Amphibian conservation in the face of land-use change and global warming

Pamela González del Pliego Castañeda

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Faculty of Science
Department of Animal and Plant Sciences

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Abstract

The loss of tropical forests has caused dramatic declines in biodiversity. Yet some tropical regions are undergoing large-scale secondary forest regeneration on abandoned farmland, and these recovering forests are increasingly recognised as being important for reversing biodiversity losses. A key issue studied in this thesis is whether these secondary forests will remain valuable for biodiversity under climate change, doing so focusing on amphibians within the Tropical Andes of Colombia. First, I measured the abundance of live (e.g. bromeliads) and dead (e.g. deadwood) microhabitats used by amphibians across primary, and young (4-18 yrs) and old secondary forests (19-35 yrs), and explored whether these microhabitats provide thermally buffered microclimates. I showed that microhabitats in secondary forests recovered towards primary forest levels and served as thermal buffers that reduce exposure to extreme temperatures. One of the main species' traits determining survival at higher temperature is the thermal point at which an animal loses its ability to escape from deadly conditions (critical thermal maximum – CTmax). I measured the CTmax from 14 *Pristimantis* frog species to demonstrate that physiological thermal tolerance is strongly related to phylogeny, but not affected by environmental factors, suggesting low levels of environmental selection. I then used individuals' current vulnerability and showed that species' in young, but not mature, secondary forests will become more threatened under global warming because they are exposed to higher temperatures. These results underscore the value of secondary forests in offering critical, thermally buffered microhabitats, suggesting that most amphibian species could maintain their current thermal niche if temperature increase remains below +2°C. Nevertheless, *c.*40% of amphibians are currently threatened with extinction, and *c.*25% are data deficient (DD) and may also be at risk. Using a global trait database and a fully sampled phylogeny, I predicted the threatened status of global DD species. I found that between almost half of DD species are threatened, mainly across Southeast Asia and the Neotropics, suggesting that DD species require urgent conservation.

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Chapter II was the result of my research and all the writing and analyses were carried out by me. However, this is the overall contribution of all authors: P.G., B.R.S., R.P.F. and D.P.E. conceived the ideas and designed methodology. P.G. and E.W.B collected microhabitat data. P.G. collected microclimate data, analysed the data and led the writing of the manuscript. P.W. and C.W. collected the carbon data. B.R.S., R.P.F., D.P.E., J.G., C.A.M.U. and T.H. revised the manuscript critically and gave important intellectual input.

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Chapter I

General Introduction

Millions of diverse life forms have evolved since the first life form appeared on Earth about 3.7 billion years ago (Nutman et al. 2016). Over the past few centuries, however, human beings have driven a dramatic loss of biodiversity, which has led to a global extinction crisis (Ceballos et al. 2017). The main pressures on global biodiversity are habitat loss, degradation and fragmentation, over-exploitation, climate change, pollution, and invasive alien species (CBD 2014). These pressures continue to increase, creating large population declines and extirpations, which in turn, have negative effects on ecosystem services that are essential to civilisation (CBD 2014, Ceballos et al. 2017).

The main driver of global biodiversity loss is land-use change, either through habitat loss, fragmentation or degradation (MEA 2005). The drivers vary across ecosystems, but for terrestrial ecosystems, land-use change is mainly driven by the conversion of wild lands to agriculture (CBD 2014). For example, more than 80 million hectares of tropical forests were lost during the 1980s and 1990s, and most of these were converted to agriculture (Gibbs et al. 2010). This rate of tropical conversion continues to increase across the world, threatening a wide range of species (Hansen et al. 2013).

The highest rates of deforestation take place in the tropics, which has the highest biodiversity globally (Hansen et al. 2013, Brown 2014). In fact, there was an increase in forest loss of 2,101 km^2 per year in the tropics from 2000 to 2012 (Hansen et al. 2013). Deforestation severely fragments the landscape and leads to a dramatic decrease in species richness (Foster et al. 2011, Gibson et al. 2011). The negative effects of deforestation vary across taxonomic groups, region and human impact types (Barlow et al. 2007, Gibson et al. 2011). However, most taxonomic groups are negatively affected by human disturbance, such as the conversion of forest to agricultural land (Gibson et al. 2011). Even with low levels of forest disturbance species are lost, especially highly sensitive, endemic and rare species (Barlow et al. 2016). For example, in Brazil, one of the world’s most biodiverse countries, a 20% loss of primary forest resulted in a 39 – 54% loss of conservation value (Barlow et al. 2016). Furthermore, disturbances such as selective logging also create edge and isolation effects, which in turn increase desiccation, tree mortality, and the likelihood of forest fires (Laurance 2004), ultimately threatening biodiversity (Laurance et al. 2002).

Alongside this trend towards greater deforestation, there has been a concurrent phase of land abandonment in areas of marginal suitability for agriculture during the past decades in some regions of the tropics (Guariguata and Ostertag 2001, Sanchez-Cuervo and Aide 2013). Following land abandonment, natural forests begin to regenerate in many of these areas, with residual seed banks and dispersed seeds underpinning this process (Lindsell et al. 2015). For example, between 2001 and 2010 Colombia and Venezuela were the two countries in South America with the largest net gains in woody vegetation of +16,963 km^2 and +5,830 km^2 respectively (Aide et al. 2013). In the same time period, deforestation occurred mainly at low elevations (<250 m), and most reforestation occurred in montane areas between 300 and 1000 m. In fact, large gains of woody vegetation occurred in the Colombian Andes (Aide et al. 2013). Over time, secondary forests recover carbon stocks (Poorter et al. 2016) and are able to reverse biodiversity loss (Martin et al. 2013, Gilroy et al. 2014), even if these levels are lower compared to undisturbed areas (Gibson et al. 2011). Moreover, secondary forests can also provide ecosystem services at levels similar to mature forests (Chazdon 2008). Although tropical regions are experiencing high rates of secondary forest regrowth, they still experience a net forest loss (Hansen et al. 2013): between 1980 and 2000, more than half of the new agricultural land across the tropics came at the expense of intact forests, and only 28% came from disturbed forests (Gibbs et al. 2010).

Global climate change is another major threat to biodiversity (CBD 2014) and is set to exacerbate future extinction risk, especially when coupled with the effects of land-use change. The rate of warming has increased significantly over the last 50 years (IPCC 2014) and is severely threatening biodiversity globally, with negative impacts now spanning continents, ecosystems and species (Rosenzweig et al. 2008, Sekercioglu et al. 2008, Freeman and Class Freeman 2014, IPCC 2014, Urban 2015, Scheffers et al. 2016). Moreover, extreme weather events (e.g., droughts, heat-waves and El Niño events) have become more frequent and intense across many regions (IPCC 2014, Diffenbaugh et al. 2017). These unprecedented extreme weather events increase the likelihood of severe, pervasive and irreversible impacts on ecosystems (IPCC 2014). Unfortunately, climate change is projected to become a more significant threat in the coming decades (CBD 2014). The impacts of global warming on organisms will depend among other factors on their level of exposure combined with their physiological sensitivity and tolerance to high temperatures (Deutsch et al. 2008, Freckleton and Jetz 2009, Scheffers et al. 2014, Sunday et al. 2014, Garcia-Robledo et al. 2016). Consequently, species' critical thermal maximum (CT_{max}), the thermal point at which an animal loses its ability to escape from deadly conditions (Cowles and Bogert 1944), is a vital trait in understanding thermal stress and vulnerability to increasing temperatures (Kearney et al. 2009, Huey et al. 2012).

Across all the ecophysiological variables, body temperature is perhaps the most important trait affecting the thermal physiology and performance of ectotherms (e.g. fecundity and growth rates) (Angilletta et al. 2002). Ectotherms need to maintain an optimum body temperature for ideal performance and thus respond to the thermal environment in different ways, including with: A) *a rapid and reversible response*; B) *a longer time scale response;* C) *a response over multiple generations*. The following is a more detailed explanation of these responses.

Rapid and reversible response: with this rapid response, an individual uses behaviour, physiology or both to regulate its body temperature (Angilletta et al. 2002). Ectotherms can maintain, increase or decrease their body temperature by using thermoregulatory behaviours, such as finding shelter in buffered microhabitats, sun basking, among others. However, to thermoregulate via microhabitats, these must be readily available and offer suitable microclimates, and the individual must have sufficient time and energy to find and move to these microhabitats. The availability of the microclimates depends on the type of habitat where the microhabitats are located and the type of microhabitat, with primary forests having higher microhabitat abundance and complexity (Gonzalez del Pliego et al. 2016). Behavioural thermoregulation can be a perfect alternative to undergoing acclimatisation, especially for tropical frogs living in near-constant temperature environments, because temperatures remain relatively constant throughout the year (Duellman and Trueb 1994; Navas 1997).

Longer time scale response: this response takes place within the lifetime of an individual and may or may not be reversible. An individual can change its physiology (e.g. upper thermal limits) by using adaptive shifts and acclimation of thermal physiology to alter their performance function, mainly at a cellular level (Angilletta et al. 2009). Ectotherms have shown phenotypic plasticity associated with CT_{max} (for example, seasonal acclimatisation) (Simon, Ribeiro et al. 2015), and acclimation potential to new thermal limits. Perfect acclimation, however, is rarely seen in ectotherms (e.g. crocodiles; Glanville and Seebacher 2006). The acclimation ability of ectotherms varies across species, for example, *Pristimantis* frogs are more able to acclimate or adapt to warmer climates than to cooler climates because of their high levels of genetic diversity (Brattstrom and Lawrence 1962; Christian and Bedford 1995; Lynch 1999; Laurila, Karttunen et al. 2002; Lynch 1999; García-R, Crawford et al. 2012). The acclimation potential also varies across physiological variables. For example, plastic changes can increase the thermal limits in many terrestrial ectotherms, but tend to have less effect on upper limits than lower limits (Hoffmann, Chown et al. 2013). However, empirical data on critical thermal limits of most tropical montane taxa remain unknown (von May 2017).

Potential for acclimation in ectotherms is generally limited and will be insufficient to buffer most species against climate warming (Gunderson and Stillman 2015). It is likely that many ectotherms have limited potential to keep pace with current rates of temperature increase (Parmesan, Root et al. 2000; Hoffmann, Chown et al. 2013). Moreover, the acclimation ability of ectotherms might reflect an evolutionary trade-off with the evolution of thermal tolerances, rather than adaptation to the thermal habitat of species (Angilletta et al. 2002).

Multiple generations scale: this is a non-reversible response that takes place across multiple generations. It relies on the evolution of key parameters of performance (e.g. increasing or decreasing the CT_{max} of species) in response to the thermal environment through natural selection (Angilletta et al. 2002). Lability of thermal physiology, however, varies among taxa (Angilletta et al. 2002). For example, the CT_{max} of ectotherms is highly related to the maximum temperature of the warmest month of their environment (Araujo, Ferri-Yanez et al. 2013) and it is largely conserved across lineages, while tolerance to cold (CT_{min}) varies between and within species (Araujo, Ferri-Yanez et al. 2013). As far as we are aware, the rate of adaptation to stressful conditions has not been shown to be enhanced by heat exposure in ectotherms, although heat stress may enhance the expression of genetic variants contributing to loss of fitness following inbreeding (Chen and Wagner 2012). There has been intermittent interest in the past few decades as to whether exposure to heat stress in one generation influences responses to heat in the next generation. It remains unclear, however, if high temperatures can influence these (environmental/genetic variance) processes (Hoffmann, Chown et al. 2013).

Although an understanding of how species can respond to the environment helps us to determine their vulnerability, solving the biodiversity crisis is a complex process that requires reducing land-use change driven extinction risks while simultaneously protecting species from future climate change and other threats. With the rising global population and increasing demand for resources, a central challenge for conservation is how to preserve biodiversity, natural ecosystems and the services they provide while meeting these demands (CBD 2014). Moreover, negative effects of threats such as habitat loss, climate change and increasing population size have synergistic effects that increase the global number of species at risk (MEA 2005, Scheffers et al. 2016, Cheptou et al. 2017). In this thesis I look at the regional interface between two of the major biodiversity pressures: land-use and climate change.

I chose amphibians as my study taxonomic group because globally they are highly threatened by habitat loss, infectious disease and climate change (IUCN 2015). Currently it is estimated that *c.*41% of total amphibian species are threatened with extinction (IUCN 2017). These species have suffered worldwide declines, many of which are related to global warming and chytrid fungus (La Marca et al. 2005, Pounds et al. 2006, Bielby et al. 2008). Amphibians are present in every continent except Antarctica, with their highest richness in the tropics. To date, there are 7,720 amphibian species described worldwide (Amphibiaweb; 12 September 2017), although we believe the true species richness to be much higher (Glaw and Köhler 1998, Köhler et al. 2005). As such, more amphibian species could be at risk, and this makes them an ideal study organism and a high conservation priority.

Thesis Overview

The over-arching goal of this research was to provide information that could benefit future conservation efforts for amphibians. I present three chapters based on data collected over three field seasons (2014-2015). I also present a desk-based data chapter with a global vision. Following this introductory chapter, Chapter II focuses on secondary forests, as they are set to become the most widespread vegetation type in the world (Hurtt et al. 2011). I established the ability of secondary forests to offer physical and thermal shelter for amphibians. I show that secondary forests have similar microhabitat composition to primary forests. I also examine the relationship between carbon stock recovery and microhabitat recovery. Finally, I assessed the ability of microhabitats to buffer climate in secondary forests compared to primary forests.

Chapters III and IV are then based on results from a previous paper where we found that land-use change has a strong impact on community composition of amphibians (Basham et al. 2016). In these chapters, I use species' critical thermal maximum (CT_{max} – the thermal point at which an animal loses its ability to escape from deadly conditions) to address the impact of climate change on amphibians (Cowles and Bogert 1944, Kearney et al. 2009, Huey et al. 2012). Variation in species' traits, including CT_{max}, is a function of both environmental exposure and the evolutionary history of species (Freckleton and Jetz 2009, Leal and Gunderson 2012, Gutiérrez-Pesquera et al. 2016). Species' vulnerability to climate change is also dependent on the environmental regimes they are exposed to and their sensitivity (Williams et al. 2008). Thus, in Chapter III, I used phylogenetic analyses to understand how phylogeny and morphology determine species vulnerability to global warming. I also showed that physiological thermal tolerance is not affected by environmental factors. In Chapter IV, I assessed amphibian vulnerability to global warming by incorporating phylogeny, level of exposure, physiological sensitivity and tolerance to high temperatures across a secondary forests in the Tropical Andes. I also explored how climatically buffered microhabitats can contribute to reduce amphibian vulnerability under present and future climate conditions.

Besides being impacted by habitat loss and climate change, amphibians are also severely threatened by over-exploitation, pollution and invasive species at a global scale. This makes amphibians the most threatened vertebrate group assessed to date (IUCN 2017). Crucially, for *c.* 25% of described amphibians there is not enough information to assess their threat status, and so they are categorised as data deficient species (DD). In Chapter V, I address this crucial gap in knowledge. Here I undertake a global amphibian analysis and aim to predict the threatened status for all DD amphibian species. To do so, I use amphibian phylogenetic relationships because species that are evolutionarily related are likely to share similar threat levels (Jetz and Freckleton 2015). The largest amphibian phylogeny published to date includes over 2,800 amphibian species (Pyron and Weins 2011). However, there is now an amphibian phylogeny for over 7,000 species (Jetz and Pyron *in review*) currently under review in Nature Ecology and Evolution. Despite not being yet published, they shared their phylogeny for this thesis and it was used in Chapter V. The results of this chapter can help in targeting conservation efforts to taxonomic groups and regions. Finally, in Chapter VI, I summarise the main findings of this research and draw general conclusions about current and future amphibian conservation.

Chapter II

Thermally buffered microhabitats recovery in tropical secondary forests following land abandonment

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Abstract

Given the dramatic loss of tropical forests and accelerating climate change, secondary forest regeneration is increasingly recognised as being an important method for reversing losses in biodiversity and carbon stocks. The recolonisation of biodiversity within secondary forests depends in part upon the recovery of forest structure, including the range of microhabitats used by diverse local communities. Here, we investigate the return of critical microhabitats along a successional gradient of secondary forest in the Tropical Andes of Colombia. We measured the abundance of live (bromeliads, tree ferns and moss) and dead (deadwood and leaf litter) microhabitats across three landscapes, each encompassing primary, and young (4 -18 yr old) and old secondary forests (19 - 35 yr old). Considering the increasing rate of climate warming in the region, we also explored whether these microhabitats provide thermally buffered microclimates. We found that secondary forests have different composition and lower complexity of microhabitats than primary forests, but microhabitats appear to be recovering towards primary forest levels. Furthermore, in all forest types, microhabitats had lower maximum temperatures and higher minimum temperatures, thereby serving as thermal buffers that reduced exposure to extreme temperatures. These benefits exist despite ambient temperatures in secondary forests surpassing those of primary forests by 1-2°C on average. The protection of secondary forest and promotion of further forest regrowth in the Tropical Andes should represent an urgent investment for conservation, and the value of these forests for offering critical microhabitats and buffered microclimates under climate change should not be overlooked.

Introduction

Land-use change is a primary driver of global biodiversity loss (MEA 2005) and carbon emissions (Grace et al. 2014). During the 1980s and 1990s, more than 80 million hectares (ha) of tropical forests were converted into cropland and pasture (Gibbs et al. 2010), with the rate of conversion continuing to increase in many tropical regions (Hansen et al. 2013). The conversion of tropical forest to agriculture severely fragments the landscape, dramatically reducing species richness (Foster et al. 2011, Gibson et al. 2011), and causing edge and isolation effects that further increases extinction risk (Ferraz et al. 2003, Schnell et al. 2013).

Despite these trends, there has been a concurrent phase of land abandonment in areas of marginal suitability for agriculture during the past decades in some regions of the tropics (Guariguata and Ostertag 2001, Sanchez-Cuervo and Aide 2013). Seventy percent of land abandonment has occurred in hilly or montane areas of Central America, the Andes, Vietnam, and the Philippines (Asner et al. 2009), but with some also occurring in flat regions such as the Amazon and Madagascar (Asner et al. 2009, Rodríguez et al. 2012).

Following land abandonment, natural forests begin to regenerate in many of these areas, with residual seed banks and dispersed seeds underpinning this process (Lindsell et al. 2015). Over time, carbon stocks in secondary forests gradually recover towards levels found in primary forests (Martin et al. 2013, Gilroy et al. 2014). Secondary forests are also rapidly (re-)colonized by animal biotas (Martin et al. 2013, Gilroy et al. 2014, Queiroz et al. 2014), including some species threatened with extinction (Gilroy et al. 2014) and animal groups that are more prone to extreme temperatures, such as amphibians (Basham et al. 2016).

The precise nature of biodiversity recovery depends on changes in forest structure, including foliage density and tree height, as well as the diversity of small-scale habitat features called microhabitats (MacArthur et al. 1962). Microhabitats are important to a wide range of taxa, for example, logs and tree cavities provide microhabitats for amphibians, birds, small mammals, and invertebrates (Stapp 1997, Cadavid et al. 2005, Grüebler et al. 2014); epiphytic plants such as bromeliads and bird's nest ferns (*Asplenium*) offer shelter and breeding habitat for amphibian and invertebrate communities (Ellwood and Foster 2004, Urbina and Galeano 2009, Silva et al. 2011, Jocque and Field 2014, McCracken and Forstner 2014, Scheffers et al. 2014b); and leaf-litter and soil are commonly used by amphibians and invertebrates (Stapp 1997, McGlynn and Kirksey 2000, Cadavid et al. 2005, Urbina and Galeano 2009, Wanger et al. 2010). Microhabitats, along with providing space for breeding and foraging, also provide buffered shelter during extreme weather events, although the latter has only been recorded in primary old-growth forest (Scheffers et al. 2014a), not secondary forests. Thus, the extent of microhabitat recovery in secondary forests is an unknown, but particularly important process to biodiversity recovery in these areas.

In addition to land-use change, biodiversity is also threatened by climate change. Over the last 50 years, the rate of warming has increased significantly while extreme weather events (e.g., droughts and heat-waves) have become more frequent and intense (IPCC 2014), severely threatening biodiversity globally (Sekercioglu et al. 2008, Chen et al. 2009, Chen et al. 2011, Hannah 2011, Maclean and Wilson 2011, Freeman and Class Freeman 2014). Carbon enhancements under carbon-based payments for ecosystem service schemes (e.g., United Nations Reducing Emissions from Deforestation and Forest Degradation, REDD+) have been implemented to reduce carbon emissions by financially incentivising forest recovery and restoration. Although these programmes offer some biodiversity co-benefits (CBD 2011, Gilroy et al. 2014), the extent to which carbon accumulation correlates positively with the recovery of microhabitats and microclimates is uncertain. Addressing this question is therefore important for understanding if and how species that depend on such environments will benefit from carbon payments.

Species have limited options in responding to climate change: become extinct; cope or adapt in situ; or shift their ranges to track optimal climates (Lawler et al. 2013), which has already occurred with a large number of species (Parmesan and Yohe 2003, Hickling et al. 2006). Importantly, for the numerous species that are unable to track their optimal climate niches (Chen et al. 2009), the presence of microhabitats could provide a microclimatic shelter during extreme weather events. Thus, the ability of microhabitats to buffer increased ambient temperatures in secondary forests will likely determine the survival of these species.

Here, we aim to answer: (1) How similar is microhabitat composition in secondary forests to that found in primary forests? (2) How much time since land abandonment is required for the recovery of critical microhabitats? (3) Is there a positive relationship between carbon stock recovery and microhabitat recovery? And (4) how well do microhabitats in secondary forests buffer climate compared to primary forests? Here we answer these key questions by focusing on a successional gradient of secondary forest in the Tropical Andes, which is a global hotspot of extinction risk and species endemism.

Methods

Study area

We chose three study landscapes located within the departments of Antioquia, Risaralda and Chocó, along the Western cordillera of the Colombian Andes (long: -75.8895 to -76.0825, lat: 5.2396 to 5.8251) (Appendix 1, Fig. S1; following (Gilroy et al. 2014)). Each site encompasses primary forests, naturally regenerating secondary forests and an agricultural matrix dominated by cattle pasture, with other crops present under very limited cover (<10 hectares in total, including maize and plantations of tamarillo *Solanum betaceum* (Gilroy et al. 2014). The study area covered an altitudinal range of 1,290-2,680 m above sea level. This region supports one of the highest global diversities of threatened and endemic taxa (Myers et al. 2000, Orme et al. 2005), and is characterised by a long history of cattle farming (Gilroy et al. 2014).

Across these three landscapes (Appendix I, Fig. S1B, from north to south: Reserva Tangaras, Chocó; Reserva Mesenia-Paramillo, Antioquia; and Cerro Montezuma, Risaralda), we created 29 sampling squares of 400 m x 400 m and spaced by >400 m apart, with squares representing one land-use type (naturally-regenerating young secondary forest from 4 to 18 yr old, old secondary forest from 19 to 35 yr old, and primary old-growth forest). In each square, data were sampled from three sampling points, each spaced by 200 m, giving 87 sampling points in total (following (Gilroy et al. 2014)).

Microhabitats

We placed a 25 m x 6 m plot at each of our 87 sampling points, within which we sampled microhabitat abundance, biomass and volume. These three microhabitat metrics will be hereafter referred to as abundance. Elevation was measured in the plot centre. We measured three live (bromeliad, fern, moss) and two dead (deadwood, leaf litter) microhabitats across each plot. Logistic field limitations allowed us to have only 74 plots for dead microhabitats and carbon.

Live vegetation:

Bromeliads - Epiphytes are particularly vulnerable to forest degradation (Turner et al. 1996), but are important microhabitats for amphibians and invertebrates (Jocque and Field 2014, Scheffers et al. 2014b). Bromeliads are one of the most abundant epiphytes in the Tropical Andes (Benzing 2000). We recorded the total number of bromeliads in our plot. Total number of plots (Ntotal) = 87 plots.

Tree ferns – Understorey tree ferns (Order Polypodiales) have a single erect trunk and very large fronds, making them important microhabitats for amphibians, mites, spiders and several arthropods including beetles (Shuter and Westoby 1992, Richards 2007, Li et al. 2011, Fountain-Jones et al. 2012). We measured height and diameter at breast height (dbh) of all ferns >5 cm dbh (following (Gilroy et al. 2014). We estimated tree fern biomass using the allometric equation of (Tiepolo et al. 2002). Ntotal = 87 plots.

Moss – Moss is an important microhabitat for insects and amphibians (Tarkowska-Kukuryk and Mieczan 2014, Lee-Yaw et al. 2015). In each plot, we delimited fifteen 1 m^2 quadrats (following (Urbina and Galeano 2009)). Moss height and density defines how easy species move through it (Lee-Yaw et al. 2015)). Therefore, moss was assessed in such a way as to retain moss original structure during measurement. To do this, we derived a metric of moss volume by multiplying depth (an average of four measurements per quadrat) and the percentage of moss coverage (Maanavilja et al. 2014). Ntotal=15 quadrats per plot*87 plots = 1,305 quadrats.

Dead vegetation:

Deadwood – Deadwood is a crucial requirement for many arthropod species since it provides a place to hide and forage (Lassauce et al. 2011, Bluhm et al. 2015), while the volume of deadwood can indicate a higher abundance of birds and beetles (Winter et al. 2005). At each sampling point we established a smaller 15 m x 5 m plot, within which we estimated the biomass of all standing or fallen dead trees (snags) >5 cm dbh (Gilroy et al. 2014). For snags >10 m high we used allometric equations to estimate biomass, applying a deadwood density of 0.31 g cm^{-3} taken from the literature (Gibbon et al. 2010). For snags <10 m high, we assumed the tree was a cylinder and estimated biomass by multiplying volume with wood density. The diameter and length of all pieces of fallen deadwood (coarse woody debris) of $\geq$5 cm diameter was also recorded, converted into volume and used to estimate biomass (assuming a cylindrical shape). Ntotal = 74 plots.

Leaf litter – Leaf litter is an essential microhabitat for amphibians and invertebrates, providing physical shelter and foraging space (Urbina and Galeano 2009, dos Santos Bastos and Harada 2011, Queiroz et al. 2013). We measured litter dry biomass since the amount (weight) of leaf litter is one of the most important characteristics to determine species abundance and richness (e.g. ants (dos Santos Bastos and Harada 2011, Queiroz et al. 2013)). Within each smaller 15 m x 5 m plot, we collected all leaf litter, grass and small plants (<0.5m in height) from 4 x 0.25 m^2 quadrats (Gilroy et al. 2014), following (Queiroz et al. 2013). We weighed these samples to the nearest 0.1 g, then used the fresh:dry weight ratio of an oven-dried subsample (10-20%) to estimate the dry biomass of litter (Nascimento and Laurance 2002). Ntotal = 74 m^2.

