficient or Not Evaluated (Sabagh, Ferreira and Rocha 2017).

Although the genus *Crossodactylodes* was described in 1938, it remains poorly investigated and is referred to only in a small fraction of papers from the growing literature on Neotropical amphibians (Teixeira *et al.* 2013; Sabagh, Ferreira and Rocha 2017). Barata *et al.* (2013) made the first natural history observations on the genus and provided novel observations on *Crossodactylodes itambe*: the population is locally abundant, completely dependent on a single species of bromeliad for reproduction, and the presence of egg masses and tadpoles inside bromeliads indicate successful breeding at the only known site.

Crossodactylodes itambe (Figure 1.1) extends the distribution of the genus by about 325 km to the northwest from where congeners were previously known, occurring in a non-costal mountain range (the Espinhaço Range). The species extent of occurrence (estimated at less than ca. 0.5 km^2, Barata *et al.* 2013) is fully located within the Pico do Itambé State Park in the southern part of the Espinhaço Range. With 4,696 ha, this protected area is equivalent to categories I and II of the International Union for Conservation of Nature (IUCN) (Dudley 2008). At 2062 m a.s.l. the Itambé summit is the highest point of the Espinhaço Range and offers a unique ecosystem with specific local features that provide the microclimate and microhabitat required by *C. itambe*. *Crossodactylodes itambe* is restricted to the Itambé summit, above 1700 m in elevation, and is known to occupy a single species of bromeliad, *Vriesea medusa* – a night-blooming bromeliad species, endemic to two locations within the Espinhaço Range (Versieux 2008; Versieux *et al.* 2010).

Figure 1.1 The bromeligenous frog *Crossodactylodes itambe*
Crossodactylodes itambe (A) is a small-sized bromeligenous frog (B) endemic to the Itambé summit of the Espinhaço Range of Brazil, restricted to a single species of bromeliad, *Vriesea medusa* (D), where they lay their eggs (C) and complete the life cycle.

1.3 Amphibian population declines and species vulnerability in the Neotropics

Substantial evidence has shown that amphibians are declining worldwide (Wake 1991; Houlahan *et al.* 2000; Collins and Storfer 2003; Beebee and Griffiths 2005;

Mendelson *et al.* 2006). Amphibian declines are widespread in Latin America and at least 13 countries have experienced declines or extinctions over the past 20 years (Young *et al.* 2001). Populations are declining due to fungal diseases (Pounds *et al.* 2006; Lips *et al.* 2008), climate change (Griffiths, Sewell and McCrea 2010; Shoo *et al.* 2011) and habitat fragmentation (Becker *et al.* 2007). Neotropical species are much more affected than those in the Afrotropical and Indomalaya Realms (Stuart *et al.* 2004), but relatively little is known about the status of amphibian populations in South American countries due to insufficient data on species distribution and population dynamics (Young *et al.* 2001; Eterovick *et al.* 2005). In Brazil, for example, few papers have addressed amphibian population declines (Heyer *et al.* 1988; Weygoldt 1989; Guix *et al.* 1998; Papp and Papp 2000; Eterovick *et al.* 2005).

In the last two decades, populations of many species of frogs have declined in relatively undisturbed habitats at high altitudes throughout the world (Pounds 2001; Young *et al.* 2001; Hero, Williams and Magnusson 2005; La Marca *et al.* 2005). Sudden disappearances of montane species were recorded in Costa Rica, Ecuador and Venezuela (Stuart *et al.* 2004) and population declines reported in the Andes occurred in areas over 500 m and 1000 m a.s.l. (Young *et al.* 2001). La Marca *et al.* (2005) reported population declines of Harlequin frogs in elevations above 1000 m of altitude and declines were of greater magnitude in highland areas when compared to the lowlands. Lips, Reeve and Witters (2003) showed that declining populations of amphibians shared restricted elevational ranges, especially at moderate- to high-elevation. In Brazil, records of amphibian declines include species in apparently undisturbed areas at mid- or high-elevations (Eterovick *et al.* 2005).

Tropical mountain ecosystems are strongly associated with climatic regulation (Pounds, Fogden and Campbell 1999; Still, Foster and Schneider 1999) and usually have a high proportion of endemic species (Still, Foster and Schneider 1999; Leite, Eterovick and Juncá 2008). According to Foden *et al.* (2008), 52% of amphibians are vulnerable to climate change, and recent estimates shows that 11–15% of species are both highly vulnerable to climate change and threatened with extinction in the IUCN Red List (Foden *et al.* 2013). Amphibian species occurring on tropical mountains are therefore considered to be particularly at risk and population declines are likely to accelerate since most amphibians occur in the tropics and have small geographic ranges (Wake and Vredenburg 2008). In Brazil, the number of small-ranged species is increasing exponentially and is highly concentrated in the coastal hotspot of the Atlantic Rainforest (Pimm and Jenkins 2010; Pimm *et al.* 2010; Scheffers *et al.* 2012).

Vulnerability of montane endemic species to population declines is particularly worrying in the case of bromeligenous frogs. All species that typically occur at high altitudes (> 2000 m a.s.l.) are currently classified as Data Deficient (DD), Near Threat (NT) or Endangered (EN) (Sabagh, Ferreira and Rocha 2017). To date, three *Crossodactylodes* species have been evaluated by the IUCN Red List. *Crossodactylodes bokermanni* and *C. izecksohni* are classified as NT, but are close to qualifying for Vulnerable (VU), with declining populations living in restricted fragments of the Atlantic Rainforest (Silvano and Peixoto 2004a; Silvano and Peixoto 2004b); and *C. pintoi* is classified as DD, although it was last recorded in 1909 (Peixoto and Carvalho-e-Silva 2004). This figure is no different for bromeligenous frogs, with 41% of the species listed as threatened and 35% with their status assessed as unknown (Sabagh, Ferreira and Rocha 2017).

Range size is an important feature to evaluate extinction risk according to the IUCN Red List assessments (IUCN Standards And Petitions Subcommittee 2017), but there is still a lot of uncertainty around species with small geographical ranges (Mace *et al.* 2008). Not surprisingly, conservation practice towards species-level and population assessments are currently limited by the lack of data (Geijzendorffer *et al.* 2015). Conservation planning usually assumes that we know the species to be conserved as well as where they occur (Pimm and Jenkins 2010), but incomplete information can jeopardize an effective allocation of conservation efforts (Scheffers *et al.* 2012).

1.4 Implications for amphibian conservation in Latin America

Alarming numbers of amphibian declines have concerned ecologists in the late 1980s (Wake 1998), with accelerating rates of extinction (Ceballos *et al.* 2015). Amphibians are the most endangered group of vertebrate in the world with 41% considered under threat (Stuart *et al.* 2004; Wake and Vredenburg 2008) and 34 species already extinct (IUCN 2018). In Brazil, among the 838 species evaluated by the IUCN, at least 60 are included in a threat category (IUCN 2018). Moreover, amphibians also have the highest proportion of DD species when compared to reptiles (Böhm *et al.* 2013), mammals or birds (Hoffmann *et al.* 2010).

Nearly 31% of amphibians in Brazil are considered as DD (Morais *et al.* 2013), a number that exceeds 80% when considering recently described species (Brito 2010). On top of that, a global analysis demonstrated that a great proportion of DD anurans and range-restricted species are not covered by existing protected areas (Nori and Loyola 2015; Nori *et al.* 2015). These numbers indicate the high level of uncertainty regarding amphibian conservation status in Brazil (Trindade-Filho *et al.* 2012; Morais *et al.* 2013), and also the low degree to which DD species are protected (Barata *et al.* 2016).

The lack of knowledge about species and their distribution hinders assessment of their status, which is especially true in tropical areas (Collen *et al.* 2008). Overall, there is a shortfall of conservation studies regarding tropical amphibians (Ficetola 2015) and there are fewer papers in conservation journals focusing on amphibians when compared to other vertebrates, such as mammals and birds (Griffiths and Dos Santos 2012). In Brazil, studies on amphibians remain largely underrepresented (Silvano and Segalla 2005) and basic research is urgently needed, especially on species richness and taxonomy (Verdade *et al.* 2012). Scientific knowledge is considered insufficient for most DD anurans endemic to the Cerrado in south-eastern Brazil, and population ecology studies continue to be less represented (Barata *et al.* 2016). These knowledge gaps prevent species conservation assessments and result in threaten categories that are inconsistent between national and international lists (Morais *et al.* 2012). Basic understanding is therefore crucial to ultimately inform conservation planning.

Following the global amphibian decline crisis, Amphibian Conservation Action Plans (ACAP) were developed to coordinate and facilitate conservation programs for amphibians, and researchers also organized the Amphibian Survival Alliance (Mendelson *et al.* 2006). At the same time, workshops in Latin America lead to the compilation of priorities for amphibian conservation (Young *et al.* 2001) and this global effort prompted Brazilian herpetologists to create the Brazilian ACAP (Verdade *et al.* 2012). These global and national efforts led the National Research Centre for Reptile and Amphibian Conservation (a centre within the Brazilian government environmental agency) to facilitate workshops that discussed specific actions for herpetofauna conservation and created an ACAP that was focused on a regional scale. The ACAP at South Espinhaço Range was proposed in 2012 (hereby ACAP Espinhaço) and covers an area of 31.814 km^2, including the Itambé summit where the only known population of *C. itambe* is currently recorded.

The ACAP Espinhaço aimed to improve scientific knowledge and deliver conservation actions for target species that were considered endemic, DD or under threat (NT, EN, VU) (RAN/ICMBio 2012). Guidelines were established during regional workshops at ACAP Espinhaço, such as evaluating the impact of climate change, searching for new populations, surveys of non-sampled areas within the Espinhaço Range, and identifying priority areas for species conservation. *Crossodactylodes itambe* is listed as a target species in the plan, for which specific actions were proposed, such as long-term monitoring studies. Although species monitoring has regionally improved in Brazil (Verdade *et al.* 2012), understanding how populations respond to environmental changes was considered crucial in both national and regional conservation planning (RAN/ICMBio 2012; Verdade *et al.* 2012).

1.5 Aims and objectives

This thesis is investigating the ecology and population dynamics of a single species of bromeligenous frog, *Crossodactylodes itambe*, endemic to a highland area in the Atlantic Rainforest of Brazil. Considering the aspects of species richness and declines outlined in this section, the lack of scientific knowledge on species ecology and distribution, as well as the need to establish conservation priorities in Brazil, this thesis aimed to:

- Expand our understanding of amphibian monitoring and population declines in tropical areas and, more specifically, improve scientific knowledge on the ecology, distribution and natural history of *Crossodactylodes itambe*; assess species conservation status and contribute to national and international lists of threatened species; and establish priority actions for the conservation of *Crossodactylodes itambe* and other bromeligenous frogs.

More specifically, this thesis was set out to answer the following questions:

1) What are the predictors of abundance of *Crossodactylodes itambe* at the Itambé summit and how is abundance affected by climatic and habitat variables at a local scale?

2) Is *Crossodactylodes itambe* naturally rare, occurring in a single locality, or is it simply under-sampled within the South Espinhaço Range? What are the variables influencing species distribution and where can we find new populations?

3) Can we detect declines in this range-restricted population using data from long-term monitoring data? If so, what would be considered a robust sampling design, that could not only precisely estimate species occupancy, but also detect changes in the population over time?

4) Is this species declining? Is occupancy probability of *Crossodactylodes itambe* changing over time and, if so, are changes in occupancy stable, declining or increasing?

5) What are the drivers of dynamics for *Crossodactylodes itambe* and which variables affect extinction and colonization probabilities at a local scale?

1.6 Thesis structure

To approach these questions this thesis covers aspects of ecology and natural history of *Crossodactylodes itambe*, outlined in the following way:

In **Chapter 2** I provide basic information on species ecology, using a Generalized Linear Model to investigate predictors of abundance. I discuss habitat use and bromeliad selection and compare results with other bromeligenous frogs, whenever possible. In this chapter I provide the first ecological study for this rare species and novel data for the whole genus *Crossodactylodes*. Based on results I also complete the first species assessment and suggest a threat category according to the IUCN.

Chapter 3 aimed to define a geographical range for species occurrence and distribution. I developed a Species Distribution Model using bromeliads as surrogates for frog occurrence and I also searched for new populations based on results of habitat suitability. I discuss the applicability of this modelling approach, demonstrating its potential use to model other bromeligenous frogs, for whose distribution data is also uncertain. I also discuss on how far the species is indeed dependent on a single species of bromeliad and if species can use other bromeliads available elsewhere.

In **Chapter 4** I combined a Power Analysis with Occupancy Models to investigate the statistical power of sampling designs that use long-term monitoring data to detect changes in amphibian populations. I argue for the benefits of power analysis to achieve specific monitoring goals, and how population declines might not be detected if sampling designs lack reliability. I discuss the challenges of monitoring rare and endemic species and provide a monitoring protocol specifically designed to detect occupancy changes for *Crossodactylodes itambe*.

For **Chapter 5** I collected data using the suggested sampling design. I compare population trends over the years using a Multi-season Occupancy Model. The main goal of this chapter was to define if the population is stable, declining or increasing, by comparing occupancy estimates over a 4-year period. I discuss occupancy trend for this population at a finer scale, demonstrating that declines of mountaintop amphibians can only be detected on specific elevational ranges.

Chapter 6 I also used a Multi-season Occupancy Model and aimed to define the drivers of population dynamics, to understand aspects of species extinction and colonization probabilities. I also evaluate the effects of fire on colonization and extinction rates, after a stochastic fire event. I compare habitat and climate drivers at a local scale and show how differently those variables can affect colonization and extinction rates on this mountaintop population of bromeligenous frog.

While chapters 2–3 touch on the lack of basic knowledge for species assessment and deliver basic information on species ecology and distribution, chapters 4–6 cover the shortfall of amphibian populations declines in tropical regions, for which we also have very limited data, and provides the first population assessment of a bromeligenous species. Finally, in **Chapter 7** I provide a general discussion on the results, highlighting the challenges of monitoring rare and range-restricted species and how to contribute to species management at a finer scale and future directions for conservation of bromeligenous frogs

Chapter 2 Predictors of abundance of a rare bromeliad-dwelling frog in the Espinhaço Mountain Range of Brazil

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2.1 Abstract

Although tank bromeliads are used by many anuran species, bromeligenous frogs (species strictly dependent on bromeliads for reproduction) occur less frequently and are poorly understood. *Crossodactylodes* are small frogs confined to bromeliads where they lay their eggs and complete their life cycle without leaving the plant. The genus comprises five species and there is little information on their natural history. We focused on *Crossodactylodes itambe* – a species confined to a single summit of < 0.5 km^2 at 1700 m above sea level in the Espinhaço Mountain Range of Brazil. We surveyed frogs in 75 individual bromeliads during two consecutive years and used a Generalized Linear Model to investigate the drivers of species abundance related to habitat structure and local climate. We recorded 446 adults, 267 tadpoles and 40 juveniles over the two years. Most bromeliads contained one adult frog and the mean number of tadpoles was 2–3. The structure of bromeliads influenced species abundance more than local climate. We found that bromeliad size, volume of central tank, and presence/absence of invertebrates and water influenced abundance of frogs. Abundance increased in larger bromeliads at higher elevation. Changes in the structure and size of bromeliads might therefore affect abundance. We provide the first species assessment and evaluation of threat categories for this poorly known species.

Keywords: Anurans, Bromeliads, Habitat use, Phytotelm, Tropical mountains, *Vriesea medusa.*

2.2 Introduction

Bromeliads are arboreal or terrestrial plants that collect water, leaf litter and detritus in a central tank – also known as a phytotelm (Lehtinen 2004). Because of their complex structure, bromeliads provide a wide range of microhabitats and contain a diverse associated fauna (Rocha *et al.* 2000; Armbruster, Hutchinson and Cotgreave 2002; Teixeira, Schineider and Almeida 2002; Jabiol *et al.* 2009; Silva, Carvalho and Bittencourt-Silva 2011; Brouard *et al.* 2012; Dézerald *et al.* 2014). Anuran amphibians represent the highest animal biomass and the richest vertebrate group associated with bromeliads (Oliveira, Rocha and Bagnall 1997; Silva, Carvalho and Bittencourt-Silva 2011). These plants are used by many anuran species (Teixeira, Schineider and Almeida 2002; Lacerda *et al.* 2009; Pertel, Teixeira and Ferreira 2010; Silva, Carvalho and Bittencourt-Silva 2011), but most of them are non-resident and are only occasionally observed. For example, species such as *Elachistocleis ovalis* (Andrade, Albertim and Moura 2009), *Aparasphenodon brunoi* (Teixeira, Schineider and Almeida 2002) and *Scinax cuspidatus* (Domingos *et al.* 2015) use bromeliads during periods of inactivity or as shelter. In contrast, and less frequently, some anurans complete their entire life cycle within bromeliads, and are defined as bromeligenous (sensu Peixoto 1995).

In Brazil there are at least 50 bromeligenous anurans (Sabagh, Ferreira and Rocha 2017) and the best documented examples are *Phyllodytes* species (Giaretta 1996; Teixeira *et al.* 1997; Eterovick 1999; Ferreira, Schineider and Teixeira 2012; Cunha and Napoli 2016; Mageski *et al.* 2016; Motta-Tavares *et al.* 2016; Ruano-Fajardo, Toledo and Mott 2016) and the *Scinax perpusillus* group (Peixoto 1995; Oliveira and Navas 2004; Alves-Silva and Silva 2009; Lacerda *et al.* 2009; Sabagh *et al.* 2012). Less well-documented, the genus *Crossodactylodes* comprises five species endemic to the Atlantic Rainforest in Brazil: *Crossodactylodes pintoi* (Cochran 1938), *C. bokermanni* and *C. izecksohni* (Peixoto 1982), *C. septentrionalis* (Teixeira *et al.* 2013) and *C. itambe* (Barata *et al.* 2013). *Crossodactylodes* species have highly restricted distributions and most of them are only recorded from type localities (Bokermann 1966; Peixoto 1981; Peixoto 1982; Barata *et al.* 2013; Teixeira *et al.* 2013). These small frogs are strictly dependent on bromeliads where they lay their eggs (Peixoto 1981; Santos *et al.* 2017) and spend the entire life cycle within the plant (Peixoto 1981; Barata *et al.* 2013; Teixeira *et al.* 2013).

Although the genus was described in 1938 (Cochran 1938), little is known about the biology of *Crossodactylodes* species, but some aspects of natural history have been revealed in recently described species (Barata *et al.* 2013; Teixeira *et al.* 2013; Santos *et al.* 2017). Overall, almost half of the bromeligenous frogs occurring in Brazil are considered as threatened and for 35% the conservation status is still unknown (Sabagh, Ferreira and Rocha 2017). Until now, three *Crossodactylodes* species have been evaluated by the IUCN Red List; two Near Threatened species (*C. bokermanni* and *C. izecksohni*) are close to qualifying for Vulnerable, with declining populations living in restricted areas (Silvano and Peixoto 2004a; Silvano and Peixoto 2004b), and one Data Deficient species (*C. pintoi*) was last recorded in 1909 (Peixoto and Carvalho-e-Silva 2004). We focused this study on *Crossodactylodes itambe*: a bromeligenous species only known from the type locality in an area of < 0.5 km^2 at 1700 m above sea level (Barata *et al.* 2013). Adults and tadpoles of *C. itambe* are reported to exclusively use a single species of bromeliad, *Vriesea medusa* (Barata *et al.* 2013; Santos *et al.* 2017) – a night-blooming tank bromeliad also with a small distributional range (Versieux 2008).

The bromeliad characteristics that determine abundance or presence of anurans are poorly understood and vary between and among species groups. For bromeligenous frogs there is a strong indication of habitat selection (Mageski *et al.* 2016; Sabagh, Ferreira and Rocha 2017) and different species show different habitat preferences and use (Eterovick 1999; Schineider and Teixeira 2001; Oliveira and Navas 2004; Cunha and Napoli 2016; Motta-Tavares *et al.* 2016). Nonetheless, these aspects have never been investigated for *Crossodactylodes* species. Given the highly restricted distribution of both *C. itambe* and its host bromeliad *V. medusa*, we ask the question: what are specific habitat characteristics that influence species abundance at the study site? Here we describe the effects of habitat structure and local climate on abundance within the only known population of *C. itambe*. We also provide the first species assessment and recommend the threat category to which this rare bromeliad-dwelling species should be allocated according to the IUCN guidelines (IUCN Standards And Petitions Subcommittee 2017).

2.3 Materials and methods

2.3.1 Study Area and data sampling

Pico do Itambé State Park (equivalent to IUCN categories I and II; Dudley 2008) is located in the state of Minas Gerais, south-eastern Brazil (18°23′S 43°20′W; Figure 2.1A), encompassing 4,696 ha and including the Itambé summit. The area is characterized by open field habitats with vegetation growing on rocky outcrops above 800 m (defined as 'campos rupestres'; Eiten 1978). The Itambé summit is the highest point of the Espinhaço Range – a mountain chain that geographically divides two hotspot biomes: the Cerrado (to the west) and the Atlantic Rainforest (to the east). The Itambé summit receives the Atlantic moist wind, which increases rainfall and mist, but the area is also influenced by the Cerrado, which provides open grassland habitats with herbs and shrubs covering an extensive outcrop.

This ecotone area hosts 14% of Brazilian vascular plants in less than 1% of the country's surface (Silveira *et al.* 2016) and is severely threatened by human activity (Alves *et al.* 2014; Monteiro *et al.* 2016), including climate change (Bitencourt *et al.* 2016). To the best of our knowledge, *Crossodactylodes itambe* is restricted to campos rupestres at the Espinhaço Range, living in a single species of tank bromeliad *Vriesea medusa* in high elevation areas. *Vriesea medusa* grows on rocky outcrops, is 1.6–2.2 m tall with a funnel-like central tank comprising a rosette of 55–80 cm diameter (c. 70 cm high) (Versieux 2008).

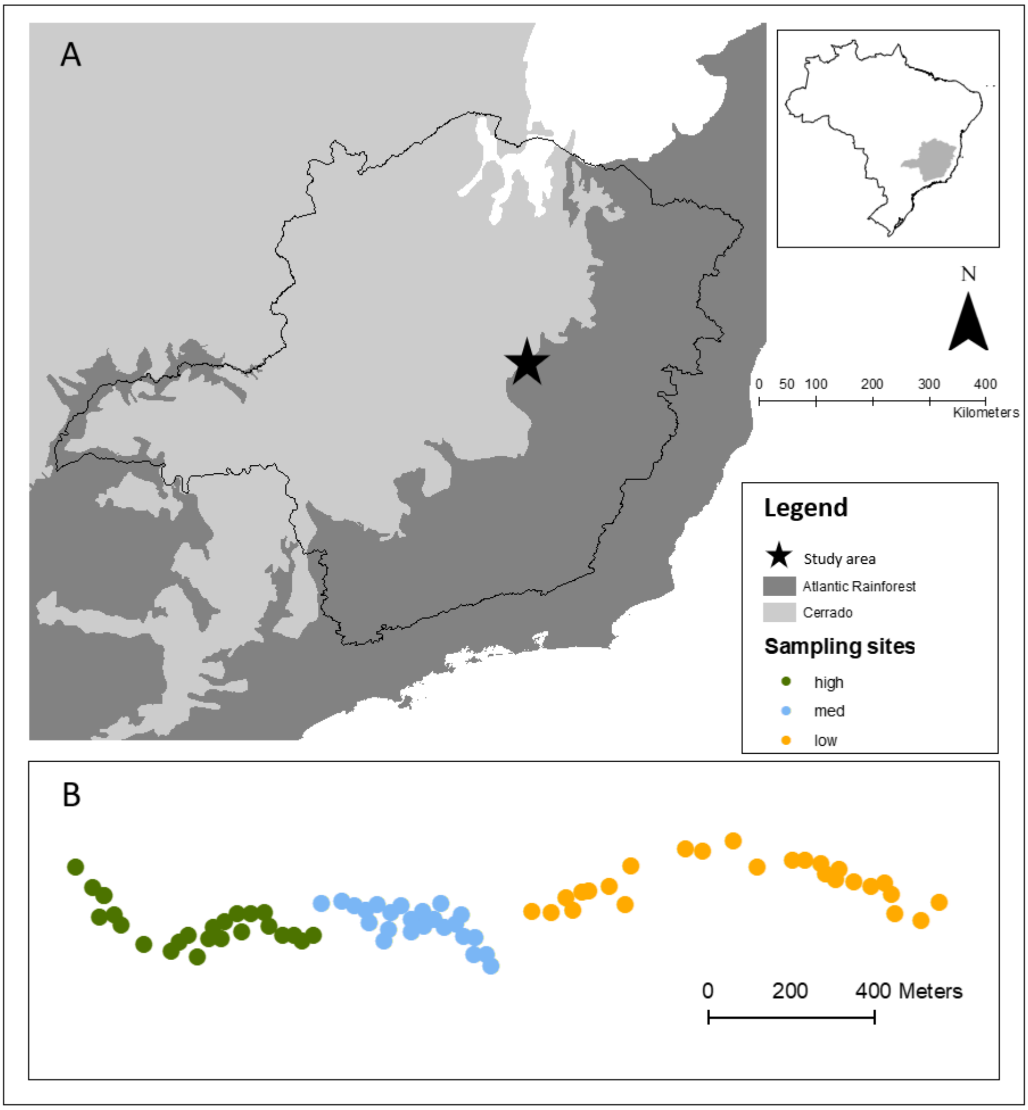

Figure 2.1 Study area and sampling sites at Pico do Itambé State Park, Brazil Location of study area in Minas Gerais state at south-eastern Brazil showing (A) the limits of the Cerrado and the Atlantic Rainforest. Sampling sites (B) are grouped in three elevation categories (given in meters above sea level): low (1704–1815 m), medium (1838–1925 m) and high (1998–2062 m).

Considering the restricted distribution of *C. itambe* on the Itambé summit (Barata *et al.* 2013), we used individual bromeliads occurring between 1700 m and 2062 m above sea level (a.s.l.) as sampling sites. We randomly selected 75 bromeliads, which we tagged with individual numbered labels that allowed repeated visits over different years. Sampling sites were at least 25 meters apart from each other and were grouped according to three elevation categories (Figure 2.1B) delimited by topography: low (1704–1815 m a.s.l.), medium (1838–1925 m a.s.l.) and high (1998–2062 m a.s.l.). The 75 sampling sites comprised 25 sites at each elevation category. To investigate frog abundance in relation to habitat structure, we characterized each sampling sites according to: elevation (in meters a.s.l.), size (given by bromeliad height x width in centimetres), volume of the central tank (hereafter rosette, given by height x width in centimetres) and number of neighbouring bromeliads touching the edge of the labelled bromeliad.

We searched for *C. itambe* using visual encounter surveys (Crump and Scott 1994) in two consecutive years (February to May 2015; February to June 2016), encompassing both wet (February to March) and dry (April to June) seasons. Each monthly survey consisted of 4–6 consecutive night visits made by one team of two investigators, starting after dusk. During our study period, we only surveyed sites that were individually tagged. Because it requires some experience to detect frogs in bromeliads (Chapter 4) only one trained observer searched for the species. By standardizing our survey to a single observer (detectability of $p = 0.6$, see Chapter 4 for details), we believe we eliminated the effect of observer bias in detection.

For each bromeliad studied and on each sampling occasion, we recorded total number of individuals and stage of development (adults, juveniles and tadpoles). Due to reduced size and coloration of *C. itambe*, we did not mark individuals and males were not distinguished from females as sex cannot be determined without capture and close examination of sexually dimorphic characteristics. To investigate changes in abundance in relation to sampling occasion, we recorded time of survey, presence and absence of water in the rosette, and presence and absence of invertebrates (potential predators of tadpoles and/or competitors for resources). We also installed data loggers at the three different elevation ranges to record daily climatic variables: maximum (max) and minimum (min) temperature, mean temperature, and relative humidity (RH). Monthly rainfall and UV radiation were recorded by a weather station located in the study area (at the top of Itambé summit).

2.3.2 Data analyses

Although we registered adults, tadpoles and juveniles (hereafter, total counts) during our study period, our data analysis included only records from adults. Therefore, the total number of adults occupying a bromeliad in a given sampling occasion (i.e., abundance) was considered as our response variable and we investigated predictors of abundance according to sampling occasion and habitat characteristics. Although our sampling sites were considered independent, for the purpose of our analysis, we did not assume population was closed. Additionally, as variables that are survey-specific (i.e., vary by both site and sampling occasion, such as presence/absence of invertebrates) cannot be fitted as predictors of abundance in models that account for detectability (Royle 2004), we used a Generalized Linear Model (GLM) to identify predictors of species abundance in the study area.

Our models included explanatory variables relating to sampling occasion (presence/absence of water and invertebrates at the time observation was made, year and season); local climate (max-min temperature, rainfall, UV radiation and RH); and habitat structure (size, volume, elevation as a continuous variable, and number of neighbours). Exploratory analyses showed that variables were not correlated (Pearson correlation coefficient all < 0.5 and P > 0.05) so they were treated as independent predictors in the models.

We fitted models using a Poisson distribution (in abundance data the mean was equal to the variance; = 0.2) and model selection was performed using the Akaike's Information Criterion (AIC) (Burnham and Anderson 2002). Models with ΔAIC < 2 were interpreted as having strong support; models with a ΔAIC of > 2 were considered to have less support (Burnham and Anderson 2002). We tested the goodness of fit of the models using a chi-square test (model was considered good with P > 0.05). We reported the results of GLM after model averaging using the parameter estimates (β), unconditional standard errors (SE), and upper and lower confidence intervals (CI). Statistical analyses were performed using R (R Core Team 2017) with the MuMIn package (Barton 2016).

2.4 Results

We counted a total number of 446 adults, 267 tadpoles and 40 juveniles over two years of sampling in the 75 bromeliads we surveyed (Figure 2.2). Considering all sampling sites, the total count of adults, tadpoles and juveniles was similar for both years (n = 398 in 2015, n = 355 in 2016; Figure 2.2) and so was the total count between seasons (n = 310 wet season; n = 443 dry season, for two years). In almost 80% of occupied bromeliads (n = 273, out of 349 occasions), we recorded only one adult (mean = 1.3, SD = 0.6), and the maximum number of adults recorded in a single bromeliad was four. Nevertheless, this distribution was random rather than over-dispersed (mean and variance = 0.2; Poisson test P = 0.94). The mean number of tadpoles in occupied bromeliads for each year was 2–3, with a maximum of eight individuals (mean = 2.4, SD = 1.7). We had more counts of adults, tadpoles and juveniles at high elevations, between 1998-2062 m a.s.l. (*n* = 486; 65% of total counts). Only 4% of our counts were made at low altitude, below 1815 m a.s.l., and were exclusively adults (*n* = 33) with no tadpoles or juveniles occurring at low elevation.

Figure 2.2 Total counts of individuals of *Crossodactylodes itambe*
Counts of individuals are given for two consecutive years, according to stage of development (adult, tadpole or juvenile) in four surveys (February to May 2015; February to June 2016).

Only two models were considered to have a good fit (i.e., ΔAIC < 2) and both included habitat and climatic variables, as well as presence/absence of invertebrates and water (Table 2.1). The third model had a ΔAIC > 2 and little explanatory power, but variables describing abundance were similar to those included in the selected models, showing consistency in model selection (Table 2.1). Our best model included a wide set of explanatory variables and explained 48% of adult abundance, while the second model explained 34% (Table 2.1).

Table 2.1 Summary of Generalized Linear Models for abundance of *Crossodactylodes itambe*

Top three GLM results showing the predicted variables with estimated parameter (β) and standard error (SE); and parameters of model selection with number of parameters within each model (N Pars), Akaike's Information Criterion (AIC), ΔAIC and AIC weights (AIC *w*). Models are ranked by AIC values and significant variables are highlighted in bold.

	Model rank					
	1		2		3	
Estimates for predicted variables						
Variable	β	±SE	β	±SE	β	±SE
Intercept	-2.48	0.21	-2.48	0.24	-2.48	0.24
Invertebrates	**-0.35**	**0.1**	**-0.35**	**0.1**	**-0.35**	**0.1**
Water	**0.55**	**0.2**	**0.56**	**0.2**	**0.58**	**0.2**
Elevation	**0.51**	**0.06**	**0.49**	**0.07**	**0.48**	**0.07**
Size	0.11	0.06	0.11	0.06	0.11	0.06
Neighbours	-0.1	0.05	-0.1	0.05	-0.1	0.05
Volume	**0.24**	**0.07**	**0.24**	**0.07**	**0.24**	**0.07**
Year	**0.33**	**0.14**	**0.38**	**0.15**	0.31	0.18
RH	-0.1	0.06	**-0.14**	**0.07**	**-0.15**	**0.07**
T max	0.11	0.06	0.09	0.06	0.1	0.06
T min	-0.12	0.06	**-0.18**	**0.08**	**-0.18**	**0.08**
Elevation: size	**0.28**	**0.06**	**0.28**	**0.06**	**0.28**	**0.06**
Season	-		0.16	0.14	0.21	0.16
Pluviosity	-		-		-0.05	0.08
Parameters of model selection						
N Pars	12		13		14	
AIC	2313.4		2314.1		2315.7	
ΔAIC	0		0.71		2.27	
AIC *w*	0.48		0.34		0.15	

We observed a positive and significant relationship between abundance and the presence of water inside the rosette, and there was a negative relationship with the presence of invertebrates in the bromeliad (Figure 2.3) – both variables changing with survey occasion.

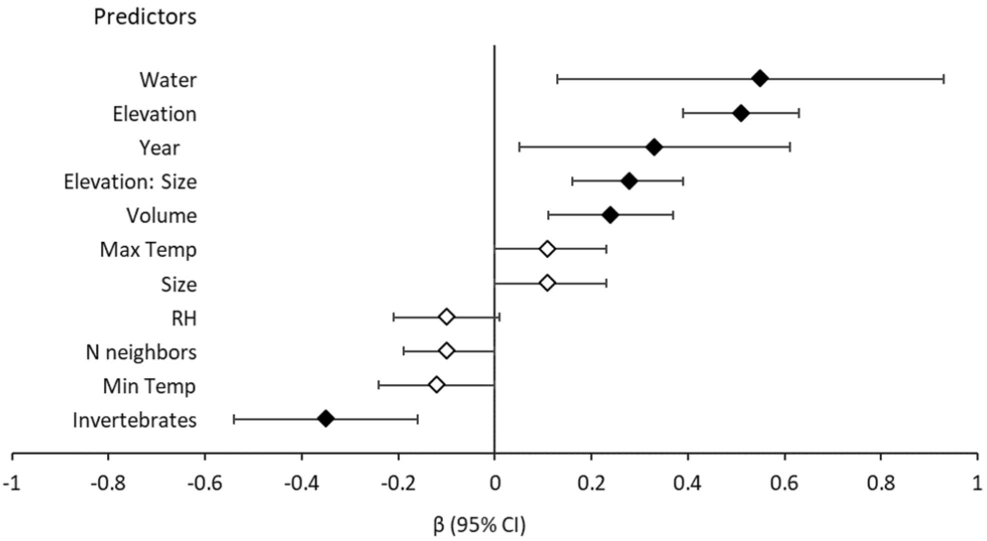

Figure 2.3 Parameter estimates from best fitting model explaining the abundance of *Crossodactylodes itambe*
Variation in parameter estimate (β) with 95% confidence interval for variables included in the best fitting model, after model averaging and selection. Significance of predictors of frog abundance is given when confidence intervals do not cross zero (black diamonds = significant effect). (Water = presence of water; Elevation: Size = interaction of elevation and size of bromeliad; Volume = volume of central tank; Max Temp = maximum temperature; Size = size of bromeliad; RH = relative humidity; N neighbour = number of neighbours; Min Temp = minimum temperature; Invertebrates = presence of invertebrates).

Elevation and size of the bromeliad are considered habitat variables and the interaction between those two had a positive effect on frog abundance at sampled sites (Figure 2.4). Although bromeliad size had high relative importance in our models, the effect of this variable alone was not considered significant (Figure 2.3).

Volume of rosette had a positive effect on adult abundance. Although included in the best model, climatic variables did not significantly explain abundance of adults inside the bromeliad (Figure 2.3; Table 2.1).

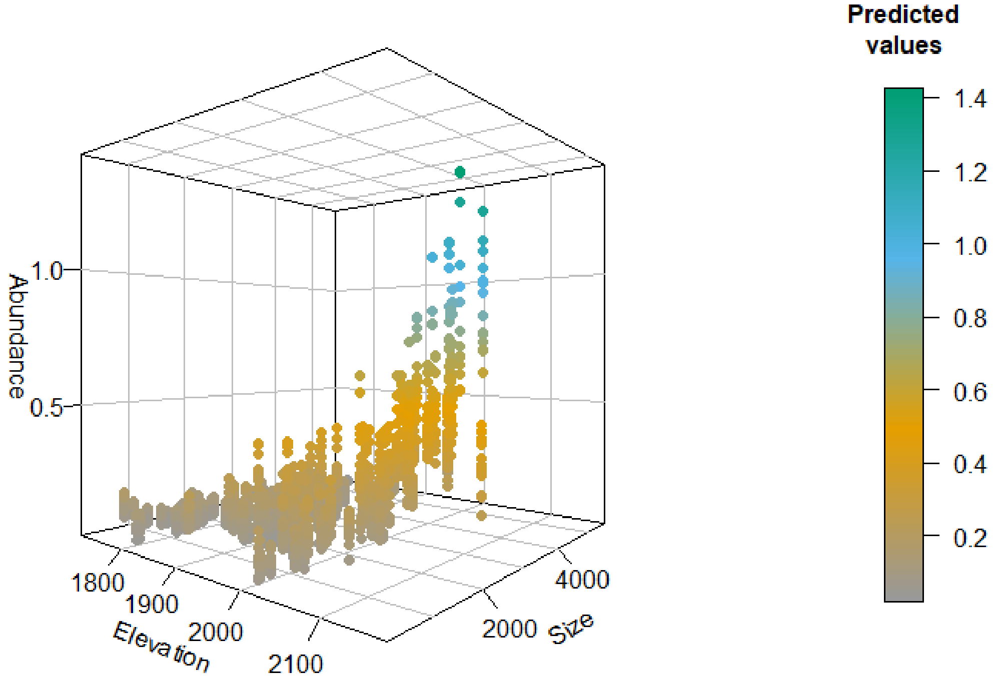

Figure 2.4 Relationship between species abundance and the interaction of elevation and size

Predicted values of abundance are based on total counts of adults per bromeliad, elevation is given in meters above sea level and bromeliad size is given by bromeliad height x width in centimetres.

2.5 Discussion

Although a quarter of bromeligenous frogs are reported to use a single species of bromeliad, further investigations of habitat selection are still needed (Sabagh et al., 2017). We only recorded *C. itambe* using *V. medusa*, confirming previous suggestions that this frog species is restricted to a single bromeliad (Barata *et al.* 2013; Santos *et al.* 2017). While *C. bokermanni* was also reported to occupy a single species of *Vriesea*, *C.*

izecksohni was recorded using different *Vriesea* species (Sabagh, Ferreira and Rocha 2017). Different species of bromeliads are used by bromeligenous frogs (Alves-Silva and Silva 2009; Lacerda *et al.* 2009; Mageski *et al.* 2016; Motta-Tavares *et al.* 2016; Sabagh, Ferreira and Rocha 2017); however, *Vriesea* sp. is one of the most common bromeliads used (often preferred) by *Phyllodytes luteolus* and *Scinax perpusillus* (Eterovick 1999; Schineider and Teixeira 2001; Oliveira and Navas 2004; Ferreira, Schineider and Teixeira 2012). *Vriesea* sp. are preferred for a variety of reasons, such as the large number of leaf axils (Schineider and Teixeira 2001; Mageski *et al.* 2016), complexity of their structure (Eterovick 1999; Ferreira, Schineider and Teixeira 2012), or position close to the ground (Oliveira and Navas 2004).

For *C. itambe* elevation may influence the distribution of adults (Barata *et al.* 2013) and tadpoles (Santos *et al.* 2017), which is also affecting species occupancy (Chapter 4) and population dynamics (Chapter 5–6). Indeed, we found elevation to be an important determinant of species abundance. All other four *Crossodactylodes* species are restricted to high elevations, from 650 m to 1200 m a.s.l. (Peixoto and Carvalho-e-Silva 2004; Silvano and Peixoto 2004b; Silvano and Peixoto 2004a; Teixeira *et al.* 2013). However, habitat use has never been investigated for other *Crossodactylodes* species. Santos *et al.* (2017) suggested that preferential breeding sites for *C. itambe* occurred above 1977 m a.s.l. We found that the interaction of elevation and bromeliad size was positive and significant, showing an increase in the number of individuals occurring in larger bromeliads at higher elevations.

Bromeliad selection by *S. perpusillus* males was mostly influenced by plant size (Oliveira and Navas 2004), but for *C. itambe* size alone was not a significant predictor. Larger bromeliads usually contain a larger number of leaves, which can be correlated with water storage capacity (Cogliatti-Carvalho *et al.* 2010; Freschi *et al.* 2010) and may offer some advantage to reduce predation (Ferreira, Schineider and Teixeira 2012) and avoid competition (Schineider and Teixeira 2001; Cogliatti-Carvalho *et al.* 2010). We found that the volume of the rosette positively influenced abundance. Larger tanks can hold more nutrients for tadpole development and are less susceptible to unpredictable droughts that can cause mortality of the offspring (Lehtinen 2004). *Vriesea* is the most diverse genus within Bromeliaceae in the state of Minas Gerais (Versieux and Wendt 2007) and varies widely in water storage capacity (Alves, Rocha and Van Sluys 1996; Schineider and Teixeira 2001; Cogliatti-Carvalho *et al.* 2010). Because it holds a large rosette, *V. medusa* can retain large volumes of water from mist and rain, making it available throughout the year and reducing chances of bromeliad tank desiccation.

Tank bromeliads are capable of holding water even in dry environments (Cogliatti-Carvalho et al., 2010). We observed that most bromeliads retained water during periods of reduced rainfall (from April to June). Holding water in the dry season may favour tadpole development and survival throughout the year and may also reduce the influence of seasonality in species abundance. Although year was an important predictor of abundance, number of individuals did not seem to vary with season, as we had similar numbers in both dry and wet seasons. In fact, season and rainfall were not important variables in our model and other climatic variables were also unrelated to abundance. Similar patterns occur in bromeligenous species *P. luteolus* (Schineider and Teixeira 2001; Ferreira, Schineider and Teixeira 2012), for which reproduction was not influenced by thermal fluctuations throughout the seasons (Oliveira and Navas 2004).

The presence of water is vital for bromeligenous frogs, and we believe that the simple presence of water inside the bromeliad is as important as water volume – we observed an increasing number of frogs in bromeliads where water was available. Indeed, only on rare occasions did we record individuals in bromeliads with no water (8% of total counts). Although preferences for bromeliads with large water reservoirs were recorded for two other bromeligenous species (Oliveira and Navas 2004; Mageski *et al.* 2016), water depth and volume stored in the rosette were not important for *P. melanomystax* (Cunha and Napoli 2016) and *P. luteolus* (Eterovick 1999; Schineider and Teixeira 2001). Therefore, we believe that the simple presence of water (not the volume stored by the tank itself) might be considered as the main factor influencing the abundance of *C. itambe*.

We found that presence of invertebrates can have a negative effect on the abundance of *C. itambe* in the bromeliad. Microhabitats with water bugs are avoided by tadpoles in streams (Eterovick and Barata 2006), but this was not investigated for bromeligenous tadpoles. In spite of being an unpredictable environment (e.g., food availability or desiccation risks), a phytotelm has fewer predators than ponds and streams (Schiesari, Gordo and Hödl 2003; Lehtinen 2004). Although invertebrates were not identified to genus level, we observed bromeliads being frequently used by spiders – which are known to prey upon small frogs and tadpoles (Menin, Rodrigues and Azevedo 2005). Our data suggest that bromeliads with presence of invertebrates might be avoided by breeding adults and, therefore, preferred for tadpole development.

For bromeligenous species, clusters of bromeliads increase the number of available tanks and provide access to several oviposition sites (Oliveira and Navas 2004; Cunha and Napoli 2016); which are also preferentially used by males of *S. perpusillus* (Oliveira and Navas 2004) and calling males of *P. melanomystax* (Cunha and Napoli 2016). Surprisingly, we found that abundance of frogs was not influenced by the number of neighbouring bromeliads. We recorded up to four adults and eight tadpoles inside a bromeliad, which is similar to *P. luteolus* (Schineider and Teixeira 2001) and *P. melanomystax* (Cunha and Napoli 2016). For *C. itambe*, clusters of adults with varying number of tadpoles and/or eggs using the same bromeliad was previously reported (Santos *et al.* 2017). Nonetheless, we usually observed one individual in each bromeliad (80% of our records) – which was also recorded for other bromeligenous frogs (Schineider and Teixeira 2001; Ferreira, Schineider and Teixeira 2012; Motta-Tavares *et al.* 2016).

Over 60% of bromeliads occurring at the Espinhaço Range are endemic, including *V. medusa* (Versieux and Wendt 2007). Over the past centuries, fire was used by dairy farmers to manage natural pasture in our study site (Versieux 2008). Although burning has not been commonly practiced since the Itambé summit became a protected area, occasional fires have recently occurred. Bromeligenous frogs are more likely to be threatened by the loss of their breeding sites (Sabagh, Ferreira and Rocha 2017) and we found that, for *C. itambe*, the structure of bromeliads had a greater influence determining adult abundance than local climate. Therefore, fire may change the structure of bromeliad and have a negative effect on population abundance, which can potentially cause extinction of local populations. Although we found that the species is locally abundant in the study area, extent of occurrence is limited to 0.5 km^2 (Barata *et al.* 2013) and no other population is known so far (Chapter 3). Based on IUCN criteria of geographic range, population number and decline in habitat quality, *Crossodactylodes itambe* could be classified as Critically Endangered (CR) under criteria B1ab(iii) and B2ab(iii), or Vulnerable (VU) under criteria A1c and D2 – both considered high levels of threat categories.

2.6 Acknowledgements

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Chapter 3 Bromeliad as biodiversity surrogates: overcoming the lack of distribution data on bromeligenous frogs

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Manuscript in preparation

3.1 Abstract

Bromeligenous frogs spend their entire life cycle inside bromeliads and the distributional range of most species is still unknown. We aimed to search for new populations and investigate the geographical extend of *Crossodactylodes itambe*, for which limited data are available. The unique life history traits of bromeligenous frogs create an exceptional opportunity for modelling species distribution and we used bromeliads as surrogates to indirectly predict the distribution of our target species. We used Maxent to predict suitable areas based on climate and topographic profiles, combined with 21 occurrence records of bromeliads. We created 12 models using bioclimatic variables, altitude and soil type. Maxent was an effective tool in predicting new occurrences with few records. Models predicted similar areas and had high and significant success rates. We found bromeliads in all visited areas, but we did not find a new population of *C. itambe*, indicating that the species is naturally rare with a small range. However, we discovered a new species of *Crossodactylodes* in one of the areas indicated to have high suitability, demonstrating that highland areas still suffer from insufficient survey effort. Our modelling approach successfully predicted the occurrence of a habitat specialist amphibian and could be broadly extended to DD bromeligenous frogs that are presumed to occur more widely, potentially resulting in the discovery of new populations or even new species.

Keywords: Bromeligenous frogs, Few occurrence data, Maxent, New population, New species, Species distribution model.

3.2 Introduction

Most range-restricted species are rare, concentrated in tropical areas (Pimm and Jenkins 2010) and threatened with extinction (Morais *et al.* 2012). Giving these attributes, range-restricted species are also hard to find and difficult to study. A quarter of amphibians in the world have very small geographical ranges (< 10^3 km^2) (Pimm *et al.* 2014) – in Brazil, for instance, almost 40% of amphibians have a range of less than 20,000 km^2 (Pimm *et al.* 2010). Range restrictions make amphibians particularly vulnerable to extinction (Wake and Vredenburg 2008). With 41% of species under risk of extinction worldwide (Stuart *et al.* 2004; Wake and Vredenburg 2008), amphibians are more threatened than either mammals, birds (Hoffmann *et al.* 2010) or reptiles (Böhm *et al.* 2013), with an increasing number of species moving to higher threat categories in the past few decades (Hoffmann *et al.* 2010).

Range-restricted species can be habitat specialists, enhancing species vulnerability (Kunin and Gaston 1997), and also have reduced dispersion capabilities, such as many bromeligenous frogs. These rare amphibians are associated with bromeliads throughout their entire life cycle without leaving the plant (Peixoto 1995), and are mostly known from very few locations, usually in montane areas of South America (Sabagh, Ferreira and Rocha 2017). In extreme cases, bromeligenous frogs are not only endemic, but also restricted to a single species of bromeliad, for example: *Anomaloglossus roraima* with one known population recorded at 2700 m above sea level (a.s.l.) at Mount Roraima in Venezuela and restricted to *Brocchinia tatei* (Kok, Willaert and Means 2013); *Tepuihyla exophthalma* from the tepuis of Guyana, restricted to an unidentified bromeliad species from the genus *Brocchinia*, occurring between 585–1550 m a.s.l. (Smith and Noonan 2001; MacCulloch and Lathrop 2005), and *Crossodactylodes itambe* from above 1700 m at the Itambé summit in the Atlantic Rainforest of Brazil, restricted to *Vriesea medusa* (Barata *et al.* 2013).

Species with a larger number of known populations are more likely to thrive (Pimm and Jenkins 2010) and range-restricted amphibians with relatively few known populations are probably at greater risk (Wake and Vredenburg 2008). This is the case for most bromeligenous frogs, which already have more than 70% of species under threat (Sabagh, Ferreira and Rocha 2017). These species are amongst those of higher conservation concern, but basic natural history information is so poorly available that they are listed as Data Deficient by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (IUCN 2018). Although the IUCN relies on geographical range to complete their assessments, there is a lot of uncertainty around species with small geographical ranges (Mace *et al.* 2008). Therefore, understanding where new populations are more likely to be found is urgently needed to define whether these species are indeed naturally rare or simply under-sampled.

Species distribution models (SDM) have emerged as an effective tool in spatial ecology with a number of applications (for a comprehensive review see Guisan and Thuiller 2005; Elith and Leathwick 2009) and are used for conservation planning (Rose *et al.* 2015); for example, to evaluate spatial scale for decision-making (Ferraz *et al.* 2012) and to define key priority sites for species conservation (Thorn *et al.* 2009).

In tropical landscapes, one of the most promising uses of SDM is identifying under-sampled locations to prioritize for surveys of range-restricted species (Raxworthy *et al.* 2003). This approach has been applied across different geographic regions and many taxonomic groups (Pearson *et al.* 2007; Marini *et al.* 2010; Chunco *et al.* 2013; Sarkinen, Gonzáles and Knapp 2013), resulting in better understanding about species distribution, discovering new populations, and even finding new species.

Species distribution models relate species occurrence records to the spatial characteristics of those locations to identify predictors of potential areas with suitable characteristics (Araújo and Guisan 2006; Elith *et al.* 2006; Elith *et al.* 2011). Although small sample sizes may compromise the power of predictions (Stockwell and Peterson 2002; Wisz *et al.* 2008), the correct choice of algorithm can increase the predictive power of a model (Elith *et al.* 2006; Pearson *et al.* 2006; Williams *et al.* 2009), with algorithms, such as Maxent, performing well even with sample sizes below 20 records (Pearson *et al.* 2007; Papeş and Gaubert 2007; Williams *et al.* 2009; Marini *et al.* 2010; Le Lay *et al.* 2010; Chunco *et al.* 2013). For instance, Chunco *et al.* (2013) modelled the distribution of a rare and threatened newt using nine occurrence records and successfully characterized species habitat and confirmed species restriction to high elevation areas of Laos.

With very limited distribution data available, bromeligenous frogs will be challenging for modelling. However, SDM can be generated by 'borrowing' information from a species that is more abundant yet associated with the species of primary concern (Edwards *et al.* 2005). Because bromeligenous frogs are strongly associated with their host plants, bromeliads can be used as surrogates for frog occurrence, and even though there is little information on the target species, models can be produced using occurrence records of bromeliads that are widely distributed. Our target species, *Crossodactylodes itambe*, is a range-restricted bromeligenous frog from Brazil that is locally abundant (Chapter 2) and highly detectable (Chapter 4). Species extent of occurrence is reduced to < 0.5 km^2 in one site above 1700 m of altitude and frogs are strictly dependent on a single species of bromeliad (Barata *et al.* 2013). No other population is known, and the species qualifies for high threat categories under the IUCN criteria of geographic range and population number (Chapter 2, Appendix I).

Using occurrence data of bromeliad species from the genus *Vriesea*, known to be used by *C. itambe,* and we asked the following questions: is *C. itambe* naturally rare and a micro-endemic or is it simply under-sampled within our study area? Is our target restricted to a single species of bromeliad or can it occupy other bromeliads species available elsewhere? Finally, considering the limited data available, could our modelling approach successfully work for other habitat specialist amphibian species? To answer these questions, we aimed to 1) use SDM to define potential areas for bromeliad occurrence; 2) search for new populations of *C. itambe* within predicted areas; and 3) define species distribution and characterize habitat restrictions. Our research questions are therefore not only related to the geographical extent of bromeligenous frogs and their levels of threat, but also to the general implications of survey effort of montane endemic species and habitat-specialist amphibians. Herein, we present a SDM using bromeliads as surrogates to indirectly predict the distribution of its host species, for which limited data are available. The success of our approach can lead to promising applications of SDM to endemic habitat specialist species, such as threatened bromeligenous frogs.

3.3 Materials and methods

3.3.1 Target species and study area

Our target species is the small-ranged bromeligenous frog *Crossodactylodes itambe*, only known from one locality in an open rocky outcrop of the Espinhaço Mountain Range, at the Itambé State Park, Minas Gerais state, south-eastern Brazil (18°23′S 43°20′W; datum WGS 84). The genus *Crossodactylodes* comprises five small-sized species of frogs with highly restricted distributions, most of them endemic to highland areas in the Atlantic Rainforest (Bokermann 1966; Peixoto 1982; Teixeira *et al.* 2013). One distinct trait of the whole genus is their association with bromeliads, where they lay their eggs (Peixoto 1981; Santos *et al.* 2017) and they complete their life cycle without leaving the plant (Peixoto 1982; Barata *et al.* 2013; Teixeira *et al.* 2013). For *C. itambe* occupancy is positively correlated with elevation (Chapters 4–6) and abundance is influenced by habitat structure, especially the interaction of bromeliad size and elevation (Chapter 2).

Tank bromeliads are large arboreal or terrestrial plants with a central tank that collect water, leaf litter and detritus, and provide a wide range of microhabitats for numerous species (Rocha *et al.* 2000; Armbruster, Hutchinson and Cotgreave 2002;

Jabiol *et al.* 2009; Brouard *et al.* 2012; Dézerald *et al.* 2014), including many amphibians (Teixeira, Schineider and Almeida 2002; Silva, Carvalho and Bittencourt-Silva 2011). According to the literature, *C. itambe* lives in a single species of tank bromeliad, *Vriesea medusa* (Barata *et al.* 2013; Santos *et al.* 2017), which is also endemic to the Espinhaço Range and only known to occur on two mountaintops (Versieux 2008; Versieux *et al.* 2010).

The Espinhaço Range is a 1200 km mountain chain and is considered the geographical divider of two hotspots biomes: the Atlantic Rainforest to the east, and the Cerrado to the west. The highest point of the Espinhaço Range is the Itambé summit at 2062 m a.s.l., in the state of Minas Gerais, where our target species is currently recorded. This mountain chain is a megadiverse area and a centre of amphibian endemism (Leite, Eterovick and Juncá 2008), but is also severely threatened by human activity (Alves *et al.* 2014; Monteiro *et al.* 2016) and climate change (Bitencourt *et al.* 2016). The Espinhaço Range is recognized as a priority area for biodiversity conservation (Drummond *et al.* 2005) and the southern portion of its range is considered a UNESCO Biosphere Reserve and shelters a mosaic of protected areas of different sizes and shapes (hereafter, Espinhaço Mosaic).

Given the habitat requirements, small body size and potentially poor dispersal capability of *C. itambe*, we restricted the geographical scale of our models to a smaller subset within the Espinhaço Range. Such a scale reflects the purpose of the analysis and corresponds to the scale at which the animal perceives their environments (Elith and Leathwick 2009; Rose *et al.* 2015). Hence, our main goal was to find new populations of C. *itambe* in our study area, within the limits of the Biosphere Reserve and the Espinhaço Mosaic (Figure 3.1A). This area contains locations where our target species is likely to occur, producing models without extrapolating predictions to areas outside the probable range of the species (Merow, Smith and Silander 2013).

