Thermal adaptation of amphibians in tropical mountains. Consequences of global warming

Adaptaciones térmicas de anfibios en montañas tropicales: consecuencias del calentamiento global

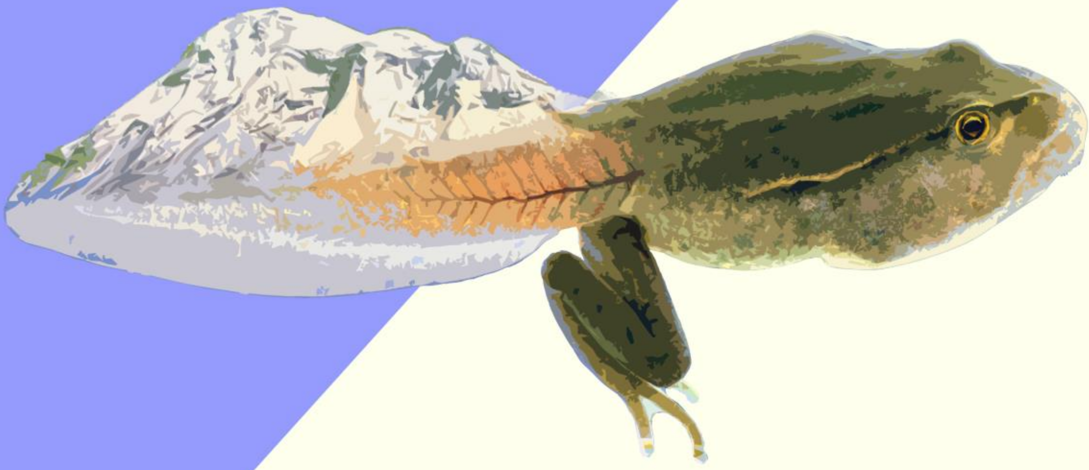
Adaptacions tèrmiques d'amfibis en muntanyes tropicals: conseqüències de l'escalfament global

Pol Pintanel Costa

ADVERTIMENT. La consulta d’aquesta tesi queda condicionada a l’acceptació de les següents condicions d'ús: La difusió d’aquesta tesi per mitjà del servei TDX (**www.tdx.cat**) i a través del Dipòsit Digital de la UB (**diposit.ub.edu**) ha estat autoritzada pels titulars dels drets de propietat intel·lectual únicament per a usos privats emmarcats en activitats d’investigació i docència. No s’autoritza la seva reproducció amb finalitats de lucre ni la seva difusió i posada a disposició des d’un lloc aliè al servei TDX ni al Dipòsit Digital de la UB. No s’autoritza la presentació del seu contingut en una finestra o marc aliè a TDX o al Dipòsit Digital de la UB (framing). Aquesta reserva de drets afecta tant al resum de presentació de la tesi com als seus continguts. En la utilització o cita de parts de la tesi és obligat indicar el nom de la persona autora.

ADVERTENCIA. La consulta de esta tesis queda condicionada a la aceptación de las siguientes condiciones de uso: La difusión de esta tesis por medio del servicio TDR (**www.tdx.cat**) y a través del Repositorio Digital de la UB (**diposit.ub.edu**) ha sido autorizada por los titulares de los derechos de propiedad intelectual únicamente para usos privados enmarcados en actividades de investigación y docencia. No se autoriza su reproducción con finalidades de lucro ni su difusión y puesta a disposición desde un sitio ajeno al servicio TDR o al Repositorio Digital de la UB. No se autoriza la presentación de su contenido en una ventana o marco ajeno a TDR o al Repositorio Digital de la UB (framing). Esta reserva de derechos afecta tanto al resumen de presentación de la tesis como a sus contenidos. En la utilización o cita de partes de la tesis es obligado indicar el nombre de la persona autora.

WARNING. On having consulted this thesis you’re accepting the following use conditions: Spreading this thesis by the TDX (**www.tdx.cat**) service and by the UB Digital Repository (**diposit.ub.edu**) has been authorized by the titular of the intellectual property rights only for private uses placed in investigation and teaching activities. Reproduction with lucrative aims is not authorized nor its spreading and availability from a site foreign to the TDX service or to the UB Digital Repository. Introducing its content in a window or frame foreign to the TDX service or to the UB Digital Repository is not authorized (framing). Those rights affect to the presentation summary of the thesis as well as to its contents. In the using or citation of parts of the thesis it’s obliged to indicate the name of the author.



Thermal adaptation of amphibians in tropical mountains

Consequences of global warming

Pol Pintanel

Tesi doctoral 2018

Thermal adaptation of amphibians in tropical mountains

Consequences of global warming

Adaptaciones térmicas de anfibios en montañas tropicales

Consecuencias del calentamiento global

Adaptacions tèrmiques d'amfibis en muntanyes tropicals

Conseqüències de l'escalfament global

Pol Pintanel

Tesi doctoral

2018

UNIVERSITAT DE BARCELONA

Departament de Biologia Evolutiva,
Ecologia i Ciències Ambientals

Estación Biológica Doñana
CSIC

Department of Evolutionary Ecology

Thermal adaptation of amphibians in tropical mountains

Consequences of global warming

Memòria presentada per **Pol Pintanel Costa** per optar

al grau de **Doctor** per la **Universitat de Barcelona**

Programa de doctorat en Biodiversitat

El doctorand:
Pol Pintanel Costa

El director de tesi:
Miguel Tejedo Madueño

El tutor i codirector de tesi:
Gustavo A. Llorente Cabrera

Barcelona, 2018

Aquesta tesi ha estat desenvolupada parcialment entre el Departament de BEECA de la Facultat de Biologia de la Universitat de Barcelona, el Departament de Ecologia Evolutiva de la Estación Biológica de Doñana (EBD-CSIC) i amb el suport del projecte la ‘Balsa de los Sapos’ i el Laboratori d’Ecofisiologia tèrmica a la Pontifícia Universidad Católica del Ecuador.

Part del treball va ser finançat per la beca MAEC-AECID de la Agencia Española de Cooperación Internacional para el Desarrollo durant l’any 2015-2016.

Acknowledgements

Agradecimientos, Agraïments

Muchos de los que están leyendo esto no avanzarán en profundizar más en la tesis. Creo que no soy el único que cuando abro una tesis lo primero que hace es mirar los 'dibujitos', para luego acabar curioseando en esta interesante sección. ¿Qué buscan los lectores cuando leen esta sección de tesis? ¿Su nombre? ¡No, qué va! ¿Anécdotas divertidas que ocurrieron durante la realización de la tesis? ¿Tampoco? Y entonces, ¿qué?

En realidad no parece que exista una ley clara de cómo o qué escribir en agradecimientos de una tesis doctoral, aparte de mostrar gratitud a la gente que te ha ayudado y acompañado este tiempo. Es más, podría –por ejemplo- escribir en Comic Sans durante un rato y no creo que nadie diría nada. También alargar los agradecimientos hasta que fueran más largos que la propia tesis (una misión prácticamente imposible para la mayoría de los compañeros herpetólogos de Barcelona en estos últimos años) o que los agradecimientos se tornaran en desagradecimientos. Por ejemplo, quejarme del (no) funcionamiento del aire acondicionado durante este agotador agosto escribiendo la tesis o del ninguneo de las instituciones públicas a los estudiantes de doctorado, especialmente cuando (ya) no disponen de beca.

En este punto el lector puede, simplemente, buscar su nombre (o grupo) en toda esta sopa de letras o aceptar lo que escribo y seguir leyendo. Uno no escribe una tesis doctoral cada año, si no es que es militante de algún partido político con algunos hilillos de corrupción, y, por ello, déjenme hacer el uso que quiera de estas pocas páginas.

Podríamos entender este espacio como parte del nicho biótico que ayuda a definir la tesis realizada. Si siguen leyendo verán que esta es la sección en que más tiempo dedico a interacciones bióticas en gradientes altitudinales y (también) latitudinales. Pensarán que todas las interacciones han sido beneficiosas para dicha tesis, ¡pues no!, p. ej. algunos puntos de muestreo han sido destruidos de forma indirecta (e.g. construcción de carreteras) o directa (intencionadamente) por las acciones humanas y en menor medida, ambientales (¡demasiados aludes en solo dos años!), lo que llevó a la pérdida de puntos de reproducción para anfibios y de algunos (muchos) dataloggers. En un caso un hombre se presentó, machete en mano, para preguntar que estábamos haciendo en su (nueva) piscina para la cría de tilapias. No obstante, dedicaré las siguientes líneas a las interacciones beneficiosas que se produjeron a lo largo de la tesis que, tanto de forma directa como indirecta, han sido imprescindibles.

El lector que no me conozca debe estar empezándose a preguntar ¿Por qué lleva escrita más de una página y aún no ha empezado a agradecer a nadie? A ese lector le diré que suele ser normal que hable demasiado y me vaya un poco por las ramas. También existe un poco de desahogo por mi parte después del estrés acumulado, especialmente estos últimos meses en los que la tesis parecía (de hecho, mientras escribo, aún parece) inacabable, además de cierto temor en dedicar poco o demasiado tiempo a alguien o, directamente, en olvidarme en citarlo y por ello, pido perdón de antemano por cualquier omisión.

Empezaré con los individuos más generalistas, aunque con ello no me refiero a '*jack-of-all-trades a master of none*' (o 'quién mucho abarca poco aprieta'; –para que mis abuelas puedan entenderlo-), si se fijan en el capítulo 3 dicha relación con la *performance* (rendimiento) es inexistente. Me refiero más bien a aquellos que colaboraron a lo largo de toda la distribución espacial de la tesis y que, aunque los agradecimientos tal vez les sean redundantes es imperativo hacerlos. Por lo tanto, no podría sino empezar con Miguel Tejedo, quién me brindó la oportunidad de empezar este bonito, pero estresante -¿pero bonito? Sí-, pero un poco estresante viaje. En este caso, a diferencia de otras tesis, el viaje no solo fue metafórico, me atrevería a decir que durante estos años de tesis he recorrido lo equivalente a tres vueltas enteras al mundo; entre Quito, Barcelona y, aunque menos, Sevilla. Fue de hecho MT durante alguno de los viajes -también los metafóricos-, quién me aconsejó la lectura de una sección que, al menos yo, suele pasar por alto cuando tiene que revisar literatura científica, el prefacio, concretamente del libro Aerografía de E. H. Rapoport (1975), con el que un servidor se ha basado para empezar estos agradecimientos. Me gustaría agradecer también a Gustavo Llorente, el director (y tutor) críptico: tiene una distribución temporal amplia en la universidad pero solo los más expertos consiguen encontrarlo. No obstante, en las tardes, existe una franja temporal en las que es posible reunirse con él para para hablar y discutir, siempre de ciencia claro. Por último, dentro de este párrafo dedicado a la dirección de la tesis -¿Aún no había quedado claro?- me gustaría dedicar unas palabras a Andrés Merino, el director fantasma, no hagan aún ninguna conjetura a partir de ese adjetivo. Me refiero a que, aunque no es oficialmente director, su incansable trabajo lo ha hecho imprescindible para la realización de la tesis, tanto como investigador como en la gincana que representa la burocracia en Ecuador.

Continuant cronològicament amb els companys i amics de Barcelona i voltants. Als de l'institut Montserrat; Sergi, Edu, Saldaña, Simó, Gina, Isabel, Judit, Andrea i Joan i també de els de Móra; Leo, Loscos, Pep, Manelet, Silvia i tants d'altres. Sempre escoltant a un servidor –tampoc era per tant- parlar de les 'boles saltarines' i els seus 'CROACKs'. Als companys de la carrera de biocoses; Eudald, Roger, Youssef, Clau, Marta, Rosa, Sayol, Zango... on parar? Tanta gent, tant de temps! Als herpetòlegs; l'Eudald i el Feeliiipe (ha de que sonar com "Mi, Do#, La" amb tempo *Grave*, sinó, no funciona) per tants moments al despatx de feina i divagar, pels litres de cervesa i cafè i per la barbacoa que ens farà un dia el Felipe! Aquí també he d'afegir als iaio-rana, Mark, Olatz, Núria, Montori i Xavi, i a les noves incorporacions paraguaies -¿para qué? paraguayes-, Katia i Freddy, i les estudiants que han passat per aquí, a la Xènia i la Mora, per tantes bones estones compartides durant aquests anys. No em puc deixar al 'gordito' que ve per les tardes (casi nit), que segur que serà la primera cosa que busqui a la tesi! –¡puto pelomocho!- pensarà. Mai havia pensat que quedar-se fins tan tard podria ser tan divertit i poc productiu. A la resta de compis del departament de –agafeu aire- Biologia Evolutiva, Ecologia i Ciències Ambientals; Alberto, Cesc, Oriol, Moises, Vir, Leia... impossible nombrar-los a tots i cada un d'ells. Molts ocellaires, no?! Pels dinars i aquells moments de xerrera a l'entrada del despatx.

Llega el momento de agradecer a los panas ecuatorianosf. Tal vez, estimado lector, si no conoce mucho a los quiteños, *de leyff* pensará que la 'f' al final es un error tipográfico. ¡No puesf! Después de tanto tiempo empecé a pensar que lo hacían debido a que están medio locos ¡Qué magníficos locos pero!, ya que alguien cuerdo no viviría a esas altitudes, con terremotos constantes y con volcanes activos a su alrededor*. En realidad, según leí en una nota del Comercio (un periódico Ecuatoriano), parece que deriva de la palabra 'pues'. Así que cuando, estimado lector quiteño, diga 'puesf' está implícitamente repitiendo la palabra 'pues' dos veces. Pero volviendo a lo importante, ir y volver al Ecuador me ha permitido conocer a un montón de magníficas personas de mil y un lugares. Suerte de encontrarme al llegar a los excompañeros de Master, Victor –niño muy bueno- y Nayive y su magnífica familia, Yonny y su hijazo Santiago, y más tarde a la catalanovalenciana Núria, ¡qué tan buenos momentos! ¿Por qué no acampamos aquí en medio de la nieve? Eso también va por Javi y la señorita Torres -*que va a llové*!-.

---

* a A. von Humboldt se le atribuye la frase: '*Los ecuatorianos son seres raros y únicos: duermen tranquilos en medio de crujientes volcanes, viven pobres en medio de incomparables riquezas y se alegran con música triste*'

Mucho párrafo y aún no se ha mencionado a ningún ecuatoriano –pu##o pol-, todo llegará. Se hace complicado intentar juntar tanta gente en un orden espaciotemporal que no acabe colapsando. En fin, agradecer a la gente del museo QCAZ o –vuelvan a tomar aire- de Zoología de la Pontificia Universidad Católica del Ecuador, empezando por su responsable (o *curador*) en el área de anfibios, Santiago Ron, por acogerme junto con Andrés y Omar Torres en su inmenso equipo de herpetólogos. Cuantas bielas y salidas de campo –y algún partido de fútbol a 2500 m- con tantos buenos profesionales. Tantos, que voy a centrarme en los que tuvieron un papel directo en esta tesis; gracias a Yerka, Pollito y Diego que ayudaron en la identificación de las diferentes especies y a Francy Mora, Daniela Pareja, Jambato, Nadia, Javo y Daniel (Perro) que ayudaron en algunas de las diferentes salidas de campo. A Denisse Galarza, su madre y su gatito por esas primeras Navidades. También a todos los compañeros de la 'Balsa de los Sapos' en la misma Universidad, cuya ayuda y compañerismo fue importantísima para avanzar en la tesis; especialmente a Freddy, imprescindible, y su a magnífica familia, también a Flor, Javi, Andrea (Morena) y Tatiana y a los diferentes estudiantes que pasaron por el subsuelo; Majo -¡hija de Colón!-, Paito y sus odonatos, las fotógrafas de renacuajos Gaby y Denisse, David Jácome y Mayra Castro. A mi amigo LuisMi, ha sido un placer trabajar y compartir unos meses contigo. To Phil, I hope we never have to run from the police again.

El Ministerio del Ambiente de Ecuador proporcionó los permisos para realizar este trabajo. Me gustaría agradecer a las personas que trabajan en los diferentes parques naturales que visitamos, tanto públicos como privados, por su ayuda y amabilidad. Una especial mención a Lucero y Medardo por hacer tan perfecta la estadía en Zanjarajuno (Puyo-Tena).

Com diu la dita, *volta el mon però torna al Born*. Per descomptat vull agrair-li a la meva família a qui els hi dec tot.

Finalmente a mi Fofilito, compañera de viaje, despacho, gordura y vida. Por el cranear y el frikear. Pel prou i la miqueta si us plau, con ese cantarcito quiteño. Por darme a conocer una nueva familia. Por hacerme la vida full mejor.

General Abstract

Temperature is likely to be one of the most important abiotic factors given how it affects the physiology of the whole organism and as a consequence, it has an essential role in ecology and evolution. However, how the geographical (and temporal) variation of temperature is related to physiology still raises many questions. Several macrophysiological hypotheses have been proposed to explain the variation patterns of thermal physiological parameters across ecological gradients. Among them, Janzen's (1967) 'seasonality' hypothesis is probably one of the most relevant as it has awakened a great interest in other areas besides physiology itself, such as biogeography, ecology, evolution and conservation biology. Janzen proposed that tropical species are specialists to thermally stable environments and therefore would be more limited to dispersing altitudinally (up or down mountain) than temperate species because of evolved physiological barriers. These biologically based dispersal constraints may be act as a selective mechanism promoting isolation in the populations and thus fuelling speciation rates in tropical mountain ranges, considered the most diverse hotspots in the world.

The present thesis explores the evolution in the thermal sensitivity of amphibians across a broad tropical elevational range (4000 meters) in the tropical Ecuadorian Andes and propose the likely environmental causes (altitude and microenvironment) driving the extraordinary diversity in physiological parameters across the gradient. This information also provides essential insight for predicting which species or populations are most vulnerable to global warming. Through estimates of thermal sensitivity in larval and adult amphibians we show, through comparative methods, how thermal sensitivity and tolerance limits diverge along the elevational gradient. We demonstrate that environmental variation at the individual scale is important when testing some of the main macrophysiological hypotheses being better predictors of thermal physiological diversity in amphibians. We demonstrate that amphibians' thermal physiology is strongly influenced by their thermal environment but also some of its variation may be limited by inherent constraints. A further main finding is the empirical demonstration that physiological barriers in tropical mountains are 'higher' upwards than downwards, refining Janzen´s paradigm that altitude functions as a barrier through cold tolerance whereas warm evolution occurs contrarily only transversally (horizontally) through habitat selection. Finally, this thesis suggests that lowland tropical amphibians are more vulnerable to an increase of temperature than their upland counterparts, because they are currently experiencing environmental temperatures close to their physiological optima and heat tolerance. Yet, the use of microclimatic information predicts how, in some cases, highland species may be also vulnerable to suffer heat stress and will therefore need to search for thermal shelters to avoid extreme heat events.

Resum General

La temperatura es probablement un dels factors abiòtics més importants, ja que afecta la fisiologia de tot l'organisme i, com a conseqüència, té un paper essencial en ecologia i evolució. No obstant això, com la variació geogràfica (i temporal) de la temperatura està relacionada amb la fisiologia encara planteja moltes preguntes. S'han proposat diverses hipòtesis macrofisiològiques per explicar els patrons de variació dels paràmetres fisiològics tèrmics a través de gradients ecològics. Entre elles, la hipòtesi de "estacionalitat" de Janzen (1967) és probablement una de les més rellevants, ja que ha despertat un gran interès en altres àrees més enllà de la pròpia fisiologia, com la biogeografia, l'ecologia, l'evolució i la biologia de la conservació. Janzen va proposar que les espècies tropicals són fisiològicament especialistes ja que viuen en ambients amb temperatures estables i, per tant, estarien menys capacitades que les espècies temperades a dispersar-se altitudinalment (muntanya amunt o avall), a causa de les barreres fisiològiques a les que estan evolutivament limitades. Aquesta restricció en la seva dispersió es considera un mecanisme selectiu que promou l'aïllament de les poblacions i, per tant, incrementa les taxes d'especiació en les zones muntanyoses tropicals, considerades les zones més biodiverses del món.

Aquesta tesi explora l'evolució de la sensibilitat tèrmica dels amfibis al llarg d'un ampli gradient altitudinal tropical (4000 metres) als Andes equatorians tropicals i proposa algunes de les possibles causes ambientals (altitud i microambient) que promouen la extraordinària diversitat en els paràmetres fisiològics. Aquestes dades ens proporcionen, també, una informació essencial per predir quines espècies o poblacions serán més vulnerables a l'escalfament global. Mitjançant estimacions de sensibilitat tèrmica en larves i amfibis adults

mostrem, a través de mètodes comparatius, com els límits de sensibilitat tèrmica (CTmax i CTmin) divergeixen al llarg del gradient altitudinal. Demostrem que la variació ambiental a escala individual és molt important quan es comproven algunes de les principals hipòtesis macrofisiològiques, ja que són un millor predictor de la diversitat fisiològica tèrmica en els amfibis. Demostrem que la fisiologia tèrmica dels amfibis està fortament influenciada per la temperatura a la que estan exposats però, també, algunes de les seves variacions poden estar limitades inherentment. Altres resultats principals són la demostració empírica que les barreres fisiològiques en les muntanyes tropicals són "més altes" cap amunt que cap avall, de manera que es redefineix el paradigma de Janzen: les barreres altitudinals funcionen a través de l'adaptació al fred (per tant cap amunt), mentre que l'evolució al calor es produeix de forma horitzontal, a través de la selecció d'hàbitats. Finalment, aquesta tesi suggereix que els amfibis tropicals de zones baixes, en comparació als de zones altes, són més vulnerables al augment de les temperatures, ja que actualment experimenten temperatures ambientals properes al seu òptim fisiològic (Topt) i tolerància a la calor (CTmax). Tanmateix, l'ús de la informació microclimàtica prediu que, en alguns casos, les espècies d’alta muntanya podrien ser també vulnerables a patir estrès tèrmic i, per tant, hauran de buscar refugi tèrmics per tal d’evitar els extrems de calor.

Table of Contents

General Introduction

Back cover: *Epipedobates darwinwallacei* (Cisneros-Heredia & Yánez-Muñoz, 2010)

General Introduction

Temperature is likely to be one of the most important abiotic factor given how it affects the physiology of the whole organism and thus, has an essential role in ecology and evolution (Angilletta, 2009). However, how the geographical (and temporal) variation of temperature is related to physiology still raises many questions (Chown *et al.*, 2004; Chown & Gaston, 2016). Several hypotheses, both ecological and evolutionary have been proposed to explain the variation patterns of thermal physiology across ecological gradients (summarized in Gaston *et al.*, 2009). Among them, Janzen's (1967) 'seasonality' hypothesis (Ghalambor *et al.*, 2006; Sheldon *et al.*, 2018) is probably one of the most relevant to explain variation in thermal tolerance combining both latitudinal and altitudinal gradients.

In his article, *Why mountain passes are higher in the tropics?*, Janzen (1967) proposed that tropical species would be more physiologically limited to disperse altitudinally than species from temperate zones (Ghalambor *et al.*, 2006). This statement was based on two assumptions: (1) Since temperature decreases with elevation (Dillon *et al.*, 2006) and thermal variation is lower in tropical environments due to a lack of seasonality, temperatures to which organisms are exposed in tropical mountains will overlap in a lesser degree (greater climatic stratification) than in similar altitudinal gradients located in temperate zones (**Fig. I.1**). (2) Organisms, especially ectotherms, will be physiologically adapted to the thermal regimes to which they are exposed to. Therefore, tropical species will have narrower ranges of thermal tolerance (i.e. specialists) than temperate species (i.e. generalists), since they experience less thermal variation.

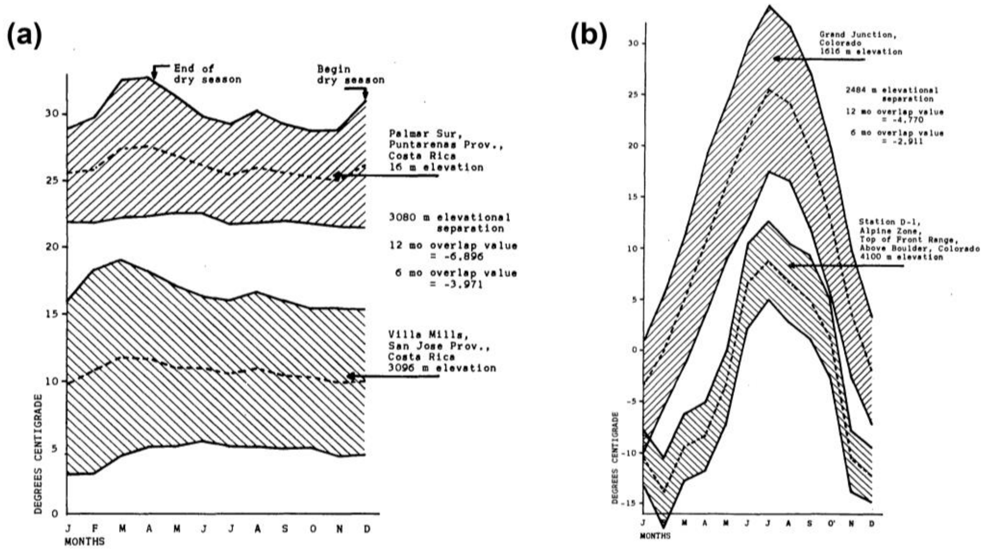


Figure I.1: Representation of two temperature regimes for a (**a**) tropical and (**b**) temperate site. The regimes above and below represent the lowest and highest elevation sites respectively. Solid lines trace the monthly daily maxima and minima. The dotted lines trace the mean temperature (modified from Janzen, 1967).

Janzen’s hypothesis was later on generalized through latitudinal and altitudinal gradients in the 'climate variability hypothesis', which predicts a positive relationship between variation of temperature at which organisms are exposed to and their ranges of thermal tolerance (Stevens, 1989, 1992; Bozinovic *et al.*, 2011). Empirical evidences suggest that these increments in thermal tolerance ranges basically depend on a lesser geographical variation in heat tolerance than cold tolerance (Gaston *et al.*, 2009; Araújo *et al.*, 2013; Bozinovic *et al.*, 2014). This pattern of asymmetric variation in thermal tolerance limits is known as the 'Brett’s rule' (Brett, 1956; Gaston *et al.*, 2009) or the 'heat-invariant hypothesis' (Bozinovic *et al.*, 2014) and has been extensively corroborated in latitudinal (Addo-Bediako *et al.*, 2000; Cruz *et al.*, 2005; Ghalambor *et al.*, 2006; Sunday *et al.*, 2012; Hoffmann *et al.*, 2013) and tropical altitudinal gradients (Gaston & Chown, 1999; Ghalambor *et al.*, 2006; Shah *et al.*, 2017). However, some exceptions have been found for aquatic environments, both marine (Sunday *et al.*, 2012) and terrestrial (Calosi *et al.*, 2010). It has been suggested that these differences in the spatial variation of thermal tolerance could be due to both evolutionary and ecological factors. Heat tolerance could be evolutionarily limited, since it has less genetic variation than cold tolerance (Beacham & Withler, 1991; Blackburn *et al.*, 2014) and lower evolutionary rates (Muñoz *et al.*, 2014; von May *et al.*, 2017). In addition, as the environments are more thermally heterogeneous during daytime than at night (Sarmiento, 1986; Ghalambor *et al.*, 2006), thermoregulation (both passive and active) would be more effective at avoiding exposure to maximum daytime temperatures rather than nocturnal minimums and would, therefore, limit the evolution to tolerate heat ('Bogert effect'; Bogert, 1949; Huey *et al.*, 2003).

Janzen's ideas have also awakened great interest in other areas besides physiology, such as biogeography, ecology, evolution and conservation biology (Bonebrake, 2013; Sheldon *et al.*, 2018). For instance, the limitations to geographical dispersion (or barriers) in the tropics, compared to temperate zones, have been related to lower ranges of geographical distribution with altitude and latitude ('Rapoport's rule'; Stevens, 1989; Stevens, 1992) and with higher speciation rates (Cadena *et al.*, 2012; Gill *et al.*, 2016). In addition, it has been proposed that, since tropical species are physiologically specialized given their presumed adaptation to climate-stable environments, the instability posed by climate change will have stronger deleterious effects on tropical than on temperate organisms (Deutsch *et al.*, 2008; Tewksbury *et al.*, 2008). Thus, in the same way that physiological barriers are 'higher in the tropics' so it would be the effects of climate change (Perez *et al.*, 2016).

At present time, the rapid anthropic climate change is one of the greatest threats posed to global biodiversity and to human being themselves (Thomas *et al.*, 2004; Dawson *et al.*, 2011). Species and populations may respond to the increase in temperatures, which can be summarized by: moving (changes in space or time), adapting (plastic or evolutionary changes) or dying (becoming extinct) (Parmesan, 2006). For example, changes have been noted in phenology (Visser & Both, 2005) and distribution (Chen *et al.*, 2011a; Lenoir & Svenning, 2013) of species in response to reduce exposure to high temperatures. However, in addition to the direct negative effect of increased temperatures on the populations, there are other indirect negative effects associated with climate change (Cahill *et al.*, 2012). For example, higher temperatures can produce changes in biotic interactions (e.g. Seimon *et al.*, 2007; Tylianakis *et al.*, 2008; Schweiger *et al.*, 2012) or even interact synergistically with other anthropic impacts (Hof *et al.*, 2011).

Estimating physiology: Thermal sensitivity

To analyse how the variation of an organism's body temperature describes the performance of any biological function (e.g. locomotion, growth, reproduction), thermal performance curves (TPC) are a very useful tool (Huey & Stevenson, 1979; Angilletta *et al.*, 2002; Angilletta, 2006). The relationship between the performance of the biological function and temperature is characterized by a progressive increase from low temperatures until reaching a maximum (Zmax) at the optimum temperature (Topt). Temperatures above this optimum (usually) produce a drastic decline in performance, producing a non-linear curve with a negative asymmetric shape that should represent organismal thermal sensitivity (**Fig. I.2**). TPC curves provide estimates of several functionally relevant traits; optimal temperature (Topt), maximum performance (Zmax), performance breadth (Bi), thermal tolerance limits (CTmax and CTmin) and the level of performance's asymmetry (e.g. physiological heating tolerance = CTmax – Topt, Payne *et al*. 2016). These physiological parameters can be used, for instance, to analyse how evolutionary and ecological factors modulate their shape and improve predictions of the effects of climate change on organisms, especially those whose body temperature depends on the environment (i.e. ectotherms) (Angilletta *et al.*, 2002; Kearney & Porter, 2009; Sinclair *et al.*, 2016).

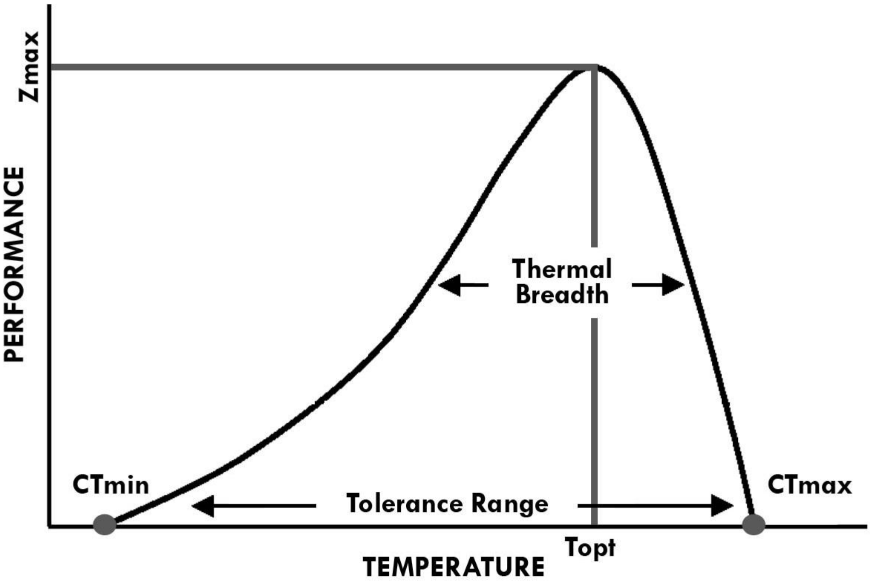


Figure I.2: Representation of the relationship between body temperature and performance in ectotherms (modified from Pintanel *et al.*, 2017). Optimum temperature (Topt) is the temperature that maximizes the performance of the function (Zmax). Critical minimum temperature (CTmin) and critical maximum temperature (CTmax) define the thermal tolerance range in which the performance is possible. Thermal breadth is the range of body temperatures that permits performance equal to or greater than an arbitrary level (e.g. 50, 80, 95 %).

Study model: Andean amphibians

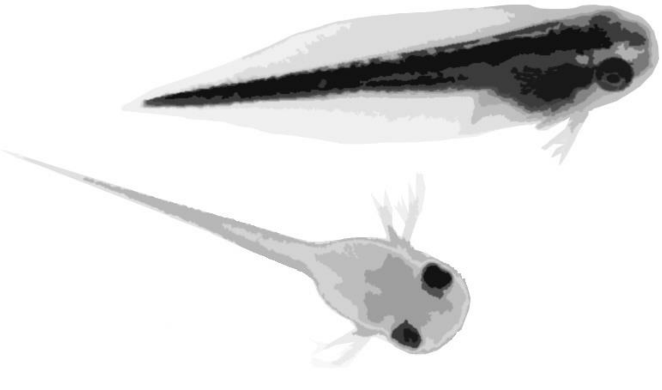
In this thesis, we used the community of amphibians inhabiting a wide elevational gradient of the Andes mountain range in Ecuador, as a study model (see **Fig. I.3**). The Andes are one of the main hotspots of amphibian richness, diversity and endemism (Myers *et al.*, 2000). Specifically, Ecuador contains a total of 600 amphibian species distributed along approximately 4200 elevational range, being the fourth richest country in absolute species richness after Brazil, Colombia and Peru, but due to its relatively small size makes Ecuador the country the largest number of amphibians per surface area ~2440 species per million km², three times more than the next richest country, Colombia (Ron *et al.*, 2018). In addition, new species of amphibians are registered every year. For instance, since this thesis started in October 2014, 41 new species have been described (see **Box I.1**).

Box I.1: Described species in Ecuador during the realization of this thesis, from October 2014 to September 2018 (41 species, ~10 species/year).

From October 2014 (6 sspp)
Pristimantis marcoreyesi (Reyes-Puig *et al.*, 2014)
Pristimantis miktos (Ortega-Andrade & Venegas, 2014)
Pristimantis paquishae (Brito *et al.*, 2014)
Pristimantis punzan (Reyes-Puig *et al.*, 2014)
Pristimantis puruscafeum (Reyes-Puig *et al.*, 2014)
Pristimantis roni (Yánez-Muñoz *et al.*, 2014)
2015 (10 sspp)
Hyloscirtus mashpi (Guayasamín *et al.* 2015a)
Pristimantis cedros (Hutter & Guayasamín, 2015)
Pristimantis enigmaticus (Ortega-Andrade *et al.*, 2015)
Pristimantis limoncochensis (Ortega-Andrade *et al.*, 2015)
Pristimantis mutabilis (Guayasamín *et al.* 2015b)
Pristimantis omeviridis (Ortega-Andrade *et al.*, 2015)
Pristimantis pahuma (Hutter & Guayasamín, 2015)
Pristimantis pichincha (Yánez-Muñoz *et al.*, 2015)
Pristimantis pinchaque (Reyes-Puig *et al.*, 2015)
Pristimantis sacharuna (Reyes-Puig *et al.*, 2015)
2016 (9 sspp)
Pristimantis allpapuyu (Yánez-Muñoz *et al.*, 2016)
Pristimantis buenaventura (Arteaga *et al.*, 2016)
Pristimantis hampatusami (Yánez-Muñoz *et al.*, 2016)
Pristimantis kuri (Yánez-Muñoz *et al.*, 2016)
Pristimantis llanganati (Navarrete *et al.*, 2016)
Pristimantis nietoi (Arteaga *et al.*, 2016)
Pristimantis prometeii (Székely *et al.*, 2016)
Pristimantis tinguichaca (Brito *et al.*, 2016)
Pristimantis yanezi (Navarrete *et al.*, 2016)
2017 (10 sspp)
Chiasmocleis parkeri (Almendáriz *et al.*, 2017)
Hyalinobatrachium yaku (Guayasamín *et al.*, 2017a)
Pristimantis albujai (Brito *et al.*, 2017b)
Pristimantis churuwiai (Brito *et al.*, 2017b)
Pristimantis ecuadorensis (Guayasamín *et al.*, 2017b)
Pristimantis muranunka (Brito *et al.*, 2017a)
Pristimantis nimbus (Urgiles *et al.*, 2017)
Pristimantis sambalan (Brito *et al.*, 2017b)
Pristimantis saturninoi (Brito *et al.*, 2017b)
Pristimantis yantzaza (Valencia *et al.* 2017)
2018, until September (6 sspp)
Amazophrynella siona (Rojas *et al.*, 2018)
Pristimantis barrigai (Brito & Almendáriz, 2018)
Pristimantis caniari (Ramírez-Jaramillo *et al.*, 2018)
Pristimantis erythros (Sánchez-Nivicela *et al.*, 2018)
Pristimantis tiktik (Székely *et al.*, 2018)
Scinax tsachila (Ron *et al.*, 2018)

Amphibians are considered to be one of the most threatened group of vertebrates (Stuart *et al.*, 2008; Hoffmann *et al.*, 2010). Among the main threats to the diversity and abundance of amphibians are the destruction, contamination and modification of habitat, the increase of ultraviolet radiation, the introduction of new species and the increase in temperatures (Stuart *et al.*, 2008; Ron *et al.*, 2011; Menéndez-Guerrero & Graham, 2013). Amphibians have a series of biological characteristics that make them especially susceptible to environmental change such as their permeable skin, high humidity dependence, ectothermy and their biphasic development cycle, aquatic during embryo and larval stages and terrestrial during juvenile and adult stages (with important exceptions such as the Strabomantidae direct-developing frogs displaying terrestrial development for embryos and larvae) (Hopkins, 2007; Wells, 2007).