Live non-soil carbon stocks

To calculate live non-soil carbon stocks, within each smaller 15 m x 5 m plot we also measured the diameter at breast height (dbh) of all live trees >5 cm dbh, and measured wood specific gravity using tree cores extracted with an increment borer (two threads, 5.15mm diameter; Haglöf, Sweden) (Gilroy et al. 2014). We used these values in four allometric biomass estimation equations taken from harvested tree studies (Chave et al. 2005, Alvarez et al. 2012, Feldpausch et al. 2012). We took the mean of the four estimates as the final aboveground estimate for each tree. Palm growth form differs from that of other trees, and so to calculate palm biomass we measured diameter and estimated height, and then used the allometric equation of Sierra et al. (2007). Root biomass was estimated using a published upland forest root:shoot ratio of 0.26 (Cairns et al. 1997). All vines with a dbh of $\geq$2 cm that were $\leq$1 m from the long edges of the plot were also measured and vine biomass was estimated using an equation developed in Colombian montane forest (Sierra et al. 2007). We summed the biomass pools for each plot before multiplying by 0.5 to give an estimate of the total live non-soil carbon stock (Gibbon et al. 2010). Ntotal = 74 plots.

Temperature data

We used 179 iButton data loggers (model: DS1921G-F5; accuracy: 0.5 ^{o}C) to record understorey ambient, canopy and microhabitat temperature, and we also set nine iButton hygrochron loggers (model: DS1923; accuracy: 0.1^{o}C) as a backup to record understorey ambient temperature across all forest types. We placed the iButtons in 17 plots from Cerro Montezuma and 16 plots from Reserva Mesenia-Paramillo, with placement spanning young and old secondary, and primary forests (Appendix I, Table S1). All loggers recorded data every two hours from February 2013 to February 2014.

Each iButton was placed inside a re-sealable zipper storage bag (50 mm X 50 mm) to shelter them from precipitation and enclosed within a metal mesh to guard them from rodents. One iButton logger per plot was hung approximately 1 m above the ground to measure understorey ambient temperature (Scheffers et al. 2013a). To identify the (near-)maximum potential ambient air temperature for our study plots, we hung a second iButton in the upper canopy cover of trees between ten and thirteen meters above the ground (Scheffers et al. 2013b), as close as possible to directly above the understorey ambient logger depending on canopy accessibility. To minimize exposure to direct solar radiation, canopy, understorey and humidity loggers were secured under a plastic funnel, with all funnels suspended facing north (controlling for diurnal variation in sun position).

Microhabitat temperature loggers were deployed thus: (1) approximately 5 cm under leaf litter (ntotal=33); (2) between the leaves of bromeliads (ntotal=26); (3) inside holes at the base of trees (tree circumference > 8cm dbh) (ntotal=33); (4) within the roots of ferns (fern circumference > 8cm dbh) (ntotal=33); and (5) at approximately 20 cm depth in soil (ntotal=9). All loggers were placed within 8 m of the understorey ambient logger (most within one and four metres). Only one iButton was placed within a particular microhabitat for each plot.

Statistical Analyses

Microhabitat composition

To compare microhabitat composition among secondary and primary forests we used nonmetric multidimensional scaling ordination (NMDS, function “metaMDS” from the vegan package). This technique uses microhabitat abundance data and makes no prior assumptions about habitat-level structuring in the data. To evaluate whether composition differed significantly across the forest types, we transformed our data into a similarity matrix (Bray-Curtis index) and performed an analysis of similarity (ANOSIM). ANOSIM uses ranks of dissimilarities and this test is free from any normality assumptions. A post-hoc permutation test (999 replications) was run to detect which pairs of groups significantly differed. The community analysis was performed using PRIMER 7 (Version 7.0.7; Clarke, KR, Gorley, RN, 2015). We also extract NMDS axis 1 and 2 to create a microhabitat composition metric.

Microhabitat complexity recovery

To compare microhabitat data to a notionally common scale we normalized the data for each microhabitat by rescaling the range from 0 to 1. To create a microhabitat complexity metric we added the normalized data for each microhabitat by forest type. This complexity metric represents a truthful microhabitat abundance value because it sums each microhabitat value, in comparison with the composition metric (derived from NMDS axes) which represents the relationship or similitude of microhabitat data among forest types in a graphical manner. To compare microhabitat complexity of secondary and primary forests, we obtained the ratio of young and old secondary forest complexity compared to primary forest complexity (considering the latter our benchmark of 100 percent). We then performed a one-way analysis of variance (ANOVA; function “aov”) with forest type as our grouping variable.

To determine whether there were differences in the abundance of each microhabitat across forest types, we performed linear mixed effect models (function “lmer”), with sampling square, elevation, and landscape as random factors. All data were log transformed before each analysis, and residuals were assessed for each model considering model assumptions.

Carbon-microhabitat co-benefits

Due to the ecological meaningfulness of carbon and the high correlation between carbon sequestration and forest age, we assessed the relationship between carbon and microhabitat recovery using total live non-soil carbon as our predictive variable. We did so for each of our microhabitats using the normalized microhabitat data, microhabitat complexity metric, and microhabitat composition metric (using NMDS axes 1 and 2). We used quantile regression (function “rq”), from the quantreg package, because our data showed a larger number of outliers than normal, and quantile regressions give more robust estimates against outliers in the response measurements. For the same reason, we analysed the 10th, 50th, and 90th percentile to discover more useful predictive relationships between our variables. All microhabitat data were log transformed prior to analyses.

Microclimates

Our data showed no seasonality patterns, and therefore was not subdivided. Temperature maxima and minima have been successfully used to measure microhabitat thermal buffering effect (Shi et al. 2014). Therefore, weekly maximum, minimum and mean temperatures were taken from each iButton per microhabitat per forest type. We used linear mixed effect models (“lme”) to determine temperature differences among forest types, including forest type and date as fixed effects, and as random effect we placed iButton identity nested within transect and elevation (the two landscapes in which we placed iButtons do not overlap in elevation, hence we do not need to include landscape as a random factor). P-values for each model were determined comparing the model versus the null model ("anova"). Significant differences between forest types were assessed via post-hoc Tukey comparisons.

To obtain the rate of increase in microhabitat temperature for every 1^{o}C increase in understorey ambient temperature we used the temperature data collected every 2 hours. Linear models ("lm") were employed to generate a relationship between each microhabitat metric (i.e. bromeliad max, bromeliad min, bromeliad mean, etc.) depending on understorey ambient temperature (ambient max, ambient min, or ambient mean). Residual plots were checked to confirm model assumptions were met. Unless stated otherwise, all statistical analyses were performed in R (Version 3.1.2).

Results

Microhabitat composition

Ordination plots showed that microhabitat composition in secondary forests differed significantly from primary forests (Fig. 1.1A; ANOSIM, R = 0.53, p = 0.001). There was some degree of overlap in the microhabitat composition between forest types (Fig. 1.1A), and pairwise comparisons revealed no significant differences between young secondary and old secondary forest (R = 0.05, p = 0.25). In contrast, there was significant dissimilarity between old secondary and primary forest (R = 0.36, p = 0.001). In the ordination plot, young secondary forests were the furthest away from primary forests, making them the least similar in microhabitat composition (R = 0.66, p = 0.001). Recovery of secondary forest thus regenerates increasingly similar microhabitat composition to primary forest over time.

Microhabitat complexity recovery

Considering bromeliads, tree ferns, moss, deadwood and leaf litter, primary forests held a significantly higher microhabitat complexity than young secondary forests ($F_{2,367} = 3.57$, $p < 0.05$). Old secondary forest was intermediate between these forest types, and did not differ significantly from either primary forest or young secondary forest (Fig. 1.1B). There was, however, no significant difference between young and old secondary forest in their ratio of complexity compared to primary forest (Fig. 1.1C; $F_{1,8} = 0.07$, p = 0.79).

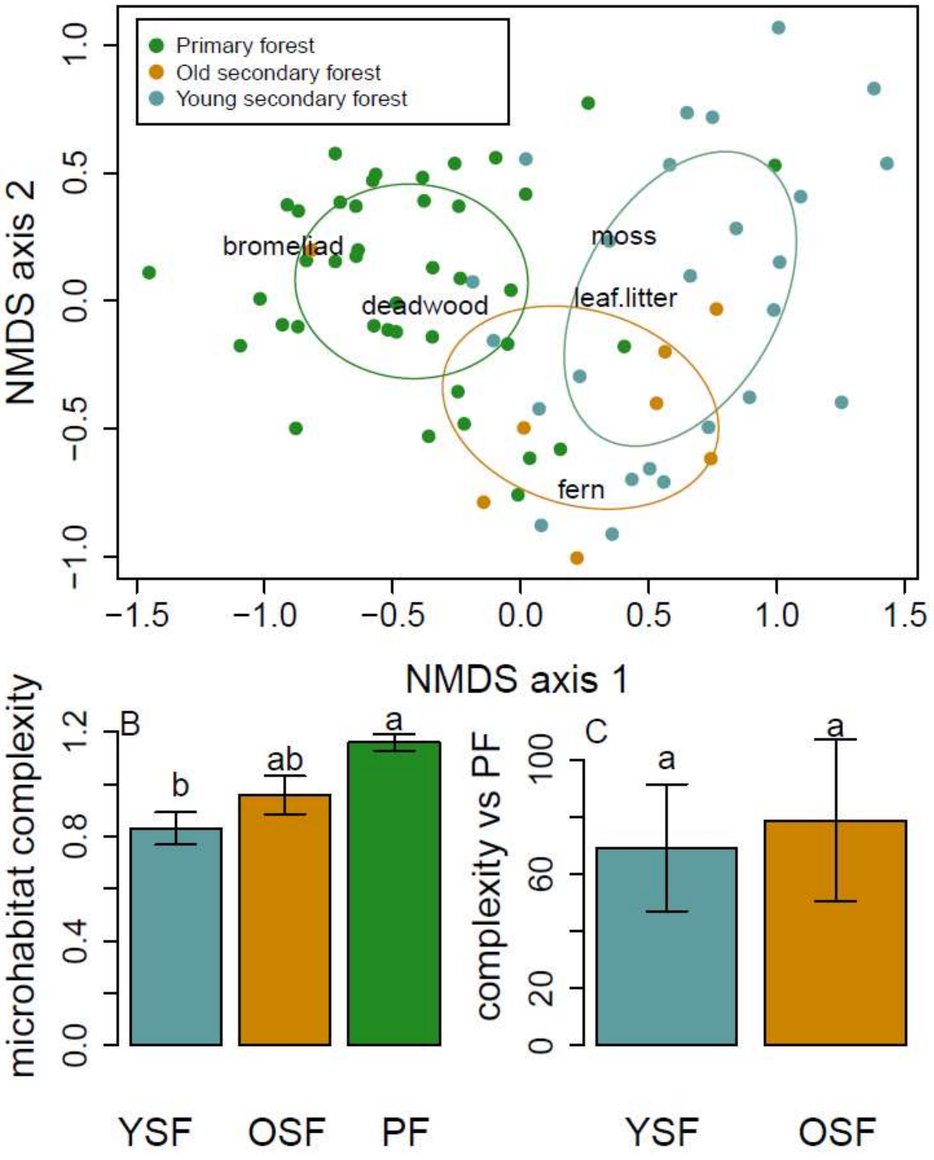

Fig. 1.1. (A) Nonmetric multidimensional scaling (NMDS) of microhabitat composition among forest types using microhabitat abundance, biomass and volume. Young secondary forests are <19 years old, old secondary forests are >19 years old, and primary forest is undisturbed old-growth. Ellipses represent a grouping function depending on the standard deviation of points with a 95% confidence interval. (B) Recovery of microhabitat complexity among forest types. (C) Secondary forest ratio of microhabitat complexity compared to primary forest (considered as 100%). YSF: young secondary forest; OSF: old secondary forest; PF: primary forest. Values represent normalized data from mean abundance of microhabitats. Error bars represent standard error. Different superscripts represent significant differences ($p<0.05$).

Live vegetation - There was a significant difference between forest types in number of bromeliads ($F = 36.5$, $p < 0.0001$; Table 1.3): old secondary had three times more bromeliads than young secondary forest, and primary forest had three times more bromeliads than old secondary (Appendix I, Fig S2A). Therefore, secondary forests need more than 35 years of regeneration to fully recover this key microhabitat. Conversely, there was no significant difference between forest types in tree fern biomass ($F = 1.96$, $p = 0.15$; Appendix I, Fig S2B; Table 1.3) or moss volume (Appendix I, Fig S2C; $F = 1.32$, $p = 0.27$; Table 1.3). Thus, even after a short period of time (4 to 18 years) of forest regeneration, tree ferns and moss volume were recovered.

Dead vegetation - There was significant variation between forest types in deadwood biomass ($F = 8.57$, $p < 0.001$; Table 1.3): young secondary forests (which did not differ significantly from old secondary forests) had over four-fold less deadwood biomass than primary forest (Appendix I, Fig S2D). Nevertheless, old secondary forest had accumulated almost half (44%) of the total deadwood volume found in primary forests just 35 years post-land abandonment (Appendix I, Fig S2D). Leaf litter biomass did not differ significantly between forest types ($F = 5.54$, $p < 0.05$; Appendix I, Fig S2E; Table 1.3), thus this critical microhabitat recovered even in the early stages of forest regrowth.

Carbon-microhabitat co-benefits

Live vegetation – Bromeliad abundance showed a positive relationship with carbon stock increase. They increased significantly with carbon stock at the 10th ($t = 4.79$, $p < 0.001$), 50th ($t = 3.78$, $p < 0.001$), and 90th ($t = 2.54$, $p = 0.01$) quantiles (Table 1.1; Fig. 1.2A). Tree fern biomass and moss volume showed no significant relationship with carbon stock at any of the quantiles (Table 1.1; Fig. 1.2C).

Dead vegetation - Deadwood volume showed a positive relationship with carbon stock increase. Deadwood volume increased significantly with carbon at the 10th ($t = 2.97$, $p = 0.003$), 50th ($t = 2.08$, $p = 0.04$), and 90th ($t = 4.4$, $p < 0.001$) quantiles (Fig. 1.2D). Conversely, there was no relationship between leaf litter biomass and carbon stock at any of the quantiles ($p > 0.19$; Table 1.1; Fig. 1.2E).

Microhabitat complexity and composition – Microhabitat complexity showed a positive increase with carbon at the 50th quantile (t = 2.82, p = 0.005), whereas the 10th and 90th quantiles showed no relationship (t > 0.80, p > 0.20; Table 1.1; Fig. 1.2F). Microhabitat composition derived from NMDS axis 1 showed a negative relation with carbon at the 10th (t = -4.23, p < 0.001), 50th (t = -4.36, p < 0.001), and 90th (t = -2.82, p = 0.006) quantiles (Table 1.1; Fig. 1.2G). Forest types, therefore, can be distinguished by their carbon stock accumulation. Conversely, there was no relationship between NMDS axis 2 and carbon stock (p > 0.19; Table 1.1; Fig. 1.2H).

Table 1.3. Microhabitat abundance, biomass and volume across forest type. Values shown are mean ± SE. Key to abbreviations: PF, primary forest; OSF, old secondary forest (19 - 35 yr); YSF, young secondary forest (4 - 18 yr).

Microhabitat	PF	OSF	YSF
Bromeliad abundance	100.87 ± 13.24	30 ± 20.71	10.16 ± 3.18
Tree fern biomass	29.13 ± 4.68	52.04 ± 14.94	25.36 ± 5.35
Moss volume	8.11 ± 1.54	2.9 ± 0.91	9.9 ± 2.48
Deadwood biomass	18.54 ± 3.76	8.18 ± 2.49	4.38 ± 1.07
Leaf litter biomass	3.41 ± 0.17	3.54 ± 0.61	3.5 ± 0.24

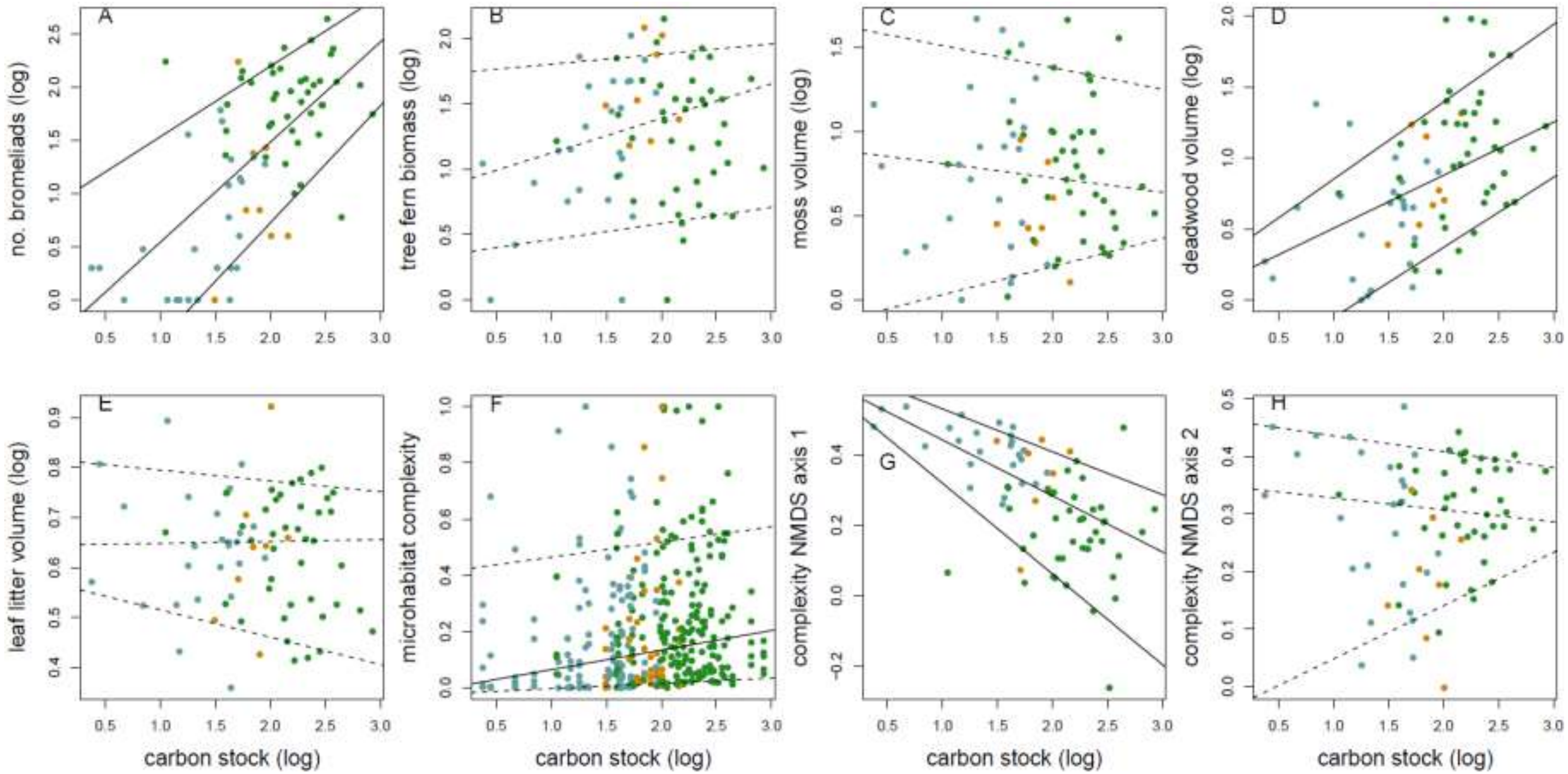
A
B
C
D
E
F
G
H
no. bromeliads (log)
tree fern biomass (log)
moss volume (log)
deadwood volume (log)
leaf litter volume (log)
microhabitat complexity
complexity NMDS axis 1
complexity NMDS axis 2
carbon stock (log)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0.4
0.6
0.8
0.9
-0.2

Fig. 1.2. Carbon and microhabitat co-benefits for bromeliads (A), tree ferns (B), moss (C), deadwood (D), leaf litter (E), microhabitat complexity (F), and NMDS axis 1 (G), and axis 2 (H). Quantile regressions of microhabitat abundance data between young secondary forest (<19 yr old ; blue points), old secondary forest (19-35 yr old; orange points), and primary forest (dark green points). Lines represent 10th (bottom line), 50th (middle line), and 90th (top line) quantiles. Solid lines represent significant relationships ($p<0.05$). Dash lines represent non-significant relationships.

Microclimates

The maximum ambient temperature in the understorey was lower than in the canopy in all forest types (Table 1.2; Fig. 1.3A and 1.3B). The daily maximum ambient temperature in the primary forest understorey was cooler than that in young secondary forests (z value = 2.98, $p = 0.007$; Fig. 1.3B2 and 1.3B4). However, the maximum ambient temperature in the understorey in old secondary forests was not significantly different from primary or young secondary forests ($p > 0.29$; Table 1.2).

Daily maximum temperatures within microhabitats were on average cooler than understorey ambient and canopy, whereas the daily minimum temperature within microhabitat was on average warmer than understorey and canopy ambient (except for bromeliads; Fig. 1.3C). Thus, these microhabitats are reducing exposure and serving as thermal buffers by keeping cooler temperatures when it is warm and warmer temperatures when it is cold. During the day, microhabitats thermally buffered the understorey ambient temperature maxima by 0.78°C to 2.27°C in primary forest, by 0.38°C to 2.15°C in old secondary forest, and by 1.67°C to 2.31°C in young secondary forest. At night, microhabitats buffered the understorey ambient temperature minima by 0.07°C to 0.23°C in primary forest and by 0.64°C to 0.97°C in old secondary forest. In young secondary forest, only leaf litter and tree holes were warmer than understorey ambient night-time temperature by 0.09°C and 0.54°C, respectively (Table 1.2; Fig. 1.3). In contrast, soil minimum and mean temperature was significantly lower in primary and old secondary forests compared to young secondary forests ($p < 0.04$; Table 1.2; Fig. 1.3G). All microhabitats warmed at a slower rate (<1°C microhabitat : 1°C ambient) than understorey ambient (Table 1.2; Fig. 1.3).

Table 1.1. Carbon-microhabitat co-benefits. Summary from quantile regressions at the 90%, 50%, and 10% quantiles from abundances of five microhabitats (Fig. 1.2A-H), microhabitat complexity as the sum of all normalized microhabitat data, and microhabitat complexity derived from NMDS axes scores (see Fig. 1.1), along a gradient of young secondary forest, old secondary forest, and primary forest in the Colombian Andes. Bold p values represent significant relationships (p<0.05). See text for detailed methods.

Microhabitat	quantile	t value	p value
Bromeliad	90%	2.54	**0.01**
	50%	3.78	**<0.001**
	10%	4.79	**<0.001**
Tree fern	90%	0.34	0.73
	50%	1.18	0.24
	10%	0.77	0.44
Moss	90%	-0.60	0.54
	50%	-0.46	0.64
	10%	1.02	0.30
Deadwood	90%	4.00	**<0.001**
	50%	2.08	**0.04**
	10%	2.97	**0.003**
Leaf litter	90%	-0.49	0.62
	50%	0.06	0.94
	10%	-1.29	0.19
Complexity	90%	0.80	0.42
	50%	2.82	**0.005**
	10%	1.27	0.20
Composition	90%	-4.23	**<0.001**
	50%	-4.36	**<0.001**
	10%	-2.82	**0.006**
Composition	90%	-0.94	0.34
	50%	-0.45	0.65
	10%	1.31	0.19

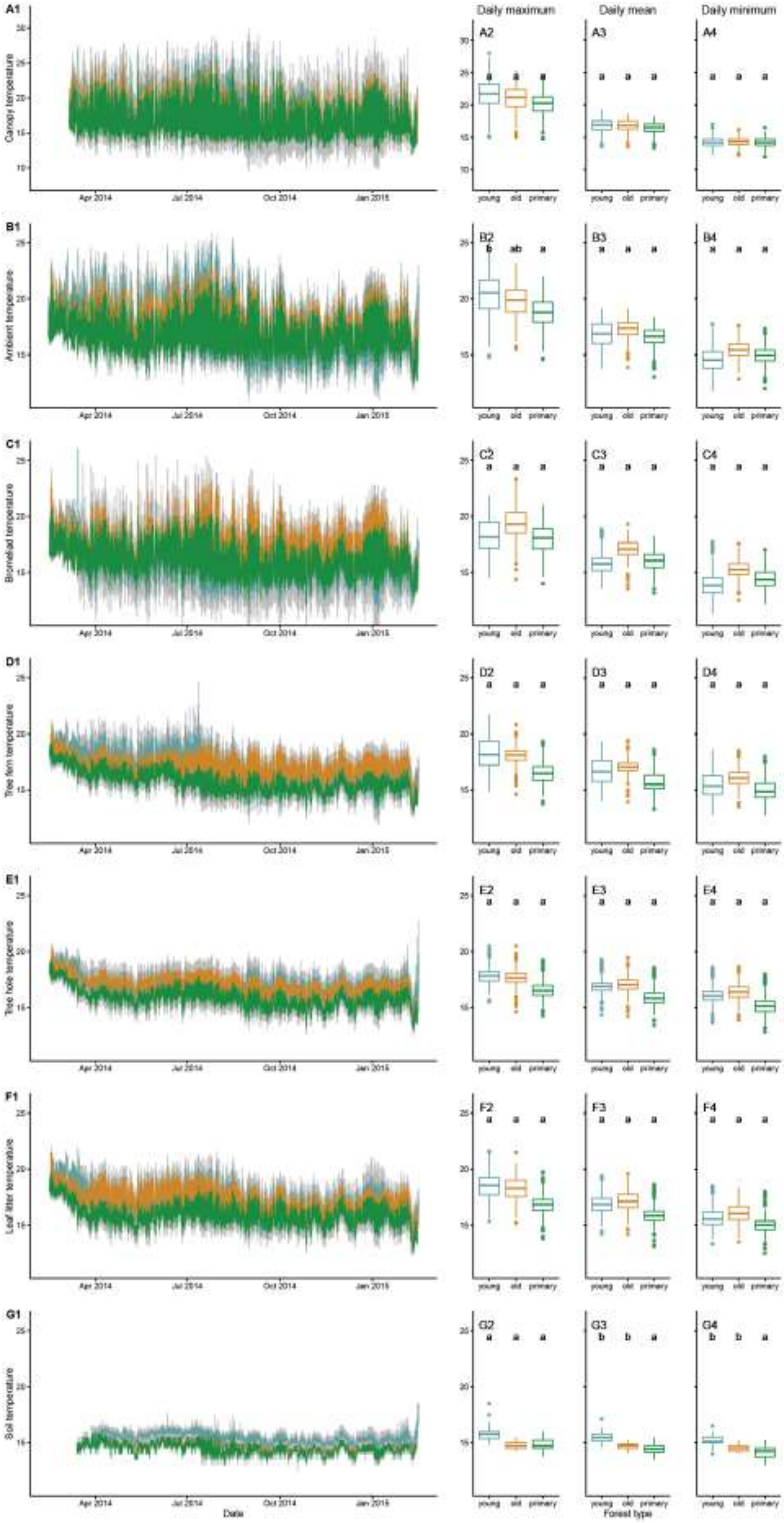
Daily maximum
Daily mean
Daily minimum
A1
Canopy temperature
A2
A3
A4
a a a
a a a
a a a
B1
Ambient temperature
B2
b ab a
B3
a a a
B4
a a a
C1
Bromeliad temperature
C2
a a a
C3
a a a
C4
a a a
D1
Tree fern temperature
D2
a a a
D3
a a a
D4
a a a
E1
Tree hole temperature
E2
a a a
E3
a a a
E4
a a a
F1
Leaf litter temperature
F2
a a a
F3
a a a
F4
a a a
G1
Soil temperature
G2
a a a
G3
b b a
G4
b b a
10
15
20
25
30
Apr 2014
Jul 2014
Oct 2014
Jan 2015
Date
young
old
primary
Forest type

Fig. 1.3. Annual temperature variation recorded every two hours (A1-G1), mean maximum temperature (A2-G2), mean average temperature (A3-G3), and mean minimum temperature (A4-G4) from Feb 2014 to Feb 2015 for canopy (A), understorey ambient (B), bromeliad (C), tree fern (D), tree hole (E), leaf litter (F) and soil (G) along a naturally regenerating young secondary forest (YSF, <19 yr; blue), old secondary forest (OSF, 19-35 yr; orange), and primary forest (PF; dark green) in the Colombian Andes. The bottom and top of the boxplots represent the first and third quartiles, respectively, the bold line represents the median, and the points represent outliers from all the studied time series. Similar superscripts represent no significant differences ($p<0.05$).