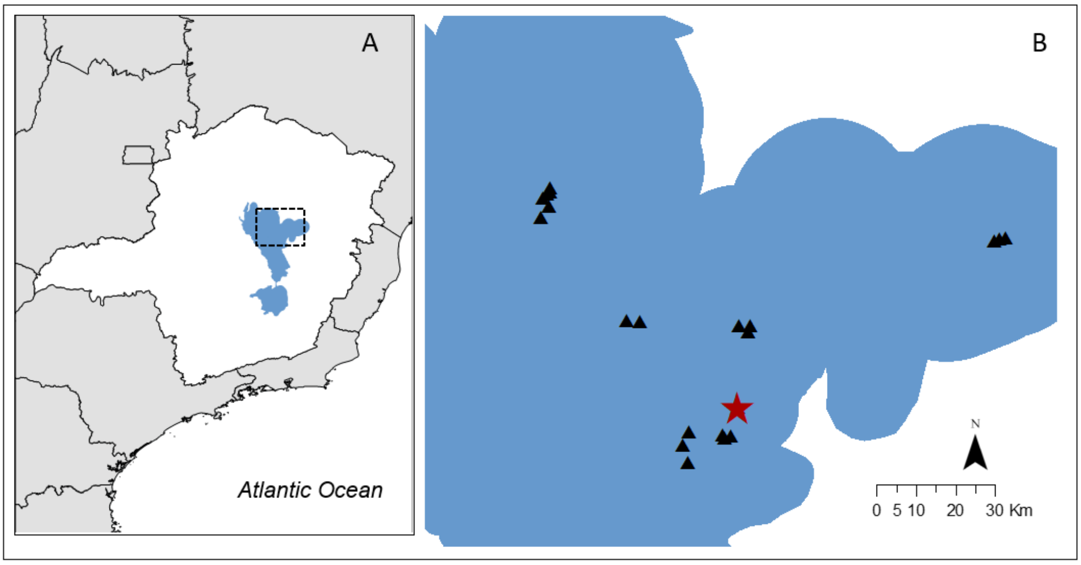

Figure 3.1 The Biosphere Reserve and the Espinhaço Mosaic, at south-eastern Brazil
Study area with (A) the location of the Biosphere Reserve and the Espinhaço Mosaic (blue area); and (B) filtered occurrence records (black triangles, $n = 21$) of the bromeliad *Vriesea* sp. used in our models. Red star shows the known population of *Crossodactylodes itambe*.

3.3.2 Occurrence and environmental data

The combination of unique life history traits of bromeligenous frogs create an exceptional opportunity for modelling species distribution. Since there are few records of the occurrence of C. *itambe*, we used bromeliads as a surrogate for species presence and we therefore modelled not our target species, but the environmental requirements for occurrence of bromeliads. *Vriesea* is the most diverse genus of bromeliads in the state of Minas Gerais (Versieux and Wendt 2006) and contains some of the largest species. Considering that the structure of the plant is an important predictor of abundance for *C. itambe* (Chapter 2), at least five other species of *Vriesea*, with similar size and structure, could be used to generate our models. We therefore used occurrence records from species of the genus *Vriesea*, assuming the structure of the plant, but not a specific species, would be appropriated for frog occurrence.

To obtain occurrence records of the bromeliads, we searched for *Vriesea* species in nine locations within our study area, ranging from 1029 m to 1592 m a.s.l., inside and outside protected areas. We selected these locations using Google Earth, comparing the surrounding landscape with the Itambé summit, where *C. itambe* originally occurs. We identified similar areas based on vegetation cover and substrate type, and selected locations based on access. More specifically, with Google Earth images at 100 m scale, we looked for extensive outcrops lacking vegetation cover and/or thinly covered by open grassland habitats with few herbs and shrubs. We then defined tracks, roads and paths that could lead us to these locations. In 2015, we surveyed these areas and gathered occurrence records of *Vriesea* sp. during a 6-day expedition. By the end of our survey we had a total of 133 occurrences, with precise GPS coordinates.

Bromeliad occurrence records were, inevitably, very close to each other (in some occasions < 100 m), which could increase the chances of model overfitting by including records that are not spatially independent (Shcheglovitova and Anderson 2013). Independence of occurrence records is highly recommended (Guisan and Thuiller 2005) and we combined clustered data into a single dataset to avoid spatial autocorrelation. Even if the number of occurrence records is reduced, for small spatial scales quality is better than quantity (Engler, Guisan and Rechsteiner 2004). Therefore, we only used records of bromeliads that were at least 1 km apart from each other. We filtered these records with ArcGIS, creating a 1 km buffer in every occurrence data and identifying overlapping occurrences that were later excluded. We completed this analysis and had a total of 21 unique records that could be used in our models (Figure 3.1B).

Models using bioclimatic variables (such as annual mean temperature and isothermality, among others) can be used to predict climatic suitable conditions for rare species (Marino *et al.* 2011; Chunco *et al.* 2013). For environmental data we used 19 bioclimatic variables and altitude, which we downloaded from WorldClim Global Climate Data (30 seconds resolution, ~1 km^2), and soil type downloaded from the Department of Soils and AgroSciences at the Federal University of Viçosa, in Brazil. Using ArcGIS, we standardized all environmental variables, using the same extent and cell size, and defined their geographical boundaries accordingly to study area (i.e., within the limits of the Biosphere Reserve and the Espinhaço Mosaic).

We tested for correlation between climatic variables performing a Principal Component Analysis (PCA) in ArcGIS, and correlated variables were excluded from our final models for comparisons. Outputs from PCA provides the variance explained by Eigenvectors coefficients (indicate the relative weight of each variable in the component), percentage of Eigenvalues and accumulative Eigenvalues, which we interpreted as the contribution of each axis as the principal components of our analysis. Finally, we built our distribution models with 1) all climatic variables and altitude, 2) all climatic variables, altitude and soil type, and 3) soil type and altitude combined with selected uncorrelated climatic variables.

3.3.3 Model settings

Potentially suitable areas for bromeliads and associated frogs were identified based on climate and topographic profiles, combined with 21 occurrence records of bromeliads to which *C. itambe* is possibly constrained. We used the machine learning algorithm Maxent version 3.4.1 (Phillips, Anderson and Schapire 2006). Maxent is a maximum entropy-based machine-learning method used for making predictions when presence-only data is available, and estimates the probability distribution for a species' occurrence given the constraints derived from the available data (Phillips, Anderson and Schapire 2006). The accuracy of models can decline considerably with few records (Stockwell and Peterson 2002; Wisz *et al.* 2008); however, Maxent performs relatively well when compared to other algorithms (Elith *et al.* 2006; Wisz *et al.* 2008), even with a sample size as low as five (Hernández *et al.* 2006; Pearson *et al.* 2007).

We built 12 different models, with slightly different settings and including a model with default values from Maxent (Table 3.1). All models were built to create a response curve and predictions, using a Jackknife to measure variable importance. Models were set up with a logistic output and the number of maximum iterations was set to 5000. In SDM the training dataset refers to the occurrence records and predictors used to build the model and distinguish from the test dataset which is used independently to test the predictive success of a model (Pearson *et al.* 2007; Elith and Leathwick 2009). Our first model contained all climatic variables and altitude, and we applied Maxent settings by default defining a random seed with a test percentage of 25%, using the remaining locations as training dataset and taking the average of 15 subsamples (Table 3.1, model 1).

Table 3.1 Settings details for different models built with Maxent

Details of model parametrization with variables (climatic = all bioclimatic variables, selected = bioclimatic variables selected after PCA, soil = soil type), run type (subsample or cross-validation), threshold method (T10 = 10 percentile training presence; MTP = minimum training presence), regularization multiplier (R; varying from 1 to 4) and model feature (LQH = linear, quadratic, hinge; H = hinge only) († default model; * climatic selected variables: temperature seasonality and annual precipitation). Highlighted in bold are models presented in the results.

Parametrization					
Model	Variables	Run type	Threshold	R	Feature
1†	**climatic + altitude**	**Subsample**	**none**	**1**	**auto**
2	climatic + altitude	Cross-validation	T10	2	LQH
3	climatic + altitude	Cross-validation	T10	2	H
4	climatic + altitude	Cross-validation	T10	4	LQH
5	climatic + altitude	Cross-validation	T10	4	H
6	climatic + altitude	Cross-validation	MTP	2	LQH
7	climatic + altitude	Cross-validation	MTP	2	H
8	climatic + altitude	Cross-validation	MTP	4	LQH
9	**climatic + altitude**	**Cross-validation**	**MTP**	**4**	**H**
10	climatic + altitude + soil	Cross-validation	MTP	4	H
11	**selected* + altitude + soil**	**Cross-validation**	**MTP**	**2**	**H**
12	selected* + altitude + soil	Cross-validation	MTP	4	H

For the following models we modified Maxent settings for small samples (Pearson *et al.* 2007; Shcheglovitova and Anderson 2013; Radosavljevic and Anderson 2014). Because both training and test datasets become very small using a subsample, we built models implementing a Jackknife cross-validation (or 'leave-one-out') procedure, described by Pearson *et al.* (2007). We built eight models using all climatic variables and altitude (Table 3.1; models 2–9), where we applied different threshold rules and a different regularization multiplier (Shcheglovitova and Anderson 2013; Radosavljevic and Anderson 2014). Thresholds in our models varied between the 10 percentile training presence (T10) and the minimum training presence (MTP), and regularization multiplier varied from 2 to 4 (default model was 1). We implemented the default model with automatic features, but for the following models features settings could be either a combination of linear, quadratic and/or hinge (LQH) or hinge only (H). We then compared the prediction and standard deviation of these models to select the best settings.

Finally, we ran three additional models where we applied a cross-validation (leave-one-out procedure) with threshold of MTP and hinge feature, varying the regularization multiplier between 2 and 4 (Table 3.1, models 10–12). For these models, we included an additional layer of soil type: while model 10 contained all climatic variables, models 11 and 12 contained only the climatic variables that contributed the most for the first and second axis of principal components and that were not correlated according to the correlation matrix output from the PCA (values < 0.5).

3.3.4 Model testing and validation

The area under the receive-operator curve (AUC) is usually used to evaluate models, however, AUC values should be used with caution for presence only methods (Merow, Smith and Silander 2013). Therefore, to test the predictive performance of our models we used a Jackknife approach described by Pearson *et al.* (2007), which performs well and is approximately correct with small sample sizes. The test compares success-failure for each model to the proportion of the study area predicted present (i.e., performance is assessed based on the ability of each model to predict the single locality excluded from the training data in the leave-one-out procedure, Pearson *et al.* 2007). A P-value was calculated based on the predictions of our models (R script available at http://www.ucl.ac.uk/cber/pearson).

Finally, to validate our models we visited four areas indicated in our predictions which were chosen based on a combination of high suitability for bromeliad occurrence and low standard deviations (≤ 0.05) predicted in the models. We also used expert knowledge to select areas to be visited for model validation. We visited the four high-suitability areas during the day to verify the presence of bromeliads, each area was extensively surveyed for bromeliads during 2–4 consecutive days. Because *C. itambe* is active at night (Barata, Griffiths and Ferreira 2018), if bromeliads were indeed available within an area, this location was resurveyed after dusk to search for *Crossodactylodes itambe*.

3.4 Results

3.4.1 Model performance and explanatory variables

Based on 21 occurrence records of bromeliads, we ran 12 Maxent models that identified areas of high suitability and that showed high consistency in the predictions across the different models (Figure S3.1). Models using a cross-validation ('leave-one-out' procedure: models 2–12; Table 3.1) performed better than the default model based on a subsample (model 1; Table 3.1), and reduced overfitting (i.e., improved the ability of a model to generalize predictions). Models built with different threshold rules (T10 or MTP) provided similar results and same predicted area (Figure S3.1). As we expected, high values of regularization multiplier (in our case, maximum parameter value was set to 4), avoided model overfitting and the same was observed when using the hinge (H) feature (Table 3.1, Figure S3.1). We found that predictions in the north-eastern part of the study area were overall consistent, and the most noticeable variation in the predictions of suitable habitat by our models was in the south of our study area (Figure S3.1).

Here we present and discuss three models with slightly different results, highlighting differences in predicted area when using different settings and choosing specific climatic variables (Figure 3.2). Model 1 was clearly overfitted to the data, since predictions of suitability fell within the original few occurrence records used to build the model. Model 9 used a selection of settings which we considered best, with a cross-validation ('leave-one-out' procedure), MTP threshold, regularization multiplier of 4 and hinge feature. Model 11 was also built with cross-validation (‘leave-one-out’ procedure), MTP and hinge feature, but we used a lower regularization multiplier (= 2) and included only selected climatic variables after the PCA (temperature seasonality and annual precipitation), and topographic layers of altitude and soil type.

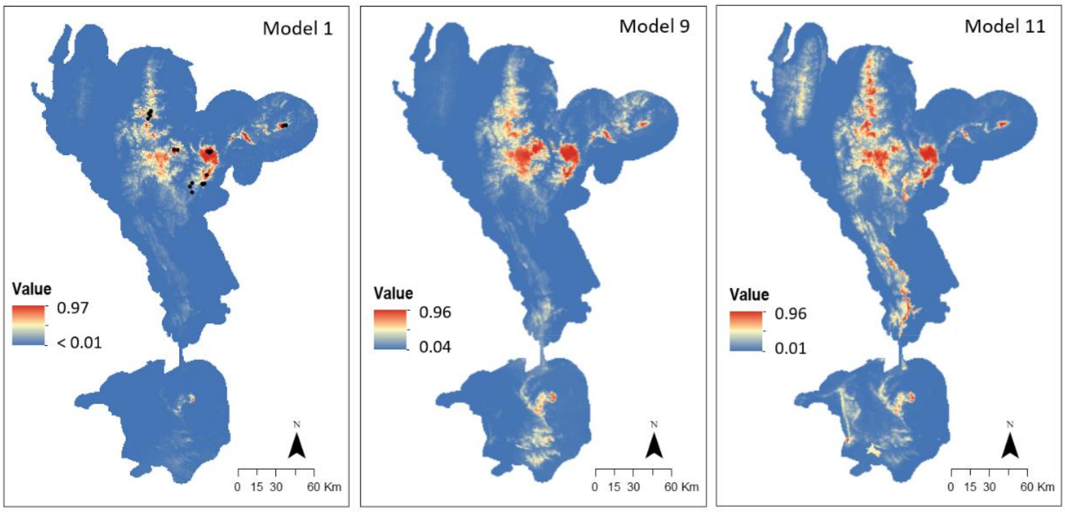

Figure 3.2 Habitat suitability for the occurrence of *Vriesea* sp. in the study area, with outputs from different model settings

Models were built under a combination of different settings and variables (see Table 3.1 for details). Black dots in model 1 are highlighting occurrence records used to build all models. Value represents habitat suitability (on a scale from 1 to 0), with warm colours indicating high suitability.

According to the PCA, the variables contributing to the first and second components were ‘temperature seasonality’ and ‘annual precipitation’, explaining 97% of variation (Table 3.2, Figure S3.2). These climatic variables were the ones selected to build model 11, which gave us broader predictions when compared to models that used all climatic variables (Figure 3.2).

Table 3.2 Results of a Principal Component Analysis of climatic variables
Variance is explained by Eigenvalues, percentage of Eigenvalues and accumulative Eigenvalues. In bold are variables associated with the first and second principal components (PC1 and PC2), selected to build models 11 and 12.

	PCA loadings		
	PC1	PC2	PC3
Variables			
Annual Mean Temperature	-0.05	0.05	0.24
Mean Temperature Diurnal Range	-0.01	0.02	0.09
Isothermality	-0.01	0.00	0.02
Temperature Seasonality	0.22	**0.97**	-0.03
Max Temperature of Warmest Month	-0.05	0.05	0.27
Min Temperature of Coldest Month	-0.05	0.02	0.18
Temperature Annual Range	0.00	0.04	0.09
Mean Temperature of Wettest Quarter	-0.05	0.06	0.23
Mean Temperature of Driest Quarter	-0.05	0.03	0.24
Mean Temperature of Warmest Quarter	-0.04	0.06	0.23
Mean Temperature of Coldest Quarter	-0.05	0.04	0.24
Annual Precipitation	**0.81**	-0.17	-0.02
Precipitation of Wettest Month	0.16	-0.06	0.53
Precipitation of Driest Month	0.01	0.01	-0.07
Precipitation Seasonality	-0.01	0.00	0.07
Precipitation of Wettest Quarter	0.38	-0.09	0.37
Precipitation of Driest Quarter	0.04	0.02	-0.19
Precipitation of Warmest Quarter	0.33	-0.05	-0.34
Precipitation of Coldest Quarter	0.04	-0.01	-0.08
Variance explained			
Eigenvalue	14177.70	5500.46	338.17
Percent of Eigenvalues	70.08	27.19	1.67
Accumulative of Eigenvalues	70.08	97.27	98.94

While temperature seasonality represents change over the year and had a negative influence in our predictions, annual precipitation is the sum of all monthly rainfall and can be used to interpret the importance of water availability to a species distribution – in our case it was positive for the bromeliad. Nonetheless, the selected climatic variables (temperature seasonality and annual precipitation) contributed to less than 20% of our predictions, which were mostly influenced by altitude and soil type. Overall, suitable areas can be described as high elevational sites with appropriate soil type, with reduced seasonal variation in temperature and high levels of rainfall.

3.4.2 Ground-truthing model predictions

To select the areas to be visited we not only used the predictions indicated by our models, but we also considered the predictive performance of selected models (given by the Jackknife approach), standard deviation (SD) of predictions, and expert knowledge. Results from Jackknife showed a high and significant success rates of both model 9 ($r = 0.95$, $P < 0.05$) and model 11 ($r = 0.9$, $P < 0.05$). The SD of models containing all climatic variables was lower (especially model 9), with high SD restricted to few areas when compared to the model including the two selected climatic variables (Figure S3.3).

Overall, predictions indicated high suitability of similar areas, but low SD were associated with areas to the east, especially when compared to areas to the south where predictions were less certain (Figure 3.3). Areas to the east not only had a high predicted suitability with lower SD, but are also under influence of the Atlantic Rainforest, which is the same biome where original population of *C. itambe* is currently recorded. Therefore, we opted to survey areas within this biome and located to the north-east of our study area.

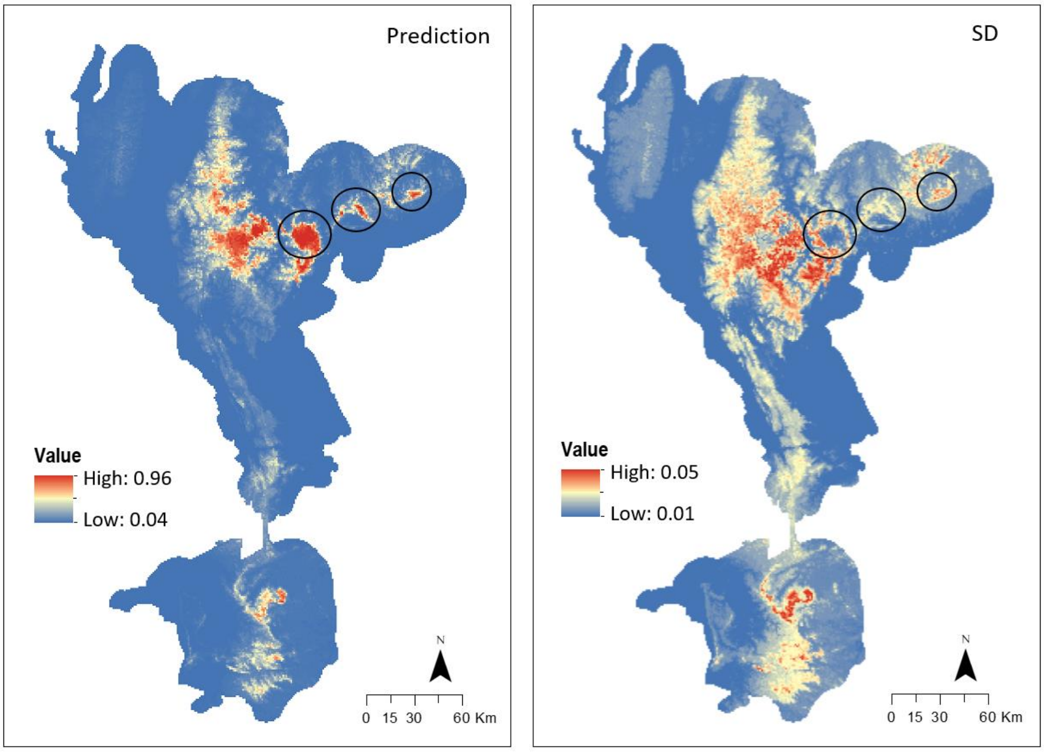

Figure 3.3 Suitable areas predicted for bromeliad occurrence in the study area
Suitable areas for the occurrence of *Vriesea* sp. and associated standard deviations of model 9. Solid circles are indicating areas that presented both high suitability (Prediction) and low standard deviations (SD).

We visited four areas with high suitability to validate the distribution of bromeliads (two inside and two outside protected areas). The areas differed in the total number of bromeliads available and elevational range, varying from 1029 m to 1592 m a.s.l., with bromeliads with larger tanks occurring inside protected areas (= 77.6 cm^2 inside protected areas; and = 37.7 cm^2 outside protected areas). We recorded a total of 684 bromeliads in the four areas, which were also visited during the night to survey for *C. itambe*. Although every recorded bromeliad was surveyed during the night, we only found frogs in one of the four predicted areas: a forested patch inside a protected area (Serra Negra state park; Figure 3.4), about 60 km from the original record of *C. itambe* at the Itambé summit.

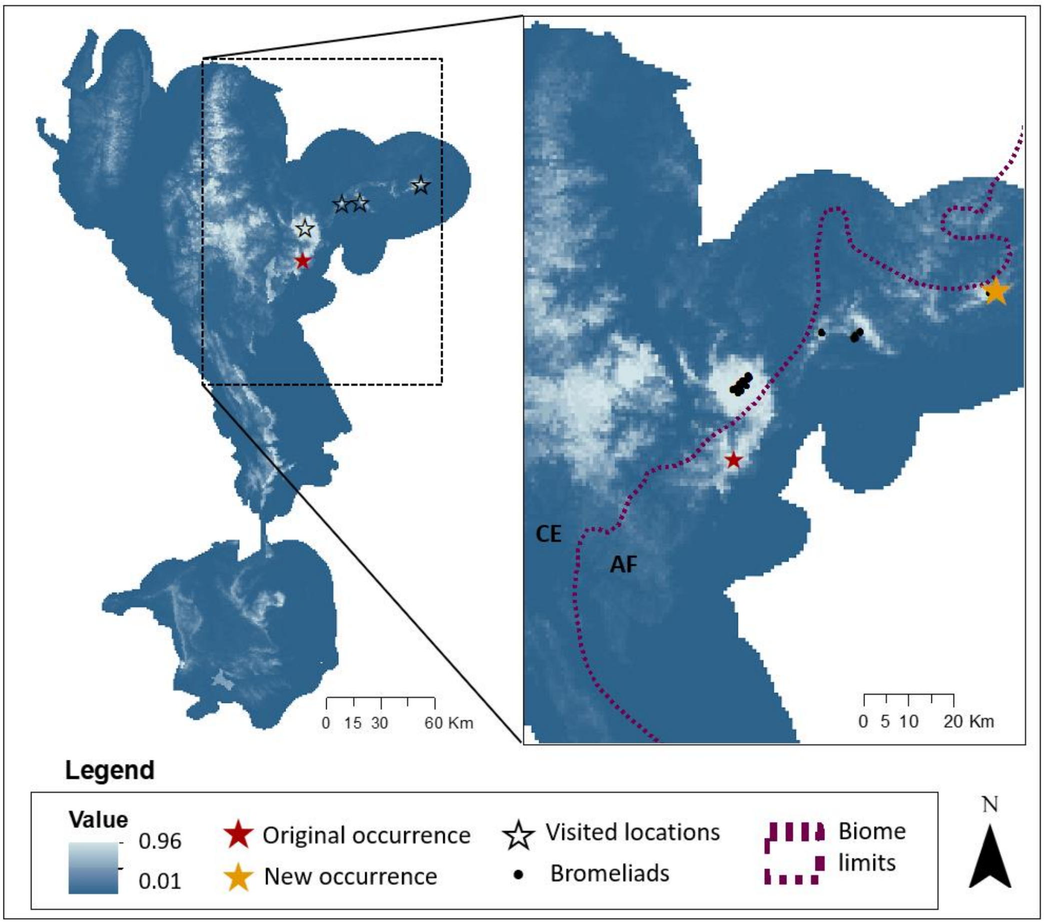

Figure 3.4 High suitability areas indicated by the species distribution model and visited locations within the Espinhaço Mosaic
Areas of high suitability are based on model 11, showing the original occurrence of *Crossodactylodes itambe* and four visited locations. Detailed map shows the limits of the Cerrado (CE) and the Atlantic Rainforest (AF) with bromeliads recorded ($n = 684$) and the occurrence of the new *Crossodactylodes* species.

Serra Negra (SN) is one of the highest elevations recorded among all areas we visited (1519–1620 m a.s.l.) and falls completely within the domains of the Atlantic Rainforest, to the east in our study area (Figure 3.4). Further examination of sampled individuals in the lab revealed that our new record was not a new population of *C. itambe*, but, is in fact a new species of the genus *Crossodactylodes*.

3.5 Discussion

Using bromeliads as surrogates for the occurrence of bromeligenous frogs we were able to successfully predict areas of high suitability for host plants and find a new population of a *Crossodactylodes* species. Although SDMs have been used to identify priorities for amphibians (Urbina-Cardona and Flores-Vilela 2010; Chunco *et al.* 2013; Fong, Dávila and López-Iborra 2015), data availability will be challenging for species only known to occur in a single location, like most bromeligenous frogs. Bromeliad occurrence will often provide a larger dataset and in our case resulted in models that performed well and predicted similar areas. To gather bromeliad records we covered an extensive area in only six days of survey; on the other hand, frogs are active during the night (Barata, Griffiths and Ferreira 2018) and require at least four visits to be precisely detected by unexperienced observers (Chapter 4). In our case, because night surveys require greater logistical and financial support, obtaining presence data for bromeliads was less time consuming and more cost effective – allowing predictions to be made for this poorly known amphibian.

Maxent was an effective tool in predicting new occurrences with few records (Chunco *et al.* 2013; Sarkinen, Gonzáles and Knapp 2013; McCune 2016). Although our aim was not to test model performance, overall, the software customized settings avoided overfitting and decreased SD. The recommended cross-validation using a Jackknife (Pearson *et al.* 2007) and MTP threshold (Shcheglovitova and Anderson 2013; Radosavljevic and Anderson 2014) proved to be useful for compensating for the scant data. Although hinge feature is more complex than default values (Merow, Smith and Silander 2013), together with a high regularization, it may prevent models from overfitting when using a small occurrence dataset (Shcheglovitova and Anderson 2013). To predict areas of suitability for host plants of bromeligenous frogs, we would recommend these settings and the use of Maxent.

For model validation, expert knowledge was an important factor to decide which areas were to be surveyed. *Vriesea* species exhibit a coastal distribution within the Atlantic Rainforest (Versieux and Wendt 2006) and *Crossodactylodes* species are usually recorded in the same biome. If new populations (or even new species) of *Crossodactylodes* are to be found in the Espinhaço Range, it is more likely that discoveries will fall within the domains of this biome, and possibly inside protected areas, considering the high rate of deforestation recorded in the Atlantic Rainforest (Ribeiro *et al.* 2009).

Although we recorded bromeliads in every area we visited, they varied in number, quality and size. For *C. itambe*, structure of the bromeliad is an important factor determining species abundance (Chapter 2) and has an important effect on the colonization rates of *C. itambe,* increasing inside larger plants and with higher density (Chapter 6). Outside protected areas the density of plants was lower, size of bromeliad was reduced, and the structure of plants was damaged, either by fire and/or by cattle trampling. Because habitat was reduced in quality or availability, populations occurring outside protected areas might have been reduced or have even gone extinct in the past. It is impossible to determine if *C. itambe* (or other species in the genus) was once widespread throughout our study area or even able to use other species of bromeliads. Fortunately, *Crossodactylodes itambe* and the new species occur in protected areas and potential threats are likely to be reduced. However, stochastic events, such as fire, might still occur and managing this risk should be a priority. Further active management actions could also benefit the species, such as controlling tourism, limiting access to plants and avoiding bromeliad collection.

Within our study area, we recorded one of the highest altitudes at SN state park (where the new species of *Crossodactylodes* was found). *Crossodactylodes* species are restricted to montane habitats in altitudes between 650 m and 2062 m a.s.l. (Peixoto 1982; Barata *et al.* 2013) and endemism of *Vriesea* sp. is particularly high in upland habitats (Versieux and Wendt 2006). Sabagh, Ferreira and Rocha (2017) called researchers' attention to report the bromeliad species used by bromeligenous frogs, which is still unknown for most species. *Crossodactylodes itambe* seems to be restricted to a single species of bromeliad, which might also be true for other *Crossodactylodes* species and many more bromeligenous frogs. Our data give further support that *C. itambe* is indeed restricted to high elevations, possibly occupying bromeliads that tolerate environments with low temperature variation and higher water availability. Soil type may be an important feature for bromeliads to persist (Versieux and Wendt 2006) and, together with altitude this had a positive relationship with predicted area, contributing to more than 80% of bromeliad prediction. These variables should therefore be considered when modelling areas for new populations of mountaintop restricted bromeligenous frogs.

Range-restricted species might be naturally rare or can be a result of deficiencies in survey effort, but distinguishing between these two can be difficult. In Brazil, access to roads leads to a strong spatial bias in data collection (Oliveira *et al.* 2016) and, within our study area, amphibian survey effort is unequally distributed (Barata, Correia and Ferreira 2016). Although we focused our surveys in areas of high predictability, we only found frogs in one of the four areas searched, indicating that our target species is naturally rare and demonstrating that montane regions still need a sampling effort that covers a wider geographical range to uncover their full biodiversity. Although *C. itambe* may suffer from insufficient geographical survey effort, it is likely that the genus as whole has a genuinely restricted distribution, including the newly discovered *Crossodactylodes* species.

Recently described species are range-restricted (Pimm and Jenkins 2010; Mora *et al.* 2011; Roberts, Taylor and Joppa 2016) and usually occur in a few locations (Costello, May and Stork 2013); they are mostly concentrated in the tropics (Giam *et al.* 2012) and are likely to be threatened (Giam *et al.* 2012; Pimm *et al.* 2014; Roberts, Taylor and Joppa 2016). The five *Crossodactylodes* species are known from single locations, but at least two near threatened species (*C. bokermanni* and *C. izecksohni*) are expected to occur more widely (Silvano and Peixoto 2004a; Silvano and Peixoto 2004b). Further intensive surveys should be conducted to define species extent of occurrence, habitat restrictions and patterns of rarity, and we demonstrate that using bromeliads as surrogates to predict frog occurrence is a feasible and cost-effective approach to exploring distribution patterns of bromeligenous frogs.

Amphibians are being described at an extraordinary rate (Wake and Vredenburg 2008) and a large number of undescribed species have been estimated for Brazil (Scheffers *et al.* 2012) – including in our study area, where 31% of amphibians are predicted as remaining to be described (Giam *et al.* 2012). In 2010 an increase of 15% in amphibian species richness was predicted for the country (Pimm *et al.* 2010) – an estimate that has already been exceeded (Segalla *et al.* 2016). New species are likely to be small bodied, less colourful, with elusive life histories and cryptic behaviour (Scheffers *et al.* 2012), which is the case of most bromeligenous frogs. For *Crossodactylodes* species, in particular, there is a large gap in species discoveries: the genus was first described in 1938 (Cochran 1938), but it took 40 years for new species to be added (Peixoto 1982) including two very recent discoveries (Barata *et al.* 2013; Teixeira *et al.* 2013). As previously suggested, inventories in remote areas might result in the discovery of new species (Leite, Eterovick and Juncá 2008), such as the remarkable finding of seven new micro-endemic mountaintop amphibians in the Atlantic Rainforest (Ribeiro *et al.* 2015).

Finally, our modelling approach successfully predicted the occurrence of a habitat specialist amphibian and could be broadly extended for other bromeligenous frogs in the Neotropics, especially in Brazil, where 50% of bromeligenous frogs are known to occur (Sabagh, Ferreira and Rocha 2017). This could include other *Crossodactylodes* species only known from one location. Although we have not found a new population of *C. itambe*, we are now more certain about the species geographical range, patterns of rarity and need of protection. Considering that severe declines have been reported for tropical montane amphibians (Young *et al.* 2001; Lips, Reeve and Witters 2003; La Marca *et al.* 2005; Lips *et al.* 2005), understanding the geographical extent of bromeligenous frogs (where they are and what make them restricted) is extremely important. At least 15 threatened and DD bromeligenous frogs are presumed to occur more widely, requiring further investigation on their extent of occurrence (IUCN 2018). Detailed studies on individual species might change our knowledge on the geographical extent of bromeligenous frogs, however, it is likely that these species will have small distributional ranges.

3.6 Acknowledgements

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3.7 Supplementary information

Figure S3.1 Model parametrization with different settings

Details of model settings and variables are given in Table 3.1: default model (1); cross-validation with all climatic variables and altitude with T10 (2–5) and MTP (6–9); cross-validation with all climatic variables, altitude and soil type combined with best settings configuration (10), and cross-validation with selected climatic variables, altitude and soil type with best settings configuration (11–12). Warmer colours indicate high suitability: red maximum values, and blue lower values (or low suitability).

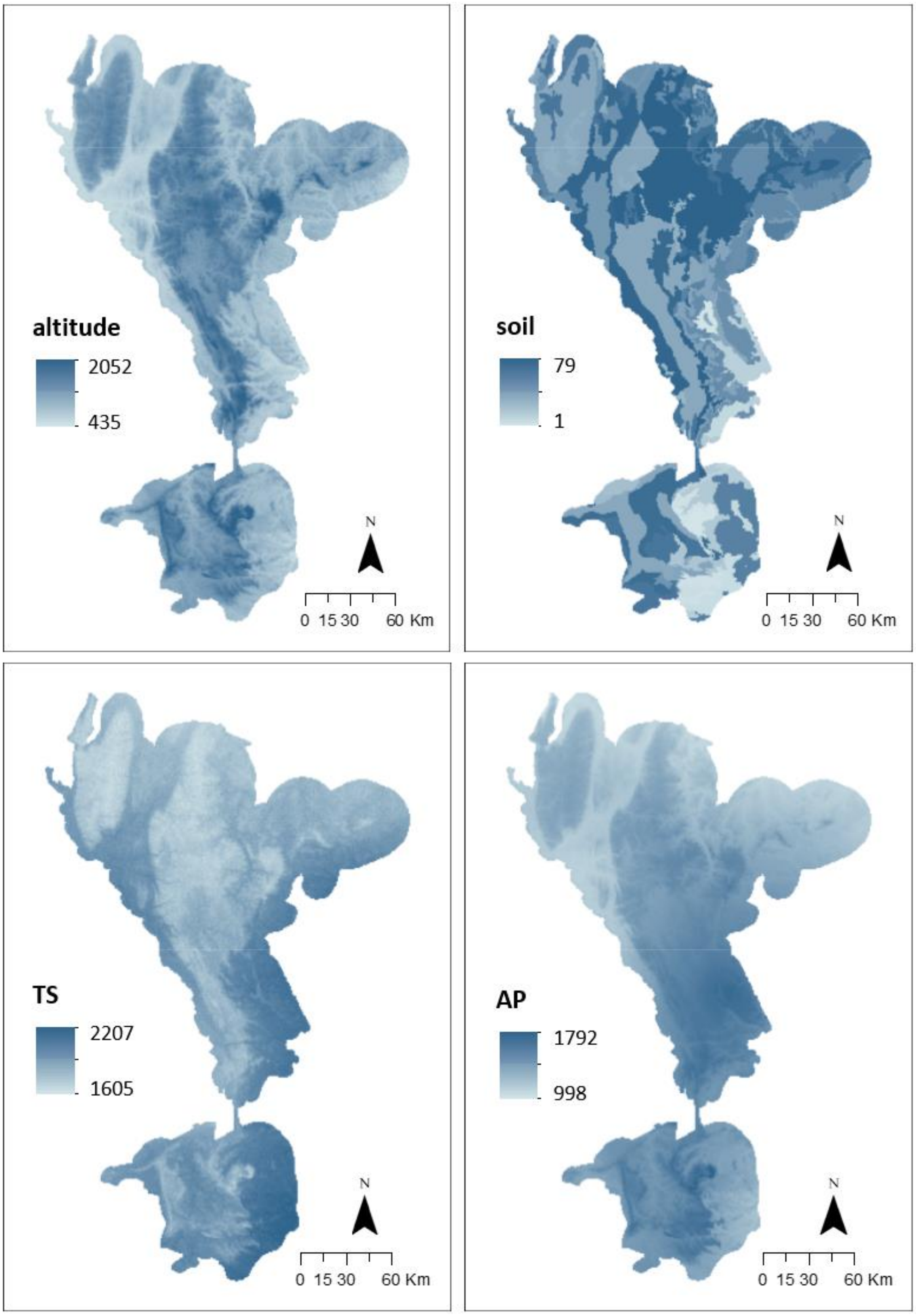

Figure S3.2 Environmental layers and bioclimatic variables used for predictions in models 11 and 12

Topographic layers of altitude and soil type, and climatic variables selected after the PCA (TS = temperature seasonality; AP = annual precipitation).

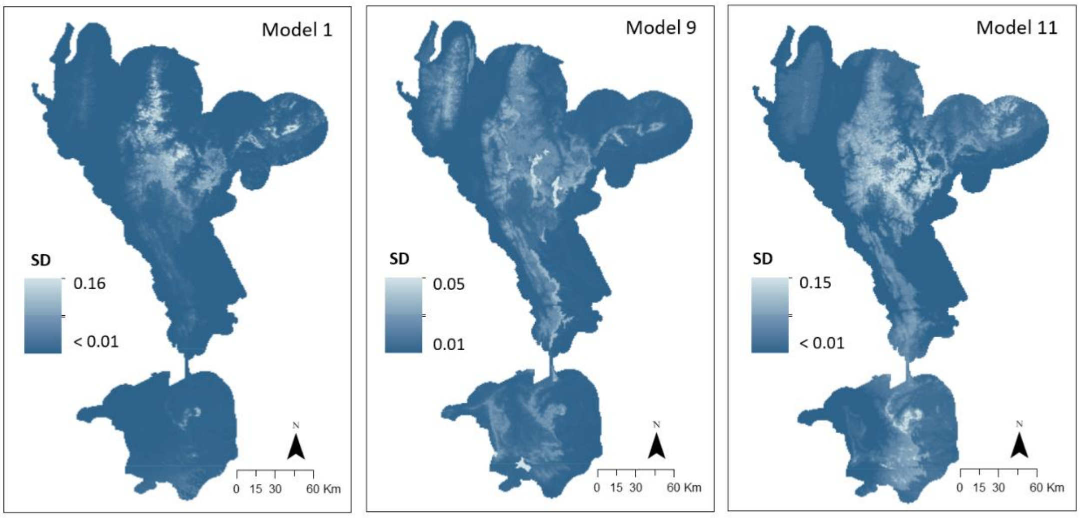

Figure S3.3 Standard deviations of three different models

Standard deviations (SD) of three habitat suitability models: model 1 (default model), model 9 (including all climatic variables) and model 11 (including only climatic variables selected after PCA).

Chapter 4 Optimizing survey designs to detect occupancy changes in a rare amphibian population

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4.1 Abstract

Biodiversity conservation requires reliable species assessments and rigorously designed surveys. However, determining the survey effort required to reliably detect population changes can be challenging for rare, cryptic and elusive species. We used a tropical bromeliad dwelling frog as a model system to explore a cost-effective sampling design that optimizes the chances to detect a population decline. Few visits were sufficient to estimate occupancy and detectability with good precision, and to detect a 30% change in occupancy with 80% power. Detectability was influenced by observer expertise, which also had an effect on the sampling design – less experienced observers require a larger number of sampling occasions to detect target species. Even when the sampling design provides precise estimates, only moderate to large changes in occupancy will be detected with reliable power. Detecting a population change of 15% or less requires a large number of sites to be surveyed, which might be unachievable for range-restricted species occurring in relatively few sites. Unless there is high initial occupancy, rare and cryptic species will be particularly challenging when it comes to detecting small population changes. This may be a particular issue for long-term monitoring of amphibians which often display low detectability and wide natural fluctuations.

Keywords: Cost-efficient designs, Detection probability, Population decline, Power analysis, Species assessment, Survey effort.

4.2 Introduction

The global biodiversity crisis has driven the development of increasingly sophisticated databases, such as the Living Planet Index (Collen *et al.* 2009) and the IUCN Red List for Threatened Species (IUCN 2018), which require reliable baseline information on species, habitats and population trends. Although monitoring data is of increasing value to conservation managers, population and status assessments are currently limited by the lack of data (Proença *et al.* 2017), resulting in poor evidence for conservation practitioners. Monitoring programmes must inform decision-making through the application of reliable survey design and statistical analysis – otherwise it will be an ineffective use of resources. Conservationists must therefore develop projects with clear objectives (Legg and Nagy 2006) and provide appropriate sampling designs (Field, Tyre and Possingham 2005; Kéry and Schmidt 2008) with sufficient statistical power to reliably describe population trends (Field *et al.* 2007; Meyer *et al.* 2010; Loos *et al.* 2014). Nonetheless, issues of sampling design are widely ignored and still a challenge for species monitoring and modelling (Bailey, MacKenzie and Nichols 2014).

Occupancy modelling is increasingly being applied in monitoring programmes to assess the determinants of population changes for different taxonomic groups (Adams *et al.* 2013; Ahumada, Hurtado and Lizcano 2013). The model estimates site occupancy and detection probabilities in an unbiased way (MacKenzie *et al.* 2002; MacKenzie *et al.* 2003) and may also be used as a proxy for abundance (Kéry and Schmidt 2008). Although sampling designs for occupancy models have been explored theoretically (MacKenzie and Royle 2005; Bailey *et al.* 2007; Guillera-Arroita, Ridout and Morgan 2010; Guillera-Arroita and Lahoz-Monfort 2012), few studies have used empirical data to investigate the survey effort required for the reliable inference of absence (Pellet and Schmidt 2005; Sewell, Beebee and Griffiths 2010; McGrath *et al.* 2015) or to explore the precision and accuracy of occupancy estimates (Courtois *et al.* 2013; Moore *et al.* 2014; Shannon, Lewis and Gerber 2014). In the context of occupancy monitoring, studies have also considered statistical power using empirical data (Meyer *et al.* 2010; Otto and Roloff 2011a; Otto and Roloff 2011b; Thorn *et al.* 2011; Sewell *et al.* 2012; Shannon, Lewis and Gerber 2014; Steenweg *et al.* 2016). Statistical power considers the number of samples, variability in the data and the expected rate of change (Gerrodette 1987) to evaluate the probability of detecting a change in the estimated parameter when that change actually occurs (e.g., increase or decrease in occupancy). Power analysis has long been recognized as a useful tool for study design, especially when dealing with early stages of monitoring planning (Di Stefano 2001; Legg and Nagy 2006; Field *et al.* 2007; Guillera-Arroita and Lahoz-Monfort 2012).

Evaluating changes in populations at risk is particularly important in the case of amphibians, which are currently more threatened than birds or mammals and show accelerating rates of extinction (Ceballos *et al.* 2015). However, amphibians are often rare, cryptic or elusive and can display considerable natural population fluctuations (Green 2003), which can make long-term monitoring difficult. Significant advances in amphibian monitoring have been developed, such as the improvement of novel research methods (e.g., environmental DNA (Biggs *et al.* 2015)), application of advanced data analysis (e.g., occupancy models (Adams *et al.* 2013)) and evaluation of national monitoring schemes (e.g., National Amphibian and Reptile Scheme (Sewell, Beebee and Griffiths 2010)). Nonetheless, these developments are often limited by the availability of funding, which contributes further to difficulties in assessing population changes.

In this study we used a tropical bromeliad frog, a rare and threatened amphibian species, as a model system to assess sampling design and the statistical power associated with detecting population changes. The endemic *Crossodactylodes itambe* (Barata *et al.* 2013; Santos *et al.* 2017) is only found at the Itambé summit, south-eastern Brazil, living exclusively inside bromeliads on a high elevation rocky outcrop and with an extent of occurrence of less than ca. 0.5 km^2. *Crossodactylodes itambe* is included in the Brazilian Amphibian Conservation Action Plan (RAN/ICMBio 2012), which suggests the implementation of a long-term monitoring programme for threatened species that are also rare and elusive. Our aim was to design a monitoring protocol that improves the chance of detecting a population change, which could also allow better allocation of survey effort and financial resources. We therefore addressed three questions fundamental to any monitoring programme: (1) Is the currently used sampling design providing precise estimates of occupancy and detectability? (2) Is this sampling design providing sufficient power to detect changes in occupancy over time? (3) How can we improve statistical power to detect small changes in populations? The frog-bromeliad system therefore provides an opportunity to explore issues of sampling and statistical power that would prove unwieldy on a larger landscape scale and we present a rigorous assessment that could benefit future monitoring programmes in their earlier stages.

4.3 Materials and methods

4.3.1 Study system and sampling design

The Itambé summit is the highest point of the Espinhaço Mountain Range at 2062 m above sea level (a.s.l.) and is located in south-eastern Brazil, in Minas Gerais state. The area is characterized by open field habitats with vegetation growing in humid rocky outcrops. *Crossodactylodes itambe* is restricted to 1700 m a.s.l. and occupies a single species of bromeliad (*Vriesea medusa*), where it spend its entire life cycle (Barata *et al.* 2013). Individuals have never been observed outside bromeliads and are mostly inactive inside the plant (Barata, Griffiths and Ferreira 2018). Although a territorial behaviour may occur (Santos *et al.* 2017), dispersal may be confined to rain storms when it is difficult to make observations. Considering field observations, life history of the genus and the small size of individuals (Barata *et al.* 2013; Santos *et al.* 2017) we believe that species dispersal capability is low and we therefore considered individual bromeliads as independent sampling sites.

To ensure independence within and between survey periods, sampled bromeliads were at least 25 m apart. We divided the study area into three altitudinal zones: low (1704–1815 m a.s.l.), medium (1838–1925 m a.s.l.) and high (1998–2062 m a.s.l.); which was delimited by the topography of the area and the species current distribution. Within these zones, we randomly tagged individual bromeliads using numbered labels that allowed repeated visits. In 2014 we tagged 123 bromeliads, and we added 20 new bromeliads in the following year. In 2015, the 143 sampling sites were equally distributed among the altitudinal zones (47 bromeliads at high elevation, 48 at the medium and low zones).

In February 2014 we surveyed our sites on four sampling occasions (i.e., four consecutive nights). We considered this year as a pilot study to test the feasibility of our sampling design. The following year, we increased the number of sampling occasions (4–6 consecutive nights) and repeated this survey effort monthly from February to May, representing wet and dry seasons. Monthly surveys were separated by 15–25 days. We surveyed frogs using visual encounter surveys, developed by two teams of two observers each, starting after dusk. To standardize our surveys, only one person of each team was allowed to record species presence/absence, and both received training in observing the target species. We recorded species presence and absence, and we used adults, juveniles and tadpoles as evidence of species presence at a site.

We considered repeated nights as independent sampling occasions. For each month, within a short survey window, we assumed individuals did not leave the site between sampling occasions and we targeted bromeliads with the numbered labels. However, some tagged plants had neighbouring bromeliads touching their edges (forming a patch of several conspicuous bromeliads) and, because of the time elapsed between each month of survey, we considered that frogs may move to a neighbouring bromeliad during this time. Therefore, we also searched for frogs inside the neighbouring bromeliads. For every sampling occasion, we first surveyed the tagged site, making notes of species presence/absence, and we then searched neighbouring bromeliads (all neighbours were searched, irrespective of presence/absence in the tagged site).

4.3.2 Modelling species occupancy

Occupancy modelling is based on the patterns of detection and non-detection and estimates both site occupancy (i.e., the probability of a randomly selected site being occupied by a species) and detection probabilities, accounting for imperfect detection (MacKenzie *et al.* 2002). Some assumptions are required for the standard single season occupancy model (MacKenzie *et al.* 2002; MacKenzie *et al.* 2003). The model assumes that there are no false positives, but failure to detect the species indicates either that the site is truly unoccupied or that it is occupied but species was missed during the survey. Also, the detection of a species at a site is independent of detections of the species at all other sites. Finally, each site is either occupied through the entire season or unoccupied throughout. To avoid violating these assumptions we used detection histories from tagged and/or neighbouring bromeliads, organizing the datasets using three different approaches: months, seasons and complete dataset (i.e., whole year of sampling). Therefore, seasons were based on the time elapsed between surveys (i.e., month, year or season).

For monthly datasets (one month in 2014; four months in 2015), we estimated occupancy and detection probabilities using the detection histories from tagged sites only (assuming individuals did not leave the site during sampling occasions). However, because of the time interval between months, we assumed individuals might have moved within the patch from one survey to the other. In this last scenario, for the 2015 dataset, we grouped detection histories from tagged and neighbouring bromeliads into a single dataset: (1) to estimate parameters for seasons, where we grouped two months of data (wet season: Feb–Mar; dry season: Apr–May); and (2) to compare estimated occupancy and detectability between years, grouping all four monthly surveys (Feb–May 2015). Therefore, to compare variation in occupancy and detectability between months, seasons and years, we fitted single season models which assumed a constant occupancy and detection probability across sites (hereafter, constant models). We also estimated parameters for each altitudinal zone to account for changes related to elevation. Because we aimed to explore aspects of sampling design (and also to simplify the analysis), we did not use the dynamic occupancy model, which could estimate colonization and extinction processes in the population (MacKenzie *et al.* 2003).

The occupancy model can accommodate covariates which may be either site or survey specific (MacKenzie *et al.* 2002; MacKenzie *et al.* 2003). We also developed models testing a priori hypotheses focusing on the drivers of occupancy and detectability (based on species traits and expert knowledge). Because the February 2014 dataset was considered a pilot study, we only tested models for the complete 2015 dataset. We incorporated covariates potentially related to sampling design. We used survey-specific covariates: time of observation (given by time after dusk);

observer (due to the experience of investigator, given as a categorical variable), and site-specific covariates: number of leaves in bromeliad; number of neighbours; size of bromeliad (given by: height x width); volume of rosette (given by: height x width); and elevation (meters a.s.l.). Correlated covariates were excluded from the model selection.

We used a stepwise model selection approach to build our model, where we combined covariates for both parameters. We first established models that included only covariates of detectability (in this case, occupancy was kept constant). We then selected the best models and incorporated covariates of occupancy, combining them with detectability covariates previously appointed by model selection. We used the Akaike's Information Criterion (AIC) to rank candidate models and to calculate Akaike weights (Burnham and Anderson 2002). Models were ranked by their AIC (model with the lowest values has the best fit) and weighted as the probability of being the best model in the set, indicating relative support of a model. We selected best models based on ΔAIC: models with ΔAIC < 2 had strong support while models with a ΔAIC of > 2 were considered to have less support (Burnham and Anderson 2002). Occupancy models and model selection were performed in R (R Core Team 2017), using the package Unmarked (Fiske and Chandler 2011).

4.3.3 Optimal survey design and statistical power

We used estimates of occupancy and detectability from constant models to explore the number of surveys required to detect the species in a given site and to compare statistical power of sampling designs from our pilot study and the following year. However, because models with covariates were fitted for the 2015 dataset, we used occupancy and detectability estimates from our best model to calculate improvements in power in relation to sampling design. We first evaluated the survey design used in our pilot study. We used estimated detection probabilities to predict the minimum number of occasions (K) required to determine that the species is truly absent from a site. We explored the values of K based on the expression (Pellet and Schmidt 2005; Sewell, Beebee and Griffiths 2010; McGrath *et al.* 2015):

$$\mathrm{K} = \log(1 - \mathrm{p}^*)/\log(1 - p),$$

where p denotes the detection probability, and p* is the probability of detecting a species at an occupied site least once (set to be 0.8, 0.9 and 0.95). We applied the suggested number of visits in our surveys in the following year and performed the same calculations for the 2015 monthly dataset to account for changes in the number of K required from one survey to the other.

We then used the predicted K to investigate the precision of our estimates as a criterion for sampling design. We used R functions available in Guillera-Arroita, Ridout and Morgan (2010) that simulate data for a given set of parameter values and sampling design to allow the quality of the estimators to be assessed under different combinations of survey effort. These functions can be applied to the single season single-species occupancy models with constant probabilities, and firstly, generate simulated histories, calculating the corresponding maximum-likelihood parameter estimates (MLE) of occupancy and detectability and evaluating the estimator performance. Secondly, the functions display the distribution of the MLEs obtained for the given design and values of occupancy and detectability (Guillera-Arroita, Ridout and Morgan 2010).

To evaluate the performance of our initial sampling design, using occupancy and detectability from our pilot study, we simulated single season occupancy models varying the number of sampling occasions (according to the previous calculations of K) and with a fixed number of sites from our pilot study. We also performed simulations with different survey efforts (i.e., a combination of varying number of occasions and sites) to explore an 'optimum survey design', which maximizes the quality of the estimators (improve precision of estimated parameters with only a few visits). We proceeded to evaluate the statistical power of our sampling design. We first compared statistical power between years (2014 and 2015 datasets). Using occupancy and detectability estimates, we calculated power as a function of the change in occupancy. Power is related to error types, the effect size, the sample size and the sample variance (Gerrodette 1987).

In this study we considered the change in occupancy as an effect size, which compares the state of occupancy in two different points in time and represents an increase or decrease from the initial state (for example, an effect size of 30% means that occupancy decreased from 70% to 40%). Statistical tests can give rise to two types of error: a Type I error occurs if a change is detected when in reality there is no change, and a Type II error arises when the test fails to detect a change that is present. The probability of a Type I error is denoted by α and the probability of a Type II error by β. A significance level conventionally chosen is 0.05 for α and 0.8 for β (known as the five-eighty convention; Di Stefano 2003). However, since power is given by G = 1 – β, levels of significance should reflect the relative seriousness of committing Type I and II errors (Di Stefano 2001; Guillera-Arroita and Lahoz-Monfort 2012). Considering our monitoring goal, we assumed that making a Type II error would be highly costly (i.e., not detecting a change in occupancy when there is one) and we therefore used higher levels of α (0.1).

For power analysis we used calculations with a Wald test in the probabilistic scale (Guillera-Arroita and Lahoz-Monfort 2012). We used estimated parameters from our best model. We explored power as a function of survey effort and observer experience, varying the number of occasions, sites and detectability under different effect sizes (from 0.15 to 0.3). To complete our analyses, we investigated statistical power varying the number of sites (50–300 sites), including our current sampling design (143 sites). For this analysis, we built a two-tailed power curve as a function of effect size, keeping occupancy, detectability and the number of occasions constant. Finally, to demonstrate the influence of significance levels in sampling design, we calculated the number of sites needed to achieve a given power (from 0.8 to 0.95) under different levels of significance (from 0.05 to 0.2). We also performed a Wald test (5000 iterations) to verify the actual performance under different numbers of sites. We used this approach to explore the power of sampling designs and evaluate the pros and cons of different survey efforts when suggesting a long-term monitoring protocol.

4.4 Results

Crossodactylodes itambe had a high detection probability, with little variation in relation to elevation, among months and between seasons (Figure 4.1; Table S4.1) – although Figure 4.1 does suggest lower detectability in February 2015. Overall, there was a 40–65% chance of detecting the species in a bromeliad if that site was occupied. Occupancy also did not vary between months or seasons (Figure 4.1; Table S4.1) but varied with elevation. While 66% of bromeliads were estimated to be occupied at high and medium elevation, only 14% were estimated to be occupied at the lowest altitude.