In amphibians, responses to global warming vary throughout their ontogenetic development, due to possible differences in their physiological capacity, behavioural responses or exposure to different climatic conditions (Huey *et al.*, 2012; Sinclair *et al.*, 2016). For example, during the metamorphosis climax at the transition of aquatic tadpoles to terrestrial juveniles, thermal tolerances decreases drastically (e.g. Floyd, 1983). In addition, the high conductivity and thermal capacity of water, together with its lower thermal heterogeneity, when compared to air (Spotila *et al.*, 1992), limits the ability to regulate body temperature more in tadpoles as opposed to adults, especially to face extreme temperatures in the ponds. Moreover, adults have the ability to reduce body temperature by losing water through evaporation (Tracy, 1976), although the nocturnal daily activity of most adult frog species reduces thermoregulation scope as a compensatory mechanism to counter high temperatures. Most relevant for the amphibians during their terrestrial cycle is water relations may impose limits to thermoregulation, restricting many amphibians to moist microhabitats, which in turn may limit the opportunities for body temperature regulation (Navas, 2006; Hillman *et al.*, 2009; Navas *et al.*, 2013). All this, together with the fact that amphibians are considered bad dispersers (Smith & Green, 2005; Buckley & Jetz, 2007), makes amphibians highly dependent on their physiology to adapt to environmental variation and, therefore, an ideal model group to analyse thermal adaptation in tropical altitudinal gradients.



General Objectives

Back cover: *Dendropsophus minutus* (Peters, 1872)

General Objectives

This doctoral thesis explores the evolution of thermal sensitivity and the environmental determinants that promote interspecific variation in the thermal sensitivity of amphibians across a tropical altitudinal gradient. It also provides essential information to predict which species or populations are most vulnerable to global warming. For this, we first characterized the terrestrial (**chapter 1**) and aquatic thermal environment (**chapter 2-4**) which amphibians are currently exposed to, and estimated their thermal sensitivity, tolerance limits (**chapters 1 and 2**) and performance curves (**chapters 3 and 4**), along a tropical elevational gradient of 4200 meters in the Andes of Ecuador (see **Fig. I.3, Box I.2**).

The Andes is one of the most fascinating regions to explore thermal adaptation in amphibians due to their extraordinary amphibian biodiversity (Ron *et al.*, 2018), and their present and historical climatic and geological complexity (Hoorn *et al.*, 2010; Hazzi *et al.*, 2018). The lack of seasonality in the tropics which present smaller annual variation, as opposed to temperate areas, predicts higher thermal barriers in the former (Janzen, 1967), as species would evolve, through thermal adaptation, to become thermal specialist to the temperature at which they are exposed to (Stevens, 1989; Ghalambor *et al.*, 2006). This hypothesis, although initially proposed for elevational ranges, may be generalized to explain how thermal sensitivity correlates to any environmental variation (Stevens, 1992). Following this argumentation, we can predict that ectotherms inhabiting open habitats with greater daily variation and higher maximum temperatures should be more thermally generalists and heat-tolerant

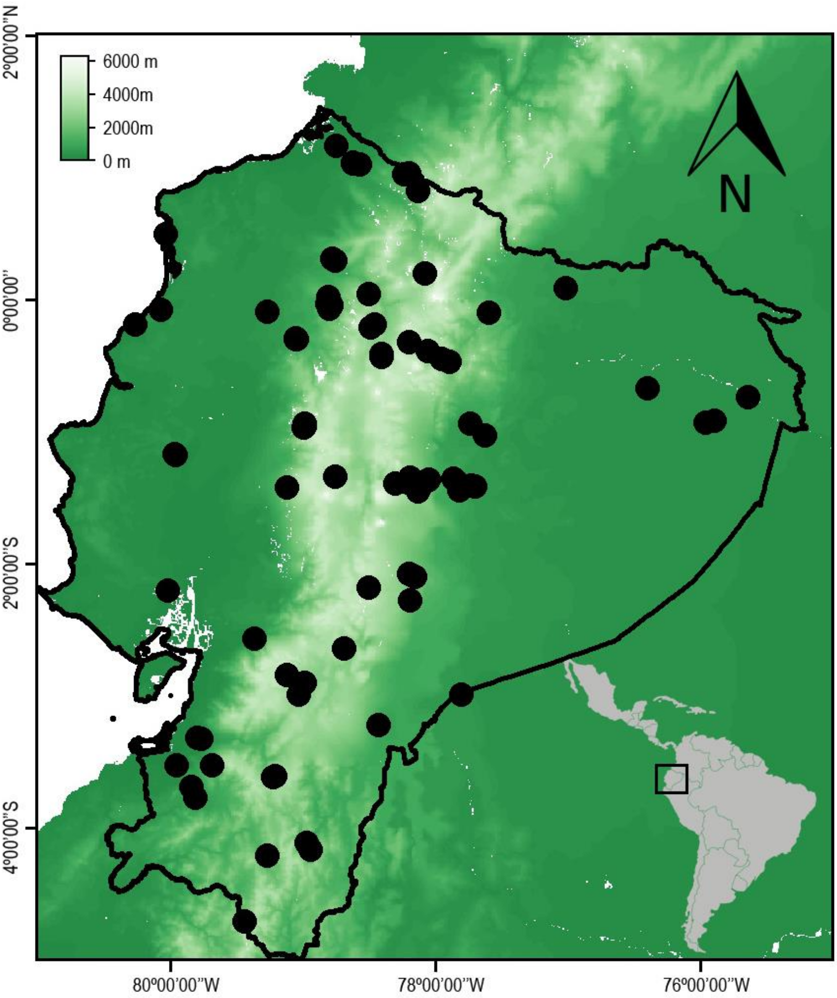


Figure I.3: Sampled sites within the study area in continental Ecuador (lined in black). Green gradient indicate elevation.

than species from forested or shaded areas (Huey *et al.*, 2009; Frishkoff *et al.*, 2015; Bonebrake *et al.*, 2016; Gutiérrez-Pesquera *et al.*, 2016). In **chapters 1 and 2**, we examined how spatial variation of temperature, both at large (altitude) and local scales (habitat), promotes the variation of thermal tolerances (i.e. CTmax and CTmin; **Box I.2a**). According to the 'heat invariability' hypothesis (Brett, 1956; Araújo *et al.*, 2013; Bozinovic *et al.*, 2014), we would expect that the evolution of thermal limits through contrasting thermal geographical variation would be lower to tolerate heat (i.e. CTmax) than to cold (i.e. CTmin), and therefore, we examined whether the predicted pattern is due to differentiated evolutionary rates for higher and lower thermal tolerances.

Although Janzen's hypothesis (1967) was proposed 50 years ago, no study to date has explicitly corroborated the existence and/or directionality of the postulated existence of physiological 'barriers'. In order to test this, in **chapter 2** we explored the evolutionary ancestral dispersion of amphibians through ancestral trait reconstruction, and tested whether estimated evolutionary elevational transitions was tightly associated with variation in thermal tolerance limits to confirm the possible existence of such physiological 'barriers' in our examined tropical mountain gradient. If this was to be the case, and assuming the existence of bi-directionality (upward and downward barriers), we would expect both lowland colonization to be associated to the evolution of greater heat tolerances as peak temperature increase at lower altitudes and similar evolution of cold tolerances associated to highland colonization of lowland lineages as environmental minimum temperature drops.

In **chapter 3**, we also examined how the spatial variation of temperature promotes variation in thermal physiology, although for this chapter we estimated thermal performance curves (TPC) for larval growth (**Fig. I.2, Box I.2b**). As a process involving many other physiological parameters also associated to temperature (Freitas *et al.*, 2010), larval growth may be a good indicator of the fitness of the species. In addition, to examine how environmental variation promotes thermal adaptation (and therefore modulates the shape of the curve), we explored four different trade-offs that may limit these adaptations ('hotter is better', 'generalist-specialist', 'hotter is narrower' and 'phylogenetic heating tolerance' hypothesis). For example, the 'jack-of-all temperatures a master of none' (or 'generalist-specialist' tradeoff) hypothesis, predicts that thermal

Box I.2: Procedures used herein to characterize (**a**) physiological thermal tolerance and (**b**) larval growth performance as function of temperature in amphibians.

HOW DID WE ANALYSE THERMAL PHYSIOLOGY IN AMPHIBIANS?

(a) Thermal bath (HUBER K15-cc-NR) used to estimate maximum and minimum thermal tolerances (CTmax and CTmin). We increased or decreased the temperature at a constant rate of 0.25 ^{0}C / min at a starting temperature of 20 ^{0}C (more info in **chapters 1 and 2**).

(b) Experimental bath with controlled temperature (9, 15, 20, 23.5, 27, 29, 31, 33 or 35 ^{0}C) used to estimate thermal performance curves for larval growth performance. Experimental temperatures were kept constant using a thermal resistance (U201431698; see top right in **b**) or a TECO TK1000 chillers. Oxygen was supplied by mechanical aeration for each individual to avoid eutrophication (more info in **chapters 3 and 4**).

species, which can perform their function over a wide range of temperatures, should have lower maximum performances than thermal specialists (Huey & Hertz, 1984). Another widely supported hypothesis is the 'hotter is better'. This hypothesis predicts that species adapted to heat will have a higher performances than those adapted to cold (Huey & Kingsolver, 1989; Savage *et al.*, 2004; Frazier *et al.*, 2006; Martin & Huey, 2008; Knies *et al.*, 2009), since adaptation is unable to overcome the depressant effects of low temperatures (Bennett, 1987).

In addition, the environmental and physiological information obtained in the previous chapters (**chapters 1 to 3**), allowed us to identify the communities most vulnerable to climate change. One possibility to generate reliable vulnerability assessment is by estimating how close current environmental temperatures are to surpass amphibians' thermal physiology (Deutsch *et al.*, 2008). Current evidences suggest that tropical ectotherms, in comparison to temperate, would be more vulnerable to an increase in temperatures (Deutsch *et al.*, 2008; Tewksbury *et al.*, 2008; Huey *et al.*, 2009; Duarte *et al.*, 2012; Sunday *et al.*, 2014), especially in lowland areas where their heat-tolerance is close to the environmental temperature to which they are exposed to (Sunday *et al.*, 2014; von May *et al.*, 2017). If organisms cannot maintain their body temperature below an optimum threshold for survival, some species will be forced to migrate to higher altitudes or latitudes (Chen *et al.*, 2009; Lenoir & Svenning, 2013). In the tropics, however, changes in altitude seem more likely than in latitude (Colwell *et al.*, 2008).

A second widely approach to predict ectotherm vulnerability due to climate-related changes, is by examining both species range contractions (i.e. extinctions) and expansions (e.g. colonization of uplands), which are based on species distribution models (SDM) (Elith *et al.*, 2010; Pacifici *et al.*, 2015). To date, correlative models are the most widely used to predict the potential effects of global warming on the distribution of species, due to the wide availability of databases which allow a rapid assessment of vulnerability in a large number of organisms (Elith *et al.*, 2006; Kearney & Porter, 2009; Pacifici *et al.*, 2015). However, since these models are based on observed distribution data (realized niche), they do not allow to extrapolate their predictions to new environments outside the climatic range used to adjust the model, both in space and time (Kearney & Porter, 2009; Buckley & Kingsolver, 2012; Veloz *et al.*, 2012; Pacifici *et al.*, 2015). Thus, any realistic vulnerability assessment will require a deep knowledge of the physiological boundaries reflecting their biological fundamental niche to enhance the forecasting of responses to environmental change (Williams *et al.*, 2008). Unfortunately, these mechanistic approaches imply experimental laboratory analyses which are largely time and resource consuming, and therefore, limits the number of species to assess.

In order to implement these mechanistic approaches, in **chapter 4** we used the available physiological and microenvironmental information obtained mainly in **chapter 3**, to develop models to predict the effects of global warming on the altitudinal distribution of a selected clade of the Dendrobatidae frog family. Unlike correlative models, predictions using mechanistic models can be extrapolated to new environments because they are based on the species fundamental niche (physiological) rather than the realized one (observed) (Kearney & Porter, 2009; Elith *et al.*, 2010; Buckley & Kingsolver, 2012). However, the reliability to predict future changes depends, to a large extent, on the ability of physiology to predict the observed niche (Buckley *et al.*, 2010; Evans *et al.*, 2015). Other abiotic and biotic factors (both current and historical) may be responsible for the current distribution range of the species. Thereby, we first examined the ability of the model to predict the observed distribution.

Chapter 1

Altitudinal variation in thermal tolerance limits and vulnerability to thermal impacts in Andean *Pristimantis* frogs. Does spatial scale matter?

POL PINTANEL, MIGUEL TEJEDO, SANTIAGO R. RON, GUSTAVO A. LLORENTE, ANDRÉS MERINO-VITERI

Abstract

Two main predictions have currently arisen in macrophysiology: phenotypic variation in upper thermal limits is less spatially variable than lower lethal limits at a range of scales and higher level of heat impacts is expected to occur in low altitudinal and latitudinal world areas. However, most of these geographical trends fail to account microenvironmental heterogeneity at the organismal scale. We examined variability in critical thermal limits (CTmax and CTmin), maximum and minimum temperatures (tmax, tmin), and vulnerability to heat and cold acute thermal stress, in species of *Pristimantis* frogs both at a large scale, through a tropical altitudinal range in Andes of Ecuador (4230 masl), and at the local scale, by comparing species inhabiting thermally contrasting microhabitats (open areas versus forest). Increasing altitude promotes faster variation in CTmin and tmin than that found for CTmax and tmax. However, a contrary pattern prompts when taking into account local habitat variation, higher CTmax and tmax in open environments but identical CTmin and tmin through environments. Vulnerability to suffer high temperature thermal stress increases inversely with elevation when employing macroclimatic predictors (WorldClim), as it is generally predicted by theory. This contrast with the trend obtained using microenvironmental temperatures (dataloggers) which revealed no relationship between elevation and the risk to suffer thermal stress. This study casts the importance of using thermal data at the scale of an organism on studies in macrophysiology. Using microclimatic data, we found that CTs mirrored the variation found on extreme temperatures and, also, the risk of suffering heating stress was invariant on elevation. Those results contrasted with the trend obtained employing macroclimatic temperatures which, on the other hand, followed the ones predicted by theory.

Back cover: *Pristimantis aff. curtipes* (Boulenger, 1882)

Introduction

Macrophysiology examine the large-scale pattern (temporal and spatial) of physiological trait variation in order to unravel the mechanisms driving physiological diversity (Chown *et al.*, 2004; Chown & Gaston, 2016). Most relevant traits are those related to the variation in thermal tolerance limits (e.g. CTs: CTmax and CTmin) and because their tight relationship with environmental temperature (e.g. Gaston *et al.*, 2009), delimit the temperature range within which ectotherms can perform their vital rates, disperse and survive, thus determining their fundamental niche and, ultimately, their potential distribution (Angilletta, 2009). Two main large geographical patterns of variation in CTs have currently arisen: First, phenotypic variation in upper thermal limits is less spatially variable than lower lethal limits at a range of scales, the heat invariant hypothesis (Brett, 1956; Bozinovic *et al.*, 2014) which predicts larger amount of variance and faster decline of cold tolerance limits with raising altitudes and latitudes (see **Fig. 1.1b**). This pattern is well documented in terrestrial ectotherms in both latitudinal (e.g. Addo-Bediako *et al.*, 2000; Sunday *et al.*, 2011, Cruz *et al.* 2005) and altitudinal (e.g. Gaston & Chown, 2009; Muñoz *et al.*, 2014; Sunday *et al.* 2014; von May *et al.*, 2017) gradients, and in aquatic ectotherms in latitudinal gradients (e.g. Calosi *et al.* 2010; Gutiérrez-Pesquera *et al.*, 2016; but see Sunday *et al.* 2011). This slower spatial variation in CTmax is argued to be due to constrained evolutionary potential with low additive genetic variance (Beacham & Withler 1999, Blackburn *et al.* 2014), lower evolutionary rates and greater potential to behavioural thermoregulation than CTmin (Muñoz *et al.* 2015, von May *et al.* 2017). In addition, the lower variation in maximum than cold temperatures (Buckley & Huey, 2016) and weaker relationship of CTmax with peak temperatures (Araújo *et al.* 2013) do suggest that other factors may also explain CTmax variation. Second, higher level of heat impacts is expected to occur in low altitudinal and latitudinal world areas owing to a reduced warming tolerance, calculated as the difference between CTmax and maximum habitat temperature (Colwell *et al.*, 2008; Deutsch *et al.*, 2008; Sunday *et al.*, 2011, but see Overgaard, Kearney & Hoffmann, 2014; Sunday *et al.*, 2014).

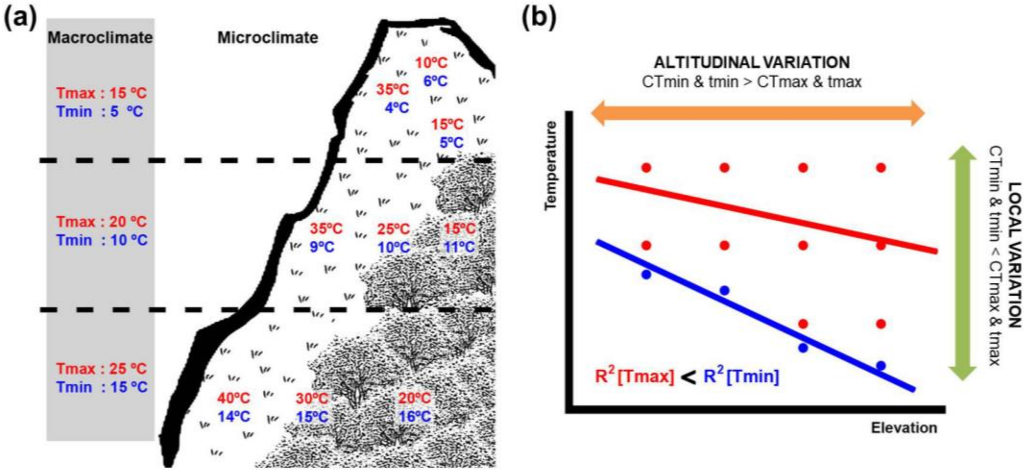


Figure 1.1: Hypothetical variation of extreme temperatures (tmax and tmin) along a tropical elevational gradient. (**a**) Macroenvironmental estimates of temperature, (e.g. WorldClim) basically grounded on lapse rate variation, mismatch the microenvironmental variation of temperatures to which organisms are actually exposed. These individual-level climatic conditions are obtained by dataloggers deployed in microsites occupied as shelters by frogs (see Methods). (**b**) Although macrophysiology prediction poses more variability in CTmin (and their climatic predictor tmin), at the broad geographical scale (altitudinal gradient), we predict that CTmax and tmax are locally more variable and, therefore, exbiting greater dispersion.

However, most of these geographical trends fail to account for microenvironmental heterogeneity at the scale of an organism that may be determinant. Current evidences reveal greater amount of variation in CTs according to the expected local thermal impacts. For instances, greater variation in cold resistance is shown by high latitudes insects (Addo-Bediako *et al.*, 2000; Chown *et al.*, 2002), whereas greater upper thermal tolerance variation is found in lowland tropical environments where heat impacts are expected. This is the case of terrestrial lizards and ants which exhibit greater CTmax variation than CTmin, with low heat resistance for understory forest species than open canopy ones (Huey *et al.*, 2009; Kaspari *et al.* 2015). Similarly, lowland tropical amphibian tadpoles exhibit high CTmax variability while CTmin remain similar among aquatic habitats (i.e. open and forested ponds), which largely varies in maximum peak temperatures but not in minimum temperatures (Gutiérrez-Pesquera *et al.*, 2016; B. Madalozzo, M. Tejedo, L.M. Gutiérrez-Pesquera *et al.*, unpublished data). All these evidences suggest that local climatic conditions may afford much of CTs variation through species thermal habitat preferences drove by thermal adaptation (**Fig. 1.1**; Kaspari *et al.* 2015; Gutiérrez-Pesquera *et al.* 2016; Pincebourde & Suppo, 2016).

This potential disparity in the level of CTs variability at the local scale can be of major concern when assessing vulnerability to thermal stress on the entire geographical range of species at the present and coming decades. Most of global warming vulnerability assessments rely on macroenvironmental temperatures that fail to account thermal variation at the individual scale and then posed geographical trends that may ultimately result inaccurate. Increasingly more studies have begun to exhort the use of microclimatic predictors, since a mismatch exist between the fine spatial scales at which organisms are environmentally exposed to and the coarse scale of easily available climate data such as Hijman *et al.*'s (2005) WorldClim climatic layers (e.g. Graae *et al.*, 2012; Navas *et al.* 2013, Gutiérrez-Pesquera *et al.*, 2016; Pincebourde *et al.* 2016) revealing contrasting patterns to those previously predicted, that ectotherms at high altitude and latitude may be also at risk (Duarte *et al.*, 2012; Buckley *et al.*, 2013; Sunday *et al.*, 2014).

Here, the extent of variation in CTs and vulnerability to thermal stress through large and local spatial scales was examined on the specious *Pristimantis* frogs (Anura: Strabomantidae) along the mountain gradient in the tropical Andes of Ecuador. Reduced seasonality in tropical mountains are predicted to cause physiological barriers by the specialization on narrow climatic conditions, especially temperature (Janzen, 1967; Stevens, 1989) and therefore, CTs are expected to evolve by thermal adaptation to fit the extreme temperature to which organisms are exposed (Ghalambor *et al.* 2006). Mountain air temperatures change strikingly along tropical elevation following a lapse rate decrease (Sarmiento, 1986), in a similar way as organisms' body temperatures do (Feder & Lynch, 1982; Navas *et al.*, 2013). Additionally, we predict that within similar altitudes, air peak temperatures also will vary between biomes (e.g. forested and open environments, Jose *et al.*, 1996; Bader *et al.*, 2007).

Pristimantis is the most specious and diverse genus among terrestrial vertebrates, which constitute one of the most impressive animal radiation with roughly 490 species (AmphibiaWeb, 2017). They mostly distribute through tropical Andes ranges from lowland rainforest to the cold paramos at 4500 masl (Hedges *et al.*, 2008; Meza-Joya & Torres, 2016). These terrestrial breeding frogs are nocturnal thermoconformers with low thermoregulatory ability (Navas, 1996a, 1997) and are usually distributed in small altitudinal ranges (e.g. Bernal & Lynch, 2008) and then, potentially exposed to a narrow range of operative temperatures. In addition, they occupy a wide array of forested habitats, such as moist lowland and montane forest, but also are widespread in open habitats: pasture, grassland and páramos (Lynch & Duellman, 1997) that are exposed to contrasting thermal regimes (Bader *et al.*, 2007; Tuff *et al.*, 2016). All these ecological diversity makes *Pristimantis* frogs a suitable model system to examine CTs variation at both, large scale (altitudinal gradients) but also at the local scale (between thermally contrasting biomes), to test first whether both thermal limits evolve at different rates and, second, the role of large and local environmental conditions on assessing the risk to suffer heat and cold impacts. A recent analysis of several Strabomantidae clades, including 10 species of Peruvian *Pristimantis*, has shown altitudinal variation in the thermal tolerance limits along its elevational gradient with faster rates of change for cold than for heat tolerances (von May *et al.*, 2017). Also, von May *et al.* found that lowland amphibian species might be more vulnerable to an increase of temperatures than high-elevation species. In this research, we study altitudinal (large scale) and habitat (local scale) variation in thermal tolerance among *Pristimantis* frog species from Ecuador within a 4000 meter elevational range. We especially focus in testing, through all the altitudinal range, whether *Pristimantis* frogs exhibit a physiological habitat preference in the use of thermally contrasting environments (forest and open habitats). We expect that physiological resistance limits will track local thermal extremes that, ultimately, may ease species isolation and thus explain the extraordinary specious radiation of *Pristimantis*. We suggest that habitat preference may be a causal mechanism of thermal evolution in these frogs, determining additional physiological barriers to dispersion and increasing the potential for genetic isolation.

Materials and Methods

Study sites, thermal variability and habitats

Our sampling area was distributed through the Andes mountain of Ecuador (between latitudes 1^0N–4^0S and elevations 23–4130 masl) from November 2014 to March 2017. The selected sample locations covered the entire altitudinal range and the main habitat types (forest and open land) occupied by *Pristimantis* frogs, in order to examine how upper and lower thermal resistance limits and vulnerability to receive thermal impacts varies at both, broad (altitude) and local (habitat) scales.

In order to match species thermal limits variation and extreme cold and heat peak temperatures to which *Pristimantis* frogs are exposed, we characterized the thermal microhabitat where species were sampled, by monitoring microclimatic temperatures over a period that ranged from 4 to 542 days (see **Table S1.1**). We used HOBO Pendant temperature dataloggers that obtain continuous record of temperature (every 15 minutes). In some instances, we could not recover all the loggers due to landslides or other unexpected losses. In that case, we alternatively employed loggers located at the same habitat and similar altitudes than sampling for the species (**Table S1.1, S1.2**). Although *Pristimantis* frogs employ many microhabitats (Navas, 1996c; Carvajalino-Fernández *et al.*, 2011; Navas *et al.*, 2013), we located loggers in the available microsites actually used by frogs as shelters during the daytime (inside bromeliads and under leafs, trunks or rocks). We assume that these shelters will be probably selected by frogs in order to avoid the environmental extreme peak heat and cold temperatures. We assigned species habitat type into restricted to forested habitats and open habitats exploiters (or generalist species) (e.g. grasslands, potreros, paramos) obtained from our field surveys and complemented with publications and well-supported observations on museums (**Table S1.2**; **Suppl. Material 1.1**). For each datalogger, we obtained three microclimatic variables: mean temperature (tmean), minimum temperature (tmin) and maximum temperature (tmax). The difference between tmax and tmin gives the absolute range of temperature (ar = tmax - tmin). Additionally, we also gathered macroclimatic measurements for the same coordinates where the loggers were located by extracting the following thermal variables: bio1 - TMEAN, bio5 - TMAX and bio6 – TMIN (see **Table S1.1**) from the WorldClim layers (1 km^2 spatial resolutions; Hijmans *et al.*, 2005).

Estimates of Critical Thermal Limits and vulnerability to thermal stress

To determine how thermal limits vary among *Pristimantis* species along elevation and habitat, we measured 148 individuals (75 CTmax and 73 CTmin), representing 22 evolutionarily significant units (ESU; Conner & Hartl, 2004) distributed among 23 populations. We treated those populations as ESU because they showed enough genetic divergence, however additional analyses may be required before considered different species. We also included one more species of the *Craugastor* genus in the analysis for both CTs. However, only for 20 populations we obtained both thermal limits (more information in **Table S1.2**).

The frogs were placed at a constant temperature of 20 °C with a photoperiod of 12L : 12D for at least three days before conducting the tolerance assays in order to reduce field acclimation noise and facilitate comparisons (see Brattstrom, 1968). Each tested individual was placed in a plastic cup with a thin layer of water (less than 1mm) in a 15 L HUBER K15-cc-NR bath at a starting temperature of 20 °C. We increased or decreased the temperature at a constant rate of 0.25 °C / min using the dynamic method of Lutterschmidt and Hutchison (1997a) until the frogs did not respond to any physically stimuli with total immobility. This was defined as the end point because it is reproducible for both thermal limits. At this endpoint, CTmax and CTmin were measured as the frog body temperature taken with a Miller & Weber quick-recording thermometer (to the nearest 0.1 ^{0}C). After a tolerance limit was determined, we immediately transferred the frogs to a plastic cup with a thin layer of water at the acclimation temperature, allowing for recovery within two hours. Each individual was tested only once. After the test each frog was wet weighed to the nearest 0,001 g. Finally, we calculated the thermal tolerance range (TR) as the difference between CTmax and CTmin for each species.

One way to determine whether thermal selection is prone to drive thermal tolerance limits through altitude and between habitats is by assessing the risk of species to suffer heat or cold impacts. An operative metric to estimate the eventual occurrence of acute heat stress is the warming tolerance (i.e. the difference between CTmax and the maximum exposure temperature taken at the micro- (tmax), or macro climatic scale (TMAX); Deutsch *et al.*, 2008; Duarte *et al.*, 2012). Similarly, we can define cooling tolerance as the risk to suffer cold shocks and measure it as the difference between CTmin and the minimum exposure temperature, taken at the micro (tmin) or macro climatic scale (TMIN) (Sunday *et al.* 2014, Gutiérrez Pesquera *et al.* 2016).

Phylogenetic reconstruction

We obtained a phylogeny for all analyzed species. The phylogeny was based on newly generated DNA sequences for mitochondrial genes 12S rRNA (12S), tRNA-Valine, and 16S rRNA (16S). DNA was extracted from muscle or liver tissue preserved in 95% ethanol or tissue storage buffer, using standard phenol–chloroform extraction protocols (Sambrook *et al.* 1989). We used a polymerase chain reaction (PCR) to amplify DNA fragments. PCR was performed under standard protocols and amplicons were sequenced by the Macrogen Sequencing Team (Macrogen Inc., Seoul, Korea). The combined DNA matrix had up to 2608 bp.

The newly generated DNA sequences will be available on GenBank after publication. We also included available sequences from GenBank. *Craugastor longirostris*, *Eleutherodactylus atkinsi*, *Leptodactylus melanonotus*, *Mannophryne trinitatis*, and *Odontophrynus occidentalis* were included as outgroups. The forward and reverse chromatograms were assembled in Geneious 9.1.8 (Kearse *et al.*, 2012) and edited manually as required. Alignment was done with MAFFT 7.2 software with the L-INS-i algorithm (Katoh & Standley, 2013).

Phylogenetic trees were obtained using maximum likelihood with software GARLI 2.0 (Zwickl, 2006). We made 40 independent searches, 20 starting from random trees and 20 from stepwise addition trees. The number of generations without topology improvement required for termination (genthreshfortopoterm) was set to 15000. Other settings were set on default values. Node support was assessed with 200 pseudoreplicate non-parametric bootstraps (npb), configured with the same settings of the full search, but with two replicates per run.

Statistical analyses

To estimate the phylogenetic dependence of the data we used the Pagel's lambda (Pagel, 1999) and Bloomberg's K (Blomberg *et al.*, 2003) approach in the R package 'phytools' (Revell, 2012). Pagel's lambda varies from 0 (phylogenetic independence) to 1 (strong phylogenetic signal). On the other hand, K varies from 0 to infinite with values <1 indicating that closely related species resemble each other less than expected under the Brownian motion model of trait evolution while values >1 indicate that closely related species are more similar than predicted by the model.

We also used the function 'ratebytree' from the R-package 'phytools' (Revell, 2012) to evaluate whether differences in the rate of evolutionary change vary among critical thermal limits. This function allows the comparison of phenotypic evolution of continuous traits between trees under different models of evolution, 'random walk' (Brownian Motion, BM) and the adaptive models Ornstein-Ehlenbeck (OU), and Early Burst, (EB) (Revell *et al.*, 2018). Therefore, we first fitted the three different models of evolution to each physiological trait (CTmax and CTmin) using the 'fitContinuous' function from the R package geiger (Harmon *et al.*, 2008). We used the Akaike information criterion corrected (AICc; Sugiura, 1978) for small sample size to identify the best model (Burnham & Anderson, 2002). If OU or EB models were not significantly better ($\Delta AIC < 2$; Burnham & Anderson, 2002) than the simpler model (i.e. BM), we kept the simpler model. Once we determined the best evolutionary model for each variable (see **Table S1.3**), we performed the test for the whole dataset. However, we repeated the analysis with a reduced dataset of 20 populations exemplifying both CTs estimates for consistency purposes.

We explored the assumption that open areas are more thermally variable than forested areas along the tropical mountain gradient. We used an ANCOVA to test the effect of elevation and habitat type on maximum temperature (tmax), minimum temperature (tmin) and daily thermal range (ar = tmax – tmin). We also analysed how microenvironment modulates macroenvironmental temperatures. We used an ANOVA to test the effect of habitat (forest versus open) on the differences between extreme micro and macroenvironmental temperatures (tmax – Tmax and tmin – Tmin).

Table 1.1: Microenvironmental (a-c) and macroenvironmental (d-e) maximum, minimum and absolute range of temperatures (depenent variables) in relation to elevation and habitat.

Climatic data		**Df**	**Sum Sq**	**F value**	**P (>F)**
a. tmax					
(R^2 = 0.634)	Altitude (A)	1	414,39	25,681	**<0.001**
	Habitat (H)	1	178.37	11.054	**0.005**
	Residuals	14	225,91		
b. tmin					
(R^2 = 0.955)	Altitude (A)	1	319.77	116.589	**<0.001**
	Habitat (H)	1	12.23	4.458	0,055
	A x H	1	15.77	5.749	**0.032**
	Residuals	13	35.66		
c. ar (tmax-tmin)					
(R^2 = 0.547)	Altitude	1	72,123	4,232	0,058
	Habitat	1	199,732	11,719	**0,004**
	Residuals	14	238,605		
d. TMAX					
(R^2 = 0.94)	Altitude	1	549,72	221.337	**0.001**
	Habitat	1	4.88	1.965	0.183
	Residuals	14	34,77		
e. TMIN					
(R^2 = 0.98)	Altitude	1	626,04	707.149	**<0.001**
	Habitat	1	0,91	1.03	0,327
	Residuals	14	12,39		
f. AR (TMAX-TMIN)					
(R^2 = 0.321)	Altitude	1	2,48	1.901	0.19
	Habitat	1	1.572	1.206	0.291
	Residuals	14	18.26		

To test the effects of elevation and habitat on thermal physiology variables (CTmax, CTmin and TR) and vulnerability risk (warming tolerance and cooling tolerance), we used a phylogenetic generalized least squares (pgls) analyses using 'pgls' function in the R package caper (Freckleton *et al.*, 2002). We found an effect of body size on thermal tolerance limits, however regardless if weight was included or not, there was no change in the results and, hence, was not included in the models shown here (but see **Table S1.4, S1.5**). Finally, we evaluated the correlations between thermal physiology variables (CTmax, CTmin and TR) with environmental extreme temperatures (TMAX, TMIN and AR respectively) at both, macroclimatic (WorldClim) and microclimatic (dataloggers). All values in the main text are expressed as mean and standard error. All analyses were done in R (R Core Team, 2014).