Table 1.2. Mean comparisons of daily maximum, daily mean, and daily minimum environmental temperature, and the rate of temperature increase for every 1^{o}C increase in understorey ambient temperature per forest type. Values shown are mean ± SE. Superscripts reveal pairwise differences at $p < 0.05$. Key to abbreviations: PF, primary forest; OSF, old secondary forest (19 - 35 yr); YSF, young secondary forest (4 - 18 yr). Bold p values represent that temperature is significantly driven by the factors included in the model. Table 1.2 continues on next page.

	Forest type	PF	OSF	YSF	L. ratio	P	df
Canopy	Max	20.19 ±0.09	21.06 ±0.01	21.71 ±0.12	21.72	**<0.001**	458
	Mean	16.46 ±0.04	16.82 ±0.04	16.86 ±0.05	51.39	**<0.001**	458
	Min	14.21 ±0.03	14.31 ±0.03	14.22 ±0.04	16.39	**<0.001**	458
	Rate	1.368	1.362	1.140			
Understorey ambient	Max	18.78 ±0.06^{B}	19.79 ±0.07^{AB}	20.18 ±0.09^{A}	57.85	**<0.001**	2054
	Mean	16.60 ±0.04	17.29 ±0.04	16.87 ±0.05	110.60	**<0.001**	2054
	Min	14.95 ±0.04	15.43 ±0.04	15.58 ±0.05	61.58	**<0.001**	2054
	Rate	-	-	-			
Bromeliad	Max	18.00 ±0.06	19.41 ±0.07	18.39 ±0.08	30.64	**<0.001**	1009
	Mean	16.02 ±0.04	17.07 ±0.04	15.85 ±0.05	36.61	**<0.001**	1009
	Min	14.45 ±0.04	15.25 ±0.04	13.94 ±0.05	6.18	0.102	1009
	Rate	0.927	0.901	0.754			

		PSF	OSF	YSF	L ratio	P	df
Tree fern	Max	16.51 ±0.04	18.04 ±0.03	18.23 ±0.06	33.36	**<0.001**	1350
	Mean	15.72 ±0.04	17.06 ±0.03	16.70 ±0.05	120.16	**<0.001**	1350
	Min	15.02 ±0.04	16.10 ±0.03	15.45 ±0.06	25.95	**<0.001**	1350
	Rate	0.463	0.473	0.546			
Tree hole	Max	16.54 ±0.03	17.64 ±0.03	17.87 ±0.03	12.07	**0.03**	1413
	Mean	15.87 ±0.04	17.05 ±0.03	16.92 ±0.03	175.43	**<0.001**	1413
	Min	15.18 ±0.04	16.40 ±0.03	16.12 ±0.04	46.44	**<0.001**	1413
	Rate	0.448	0.356	0.303			
Leaf litter	Max	16.85 ±0.04	18.24 ±0.05	18.51 ±0.05	15.09	0.01	1384
	Mean	15.91 ±0.04	17.12 ±0.04	16.89 ±0.04	87.48	**<0.001**	1384
	Min	15.05 ±0.04	16.07 ±0.04	15.67 ±0.04	20.86	**<0.001**	1384
	Rate	0.547	0.560	0.503			
Soil	Max	14.83 ±0.02	14.79 ±0.01	15.71 ±0.02	10.42	0.06	373
	Mean	14.45 ±0.02^{B}	14.70 ±0.01^{B}	15.45 ±0.02^{A}	9.25	**0.009**	1384
	Min	14.13 ±0.02^{B}	14.60 ±0.01^{B}	15.20 ±0.02^{A}	11.25	**0.04**	1384
	Rate	0.124	0.043	0.124			

Discussion

The regrowth of secondary forest on abandoned farmlands is prevalent across the tropics, and these forests are important for biodiversity and carbon stock recovery (Gilroy et al. 2014). Our study suggests that as abandoned lands regenerate as forests, their functional value also increases through the accumulation and diversification of critical microhabitats. Specifically, we document a chronological recovery of microhabitat complexity and a general trend toward niche diversification in older secondary forests. While we found that forest types have different microhabitat composition, secondary forest approached primary forest levels of microhabitat complexity over time. In turn, recovered microhabitats buffered against extreme low and high temperatures in a similar manner to those in primary forest. Moreover, the high correlation between carbon stocks and forest age suggests positive carbon-microhabitat co-benefits as forests regrow. Thus, investment to protect or enhance carbon stocks in secondary forests under carbon-based payments for ecosystem services (PES, e.g., REDD+) will also support an abundance of thermally buffered microhabitats.

Microhabitat composition

The extent to which secondary forests support biodiversity is strongly linked to the presence and abundance of critical microhabitats (Michel and Winter 2009). Our study indicates that microhabitat composition in primary forests differs from secondary forests, and that these differences tend to be most pronounced between young secondary and primary forests. This underlines the need to protect primary forests, in order to avoid loss of specialist species that are strongly dependent on certain microhabitats. Moreover, young and old secondary forests did display some similarities in microhabitat composition, suggesting that even from early stages of forest development some microhabitats are already available for biodiversity (Cadavid et al. 2005, Urbina-Cardona et al. 2006).

Microhabitat complexity recovery across habitat types

We found a highly variable, yet predictable, recovery of microhabitats across forest types (Bittner and Breckle 1995, Oldekop et al. 2012, Woziwoda et al. 2014). Nevertheless, microhabitat complexity in our study recovered chronologically from young to old secondary forests and this increase in ecological complexity has documented benefits for vertebrates and invertebrate diversity and abundance even in young secondary forests (Lassauce et al. 2011, Jocque and Field 2014, Scheffers et al. 2014b, Bluhm et al. 2015). As such, our study provides strong support for allocating secondary growth forests as an important conservation tool for recovering biodiversity and reversing extinction risk (Chazdon 2014, Queiroz et al. 2014).

There is a clear transition from young secondary forests rich in moss and leaf litter to primary forests that also contain more than ten times the number of bromeliads and more than four times the deadwood biomass than young secondary forests. Importantly, bromeliad abundance and deadwood biomass levels in secondary forest reach almost half of those found in primary forest within 35 years following land abandonment (Appendix I, Fig. S2A and S2D). The high elevation of our study sites likely supports a faster recovery rate of bromeliads than lower altitude forests. For example, we found similar recovery rates to those documented in the Venezuelan Andes (Barthlott et al. 2001), whereas Australian tropical rainforests (<900 m a.s.l.) showed a slower rate of recovery of epiphyte richness (Shoo et al. 2016), lowland rainforests in Panama (140 m a.s.l.) have not shown any epiphytic recovery after 35 years of forest growth (Woods and DeWalt 2013), and a pan-tropical meta-analysis showed very slow recovery of epiphytic richness, especially of species that are found in undisturbed forest (Martin et al. 2013). Such a dichotomy probably reflects bromeliad requirements for cooler, wetter climates like the Andes in comparison with more seasonal and hotter lowlands (Ruiz et al. 2012). As amplifiers of biodiversity, bromeliad recovery in secondary forests may offer additional niche space. Bromeliad abundance and deadwood volume are strongly linked to forest age (Barthlott et al. 2001, Woods and DeWalt 2013, McGarvey et al. 2015), and translocation of bromeliads into medium-aged secondary forests may be a useful tool in applied forest management (Donnelly 1989).

Linking forest carbon and microhabitat recovery

Climate change scenarios predict increasing temperatures and variable rainfall in the Tropical Andes, which may hinder forest and microhabitat recovery (IPCC 2014). Carbon-based payments for ecosystem services (PES) initiatives seek to maximize carbon storage and sequestration as part of a global scheme for climate change mediation, but may also offer important co-benefits for localized biodiversity conservation (Strassburg et al. 2010, Phelps et al. 2012, Gilroy et al. 2014). Our study showed a strong positive relationship between carbon stock and microhabitat complexity, which underpins ecological resilience and increases the benefits to biodiversity (CBD 2011). Recovery potential appears to be high in secondary forests across the Neotropics (from Mexico to Chile), with considerable capacity for carbon sequestration and storage (Poorter et al. 2016). Although, secondary forests have lower carbon stocks and biodiversity than primary forests (Poorter et al. 2016), recovery of these stocks may now be linked to microhabitat complexity recovery. This also underscores that the Tropical Andes, which is a global hotspot of endemism and extinction risk, are of great interest for promoting carbon enhancements via natural forest regeneration – particularly given that it would be cheap to do so in this region (see (Gilroy et al. 2014)), and that such projects would offer strong carbon, microhabitat, and biodiversity benefits.

Are secondary forests climate change ready?

Microhabitats in secondary forest showed temperature-buffering abilities similar in magnitude to those found in primary forests, suggesting that all forest types provide species with climate microrefugia. This is particularly important in young secondary forests since their daily maximum temperatures are higher than in primary forests. The low rate of temperature increase in microhabitats also shows that species are thermally protected for longer periods of time under extreme temperature increases than would otherwise be the case in understorey ambient temperatures (see also (Scheffers et al. 2014a) for primary forests). Our findings suggest that microhabitats will become an increasingly important resource to help ectotherm communities mitigate the negative impacts of climate change (Huey and Tewksbury 2009), especially in the Tropical Andes where extreme weather events have become more intense and frequent and overall temperatures have increased (Ruiz et al. 2012).

Of concern, much tropical montane vegetation depends on specific environmental characteristics to thrive and is highly sensitive to climate change (Morueta-Holme et al. 2015). Climate change could therefore negatively affect the abundance of critical living microhabitats, and perhaps more so in recovering secondary forests. For instance, mosses and bromeliads need constant moisture to grow (Nadkarni 2000, Merrifield and Royce 2002), such that a drastic decline in moisture could reduce their abundance. This could in turn, threaten many bird, amphibian and invertebrate species (Merrifield and Royce 2002, Panizzo 2011, Scheffers et al. 2014b, Silva and Piratelli 2014).

Survey limitations

Our study sites are restricted to naturally regenerating secondary forests in an agricultural matrix dominated by pasture. It is therefore difficult to extrapolate our results to other agricultural systems, as different taxa may respond differently to particular crops (Edwards et al. 2014, Jordani et al. 2015). Our results are also restricted to secondary forests near primary forest, whether small isolated fragments of secondary forests that can have lower buffering potential would show the same results is unknown (Ewers and Banks-Leite 2013). Moreover, whether similar recovery rates would occur in isolated secondary forests and whether microhabitat recovery would be so rapid in other ecosystems outside of the Tropical Andes, especially in hotter and drier lowlands, are both critical unanswered questions.

Conclusions

Old secondary forests have high carbon storage potential, and our results suggest that the abundance of critical microhabitats in secondary forests is recovering, sometimes to levels found in a primary forest. A higher abundance of microhabitats increases landscape resilience by returning structure to forests and as such enhances ecological integrity (Ruiz-Jaen and Aide 2004). This provides habitat for a range of local species, including many at risk of extinction. While protecting primary forests remains a critical conservation goal (Gibson et al. 2011), we live in an era of increasingly human-dominated landscapes (Lewis et al. 2015). While we acknowledge the importance of primary forests, the protection or promotion of secondary forest regrowth is often a more economically feasible strategy of protecting microhabitat rich and climate change resilient forests (Fisher et al. 2011). The critical role of secondary forests should not be overlooked as we seek solutions to the biodiversity crisis, both now and under future climate change.

Chapter III

Phylogeny and morphology constrain thermal tolerances in tropical amphibians

Abstract

Global warming is a great threat to biodiversity with negative impacts spanning the entire biological hierarchy. One of the main species' traits determining survival at higher temperature is the thermal point at which an animal loses its ability to escape from deadly conditions (critical thermal maximum – CT_{max}). Variation in CT_{max} across species is the outcome of environmental and evolutionary factors, but studies do not typically measure the degree to which environment or phylogeny influences the variation in trait values. Here, we aim to elucidate whether local environmental variables or phylogeny influence CT_{max} in highly climate change-threatened amphibians in the Tropical Andes. We measured CT_{max} from 12 *Pristimantis* frog species encountered in primary and secondary forests and cattle pasture. We recorded their habitat, elevation and the range of environmental temperatures they experienced over a year. Using phylogenetic analyses, we demonstrate that physiological thermal tolerance is strongly related to phylogeny, positively related to body length, but not affected by environmental factors. We suggest that phylogeny and morphology interact to determine vulnerability to global warming.

Introduction

The rate of global warming is accelerating and is severely threatening biodiversity globally (Maclean and Wilson 2011, Freeman and Class Freeman 2014), with observed impacts of climate change now spanning the entire hierarchy of biological organization (Scheffers et al. 2016). The impacts of global warming on organisms will depend, among other factors, on their level of exposure combined with their physiological sensitivity and tolerance to high temperatures (Deutsch et al. 2008, Freckleton and Jetz 2009, Scheffers et al. 2014a, Sunday et al. 2014, Garcia-Robledo et al. 2016). Consequently, species' critical thermal maximum (CT_{max}), the thermal point at which an animal loses its ability to escape from deadly conditions (Cowles and Bogert 1944), is a vital trait in understanding thermal stress and vulnerability to increasing temperatures (Kearney et al. 2009, Huey et al. 2012).

Variation in species' traits, including CT_{max}, is a function of both environmental exposure and the evolutionary history of species (Freckleton and Jetz 2009, Leal and Gunderson 2012, Gutiérrez-Pesquera et al. 2016). In vertebrate ectotherms, for example, CT_{max} has been considered a stable evolutionary trait (Araujo et al. 2013). Yet, there are also strong environmental effects, where acclimation to extraordinarily hot and/or cold temperatures can move the thermal ceiling up and down, respectively (Phillips et al. 2015, Llewelyn et al. 2016a). However, in this nature versus nurture scenario, it is generally unknown whether environment or phylogeny is more important in determining trait variation across species (Peres-Neto 2006, Freckleton and Jetz 2009).

A key question is whether upper thermal tolerances can adjust through plastic and/or evolutionary responses, which would aid in predicting species' potential responses to global warming (Hoffmann et al. 2003, Williams et al. 2008, Kellermann et al. 2009, Wiens et al. 2010). Phylogenetic comparative analyses may provide the best method to answer this question (Cooper et al. 2010). While several methods exist to determine the evolutionary model of traits, models can produce different results due to different methodologies and underlying assumptions (Cooper et al. 2010). Therefore, analyses on trait variation must have an explicit framework and a clear underlying evolutionary model (Cooper et al. 2010).

Here, we used a phylogenetic inertia model to assess trait evolution. Phylogenetic inertia occurs when the rate of evolution of a trait is too slow to match the rate of change of an external driver (e.g. environmental change) (Hansen 1997, Labra et al. 2009). A strong phylogenetic signal might suggest that CT_{max} is conserved across the phylogeny, thus making local adaptation to environmental factors challenging because changes in CT_{max} would have to go through a long evolutionary process. Alternatively, a weak phylogenetic signal could suggest strong phenotypic plasticity in CT_{max} to changing environmental conditions. Here, we aim to incorporate phylogenetic information into comparative analyses to reveal correlations between CT_{max} and environmental variables (Freckleton and Jetz 2009, Kellermann et al. 2012a).

We chose amphibians as our study organism because they are highly threatened by habitat loss, infectious disease and climate change (IUCN 2015). Since impacts of habitat loss and degradation on local temperature (González del Pliego et al. 2016) are likely to synergize with climate change, we chose a disturbance-climate gradient, including secondary forests. These are likely to become the most widespread habitat type in the future (Hurtt et al. 2011), and each of these habitats will exert different levels of thermal stress, which may define the capacity to evolve upper thermal limits (Blackburn et al. 2014). Information on phylogenetic constraints in thermal limits will help further assess this threatened taxon.

The spatial scale (resolution) of environmental variables used can result in some variables not representing accurately the actual conditions experienced by species and/or a geographical or environmental bias, all of which can ultimately decrease the phylogenetic signal of CT_{max} (Menke et al. 2009, Cooper et al. 2011). In broad-scale studies, environmental variables that might influence a species' phenotype tend to be averaged across the species' broad geographic range. By contrast, phenotypic plasticity occurs within populations at local spatial scales (Llewelyn et al. 2016b). Thus studies that test for phenotypic responses across large spatial extents must overcome a scaling issue whereby environmental variables (e.g., averaged maximum temperature within a grid cell) occur at different scales than that at which selection for phenotypes occurs. This is one of few studies to use a small-scale approach to ask whether local environmental or phylogenetic factors shape the evolution and distribution of local physiology. We hypothesize that: 1) local *environmental* variables will influence CT_{max} variation, but to a lesser extent than phylogeny because tropical species have small geographical ranges, occupy a narrow range of climatic conditions and experience less environmental variability (Cooper et al. 2011); 2) *phylogeny* will be strongly related to upper thermal limits, since they are a stable evolutionary trait in ectothermic vertebrates (Araujo et al. 2013); and 3) *morphologically* larger species will show higher CT_{max} as body size has been shown to influence species' CT_{max} (Ribeiro et al. 2012). We tested these hypotheses in *Pristimantis* frogs in the Tropical Andes, which is a global hotspot of extinction risk and species endemism.

Methods

Study site

Our study area is located on the western slope of the Western cordillera of the Colombian Andes, in the Reserva Mesenia-Paramillo, Antioquia (N: 05° 29' 45.8'' to 05° 12' 54.3'', W: 75° 53' 20.8''). The study area covered an altitudinal range of 2,150 m - 2,690 m above sea level and it encompassed primary forest, naturally regenerating secondary forests and cattle pasture. This region supports one of the highest global diversities of threatened and endemic amphibians (Myers et al. 2000, Orme et al. 2005).

Study Organisms

Between July and August 2014 we collected frogs from 12 species of the genus *Pristimantis* (Craugastoridae, Anura). These small frogs (range 10 – 37 mm) have the highest abundance in our study area (Acosta-Galvis and Cuentas 2016). *Pristimantis* is the most species-rich genus of vertebrates, with over 500 species (AmphibiaWeb 2016, Frost 2016), hence, our results may be extended to this wide range of frog species. We excluded obvious juveniles by collecting individuals with a length cloaca-rostrum (LCR) ≥10 mm (Basham et al. 2016). Frogs were sampled in 25 m x 10 m transects, each separated by at least 200-300 m, in primary forest (24 transects; 130 individuals), old secondary forest (7 transects; 38 individuals), young secondary forest (16 transects; 35 individuals), and cattle pasture (15 transects; 19 individuals; Appendix II, Table S1).

Trait variation - Critical thermal maximum (CT_{max})

We used the loss of righting response (LRR) as an indicator of CT_{max} (Navas et al. 2007, Catenazzi et al. 2014). In total, 222 frogs were acclimated at similar temperature for 3 to 5 days before experiments (Catenazzi et al. 2014). Experiments were performed at 2200 m a.s.l. We placed the frogs in a transparent plastic cup with a thin layer of water (1.5 ml) to prevent desiccation (Navas et al. 2007), and cups were positioned in a water bath. The temperature of the water started at 17°C (Catenazzi et al. 2014) and increased by 0.45°C per minute on average.

We used the probe of a two-channel digital thermometer (accuracy: ±0.1°C) to gently but often turn the frogs over on their backs. We incited individuals to righten by touching their flanks and inner thighs. After 5 s, if the frog was incapable of rightening, we registered its temperature by touching one flank with the probe (K-type) (Navas et al. 2007, Catenazzi et al. 2014). We considered this temperature as the core body temperature, since small-sized frogs have high heat transfer (Catenazzi et al. 2014) and their body temperature is only slightly above environmental temperature (Vitt and Caldwell 2009). The frogs were immediately placed in a cool container (Navas et al. 2007), and observed for 24 hrs to verify they were healthy, and ready to be released (there were no mortalities in our study, and all individuals recovered fully). Experimental protocols received ANLA (National Agency for Environmental Licences) approval (#1579). In addition, we recorded individuals’ body length by measuring the length from cloaca to the rostrum.

Measuring environmental exposure

We used the following environmental variables that may be related to CT_{max} variation:

Habitat type and elevation

Habitat type and elevation can be important factors influencing variation in CT_{max} (Simon et al. 2015, Garcia-Robledo et al. 2016). We categorized habitat type into

4 categories: primary forest (pristine forest), old secondary forest (≥19 years old), young secondary forest (<19 years old), and cattle pasture (forest ages were acquired from residents that have known the area for > 30 years). As well as recording the habitat type per individual, we used a GPS to record the elevation where each individual was found.

Temperature

To determine if temperature is shaping the evolution of CT_{max}, we monitored individuals' temperature exposure. Temperature variables, such as maximum temperature and climatic niche breadth (temperature conditions where species are living), are linked to thermal limits, and are important factors determining the impacts of climate warming on ectotherms (Deutsch et al. 2008, Duarte et al. 2012, Kellermann et al. 2012a). For example, in ectotherms CT_{max} has been related to thermal variability (Clusella-Trullas et al. 2011). We recorded temperature exposure from March 2014 to February 2015 using iButtons temperature loggers (model: DS1921G-F5; accuracy: 0.5°C), by capturing understorey ambient temperature, which is buffered by above-ground vegetation. The iButtons were placed approximately 1 m above the ground (Scheffers et al. 2013a), recording the temperature ectotherms would experience at understorey level. We placed the iButtons in 18 transects spanning all the different habitats across the range of elevations (6 in primary forest, 4 in old secondary, 6 in young secondary forest, and 2 in cattle pasture). We then calculated two temperature variables for each individual: 1) Annual mean maximum temperature, which is the mean daily maximum temperatures recorded; and 2) Climatic niche breadth, which was calculated by subtracting the mean daily minimum temperature from the mean daily maximum temperature. We used the temperature data from the closest temperature logger to where each individual was found accounting for habitat type and elevation (mean difference = 431 m +/- 34 SE, range = 0 to 1800 m).

Phylogeny

We built a phylogeny for 12 species of *Pristimantis* frogs. Sequences of nucleotides were retrieved from GeneBank (Benson et al. 2013) when available for the nuclear markers Tyrosinase (TYR) and Recombination-activating genes (RAG1), also for the mitochondrial marker 16S rRNA gene (16S). All these genes have been used successfully for amphibian phylogenies (van der Meijden et al. 2007, Pyron and Wiens 2011). For one species (*Pristimantis baiotis*) no sequences were available in GenBank, hence we used the sequences from a sister-taxon (from (Lynch 1998)) to estimate its position in the phylogeny. The number of genes available for each species is shown in Table 2.1.

For each gene, sequences were aligned using MUSCLE (Edgar 2004) and concatenated. The resulting alignment was trimmed to eliminate poorly aligning positions or lacking of data for more than 50% of the species using Gblocks v.0.91 (parameters: -t=c -b2=b1 -b5=h; (Castresana 2000)). This was used to construct a maximum likelihood phylogenetic tree using PhyML (Guindon and Gascuel 2003) with the GTR + G + I nucleotide substitution evolution model and 100 bootstrap pseudoreplicates. The concatenated alignment length had 1,503 base pairs (bp), and we had a very high overall support value (mean bootstrap values across all branches) of 86% for our phylogeny (Appendix II, Fig. S1). We divided the species into three groups and assigned them different colours for ease of interpretation (black, red and blue groups). However, colour does not represent a monophyletic group, specifically for the blue group.

Table 2.1. Genes used to construct the phylogenetic relationship between each *Pristimantis* species. Tyrosinase (TYR), recombination-activating gene 1 (RAG1), and the large subunit of the mitochondrial ribosome gene (16S).

	TYR	**RAG-1**	**16S**	**Genes per species**
P. achatinus	x	x		2
P. baiotis	x	x	x	3
P. brevifrons	x		x	2
P. calcaratus	x	x	x	3
P. erythropleura	x	x	x	3
P. juanchoi	x	x		2
P. kelephus	x	x	x	3
P. myops	x	x	x	3
P. orpacobates	x	x		2
P. ptochus	x			1
P. quantus	x	x	x	3
P. zophus	x	x	x	3
Species per gene	12	10	8	

Statistical analyses

Environment or phylogeny shapes local CT_{max}?

We tested for phylogenetic inertia to understand whether the environment will change more quickly than species' thermal tolerances are able to evolve. We use a phylogenetic signal (V) to measure this trait evolution, as it represents the tendency of related species to resemble each other more than at random (Blomberg and Garland 2002). We tested the relationships between CT_{max} and habitat, elevation, temperature variables, and body size by fitting a linear mixed effect model that allows phylogenetic covariance matrices. We used the function lmekin in the R package coxme (Therneau 2015) in R (version 3.3.1(R 2016)). This model produces a z-value that represents the contribution of each environmental factor and of body size. Our model included residual variance associated with both the individual measurement (e.g. species-specific variation in CT_{max}) and with phylogeny. We specified the variance-covariance structure of the residual variance as a vector of errors (e) (following Freckleton and Rees *in press*). We estimated the errors assuming they follow a multivariate normal distribution with a variance-covariance matrix with three components**.** The first component is the phylogenetic signal, representing the variance among species means that results from phylogenetic dependence (V). This component shows the covariance between each pair of tips, calculated using the branch lengths of the phylogeny. Thus, it represents the phylogenetic contribution. The second describes variation in the species means that is independent of phylogeny (S). The final variance is that between replicate experimental units independent of phylogeny or species identity, i.e. the error variance (I) (Freckleton and Rees *in press*).