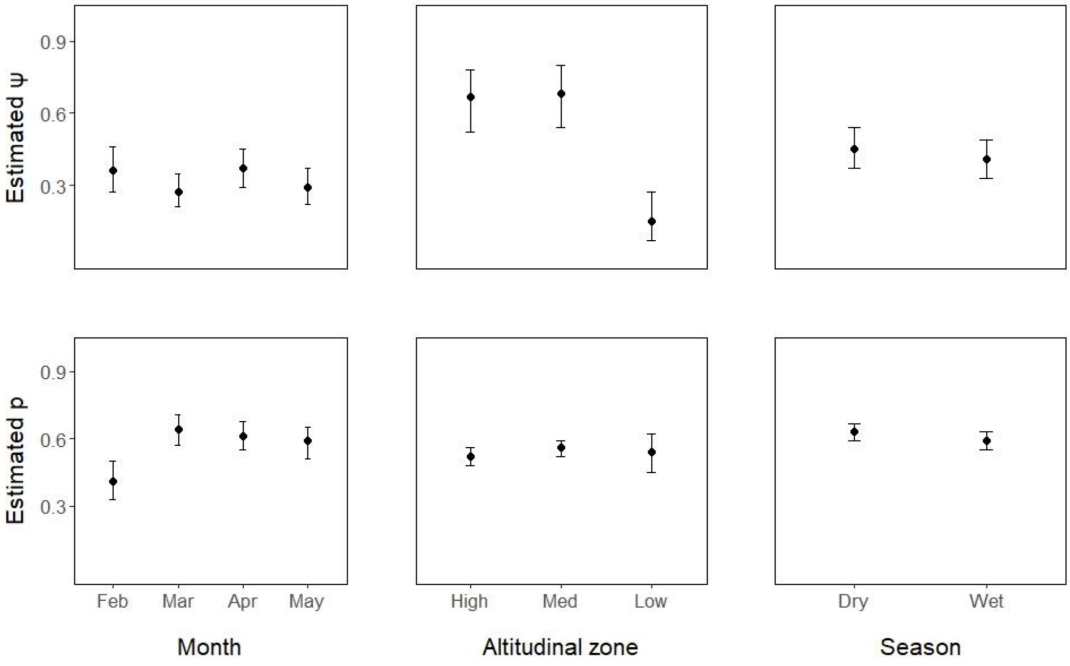

Figure 4.1 Estimated occupancy and detection probabilities for *Crossodactylodes itambe* varying with months, altitude and season

Occupancy (ψ) and detection (*p*) probabilities for 2015 monthly dataset using site only, varying with months (February to May), altitudinal zone (high, medium and low) and season (dry and wet). Vertical bars represent 95% confidence intervals.

Stepwise model selection indicated that observer experience was the best covariate explaining detectability (AIC weight 0.96). We tested six models combining observer experience with occupancy covariates (size of bromeliad and elevation), but also including a constant model (Table S4.2). The best-fitting model had elevation as an important covariate explaining occupancy (ΔAIC < 2, AIC weight 1). Detection probability was explained by observer experience and detectability varied significantly among observer (observer A: 0.61, 95% CI 0.57–0.65; observer B: 0.38, 95% CI 0.32–0.43). Other covariates had weak support and were unlikely to explain estimated parameters (Table S4.2).

The number of sampling occasions required to determine species presence at occupied sites varied according to the desired confidence level (Table S4.3). Based on our estimates of occupancy and detectability, the required number of visits for our 2014 pilot study varied from two to four. Simulations showed that three visits are enough to provide reliable estimates of occupancy and detectability (Figure 4.2). The required number of visits in the 2015 dataset varied from two to six (Table S4.3). Further simulations demonstrated that a reduced number of sites (n = 50) would require a high number of sampling occasions to improve precision (Figure S4.1). On the other hand, a large sample (with 150 sites) would require as few as two sampling occasions to produce reliable estimates. In any scenario, there is no improvement in precision after four sampling occasions (Figure S4.1).

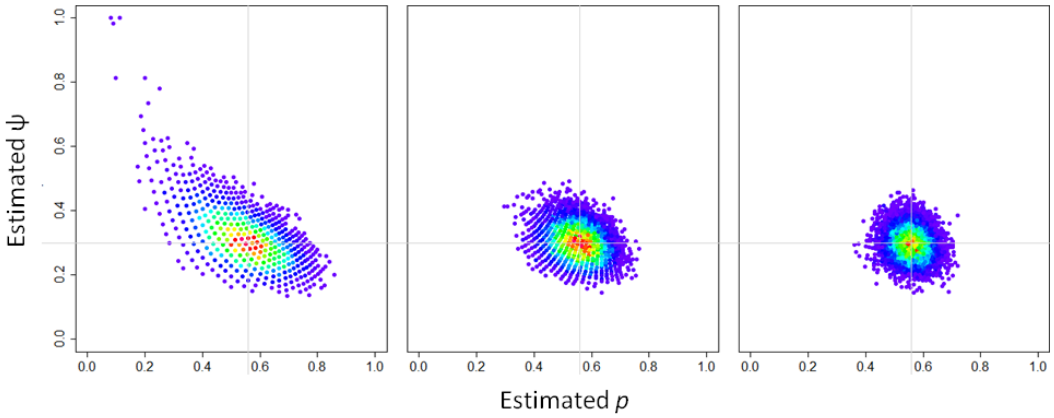

Figure 4.2 Distribution of the maximum likelihood estimates with varying number of visits
Distribution of MLE for 2014 dataset (ψ = 0.3, *p* = 0.56); number of visits vary according to predicted number of occasions and with a constant number of sites (S = 123). From left to right panels differ according to number of visits (K = 2, 3 and 4, respectively), based on previous calculations.

There was an increase in statistical power between years (Figure S4.2). While our pilot study (with 123 sites and 4 visits) had an 80% chance to detect a 50% change in occupancy, in 2015 our increased survey effort had the same chance to detect a 30% change in occupancy. Calculations varying the number of sampling sites, sampling occasions and detectability showed how the power to detect a smaller change in occupancy (from 15–30%) could be increased. There was constant statistical power after three visits and the power to detect a change did not increase with detectability over 0.5 (Figure 4.3). Nonetheless, we observed an increase in power by increasing the number of sites (Figure 4.3).

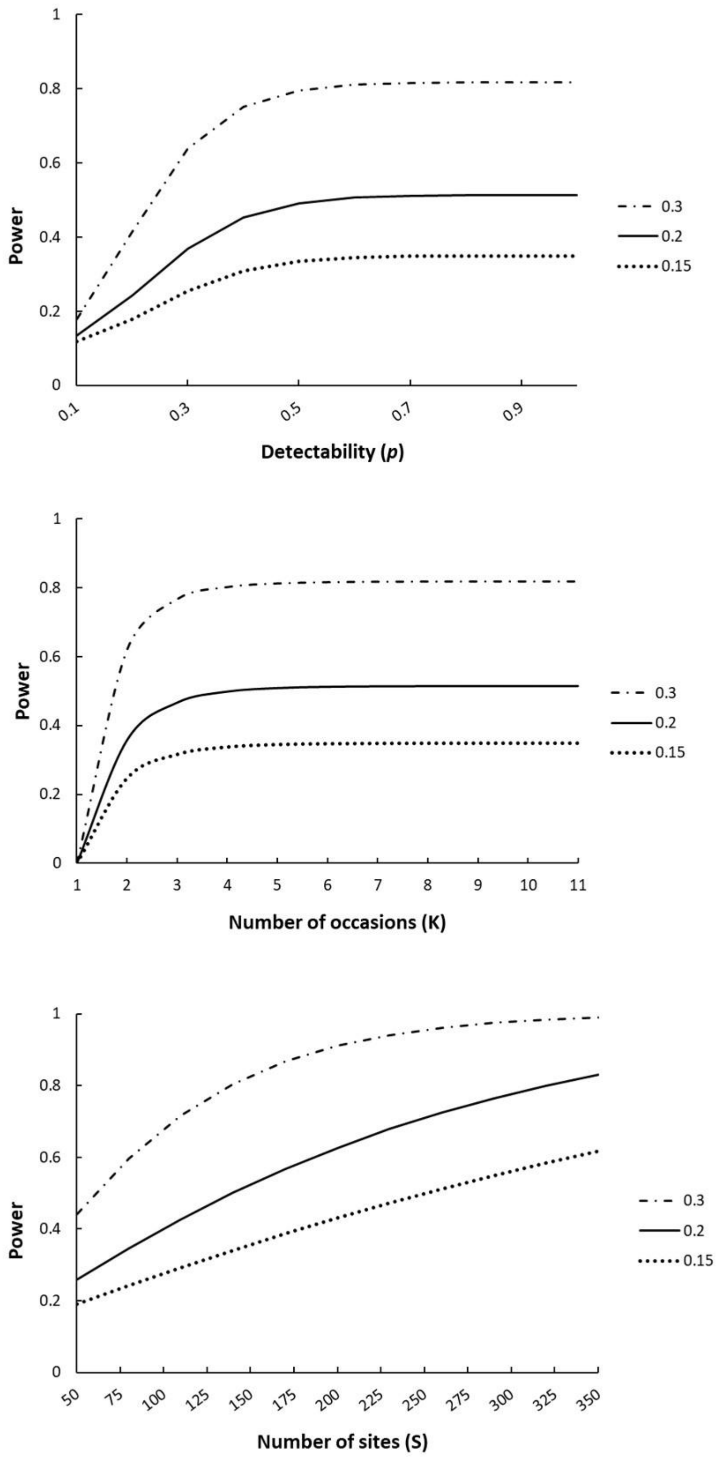

Figure 4.3 Detection, occasions and sites needed to increase statistical power under different effect sizes

Power to detect a change in occupancy under effect sizes of 0.3, 0.2 and 0.15, with respect to species detectability (*p*), number of sampling occasions (K) and number of sampling sites (S).

The sampling design currently used had 82% power to detect a change of 30% in occupancy; smaller changes had less statistical power, with 53% and 36% chances to detect changes of 20% and 15%, respectively. We found that doubling the number of sites would detect a 20% change in occupancy, with the same statistical power (0.8; Figure 4.4). However, the number of sites needed depended on the significance level and the effect size (Table S4.4). For example, to detect a 15% change in occupancy in *C. itambe* at a significance level of 0.1 would require 565 sampling sites.

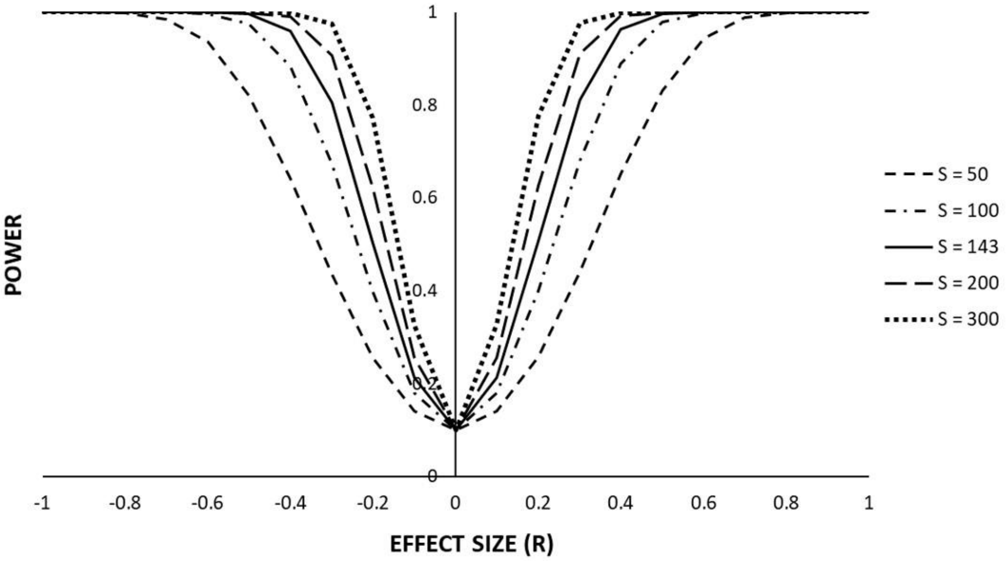

Figure 4.4 Statistical power of different sampling designs
Power is given as function of change in occupancy (effect size, R) under different sampling designs, based on estimates of best fitting model ψ(alt) *p*(obs) for 2015 dataset (ψ = 0.49; *p* = 0.61; *α* = 0.1). S = number of sites surveyed.

4.5 Discussion

Although cost-effective sampling designs can be based on simulated data (MacKenzie and Royle 2005; Guillera-Arroita, Ridout and Morgan 2010), many aspects require customization using real data and sampling needs (Bailey, MacKenzie and Nichols 2014). Sampling designs depend on detection probabilities (Wintle *et al.* 2004; MacKenzie and Royle 2005); in the case of amphibians, detection may change considerably in relation to season, such as an increase of frogs during rainy periods (Guimarães, Doherty Jr. and Munguía-Steyer 2014) or a decrease of salamanders over the summer (Otto and Roloff 2011b). We observed little variation in detectability between seasons or among months (except for February, which was slightly lower), which means *C. itambe* can be detected regardless of the sampling period. Potential seasonal variation should be accounted for when designing surveys, especially for species that are detectable only during short time-frames (e.g., seasonal breeding frogs). Surveys should therefore target periods when detectability is likely to be high (Sewell, Beebee and Griffiths 2010; Otto and Roloff 2011b), particularly when dealing with elusive species.

The detection probability of *C. itambe* was strongly influenced by observer expertise, which therefore had an effect on our sampling design. Less experienced observers require a larger number of sampling occasions in order to compensate for their lower species detection rates. In our case, species misidentifications by different observers – which can lead to false positives and introduce bias in occupancy estimates (Royle and Link 2006; Fitzpatrick *et al.* 2009; Miller *et al.* 2011) – were considered unlikely. Other amphibian species using bromeliads in the study area are rare; indeed, other frog species are morphologically distinct from *C. itambe* and do not use the bromeliads for breeding. Additionally, because detectability is influenced by abundance (Moore *et al.* 2011; Garrard *et al.* 2013), local density of individuals is also relevant to observer experience. Volunteers failed to detect low-density populations of invasive pests when compared to experts (Fitzpatrick *et al.* 2009), which might have implications when designing surveys for rare and cryptic species. Despite being considered range-restricted, *C. itambe* showed moderate levels of occupancy – meaning the species is rare, but with high local occurrence – providing a good opportunity for reliable monitoring at the local scale.

Observer experience is an important source of sampling variation (Grant *et al.* 2005; Fitzpatrick *et al.* 2009) and accounting for differences in detectability among observers can improve the survey design and avoid inefficient sampling (Burton *et al.* 2012; Erb, McShea and Guralnick 2012; Bornand *et al.* 2014). Although differences between observers have been previously reported (Grant *et al.* 2005; Moore *et al.* 2011; Burton *et al.* 2012; Garrard *et al.* 2013; Bornand *et al.* 2014), the impact of such variation on the quality of biodiversity data is poorly understood (Austen *et al.* 2016). We attempted to minimize data heterogeneity by training the observers, who could also gain experience with time. Although training can reduce bias and variability (Grant *et al.* 2005), in our case, even after training, there remained a difference in detection when accounting for expertise. Therefore, training did not eliminate the importance of experience in monitoring the species. Consequently, inter-observer variation should be acknowledged when designing a survey and included in the model selection when estimating the parameters of interest.

When designing surveys, the trade-off between the number of sampling occasions and the number of sites needs to be assessed. While a reduced number of sites required a higher number of sampling occasions to maintain precision and accuracy of the parameters we estimated, an increased number of sites needed only a few visits. Precision is gained from increasing sampling occasions (Wintle *et al.* 2004; MacKenzie and Royle 2005; Moore *et al.* 2014; See and Holmes 2015) and, as observed in other amphibians, we found that the number of visits required increased with the level of certainty needed (Pellet and Schmidt 2005; Sewell, Beebee and Griffiths 2010; Sewell *et al.* 2012). Thus, the minimum number of sampling occasions must (1) ensure recorded absences from a site are reliable; and (2) deliver precise estimates of occupancy and detectability. However, there was a limit to increasing precision with no improvement after three to four visits, presumably because there is no real uncertainty remaining about whether the site is occupied. In our case, relatively few visits were sufficient to estimate parameters with good precision, which can reduce the costs of the monitoring programme.

In some aspects *C. itambe* may be an unusual model for a rare and threatened species. Estimates of amphibian detection are frequently low (Rinehart *et al.* 2009; Sewell, Beebee and Griffiths 2010; Courtois *et al.* 2013; Guimarães, Doherty Jr. and Munguía-Steyer 2014) and this is particularly challenging for population monitoring. Although strong inferences on population trends are mostly needed for rare and cryptic species, these are the very taxa that display low detectability or occupancy rates (or both). Amphibian detectability can be improved by conducting surveys with multiple observers or repeating occasions in a single night (MacKenzie *et al.* 2003), increasing the number of traps and/or reducing the sampling area (Guimarães, Doherty Jr. and Munguía-Steyer 2014), combining different sampling methods (Bailey, Simons and Pollock 2004; Sewell, Beebee and Griffiths 2010; Courtois *et al.* 2013) or surveying under ideal weather conditions (Pellet and Schmidt 2005). However, rare and cryptic species may be more costly to get precise estimates because of the increased sampling effort needed. Therefore, explorations of sampling design should be developed during the early stages of a monitoring programme and designed to be both species and habitat specific.

If a monitoring programme aims to detect changes in a given population, the sampling design should be able to distinguish real trends from stochastic fluctuations (Field, Tyre and Possingham 2005). As in other studies, we showed that power increases with sample size, but also depends on the level of significance and the effect size considered (Pollock 2006; Loos *et al.* 2014). Appropriate levels for α and β depend on the goals of a study and should not be set arbitrarily (Di Stefano 2003). Our results show the impact of this choice in the sampling design. For monitoring programmes, we suggest statistical power to be investigated with $\alpha = 0.1$ as previously applied in sampling designs (Thorn *et al.* 2011) to avoid the negative consequences of not detecting a change in occupancy when in fact there is one (i.e., committing a Type II error). Although relatively few visits were required to deliver good precision in our study system, the same sampling design can yield good statistical power, but it was limited to detecting changes of at least 30% in occupancy. In our case, the sampling effort needed to detect a 15% change would require an unrealistic number of sampling sites. Nonetheless, we must consider the effect size expected to be seen when monitoring a target species – which should be based on previous knowledge, such as pre-existing data or ecological theory (Di Stefano 2001). Although the effect size achieved by our current sampling design is not ideal, we considered it acceptable for this population.

Very often sampling designs are unlikely to provide sufficient power to detect small changes in estimated parameters (Thorn *et al.* 2011; Sewell *et al.* 2012; Loos *et al.* 2014) especially for less detectable species (Field *et al.* 2005). Statistical power to detect small changes can be gained by changing the sampling design, usually (if not always) by increasing sampling effort (Field, Tyre and Possingham 2005; Otto and Roloff 2011a; Thorn *et al.* 2011; Sewell *et al.* 2012; Courtois *et al.* 2013; Ellis, Ivan and Schwartz 2013; Loos *et al.* 2014; Steenweg *et al.* 2016). In our case, increasing the number of sampling occasions had a small effect on statistical power. In fact, relatively few observations are needed to maximize the power to detect trends (Field, Tyre and Possingham 2005) and there is no improvement after a given number of occasions (Sewell *et al.* 2012; Ellis, Ivan and Schwartz 2013; Steenweg *et al.* 2016). For amphibians, detectability was shown to affect the power to detect occupancy changes (Courtois *et al.* 2013). However, after detectability reached 0.5, we found no further increase in statistical power. In the case of *C. itambe*, increasing the number of sampling sites is the only strategy to improve power to detect small changes, which was also suggested for bats (Meyer *et al.* 2010), amphibians (Otto and Roloff 2011a;

Courtois *et al.* 2013), reptiles (Sewell *et al.* 2012) and large mammals (Whittington *et al.* 2015).

As demonstrated by the improved statistical power between years, higher initial occupancy probability yields larger statistical power (Guillera-Arroita and Lahoz-Monfort 2012) – an effect previously shown for amphibians (Sewell *et al.* 2012; Courtois *et al.* 2013). Species with lower initial occupancy rates, such as rare species, will therefore require more sites (Courtois *et al.* 2013). The definition of sampling sites can vary from a single unit to a patch of potential breeding habitat (Bailey, MacKenzie and Nichols 2014). Because spatial correlation can reduce power (Whittington *et al.* 2015), distance between sites must respect species distribution, home range and dispersal capabilities. If sites are close, surveys can be done by multiple observers on the same night – a design that could be applied for pond-breeding amphibians with moderate detectability.

For territorial anurans, for example, sites could be closely located (e.g., sub-transects in the same stream) and visited during a short survey window, when detectability is higher. The number of sites can also be increased by placing automated recording units and/or increasing the number of traps. A removal sampling design can be applied (although this might be less robust to model assumptions, see MacKenzie and Royle 2005), as well as a double sampling design, for which a high detection probability is required (MacKenzie and Royle 2005). Nevertheless, as we demonstrated here, for species restricted to a small number of remaining sites, obtaining sufficient statistical power to distinguish real population changes from natural fluctuations may be an unachievable goal.

In many cases, increasing the number of sites may pose a problem for monitoring species with highly restricted distributions and which occur in relatively few sites. Even when the sampling design provides precise estimates and reliable power, we can only realistically detect moderate to large declines in the population and smaller changes will not be detected. Unless there is high abundance and moderate initial occupancy, rare and cryptic species will be particularly challenging when it comes to detecting population changes. Our data show the importance of considering inter-observer variation in detection probabilities and we emphasize that future monitoring should consider the role of observer variability when estimating occupancy and detectability.

For monitoring programmes in their initial stages, we recommend a pilot study to optimize the sampling design of the main study. Although we have used specific data from a single case study, the same modelling and calculations can be applied to any target species. This can be particularly useful for targeted species, for example, in the Brazilian Amphibian Conservation Action Plan (RAN/ICMBio 2012), where long-term monitoring studies are proposed. Some existing tools are available to evaluate estimator bias and variance of the estimated parameters from a given sampling design (e.g., GenPres (Bailey *et al.* 2007) and SODA (Guillera-Arroita, Ridout and Morgan 2010)), which should make the analytical process straightforward for conservation practitioners. Failing to deliver precise estimates and appropriate levels of statistical power will lead to cost-ineffective surveys designs as well as spurious conclusions about population trends.

4.6 Acknowledgements

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4.7 Supplementary information

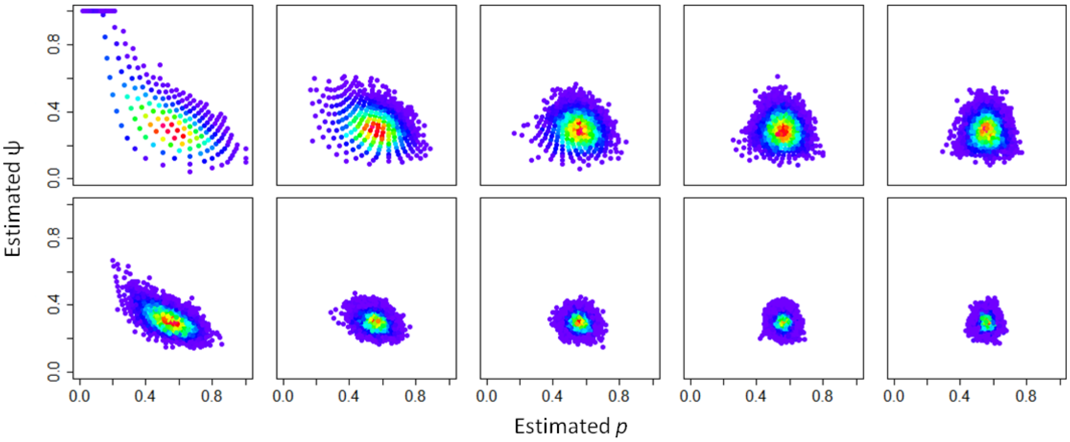

Figure S4.1 Maximum likelihood estimates with varying number of occasions and sites Distribution of MLE with varying number of occasions (2–6 visits) and with a constant number of sites (first line, S = 50; second line S = 150). Estimates are based on a constant model ψ(.) *p*(.) using detection history of sites and neighbours from 2014 dataset, where ψ = 0.3, *p* = 0.56 (10000 interactions).

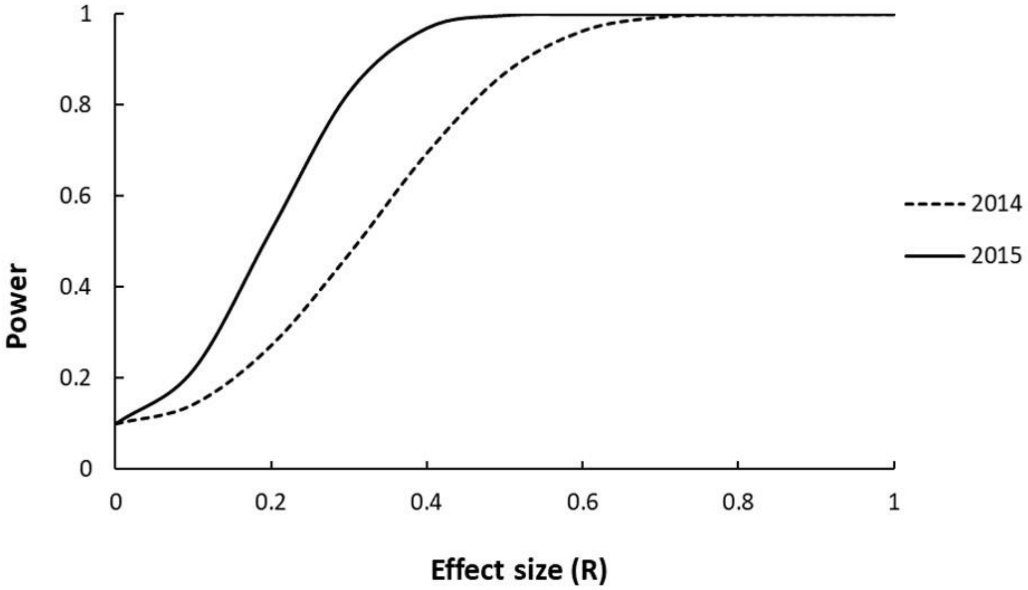

Figure S4.2 Differences in statistical power between surveyed years

Power is given in relation to the absolute change in occupancy (effect size, R) for two years: 2014 (123 sampling sites, 4 sampling occasions, $\psi = 0.3$, $p = 0.56$), and 2015 (143 sampling sites, 21 sampling occasions, $\psi = 0.49$, $p = 0.54$) ($\alpha = 0.1$ for both datasets).

Table S4.1 Naïve occupancy, estimated occupancy and detection probabilities for *Crossodactylodes itambe*

Occupancy (ψ) and detection (p) are based on a constant model ψ(.) p(.) for **A)** 2014 and 2015 monthly dataset using site only and **B)** for 2015 complete dataset using patch data (sites and neighbours), giving 95% confidence interval (CI), number of sampling sites (S) and number of occasions (K); where p^* is the probability of detecting a species at an occupied site at least once, given by: $p^* = 1 - (1 - p)^K$

A)

	naïve ψ	ψ	CI	*p*	CI	*p**	S	K
2014 monthly dataset								
Feb	0.27	0.30	0.22–0.40	0.56	0.45–0.66	0.96	123	4
2015 monthly dataset								
Feb	0.25	0.36	0.27–0.46	0.41	0.33–0.50	0.93	131	5
Mar	0.25	0.27	0.21–0.35	0.64	0.57–0.71	1.00	143	6
Apr	0.36	0.37	0.29–0.45	0.62	0.55–0.68	0.99	143	5
May	0.29	0.29	0.22–0.37	0.59	0.51–0.65	0.99	143	5

B)

	Naïve ψ	ψ	CI	*p*	CI	*p**	S	K
2015 complete dataset								
2015	0.50	0.50	0.41–0.58	0.54	0.51–0.57	1.00	143	21
Season								
Dry	0.45	0.45	0.37–0.54	0.63	0.59–0.67	1.00	143	11
Wet	0.40	0.41	0.33–0.59	0.59	0.55–0.63	1.00	143	10
Elevation gradient								
High	0.67	0.67	0.52–0.78	0.52	0.48–0.56	1.00	48	19
Med	0.68	0.68	0.54–0.80	0.56	0.52–0.59	1.00	47	20
Low	0.14	0.14	0.07–0.27	0.54	0.45–0.62	1.00	48	18

Table S4.2 Fitted models for 2015 complete dataset (detection history from sites and neighbouring bromeliads) for *Crossodactylodes itambe*

Fitted models with **A)** covariates of detectability (p) and constant occupancy (ψ); and **B)** including covariates for both parameters. N Pars = number of parameters; ΔAIC is the difference between the model with the lowest AIC and the given model; AIC w is AIC weight. Constant model is also included, given by $\psi(.)\ p(.)$; covariates of detectability are: obs = observer experience, neig = number of neighbours in the patch, leaf = number of leaves in bromeliad, time = time of observation, vol = volume of rosette; covariates of occupancy are: alt = elevation, size = size of bromeliad.

A)

Model	N Pars	AIC	ΔAIC	AIC w	Cumulative Weight
$\psi(.)\ p(\text{obs})$	3	1906.82	0.00	0.96	0.96
$\psi(.)\ p(\text{neig})$	3	1912.98	6.16	0.04	1.00
$\psi(.)\ p(.)$	2	1924.19	17.37	< 0.01	1.00
$\psi(.)\ p(\text{leaf})$	3	1924.23	17.42	< 0.01	1.00
$\psi(.)\ p(\text{time})$	3	1925.29	18.48	< 0.01	1.00
$\psi(.)\ p(\text{vol})$	3	1925.58	18.76	< 0.01	1.00

B)

Model	N Pars	AIC	ΔAIC	AIC w	Cumulative Weight
$\psi(\text{alt})\ p(\text{obs})$	4	1882.82	0.00	1.00	1.00
$\psi(\text{alt})\ p(.)$	3	1900.19	17.37	< 0.01	1.00
$\psi(\text{size})\ p(\text{obs})$	4	1904.36	21.54	< 0.01	1.00
$\psi(.)\ p(\text{obs})$	3	1906.82	24.00	< 0.01	1.00
$\psi(\text{size})\ p(.)$	3	1921.74	38.92	< 0.01	1.00
$\psi(.)\ p(.)$	2	1924.19	41.37	< 0.01	1.00

Table S4.3 Number of surveys required to determine species presence at occupied site given 80%, 90% or 95% certainty

S = number of sites surveyed; K =number of visits; ψ = estimated occupancy; *p* = estimated detectability for each dataset. Estimates are based on constant model ψ(.) *p*(.) using monthly detection history of sites only. Minimum and maximum number of surveys needed are given in bold.

	S	K	ψ	*p*	N survey needed		
					0.8	0.9	0.95
2014	123	4	0.3	0.56	2.0	2.8	3.6
Feb	131	5	0.36	0.41	3.1	4.4	**5.7**
Mar	143	6	0.27	0.64	**1.6**	2.3	2.9
Apr	143	5	0.37	0.62	1.7	2.4	3.1
May	143	5	0.29	0.59	1.8	2.6	3.4

Table S4.4 Number of sampling sites needed to achieve statistical power from 80–95% Power (G) is given for different effect sizes (R; from 0.5–0.15) and with varying significance level (α; from 0.05–0.2). Calculations are based on estimates of best model for 2015 complete dataset ($\psi = 0.49$, $p = 0.61$, 21 occasions, 143 sites).

G	α	R			
		0.5	0.3	0.2	0.15
0.8	0.05	57	173	400	717
	0.1	45	136	315	565
	0.2	33	100	230	412
0.85	0.05	66	198	457	820
	0.1	53	159	366	656
	0.2	39	119	274	491
0.9	0.05	77	232	535	959
	0.1	63	189	436	782
	0.2	48	145	334	600
0.95	0.05	95	286	661	1186
	0.1	79	239	551	988
	0.2	63	189	436	782

Chapter 5 Occupancy trends of an endemic amphibian from the Atlantic Rainforest

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5.1 Abstract

Amphibian population declines are globally widespread, including tropical areas and the threatened Atlantic Rainforest. However, detailed information from long-term monitoring studies is still unavailable for most tropical species. We designed and evaluated a long-term monitoring protocol that aimed to detect a population change for the Itambé bromeliad frog, a threatened and endemic amphibian species living inside bromeliads on a highland area of Brazil. Here we report a 4-year population trend using a single-species dynamic occupancy model and give estimates of colonization and extinction over time and among seasons. Elevation was a significant predictor of population dynamics. For this species, sites at higher elevation are more likely to be occupied, are easier to colonize and are less likely to become extinct. Season had a significant effect on colonization rates, which was slightly higher during the wet season. Overall, colonization and extinction rates were low, and species might have reduced dispersal capabilities. Nonetheless extinction rates were almost 10 times above colonization rates. The direction of change in occupancy differed between elevation categories and we detected a decline in the population in sites at lower elevation, between 1704–1815 m above sea level. We were unable to detect a significant decline at higher elevation sites and a decline was only detected at a very limited elevational range.

Keywords: Amphibian decline, Bromeliads, Dynamic occupancy, Population decline, Rare species, Threatened species.

5.2 Introduction

The Atlantic Rainforest is a biodiversity hotspot (Myers *et al.* 2000) with high levels of endemism of amphibians (Haddad *et al.* 2013) and plants (Joppa *et al.* 2011), currently reduced to forest remnants and poorly covered by protected areas in Brazil (Ribeiro *et al.* 2009). Amphibian species in this biome can be negatively affected by land cover change (Ribeiro *et al.* 2018) and are also threatened by climatic instability (Vasconcelos, Nascimento and Prado 2018). More than 40% of amphibian species are declining worldwide (Stuart *et al.* 2004) and this level of threat far exceeds that for any other vertebrate group (Wake and Vredenburg 2008; Hoffmann *et al.* 2010; Böhm *et al.* 2013). Severe population declines and species extinctions have been reported in the Neotropics (Lips, Reeve and Witters 2003; Eterovick *et al.* 2005; La Marca *et al.* 2005; Lips *et al.* 2005; Whitfield, Lips and Donnelly 2016), but relatively little is known about the status of amphibian populations in Brazil (Silvano and Segalla 2005; Verdade *et al.* 2012), especially due to the lack of data on species distribution and population dynamics (Eterovick *et al.* 2005).

Most studies addressing amphibian declines in Brazil are from the Atlantic Rainforest (Heyer *et al.* 1988; Weygoldt 1989; Guix *et al.* 1998; Papp and Papp 2000; Eterovick *et al.* 2005), where there is high amphibian species richness and a large number of threatened species (Hoffmann *et al.* 2010; Pimm *et al.* 2014). Tropical amphibian declines have been particularly critical at higher elevations (Young *et al.* 2001; Wake and Vredenburg 2008), such as the emblematic Harlequin frogs that rapidly vanished from throughout their original range (Lips 1998; La Marca *et al.*

2005; Lips *et al.* 2006; Lips *et al.* 2008; Crawford, Lips and Bermingham 2010; McCaffery, Richards-Zawacki and Lips 2015). Tropical mountains usually have a high proportion of endemic species (Beniston 2003; Leite, Eterovick and Juncá 2008), and some amphibians are only found in only a few highland areas in the Atlantic Rainforest (Ribeiro *et al.* 2015). Amphibians restricted to mountaintops can be susceptible to microclimate variations (Pounds, Fogden and Campbell 1999; Whitfield, Lips and Donnelly 2016) and may collapse under the global warming scenario (Stuart *et al.* 2004; Wake 2012), escalating the current number of amphibians species that are both highly vulnerable to climate change and threatened with extinction (Foden *et al.* 2013).

Although amphibians have received increasing attention since the start of the decline crisis, population trends are still unknown for almost 30% of species (Stuart *et al.* 2004). To make reliable conclusions about changes in population status, quantitative evidence from long-term monitoring is needed (Green 2003), but such studies are often expensive, lack clear objectives and have insufficient statistical power (Legg and Nagy 2006; Field *et al.* 2007; Loos *et al.* 2014). We developed a monitoring protocol (Chapter 4) that improves the chance of detecting a population change in our target species, the Itambé bromeliad frog (Barata *et al.* 2013), which also provides a cost-effective allocation of financial resources. Presence-absence data are usually easier to obtain and less costly in terms of time and effort than methods used for abundance estimation (MacKenzie and Royle 2005; Conner *et al.* 2016). Additionally, disregarding species detectability may also overestimate population declines (Cruickshank et al. 2017). Our sampling design was based on an occupancy model (MacKenzie *et al.* 2002) and uses presence/absence data and accounts for imperfect detection, and few visits are necessary to estimate parameters precisely and detect a moderate population change with good statistical power (Chapter 4).

In a monitoring context, temporal variation in occupancy probabilities may describe trends over time as site occupancy changes between seasons (MacKenzie, Nichols and Yoccoz 2006). This dynamic occupancy model accounts for processes involving both extinctions at occupied sites and colonization of unoccupied sites, and changes in occupancy can be modelled as a function of site colonization and extinction rates (MacKenzie *et al.* 2003). The use of site occupancy models for monitoring population status has rapidly increased over recent years (Pellet and Schmidt 2005; Mattfeldt, Bailey and Grant 2009; Rinehart *et al.* 2009; Walls, Waddle and Dorazio 2011; Sewell *et al.* 2012) and is widely applied in national monitoring programs (Weir, Fiske and Royle 2009; Adams *et al.* 2013; Buckley, Beebee and Schmidt 2014; Weir, Royle and Gazenski 2014; Villena *et al.* 2016), which rely on long time series and large datasets. However, in the Neotropics, such studies are rare and extensive databases are usually unavailable (Collen *et al.* 2008).

The Itambé bromeliad frog (*Crossodactylodes itambe*), belongs to a rare group of amphibians that spend their entire life cycle inside a bromeliad, where they lay their eggs and complete their development (also known as bromeligenous frogs, sensu Peixoto 1995). Bromeligenous frogs are mostly recorded at high elevation areas of South America with very restricted distributions (Sabagh, Ferreira and Rocha

2017). *Crossodactylodes itambe* is endemic to a single mountaintop in the Atlantic Rainforest, above 1700 m in elevation, and has a narrow geographical range (Chapter 3). Bromeligenous frogs occurring at altitudes > 1000 m above sea level (a.s.l.) are currently under threat (Sabagh, Ferreira and Rocha 2017) and, although *C. itambe* has not been evaluated by the IUCN, the species could be classified as vulnerable or critically endangered (Chapter 2, Appendix I). Unfortunately, there is no assessment of population trends for *C. itambe* – in fact, there is little information on ecology and natural history for the whole genus and many bromeligenous frogs.

Our main goal was to define a population trend for the Itambé bromeliad frog, indicating whether declines have been occurring in this mountaintop population over the studied period. In this chapter, we have not directly assessed the drivers of population dynamics (but see Chapter 6 for details), instead we focused on describing an occupancy change over the years. Our monitoring protocol was developed to detect a 30% population change with high statistical power (Chapter 4). Here we report a 4-year evaluation of population trends using a single-species dynamic occupancy model. We also estimate colonization and extinction over time and among seasons. Considering the species is a mountaintop endemic, we hypothesized that the dynamic process of colonization and extinction is influenced by elevation, as previously demonstrated for occupancy estimates (Chapter 4). To the best of our knowledge, we provide the first population trend for a mountaintop endemic bromeligenous frog.

5.3 Materials and methods

5.3.1 Study area and species

This study was conducted at the Itambé summit, inside a protected area in south-eastern Brazil (Pico do Itambé State Park, 18°23′S 43°20′W; datum WGS 84), at the southern portion of the Espinhaço Mountain Range, within the domains of the Atlantic Rainforest. The Espinhaço Mountain Range shelters 14% of Brazilian vascular plants in less than 1% of the country's surface (Silveira *et al.* 2016) and is severely threatened by human activity (Alves *et al.* 2014; Monteiro *et al.* 2016). The southern portion of the Espinhaço Mountain Range is a UNESCO Biosphere Reserve, a centre of plant diversity (Davis, Heywood and Hamilton 1995), one of the Global 200 Ecoregions (Olson *et al.* 2001), an Important Bird Area for endemic species (Mugica *et al.* 2009), and a centre for amphibian endemism (Leite, Eterovick and Juncá 2008). Because of its biological importance, the area is nationally recognized as a priority area for biodiversity conservation (Drummond *et al.* 2005).

Reaching 2062 m a.s.l., the Itambé summit is the highest elevation recorded at the Espinhaço Mountain Range and captures the Atlantic Rainforest moist wind, which increases rainfall and mist. Although the protected area encompasses more than 4000 ha, our study was restricted to a small portion of the mountaintop, above 1700 m, where the Itambé bromeliad frog occurs. *Crossodactylodes itambe* is a small-sized frog, endemic to the Itambé summit and with estimated area of occurrence of less than 0.5 km^2 (Barata *et al.* 2013). *Crossodactylodes itambe* uses a single species of bromeliad, *Vriesea medusa*, which is also endemic to the Espinhaço Mountain Range and known to occur in only two locations (Versieux and Wendt 2007), including the Itambé summit.

Bromeliads are terrestrial or arboreal plants native to the Neotropics and widespread in South America (Benzing 2000). These plants have a complex architecture, with multiple sized interlocking leaves forming a circular central tank that collects water, leaf litter and detritus, providing shelter to many amphibian species (Rocha *et al.* 2000; Teixeira, Schineider and Almeida 2002; Silva, Carvalho and Bittencourt-Silva 2011). At the Itambé summit, bromeliads are very abundant and grow in a rocky substrate, covered by an extensive open grassland habitat, with increasing density towards to the summit.

5.3.2 Data collection

Our sampling design considered the local characteristics of our study system and the biological traits of the species (i.e., body size, species range, habitat restrictions, and presumed low dispersal capabilities). We considered individual bromeliads as independent sampling sites and we tagged 143 bromeliads with an identification number that allowed repeated visits. Sampling sites are distributed in a restricted elevational range, from 1700 m a.s.l. to the summit, at 2062 m a.s.l. We surveyed frogs over four consecutive years (from 2014 to 2017), during wet and dry seasons, and each year consisted of monthly surveys. During the first and last years, we surveyed only one month (February 2014; June 2017), and we surveyed four months in 2015 (February–May) and 2016 (February–June). Over the study period, we had a total sampling effort of ten months, five months representing each season.

Because *Crossodactylodes itambe* is active at night (Barata, Griffiths and Ferreira 2018), we searched for frogs in bromeliads using visual encounters, starting after dusk, and each monthly survey consisted of 4–6 visits over consecutive nights.

All bromeliads were surveyed on the same night by two teams of two observers. To standardize our surveys, only one person of each team was surveying species presence/absence while the other was taking notes of covariates. One observer was considered to have experience in recording the species (> 5 years working in the study area), but other observers, who were less experienced (< 5 years of experience), received training in observing the target species prior to our surveys, to avoid differences in detection (Chapter 4). Although tadpoles and juveniles can share the same bromeliad with adults (Santos *et al.* 2017), we only considered records of adults during our surveys and therefore presence was defined by one or more adults occupying a single bromeliad. For each surveyed month, we created a detection history of presence/absence of adult frogs at a site by recording whether there were one or more detections (1) or no detections (0) for each bromeliad visited over consecutive night visits.

5.3.3 Data analyses

We used a single-species multi-season occupancy model (hereafter dynamic model), where seasonal changes in the probability of occupancy (ψ), colonization (γ) and extinction (ε) are explicitly modelled while accounting for imperfect detection (MacKenzie *et al.* 2003). While site occupancy is defined as the probability of a randomly selected site being occupied by a species, colonization and extinction rates represent probabilities of a site changing between occupied and unoccupied status between consecutive seasons. Specifically, colonization is defined as the probability of a site unoccupied at time *t* becoming occupied at the following season, *t*+1; and extinction is defined as the probability of a previously occupied site during season *t* becoming unoccupied at *t*+1 (MacKenzie *et al.* 2003). The modelling approach takes detectability (*p*) into consideration, which is defined as the probability of a species being detected at a site if that site is indeed occupied (MacKenzie *et al.* 2003).

Dynamic occupancy models use a robust design, which assumes the population is closed within a specific season (i.e., no immigration and/or emigration within our 4–6 night visits), but is open between one season and the next (MacKenzie *et al.* 2003). We considered each month as a primary sampling period (i.e., a season, as defined by MacKenzie *et al.* 2003) and, to avoid misleading estimates of monthly extinction and colonization rates, we added missing values for months we did not sample and for which we did not have any observations. At the end of our 4-year study we had a total of 10 sampled months and, considering additional missing values, we had a total of 41 primary sampling periods. We therefore looked at a monthly dynamic process and we used the term 'time series' to refer to Mackenzie's seasons and to avoid confusion with the actual definition of wet/dry seasons, which we also investigated.

Dynamic models can accommodate covariates that explain estimated parameters of detection, occupancy, colonization and/or extinction. Previous work (Chapter 4) showed that observer experience affects detection probabilities and occupancy rates are positively affected by elevation. We therefore used observer experience and elevation as fixed covariates for estimated parameters, and all models had observer experience as a covariate of detection and elevation as a covariate of occupancy.

We then fitted models with covariates for colonization and extinction rates. To investigate if elevation was also influencing population dynamics, we used this covariate for colonization and extinction parameters. We fitted additional models that allowed extinction and colonization to (1) vary between season (i.e., wet and dry); and (2) vary among months (i.e., time series). Finally, we fitted models of colonization and extinction that combined elevation with either month or season. We performed model selection using Akaike's Information Criterion (AIC) with all candidate models and including a constant model (no covariates fitted for colonization/extinction) for comparison. Models were ranked by their AIC and we also used AIC weights to indicate the relative support of different fitted models. Models with ΔAIC < 2 were considered to have strong support while models with a ΔAIC of > 2 were considered to have less support (Burnham and Anderson 2002).

We predicted colonization and extinction rates at each sampling site based on the best fitting model. The predict function returns estimates of parameters at specific covariate values, enabling us to plot predicted colonization and extinction rates against elevation. For visual interpretation, we also mapped the estimated probabilities of colonization and extinction at points that were not sampled using a spatial interpolation, which was calculated using inverse distance weighting (IDW).

IDW estimates values as a weighted average of the predicted values at nearby sampled points: the closer a sampling point is to the centre of the site being estimated, the more influence, or weight, it has in the averaging process. To calculate interpolation by IDW we used ArcGIS, available with the Spatial Analyst tool.

Finally, to estimate occupancy trends over our time series, we used the smoothing method described by Weir, Fiske and Royle (2009), which is recommended when looking at estimated occupancy change at sites that are repeatedly surveyed. This method estimates the proportion of sampled sites that are occupied at each time and uses a less parametrized model to estimate a sample trend that reveals more complex patterns (Weir, Fiske and Royle 2009). Therefore, we define population trend as the smoothed trajectory of occupancy over our time series. We first obtained an estimate of the site-specific trajectory for each time series and then averaged site-specific trajectories over all sites to get an overall estimate of the proportion of sites occupied at each time. We calculated 95% confidence intervals for smoothed estimates based on standard errors obtained from 1000 non-parametric bootstrap iterations from derived parameter estimates. Finally, we used a simple linear regression to test for significance of the trend and we report P-values (P) and the slope of beta coefficients, given by β.

Because elevation was an important factor determining species occupancy (Chapter 4), we used the same modelling approach to fit models at different elevation categories, which allowed us to investigate population trends at a finer scale. We first created separate datasets for three elevation categories, which were defined based on the topography of the area: high (ranging from 1998–2062 m a.s.l., with 48 sampling sites); medium (from 1838–1925 m a.s.l., 47 sites); and low (ranging from 1704–1815 m a.s.l., 48 sampling sites). Keeping initial occupancy constant, we fitted the same models for every elevation category and compared them using AIC values, as previously described. We were mostly interested in understanding if different population trends were occurring in different elevation categories, so we calculated the smoothed trends for comparison. We report estimates of occupancy changing over our time series, and a single estimate of colonization and extinction. Dynamic occupancy models and analyses (model fit, model selection and predictions) we made using R (R Core Team 2017) and its extension package Unmarked (Fiske and Chandler 2011; Kéry and Chandler 2012). In all analyses, elevation was z-transformed to standardize means and variances.

5.4 Results

During our study period, the number of bromeliads surveyed varied from 122 to 143, with an average of 40 visits per bromeliad. The vast majority of visits were negative, with frogs detected only 810 times out of over 8000 sampling occasions. Nonetheless, detectability was considered high (p = 0.60 ± 0.02). Model selection demonstrated little evidence that either colonization or extinction changed over time (i.e., no influence of month on estimated parameters, Table 5.1). Elevation was a significant covariate influencing the colonization rates of the Itambé bromeliad frog and increased in bromeliads at higher elevation (Figure 5.1).

Table 5.1 Fitted dynamic occupancy models for the Itambé bromeliad frog

Colonization (γ) and extinction (ε) rates explained by elevation alone (elev) and in combination with season (ssn) or month, combined with fixed covariates for occupancy (ψ) and detectability (*p*), where obs is the difference in observer experience. Pars = number of parameters; AIC = Akaike's Information Criterion; ΔAIC is the difference between the model with the lowest AIC and the given model; AIC *w* = AIC weight.

Models	Pars	AIC	ΔAIC	AIC *w*	Cumulative weight
ψ(elev) γ(elev+ssn) ε(elev+ssn) *p*(obs)	11	2877.19	0	0.94	0.94
ψ(elev) γ(elev) ε(elev) *p*(obs)	9	2882.87	5.68	0.05	1.00
ψ(elev) γ(elev+month) ε(elev+month) *p*(obs)	11	2887.8	10.61	<0.01	1.00
ψ(elev) γ(month) ε(month) *p*(obs)	9	2907.83	30.64	<0.01	1.00
ψ(elev) γ(ssn) ε(ssn) *p*(obs)	9	2908.3	31.11	<0.01	1.00
ψ(elev) γ(.) ε(.) *p*(obs)	7	2914.53	37.34	<0.01	1.00

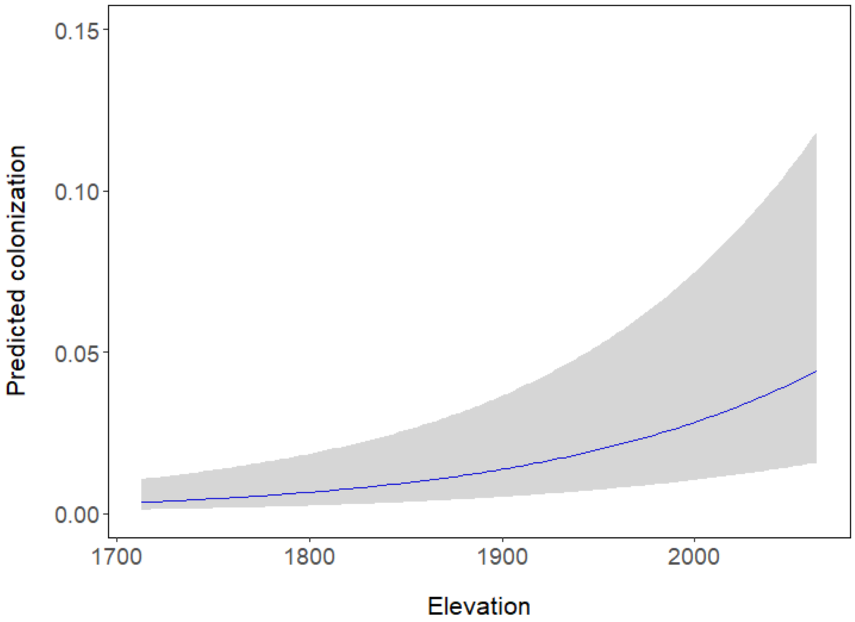

Figure 5.1 Predicted probability of colonization in relation to elevation for the Itambé bromeliad frog

Predicted probability of colonization (blue line) based on best fitting model for all sites: ψ(elev) γ(elev+ssn) ε(elev+ssn) *p*(obs). Elevation is given in meters a.s.l. and shaded areas represent 95% confidence intervals of predicted values.

Although the best supported model for all sites included elevation for both colonization and extinction (Table 5.1), this covariate was not significant in explaining extinction rates (P = 0.8), which had little variation among all sites. This pattern was also observed by the mapped distribution of fitted interpolation showing colonization and extinction rates beyond sampling sites (Figure S5.1). However, considering each elevation category separately, we observed that elevation was a significant predictor of extinction rates at high and medium elevation (Figure 5.2, Table 5.2).

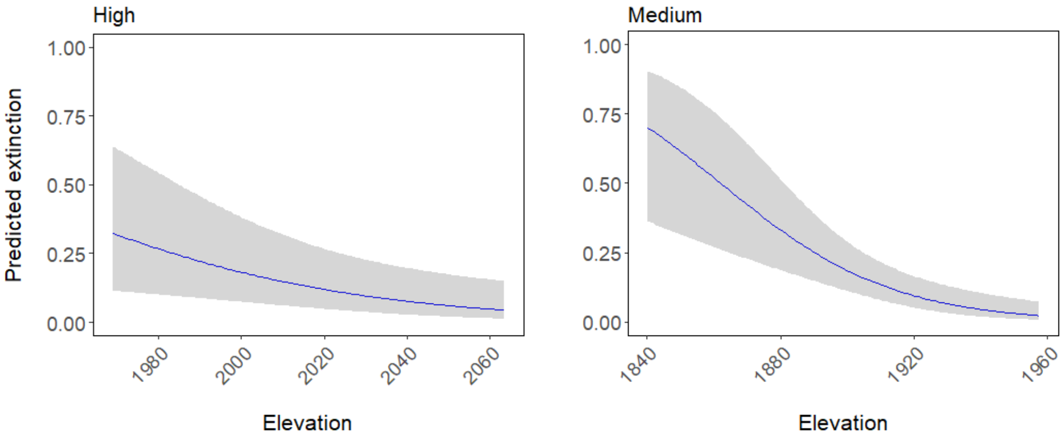

Figure 5.2 Predicted probability of extinction in relation to elevation at high and medium categories for the Itambé bromeliad frog

Estimated parameters were based on best fitting model for sites at high elevation: ψ(.) γ(elev+ssn) ε(elev+ssn) *p*(obs), and for sites at medium elevation: ψ(.) γ(elev) ε(elev) *p*(obs). Predicted probability of extinction is given by blue lines and elevation is given in meters a.s.l. Shaded areas represent 95% confidence intervals of predicted values.

Table 5.2 Dynamic occupancy models for the Itambé bromeliad frog at different elevation categories

Dynamic models with occupancy (ψ), colonization (γ), extinction (ε) and detectability (p) fitted at different elevation categories (High, Medium and Low). Pars = number of parameters; AIC = Akaike's Information Criterion; ΔAIC is the difference between the model with the lowest AIC and the given model; AIC *w* = AIC weight; elevation = elev; season = ssn; month; obs = observer experience. Models highlighted in bold had better support with ΔAIC < 2.

Models	Pars	AIC	ΔAIC	AIC *w*	Cumulative weight
High					
ψ(.) γ(elev+ssn) ε(elev+ssn) p(obs)	**10**	**1294.82**	**0**	**0.570**	**0.57**
ψ(.) γ(elev) ε(elev) p(obs)	**8**	**1296.12**	**1.3**	**0.297**	**0.87**
ψ(.) γ(elev+month) ε(elev+month) p(obs)	10	1297.87	3.06	0.124	0.99
ψ(.) γ(ssn) ε(ssn) p(obs)	8	1304.94	10.12	0.004	0.99
ψ(.) γ(.) ε(.) p(obs)	6	1305.05	10.23	0.003	1.00
ψ(.) γ(month) ε(month) p(obs)	8	1305.59	10.77	0.003	1.00
Medium					
ψ(.) γ(elev) ε(elev) p(obs)	**8**	**1262.93**	**0**	**0.516**	**0.52**
ψ(.) γ(elev+ssn) ε(elev+ssn) p(obs)	**10**	**1264.26**	**1.33**	**0.265**	**0.78**
ψ(.) γ(elev+month) ε(elev+month) p(obs)	**10**	**1264.88**	**1.95**	**0.194**	**0.98**
ψ(.) γ(month) ε(month) p(obs)	8	1270.1	7.17	0.014	0.99
ψ(.) γ(ssn) ε(ssn) p(obs)	8	1271.99	9.07	0.006	1.00
ψ(.) γ(.) ε(.) p(obs)	6	1272.42	9.49	0.005	1.00
Low					
ψ(.) γ(.) ε(.) p(obs)	**6**	**260.23**	**0**	**0.660**	**0.66**
ψ(.) γ(ssn) ε(ssn) p(obs)	8	262.87	2.63	0.180	0.84
ψ(.) γ(elev) ε(elev) p(obs)	8	263.87	3.64	0.110	0.95
ψ(.) γ(elev+ssn) ε(elev+ssn) p(obs)	10	266.49	6.26	0.029	0.98
ψ(.) γ(elev+month) ε(elev+month) p(obs)	10	267.04	6.81	0.022	1.00
ψ(.) γ(month) ε(month) p(obs)	8	284.21	23.98	<0.01	1.00

In addition to elevation, the best fitting model for this species at all sites included differences between wet and dry seasons, which had strong support (AIC weight = 0.94, Table 5.1). Although predicted values of extinction were similar between dry and wet seasons, predicted colonization rates were significantly different (t-test $P < 0.05$), with slightly higher values observed during the wet season (Figure 5.3). Although the best fitting model for all sites together included covariates of season and elevation combined, season was not included in best fitting models for all elevation categories (Table 5.2). Season was important for sites at high and medium elevation categories. At higher elevation, we found that 57% of the support was from the best fitting model, which included elevation and seasons combined. At medium category, the best fitting model had only elevation as an explanatory variable, while at low elevation the model with constant colonization and extinction rates performed best (Table 5.2).