Results

Phylogenetic signal and rate of change on thermal limits

We identified at least three putative new species (**Fig. S1.1**, **Table S1.2**). When each pair of cryptic taxa was compared, the populations of higher elevation displayed lower CTmax and CTmin values than those from lower elevation. We found strong phylogenetic signal for CTmax ($\lambda = 0.807$; $K = 0.911$) and CTmin ($\lambda = 1$; $K = 1.032$). Brownian motion (BM) model of evolution was the most supported model for both CTs (**Table S1.3**). Finally, we found no differences on the rate of evolutionary change for the thermal tolerance limits under the BM model of evolution using either the reduced or the whole dataset (only results of whole dataset are shown; $\sigma^2_{CTmax} = 14.798$, $\sigma^2_{CTmin} = 20.44$, LRT = 0.571, $p = 0.45$).

Critical thermal limits and environmental thermal variation at large (altitude) and local (habitat) scale

Microenvironmental extreme temperatures (dataloggers) are better predictors than macroenvironmental temperatures (WorldClim) for CTmax (PGLS: $F_{1,20} = 38.82$, $P < 0.001$, $R^2 = 0.643$, AIC = 84.886 and $F_{1,20} = 0.742$, $P = 0.399$, $R^2 = 0.012$, AIC = 101.475 respectively) and thermal tolerance range (TR;

PGLS: $F_{1,18}$ = 26.47, P < 0.001, R^2 = 0.5733, AIC = 97.431 and $F_{1,18}$ = 0.025, P = 0.876, R^2 = 0.054, AIC = 115.385 respectively), but no for CTmin (PGLS: $F_{1,20}$ = 64.09, P < 0.001, R^2 = 0.75, AIC = 89.909 and $F_{1,20}$ = 53.05, P < 0.001, R^2 = 0.712, AIC = 88.706 respectively). Both critical thermal limits and extreme maximum and minimum temperatures exhibit contrasting pattern of variation at large and local scales (**Fig. 1.2, 1.3**; **Tables 1.1, 1.2**). Overall, greater amount of variability is attained by CTmin than CTmax with altitude, especially for open environment species (Bartlett´s test, $F_{1,23}$ = 8.45, P = 0.008; **Table S1.7**). However, both thermal limits equally vary with altitude when we compare forest species (Bartlett´s test, $F_{1,17}$ = 0.12, P = 0.728; **Table S1.7**). Altitude explained greater amount of variation in CTmin and tmin (PGLS: R^2 = 0.79 and R^2 = 0.94 respectively) than in CTmax and tmax (PGLS: R^2 = 0.27 and R^2 = 0.39 respectively). CTmin decline with altitude at a faster rate (PGLS: -0.003 ±0.0003; **Fig. 1.2b**) than CTmax (-0.0007 ±0.0004; **Fig. 1.2a**) (heterogeneity of slopes test: $F_{1,40}$ = 10.23, P = 0.0027). Similarly, tmin decreased at faster rate (-0.006 ±0.0003; **Fig. 1.2e**) than tmax (-0.003 ± 0.001; **Fig. 1.2d**) (heterogeneity of slopes test: $F_{1,40}$ = 16.83, P = 0.0002). Interestingly, this asymmetric decline with altitude in extreme temperatures taken at the individual level, is not found at the

Table 1.2: Physiological variables in relation to elevation of the population sample point and habitat (open versus forest) in direct-developing frogs using a PGLS approach.

Physiological traits		**Estimate**	**Std. Error**	**t value**	**P (> |t|)**
a. CTmax	**Intercept**	35.855	0.721	49.7	**<0.001**
(R^2 = 0.61, λ= 0, n = 22)	**Altitude**	-0.0017	0.0003	-5.186	**<0.001**
	Habitat_open	3.359	0.766	4.3843	**<0.001**
b. CTmin	**Intercept**	9.028	0.837	10.78	**<0.001**
(R^2 = 0.836, λ = 0.438, n = 22)	**Altitude**	-0.0027	0.0003	-8.22	**<0.001**
	Habitat_open	-1.4	0.719	-1.948	0.0663
c. TR	**Intercept**	9.125	0.695	13.125	**<0.001**
(R^2 =0.851, λ= 0, n = 20)	**Altitude**	-0.0027	0.0003	-8.831	**<0.001**
	Habitat_open	-1.734	0.746	-2.326	**0.0327**

macroclimate (TMAX and TMIN) (heterogeneity of slopes test: $F_{1,40} = 0.399$, $P = 0.531$).

We found a contrary pattern of CTs and extreme thermal variation when we looked at the local scale, by comparing open and forest microenvironments.

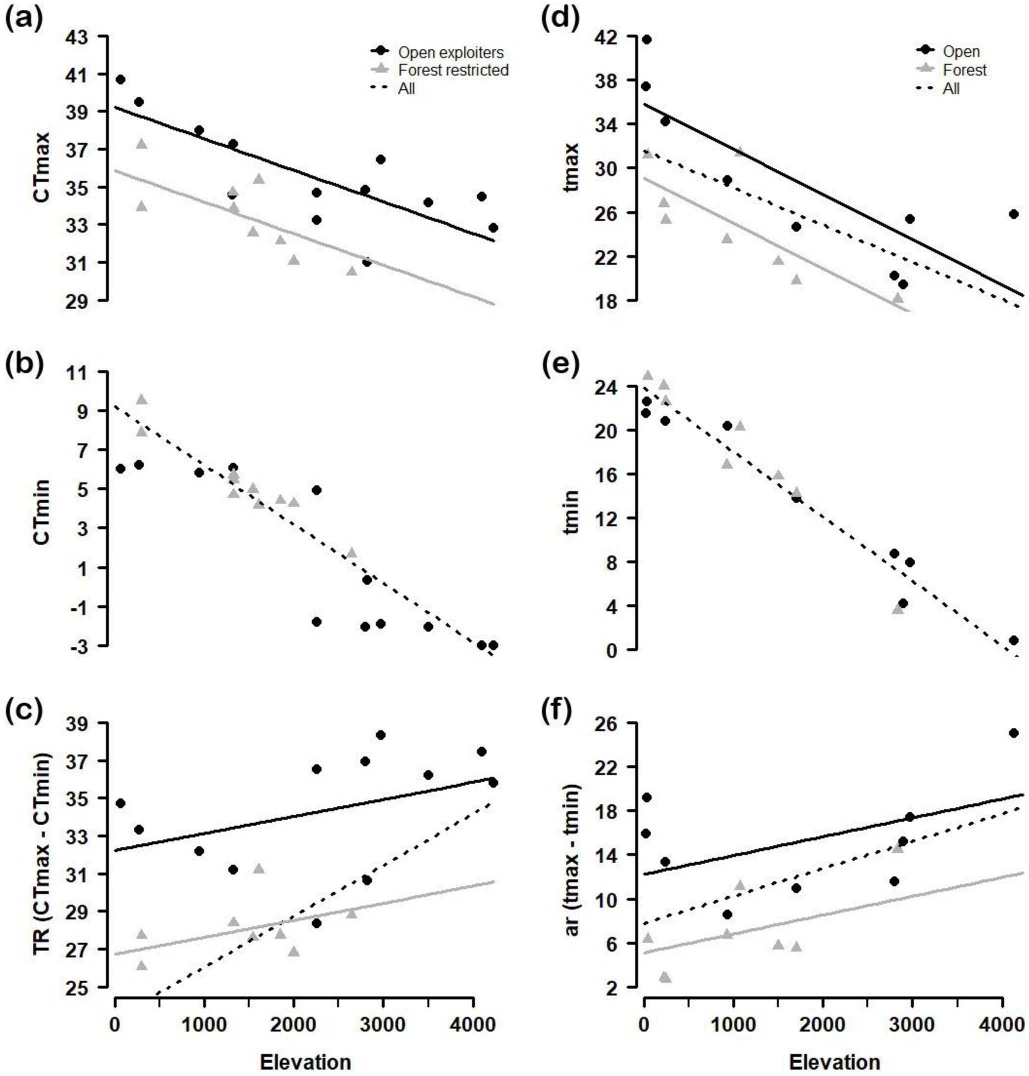


Figure 1.2: Relationship between physiological variables (CTmax, CTmin and TR; **a-c**) and microenvironmental temperatures (tmax, tmin and ar; **d-e**) with elevation. The slopes of the regression lines for the thermal variables reflect the significant phylogenetic corrections in each model.

Linear models showed that microenvironment explain a significant source of variation in CTmax and tmax but not in CTmin and tmin (**Fig. 1.2**; **Table 1.1**, **1.2**). Those frogs inhabiting open environments have higher CTmax (**Table 1.2a, Fig. 1.2a**; PGLS means: 35.768 ±0.106 and 32.73 ±0.676 respectively) and were exposed to warmer tmax than frogs limited to forest habitats (**Fig. 1.2d, 1.3a**; **Table 1.1a**), however, not environmental distinction occurred in CTmin (**Table 1.2b**, **Fig. 1.2b**) and tmin (**Fig. 1.2e**, **1.3b**; **Table 1.1b**). This environmental signal on CTmax variation appears to be mediated by maximum tmax. When tmax is introduced in the model, the difference in CTmax between forest and open environments disappears, thus suggesting that contrasting tmax to which species are exposed and have to face, may be a mechanistic explanation of the evolution of CTmax between both environments (PGLS: $F_{1,18} = 0.676$, $P = 0.422$; **Fig. S1.4**, **Table S1.6**).

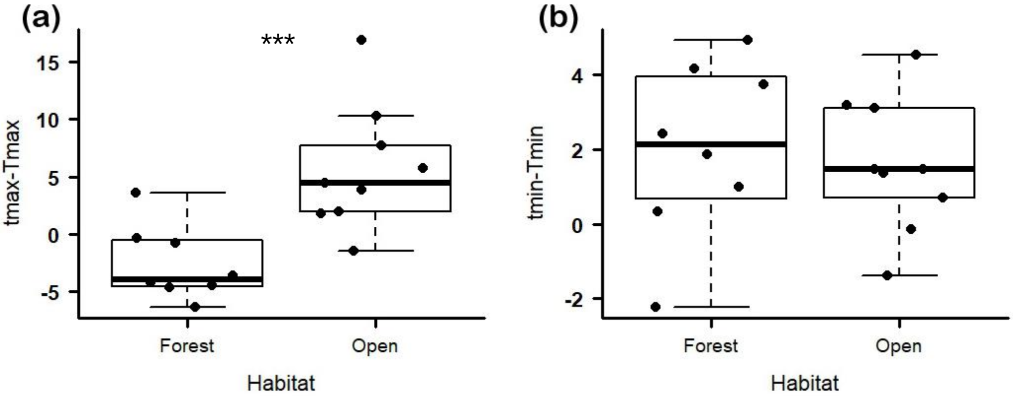


Figure 1.3: Differences between maximum (**a**) and minimum (**b**) temperatures estimated with dataloggers and WorldClim (tmax-TMAX and tmin-TMIN respectively) in different habitats. Central bars represent medians and boxes represent quartiles.

Both thermal tolerance range and the absolute range of temperatures were broader in opened areas than in forested habitats and both ranges increased with altitude (**Fig. 1.2c, 1.2f**; **Table 1.1c, 1.2c**). Thermal tolerance limits did not exhibit a physiological trade-off (PGLS: $F_{1,18} = 0.077$, $P = 0.785$), nonetheless, when we included habitat in the model, thermal tolerance limits covaried positively with elevation (PGLS: $F_{1,17} = 13.837$, $P = 0.002$; **Fig. S1.2**) suggesting a trade-off in thermal resistance. Increased cold tolerance implies a reduction in heat resistance that was parallel in both habitats although higher heat resistance was found in open habitats for similar cold resistance.

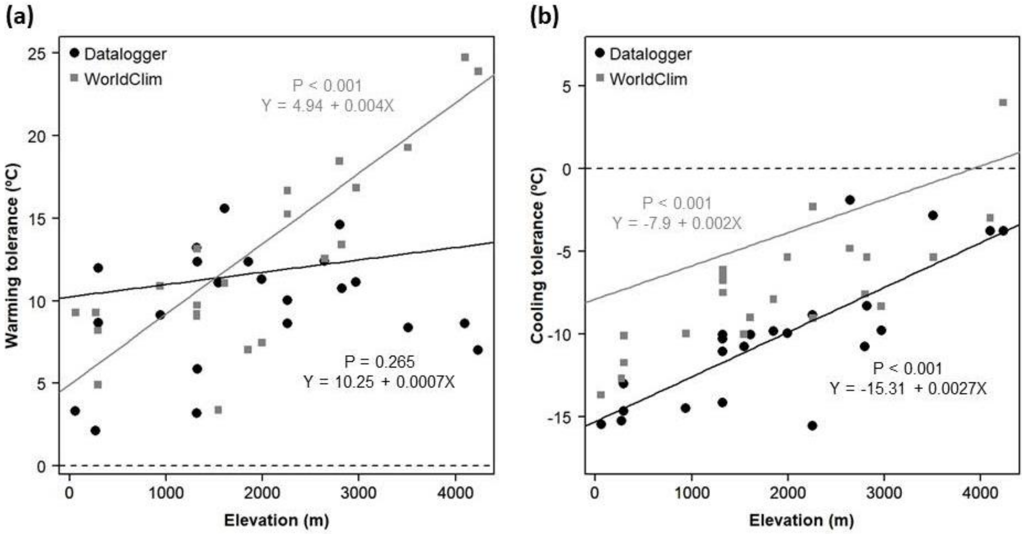


Figure 1.4: Phylogenetic generalized least squares for warming tolerance and cooling tolerance using macroclimatic (black) and microclimatic (grey) predictors. Dashed lines represent a lethal threshold (WT or CT = 0). Note that, contrary to warming tolerance, for cooling tolerance lower values represent less risk of cooling stress.

Increasing altitude determines higher risk to suffer cold impacts because tmin decreases with altitude at a faster rate than CTmin (heterogeneity of slopes test: $F_{1,40} = 51.21$, $P < 0.001$). Likewise, faster decreases is found when employing macroclimate TMIN (heterogeneity of slopes test: $F_{1,40} = 20.17$, $P < 0.001$; **Fig. S1.3**). Contrarily, tmax does decrease at the same rate than CTmax and then not altitudinal trend could be expected in heating risk (heterogeneity of slopes test, $F_{1,40} = 0.67$, $P = 0.419$). However, a contrasting altitudinal decline in heating risk with altitude is obtained when employing macroclimatic TMAX, since it decreases at a faster rate than CTmax (heterogeneity of slopes: $F_{1,40} = 37.28$, $P < 0.001$; **Fig. S1.4**). Then, macro and micro climatic estimates provides divergent patterns with altitude when assessing frog vulnerability to suffer heat stress (**Fig. 1.4**; **Table S1.4**).

Discussion

In this research, we aim to examine whether two macrophysiology rules: 1) greater altitudinal variation in lower than upper thermal limits and, 2) greater risk of heat impact at lower altitudes, are sustained in a tropical mountain frog community when considering species selection of thermally contrasting environments (open and forest) and by assessing climatic conditions at the organismal level.

Increasing altitude promotes faster variation in CTmin than in CTmax as generally predicted in ectotherms (Gaston & Chown 1999, Muñoz *et al.* 2015, von May *et al.* 2017). However, a contrary pattern prompts when taking into account local habitat variation. At the same elevation, we found higher variation on CTmax in open than in forested environments but identical CTmin. This contrasting pattern, depending on the spatial scale, mirrored the pattern found in microenvironmental extreme temperatures, thus suggesting the role of species thermal selection on habitats exposed to contrasting maximum and minimum peak temperatures. This is of major concern in predicting and mitigating effects to challenge increase in temperatures in the coming decades. Tropical mountain organisms, especially those inhabiting the lowlands, are considered highly endangered because the actual high temperatures to which they are exposed to (Deutsch *et al.* 2008; Duarte *et al.* 2012), and subsequent uplifts are predicted as the main mitigating mechanisms (Colwell et al 2008). Janzen (1967) suggested that tropical mountain passes are physiologically 'higher' than temperate mountains. Here, we propose that, in aseasonal tropical mountains, physiological barriers proposed in the altitudinal axis may also exist transversally within altitude throughout habitat selection (see Gutiérrez-Pesquera *et al.*, 2016;

Nowakowski *et al.*, 2017). Contrasting thermal habitats would ultimately promote a matching between species thermal resistance limits and their extreme temperatures to which they are exposed. These two-dimensional physiological barriers may increase the potential for genetic isolation of populations and thus it may be a determinant evolutionary engine promoting species radiation in the tropics (Janzen 1967; Ghalambor *et al.* 2006) and specifically in this extreme specious group of terrestrial breeding frogs.

Our results found strong phylogenetic signal for both thermal tolerance limits. Although CTmax is considered to be an evolutionary conserved trait, compared to CTmin (e.g. Klok & Chown, 2003; Araújo *et al.*, 2013; Hoffmann *et al.*, 2013 but see Muñoz *et al.* 2014 ; von May *et al.*, 2017), we did not find significant differences on the evolutionary rate of heat and cold tolerance and, therefore, heat tolerances may not be more limited to evolve than cold tolerances. Also, we did not find a physiological trade-off between thermal limits that could constrain thermal evolution as been seen in other terrestrial ectotherms (see Alford *et al.*, 2012; Kellermann *et al.*, 2012a). However, when we included the effect of habitat in the model, we observed a correlated trend and, therefore, a trade-off between thermal limits (**Fig. S1.2**). Thus, it seems that environmental conditions promoting the evolution of physiological resistance matching local extreme temperatures are not constrained when considering that ancestral species could live in habitats different from the descendant ones. Secondly, for some cryptic populations of *Pristimantis* distributed at different elevations, thermal tolerances differ up to more than 3 ^{0}C for CTmin (*P. laticlavius*) and 2 ^{0}C for CTmax (*P. achatinus*) suggesting local adaptation. Adaptation to warmer temperatures can rapidly occur in ectotherms in short time-scales (Skelly & Freidenburg, 2001; Logan *et al.*, 2014). In fact, heat tolerance on different organisms can increase in response to experimental selection (Donaldson & Olson, 1957; Gilchrist & Huey, 1999), however, studies on terrestrial vertebrates are lacking.

Second main result revealed that *Pristimantis* frogs are not less vulnerable to receive heat impacts with altitude when we examine extreme exposures achieved at the individual climatic conditions. This contrast with the general trend obtained employing macroclimatic predictors, where lower risk of heat impacts is found for high altitude frogs as it is, otherwise, generally predicted by theory (e.g. Colwell *et al.*, 2008; Deutsch *et al.*, 2008; Sunday *et al.*, 2011). This casts doubts on the reliability of macroclimatic repositories in order to examine geographical trends in both physiological traits and vulnerability assessments since those ignore the potential of habitat to buffer maximum temperatures. This finding has an important implication; vulnerability predictions that obviate habitat heterogeneity will likely lack accuracy (Sunday *et al.* 2014). More field based studies are still necessary to improve our knowledge on thermal adaptation and vulnerability on ectotherms at local scales.

Chapter 2

Are tropical thermal barriers ‘higher’ on the way up or down?

POL PINTANEL, LUIS MIGUEL GUTIERREZ-PESQUERA, FREDDY ALMEIDA-REINOSO, GUSTAVO A. LLORENTE, ANDRÉS MERINO-VITERI, MIGUEL TEJEDO

Abstract

Janzen (1967) proposed that tropical species are specialists to thermally stable environments and thus, they would be more limited to dispersing altitudinally (up or down mountain) than temperate species because of evolved physiological barriers. Yet the relative role that physiological barriers, such as thermal tolerance limits, CTmax and CTmin, may play in tropical ectotherm dispersing to either lower o higher elevations is largely untested. Here, we estimated thermal tolerance limits of 75 species of amphibian tadpoles from an aseasonal tropical mountain range of the Ecuadorian Andes, distributed along a 3500 m elevational range, to test whether moving up or down may be more physiologically challenging. Our results by using ancestral reconstruction of evolutionary trajectories, suggest that moving upwards is physiologically more challenging than dispersing downwards; since moving up implied a drastic evolution in cold tolerances. By contrast, higher heat tolerances were not required when species moved downwards. This contrasting evolutionary pattern may result from divergent local habitat selection on both thermal limits to face environmental thermal extremes (tmax and tmin). Specifically, within the same altitudinal window, exposure to extreme maximum temperatures can be avoided through habitat shifts from temporary ponds to permanent ponds or streams, while minimum peak temperatures remained invariable between habitats. Thereby we propose habitat selection as a main driving mechanism in the evolution of CTmax whereas altitude does with cold resistance.

Back cover: *Boana rosenbergi* (Boulenger, 1898)

Introduction

Temperature is thought to be the single most important factor limiting fauna and flora in the tropical mountainous areas (Navas, 2002; Angilletta, 2009). Janzen (1967) seasonality hypothesis predicts that species adapted to less variable tropical mountain climates would be more limited to dispersing altitudinally (up or down mountain) than temperate species because of the existence of physiological barriers. This argument grounds in the two following assumptions; first, thermal stability within specific altitudes in the tropical mountains, due to no temporal seasonal changes, being their thermal variation basically a linear decline of temperatures with elevation (adiabatic lapse rate) (Jankowski *et al.*, 2013). Second, and because this thermal stability, ectotherms will be locally adapted leading to the evolution of thermal specialist to the thermal extremes and modal temperatures encountered at specific altitudes. These site-selection processes would be especially strengthened in those environments where thermoregulatory options are limited in order to avoid stressful temperatures (e.g. canopy forest and aquatic environments) (Angilletta *et al.*, 2006; Huey *et al.*, 2009). As a consequence, thermal selection may reduce survival of dispersing non-adapted organisms in the new climate encountered at alien altitudes and thus leading to the evolution of physiological barriers that, in addition to topographical barriers (e.g. Hazzi *et al.*, 2018), may further restrict tropical organismal dispersal (Gill *et al.*, 2016; Zuloaga & Kerr, 2017).

Posed 50 years ago, Janzen´s prediction on reduced dispersal capability in tropical organisms by means of physiological barriers (Sheldon *et al.*, 2018), have currently employed as a mechanistic explanation of, for instances, increased rates of speciation in the tropics (Ricklefs, 2006; Martin *et al.*, 2009), their smaller altitudinal range sizes (Sheldon *et al.*, 2011; Cadena *et al.*, 2012; Gill *et al.*, 2016) and, finally, the predicted uplifts dispersal of tropical lowland ectotherms, to track thermal climatic niche due to increased heating (Colwell *et al.*, 2008; Chen *et al.*, 2011a; Forero-Medina *et al.*, 2011; Buckley *et al.*, 2013). However, to our knowledge, no study has conducted an experimental corroboration of the existence of these physiological barriers in any tropical ectothermic clade and, particularly, whether these physiological barriers will be equally effective to limit up and downlift dispersal, namely whether species would be more physiologically constrained to move to higher or to lower elevations. Although not explicitly stated, we can assume that Janzen´s physiological barriers would limit movements either for lower elevation organisms crossing mountain passes (as literally the paper title suggests) but also for higher elevation organisms crossing the lowland valleys. Thus, the reduced dispersal ability grounded on physiological barriers will be symmetrical and bidirectional imposing physiological impairing to face colder temperatures to lowland travellers and heat damages for, cold adapted, high altitude travellers.

A previous contrasted tenet in macrophysiological research (Chown & Gaston, 2016) is less geographic variation in upper than lower thermal limits (‘heat-invariant hypothesis’; Bozinovic *et al.*, 2014) well documented in in latitudinal gradients for terrestrial (Addo-Bediako *et al.*, 2000; Cruz *et al.*, 2005; Sunday *et al.*, 2011) and in aquatic ectotherms (Brett, 1956; Calosi *et al.*, 2010; Gutiérrez-Pesquera *et al.*, 2016; but see Sunday et al. 2011). Altitudinal variation, although less reported, has also been shown (e.g. Gaston & Chown, 1999; Muñoz *et al.*, 2014; Sunday *et al.*, 2014; Chapter 1). This asymmetry in the geography of thermal limits variation may have implications in the strength of physiological barriers through mountains, especially when focusing in aseasonal tropical ranges. By assuming that thermal resistance limits are driven by thermal selection through organismal exposure to extreme temperatures (Angilletta *et al.*, 2002; Angilletta, 2009), we could expect that cold peak temperatures will change faster than maximum temperatures (Gutiérrez-Pesquera *et al.*, 2016). However, mapping the clinal variation in physiological limits with environmental climatic predictors has rarely conducted at the individual scale, at the actual exposure level of organisms (but see Potter *et al.*, 2013; Gutiérrez-Pesquera *et al.*, 2016; Katzenberger *et al.*, 2018). Microenvironmental climatic surveys are crucial to define the thermal selective pressures that organisms experience which are bypassed at coarser climatic scales (Pincebourde *et al.*, 2016). For instance, organisms inhabiting canopy covered habitats will experience lower and more constant temperatures than those from openlands and thus, this divergence in thermal exposure may promote physiological adaptation locally (Duarte *et al.*, 2012; Kaspari *et al.*, 2015; Kaspari *et al.*, 2016; Chapter 1).

Amphibians are an excellent group of ectothermic vertebrates to test for evolution of thermal physiology in tropical elevations and the eventual physiological barriers to dispersion. We chose the amphibian community that inhabits the Andes mountain ranges in Ecuador because of their extraordinary species richness and diversity (600 spp, 253 endemics, in a range of at 250.000 km2; Ron *et al.*, 2018), which might have been promoted by the geological and climatic complexity of the region (Hoorn *et al.*, 2010; Hazzi *et al.*, 2018). Being distributed along a whole 4000 m elevational range and occupying all terrestrial and aquatic environments, Andean amphibians experience strinkly differents thermal pressures. In amphibians, research on elevational gradients has been conducted in the adult terrestrial stage (Brattstrom, 1968; Snyder & Weathers, 1975; Christian *et al.*, 1988; John-Alder *et al.*, 1988; Ghalambor *et al.*, 2006; von May *et al.*, 2017) yet, to our knowledge, this is the first attempt to characterize the altitudinal variation in the critical thermal limits of tadpoles. Aquatic larval stages are capable of behaviourally thermoregulate (e.g. Hutchison & Hill, 1978; Balogová & Gvoždík, 2015). However thermoregulation may be limited due to the high heat capacity and conductivity of water in comparison to air temperatures

(Erskine & Spotila, 1977; Hillman *et al.*, 2009). Thus, tadpoles’ thermal tolerances should match local thermal conditions (Gutiérrez-Pesquera *et al.*, 2016).

In order to examine the existence of altitudinal physiological barriers, its potential bidirectional pattern and their correspondence with environmental organismal level exposure, we obtained critical thermal limits and thermal tolerance ranges of 75 species of amphibian tadpoles from an aseasonal tropical mountain range of the Andes of Ecuador, distributed along a ~3500 m elevational range, to test whether moving up or down may be more physiologically challenging by applying a phylogenetic-based framework. First, we examined the historic directionality on amphibian distributional shifts on elevation, by using a phylogenetic reconstruction of ancestral transitions. To then, test whether variation in heat and cold tolerance are consistent with past thermal adaptation in elevation (Beaulieu *et al.*, 2012) and thus, if distribution on elevational ranges in amphibians are more physiologically limited upward (CTmin) or downward (CTmax). Finally, we evaluated if thermal tolerance limits exhibit different evolutionary rates in response to divergent selective pressures.

Material and Methods

Study sites

This study was conducted in the Andes of Ecuador (between latitudes 1.2^{0}N – 4.8^{0}S and elevations 23 - 3630 masl). Larval amphibians occupy nearly all available aquatic environments, from opened areas near the coast to rivers and high elevation pasture-like paramos with (**Table S2.1-S2.3**). Tadpole sampling was carried out between June 2014 and April 2016. We analysed thermal tolerance limits of 75 species of tadpoles (**Fig. 2.1**; **Table S2.1**), collected from their natural habitat and transported to the experimental facilities in the Pontificia Universidad Católica del Ecuador. However, six species (*Atelopus elegans*, *Atelopus spumarius complex* ‘limón’, *Engystomops guayaco*, *Epipedobates* *machalilla*, *Epipedobates tricolor* and *Hyloxalus nexipus*) were obtained through captive breeding from the 'Balsa de los Sapos' initiative facilities in the same university. In these cases, we assumed that the results did not differ from those obtained in natural populations given the limited number of generations spent in captivity in the laboratory (F1 or F2). Finally, for each species, we also compiled a distributional range on elevation (maximum and minimum) database of the Andean species studied here (**Fig. 2.1**; **Table S2.2**).

Thermal data

We used Hobo Pendant temperature data loggers to obtain a continuous record of water temperature at each sampling site. Temperature was recorded every 15 min. We analysed mean (tmean), maximum (tmax), minimum (tmin) and mean daily range (dr) temperatures from each aquatic habitat. We also calculated the absolute thermal range (ar) as the difference between tmax - tmin. The number of sampling days ranged from 2-456 days (**Table S2.3**). We finally sorted each sampling site into four different habitat categories according to possible differences in their thermal regimes (river, permanent ponds, shaded temporal ponds and open ponds). However, since tadpoles can be found in different aquatic environments, we decided to include only the two most restrictive levels of habitat type, which are river restricted species and pond-like exploiter species (**Table S2.1**). We employed ANCOVA to determine the relationship of thermal data and habitat with altitude using the basic R package.

Estimates of thermal tolerance

We estimated thermal limits for 1890 specimens from 75 species (**Table S2.1**) using the Hutchison's dynamic method (Lutterschmidt & Hutchison, 1997b) that consists in exposing each animal to a constant heating / cooling rate ($\Delta T = 0.25$ °C min^{-1}) using a thermal bath (HUBER K15-cc-NR) until an end point is attained. The end-point was signalled for both thermal limits, as the point at

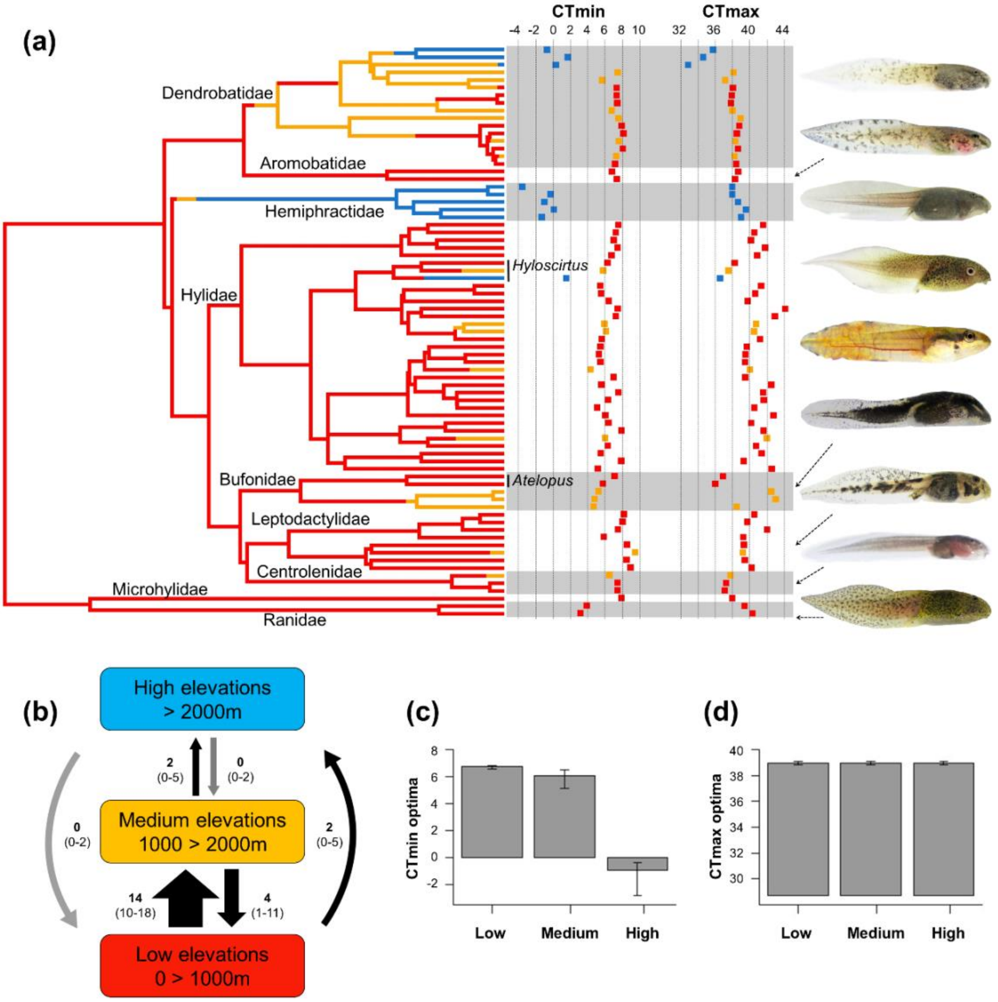


Figure 2.1: Ancestral reconstruction of elevational distribution and the evolution of thermal tolerance limits. (**a**) An example of a reconstruction of shifts on elevational distribution using a randomly selected tree, with estimated CTmax and CTmin (mean) for each species; each elevational distribution is given a different colour (see (**b**) for colour assignation). (**b**) Number of transitions between elevational categories; values represent the median number of transition between elevations and the quantile 2.5 and 97.5. Arrow size are relative to number of transitions between elevations, grey indicate a median of zero transitions. The estimated CTmax (**c**) and CTmin (**d**) optima (barplot; median and the 97.5 and 2.5% confidence intervals) for each elevation category under the OUM and BM1 model of evolution respectively.

which the tadpole mobility ceases completely and fails to respond to external stimuli. Whenever possible, we used a minimum sample of 15 individual tadpole replicates per species and thermal tolerance limit (for some species the available number of individuals examined was lower, **Table S2.1**). Each tadpole was weighed and staged (Gosner, 1960) immediately after the test. Only individuals between stages 25 and 38 were tested. Previous studies demonstrate that on later stages, where the metamorphic climax is approaching, organisms tend to lower their thermal tolerances (Floyd, 1983).

Before the experiments, larvae were acclimated at 20 ^{0}C with a photoperiod of 12L:12D for at least three days in order to stabilize thermal tolerance estimates of field sampled individuals living at diverse environmental temperatures (Hutchison, 1961; Brattstrom, 1968). During that time tadpoles were maintained in containers in small densities and fed *ad libittum*. During the assays each tadpole was placed individually in separate containers filled with 100ml of dechlorinated tap water immersed in the thermal bath at a starting temperature of 20^{0}C. Once the end point of tadpoles was attained, we measured water temperature with a Miller & Weber quick-recording thermometer (0.1^0 C accuracy) placed beside the tadpole, we assumed that body temperature equalled water temperature because of tadpoles' small size (Lutterschmidt & Hutchison, 1997a).

Although we only included a single population for each species, we assumed that response variation among species is larger than variation within species (see Klok & Chown, 2003; Gutiérrez-Pesquera *et al.*, 2016). Species' tolerance range was calculated as the difference of CTmax - CTmin. Finally, to determine whether species inhabiting lowlands or mountaintops are exposed to extreme thermal conditions and, therefore, should be exposed to stronger thermal selection, we evaluated the risk of each species to suffer thermal stress. To do so, we estimated warming tolerances (wt) as the difference of CTmax and tmax (Deutsch *et al.*, 2008; Duarte *et al.*, 2012) and cooling tolerance (ct) as the difference CTmin and tmin (Gutiérrez-Pesquera *et al.*, 2016).

Phylogenetic hypotheses

For the phylogenetic analyses, we used the package ape in R (Paradis *et al.*, 2004) to reconstruct a consensus and 1000 randomly selected phylogenetic trees, using the most comprehensive and recent study on amphibians (Jetz & Pyron, 2018). We used the position of a known sister taxon for four species not included in this phylogeny (**Fig. 2.1**; **Table S2.1**).

Physiological and environmental variation with elevation

We tested for phylogenetic signal in each trait using the most common indices for continuous traits, Pagel's λ and Bloomberg’s K. We used the fitContinuous function in geiger (Harmon *et al.*, 2008) for both analyses. Since we detected a strong phylogenetic signal with both methods ($\lambda = 0.957$, $p < 0.001$ (CTmin) and $\lambda = 1$, $p < 0.001$ (CTmax); **Table S2.12**) a phylogenetic generalized least squares (PGLS) approach was applied to determine the association between thermal physiology and vulnerability variables (CTmax, CTmin and TR, wt and ct), with ecological variables, altitude and habitat type (rivers and ponds), under Brownian motion of evolution using the R-package ‘caper’ (Orme, 2013) through likelihood maximum estimations of Pagel’s lambda. We decided to employ the elevational midpoint of the species distribution, since it did not differ from population elevation point, when explaining physiological traits variation (see **Table S2.8, S2.10**). Also, we used simple PGLS regressions to examine the relationship between the physiological variables (CTmax, CTmin and TR) and the environmental temperature (tmax, tmin, tmean dr). To test whether a physiological trade-off between thermal limits exists, we correlated CTmax and CTmin. For all the comparative analyses we used the consensus phylogenetic tree, however we reran the analyses with 100 randomly selected trees to account for phylogenetic uncertainty (e.g. Sayol *et al.*, 2016). Tadpole mass was not included in our analysis because, although it was significant for simple regression with CTmin, it did not explain significant variation or change the results when included in the models (**Table S2.8, S2.11**).