We also calculated Pagel's lambda (λ) for ease of interpretation of a phylogenetic signal for both CT_{max} and body length using phylosig function from the R package phytools (Revell 2012). Values of Pagel's lambda range from 0 (phylogenetic independence) and 1 (strong phylogenetic signal, species' traits covary in direct proportion to their shared evolutionary history).

Results

Critical thermal maximum (CT_{max})

The mean CT_{max} (±1SE) across all species was 22.73±0.33. Species closer together in the phylogeny did not show a strong pattern of CT_{max} similarity (Fig. 2.1A). The black phylogenetic group showed the highest level of deviation from the overall mean, encompassing both the highest and lowest CT_{max} values (Fig. 2.1A; black symbols). However, mean values of CT_{max} were similar across the different phylogenetic groups, with a mean CT_{max} for the black group of 22.77±0.99SE; red group of 22.72±0.22SE; and blue group of 22.67±0.39SE. The red group showed the lowest deviation from the overall CT_{max} mean (Fig. 2.1A), supported by the lowest standard error across all groups.

Environmental exposure

Our sampling encompassed different habitats (pasture, young and old secondary and primary forest), elevational ranges (2,150 m - 2,690 m) and temperature gradients. Maximum temperatures spanned from 15.30^{o}C to 24.95^{o}C, and temperature decreased 1.5^{o}C for every 100 m increase in elevation, in part due to the confounding effect of habitat type: The lowest elevation belonged to cattle pasture, whereas the highest elevations were recorded in primary forests. The lowest temperature recorded was in primary forests, with a mean annual maximum temperature of 15.89^{o}C, followed by old secondary forest = 17.12^{o}C, young secondary forest = 21^{o}C, and the highest mean annual maximum temperature recorded was in cattle pasture = 24.87^{o}C. Since cattle pasture showed more extreme temperatures, the highest values of climatic niche breadth also belonged to species that are present in cattle pasture.

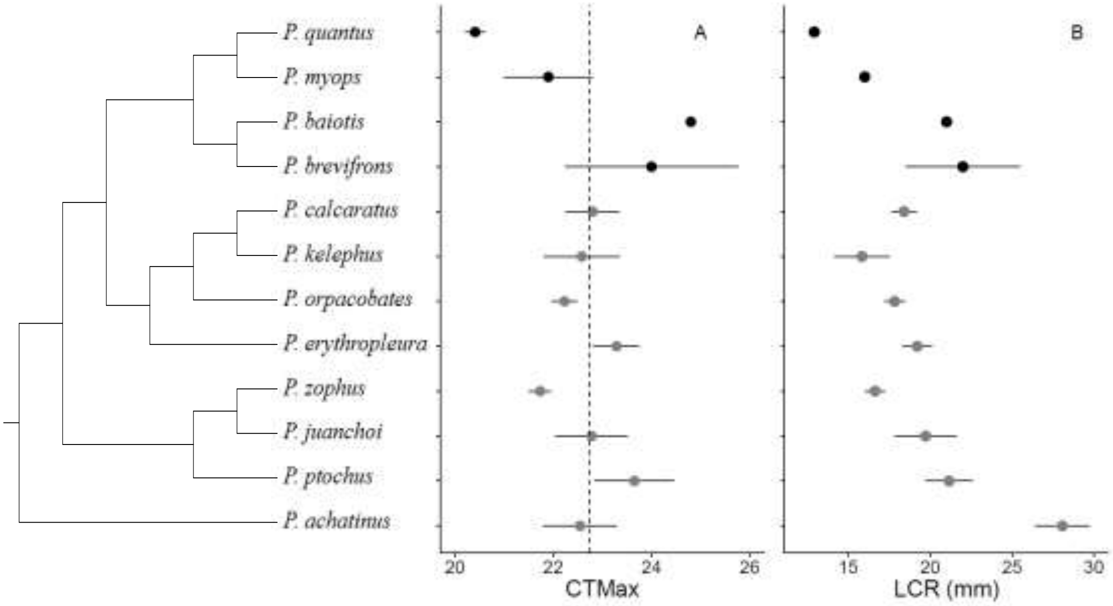

Figure 2.1. *Pristimantis* frog phylogeny separated by colours into three groups. (A) Mean CT_{max} ± SE per species. Dash line represents mean CT_{max} across all species. (B) Mean LCR ± SE per species. Colours represent species´ position in the phylogeny.

Environment or phylogeny shapes local CTmax?

We used the CT_{max} of 222 captured frog individuals in our model to test for phylogenetic inertia. Habitat type, elevation, maximum temperatures and climatic niche breadth showed similar patterns across the phylogeny (Appendix II, Fig. S2).

Hypothesis 1) local environmental variables will influence CT_{max} variation, but to a lesser extent than phylogeny: We found no evidence suggesting that local environmental variables were influencing CT_{max} variation. There were no differences in CT_{max} across habitat types ($z = 0.01$, $p = 0.78$; Fig. 2.2A), elevation ($z = 1.06$, $p = 0.29$; Fig. 2.2B), maximum temperature ($z = 0.17$, $p = 0.87$; Fig. 2.2C) or climatic niche breadth ($z = 0.06$, $p = 0.95$; Fig. 2.2D) accounting for phylogeny. We did not find any differences in CT_{max} even when including the three different clades as a factor ($z = 0.47$, $p = 0.64$).

Hypothesis 2) phylogeny will be strongly related to upper thermal limits: Phylogeny was strongly related to species' upper thermal limits. We found a strong phylogenetic signal ($V = 0.38$; Table 2.2; $\lambda = 0.80$), and the second and third error components had low variances ($S = 0.03$; $I = 0.04$; Table 2.2), indicating a strong model since the error variance and the variance among species were very low.

Hypothesis 3) larger species will show higher CT_{max}: Body length (LCR) was positively related to individuals' CT_{max} ($F = 49.47_{1,220}$, $r^2 = 0.18$, $p < 0.001$; Fig. 2.2E) and showed a strong phylogenetic signal ($\lambda = 0.88$). The removal of body length from the mixed-effect model resulted in an increase of the phylogenetical signal ($V = 0.69$), with the second error decreasing ($S = 0.01$) and the third error increasing slightly ($I = 0.14$).

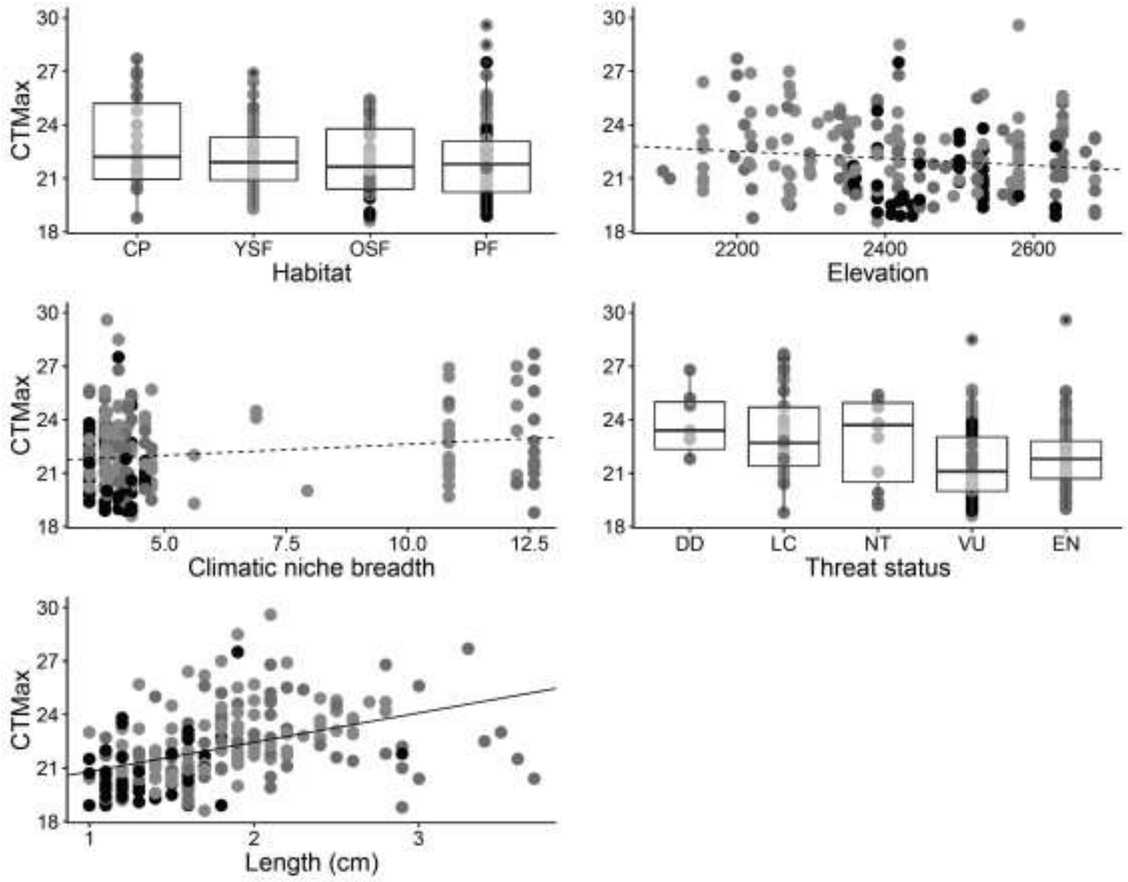

Figure 2.2. Relationship between *Pristimantis* frogs' CT$_{max}$ and (A) habitat (CP: cattle pasture; YSF: Young secondary forests; OSF: Old secondary forests; PF: Primary forests), (B) elevation, (C) maximum temperature, (D) climatic niche breadth, and (E) body size (length cloaca-rostrum). Colour of the points represents their position in the phylogeny (see Fig. 2.1). Solid lines represent significant relationships ($p < 0.05$).

Table 2.2. Summary of linear mixed effect model of environmental and phylogenetic factors on CT_{max} variation of 222 frog individuals.

		value (se)	z	p
Fixed	Cattle pasture (Intercept)	20.43 (4.45)	4.59	<0.001
	Young secondary forest	0.19 (0.76)	0.25	0.80
	Old secondary forest	0.62 (1.04)	0.60	0.55
	Primary forest	0.78 (1.08)	0.73	0.47
	Elevation	-0.0007 (0.001)	-0.44	0.66
	Max temperature	0.019 (0.11)	0.17	0.87
	Climatic niche breadth	0.045 (0.11)	0.39	0.70
	LCR (body size)	1.39 (0.27)	5.08	**<0.001**
Random			Std Dev	Variance
	Phylogenetic signal (V)		0.62	**0.38**
	Variation in species means (S)		0.19	0.037
	Error variance (I)		0.22	0.04

Discussion

In this article, we studied evolutionary patterns of physiology, which help us to understand potential responses of amphibians to the dangers posed by climate change. This study uniquely uses a small spatial-scale approach, which guarantees that the environmental variables measured represent the real environment experienced by species, to assess local physiology combined with phylogenetic and local environmental factors. We found that local CT_{max} was not driven by any environmental factor assessed, whereas frog body length was positively related to CT_{max}. Moreover, we found a strong phylogenetic signal in species' CT_{max} suggesting evolutionary constraints on this physiological trait. Thus, there is minimal potential for this group to locally adapt to keep pace with changes in temperature because such changes in CT_{max} would require evolutionary processes.

Local environmental variables did not influence CTmax variation

Our results did not support our first *environmental selection hypothesis* which is in accordance with other studies showing that environmental factors, such as temperature and elevation, have minimum influence on the local thermal tolerance of lizards (Leal and Gunderson 2012); Munoz et al 2016), beetles (Garcia-Robledo et al. 2016), and *Drosophila* in wet environments (Kellermann et al. 2012a). This suggests that species are living in environments to which they are preadapted, rather than adapting their thermal tolerance to a new environment (Kellermann et al. 2012a). Thus, species might have limited potential for local adaptation or may not be able to use physiological plasticity to keep pace with temperature increases predicted under climate change (Angilletta 2009, Sinervo et al. 2010, van Heerwaarden et al. 2016), particularly in the tropics (Tewksbury et al. 2008, Huey et al. 2009). However, other factors such as humidity and microhabitat abundance could be having an effect on species distribution. For example, species living in three different habitat types, such as *P. zophus* and *P. orpacobates*, can live across a broader range of temperatures and forest characteristics, considering that young secondary forests have higher mean annual maximum temperatures and lower microhabitat complexity than primary forests (González del Pliego et al. 2016).

The lack of correlation between CT_{max} and environmental variables at this regional level could be partially explained by the tendency of phylogenetically related amphibian species to live in similar combinations of climatic variables (Hof et al. 2010). This is achieved due to the strong spatial autocorrelation existing in environmental variables and species' low dispersal ability that creates spatial proximity of these closely related species (Freckleton and Jetz 2009). Moreover, other factors could play a large role in driving species' CT_{max}. For example, Kellerman *et al.* (2012) found that in *Drosophila* CT_{max} increased as precipitation decreased. Thus, water-related environmental factors might be more important in driving CT_{max} than high temperatures alone, especially in very wet environments (Kellermann et al. 2012a), such as the Tropical Andes. Nevertheless, an increase in temperature will not be the only effect of climate warming: lower humidity and reduced cloud cover could also stress ectotherms, especially water-dependant tropical amphibians (Pounds et al. 1999).

Phylogeny is strongly related to CTmax

Confirming our second *phylogeny hypothesis*, we observed a strong correlation between phylogeny and local CT_{max}, previously reported for other ectotherms (Grigg and Buckley 2013), and suspect that with a larger number of species analysed we would have uncovered an even stronger phylogenetic signal (Kellermann et al. 2012a). Our results concur with several studies showing that CT_{max} is a stable evolutionary trait in ectothermic vertebrates rather than being determined by ecological filtering, dispersal, or local adaptation (Labra et al. 2009, Bonino et al. 2011, Araujo et al. 2013, Grigg and Buckley 2013, Muñoz et al. 2014, Muñoz et al. 2016). This suggests that the evolution of CT_{max}, even at a local spatial scale, follows a model of phylogenetic inertia, rather than a model free from phylogenetic associations. Moreover, evolutionary responses are slow, hence species might not be able to evolve higher CT_{max} rapidly enough to meet the pace of environmental warming (Grigg and Buckley 2013, Muñoz et al. 2016).

The phylogenetically conserved thermal tolerance that we found (strong phylogenetic signal, $\lambda = 0.80$) suggest that these species are unlikely to adapt locally. This could be explained by the evolution of local CT_{max} being constrained by hard physiological boundaries (Araujo et al. 2013). Therefore, evolution is likely to complement, rather than replace, projected ecological changes (Parmesan 2006) and local evolutionary responses are unlikely to mitigate the negative impacts of future global warming, as has been suggested for marine environments (Hoegh-Guldberg et al. 2002, Donner et al. 2005). This will be aggravated by the fact that many montane species living in these areas of high phylogenetic and species diversity may be particularly susceptible to rapid anthropogenic climate change (Kozak and Wiens 2010).

Body length is positively related to CTmax

Supporting our third *morphology hypothesis*, we found that body size was positively correlated with CT_{max}, as has been recorded in other ectotherms (Ribeiro et al. 2012). Individual body length had a stronger effect than environmental factors and when added into the model the phylogenetic signal decreased. This means that body size length had a stronger influence on species' CT_{max} than any of the four environmental variables considered, and that there is an autocorrelation between body length and phylogeny, which has been previously shown in Freckleton and Jetz (2009). Moreover, the similar pattern that species' CT_{max} and body size showed (Fig. 2.1) suggests that lability in CT_{max} could be mediated by body size.

In ectotherms, many ecological and physiological processes (e.g. fecundity) are directly related to body temperature, and thus body size (Peters 1983, Brown 1995, Hone and Benton 2005). Achieving optimal body temperature through behavioural thermoregulation can play a key role in avoiding heat stress (Kellermann et al. 2012a) and limit adaptation for elevated upper physiological limits (Huey et al. 2003, Angilletta 2009). Indeed, thermoregulation could explain the similar CT_{max} we found across environmental variables.

The positive relationship between CT_{max} and body size could also bring further implications. There is cumulative evidence suggesting that reductions in body size is a major response to climate change (Gardner et al. 2011, Caruso et al. 2014), but there is also evidence suggesting that animal lineages have evolved toward larger sizes over time (Cope's rule) (Rensch 1948, Heim et al. 2015). A reduction in body size would decrease species' CT_{max}, whereas evolving larger body sizes would increase their maximum thermal tolerance. Our study reinforces that evolving larger body sizes would be favoured under global warming scenarios, as higher CT_{max} would aid species to counter climate change.

Conclusions

Here we present a study that simultaneously compares physiology and local environmental conditions in an evolutionary framework. Our study captured high variation in CT_{max}, because we focused on sampling across different habitats, elevational ranges and temperature gradients. The fact that we found a strong phylogenetic signal, despite our local scale approach, suggests that phylogenetic factors are indeed involved in shaping species' thermal tolerances. We conclude that species are unlikely to increase their upper thermal limits via plastic responses and evolutionary responses may not be fast enough to keep pace with global warming. Working with the most speciose genus of frogs globally could allow us to infer that species from this genus will show similar vulnerability to global warming, as the species from this study inhabit a wide range of habitat types that are increasingly found across the world. They also encompass a large altitudinal range from 0 m a.s.l. (for *P. achatinus*) to 3200 m a.s.l. (for *P. brevifrons*) (Acosta-Galvis and Cuentas 2016). Global warming will be especially harmful for tropical species, since tropical organisms are highly sensitive to temperature change, and they can approach near-lethal temperatures much faster than species from temperate climates (Deutsch et al. 2008). If species cannot adapt their CT_{max} to rising ambient temperatures, increasing their body size would result in a CT_{max} increase, which could aid them to survive under future global warming.

Chapter IV

Static thermal tolerances suggest limited adaptive potential in Andean frogs to land-use and climate change

Abstract

Global warming is having negative impacts spanning the entire biological hierarchy. Species' physiological sensitivity to temperature change and the environmental regimes they are exposed to are crucial to determine vulnerability to global warming. Here we ask how species' vulnerability varies across habitats and elevations, and how climatically buffered microhabitats can contribute to reduce their vulnerability. We measured amphibian thermal sensitivity (critical thermal maximum – CTmax) of fourteen frog species of the genus Pristimantis inhabiting young and old secondary, and primary forests in the Tropical Andes. We measured exposure to temperature stress by monitoring temperature in the canopy, understorey and within five frequently used microhabitats. To measure thermal sensitivity, we obtained the loss of righting response for each individual. Using individuals' thermal sensitivity and exposure, we determined their current vulnerability across habitats, elevations and microhabitats. We then ask how this vulnerability varies under four warming scenarios: 1.5^{o}C, 2^{o}C, 3^{o}C and 5^{o}C increase. We found a constant CTmax across species regardless of the habitat and elevation in which they occur, suggesting low levels of environmental selection. We also showed that species in young secondary forests will become more threatened under global warming because they are exposed to higher temperatures. Through continued use of microhabitats, most species could maintain their current thermal niche if the temperature increase does not surpass +2°C, as was agreed in the 2015 Paris Agreement (COP21). Managing landscapes by preserving primary forests and allowing regrowth of secondary forest would aid in the survival of this group and offer thermally buffered microhabitats.

Introduction

The rate of global warming is accelerating and is driving a global extinction crisis, with negative impacts now spanning continents, ecosystems and species (Rosenzweig et al. 2008, Sekercioglu et al. 2008, Freeman and Class Freeman 2014, Urban 2015, Scheffers et al. 2016). Species from high mountain environments will be particularly vulnerable due to the disappearance of specific thermal environments and systematically stronger warming rates, which are amplified with elevation (Williams et al. 2003, Mountain Research Initiative 2015). For example, the warming rate in the Tropical Andes has tripled since 1939 (Vuille and Bradley 2000). Tropical organisms, living in near-constant temperatures, may also be severely impacted due to their small thermal ranges (Deutsch et al. 2008, Dillon et al. 2010, Khaliq et al. 2014). As warming accelerates, species will have to shift their distributions to more favourable environments, adapt *in situ*, or acclimate via pre-existing phenotypic plasticity (IPCC 2014, McDonnell and Chapman 2015). For populations unable to move to more favourable environments, physical shelters that can house a wide range of taxa, such as epiphytes, tree holes and leaf litter can provide refuge during extreme warming events, such as unusually hot or dry conditions (Scheffers et al. 2014a, Woods et al. 2015, González del Pliego et al. 2016). The impact of global warming on the long-term persistence of species will depend largely upon extreme warming events more than mean daily temperatures (Parmesan et al. 2000).

Negative effects of global warming on biodiversity are amplified by other threats, especially land-use change (Brook et al. 2008, Mantyka-Pringle et al. 2012), which results in an immediate change in local climate towards hotter and drier conditions. The quality of habitat is therefore a critical consideration in determining how species might cope to future changes in climate. For example, tropical species suffer the highest rates of deforestation globally (Hansen et al. 2013, FAO 2015). Yet, these areas are also experiencing high rates of secondary forest regrowth on abandoned farmland. Mountainous and dry environments of the Latin American tropics alone, accumulated 362,430 km^2 of woody vegetation recovery between 2001 and 2010 (Aide et al. 2013). Young secondary forests (YSF) have higher maximum temperatures than primary forests (PF), but as they age, they provide lower ambient temperatures and higher densities of buffered microhabitats (González del Pliego et al. 2016). This regrowth benefits threatened and endemic tropical biodiversity (Rudel et al. 2009, Gilroy et al. 2014), but a key question remains: will secondary forests provide sufficient refuge under climate warming (Schonbeck et al. 2015).

Species' vulnerability to climate change depends on the environmental regimes they are exposed to and their sensitivity, determined by physiological responses (Williams et al. 2008). Understanding how a species' tolerance to high temperatures (i.e. species critical thermal maximum – CT_{max}) changes across elevation and habitat would reveal if species are able to adapt their physiology across these gradients. For example, CT_{max} can be higher in lowland species versus high-altitude tropical species, and in species living in pasture versus forests (Garcia-Robledo et al. 2016, Nowakowski et al. 2016). Determining which species currently live closest to their CT_{max} will help predict how global warming will affect where species occur and how well they perform in warmer ecosystems.

Forest fragments and secondary forest regrowth (Ewers and Banks-Leite 2013, González del Pliego et al. 2016, Vogado et al. 2016) often have highly variable temperatures, which might be expected to select for increased levels of physiological plasticity over short periods of time (Frishkoff et al. 2015). Short-term selection for high heat tolerance (heat hardened; (Frishkoff et al. 2015), has been observed in various taxa exposed to extreme climates (Phillips et al. 2015). However, this must be balanced by the overall interaction between temperature and precipitation, the latter of which tends to buffer the severity of thermal stress—possibly reducing the overall synergistic effects of climate and land-use change (Mantyka-Pringle et al. 2012, Chan et al. 2016). Precipitation may also alleviate negative effects of extreme thermal stress on animal communities (McCain and Colwell 2011, Kellermann et al. 2012b, Mantyka-Pringle et al. 2012).

Across an elevation gradient in the western Colombian Andes, we assessed the physiological response of frogs to extreme heat, testing whether CT_{max} is selected by environmental conditions, such as habitat degradation (i.e. primary versus secondary forest). We thus assessed species vulnerability to global warming by answering the following questions: (1) Does amphibian CT_{max} vary across primary and secondary forests? (2) Is the CT_{max} of amphibians lower than ambient temperatures in secondary forests, thus physiologically constraining them? (3) To what degree do different microhabitats reduce the impact of extreme weather events on amphibians? (4) Will microhabitats buffer temperatures in secondary forests as temperature increases?

Methods

Study site and regional climate

We worked on the western slope of the western cordillera of the Colombian Andes, in the Reserva Mesenia-Paramillo, Antioquia (N: 05^{o} 29’ 45.8’’ to 05^{o} 12’ 54.3’’, W: 75^{o} 53’ 20.8’’) (Appendix III, Fig. S1). The study area covered an altitudinal range of 2,150 m - 2,690 m above sea level and it encompasses primary forest, naturally regenerating secondary forests and an agricultural matrix dominated by cattle pasture. This region supports one of the highest global diversities of threatened and endemic amphibians (Myers et al. 2000, Orme et al. 2005). Our site incurs annual rainfall ranges between 5,000 mm to 12,000 mm/year and relative humidity is 97% on average. The Intertropical Convergence Zone (ITZC) creates an annual cycle of precipitation consisting of rainy seasons (Apr-May and Oct-Nov), and ‘dry’ (less rainy) seasons (Dec-Feb and Jun-Aug) (Poveda et al. 2005).

Study Organisms

Between July and August 2014, we collected frogs from fourteen species, with a length cloaca-rostrum (LCR) ≥10 mm. Phylogenetic effects were reduced by sampling species exclusively from the genus *Pristimantis* (Craugastoridae, Anura). These small frogs (range 10 – 37 mm) have the highest abundance in our study area and they are all nocturnal, semi-arboreal and share similar microhabitats (Acosta-Galvis and Cuentas 2016). Frogs were sampled in 25 m x 10 m transects each separated by at least 200-300 m in primary forest (20 transects; 175 individuals), old secondary forest (OSF) ≥19 years old (4 transects; 35 individuals), and young secondary forest <19 years old (11 transects; 35 individuals). Sampled frogs were identified to species by a regional expert (ARA-G) and LCR measured.

Sensitivity - Critical thermal maximum (CTmax)

We use the loss of righting response (LRR) as our CT_{max} target (Navas et al. 2007, Catenazzi et al. 2014). Phylogenetic effects on this trait have been previously established for these species, revealing that CT_{max} is strongly related to phylogeny, influenced by body length, but not affected by elevation or habitat type (González del Pliego et al., in review). Thus, in this study, while we consider phylogeny we do not discuss phylogenetic effects on CT_{max}. Frogs were acclimated in the field laboratory for 3 to 5 days before experiments (Catenazzi et al. 2014). We placed a frog in a transparent plastic cup with a thin layer of water (1.5 ml) to prevent desiccation (Navas et al. 2007), and cups were positioned in a water bath. The temperature of the water bath started at 17°C (Catenazzi et al. 2014), and was increased by hand by ~0.45°C per minute on average.