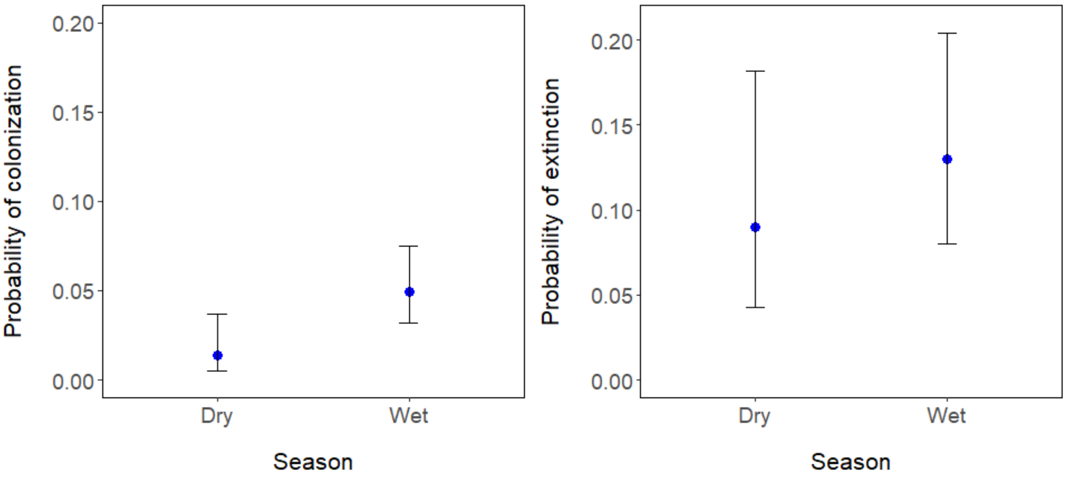

Figure 5.3 Differences in the predicted probability of colonization and extinction for the Itambé bromeliad frog between dry and wet seasons
Average values of predicted probability of colonization and extinction (blue dots) during dry and wet seasons, with 95% confidence intervals (vertical bars). Predicted values were based on the best fitting model for all sites: ψ(elev) γ(elev+ssn) ε(elev+ssn) p(obs).

The best supported model for all elevation categories that included elevation and season (top model, Table 5.1) indicated an initial occupancy estimate of $\psi = 0.24$ (SE = 0.04), and we found that extinction probability was proportionally higher than the probability of colonization (ε = 0.09 ± 0.03 and γ = 0.01 ± 0.01). The smoothed trajectory of occupancy rates was calculated for each surveyed month (Figure 5.4) and this dynamic process of higher extinction rates and lower colonization rates did not result in a significant decrease in occupancy estimates for subsequent months (β = -0.003; P = 0.3; Figure S5.2). Although there was no significant decline in occupancy trend for all sites together, we observed different trends when investigating sites at different elevation categories (Figure S5.2).

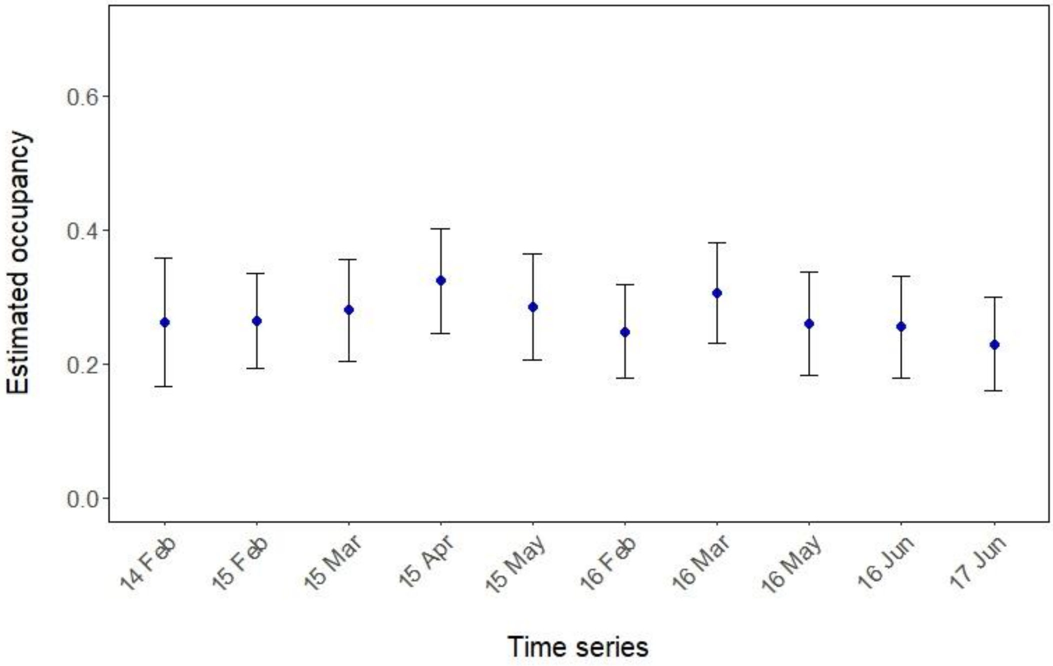

Figure 5.4 Trend in occupancy over time for the Itambé bromeliad frog
Occupancy probabilities from a dynamic occupancy model showing smoothed values of estimated occupancy (blue dots) and 95% confidence intervals (vertical bars) from February 2014 to June 2017 (time series).

Occupancy trend was increasing at sites at higher elevation (Figure 5.5), but this relationship was not considered significant (β = 0.005; P = 0.35; Figure S5.2). We observed a sharp decline in February 2016 for sites at medium elevation (Figure 5.5).

We also found a negative relationship between the smoothed values of occupancy and each surveyed month; however, this relationship was not considered significant ($\beta = -0.011$; $P = 0.08$; Figure S5.2). Occupancy change was only considered significant at the lower elevation category and smoothed occupancy values had a negative relationship in relation to surveyed month ($\beta = -0.003$; $P < 0.05$; Figure S5.2), indicating a population decline at sites at lower elevation (Figure 5.5).

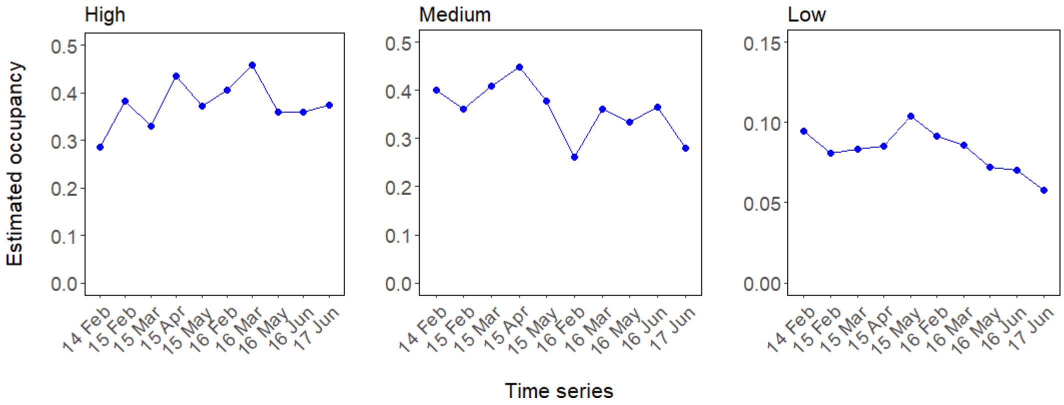

Figure 5.5 Trend in occupancy for the Itambé bromeliad frog at different elevation categories
Estimated occupancy probability from February 2014 to June 2017 (time series), at different elevation categories (High, Medium and Low). Population trend is given by smoothed occupancy estimates (estimated occupancy), based on best fitting dynamic model for each elevation category (see Table 5.2 for details).

5.5 Discussion

We found that elevation was a significant predictor of population dynamics, significantly affecting the colonization rates of the Itambé bromeliad frog. Previous studies indicated that elevation is a good predictor of amphibian occupancy (Ray *et al.* 2016), especially for montane species (Gould *et al.* 2012; Sarkinen, Gonzáles and Knapp 2013; Kroschel *et al.* 2014; Hossack *et al.* 2015; Băncilă *et al.* 2017). For this mountaintop endemic amphibian species, elevation affects species occupancy (Chapter 4), plays an important role determining species distribution (Chapter 3), and we demonstrate that colonization rates increased with elevation, presenting a similar pattern for predicted occupancy. The effects of elevation on extinction rates were not explicit when considering all sites and were only detected when analysing each elevation category separately, showing that extinction rates decreased at high and medium elevations. For the Itambé bromeliad frog, sites at higher elevation are (1) more likely to be occupied, (2) are easier to colonize and (3) are less likely to become extinct.

Most importantly, the relationship between population dynamics and elevation had an impact on the direction of the trend observed for population change at different elevation categories. Likewise, Kroschel *et al.* (2014) reported differences in occupancy decline related to elevation, concluding that the population of the Cheat Mountain Salamander, declined at mid- and high elevations over a 32-year period. Considering all sites, we found that the slope of overall change in occupancy is negative, but differences over the studied period were not significant. Initial occupancy estimates at low and medium elevation categories differed markedly (0.08 ± 0.05 and 0.40 ± 0.09, respectively) and we also observed a downward trend in occupancy. However, the same pattern did not hold true at the highest elevation, where we observed an upward trend, with occupancy increasing in sites at higher elevation. Although different population trends were observed at different elevation categories, a significant decline in occupancy was only detected at low elevation sites.

At lower sites, elevation did not have an important role in shaping population dynamics, which indicates that factors other than elevation are explaining colonization and extinction rate at these sites. Together with soil type and microclimatic conditions, elevation plays an important role in shaping habitat structure in our study area (Silveira *et al.* 2016) and we acknowledge that elevation may have a complex association with other local factors. For example, we know that the interaction of elevation with bromeliad size is important for species abundance (Chapter 2). We also observed that bromeliad density increased with elevation, which could enhance the opportunity for colonization of new sites available and/or decrease local extinction at higher elevation sites. A history of trampling and fire in the lowlands, for instance, might have shaped landscape characteristics in our study area, including the limited number of bromeliads available. With our data, however, it is hard to identify the underlying factors that could be associated with differences in extinction and colonization rates observed between elevation categories.

We detected a sharp decline in February 2016, which was followed by a fast recovery. This decline could be attributed to a fire that affected 80% of the park's area by the end of 2015 and affected bromeliads within species range, including tagged plants that were being monitored during our study. Although many plant species at the Espinhaço Mountain Range are resilient to fire (Silveira *et al.* 2016), bromeliads can have a low recovery rate (Alves, Rocha and Van Sluys 1996), abundance of plants can decrease dramatically after fire (Rocha *et al.* 1996) and there is a considerable loss of green biomass after being burnt (Ariani *et al.* 2004). In a coastal habitat of Brazil, frogs that are less dependent on bromeliads can experience a possible population recovery after a burn (Rocha *et al.* 2008), but fire can have a negative impact on bromeligenous frogs resulting in declining populations (Papp and Papp 2000). Because the abundance of the Itambé bromeliad frog is directly affected by habitat characteristics (Chapter 2), we believe that fire might have affected species abundance, population size and/or dynamic processes in a way that is still unknown (see Chapter 6 for details).

Overall, we found low values of extinction and colonization probabilities for the Itambé bromeliad frog, showing that monthly turnover rates, explained by elevation and season, occur slowly. These rates might indicate that the species is either territorial or has reduced dispersal capabilities – or even both. Indeed, we have not observed movement of individuals between bromeliads during our study, demonstrating the rarity of this type of event. Extinction probabilities exceeded colonization probabilities, which was also observed in previous studies of amphibians (Ray *et al.* 2016) and mammals (Farris *et al.* 2017). Studying the population dynamics of the Northern leopard frog, Randall *et al.* (2015) suggested that extinction rate was greater than colonization because some of the newly occupied sites were not capable of maintaining frogs throughout the year, which the authors attributed to conditions associated with summer and winter. The same could be true for the Itambé bromeliad frog if newly colonized sites are unable to maintain individuals, with sites going extinct at a higher rate than they are colonized.

Finally, the best fitting model included season as a good predictor of colonization and extinction rates (which was also true for fitting models at high elevation category). In our study area, seasons consist of markedly dry winters and wet summers (Silveira *et al.* 2016), which can change availability of sites. For amphibians, permanent and seasonal wetlands have notably different occupancy rates (Gould *et al.* 2012) and wetlands prone to drying can have a higher extinction probability (Mattfeldt, Bailey and Grant 2009). However, because of plant structure, bromeliads can store water in dry environments (Cogliatti-Carvalho *et al.* 2010) or dry season (Chapter 2), reducing the chances of bromeliad desiccation. Therefore, constant water availability over the dry season could reduce the effects of this predictor on extinction rates. On the contrary, season affected colonization rates and was slightly higher during the wet season. Peterman *et al.* (2013) suggested that dispersal costs of *Rana sylvatica* are lower in wet years, increasing survival/colonization rates. Increased colonization over the wet season might therefore indicate movement between bromeliads during periods of increased rainfall over summer, but unfortunately, there is no information on the dispersal of individuals of the Itambé bromeliad frog.

5.6 Conclusions

We present the first multi-season assessment of occupancy trends for a bromeligenous frog, restricted to a high elevation area in the Atlantic Rainforest of Brazil. Elevation was an important predictor of population dynamics, which might also be true for other bromeligenous frogs from tropical mountains in South America.

Colonization rates were positively related to elevation, and increased at higher elevation, and the opposite was found for extinction rates at medium and high elevation categories. Season can have different effects on amphibian population dynamics (Mattfeldt, Bailey and Grant 2009; Peterman *et al.* 2013; Randall *et al.* 2015; Ray *et al.* 2016) and, considering that season had a significant effect on colonization rates, changes in seasonal climatic patterns are expected to have an impact on this population. Nevertheless, the underlying factors driving colonization and extinction processes are still unknown for bromeligenous frogs and population dynamics are likely to be affected by fine scale processes related, for example, to habitat structure and local weather conditions. Given the reduced colonization and extinction rates from one month to the next, the Itambé bromeliad frog is likely to have limited dispersal capabilities.

Like other bromeligenous frogs, the Itambé bromeliad frog is restricted to high elevation areas and threatened with extinction (Sabagh, Ferreira and Rocha, 2017; Appendix I). However, population declines of rare and elusive species are hard to detect (Ward *et al.* 2017). Our results are based on a sampling design that aimed to detect moderate to large changes in occupancy with reliable statistical power (Chapter 4). Nonetheless, we were unable to detect significant population change, except at lower elevation sites where we found a declining trend in occupancy. We acknowledge that the relatively short time period monitored during our study may not be representative of longer population trends, but long-term studies investigating population fluctuations and declines are virtually absent in Brazil (Silvano and

Segalla 2005; Verdade *et al.* 2012), and similar studies describing changes in occupancy are unavailable for comparison.

Most notably, our results indicate that declines in occupancy might go unnoticed for species that are closely associated with elevation, unless they are investigated at a finer scale. For the Itambé bromeliad frog, population trends differed between elevation categories, showing signs of declines at a very limited elevational range. Therefore, for small-ranged species restricted to high elevations, downscaling the ecological processes driving colonization and extinction is recommended, especially if the aim is to detect subtle declines in a population.

5.7 Supplementary information

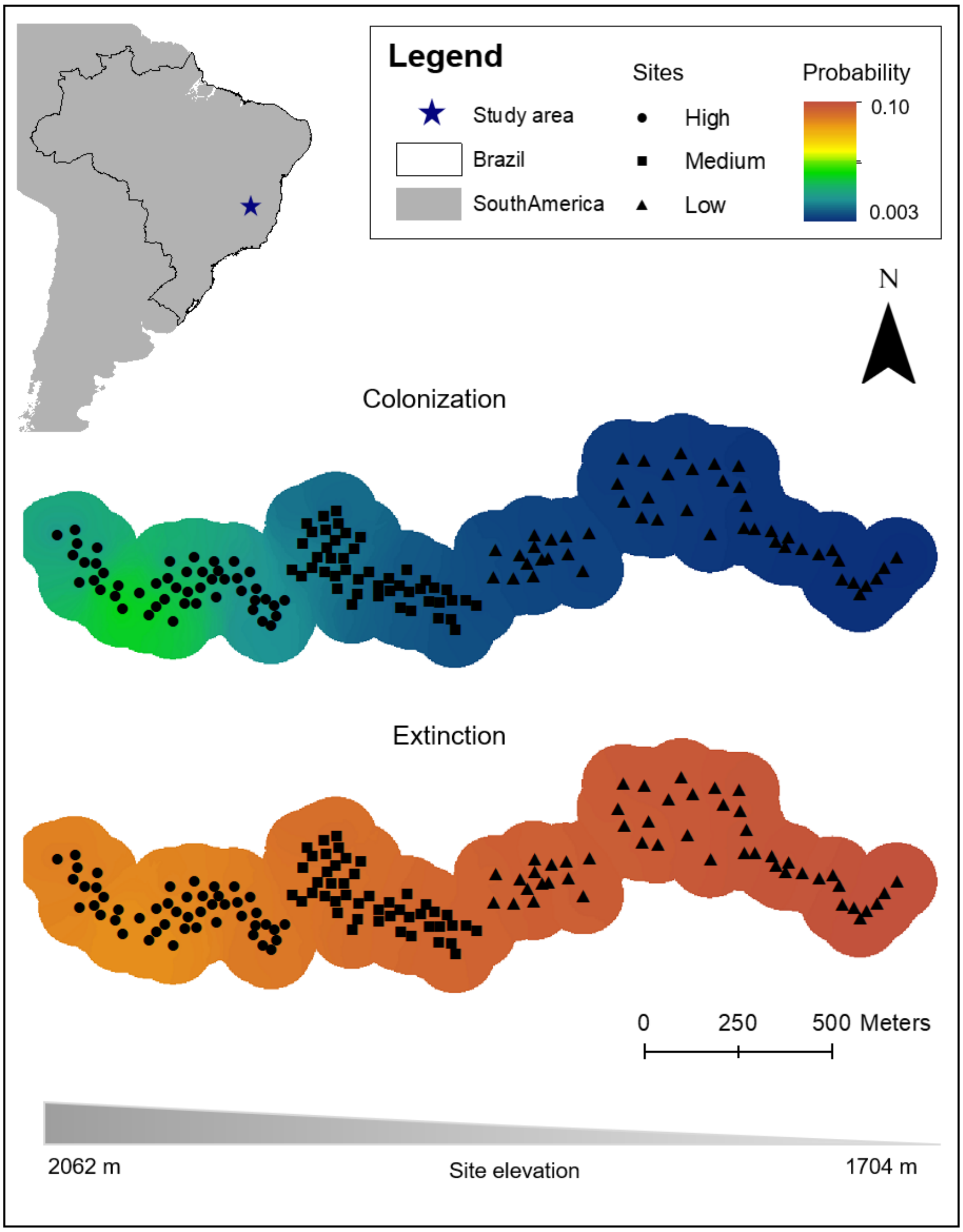

Figure S5.1 Mapped distribution of fitted probabilities of colonization and extinction for *Crossodactylodes itambe* at the Itambé summit, south-eastern Brazil

Fitted probabilities beyond sampled sites using spatial interpolation of colonization and extinction rates. Sampling sites are distributed at different elevation categories, given by meters above sea level: High (1998–2062 m), Medium (1838–1925 m) and Low (1704–1815 m).

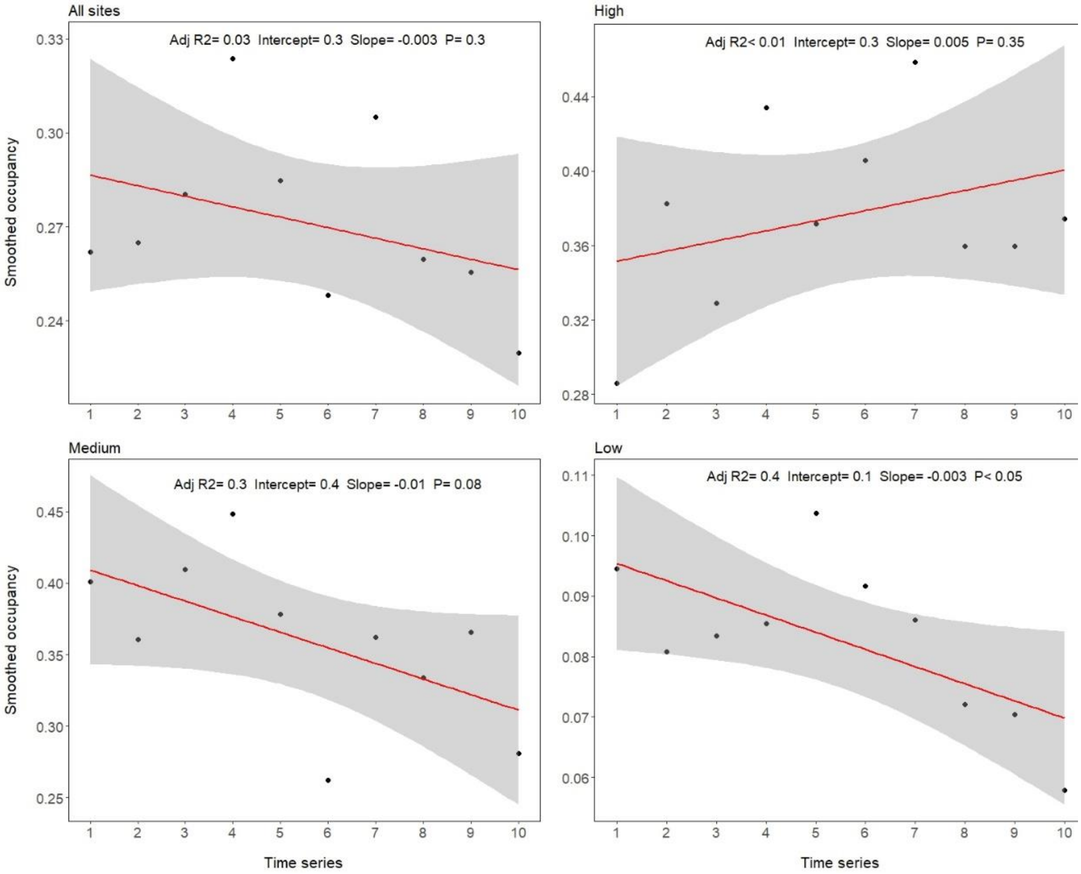

Figure S5.2 Linear regression model output for population trend of the Itambé bromeliad frog

Smoothed occupancy estimates are given as a function of surveyed month (time series) for all sites and at each elevation category (High, Medium, Low). Black dots are estimated values of occupancy, red line represents the direction of the linear trend and shaded area is giving 95% confidence interval.

Chapter 6 Drivers of population dynamics and effects of fire on a threatened amphibian species informed by occupancy modelling

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6.1 Abstract

The persistence of populations in time requires a balance in the dynamic process of extinction and colonization rates, which has been increasingly investigated using multi-season occupancy models. Such studies are rare for amphibians in tropical areas and are specially challenging for rare and range-restricted species. We explicitly modelled occupancy, colonization and extinction rates of an endemic bromeligenous frog, restricted to a single mountain in the Atlantic Rainforest of Brazil. Using a standardized sampling design, we describe the role of habitat structure and seasonal variation in local weather as factors affecting population dynamics. We also investigated the effects of fire on colonization and extinction rates, using data from pre- and post-fire events. Extinction was exclusively affected by seasonal variation in local weather conditions and the effects of relative humidity were not considered significant. Colonization was influenced by the characteristics of the habitat. Species showed preference to colonize bromeliads with larger tanks and individuals are more likely to find an appropriate site to be colonized when density of plants is high. Fire negatively affected population dynamics, significantly decreasing colonization rates in burnt sites. Habitat requirements needed to support a stable population are probably similar among other bromeligenous frogs. Negative responses of a fire event should be expected for other bromeligenous species, especially due to changes in plant structure.

Keywords: Bromeligenous frogs, Multi-season occupancy model, Population dynamics, fire.

6.2 Introduction

Species abundances and distributions vary in space and time, and the persistence of populations requires a balance between local extinction and colonization. Inferences on population dynamics can be achieved by explicitly modelling occupancy over multiple seasons, also providing estimates of extinction and colonization parameters when detection is imperfect (MacKenzie *et al.* 2003). This dynamic process is an important topic in ecology and there has been an increasing number of multi-season occupancy studies published over the past years, including for amphibians (Mattfeldt, Bailey and Grant 2009; Cayuela *et al.* 2012; Walls *et al.* 2013; Hamer *et al.* 2016; Johnson *et al.* 2016; Ray *et al.* 2016; Kay *et al.* 2017). However, studies on amphibian ecology and population dynamics, such as multi-season occupancy models, are still insufficient in tropical areas, especially when considering the effects of imperfect detection (Guimarães, Doherty Jr. and Munguía-Steyer 2014; Ficetola 2015).

Developing long-term ecological studies of rare, elusive and range-restricted species is challenging (Ward *et al.* 2017), but still extremely necessary. We designed a monitoring protocol to investigate patterns of occupancy, colonization and extinction for a micro-endemic species of frog, with a restricted population occurring at a high elevation site in the Atlantic Rainforest of Brazil. Our protocol was designed to detect changes in the population over time (Chapter 4), but it also allowed investigation of drivers of population dynamics. Our target species, the Itambé bromeliad frog (*Crossodactylodes itambe*), belongs to a rare group of bromeligenous frogs (sensu

Peixoto 1995) that are known to occupy bromeliads throughout their entire life cycle. *Crossodactylodes itambe* occupies a single species of bromeliad at elevations above 1700 m, with an estimated area of occurrence of < 0.5 km^2 (Barata *et al.* 2013). Because the species is restricted to bromeliads within a reduced geographical range, we used these plants as sampling sites, which makes our study system a good biological model to investigate extinction and colonization processes at a local scale.

Multi-season occupancy models can accommodate predictors that explain occupancy, extinction and colonization rates (MacKenzie *et al.* 2003), and results can support decision making and inform specific requirements for species persistence at small scales (Gould *et al.* 2012; Scherer, Muths and Noon 2012; Liang *et al.* 2017). Amphibian population dynamics varies strongly among species and across regions (Lehtinen and Witter 2014; Hossack *et al.* 2015; Wolf *et al.* 2016), and are influenced by different predictors (Mattfeldt, Bailey and Grant 2009; Peterman *et al.* 2013; Randall *et al.* 2015; Ray *et al.* 2016). For instance, occupancy of *C. itambe* increases with elevation and detectability is influenced by observer experience (Chapter 4), and the abundance of adult frogs is explained by the structure of the plant (Chapter 2). However, the dynamic processes of colonization and extinction were assumed to be primarily related to elevation (Chapter 5), needing further investigation.

We collected data one year before a fire burned part of our study area, and we continued data collection after the fire event, monitoring the same bromeliads, even if they were damaged by the fire. Because of our standard sampling design, we were able to gather pre- and post-fire data, presenting a rare opportunity to evaluate the impacts of a fire on population dynamics. Most studies evaluating fire effects on amphibians are either from temperate forests (Hossack and Corn 2007; Chelgren *et al.* 2011; Hossack, Lowe and Corn 2013), from low-intensity fire regimes (Driscoll and Roberts 1998; Russell, Lear and Guynn 1999; Schurbon and Fauth 2003; Bishop and Haas 2005; Gorman, Haas and Bishop 2009; Driscoll *et al.* 2012), or focused on aquatic ecosystems (Westgate *et al.* 2018). While some amphibians are resilient to wildfire (Hossack and Corn 2007; Westgate, Driscoll and Lindenmayer 2012; Westgate *et al.* 2018), other species can be negatively affected (Driscoll and Roberts 1998; Papp and Papp 2000; Schurbon and Fauth 2003). Although the effects of fire have been investigated for amphibian community composition (McCoy *et al.* 2013) and species richness (Drummond, Moura and Pires 2018), fire impact on amphibian occupancy and population dynamics is still poorly investigated (Hossack and Corn 2007; Gorman, Haas and Bishop 2009; Chelgren *et al.* 2011; Hossack, Lowe and Corn 2013).

In this study we aimed to describe the role of habitat structure and seasonal variation in local weather conditions as factors affecting the population dynamics of a mountaintop endemic bromeligenous amphibian species. We also investigated the effects of fire on colonization and extinction rates using data from pre- and post-fire events. We analysed species detection history using a multi-season occupancy model to obtain estimates of local extinction and colonization. Extinction refers to the probability that an occupied bromeliad in a given season was not used by frogs in the following season, and colonization refers to the probability that a bromeliad with no frogs in the previous season was being used by the species in the following season.

We hypothesized that (1) habitat structure and local weather conditions can affect local colonization and extinction rates; (2) estimated parameters of colonization and extinction differ significantly across different sites; and (3) fire can influence both rates, changing significantly after bromeliads being burnt.

6.3 Materials and methods

6.3.1 Study system and sampling design

This study was conducted at the Itambé summit, inside a protected area in south eastern Brazil (Pico do Itambé State Park, 18°23'S, 43°20'W), located at the southern portion of the Espinhaço Mountain Range and within the domains of the Atlantic Rainforest. The Itambé summit is the highest elevation recorded in the Espinhaço Range, at 2062 m above sea level (a.s.l.). Our study was conducted at the top of the mountain, above 1700 m in elevation, the only area where *C. itambe* is known to occur (Chapter 3). Species from the genus *Crossodactylodes* occur in high elevation areas of the Atlantic Rainforest and are likely to have very small geographical range. There is little information on the ecology of all five *Crossodactylodes* species: they are mostly known at type locality only, have small body sizes (usually up to 3 cm) (Peixoto 1982; Barata *et al.* 2013; Teixeira *et al.* 2013) and are defined as bromeligenous, as they occupy bromeliads throughout their life cycle without leaving the plant (sensu Peixoto 1995).

Bromeliads are arboreal and terrestrial flowering plants, widespread in the Neotropics (Benzing 2000), with a unique leaf structure that allows water to be collected in a central tank, providing shelter and resources to several different species, including many amphibians. To the best of our knowledge, *C. itambe* uses a single species of bromeliad, *Vriesea medusa* (Barata *et al.* 2013; Santos *et al.* 2017), which is also endemic to the Espinhaço Range and known to occur in only two localities (Versieux and Wendt 2007). At the Itambé summit, bromeliads are very abundant and grow in a rocky substrate in open humid areas at high elevations. Considering the small size of *C. itambe* (Barata *et al.* 2013), its specific habitat requirements (Chapter 2), and presumed low dispersal capabilities (Chapter 5), we considered individual bromeliads as sampling sites and we marked plants with an identification number that allowed repeated visits over consecutive nights. Sampling sites were at least 25 m apart from each other to ensure independence between visits. We marked a total of 143 sites (i.e., individual bromeliads), starting at 1700 m a.s.l. to the top of the summit at 2062 m a.s.l.

We monitored the population using the same protocol over two consecutive years (2015 and 2016), during which we sampled the same habitat and weather variables. Sampling was carried out during four months in each year (Feb–May 2015; Feb–Jun 2016), during wet and dry seasons. Monthly surveys were separated by 20–30 days. Because *C. itambe* is active at night (Barata, Griffiths and Ferreira 2018), we searched for frogs using a visual encounter survey, starting after dusk using two teams of two observers. Observer expertise can affect species detection probabilities (Chapter 4) and therefore only one observer was allowed to survey the species, while the other was taking notes. To create a detection history, each month of survey consisted of 4–6 consecutive visits to the same sampling sites, and all sites were surveyed over the same night. Although bromeliads can be shared by adult frogs and tadpoles (Santos *et al.* 2017), we defined species presence by the occurrence of one or more adults occupying a site.

6.3.2 Multi-season occupancy model for a single species

We used a multi-season occupancy modelling (hereafter, dynamic occupancy model) for a single species, where seasonal changes in the probability of occupancy (ψ), colonization (γ) and extinction (ε) are explicitly modelled, while accounting for detectability (MacKenzie *et al.* 2003). Detectability is the probability of a species being detected at a site if that site is indeed occupied and provides a balanced estimate of occupancy and dynamic parameters. While occupancy is given by the probability of a randomly selected site being occupied by a species, colonization and extinction rates take into consideration the dynamic processes representing probabilities of a site changing between being occupied and unoccupied over consecutive seasons. Specifically, colonization is defined as the probability of an unoccupied site at time t becoming occupied in the following season, $t + 1$, and extinction is the probability of a site previously occupied during season t becoming unoccupied at $t + 1$ (MacKenzie *et al.* 2003).

Our study system provides a good opportunity to develop a dynamic model with a robust design that reduces the likelihood of violating the statistical assumptions of the model. Dynamic occupancy models assume that the population is closed during surveys (defined as secondary sampling period), but open between seasons (defined as primary sampling period) (MacKenzie *et al.* 2003). For our population, night visits represent the secondary sampling period and we assumed there was no immigration and/or emigration within our 4–6 visits in the same month. However, we assumed that individuals might have moved from one bromeliad to another between surveyed months. We considered each month as a unique season (i.e., primary sampling period) and we added missing values for months we did not survey to avoid misleading estimates of monthly extinction and colonization rates. We therefore had a total of 16 seasons, of which we surveyed four months at each year (i.e., eight surveyed seasons). For each season, we created a detection history of presence/absences of adults at a site by recording whether the site was occupied, with one or more detections of adult frogs (1), and/or unoccupied, with no detections (0) during consecutive visits.

The dynamic model can incorporate information from different predictors which are used to explain estimated parameters. A predictor can be (1) site-specific, when it is characteristic of the site and remains constant within seasons (used to model detection, occupancy, colonization and extinction parameters); (2) survey-specific, when information varies from one visit to the other (used to explain changes in detectability); and (3) season-specific, if it varies between seasons (used to model local colonization and extinction rates). We collected data on variables related to location of the bromeliad and the structure of the plant (site-specific habitat predictors), as well as related to local weather (season-specific climatic predictors). To characterize weather conditions, we used data from the closest weather station, located 35 km from our study area. The weather station automatically recorded daily climatic data and we used the averaged values for each surveyed month. We used the following weather predictors: mean air temperature (^{o}C), maximum air temperature (^{o}C), minimum air temperature (^{o}C), relative humidity (%), and rainfall (mm) (Table S6.1).

We had eight habitat predictors: elevation, number of neighbouring bromeliads, size of plant, size of central tank, number of leaves in a bromeliad, water temperature, air temperature, and density of bromeliads (Table S6.1). Elevation is given in metres a.s.l. and was recorded with a barometric GPS at each sampling site. Due to the reproductive characteristics of the bromeliad, marked sites usually had a varying number of neighbouring bromeliads, forming a patch of several conspicuous plants. Thus, number of neighbours at each site is given by a total count of individual bromeliads touching the edge of the marked plant (we considered an individual central tank as a unique plant). Plants also varied in size and according to the size of the central tank – both measures given by height x width in centimetres. We used size of central tank as a proximate value for the volume of water potentially retained by the tank (hereafter, volume of central tank). Number of leaves in a plant is given by the average number of leaves per plant in each year. We also measured air and water temperature in every site and during all surveyed seasons, and we calculated average values to characterize each site. Finally, we calculated the density of plants at each site by recording the total number of bromeliads occurring within 3 m of a marked plant.

We used presence/absence of tadpoles as a fixed variable (i.e., this predictor was included in every model of colonization and extinction). We assumed that tadpole presence/absence would improve model fit and could potentially influence both extinction and colonization rates. For *C. itambe*, tadpoles are known to share bromeliads with adults and juveniles (Santos *et al.* 2017), and for every visit, we recorded whether the site was occupied or unoccupied by one or more tadpoles. We calculated the frequency of records at sites where tadpoles were recorded. Tadpoles were considered to be present at a site if this frequency exceeded 10%, and absent otherwise. A summary of predictors used to estimate biological parameters of occupancy, colonization and extinction is given in Table S6.1.

6.3.3 Data analysis framework and model fitting

Statistical analyses were performed in R (R Core Team 2017). We fitted models with standardized predictors and we tested correlation of continuous variables using a Pearson correlation test with the Hmisc package (Harrell 2017). Predictors were considered correlated when Pearson's $r > 0.6$ and, in such cases, we used only the variable of greater ecological meaning and ease to interpret. Number of leaves in a bromeliad was positively correlated with plant size ($r = 0.65$), and elevation was negatively correlated with site-specific air temperature ($r = -0.89$) (Figure S6.1). We therefore kept size and elevation only, excluding number of leaves and air temperature from model fitting. We used a stepwise model selection to gradually increase model complexity, fitting covariates for each biological parameter separately and performing model selection using Akaike's Information Criterion (AIC). We ranked models by their AIC (models with the lowest values having the best fit) and weighted as the probability of being the best model in the set, indicating relative support of a model. We selected best models based on ΔAIC: models with ΔAIC ≤ 2 had strong support while models with ΔAIC ≥ 2 were considered to have less support (Burnham and Anderson 2002).

We first fitted models that included predictors of detectability only, leaving other biological parameters (occupancy, colonization and extinction) constant. We used observer as a predictor of detection (Chapter 4), but we also fitted models with time of observation (given by minutes after dusk) and Julian date (Table S6.1). Next, using the best predictor of detection from the model with the lowest AIC value, we fitted models to estimate initial occupancy, keeping colonization and extinction constant. For occupancy we used elevation and plant size, fitting models with elevation only and in combination with size, including the interaction of both variables (Chapter 2). Finally, using the best predictor of detection and the best predictor of occupancy based on the model with the lowest AIC value, we fitted models for colonization and extinction and we used variables related to habitat structure and local weather conditions.

For colonization and extinction, we first fitted three sets of models containing: (1) all variables relating to habitat structure (number of neighbours, size of plant, volume of central tank, water temperature, and density of bromeliads); (2) all variables relating to local weather (mean air temperature, maximum air temperature, minimum air temperature, relative humidity and rainfall); and (3) a combination of all habitat and weather variables. With this approach, we were interested to find the best structure of the model affecting each estimated parameter. We selected the best fitting model structure based on the lowest AIC values and we then fitted colonization and extinction with specific predictors (i.e., variables of either habitat structure or weather condition). We then fitted extinction first and colonization second (Gálvez *et al.* 2018) and to avoid overfitting (Burnham and Anderson 2002) we added only one variable each for colonization and extinction. Predictors of both colonization and extinction were compared based on AIC values and best fitting models were selected with ΔAIC < 2. To build our final set of models, we combined the best predictors of detection and occupancy with the variables that were best at explaining extinction and colonization. Final model selection also included a constant model (i.e., with no variables) for comparison.

We investigated the effects of fire on population dynamics by adding this variable into our final set of best fitting models. Fire was considered as a site-specific covariate and bromeliads could be classified as either affected or not affected by fire. We incorporated fire as a predictor of colonization and extinction rates, and we did a model selection comparing models from the final set of best fitting models (without fire) and models including fire as a predictor of population dynamics. Finally, we predicted values for colonization and extinction at each site based on best fitting model using the predict function. The predict function returns estimates of parameters at specific values, enabling us to plot predicted colonization and extinction rates against the variables indicated by model selection. Dynamic occupancy models and analyses (model fit, model selection and predictions) were made using the package Unmarked (Fiske and Chandler 2011; Kéry and Chandler 2012).

6.4 Results

Tadpole presence/absence improved model performance and affected estimates of colonization and extinction (Figure 6.1). Presence of a tadpole inside a bromeliad increased colonization rates, which was lower (γ = 0.10) when tadpoles were absent (Figure 6.1). In contrast, tadpole absence increased extinction probabilities and was close to 35% when tadpoles were not present (Figure 6.1).

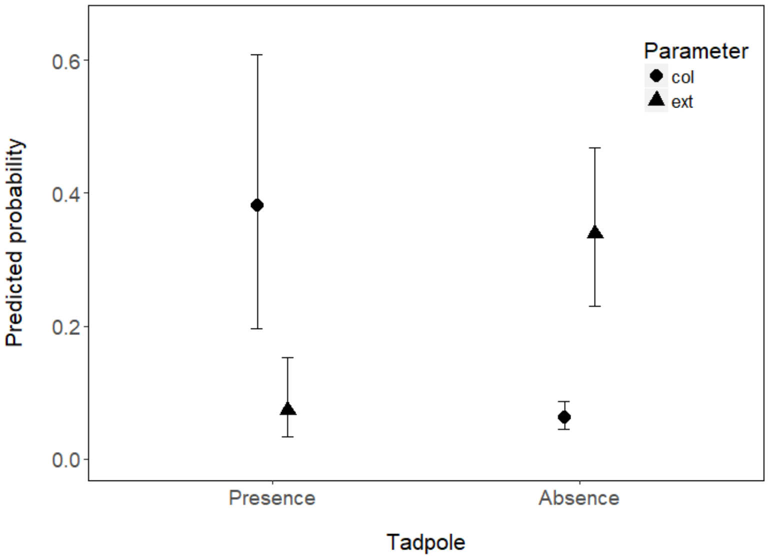

Figure 6.1 Predicted colonization and extinction for *Crossodactylodes itambe* in relation to the presence and absence of tadpoles
Probabilities of colonization (col) and extinction (ext) are based on the best fitting model: ψ(elev*size) γ(dens+vol) ε(rh) p(obs) with tadpole used as fixed predictor of colonization and extinction rate. Vertical bars represent 95% confidence intervals.

The best fitting model for detectability included the observer (Table 6.1), with significant differences in detection in relation to experience (Figure 6.2). Using observer as a fixed predictor for detection, we found that occupancy was mostly influenced by elevation and its interaction with bromeliad size (Table 6.1). Initial occupancy (0.23 ± 0.04) was positively correlated with elevation, increasing significantly at higher elevation sites (Figure 6.3).

Table 6.1 Best fitting models with predictors of detection, occupancy, colonization and extinction for *Crossodactylodes itambe* at the Itambé summit

Results for the top models during a stepwise procedure for the probabilities of detection (p), occupancy (ψ), colonization (γ) and extinction (ε). Pars = number of parameters; AIC = Akaike's Information Criterion; ΔAIC is the difference between the model with the lowest AIC and the given model; AIC w is AIC weight (see Table S6.1 for predictors included in each model).

Model	**Pars**	**AIC**	**ΔAIC**	**AIC *w***	**Cumulative weight**
Detection model with constant parameters					
ψ(.) γ(.) ε(.) p(obs)	6	2474.1	0	1.00	1.00
ψ(.) γ(.) ε(.) p(hour)	5	2490.81	16.71	0.00	1.00
Occupancy model fitted with p(obs) and constant colonization and extinction					
ψ(elev*size) γ(.) ε(.) p(obs)	9	2458.34	0	0.55	0.55
ψ(elev) γ(.) ε(.) p(obs)	7	2459.38	1.03	0.33	0.88
ψ(elev+size) γ(.) ε(.) p(obs)	8	2461.37	3.02	0.12	1.00
*Extinction fitted with fixed ψ(elev*size) p(obs) and constant colonization*					
ψ(elev*size) γ(.) ε(rh) p(obs)	11	2394.22	0	0.56	0.56
ψ(elev*size) γ(.) ε(rain) p(obs)	11	2397.11	2.89	0.13	0.69
*Colonization fitted with fixed ψ(elev*size) p(obs) and constant extinction*					
ψ(elev*size) γ(dens) ε(.) p(obs)	11	2388.57	0	0.41	0.41
ψ(elev*size) γ(vol) ε(.) p(obs)	11	2389.02	0.45	0.33	0.75
ψ(elev*size) γ(neig) ε(.) p(obs)	11	2391.31	2.74	0.11	0.85
Final model set					
ψ(elev*size) γ(dens+vol) ε(rh) p(obs)	14	2365.16	0	0.68	0.68
ψ(elev*size) γ(dens) ε(rh) p(obs)	13	2367.53	2.37	0.21	0.89
ψ (elev*size) γ(vol) ε(rh) p(obs)	13	2368.88	3.72	0.11	1.00

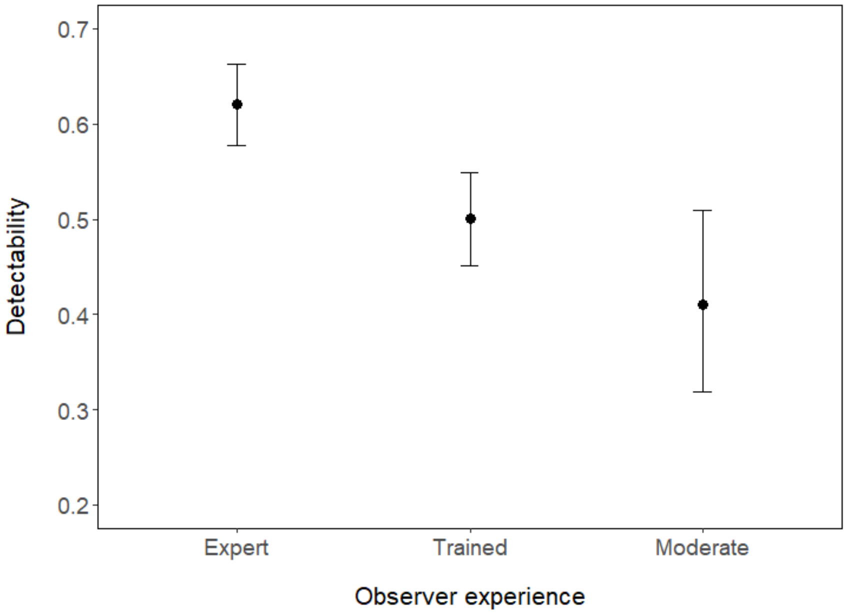

Figure 6.2 Detectability of *Crossodactylodes itambe* in relation to observer experience Predicted probability of detection is based on best fitted model: ψ(elev*size) γ(dens+vol) ε(rh) *p*(obs). Expert = high level of experience, > 2 years; Trained = medium level of experience, trained for 2 years; Moderate = moderate level of training, < 2 years of experience. Vertical bars represent 95% confidence intervals for the estimated parameter.

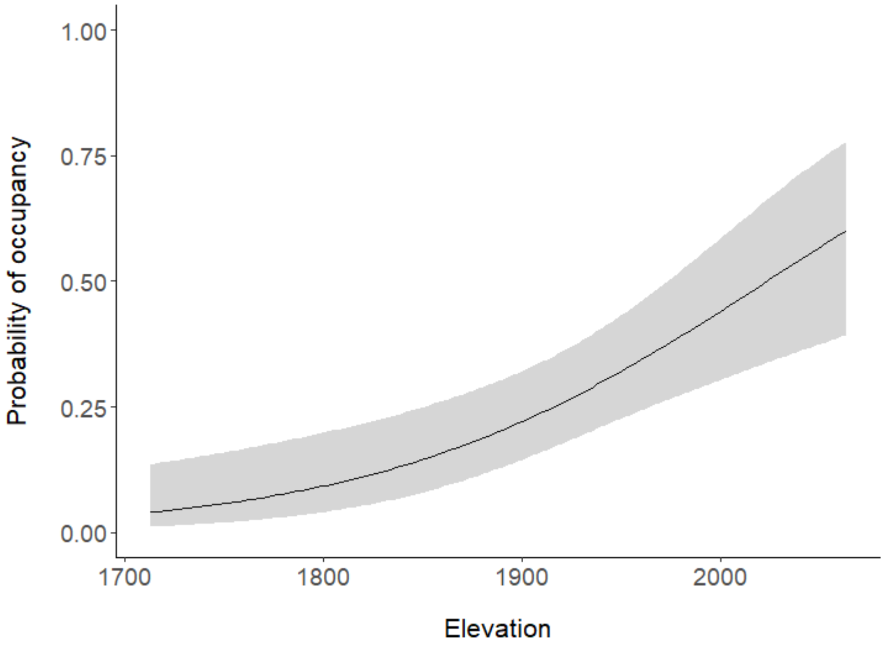

Figure 6.3 Probability of occupancy for *Crossodactylodes itambe* in relation to elevation Predicted values of occupancy are based on best fitting model: ψ(elev*size) γ(dens+vol) ε(rh) *p*(obs). Elevation is given in meters above sea level and shaded areas represent 95% confidence intervals of predicted values.

When comparing different sets of models for extinction, we found that 78% of the support in the data was from models that included seasonal changes in local weather (Table 6.2) and we therefore fitted extinction models with weather predictors only. For colonization, the best fitting model included habitat structure, showing high support in explaining the data (Table 6.2). We therefore fitted colonization with habitat predictors only.

Table 6.2 Model structure for extinction and colonization rates of *Crossodactylodes itambe* at the Itambé summit

Predictors of seasonal weather conditions (climate) and habitat structure (habitat) for colonization (γ) and extinction (ε) probabilities of *Crossodactylodes itambe*. Pars = number of parameters; AIC = Akaike's Information Criterion; ΔAIC is the difference between the model with the lowest AIC and the given model; AIC *w* is AIC weight, ψ = occupancy, *p* = detection (see Table S6.1 for predictors included in each model).

Model	**Pars**	**AIC**	**ΔAIC**	**AIC *w***	**Cumulative weight**
Model structure for extinction					
ψ(elev*size) γ(.) ε(climate) *p*(obs)	15	2399.33	0	0.78	0.78
ψ(elev*size) γ(.) ε(habitat) *p*(obs)	15	2402.17	2.84	0.19	0.97
ψ(elev*size) γ(.) ε(climate+habitat) *p*(obs)	20	2405.9	6.57	0.03	1.00
ψ(elev*size) γ(.) ε(.) *p*(obs)	9	2458.34	59.01	< 0.01	1.00
ψ(.) γ(.) ε(.) *p*(obs)	6	2474.1	74.76	< 0.01	1.00
ψ(.) γ(.) ε(.) *p*(.)	4	2493.34	94	< 0.01	1.00
Model structure for colonization					
ψ(elev*size) γ(habitat) ε(.) *p*(obs)	15	2387.61	0	1.00	1.00
ψ(elev*size) γ(.) ε(.) *p*(obs)	9	2458.34	70.74	< 0.01	1.00
ψ(.) γ(.) ε(.) *p*(obs)	6	2474.1	86.49	< 0.01	1.00
ψ(.) γ(.) ε(.) *p*(.)	4	2493.34	105.73	< 0.01	1.00
ψ(elev*size) γ(climate) ε(.) *p*(obs)	15	2540.76	153.15	< 0.01	1.00
ψ(elev*size) γ(climate+habitat) ε(.) *p*(obs)	20	2544.73	157.12	< 0.01	1.00

We fitted 29 models, including a constant model. Keeping colonization constant and including weather predictors for extinction, we found that the cumulative AIC weight of best fitting model for extinction rates was 0.56 (Table 6.1) and only included relative humidity. For colonization cumulative AIC weight of best fitting models was 0.75, which also had ΔAIC < 2 (Table 6.1) and included density of bromeliad and volume of central tank.

Using predictors from the best supported models of detection and occupancy, we combined the best fitted predictors of extinction (relative humidity) and colonization (volume and density). The final set of top ranked models showed that relative humidity is important for extinction probabilities, and colonization was mostly influenced by the combination of volume and density (Table 6.1), which resulted in similar estimates of colonization (γ = 0.58). Colonization rates had a significant influence of habitat predictors and increased with both density and volume of the central tank (Figure 6.4). Although relative humidity was not considered significant (P = 0.1), models including this predictor performed better than models with constant extinction probabilities (i.e., models with no predictors of extinction).

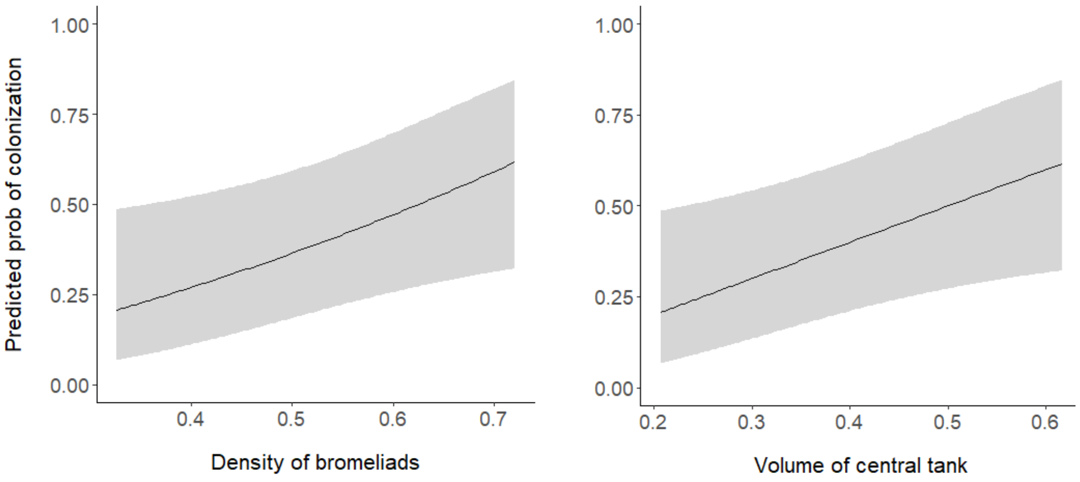

Figure 6.4 Predicted probability of colonization for *Crossodactylodes itambe* in relation to habitat structure
Probability (prob) of colonization in relation to density of bromeliads (left) and volume of central tank (right). Predicted values are based on best fitting model: ψ(elev*size) γ(dens+vol) ε(rh) p(obs). Shaded areas represent 95% confidence intervals.

To evaluate the effects of fire on population dynamics, we fitted 73 models including fire as a predictor of both colonization and extinction rates. Models with fire performed better than models without the effects of this variable, especially for colonization (Table 6.3). Although best fitting model did not include the effects of fire on extinction rates, this predictor was included in fitting models considered to have a good support (i.e., ΔAIC < 2; Table 6.3).

Table 6.3 Best fitting models with the effects of fire on colonization and extinction rates of *Crossodactylodes itambe* at the Itambé summit

Model selection results including the effect of fire as predictor of colonization (γ) and extinction (ε) probabilities for *Crossodactylodes itambe*. Pars = number of parameters; AIC = Akaike's Information Criterion; ΔAIC is the difference between the model with the lowest AIC and the given model; AIC *w* is AIC weight, ψ = occupancy, *p* = detection (see Table S6.1 for predictors included in the model).

Model	**Pars**	**AIC**	**ΔAIC**	**AIC *w***	**Cumulative weight**
ψ(elev*size) γ(fire+dens+vol) ε(rh) *p*(obs)	15	2355.77	0	0.27	0.27
ψ(elev*size) γ(fire+dens+vol) ε(fire+rh) *p*(obs)	16	2356.42	0.65	0.19	0.46
ψ(elev*size) γ(fire+dens) ε(rh) *p*(obs)	14	2356.64	0.88	0.17	0.63
ψ(elev*size) γ(fire+vol) ε(rh) *p*(obs)	14	2356.99	1.23	0.14	0.77
ψ(elev*size) γ(fire+dens) ε(fire+rh) *p*(obs)	15	2357.22	1.45	0.13	0.9
ψ(elev*size) γ(fire+vol) ε(fire+rh) p(obs)	15	2357.81	2.04	0.096	0.99

Extinction rates, however, were not statistically different and fire, combined with relative humidity, did not seem to affect extinction rates in unburnt sites (P = 0.2) (Figure 6.5). Fire had a significant negative effect on colonization rates, which decreased in burnt bromeliads (Figure 6.5). Overall, predicted colonization rates decreased in bromeliads damaged by fire and increased in plants with larger central tanks (Figure 6.6).