Evolutionary rates of thermal tolerance change

We compared the rate of evolution for both resistance limits using the function ‘ratebytree’ from the R package ‘phytools (Revell, 2012). This method allows comparisons of the rate of evolution of different characters under two adaptive models (Ornstein-Uhlenbeck, OU and Early-Burst, EB) and a simpler model (Brownian Motion, BM)(Revell *et al.*, 2018). We first searched which of those models best fits our data using the function ‘fitContinuous’ in geiger (Harmon *et al.*, 2008). We used the simplest model (i.e. BM) if evolutionary models were not significantly better. For the analysis we used the consensus tree, however, we repeated the analysis with 1000 phylogenies in order to reduce phylogenetic uncertainty.

Ancestral reconstruction and evolution of thermal tolerance limits

To assess the existence of altitudinal physiological barriers and their possible association to the evolutionary changes in CTmax and CTmin with elevation, we used the elevational range of species to reconstruct transitions between lowland and mountaintops using stochastic character mapping (SCM) with the function ‘simmap’ from the R-package phytools (Revell, 2012). We defined three different altitudinal categories according to the species’ midpoint distribution on elevation (low, 0-999m; medium 1000-1999m; high >2000m), yet, preliminary analyses showed that results were similar even when using different categories. We used 1000 phylogenies to reduce the potential effects of phylogenetic uncertainty (e.g. Sayol *et al.*, 2016). We finally estimated the amount and directionality (uplift and downlift) of the evolutionary transitions. These reconstructions were then used to test which evolutionary model could better explain the evolution of CTs on elevation using the R-package OUwie (Beaulieu *et al.*, 2012; Beaulieu & O'Meara, 2016). We fitted two random (i.e. Brownian motion, BM) and two adaptive (i.e. Ornstein–Uhlenbeck, OU) models of evolution that could either include a single optimum (BM1 and OU1 respectively) or different optima for each elevation category (BMS and OUM respectively)(O'Meara *et al.*, 2006; Thomas *et al.*, 2006). However, when using the 'BMS' model, some of the trees gave evolutionary optima that were outside the range of existing values of thermal tolerance and we therefore excluded this model for further analyses (also see Muñoz *et al.*, 2016). Finally, the best model was selected according to its lowest AICc scores.

Results

Thermal habitat variation

The temperature of tropical aquatic environments decreases linearly with elevation (Linear Model (LM): (tmin) $t_{44} = -19.49$, $p < 0.001$, $R^2 = 0.894$; (tmean) $t_{44} = -19.24$, $p < 0.001$, $R^2 = 0.891$ and (tmax) $t_{44} = -6.496$, $p < 0.001$, $R^2 = 0.478$; **Table S2.6**). However, we did not find a significant relationship between mean daily range (dr) and absolute range (ar) with altitude (LM: $t_{44} = -0.355$, $p = 0.724$ and $t_{44} = 0.220$, $p = 0.827$ respectively; **Table S2.6**). On the contrary, we found significant differences on tmax (LM: $F_{3,41} = 7.243$, $p < 0.001$), tmean (LM: $F_{3,41} = 433.62$, $p < 0.001$), ar (LM: $F_{3,41} = 5.677$, $p = 0.002$) and dr (LM: $F_{3,41} = 8.376$, $p < 0.001$) with habitat, but not for tmin (LM: $F_{3,41} = 0.677$, $p = 0.571$)(**Table S2.6**). Rivers and permanent ponds usually have lower maximum temperatures and smaller ranges of thermal variation (both absolute and daily range) than the rest of habitats (**Table S2.6, S2.7**; **Fig. S2.1**). The maximum temperature (38.5 ^{0}C) was recorded in a lowland open temporary pond while minimum temperature (6.2 ^{0}C) was recorded in a highland open temporary pond in paramo pastures (**Table S2.3**).

Table 2.1: Physiological traits (dependent variables) in relation to species elevational midpoint and habitat (river or pond).

Physiological traits		**Estimate (±SE)**	**tvalue**	**Pr (>|t|)**
1. CTmax	**Intercept**	40.263 (±0.55)	73.186	**<0.001**
	Elevation	-0.0009 (±0.0003)	-3.382	**0.001**
	Habitat (river)	-2.785 (±0.492)	-5.66	**<0.001**
(N=75; Pvalue <0.001; R2=0.399; λ=0.642)				
2. CTmin	**Intercept**	7.588 (±0.711)	10.677	**<0.001**
	Elevation	-0.002 (±0.0003)	-8.565	**<0.001**
	Habitat (river)	-0.189 (±0.549)	-0.344	0.732
(N=75; Pvalue < 0.001; R2=0.502; λ=0.874)				
3. TR	**Intercept**	32.613 (±0.887)	36.751	**<0.001**
	Elevation	0.0016 (±0.0004)	4.376	**<0.001**
	Habitat (river)	-2.47 (±0.691)	-3.574	**<0.001**
(N=75; Pvalue < 0.001; R2=0.256; λ=0.863)				

The effects of environmental variation on thermal tolerance traits and estimates of warming and cooling tolerance

The PGLS analyses revealed that both critical thermal limits decreased with elevation (PGLS: (CTmax) $F_{1,73}$ = 12.87, p < 0.001; (CTmin) $F_{1,73}$ = 86.94, p < 0.001; **Fig. 2.1, 2.2**; **Fig. S2.2**). Habitat was a significant predictor for CTmax but not for CTmin (**Fig. 2.2**; **Table 2.1**; **Fig. S2.2**). River species, characterized by low and constant temperatures, had significantly lower CTmax than pond species

(**Table 2.1**). We found a positive relationship between CTmax and CTmin (PGLS: $F_{1,73} = 12.92$, $p < 0.001$; **Fig. 2.3**). We also found a significant effect of habitat when included in the model (PGLS: $t_{72} = -5.685$, $p < 0.001$; **Fig. 2.3**); for the same

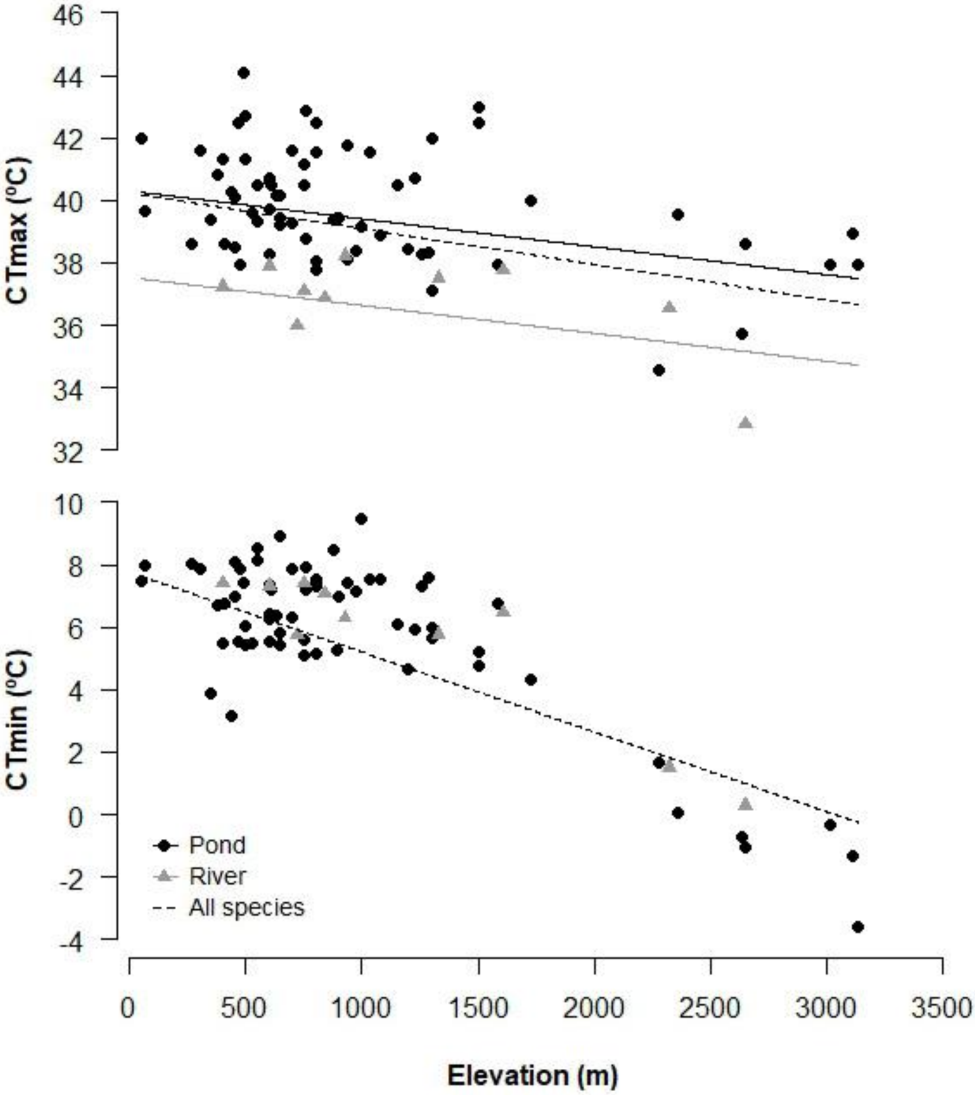


Figure 2.2: Upper and lower thermal tolerance limits (CTmax and CTmin) variation in tadpoles with mid-elevation of the species' distribution using PGLS. 'Pond' (black) corresponds to pond-like exploiter species and 'River' (grey) for river-restricted species.

CTmin, river-restricted species had lower CTmax than the rest of pond breeding species. Thermal tolerance ranges increases with elevation (PGLS, $F_{1,73} = 9.45$, $P = 0.003$; **Fig. S2.3**) and also showed a marginal tendency with species altitudinal range (PGLS, $F_{1,68} = 3.811$, $P = 0.055$; **Fig. S2.3**). Finally, river species tend to have lower thermal ranges than their conspecifics when accounted for elevation (**Table 2.1**).

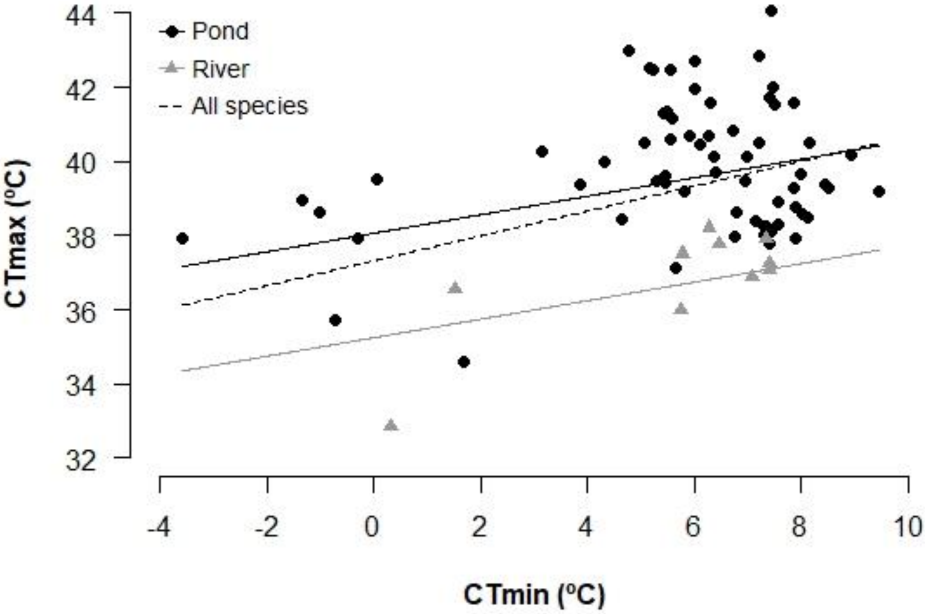


Figure 2.3: Phylogenetic generalized least squares for the CTmax-CTmin tradeoff.

Both CTmax and CTmin correlated with maximum and minimum water temperatures respectively (PGLS: ($F_{1,67} = 27.75$; $P < 0.001$ and $F_{1,67} = 27.21$; $P < 0.001$ respectively). However, TR was not predicted by with neither daily range (PGLS: $F_{1,67} = 0.029$; $P = 0.866$) or absolute range (PGLS: $F_{1,67} = 0.775$; $P = 0.382$)(**Table S2.9**).

The analyses of warming and cooling tolerance showed a positive correlation with altitude for both vulnerability estimates (PGLS: $F_{1,67} = 21.5$, $P <$

0.001 and $F_{1,67} = 18.81$, $P < 0.001$ respectively; **Table S2.8**). Nonetheless, none of the highland species appear to be under risk of suffering from acute cold stress under current conditions (ct < - 6.98 ^{0}C), yet at least three low elevation species (*Epipedobates machalilla* and both *Rana sp.* included) turned out to be highly vulnerable to suffer heat impacts (wt < 2 ^{0}C).

Comparing evolutionary rates of thermal tolerance limits

Evolutionary models were not significantly better than the simpler model (i.e. BM) and, hence, we compared the evolutionary rate among CTmax and CTmin under the BM model of evolution (see Table S13). We found that CTmin evolves at higher rates than CTmax ($\sigma 2 = 7.49$ and $\sigma 2 = 3.7$, respectively; LRT = 9.143; P = 0.0025). However, we only found a slight tendency (P = 0.108) when we reran the analysis with 1000 phylogenetic trees (**Table S2.14**).

Reconstructions thermal tolerance evolution on elevation

Evolutionary reconstructions of elevational distribution of tadpoles revealed several independent transitions between lowland and mid elevation species (**Fig. 2.1**). However, amphibian colonization of Andes mountaintops (> 2000 masl) seems to be a non-return trip, as median number of transitions to lower elevations is null (**Fig. 2.1b**). The best supported evolutionary model for CTmax was BM1, a random model (i.e. Brownian Motion) that include one single optimum for all selective regimes (elevation distribution midpoint) (**Table S2.15**), which is contrary to the hypothesis that species should evolve higher heat tolerances to inhabit lowlands (**Fig. 2.1c**). However, for CTmin, the best supported model was OUM, a random model that assumes the existence of different optima for each selective regime (**Fig. 2.1c**; **Table S2.15**). The estimates of CTmin optima are consistent with the hypothesis that species evolved lower cold tolerances when colonizing mountaintops ($P\ [CTmin_{high\text{-}elevation} > CTmin_{other}] <$

0.001; **Fig. 2.1c**). Also, estimates of CTmin optima for mid-elevation species were lower (i.e. more cold resistant) than low elevation counterparts (P [$\mathrm{CTmin}_{\text{mid-elevation}}$ > $\mathrm{CTmin}_{\text{low-elevation}}$] = 0.023; **Fig. 2.1c**).

Discussion

Our results indicate that in Andean tropical mountains amphibians to move to higher elevations is physiologically more challenging than dispersion to lower elevations. Apparently, cold temperatures were much more likely to be an important selective force in the evolution of CTmin of upland frog species while CTmax was mostly invariant along all the elevational range. Despite tadpole community are exposed to maximum peak temperatures differing by 25 ^{0}C (38.5^{0}C at a 32 m lowland pond to 13.5 ^{0}C in a stream at 2830 m), CTmax values were remarkably similar across altitudes. For example, *Epipedobates machalilla*, which inhabits temporal shadowed ponds near sea level, presents similar CTmax to *Gastrotheca pseustes*, the species registered at the highest elevation in this study (38.6 ^{0}C and 38.61 ^{0}C; respectively, **Fig. 2.1**). In contrast, for these same species, CTmin values differed 11.6 ^{0}C (**Table S2.1**).

At least six families of anurans (i.e. Bufonidae, Centrolenidae, Strabomantidae, Dendrobatidae, Hemipractidae, Hylidae and Telmatobidae) have independently colonized Andean high-elevations (Ron *et al.*, 2011) through dramatic evolutionary shifts in both in thermal resistance limits (present results) and thermal sensitivity to cold (van Berkum, 1988; Navas, 1996b; Chapter 3 and 4). Similar temperature-related shifts in amphibian climatic niche have also been reported for Andean dendrobatids (Graham *et al.*, 2004) and Holarctic hylid frogs, supposedly derived from central American tropical ancestors (Smith *et al.*, 2005). In both cases, these new environments are supposedly characterized by greater daily/absolute thermal variability and lower minimum temperatures (but see results). Compared to latitudinal gradients, altitudinal clines integrate substantial climatic variation over much shorter geographical distances (Sørensen *et al.*, 2005; Körner, 2007). Thus, physiological thermal variation found for ectothermic species on latitudinal gradients (e.g. Addo-Bediako *et al.*, 2000; Sunday *et al.*, 2011) and, particularly in amphibians tadpoles (Gutiérrez-Pesquera *et al.*, 2016), matched the physiological thermal variation found for an altitudinal transect between 0-4000 m within a similar latitude. This amazing evolutionary divergence has occurred for some lineages even when critical thermal limits are considered highly conservatives (Wiens *et al.*, 2006; Olalla-Tárraga *et al.*, 2011; Hoffmann *et al.*, 2013). Our data supports this view, as both cold and heat tolerance exhibit strong phylogenetic signal, with a few exceptions in assemblages of species that have drastically shifted their cold tolerance (‘escapee’ species) to colonize higher elevations (for latitudinal gradients see: Olalla-Tárraga *et al*., 2011).

Our analyses also highlights that aquatic habitat may impose local variability in maximum peak temperatures and resistances but not influence CTmin and tmin. This similar contrasting pattern appears in other terrestrial ectotherms (Frishkoff *et al.*, 2015; Kaspari *et al.*, 2015; Nowakowski *et al.*, 2017; Chapter 1). This divergence on the variation of extreme environmental temperatures (i.e. tmax and tmin) should pose different selective pressures on critical thermal limits. If maximum temperatures of lotic habitats (i.e. rivers) are lower and more constant than most lentic habitats (e.g. ponds, puddles), it is not surprising that species that are exclusive river breeders (e.g. Centrolenidae family, *Atelopus* and *Hyloscirtus* genus; see **Fig. 2.1a**) show lower CTmax than species that can exploit open temporal ponds as found in other subtropical and lowland tropical amphibian tadpole communities (Duarte *et al.*, 2012; Gutiérrez-Pesquera *et al.*, 2016). Thus, finding that colonization of low elevations is not linked to CTmax evolution but to heat buffering via habitat selection as a driving mechanism, ultimately determining a pattern of physiological niche conservatism in upper thermal resistance through altitudinal gradients (Araújo *et al.*, 2013). If habitat, through either active or passive selection, is responsible for heat resistance invariability, addressing the possible effects of habitat modification is crucial for future research on vulnerability to climate warming.

Contrary to maximum temperatures, minimum temperatures do not vary across habitats. In fact, most tmin variation, about 90%, is explained by elevation alone (**Table S2.5**). This implies that physiological barriers to face cold extremes at higher altitudes cannot be ameliorated by thermoregulation (see for lizards: Muñoz *et al.,* 2014; 2016; insects: Gill et al., 2016). Therefore, ectotherms cannot shield extreme minimum temperatures leaving no other option but to adapt physiologically, which might be an explanation for the fast rate of CTmin evolution (Araújo *et al.*, 2013; Muñoz *et al.*, 2014 but see von May et al., 2017; chapter 1). Other evolutionary constraint may also afford for CTmin variation. For instance, both species of Ranidae found in Ecuadorian coastal lowlands (i.e. *Rana bwana* and *Rana vaillanti*) with altitudinal distributions that do not exceed 900 meters (**Table S2.2**), exhibit relatively low CTmin values (< 3,8 ^{0}C) although these species never experience low environmental temperatures (tmin > 24^{0}C) (**Fig. 2.1**; **Table S2.1**), This may simply reflect a significant degree of niche conservatism in *Rana*'s thermal physiology which can tolerate very low temperatures (e.g. Menke & Claussen, 1982) given how this clade of frogs originated in northern latitudes (Hua & Wiens, 2010). Also, ontogeny may limit evolution of thermal resistance in tadpoles but studies are still scarce (Menke & Claussen, 1982; Floyd, 1983). Furthermore, we find a physiological trade-off between thermal limits, especially when including habitat in the model. However, evolution of thermal tolerances does not seem to be constrained since species that can exploit open ponds have, for the same CTmin, higher CTmax than river-restricted species. Yet this may reflect how, for some species, variation of physiological resistance is not related to thermal adaptation but to a physiological trade-off.

Even though moving to higher elevations may be more physiologically challenging, we found that transitions to higher elevations, as opposed to lower elevations, are more frequent (**Fig. 2.1b**). This result coincides with a recent study in birds (Quintero & Jetz, 2018) which found that diversification rates increase with elevation, suggesting that frequent extinctions and immigrations from lower areas is a key mechanism for diversity in high-elevation habitats. Altitude may promote diversification rates yielding increased beta diversity and species turnover, especially at low latitudes (Baselga *et al.*, 2012). Results revealed that, although moving down seems to be less physiologically challenging, no high elevation clades have gone downwards. Even transitions from medium to low altitudes is a minimum fraction from that exhibited from low to medium elevations (4 and 14 transitions respectively, **Fig, 2.1b**). Several argumentations may afford for this asymmetric pattern of geographical transitions. First, uplift and downward dispersal has an additional limitation to climatic disparity that is the spatial asymmetry in lateral area with greater land range at low than at high elevations (Colwell & Rangel, 2010). This lateral area effect may be a scale based cause for the generally decrease in species richness at the mountaintops (Lomolino, 2001; Rahbek, 2005). Our mountaintop frog community over 2000 m, represented 12 % of the total number of analysed species, a figure that fits to the total amphibian community of Ecuador (Ron *et al.*, 2018) and Colombia (Bernal & Lynch, 2008). Also, the richer lowland communities imply an increase of biotic interactions that could impede downward shifts (Schemske *et al.*, 2009). Altitudinal species turnover has been argued to result from interactions between biotic and abiotic factors, which may play a primary role on distribution to lower and higher elevations respectively (Schemske *et al.*, 2009; Jankowski *et al.*, 2013; Wisz *et al.*, 2013). For instance, some species may be excluded from particular elevations by competitively superior species, while the elevational range of the latter species may be limited by climatic factors.

Historical climatic variation should also have conditioned species dispersal. The Andean uplifts that dates from Miocene (Hoorn *et al.*, 2010) and last glaciations of the Pleistocene (Torres *et al.*, 2013; Flantua *et al.*, 2014) may have impeded, physically or physiologically, upward distribution until recently. In fact, vegetation currently found in high elevation Andes was not established until the last glaciations (van der Hammen, 1974; Torres *et al.*, 2013; Flantua *et al.*, 2014). Moreover, last glaciations can be responsible for the large decrease of CTmin in upland species (see **Fig. 2.1**). Especifically, high elevation ectotherms may have remained in locations where previous temperatures from glaciations were much lower than their lowland counterparts (6-7ºC and 3ºC respectively; van der Hammen, 1974), explaining the current disparity found between environmental temperatures and cold tolerance.

However, there are some clades that have presumable experienced downlift such as Hemiphractidae *Gastrotheca* frogs, that have suffered a transition from biphasic to gastric brooding breeding system associated to the downlift transition (Wiens *et al.*, 2007; Duellman & Trueb, 2015). This pattern of elevational transitions are also found in other families such as river-breeder glass frogs, Centrolenidae, where ancestral altitudinal reconstructions suggest that glassfrogs were present in mid-elevation habitats ancestrally, and that lower and higher elevation habitats were colonised more recently (Hutter *et al.*, 2013). These authors state that niche climatic conservatism is responsible for the elevational pattern of glass frogs because they found that clades with slow rates of climatic-niche evolution have slow rates of elevational change. A similar argument to explain lowland origin in dendrobatid frogs is afforded by Santos *et* *al.* (2009). However, other ectotherms appear to exhibit downlift transitions from mid altitude (Elias *et al.*, 2009).

Acknowledging the factors that promoted or constrained past migrations may improve our forecast to climate warming. Although none of the analysed species seem to be exposed to maximum temperatures higher than their heat tolerance (CTmax), our results suggest that some populations from low altitudes will suffer short term thermal stress due to exposure to hotter environments (Deutsch *et al.*, 2008; Tewksbury *et al.*, 2008) which may force to retreat uphill (Colwell *et al.*, 2008). The fact that distributional shifts required of higher cold tolerances (i.e. lower CTmin) may suggest that lowland amphibians are constrained to migrate upwards. However, because of the stasis of CTmax in contrast to the high labiality in CTmin, the several historical upward migrations observed, makes moving up the most likely option. In contrast, although upland species showed wider warming tolerance, the increase of temperatures may (for instance) increase nonlethal stress (Ruel & Ayres, 1999) or alter biotic interactions (Sinervo *et al.*, 2010), with the eventual upward migration of lowland species, which may threaten their survival in the long/medium term.

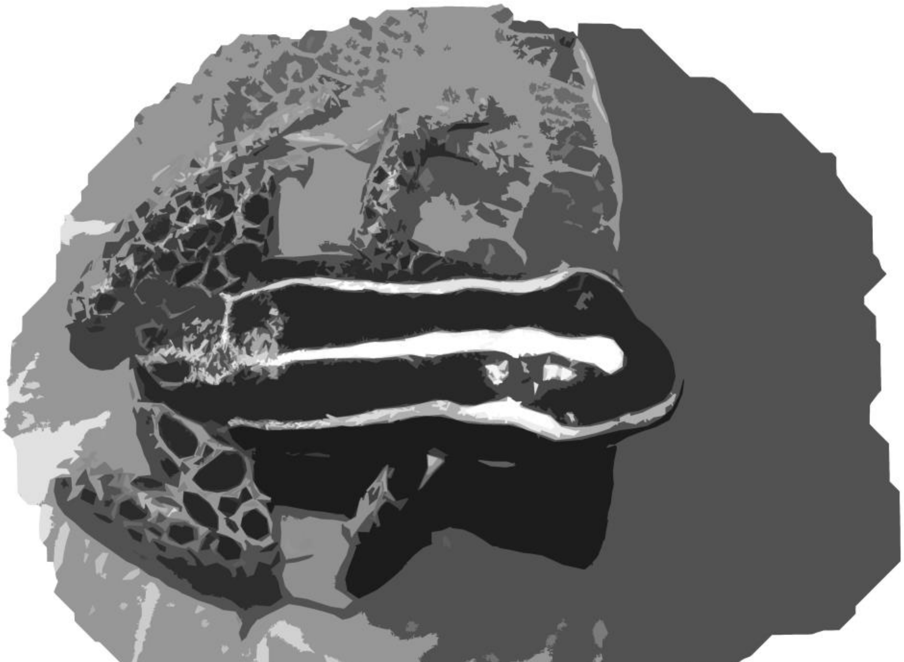
Concluding remarks

Janzen hypothesis predict narrower thermal tolerance in tropical ectotherms and thus, physiologically limiting their dispersal potential and effectiveness to settle in alien altitudes where they are not thermally adapted (physiological barrier hypothesis; Janzen, 1967; Colwell *et al.*, 2008). Our results, using ancestral reconstructions on a tropical elevational range, revealed a contrasting pattern of lability and stasis for thermal tolerance limits. Cold resistance exhibit multiple optima whereas stasis single optimum is found for CTmax, suggesting that physiological barriers are higher upwards than downwards. This asymmetric evolutionary pattern with altitude is, in part, a product of habitat selection that ultimately provide a stasis in the evolution of upper thermal resistance. This pattern is also found in other ectotherms such as lizards (Muñoz *et al.*, 2014; Muñoz *et al.*, 2016), and are highly relevant for those approaches examining climatic niche evolution in ectotherms, which does not take into account direct biological trait information such as physiology or behavioural thermoregulatory accommodations (e.g. Hutter *et al.*, 2013). However and paradoxically, altitudinal transitions downwards, especially from mountaintops, are scarce when compared to uplifts. Other causes unrelated to the current climate such as historic climate changes, Andean uplifts, topographic complexity and biotic interactions could be responsible for this paradox. In light of the distributional shifts predicted in the present scenario of global warming (Colwell *et al.*, 2008; Chen *et al.*, 2011a), we believe that understanding the causes for past shifts is essential to forecast future ones.

Chapter 3

Evolution of thermal sensitivity in an amphibian tropical mountain community

POL PINTANEL, MIGUEL TEJEDO, GUSTAVO A. LLORENTE, ANDRÉS MERINO-VITERI



Abstract

Despite growing interest in the evolution of thermal physiology in tropical mountains ectotherms, due to the increased concern of global warming, understanding how environmental variation in temperature influences their physiology remains almost unknown. Herein, we investigated variation in thermal sensitivity in larval amphibian growth rates, their evolution and constraints and, finally, by examining microenvironmental temperatures, providing a vulnerability assessment to global warming. We estimated in 28 amphibian species through an elevational gradient (0 - 3500 masl) in Ecuadorian Andes, thermal performance curves (TPC) parameters for growth performance: optimum temperature (Topt), maximum relative growth rate performance (Zmax) and thermal breadth (Bi); and critical thermal limits, both upper (CTmax) and lower (CTmin) and characterized the water temperature of their breeding habitats they inhabit. Results showed that optimum temperature covaries negatively with elevation being its main microclimatic predictors maximum (tmax) and mean (tmean) water temperature. However, we did not find an increase of thermal breadth with elevation as previously observed, and only a marginally significant trend with daily thermal variation that is, otherwise, the single microclimatic predictor of maximum growth performance. Testing the evolution of TPCs revealed that warm-adapted species grow faster than cold-adapted ones as predicted by the 'hotter is better' hypothesis, but there are no support for the specialist-generalist trade-off and hotter is narrower hypothesis. Finally, the 'physiological heating tolerance' (PHT) prediction is confirmed since species with warmer Topt have smaller safety physiological margins (CTmax - Topt). This indirectly implies that warm adapted species are prone to suffer lethal heat damage by small increases in pond temperatures. These warm adapted species, mainly located in of low and middle altitudes would be probably at risk of thermal damages and would have to uplift to higher altitudes or shift to cooler microenvironments (e.g. from open to forest ponds) to avoid these chronic thermal impacts that will probably increase in the coming decades.

Back cover: *Ranitomeya ventrimaculata* (Shreve, 1935)

Introduction

The interest in how temperature affects physiological performance in ectotherms and how it may drive their evolution has become a central issue in evolutionary and conservation biology (e.g. Huey & Kingsolver, 1989; Angilletta *et al.*, 2002; Angilletta, 2009; Sinclair *et al.*, 2016). Current research seeks for a better assessment of the potential of organisms to face *in situ* current and upcoming increase of temperatures, by modelling potential range shifts to newly suitable areas (Chen *et al.*, 2011a). Thus, informing of species vulnerability to extinction assessments due to climatic change (e.g. Thomas *et al.*, 2004; Deutsch *et al.*, 2008; Loarie *et al.*, 2008; Sinervo *et al.*, 2010; Kingsolver *et al.*, 2011) and how community structure will change in the coming decades (Lawler *et al.*, 2009; Gilman *et al.*, 2010).

A useful tool to examine organismal thermal sensitivity is the Thermal Performance Curve (TPC), a continuous, nonlinear, reaction norm in which an ectothermic species' performance (e.g. growth, development, locomotor ability) is described as a function of body temperature (Huey & Stevenson, 1979; Angilletta *et al.*, 2002; Angilletta, 2009). TPCs are typically concave in shape, with a central or left-skewed distribution (Huey & Stevenson, 1979; Angilletta, 2009). The shape of the curve can be described by the following parameters: the optimum temperature (Topt), the temperature that maximizes the performance of a trait (Zmax); the thermal breadth (B_i), or range of temperatures in which performance is above an arbitrary percentage 'i' to respect to maximum Zmax; and, finally, the maximum (CTmax) and minimum (CTmin) thermal tolerance limits which are the maximum and minimum range of temperatures (TR = B_0) in which the function can be performed (see **Fig. 3.1**). Variations of those parameters, either by phenotypic plasticity or adaptive evolution, do modify the shape and height of TPCs (Huey & Kingsolver, 1989; Izem & Kingsolver, 2005;

Martin & Huey, 2008; Knies *et al.*, 2009). Thus TPC variation, at the individual, population and species levels, should reflect the thermal conditions (average and variance) that organisms are exposed to (Angilletta *et al.*, 2002). First, Topt should correlate with the most frequent body temperature of the organism (Hertz *et al.*, 1983; Frazier *et al.*, 2006; Deutsch *et al.*, 2008; Huey *et al.*, 2012) producing an horizontal variation in the TPC shape (hotter-colder, **Fig. 3.1a**); second, thermal breadth (Bi) should be correlated to body temperature variation (van Berkum, 1986) altering the breadth width (broader-narrower, **Fig. 3.1c**). Finally, variation in maximum performance (Zmax) may produce vertical shifts in TPC's height (faster-slower, **Fig. 3.1b**). This latter response is predicted to be related with time-constrains to organismal activity such as seasonality, because cold-season constrains the time available for activity (Yamahira *et al.*, 2007; Conover *et al.*, 2009), which yields to mechanisms of compensation for more environmentally favourable activity periods ('metabolic cold adaptation hypothesis'; Scholander *et al.*, 1953). In the tropics, where seasonality is almost null, we do not expect this mode of TPC variation, but we hypothesize that vertical increase in performance would be selected in species breeding in temporary ponds compared to those breeding in permanent ponds or rivers because water availability may constrain the time available for completing the metamorphosis (Richter-Boix *et al.*, 2011).