We used the probe of a two-channel digital thermometer (accuracy: ±0.1°C) to gently, but often, turn the frogs over on their backs. We incited individuals to righten by touching their flanks and inner thighs. After 5 s, if the frog was incapable of rightening, we registered its temperature by touching one flank with the probe (K-type; (Navas et al. 2007, Catenazzi et al. 2014). We considered this temperature as the core body temperature, since small-sized frogs have a great heat transfer (Catenazzi et al. 2014) and their body temperature is only slightly above environmental temperature (Vitt and Caldwell 2009). The frogs were immediately placed in a cool container (Navas et al. 2007), and observed for 24 hrs to verify they were healthy and ready to be released (there were no mortalities in our study, and all individuals recovered fully).

Exposure – Environmental temperatures

To record the temperature at which sampled individuals are exposed to on a daily basis, we measured canopy and understorey ambient, and microhabitat temperatures using 90 iButton temperature loggers (model: DS1921G-F5; accuracy: 0.5°C) from March 2013 to February 2014. Loggers were placed within 12 amphibian sample transects. Each iButton was placed inside a re-sealable zipper storage bag (50 mm X 50 mm) to shelter it from precipitation and enclosed within a metal mesh to guard it from rodents.

iButtons used to sample ambient air were secured under a plastic funnel to minimize exposure to direct solar radiation.

To capture ambient temperature that is buffered by relative amounts of above-ground vegetation, we monitored understorey ambient with iButtons placed approximately 1.5 m above the ground (Scheffers et al. 2013a) in 16 transects (6 in PF, 4 in OSF, and 6 in YSF). To capture ambient that is not buffered by above-ground vegetation, we hung a second iButton in the upper canopy of trees between ten and thirteen meters above the ground (Scheffers et al. 2013b) as close as possible to the previous iButton (ntotal=6).

Microhabitat temperature loggers were also deployed within microhabitats that are commonly used by frogs on the same transects as ambient loggers. We monitored temperature via data loggers placed: (1) at approximately 20 cm depth in soil (ntotal=5); (2) approximately 5 cm under leaf litter (ntotal=16); (3) within the roots of ferns (fern circumference > 8cm DBH) (ntotal=16); (4) inside holes at the base of trees (tree circumference > 8cm DBH) (ntotal=16); and (5) between the leaves of bromeliads (ntotal=13). All loggers were placed within 8 m of the understorey ambient logger. Only one iButton was placed within a particular microhabitat per transect.

Statistical Analyses

Sensitivity

We performed a linear model to assess the impact of elevation on individual's CT_{max}. Also, to determine any phylogenetic signal from CT_{max} we calculated Pagel's lambda (λ) using phylosig function from the R package phytools (Revell 2012). Lambda values tend to range from 1 (strong phylogenetic signal) to 0 (no phylogenetic signal). Furthermore, we used linear models to determine the relationships between CT_{max} and (1) number of acclimation days and (2) body size – length cloaca-rostrum, because these variables might have an influence on variation in CT_{max} (Hutchison and Rowlan 1974, Ribeiro et al. 2012). We also used linear models to explore relationships across (1) amphibian body size and elevational gradient and (2) warming rate for each CT_{max} experiment (the temperature change by time for each thermal experiment) and elevational gradient.

Exposure

We used piecewise regression to explore the relationships between maximum and minimum understorey ambient temperature across all sixteen transects and elevation (‘segmented’ package). This method was used to identify the elevational point at which the slope of the temperature changed since there were clearly two different relationships in our data (Muggeo and Adelfio 2011). We tested whether the *x* variable (i.e. elevation) was partitioned into two groups and a regression model was fitted to each group. For maximum understorey ambient temperatures, we used (1) absolute maximum (maximum temperature ever recorded for specific elevations), (2) climatic maximum extremes (extreme outlier events), and (3) mean daily maximum, with (4) average daily mean used as a reference. For minimum ambient temperatures, we used (1) average daily mean as a reference, (2) average daily minimum, (3) climatic minimum extremes (lower extreme outlier events), and (4) absolute minimum temperature recorded for each elevation. We defined extreme temperature events (or extreme outliers) as data values that are 1.5 times the interquartile range of our data (the difference in the response variable between its first and third quartiles) above the third quartile (Scheffers et al. 2014a).

Vulnerability to extreme weather events – Warming tolerance

We assess the vulnerability of species to extreme weather events because these events may substantially increase the susceptibility to warm conditions and the decline of species in our study area (Welbergen et al. 2008, Scheffers et al. 2013a). To determine exposure to extreme temperature, for each individual we examined extreme weather events from the canopy, understorey, and microhabitats from the iButtons located closest to that individual, considering both elevation and habitat type. Extreme weather events were calculated as described above.

We then calculated warming tolerance (WT), as the degrees between extreme temperature and CT_{max} (Deutsch et al. 2008).

$$WT = CT_{max} - T_{ex}$$

Where WT is calculated as each individual's critical thermal maximum (CT_{max}) minus the average temperature of extreme weather events that each individual experience (T_{ex}), across canopy, understorey or the different microhabitats. Thus, warming tolerance indicates whether individuals have sufficient thermal tolerance to cope with extreme temperatures (i.e. whether exposure is close to lethal limits). Therefore, positive numbers indicate 'safety' whereas negative values indicate 'danger'.

Vulnerability across elevation and habitat types

To determine the relationship between warming tolerance and elevation we performed a piecewise regression since there were clearly two different relationships in our data. Our model used 2321 m a.s.l as the break point. To understand whether warming tolerance of amphibians in canopy, understorey ambient and the different microhabitats differed between young, old secondary and primary forests we used a phylogenetic approach to account for trait evolution. We used the phylogeny from González del Pliego et al. (in review). However, their phylogenetic tree contained eleven of the fourteen species we sampled, with the remaining three species lacking genetic information. We use a phylogenetic signal (V) to measure this trait evolution, as it represents the tendency of related species to resemble each other more than at random (Blomberg and Garland 2002). We tested the relationships between warming tolerance and forest types by fitting a linear mixed effect model that allows phylogenetic covariance matrices. We used the function lmekin in the R package coxme (Therneau 2015). This model produces a z-value that represents the contribution of forest type (shown in Table 3.2). Our model included residual variance associated with both the individual measurement (e.g. species-specific variation in CT_{max}) and with phylogeny (shown in Appendix III, Table S1). We specified the variance-covariance structure of the residual variance as a vector of errors (e) (following Freckleton and Rees *in press*). We estimated the errors assuming they follow a multivariate normal distribution with a variance-covariance matrix with three components**.** The first component is the phylogenetic signal, representing the variance among species means that results from phylogenetic dependence (V). This component shows the covariance between each pair of tips, calculated using the branch lengths of the phylogeny. Thus, it represents the phylogenetic contribution. The second describes variation in the species means that is independent of phylogeny (S). The final variance is that between replicate experimental units independent of phylogeny or species identity, i.e. the error variance (I) (Freckleton and Rees *in press*). We re-analysed these data grouping YSF and OSF, to confirm that differences prevail despite using two categories for secondary forests. Results are shown in Appendix III, Table S2. We also calculated Pagel's lambda (λ – as described above) to determine any phylogenetic signal from warming tolerance.

Vulnerability under future warming

Barring a successful implementation of the COP21 target to limit warming to well below 2°C, in our study area, ambient temperatures are projected to increase by 3 - 4°C by the end of this century (IPCC 2014). Therefore, we selected four different climate change scenarios to determine the impact on species sensitivity: (1) 1.5°C increase reflecting the COP21 aspirational limit of well below 2°C, (2) 2°C increase pre-COP21 best-case scenario targets; (3) 3°C increase as a more feasible scenario (IPCC 2014); and (4) a worst case scenario of a 5°C increase. All analyses were conducted using R (version 3.3.1(R 2016)).

Results

Community composition

Primary forest had the highest abundance and the highest species richness and young secondary forest had the lowest species richness (Appendix III, Table S3). We found 11 species in primary forest, 9 in old secondary forest, and 4 in young secondary forest. Young secondary forest was the only habitat that had no specialist species (species found exclusively in one habitat), whereas old secondary forest had one and primary forest had five specialist species.

Sensitivity

Species' critical thermal maxima was constant across elevation ($F_{1, 243} = 0.21$, $p = 0.64$; Fig. 3.1B), showed a strong phylogenetic signal ($\lambda = 0.85$), and was not driven by number of acclimation days ($F_{1, 242} = 0.36$, $p = 0.54$; Appendix III, Fig. S2A). However, CT_{max} was driven by the size of the individual ($F_{1, 243} = 60.40$, $r^2 = 0.19$, $p < 0.001$; Appendix III, Fig. S2B), yet body size did not change with elevation ($F_{1, 243} = 0.24$, $p = 0.61$; Appendix III, Fig. S3). We found no relationship between the warming rate for each experiment and the elevational gradient ($F_{1, 243} = 0.49$, $r^2 = 0.002$, $p = 0.48$), thus we are confident that CT_{max} values are not an artefact of our methodology.

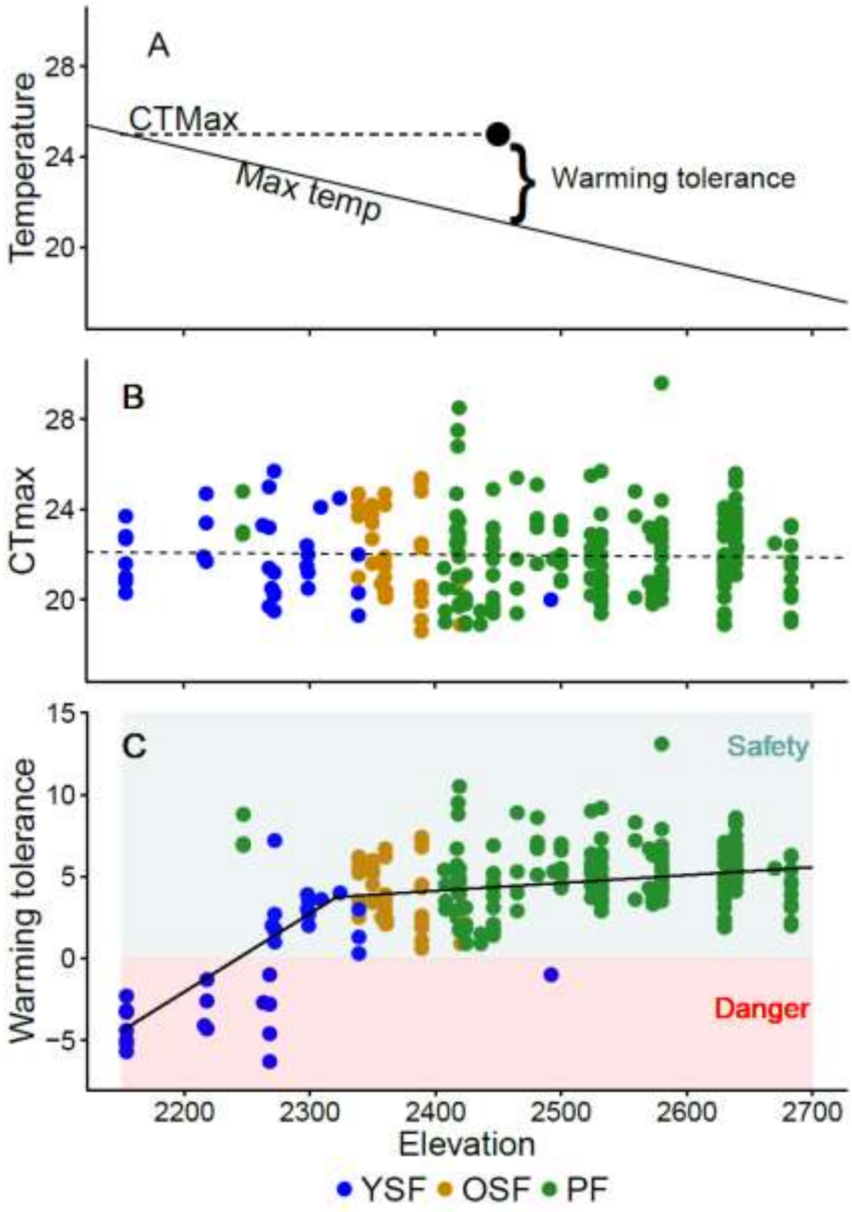

Fig. 3.1. Graphical representation of warming tolerance (A). Linear model from critical thermal maxima (B) and piecewise regression from warming tolerance from understorey ambient (C) from *Pristimantis* species across an elevational gradient in young secondary (blue), old secondary (orange), and primary forests (green) in the Tropical Andes. Each dot represents an individual. Dashed line represents non-significant relationship ($p>0.05$); solid line represents significant relationship ($p<0.05$)

Exposure

The highest extreme temperatures were recorded in YSF, whereas the lower extreme temperatures were recorded in PF. This is true for canopy, understorey ambient and all microhabitats (Table 3.1). Moreover, minimum temperatures were more constant across elevations than its counterpart, maximum temperatures (Fig. 3.3).

Vulnerability

The warming tolerance of species was strongly driven by forest type ($p<0.001$; Fig. 3.1C), with frogs in YSF being considerably more vulnerable than those found in OSF and PF (Table 3.2). Our data showed that species warming tolerance would be extremely low if species were to experience canopy temperatures (Fig. 3.2A). In the understorey ambient environment, warming tolerance increased compared to the canopy level (Fig. 3.2B); however, most species in YSF are currently experiencing extreme temperatures that exceed their thermal tolerance. Nevertheless, across all forest types, after incorporating the thermal buffering capacity of microhabitats, all species were safe under current levels of exposure (Fig. 3.2C to 3.2G). Microhabitats in YSF were 5.58°C cooler on average than understorey ambient temperature, they were 2.03°C cooler on average in old secondary than understorey ambient, and in PF they were 1.56°C cooler on average than understorey ambient (Table 3.1; Fig. 3.2C to 3.2G).

Table 3.1. Mean extreme weather event temperatures (°C) of canopy, understorey ambient and different microhabitats across young, old secondary and primary forests in Tropical Andes.

	YSF	OSF	PF
Canopy	21.33	20.05	18.0
Understorey	22.58	18.12	16.96
Bromeliad	18.0	17.5	17.0
Tree fern	17.0	16.0	15.0
Tree hole	16.5	15.5	15.0
Leaf litter	17.0	16.5	15.5
Soil	16.5	15.0	14.5

Table 3.2. Summary of linear mixed effect models accounting for phylogenetic effect of current warming tolerance of *Pristimantis* species in canopy, understorey ambient and different microhabitats across all pairwise comparisons between young, old secondary and primary forests in Tropical Andes. Bold values represent significant relationships (p<0.05). See text for detailed methods. See Appendix III, Table S1 for the phylogenetic variances.

	z score; p value	YSF vs PF	OSF vs PF	YSF vs OSF
Canopy	z	**32.29**	**13.81**	**7.93**
	p	<0.001	<0.001	<0.001
Understorey	z	**10.91**	1.60	**7.99**
	p	<0.001	0.11	<0.001
Bromeliad	z	**3.64**	**2.34**	1.09
	p	<0.001	0.01	0.28
Tree fern	z	**6.56**	**3.83**	**2.26**
	p	<0.0001	<0.001	0.02
Tree hole	z	**5.10**	**2.34**	**2.26**
	p	<0.001	0.01	0.02
Leaf litter	z	**5.10**	**3.83**	1.09
	p	<0.001	<0.001	0.28
Soil	z	**6.56**	**2.34**	**3.44**
	p	<0.001	0.01	<0.001

Vulnerability under future warming

Species warming tolerance in the canopy was below zero (in the danger area) across all species even in our most conservative warming scenario (1.5°C increase; Fig. 3.2A). In the understorey ambient environment, species warming tolerance decreased across all habitat types under all four future warming scenarios: 1.5$^{\mathrm{o}}$C, 2$^{\mathrm{o}}$C, 3$^{\mathrm{o}}$C and 5$^{\mathrm{o}}$C increase. With a 3°C increase in temperature, all (4/4) species from YSF, most species (5/9) from OSF, and five species (5/11) from PF will become vulnerable to extreme weather events when exposed to understorey ambient temperatures (Fig. 3.2B). Moreover, if the aspirational COP21 limits are met, and there is only a 1.5°C increase in temperature, still all (4/4) species from YSF, three species (3/9) from OSF, and four species (4/11) from PF will become vulnerable to extreme weather events when exposed to understorey ambient temperatures. Considering the worst-case scenario, an increase of 5°C will put all species from all habitats in peril (except *P. permixtus*; Fig. 3.2B).

Under a 1.5°C increase in temperature, if species actively search for thermally buffered microhabitats, they will be able to use safe microclimates across all habitat types (Fig. 3.2C to 3.2G). A 2°C and 3°C increase in temperature would result in most microhabitats, except bromeliads (Fig. 3.2C), offering climatic refuge for all species during extreme events. A 5°C increase in temperature would only provide thermally buffered microhabitats for primary forest specialists (excluding bromeliads; Fig. 3.2C) and thermally buffered microhabitats would be lost for species that live in YSF and OSF. Moreover, soil will continue to provide a climatic refuge, unless temperature increases to 5°C (Fig. 3.2H).

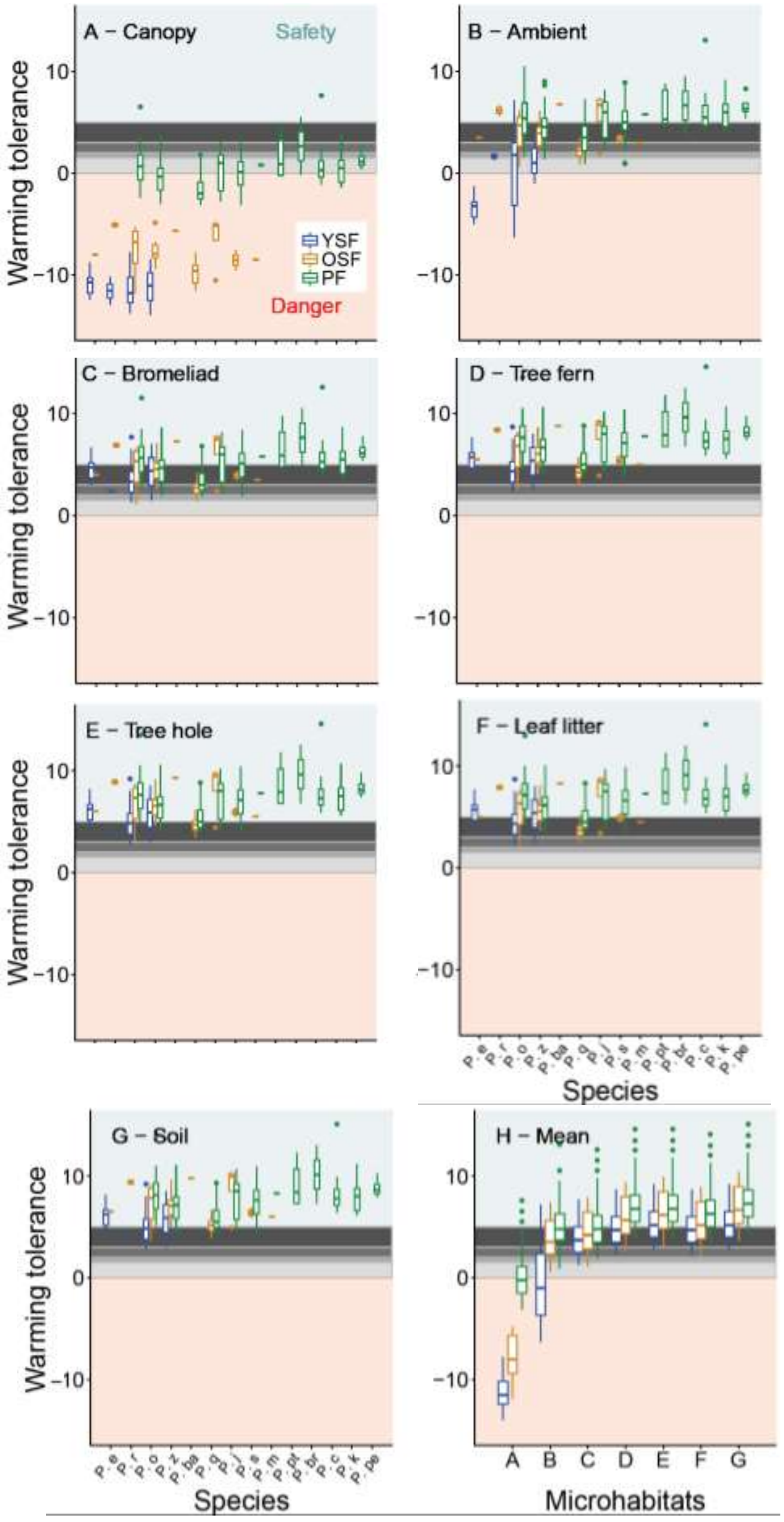
A – Canopy
Safety
Danger
YSF
OSF
PF
B – Ambient
C – Bromeliad
D – Tree fern
E – Tree hole
F – Leaf litter
G – Soil
H – Mean
Warming tolerance
10
0
−10
P. e
P. r
P. o
P. z
P. ba
P. q
P. j
P. s
P. m
P. pt
P. br
P. c
P. k
P. pe
Species
A
B
C
D
E
F
G
Microhabitats

Fig. 3.2. Amphibian warming tolerance as a function of average extreme weather event temperatures in young secondary (blue), old secondary (orange), and primary forests (green) from (A) canopy, (B) understorey ambient and (C-G) different microhabitats, and species CT_{max} (critical thermal maxima) along an elevational gradient in the Tropical Andes. (H) Mean amphibian warming tolerance per habitat across the ambient air and microhabitats previously described (A to G). The grey bars represent the temperature increase for different global warming scenarios: Light grey: 1.5°C; medium light grey: 2°C; medium dark grey: 3°C; dark grey: 5°C. Species: P.e: *Pristimantis erythropleura*; P. r: *P. ruedai*; P. o: *P. orpacobates*; P. z: *P. zophus*; P.ba: *P. baiotis*; P. q: *P. quantus*; P. j: *P. juanchoi*; P. s: *P. sp*.; P. m: *P. myops*; P. pt: *P. ptochus*; P. br: *P. brevifrons*; P. c: *P. calcaratus*; P. k: *P. kelephus*; P. pe: *P. permixtus*. Values below zero represent thermal danger, whereas values above zero represent thermal safety.

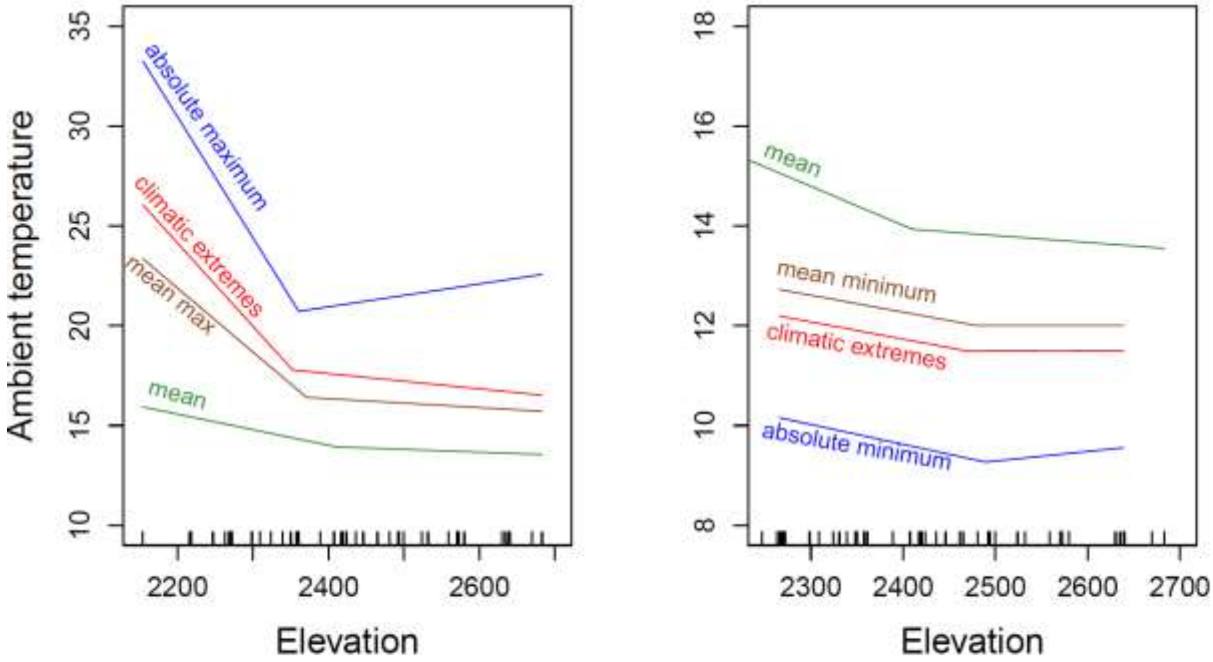

Fig. 3.3. Piecewise regression between elevation and understorey ambient temperature as (left panel) the absolute maximum temperature recorded for each elevation, climatic maximum extremes (upper extreme outlier events), mean daily maximum, average daily mean, and (right panel) average daily mean, average daily minimum, climatic minimum extremes (lower extreme outlier events), and absolute minimum temperature recorded for each elevation.

Discussion

In this paper, we found that the vulnerability of frog species to heat extremes (CT_{max}) was indifferent across habitats and elevations. However, microhabitats contribute to reduce their exposure to hostile temperature. The similar CT_{max} across all the frog species suggest that current levels of exposure are not sufficiently extreme to drive large differences in heat tolerance in our system, which may also be due to the buffering effect of high precipitation across elevation and the strong phylogenetic signal on CT_{max}. When placing CT_{max} in context to habitat-specific temperature, we found that species in young secondary forests displayed the lowest warming tolerance, which continued to worsen under future warming scenarios. Importantly, microhabitats can reduce climate exposure so long as future temperature increases do not surpass +2°C.

Sensitivity – CTmax

Contrary to other studies, we found no variation in species' CT_{max} across secondary and primary forest, with CT_{max} remaining almost constant across the elevation-climate gradient. CT_{max} in ectotherms is known to vary by several degrees across related species, elevations and habitat types (Araujo et al. 2013, Catenazzi et al. 2014, Muñoz et al. 2016, Nowakowski et al. 2016). For example, in the tropics, high altitude species have lower CT_{max} than low altitude species, and forest-restricted species have lower CT_{max} than species from warmer pastures and cultivated areas (Garcia-Robledo et al. 2016, Nowakowski et al. 2016). The constant CT_{max} observed suggests that heat tolerance is highly constrained across lineages, supported by the strong phylogenetic signal that we found on $CT_{max.}$ This minimizes the potential for thermal adaptation to current temperatures (Kellermann et al. 2012b, Araujo et al. 2013). Moreover, if species are using thermoregulation (e.g. using microhabitats to avoid the warmest hours), they could be reducing the need for local physiological adaptation to the thermal environment (Bogert 1949, 1959, Huey et al. 2003) because they would be experiencing similar nocturnal (minimum) temperatures (Fig. 3.3).