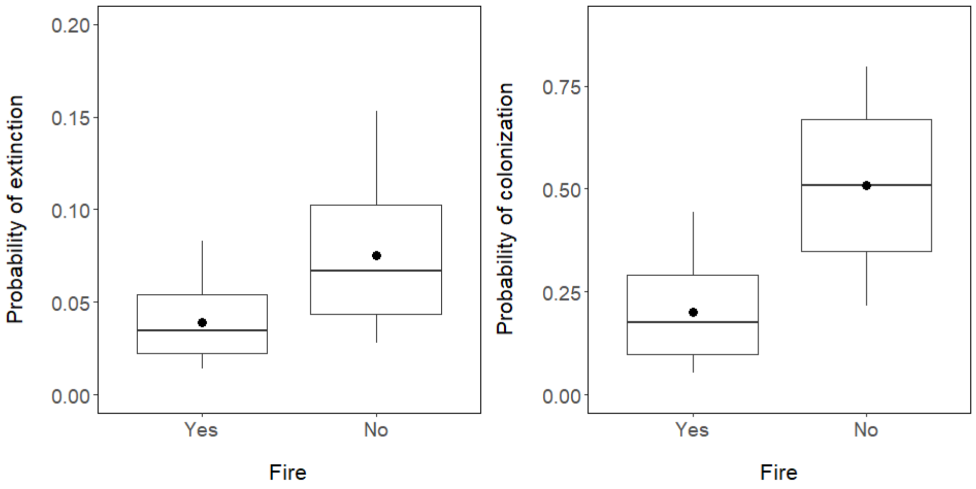

Figure 6.5 Differences in the predicted probability of colonization and extinction for *Crossodactylodes itambe* in relation to fire

Bromeliads were either affected (yes) or not affected (no) by the fire. Predicted values were based on the dynamic model: ψ(elev*size) γ(fire+dens+vol) ε(fire+rh) *p*(obs), showing median values (horizontal bars), mean values (blue dots) and maximum and minimum range (vertical bars).

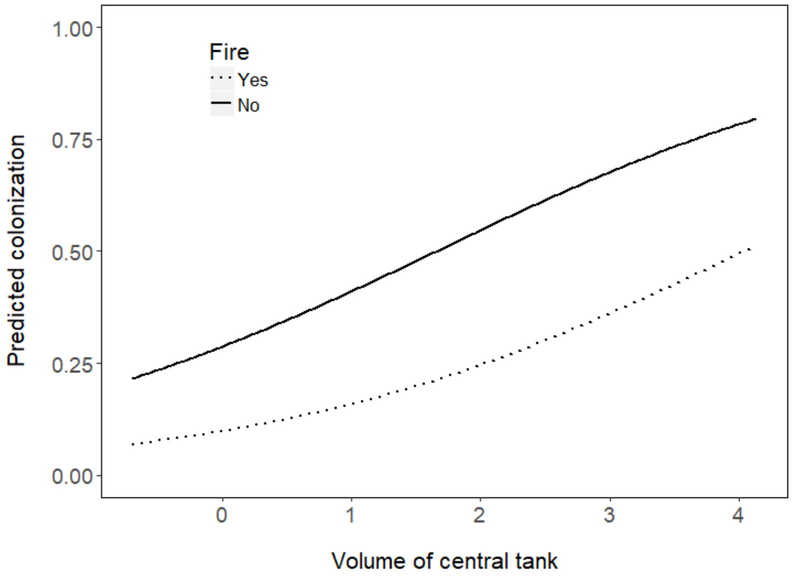

Figure 6.6 Predicted colonization rates for *Crossodactylodes itambe* in relation to volume of central tank, for bromeliads affected by the fire

Predicted values were based on best fitting dynamic occupancy model: ψ(elev*size) γ(fire+dens+vol) ε(rh) *p*(obs). Bromeliads were either affected (yes) or not affected (no) by the fire.

6.5 Discussion

6.5.1 Drivers of population dynamics

For *Crossodactylodes itambe* different sets of predictors can drive population dynamics in different ways. While extinction was exclusively affected by seasonal variation in local weather conditions, colonization was mostly influenced by the characteristics of the habitat. Habitat and landscape characteristics are important predictors of amphibian population dynamics (Werner *et al.* 2009; Gould *et al.* 2012; Anderson *et al.* 2015; Hamer *et al.* 2016; Kay *et al.* 2017) as well as seasonality and hydroperiods (Mattfeldt, Bailey and Grant 2009; Walls *et al.* 2013; Randall *et al.* 2015). For amphibian breeding dynamics, models incorporating climate drivers outperformed models of those that were exclusively habitat based (Ray *et al.* 2016). For the abundance of *C. itambe*, the structure of bromeliads was more important than local climate (Chapter 2) and we found that colonization rates were also less responsive to seasonal weather variation than extinction, a pattern that was also observed for other amphibian species in montane areas (Ray *et al.* 2016).

Colonization increased with bromeliad density and in bromeliads with larger tanks, which could be partially explained by the strict life cycle and habitat requirements of *C. itambe,* suggesting that larger plants are better habitat for the species. Preference for clustered bromeliads by bromeligenous frogs was previously attributed to the increased availability of tanks used as oviposition sites (Oliveira and Navas 2004; Cunha and Napoli 2016). In our study area, the density of *V. medusa* is remarkably high. While in low areas we recorded about three bromeliads per 100 m^2, at medium and higher elevations density was 20–30 bromeliads per 100 m^2. Increased plant density at higher elevations facilitates colonization of new sites, possibly during the wet season when colonization rates for *C. itambe* are higher (Chapter 5). The number of bromeliads available nearby an occupied plant is therefore crucial for colonization and individuals are more likely to find an appropriate site to be colonized when density of bromeliads is high.

Larger tanks can hold water during periods of drought (Cogliatti-Carvalho *et al.* 2010) and provide more nutrients for tadpole development (Lehtinen 2004). *Vriesea medusa* has a large funnel-shaped central tank (Versieux 2008) which can retain large volumes of water. Abundance of *C. itambe* is affected by the volume of the central tank (Chapter 2) and, not surprisingly, colonization rates increased in bromeliads with larger tanks that can store large volumes of water and are less susceptible to drying out. In contrast, volume had no effect on extinction rates, perhaps because frogs remain in the bromeliad even if the water level is low – a conclusion that is reinforced by the fact that extinction rates do not change during the dry season (Chapter 5). Reproduction might fail if the bromeliad cannot hold water for a sufficient period during the dry season and our results indicate that the species prefers larger tanks, which allows the colonization of a new site.

Extinction rates of *C. itambe* were related to seasonal variation in local weather. Differences in climatic conditions have been used to explain amphibian occupancy, population dynamics and breeding probabilities (Werner *et al.* 2009; Cayuela *et al.* 2016; Ray *et al.* 2016), and are usually associated with seasonality and hydroperiods (Mattfeldt, Bailey and Grant 2009; Walls *et al.* 2013; Randall *et al.* 2015; Hamer *et al.* 2016; Basile *et al.* 2017). Although periods of reduced rainfall can increase extinction rates of frogs (Cayuela *et al.* 2012), the structure of the bromeliad allows water to be retained during dry seasons, probably reducing the effect of this predictor on extinction rates. Temperature and humidity can also affect reproduction and population dynamics of reptiles and amphibians (Walther *et al.* 2002). In our study area, we observed that relative humidity was constantly close to 80% at sites at higher elevation, and air temperature was about 2 degrees lower on sites between 1800 to 2060 m a.s.l. when compared to sites located below 1800 m in elevation. However, we found that the effects of relative humidity on extinction rates of *C. itambe* were not significant.

It is worth emphasizing that extinction rates do not necessarily represent an extinction of the local population, instead they can reflect reproductive failures at a particular site. Most importantly, our result shows that the weather conditions in a season can affect the occupancy state of a site in the following period, increasing or decreasing extinction rates according to previous variations in weather. This demonstrates the importance of stable local weather conditions to the population dynamics of *C. itambe*, and possibly to other bromeligenous frogs. This is particularly worrying for tropical montane species which are severely threatened by climate change (Parmesan 2006) – as climate regulates vegetation structure, the endemic flora of the Espinhaço Range could be dramatically reduced due to global warming (Bitencourt *et al.* 2016).

6.5.2 Effects of fire on habitat structure and colonization rates

Colonization rates of burnt bromeliads were significantly lower than in unburnt sites. Habitat characteristics were fundamental for successful colonization and fire can therefore have an immediate/direct effect on this population by influencing the number and density of plants available or changing the structure of the plant. Overall, amphibian responses to fire are mostly related to the frequency of burns in the ecosystem and changes in vegetation structure (Schurbon and Fauth 2003; Westgate, Driscoll and Lindenmayer 2012; Hossack, Lowe and Corn 2013; McCoy *et al.* 2013), such as density of breeding sites (Westgate *et al.* 2018). McCaffery et al. (2015) showed the importance of habitat heterogeneity to population dynamics of a pond-breeding amphibian, demonstrating that loss of pond habitat decreased population growth rate. In our study area, the fire of 2015 affected 80% of the protected area and about 33% of our sites, destroying breeding habitats and reducing habitat quality, especially at lower elevation.

Density of bromeliads can change considerably after a fire event (Rocha *et al.* 1996; Ariani *et al.* 2004), which can partially explain the effects of fire on colonization rates of *C. itambe*, since density of plants had a positive effect on colonization. In a coastal area of Brazil, fire not only reduced the number of bromeliads available (Rocha *et al.* 1996; Ariani *et al.* 2004), but also decreased density to 30% 15 months after the fire event (Alves, Rocha and Van Sluys 1996). Changes in the density may inhibit movement and reduce opportunities for colonizing new sites, potentially affecting patterns of occupancy over time and partially explaining declines detected in the population at lower elevations (Chapter 5), where most bromeliads were burnt. Moreover, fire can directly damage the structure of the bromeliad, negatively affecting the volume of water retained by the plant. After a fire event, bromeliads from the genus *Vriesea* can reduce volume of water by approximately 28% (Alves, Rocha and Van Sluys 1996), which could affect not only colonization rates, as we found in our results, but also species abundance (Chapter 2).

We observed that burnt bromeliads still retained some water, which can prevent the complete destruction of the plant structure (Ariani *et al.* 2004), allowing the bromeliad to act as a refuge for frogs during the fire. Although bromeliads can recover in size one year after a fire, complete recovery can be slow with no substantial formation of new tanks 15 months post-burn (Alves, Rocha and Van Sluys 1996). Furthermore, frequent fires are likely to kill plants or at least prevent them to reach larger sizes, which are both detrimental for *C. itambe*. Recolonization of sites will be dependent on a successful recovery of the bromeliad population combined with a gradual increase in colonization rates from individuals that survived the fire and/or colonized areas that were not affected. In our case, surviving bromeliads played an essential role in sustaining the population of *C. itambe*, offering suitable conditions for future recolonization and species persistence. For this species, in particular, caution is needed to avoid frequent fires in locations that support high densities of bromeliads, especially at mid and high elevations.

6.5.3 Conservation implications for montane bromeligenous frogs

Species response to fire impacts may vary according to species traits and life history (Westgate, Driscoll and Lindenmayer 2012). For example, frogs that use bromeliads for shelter only are likely to recover after a fire event, because they are not completely dependent on bromeliads for reproduction (Rocha *et al.* 2008). In contrast, the structure of the central tank might be unsuitable after the fire, drastically reducing the population of bromeligenous frogs from a coastal area of Brazil (Papp and Papp 2000). Although colonization and extinction rates might differ among species, bromeligenous frogs share a unique life history and the habitat requirements needed to support stable populations of other endemic mountaintop species are likely to be similar to our findings. Therefore, for bromeligenous frogs, conservation actions should focus on maintaining habitat integrity, mainly by conserving plant structure and local density.

Overall, our data provide evidence of the impacts that habitat structure and weather fluctuations have on local population dynamics of this endemic bromeligenous frog. Habitat structure affected colonization rates, demonstrating the close dependency of bromeligenous frogs on habitat quality and bromeliad availability. Extinction, on the other hand, was exclusively affected by seasonal weather variation, showing that a stable climatic condition is an important feature for species persistence. Because bromeligenous frogs are extremely dependent on bromeliads for reproduction, negative responses to changes in bromeliad structure and density should also be expected for other species with similar requirements. This was confirmed by the negative effect that fire had on the population dynamics of *C. itambe*, which can be considered an ongoing threat to this endemic amphibian. Considering the dynamic processes ruling this montane bromeligenous frog, habitat conservation should be a priority action when compared to climate change mitigations in the short term.

6.6 Supplementary information

Table S6.1 Predictors of occupancy, colonization, extinction and detection included in a dynamic occupancy model for *Crossodactylodes itambe*

Predictors related to sampling occasion (survey-specific), habitat structure (site-specific) and local weather conditions (season-specific) evaluated when modelling biological parameters of occupancy (ψ), colonization (γ), extinction (ε) and detection (*p*). Fixed variables were included in all fitted models for colonization and extinction.

Predictor	Parameter	Abbreviation in models
Survey specific		
Time of observation	*p*	hour
Julian date	*p*	date
Observer	*p*	obs
Site-specific		
Elevation	ψ, γ, ε	elev
Density of bromeliads	ψ, γ, ε	dens
Number of neighbours	ψ, γ, ε	neig
Number of leaves	ψ, γ, ε	leaf
Volume of central tank	ψ, γ, ε	vol
Size of bromeliad	ψ, γ, ε	size
Water temperature	ψ, γ, ε	wtr
Air temperature	ψ, *γ*, *ε*	air
Season-specific		
Mean temperature	γ, ε	temp
Minimum temperature	γ, ε	minT
Maximum temperature	γ, ε	maxT
Relative humidity	γ, ε	rh
Accumulated rainfall	*γ*, *ε*	rain
Fixed variables		
Tadpole presence/absence	γ, ε	tad
Fire presence/absence	*γ*, *ε*	fire

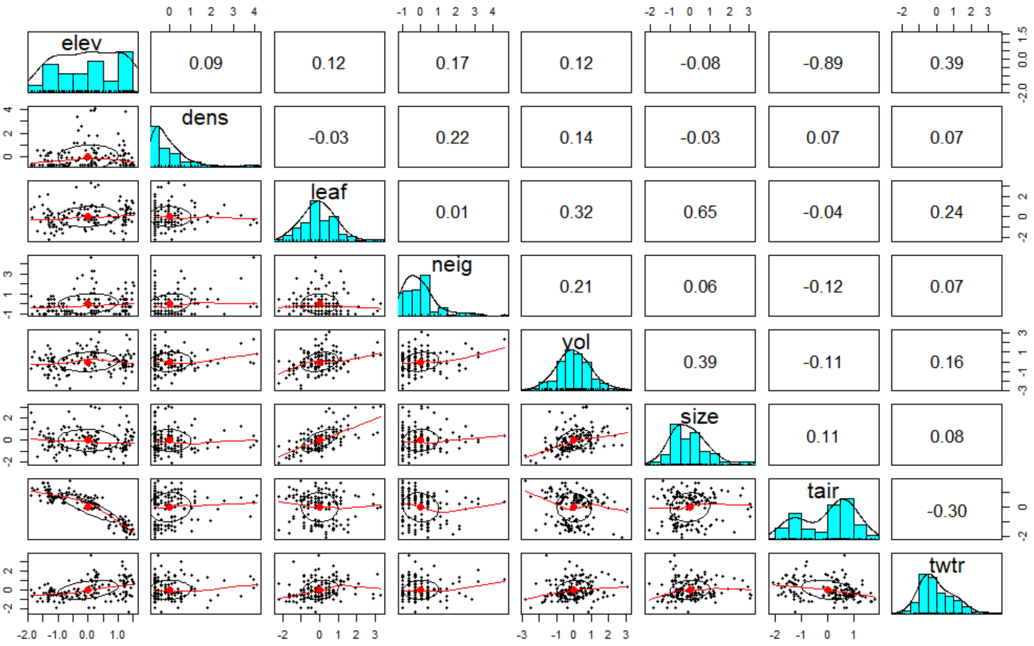

Figure S6.1 Correlation matrix for continuous variables using a Pearson correlation test
Predictors were considered correlated when Pearson's r > 0.6.

Chapter 7 General discussion

This thesis investigated the ecology and population dynamics of *Crossodactylodes itambe*, a bromeligenous frog endemic to a highland area in the Espinhaço Mountain Range of Brazil. In addition to providing valuable information on the drivers of distribution and abundance of this threatened species, this research also demonstrates how the frog-bromeliad system can provide a model for investigating key demographic parameters, such as extinction and colonization, which might be intractable to measure in patchy habitats on a larger scale.

Considering the aim of this thesis (section 1.5), the results contributed to (1) expanding our understanding of amphibian monitoring and population declines in tropical areas, (2) overcoming the lack of scientific knowledge on the ecology, distribution and natural history of *Crossodactylodes itambe*, and (3) assessing species conservation status and establishing priority actions for the conservation of bromeligenous frogs. The contribution of my research to each of these aims are outlined in this discussion.

7.1 Contribution to amphibian decline research

Over the past few years, considerable advances towards national monitoring schemes have been made in developed countries, such as the USA (Weir, Fiske and Royle 2009; Adams *et al.* 2013; Weir, Royle and Gazenski 2014; Villena *et al.* 2016) and the UK (Griffiths, Sewell and McCrea 2010; Sewell, Beebee and Griffiths 2010; Sewell *et al.* 2012). These studies have proved that occupancy modelling is a valuable tool to overcome the problem of imperfect detection (MacKenzie, Nichols and Yoccoz 2006) which is widespread in amphibian population status assessments (Guimarães, Doherty Jr. and Munguía-Steyer 2014; Ficetola 2015; Cruickshank *et al.* 2016). Nonetheless, I was not able to find long-term monitoring studies investigating amphibian population declines using occupancy analysis in Brazil – in fact, I found just a few studies using multi-season occupancy modelling for amphibians in the country (Moreira *et al.* 2016; Moreira, Moura and Maltchik 2016). While single-season analyses are slightly more common, the use of dynamic occupancy models to investigate tropical amphibian populations is still scarce (Hamer and Mahony 2010; Lehtinen *et al.* 2016).

Monitoring programs are crucial for establishing baselines for conservation and should be statistically sensitive to changes in amphibian populations, otherwise declines will go unnoticed. However, a long-term monitoring study requires not only continuous funding but also trained people and logistical support. With the power analysis I was able to change my sampling design, reducing the number of sampling nights, which consequently reduced the costs of surveys: the cost of six nights of survey, for example, could be redistributed to conduct at least two years of sampling with four nights of survey in each month. Power analysis combined with occupancy modelling can therefore inform the design and allocation of resources providing clear objectives are identified.

Detecting declines in amphibian populations is challenging, especially for rare and elusive species (Ward *et al.* 2017). The same was true for my data, in which detecting a small population change would require an impractical number of sampling sites (Chapter 4). Detecting amphibian declines in populations that often show a high degree of natural fluctuation is also problematical (Green 2003), especially when dealing with a short time series. Indeed, long-term studies investigating population fluctuations and declines are virtually absent in Brazil (Silvano and Segalla 2005; Verdade *et al.* 2012). Because my results are limited to a short time period (4 years of data), it might not be representative of longer population trends. Continuing the monitoring is therefore highly recommended.

My sampling design aimed to detect moderate to large changes in occupancy with reliable statistical power (Chapter 4) and I was unable to detect significant population declines in *C. itambe*, expect at lower elevation, where bromeliads are smaller and have lower density (Chapter 5). This decline might be attributed to changes in habitat structure, in particular, volume of the central tank and density of bromeliads, which have an effect in the colonization rates of *C. itambe* (Chapter 6). Colonization was the only parameter negatively affected by fire (Chapter 6), which can directly affect the structure of the plant (Alves, Rocha and Van Sluys 1996; Rocha *et al.* 1996; Ariani *et al.* 2004). A history of trampling and fire might have shaped landscape characteristics at the Itambé summit, influencing, for example, the limited number of bromeliads available at lower elevations.

My results suggest an upwards trend for occupancy in sites at higher elevation and a downwards trend for sites at medium and lower elevation (Chapter 5). This might indicate that the population is moving uphill and, alarmingly, that its global range could become restricted to the very top of the Itambé summit, before going extinct. An upward elevational change has been detected for a variety of taxa (Wilson *et al.* 2005; Devi *et al.* 2008; Moritz *et al.* 2008; Beggs 2012; Tingley *et al.* 2012; Neate-Clegg *et al.* 2018) and has been attributed to climate change. Given the short time scale of my study, these observations should not the attributed to anthropogenic climate change (Parmesan *et al.* 2011) and a possible upward movement of the population remains enigmatic and should be further investigated.

The modelled colonization and extinction rates for *C. itambe* demonstrate the dynamic process of settlement and vacancy of sites from one month to the other. Extinction rates can be interpreted a proxy for movement (Betts *et al.* 2008) and reflect reproductive failures at a site – not necessarily representing an extinction of the local population. Although extinction rates were partially explained by seasonal variation in local weather conditions (Chapter 6), I was not able to define the drivers of local extinction of this population. Extinction of a site could be led by several factors, such as: (1) demographics (e.g., bromeliad is saturated with several individuals, or sex ratio is unbalanced); (2) local random processes; or (3) stochastic events (e.g., heavy rains could wash down individuals from occupied bromeliads). These are hypothetical situations that deserve further investigation.

Finally, declines might be associated with disease infections that have been poorly investigated for bromeligenous frogs. Declines of amphibian populations in pristine habitats of the Atlantic Rainforest is an alarming indication of higher disease risk (Becker and Zamudio 2011). Disturbingly, high prevalence of chytrid fungus was reported in a bromeligenous frog from the Atlantic Rainforest (Ruano-Fajardo, Toledo and Mott 2016) – a disease indicated to have caused most of the historical amphibian declines observed in Brazil (Carvalho, Becker and Toledo 2017). Unfortunately, chytridiomycosis has not been tested for any *Crossodactylodes* species and there is no information available on the risks of infection for other bromeligenous frogs, except Ruano-Fajardo, Toledo and Mott (2016). This is a potential threat to montane bromeligenous frogs and deservers additional research.

7.2 Contribution to the ecology of *Crossodactylodes itambe*

There are currently 99 species of bromeligenous frogs and half of them occur in Brazil (Sabagh, Ferreira and Rocha 2017). *Crossodactylodes* is the only genus of bromeligenous frogs where all species are restricted to plants (Cochran 1938; Peixoto 1982; Barata *et al.* 2013; Teixeira *et al.* 2013) and oviposition in bromeliads is considered a synapomorphy of the genus (Fouquet *et al.* 2013; Santos *et al.* 2017). *Crossodactylodes itambe* was only recently described (Barata *et al.* 2013) and the whole genus lacks sufficient information on ecology and natural history – which is the case for most amphibian species occurring in Brazil (Trindade-Filho *et al.* 2012; Verdade *et al.* 2012; Morais *et al.* 2013), including many bromeligenous frogs (Sabagh, Ferreira and Rocha 2017).

It is clear that *C. itambe* has a high level of habitat dependency which may also be true for other bromeligenous frogs, since they share the same life history and habitat restrictions (Peixoto 1995). Abundance of adult frogs inside a bromeliad was explained by the structure of the plant, which was more important than local climate

(Chapter 2). I also found a similar pattern for colonization rates, with models of habitat outperforming models that included seasonal change in climate (Chapter 6). More specifically, *C. itambe* showed a close relationship with the size of bromeliad, the volume of the central tank and the density of plants distributed at the Itambé summit. These findings corroborate the preferences and habitat requirements observed for other species of bromeligenous frogs (Eterovick 1999; Oliveira and Navas 2004; Alves-Silva and Silva 2009; Silva, Carvalho and Bittencourt-Silva 2011; Motta-Tavares *et al.* 2016).

The frequent records of one individual per bromeliad suggest that bromeligenous frogs may be territorial (Cunha and Napoli 2016; Motta-Tavares *et al.* 2016). Parental care was previously suggested for *C. itambe* (Santos *et al.* 2017), but I did not observe individuals defending their territory either through camera trapping or occasional encounters (Barata, Griffiths and Ferreira 2018). I usually recorded one individual in each bromeliad (Chapter 2) and observed low colonization and extinction rates from one month to the other (Chapter 5), suggesting that species is either territorial or has a reduced dispersal capability – or even both. While low dispersal capabilities could be reinforced by the species' small body size (Barata *et al.* 2013), territoriality could be supported by the presence of spines on their thumbs which could be used in fighting (Santos *et al.* 2017).

Crossodactylodes itambe is naturally rare and has a very limited extent of occurrence (Chapter 3), which might also be true for other *Crossodactylodes* species and many others bromeligenous frogs. The distribution of bromeliads was mostly influenced by the topography of the area (i.e., elevation and soil type) and specific climatic conditions (temperature seasonality and annual precipitation). However, within the areas I surveyed, I also observed differences in bromeliad density and habitat structure inside and outside protected areas (Chapter 3) which could influence the quantity and quality of bromeliads available. Because habitat loss in the Atlantic Rainforest can restrict the geographical range of anurans (Vasconcelos and Doro 2016), protected areas play an important role in safeguarding current forest remnants (Ribeiro *et al.* 2009) and maintaining natural characteristics that are important for both bromeliads and frogs.

Although *C. itambe* is completely covered by a protected area, the species is declining at lower elevation sites (Chapter 5), where most bromeliad species were burnt after a fire that negatively affected colonization rates (Chapter 6). The same negative impact of fire could also be expected to affect species abundance, since habitat is an important predictor of the number of adults inside the bromeliad (Chapter 2). Historical land-use by dairy farmers could have shaped landscape characteristics at the Itambé summit, especially at lower elevations where a population decline was detected (Chapter 5, Chapter 6). Fire should be considered an imminent threat for this species and many other bromeligenous frogs that are strictly dependent on bromeliads.

7.3 Contribution to the conservation of bromeligenous frogs

Amphibian conservation priorities were highlighted for the Espinhaço Mountain Range (Barata, Correia and Ferreira 2016) and a detailed conservation action plan was proposed for threatened amphibian species (RAN/ICMBio 2012), which included *Crossodactylodes itambe*. My thesis support national efforts for monitoring amphibian populations in high altitude areas (Verdade *et al.* 2012) and contribute to at least six guidelines proposed by the Amphibian Conservation Action Plan at South Espinhaço Range (RAN/ICMBio 2012), including: (1) implementing a long-term monitoring program for *C. itambe*; (2) studying species ecology; (3) evaluating effects of fire on populations; (4) searching for new populations; (5) surveying non-sampled areas; and (6) identifying priority actions for species and habitat conservation.

High elevation areas within the Espinhaço Range shelter many amphibian species with highly restricted distributions (Barata, Correia and Ferreira 2016) and, most notably, these areas can still harbour undescribed amphibian species (Chapter 3). Despite my extensive survey effort to search for new populations (Chapter 3), the only record for this species remains at the top of the Itambé summit, above 1700 m in elevation. It is likely that bromeligenous frogs are naturally rare and have a reduced extent of occurrence. Without delimiting species distributions and extent of occurrence it will be difficult to prioritize effective conservation actions (Scheffers *et al.* 2012). The method used to find the new species (using plants as surrogates for frog occurrence) was a viable solution to overcome the lack of occurrence data for *C. itambe* and can potentially reduce the costs of field expeditions (Chapter 3). The same approach could be possible for species dependent on bromeliads and with high detection and abundance. This could include other *Crossodactylodes* species only known from one location and at least 15 threatened and Data Deficient (DD) bromeligenous frogs that are presumed to occur more widely (IUCN 2018).

Based on ecological data (Chapter 2) and on species geographical extent (Chapter 3) I completed the first IUCN assessment for *C. itambe* (Appendix I). The species was classified as Critically Endangered because it has an area of occupancy of less than 0.5 km^2 (Barata *et al.* 2013), there is only a single known population (Chapter 3) that is highly dependent on habitat quality and the structure of the host plant (Chapter 2) which has been damaged by fire (Chapter 6). Habitat conservation should be a priority action and, more specifically, the protection of specific features of plant structure (such as size of the bromeliad, volume of the central tank and density of plants), is highly recommended for species persistence. Similar recommendations are likely to benefit other *Crossodactylodes* species, for which we have no data to support conservation actions (Peixoto and Carvalho-e-Silva 2004; Silvano and Peixoto 2004a; Silvano and Peixoto 2004b), and possibly other threatened and DD species of bromeligenous frogs.

Four *Crossodactylodes* species are currently covered by protected areas: *C. itambe* (Barata *et al.* 2013), *C. bokermanni* (Silvano and Peixoto 2004a), *C. izecksohni* (Silvano and Peixoto 2004b) and the new *Crossodactylodes* species (Chapter 3). However, even inside protected areas, habitat quality is imperilled by stochastic events, such as fire, or illegal bromeliad collection, which is also a potential threat to bromeligenous frogs (Mageski *et al.* 2016). Unfortunately, local conservation policies for protected areas in the Atlantic Rainforest do not guarantee the survival of most amphibian species (Campos *et al.* 2017) and Brazilian biodiversity is threatened by the lack of resources allocated to biodiversity protection, along with the deficiency of funding for scientific research and poor law enforcement (Ferreira *et al.* 2014; Magalhães 2017). Ensuring habitat quality inside protected areas is therefore extremely important, allowing populations of both plants and frogs to thrive.

Many DD amphibians may actually warrant classification in higher extinction risk categories (Morais *et al.* 2013; Howard and Bickford 2014). The observed decline of a population that is strictly protected (Chapter 5), completely dependent on habitat quality (Chapters 2 and 6) and with limited geographical range (Chapter 3), show that other DD bromeligenous frogs are potentially at risk, even if they occur inside protected areas. Given the logistical constraints of long-term monitoring, it is unlikely that detailed information will be available before populations of many species decline (Howard and Bickford 2014). Based on evidence provided by my thesis, bromeligenous frogs for which we currently lack information are likely to be more threatened, and could be assigned as potentially threatened DD species (Jarić *et al.* 2016).

7.4 Conclusions

A large proportion of bromeligenous frogs are classified as DD (35%) or threatened with extinction (41%) (IUCN 2018). This thesis improves the biological knowledge regarding *Crossodactylodes itambe* and is possibly the most detailed source of information for the whole genus. Despite being focused on a single species, the results of this thesis provide useful insights for the conservation of DD bromeligenous frogs, for which little information are available. The conservation of a bromeligenous frog cannot be achieved without the conservation of its host plant (Sabagh, Ferreira and Rocha 2017) and maintaining habitat quality is extremely necessary for bromeligenous frogs to persist, even inside protected areas, where population declines were detected for *C. itambe*. Overall, considering the dynamic processes ruling the abundance, distribution and colonization and extinction rates of this montane bromeligenous frog, habitat conservation should be a priority action when compared to climate change mitigations in the short term.

Dealing with the lack of data will be the major challenge to investigating the ecology and distribution of *Crossodactylodes* species and many other bromeligenous frogs that are both DD and range-restricted. However, bromeligenous frogs are strictly restricted to plants, and this close relationship provides a self-contained study system that can be used to investigate fundamental ecological questions concerning population persistence in patchy habitats. Each plant is a unit with specific physical parameters that can be precisely measured at any point in time, together with information of its animal community (Richardson 1999). Natural microcosms (i.e., small contained natural systems) are strong candidate models for ecology and are as complex and biologically realistic as other natural systems (Srivastava *et al.* 2004).

The frog-bromeliad system is a great opportunity to investigate population ecology. Detailed ecological data might be difficult to gather in a larger scale and more complex ecosystem, but this is an achievable task in the small-scale frog-bromeliad system. For example, the closure assumption of a dynamic occupancy model, which is presumably hard to be achieved in most biological systems, can be easily met for species that have (1) specific life history traits and restriction to plants, (2) reduced body size and presumed low dispersal capabilities, (3) moderate to high local abundance and detection probabilities. Other bromeligenous frogs displaying these features could benefit by using occupancy analysis and a sampling design could be easily implemented.

Finally, understanding population dynamics at the microhabitat level is of particular interest for rare and endemic species (Liang *et al.* 2017) and local-scale processes may have a larger influence than landscape-level factors (Johnson *et al.* 2016; Lamb, Waddle and Qualls 2017; Liang *et al.* 2017). This is probably true for bromeligenous frogs that are range-restricted. For example, I was only able to detect a population change at a very limited geographical range, demonstrating that declines in occupancy may go undetected in species that have small ranges and are closely associated with elevation. Therefore, the ecology of bromeligenous frogs should be investigated at small-scales, taking advantage of species life history, reduced geographical range and the fine scale process driving population dynamics.

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Appendix I Red List Assessment

Draft

Crossodactylodes itambe - Barata, Santos, Leite & Garcia, 2013

ANIMALIA - CHORDATA - AMPHIBIA - ANURA - LEPTODACTYLIDAE - *Crossodactylodes - itambe*

Common Names: Itambe's Bromeliad Frog (English)
Synonyms: No Synonyms

Red List Status
CR - Critically Endangered, (IUCN version 3.1) Critically Endangered reason: has an area of occupancy and extent of occurrence less than 0.5 km^2, there is only a single known population and there is a continuing decline in the quality of the habitat.

Red List Assessment

Assessment Information

Assessor(s): IUCN SSC Amphibian Specialist Group

Facilitators/Compilers: Best, S.; Rob Ward; Izabela M. Barata

Regions: Global

Assessment Rationale

Barata *et al*. 2013 note that the known area of occupancy is restricted and at only one location. The species has not been found in other localities despite extensive surveys (Barata, pers. comm.). This, together with known fire threats, could potentially drive declines in over a very short time. The species is listed as Critically Endangered because it has an area of occupancy and extent of occurrence less than 0.5 km^2 (Barata *et al*. 2013; Santos *et al*. 2017), there is only a single known population (Barata *et al.* in prep.) and there is a continuing decline in the quality of the habitat.

Distribution

Geographic Range

Known only from the type locality in the Parque Estadual do Pico do Itambé, municipality of Santo Antônio do Itambé, state of Minas Gerais, Brazil with an EOO of less than 0.5 km^2 (Barata *et al.* 2013; Santos *et al.* 2017).

Area of Occupancy (AOO)

Estimated area of occupancy (AOO) - in km2	Justification
0.5	Area above 1713 m a.s.l. of the Itambé summit where this species is found (Barata *et al.* 2013; Santos *et al.* 2017).

Extent of Occurrence (EOO)

Estimated extent of occurrence (EOO)- in km2	EOO estimate calculated from Minimum Convex Polygon	Justification
0.5	-	Total area above 1713 m a.s.l. of the Itambé summit (Barata *et al.* 2013; Santos *et al.* 2017). It could occur more widely, but localised surveys have not revealed presence on other peaks, so it is unlikely (Barata *et al.* in prep.).

Locations Information

Number of Locations	Justification
1	Only found above 1713 m in the Pico do Itambé State Park. Four peaks above 1500 m a.s.l. were surveyed; five nights per peak with a total of > 1500 bromeliads checked for occupancy, giving a high certainty that the species was not present in other locations (Barata *et al.* in prep.).

Very restricted AOO or number of locations (triggers VU D2)

Very restricted in area of occupancy (AOO) and/or # of locations	Justification

Yes	Area above 1713 m a.s.l. of the Itambé summit where this species is found (Barata *et al.* 2013) is about 0.5km^2 and the only place it is found.

Elevation / Depth / Depth Zones

Elevation Lower Limit (in metres above sea level): 1713 (Santos *et al.* 2017)

Elevation Upper Limit (in metres above sea level): 2062 (Barata *et al.* 2013)

Map Status

Map Status	How the map was created, including data sources/methods used:	Data Sensitive?	Justification	Geographic range this applies to:	Date restriction imposed:
Done	-	-	-	-	-

Biogeographic Realms

Biogeographic Realm: Neotropical

Occurrence

Countries of Occurrence

Country	Presence	Origin	Formerly Bred	Seasonality
Brazil	Extant	Native	-	Resident
Brazil -> Minas Gerais	Extant	Native	-	Resident

Population

Found relatively easily and in a high abundance at its type locality (Barata *et al.* 2013; Barata *et al.* 2018). Bromeliad occupancy increases with elevation with approximately 50% of bromeliads at the summit occupied, but only 9% at lower elevations (Barata *et al.* 2017). During surveys in 2015 and 2016, over 400 observations of adults were made, but only 40 juveniles were observed during the same period, which may be due to lower detectability (Barata *et al.*, in prep.). There is no information on population size or indication of population trends. There have been no records of other populations since the species' description. A single population is known and a recent survey (four peaks above 1500 m a.s.l. visited; five nights per peak; > 1500 bromeliads checked) showed no records of new populations with a high certainty that the species was not present in other locations (Barata pers. comm.).

Habitats and Ecology

The species occurs at high altitudes in rocky mountain meadows. It is abundant, but restricted in distribution. Individuals were found in open field rupicolous bromeliads. The species is active by night and most individuals were found during this period inside bromeliads' tanks and leaves; usually with half of their body inside the water line. Egg masses contain only one egg and are laid attached to lateral leaves of bromeliad tanks, at the water level (Barata *et al.* 2013). Although adults were using bromeliads at lower elevations, tadpoles were not recorded. Clustered individuals can be found sharing the same bromeliad (Santos *et al.* 2017). Usually one adult is observed inside a bromeliad, but up to 6 individuals were observed sharing the plant, and the number of tadpoles varied from 1-8 (mean of two per bromeliad) (Barata *et al.* 2018). Occupancy increases with increasing elevation (Barata *et al.* 2017). There is no record of new populations. Species could occur more widely, but localised surveys have not revealed presence on other peaks, so it is unlikely (Barata pers. comm.). The species is only recorded in a single bromeliad species (*Vriesea medusa*) (Barata *et al.* 2013; Santos *et al.* 2017; Barata *et al.* 2018). Habitat use is influenced by structure of bromeliad (such as size, volume of tank and presence of water), while climatic variables have low impact on species abundance (Barata *et al.* 2018).

IUCN Habitat Classification Scheme

Habitat	Season	Suitability	Major Importance?
4.7. Grassland -> Grassland-Subtropical / Tropical High Altitude	resident	Suitable	Yes

Life History

Breeding strategy

Does the species lay eggs?
Yes, usually one egg per bromeliad (Santos *et al*. 2017).
Does the species give birth to live young?
No
Does the species exhibit parthenogenesis
No

Does the species have a free-living larval stage?
Yes. Cluster of adults with varying number of tadpoles and/or eggs can be found. Up to 4 tadpoles and 4 adults observed sharing the same bromeliad. Tadpoles are not recorded at lower elevations (Santos *et al.* 2017). In two years, the number of tadpoles

inside bromeliads varied between 1-6 (average of two tadpoles per bromeliad) (Barata *et al.* 2018).
Does the species require water for breeding?
Yes. Completely restricted to bromeliads (Barata *et al.* 2013; Santos *et al.* 2017, Barata *et al.* 2018), with a single egg developing within the tank of the bromeliad attached to lateral leaves. Parental care is suggested to occur (Santos *et al.* 2017).

Systems

System: Terrestrial

Use and Trade

General Use and Trade Information

Species not utilized: true

There are no records of this species being utilized.

Threats

Human activity, namely fires and selective removal of plant species with economic value (including bromeliads), are the main potential threats. These threats were thought to be minimal, particularly due to the species occurring within a protected area (Barata *et al.* 2013). However, a fire in 2015 reduced the number of bromeliads by half at lower elevations (Barata *et al.*, in prep). Fire can affect the structure of bromeliad, which is important to adult abundance (Barata *et al.* 2018).

Threats Classification Scheme

Threat	Timing	Scope	Severity	Impact Score
5.2.1. Biological resource use -> Gathering terrestrial plants -> Intentional use (species is the target)	Rare	-	-	Low Impact: 3
7.1.1. Natural system modifications -> Fire & fire suppression -> Increase in fire frequency/intensity	Infrequent	-	high	High Impact
Tourism (damage/degradation of bromeliads)	Common	-	low	Low Impact: 3

Conservation

Conservation Actions

Found in Parque Estadual do Pico do Itambé, Brazil. The park is considered well managed, therefore building relationships with local stakeholders to reduce fire incidence through education and prioritising the region for fire response strategies are important. The number of visitors to the area should continue to be limited to reduce degradation to the ecosystem. The species occurs in the coverage area of the National Action Plan for the Conservation of Reptiles and Amphibians threatened with extinction in the Espinhaço. The species is also part of a monitoring programme since 2011 and is the subject of a PhD study. Work is ongoing to raise awareness amongst local students about the species and the ecosystem through educational workshops. Nationally categorised as DD.

Research Needed

Long term monitoring to assess population size and trends. Surveys to determine distribution and extent of occurrence were recently developed.

Conservation Actions In-place

Occur in at least one PA	Note
Yes	Parque Estadual do Pico do Itambé, Brazil

Research Needed

Research	Note
1.2. Research -> Population size, distribution & trends	-

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Appendix II Publications

Papers published as first author:

Barata, I.M. *et al.* (2016). Downscaling the gap: Protected areas, scientific knowledge and the conservation of amphibian species in Minas Gerais, Southeastern Brazil. *South American Journal of Herpetology* **11**:34–45.

Barata, I.M., Correia, C.M. and Ferreira, G.B. (2016). Amphibian species composition and priorities for regional conservation at the Espinhaço Mosaic, Southeastern Brazil. *Herpetological Conservation and Biology* **11**:293–303.

Barata, I.M., Griffiths, R.A. and Ferreira, G.B. (2018). Activity pattern and behavior of an endemic bromeliad frog observed through camera trapping. *Herpetological Review* **49**.

Papers published in collaboration:

Santos, M.T.T. *et al.* (2017). The tadpole of the microendemic, bromeligenous *Crossodactylodes itambe* (Anura, Leptodactylidae) from the endangered 'campo rupestre' of Southeastern Brazil, with additional comments on natural history. *South American Journal of Herpetology* **12**:14–23.

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Downscaling the Gap: Protected Areas, Scientific Knowledge and the Conservation of Amphibian Species in Minas Gerais, Southeastern Brazil

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Abstract. Protected areas (PAs) cover a small proportion of the Earth's surface and most species are not covered by the current network. Amphibians are the least represented group in PAs around the world and expanding the network is still the major recommendation for species conservation. We evaluated the effectiveness of PAs in safeguarding endemic amphibians in the Cerrado biome of Minas Gerais state, southeastern Brazil. We conducted a gap analysis to highlight site-based conservation actions for target species within study site. We extracted occurrence points from the national database and calculated the intersection between the minimum convex polygon and natural vegetation remnants for each species. For each target species, we calculated the percentage of the range covered by PAs and assessed the scientific knowledge based on academic publications between 1950–2015. We recorded 206 amphibians in Minas Gerais, of which 127 occur in the Cerrado. We identified 24 target species and concluded that 80% are insufficiently protected by the current PA network. A quarter of the species have zero coverage and most species have < 30% of their range legally protected. In southwestern Minas Gerais, we recommend habitat restoration and connectivity to provide additional habitat to target species. In western Minas Gerais, the creation of PA seems to be the best solution. The distribution of target species is concentrated in the Espinhaço Mountain Range, where we recommend the establishment of biodiversity corridors. We examined 246 publications, most of which focus on taxonomy. Few species have sufficient information to have their conservation status re-assessed, with only 26.8% of publications containing specific information on conservation. Scientific knowledge must be improved for all research areas, especially species distributions and ecology, to support evidence-based conservation and management actions.

Keywords. Cerrado; DD species; Endemic species; Geographical information system; Protected areas effectiveness; Representativeness.

INTRODUCTION

Although 15.4% of the Earth's land surface is formally protected (Juffe-Bignoli et al., 2014), the Convention on Biological Diversity (Aichi Biodiversity Target 11) advocated an increase in protected area (PA) coverage to at least 17% by 2020 (CBD, 2015)—an expansion of 2.2 million km^2. Despite an increase in the number and coverage of PAs in the last few decades (Watson et al., 2014), 85% of threatened birds, mammals, and amphibians are still not adequately protected (Venter et al., 2014). Amphibians are the least represented group inside PAs around the world (Rodrigues et al., 2004); a recent estimate identifies 42% of all amphibian species as not represented in PAs or with less than 5% of their distribution covered by such areas (Nori et al., 2015). This may be a consequence of biased selection criteria to establish PAs, with emphasis on charismatic megafauna and certain ecosystems, resulting in an unbalanced representation of biodiversity within the network (Beresford et al., 2010; Sritharan and Burgess, 2011).

Amphibians represent the highest proportion of threatened species among all vertebrates (41% are threatened; Pereira et al., 2012) and are declining more rapidly than either birds or mammals (Stuart et al., 2004). Populations are declining worldwide due to fungal diseases (Pounds et al., 2006; Lips et al., 2008), use of agrochemicals (Kiesecker, 2002), climate change (Griffiths et al., 2010; Shoo et al., 2011) and habitat loss and fragmentation (Becker et al., 2007). However, knowledge on amphibian population ecology, species distributions and conservation status is lacking, especially in South American countries (Young et al., 2001). In the Brazilian savanna—the Cerrado hotspot, where 51.7% of amphibian species are endemic (Valdujo et al., 2012)—estimates of threats to amphibians have been poorly investigated (Diniz-Filho et al. 2006, 2007). In Minas Gerais, amphibian research has focused on natural history and distribution patterns, with ecological studies beginning in the late 1990's (Nascimento et al., 2009). However, in the Cerrado biome, population declines are still poorly understood (Eterovick et al., 2005).

Amphibians are not only highly threatened, but also have the highest proportion of Data Deficient species (DD; IUCN, 2014). According to Morais et al. (2013), 25%

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of DD amphibians are recorded in Brazil, which reflects the level of uncertainty about species conservation status—and also the degree to which they are protected. Although Mace et al. (2008) recommended the same degree of protection to DD species, this proposition is usually disregarded (Trindade-Filho et al., 2012) and species classified in this category receive less attention in conservation plans than those assessed as threatened (Brito, 2010; Trindade-Filho et al., 2012; Morais et al., 2013).

Considering that only 3% of the original area of the Cerrado is strictly protected (MMA/ICMBio, 2014) and the high amphibian diversity within this biome in Minas Gerais state, southeastern Brazil, herein we evaluate the effectiveness of existing PAs in safeguarding amphibian species that are endemic to Minas Gerais and occur in Cerrado. We conduct a gap analysis to pinpoint where increases in PAs are needed and also where it would be considered most feasible, based on the remaining vegetation cover and past priority recommendations for the study area. Furthermore, as DD accounts for a high proportion of evaluated species in the country, we also investigate the scientific knowledge available for DD target species, according to IUCN assessments. We highlight the shortfalls of scientific knowledge and suggest future research areas that might contribute to the conservation status of amphibians in Minas Gerais.

MATERIALS AND METHODS

We selected target species based on endemism and considered only species with their entire extent of occurrence (sensu IUCN, 2013) restricted to the state of Minas Gerais, southeastern Brazil. We checked species occurrences using the Amphibian Species of the World database (Frost, 2014), which resulted in a broad description of the geographic distribution within the country. Species listed as occurring in Minas Gerais were double checked using the International Union for Conservation of Nature database on species distributions (IUCN, 2014). Based on the Brazilian official database for major biomes (IBGE, 2014) and on the municipality in which a given species occurred, we assigned each species listed in Minas Gerais to one or more of the three biomes in the state: Cerrado, Atlantic Rainforest and Caatinga. We included species from transitional areas in both biomes of occurrence. Our aim was to produce a database of anuran species from Minas Gerais, with detailed information on species distributions and biome of occurrence. From this database, we selected endemic species of Minas Gerais state occurring in the Cerrado biome. These species were assigned to IUCN Red List categories (IUCN, 2014) and recently described species were assigned as not evaluated (NE). Since we did not consider Least Concern (LC) species in analyses, our final targets were defined as threatened, DD and NE species endemic to Minas Gerais and with occurrence in the Cerrado.

Protected area effectiveness

We extracted the occurrence points for the target species from a recent database (20,000 records) produced by "Centro Nacional de Pesquisa e Conservação de Répteis e Anfíbios – RAN". Using ArcGIS, we calculated a minimum convex polygon (MCP) for each species based on occurrence data. For species with two or less occurrence points, we assigned a buffer of 10 km, merging buffers whenever they overlapped to each other (i.e., when buffer margins touched an adjacent buffer). Since species are not likely to occur in the whole MCP area, species distribution maps can overestimate the true area of occupancy. To avoid overestimation, we considered as the potential area of occurrence only those areas with natural vegetation inside the MCP (hereafter species range). To obtain this, we overlapped each species MCP with a natural vegetation remnants layer (IBAMA/PMDBBS, 2011). Even though this does not guarantee that species will be found in all natural remnants, we believe it provides a more realistic estimate of occurrence and therefore a better estimate of the range covered by PAs.

To assess protection effectiveness, we used the percentage of each species range covered by PAs (i.e., proportional area of species range that is legally protected). To calculate this proportion, we overlapped the range of each species (area with natural vegetation inside the MCP) with the PA network layer. We only considered strictly protected areas (according to the Brazilian PA system; SNUC, 2000, equivalent to IUCN categories I–IV; Dudley, 2008) within the state of Minas Gerais. Considering the small scale of our study and given the reduced number of occurrence points, we defined protection effectiveness based on the percentage of habitat covered by a PA within a species' range:

- Not protected (NP): zero coverage and/or < 10% of range protected
- Unsatisfactorily protected (UP): 10–29% of range protected
- Partially protected (PP): 30–49% of range protected
- Satisfactorily protected (SP): 50–80% of range protected
- Protected (P): > 80% of range protected

Finally, to contrast the distribution of target species with previously recommended conservation goals, we overlapped NP species ranges, PAs and natural vegetation remnant layers with the national priority areas for biodiversity conservation (PROBIO/MMA, 2007) and state priority areas for herpetofauna conservation (Drummond et al., 2005). To conduct all spatial analyses we converted shapefiles to the same datum and projection system.

Scientific knowledge

We investigated the scientific knowledge produced between 1950–2015 for all target species, including DD species as well as threatened species and species that are yet to be evaluated by the IUCN. We used the scientific name and synonyms of each target species as search criteria in Google Scholar and only considered papers published in peer-reviewed journals. To avoid duplicates or unreliable entries, we analyzed the results and eliminated repeated references. We placed each reference into subject categories (not mutually exclusive; i.e. one paper could have multiple citations and/or be placed in more than one category), according to keywords (in parentheses): *Conservation* (conservation, decline, vulnerab*, threat*, extinct*); *Taxonomy* (taxonomy, description, morphology, new species); *Ecology* (ecolog*, vocal*, diet, temporal distribution, spatial distribution, natural history, predation, behavior); *Genetics* (genetics, cytogenetic, karyotype, phylogen*, chromosom*); and *Distribution* (distribution, geographic distribution, new record). When analyzing the content of each paper, we checked if the published data and analysis presented specific information about the species (e.g., taxonomic review, population ecology) or general information about a community (e.g., cited as comparing taxa or listed in a community). Using this approach we produced a list of publications from the last 65 years for each target species according to research area and specific content that could contribute to an evaluation of their conservation status or future conservation plans.

RESULTS

Protected areas effectiveness in the Cerrado of Minas Gerais State

We obtained records of 206 amphibian species in Minas Gerais, of which 127 occur in the Cerrado, including species occurring either at Cerrado and Caatinga or at Cerrado and Atlantic Rainforest. We recorded 35 species endemic to Minas Gerais and occurring in the Cerrado biome, representing 151 data points that were used in our analyses. Eleven of these species are classified as LC and the remaining species were considered as targets (n = 24, Table 1): two are Near Threatened (NT), 16 are DD and six are NE. The number of points retrieved differed among species and, as a consequence, the estimated species ranges differed as well, varying from 125.7–31,729.6 km^2 (Table 1). According to our classification, approximately 80% of the target species are either NP or UP by the current network (Fig. 1A; Table 1). Six species have zero coverage, representing 25% of our targets (Fig. 1B), and more than half (n = 13) are classified as UP. The remaining five species (20.8%) are PP (n = 2), SP (n = 1) or P (n = 2) (Fig. 1A–B).

The distribution of target species is highly concentrated in the Espinhaço Mountain Range, a transitional area between the borders of Cerrado and Atlantic Rainforest in eastern Minas Gerais state (Fig. 2). This region shelters one SP and one PP species, but also a high number of UP species (n = 11) and one NP species, *Physalaemus deimaticus*. The southwestern part of the state (close to Serra da Canastra National Park and Triângulo Mineiro) is another region with a considerable number of target occurrences, including the three NP species *Ischnocnema karst, I. penaxavantinho* and *Pseudopaludicola facureae* (Fig. 2). There are few records in the central-western state and the only two target species recorded in this region have zero PA cover: *Bokermannohyla ravida* and *Proceratophrys carranca* (Fig. 2). The occurrence of all NP species overlapped with priority areas for biodiversity conservation (national priorities; PROBIO/MMA, 2007) or herpetofauna conservation (state priorities; Drummond et al., 2005; Fig. 3).

Scientific knowledge on target species

We examined 173 papers that included 246 citations for all 24 target species. Most citations were about taxonomy (n = 145), representing 59% of our search, followed by ecology, with 19% of records (n = 48) (Table 2). Although the category "ecology" ranked second in general numbers, less than 17% (n = 8) were specific studies. Citations focusing on distribution (n = 22), conservation (n = 19) and genetics (n = 12) represented 21.5% of the total literature. Few papers were published in the first four decades (n = 20; from 1950 to 1990), with an increasing number from 2000 and the present (n = 135) (Fig. 4).

There are few species-specific citations (Fig. 5) and only 26.8% (66 of 246 citations) contained information on populations of target species (Table 2). Species with the highest number of specific studies are *Crossodactylus trachystomus* (n = 9), followed by *Phyllomedusa megacephala* (n = 8), and *Bokermannohyla ibitiguara* and *Pseudopaludicola mineira,* both with five records (Fig. 5). Nine species had only one specific citation, all of them on their taxonomic description (Table 2), and two species had only one record in general and specific literature search, *Odontophrynus monachus* and *I. karst,* both of which were recently described (Fig. 5).

DISCUSSION

Are protected areas enough?

The great majority of amphibian species that are endemic to Minas Gerais and occur in Cerrado are unsatisfactory or not protected by the current PA network and

Table 1. Data extracted for each target amphibian species endemic to the Cerrado in the state of Minas Gerais including: IUCN classification (DD: data deficient; NT: near threatened; NE: not evaluated); protection category (NP: not protected; UP: unsatisfactorily protected; PP: partially protected; SP: satisfactorily protected; P: protected); polygon area (total area); natural vegetation remnants inside species' polygon (considered as species' range); proportion of natural vegetation remnants inside species' polygon; natural vegetation remnants inside species' polygon covered by protected areas (PAs) (i.e., species' range inside PA); proportion of species' range inside PA; natural remnants inside PA within species' range; proportion of natural area covered inside PA within species' range; total number of points retrieved for target species; total number of points inside PA; and protected areas where target species is recorded (NP National Park, SP State Park). Area is given in km^2 and proportion is given in percentage (%). NP species are highlighted in **bold.**

Species	IUCN category	Protection category	Polygon area	Remnants inside polygon*	% remnants inside polygon	*covered by PA	% inside PA	Remnants inside PA	% remnants inside PA	Points	Points inside PA	Protected area
Bokermannohyla ibitiguara (Cardoso, 1983)	DD	P	358.75	97.68	27.2	88.50	90.6	71.3	80.5	3	1	Canastra NP
Bokermannohyla ravida (Caramaschi et al., 2001)	DD	**NP**	314.00	172.55	55.0	0.00	**0.0**	0.0	0.0	1	0	
Bokermannohyla sagarana (Leite et al., 2011)	NT	UP	125.75	121.49	96.6	18.22	15.0	18.2	100.0	6	0	
Bokeramnnohyla sazimai (Cardoso and Andrade, 1982)	DD	UP	18184.98	6162.98	33.9	1493.12	24.2	1339.7	89.7	6	1	Canastra NP
Crossodactylus trachystomus (Caramaschi and Sazima, 1985)	DD	UP	4103.24	2856.55	69.6	508.39	17.8	495.8	97.5	12	3	Serra do Cipó NP Rio Preto SP
Hylodes otavioi (Sazima and Bokermann, 1983)	DD	UP	350.93	263.17	75.0	49.57	18.8	49.6	99.1	7	1	Serra do Intendente SP
Hypsiboas botumirim (Caramaschi et al., 2009)	NE	UP	9460.11	6088.20	64.4	1244.76	20.4	3.1	0.3	5	0	
Hypsiboas cipoensis (Lutz, 1968)	NT	UP	11946.17	8302.04	69.5	883.25	10.6	877.7	99.4	8	1	Sempre-vivas NP
Ischnocnema penaxavantinho (Giaretta et al., 2007)	DD	**NP**	628.24	160.27	25.5	0.00	**0.0**	0.0	0.0	2	0	
Ischnocnema karst (Canedo et al., 2012)	NE	**NP**	293.51	51.98	17.7	0.00	0.0	**0.0**	0.0	1	0	
Leptodactylus camaquara (Sazima and Bokermann, 1978)	DD	UP	9745.70	7826.07	80.3	935.09	11.9	934.0	99.9	10	3	Serra do Cipó NP Rio Preto SP Sempre-vivas NP
Odontophrynus monachus (Caramaschi and Napoli, 2012)	NE	P	314.00	190.64	60.7	167.43	87.8	166.1	99.2	1	1	Canastra NP
Phyllomedusa megacephala (Miranda-Ribeiro, 1926)	DD	UP	9866.67	6483.08	65.7	1309.99	20.2	1077.8	82.3	7	2	Serra do Cipó NP Rio Preto SP
Physalaemus deimaticus (Sazima and Caramaschi, 1988)	DD	**NP**	292.07	233.34	79.9	0.00	**0.0**	0.0	0.0	3	0	
Physalaemus evangelistai (Bokermann, 1967)	DD	UP	2539.61	1604.60	63.2	326.22	20.3	316.7	97.1	3	0	
Proceratophrys carranca (Godinho et al., 2013)	NE	**NP**	314.00	139.88	44.5	0.00	**0.0**	0.0	0.0	1	0	
Proceratophrys cururu (Eterovick and Sazima, 1998)	DD	UP	4916.49	4368.86	88.9	1024.59	23.5	992.4	96.9	12	4	Rio Preto SP Sempre-vivas NP
Pseudopaludicola facureae (Andrade and Carvalho, 2013)	NE	**NP**	399.30	47.31	11.8	0.00	**0.0**	0.0	0.0	2	0	
Pseudopaludicola mineira (Lobo, 1994)	DD	UP	4334.15	3860.02	89.1	815.75	21.1	783.4	96.0	6	3	Serra do Cipó NP Rio Preto SP Sempre-vivas NP
Scinax cabralensis (Drummond et al., 2007)	DD	PP	618.05	403.70	65.3	163.00	40.4	163.0	100.0	2	1	Serra do Cabral SP
Scinax curicica (Pugliese et al., 2004)	DD	UP	6496.42	5045.14	77.7	889.36	17.6	847.5	95.3	7	2	Rio Preto SP Sempre-vivas NP
Scinax maracaya (Cardoso and Sazima, 1980)	DD	UP	31729.58	8701.60	27.4	2013.57	23.1	1771.0	88.0	6	1	Canastra NP
Scinax pinima (Bokermann and Sazima, 1973)	DD	SP	314.12	297.98	94.9	164.00	55.0	164.0	100.0	1	1	Serra do Cipó NP
Scinax pombali (Lourenço et al., 2013)	NE	PP	314.00	138.74	44.2	43.01	31.0	32.4	75.3	1	0	

can be considered in a vulnerable situation, with less than 30% of their range legally protected. This agrees with a recent global analysis showing that the ranges of > 80% of global DD anurans lie completely outside existing PAs (Nori and Loyola, 2015) and > 50% of all range restricted amphibian species are not represented in any PA around the world (Nori et al., 2015). Poor PA coverage is also true for other taxonomic groups: most of the world's terrestrial threatened vertebrates (Venter et al., 2014) and 20% of the Cerrado endemic and threatened species (Klink and Machado, 2005) are not adequately protected. These results highlight the need to incorporate species distribution knowledge when designating PAs and indicate, as noted by Venter et al. (2014), that new PAs must be strategically located to deliver the highest conservation benefits.