Several constrains may limit TPC shape adaptive evolution (e.g. Lynch & Gabriel, 1987; Huey & Kingsolver, 1989; Frazier *et al.*, 2006; Martin & Huey, 2008). First, the 'hotter is better' hypothesis (**Fig. 3.1d**), posits that organisms adapted to higher temperatures are predicted to have higher maximum performances than those adapted to lower temperatures (Bennett, 1987; Frazier *et al.*, 2006), based on the rate-depressing effects of low temperatures on biochemical reactions (Hamilton, 1973; Bennett, 1987). Second, the 'generalist-

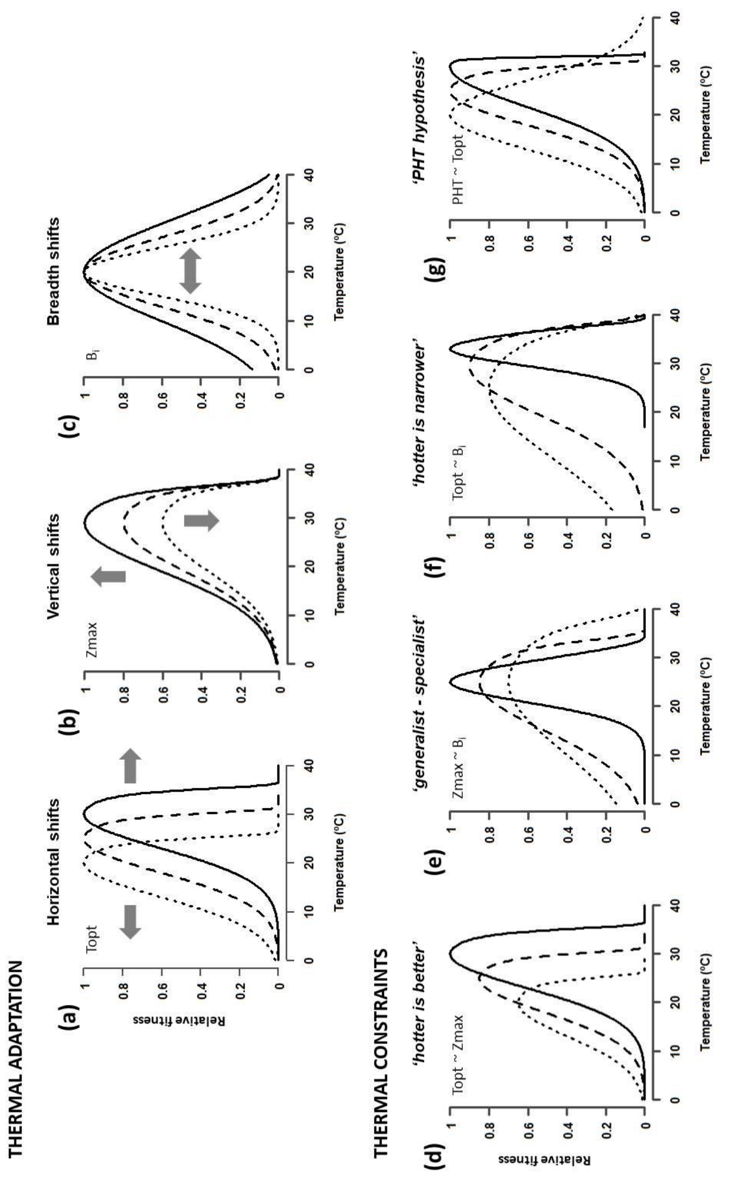


Figure 3.1: Predicted hypothesis on thermal adaptation (a-c) and interspecific trade-offs in variation of TPCs (d-g). (**a**) Horizontal shifts, hotter-colder performance. (**b**) Vertical shifts, faster-slower performance. (**c**) Breadth shifts, broader-narrower performance breadth. (**d**) 'hotter is better' predicts that adaptation to higher temperatures results in increased performance. (**e**) The generalist-specialist trade-off predicts that populations adapted to perform in a narrow temperature range (i.e. specialist) will perform better than thermal generalist species. The combination of 'hotter is better' (**d**) and 'generalist-specialist' (**e**), predicts that population adapted to higher temperatures will have narrower temperature range, (**f**) 'hotter is narrower'. (**g**) The 'physiological heat tolerance (PHT) hypothesis' suggest that adaptation to higher temperatures results in lower safety margins of acute heat stress (CTmax – Topt).

specialist' (or 'jack-of-all-temperatures a master of none'; **Fig. 3.1e**) hypothesis, suggests that organisms that performs at a broad range of temperatures (i.e. generalists) would perform worse than those adapted to a narrower range of temperatures (Huey & Hertz, 1984). This constrain is mechanistically grounded on the compromise between enzymes' flexibility and stability (Huey & Kingsolver, 1989). (3) The 'hotter is narrower' hypothesis (**Fig. 3.1f**) comes from the combination of the previous two. If organisms adapted to higher temperatures have higher maximum performances (hotter is better) and high performances are associated to narrower thermal breaths (generalist-specialist), then, we would expect that optimum temperature should be inversely related to thermal performance breadth (Frazier *et al.*, 2006; Knies *et al.*, 2009). Finally, (4) the 'physiological heating tolerance' hypothesis (PHT, *sensu* Payne *et al*., 2016; **Fig. 3.1g**) suggest that the magnitude of asymmetry in thermal performance curves (fitness drops more sharply at temperatures above the optimum; Huey & Stevenson, 1979; Izem & Kingsolver, 2005; Martin & Huey, 2008) increases in organisms adapted to higher temperatures and, therefore, the physiological safety boundary to suffer acute stress (calculated as PHT = CTmax - Topt) decreases with organisms' optimum temperature. Recently, Payne and Smith (2017) proposed that declines in performance curves proceed more rapidly with higher Topt because biological rates increase exponentially with temperature.

Latitudinal and altitudinal climatic gradients are amenable to the analysis of thermal sensitivity evolution and their constraints (Chown *et al.*, 2004; Gaston *et al.*, 2009), allowing to examine whether thermal physiology set limits to the distributional range of individuals (Sánchez-Fernández *et al.*, 2012; Overgaard *et al.*, 2014; chapter 4). Otherwise, the analysis of spatial variation in TPC parameters provide an useful tool to assess organismal vulnerability to climate change (Deutsch *et al.*, 2008; Huey *et al.*, 2009; Kearney & Porter, 2009; Sinclair *et al.*, 2016). Current studies on geographical TPC variation have mainly focused on latitudinal gradients (e.g. van Berkum, 1988; Cunningham & Read, 2002; Angert *et al.*, 2011; Krenek *et al.*, 2012; Thomas *et al.*, 2012; Stefansson *et al.*, 2013; Stevenson *et al.*, 2013; Bonino *et al.*, 2015). However, not much information has been reported on thermal sensitivity in ectotherms through altitudinal gradients. Singular exceptions are van Berkum (1986) and Navas (1996b; 1997) analyses of thermal sensitivity of locomotor performance at contrasting tropical elevational ranges in both *Anolis* lizards and several frog clades, respectively. Both studies report thermal evolution implying adaptive shifts in TPC parameters. For instances, lower Topt for those inhabiting colder highlands, and wider thermal breadths (B_{80}) for species living at more thermally variable highlands.

The evaluation of TPCs also allows the estimation of the impact of increasing temperatures on organisms under the current global warming scenario (e.g. Kearney *et al.*, 2009; Pacifici *et al.*, 2015; Sinclair *et al.*, 2016). It is predicted that lowland tropical organisms will be more vulnerable to climate warming because environmental temperature are just closer to their physiological optima than those from higher elevations (e.g. Deutsch *et al.*, 2008; Tewksbury *et al.*, 2008; Huey *et al.*, 2009; Sunday *et al.*, 2011; Sunday *et al.*, 2014), and predicted uplifts in distributional ranges have been currently reported in lowland tropical mountains organisms (Raxworthy *et al.*, 2008; Chen *et al.*, 2011b; Feeley *et al.*, 2011) that may determine lowland biotic attrition in the tropics (Colwell *et al.*, 2008; Lenoir & Svenning, 2013; Brodie *et al.*, 2017). Thus, a deep knowledge of thermal physiological sensitivity is crucial to define the level of risk to suffer heat impacts and to establish the suitability of novel ranges in a future. The most common estimates to determine the risk of organisms to suffer heat impacts, either acute or chronic is: (1) the difference between the physiological capacity to tolerate heat (i.e. CTmax) and the maximum temperature of the habitat (warming tolerance, WT = CTmax - tmax)

(Duarte *et al.*, 2012; Hoffmann *et al.*, 2013), and (2) the difference between the optimum temperature and the mean temperature of the habitat (thermal safety margin, TSM = Topt – tmean) (Deutsch *et al.*, 2008). In addition, we propose an additional estimate of chronic stress, the 'daily thermal stress' (DTS), defined as the relative amount of time that the environmental temperature is above the Topt.

In this study, we provide an analysis on interspecific thermal adaptation and inherent constrains in TPC evolution, by examining thermal sensitivity in tadpole growth rate for 27 amphibian larvae along a tropical elevational gradient (0-3500 masl) in the Andes of Ecuador. The tropical Andes are one of the most biodiverse regions of the world exhibiting great topographical and environmental complexity (Myers *et al.*, 2000; Kattan *et al.*, 2004; Hazzi *et al.*, 2018). Andean elevational ranges provide a great opportunity to study the evolution of thermal performance curves because two main reasons: First, altitudinal climatic variation occurs at a relatively small spatial scale (see Rapp & Silman, 2012), compared to larger gradients (i.e. latitudinal) that may confound historical with adaptive processes (e.g. Gutiérrez-Pesquera *et al.*, 2016). Second, tropical mountain organism are exposed to local altitudinal climatic zonation that make unfeasible phenological adjustments observed at higher latitudes (e.g. John-Alder *et al.*, 1988), and as a derived consequence, high altitude tropical ectotherms should not be temporally constrained to develop their life cycle, as it occurs in seasonal temperate, who adaptively exhibit vertical shifts in their TPCs (Berven, 1982; Conover *et al.*, 2009; Muir *et al.*, 2014). Additionally, by focusing in the aquatic larval stage, water environments exhibit low thermal variability compared to adult terrestrial environments (Spotila *et al.*, 1992). This determine that aquatic tadpoles are limited to adopt compensatory behavioural mechanisms, such as thermoregulation, to buffer thermal variation and then be forced to evolve thermal adaptations (Huey *et al.*, 2012) and thus, limited thermoregulatory scope that may buffer the aquatic tadpoles.

Main aims of this study are: (1) To examine the different modes of evolution of TPC variation. Because amphibian larvae are distributed in environments that largely vary on its temperature (both mean and variance), we expect that, under a scenario of thermal adaptation, variation of environmental temperatures will match variation on thermal sensitivity for growth performance (Lynch & Gabriel, 1987; Angilletta *et al.*, 2002). For instance, Topt should be correlated to mean environmental temperature (horitzontal variation, **Fig. 3.1a**; Hertz *et al.*, 1983; Huey *et al.*, 2012); thermal breadth should be correlated with environmental thermal variation (breadth variation, Fig. 3.1c; van Berkum, 1986; Navas, 1996b) and, finally, maximum performance should increase on environments with restricted time for growth (vertical variation, **Fig. 3.1b**; Yamahira *et al.*, 2007; Richter-Boix *et al.*, 2011). (2) As variation in TPCs might, inherently, be constrained, we also analysed the following constrain hypotheses; 'hotter is better', 'generalist-specialist', 'hotter is narrower' and the 'PHT' hypothesis (e.g. Huey & Kingsolver, 1989; Frazier *et al.*, 2006; Martin & Huey, 2008) (see **Fig. 3.1d-g**). (3) Furthermore, we analysed the correlations between TPC parameters of growth performance with thermal tolerance limits (i.e. CTmax, CTmin) and thermal range (TR = CTmax - CTmin). For instance, Topt is expected to be related to CTmax but not to CTmin (Huey *et al.*, 2009) and thermal breadth (B_{50}) should be positively correlated to thermal tolerance range, as both parameters are related to organismal performance breadth. (4) Finally, we analysed the hypothesis that tropical lowland ectotherms are more prone to suffer thermal stress because environmental temperatures are closer or even exceed their Topt (e.g. Sunday *et al.*, 2014) and, therefore, we provide estimates of species vulnerability (both acute and chronic) under predicted future impacts of global warming (Evans *et al.*, 2015).

Material and methods

Study organisms and thermal performance curves

We sampled 28 tropical amphibian species during their aquatic tadpole stage, across an altitudinal gradient in Ecuador (between latitudes 1^0N - 4^0S and elevations 23 - 3631 masl), (see **Table S3.1**). Most of the specimens were collected from their natural habitats since 2014 to 2016. Four species (*Atelopus elegans*, *Epipedobates machalilla*, *Epipedobates tricolor* and *Hyloxalus nexipus*) were, however, obtained from the captive breeding 'Balsa de los Sapos' initiative at the Pontificia Universidad Católica del Ecuador (PUCE, Quito, http://bioweb.puce.edu.ec/QCAZ/contenido/BalsaSaposNuestrosInicios). Because those specimens were first or second generation breeding, we assumed that the physiological parameters did not largely differ from those obtained directly from natural populations (for insects see Griffiths *et al.*, 2005; Kellermann *et al.*, 2012b).

To assess thermal sensitivity variation we chose tadpole growing performance as a good proxy for fitness, since growth is an integrative process resulting from the interaction among other temperature dependent physiological parameters (Freitas *et al.*, 2010). Growth experiments were conducted in a lab with a room temperature of 19-20 ^{0}C. Previous to start growth experiments both field collected and captive breeding tadpoles were acclimated at this temperature for 2-3 days. Once this acclimating period tadpoles were randomly assigned to eight constant temperatures treatments (9, 15, 20, 23.5, 27, 29, 31 and 33 ^{0}C) and a photoperiod of 12L:12D, being individually maintained for 10 days in plastic cups with 400 ml of dechlorinated water, aerated with an air pump system and fed *ad libitum*. Water and food was completely renewed four times during the experiment (each 2-3 days) and individuals were checked daily for survival. For some species we eliminated those treatments (or reduced the number of individuals of those) that were manifestly lethal, while for some thermophilic species (i.e. lowland open-pond breeders), we included an additional hotter treatment (35 ^{0}C). To keep constant thermal regimes during the experiment, we used portable fluid heaters with regulation adjustment (U201431698). To obtain colder temperature treatments (9 ^{0}C and 15 ^{0}C), we used TECO TK 1000 chillers. During the experiments consigned temperature treatments oscillated in a range of 0.2-0.4 ^{0}C). Tadpoles were weighed and assigned their stage of development (Gosner, 1960) just before and after the experiment. Then, we estimated relative growth rate (GRr), calculated (following Travis, 1980) as:

$$\mathrm{GRr} = {W_\mathrm{f} - W_\mathrm{i}}\Big/{W_\mathrm{i} \times \mathrm{N_{days}}}$$

W_i and W_f were the initial and final weight of the individual respectively and $\mathrm{N_{days}}$ was the number of days elapsed from the start of the experiment. We considered negative values of relative growth rate as non-growth and therefore analysed as zero (Overgaard *et al.*, 2014). Because anuran larval growth rate decay near metamorphosis (Harris, 1999; Richter-Boix *et al.*, 2011), and to obtain estimates during the linear growth phase, we avoided selecting experimental tadpoles in late developmental stages (> 33 Gosner stages). Sampling tadpoles differing in size and developmental stage were homogeneously distributed across temperature treatments.

Climatic data and estimates of thermal tolerance limits

For all species analysed in their TPC, we previously obtained estimates of physiological thermal tolerance limits: CTmax, CTmin and thermal tolerance range (TR = CTmax - CTmin) from individuals taken for the same locality (see methods in chapter 2).

We obtained microclimatic habitat temperature for each sampling site by deploying dataloggers (HOBO pendant) at the deepest bottom of each aquatic environment. Loggers were programmed to sample every 15 minutes during a period of 15 to 435 days (see **Table S3.1**). We analysed mean (tmean), maximum (tmax) and minimum (tmin) temperatures, average daily range (dr) and absolute range (ar = tmax - tmin) from each aquatic environment with the exception of two species, *E. tricolor* and *H. phyllognatus* (grp.), from which we could not gather thermal predictors.

Vulnerability to global warming

To evaluate the risk of species vulnerability to suffer chronic and acute thermal stress, we obtained three different estimates: one for acute (warming tolerance, 'WT') and two for chronic thermal stress (thermal safety margin, 'TSM' and daily thermal stress, 'DTS'). WT was defined as the difference between CTmax and maximum temperature (tmax) (Duarte *et al.*, 2012; Hoffmann *et al.*, 2013), while TSM as the difference between Topt and mean temperature (tmean) (Deutsch *et al.*, 2008). We also included another chronic estimator of thermal stress, daily thermal stress (DTS), defined as the percentage of time that environmental temperatures are above the Topt. For DTS, values range between 0, indicating no thermal stress, and 1, indicating all-day chronic stress. We consider those estimates as conservative since environmental temperatures are obtained from dataloggers deployed at the pond coolest bottom and then tadpoles could not select pond areas where water temperatures were lower, either for maximum and mean temperatures.

Phylogeny reconstruction

To control for phylogenetic relatedness in our analyses, we used the consensus tree of the most recent published phylogeny of amphibians by Jetz and Pyron (2018) using the ape package in R (Paradis *et al.*, 2004). For *Rhinella* *horribilis*, not included in Jetz and Pyron's phylogeny, we used the position of its sister species *R. marina* (**Fig S3.2**).

Analysis of thermal performance curves for growth rate

To fit species growth performance with temperature, we adjusted a nonlinear mixed effect model according to Angilletta (2006) with the R-package 'nlme' (Pinheiro *et al.*, 2018) using four different models: quadratic, cubic, Gaussian and Gaussian-Gompertz (Frazier *et al.*, 2006; Martin & Huey, 2008). The Akaike information criterion (AIC) was used to identify the best model (Burnham & Anderson, 2002). Then, we extracted the TPC parameters from this model: Topt was estimated as the temperature when performance is maximized (Zmax); thermal breadths were estimated as the range of temperatures in which performance was equal to or greater than 50 or 80% of the Zmax (B_{50} and B_{80}, respectively). Because the results did not differ using one or another thermal breadth, we only show here the results with B_{50}. Finally, to test the hypothesis that performance curves decline more sharply at higher Topt, we calculated the boundary of temperatures to suffer heat stress or physiological heating tolerance (PHT; Payne *et al.*, 2016) for all species as the difference between CTmax and Topt. Maximum growth performance (Zmax) was log-transformed to provide normality (hereafter referred as Zmax).

Phylogenetic comparative analyses

We used Pearson correlations to analyse the relationships between growth TPC parameters (Topt, Zmax and B_{50}), thermal resistance limits (CTmax, CTmin and TR), physiological heating tolerance (PHT), vulnerability indexes (WT, TSM and DTS) and elevation at the species sample point. To analyse if breeding habitat has an effect on TPC parameters, we also performed ANOVAs with habitat

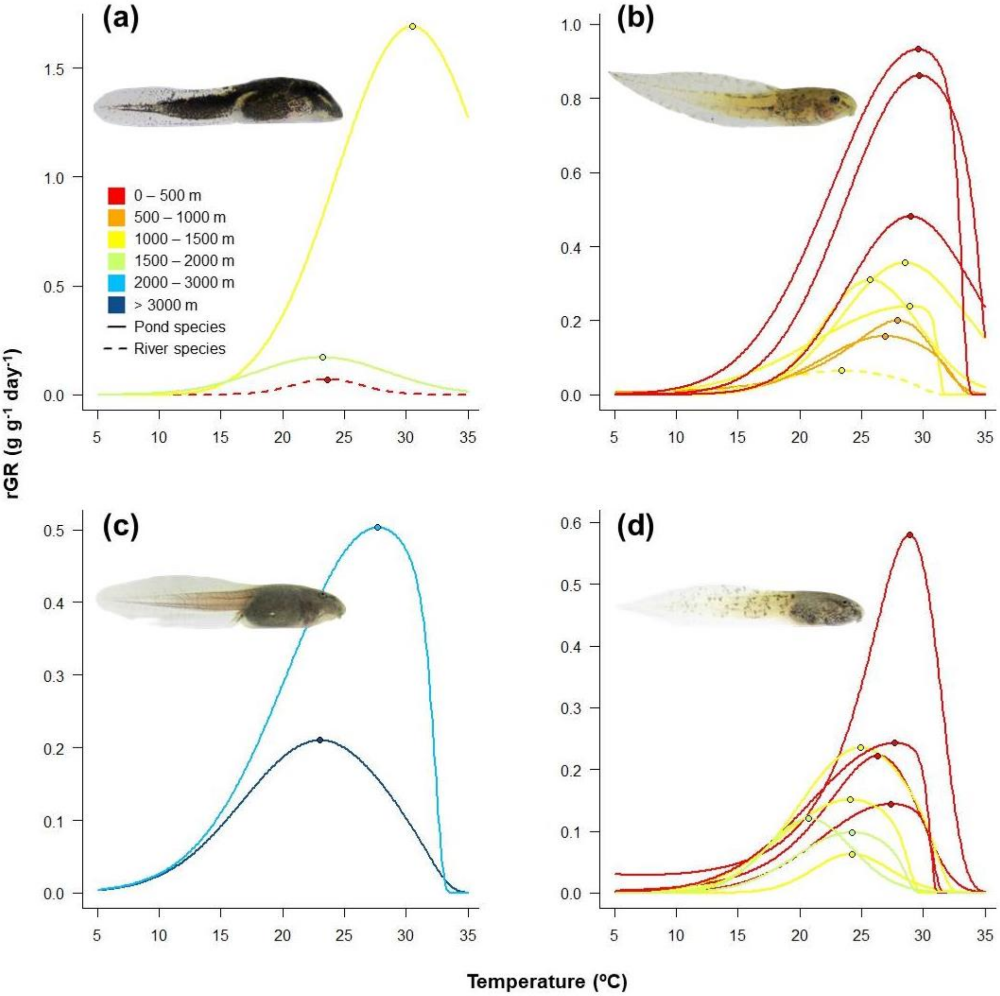


Figure 3.2: Thermal performance curves for relative larval growth rate within four amphibian families: (a) Bufonidae, (b) Hylidae, (c) Hemiphractidae (*Gastrotheca* genus) and (d) Dendrobatidae. Open circle represents Topt at maximum performance.

(river versus pond) as main factor. In order to account for phylogenetic correction for all the above analyses, we used a phylogenetic generalized least squares (PGLS) approach with the function pgls in the R-package caper (Orme, 2013), which accounts for phylogeny through maximum estimation of Pagel's lambda (Pagel, 1999). We also used univariate PGLS regressions to examine the relationship between Topt, Zmax and B_{50} and microclimatic thermal variables.

We further selected the best model using the Akaike information criterion (AIC) (Burnham & Anderson, 2002). All analyses were performed in R (R Core Team, 2014).

Results

Thermal adaptation in tropical elevations

Based on its lowest AIC, species thermal performance curves were best fitted by the Gaussian-Gompertz or by the Gaussian models (**Fig. 3.2**, **Table S3.2**). Conventional and phylogenetic correlations did not differ because the phylogenetic signals of the TPC parameters were generally low (**Table 3.1**, **S3.3**). Results are expressed with mean (±SE).

Optimum temperature was negatively correlated with elevation and, therefore, lowland species had higher Topt than uplands (**Table 3.1**; **Fig. 3.2**). Furthermore, Topt slightly differed with habitat, stream-breeder species had lower Topt than pond-breeders (24.2 ^{0}C (± 0.72), n = 3 and 26.72 ^{0}C (±0.49), n = 25, respectively; $F_{1,26}$ = 4.036; P = 0.055). Both results are consistent with the positive correlation between optimum temperature and mean and maximum environmental temperatures (tmean: $F_{1,24}$ = 13.88; P = 0.0011; R^2 = 0.34 and tmax: $F_{1,24}$ = 12.48; P = 0.0017; R^2 = 0.315; **Table 3.1**), being Topt generally higher than mean temperature (**Table 3.2**, **Fig. S3.1a,b**). The prediction that thermal breadth (i.e. B_{50}) increases with elevation and environmental thermal variation showed only a statistically marginal tendency (P = 0.091 and P = 0.072, respectively) (see **Tables 3.1, 3.2**) and was not related with habitat (river: 9.03 ^{0}C (±1.5) and pond: 10.25 (±0.46); $F_{1,26}$ = 1.16; P = 0.29). Maximum growth performance (Zmax) was not related to elevation ($F_{1,26}$ = 0.828, P = 0.371, R^2 = -0.006) however it was positively correlated with daily thermal range ($F_{1,24}$ = 5.55, P = 0.027, R^2 = 0.154; **Table 3.2**) and differed with habitat (river: -2.44

Table 3.1: Conventional, non-phylogenetic correlation (below the diagonal) (Pearson R, P-value) and simple regression (R^2, P-value, Pagel's λ) using a PGLS approach (above the diagonal) between physiological traits. Values highlighted in grey indicate significant regressions after applying Bonferroni correction (Rice, 1989). PHT: physiological heating tolerance; Topt: optimum temperature; Zmax: maximum performance; B_{50}: thermal breath (50); maxB50 and minB50: maximum and minimum temperature for B_{50}; CTmax, CTmin: maximum and minimum critical thermal tolerances; TR: thermal tolerance range.

Trait	PHT	Topt	Zmax	B50	maxB50	minB50	Ctmax	Ctmin	TR	elevation
PHT	-	**(0.43, 0, 0.32)**	(0.1, 0.057, 0)	(0.04, 0.16, 0)	(0.02, 0.22, 0)	**(0.23, 0.0053, 0)**	(0, 0.92, 0)	(0.02, 0.24, 0)	(0, 0.31, 0)	(0.07, 0.096,0)
Topt	**(-0.6, 0.0007)**	-	**(0.57, 0, 0.24)**	(0, 0.54, 0,063)	**(0.74, 0, 0.56)**	**(0.61, 0, 0)**	**(0.64, 0, 0)**	(0.08, 0.08, 0.14)	(0.02, 0.23, 0)	**(0.24, 0.0046, 0.29)**
Zmax	(-0.28, 0.057)	**(0.67, 0)**	-	(0.05, 0.12, 0)	**(0.61, 0, 0)**	**(0.29, 0.002, 0)**	**(0.44, 0, 0)**	(0, 0.96, 0)	**(0.16, 0.02, 0)**	(0, 0.37, 0)
B50	(0.27, 0.16)	(0.13, 0.51)	(0.29, 0.12)	-	**(0.23,0.0057, 0)**	**(0.15, 0.024, 0.38)**	(0.08, 0.07, 0.35)	**(0.23, 0.006, 0)**	**(0.47, 0, 0)**	(0.07, 0.091, 0.02)
maxB50	(-0.24, 0.22)	**(0.85, 0)**	**(0.75, 0)**	**(0.51, 0.0057)**	-	**(0.32, 0.001, 0.38)**	**(0.79, 0, 0)**	(0, 0.52, 0.3)	**(0.21, 0.009, 0.13)**	(0.07, 0.091, 0.42)
minB50	**(-0.51, 0.0055)**	**(0.79, 0)**	**(0.53, 0.002)**	**(-0.4, 0.034)**	**(0.58, 0.001)**	-	**(0.35, 0.0006, 0)**	**(0.28, 0.002, 0.04)**	(0, 0.52, 0.08)	**(0.33, 0.0009, 0.03)**
Ctmax	(-0.02, 0.92)	**(0.81, 0)**	**(0.63, 0)**	(0.36, 0.059)	**(0.89, 0)**	**(0.61, 0.0006)**	-	-	-	-
Ctmin	(-0.23, 0.24)	(0.32,0.1)	(0.004, 0.96)	**(-0.5, 0.006)**	(0.08, 0.7)	**(0.56, 0.0022)**	(0.23, 0.24)	-	-	-
TR	(0.2, 0.31)	(0.23, 0.23)	**(0.4, 0.021)**	**(0.7, 0)**	**(0.51, 0.0055)**	(-0.12, 0.55)	**(0.44, 0.02)**	**(-0.78, 0)**	-	-
elevation	(0.32, 0.096)	**(-0.48, 0.005)**	(-0.18, 0.37)	(0.33,0.091)	(-0.26, 0.17)	**(-0.59, 0.0009)**	**(-0.37, 0.025)**	**(-0.77, 0.0002)**	**(0.48, 0)**	-

Table 3.2: Simple regression models (PGLS) used to predict physiological variables estimated from TPCs with environmental variables of the breeding-site (n = 26; tmax = maximum temperature; tmin = minimum temperature; tmean = mean temperature; dr = daily thermal range and ar = absolute range, tmax-tmin). Models are ranked by AIC and only models with ΔAIC < 2 are presented (see **Table S3.4** for completed list of the models).

Physiological trait	Environmental predictor	λ	R^2	AIC	ΔAIC	W_i	Slope	Fvalue	P
Topt	tmean	0.191	0.34	111.421	0	0.602	0.384	13.88	0.0011
	tmax	0.055	0.315	112.593	1.172	0.335	0.272	12.48	0.0017
Zmax	dr	0	0.154	60.998	0	0.532	0.196	5.551	0.027
	tmax	0.013	0.092	62.829	1.83	0.213	0.056	3.545	0.072
B50	dr	0.125	0.093	117.161	0	0.402	0.461	3.552	0.072
	tmin	0.074	0.046	118.63	1.469	0.193	-0.144	2.203	0.151
	ar	0.446	0.019	118.631	1.471	0.193	0.117	1.488	0.234

(±0.26) and pond: -1.31 (±0.16); $F_{1,26}$ = 6.77, P = 0.015). All of this is consistent with the hypothesis that tadpoles living in thermally variable habitats (e.g. temporary ponds) would have higher growth performances to improve their survival in uncertain in durability breeding habitats (see **Fig. 3.2a-b**).

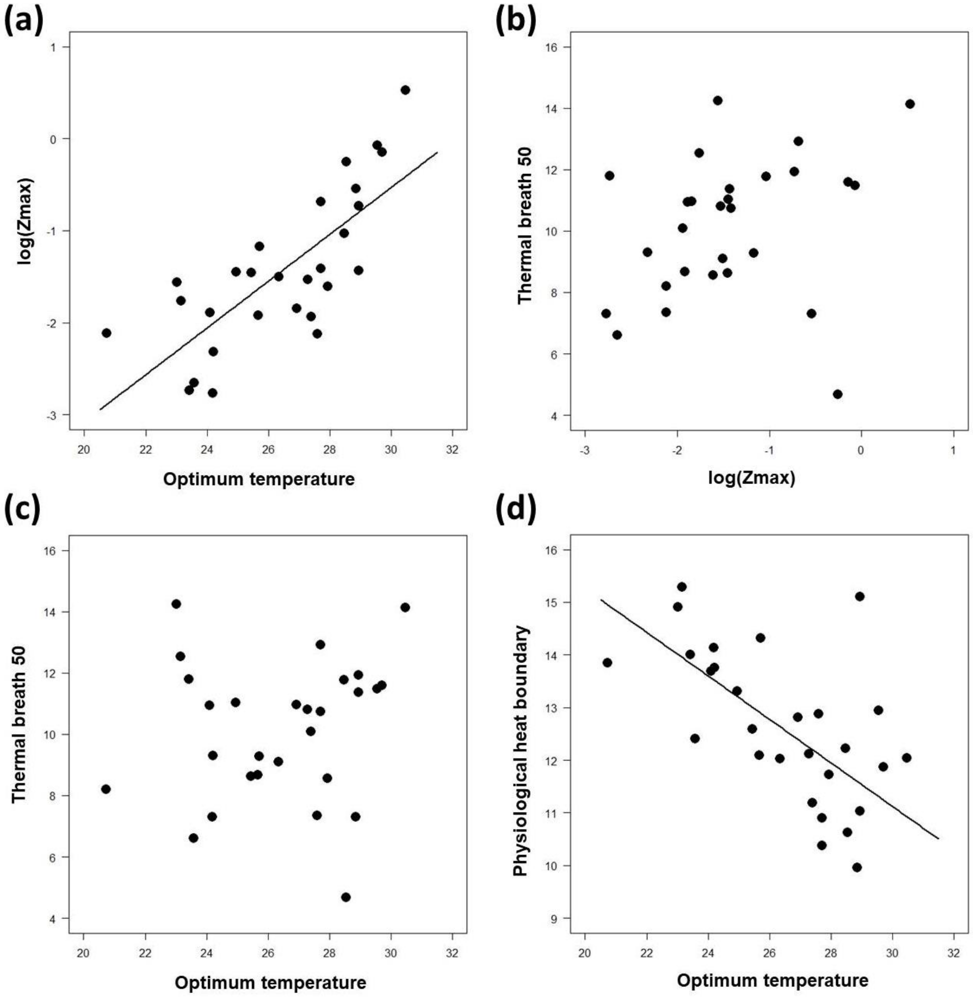


Figure 3.3: Testing for interspecific constrains hypotheses among the three parameters describing tadpoles' thermal performance: (a) 'hotter is better', (b) specialist-generalist and (c) 'hotter is narrower' and for (d) physiological heating tolerance (PHT) hypothesis. All variables are in ºC, except maximum performance (Zmax) that is in $gg^{-1}day^{-1}$. Fitted lines represent a significant PGLS regression.

Relationship between physiological thermal traits

Species with higher optimal temperature showed higher performances validating the 'hotter is better' hypothesis (**Fig. 3.2, 3.3a**; **Table 3.1**). However, thermal performance breath (B_{50}) was neither related to maximal growth performance (Zmax), 'generalist-specialist', nor optimum temperature (Topt), 'hotter is narrower' hypotheses (**Fig. 3.3b-c**; **Table 3.1**). Finally, a significant relationship between Topt and physiological heating tolerance (PHT = CTmax - Topt) was found, confirming that safety margins for acute stress decrease with Topt (**Fig. 3.3d**; **Table 3.1**).

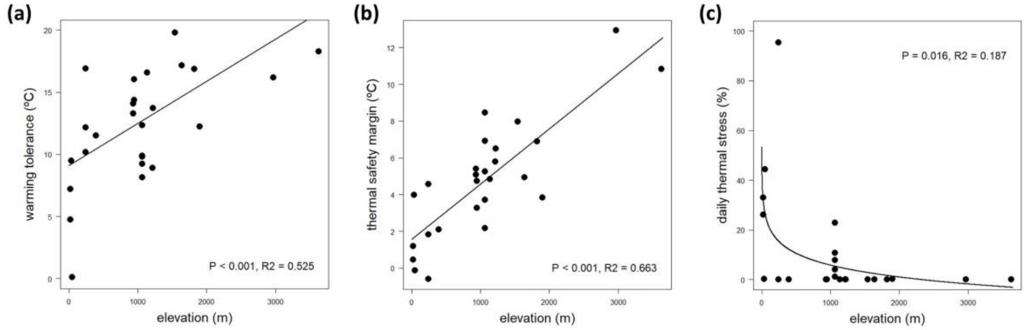


Figure 3.4: Variation of thermal stress along a tropical elevational range. Estimates of thermal acute stress (**a**, WT) and chronic stress (**b**, TSM and **c**, DTS). Lines show significant phylogenetic linear (**a-b**) and logarithmic (**c**) regressions (PGLS).

For the correlations between TPC for growth parameters and critical thermal limits, Topt and Zmax were positively correlated with CTmax (**Table 3.1**). Thermal breadth (B_{50}) was also correlated with CTmin and Thermal tolerance range (TR) (**Table 3.1**). Zmax was also positively correlated to TR although marginally significant after Bonferroni correction (**Table 3.1**).

Vulnerability to suffer heat impacts due to global warming

Warming tolerances were all positive thus suggesting that analysed species are not currently suffering acute thermal stress (**Fig. 3.4a**). In contrast, our measures of chronic stress (i.e. TSM and DTS) suggest that lowland amphibians are already experiencing temperatures that could have negative consequences in its growth performance (**Fig. 3.4b-c**). Indeed, we found a relationship between the three estimates of heat thermal stress and elevation (PGLS: (WT) $F_{1,24} = 28.63$, $p < 0.001$; (TSM) $F_{1,24} = 50.11$, $p < 0.001$; (DTS) logarithmic regression, $F_{1,24} = 6.735$, $p = 0.0159$; **Fig. 3.4**).

Discussion

We examined the climatic gradient of tropical Andes, an aseasonal and limited geographical area, as a potential selective engine for the evolution thermal diversification in amphibians. These ectothermic vertebrates have reduced dispersal and, especially in the tropics, very narrow elevational ranges (Berven & Grudzien, 1990; Funk *et al.*, 2005; Smith & Green, 2005; Ron *et al.*, 2018). This pattern may be compatible with the existence of physiological barriers to dispersion (Janzen, 1967; Ghalambor *et al.*, 2006), which in turn may result in greater geographical isolation and local adaptation (Ghalambor *et al.*, 2006; Martin *et al.*, 2009) and lastly promoting speciation (e.g. Gill *et al.*, 2016). This study suggests that thermal performance in larval amphibians is strongly influenced by their thermal environment and therefore might determine where species can live. For instances, Topt decreased with elevation and with habitat, which is consistent with variation in mean and maximum environmental temperature, and in accordance with previous studies (Frazier *et al.*, 2006; Deutsch *et al.*, 2008; Huey *et al.*, 2012; Payne *et al.*, 2016). However, the hypothesis that thermal breadth (B_{50}) increases with elevation (van Berkum, 1986; Navas, 1996b, 1997) and climatic predictors related to niche's thermal breadth, such as absolute thermal range and daily thermal range, only showed a tendency.

Variation in maximum growth performance was, however, correlated with daily thermal range (which in turn is a good predictor of habitat; see chapter 1, 2 and 4). This finding is consistent with the hypothesis that growth rate may increase in time-constrained habitats for growing (i.e. temporary ponds more prone to desiccation). In fact, species inhabiting temporary habitats, with higher daily range of temperatures (see chapter 2 and 4), may lead to selection for increased growth rates and its correlated development rates, a main predictor of timing to metamorphosis (Smith-Gill & Berven, 1979), to allow the completion of metamorphosis under the time constrain, before the drying of the habitat (e.g. Denver *et al.*, 1998; Richter-Boix *et al.*, 2011). Another alternative explanation proposed to be driving variation in maximum growth performance, while not analysed here, is the presence and abundance of predators (e.g. Munch & Conover, 2003; Laurila *et al.*, 2008) which in turn, may also be related to temperature and type of habitat (Pearman, 1995; Wellborn *et al.*, 1996).