Local thermal conditions can drive the evolution of thermal tolerance limits (acclimation hypothesis; (Leroi et al. 1994, Angilletta 2009), and CTmax can also vary with large fluctuations in temperature (e.g. distinct seasons; (Clusella-Trullas et al. 2011, Sanabria et al. 2012, Chan et al. 2016). Temperature variability favours organisms with broad temperature tolerance, whereas small fluctuations in annual temperatures, such as those in the forest types in our study area, favours narrow physiological thermal tolerances (physiological specialization; (Chan et al. 2016). Yet, negative impacts from temperature are stronger in physiologically specialized species (Ghalambor et al. 2006). Acclimation is difficult in ectotherms from high-altitude environments, such as our study area, due to a lack of metabolic responses and its high energetic cost (e.g. reduced survivorship and fecundity) (Navas 1996, Angilletta 2009). The similar CT_{max} that we found across species and the potential lack of metabolic acclimation suggests that no physiological adaptation (e.g. acclimation) is occurring at current levels of exposure (Navas 1997). Indeed, perfect acclimation is rarely seen in ectotherms (e.g. crocodiles; (Glanville and Seebacher 2006)). Our results show this assemblage have limited adaptive potential in terms of its physiological plasticity and might be in peril of overheating under climate change (Araujo et al. 2013, Muñoz et al. 2016).

The effects of maximum ambient temperatures on CT_{max} could instead be buffered by annual precipitation, because the CT_{max} of species decreases as precipitation increases (Kellermann et al. 2012b). When annual precipitation is low (<1000 mm), maximum temperature may be strongly related to CT_{max}, but the opposite may happen with high annual precipitation. Thus, precipitation might be more important than maximum temperature in driving CT_{max} (Kellermann et al. 2012b). Consequently, the high precipitation in our study area could be buffering the relationship between temperature and CT_{max}, and reducing the adaptive response of these populations to hot temperatures (Kellermann et al. 2012b), especially in YSF. Because these populations have likely been thermally insulated by precipitation, there has never been a temperature-driven environmental filtering event. This in turn could lead to populations in YSF being maladapted to hot temperatures (Angilletta 2009) and might be moving upslope to more optimal cooler temperatures (Chan et al. 2016) where they can enjoy higher physiological performance (Araujo et al. 2013, Logan et al. 2014). Moreover, precipitation in the western cordillera of the Colombian Andes has been predicted to decrease by up to 50% by 2100, which, together with increasing temperatures, may amplify the negative effects of habitat loss (Mantyka-Pringle et al. 2012, IPCC 2014). For example, a decrease on connectivity across primary forests along with warmer and drier conditions could decrease the dispersal ability of species (Hanski 2011).

Exposure and Vulnerability

Results from the piecewise regression showed that warming tolerances increased very steeply with elevation up to the break point (2321 m a.s.l.), after which warming tolerance remained constant across elevation. Considering the near-constant CT_{max} across the elevational gradient (Fig. 3.1B), the dramatic shift in warming tolerance (Fig. 3.1C) can only be driven by local habitat temperatures. This suggests that the vulnerability of frogs is driven more by exposure than by their sensitivity. Because temperatures within forest type play an important role determining species exposure and vulnerability to climatic extremes, differences in community composition after tropical deforestation may be driven by temperatures acting as an ecological filter (Frishkoff et al. 2015). For example, forest vegetation decreases temperature on the ground across all habitat types (Fig. 3.2B). This means that species living in denser, more structurally complex forests (PF) experience lower temperature than species living in less complex forests (YSF), regardless of elevation. Lower warming tolerance in YSF means that frogs are considerably more vulnerable than those found in OSF and PF. Most mountain tops in our study area still retain PF, and canopy-protected primary forest species have larger warming tolerance and thus should be less impacted by peak temperatures (Duarte et al. 2012).

Although ambient temperatures already surpass some species' thermal tolerance, microhabitats effectively buffer extreme weather events across all forest types (Scheffers et al. 2014a). Exposure to climatic extremes will become more severe and frequent as a consequence of global warming (Butt et al. 2016). However, species can avoid thermal stress by using these thermally buffered microhabitats to reduce their exposure to climatic events (Scheffers et al. 2013a). For example, we found that bromeliads and soil reduced exposure to climatic extremes by 14% and 90% on average, respectively. Microhabitats are available across all forest types, yet are less abundant in YSF, where they are potentially more necessary as YSF have higher annual mean maximum temperatures (González del Pliego et al. 2016). As such, microhabitats can play an important role in moderating the ecophysiological impacts of climate change on ectotherm communities (Huey and Tewksbury 2009).

Vulnerability under future warming

During warming events, the ability of different species to find refuge in thermally buffered microhabitats will depend on the overall temperature increase (Fig. 3.2C to 3.2G). By limiting global warming to well below 2°C, as was agreed in the 2015 Paris Agreement (COP21), few species will be affected across all habitat types (Fig. 3.2). If temperature increases by 2°C, most microhabitats, except bromeliads, would still offer climatic refuge during extreme events, whereas a 3°C increase would increase exposure to climatic extremes by several orders of magnitude (Fig. 3.2). Our worst case scenario, a 5°C increase, would result in all species (except for *P. permixtus*) having a warming tolerance below the ambient temperature, and thermally buffered microhabitats would only be available for forests specialists (Fig. 3.2B to 3.2G). This in turn could cause future local extinction from acute thermal stress. Warmer conditions could also increase species' refuge use and burying time, decreasing time for foraging, food intake, reproductive and social activities (Gvozdik 2002, Rohr and Madison 2003, Rohr and Palmer 2005, Martin et al. 2010). This, in turn, could reduce locomotion capacity, fecundity, recruitment, fitness, and body size (Reading 2007, Martin et al. 2010, Lowe 2012), with the latter being strongly positively related to CT_{max} (Appendix III, Fig. S2B).

The impacts of land-use change and global warming will continue to create major challenges for species survival. Multiple variables affecting the biological responses of ectotherms - such as deforestation, increasing temperatures and decreasing precipitation - increase the uncertainty and complexity of predicting the impacts of climate change (Clusella-Trullas et al. 2011). The similar CT_{max} across species reflects a potential buffering effect from strong precipitation and a lack of acclimation to new thermal environments. Species in young secondary forests face greater exposure to extreme temperatures and therefore are more vulnerable under global warming. By adapting their behaviour, most species could avoid changing their distributions if the temperature increase does not surpass 2°C. Managing the landscape by preserving and re-connecting primary forests and allowing the natural regrowth of secondary forests over longer timeframes would create larger areas of potential suitable environments and thermally buffered microhabitats. This can aid in the survival of amphibians, of which 40% of species are currently considered at risk of extinction via the combined effects of climate change and deforestation (IUCN 2015). Thus, there may be greater uncertainty in assessing the vulnerability of Andean communities than previously thought.

Chapter V

Preserving data deficient amphibians: Using fully-sampled phylogeny and trait-based approaches to predict threat status

Abstract

When assessing the threat status of species globally, many species remain not included owing to lack of data. Of special interest, *c.*40% of amphibians are currently threatened with extinction, and *c.*25% are 'Data Deficient' (DD) and may also be at risk. Here we applied a spatial-phylogenetic statistical framework to the task of predicting extinction risk for amphibian species around the globe that are currently listed as DD by the International Union for the Conservation of Nature (IUCN). We used a global trait database, including phylogenetically imputed body mass, and a fully sampled phylogeny with the aim of providing initial baseline predictions of threat status for data-deficient species. We found that almost half of DD species are threatened with extinction (48%), mainly across Southeast Asia, the Central Africa region and the Neotropics. Additionally, we suspect that from this, 100 species are likely to be Endangered or Critically Endangered. This suggests that Data Deficient species require urgent conservation strategies.

Introduction

Habitat loss, climate change, increasing human population size, wildlife trade and synergies between these are increasing the number of species at risk globally (MEA 2005, Scheffers et al. 2016, Cheptou et al. 2017). To determine the local and global extinction risk of species, the International Union for the Conservation of Nature (IUCN) created the 'Red List' (IUCN 1994). Through this list, species are assessed through expert assessment of species' extent of occurrence, area of occupancy and population trend, and assigned to one of 9 categories (Table 4.1). Excluding species which are extinct or for which there is too little information, there are 5 categories of threat on an ordinal scale. Each category has a unique meaning, but distances between categories are unknown (Agresti 2013). The Red List has been linked to several decision-making processes, including global evaluations of policy goals and helping to track progress towards the Convention on Biological Biodiversity (CBD) Biodiversity 2010 targets (Walpole et al. 2009, Butchart et al. 2010).

Species with limited information (e.g. lacking population trend data) cannot be evaluated. They are therefore considered as data deficient (DD). It is unlikely that most DD species will be assessed because relative to other taxa amphibians remain grossly underfunded (ASG and ASA 2014). DD species are, therefore, rarely included in conservation planning (Sousa-Baena et al. 2014), whereas, Critically endangered (CR), Endangered (EN) and Vulnerable (VU) species are prioritized for resource allocation for species recovery (Possingham et al. 2002). For example, the conservation priorities of the Convention on Biological Diversity (CBD, target 12) rely on threatened species, without considering DD species (CBD 2017).

Data deficient species increase the uncertainty in extinction risk patterns across taxa and regions, which in turn, hampers policy, conservation research and effort (Butchart and Bird 2010, Trindade-Filho et al. 2012). DD species might also be more threatened than we know and are much more likely to be under threat than those that have already been assessed (Howard and Bickford 2014, Jetz and Freckleton 2015).

Table 4.1. Categories of the IUCN Red List and definitions.

Categories	Definition
Extinct (EX)	There is no reasonable doubt that the last individual has died.
Extinct in the wild (EW)	It is known only to survive in cultivation, in captivity or as a naturalized population (or populations) well outside the past range.
Critically endangered (CR)	It meets any of the criteria A to E* for Critically Endangered, as such it is facing an extremely high risk of extinction in the wild.
Endangered (EN)	It meets any of the criteria A to E* for Endangered, and it is therefore considered to be facing a very high risk of extinction in the wild.
Vulnerable (VU)	It meets any of the criteria A to E* for Vulnerable, and thus it is facing a high risk of extinction in the wild.
Near threatened (NT)	It has been evaluated but does not qualify as threatened now, but is close to qualifying for or is likely to qualify for a threatened category in the near future.
Least concerned (LC)	It has been evaluated and does not qualify as Threatened. Widespread and abundant taxa are included in this category.
Data deficient (DD)	There is inadequate information to make a direct, or indirect, assessment of its risk of extinction based on its distribution and/or population status.
Not evaluated (NE)	The taxon has not yet been evaluated against the Red List criteria.

*Criteria: A) population size reduction; B) geographic range in the in the form of extent of occurrence or area of occupancy; C) small population size and decline; D) very small or restricted population; E) quantitative analysis.

Across the terrestrial vertebrates, amphibians are more threatened and are declining more rapidly than either birds or mammals (Stuart et al. 2004, IUCN 2017). They also have a higher proportion of DD species (21.3%), than birds (0.59%), mammals (14%) but similar to reptiles (21%) (Böhm et al. 2013, IUCN 2017). The high proportion of potentially threatened DD species highlights the need to better understand the extinction risk of these species, especially amphibians.

There are numerous drivers and characteristics that interact and influence amphibian extinction and decline (Bielby et al. 2008, Sodhi et al. 2008). For example, the range size of amphibians has been considered the most important predictor in analyses of extinction risk, (Sodhi et al. 2008, Howard and Bickford 2014, Veron et al. 2016). Thus if the geographical ranges of amphibians are known then it is potentially possible to make predictions of threat status even if other information is lacking. Moreover, amphibian declines have ecological, geographical and taxonomic patterns (Stuart et al. 2004) meaning that some biological, geographical and phylogenetic attributes increase extinction risk (Bielby et al. 2006). For example, species with aquatic life-stages are particularly susceptible to decline (Bielby et al. 2008) and Neotropical species are more affected than Afrotropical and Indomalayan species (Stuart et al. 2004). Extinction risk is also phylogenetically non-random in frogs (Bielby et al. 2006), thus accounting for phylogeny is essential in extinction risk analyses of this group. If DD species are threatened but they are also phylogenetically clumped we could lose whole branches of the tree of life (Howard and Bickford 2014). By preserving phylogenetic diversity we can also have positive impacts on preserving functional diversity and ecosystem services (Veron et al. 2016).

The aim of this chapter is to understand the attributes that contribute to global amphibian extinction risk, which in turn, can help in targeting conservation effort to specific species (or taxonomic group) and regions. Predictive studies of risk in DD species have used a diversity of methods, such as spatial-phylogenetic frameworks (Safi and Pettorelli 2010, Jetz and Freckleton 2015), machine learning (Bland and Bohm 2016), and rule-based methods (Veron et al. 2016). Although recent studies have focused on predicting the likely status of DD species based on available data (e.g. range size, body size, biology, phylogeny), so far no studies have used a trait-based approach along with the fully-sampled phylogeny to assess the global amphibian threat status. Specifically, the aims of this study are: 1) Predict the global threat status of Data Deficient amphibian species; and 2) Assess which regions are most at risk considering the lack of species knowledge and high extinction risk.

Methods

Data

We collected data on 7,677 amphibian species from 3 orders and 75 families, of which, 4,906 have been assessed by the IUCN. Of these, 2,062 are deemed threatened (i.e. categories 'Vulnerable', 'Endangered', 'Critically Endangered' and 'Extinct'), 1,426 species recognized but not assessed (category 'Data Deficient') and 1,345 species not recognized by the IUCN (which were also considered as 'Data Deficient'). The data deficient species were split through the different orders as follow: Anura, 2,439 species; Caudata 190; and Gymnophiona 142 species. From these, 23 families had no DD species, and one family had only DD species. From the total amphibian species, 7,180 species could be placed in the amphibian super tree phylogeny (Jetz and Pyron *in review*). This fully sampled amphibian phylogeny incorporates for the first time nearly all extant amphibian diversity: 7,238 species. They used an approach that uses taxonomic constraints for the placement of species without genetic data (that is geographically uneven distributed) and captures the resulting branch length uncertainty arising in a posterior distribution (Jetz and Pyron *in review*).

Life history traits

Life history data for 30 traits were collected for all amphibian species. We excluded traits that had more than 40% of missing values, and also excluded traits that had high collinearity ($r^2 > 0.80$). This resulted in a highly complete database of ten life history traits (Appendix IV, Table S1), whose strong importance for predicting threat status has been previously illustrated (Sodhi et al. 2008)(Appendix IV, Table S1). We used traits such as body size, which is regarded as the second most important factor to determine threat status (Sodhi et al. 2008).

Geographical ranges

We used range size, because it is the largest contributor to extinction risk (Sodhi et al. 2008, Howard and Bickford 2014, Veron et al. 2016) and it is used by the IUCN to assess threat status. We obtained the geographical range from 6,445 species from the IUCN and amphibiaweb (AmphibiaWeb 2016, IUCN 2017). We calculated the centroid of each range to obtain mean latitude and mean longitude of the distribution of each species. We then projected all the geographical ranges to Mollweide equal-area projection, which preserves the real area ratio, and estimated the total area per species (km^2). Spatial data was normalized prior to analysis.

Imputation of amphibian traits

The trait database, although highly completed, still had missing data due to the lack of knowledge of species ecology, which is a particularly common problem in methods for phylogenetic comparative studies (Howard and Bickford 2014, Goolsby et al. 2017). To address this, we used Rphylopars to impute the missing values of the trait database (Goolsby et al. 2017). Rphylopars is a tool for phylogenetic imputation of missing data, it uses the relationships across traits as well as the phylogenetic position of each species to impute new data (Goolsby et al. 2017). However, if the traits do not show a strong phylogenetic signal, then the imputed values can be unreliable. Thus we first calculated Pagel's lambda (λ) for all the traits using phylosig function from the R package phytools (Revell 2012). Lambda values tend to range from 1 (strong phylogenetic signal) to 0 (no phylogenetic signal). We then imputed values for traits that had a strong phylogenetic signal (>0.60; Appendix IV, Table S1). All subsequent analyses were performed on imputed data.

Predicting threat status – GLS approach

To predict the threat status of data deficient, we used the generalized least-squares (GLS) approach described in (Freckleton and Jetz 2009) and implemented in (Jetz and Freckleton 2015). Traits may often be significantly affected by phylogenetic factors (Freckleton and Jetz 2009). Therefore, predictions from our model were generated by using the trait values together with the degree of phylogenetic similarity for species (following Jetz and Freckleton 2015). The phylogenetic component allows for similar species to share similar levels of threat because they are evolutionarily related (Jetz and Freckleton 2015).The phylogenetic similarity was defined by a variance-covariance matrix from the full phylogeny (Jetz and Freckleton 2015). The response variable in our model (Red List categories) is an ordinal scale. However, we considered it as a continuous variable ranging from 'Least Concern', 1, to 'Extinct', 6, retaining the ordinal nature of the IUCN scale. We used this approach as analyses that consider the response variable as an ordinal value are unable to address the phylogenetic covariance (Jetz and Freckleton 2015). We then calculated the threat probabilities for data deficient species by using equation 2.1 in (Jetz and Freckleton 2015). This is the probability that the predicted value of species *i* is greater than 2.5 (between 'Near Threatened' and 'Vulnerable').

Rphylopars approach

To validate our model we used we used Rphylopars (Goolsby et al. 2017) to predict the threatened status of data deficient species. To assess the differences across these two approaches, we did a linear model across the results of both models.

We also validated our model, by using the GLS approach on fully assessed species. We randomly deleted the threat status for 20% of fully assessed species and we predict their threatened status. We then compared the real values versus the predicted values.

Differences across families

To test for differences on threatened status across families we performed a sign test across the 51 families that had both assessed and data deficient species using the function ('SIGN.test') from the BSDA package in R (Arnholt and Evans 2017). We also performed analyses of variance ('aov') to determine if threat status was significantly different between assessed and data deficient species, across orders and families. All analyses were carried out in R version 3.3.1 (R 2016).

Model limitations

We think out model might be slightly more optimistic than reality. This framework will become more robust by incorporating: 1) a human encroachment index; b) data with improved knowledge of land-cover; and c) a variance-covariance matrix of spatial distances (Jetz and Freckleton 2015). Species that live in the same place, should be similar to each other, thus accounting for spatial structure should be incorporated (Jetz and Freckleton 2015). To account for spatial structure, for future analyses, we would need to calculate the distances between the centroids of the ranges of each pair of species following (Freckleton and Jetz 2009). Another important limitation is the lack of geographic ranges for many species. Our database contained life history traits for an extra 735 species; however, these species had no geographic data, and thus we could not estimate their threat status. As ecological traits have a strong phylogenetic signal, missing data could be imputed, however, geographic ranges do not share this same characteristic. It is then unfortunate that this global assessment is still missing some species. Moreover, we decided not to use environmental variables (e.g. temperature) even if mean annual temperature can be strongly negatively correlated with clutch size, which in turn is related to geographical range size and this is related to extinction risk (Cooper et al. 2008). This was decided because temperature has shown to explain little (less than 5%) about amphibian extinction risk (Cooper et al. 2008, Sodhi et al. 2008).

Results

Our GLS validation approach showed that our model correctly predicted 61% of the threat status for assessed species. It underestimated 25% of the species and overestimated 14%. Although precise prediction were below 70%, we suspect this model is still a strong one, as the predictions from the GLS approach and the Rphylopars approach were extremely similar (r^2=0.99, p< 0.001). This suggests that with the traits that we used, our predictions for the threat status are precise, regardless of the approach. We are confident that by adding more data to our model, it will become more robust (see model limitations).

Range size and body size were strong correlates of threat status. As the IUCN uses range size to estimate threat status, this association was not unexpected (IUCN 2017). The strong correlation with body size was also expected (Cooper et al. 2008, Sodhi et al. 2008), with smaller species tending to have slightly higher extinction risk.

Threat status between assessed and data deficient species

According to this study, it is extremely likely that well over 750 additional amphibian species (48% of the data deficient species) are threatened with extinction. Additionally, we suspect that from this, 100 species are likely to be Endangered or Critically Endangered. Compared to current assessed amphibian species, the percentage of species predicted to be threatened with extinction is higher for the data deficient species (48% versus 41%). However, data deficient species showed slightly lower threat probabilities than fully assessed species (Fig. 4.1). In fact assessed amphibian species had a mean threat status of 2.31±0.02, which was higher than the predicted threat status 2.17±0.02 for data deficient species (F_{6443}= 12.61, p< 0.001). These data deficient species are found across all continents and at different latitudes, but are mainly found across the tropics (Fig. 4.2).

When considering all three orders of amphibians, we found that both fully assessed and data deficient species had a mean threat status between NT and V (between 2 and 3). Moreover, within the different orders (Fig. 4.3), we found no difference across assessed and data deficient species in the order Caudata (F= 1.72, df= 591, p= 0.19) and Anura (F= 2.98, df= 5591, p= 0.08). We found that for the order

Gymnophiona, assessed species had a higher threat status 1.41±0.12 than the predicted threat status 1.21±0.03 for data deficient species (F= 4.13, df= 137, p= 0.04). When considering families, we found no significant differences across assessed species and data deficient species for Caudata (F= 0.27, df= 591, p= 0.84), Gymnophiona (F= 1.72, df= 137, p= 0.09) or Anura (F= 0.82, df= 5591, p= 0.76). This was supported by our sign test, showing that assessed and predicted threatened status was similar across families (S= 24, p= 0.77).

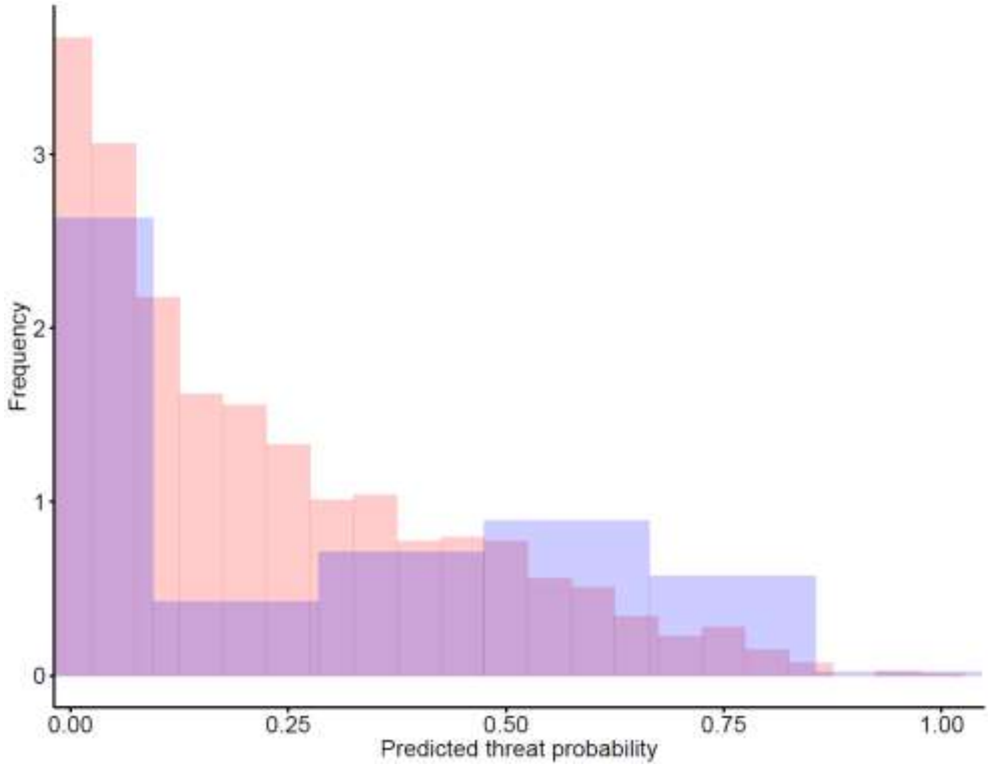

Figure 4.1. Fitted/predicted threat probabilities for fully assessed amphibian species (blue) and data deficient species (red).

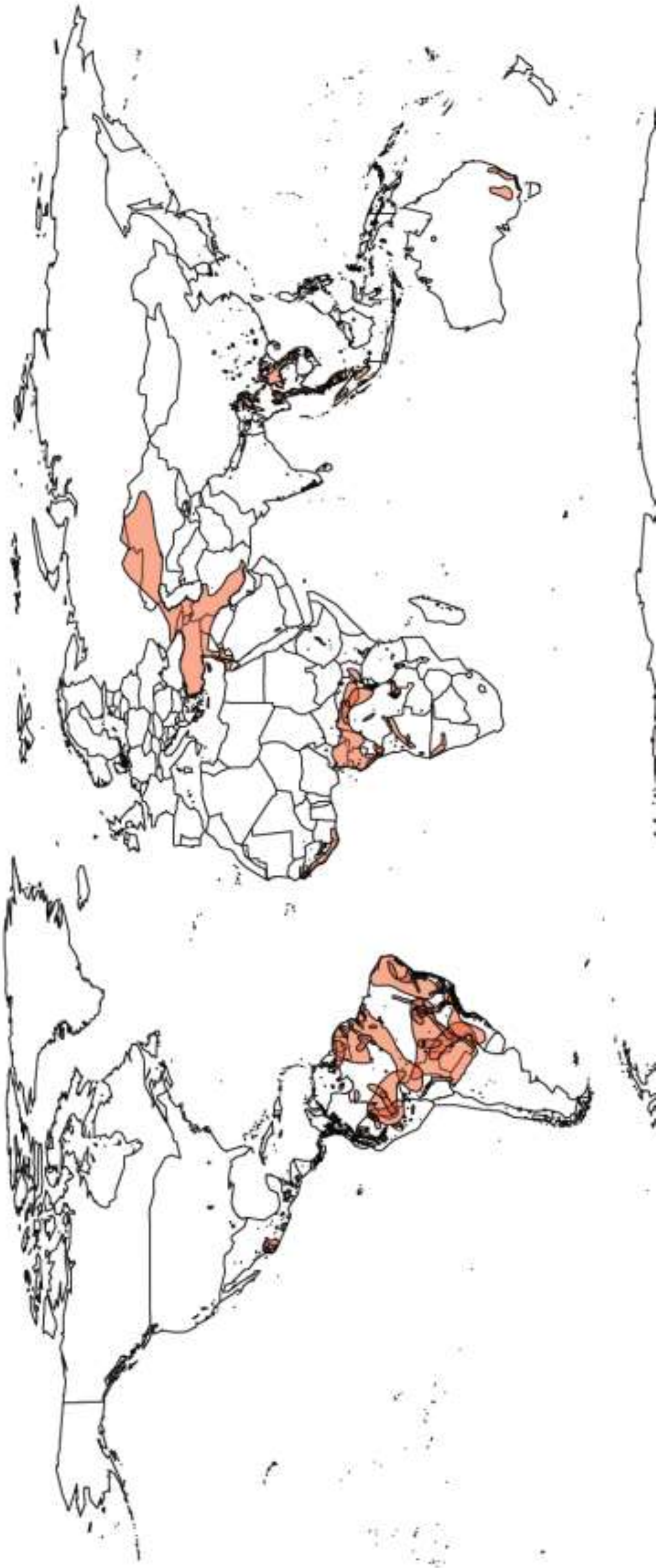

Figure 4.2. Distribution of data deficient species. Polygons represent the full geographic range for each data deficient species. Higher species richness is represented by a darker red.