Our results reinforce previous recommendations to include DD species in conservation planning, especially

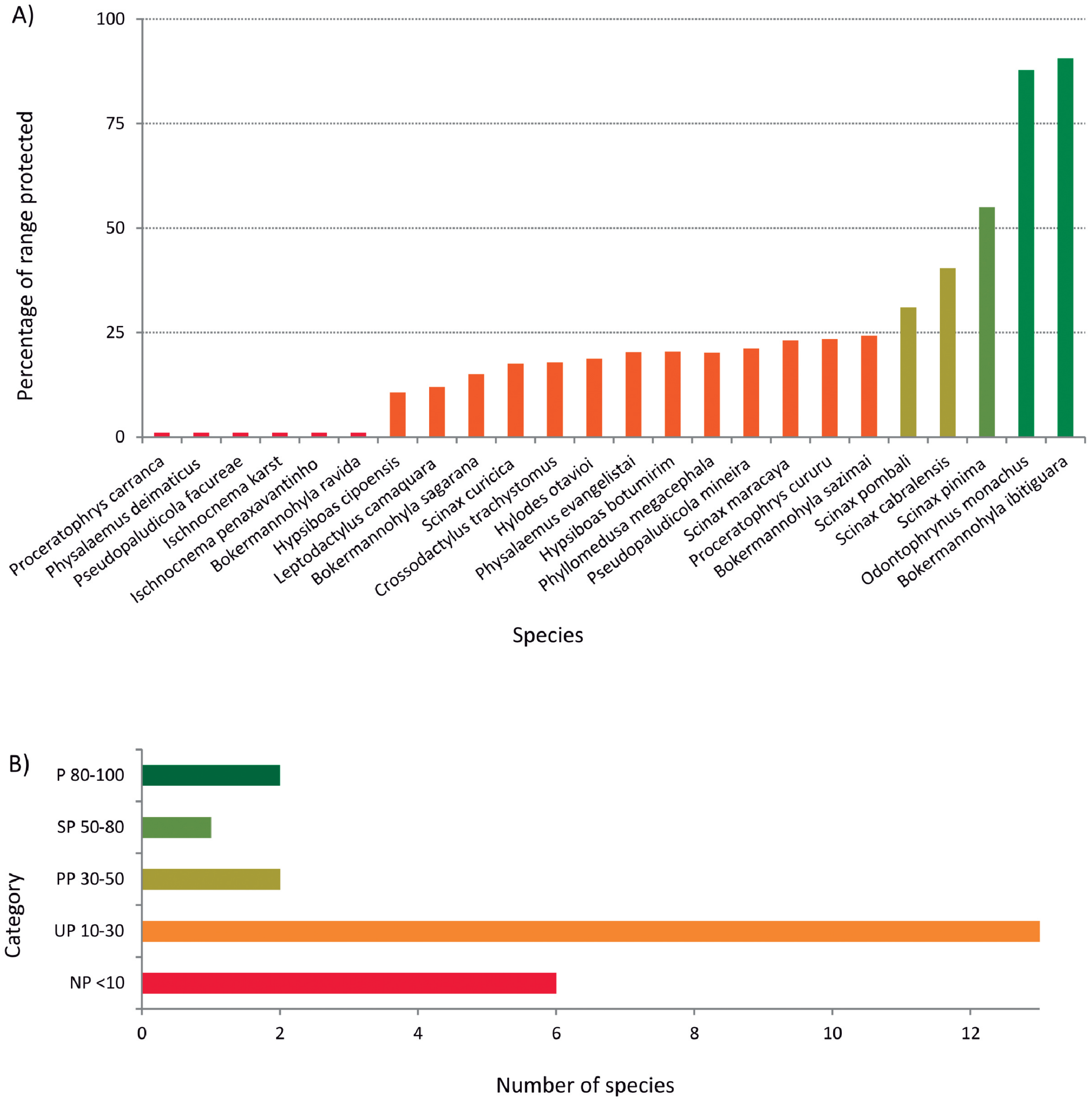

Figure 1. **(A)** Percentage of species ranges inside protected areas and **(B)** number of species in each protection category according to the percentage of species ranges covered by protected areas for Cerrado amphibian species endemic to Minas Gerais state, southeastern Brazil. NP: not protected, < 10% of range covered; UP: unsatisfactory protected, 10–30% of range covered; PP: partially protected, 30–50% of range covered; SP: satisfactorily protected, 50–80%; P: protected, > 80% of range covered.

with regard to the degree to which they might be threatened in the future (Trindade-Filho et al., 2012; Morais et al., 2013; Nori and Loyola, 2015; Nori et al., 2015). Trindade-Filho et al. (2012) demonstrated that the inclusion of DD species can impact and change the spatial configuration of protected areas network, while Brito (2010) argued that protecting localities with DD species might also protect sites with species unknown to science. Occurrence points for target species might be considered an imperfect representation of their true extent of occurrence, since the distributional range of several DD species is not completely known. While we acknowledge the limitations of using data points to represent the distribution of our targets, we believe our analysis provides the most up-to-date picture of the current scenario for target species in the Cerrado of Minas Gerais.

Considering the high rates of habitat loss and the low percentage of protected habitats in the Cerrado, establishment of new PAs and/or expansion of existing ones is likely to benefit not only the target species within

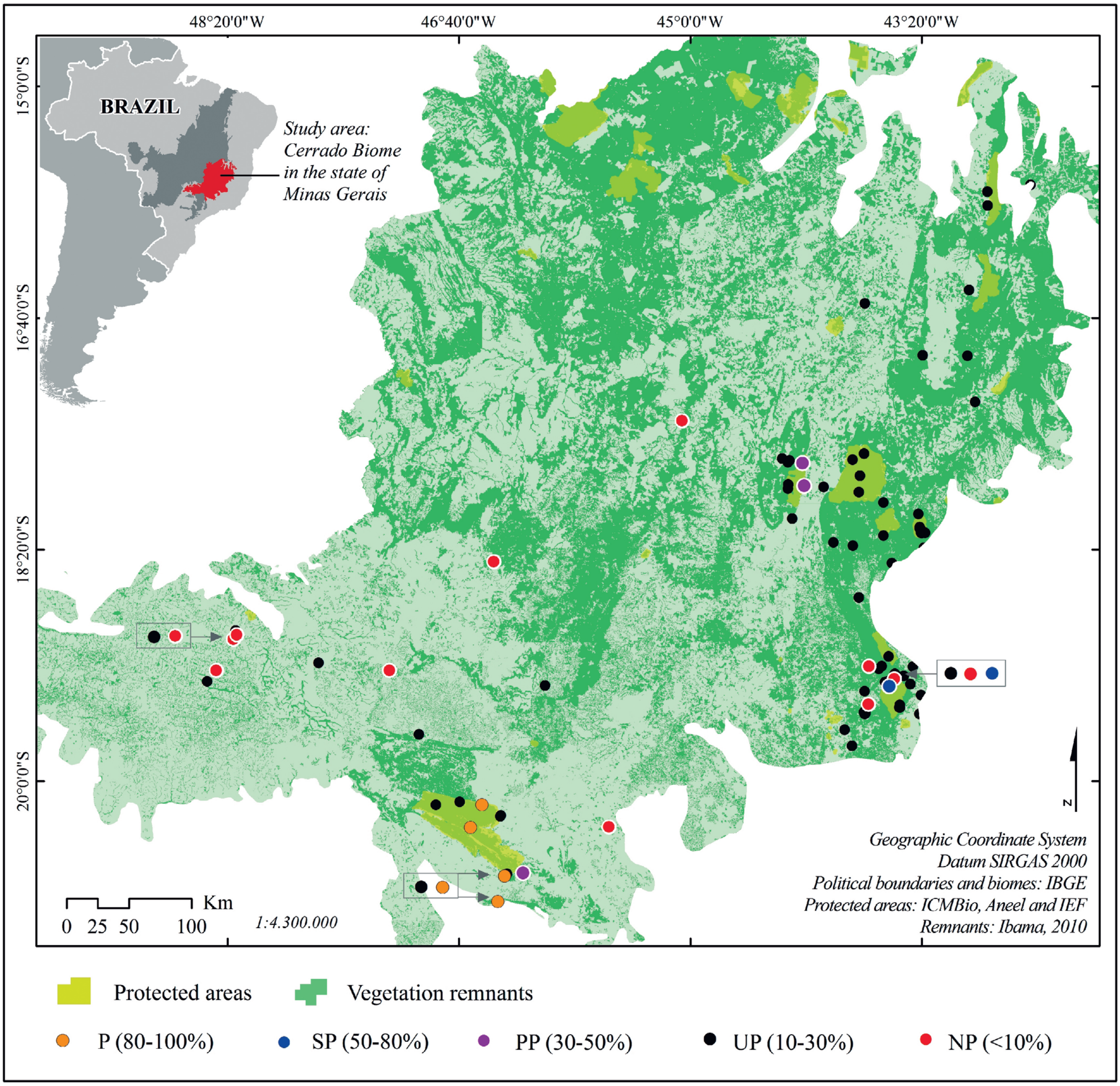

Figure 2. Distribution records of target amphibian species within study area (Cerrado biome in Minas Gerais state, southeastern Brazil), along with protected areas (light green) and vegetation remnants (dark green). Species are labelled according to protection category. NP: not protected, < 10% of range covered; UP: unsatisfactory protected, 10–30% of range covered; PP: partially protected, 30–50% of range covered; SP: satisfactorily protected, 50–80%; P: protected, > 80% of range covered.

this study, but Cerrado biodiversity in general. Although site protection persists as the main conservation recommendation for most globally threatened tetrapods (Boyd et al., 2008), the financial, political, and social costs of establishing strict PAs make this solution feasible only in some cases. Thus, additional strategies must be considered together with PA network expansion, as well as refining the information to where and how conservation efforts should be allocated.

Conservation goals for target species

Our data clearly show that improvements in the proportion of species ranges that is protected are still required. The decision regarding which species to target with conservation actions is complex and potentially controversial. Evidently, NP species are in a critical situation; however, some NP species are of higher concern than others because they lack continuous natural habitats inside their

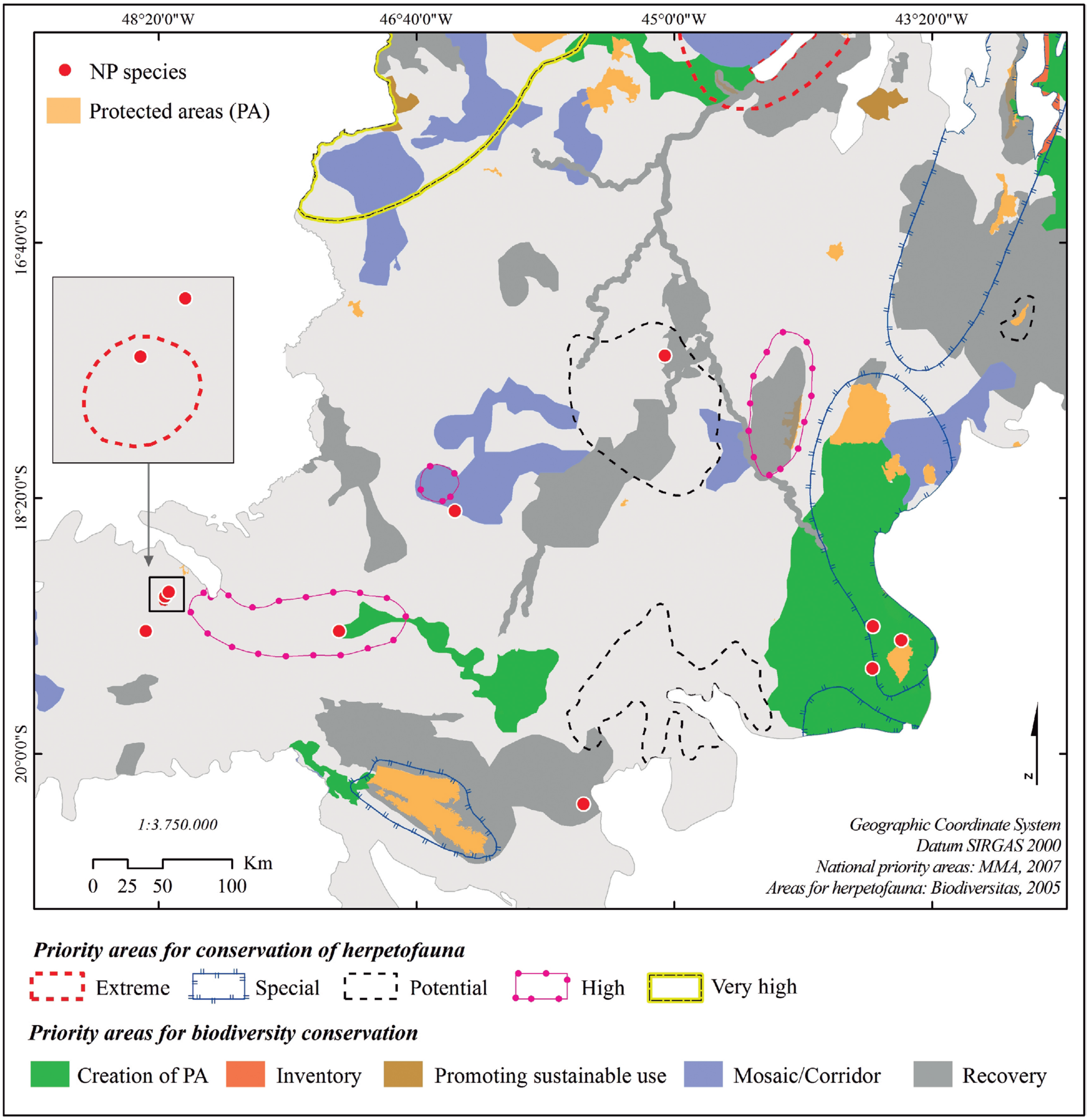

Figure 3. Distribution of Cerrado amphibian species endemic to Minas Gerais state, southeastern Brazil that are not protected (NP) overlapped with protected areas (pale orange), priority areas, and actions recommended for conservation planning.

Table 2. Summary of scientific papers on target amphibian species endemic to the Cerrado in the state of Minas Gerais. The table reports year of description (Year), total number of citations obtained in the general search within each category and number of specific articles on target species (in parenthesis) for each category, and total number of citations (Total citations) and total number of specific studies (Specific papers) for each species and category.

Species	Year	Category										Total citations	Specific papers
		Conservation		Distribution		Ecology		Genetics		Taxonomy			
Bokermannohyla ibitiguara	1983	0	(0)	1	(0)	6	(3)	2	(1)	13	(1)	22	5
Bokermannohyla ravida	2001	0	(0)	0	(0)	0	(0)	0	(0)	5	(1)	5	1
Bokermannohyla sagarana	2011	0	(0)	0	(0)	0	(0)	0	(0)	3	(1)	3	1
Bokermannohyla sazimai	1982	0	(0)	4	(0)	0	(0)	0	(0)	7	(1)	11	1
Crossodactylus trachystomus	1985	3	(1)	1	(1)	7	(1)	0	(0)	7	(6)	18	9
Hylodes otavioi	1983	2	(0)	1	(0)	0	(0)	0	(0)	11	(1)	14	1
Hypsiboas botumirim	2009	0	(0)	0	(0)	0	(0)	0	(0)	4	(1)	4	1
Hypsiboas cipoensis	1968	0	(0)	1	(0)	3	(0)	0	(0)	9	(2)	13	2
Ischnocnema penaxavantinho	2007	0	(0)	1	(0)	2	(1)	0	(0)	3	(1)	6	2
Ischnocnema karst	2012	0	(0)	0	(0)	0	(0)	0	(0)	1	(1)	1	1
Leptodactylus camaquara	1978	2	(0)	1	(1)	3	(0)	0	(0)	7	(2)	13	3
Odontophrynus monachus	2012	0	(0)	0	(0)	0	(0)	0	(0)	1	(1)	1	1
Phyllomedusa megacephala	1926	3	(0)	2	(1)	4	(1)	3	(3)	5	(3)	17	8
Physalaemus deimaticus	1988	1	(0)	2	(0)	2	(0)	2	(1)	9	(2)	16	3
Physalaemus evangelistai	1967	1	(0)	1	(1)	3	(0)	1	(0)	4	(3)	10	4
Proceratophrys carranca	2013	0	(0)	0	(0)	2	(0)	0	(0)	4	(2)	6	2
Proceratophrys cururu	1998	2	(0)	2	(1)	6	(0)	0	(0)	9	(2)	19	3
Pseudopaludicola facureae	2013	0	(0)	0	(0)	0	(0)	1	(1)	5	(2)	6	3
Pseudopaludicola mineira	1994	2	(0)	1	(0)	4	(1)	2	(2)	6	(2)	15	5
Scinax cabralensis	2007	0	(0)	1	(0)	0	(0)	0	(0)	5	(1)	6	1
Scinax curicica	2004	2	(0)	3	(1)	5	(0)	1	(1)	9	(2)	20	4
Scinax maracaya	1980	0	(0)	0	(0)	1	(1)	0	(0)	13	(1)	14	2
Scinax pinima	1973	1	(0)	0	(0)	0	(0)	0	(0)	3	(2)	4	2
Scinax pombali	2013	0	(0)	0	(0)	0	(0)	0	(0)	2	(1)	2	1
Total		**19**	**1**	**22**	**6**	**48**	**8**	**12**	**9**	**145**	**42**	**246**	**66**

distributions, which means it is likely to be more costly to promote their conservation. This is the case of *Pseudopaludicola facureae, Ischnocnema karst* and *I. penaxavantinho*—all NP species with only 11.8, 17.7, and 25.5% of natural vegetation remnants inside their MCP, respectively. These species occur in southwestern Minas Gerais where national and state priority recommendations are species inventory, creation of PA and corridors, and expansion of existing PA (Serra da Canastra National Park; Drummond et al., 2005; PROBIO/MMA, 2007). Considering the low percentage of available natural habitats for these species, we suggest that habitat restoration and connectivity are more feasible and have better chances of success, together with the creation of private reserves and law enforcement to guarantee riparian habitats protection.

The scenario is different for *Physalaemus deimaticus,* in southern Espinhaço Range, close to Serra do Cipó National Park, but with zero coverage by PAs and 70% of native habitat remnants inside its MCP. For this species, expansion of the existing PAs might be feasible; nevertheless, we suggest further investigation on species range, surveying nearby protected areas (Serra do Cipó National Park and Serra do Intendente State Park) to search for new populations. According to Eterovick et al. (2005), the species is known from Serra do Cipó National Park; however, the occurrence points provided show an overlap of less than 0.1% with PA boundaries.

In addition to NP species, several UP species are also recorded in the Espinhaço Range, where there is still a high percentage of natural vegetation cover outside PAs.

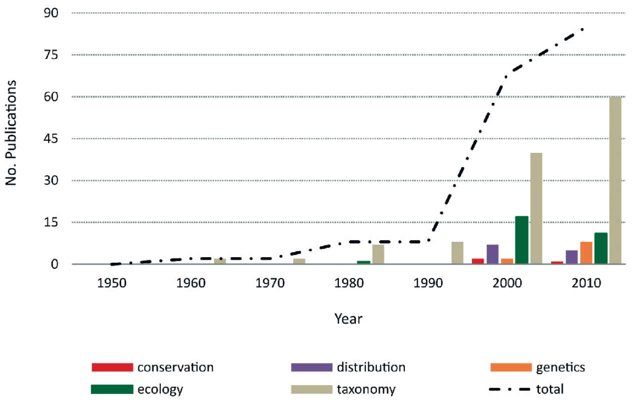

Figure 4. Number of scientific publications 1950–2015 (note: 2010 includes only 2010–present), for each target species of Cerrado amphibians endemic to Minas Gerais state, southeastern Brazil. An evident increase from the late 1990s is shown by the dash-dot line.

For example, *Bokermannohyla sagarana* has only 15% of its range protected by Serra do Cabral State Park, but has 96.6% of native remnants inside its MCP; or *Pseudopaludicola mineira* and *Proceratophrys cururu* that have less than 20% of their distributions inside PAs and almost 90% of native habitats within their polygons. Previous recommendations for this region include species inventory, habitat restoration, expansion of existing PAs, and establishment of biodiversity corridors (Drummond et al., 2005; PROBIO/MMA, 2007). Nevertheless, when compared to other regions in the state, the south Espinhaço Range has a considerable number of PAs and, according to Silva et al. (2008), the creation of new PAs in this region is highly costly. Therefore, we believe the most reasonable conservation actions would be the establishment of biodiversity corridors connecting existing PAs.

Creation of PAs still stands as probably the best solution for the protection of *Proceratophrys carranca* and *Bokermannohyla ravida,* two NP species known only from their type localities in western Minas Gerais and with 44% and 55% of natural cover inside their MCPs (respectively). Previous recommendations for this region include habitat restoration and creation of biodiversity corridors (Drummond et al., 2005), but there are few opportunities for connectivity with other PAs within a 100 km radius for both species. The region is considered a priority area for herpetofauna conservation (Drummond et al., 2005), has relatively low human pressures and high vegetation

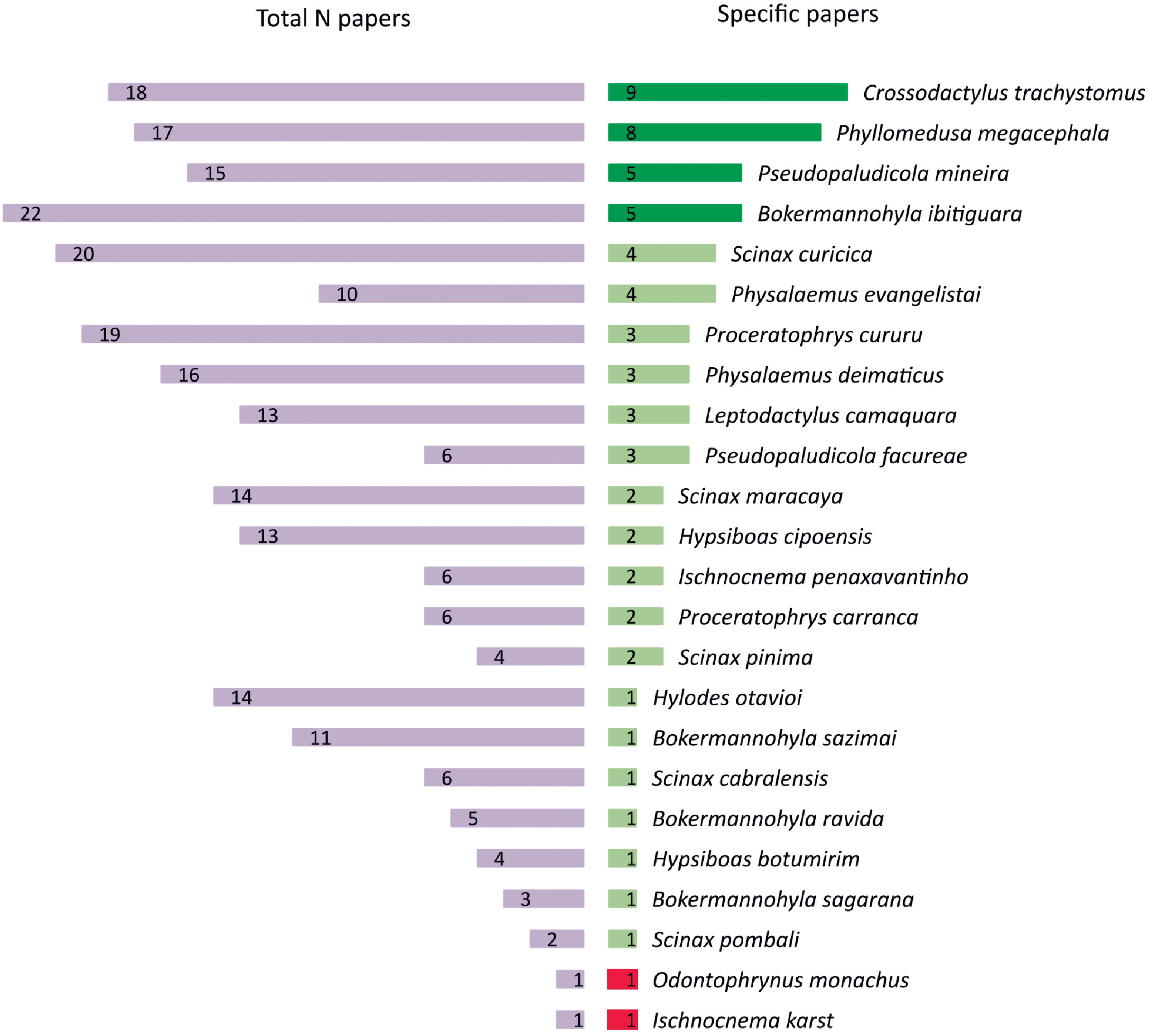

Figure 5. Number of publications recorded for each target species (all categories combined) of Cerrado amphibians endemic to Minas Gerais state, southeastern Brazil, showing the proportion of total records (left, pale purple) and specific papers (right, light green). Targets species with more than five publications are highlighted (dark green), as are critical species with only one record provided in total and specific numbers (light red).

cover, indicating that PA establishment is viable and would result in at least one population of each species to be safeguarded.

Improving scientific knowledge

Scientific knowledge continues to be insufficient for most DD anuran species endemic to the Cerrado of Minas Gerais and all research categories must be improved to allow the redefinition of each species' status and ultimately inform conservation and management actions. In the state of Minas Gerais, three anurans are critically endangered and seven are vulnerable (all from the Atlantic Rainforest), while 71 are still classified as DD (Nascimento et al., 2009). However, threatened species categories are inconsistent between national and international lists (Morais et al., 2012). Detailed information is needed about the processes affecting the species, without which an accurate assignment of species status is impossible. In general, we still lack sufficient knowledge on most target species. Nevertheless, based on the current literature, we believe some of these species might already have sufficient information to be re-assessed, such as *Crossodactylus trachystomus, Phyllomedusa megacephala, Bokermannohyla ibitiguara, Pseudopaludicola mineira* and *Scinax curicica*.

All target species, especially DD species, need further research on their geographical distribution, but some of them must be prioritized, such as recently described species (e.g., *Proceratophrys carranca*), amphibians with only one or two known occurrence points (e.g., *Bokermannohyla ravida*) and *Physalaemus deimaticus*. This would increase known species ranges and help future reviews on effective protection options. For species with 5–10 occurrence points, the modeling method proposed by Teixeira et al. (2015) for Brazilian DD amphibian species in forested habitats might be a useful tool to combine occurrence data, environmental suitability, and connectivity to prioritize sites for field surveys. Additionally, considering the shortage of distribution and status data in the country, we recommend that researchers share their data with conservation planners and practitioners.

Population ecology studies were less represented in our analysis. Controversially, Griffiths and Dos Santos (2012) revealed that the main topic of papers published in conservation journals was population biology, but the most popular taxa were mammals, birds, invertebrates, and plants. However, our analysis focused on amphibians only, a group with a very few specific articles published in main conservation journals (Griffiths and Dos Santos, 2012). Likewise, ecological research focusing on amphibians in Minas Gerais has been developed recently (Nascimento et al., 2009), with an increasing number of professionals in the area. These factors might respond to the expected low number of population-based studies for our target species. While we acknowledge the drawbacks of developing population ecology studies of rare and range-restricted amphibian species, efforts to do so should be increased, since most of the knowledge required to assess conservation status comes from this type of analysis. Ecological studies should focus on target species with better documented occurrences (e.g., *Proceratophrys cururu, Leptodactylus camaquara,* and *Hypsiboas cipoensis* in the Espinhaço Range; *Bokermannohyla sazimai* and *Scinax maracaya* in Serra da Canastra). Nonetheless, the first step in an ecological approach for species with few data points is to improve knowledge on their distributions.

Since collaboration is required to combine expertise and techniques that can address further conservation problems (Griffiths and Dos Santos, 2012), we also encourage collaborative research on different research subjects, such as genetics and conservation, ecology, and declines. Furthermore, although declines have been a major topic for amphibian conservation worldwide in the last years, research on declines has not been a focus between 2000–2010, at least for the target species in this study. According to Eterovick et al. (2005), in the Cerrado biome a minimum of 20 declining species are lacking ecological or monitoring studies, both of which are urgently needed to provide policy makers with specific recommendations for amphibian conservation.

CONCLUDING REMARKS

The current network of protected areas is insufficient to safeguard range-restricted and poorly-known anuran species within the Cerrado biome of Minas Gerais state. We have produced a reliable database for a subset of the endemic amphibians of Minas Gerais and provided recommendations on future research and conservation efforts. Our main recommendations concern PA establishment in the western part of the state to safeguard populations of NP species; creation and maintenance of biodiversity corridors between existing PAs and riparian habitat protection to connect UP species populations in the Espinhaço Range; and, finally, habitat restoration in southwestern Minas Gerais to provide additional habitat for NP species within this region. Although scientific knowledge on target species needs much improvement, we provided an update on species range distributions that may be useful in future assessments. Additionally, we have shown that some of the DD species might have enough information to be re-evaluated. More importantly, we expect the results and recommendations provided here to guide state and national agendas, contributing to well-informed conservation actions, improved allocation of resources, better management of PAs, and more reliable species assessments.

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AMPHIBIAN SPECIES COMPOSITION AND PRIORITIES FOR REGIONAL CONSERVATION AT THE ESPINHAÇO MOSAIC, SOUTHEASTERN BRAZIL

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***Abstract*.—The southern portion of the Espinhaço Range in Brazil is recognized worldwide as a priority area for biodiversity conservation, and it contains a high number of endemic anuran species. We conducted field surveys and compiled published data on amphibian community composition from seven sites within Espinhaço Mosaic (EM; 910,000 ha) to explore the contribution of this area to amphibian species richness in the southern Espinhaço Range. We aimed to describe local and regional community composition and to identify priorities for future amphibian surveys and inventories in the study area. We consider the EM a species-rich area sheltering 73 anuran species, which represents 36.5% of the amphibians known for the state of Minas Gerais, 57.5% of those in the Cerrado biome, and almost 70% of the species in the Espinhaço Range. Unequal sampling effort is a major concern in the study area, and species richness in under-sampled sites might increase as new assessments are conducted. Therefore, sites for which no data are available should be prioritized for species inventories. Although an increase in sampling effort is likely to reduce the proportion of exclusive species (i.e., species known to occur in only one of the seven investigated sites), we conclude that the levels of endemicity indicate a high number of narrowly distributed (micro-endemic) species. We believe anuran community composition and similarities in composition among the sites investigated are influenced by the gradient between the Cerrado and Atlantic Rainforest biomes, which deserves further investigation.**

Key Words.—anurans; biodiversity; cluster analysis; community composition; endemism; Espinhaço Range; species richness

INTRODUCTION

The Brazilian list of amphibians comprises 1,026 living species of the nearly 7,348 known species in the world (Frost, D.R. 2015. Amphibian species of the world: an online reference. Version 6.0. Available from http://research.amnh.org/herpetology/amphibia/index.html. [Accessed 10 May 2015]; Segalla, M.V., U. Caramaschi, C.A.G. Cruz, P.C.A. Garcia, T.L. Grant, C.F.B. Haddad, and J. Langone. 2015. Brazilian amphibians – list of species. Available online at: http://www.sbherpetologia.org.br [Accessed 9 January 2015]), a number that exceeds the latest estimates of amphibian species richness for the country (Pimm et al. 2010). Two biomes of particular interest for biodiversity conservation are the Cerrado and Atlantic Rainforest, both of which have high levels of endemism and are severely threatened by habitat loss (Myers et al. 2000). Valdujo et al. (2012) recorded 209 species from at least one locality within the Cerrado, including 108 endemics (51.7%), whereas Haddad et al. (2013) reported more than 500 amphibian species within the Atlantic Rainforest, and 88% endemism. The Espinhaço Range is the geographical divisor of these hotspots (the Cerrado to the west and Atlantic Rainforest to the east) and its unique geological conditions contribute to a high level of endemism for several taxa (Gontijo 2008), including amphibians (Leite et al. 2008; Leite 2012). According to Valdujo et al. (2012) some endemic anuran species occur only on the western slope and summit (Cerrado) of the Espinhaço Range, while others occur exclusively in a few localities on the eastern slope (Atlantic Rainforest).

The Espinhaço Range is nationally and regionally recognized as a priority area for biodiversity conservation (Projeto de Conservação e Utilização Sustentável da Diversidade Biológica Brasileira/ Ministério do Meio Ambiente [PROBIO/MMA] 2007; Drummond et al. 2005). The southern portion of the Espinhaço Range is a UNESCO Biosphere Reserve, a center of plant diversity (Davis et al. 1995), one of the Global 200 Ecoregions (Olson and Dinerstein 2001), an Important Bird Area for endemic species (Develey and Goerck 2009), and a center for amphibian endemism (Leite et al. 2008). In the southern Espinhaço Range, the landscape is characterized by several fragments of Cerrado and Atlantic Rainforest, some of which are

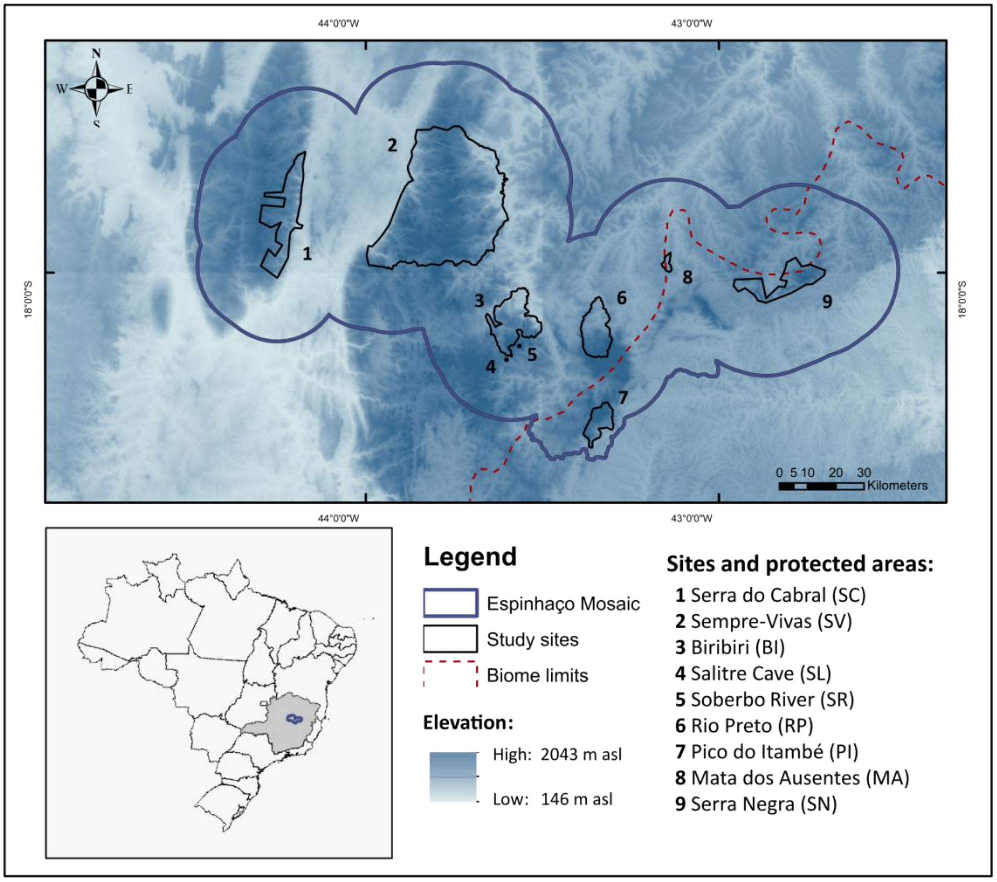

FIGURE 1. Study sites within the Espinhaço Mosaic, at South Espinhaço Range, Minas Gerais State (shaded area on inset map), southeastern Brazil. Sites are numbered; biome boundary (dotted red line) delimits Cerrado biome west of the boundary and Atlantic Rainforest biome to the east. Elevational bands also presented (meters above sea level).

legally protected by state and national authorities, composing a mosaic of protected areas of different sizes and shapes. In 2010 Brazilian authorities recognized the Espinhaço Mosaic in the state of Minas Gerais, southeastern Brazil (Mosaico Espinhaço: Alto Jequitinhonha-Serra do Cabral; hereby EM), which is listed as one of the 20 mosaics in Brazil (Gidsicki 2013).

Due to its level of diversity and threat, a national action plan was recently proposed for the conservation of threatened amphibian and reptile species in the southern Espinhaço Range (PAN Espinhaço 2012). However, an effective conservation plan requires at least some understanding of the target species (Brito 2004), and very often knowledge about biodiversity spatial patterns is crucial to regional conservation planning (Gaston and Rodrigues 2003). Practical decisions are usually made at regional or local scales (Bini et al. 2006), but unfortunately local data are lacking for several regions in the world, especially in the tropics (Collen et al. 2008). Conducting biodiversity surveys in such areas is the only way to overcome this data gap, with the added benefit of potentially finding species new to science and improving the understanding of the geographic distributions of species (Rondinini et al. 2005).

In this study we investigated the amphibian species richness and community composition in the EM. We conducted field surveys and compiled data on amphibian community composition for seven sites (five protected areas and two adjacent natural areas) at southern Espinhaço Range, all within EM. Furthermore, we explored the contribution of studied sites to amphibian species richness within the Espinhaço Range, and we also identified priorities for amphibian surveys and inventories in the state of Minas Gerais, especially in the EM.

MATERIALS AND METHODS

Our study area, the EM, is located at the southern portion of the Espinhaço Range, in the state of Minas Gerais, southeastern Brazil (Fig. 1). It covers an area of 910,000 ha and includes seven protected areas (IUCN categories I and IV; Dudley 2008) that we defined as our study sites. We also included two additional sites, which are not protected areas but are located within EM (Fig. 1; therefore, a total of nine sites within study area). From 2010 to 2015, we surveyed four of the above sites: Sempre-Vivas National Park (SV), Pico do Itambé State Park (PI), Soberbo River (SR) and Salitre Cave (SL).

We followed the Rede ComCerrado sampling protocol (available from www.conservacao.bio.br/comcerrado/protocolos [Accessed 20 May 2014]) to survey anuran species at SV (municipality of Diamantina, Minas Gerais state, southeastern Brazil, 17°52'S, 43°45'W). We selected 10 sampling units and we conducted visual encounter surveys (Crump and Scott 1994) during the wet season, in October 2010 and May 2011 (16 nights). At PI (municipality of Santo Antônio do Itambé, Minas Gerais state, southeastern Brazil, 18°24'S, 43°19'W) we surveyed all available microhabitats from 1,230 to 2,060 m above sea level (asl), using night visual encounter surveys (Crump and Scott 1994) during wet and dry seasons (19 nights, from September 2010 to October 2011). We conducted monthly surveys at SR (municipality of Diamantina, Minas Gerais state, southeastern Brazil, 18°15'S, 43°36'W, 1,113 m asl) from April 2010 to March 2011 (26 nights). At this site we placed linear transects (Heyer et al. 1994) in sections of 100 m along the river, using night visual encounter (Crump and Scott 1994) and acoustic surveys (Zimmerman 1994). We used the same methods to survey anurans at SL (municipality of Diamantina, Minas Gerais state, southeastern Brazil, 18°41'S, 43°11'W) during a rapid assessment in dry and wet seasons (February and June 2015, 15 nights), with the survey effort randomly distributed in 25 sampling units. Specimens are available for examination at the herpetological collection of Museu de Ciências Naturais, Pontifícia Universidade Católica de Minas Gerais (Belo Horizonte, Minas Gerais), and Coleção Herpetológica do Laboratório de Zoologia dos Vertebrados, Universidade Federal de Ouro Preto (Ouro Preto, Minas Gerais). We estimated species richness using Jackknife I and evaluated inventory completeness by plotting species richness observed (SOBS) against sampling effort. We obtained both Jackknife I and SOBS from the software EstimateS (Colwell 2013).

In June 2015 we performed literature searches for publications containing amphibian species lists for the following sites: Biribiri State Park (BI), Rio Preto State Park (RP), Serra do Cabral State Park (SC), Serra Negra State Park (SN), Pico do Itambé State Park (PI), Mata dos Ausentes Ecological Station (MA), and Sempre-Vivas National Park (SV). Our search aimed to list all species recorded for each of these sites. We searched for scientific publications (papers, reviews, and books), but also reports, conference abstracts, management plans, theses, and monographs. We used specific keywords during our search (both in English and Portuguese), combined in different ways: keyword related to taxon (e.g., amphibia, anura, herpetofauna); keyword related to study area (e.g., protected area's name, mosaic name and synonyms, and Espinhaço Range); and (when necessary) a keyword related to our aim (e.g., species list, inventory, species richness, and community composition). We searched peer-reviewed references with the Thomson ISI research tool (Web of Science database, available from http://ipscience.thomsonreuters.com [Accessed 10 February 2015]) with the following parameters: all documents types, all languages, all databases; from 1950–2015, and keywords entered in the title and abstract. We searched for other references using Google Scholar (available from https://scholar.google.co.uk [Accessed 10 February 2015]), and to identify management plans available we contacted protected area managers and state administration offices. We classified all species according to their IUCN category (IUCN. 2015. The IUCN Red List of Threatened Species. Version 2012.2. Available from www.iucnredlist.org [Accessed 1 March 2015]).

We generated a species list for each site, combining our survey and literature search when both sources were available. This approach provided us not only with species richness at each site, but also the regional species pool for the study area. We used this compilation to evaluate the contribution of EM to the anuran species richness for the state of Minas Gerais and for the Espinhaço Range as a whole. To evaluate the similarity between the anuran assemblages at each site, we used hierarchical cluster analysis, which combines similar objects in groups using a similarity or distance measure (Quinn and Keough 2007). We conducted this exploratory analysis in R (R Core Team 2014) using species presence/absence data. We used UPGMA as the linking method and Euclidian distance as the distance measure (Quinn and Keough 2007). To avoid uncertainties in characterizing community composition at each site and to provide a more conservative exploratory analysis, we excluded records from the cluster analysis that were not identified to species level (e.g., *Hypsiboas* sp.), and we followed recent taxonomic reviews that grouped species (e.g., we grouped records of *Elachistocleis* sp. into *Elachistocleis cesarii* according to Caramaschi 2010).

RESULTS

We surveyed four sites (PI, SL, SV, and SR) and compiled data from literature for four sites (BI, PI, RP, and SC). Overall, we gathered data from seven sites within the EM (data from one of the sites came from both surveys and literature), among which five are protected areas. We recorded 15 anuran species in 26 nights at SR, which represents 72.25% of estimated richness (18.8; Fig. 2). We recorded 28 anurans during

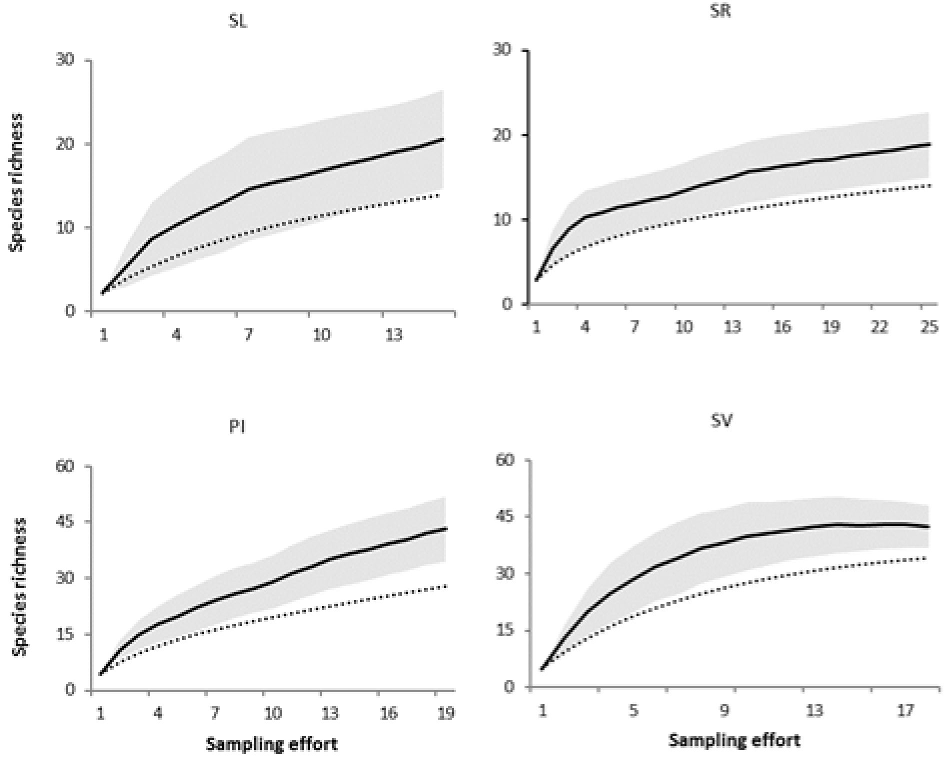

FIGURE 2. Species accumulation curves for surveyed sites: Soberbo River (SR), Pico do Itambe State Park (PI), Sempre-Vivas National Park (SV), and Salitre Cave (SL), Espinhaço Mosaic, Brazil. Estimated species richness (solid line) is shown with its 95% confidence interval (shaded gray). Observed species richness is represented by dotted line. Sampling effort is represented by number of survey nights.

19 sampling nights at PI, adding 11 new species to the list available in the literature. Estimated species richness at this site was 43.16 (considering only sampled data; 65% of estimated species richness; Fig. 2). At SV we recorded 34 species in 16 nights, which represents 80% of estimated richness (42.5; Fig. 2). Our sampling effort at SL was 15 nights and we observed 14 anuran species, representing 68% of those estimated to occur at this site (20.53; Fig. 2).

We found 11 publications containing amphibian species lists for four sites (all protected areas): BI, PI, RP, and SC (Table 1). We had no records or additional information for SV, SN, and MA (Table 1) and, therefore, these sites were not included in our exploratory analysis. Sampling effort (represented as total number of night surveys) differed among sites, as well as total anuran species richness (Table 1). Based on the literature and our inventories, we listed 73 anuran species within the EM; however, nine were not identified to species level (Appendix). If we exclude these species, the total number drops to 64, among which 21 were restricted to only one site (i.e., exclusive species; Fig. 3; Appendix). The highest richness was found at RP with 46 species, followed by PI with 44 species (Table 1; Fig. 3). None of the species recorded are considered in the national or regional lists of threatened amphibians (Machado et al. 2008; Drummond et al. 2008). Only four species were not evaluated by the IUCN (IUCN. 2015. *op. cit.*) and we recorded 10 species designated as Data Deficient by the IUCN (Appendix). Among the latter, six are endemic to the Espinhaço Range and three are known for type locality (Appendix). Two species are considered near threatened (NT): *Bokermannohyla sagarana* and *Hypsiboas cipoensis*. The cluster analysis indicated that our sites form distinct groups according to their anuran community composition (Fig. 4). Our analysis demonstrated that RP and PI harbor similar amphibian communities, whereas SV and SC formed a different group with distinct anuran assemblages, closely positioned to the third group with BI, SL, and SB (Fig. 4).

DISCUSSION

In this study we provide species lists for three previously unsurveyed sites within the Espinhaço Range

TABLE 1. Information and data sources for sites evaluated in the present study of amphibian species richness and community composition at Espinhaço Mosaic, Brazil. Species richness values are from surveys in present study, literature cited, or both combined. Sampling effort is represented by number of nights. Biomes are Cerrado (CE) and Atlantic Rainforest (RF); data not available is given as na.

Code	Study sites	Area (ha)	Biome	Species richness	Sampling effort	References
BI	Biribiri State Park	16,999	CE	24	10	IEF 2004a
MA	Mata dos Ausentes	490	CE	na	na	na
PI	Pico do Itambé State Park	4,696	RF	44	29	Present study; Barata et al. 2013; IEF 2004b
RP	Rio Preto State Park	12,185	RF/CE	46	121	Correia 2015; Oliveira and Eterovick 2009, 2010 Leite et al. 2006; IEF 2004c
SL	Gruta do Salitre	100	CE	14	15	Present study
SC	Serra do Cabral State Park	22,494	CE	34	12	IEF 2015; Drummond et al. 2007; Leite et al. 2011
SN	Serra Negra State Park	13,654	RF/CE	na	na	na
SV	Sempre-Vivas National Park	124,154	CE	34	16	Present study
SR	Soberbo River	na	CE	15	26	Present study

(SV, SL, and SR) and we also complement the species list for Pico do Itambé State Park. Despite the large number of anuran species recorded, our estimates of species richness indicated the need to increase sampling effort, which is also evident from most of the observed richness accumulation curves. This result suggests that further assessments are likely to increase species richness, especially at sites with a lower number of surveys, such as at SR and SL. It is noteworthy though, that at PI, for which our survey results and literature data were both available, the joint species richness is similar to the estimated richness based solely on our field surveys. Uneven surveys are a problem within the entire Espinhaço Range, where the number of species recorded at the northern mountain Range (Bahia State) is less than those recorded in the southern portion (Leite et al. 2008). Even in Minas Gerais, survey effort is concentrated at Serra do Cipó and Quadrilátero Ferrífero (Nascimento et al. 2009). It should thus be a major priority to survey anuran species in Espinhaço sites (protected areas and elsewhere) for which no data are available, such as SN and MA.

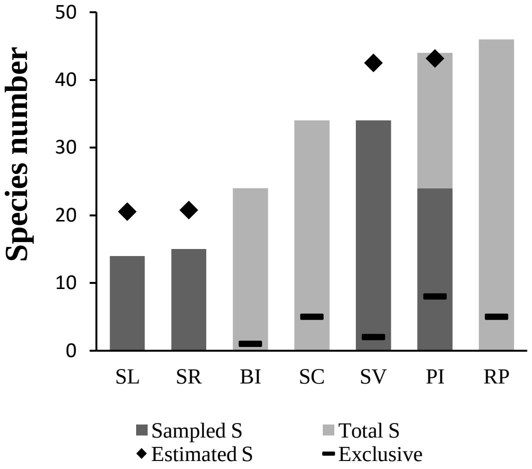

FIGURE 3. Species richness at the seven study sites with data: Salitre Cave (SL), Soberbo River (SR), Biribiri State Park (BI), Serra do Cabral State Park (SC), Sempre-Vivas National Park (SV), Pico do Itambe State Park (PI), and Rio Preto State Park (RP), Espinhaço Mosaic, Brazil. Data are from surveyed sites (Sampled S), literature search and survey data combined (Total S), and number of exclusive species (i.e., species reported for only one site). For surveyed sites, estimated species richness (Estimated S) is also given (black diamonds). Original data are provided in Appendix.

Our results provide a compilation of available data on regional anuran species richness and community composition for the southern portion of the Espinhaço Range (especially at EM). We also contribute to the geographic knowledge of the distribution of several species known to this mountain range. This information can be useful in further assessments of the conservation status of Data Deficient anurans endemic to the Cerrado, which are mainly concentrated in the EM according to Barata et al. (2016). With 73 species recorded, we consider the EM a species-rich area, harboring an amphibian community representative of both the Cerrado and Atlantic Rainforest biomes, and the Espinhaço Range. Approximately 200 amphibian species are recorded from Minas Gerais (Nascimento et al. 2009), among which 127 are reported within the Cerrado (Barata et al. 2016). More than 105 anurans occur in the Espinhaço Range (Leite et al. 2008), although this number might be slightly greater (Leite 2012), and include many endemic species. Therefore,

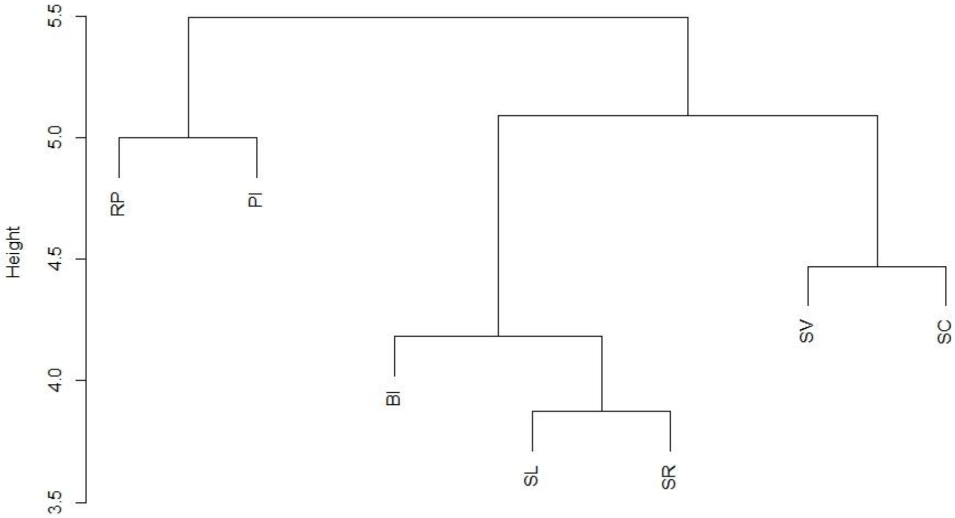

FIGURE 4. Cluster analysis showing similarities in anuran composition among seven study sites: Salitre Cave (SL), Soberbo River (SR), Biribiri State Park (BI), Serra do Cabral State Park (SC), Sempre-Vivas National Park (SV), Pico do Itambe State Park (PI), and Rio Preto State Park (RP), Espinhaço Mosaic, Brazil.

our compilation represents 36.5% of amphibian species from Minas Gerais, 57.5% of the Cerrado in the state, and almost 70% of the species known from Espinhaço Range. Not only is regional diversity high, but also local species richness is high in most sites. For example, RP alone harbors 42% of the amphibian species from Espinhaço Range, which highlights the contribution of this protected area in conserving regional species richness. Although this impressive amphibian diversity is recorded in the EM (mainly inside protected areas), this is not the usual pattern recorded in the state of Minas Gerais, where several Data Deficient endemic anuran species still lack coverage by protected areas (Barata et al. 2016).