The absence or low correlation between physiological parameters and environmental temperatures might be due to a low statistical power (Error Type II) by attributable to a low number of examined species (although N=28, is bigger enough to previous reported analyses of TPCs, (n = 12, plant populations, Angert *et al*. 2011; n = 10, *Drosophila* species, Overgaard *et al.* 2014; n = 15 lizards, Bonino *et al*. 2015; n = 13, *Drosophila* species, Schou *et al*. 2017). Another possibility is our relatively small sample of aquatic habitats monitored for each species. For instance, some species can be found in a broad range of environments that could greatly differ in their temperature (e.g. Kearney *et al.*, 2009; Hannah *et al.*, 2014; Sunday *et al.*, 2014) and, therefore, a single habitat thermal record for each species may only partially explain variation of thermal sensitivity. Also, TPCs can vary on space and time and according to the methodology used to estimate it (widely discussed in Sinclair *et al.*, 2016). For instance, the use of constant regimes of temperatures, in contrast to more realistic fluctuating conditions, for estimates of TPC parameters can modify measurements of Topt and CTmax (e.g. Niehaus *et al.*, 2012; Kingsolver & Woods, 2016). Further, wild-derived estimates of performance may better correlate with environmental temperatures than the estimates in laboratory (Payne *et al.*, 2016) but might also be more thermally restricted (Huey, 1982). However, thermal adaptation is unable to overcome inherent constrains that may limit it (Huey & Kingsolver, 1989; Martin & Huey, 2008).

Accordingly, two main constraints, the 'hotter is better' thermodynamic constraint (Huey & Kingsolver, 1989) and the 'physiological heating tolerance' hypothesis (Payne *et al.*, 2016) could limit the evolution of thermal sensitivity in our tropical mountain tadpole community. As predicted by 'hotter is better' hypothesis, Topt correlate positively with Zmax (Frazier *et al.*, 2006; Knies *et al.*, 2009; Angilletta *et al.*, 2010) which is suggested to be related to thermodynamic depression of biochemical reactions at low temperatures (Hamilton, 1973; Bennett, 1987). Moreover, we also found that Topt correlates negatively with physiological heating tolerance. Thus, while Topt is correlated to CTmax, an increase of Topt do not imply the same increase to CTmax and therefore, the susceptibility to suffer acute heat damaeges once Topt is exceeded increases for species adapted to higher temperatures (Payne *et al.*, 2016). This constrain may have important implications on assessments to organism vulnerability since it would suggest that physiological safety margin to suffer acute stress is smaller for species that are actually exposed to higher environmental temperatures (see below; Deutsch *et al.*, 2008; Tewksbury *et al.*, 2008; Huey *et al.*, 2009).

The present work support the extended idea that, in tropical mountain ranges, lowland species may be more sensitive to an increase of temperatures, both extreme and mean temperatures, than high-elevation species (Colwell *et al.*, 2008; Huey *et al.*, 2009; Sunday *et al.*, 2014; von May *et al.*, 2017). However, none of the species appears to be actually exposed to acute stress, since maximum environmental temperatures are ever below CTmax. Furthermore, mean pond temperatures are generally lower than the physiological optimal temperatures for species growth (Knies *et al.*, 2009; Nilsson-Örtman *et al.*, 2013). Martin and Huey (2008) proposed that, since TPCs are highly asymmetric with a drastic drop at temperatures above the optimum (Huey & Stevenson, 1979; Izem & Kingsolver, 2005), organisms may occupy environments with temperatures below optimum which could buffer the reduction of fitness (the 'suboptimal is optimal'; Martin & Huey, 2008) because an increase of few degrees above Topt may be fatal for the organism due to Jensen´s inequality, the mathematical property of nonlinear curves such as TPC (Ruel & Ayres, 1999).

Coincident with the increase of the thermal safety margins with elevation, we also found an increase in the physiological heating tolerance (Payne *et al.*, 2016; Payne & Smith, 2017). This result is worrisome since it may imply that lowland populations are more exposed to suffer chronic stress and, in addition, their physiological boundary to buffer acute stress (i.e. PHT) are lower, which could have disastrous synergic effects on the persistence of these populations. One mechanism to mitigate the potentially catastrophic effects of increase heating, both means and extreme is the microenvironmental buffering that may result crucial to avoid heat impacts (see Oyamaguchi *et al.*, 2018). However, the populations analysed at lowlands may represent the warm edge of the whole distributional range of the species. Local temperatures within the species' range may be different from those at the range limit, causing local extinctions at the warmest edges but not within the whole distribution (Cahill *et al.*, 2014). Furthermore, the increase of thermal safety margins with elevation has another consequence. While upland species do not seem to be vulnerable to global warming at short periods of time, given how TSMs are over 10^0C, it may also suggest that upland larval amphibians are actually living in aquatic environments that are thermally suboptimal for growth (see chapter 4).

Given that the Andes experienced large climate variation due to multiple uplifts since the Miocene (Gregory-Wodzicki, 2000; Hoorn *et al.*, 2010), along with the climate changes of the Pleistocene (Hooghiemstra *et al.*, 2006; Torres *et al.*, 2013; Flantua *et al.*, 2014), amphibians might have persisted in environments that are no longer optimum, causing the actual mismatch observed in this study between physiological parameters and climatic predictors. Another, not mutually exclusive, hypothesis is that species uplifts to mountaintops are recent (see Quintero & Jetz, 2018; chapter 2) and thus physiological adaptations have not yet paralleled current environmental variation. Finally, we have to consider that non-intrinsic features such as physiology and temperature are the main drivers of the patter of distribution of organism. Biotic rather than environmental abiotic factors may be more important in defining distributional ranges in equatorial areas (Dobzhansky, 1950; MacArthur, 1969; Schemske *et al.*, 2009; Jankowski *et al.*, 2013; Wisz *et al.*, 2013), since species richness and abundance tend to increase at low altitudes and latitudes (Hawkins *et al.*, 2003). Solving those questions will require further studies integrating physiological and behavioural approaches (e.g. larval thermoregulatory behaviour and adult selection of breeding habitats) under an evolutionary context and could aid in assessing vulnerability of mountaintop ectotherms to climate change (see chapter 2 and chapter 4).

Chapter 4

Does thermal physiology predict realized species niche? A test with dendrobatid frogs in an altitudinal tropical gradient

POL PINTANEL, MIGUEL TEJEDO, URTZI ENRIQUEZ-URTZELAI, GUSTAVO A. LLORENTE, ANDRÉS MERINO-VITERI

Abstract

Understanding how temperature regulates species distribution is of major concern to test predictions on future range shifts and species vulnerability in response to climate change. High risky tropical mountain ectotherms, currently living closer to their optimal temperatures, are predicted to be forced to upslope shifts to avoid thermal damages. However, empirical support for this predicted trend is scarce and still remains a major challenge since alternative spatial modelling approaches are mostly based on correlative climatic approach neglecting fundamental niches and organismal level microenvironmental buffering. Herein, we investigated whether physiology can predict current elevational distribution in nine closely related Dendrobatid frogs distributed along a tropical elevational gradient (~ 2000 m) by relying in modelling microenvironmental thermal variation of their aquatic larval environments and thermal sensitivity of larval growth performance to, then, forecast future effects of global warming. One conclusion is that temperature is unlikely to be the only main factor constraining altitudinal distribution in this clade. Even though optimum temperature and heat and cold resistance (survival at 9 ^{0}C) are correlated to current elevational species distribution, we found that between-species predicted range of altitudinal distribution overlap in a greater extent than actual ranges, thus suggesting that species variation in thermal sensitivity is not the unique factor limiting species ranges. Specifically, optimum temperatures of species' growth rate along elevation vary less than changes in mean environmental temperature and, therefore, our analyses suggest that upland populations may have to settle for suboptimal performance. Finally, our models under a future scenario of global warming suggest an essential role of habitat buffering from thermal stress, even for high-elevation species.

Back cover: *Epipedobates anthonyi* (Noble, 1921)

Introduction

Temperature is one of the main environmental factors influencing rates of physiological and biochemical processes (Angilletta, 2009) and, therefore, is one of the most important factors defining the fundamental niche of organisms (Hutchinson, 1957; Kearney & Porter, 2004; Soberón & Peterson, 2005). However, understanding how temperature regulates species distribution still remains a major challenge (Bozinovic *et al.*, 2011). Species distribution models (SDMs) are widely used to explore the association between climatic factors and current and future distribution of species under a climatic change scenario (Buckley *et al.*, 2010). The increasing need to evaluate the effects of a changing climate on species distributions has led to incorporate the role of thermal physiology to improve the accuracy on SDMs and conservation initiatives based on this data (Huey *et al.*, 2012; Helmuth *et al.*, 2014; Sunday *et al.*, 2014; Evans *et al.*, 2015). Yet, physiological data is principally used to identify places were species will be thermally unsuitable for performance (i.e. too high/low temperatures) rather than those that are suitable for optimum thermal conditions to perform (Huey *et al.*, 2009; Sinervo *et al.*, 2010) because of the many non-climatic factors (e.g. dispersal capacity, establishment ability, biotic interactions) that intervene in defining the realized habitat for the species (Soberón & Peterson, 2005; Araújo & Luoto, 2007; Wisz *et al.*, 2013). For instance, using thermal physiological data in SDM can provide information of thermal constraints limiting species distribution yet, excluding other non-thermal factors that may affect it. Hence, there are concerns regarding on the reliability of such models to forecast impacts of climate change on biodiversity (Diamond *et al.*, 2012b; Woodin *et al.*, 2013) since their effectiveness largely depends on which extent observed distribution of species (realized niche) is controlled by climatic factors (fundamental niche). Still, only few studies have investigated the relationship between thermal physiology and current distribution limits at interspecific level (but see Sánchez-Fernández *et al.*, 2012; Overgaard *et al.*, 2014).

The ability to maintain physiological function at optimum rates and to cope with extreme temperatures must play a main role in determining species distribution (Olalla-Tárraga *et al.*, 2011; Cahill *et al.*, 2014; Overgaard *et al.*, 2014). However, physiological traits often fail to predict species distributional ranges at large ecological scales (Sunday *et al.*, 2012; Gouveia *et al.*, 2014). The use of different spatial resolutions could lead to contrasting predictions instead (Randin *et al.*, 2009; Helmuth *et al.*, 2014). For instance, species that exploit hot environments (i.e. open habitats) are usually better adapted to tolerate higher temperatures than those restricted to colder retreats (i.e. canopy habitats) in terrestrial (Huey *et al.*, 2009; Frishkoff *et al.*, 2015; Bonebrake *et al.*, 2016) and aquatic species (Duarte *et al.*, 2012; Gutiérrez-Pesquera *et al.*, 2016) which could be overlooked at larger scales. Thus, the choice of accurate spatial and temporal scales is crucial to effectively capture the environmental temperature experienced by organisms (Potter *et al.*, 2013; Pincebourde *et al.*, 2016), especially for those small aquatic ectotherms with limited capacity for behavioural thermoregulation, whose body temperatures often track environmental temperatures (Spotila *et al.*, 1992). As climate warms, those microclimatic approaches can better predict local climatic condition that may limit species range shifts (expansions and contractions) or, instead, allow them to face local increased heating stress (Sunday *et al.*, 2014; Suggitt *et al.*, 2018). A way to determine if ectotherms are able to withstand temperature increase is by determining how close future temperatures will be to the species optimal or critical temperature (thermal safety margin and warming tolerance respectively; *sensu* Deutsch *et al.*, 2008) and/or the amount of time that organisms are exposed to stressful temperatures (daily thermal stress; chapter 3).

Because the extant of the altitudinal climatic zonation in tropical mountains, that may impose an additional physiological barrier to organismal dispersion (Janzen, 1967), the geographical isolation, limited range size and the potentially local physiological adaptations, make tropical montane species potentially the most threatened under impeding climate change (Colwell *et al.*, 2008; La Sorte & Jetz, 2010). In fact, tropical species, especially lowland populations, are more likely predicted to be vulnerable to an increase in temperature as they are exposed to temperatures close to their physiological optimum (Deutsch *et al.*, 2008; Huey *et al.*, 2009). The predicted rise in environmental temperature may determine both a poleward and upslope shifts in range (Colwell *et al.*, 2008; Chen *et al.*, 2011a). However, in the tropics, the shallow latitudinal thermal gradient makes upward shifts the most likely option to occur.

Herein, we integrated data on thermal sensitivity in amphibian larval growth rate along with microenvironmental information of the aquatic habitats available for breeding, to explore their link with current elevational distribution range in the Neotropical Andes, one of the most species-rich mountain range in the world (Myers *et al.*, 2000). We selected growth rate because is predicted to be a good proxy for fitness (e.g. Deutsch *et al.*, 2008; Kearney & Porter, 2009) because it results from the interaction among many temperature dependent parameters (Freitas *et al.*, 2010). To do so, we selected the frogs of the family Dendrobatidae, widely distributed on elevation (from sea level to almost 4000 masl; Coloma, 1995) and, as result, the temperature they experience should pose different selective pressures and provide the opportunity for physiological adaptation to different thermal conditions. Previous work by Graham *et al.* (2004), employing environmental niche modelling, found that sister clades within the *Epipedobates* genus generally segregates on a consistent pattern of elevation and latitude divergence, suggesting an important role of environmentally mediated divergence in its speciation.

To capture potential variation in thermal habitat along the elevational range, we first combined microclimatic data taken *in situ* with WorldClim climatic layers (Hijmans *et al.*, 2005) to model three different thermal regimes of aquatic microclimates. Then, we applied a mechanistic model to predict each species' physiological elevational range and compared it to their observed range. Finally, we projected future models to examine how elevation and microenvironment could influence the species' vulnerability to climate change.

Material and methods

Selection of species

We sampled nine Ecuadorian frogs of the family Dendrobatidae found across a broad altitudinal range in Ecuador (between latitudes 1^0N - 4^0S and elevations 38 - 1900 masl), five out of the six species of *Epipedobates* present in the coastal side of Ecuador, and four *Hyloxalus* present in the Amazonian slope out of the 27 found in whole Ecuador (Ron *et al.*, 2018; for more information see **Table S4.1** and **Fig. S4.4**). Most of the specimens were collected from their natural aquatic habitats from December 2014 to April 2016. However, three species (*Epipedobates machalilla*, *Epipedobates tricolor* and *Hyloxalus nexipus*) were obtained from captive breeding in the 'Balsa de los Sapos' initiative at Pontificia Universidad Católica del Ecuador. While those captive specimens were first or second generation breeds, we assumed that physiological performance in these species was not affected.

Thermal performance curves for growth and survival (TPC)

To assess how tadpole growing performance and survival were affected by temperature, we set up groups of 10-15 individuals, whenever possible, that were raised under different constant temperature treatments (9, 15, 20, 23.5, 27, 29, 31 and 33 ^{0}C) in a lab kept at 19-20^{0}C under a photoperiod of 12L:12D during 10 days with food *ad libitum*. Both water and food was completely renewed three times during the experiment, each 2-3 days, and all the individuals were daily checked to ascertain survival. For *Hyloxalus pulchellus* we did not include the 33^{0}C treatment since it was undoubtedly lethal. We used portable fluid heaters with regulation adjustment (U201431698) to keep the temperature treatments equal or over the room air temperature (20-33^{0}C). However, for colder treatments (9 and 15 ^{0}C), we used TECO TK 1000 chillers. Tadpoles were randomly assigned to each temperature treatment and were individually maintained in plastic cups with 400 ml of dechlorinated water and aerated with an air pump system. We weighed and analysed the stage of development of the tadpoles (Gosner, 1960) just before and after the experiment and estimate relative growth rate (GRr) as; $\text{Weight}_{\text{final}}$ – $\text{Weight}_{\text{initial}}$ / $\text{Weight}_{\text{final}}$ * number of days. We considered negative values as non-growth and therefore analysed as zero (Overgaard *et al.*, 2014) All tadpoles that died during the first day, were removed from the analysed sample to avoid possible confounding effects. Because anuran larval growth is non-linear, experiencing an exponential decay in relative growth rate close to metamorphosis (Harris, 1999; Richter-Boix *et al.*, 2011), we chose experimental tadpoles from either field or captive breeding sources in early developmental stages (< 33 Gosner stages). Sampling tadpoles differing in size and developmental stage were homogeneously distributed across temperature treatments.

To fit TPCs, we build a nonlinear mixed effect model to quantify growth performance for each species across all treatment temperatures using the nlme R-package (Pinheiro *et al.*, 2018). We adjusted the TPCs according to Angilletta (2006). We fitted four different models (Quadratic, Cubic, Gaussian and Gaussian-Gompertz) to estimate each species' TPC (Frazier *et al.*, 2006; Martin & Huey, 2008). We used the Akaike information criterion (AIC) to identify the best model (Burnham & Anderson, 2002). TPC parameters were estimated for each species using the fitted curves. Topt was estimated as the temperature when the performance was maximized (Zmax).

Estimates of (micro and macro) habitat temperatures along elevation

Species distributional data for the Ecuadorian *Epipedobates* and Amazonian-side *Hyloxalus*, was obtained from the Museo de Zoología of the Pontificia Universidad Católica del Ecuador database (**Fig. S4.4**; Ron, 2018). To reduce sampling bias, we only kept georeferenced points that were separated by more than 1 km between them using the 'distGeo' function in the geosphere package (Hijmans, 2017b).

We gathered current and future macroclimate data (WorldClim) across the species' distributional georeferenced points (Hijmans *et al.*, 2005). To estimate shifts of each species' thermal suitability under different climate warming scenarios, we examined future projections for two time periods (2050 and 2070). We applied low (RCP 4.5) and high (RCP 8.5) emission scenarios from the CCSM4 global circulation model. We used the 'extract' function of the package raster (Hijmans, 2017a) to obtain the climatic information from WorldClim spatial resolution. We also extracted the elevation data with the function 'elevation' in the R-package rgbif (Chamberlain, 2017).

We also gathered *in situ* microclimatic data from 38 aquatic environments in which different species of Ecuadorian amphibian breed, distributed along the altitudinal ranges (23 – 3631 masl) and through different aquatic habitats (see below). We recorded temperature every 15 minutes from dataloggers (HOBO pendant) deployed at the bottom of each water body. We analysed mean (tmean), maximum (tmax) and minimum (tmin) daily water temperatures. The number of sampling days ranged from 2 to 546 days (see **Table S4.2**). Finally, we categorized the water bodies in three different thermal variability regimes according to their mean daily thermal range (dr = tmax - tmin; *low* (0.05 – 1 ^{0}C), river and permanent deep ponds, *medium* (1 - 2.25 ^{0}C), forested ponds and permanent opened ponds, and *high* (2.4 – 9 ^{0}C), temporal opened ponds). Altitude was a strong predictor of microclimatic temperatures, tmean, tmax and tmin ($R^2 > 0.75$, $P < 0.001$, N =38). Contrarily, daily range was independent of altitude for all locations and within any thermal range categories (pooled sample, $R^2 = 0.02$, n = 38, P = 0.891), therefore, we considered that dr was the variable that best discriminates between thermally contrasting habitats eliminating the confounding effect of decreasing temperatures with altitude due to the adiabatic lapse rate (Sarmiento, 1986; Dillon *et al.*, 2006). Although dendrobatid tadpoles are rarely found in open temporary ponds, we decided to include the category 'high' to embrace the full range of environmental temperatures potentially available for frogs and to include the effect of human-modified habitat conversion.

Modelling microclimatic temperatures through species range distribution

To estimate microclimatic temperatures experienced along the species distributional range, we fitted GLM models for tmax and tmin (see **Supplementary material**). Then, we extracted the coefficients for intercept and for each explanatory variable and interpolated these model parameters with current and future temperatures across studied species distributional points, to obtain maximum and minimum mean modelled microenvironmental temperatures (see **Supplementary Material**). Because the low latitude of our sampling locations (ranging 1.165 N, 4.164 S), temperatures are nearly constant without any relevant seasonality throughout the year. Microenvironmental thermal variation is much higher than annual variability (see **Fig. S4.2**). However, to estimate a real daily thermal variation we interpolated estimated maximum and minimum mean microenvironmental temperatures from three current microhabitats with contrasting temperatures (river, forested pond and opened pond) with data for a range of 78-100 days (see **Supplementary Material 4.1**, **Fig. S4.3**).

Predicting altitudinal distribution and thermal stress with TPCs

We assumed that to enhance larval growth rate species will better select temperature ranges between the optimum temperature, as maximum preferred temperature, and avoid temperatures in which performance is lower than 50% (B_{50}) as a colder edge. We assumed that this thermal range better reflects the optimum growth range since ectotherms appears to prefer temperatures that maximizes its fitness, while avoiding temperatures above its Topt (I. Sanuy, A. Merino-Viteri, P. Pintanel, M. Tejedo et al. unpublished data; also see Martin & Huey, 2008). This selection of temperatures is basically grounded on the fact that, due to the left-skewness of TPCs, an increase of few degrees above Topt may be lethal for the organism (Ruel & Ayres, 1999). To quantify the exposure to stressful temperatures, we used the higher extreme of B_{50} ($maxB_{50}$) as the temperature that starts to be stressful for tadpoles on the basis that we found high mortality in TPC experiments (see **Fig. 4.1**). Cold stress temperatures were more difficult to estimate because some species survived throughout the ten-day experiment at the lowest temperature treatment (9 ^{0}C) growing only 5% of its maximal growth performance. Nevertheless, in order to discuss the results, we extracted lethal time (from 1 to 10 days) at 9 ^{0}C for each species.

Therefore, we calculated the percentage of potential hours of maximum performance for each species, as the sum of hours in which the performance is maximum minus the hours of heat stress (> $maxB_{50}$) with respect to the total. Previous studies comparing constant and fluctuant temperatures (e.g. Bevelhimer & Bennett, 2000; Niehaus *et al.*, 2012) suggest that even short daily

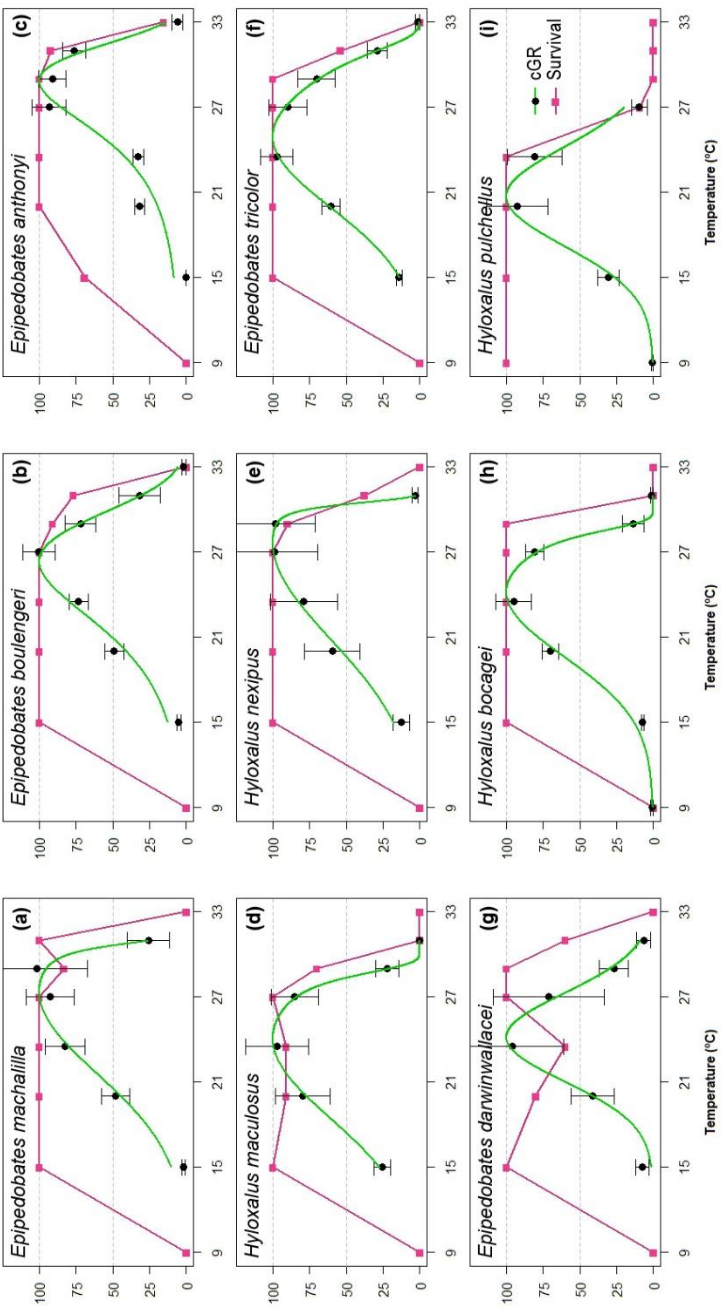


Figure 4.1: Tadpole growth rate (green) and survival (purple) across different temperatures for five *Epipedobates* species (**a-c**, **f-g**) and four *Hyloxalus* species (**d-e**, **h-i**). Values represent the % in respect to the maximum for both, survival and growth rate (±SE).

exposures to high temperatures may have negative effects on performance. To quantify the impacts of climate change, we projected present and future heat stress temperatures for each occurrence point according to the elevation and habitat.

Statistical analyses and phylogenetic reconstruction

To reconstruct the phylogenetic tree we used the most comprehensive and recent amphibian phylogeny of Jetz and Pyron (2018) including all studied species, with the ape package in R (Paradis *et al.*, 2004).

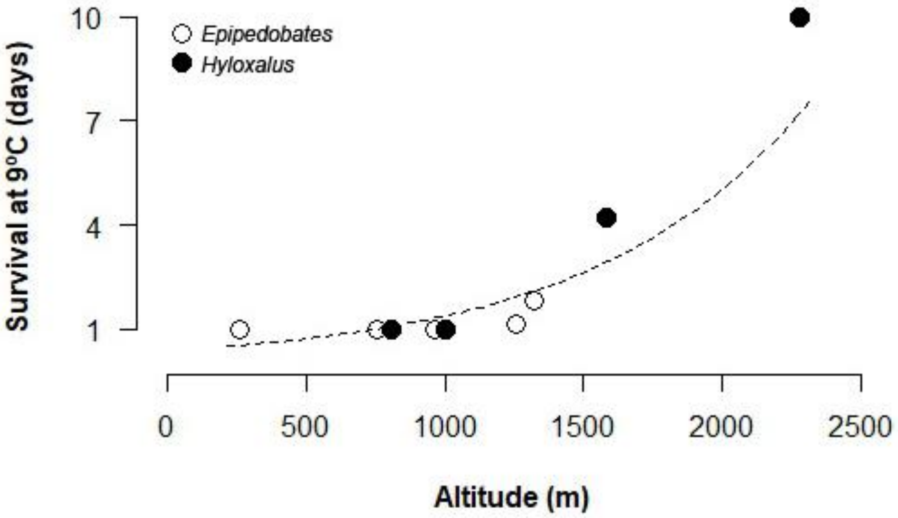


Figure 4.2: Exponential variation of days of tadpoles survival with the elevational centroid of the species' distribution (PGLS: $t = 5.29$, $R^2 = 0.77$, $P = 0.001$).

We tested the correlation between elevation with Topt and with estimates of tolerance to cold, measured as mean time of survival at 9 ^{0}C, and tolerance to heat, measured as the temperature threshold, max B_{50}, by applying the Phylogenetic Generalized Least Squares (PGLS) approach with the R-package caper (Orme, 2013). Unless specified, we used mid-elevation of the species distribution for all the statistical analyses, because using elevation of the sampled population gave similar results. We also repeated the analysis with the mean temperature (TMEAN) (WorldClim; Hijmans *et al.*, 2005) of the sample point as an elevational proxy. Since all thermal data (tmax, tmean and tmin) were highly correlated with each other and with the elevation, we decided to use tmean because many studies suggest that it might be a main driver in TPCs variation (e.g. Frazier *et al.*, 2006; Deutsch *et al.*, 2008; Huey *et al.*, 2012; also see Chapter 3). Furthermore, we performed a GLM with Poisson distribution to compare the lethal time (from 1 to 10 days) at 9 ^{0}C for each species, including the species and genera as random factors. All analyses were done in R v3.4.3 (R Core Team, 2014).

Results

Thermal physiology in altitude

The function that best fitted our data was the Gaussian-Gompertz (see Frazier *et al.*, 2006; Martin & Huey, 2008). However, for *H. pulchellus* and *E. darwinwallacei*, we fitted the data using a Gaussian function according to its lower AIC (see **Fig. 4.1**; **Table S3.2**).

Survival of tadpoles was greater at intermediate temperatures (ca. 90%) and decreased either at the lowest (9^{0}C) and highest temperatures (29, 31 and 33^{0}C), resulting generally in an inverted 'U' (**Fig. 4.1**). All individuals maintained at 9^{0}C died except for high altitude *Hyloxalus pulchellus*. We found significant differences on the survival time between genera (χ^2 = 103.63, df = 70, p < 0.001) and species (χ^2 = 5.23, df = 63, p < 0.001) at 9 ^{0}C. Tukey's post-hoc analysis showed that *H. bocagei* survived less time than *H. pulchellus* but more than the rest of species (see **Table S4.4**). Also, the PGLS analysis revealed that time of survival at 9 ^{0}C was exponentially correlated to elevation (**Fig. 4.2**). Conversely, *H. pulchellus* did not survive treatments over 27 ^{0}C (**Fig. 4.1i**). Most *E. anthonyi* tadpoles died in the 33 ^{0}C treatment (**Fig. 4.1c**). PGLS analysis also revealed that capacity to tolerate heat stress was negatively correlated to elevation (t = -3.55, R^2 = 0.591, p = 0.0094) being lowland species more tolerant to heat chronic stress.

Optimal temperature (Topt) was negatively correlated with elevation (for sample point: t=-5.44, R^2 = 0.782, p < 0.001; for centroid of the distribution: t = -3.218, R^2 = 0.539, p = 0.015) and mean temperature (**Fig. 4.3**). However, optimum temperature decreased with altitude in a lesser degree than the variation of tmean (for sample point: slope = 0.691 ± 0.139; for centroid of the distribution see **Fig. 4.3**). In fact, expected biological niche models for high-elevation species were lower than observed altitudinal ranges. On the other hand, expected physiological ranges for lowland species exceeded their observed maximum altitude, except for *Epipedobates anthonyi*. Overall, dendrobatids showed physiological ranges which greatly overlap in space in comparison to observed elevational ranges (**Fig. 4.4**; **Fig. S4.6**).

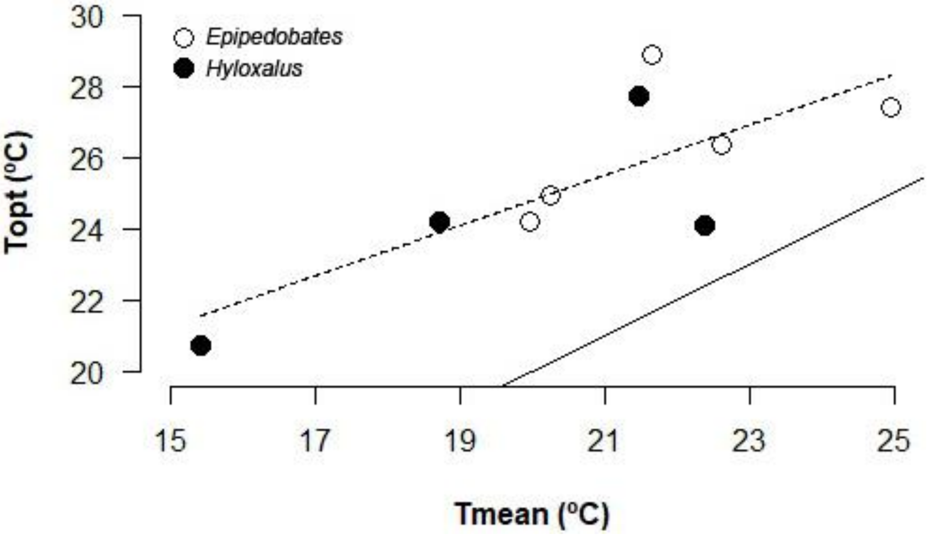


Figure 4.3: Variation of optimum temperature (Topt) with the mean temperature (TMEAN; WorldClim) of mid-elevational distribution (PGLS: Y = 10.66 + 0.71X, R^2 = 0.54, t = 3.22, P = 0.015). Solid line represents Topt = Tmean.

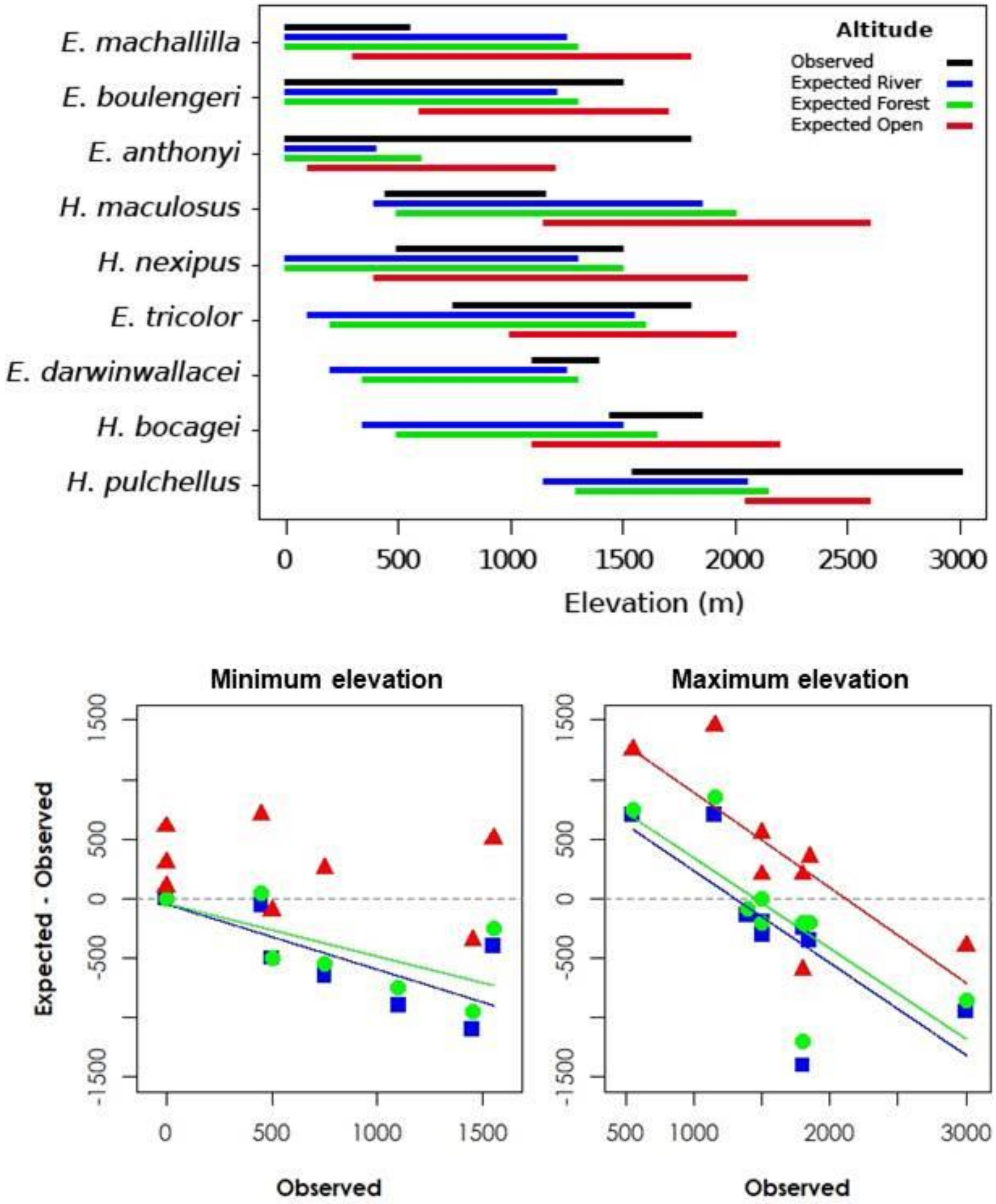


Figure 4.4: Observed and expected elevational range for dendrobatid species (above). Expected values correspond to elevation in which potential hours of maximum performance was higher than 50% (see **Fig. S4.5**). Graphs below represent mismatch between observed and expected elevational range for minimum (left) and maximum (right) elevation extreme: continuous line represents significant regression for each species and microenvironment (see above for colour assignations); dotted lines represent zero difference.