Regions most at risk

Geographically, the majority of the threatened species, both assessed and predicted, are located in the tropics (Appendix IV, Fig. S1). Across the tropics, there were three regions that held most of the species predicted to be threatened: the Neotropics, the Central Africa region, and Southeast Asia (Fig. 4.4 and Appendix IV, Fig. S1). The Neotropics actually hold the largest amount of species richness in the world, both threatened and non-threatened, and assessed or data deficient. The highest concentration of threatened species within the Neotropics reaches from the south of Mexico through Central America to the Tropical range of the Andes (Fig. 4.4 and Appendix IV, Fig. S1). Within the Neotropics, the distribution of predicted threatened species is in most cases, similar to that of assessed threatened species (Fig. 4.4). This is not the case for the species in Central Africa, where most of the predicted threatened species ranges do not overlap with that of the assessed threatened species (Fig. 4.4). In Southeast Asia, although some of the assessed and predicted threatened ranges of species overlap, there are also areas or countries (e.g. Papua New Guinea) were there is little or no overlap (Fig. 4.4). There are also several countries such as Australia (mainly East Australia), Madagascar, Cuba and Jamaica that although they have a large number of assessed threatened species; they have a low or null number of predicted threatened data deficient species (Fig. 4.4).

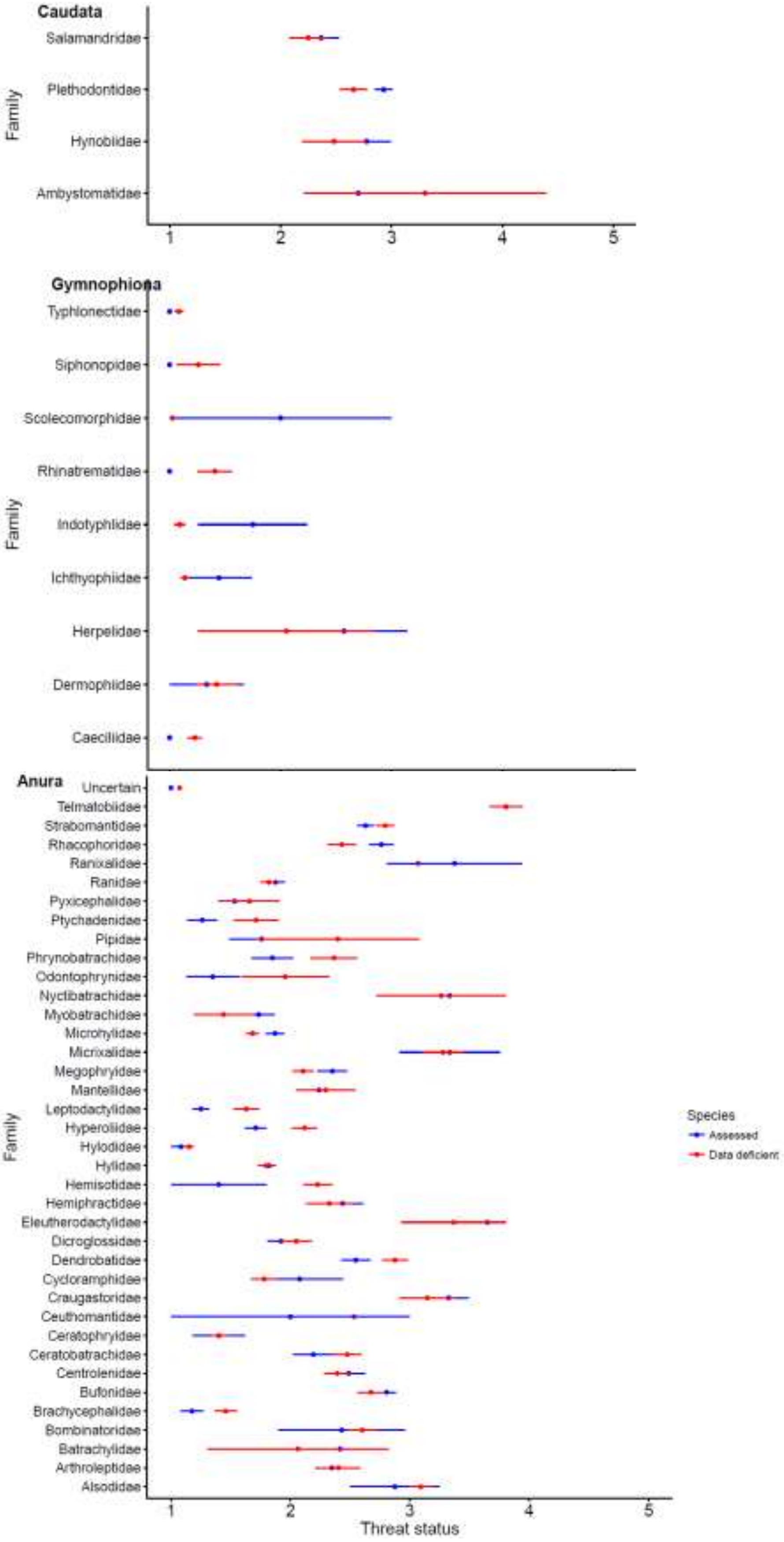

Figure 4.3. Mean threat status (±se) of fully assessed species (blue points) and predicted threat status for data deficient species (red points).

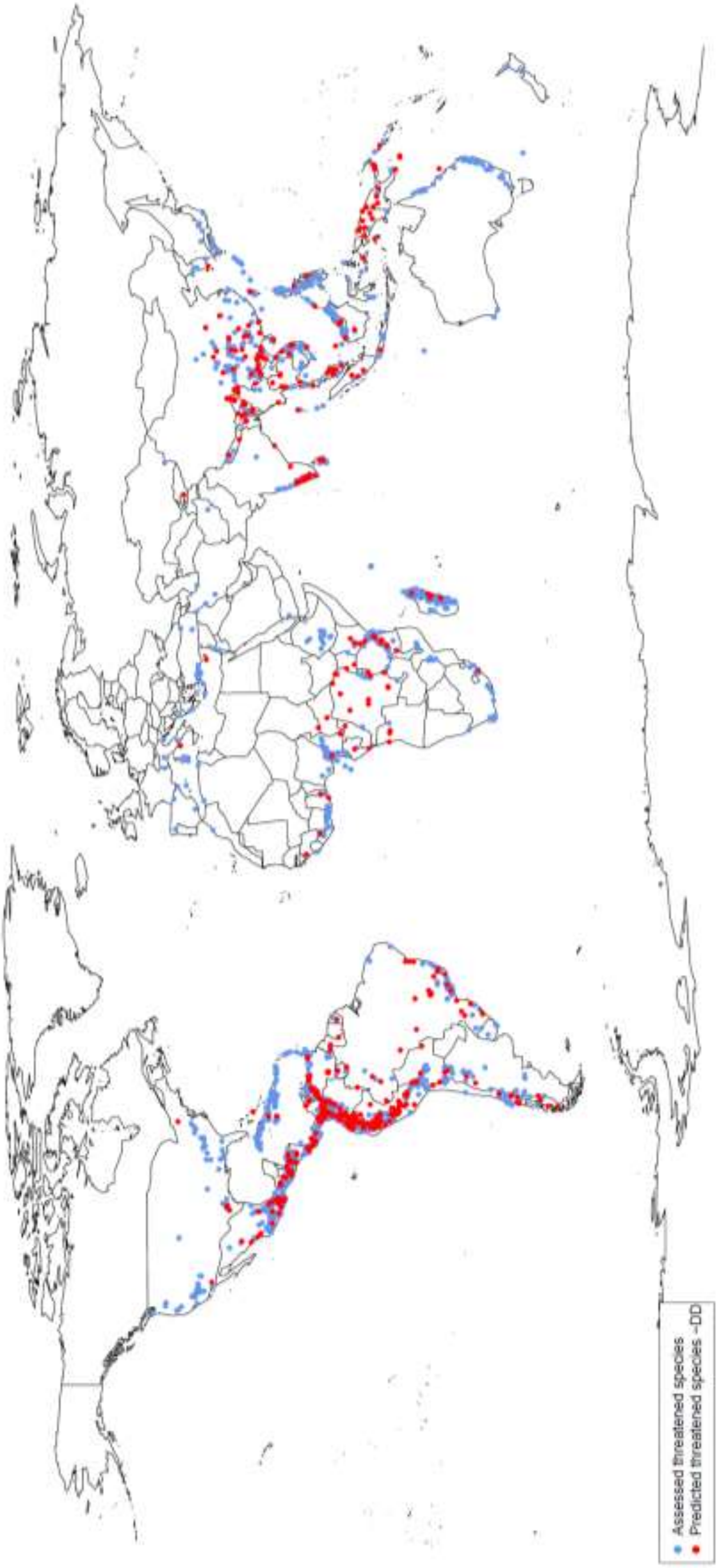
● Assessed threatened species
● Predicted threatened species –DD

Figure 4.4. Global distribution of threatened species for (blue) fully assessed species and (red) predicted species. Each dot represents the centroids of the ranges of each species.

Discussion

In this study, we have for the first time predicted the threatened status of global data deficient amphibian species using a phylogenetic approach. We showed that DD species are much more likely to be under threat than those that have already been assessed. Moreover, the geographical distribution of DD species that are predicted to be threatened is in most cases similar to that of assessed threatened species. This may have important implications for global amphibian conservation strategies.

According to this study, it is extremely likely that well over 750 additional amphibian species (48% of the data deficient species) are threatened with extinction. From this, 100 species are likely to be Endangered or Critically Endangered. These results are similar to those found by Howard and Bickford (2014) and Morais *et al.* (2013). Both these studies showed that the percentage of data deficient species that are threatened with extinction is higher than that of their fully assessed counterparts. Our results, however, are slightly more optimistic, with 48% of species predicted to be threatened versus 63% (Howard and Bickford 2014) and 57% for Brazilian species (Morais et al. 2013). However, the previous studies did not use phylogenetic information. It has been shown that amphibian declines exhibit taxonomic and regional patterns (Stuart et al. 2004). It was, thus, very important to consider both phylogeny and range size. We also found a strong phylogenetic signal for most of the traits used (Appendix IV, Table S1). Indeed, the lower percentage of threatened species that we found compared to other studies could be partially due to the phylogenetically related IUCN category across species, as has been shown for mammals (Jetz and Freckleton 2015).

We found that most of the species predicted to be threatened are located in the Neotropics, the Central Africa region and Southeast Asia. This is similar to that found in other studies, even if this study analysed 14% more amphibian species compared to previous studies (Howard and Bickford 2014). The Neotropics encompass more than half the world's amphibian species, with Brazil and Colombia holding the highest global amphibian richness — 1046 and 780 respectively (AmphibiaWeb 2016). Currently, the top five countries in this regard are all located within the Neotropics (AmphibiaWeb 2016).

The largest amount of threatened amphibians, both assessed and predicted, are also located in the Neotropics (Latin America). One of the main reasons for this high rate of threatened species is deforestation. Land-use change is currently the largest threat for amphibians (CBD 2014). The tropical Andes has lost about 75% of its original intact forest coverage, while a large part of its enduring forest landscapes are severely fragmented (Amézquita et al. 2016). Moreover, the tropical Andes is considered the most critically important and most endangered of the world's Hotspots. They contain few protected areas (with only 16% of the remaining 25% of original area protected) and limited forest connectivity (Amézquita et al. 2016). However, assessed and predicted threatened species in this region have similar geographic ranges (Fig. 4.4). Thus, conservation initiatives in the Neotropics aimed to protect current threatened species would have a positive effect on predicted threatened species.

In the Central Africa region, assessed and predicted threatened amphibian geographic ranges do not overlap as well as in the Neotropics. The presence of predicted threatened species in this central region might be caused by high inaccessibility caused by human conflict and political isolation (AmphibiaWeb 2016). The next area of concern is Southeast Asia, where countries like the Philippines have 49% of its amphibians listed as Threatened, and Sri Lanka has almost 70% (AmphibiaWeb 2016, IUCN 2017). Also, countries such as Papua New Guinea and Thailand have a low number of threatened species but this is probable only due to a lack of data (Fig. 4.4). Interestingly, Papua New Guinea and Sri Lanka are among the top four countries for new species discovery, along with Brazil and Peru (AmphibiaWeb 2016).

Amphibians continue to have a high rate of newly discovered species suggesting that amphibian true richness is higher than we currently estimate (Glaw and Köhler 1998, Köhler et al. 2005). Currently, the rate of new amphibian discoveries is much higher than any other vertebrate group (Parra et al. 2007). During the last few months approximately 50 new amphibian species have been described and today there are 7720 amphibian species described in the world (AmphibiaWeb; 12 September 2017). Moreover, the range sizes of newly described species is less robustly defined than the range size of the species described a long time ago (Morais et al. 2013). Because geographic range size is highly correlated to threatened status (IUCN 2017), there could be a relation between newly described species and threatened status worth studying in the future.

The number of threatened amphibian species continues to rise while amphibian declines continue worldwide (Pounds et al. 2006). These results agree with the global consensus that species most at risk are in the tropics (Fig. 4.2 and Fig. 4.4)(Collins and Halliday 2005). Amphibian declines must be understood and quickly reversed or hundreds of amphibian species will become extinct over the next few decades (Stuart et al. 2004). Among other pressures, climate change has been proven to be a key factor in the disappearances of many species due to interaction with other factor (e.g. chytrid fungus) (Pounds et al. 2006, Hof et al. 2011). Amphibian threats are likely to be regionally located as the highest impacts of land-use change and climate change coincide (Hof et al. 2011). Regions with the highest species richness are also more affected by threats than low richness areas (Hof et al. 2011). Unfortunately, the regions of the world that harbour the highest amphibian richness are also experiencing the greatest rates of forest loss and increases in human population size, as well as greater fertiliser use, agricultural production, creation of new croplands and irrigation (Collins and Halliday 2005).

In this study, we used ecological traits and range size, whose strong importance for predicting threat status has previously been illustrated (Sodhi et al. 2008). Thus, quantifying the influence of traits on extinction risk was not the scope of this research. The general aim was to demonstrate that information could be available to make initial predictions about the probable conservation status of species that have not been formally assessed. Our predictions represent a very large increase of amphibian species at risk compared to the IUCN data. Many new species could thus be added to the threatened categories of the Red List, with potentially great consequences for geographical conservation prioritisation. Despite the methods, there is a consensus that while the fate of DD species is uncertain, they are currently under high pressure, have high extinction risk and require urgent attention (Morais et al. 2013, Howard and Bickford 2014, Nori and Loyola 2015). We need to move quickly to consider amphibians as a high conservation priority and integrate DD species into conservation strategies.

Chapter VI

General discussion

The ongoing extinction crisis shows that biodiversity cannot support current human pressures (IUCN 2017). The incidence of irreversible extinctions continues to grow, with an average loss rate of up to 100 times higher than the background rate for vertebrate species (Ceballos et al. 2015). The loss of biodiversity and natural systems also represents a serious threat to our health and wellbeing by threatening the ecosystem services they currently provide for free. For example, mangrove forests provide coastal protection from flooding and erosion caused by sea-level rise (IUCN 2017). Nonetheless, contemporary societies continue to be built on the exploitation of the natural environment, so what is the best way to protect biodiversity? The main issue in solving this problem is that there is no straightforward solution (DeFries and Nagendra 2017). Ecosystems are inherently complex and it is close to impossible to foresee the consequences of any interventions across spatial, temporal, and administrative frameworks (DeFries and Nagendra 2017). For example, a forest provides different services depending on the spatial scale considered. At a local level, a forest provides services such as timber for cooking, bush meat and drinking water. From a more global perspective, a forest could be more valuable for its aesthetic value, flood protection and carbon sequestration. With such discrepancy, how are we going to solve the biodiversity crisis?

To solve this crisis, it is not enough for governments to pledge to "achieve a significant reduction in the rate of biological diversity loss by 2010", and then not meet the pledge (CBD 2017). Solving a global crisis requires a global approach. Not only is it necessary to reduce the rate of biodiversity loss, extinction needs to be stopped altogether while restoring the ecological integrity that has been damaged across all ecosystems. In this sense, secondary forests can play a key role in the restoration of ecosystems. Fortunately, the regrowth of secondary forest on abandoned farmlands is prevalent across the tropics, and these forests have been shown to be important for biodiversity and carbon stock recovery (Gilroy et al. 2014). In Chapter II, I show that as abandoned lands regenerate into forests, their functional value also increases through the accumulation and diversification of critical microhabitats. These microhabitats are vital for the recovery of biodiversity. Even in cases where different forest types have different microhabitat composition, over time secondary forests can approach primary forest levels of microhabitat complexity. This high abundance of microhabitats increases landscape resilience by returning structure to forests and, as such, aids in the recovery of ecological integrity (Ruiz-Jaen and Aide 2004). Additionally in Chapter II, I show that these recovered microhabitats buffer against low and high temperature extremes in a similar manner to those in primary forest. As such, they serve as thermal buffers that reduce exposure to extreme temperatures, which is extremely valuable considering global warming is another major pressure on biodiversity (CBD 2014). Moreover, these benefits exist despite ambient temperatures in secondary forests surpassing those of primary forests by 1-2^{o}C on average. The protection of secondary forest and promotion of further forest regrowth in the Tropical Andes should represent an urgent investment for conservation. Worldwide, the critical role of secondary forests should not be overlooked as we seek solutions to the biodiversity crisis, both now and under future climate change.

Land-use change is currently the major threat to amphibians; however, the threat of climate change is expected to be even greater. We know that amphibian thermal responses to climate change are limited and evolutionarily constrained (Chapter III). Our results concur with several studies showing that CT_{max} is a stable evolutionary trait in ectothermic vertebrates rather than being determined by ecological filtering, dispersal or local adaptation (Labra et al. 2009, Bonino et al. 2011, Araujo et al. 2013, Grigg and Buckley 2013, Muñoz et al. 2014, Muñoz et al. 2016). This suggests that the evolution of CT_{max}, even at a local spatial scale, follows a model of phylogenetic inertia, rather than a model free from phylogenetic associations. Moreover, evolutionary responses are slow, hence species might not be able to evolve higher CT_{max} rapidly enough to meet the pace of environmental warming (Grigg and Buckley 2013, Muñoz et al. 2016). Therefore, we need to make additional efforts to minimise the negative influence of other factors, especially land-use change via deforestation and degradation, so as to ensure that ecosystems are less vulnerable and more resilient to the increasing threat posed by climate change (IUCN 2017). By protecting secondary forests, which are expected to become the most widespread land-use type in the near future (Hurtt et al. 2011), we are protecting the thermally buffered microhabitats that will be crucial under climate change.

The rate of warming is accelerating and extreme weather events (e.g., droughts and heat-waves) have become more frequent and intense (IPCC 2014). Amphibians will be greatly affected by this as they are highly sensitive to temperature change (Deutsch et al. 2008). For example, sensitivity to temperature can be greater in high-altitude tropical species versus lowland species, and in species living in forests versus pasture (Garcia-Robledo et al. 2016, Nowakowski et al. 2016). Determining which species currently live closest to their CT_{max} will help predict how global warming will affect where species occur and how well they perform in warmer ecosystems. Unfortunately, *Pristimantis* species have minimal potential to locally adapt their physiology to keep pace with changes in temperature, shown by their evolutionary constrained CT_{max} (Chapter III). Our results showed that this group of amphibians has limited adaptive potential in terms of its physiological plasticity, thus they are unlikely to increase their upper thermal limits via plastic responses and evolutionary responses may not be fast enough to keep pace with global warming, hence they might be in peril of overheating under climate change (Araujo et al. 2013, Muñoz et al. 2016). *Pristimantis* is the most speciose genus of frogs, and species from this genus might share this high vulnerability to global warming, irrespective of their habitats and elevations (Chapter IV). Fortunately, microhabitats contribute to reduce their exposure to hostile temperatures. This means that by protecting or enhancing microhabitat recovery, we can reduce climate exposure, as long as future temperature increases do not surpass +2°C. This would allow species to avoid changing their distributions, contrary to the case for a large number of species such as butterflies and mammals, which are currently shifting their ranges to track optimal climates (Parmesan and Yohe 2003, Hickling et al. 2006).

Even when a large number of species are either moving towards the poles or to higher elevation, shifts in ranges for amphibians are scarce (Hickling et al. 2006). This may be due to the low dispersal ability of most frogs and their small home ranges (Ovaska 1992, García-R et al. 2005). Tracking their optimal climate is thus quite unlikely for most amphibian species. Accordingly, amphibians are likely to remain *in situ* or move at a very slow pace. Therefore, implementing networks of protected areas that are also climate resilient could be one of the most cost-effective solutions for *in situ* conservation of species and ecosystems. Connectivity strategies should account for: a) possible land-use changes in the region; b) species richness and gene pools; and c) climate change scenarios to also create future climatic connectivity.

One way to ensure future climatic connectivity is to consider the thermal tolerance of species and to create connectivity based on their physiology. CT_{max} can provide this valuable information. Acquiring the maximum thermal tolerance of different species along with climate change predictions will be of great use. We must, however, remember that CT_{max} varies across species and sometimes within individuals, thus, climate change will affect species in different ways. Efforts continue to acquire thermal tolerances for more species, but for species for which we currently have CT_{max} data we can already start building future climatic connectivity maps.

Nonetheless, there is still some discussion regarding the accuracy and utility of CT_{max} in conjunction with climate change predictions. In small populations of Drosophila, for example, experiments indicate a reduced adaptive response under heat stress (Willi and Hoffmann 2009). Studies like this provoke ongoing discussions as to whether environmental conditions can trigger cross-generation effects that help in adaptation processes (Hoffmann, Chown et al. 2013).On the other hand, one of the stronger studies supporting the worthiness of CT_{max} is by Sunday et al. (2014). They compiled thermal tolerance limits of a wide range of amphibian and reptile species across different latitudes and elevations. They showed that CT_{max} varied slightly with latitude and showed a dome-shaped relationship peaking between 20° and 40°. However, they found a strong relationship when considering CT_{max} and operative temperatures, suggesting that species' vulnerability is strongly related to the temperatures that the individuals are exposed to, which concurs with my results from Chapter III and IV. Sunday et al. (2014) also showed that most ectotherms do not have a physiological thermal-safety margin and thus they will have to rely on behaviour to avoid overheating during periods of greatest warmth. For amphibians to be able to use thermoregulatory behaviour, microhabitats and microclimates must be readily available (see Chapter II).

Managing the landscape by preserving and re-connecting primary forests and allowing the natural regrowth of secondary forests over longer timeframes would create larger areas of potential suitable environments and thermally buffered microhabitats. This can aid in the survival of amphibians, of which *c.* 41% of assessed species are currently considered at risk of extinction via the combined effects of climate change and deforestation (IUCN 2017).

The impacts of land-use change and global warming will continue to create major challenges for species survival. For amphibians specifically, one of the biggest challenges for their conservation is the lack of information, with *c.* 25% of amphibians being data deficient (DD). Multiple variables affecting their biological responses - such as deforestation, increasing temperatures and decreasing precipitation - increase the uncertainty and complexity of predicting their extinction risk (Clusella-Trullas et al. 2011). Currently, almost half of the current DD species are likely to be threatened with extinction (Chapter V), which is higher than their assessed counterparts (IUCN 2017). This means that DD species require urgent conservation. It is critical that DD species are not ignored in conservation threat assessments or global amphibian conservation strategies.

Considering the findings of chapter V and in order to protect amphibians globally, regions such as the Neotropics and some areas in Central Africa and Southeast Asia should be high conservation priorities. The Neotropics and Southeast Asia have the highest rates of threatened amphibians, both assessed and predicted. Moreover, many areas on Central Africa, Asia and Latin-America have been largely inaccessible due to human conflict and political isolation. Furthermore, within the Neotropics, the Andes have had a very high rate of deforestation, losing 75% of their historical forest cover (Mittermeier et al. 2004), while Southeast Asia has the highest relative rate of deforestation of any major tropical region (Achard et al. 2002). Creating forest connectivity across these regions by protecting both primary and secondary forests would be the best strategy for protecting global amphibians.

Integrating ecosystem services and socioeconomic challenges

A core mechanism for achieving the desired protection is likely to be payments for ecosystem services (PES), which aim to preserve or maintain the ecosystem services provided by natural resources (Smith et al. 2013). On both local and global scales, such schemes are designed to offer payments to people or nations to retain natural habitats that provide positive externalities for the benefit of people who do not presently pay for them (UN-REDD 2017). While there are still many inherent problems within PES such as diverted funds, for the last couple of decades PES programs have been increasingly used as conservation instruments around the world (Chan et al. 2017). Probably the most advanced, and of particular importance, are carbon-based PES schemes. These include REDD+, which is a key mechanism for slowing the rate of global climate change (UN-REDD 2017). As such, these schemes can pay to protect existing forests from deforestation and degradation and to recover forests where they have been lost.

In Chapter II, I show that old secondary forests have high carbon storage potential. The high correlation between carbon stocks and forest age suggests positive carbon-microhabitat co-benefits as forests regrow. As such, investing to protect or enhance carbon stocks in secondary forests under carbon-based payments for ecosystem services (PES, e.g., REDD+) will create protected areas and have strong positive effects on the abundance of thermally buffered microhabitats and biodiversity recovery.

In regions where carbon-based payments cannot be implemented in primary or secondary forests, a particularly effective strategy is to cheaply purchase forests or land that has been heavily degraded via intensive logging or abandoned following farming. This would work as the protection or promotion of secondary forest regrowth is often a more economically feasible strategy of protecting microhabitat rich and climate change resilient forests (Fisher et al. 2011). By allowing the regeneration of secondary forests, we would be protecting biodiversity, ecosystems and therefore ecosystem services. For example, implementation of conservation actions such as those carried out by the charity World Land Trust (WLT) would be particularly beneficial as their strategy focuses on the conservation of threatened habitats through land purchase. This is an effective strategy as funding the purchase or leasing of threatened land to create nature reserves will in turn protect habitats, their wildlife and the ecosystem services they provide (WLT 2017).