We recorded 21 species exclusively at only one of the seven study sites, among which three are widespread in Brazil. Three other species of the 21 are representative of the Cerrado, nine are representative from the Atlantic Rainforest, two are exclusive to the Espinhaço Range, and four are known from type localities only (Appendix). The high proportion of species exclusive to one site (29%) might partially be a consequence of the differences in sampling effort and approaches used; we surveyed some sites intensively during a few days, we surveyed others on different occasions across a period of several months. Although short surveys are efficient for obtaining a general knowledge about the amphibian community, some species may go undetected due to rarity or inactivity during the survey period. Although Heyer et al. (1994) recommend intensive sampling during the wet season, temporal variation can also be a strong factor in determining species distribution in the tropics (Conte and Machado 2005; Borges and Juliano 2007). For example, species with low abundance can be missed at a site if surveys are not well distributed over time (e.g., over a couple of years). A larger effort on site with high levels of endemism, such as the Espinhaço Range, could lead to new discoveries. According to Pimm et al. (2010) unknown species will be rare and threatened with extinction, and science may not discover them before they go extinct. Leite et al. (2008) suggested that the investigation of unexplored areas above 1,700 m elevation could result in the discovery of new species. This idea is corroborated by the recent description of the mountain endemic Itambe's Bromeliad Frog (*Crossodactylodes itambe*; Barata et al. 2013).

Although an increase in sampling effort is likely to reduce the proportion of exclusive species, we believe the levels of endemicity recorded indicate a high number of narrowly distributed species inside the EM region. For example, among the exclusive species, we considered four as micro-endemic because they have been recorded only at the type locality and have highly restricted distributions (small ranged and few known populations): *Bokermannohyla* cf. *diamantina*, *B. sagarana*, *Scinax cabralensis*, and *Crossodactylodes itambe*. Micro-endemic species did not have their geographic distributions extended, demonstrating that the EM, does indeed, hold true micro-endemic species. These species are completely contained within protected reserves (equivalent to IUCN categories I and IV), being more likely to be safeguarded from habitat alteration and land use changes, such as fire and grazing. Although human induced impacts are not expected, species with small ranges are more vulnerable and prone to extinction due to adverse natural events (e.g., such as natural fire and drought; Barata et al. 2013) than wide ranging species. This emphasizes the need for adequate management of protected areas.

Community composition at EM exhibited a pattern of distribution reflecting the Atlantic Rainforest and Cerrado gradient from east to west. The first group is composed of two protected areas located at the east of EM (RP and PI), which experience a higher influence from Atlantic Rainforest habitats when compared to the opposite group (SV and SC) of western protected areas that receive higher influence from the Cerrado. This spatial pattern was evident in our cluster analysis. The number of Cerrado to Rainforest species represented at each site is higher at SV and SC (seven and six species from the Cerrado and zero and four species from the Rainforest, respectively) when compared with RP and PI, which are mainly represented by Rainforest and Espinhaço species (eight and 11 species from the Rainforest, four and eight species from the Cerrado, respectively). By contrast, among the 23 species shared between SV and SC (the Cerrado Group), there are no species characteristic of the Atlantic Rainforest. Of the 28 species shared between RP and PI (the Rainforest Group), only three are Cerrado-related species. According to Valdujo et al. (2012) species occurring in the Cerrado and its domains have a highly structured spatial pattern in which Atlantic Forest species are restricted to southeastern portions of the savanna ecosystem. Therefore, in the Cerrado, it is expected that more species are shared with the Atlantic Rainforest as you move further to its eastern limits.

It could be argued that groups identified in the cluster analysis are strongly influenced by species richness in each site, which for our data would be of some concern due to the uneven sampling effort. Even though species richness may be affecting the clusters, it also indicates a geographic pattern in the anuran communities. Therefore, we believe community composition and similarities between sites are at least partially influenced by the Cerrado-Rainforest gradient. As data from future inventories becomes available, we can improve this exploratory analysis to facilitate understanding of the effects of the ecosystem gradient on anuran community composition within the study region. Furthermore, our data suggest that species richness at less-sampled sites might increase as new assessments are conducted, showing the need to equalize sampling effort in surveyed areas. Implementing these two broad recommendations (i.e., survey new sites and equalize sampling effort) would allow a better understanding of community composition patterns across the Espinhaço Range and the influence of the Cerrado-Rainforest gradient on community composition. Moreover, we suggest that sites with larger sampling effort (such as PI and RP) should be considered for focused-ecological studies, as investing in more species surveys in these sites seems unreasonable when other sites (especially protected areas) in the region do not even have a species list. Investigating species richness of unsurveyed sites can help us to better develop conservation actions and can also facilitate future studies on ecology, distribution, and taxonomy of anuran species in the Espinhaço Range.

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Appendix Table. Anuran species occurring at the seven study sites using data from literature (four sites) and field surveys in present study (four sites) within the Espinhaço Mosaic, Brazil: Salitre Cave (SL), Soberbo River (SR), Sempre-Vivas National Park (SV), Rio Preto State Park (RP), Pico do Itambé State Park (PI), Biribiri State Park (BI), and Serra do Cabral (SC). For species occurring exclusively at one site (Exclusive), name of that site is listed. Distribution refers to species occurrence in Brazil: Cerrado biome (CE), Atlantic Rainforest biome (AF), Espinhaço Range (ES), widespread (W), type locality (T). Conservation status according to IUCN: Least Concern (LC), Data Deficient (DD), Near Threatened (NT).

	Study sites							Total			IUCN
Species	SL	SR	SV	RP	PI	BI	SC	sites	Exclusive	Distribution	Status
Brachycephalidae											
Ischnocnema juipoca				x			x	2		CE, RF	LC
Bufonidae											
Rhinella cruficer	x			x	x	x		4		RF	LC
R. mirandaribeiroi			x					1	SV	CE	
R. rubescens	x	x	x	x	x	x	x	7		CE	LC
R. schneideri			x	x			x	3		W	LC
R. sp.				x				1			
Centrolenidae											
Vitreorana eurygnatha					x			1	PI	RF	LC
V. sp.				x				1			
Craugastoridae											
Haddadus binotatus					x			1	PI	RF	LC
Cycloramphidae											
Thoropa megatympanum	x	x	x	x	x	x	x	7		ES	LC
Dendrobatidae											
Ameerega flavopicta							x	1	SC	CE	LC
Hylidae											
Bokermannohyla alvarengai	x	x	x	x	x			5		ES	LC
B. gr. *circumdata*	x			x	x		x	4		RF	LC
B. cf. *diamantina*					x			1	PI	T	DD
B. nanuzae	x	x		x	x	x		5		ES	LC
B. sagarana							x	1	SC	T	NT
B. saxicola		x	x	x	x	x	x	6		ES	LC
B. sp.					x			1			
Dendropsophus branneri					x			1	PI	RF	LC
D. elegans				x	x			2		RF	LC
D. minutus	x		x	x	x	x	x	6		W	LC
D. rubicundulus			x				x	2		CE	LC
Hypsiboas albomarginatus				x				1	RP	RF	LC
H. albopunctatus	x	x	x	x	x	x	x	7		W	LC
H. botumirim		x	x	x	x			4		T	NE
H. cipoensis			x	x				2		ES	NT
H. crepitans			x		x	x	x	4			LC
H. faber				x	x	x		3		CE, RF	LC
H. lundii				x				1	RP	CE	LC
H. polytaenius					x	x		2		RF	LC
H. sp.			x					1			
Phyllomedusa megacephala				x			x	2		T	DD
P. sp.					x			1			
Scinax aff. *berthae*					x			1	PI	W	LC
S. gr. *catharinae*		x		x	x	x	x	5		RF	LC
S. cabralensis							x	1	SC	T	DD
S. curicica		x	x	x	x		x	5		ES	DD
S. aff. duartei				x	x			2		RF	LC
S. eurydice					x			1	PI	RF	LC
S. fuscomarginatus				x	x	x	x	4		W	LC
S. fuscovarius			x	x	x	x	x	5		W	LC
S. aff. machadoi				x				1	RP	ES	LC
S. gr. *ruber*	x		x	x				3		W	LC
S. aff. *similis*							x	1	SC	RF	LC
S. squalirostris			x	x	x		x	4		CE, RF	LC
S. sp.			x		x			2			

Trachycephalus typhonius			x	x			x	3		W	LC
Hylodidae											
Crossodactylus trachystomus				x	x	x		3		ES	DD
Leptodactylidae											
Adenomera sp.					x			1			
Crossodactylodes itambe					x			1	PI	T	NE
Leptodactylus camaquara		x	x	x	x	x		5		ES	DD
L. cunicularius				x		x		2	BI	RF	LC
L. furnarius			x	x		x	x	4		CE, RF	LC
L. fuscus			x	x	x		x	4		W	LC
L. jolyi			x	x	x		x	4		CE, RF	DD
L. labyrinthicus		x	x	x	x	x	x	6		CE, RF	LC
L. latrans		x	x		x	x	x	5		W	LC
L. mystacinus			x	x				2	SV	W	LC
L. syphax				x				1	RP	W	LC
L. sp.					x			1			
Physalaemus centralis			x				x	2		CE	LC
P. cuvieri	x	x	x	x	x	x	x	7		W	LC
P. evangelistai				x				1	RP	ES	DD
P. marmoratus	x		x			x	x	4		CE	LC
P. cf. *signifer*					x			1	PI	RF	LC
Pseudopaludicola mineira	x	x	x	x	x		x	6		ES	DD
Ps. saltica		x	x	x	x	x		5		CE	LC
Ps. murundu				x			x	2	SC	RF	NE
Ps. sp.				x				1			
Microhylidae											
Dermatonotus muelleri			x				x	2		W	LC
Elachistocleis cesari	x		x	x	x	x	x	6		CE	NE
Odontophrynidae											
Odontophrynus americanus	x					x	x	3		W	LC
Proceratophrys cururu			x	x	x			3		ES	DD
TOTAL	14	15	34	46	44	24	34		21		

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Activity Pattern and Behavior of an Endemic Bromeliad Frog Observed through Camera Trapping

Camera trapping has been widely used to assess the occurrence, abundance, distribution and behavior of species and communities (O'Connell et al. 2011; Meek et al. 2014). Although the use of camera traps has expanded considerably to investigate a diverse range of mammal species, the effectiveness of this method to evaluate other faunal groups is relatively poorly investigated (Ariefiandy et al. 2013; Welbourne et al. 2015; Adams et al. 2017; Laughlin et al. 2017). In fact, just a small proportion of studies (< 2%) cover any ecological aspects of amphibians and reptiles (Burton et al. 2015; Welbourne et al. 2017). Most digital camera traps are triggered by a passive infrared (PIR) sensor that detect differences in the surface temperature of objects in the detection zone; consequently, they are regarded as less reliable for ectotherms (Ariefiandy et al. 2013). However, improvements in camera technology to detect small animals (Welbourne 2013; Hobbs and Brehme 2017) and use of time-lapse mode can improve the detection of ectothermic vertebrates (Welbourne et al. 2017).

Camera traps have been used to characterize reptile communities (Welbourne et al. 2015; Adams et al. 2017) and were previously applied to collect data on the vulnerable Komodo Dragon (Ariefiandy et al. 2013); to monitor activity patterns of the endangered Grassland Earless Dragon in Australia (McGrath et al. 2012); and to identify individuals in reptile assemblages (Welbourne 2013; Bennett and Clements 2014). For amphibians, camera traps have been successfully used to assess movements (Pagnucco et al. 2011; Crosby 2014) and oviposition behavior (Ramsdell 2013); identify potential predators (Velo-Antón and Cordero-Rivera 2017); and describe activity patterns (Hoffman et al. 2010; Engbrecht and Lannoo 2012). Most camera trap implementations are species-specific (Bennett and Clements 2014), and for amphibians they have focused on both newts (Pagnucco et al. 2011; Crosby 2014; Velo-Antón and Cordero-Rivera 2017) and frogs (Hoffman et al. 2010; Engbrecht and Lannoo 2012; Ramsdell 2013; Laughlin et al. 2017). Although most studies using camera traps come from Asia and the Americas (Burton et al. 2015), the majority of studies on amphibians have been conducted in North America (Hoffman et al. 2010; Pagnucco et al. 2011; Engbrecht and Lannoo 2012; Ramsdell 2013; Crosby 2014; Laughlin et al. 2017).

In this study we describe activity patterns and behavior of the rare and elusive *Crossodactylodes itambe*—a micro-endemic frog strictly dependent on bromeliads, where they spend their entire life cycle (Barata et al. 2013; Santos et al. 2017). There are five species in the genus—each restricted to a single location and occurring in high elevation areas of the Atlantic Rainforest in Brazil—and there is still very little information on the ecology and natural history of this group. Using camera trapping and video recording, we investigate activity patterns of this poorly known *Crossodactylodes* species and report periods of activity with descriptions of behavior. We also discuss the application of camera traps to study bromeliad-dwelling frogs and how this technique can be used to enhance our understanding of the group's ecology and natural history.

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METHODS

Crossodactylodes itambe is a small frog species (average 15.7 mm; Fig. 1A–B) only known from the type locality in an area of < 0.5 km^2 at 1700 m above sea level (Barata et al. 2013). Adults and tadpoles are reported to exclusively use a single species of bromeliad, *Vriesea medusa* (Barata et al. 2013; Santos et al. 2017), where they spend their entire life cycle without leaving the plant. Bromeliads are flowering terrestrial or arboreal plants characterized by multiple sized interlocking leaves forming a circular central tank (hereby, rosette) that collects and holds water, leaf litter and detritus. *Vriesea medusa* is a night-blooming flowering bromeliad (Fig. 1B–C) with a funnel-form rosette c. 70 cm high (Versieux 2008) that can hold a large amount of water even during periods of low rainfall. Both the bromeliad and frog are known to occur at the summit of Pico do Itambé State Park; a protected area with 4700 ha located in the state of Minas Gerais, southeastern Brazil (18°23'S, 43°20'W). This system provides an opportunity to explore the use of camera traps to study amphibians, especially because the sampling unit (each

PHOTOS BY I. M. BARATA

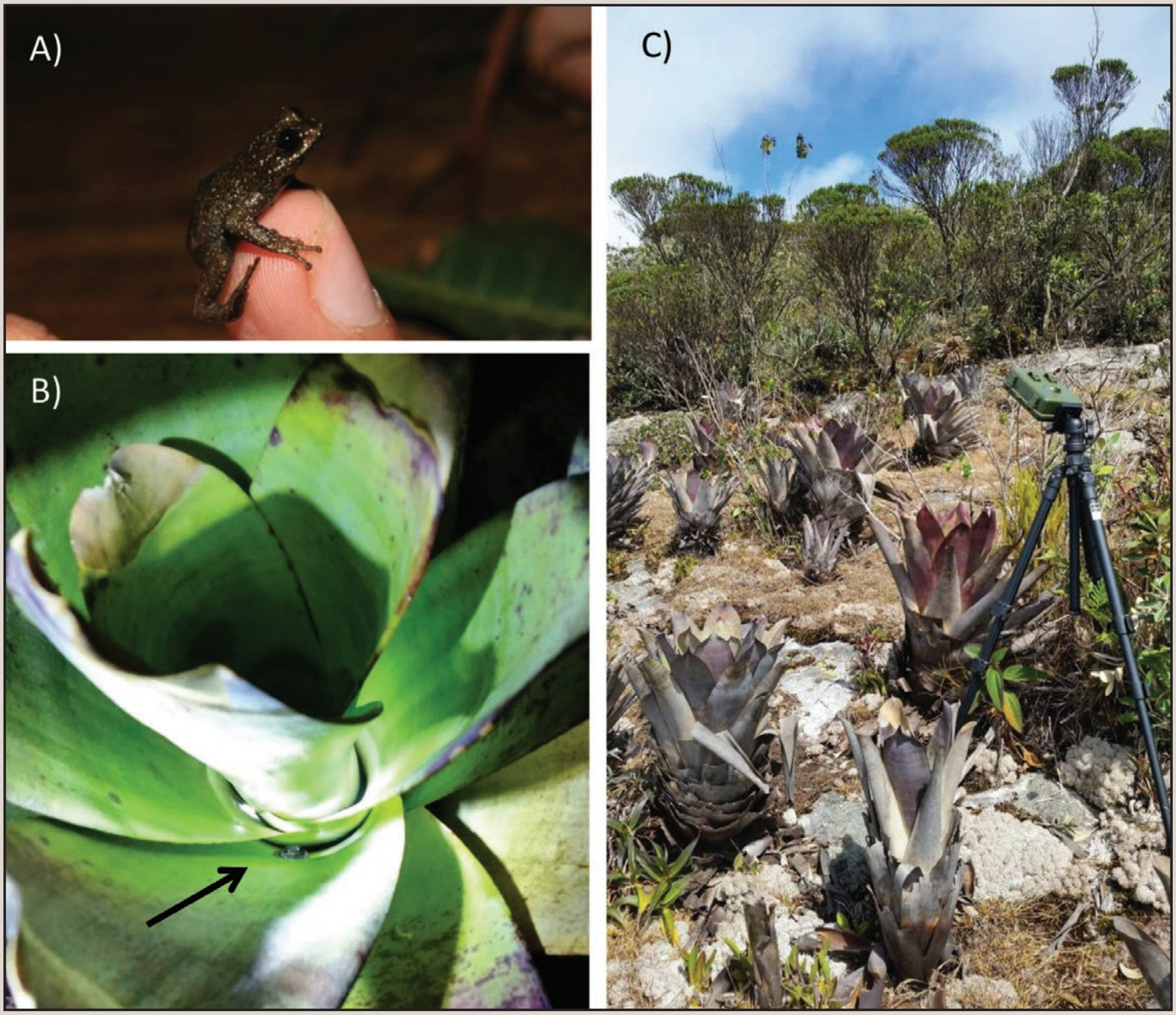

Fig. 1. Adult male of *Crossodactylodes itambe* (A), occupying the axil leaf of a bromeliad *Vriesea medusa*, as indicated by the black arrow (B). Camera trap deployment is shown in detail (C) with the tripod and camera facing the bromeliad at a focal distance of at least 30 cm.

bromeliad) is self-contained and frog movement is restricted to the plant.

To describe activity patterns and how frogs use the bromeliad we installed a camera trap (Bushnell Nature View HD) that allowed observations of frogs for the whole night. We used an interchangeable lens, allowing a focal distance of approximately 45 cm (i.e., from the bromeliad and frogs). We mounted the camera trap onto a tripod (Induro AKB0 AT014), which was positioned close to the bromeliad with its flexible head adjusted so the camera could face down (Fig. 1C). Tripod height was about 1 m and the camera trap was at least 30 cm distant from the focal object. At each bromeliad, we tested the camera and adjusted the tripod to ensure the images would frame the largest view as possible.

In May and June 2016, we deployed one camera trap in four different bromeliads known to be occupied by *C. itambe* for one to three consecutive nights. Sampled bromeliads were at least 25 m apart from each other. We set the camera trap in time-lapse mode taking one picture per minute for 12 hours on each night (from 1700 h to 0500 h), apart from our first survey night when we set up the camera to take one picture every five minutes (but kept all other settings equal). In addition to setting the camera in time-lapse function, we also allowed it to be triggered by movements through its PIR sensor (sensitivity was set to high). Because the camera trap was too close to the focal object, we set up the infrared flash control to low and used a thin piece of cloth covering the camera flash to avoid overexposure. The camera operated with 12 batteries and was equipped with a 32 gigabyte SD card to store the images.

We considered each photograph taken as a record that could be either positive (i.e., a photo with presence of our target species) or negative (i.e., species was not recorded). For all positive records, we defined whether the camera had been triggered due to the time-lapse mode or by its PIR sensor. We did this by looking at the time of the record, as time-lapse pictures were taken on the first second of each minute (e.g. 22h 10min 01sec, 22h 11min 01sec, etc.), while pictures triggered by the PIR sensor could have been taken at any time during the survey period. We classified positive records as active or inactive, based on the sequence of movements taken by the camera. While we considered inactivity as a sequence of records where frogs remained immobile, activity was considered when any

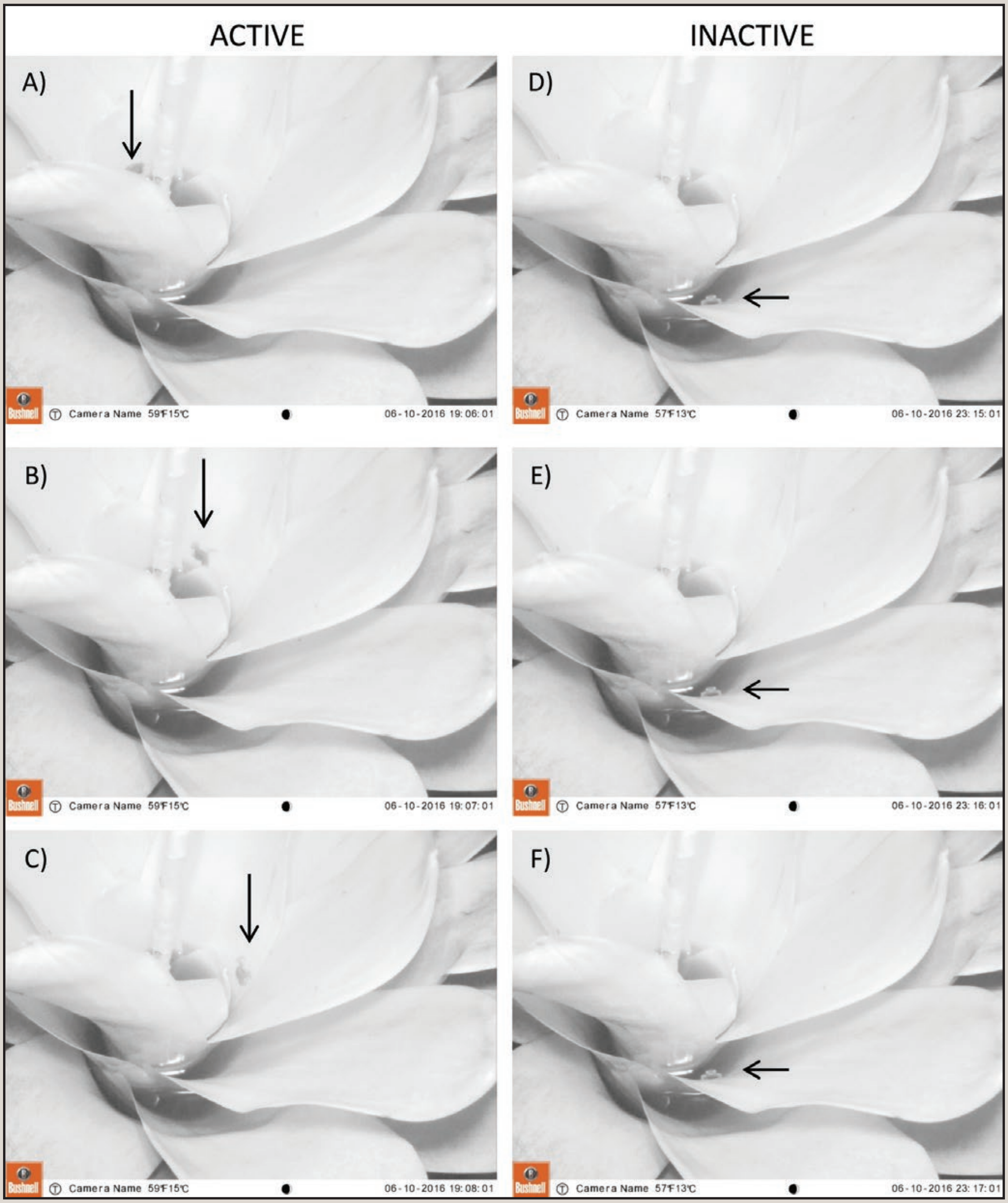

Fig. 2. Sequence of positive records taken with a camera trap on the same night of survey, showing when the species is active (A–C) and inactive (D–F). Time-lapse was set for one picture every minute, as shown by the pictures.

movement was detected between consecutive records (Fig. 2). Adults and tadpoles of *C. itambe* are rarely observed during the day (Barata et al. 2013; Santos et al. 2017) and, for that reason, we assumed frogs were inactive during the day and we only analyzed nocturnal activity patterns.

We used the R package ‘activity’ to fit a flexible circular distribution to time-of-detection data extracted from camera trap photos (Rowcliffe et al. 2014) to describe the target species’ activity pattern and its overall activity level. Activity level was estimated as the percentage of time the species was active, with standard errors obtained through nonparametric bootstrapping (Rowcliffe et al. 2014). For this analysis, we considered only the positive records classified as active and excluded data from the first survey night because the interval between photographs was distinct from all other nights. We set the number of bootstrap iterations to 10,000 and defined ‘data’ as the sampling method for bootstrapping errors.

We performed monthly visual surveys at the study area, between Feb–May 2015 and Feb–June 2016, and bromeliads were visited on four to six consecutive nights as part of a population monitoring project (Barata et al. 2017). Based on occasional encounters, we described three behaviors made during our surveys: 1) locomotion: movement (usually walking) inside the bromeliad; 2) escaping: sudden movement (going

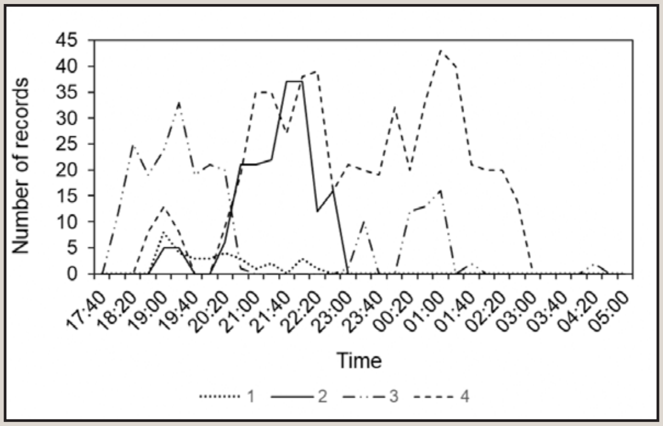

Fig. 3. Total number of records (i.e., total number of photographs taken) over time at four different bromeliads (numbered from 1 to 4) surveyed in this study, starting at 1700 h and finishing at 0500 h.

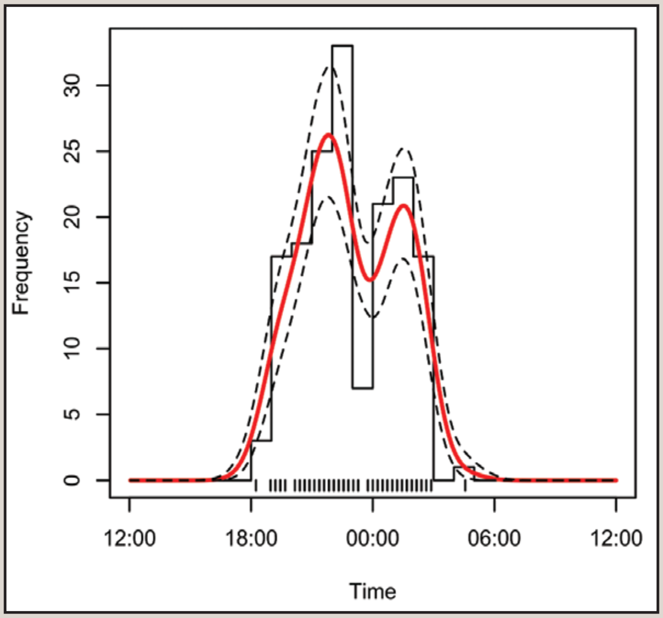

Fig. 4. Overall activity pattern of *Crossodactylodes itambe* given by the frequency of positive records (N = 993) in all surveyed bromeliads (bars), with estimated levels of activity (red line) and 95% confidence interval (dotted lines). Dashes represent a positive record at a specific time.

quickly from inactive to active) whenever frogs were disturbed; and 3) vocalizing: frog calling behavior. Notes on behaviors were only taken by one trained observer. Observations were made opportunistically (i.e., whenever these behaviors were detected during our survey) and continued until the behavior ceased. Finally, in April 2016, we video recorded the frogs in their natural habitat using a digital video camera Sony HDR-CX110E to illustrate our descriptions of behavior.

Results

In 2016, four bromeliads were surveyed using camera traps for eight nights, resulting in more than 4000 records. Despite using the same settings (except on the first survey night), the number of records over time, as well as the total records per night varied among bromeliads (Fig. 3). We had a total of 993 frog detections with the camera trap (i.e., positive records), corresponding to 24% of our records. Most of our positive records were made through time-lapse mode (83.3%). Our first positive record was at 1815 h and the last one at 0427 h, with no detections at 1700 h, 0300 h or 0500 h. Most of our positive records were made between 1900–2200 h (57.5%, N = 571), and less than 30% were made after midnight (Fig. 4). We detected two activity peaks, at 2300 h and 0100 h (Fig. 4); however, frogs were mostly inactive throughout the sampling period (Activity level = 0.26, SE = 0.02, CI = 0.22–0.30).

Through our occasional encounters we were able to record three different behaviors (illustrated in supplemental material, Fig. S1–S4). When inactive, frogs remain immobile and occupy the inner axil of the bromeliad close to the water line: the body is usually flattened, with front legs tucked under the body and head low. In most of our positive records, frogs were inactive in the leaf axils of the bromeliad, in a resting posture. When active, frogs were usually walking towards the rosette or moving to another leaf in the bromeliad. Frogs moved slowly in the bromeliad leaf, with the body raised and front legs held apart. In the camera trap, whenever active, frogs were detected moving short distances on the bromeliad leaf (Fig. 2). The only occasions where individuals moved fast were during escaping. In these cases, they jumped back into the water and did a fast dive. When diving into the bromeliad rosette, individuals usually floated in the water with stretched flat body and wide-open front and rear legs or submerged and hid within suspended sediment. We recorded a total of 34 sequences (40 min 55 sec) demonstrating these behaviors.

Within our survey period, calling behavior was rarely observed, with 10 individuals calling in 2015 and five individuals calling in 2016. The earliest calls were made at 1630 h and the latest at 2200 h. We recorded only one male calling at each site, but occasionally the same bromeliad was occupied by other individuals (usually up to two, non-calling individuals). Although we detected the species calling 15 times, we were able to directly observe this behavior on only three occasions. When calling, individuals remained close to the water line (but never submerged), with half of their body inside the water, front legs spread, and head held low at the surface of the leaf axil. Sound was produced with discrete subgular movements, with small pulses during the entire call. The call was continuous, volume was low, and maximum duration observed was 04 min 03 sec (average call duration was 02 min based on a sample size of seven records).

Discussion

Activity pattern, bromeliad use and vocalization.—We successfully investigated the activity pattern of *C. itambe* using a camera trap and our records suggest the species has an activity peak between 2100–2300 h. Although bromeliads might be used as shelter by anuran species during the day (Pertel et al. 2010; Silva, Carvalho and Bittencourt-Silva 2011), bromeligenous frogs (i.e., amphibians strictly restricted to bromeliads, where they complete their entire life cycle, *sensu* Peixoto 1995) are usually nocturnal (Cunha and Napoli 2016; Eterovick 1999; Mageski et al. 2014; Oliveira and Navas 2004). Due to our survey design, we have no records of diurnal activity; however, on only rare occasions (twice, in a very cloudy afternoon before a thunderstorm) did we observe active individuals (engaged in vocal activity) around 1600 h. Based on our data and available literature, we believe *C. itambe* is inactive during the day.

Frogs moved very short distances when active. This corroborates our observations on locomotion, which showed that frogs moved slowly inside the bromeliad. During visual encounters, individuals were never seen outside the bromeliad. From camera trapping, frogs were rarely recorded on the top of the leaf or distant from the rosette – a pattern also observed during our direct visual surveys. Although Santos et al. (2017) recorded up to four adults sharing the same bromeliad with tadpoles, using camera traps we had only one bromeliad where individuals were seen in pairs, sharing the same plant. Most of our photographs captured only one individual in the bromeliad, which seems to be the pattern for this species (Barata et al. 2018) and most bromeligenous frogs (Schineider and Teixeira 2001; Ferreira et al. 2012; Motta-Tavares et al. 2016; Santos et al. 2017).

All observations of calling males of *C. itambe* were made in the leaf axil, during occasional encounters. Cunha and Napoli (2016) observed that most calling males of the bromeligenous frog *Phyllodytes melanomystax* were preferentially positioned in the bromeliad rosette, a behavior attributed to the structure of the plant and the reduced space between leaf axils. In our case, calling males were never seen in the central tank, which was usually used only when the frog was escaping. We did not record calling activity by camera traps, but we consider this would be impractical given the subtle movements and postures associated with this behavior. In our study, only one male was recorded vocalizing in a plant. Other bromeligenous frogs were previously observed calling from the same bromeliad (Cunha and Napoli 2016) and individuals did not seem to be disturbed by other calling males (Eterovick 1999). However, for *C. itambe*, calling ceased whenever individuals were disturbed by our torches or movement.

Benefits, potential and limitations of camera traps to study amphibians.—Despite it being relatively easy for a trained observer to detect *C. itambe* during night visual surveys (Barata et al. 2017), once the species is detected it will usually quickly escape and hide among bromeliad leaves, avoiding flashlight. Camera traps were considered to have low disturbance when investigating the behavior of lizards (Bennett and Clements 2014), and we saw no evidence of camera trap affecting species behavior in bromeliads. Apart from removing bias caused by the presence of the observer, camera traps also eliminate bias in detectability caused by the difference in expertise between observers (Barata et al. 2017) because records are stored and available for independent validation.

The use of more camera trap units combined with existing analysis frameworks for time-of-detection data (e.g., Ridout and Linkie 2009; Rowcliffe et al. 2014) would allow for the collection of more robust data and the formal testing of ecological hypotheses related to activity levels. For instance, it would be possible to investigate the effect of temperature and rainfall on activity patterns, or compare levels of activity in relation to distance from hiking trails. The initial financial costs would be relatively high, but because a large amount of data on *C. itambe* can be gathered with only a few weeks of sampling, this cost could be offset by joint research projects where camera trap units are shared with other researchers. Some large mammal surveys using camera traps take place during part of the year only (for example, during dry season when sampling is more effective), potentially leaving the equipment free for other types of use (e.g., Ahumada et al. 2011; Ferreira et al. 2017).

Camera trapping provided insights into the activity patterns of *C. itambe*, but we acknowledge some limitations with the method. Firstly, the quality of some photographs was impaired by weather conditions: on some occasions the amount of mist blocked any visualization of our target—a condition that is hard to predict and difficult to mitigate. The area framed by the camera was also limited by the position of the tripod and the angle in which the camera was facing the object. In this case, observations were restricted to either a side view (showing a limited number of leaves but reaching the leaf axils close to the water), or a top view (enabling observation of the central tank and the end edge of many leaves), but never the entire plant. This resulted in a large amount of negative records (i.e., without the target species), which means frogs might have been active or inactive, but outside the camera field of view. A possible solution is to use two camera trap units surveying different parts of the same bromeliad, but this would increase the costs per plant assessed causing a trade-off between thoroughly surveying a single bromeliad or increasing the number of bromeliads surveyed in the population.

Camera traps can detect ectothermic animals if they have different temperatures from the background (Welbourne et al. 2016); however, in our study the proportion of positive records triggered by the PIR sensor is much smaller than the ones obtained through time-lapse mode. This suggests the difference in electromagnetic radiation between our target species and the bromeliad leaf (background) in most situations is below the threshold for triggering the system. Laughlin et al. (2017) suspected that positive records of arboreal frogs using camera traps were caused by the presence of an active mammal in the canopy, which triggered the PIR sensor. Even for the records triggered by the PIR sensor we cannot be completely confident that in all of them the frog actually activated the sensor, because camera traps in the field can be triggered without the presence of an animal (false triggers). Considering the current technology and available equipment, the use of camera traps to study very small ectothermic species will very likely be restricted to time-lapse mode.

Although limiting the possibilities of use, time-lapse cameras have been effectively used to study herpetofauna (Adams et al. 2017; Welbourne et al. 2017) and should be useful in self-contained habitats with high probability of use by a target species. Finally, although we captured frogs moving inside the bromeliads, we were unable to observe behaviors such as calling, reproduction or feeding with camera trapping. In a longer survey, camera traps may aid in the investigation of these behaviors but given the current technology they cannot replace direct observations in the field.

Conclusions

Camera traps were a useful tool for investigating the activity patterns of *C. itambe* without observer interference or disturbance, especially considering the escape behavior observed during direct visual surveys. Because the bromeliad is a self-contained environment, the method was successfully applied, and we recommend its use for habitat-specific species, such as bromeligenous frogs. Camera traps are now being produced with two important features: a time-lapsed trigger and a shorter focal distance. Most camera traps have a fixed focal distance restricted to a few meters, but in our case, the equipment with an interchangeable lens provided a close focal distance, which was enough to detect a small-sized species and provide good quality images. The PIR trigger system was not able to capture all events even for larger reptiles (Bennett and Clements 2014), and the use of camera traps to study very small ectothermic species will be limited to time-lapse mode. Although cameras have a high initial cost, they are considered less expensive in the long term (Welbourne et al. 2015) and might also be beneficial to obtain data from a longer time series. Although the use of camera traps to study herpetofauna has mainly been restricted to larger species of lizards and snakes (Meek et al. 2014), we have shown that within self-contained micro-habitats, using the correct equipment and the right settings will permit the study of very small frogs.

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Supplemental Material

Notes on the behavior of *Crossodactylodes itambe* made though occasional encounters. All images were extracted from video recording, which can be made available upon request.

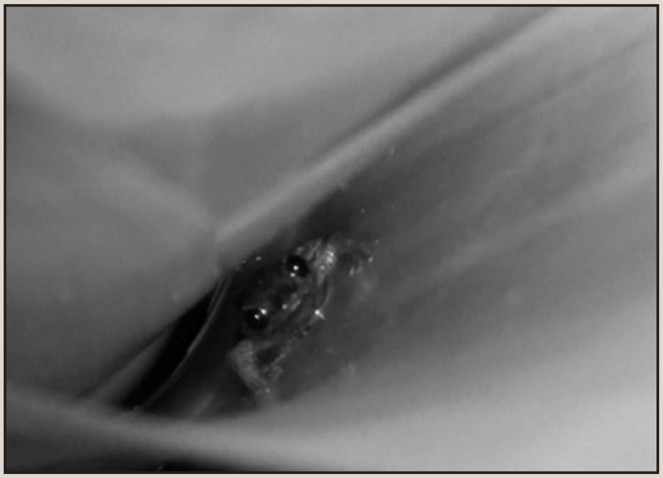

PHOTO BY M. BECHELENI

Fig. S1. Resting posture of *Crossodactylodes itambe* showing an inactive frog at the leave axil with flattened body, front legs tucked under the body and head low.

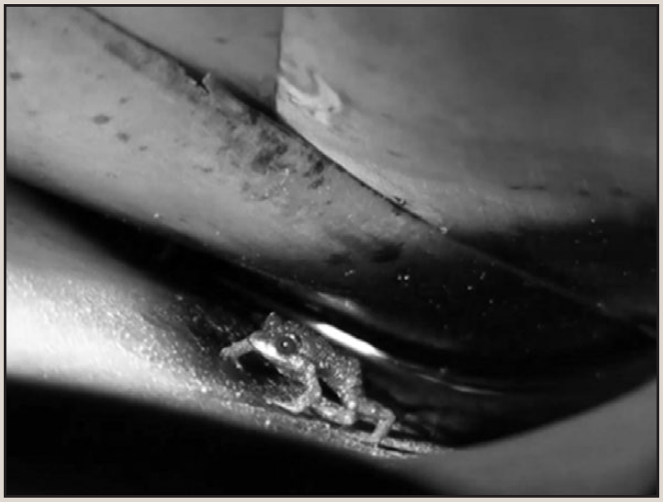

PHOTO BY M. BECHELENI

Fig. S2. Locomotion of an active *Crossodactylodes itambe* showing slow movements in the bromeliad leaf, with raised body.

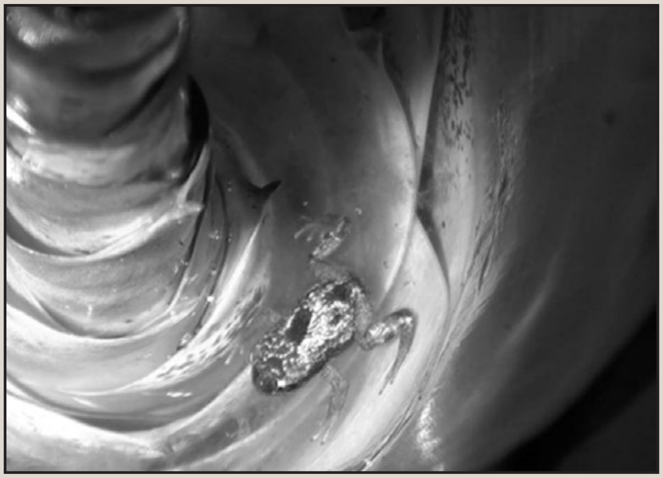

PHOTO BY M. BECHELENI

Fig. S3. Escaping behavior of *Crossodactylodes itambe* showing one individual floating in the water accumulated in the rosette, with stretched flat body and wide-open front and rear legs.

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Fig. S4. Escaping behavior of *Crossodactylodes itambe* showing a submerged individual, after escaping, hiding within suspended sediment in the bromeliad rosette.

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The Tadpole of the Microendemic, Bromeligenous *Crossodactylodes itambe* (Anura, Leptodactylidae) from the Endangered 'Campo Rupestre' of Southeastern Brazil, with Additional Comments on Natural History

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Abstract. We describe the external larval morphology of the microendemic leptodactylid frog *Crossodactylodes itambe* from the 'campo rupestre' of the Espinhaço Mountain Range in the state of Minas Gerais, Brazil. The species identity of the only previously described *Crossodactylodes* tadpole cannot be confirmed, making this the first description of a tadpole of known taxonomic identity for the genus. The tadpole of *C. itambe* differs from the previously described *Crossodactylodes* tadpole in total length, origin of dorsal fin, development of tail musculature, spiracle position, absence of inner wall of spiracle and shape, and direction of vent tube. Characters such as narrow lateral gaps of marginal papillae and the distinct medial serration on the oral face of the upper jaw are proposed as putative synapomorphies for the genus. Tadpoles of *C. itambe* occur exclusively in the axils of the rupicolous bromeliad *Vriesea medusa*. Observation on the natural history, eggs, and larvae are also reported.

Keywords. Bromeliad; Espinhaço Mountain Range; Larvae; Paratelmatobiinae; Phytotelmata.

Resumo. Nós descrevemos a morfologia larval externa do anuro leptodactilídeo *Crossodactylodes itambe*, uma espécie microendêmica do campo rupestre da Cadeia do Espinhaço do estado de Minas Gerais, Brasil. A determinação da espécie da única larva de *Crossodactylodes* previamente descrita não pode ser confirmada, tornando esta a primeira descrição de um girino do gênero com identidade taxonômica conhecida. O girino de *C. itambe* distingue-se do girino de *Crossodactylodes* previamente descrito pelo tamanho total, origem da nadadeira dorsal, desenvolvimento da musculatura da cauda, posição do espiráculo, ausência de parede interna do espiráculo e forma e direção do tubo ventral. Caracteres como as interrupções laterais estreitas de papilas marginais e o serrilhado medial distinto na face oral da maxila superior são propostos como sinapomorfias putativas para o gênero. Girinos de *C. itambe* são encontrados exclusivamente nas axilas da bromélia rupícola *Vriesea medusa*. Observações sobre história natural, ovos e larvas são fornecidas.

INTRODUCTION

The genus *Crossodactylodes* Cochran, 1938, consist of five described bromeligenous (i.e., all life cycle stages associated with bromeliads) species: *Crossodactylodes pintoi* Cochran, 1938; *C. bokermanni* Peixoto, 1982; *C. izecksohni* Peixoto, 1982; *C. septentrionalis* Teixeira Jr., Recoder, Amaro, Damasceno, Cassimiro and Rodrigues, 2013; and *C. itambe* Barata, Santos, Leite and Garcia, 2013. The genus is distributed throughout highland areas of the Atlantic Forest in the states of Rio de Janeiro, Espírito Santo, and Bahia, Brazil (Cochran, 1938; Peixoto, 1982; Teixeira Jr. et al., 2013), and one location in 'campo rupestre' (rupestrian grassland, Silveira et al., 2015) of the Espinhaço Mountain Range in the state of Minas Gerais, Brazil (Barata et al., 2013).

The only known tadpole for the genus is that of a *Crossodactylodes pintoi* (Peixoto, 1981) based on specimens from the municipality of Santa Teresa in the state of Espírito Santo, Brazil. Subsequently, two additional species were described from this location (*C. bokermanni* and *C. izecksohni*) and previous reported larvae were assigned to *C. bokermanni* or *C. izecksohni* (Peixoto, 1982). Moreover, significant variation in morphological characters of these tadpoles (e.g., length and shape of the vent tube; Peixoto, 1981), raises the possibility that the analyzed lots might contain both species. Later, an unidentified tadpole specimen from the same locality was used in

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a comparative survey of internal oral features of neobatrachian anurans (Wassersug and Heyer, 1988).

Information concerning natural history is especially scarce for species of *Crossodactylodes,* but the limited information available indicates that (1) they are associated with bromeliads on mountaintops (Peixoto, 1981; Peixoto, 1982; Barata et al., 2013; Teixeira Jr. et al., 2013), (2) females lay few large eggs attached to bromeliads (Peixoto, 1995; Barata et al., 2013), and (3) tadpoles complete their development in the water accumulated in leaf axils (Peixoto, 1981). *Crossodactylodes* tadpoles were listed as detritivorous by Peixoto (1981), but their internal oral features suggested a mixture of macrophagy and suspension feeding (Wasserssug and Heyer, 1988).

Crossodactylodes itambe is a microendemic species known only from the summit of Itambé within Parque Estadual do Pico do Itambé, municipality of Santo Antônio do Itambé in the state of Minas Gerais, Brazil, and represents the westernmost distribution of the genus (Barata et al., 2013). Herein, we describe the tadpole of this species, which represents the first larval description assuredly attributed to a single species of the genus, and report additional data from natural history observations.

MATERIALS AND METHODS

Tadpole identification

We sequenced a 645 base pairs (bp) fragment of the mitochondrial cytochrome oxidase c subunit I (COI) from one tadpole (lot UFMG 1477; GenBank accession KY362547) and four paratypes of *Crossodactylodes itambe* (UFMG 13376, 13377, 13379, 13381; GenBank accessions KY362548–KY362551, respectively). The primers employed are AnF1 and AnR1 (Lyra et al., 2017). DNA extraction, amplification and sequencing methods follow the protocols described in Blotto et al. (2013). Chromatograms obtained from the automated sequencer were read and assembled using the sequence editing software SeqScape® v 2.6 (Thermo Fisher Scientific, Waltham, MA, USA). Our sampling was complemented with COI sequences from GenBank produced by Fouquet et al. (2013) and Teixeira Jr. et al. (2013), including four specimens of *C. bokermanni* (KF534659.1, KF534658.1, KF534657.1, KF534656.1), five specimens of *C. izecksohni* (KF534655.1, KF534654.1, KF534653.1, KF534652.1, KF534651.1) and one specimen of *C. septentrionalis* (KC603985.1). Complete sequences were aligned in the MEGA7 software' CLUSTALW module under default parameters (Larkin et al., 2007; Kumar et al., 2016). Next, we calculated intraspecific and interspecific pairwise distances in MEGA7 (Kumar et al., 2016).

Study area and morphological examination

Tadpoles of *Crossodactylodes itambe* were collected from rupicolous bromeliads using a collecting aspirator (Silva and Alves-Silva, 2008) at the type locality on the summit of Itambé (18°23′52″S, 43°20′39″W; 1,769–2,063 m above sea level (asl); Datum WGS 84), in Parque Estadual do Pico do Itambé, municipality of Santo Antônio do Itambé, the southeastern part of the Espinhaço Mountain Range in Southeastern Brazil. Collecting took place over four days in November 2012.

Tadpoles were euthanized in 5% lidocaine solution and fixed and preserved in 10% commercial grade formalin. Voucher are housed in the Tadpole Collection of the Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil (voucher numbers UFMG 1477–79). Larval staging follows Gosner (1960). Descriptions of external morphology and proportions were based on five tadpoles in stages 36–39 and measurements were based on 21 specimens in stages 26–39. Terminology and measurements follow Altig and McDiarmid (1999a) for total length (TL), body length (BL), tail length (TAL), maximum tail height (MTH), internarial distance (IND), interorbital distance (IOD), tail muscle height (TMH) and tail muscle width (TMW); Lavilla and Scrocchi (1986) for body height (BH), body width (BW), body width at eye level (BWE), body width at narial level (BWN), snout–spiracular distance (SSD), eye–snout distance (ESD), eye–nostril distance (END), nostril–snout distance (NSD), eye diameter (ED), narial diamenter (ND) and oral disc width (ODW); Grosjean (2005) for dorsal fin height (DFH) and ventral fin height (VFH); adapted from Altig and Johnston (1989) for oral disc position (ODP; i.e., angle formed by the tangent of the extended line connecting the superior and inferior lips and the longitudinal plane of the tadpole, characterized as ventral if $0° < x < 30°$ and anteroventral if $31° < x < 80°$; and Pinheiro et al. (2012) for spiracle length (SL), spiracle distal edge height (SDH) and dorsal fin insertion angle (DFiA). All measurements were taken to the nearest 0.1 mm with the aid of ImageTool version 3.0 (Wilcox et al., 1996). To obtain high quality photos we used an adjustable platform to support tadpoles immersed in water (Schacht and McBrayer, 2009). Terminology for egg morphology and ovipositional mode follow Altig and McDiarmid (2007). For morphological comparisons to other *Crossodactylodes* species we used the tadpole description of Peixoto (1981) and, given the impossibility of taxonomic confirmation, refer to this tadpole below as *Crossodactylodes* sp.

Natural history observations

Observations were made in February 2014 and February–May 2015. In February 2014, we surveyed 123 bromeliads during four nights at elevations of

Table 1. Uncorrected pairwise distances among COI sequences of a tadpole from lot UFMG 1477, four paratypes of *Crossodactylodes itambe,* four specimens of *C. bokermanni,* five specimens of *C. izecksohni,* and one specimen of *C. septentrionalis*. Intraspecific distances are highlighted in gray in diagonal, distances from tadpole to other species, and interspecific distances are shown under the diagonal, and standard error estimates are shown above the diagonal. N/C = not calculated, since there is only one individual.

Intraspecific and interspecific *p*-distances					
	Tadpole	***C. itambe***	***C. bokermanni***	***C. izecksohni***	***C. septentrionalis***
Tadpole	N/C	0.001	0.014	0.011	0.012
C. itambe	0.002	0.0031 (± 0.0020)	0.014	0.011	0.012
C. bokermanni	0.146	0.144	0.0	0.013	0.014
C. izecksohni	0.115	0.116	0.161	0.0084 (± 0.0029)	0.012
C. septentrionalis	0.129	0.127	0.169	0.146	N/C

Table 2. Measurements in mm or degrees (ODP and DFiA) of *Crossodactylodes itambe* from Santo Antônio do Itambé (UFMG 1477–79), state of Minas Gerais, Brazil. Data presented as mean ± SD (range). TL: total length; BL: body length; BH: body height; BW: body width; BWE: body width at eye level; BWN: body width at narial level; TAL: tail length; MTH: maximum tail height; TMH: tail muscle height; DFH: dorsal fin height; VFH: ventral fin height; TMW: tail muscle width; SL: spiracle length; SDH: spiracle distal edge height; SSD: snout–spiracular distance; ESD: eye–snout distance; END: eye–nostril distance; NSD: nostril-snout distance; ED: eye diameter; IOD: interorbital distance; ND: narial diamenter; IND: internarial distance; ODW: oral disc width; ODP: oral disc position; DFiA: dorsal fin insertion angle.

Measurements	Stage 26–29 (*n* = 6)	Stage 30–35 (*n* = 10)	Stage 36–39 (*n* = 5)
TL	25.9 ± 2.0 (24.1–29.2)	33.2 ± 1.7 (30.3–36.4)	34.6 ± 1.6 (32.5–36.6)
BL	8.8 ± 0.6 (8.2–9.6)	11.1 ± 0.4 (10.5–11.6)	11.7 ± 0.4 (11.2–12.2)
BH	3.5 ± 0.12 (3.2–3.7)	4.3 ± 0.2 (3.9–4.5)	4.5 ± 0.3 (4.1–4.8)
BW	6.5 ± 0.5 (5.8–7.2)	8.1 ± 0.5 (7.1–8.8)	8.3 ± 0.5 (7.6–8.8)
BWE	5.9 ± 0.4 (5.3–6.2)	7.1 ± 0.4 (6.5–7.8)	7.3 ± 0.5 (6.6–7.8)
BWN	4.1 ± 0.3 (3.7–4.5)	5.0 ± 0.5 (4.2–5.9)	5.2 ± 0.3 (4.8–5.6)
TAL	17.1 ± 1.6 (15.4–19.6)	22.1 ± 1.4 (19.6–24.7)	22.9 ± 1.4 (20.8–24.5)
MTH	3.5 ± 0.2 (3.2–3.6)	4.0 ± 0.2 (3.6–4.2)	4.1 ± 0.2 (3.9–4.3)
TMH	1.8 ± 0.1 (1.7–2.0)	2.6 ± 0.2 (2.3–3.0)	2.7 ± 0.3 (2.3–3.0)
DFH	1.2 ± 0.1 (1.1–1.3)	1.3 ± 0.1 (1.2–1.5)	1.4 ± 0.2 (1.3–1.6)
VFH	1.0 ± 0.1 (1.0–1.1)	1.3 ± 0.1 (1.1–1.5)	1.3 ± 0.1 (1.2–1.5)
TMW	2.0 ± 0.2 (1.8–2.3)	2.6 ± 0.2 (2.3–2.9)	2.9 ± 0.3 (2.5–3.1)
SL	0.8 ± 0.01 (0.7–0.9)	1.1 ± 0.2 (0.9–1.5)	1.1 ± 0.2 (1.0–1.3)
SDH	0.8 ± 0.2 (0.6–1.1)	0.9 ± 0.2 (0.7–1.3)	0.9 ± 0.2 (0.8–1.2)
SSD	5.5 ± 0.3 (5.0–5.9)	6.5 ± 0.3 (6.0–6.9)	6.9 ± 0.3 (6.5–7.2)
ESD	2.6 ± 0.1 (2.4–2.8)	3.1 ± 0.2 (2.7–3.3)	3.3 ± 0.1 (3.1–3.5)
END	1.6 ± 0.1 (1.5–1.7)	1.8 ± 0.1 (1.7–2.0)	2.0 ± 0.1 (1.8–2.0)
NSD	1.0 ± 0.1 (1.0–1.2)	1.3 ± 0.1 (1.1–1.4)	1.3 ± 0.1 (1.3–1.5)
ED	0.7 ± 0.1 (0.7–0.8)	0.9 ± 0.1 (0.8–1.1)	0.9 ± 0.1 (0.9–1.0)
IOD	2.5 ± 0.1 (2.3–2.6)	3.1 ± 0.1 (3.0–3.3)	3.3 ± 0.2 (3.0–3.5)
ND	0.3 ± 0.1 (0.2–0.3)	0.3 ± 0.1 (0.3–0.4)	0.3 ± 0.1 (0.3–0.4)
IND	0.9 ± 0.1 (0.8–1.0)	1.4 ± 0.1 (1.1–1.5)	1.4 ± 0.1 (1.3–1.6)
ODW	2.6 ± 0.1 (2.4–2.8)	3.1 ± 0.1 (3.0–3.3)	3.2 ± 0.1 (3.00–3.3)
ODP	18.3 ± 2.4 (14.7–21.3)	18.6 ± 2.1 (15.1–22.1)	16.8 ± 4.2 (12.1–21.3)
DFiA	6.8 ± 1.0 (5.6–8.3)	7.1 ± 1.3 (5.5–9.0)	7.1 ± 1.7 (5.4–9.5)

1,713–2,063 m asl (the summit). In February–May 2015, the study area was divided in three elevational gradients: low (1,713–1,841 m asl), medium (1,853–1,957 m asl), and high (1,977–2,063 m asl), and we surveyed 48 bromeliads in each gradient for six nights each month.

RESULTS

Tadpole identification

The molecular analysis confirmed the identity of the tadpole as *Crossodactylodes itambe*. The distance between the tadpole and paratypes of *C. itambe* is 0.002 (SE = 0.001), whereas the distances between the tadpole and other species of *Crossodactylodes* vary from 0.115–0.146 (Table 1). Moreover, considering only the COI sequences of tadpole and paratypes of *C. itambe,* there are only three segregating sites in the COI fragment, and we identified four haplotypes, with the haplotype of the tadpole from lot UFMG 1477 being identical to that of the paratype UFMG 13379.