Climate change impacts

Our models assessing the effects of habitat and elevation on potential incidence of chronic thermal stress, revealed that habitats with *high* thermal variation such as opened ponds currently have temperatures that could be stressful for low and even for mid-elevation populations of some of analysed species (**Fig. 4.5**). Conversely, none of the river-like water environments (*low*) across each species distribution had temperatures higher than species' max B_{50}, and temperatures are not expected to exceed max B_{50} at 2070 for any population, even under the highest emission scenario (RCP 8.5) except for the low-elevation populations of *E. boulengeri* (**Fig. 4.5**).

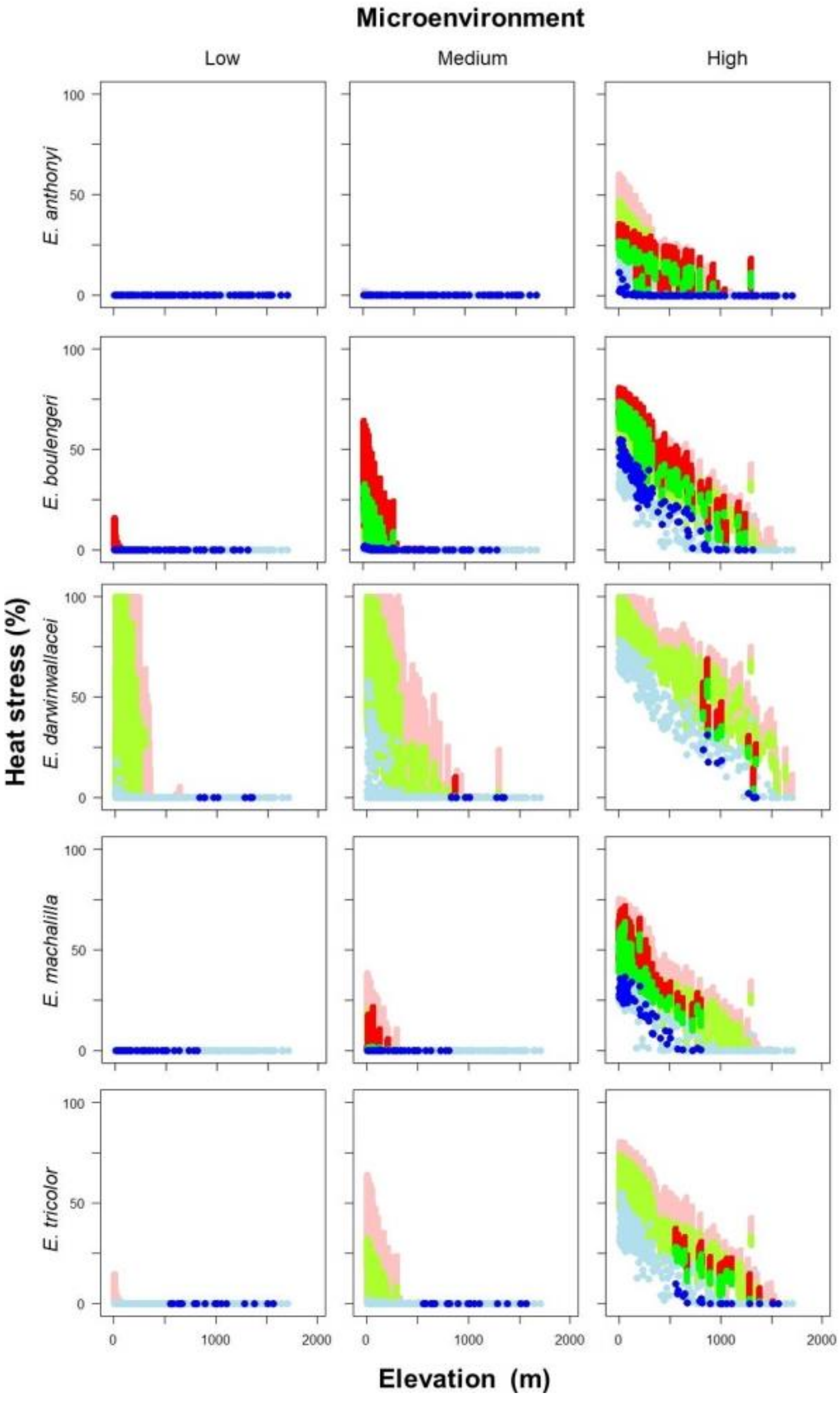
Future predictions suggest that, in *high* variation environments, even upland species could suffer from thermal stress (**Fig. 4.5**). In some cases, for lowland populations, microenvironmental temperature would exceed its physiological safety margins more than 50% of the time. In microenvironments with *medium* thermal variation such as forested ponds, heat peaks of stressful temperatures could be buffered by 2050. In 2070 only lowland populations for some species could suffer from severe thermal stress. However, these species would not be under thermal stress for more than 20% of the time.

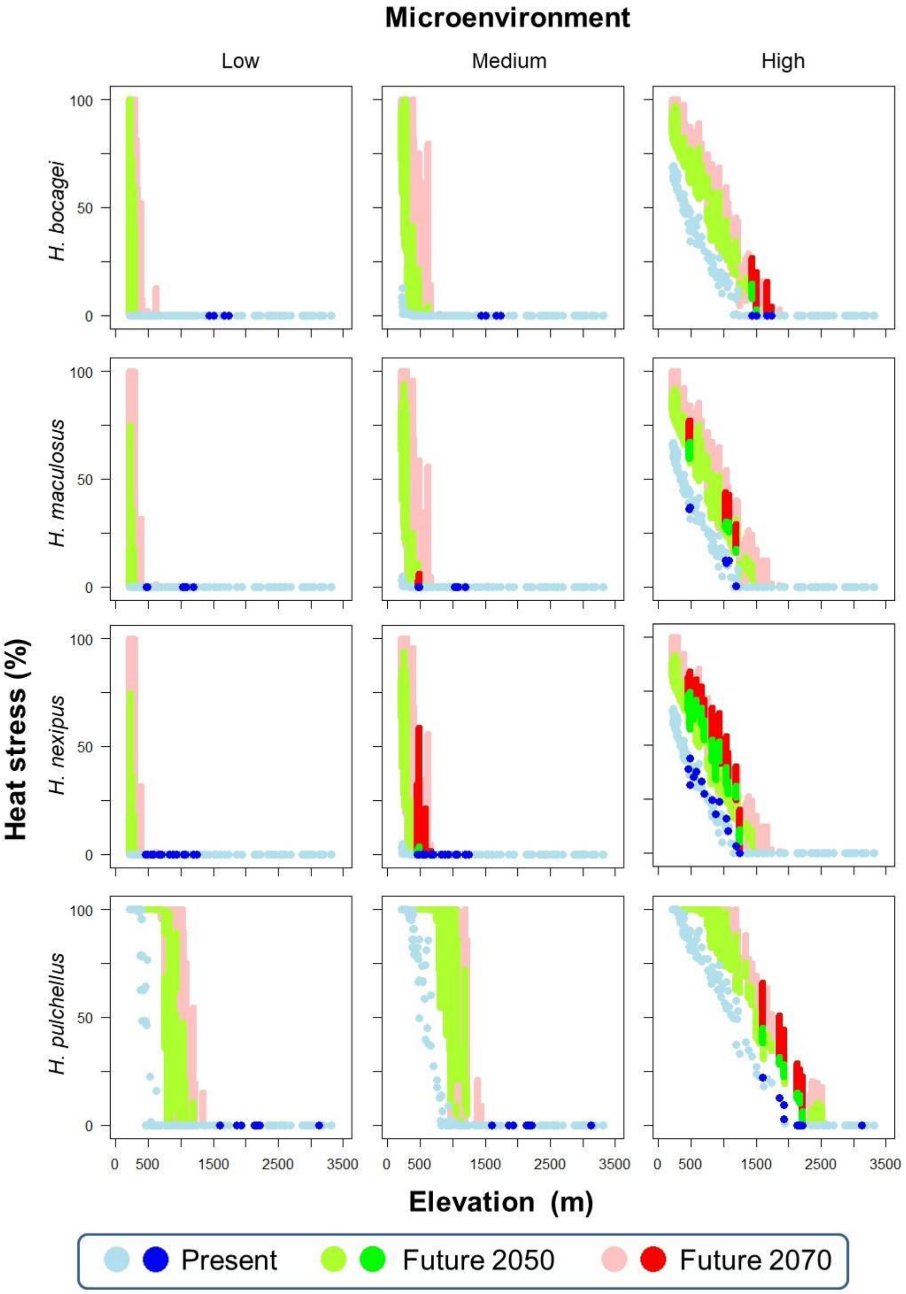
Discussion

The question regarding how temperature influences distributional patterns has been a central topic in ecology (e.g. Hutchinson, 1957; Whittaker *et al.*, 1973; Kearney & Porter, 2009), yet recently, the need to answer this question has become of the utmost importance due to the challenge that represent global warming to biodiversity (Deutsch *et al.*, 2008; Tewksbury *et al.*, 2008). Our mechanistic approach suggest that estimated elevational range, derived from TPCs parameters shows only partial predictive power on the current altitudinal distribution of analysed species which casts doubts on the reliability of physiologically based SDMs approaches. Thus, thermal fundamental niche estimates grounded on key fitness traits, such as larval growth, did not fit with realized niche of current distribution. Optimal range of temperatures for larval growth might not be the main factor determining altitudinal distribution. Our results revealed that physiological models predicted broader elevational ranges for most lowland species while predicting lower distributions than observed for high-elevation ones (**Fig. 4.4**).

A likely explanation for the low predictability of altitudinal distribution by means of thermal physiology may be based on the lower variation of species' Topt compared to the elevational variance of mean temperature (**Fig. 4.3**), which is consistent with previous studies on latitudinal variation (Deutsch *et al.*, 2008; Tewksbury *et al.*, 2008; Overgaard *et al.*, 2014) and with the 'heat invariability' hypothesis (Gaston *et al.*, 2009; Bozinovic *et al.*, 2014). Even though this hypothesis was initially attributed to extreme heat tolerances (e.g. lethal temperature, CTmax), it might be also attributed to Topt (Huey, 2010) as both parameters are highly correlated (Huey & Kingsolver, 1993; Huey *et al.*, 2009; Payne *et al.*, 2016). The low variability in Topt might revolve around the fitness benefits of high temperatures, because rates of biochemical reactions increase along with temperature (Huey & Kingsolver, 1989; Huey, 2010). Thus, we conclude that the physiologically predicted elevational range between species is more similar than would be expected if temperature was a main predictor of realized niche.

The predicted elevational ranges are partially in agreement with the current lowland species distribution, and in some of them (*E. machalilla, E. boulenger, H. maculosus*) may suggest an altitudinal uplift in expected ranges. However, expected altitudinal ranges did not fit with current distribution of high elevation species (*E. darwinwallacei, H. bocagei, H. pulchellus*). In other words,


Microenvironment
Low
Medium
High
Heat stress (%)
E. anthonyi
E. boulengeri
E. darwinwallacei
E. machalilla
E. tricolor
100
50
0
0
1000
2000
Elevation (m)



← **Figure 4.5:** Present (blue) and projected (2050, green; 2070, red) heat stress (in % of time) for all species in the study and three different microenvironments according to its daily thermal variation (low, medium and high). Bright coloration represents each species distributional points. Paler coloration represents all distributional points available for the genus.

fundamental niche based ranges fails to explain what allowed medium-high elevation dendrobatid frogs to colonize mountaintops. Aquatic environments are thermally homogeneous within sites, especially in streams and forested ponds (Huey *et al.*, 2012), therefore, behavioural thermoregulatory compensation is more limited for amphibian larvae inhabiting cold environments in the mountaintops. The absence of seasonality in the tropics may further constrain mountaintop species from exploiting warmer temporal windows as, contrarily, predicted to occur in high-latitude species (Overgaard *et al.*, 2014). Yet, thermal heterogeneity is found between sites with opened ponds showing higher mean and variable temperatures, allowing habitat selection as an alternative to exploit warmer environments. Despite this, our results suggests that, even occupying opened ponds, expected elevational range for *H. pulchellus* falls 400 meters below the maximum observed elevation (2970 m; Coloma, 1995), indicating how mountaintop species must rely on performing in suboptimal environments. On the contrary, other traits (i.e. locomotor performance, vocal behaviour) of high-elevation anurans seem to be barely thermosensitive in broad thermal ranges (Navas, 1996c; Lüddecke & Sánchez, 2002). Then, since temperature for optimal growth occurs at lower elevations than observed, two questions arise: why are highland species not distributed at lower elevations? And, how can they inhabit in environments with suboptimal temperatures?

The mismatch between predicted and observed elevational ranges could be explained by many other factors not related to current climate, such as evolutionary history, dispersal capacity/ability, biotic interactions or stochastic events of extinction (Pearson & Dawson, 2003; Rapp *et al.*, 2012) which may restrain species' distributional range. In fact, elevational gradients present important changes in ultraviolet radiation, oxygen pressure and the diversity and density of predators and competitors (Navas, 2006) which could synergically and inversely affect their distributions. Because biodiversity is greater in the lowland tropics (Lomolino, 2001; McCain, 2010), species that distribute throughout these habitats may be limited by an increase of biotic interactions (Jankowski *et al.*, 2013; Wisz *et al.*, 2013). By contrast, the absence or scarcity of predators and competitors at higher elevations might allow some species to occupy these environments even if it leads to a reduction in their performance. Another possibility would be that current environmental temperatures differ from paleoclimatic conditions. The mismatch between biological niche ranges with current distribution may be partially explained by the colder climatic conditions occurring in the Andes during the Pleistocene (200.000 to 14.000 years), whereas drops of temperatures were larger in the highlands than lowlands (Haffer, 1979; Hooghiemstra *et al.*, 2006). This may have promoted adaptations to cold tolerance in current lowland dendrobatids that are physiologically able to occupy higher elevations but these uplifts have not yet occurred by a myriad of collateral factors such as the mentioned above. The trend occurring in upland dendrobatids, such as *H. pulchellus*, may be otherwise be a consequence of a recent distributional shift (i.e. mountaintop uplifts; Quintero & Jetz, 2018; chapter 2).

Besides the above mechanisms that may explain differences between observed and predicted distributional range, several sources of variation in experimental methodology may yield contrasting estimates of thermal fundamental niches that modify our results on predicted distribution (Sinclair *et al.*, 2016). For instance, most species constitute an array of locally adapted populations and therefore, their thermal sensitivity could vary across their geographical range (e.g. Zani *et al.*, 2005; Lindgren & Laurila, 2009; Richter-Boix *et al.*, 2015). For instances, adaptive divergence on thermal tolerance traits, tracking changes of temperature, along elevational transects has been observed, especially in cold tolerance, in *Epipedobates anthonyi* (Páez-Vacas, 2016), and in other Andean amphibians (Pintanel, Tejedo, Merino-Viteri *et al.*, unpublished data). Another possibility is that high-elevation species can take better advantage of high temperatures in cold climates compared to species from lower altitudes or higher latitudes (the countergradient effect; Yamahira *et al.*, 2007; Buckley & Nufio, 2014). Nonetheless, wider experimental designs that incorporate estimates of intraspecific variation on the thermal sensitivity would probably still fail to explain why optimum temperature of high-elevation species is warmer than it would be expected.

Relying on thermal sensitivity through optimal range of growth performance may result insufficient for a comprehensive explanation of high-elevation distributions of ectotherms. The evolution of physiological traits related to tolerate extreme thermal events, instead, may better define organisms' realized niches, at least in extreme thermal environments (e.g. Bozinovic *et al.*, 2011; Clusella-Trullas *et al.*, 2011; Kingsolver *et al.*, 2011; Overgaard *et al.*, 2014; Gutiérrez-Pesquera *et al.*, 2016). Indeed, survival rate in low temperatures (9^0C) increased as species' elevational range did (**Fig. 4.3**), while their growth performance was never higher than 5% of total (**Fig. 4.1**) (also see Zani *et al.*, 2005).

Even though we did not analyse TPCs for the endangered high-elevation dendrobatid species (e.g. *Hyloxalus jacobuspetersi* or *H. vertebralis*) due to ethical concerns, we believe that results would not differ by including these taxa. In fact, tolerance to cold temperatures seems to be a main driver in successful colonization for amphibians of high Andean tropical elevations (Navas, 2006; chapter 2) and more broadly for ectotherms to colonize colder regions, both latitudinally and altitudinally (Wiens *et al.*, 2006). Interestingy, the highest altitude analysed species *H. pulchellus* exhibits the highest cold tolerance of all dendrobatids in this study (CTmin = 1.67 ^{0}C), approximately 5 ^{0}C more cold tolerant than average of the rest of species (chapter 2).

Our results revealed that ongoing global warming will probably have different effects on lowland and upland dendrobatid species. Tropical lowland species might be more threaten to warming temperatures (e.g. Deutsch *et al.*, 2008; Sunday *et al.*, 2014). Our results confirm that lowland tropical amphibian larvae are already experiencing stressful temperatures, particularly in aquatic environments with high thermal variability (e.g. open ponds; **Fig. 4.5**). Nonetheless, this group of amphibians is broadly distributed along different breeding environments (e.g. rivers, ponds and bromeliads) (see Coloma, 1995) which may allow these species to challenge heat impacts as they shift or stay in cooler breeding environments. Specifically, our climatic models in low thermal variability microenvironments (i.e. rivers and deep ponds) predict that all taxa can physiologically tolerate the future increase of temperatures even with the most extreme scenario (**Fig. 4.5**). By contrast, all species within high thermal variation habitats are expected to eventually experience stressful temperatures.

As warming continues, amphibians will increasingly rely on colder breeding microhabitats to avoid heat stress and local extinctions (Potter *et al.*, 2013; Sunday *et al.*, 2014; Suggitt *et al.*, 2018). This will be particularly decisive for lowland species. However, the availability of habitats that could protect them from an increase of temperatures may be limited in the future. First, human environmental impacts, particularly habitat conversion of cooler forested environments, will have drastic consequences on the availability of suitable environments for organisms (Cushman, 2006; Dirzo *et al.*, 2014; Mendenhall *et al.*, 2014) and important thermal shifts on the altered habitats left (Frishkoff *et al.*, 2015; Brusch *et al.*, 2016; Nowakowski *et al.*, 2017). Second, aquatic microclimates availability is dependent on precipitation and therefore, in a climate change scenario, shifts on precipitation could reduce the availability of suitable aquatic environments as it has been reported in temperate amphibian communities (McMenamin *et al.*, 2008). Even if ponds do not dry out, since deep ponds have lower and more constant temperatures than shallower ponds (Pearman, 1995; also see **Fig. S4.2**), this will reduce the availability of colder microclimates.

Finally, the increase of temperatures could force amphibians to move their ranges upward (Parmesan, 2006; Raxworthy *et al.*, 2008; Chen *et al.*, 2011a). However, since cold tolerance seem to be a main driver of colonization to higher elevations in the tropics (Navas, 2006; chapter 2), upward shifts may be constrained to an increase in minimum environmental temperatures or to evolutionary adaptations to cold. An analysis of ancestral trait reconstruction revealed that in a community of 75 Andean tadpoles, historical uplifts were associated with a parallel evolution in cold thermal tolerance (CTmin), although no variation occurred in CTmax (see chapter 2). By contrast, as climate warms, environmental temperatures would approximate the upland tadpoles' optimum temperatures which should benefit them (for latitudinal patterns see Deutsch *et al*., 2008; Tewksbury *et al*., 2008). However, and in case of an upward migration of lowland species, the novel interactions may have negative effects on upland species (Huey *et al*., 2009).

In conclusion, this study confirms that larval growth performance is not a good predictor of current elevational distribution in dendrobatid species, especially those from uplands, and therefore, it may not be a reliable predictor for climate-related shifts, both contractions (i.e. extinctions) and expansions (i.e. upward colonization). In fact, temperature appears to be weakly related to population declines and extinctions (Cahill *et al.*, 2012) except on the edges of the species distribution (Cahill *et al.*, 2014). Thus, we propose prioritizing research on the edges of ectotherms distribution in order to further understand the factors that constrain distribution on elevation in order to improve our knowledge on biogeographic patterns and, hence, better forecast climate-related impacts.

General Discussion

Back cover: *Gastrotheca sp.*

General Discussion

The study of thermal physiology within an ecological and evolutionary perspective has increased enormously in recent decades (Gaston *et al.*, 2009; Chown & Gaston, 2016), primarily because it has become a very important tool for understanding and predicting the possible effects of climate change (Deutsch *et al.*, 2008; Kearney & Porter, 2009; Evans *et al.*, 2015). In this thesis we have explored the causes that promote the evolution and interspecific variation of amphibian thermal sensitivity in a tropical altitudinal gradient. This information has also been especially relevant to understand how ectotherms will respond to environmental changes promoted by human activity. One of the most interesting result is that thermal physiological variation in amphibians through a tropical altitudinal gradient encompass a similar or even greater range to that found through latitudinal gradients, although it is restricted to a much lesser geographic distance (see Duarte *et al.*, 2012; Gutiérrez-Pesquera *et al.*, 2016).

As it happens with latitude (Janzen, 1967; Ghalambor *et al.*, 2006), the ranges of thermal tolerance increase positively with altitude (Gaston & Chown, 1999; Araújo *et al.*, 2013; Muñoz *et al.*, 2014; **chapter 1 and 2**). However, this increase in thermal tolerance range is mainly attributable to a greater decline in CTmin whereas CTmax only suffer a slightly decrease with altitude. This asymmetric pattern of spatial variation in thermal tolerance limits is known as Brett's rule or the 'heat-invariant' hypothesis (Brett, 1956; Araújo *et al.*, 2013; Bozinovic *et al.*, 2014). The evolution of species' cold tolerance, rather than heat tolerance, was suggested during 19th century by naturalists such as Alexander von Humboldt and others as a key factor for species distribution in latitude (his 'temperature-fluidity' hypothesis; Hawkins, 2001; Bonebrake, 2013), although it may also apply to other ecological gradients such as altitude (this thesis). James Dana (1853, In: Lomolino *et al.*, 2017; pp. 85), suggested that " *The cause which limits the distribution of species northward or southward from the Equator is the cold of winter rather than the heat of summer or even the mean temperature of the year* ". In **chapter 2**, we have confirmed that the evolution of cold tolerance is important for colonization of tropical mountaintops, while the colonization of tropical lowlands is not linked to evolution of higher heat tolerances (discussed below).

Different mechanisms, such as evolutionary limitations (Beacham & Withler, 1991; Blackburn *et al.*, 2014), lower rates of evolution (Muñoz *et al.*, 2014; von May *et al.*, 2017) or greater capacity to face exposure to peak maximum temperatures by thermoregulation, and the unability of behavioural compensation to buffer minimum temperatures (Huey *et al.*, 2003; Muñoz *et al.*, 2014) have been suggested to explain the invariability of CTmax in contrast to CTmin. In **chapters 1 and 2**, we examined the prediction that the CTmin presented a higher evolutionary rate than the CTmax, both in adults (terrestrial environment) and tadpoles (aquatic environment), respectively. In both cases, the results show a clear tendency of higher evolution rates for cold tolerance than for CTmax. However, contrasting evolutionary rates in both thermal limits are not a sufficient argument to confirm the prediction of the spatial inflexibility of heat tolerance in contrast to cold. In fact, although both thermal limits are conserved trait with strong phylogenetic signal both in terrestrial and aquatic stages, the absolute range of variation in CTmax are wide and of similar magnitude than CTmin ranges (for instances, in tadpoles: CTmin: 13.2^{0}C [-3.6 - 9.5 ^{0}C]; CTmax: 11.3 ^{0}C [32.8 - 44.1 ^{0}C]; **chapter 2**).

Yet, other dimension of the spatial pattern rather than altitude has to be responsible of the wide range of CTmax variation. We argue that selection of habitat, both aquatic and terrestrial, seems to have a more important role explaining this divergence in upper thermal tolerance limits. Although the terrestrial stage shows greater opportunities for active thermoregulation than the aquatic stage (Tracy, 1976; Spotila *et al.*, 1992), the inability or incapacity of adult amphibians, most of them nocturnal, to body temperature regulation (Navas, 2002; Navas *et al.*, 2013) limits the opportunities to elude thermal extremes, which may have triggered selection to evolve thermal adaptations to the environmental temperatures they are exposed to (see Duarte *et al.*, 2012; Gutiérrez-Pesquera *et al.*, 2016). Our results show that, although geographical variation in heat tolerance at large scales (i.e. altitude) are lower than cold tolerance variation (Bozinovic *et al.*, 2014), the opposite occurs on a local scale. Amphibians that inhabit zones with lower and constant temperatures (i.e. rivers and forests in both aquatic and terrestrial stages) have lower thermal tolerance ranges and lower CTmax than those found in environments with higher and more variable temperatures (i.e. open environments) (Duarte *et al.*, 2012; other ectotherms: Frishkoff *et al.*, 2015; Kaspari *et al.*, 2015). Therefore, analogous to Janzen´s (1967) *motto* mountains are 'higher' in the aseasonal tropics, we propose that habitats may also turn to be 'higher' in the tropics. Thereby, habitat thermal heterogeneity can also act as transversal physiological barrier, promoting in each environment, and additional to altitude source of variation in thermal specialization.

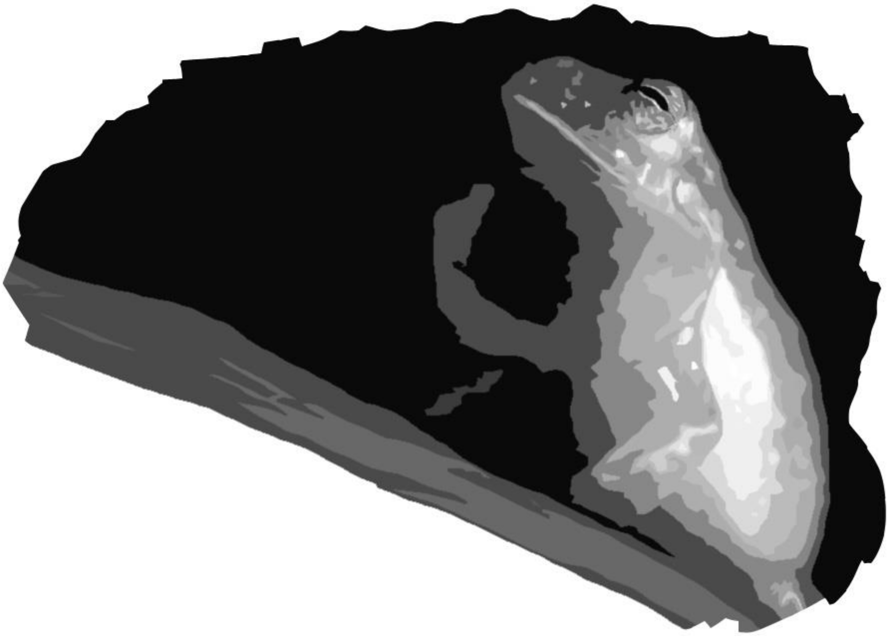
Hence, the general, macrophysiological rule, of heat-invariability observed at broad spatial ranges could simply be derived from focusing in a single dimension of spatial variation (latitude, or in our case altitude). This weak characterization of the overall spatial variation in thermal limits ignores other variables or mechanisms that confer a high explanatory power of the pattern but that vary in a more complex spatial pattern (Hawkins & Diniz-Filho, 2004). For instances, if we look to climatic predictors that does not reflect the scale at which the organisms are exposed or ignore the potential mechanisms of organismal body temperature regulation, we can miss, for instances, the observed variability occurring in the CTmax at different microhabitats (**chapter 1 and 2**) (Chown & Gaston, 2016). In fact, the use of microclimatic predictors may alter previously foretold macrophysiological patterns (Sunday *et al.*, 2014; Woods *et al.*, 2015; Chown & Gaston, 2016). For instances, the analysis of vulnerability to global warming in **chapters 1 to 4** based on micro- (dataloggers) and macro-climatic (WorldClim; Hijmans *et al.*, 2005) temperature, results in different predictions. Using macroclimatic predictors, our results suggest that tropical lowland species would be more vulnerable to heat stress, just as previously predicted (Colwell *et al.*, 2008; von May *et al.*, 2017). Contrarily, the risk of suffering thermal stress results invariant with altitude when using microclimatic predictors (**chapters 1 and 4** but not **2 and 3**). As a consequence, vulnerability assessments derived from microclimatic individually-based information, reveal that highland amphibians will also require thermal shelters to avoid extreme temperature events that may condition their persistence (see Sunday *et al.*, 2014).

Another reason that may explain why heat-tolerance is invariant in broad ecological gradients could be based on the benefits of high temperature performance (**chapter 3**). As predicted by the 'hotter is better' hypothesis, warm-adapted organisms may benefit from a greater performance in their functions than cold-adapted ones because chemical reactions increase with temperature (Hamilton, 1973; Bennett, 1987). Thus, selection should favour adaptation to high optimal temperatures within the range of body temperatures available. However, this explanation would fail to explain why species from lowlands, with greater potential to find higher temperatures, have not evolved far greater heat tolerances or optimum temperatures (Muñoz *et al.*, 2014). One possible explanation, also because chemical reactions increase with temperature, is that warm-adapted species generally exhibit a greater decrease in performance after the optimum ("physiological heating tolerance (PHT) hypothesis" in **chapter 3**;

Payne & Smith, 2017) which could imply a trade-off that may restrict the evolution of heat tolerance. In summary, warm-adapted species may benefit from greater performance ('hotter is better'), however, this may also imply a greater asymmetry in their performance rates (‘PHT’ hypothesis), and therefore lower physiological safety ranges to thermal stress (CTmax – Topt).

The second main result derived from our thesis is that the altitudinal distribution of tropical amphibians does not seem to be singly explained by temperature, or at least, it is not the only factor that limits it (also see Navas, 2006). For example, some highland species show CTmax and Topt comparable to lowland species (e.g. some species within *Gastrotheca* genus) but their distributions are limited to highlands (**chapter 1 to 4**). In addition, although our results indicate that altitudinal species distribution should be physiologically constrained by cold temperatures and therefore, limiting upwards dispersal of lowland species, we found that a greater number of elevational transitions upwards rather than downwards (**chapter 2**). These results suggest that there are other biotic or abiotic factors (historic or recent) that limit the elevational distribution of ectotherms in the tropics. For instance, the great biodiversity found in the lowland tropics may increase biotic interactions, which could restrict species’ distribution (Jankowski *et al.*, 2013; Wisz *et al.*, 2013). Likewise, other factors such as changes on precipitation, ultraviolet radiation, oxygen pressure, complex orogeny or past-climate changes in the Andes may have limited current species distributional ranges (Pearson & Dawson, 2003; Rapp *et al.*, 2012; Hazzi *et al.*, 2018). Acknowledging the causes and mechanisms that determined present upper and lower elevational range limits was beyond the scope of this thesis, however, given the growing concern over global change on species loss, understanding the mechanisms that set distributional ranges should be a central topic for future research.

In conclusion, the results in this thesis can help explain how physiological diversity has evolved in tropical mountain amphibians. We consider that these results may have important implications for the forecast of the effects of human-induced environmental changes in the future. However, the physiological diversity found is insufficient for explaining the current distribution patterns of species, which reignites the debate on which factors shape species distribution in the tropics (Dobzhansky, 1950; Janzen, 1967; MacArthur, 1969). Nonetheless, cold tolerance keeps accumulating support as a key condition for the colonization of higher latitudes but also in tropical mountaintops (Wiens *et al.*, 2006; Sunday *et al.*, 2011; von May *et al.*, 2017), which should motivate more research in the evolution of cold tolerances that has been widely neglected by ecophysiologists, more interested in the analysis of heat tolerances to provide vulnerability assessments to global warming impacts on biodiversity.



Conclusions

Back cover: *Centrolene peristictum* (Lynch & Duellman, 1973)

Conclusions

1. The variation of thermal tolerance ranges of amphibian tadpoles through an altitudinal tropical mountain gradient is similar in magnitude to those previously found in long range latitudinal gradients of temperate and tropical communities (**chapter 1 to 3**).

2. Amphibian thermal tolerance ranges correlates with environmental thermal variability encountered, in agreement with Janzen's and Steven's, climatic variability hypothesis (**chapter 1 and 2**).

3. The increase in thermal tolerance range with altitude is largely attributed to faster variation in cold tolerance (CTmin) than that found for heat tolerance (CTmax) that fits well with the 'heat-invariant' hypothesis. By contrast, at local scales (i.e. microhabitats), CTmax is more variable than CTmin. This greater, within site, CTmax variation explain the apparent heat-invariability detected at larger scales (**chapter 1 and 2**).

4. The evolutionary rate of CTmax is lower than that found in CTmin, which could imply that tropical Andean amphibians are unlikely to buffer increase in temperatures due to global warming effects through marked evolutionarily changes (**chapter 1 and 2**). The relative stasis in CTmax, may be attributed to the behavioural buffering of thermoregulation, which is more effective at limiting exposure to maximum rather than minimum extreme temperatures.

5. The incorporation of microclimatic data (datalogger) in the analyses, rather than large climatic databases (WorldClim), could lead to different conclusions on macrophysiological patterns (**chapter 1 to 4**).

6. Amphibians are more limited, in terms of thermal physiology, to move upward rather than downward in tropical mountains since elevational transitions required evolving different optima for CTmin but not for CTmax (**chapter 2**).

7. Contrary to the above finding, thermal physiology appears not to be the main mechanism determining or limiting altitudinal distribution; in fact upward migrations appeared to be more frequent rather than downward migrations (**chapter 2**). Moreover, for nine related dendrobatid frog species, their modelled predicted altitudinal distributions overlap in a greater extent than actual ranges (**chapter 3 and 4**). Hence, predicted elevational shifts by using physiologically based mechanistic models may not be reliable in all amphibian clades.

8. Lowland tropical amphibians are more vulnerable to upcoming increase of temperatures, than their upland counterparts, because they are currently experiencing environmental temperatures close to their physiological optima and heat tolerance (**chapter 1 to 4**). However, some highland species may be equally vulnerable to suffer heat stress and will therefore need to search for thermal shelters to avoid extreme heat events (**chapter 1 and 4**).

9. Warm-adapted species have higher performance rates than cold-adapted, as predicted by the 'hotter is better' hypothesis (**chapter 3**). We suggest that 'hotter is better' hypothesis may be an explanatory mechanism of the heat-invariability rule, given how selection might favour warm-adapted species as they benefit from greater performance.

10. Warm-adapted species, present lower thermal safety boundaries (CTmax – Topt) than cold adapted, as predicted by the 'physiological heating tolerance' trade-off. Because warm-adapted species at lowlands are more vulnerable to an increase of temperatures by currently living at hotter temperatures (conclusion 8), this inherent physiological constrain may exacerbate the deleterious effects of climate change in lowland communities (**chapter 3**).

References

Addo-Bediako, A., Chown, S.L. & Gaston, K.J. (2000) Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **267**, 739-745.

Alford, L., Blackburn, T.M. & Bale, J.S. (2012) Effect of latitude and acclimation on the lethal temperatures of the peach-potato aphid Myzus persicae. *Agricultural and Forest Entomology*, **14**, 69-79.

AmphibiaWeb (2017) *Information on amphibian biology and conservation. [web application]*. Available at: http://amphibiaweb.org/ (accessed jun 26, 2017).

Angert, A.L., Sheth, S.N. & Paul, J.R. (2011) Incorporating Population-Level Variation in Thermal Performance into Predictions of Geographic Range Shifts. *Integrative and Comparative Biology*,

Angilletta, M.J. (2006) Estimating and comparing thermal performance curves. *Journal of Thermal Biology*, **31**, 541-545.

Angilletta, M.J. (2009) *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford Univ Press, Oxford, UK.

Angilletta, M.J., Niewiarowski, P.H. & Navas, C.A. (2002) The evolution of thermal physiology in ectotherms. *Journal of Thermal Biology*, **27**, 249-268.

Angilletta, M.J., Huey, R.B. & Frazier, M.R. (2010) Thermodynamic Effects on Organismal Performance: Is Hotter Better? *Physiological and Biochemical Zoology*, **83**, 197-206.