The main problem under this strategy is that protected areas do not usually work in the context of social conflicts. Protected areas are more likely to be stable if they enjoy local support and the cooperation of communities living around them (Peres 1994). Through chapters II to IV, I looked at the interaction between land-use and climate change and their current and future negative effects on amphibians. In these chapters I tackled the land-use and climate change problems in a relatively simple way in comparison to their complexity. There are multiple problems bombarding ecosystems daily, including the pressure that people put on them for flood protection, carbon sequestration, drinkable water, etc. This demand for resources continues to grow and unfortunately that which is biologically required may not be socially acceptable (DeFries and Nagendra 2017). To tackle a complicated problem such as the biodiversity crisis, the first step is to gather useful data. In this thesis, I aim to create useful information regarding the main current and future pressures on amphibians, both at a local and global scale, in the hope that this can help underpin or inform strategies for amphibian conservation.

Human societies will continue to depend on the services that ecosystems provide: provisioning (e.g., food), regulating (e.g., water filtration and decomposition of wastes), supporting (e.g., soil formation), and cultural (e.g., recreation)(MEA 2005). However, if we continue with the current elevated extinction rate, in as little as three human lifetimes we will be deprived of critical ecosystem services (Ceballos et al. 2015). Ecosystems have very complex relationships between their ecological and social elements. Because of this, well-meaning conservation interventions can unfortunately have negative outcomes (Larrosa et al. 2016, Lim et al. 2017). Thus, how human reactions and ecosystem dynamics will affect conservation outcomes is hard to predict (Larrosa et al. 2016). To avoid the collapse of ecosystem services, rapid global efforts are required to conserve already threatened species and to mitigate pressures on biodiversity and ecosystems – mainly habitat loss, climate change, invasive species, overexploitation and pollution (CBD 2014). All of these have social aspects, with population growth increasing the demand for resources. Although the window to take action is closing, now more than ever global strategies can be planned and global actions can be taken to stop extinctions and restore the ecological integrity of ecosystems.

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Appendix I

Table S1. Number of iButtons placed in primary forest (PF), Old (19- 35 yr) secondary forest (OSF), and young (4 - 18 yr) secondary forest (YSF) in two sites in the Colombian Andes.

	hygrochron	canopy	ambient	bromeliad	tree fern root	tree hole	leaf litter	soil
PF	3	4	12	12	12	12	12	2
OSF	4	4	10	9	10	10	10	2
YSF	2	4	11	5	11	11	11	5

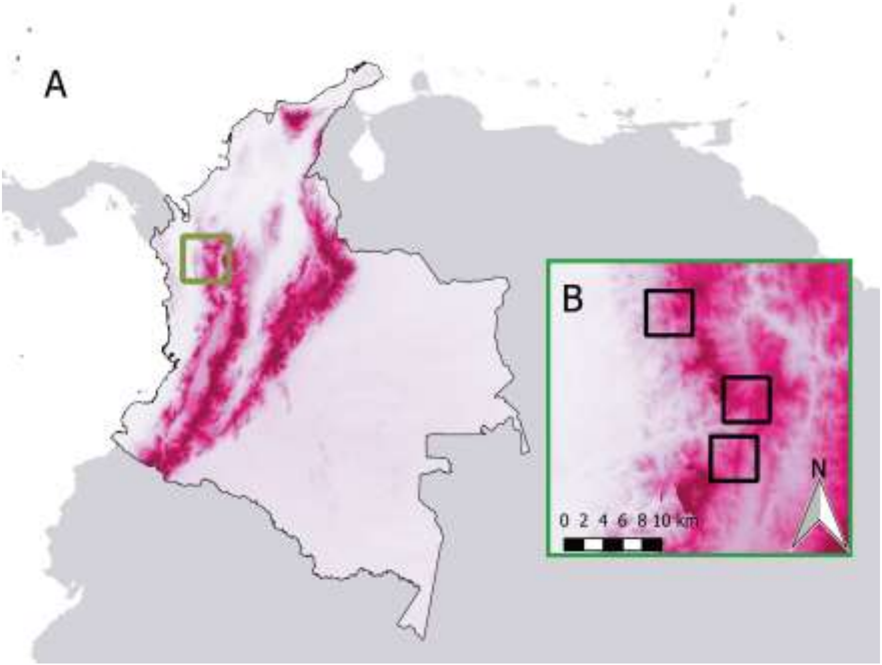

Figure S1. (A) Colombian Tropical Andes and (B - green box) our study area. Our study sites are enclosed by black boxes. Colours represent elevational gradient, with darker colours representing higher elevations.

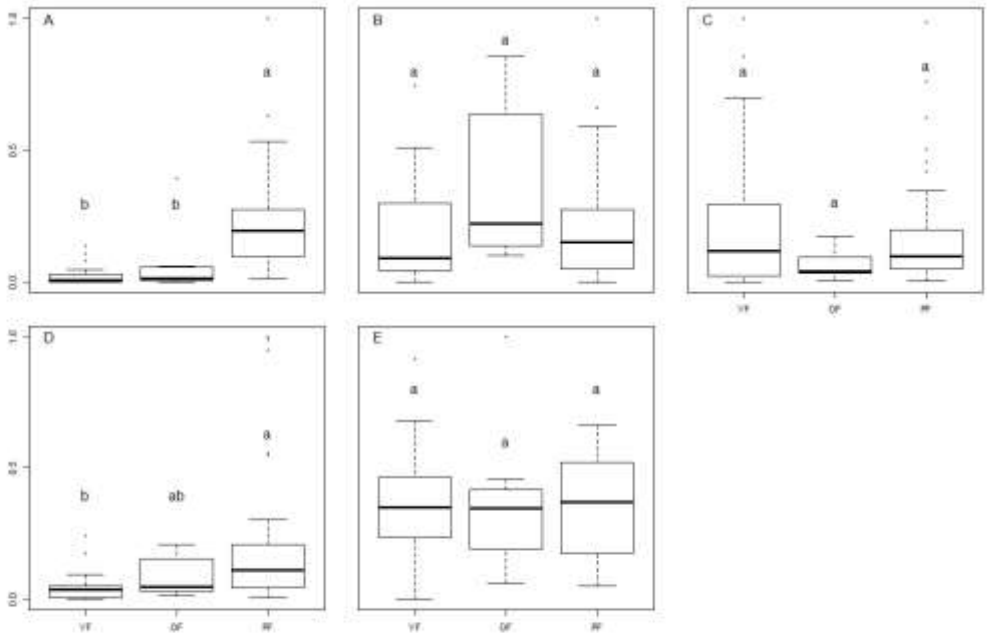

Figure S2. Microhabitat abundance (from normalized data) across young secondary forest (<19 yr old), old secondary forest (19-35 yr old) and primary forest for bromeliads (a), tree ferns (b), moss (c), deadwood (d) and leaf litter (e). The bottom and top of the boxes represent the first and third quartiles, respectively, the bold line represents the median, and the points represent outliers. Similar superscripts represent no significant differences (p<0.05).

Appendix II

Table S1. Total number of individuals collected per species and habitat type. CP: cattle pasture; YSF: young secondary forest; OSF: old secondary forest; PF: primary forest.

	CP	**YSF**	**OSF**	**PF**
P. achatinus	13	0	0	0
P. baiotis	0	0	1	0
P. brevifrons	0	0	1	2
P. calcaratus	0	0	0	15
P. erythropleura	6	12	1	0
P. juanchoi	0	0	4	7
P. kelephus	0	0	0	6
P. myops	0	0	1	1
P. orpacobates	0	20	13	21
P. ptochus	0	0	1	5
P. quantus	0	0	8	32
P. zophus	0	3	8	41
Total number of individuals	19	35	38	130

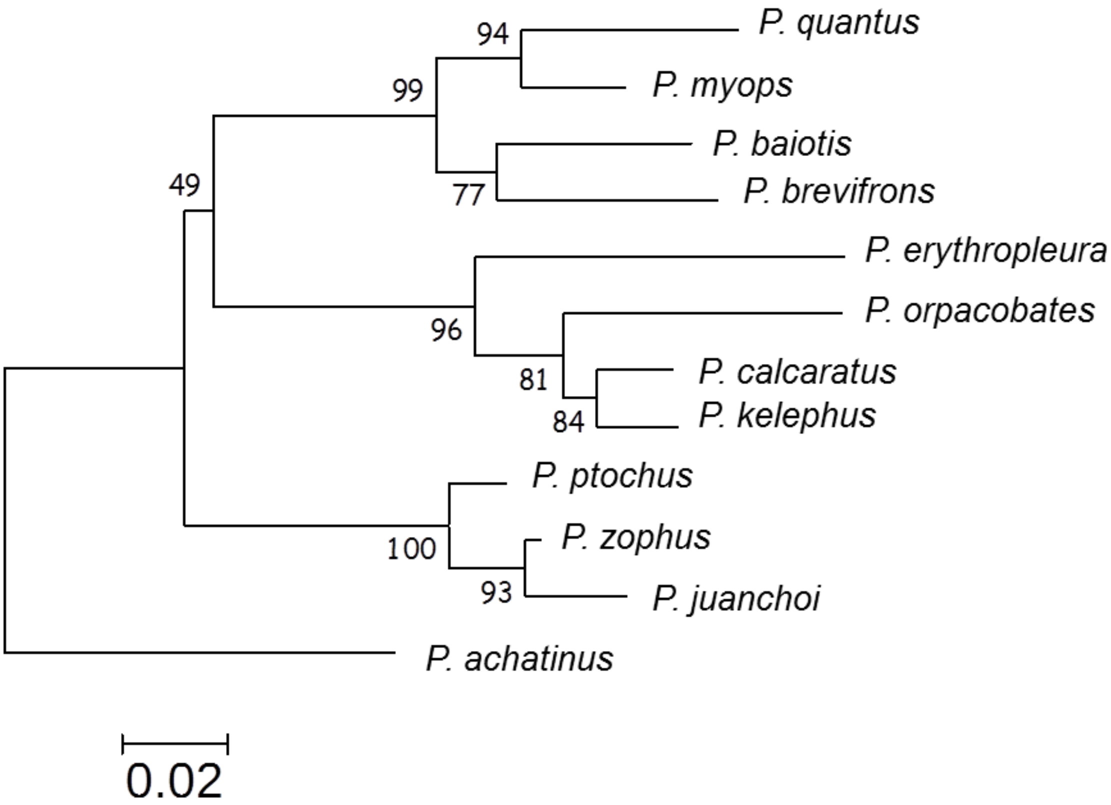

Figure S1. *Pristimantis* frog phylogeny with branch length and *bootstrap values per branch.*

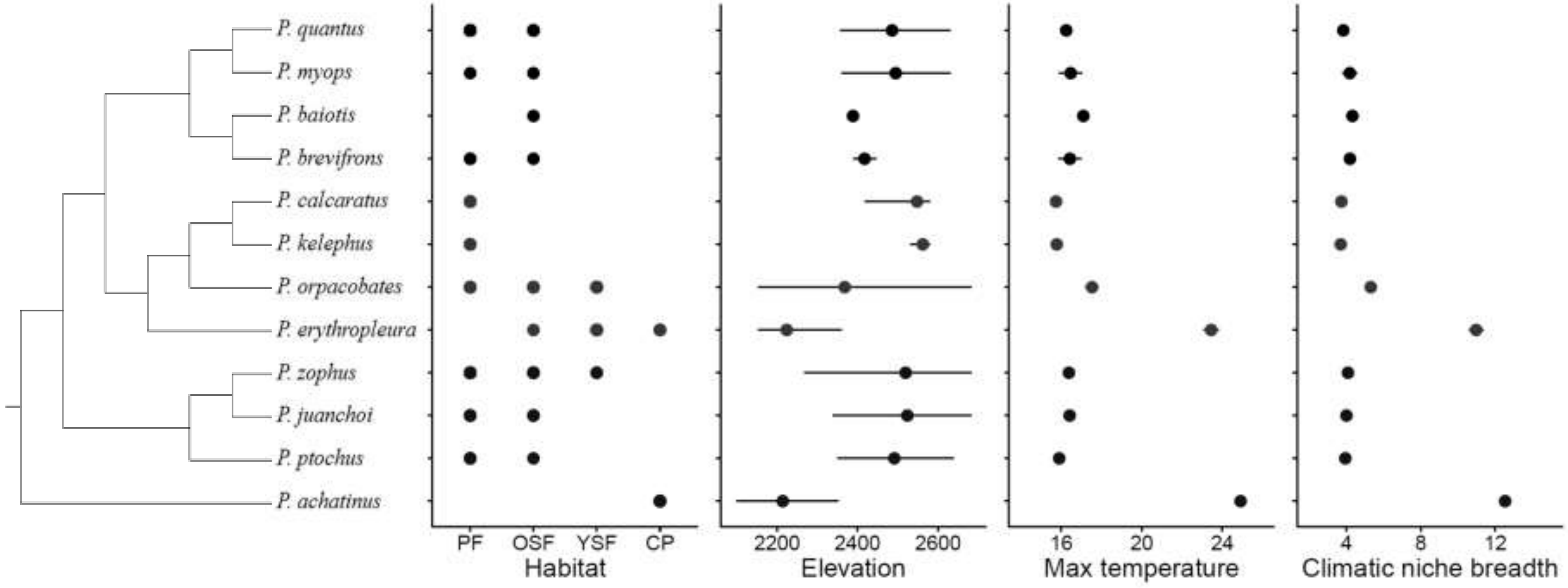
P. quantus
P. myops
P. baiotis
P. brevifrons
P. calcaratus
P. kelephus
P. orpacobates
P. erythropleura
P. zophus
P. juanchoi
P. ptochus
P. achatinus
PF
OSF
YSF
CP
Habitat
2200
2400
2600
Elevation
16
20
24
Max temperature
4
8
12
Climatic niche breadth

Figure S2. *Pristimantis* frog phylogeny, their habitat, mean (colour point), minimum and maximum (black line) elevation where they were collected, mean ± SE daily maximum temperature they experience and mean ± SE climatic niche (calculated as maximum ambient temperature minus minimum ambient temperature). Colour of the points represents their position in the phylogeny on the left. PF: primary forests; OSF: old secondary forests; YSF: young secondary forests; CP: cattle pasture.

Appendix III

Table S1. Summary of phylogenetic effect variances from the linear mixed effect models (z values and p values shown in Table 3.2) and lambda values for current warming tolerance of *Pristimantis* species in canopy, understorey ambient and different microhabitats across young, old secondary and primary forests in Tropical Andes. See text for detailed methods.

	Canopy	Understorey	Bromeliad	Tree fern	Tree hole	Leaf litter	Soil
Phylogenetic signal (V)	0.174	<0.001	0.12	0.12	0.12	0.12	0.12
Variation in species means (S)	0.003	0.002	<0.001	<0.001	<0.001	<0.001	<0.001
Error variance (I)	<0.001	0.87	<0.001	<0.001	<0.001	<0.001	<0.001
Lambda (λ)	<0.001	<0.001	0.80	0.63	0.76	0.70	0.71

Table S2. Summary of linear mixed effect models accounting for phylogenetic inertia of current warming tolerance of amphibians in canopy, understorey ambient and different microhabitats across young and old secondary (SEC) and primary forests (PF) in an elevational gradient in Tropical Andes. Bold p values represent significant differences (p<0.05). See text for detailed methods.

	z score SEC vs PF	p value
Canopy	**-32.72**	<0.001
Understorey	**-5.97**	<0.001
Bromeliad	**-3.81**	0.001
Tree fern	**-6.60**	<0.001
Tree hole	**-4.71**	<0.001
Leaf litter	**-5.71**	<0.001
Soil	**-5.59**	<0.001

Table S3. Total number of individuals collected per species and habitat type. YSF: young secondary forest; OSF: old secondary forest; PF: primary forest.

	YSF	**OSF**	**PF**
P. baiotis	0	1	0
P. brevifrons	0	0	2
P. calcaratus	0	0	15
P. erythropleura	10	1	0
P. juanchoi	0	4	7
P. kelephus	0	0	6
P. myops	0	1	1
P. orpacobates	20	10	20
P. permixtus	0	0	4
P. ptochus	0	0	5
P. quantus	0	8	32
P. ruedai	2	2	0
P. sp.	0	2	41
P. zophus	3	6	42
Total number of individuals	35	35	175

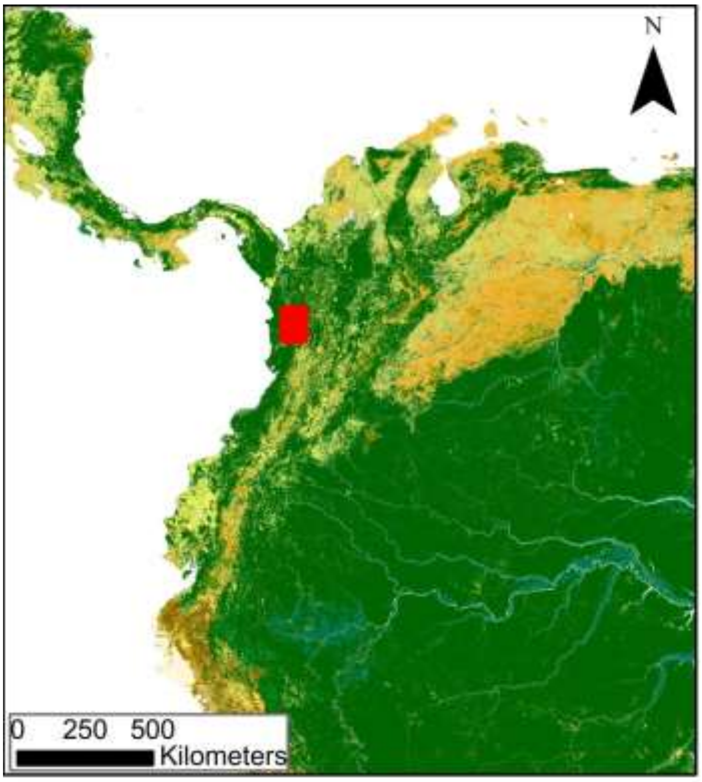

Figure S1. Map showing the extent of habitat conversion in a section of South America including the Tropical Andes. Our study area is denoted with a red box. Colours represent in green: extant natural vegetation, in pale orange: agriculture or other land uses.

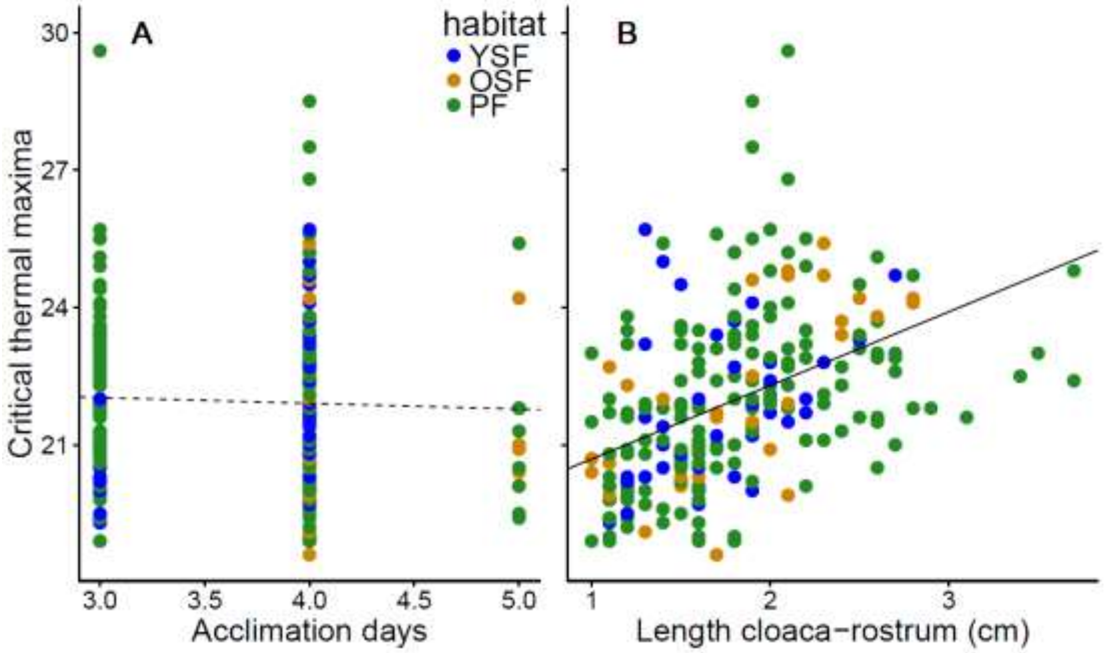

Figure S2. Relationship between critical thermal maxima (CT_{max}) and acclimation days (A), and length of individuals (B), across young secondary (blue), old secondary (orange), and primary forests (green) in the Tropical Andes. Each dot represents one individual. Dashed line represents non-significant relationship ($p>0.05$); solid line represents significant relationship ($p<0.05$)

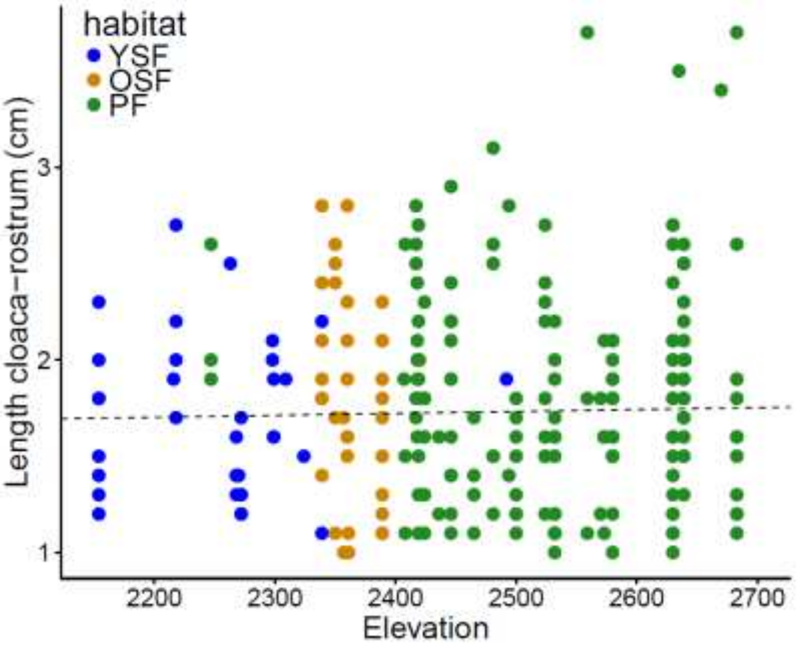

Figure S3. Relationship between length of individuals and an elevational gradient across young secondary (blue), old secondary (orange), and primary forests (green) in the Tropical Andes. Each dot represents one individual. Dashed line represents non-significant relationship ($p>0.05$).

Appendix IV

Table S1. Phylogenetic signal (lambda) for each trait that was used in the analyses. Bold traits were the ones whose values were imputed.

Traits	Lambda
Maximum length	0.75
Minimum length	0.83
Habit	0.83
Fertilization	0.86
Reproduction cycle	0.91
Reproductive mode	0.91
Egg deposition site	0.91
Parental care	0.93
Forest	0.61
Non forest	0.62
Area (km^2), Latitude, longitude	<0.30

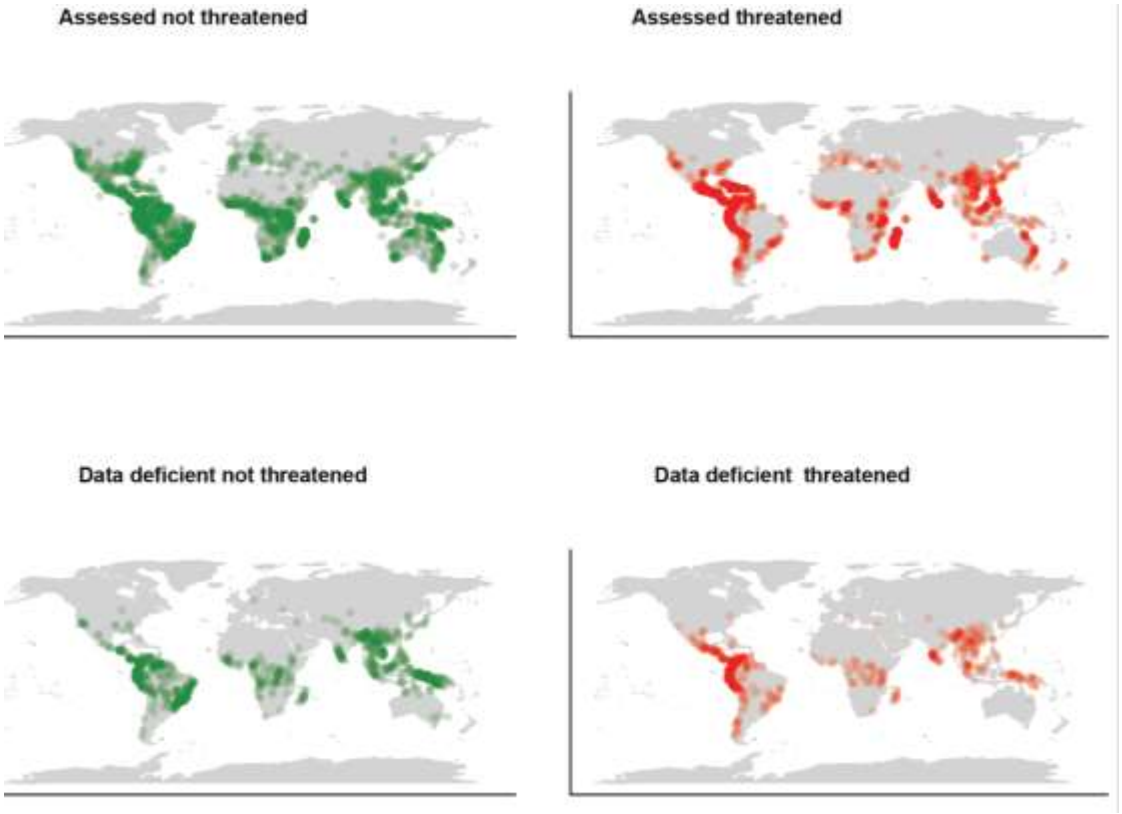

Figure S1. Global distribution of fully assessed (top panels) and data deficient (bottom panels) amphibian species across all the Red List categories. Red dots are the categories of critically endangered (CR), endangered (EN), and vulnerable (VU). Green dots represent the non-threatened species: near threatened (NT) or least concerned (LC).