Tadpole description

External morphology

Maximum total length 36.6 mm (at stage 36; Table 2). Body 0.33–0.36 times TL, strongly depressed (BH/BW = 0.49–0.56), elliptical in dorsal view, with well-marked lateral constrictions; depressed in lateral view, with ventral contour approximately straight from peribranchial region to abdomen (Fig. 1A–B). Snout semicircular in dorsal view (BWN/BWE = 0.70–0.75) and sloped in lateral view. Eyes 0.12–0.15 times BW, dorsally located (IOD/BWE = 0.40–0.49), dorsolaterally directed. Nostrils elliptical, dorsal, 0.02–0.03 times BL, located closer to snout than to eyes (NSD/ESD = 0.39–0.43), anterolaterally directed and with a continuous and elevated rim, without fleshy projection. Spiracle sinistral, lateroventral (SDH/BH = 0.18–0.24), posteriorly directed, 0.08–0.11 times BL, opening at the middle third of body (SSD/BL = 0.56–0.61); inner wall absent (Fig. 1A). Lateral line system indistinct. Intestinal switchback point located at the center of abdominal region (Fig. 1C). Vent tube medial, ventrally directed, small, extensively broad, fused to ventral fin and positioned at its margin (Fig. 1C). Oral disc 0.37–0.40 times BW, ventral (ODP = 12.1–21.3°), laterally emarginated; a single row of alternated marginal papillae with a wide dorsal gap and narrow lateral gaps (at the emargination regions); submarginal papillae absent; labial tooth row formula (LTRF) 2(2)/3, A-1=A-2, P-1=P-2>P3; jaw sheaths narrow, finely serrated, with a distinct medial serration on the oral face of the upper jaw, which has elevated surface; upper jaw sheath arc-shaped and lower sheath "V"-shaped (Fig. 1D–E). Tail low (MTH/TAL = 0.16–0.20); musculature robust (TMH/BH = 0.56–0.63), not reaching the broad, rounded tail tip; dorsal fin approximately equal in height to ventral fin (DFH/VFH = 1.03–1.14). Dorsal fin height 0.05–0.08 times TAL,

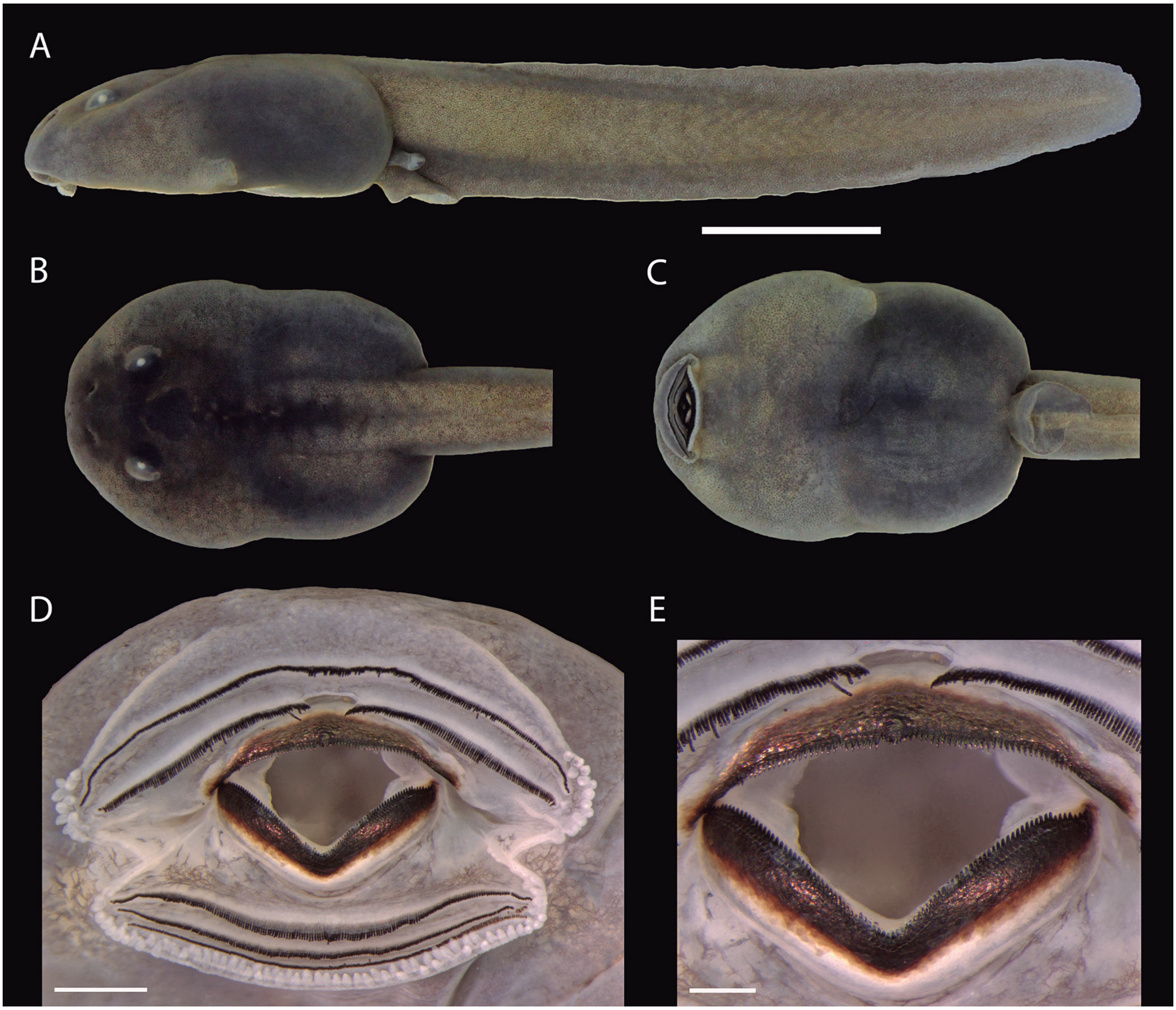

Figure 1. The tadpole of *Crossodactylodes itambe* (lot number: UFMG 1478) in stage 34 (Gosner, 1960). Body and tail shown in **(A)** lateral, **(B)** dorsal, and **(C)** ventral views (scale = 5.0 mm). **(D)** Oral disc (scale = 0.5 mm). **(E)** Detail of jaw sheaths showing the distinct medial serration on the oral face of the upper jaw (scale = 0.2 mm).

with slightly convex external margin; emerging on the anterior third of tail with a low slope (DFiA = 5.4–9.5°); maximum height at the posterior third of tail. Ventral fin height 0.05–0.07 times TAL, with slightly convex external margin; originates at level of vent tube.

Coloration

In life, body uniformly dark brown with scattered melanophores; spiracle lightly pigmented; iris uniformly dark brown; caudal musculature light brown with numerous melanophores along its entire length. This pattern is also present on the fins, which are dark brown (Fig. 2A). In preservative, the color is similar to that in life but fades over time. Body and fins become grayish brown, tail musculature yellowish, spiracle whitish, and iris darker (Fig. 1A).

Morphological variation

There is little intrapopulational variation in shape and coloration. Two individuals, stages 34 and 35, have truncated snouts in dorsal view. Two individuals, stages 30 and 33, have slightly rounded snouts in lateral view. Tadpoles showed some variation in the disposition and number of marginal papillae. Three individuals have small series of marginal papillae aligned posterolaterally and/or in the medial portion of posterior labium. Some have small biseriate (*n* = 2), or triseriate sections of marginal papillae posterolaterally (*n* = 3). These variations are apparently unrelated to ontogeny.

Natural history

Tadpoles of *Crossodactylodes itambe* were found exclusively in the rupicolous bromeliad *Vriesea medusa* Versieux, 2008, a species that is endemic to the southeastern part of the Espinhaço Mountain Range but is not restricted to the Itambé massif (Versieux et al., 2010). The bromeliads possess funnel-form rosettes, 55–80 cm diameter and approximately 70 cm high (Versieux, 2008), and usually occur in clusters of 2–5 rosettes in the study

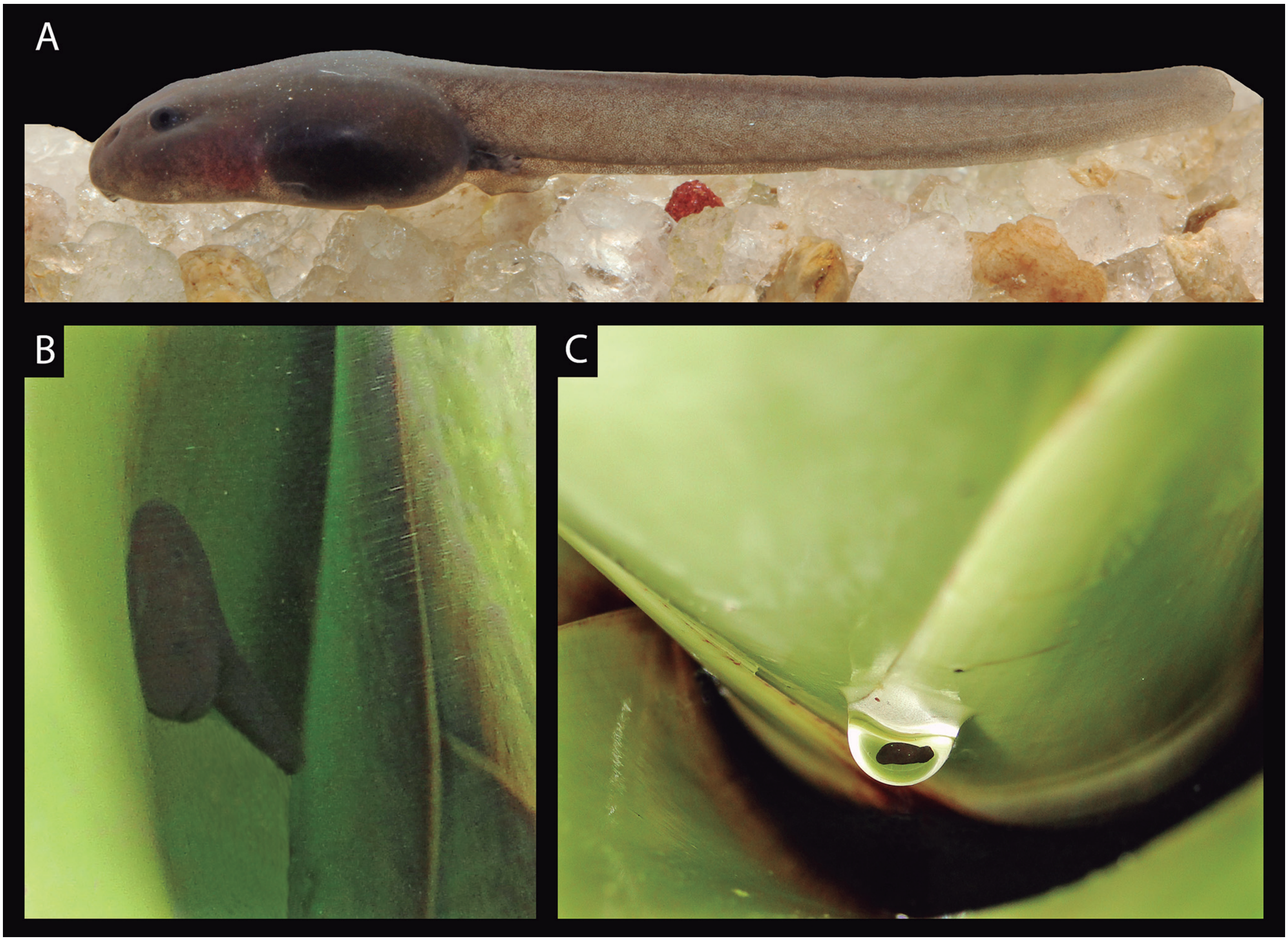

Figure 2. (A) A tadpole of *Crossodactylodes itambe* photographed in life. **(B)** The resting posture of *C. itambe* tadpole. **(C)** A single egg of *C. itambe* attached to the abaxial surface of a bromeliad leaf. (Photos: L.O. Drummond).

area. Tadpoles were found inside the axils of lateral leaves of bromeliads (Fig. 2B) where water is retained.

In February 2014, we recorded 34 tadpoles exclusively above 1,895 m, although adults were found at lower elevations. Most tadpoles were found above 1,969 m asl (22 individuals between 1,969–2,063 m asl and 12 individuals between 1,895–1,968 m asl). Surveys in February–May 2015 recorded 470 tadpoles (February, *n* = 29; March, *n* = 81; April, *n* = 163; May, *n* = 197), of which 13 were collected in the lowest elevational gradient, 166 in the mid-level gradient, and 291 at the highest gradient. The lowest elevation at which tadpoles were recorded was 1,769 m asl.

In most cases, more than one tadpole was recorded inside a given bromeliad (maximum of six individuals per bromeliad), but tadpoles never occupied the same leaf axil. Due to difficulties in sampling and observing tadpoles within their axil, we could not determine developmental stages of tadpoles within and between months. Although we conducted surveys during day and night, tadpoles were most easily observed at night. When tadpoles detected us handling or even shining a light on a bromeliad, they usually dove quickly to the bottom of the axils. The normal larval resting posture is with the snout pointing upward (Fig. 2B).

Eggs of *Crossodactylodes itambe* were found as single eggs attached to either the abaxial or adaxial surface of bromeliad leaves slightly above the surface of the water of the axil, positioned singly (Fig. 2C). Eggs are large and dark-pigmented; embryos are uniformly darkly pigmented and surrounded by two visible, tough jelly layers (an inner translucid layer and an outer opaque layer; Fig. 2C). In November 2012, we observed 13 eggs, including two in the same bromeliad but on different leaves, two at different development stages juxtaposed on the same leaf, and nine that were the only egg in the bromeliad. Of these nine eggs, four were found in a same bromeliad cluster, two in another cluster, and three were the sole occupants of their bromeliad cluster. On two occasions, we observed an adult male sitting alongside an egg; in both cases, the male dove quickly into the axil water while being photographed.

Aggregations of adults were found on the same bromeliad and/or cluster as eggs and tadpoles. Such groupings included: (1) one male and one female, six tadpoles and four eggs; (2) one male and one female, five tadpoles and two eggs; (3) one male and one female, four tadpoles and two eggs; (4) one male and one female, one juvenile, two tadpoles and one egg; (5) one male and one female, three juveniles and one tadpole; (6) one male and two females, one juvenile and one egg; (7) two males and two females and four tadpoles; (8) two females and two eggs and (9) one male and one egg. In addition to these groupings, we also recorded 12 observations of one male and one female occupying the same bromeliad with no tadpoles and/or eggs. Amplectant pairs were not observed.

The following species were syntopic with *Crossodactylodes itambe: Bokermannohyla alvarengai* (Bokermann, 1956), *Bokermannohyla nanuzae* (Bokermann and Sazima, 1973), *Bokermannohyla saxicola* (Bokermann, 1964), *Hypsiboas botumirim* Caramaschi, Cruz and Nascimento, 2009, *Scinax squalirostris* (Lutz, 1925), *Crossodactylus trachystomus* (Reinhardt and Lutken, 1862), *Rhinella rubescens* (Lutz, 1925), *Thoropa megatympanum* Caramaschii and Sazima, 1984, *Leptodactylus camaquara* Sazima and Bokermann, 1978 and *Physalaemus deimaticus* Sazima and Caramaschii, 1986. However, the only species occupying the same microhabitat as *C. itambe* in the study area (i.e., inside bromeliads) was *B. nanuzae,* but this species was not observed using bromeliads for breeding activity (no records of tadpoles, amplexus, or calling activity).

DISCUSSION

Larvae of *Crossodactylodes* correspond to tadpole Type IV (Orton, 1953), exotrophic, lentic, arboreal—a category that includes bromeligenous tadpoles (McDiarmid and Altig, 1999). The reproductive mode, consisting of eggs and exotrophic tadpoles in water in aerial plants, is reproductive mode 6 (Haddad and Prado, 2005). The only previous tadpole description for *Crossodactylodes* (Peixoto, 1981) cannot be reliably assigned to a single species (Peixoto, 1982). Consequently, the description of the tadpole of *C. itambe* represents the first of known species identity for this genus. The previously described tadpoles (Peixoto, 1981) share several morphological features with the tadpole of *C. itambe,* thereby strengthening the identification of those tadpoles to the generic level. These similarities are: depressed body with lateral constrictions; long tail with rounded tip; LTRF 2(2)/3; presence of narrow lateral gaps of marginal papillae; tail musculature not reaching the tail tip and presence of a distinct medial serration on the oral face of the upper jaw—the last two features were illustrated in Peixoto (1981).

Despite these similarities, the tadpole of *Crossodactylodes itambe* has some striking differences from the tadpole of *Crossodactylodes* sp., including total length 30.3–36.4 mm in *C. itambe* (*n* = 10, stages 30–35; 19 mm in *Crossodactylodes* sp.; n = 1, stage 35); dorsal fin emerging on the anterior third of tail (posterior third of body in *Crossodactylodes* sp.); tail musculature robust (narrow in *Crossodactylodes* sp.); spiracle position lateroventral (lateral in *Crossodactylodes* sp.); inner wall of spiracle absent (present in *Crossodactylodes* sp.); vent tube medial, ventrally directed and extensively broad (dextral, posteriorly directed and not broad in *Crossodactylodes* sp.). The last four features are seen in the illustration by Peixoto (1981).

The absence of the inner wall of the spiracle and the peculiar widening and direction of the vent tube are features exclusive to *Crossodactylodes itambe* among described tadpoles of Leptodactylidae (e.g., Altig and McDiarmid, 1999b; Rossa-Feres and Nomura, 2006; Garcia et al., 2009; Juncá and Lugli, 2009; Kolenc et al., 2009; Provete et al., 2011). The narrow lateral gaps of marginal papillae and the distinct medial serration on the oral face of the upper jaw reported for *Crossodactylodes* sp. and *C. itambe* do not occur in any other described tadpole of Paratelmatobiinae (Cardoso and Haddad, 1990; Giaretta and Castanho, 1990; Pombal and Haddad, 1999; Garcia et al., 2009; Juncá and Lugli, 2009; Domenico et al., 2014) and are, therefore, putative synapomorphies for the genus.

Despite the same amount effort surveying bromeliads at lower elevational gradients, more tadpoles were recorded at higher gradients. Furthermore, few adults were found at lower elevations (i.e., from 1,713–1,841 m asl), but tadpoles were never recorded below 1,769 m asl. This suggests that the species preferentially breeds above 1,977 m. Since bromeliads can also be found at lower elevations (from 1,500 m asl; Versieux, 2008), the optimum area of distribution for *Crossodactylodes itambe* might be related to higher elevations, specifically microclimate and local ecological features, such as bromeliad density (which increases with elevation), which seems essential for species reproduction.

Tadpoles of *Crossodactylodes itambe* are easily found at night using flashlights but are rarely observed during the day. This might be due to their period of activity (which could occur mostly at night when they are expected to move closer to the surface of the water) or their dark coloration and the low luminosity inside bromeliads during the day (making it more difficult for them to be observed). The behavior we observed of tadpoles of *C. itambe* diving towards the bottom of bromeliad axils when disturbed has also been reported for other bromeligenous tadpoles, e.g., *Scinax perpusillus* group (Silva and Alves-Silva, 2008; Lacerda et al., 2012). The use of a collecting aspirator (Silva and Alves-Silva, 2008) allowed sampling of almost the entire contents of bromeliad axils, which made it easier to count individuals than using flashlights. This method enables sampling a greater number of individuals without damaging them or the bromeliads.

The resting posture of tadpoles of *Crossodactylodes itambe,* with the snout pointing upward (Fig. 2B), has also been reported for other arboreal-developing tadpoles (Perret, 1962; Lanoo et al., 1986). The water bodies of bromeliads usually have low levels of dissolved oxygen due to their heterotrophic metabolism (Guimaraes-Souza et al., 2006), which might force tadpoles to surface for oxygen uptake, which their resting posture could facilitate. The long tail with well-developed muscles and low fins may function as a static-postural organ, serving to position the body nearer to the water's surface (Lanoo et al., 1986). The lungs of these tadpoles may also facilitate this behavior by providing buoyancy, whereas their low fins may minimize oxygen loss due to reduced cutaneous surface area (Lanoo et al., 1986).

The melanic pigmentation found in eggs of *Crossodactylodes itambe* usually occurs in species that inhabit open areas (Altig and McDiarmid, 2007) and probably offers protection from high exposure to solar radiation (Salthe and Duellman, 1973; Bastos et al., 2010). The low number of eggs found in proximity to each other within a bromeliad cluster (< 5) indicates that clutches of *C. itambe* contain just one, or perhaps a few, large isolated eggs. For many anuran assemblages, the number of eggs is negatively correlated with their diameter (Salthe, 1969; Crump, 1974; Hartmann et al., 2010), and species with more specialized reproductive modes usually lay fewer larger eggs (Hartmann et al., 2010). Other bromeliad-breeding species, such as species of *Phyllodytes* and the *Scinax perpusillus* group, lay a small number of eggs (Bokermann, 1966; Giaretta, 1996; Eterovick, 1999; Alves-Silva and Silva, 2009), a reproductive strategy that increases the chance of survival of each descendant. Lower tadpole density decreases competition for food and dissolved oxygen, and also decreases pollution of the small aquatic habitat with larval waste (Alves-Silva and Silva, 2009).

Other strategies adopted by other bromeligenous species to cope with limited resources and favour offspring survival include laying eggs with a large amount of yolk (Krugel and Richter, 1995; Peixoto, 1995; Duellman et al., 2011), or even laying unfertilized trophic eggs to provide food for tadpoles (oophagy; Lanoo et al., 1986; Weygoldt, 1987; Jungfer, 1996; Jungfer and Weygoldt, 1999; Lourenço-de-Moraes et al., 2013). According to Lanoo et al. (1986), this latter strategy is usually accompanied by a morphological adaptation in the number of tooth rows, which is usually reduced to 1/1 or less. The lack of this feature, and the low number of eggs reported for *Crossodactylodes itambe,* provide strong evidence that oophagy does not occur in this species. According to Peixoto (1981), tadpoles of *Crossodactylodes* sp. feed on organic detritus accumulated in the water of bromeliad axils. Based on internal oral features (e.g., reduction of the median ridge and presence of secretory ridges), Wassersug and Heyer (1988) suggested that they have features tending towards macrophagy, while characters related to suspension feeding are also present.

Parental investment in offspring survival in *Crossodactylodes* species might involve changes in ovipositional mode and behavior of adults (see Altig and McDiarmid, 2007). The occurrence of tadpoles of *Crossodactylodes itambe* individually in different axils suggest that adults might choose oviposition sites to decrease competition between tadpoles. Additionally, the occurrence of males near eggs might indicate that they are performing egg-guarding behavior. This may explain the hypertrophied forearms and the spines on the inner surface of the first finger of males of *C. itambe* (Barata et al., 2013). These features may also be indicative of other intraspecific social interactions, such as breeding site defense. If species of *Crossodactylodes* do indeed perform parental care, it would be consistent with Crump (1996), who states that most anurans that exhibit this behavior are small and have small clutch sizes.

Species of Paratelmatobiinae exhibit a variety of reproductive strategies: *Crossodactylodes* species lay a low number of large eggs in bromeliads (Peixoto, 1995; Barata et al., 2013); *Rupirana* lays clutches with approximately 120 eggs in shallow backwaters or ponds (Juncá and Lugli, 2009); *Paratelmatobius cardosoi* and *P. yepiranga* lay ca. 20 eggs in muddy ponds (Pombal and Haddad, 1999; Garcia et al., 2009); *P. poecilogaster* lays a terrestrial egg mass adhered to humid rocks with approximately 5–13 eggs (Pombal and Haddad, 1999); and *Scythrophrys sawayae* lays clutches with approximately 30 eggs in small, shallow ponds with muddy bottoms formed by the backwaters of streams (Garcia, 1996). Based on this information, Fouquet et al. (2013) suggested that oviposition in bromeliads is a putative synapomorphy of *Crossodactylodes,* which is reinforced by the findings of the present study. In addition, despite the lack of information on the natural history of some species of *Crossodactylodes,* the low number of eggs reported for *C. itambe* and *Crossodactylodes* sp. relative to other Paratelmatobiinae might represent another putative synapomorphy for the genus. Denser sampling of this poorly known genus is needed in order to better understanding the distribution of the morphological and natural history characters in *Crossodactylodes*.

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Conducting robust ecological analyses with climate data

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Although the number of studies discerning the impact of climate change on ecological systems continues to increase, there has been relatively little sharing of the lessons learnt when accumulating this evidence. At a recent workshop entitled 'Using climate data in ecological research' held at the UK Met Office, ecologists and climate scientists came together to discuss the robust analysis of climate data in ecology. The discussions identified three common pitfalls encountered by ecologists: 1) selection of inappropriate spatial resolutions for analysis; 2) improper use of publically available data or code; and 3) insufficient representation of the uncertainties behind the adopted approach. Here, we discuss how these pitfalls can be avoided, before suggesting ways that both ecology and climate science can move forward. Our main recommendation is that ecologists and climate scientists collaborate more closely, on grant proposals and scientific publications, and informally through online media and workshops. More sharing of data and code (e.g. via online repositories), lessons and guidance would help to reconcile differing approaches to the robust handling of data. We call on ecologists to think critically about which aspects of the climate are relevant to their study system, and to acknowledge and actively explore uncertainty in all types of climate data. And we call on climate scientists to make simple estimates of uncertainty available to the wider research community. Through steps such as these, we will improve our ability to robustly attribute observed ecological changes to climate or other factors, while providing the sort of influential, comprehensive analyses that efforts to mitigate and adapt to climate change so urgently require.

Synthesis

Climate is a key driver of ecological patterns and processes, and as such has been the subject of huge research effort over a number of decades. Yet although the literature on the subject is vast, ecologists still succumb to a number of common pitfalls when analysing climate data. In this paper we share some of the lessons and techniques for avoiding these pitfalls, before suggesting some better ways forward, namely: more collaboration, more communication, and more sharing of data and code. By working more closely together, ecologists and climatologists will generate outputs that are far more useful and tractable for society.

The fingerprint of anthropogenic climate change is increasingly evident in many of the world's ecosystems (Scheffers et al. 2016). Ecologists are therefore increasingly seeking to represent and analyse these effects for a more complete understanding of their study systems, and to inform conservation or wider interests. Even for those experienced with analysing climatic impacts, the array of options and scale of the data involved can make the process challenging. Furthermore, the assumptions or uncertainties that underlie publically available data and computer code can be poorly described, causing ecologists to use them uncritically. A recent meeting entitled: 'Using climate data in ecological research' held at the UK Met Office (Exeter, UK) sought to address some of these issues, and discuss examples of good practice (and bad). Participants noted that much of the advice on making climate analyses more robust has not been published formally in the literature, or online (but see Foden and Young 2016 for specific guidance aimed at conservation practitioners).

The aim of this Forum article is to highlight some important considerations for any ecologist concerned with the use of climate data in their analyses. We adopt the usual chronology of ecological research, proceeding from the design stage, to preparatory work, before discussing some key considerations for undertaking the analyses.

What is 'climate', and is it relevant to the ecological question?

Here, we take 'climate' to be a measure (e.g. the mean, or variability) of the weather conditions over some period of time. This measure can be derived from data spanning a few months to a few millennia. Although a period of thirty years is commonly adopted by climate scientists (Arguez and Vose 2011), ecologists tend to use the term 'climate' to refer to data spanning shorter time periods than this. Because usage and understanding of the terms 'weather' and 'climate' varies across the literature, we simply refer to 'climate' throughout this paper, rather than adopting our own distinction. Whichever term is adopted, we argue that the precision and clarity with which it is defined is of the most importance, and that the reasoning for using a particular time period should be provided.

Before considering how to include climatic effects in ecological studies, it is also worth considering if climate is actually relevant to the particular focal question at all. Listing the situations in which climate could be relevant is beyond the scope of this paper, but here we echo the views of Lawton (1999), who argued that the profound influence of climate on the distribution of species and biomes means that it should at least be considered at the design stage of most ecological studies. At a basic level this can simply be via the inclusion of one or more climate variables as a control, or the selection of field sites to control for one or more climate variables, such as gradients in temperature or precipitation. Ecologists wishing to quantify the specific role of climate within a system may however wish to adopt one of the more complex approaches we discuss below.

Which aspects of the climate are relevant?

The identification of appropriate climate data by ecologists first requires an understanding of which aspect of the climate the study organism or system responds to (if any). This is not always a straightforward process, as organisms may respond to interactions between several variables, or different variables at different life-stages. Where this information cannot be gleaned from the literature or previous work, a more exploratory approach can be adopted, and in highly complex systems this is likely to be a requirement (van de Pol et al. 2016). Because the choice of which variables to include in experiments often has a substantial effect on the eventual results (Porfirio et al. 2014), care should certainly be taken to test the sensitivity of any analytical framework to a range of predictor combinations. Operating at (or switching between) different spatial or temporal resolutions may also lead to different conclusions (Gillingham et al. 2012, Pearce-Higgins and Green 2014), as shown in the illustrative Fig. 1a where the estimated frequency of temperature threshold exceedance is sensitive to the temporal resolution of the underlying data. Responses to weather or climate can also be lagged (Fig. 1b), such that an ecological response is discerned some period of time after the climatic trigger itself (in this case, overwintering temperature). The interaction of climatic events at different temporal resolutions can also be responsible for particular ecological effects; in Fig. 1c, fire risk is approximated by the total annual precipitation – a useful correlate of longer term moisture content of the vegetation – and the temperature of the hottest month, which correlates with the probability of ignition. Note that many other factors, such as wind strength, humidity and the passage of weather fronts, are associated with fire risk, and the relative importance of these drivers is dependent on the spatial and temporal scale of analysis. Therefore in this example and more generally, there is a need to select and work at appropriate resolutions.

Also of potential importance is the duration or 'persistence' of climatic events, which can result in both positive (Fig. 1d) and negative (Fig. 1e) effects on a study species. The period of growth in plants, or other thermal conforming species (including most insects, Davies et al. 2006), can often be described by the period of time at which the temperature (often the mean temperature) is above a physiologically-relevant threshold (Fig. 1d). But persistent periods of low rainfall create the necessary conditions for a meteorological drought (Fig. 1e). The particular sequence in which multiple events occur can expose populations (or individuals) to conditions that single events acting in isolation would not achieve. In the last example (Fig. 1f), an unusually warm spring (weeks 4–6) has the counterintuitive effect of increasing the exposure of nearby ground-dwellers to the subsequent cooler conditions (i.e. the late frosts of weeks 10 and 11). All the illustrated examples could potentially be drawn from the same climate dataset, highlighting that findings will depend as much on how the data are made relevant to the research question as they do on the choice of climate data product that is analysed.

Although the impact of changes to the frequency or severity of extreme events can be as important as the impact of an overall mean trend (McDermott Long et al. 2017), extremes tend to be the subject of far less research effort in ecology (Jentsch et al. 2007). Most of the studies that have analysed extremes have focussed on the short-term impact of single events (Morecroft et al. 2002), leaving the effects of multiple events and long-term impacts understudied (Bailey and van de Pol 2016, but see Palmer et al. 2017). This is concerning given the number of species known to be sensitive to such effects (Cuoto et al. 2014), and likely reflects the inability of relatively short duration ecological data series to encompass extremes, which by definition are rare. It is therefore likely that many of the ecological effects of extremes are yet to be described.

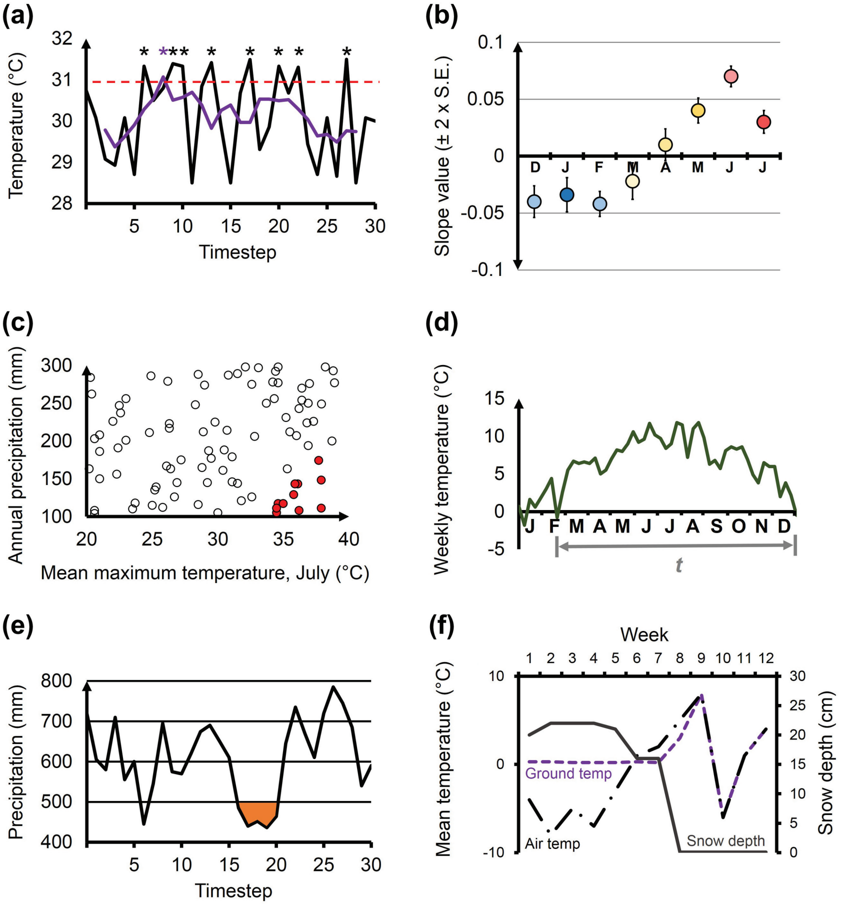

Figure 1. Making climate data relevant to ecology. In (a), the frequency of threshold exceedance is sensitive to the temporal resolution of the underlying data, with raw values (black colour) generating a different estimate to smoothed values (purple). In (b), the summer population count of an example organism is positively related to the monthly temperature means of the current summer, yet negatively related to the temperature means of the previous winter – the latter is a lagged response to conditions at that time. In (c), two climate variables calculated at different temporal resolutions contribute towards an estimate of fire risk (red circles indicating conditions of high risk). In (d), a variable describing a continual exposure to a particular set of climatic conditions has been derived – length of the frost-free growing season, *t*. In (e), both the extremity and the duration of low precipitation values have been taken into account to represent a meteorological drought (orange highlight). In (f), a sequence of events sees unusual spring warmth followed by a late frost, counterintuitively exposing ground-dwellers to cooler conditions. All examples are hypothetical and were generated using synthetic data.

Over longer time frames, some measure of the 'stability' of the climate is also important, and there are a number of metrics that seek to quantify this (Garcia et al. 2014). Examples include the climate velocity (Loarie et al. 2009), which is the velocity of species movement required to track analogous climates as the conditions change, and the timing of climatic 'departure' from current conditions, i.e. the point at which climate at a location moves beyond the historical observed range of variability (Mora et al. 2013). The principal driver for the development of this type of metric has been the multitude of studies demonstrating an exacerbating effect of climate change on extinction risk (Urban 2015), although they also offer a means of assessing species' vulnerability to climate change where good ecological data are lacking (Foden and Young 2016). A further motivation for assessing stability is to establish the existence of modern day 'refugia' (Ashcroft 2010) or 'microrefugia' (Rull 2009) from climate change; these were areas of atypical climate that buffered species from the adverse climate conditions of the past (Baker 1980). Efforts to describe the locations and beneficial effects of these refugia have been enhanced by recent progress in climate downscaling.

There will also be situations where deriving ecologically relevant climate predictors is simply not possible given the limitations of the climate data. More and continued communication and collaboration between climate scientists and ecologists would help climate scientists to identify such limitations and to orient their climatological outputs towards the user community (Dobor et al. 2015), whilst also ensuring that ecologists use and analyse climate data robustly. Ideally engagement should take place: 1) in person – during symposia, interactive workshops, and targeted sessions at conferences such as INTECOL; 2) on paper – with grant proposals and scientific publications; and 3) online – via popular media platforms and blogs.

Obtaining climate data

'Climate data' consist of one, or a blend, of the following products: point-based meteorological observations, gridded observations (including reanalysis products), satellite-derived estimates of climate, and simulations of climate derived from Global climate models or Earth system models, i.e. model data. We briefly deal with these in turn, pointing out their strengths, weaknesses and other factors that require consideration.

Point-based meteorological observations

Many ecologists will be interested in the conditions that organisms experience at the local level (centimetres to hectares). This can be at odds with the design of meteorological station networks, which are purposefully sited away from particularly unusual habitats or atypical landscape characteristics in order to be more indicative of wider atmospheric conditions (WMO 1996). The extent to which a station can be considered a useful record of the climate conditions over an area of ecological interest is a function of the distance to the station, the climatic variable of interest, and any differences in landscape characteristics that decouple the study site from the atmospheric conditions captured by the station (such as elevation, topographic slope and aspect, and distance to coast). Adjusting meteorological outputs to account for these site-level effects forms the basis for generating higher resolution climate data.

Where station data do not capture what ecologists require, other approaches to measurement have been adopted, ranging from siting a bespoke observing station within a fieldsite (Bennie et al. 2008), installing miniaturised dataloggers (Suggitt et al. 2011), thermography (Scherrer and Körner 2010), or even trapping the study organism and directly attaching or implanting monitoring equipment ('bio-logging', Ropert-Coudert and Wilson 2005). The life history and distance over which a species can move will define which of these techniques is required (if any), with smaller, thermal non-conforming species more likely to occur in atypical conditions, thereby requiring specialist monitoring. Species will often also occupy differing three-dimensional spaces within a single day; measurements taken at the soil surface or vegetation canopy are an attempt to represent the properties of these spaces more closely. On the other hand, because migratory species cross countries and even continents, these broader-ranging species are also likely to require more tailored representations of their climate (Small-Lorenz et al. 2013). At a minimum this would involve the collection of data to establish their location at critical points in their life cycle. As technology improves and all types of ecological data become more detailed, interdisciplinary collaborations will lead to the development of new, higher resolution climate metrics that can make best use of them (Potter et al. 2013).

There have been huge increases in the capabilities of meteorological sensors, data storage capacities, and channels for dissemination to the wider public (from Twitter updates by meteorological organisations, to publically accessible archives such as NOAA-NCEI; <https://www.ncei.noaa.gov/>). To assist the research community in traversing these rich but sometimes disparate sources of data (NOAA's NCEI, GHCN, WMO, UK Met Office, and many others), we advocate their collation in a global catalogue, providing a one-stop shop for those wishing to assess their availability.

Similarly, the reduction in size and cost of data loggers and automatic weather stations means that individual research groups, independent researchers, and amateur enthusiasts are now collecting large volumes of data that could be voluntarily contributed to this single online repository (e.g. MICROCLIM, <www.microclim.org.uk>), together with any historical data digitised from paper archives. Similar endeavours in other fields have been hugely successful, most prominently the GenBank genetic sequence database (Benson et al. 2011). A 'ClimBank', based on similar principles, would foster new collaborations and scientific advances through the preservation, collation and meta-analysis of existing climate data.

Gridded meteorological observations

The expense and effort of collecting direct meteorological observations, coupled with the desire to give them more ecological meaning, has led to the generation of fine-grained, spatially-gridded datasets for studies of climate change impacts (Fig. 2). Here, the resolution of gridded data can be tailored to the spatial scale of ecological response, although the ultimate accuracy of these data is constrained by the observational data that underlie them (see section on data resolution below), and the techniques used in gridding these data.

Approaches to generating gridded data vary in complexity, from simple interpolations based on latitude, longitude and elevation, to local adjustments for topography (lapse rate, solar radiation regime, cold air pooling), coastal effects, wind, latent heat exchange and snow. Generating fine-grained precipitation data often requires more underlying data than temperature, with storm tracks and wind direction to take into account, depending on the temporal resolution required. Specialised gridding routines (e.g. PRISM or equivalent) can be used to generate such grids over local areas if enough data are available, and some gaps are also filled with reanalysis products, which combine observed weather data with numerical weather prediction model output. Note that uncertainties underlie all these approaches – including that arising from the source(s) of the observation(s), the choice of local climate ('microclimate') effect(s) to include, and also the means of including them. It is important to consider the degree to which the assumptions behind simplifying relationships are valid, such as temperature lapse rate adjustments, which may assume dry or stable atmospheric conditions.

There are strong ecological motivations for generating climate data at finer spatial resolutions, because the evidence for the ecological relevance of local climate effects is strong. This is particularly true for topographic effects, which account for a large part of the variance in temperature and moisture in montane regions (Dobrowski 2011), and are therefore a particularly important control on the distributions of flora (Scherrer and Körner 2011) and fauna (Ashton et al. 2009) in these regions. Many upland or high-altitude plants also rely on the ameliorative effect of snow lie on frost risk, thus reductions in the extent or thickness of snow lie could leave these species at higher risk of extinction in the spring (Bannister et al. 2005). Other species have specific microclimatic requirements at or near their range margins, and so the inclusion of fine-scale climate information can

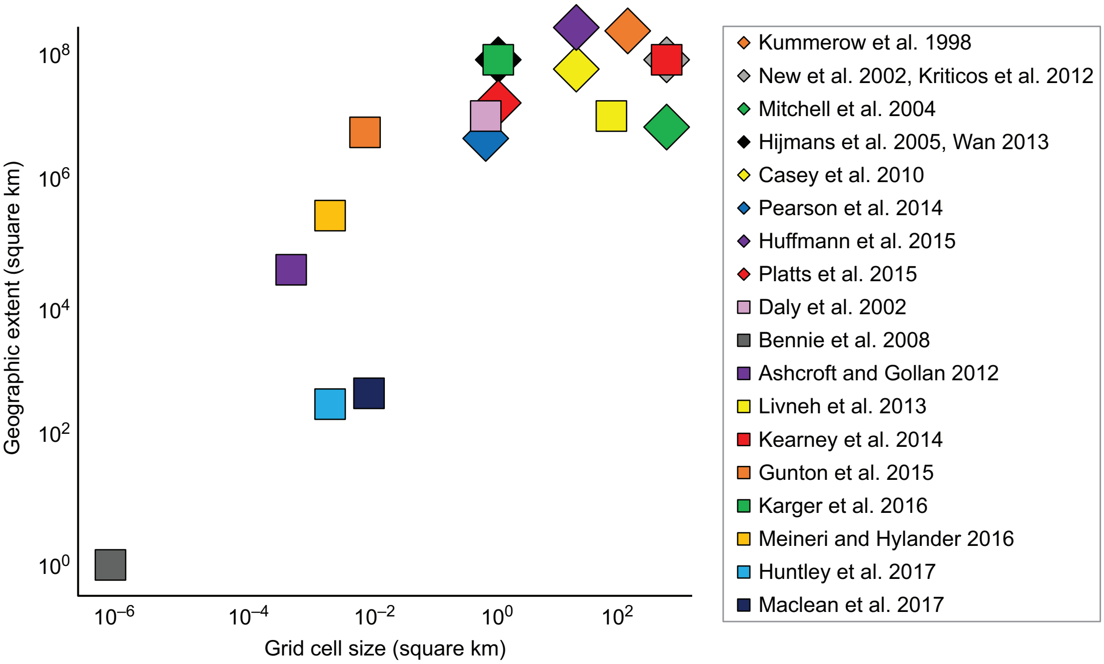

Figure 2. The spatial resolution and geographic extent of gridded climate datasets (cell size of 400 km^2 or less) available for use in ecology. Includes studies where the data or code (or both) are publically available. Diamond symbols indicate studies employing statistical interpolation only; square symbols indicate studies combining statistical interpolation with adjustments for landscape characteristics (e.g. solar input). The lead author and year of the associated journal article is provided; full references are available in the reference list. Where two separate datasets share the same x- and y-values they have also been assigned the same symbol (New et al. 2002, Kriticos et al. 2012).

improve our understanding of their range dynamics (Lawson et al. 2014, Huntley et al. 2017), and distributional shifts under climatic change (Bennie et al. 2013). Microclimate surfaces are also feeding into studies seeking to identify 'refugia' from climate change, both in palaeoecological and contemporary contexts (Suggitt et al. 2014).

Satellite-derived (blended) estimates of climate

Gaps in the spatial coverage of meteorological data can limit their usefulness in areas with fewer observations, such as rural areas or in the tropics. To overcome this, point-based surface observations have been combined with satellite observations to create blended climate data products that make the best of both formats (e.g. MODIS/Terra land surface temperature; Tropical rainfall monitoring mission, or TRMM).

Much of the effort in developing satellite-blended products has focussed on improving the utility of rainfall data for drought monitoring, and its subsequent impact on vulnerable human communities (Funk et al. 2015), although their applicability to other types of ecological research is clear (Pettorelli et al. 2014). Their use is therefore increasing, particularly in regions where the topography is complex or existing monitoring is sparse (e.g. rain gauge networks in Africa, Maidment et al. 2014), both of which can make interpolation less robust. For example, Deblauwe et al. (2016) found that blended data improved the performance and transferability of species distribution models in the tropics when compared with data derived solely from surface observations. A key constraint on the quality of these datasets in high latitudes and/or elevations is cloud cover, with time-sensitive analyses (such as phenology) particularly affected, and estimates derived for these hard to reach areas are also more difficult to ground-truth. Usage of these products will nevertheless continue to rise as the spatial and temporal resolution, coverage and accessibility of satellite observations improves.

Model-derived estimates of climate

Global climate models (GCMs) represent the patterns of weather and climate arising from the atmospheric and ocean circulations. Earth system models (ESMs) are a more recent development, and are GCMs that include more sophisticated representations of the atmospheric, terrestrial, and ocean biogeochemical cycles. Because this type of model includes a number of additional biogeochemical processes (such as interactions between land use, vegetation and the atmosphere) and interactive atmospheric chemistry, outputs from ESMs are highly relevant to research questions in ecology. The outputs from almost all the GCMs and ESMs in the latest Climate model intercomparison project (CMIP5) have been made freely available online for non-commercial use, via the Earth system grid federation (<www.pcmdi.llnl.gov>). Although the new CMIP experiments – CMIP6 – began in 2016, it will be a few years before the model data behind the next IPCC report are made available for analysis (Eyring et al. 2015).

The public availability of model data means that the ecological implications of various sources of uncertainty can be explored, such as the choice of climate model, different assumptions of climate sensitivity, and various commitments to greenhouse gas mitigation (Beaumont et al. 2008). The potential for the ecological systems themselves to act as sources of uncertainty in the global climate system is huge (e.g. via carbon cycle or land use feedbacks, Qian et al. 2016), and thus greater uptake of model data by the ecological research community is also in the interests of climate scientists. In using such data it is important to understand their limitations, to report on the source of the data and, especially, the baseline time period used in any analysis. For example, there is no facility within the experimental design for CMIP5 to account for the protection status of land, nor any potential changes in urban areas, which limits their applicability for investigating changes in land use. Climate scientists will be more aware of these types of potential pitfall, and ecologists could therefore reduce the risk of drawing erroneous conclusions by collaborating more widely.

Model data are commonly made available at the cell size typical of most GCMs/ESMs, which ranges from 0.75° to 2.8° horizontal resolution. Although this is coarse compared to the resolution of most ecological studies, the model data can be downscaled to finer cell sizes using statistical (Mitchell and Osborn 2005) or dynamical techniques (Jones et al. 2004). Many ecologists employ the 'delta' or 'change factor' method of imposing interpolated future anomalies onto finer-grained observational datasets, to generate future gridded climates that better reflect local heterogeneity (Pearson et al. 2014, Platts et al. 2015). The implicit assumption here is that the present day spatial patterning of local climate will persist under future climate change, which is valid in some landscape contexts but not in all (Maclean et al. 2017).

Considering appropriate spatial resolutions for analysis

The estimated impact of climate change on a study species can change if different spatial resolutions of climate data are employed (Trivedi et al. 2008, Gillingham et al. 2012). The question of which resolution is 'appropriate' will depend upon species' life cycle stage, movement ability (flight, mobile, static) and the component of the climate being analysed. It is also possible that species will respond to climate at a variety of scales, sometimes more than one at particular points in time, and in this case preliminary work will help to identify the critical life stage to focus on.

As highlighted above, many ecologists will be interested in how well coarse-scale models represent the climate that their study species experience(s). A recent meta-analysis of SDM use estimated that grid cell sizes are typically 1000 (for plants) to 10 000 (for animals) times larger than the size of the organism they focus on (Potter et al. 2013), highlighting the challenge of representing the biotic interactions (Pateman et al. 2012) or demographic effects (Kearney 2013) that can be important modifiers of responses to climate (Ockendon et al. 2014). These concerns have contributed toward the recent drive towards finer-scale data for use in ecology (Fig. 2). It should however be noted that there are many cases in which coarse-scale climate data are appropriate for modelling coarsely mapped response variables, such as the extinction or persistence of populations (Bennie et al. 2014), and thus the ultimate decision on which spatial resolution is appropriate will depend upon the research question.

Although the use of finer-scale, gridded climate data has improved our ecological understanding considerably, the spatial accuracy of these grids (indeed all climate grids) are a function of both: 1) the density of meteorological and satellite observations that contribute to them, and 2) the complexity of local climatic processes that operate in the region of interest (Nadeau et al. 2016). Thus the absolute value of differences between nearby cells may fall within the bounds of uncertainty in the data they are derived from, and where resulting effect sizes are found to be within this range of uncertainty, this should be acknowledged. This perhaps highlights a need for improved communication of uncertainties in gridded climate datasets by their creators in order to ensure that user communities are fully aware of how factors such as weather station density affect the climate data generated for a given study area.

Thinking critically about published data and code

An increasing emphasis on open access to data and computer code means that a huge variety of material is freely available for use by ecologists, via open source platforms such as R (<www.r-project.org>). But although these approaches and data may have been the most appropriate tools to test the researchers' original ideas, they are not always appropriate in other analytical contexts. A recent disagreement over the calculation of growth thresholds using the CMIP5 model data at daily resolution (PLoS Biology 2015) served to highlight how the views of some scientists over the appropriate use of data are not always shared by the wider community. Stated levels of precision should not be mistaken for accuracy, and where the accuracy of the data is unclear, this should be checked with the authors or custodians of the dataset. Critically, assumptions in the data or methods used (baselines and downscaling techniques) may not even be readily available. Ecologists should therefore share their analytical code when publishing data papers, so that this is available to those interested in greater methodological detail. More collaboration between ecologists and climate scientists would ensure that any methodological concerns can be headed off at an early stage of project development (Table 1).

A critical eye should also be applied to the code of others. In broad terms, 'code' represents a step-by-step record of a computational method that another scientist has developed. Because no ecologist follows another's field protocol without question, some level of critical thought should therefore also be applied when using a computational method supplied by another. This does not necessarily involve examining code line-by-line, but rather that adjustable parameters should be set and checked appropriately, and the uncertainties and assumptions behind the approach determined. The literature on how to do this is growing substantially, especially for the more popular software packages (e.g. MaxEnt, Philips and Dudík 2008, Phillips et al. 2017), and the Zoön Project for species distribution models (Lucas et al. 2016) offers a possible template for how to make code more open, shareable and accessible for all.

A recent survey of species distribution modellers noted that although the "code used to conduct the science is not formally peer-reviewed… many scientists rely on the fact that the software has appeared in a peer-reviewed article, recommendations, and personal opinion" (Joppa et al. 2013). Thus the method behind an article may not be adequately

Table 1. Four things that ecologists and climate scientists could do more of.

Problem area	Ecologists could…	Climate scientists could…
1) Communication and collaboration	…collaborate with climate scientists at an early stage of proposals, to ensure that projects are tailored to the strengths of the climate data, and do not fall victim to their weaknesses.	…talk to ecologists to identify and develop biologically meaningful climate variables to maximise the utility of climate datasets within the wider research community.
2) Handling uncertainty	…acknowledge and actively explore uncertainty in all types of climate data, not simply when using projections of future climates.	…make uncertainty estimates more widely available and interpretable for others in the research community.
3) Sharing lessons and resources	…share their own climate data and code more widely, expanding the resources available to all.	…make data products, code and guidance material easy to obtain and understand for non-specialists.
4) Selecting and using an appropriate resolution	…develop methods to account for the scale limitations of climate models, and work with climate scientists to use appropriately-downscaled climate information.	…be clearer about the appropriate spatial resolution at which to use GCM data, and work with ecologists to develop downscaling approaches that suit ecological applications.

assessed for quality. The increasing number of journals obliging authors to publish their code alongside their article represents a welcome move towards improving methodological clarity (Ince et al. 2012), even if conducting full assessments of these submissions for quality is unrealistic.

Accounting for uncertainty in climate data

The need to account for uncertainties in observed climate datasets was highlighted by Baker et al. (2016), who in a study of future climate effects found that uncertainty arising from choice of baseline climatology was often on a par with, or in fact exceeded, that arising from a GCM choice. However, although ecologists are often accustomed to dealing with the numerous sources of uncertainty in an ecological analysis (such as that arising from recording misidentifications or mislocations), they are often less aware of the uncertainty that is inherent in almost any climate data they use (IPCC 2013). Whether recognised or not, uncertainty will propagate through the many stages of processing and modelling required to derive ecologically meaningful climate data (Wilby and Dessai 2010). The level of uncertainty in observations and modelled data will depend on the characteristics of the study region, such as its topographic diversity or proximity to large water bodies, but also on both the homogeneity of the regional climate and the density of the meteorological observations taken nearby. Some datasets are provided with the uncertainty or quality control estimates enclosed (e.g. sampling and station errors in the global CRUTEM4 dataset, or MODIS quality control), and these should be utilised wherever possible.

Studies employing GCM data arguably require a greater consideration of the uncertainties involved. These uncertainties can arise from the (realistic) representation of climate variability, the alternative socioeconomic scenarios for the future, the 'structural' uncertainty arising from the physics behind different climate models used, and many other factors. The simplest means of exploring these uncertainties is via the use of more than one scenario (i.e. two or more RCPs) and multiple GCMs. Ideally analyses are rolled out across all the scenario-model combinations made available. Although averaged 'ensemble' estimates are computationally efficient, their use as inputs in analysis should be avoided wherever possible, as they can conceal large differences in projected climate (particularly for precipitation) and thus they underestimate uncertainty. The CMIP website (<www.cmip-pcmdi.llnl.gov>) provides a useful introduction to the effect of differences in the design of climate models, while also providing detailed guidance on using their outputs.

An additional consideration here is the presence of model bias, which can mean that the use of raw outputs from GCMs for certain types of impact study is not robust (e.g. accumulated time above or below a certain threshold). Scientists have overcome this problem by calibrating the projections with observed data, generating revised estimates that are more appropriate for establishing the impacts of climate change on heat stress (Hawkins et al. 2013) and river runoff (Hagemann et al. 2011). Note that biases or errors can also be inherent in any dataset of climate observations, and where these are known, these should be acknowledged, their possible effects explored, and, wherever possible, corrected for. Sensitivity analysis will reveal the degree to which conclusions are resilient to these effects.

Conclusion

The acceleration of climate change this century brings both threats and opportunities for species and ecosystems. It will be the job of ecologists to describe and make sense of these effects, and for wider society to formulate a response. We suggest a number of changes to the approaches of both ecologists and climate scientists to make successful outcomes for both disciplines more likely (Table 1). Underpinning these changes is a clear need for more interdisciplinary working and better communication among researchers. Engagement across disciplines has never been easier, with open access digital repositories, post-publication peer-review, webinars, online blogs and social media removing traditional barriers to communication. Whilst we should always be more mindful of the quality and veracity of material made available outside the peer-reviewed literature, interactions via these platforms have the potential to grow into more formal collaborations across disciplines, such as funding proposals and co-authored manuscripts. These collaborations will lead to new ways of working, new research questions to tackle, and will ultimately strengthen research findings. Scientists that adopt an interdisciplinary ethos will also find themselves well placed to address the more pressing issues of the 21st century, which due to their scope and complexity often require a broader perspective.

The challenge for ecology is to move beyond simple, indicative studies of what to expect from climate change, to a more specific, detailed approach that acknowledges issues of uncertainty and scale. In so doing, ecologists will get closer to resolving some of the fundamental questions and unknowns that remain in the discipline, while also producing the kind of informative and actionable results that are urgently required if we are to successfully mitigate and adapt to climate change.

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