Angilletta, M.J., Bennett, A.F., Guderley, H., Navas, C.A., Seebacher, F. & Wilson, R.S. (2006) Coadaptation: A Unifying Principle in Evolutionary Thermal Biology. *Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches*, **79**, 282-294.

Araújo, M.B. & Luoto, M. (2007) The importance of biotic interactions for modelling species distributions under climate change. *Global Ecology and Biogeography*, **16**, 743-753.

Araújo, M.B., Ferri-Yáñez, F., Bozinovic, F., Marquet, P.A., Valladares, F. & Chown, S.L. (2013) Heat freezes niche evolution. *Ecology Letters*, **16**, 1206-1219.

Bader, M.Y., Rietkerk, M. & Bregt, A.K. (2007) Vegetation Structure and Temperature Regimes of Tropical Alpine Treelines. *Arctic, Antarctic, and Alpine Research*, **39**, 353-364.

Balogová, M. & Gvoždík, L. (2015) Can Newts Cope with the Heat? Disparate Thermoregulatory Strategies of Two Sympatric Species in Water. *PLoS ONE*, **10**, e0128155.

Baselga, A., Gómez-Rodríguez, C. & Lobo, J.M. (2012) Historical Legacies in World Amphibian Diversity Revealed by the Turnover and Nestedness Components of Beta Diversity. *PLoS ONE*, **7**, e32341.

Beacham, T.D. & Withler, R.E. (1991) Genetic variation in mortality of chinook salmon, Oncorhynchus tshawytscha (Walbaum), challenged with high water temperatures. *Aquaculture Research*, **22**, 125-133.

Beaulieu, J.M. & O'Meara, B. (2016) *Analysis of Evolutionary Rates in an OU Framework*. R package version 1.5.

Beaulieu, J.M., Jhwueng, D.-C., Boettiger, C. & O'Meara, B.C. (2012) Modeling stabilizing selection: Expanding the Ornstein–Uhlenbeck model of adaptive evolution. *Evolution*, **66**, 2369-2383.

Bennett, A.F. (1987) Evolution of the control of body temperature: is warmer better. *Comparative physiology: Life in water and on land* (ed. by P. Dejours, L. Bolis, C. Taylor and E. Weibel), pp. 421-431. Liviana, Padova.

Bernal, M.H. & Lynch, J.D. (2008) Review and analysis of altitudinal distribution of the Andean anurans in Colombia. *Zootaxa*, **1826**, 1–25.

Berven, K.A. (1982) The genetic basis of altitudinal variation in the wood frog Rana sylvatica II. An experimental analysis of larval development. *Oecologia*, **52**, 360-369.

Berven, K.A. & Grudzien, T.A. (1990) Dispersal in the wood frog (*Rana sylvatica*): Implications for genetic population structure. *Evolution*, **44**, 2047-2056.

Bevelhimer, M. & Bennett, W. (2000) Assessing cumulative thermal stress in fish during chronic intermittent exposure to high temperatures. *Environmental Science & Policy*, **3**, 211-216.

Blackburn, S., van Heerwaarden, B., Kellermann, V. & Sgrò, C.M. (2014) Evolutionary capacity of upper thermal limits: beyond single trait assessments. *The Journal of Experimental Biology*, **217**, 1918-1924.

Blomberg, S.P., Garland, T., Jr. & Ives, A.R. (2003) Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution*, **57**, 717-45.

Bogert, C.M. (1949) Thermoregulation in Reptiles, A Factor in Evolution. *Evolution*, **3**, 195-211.

Bonebrake, T.C. (2013) Conservation implications of adaptation to tropical climates from a historical perspective. *Journal of Biogeography*, **40**, 409-414.

Bonebrake, T.C., Pickett, E.J., Tsang, T.P.N., Tak, C.Y., Vu, M.Q. & Vu, L.V. (2016) Warming threat compounds habitat degradation impacts on a tropical butterfly community in Vietnam. *Global Ecology and Conservation*, **8**, 203-211.

Bonino, M.F., Moreno Azócar, D.L., Schulte Ii, J.A., Abdala, C.S. & Cruz, F.B. (2015) Thermal sensitivity of cold climate lizards and the importance of distributional ranges. *Zoology*, **118**, 281-290.

Bozinovic, F., Calosi, P. & Spicer, J.I. (2011) Physiological Correlates of Geographic Range in Animals. *Annual Review of Ecology, Evolution, and Systematics*, **42**, 155-179.

Bozinovic, F., Orellana, M.J.M., Martel, S.I. & Bogdanovich, J.M. (2014) Testing the heat-invariant and cold-variability tolerance hypotheses across geographic gradients. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **178**, 46-50.

Brattstrom, B.H. (1968) Thermal acclimation in Anuran amphibians as a function of latitude and altitude. *Comparative Biochemistry and Physiology*, **24**, 93-111.

Brett, J.R. (1956) Some Principles in the Thermal Requirements of Fishes. *The Quarterly Review of Biology*, **31**, 75-87.

Brodie, J.F., Strimas-Mackey, M., Mohd-Azlan, J., Granados, A., Bernard, H., Giordano, A.J. & Helmy, O.E. (2017) Lowland biotic attrition revisited: body size and variation among climate change 'winners' and 'losers'. *Proceedings of the Royal Society B: Biological Sciences*, **284**

Brusch, G.A., Taylor, E.N. & Whitfield, S.M. (2016) Turn up the heat: thermal tolerances of lizards at La Selva, Costa Rica. *Oecologia*, **180**, 325-334.

Buckley, L.B. & Jetz, W. (2007) Environmental and historical constraints on global patterns of amphibian richness. *Proceedings of the Royal Society B: Biological Sciences*, **274**, 1167-1173.

Buckley, L.B. & Kingsolver, J.G. (2012) Functional and Phylogenetic Approaches to Forecasting Species' Responses to Climate Change. *Annual Review of Ecology, Evolution, and Systematics*, **43**, 205-226.

Buckley, L.B. & Nufio, C.R. (2014) Elevational clines in the temperature dependence of insect performance and implications for ecological responses to climate change. *Conservation Physiology*, **2**, cou035-cou035.

Buckley, L.B. & Huey, R.B. (2016) Temperature extremes: geographic patterns, recent changes, and implications for organismal vulnerabilities. *Global Change Biology*, **22**, 3829-3842.

Buckley, L.B., Miller, E.F. & Kingsolver, J.G. (2013) Ectotherm Thermal Stress and Specialization Across Altitude and Latitude. *Integrative and Comparative Biology*, **53**, 571-581.

Buckley, L.B., Urban, M.C., Angilletta, M.J., Crozier, L.G., Rissler, L.J. & Sears, M.W. (2010) Can mechanism inform species' distribution models? *Ecology Letters*, **13**, 1041-1054.

Burnham, K.P. & Anderson, D.R. (2002) *Model selection and multimode inference: a practical information theoretic approach*. Springer, New York.

Cadena, C.D., Kozak, K.H., Gómez, J.P., Parra, J.L., McCain, C.M., Bowie, R.C.K., Carnaval, A.C., Moritz, C., Rahbek, C., Roberts, T.E., Sanders, N.J., Schneider, C.J., VanDerWal, J., Zamudio, K.R. & Graham, C.H. (2012) Latitude, elevational climatic zonation and speciation in New World vertebrates. *Proceedings of the Royal Society of London B: Biological Sciences*, **279**, 194-201.

Cahill, A.E., Aiello-Lammens, M.E., Fisher-Reid, M.C., Hua, X., Karanewsky, C.J., Ryu, H.Y., Sbeglia, G.C., Spagnolo, F., Waldron, J.B. & Wiens, J.J. (2014) Causes of warm-edge range limits: systematic review, proximate factors and implications for climate change. *Journal of Biogeography*, **41**, 429-442.

Cahill, A.E., Aiello-Lammens, M.E., Fisher-Reid, M.C., Hua, X., Karanewsky, C.J., Yeong Ryu, H., Sbeglia, G.C., Spagnolo, F., Waldron, J.B., Warsi, O. & Wiens, J.J. (2012) How does climate change cause extinction? *Proceedings of the Royal Society B: Biological Sciences*,

Calosi, P., Bilton, D.T., Spicer, J.I., Votier, S.C. & Atfield, A. (2010) What determines a species' geographical range? Thermal biology and latitudinal range size relationships in European diving beetles (Coleoptera: Dytiscidae). *Journal of Animal Ecology*, **79**, 194-204.

Carvajalino-Fernández, J.M., Bonilla Gomez, M.A. & Arturo Navas, C. (2011) Freezing Risk in Tropical High-Elevation Anurans: An Assessment Based on the Andean Frog Pristimantis nervicus (Strobomantidae). *South American Journal of Herpetology*, **6**, 73-78.

Clusella-Trullas, S., Blackburn, T.M., Chown, S.L., Associate Editor: Carlos, M., xed, nez del, R. & Editor: Mark, A.M. (2011) Climatic Predictors of Temperature Performance Curve Parameters in Ectotherms Imply Complex Responses to Climate Change. *The American Naturalist*, **177**, 738-751.

Coloma, L.A. (1995) *Ecuadorian frogs of the genus Colostethus (Anura: Dendrobatidae)*. Natural History Museum, University of Kansas, Lawrence, Kansas.

Colwell, R.K. & Rangel, T.F. (2010) A stochastic, evolutionary model for range shifts and richness on tropical elevational gradients under Quaternary glacial cycles. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **365**, 3695-3707.

Colwell, R.K., Brehm, G., Cardelús, C.L., Gilman, A.C. & Longino, J.T. (2008) Global Warming, Elevational Range Shifts, and Lowland Biotic Attrition in the Wet Tropics. *Science*, **322**, 258-261.

Conner, J.K. & Hartl, D.L. (2004) *A primer of ecological genetics*. Sinauer Associates, Sunderland, MA.

Conover, D.O., Duffy, T.A. & Hice, L.A. (2009) The Covariance between Genetic and Environmental Influences across Ecological Gradients. *Annals of the New York Academy of Sciences*, **1168**, 100-129.

Cruz, F.B., Fitzgerald, L.A., Espinoza, R.E. & Schulte, I.J.A. (2005) The importance of phylogenetic scale in tests of Bergmann's and Rapoport's rules: lessons from a clade of South American lizards. *Journal of Evolutionary Biology*, **18**, 1559-1574.

Cunningham, S. & Read, J. (2002) Comparison of temperate and tropical rainforest tree species: photosynthetic responses to growth temperature. *Oecologia*, **133**, 112-119.

Cushman, S.A. (2006) Effects of habitat loss and fragmentation on amphibians: A review and prospectus. *Biological Conservation*, **128**, 231-240.

Chamberlain, S. (2017) rgbif: Interface to the Global 'Biodiversity' Information Facility API. In, https://CRAN.R-project.org/package=rgbif.

Chen, I.-C., Hill, J.K., Ohlemüller, R., Roy, D.B. & Thomas, C.D. (2011a) Rapid Range Shifts of Species Associated with High Levels of Climate Warming. *Science*, **333**, 1024-1026.

Chen, I.-C., Shiu, H.-J., Benedick, S., Holloway, J.D., Chey, V.K., Barlow, H.S., Hill, J.K. & Thomas, C.D. (2009) Elevation increases in moth assemblages over 42 years on a tropical mountain. *Proceedings of the National Academy of Sciences*, **106**, 1479-1483.

Chen, I.-C., Hill, J.K., Shiu, H.-J., Holloway, J.D., Benedick, S., Chey, V.K., Barlow, H.S. & Thomas, C.D. (2011b) Asymmetric boundary shifts of tropical montane Lepidoptera over four decades of climate warming. *Global Ecology and Biogeography*, **20**, 34-45.

Chown, S.L. & Gaston, K.J. (2016) Macrophysiology – progress and prospects. *Functional Ecology*, **30**, 330-344.

Chown, S.L., Addo-Bediako, A. & Gaston, K.J. (2002) Physiological variation in insects: large-scale patterns and their implications. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, **131**, 587-602.

Chown, S.L., Gaston, K.J. & Robinson, D. (2004) Macrophysiology: large-scale patterns in physiological traits and their ecological implications. *Functional Ecology*, **18**, 159-167.

Christian, K.A., Nunez, F., Clos, L. & Diaz, L. (1988) Thermal Relations of Some Tropical Frogs Along an Altitudinal Gradient. *Biotropica*, **20**, 236-239.

Dawson, T.P., Jackson, S.T., House, J.I., Prentice, I.C. & Mace, G.M. (2011) Beyond Predictions: Biodiversity Conservation in a Changing Climate. *Science*, **332**, 53-58.

Denver, R.J., Mirhadi, N. & Phillips, M. (1998) Adaptive plasticity in amphibian metamorphosis: Response of *Scaphiopus hammondii* tadpoles to habitat desiccation. *Ecology*, **79**, 1859-1872.

Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., Haak, D.C. & Martin, P.R. (2008) Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences*, **105**, 6668-6672.

Diamond, S.E., Sorger, D.M., Hulcr, J., Pelini, S.L., Toro, I.D., Hirsch, C., Oberg, E. & Dunn, R.R. (2012a) Who likes it hot? A global analysis of the climatic, ecological, and evolutionary determinants of warming tolerance in ants. *Global Change Biology*, **18**, 448-456.

Diamond, S.E., Nichols, L.M., McCoy, N., Hirsch, C., Pelini, S.L., Sanders, N.J., Ellison, A.M., Gotelli, N.J. & Dunn, R.R. (2012b) A physiological trait-based approach to predicting the responses of species to experimental climate warming. *Ecology*, **93**, 2305-2312.

Dillon, M.E., Frazier, M.R. & Dudley, R. (2006) Into thin air: Physiology and evolution of alpine insects. *Integrative and Comparative Biology*, **46**, 49-61.

Dirzo, R., Young, H.S., Galetti, M., Ceballos, G., Isaac, N.J.B. & Collen, B. (2014) Defaunation in the Anthropocene. *Science*, **345**, 401-406.

Dobzhansky, T. (1950) Evolution in the tropics. *American Scientist*, **38**, 209-221.

Donaldson, L.R. & Olson, P.R. (1957) Development of Rainbow Trout Brood Stock by Selective Breeding. *Transactions of the American Fisheries Society*, **85**, 93-101.

Duarte, H., Tejedo, M., Katzenberger, M., Marangoni, F., Baldo, D., Beltrán, J.F., Martí, D.A., Richter-Boix, A. & Gonzalez-Voyer, A. (2012) Can amphibians take the heat? Vulnerability to climate warming in subtropical and temperate larval amphibian communities. *Global Change Biology*, **18**, 412-421.

Duellman, W.E. & Trueb, L. (2015) *Marsupial frogs: Gastrotheca and allied genera*. Jhon Hopkins University Press, Baltimore, Maryland.

Elias, M., Joron, M., Willmott, K., Silva-Brandão, K.L., Kaiser, V., Arias, C.F., Piñerez, L.M.G., Uribe, S., Brower, A.V.Z., Freitas, A.V.L. & Jiggins, C.D. (2009) Out of the Andes: patterns of diversification in clearwing butterflies. *Molecular Ecology*, **18**, 1716-1729.

Elith, J., Kearney, M. & Phillips, S. (2010) The art of modelling range-shifting species. *Methods in Ecology and Evolution*, **1**, 330-342.

Elith, J., H. Graham*, C., P. Anderson, R., Dudík, M., Ferrier, S., Guisan, A., J. Hijmans, R., Huettmann, F., R. Leathwick, J., Lehmann, A., Li, J., G. Lohmann, L., A. Loiselle, B., Manion, G., Moritz, C., Nakamura, M., Nakazawa, Y., McC. M. Overton, J., Townsend Peterson, A., J. Phillips, S., Richardson, K., Scachetti-Pereira, R., E. Schapire, R., Soberón, J., Williams, S., S. Wisz, M. & E. Zimmermann, N. (2006) Novel methods improve prediction of species' distributions from occurrence data. *Ecography*, **29**, 129-151.

Erskine, D.J. & Spotila, J.R. (1977) Heat-Energy-Budget Analysis and Heat Transfer in the Largemouth Blackbass (Micropterus salmoides). *Physiological Zoology*, **50**, 157-169.

Evans, T.G., Diamond, S.E. & Kelly, M.W. (2015) Mechanistic species distribution modelling as a link between physiology and conservation. *Conservation Physiology*, **3**, cov056-cov056.

Feder, M.E. & Lynch, J.F. (1982) Effects of Latitude, Season, Elevation, and Microhabitat on Field Body Temperatures of Neotropical and Temperate Zone Salamanders. *Ecology*, **63**, 1657-1664.

Feeley, K.J., Silman, M.R., Bush, M.B., Farfan, W., Cabrera, K.G., Malhi, Y., Meir, P., Revilla, N.S., Quisiyupanqui, M.N.R. & Saatchi, S. (2011) Upslope migration of Andean trees. *Journal of Biogeography*, **38**, 783-791.

Flantua, S.G., Hooghiemstra, H., Van Boxel, J.H., Cabrera, M., González-Carranza, Z. & González-Arango, C. (2014) Connectivity dynamics since the Last Glacial Maximum in the northern Andes; a pollen-driven framework to assess potential migration. *Paleobotany and Biogeography: A Festschrift for Alan Graham in His 80th Year. Missouri Botanical Garden, St. Louis*, 98-123.

Floyd, R.B. (1983) Ontogenetic change in the temperature tolerance of larval Bufo marinus (Anura: bufonidae). *Comparative Biochemistry and Physiology Part A: Physiology*, **75**, 267-271.

Forero-Medina, G., Joppa, L. & Pimm, S.L. (2011) Constraints to Species' Elevational Range Shifts as Climate Changes. *Conservation Biology*, **25**, 163-171.

Frazier, M.R., Huey, Raymond B. & Berrigan, D. (2006) Thermodynamics Constrains the Evolution of Insect Population Growth Rates: "Warmer Is Better.". *The American Naturalist*, **168**, 512-520.

Freckleton, R.P., Harvey, P.H. & Pagel, M. (2002) Phylogenetic analysis and comparative data: A test and review of evidence. *American Naturalist*, **160**, 712-726.

Freitas, V., Cardoso, J.F.M.F., Lika, K., Peck, M.A., Campos, J., Kooijman, S.A.L.M. & van der Veer, H.W. (2010) Temperature tolerance and energetics: a dynamic energy budget-based comparison of North Atlantic marine species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **365**, 3553-3565.

Frishkoff, L.O., Hadly, E.A. & Daily, G.C. (2015) Thermal niche predicts tolerance to habitat conversion in tropical amphibians and reptiles. *Global Change Biology*, **21**, 3901-3916.

Funk, W.C., Greene, A.E., Corn, P.S. & Allendorf, F.W. (2005) High dispersal in a frog species suggests that it is vulnerable to habitat fragmentation. *Biology Letters*, **1**, 13-16.

Gaston, K.J. & Chown, S.L. (1999) Elevation and Climatic Tolerance: A Test Using Dung Beetles. *Oikos*, **86**, 584-590.

Gaston, K.J., Chown, S.L., Calosi, P., Bernardo, J., Bilton, D.T., Clarke, A., Clusella-Trullas, S., Ghalambor, C.K., Konarzewski, M., Peck, L.S., Porter, W.P., Portner, H.O., Rezende, E.L., Schulte, P.M., Spicer, J.I., Stillman, J.H., Terblanche, J.S. & van Kleunen, M. (2009) Macrophysiology: a conceptual reunification. *Am Nat*, **174**, 595-612.

Ghalambor, C.K., Huey, R.B., Martin, P.R., Tewksbury, J.J. & Wang, G. (2006) Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integrative and Comparative Biology*, **46**, 5-17.

Gilchrist, G.W. & Huey, R.B. (1999) The direct response of Drosophila melanogaster to selection on knockdown temperature. *Heredity*, **83**, 15-29.

Gilman, S.E., Urban, M.C., Tewksbury, J., Gilchrist, G.W. & Holt, R.D. (2010) A framework for community interactions under climate change. *Trends in Ecology & Evolution*, **25**, 325-331.

Gill, B.A., Kondratieff, B.C., Casner, K.L., Encalada, A.C., Flecker, A.S., Gannon, D.G., Ghalambor, C.K., Guayasamin, J.M., Poff, N.L., Simmons, M.P., Thomas, S.A., Zamudio, K.R. & Funk, W.C. (2016) Cryptic species diversity reveals biogeographic support for the ‘mountain passes are higher in the tropics’ hypothesis. *Proceedings of the Royal Society of London B: Biological Sciences*, **283**

Gosner, K.L. (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, **16**, 183-190.

Gouveia, S.F., Hortal, J., Tejedo, M., Duarte, H., Cassemiro, F.A.S., Navas, C.A. & Diniz-Filho, J.A.F. (2014) Climatic niche at physiological and macroecological scales: the thermal tolerance–geographical range interface and niche dimensionality. *Global Ecology and Biogeography*, **23**, 446-456.

Graae, B.J., De Frenne, P., Kolb, A., Brunet, J., Chabrerie, O., Verheyen, K., Pepin, N., Heinken, T., Zobel, M., Shevtsova, A., Nijs, I. & Milbau, A. (2012) On the use of weather data in ecological studies along altitudinal and latitudinal gradients. *Oikos*, **121**, 3-19.

Graham, C.H., Ron, S.R., Santos, J.C., Schneider, C.J. & Moritz, C. (2004) Integrating phylogenetics and environmental niche models to explore speciation mechanisms in dendrobatid frogs. *Evolution*, **58**, 1781-93.

Gregory-Wodzicki, K.M. (2000) Uplift history of the Central and Northern Andes: A review. *GSA Bulletin*, **112**, 1091-1105.

Griffiths, J.A., Schiffer, M. & Hoffmann, A.A. (2005) Clinal variation and laboratory adaptation in the rainforest species Drosophila birchii for stress resistance, wing size, wing shape and development time. *Journal of Evolutionary Biology*, **18**, 213-222.

Gutiérrez-Pesquera, L.M., Tejedo, M., Olalla-Tárraga, M.Á., Duarte, H., Nicieza, A. & Solé, M. (2016) Testing the climate variability hypothesis in thermal tolerance limits of tropical and temperate tadpoles. *Journal of Biogeography*, n/a-n/a.

Haffer, J. (1979) Quaternary biogeography of tropical lowland South America. *The South American herpetofauna: its origin, evolution and dispersal* (ed. by W.E. Duellman), pp. 107-140. Museum of Natural History, University of Kansas.

Hamilton, W.J., III (1973) *Life’s color code*. McGraw-Hill, New York.

Hannah, L., Flint, L., Syphard, A.D., Moritz, M.A., Buckley, L.B. & McCullough, I.M. (2014) Fine-grain modeling of species’ response to climate change: holdouts, stepping-stones, and microrefugia. *Trends in Ecology & Evolution*, **29**, 390-397.

Harmon, L.J., Weir, J.T., Brock, C.D., Glor, R.E. & Challenger, W. (2008) GEIGER: investigating evolutionary radiations. *Bioinformatics*, **24**, 129-31.

Harris, R.N. (1999) The anuran tadpole: evolution and maintenance. *Tadpoles: the biology of anuran larvae* (ed. by R.W. Mcdiarmid and R. Altig), pp. 279-294. University of Chicago Press, Chicago.

Hawkins, B.A. (2001) Ecology's oldest pattern? *Trends in Ecology & Evolution*, **16**, 470.

Hawkins, B.A. & Diniz-Filho, J.A.F. (2004) ‘Latitude’ and geographic patterns in species richness. *Ecography*, **27**, 268-272.

Hawkins, B.A., Field, R., Cornell, H.V., Currie, D.J., Guégan, J.-F., Kaufman, D.M., Kerr, J.T., Mittelbach, G.G., Oberdorff, T., O'Brien, E.M., Porter, E.E. & Turner, J.R.G. (2003) Energy, water, and broad-scale geographic patterns of species richness. *Ecology*, **84**, 3105-3117.

Hazzi, N.A., Moreno, J.S., Ortiz-Movliav, C. & Palacio, R.D. (2018) Biogeographic regions and events of isolation and diversification of the endemic biota of the tropical Andes. *Proceedings of the National Academy of Sciences*, Hedges, S.B., Duellman, W.E. & Heinicke, M.P. (2008) New World direct-developing frogs (Anura: Terrarana): Molecular phylogeny, classification, biogeography, and conservation. *Zootaxa*, **1737**, 1-182.

Helmuth, B., Russell, B.D., Connell, S.D., Dong, Y., Harley, C.D., Lima, F.P., Sará, G., Williams, G.A. & Mieszkowska, N. (2014) Beyond long-term averages: making biological sense of a rapidly changing world. *Climate Change Responses*, **1**, 6.

Hertz, P.E., Huey, R.B. & Nevo, E. (1983) Homage to Santa Anita: Thermal sensitivity of sprint speed in agamid lizards. *Evolution*, **37**, 1075-1084.

Hijmans, R.J. (2017a) raster: Geographic Data Analysis and Modeling. In, https://CRAN.R-project.org/package=raster.

Hijmans, R.J. (2017b) geosphere: Spherical Trigonometry. In, https://CRAN.R-project.org/package=geosphere.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965-1978.

Hillman, S.S., Withers, P.C., Drewes, R.C. & Hillyard, S.D. (2009) *Ecological and environmental physiology of amphibians*. Oxford University Press, Oxford, UK.

Hof, C., Araujo, M.B., Jetz, W. & Rahbek, C. (2011) Additive threats from pathogens, climate and land-use change for global amphibian diversity. *Nature*, **480**, 516-519.

Hoffmann, A.A., Chown, S.L. & Clusella-Trullas, S. (2013) Upper thermal limits in terrestrial ectotherms: how constrained are they? *Functional Ecology*, **27**, 934-949.

Hoffmann, M., Hilton-Taylor, C., Angulo, A., Böhm, M., Brooks, T.M., Butchart, S.H.M., Carpenter, K.E., Chanson, J., Collen, B., Cox, N.A., Darwall, W.R.T., Dulvy, N.K., Harrison, L.R., Katariya, V., Pollock, C.M., Quader, S., Richman, N.I., Rodrigues, A.S.L., Tognelli, M.F., Vié, J.-C., Aguiar, J.M., Allen, D.J., Allen, G.R., Amori, G., Ananjeva, N.B., Andreone, F., Andrew, P., Ortiz, A.L.A., Baillie, J.E.M., Baldi, R., Bell, B.D., Biju, S.D., Bird, J.P., Black-Decima, P., Blanc, J.J., Bolaños, F., Bolivar-G., W., Burfield, I.J., Burton, J.A., Capper, D.R., Castro, F., Catullo, G., Cavanagh, R.D., Channing, A., Chao, N.L., Chenery, A.M., Chiozza, F., Clausnitzer, V., Collar, N.J., Collett, L.C., Collette, B.B., Fernandez, C.F.C., Craig, M.T., Crosby, M.J., Cumberlidge, N., Cuttelod, A., Derocher, A.E., Diesmos, A.C., Donaldson, J.S., Duckworth, J.W., Dutson, G., Dutta, S.K., Emslie, R.H., Farjon, A., Fowler, S., Freyhof, J., Garshelis, D.L., Gerlach, J., Gower, D.J., Grant, T.D., Hammerson, G.A., Harris, R.B., Heaney, L.R., Hedges, S.B., Hero, J.-M., Hughes, B., Hussain, S.A., Icochea M., J., Inger, R.F., Ishii, N., Iskandar, D.T., Jenkins, R.K.B., Kaneko, Y., Kottelat, M., Kovacs, K.M., Kuzmin, S.L., La Marca, E., Lamoreux, J.F., Lau, M.W.N., Lavilla, E.O., Leus, K., Lewison, R.L., Lichtenstein, G., Livingstone, S.R., Lukoschek, V., Mallon, D.P., McGowan, P.J.K., McIvor, A., Moehlman, P.D., Molur, S., Alonso, A.M., Musick, J.A., Nowell, K., Nussbaum, R.A., Olech, W., Orlov, N.L., Papenfuss, T.J., Parra-Olea, G., Perrin, W.F., Polidoro, B.A., Pourkazemi, M., Racey, P.A., Ragle, J.S., Ram, M., Rathbun, G., Reynolds, R.P., Rhodin, A.G.J., Richards, S.J., Rodríguez, L.O., Ron, S.R., Rondinini, C., Rylands, A.B., Sadovy de Mitcheson, Y., Sanciangco, J.C., Sanders, K.L., Santos-Barrera, G., Schipper, J., Self-Sullivan, C., Shi, Y., Shoemaker, A., Short, F.T., Sillero-Zubiri, C., Silvano, D.L., Smith, K.G., Smith, A.T., Snoeks, J., Stattersfield, A.J., Symes, A.J., Taber, A.B., Talukdar, B.K., Temple, H.J., Timmins, R., Tobias, J.A., Tsytsulina, K., Tweddle, D., Ubeda, C., Valenti, S.V., Paul van Dijk, P., Veiga, L.M., Veloso, A., Wege, D.C., Wilkinson, M., Williamson, E.A., Xie, F., Young, B.E., Akçakaya, H.R., Bennun, L., Blackburn, T.M., Boitani, L., Dublin, H.T., da Fonseca, G.A.B., Gascon, C., Lacher, T.E., Mace, G.M., Mainka, S.A., McNeely, J.A., Mittermeier, R.A., Reid, G.M., Rodriguez, J.P., Rosenberg, A.A., Samways, M.J., Smart, J., Stein, B.A. & Stuart, S.N. (2010) The Impact of Conservation on the Status of the World's Vertebrates. *Science*, **330**, 1503-1509.

Hooghiemstra, H., Wijninga, V.M. & Cleef, A.M. (2006) The paleobotanical record of Colombia: Implications for biogeography and biodiversity. *Annals of the Missouri Botanical Garden*, **93**, 297-325.

Hoorn, C., Wesselingh, F.P., ter Steege, H., Bermudez, M.A., Mora, A., Sevink, J., Sanmartín, I., Sanchez-Meseguer, A., Anderson, C.L., Figueiredo, J.P., Jaramillo, C., Riff, D., Negri, F.R., Hooghiemstra, H., Lundberg, J., Stadler, T., Särkinen, T. & Antonelli, A. (2010) Amazonia Through Time: Andean Uplift, Climate Change, Landscape Evolution, and Biodiversity. *Science*, **330**, 927-931.

Hopkins, W.A. (2007) Amphibians as Models for Studying Environmental Change. *ILAR Journal*, **48**, 270-277.

Hua, X. & Wiens, J.J. (2010) Latitudinal variation in speciation mechanisms in frogs. *Evolution*, **64**, 429-443.

Huey, Raymond B. (1982) Temperature, physiology, and the ecology of reptiles. *Biology of the Reptilia* (ed. by C. Gans and F.H. Pough), pp. 25-74. Academic Press, New York.

Huey, R.B. (2010) Evolutionary physiology of insect thermal adaptation to cold environments. *Low temperature biology of insects* (ed. by D.L. Denlinger and R.E. Lee), pp. 223-241. Cambridge University Press, Cambridge, UK.

Huey, R.B. & Stevenson, R.D. (1979) Integrating Thermal Physiology and Ecology of Ectotherms: A Discussion of Approaches. *American Zoologist*, **19**, 357-366.

Huey, R.B. & Hertz, P.E. (1984) Is a jack-of-all-temperatures a master of none? *Evolution*, **38**, 441-444.

Huey, R.B. & Kingsolver, J.G. (1989) Evolution of thermal sensitivity of ectotherm performance. *Trends in Ecology & Evolution*, **4**, 131-135.

Huey, R.B. & Kingsolver, J.G. (1993) Evolution of Resistance to High Temperature in Ectotherms. *The American Naturalist*, **142**, S21-S46.

Huey, R.B., Hertz, P.E. & Sinervo, B. (2003) Behavioral drive versus behavioral inertia in evolution: a null model approach. *Am Nat*, **161**, 357-66.

Huey, R.B., Kearney, M.R., Krockenberger, A., Holtum, J.A.M., Jess, M. & Williams, S.E. (2012) Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **367**, 1665-1679.

Huey, R.B., Deutsch, C.A., Tewksbury, J.J., Vitt, L.J., Hertz, P.E., Álvarez Pérez, H.J. & Garland, T. (2009) Why tropical forest lizards are vulnerable to climate warming. *Proceedings of the Royal Society B: Biological Sciences*,

Hutchinson, G.E. (1957) Concluding Remarks. *Cold Spring Harbor Symposia on Quantitative Biology*, **22**, 415-427.

Hutchison, V.H. (1961) Critical Thermal Maxima in Salamanders. *Physiological Zoology*, **34**, 92-125.

Hutchison, V.H. & Hill, L.G. (1978) Thermal selection of bullfrog tadpoles (rana catesbeiana) at different stages of development and acclimation tempeatures. *Journal of Thermal Biology*, **3**, 57-60.

Hutter, C.R., Guayasamin, J.M. & Wiens, J.J. (2013) Explaining Andean megadiversity: the evolutionary and ecological causes of glassfrog elevational richness patterns. *Ecology Letters*, **16**, 1135-1144.

Izem, R. & Kingsolver, Joel G. (2005) Variation in Continuous Reaction Norms: Quantifying Directions of Biological Interest. *The American Naturalist*, **166**, 277-289.

Jankowski, J.E., Londoño, G.A., Robinson, S.K. & Chappell, M.A. (2013) Exploring the role of physiology and biotic interactions in determining elevational ranges of tropical animals. *Ecography*, **36**, 1-12.

Janzen, D.H. (1967) Why mountain passes are higher in the tropics. *American Naturalist*, **101**, 233-247.

Jetz, W. & Pyron, R.A. (2018) The interplay of past diversification and evolutionary isolation with present imperilment across the amphibian tree of life. *Nature Ecology & Evolution*, **2**, 850-858.

John-Alder, H.B., Morin, P.J. & Lawler, S. (1988) Thermal Physiology, Phenology, and Distribution of Tree Frogs. *The American Naturalist*, **132**, 506-520.

Jose, S., Gillespie, A.R., George, S.J. & Kumar, B.M. (1996) Vegetation responses along edge-to-interior gradients in a high altitude tropical forest in peninsular India. *Forest Ecology and Management*, **87**, 51-62.

Kaspari, M., Clay, N.A., Lucas, J., Yanoviak, S.P. & Kay, A. (2015) Thermal adaptation generates a diversity of thermal limits in a rainforest ant community. *Global Change Biology*, **21**, 1092-1102.

Kaspari, M., Clay, N.A., Lucas, J., Revzen, S., Kay, A. & Yanoviak, S.P. (2016) Thermal adaptation and phosphorus shape thermal performance in an assemblage of rainforest ants. *Ecology*, n/a-n/a.

Katoh, K. & Standley, D.M. (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution*, **30**, 772-780.

Kattan, G.H., Franco, P., Rojas, V. & Morales, G. (2004) Biological diversification in a complex region: a spatial analysis of faunistic diversity and biogeography of the Andes of Colombia. *Journal of Biogeography*, **31**, 1829-1839.

Katzenberger, M., Hammond, J., Tejedo, M. & Relyea, R. (2018) Source of environmental data and warming tolerance estimation in six species of North American larval anurans. *Journal of Thermal Biology*, **76**, 171-178.

Kearney, M. & Porter, W.P. (2004) Mapping the fundamental niche: Physiology, climate, and the distribution of a nocturnal lizard. *Ecology*, **85**, 3119-3131.

Kearney, M. & Porter, W. (2009) Mechanistic niche modelling: combining physiological and spatial data to predict species' ranges. *Ecology Letters*, **12**, 334-350.

Kearney, M., Shine, R. & Porter, W.P. (2009) The potential for behavioral thermoregulation to buffer "cold-blooded" animals against climate warming. *Proceedings of the National Academy of Sciences*, **106**, 3835-3840.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. & Drummond, A. (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, **28**, 1647-1649.

Kellermann, V., Overgaard, J., Hoffmann, A.A., Fløjgaard, C., Svenning, J.-C. & Loeschcke, V. (2012a) Upper thermal limits of Drosophila are linked to species distributions and strongly constrained phylogenetically. *Proceedings of the National Academy of Sciences*, **109**, 16228-16233.

Kellermann, V., Loeschcke, V., Hoffmann, A.A., Kristensen, T.N., Fløjgaard, C., David, J.R., Svenning, J.-C. & Overgaard, J. (2012b) Phylogenetic constraints in key functional traits behind species' climate niches: patterns of desiccation and cold resistance across 95 Drosophila species. *Evolution*, **66**, 3377-3389.

Kingsolver, J.G. & Woods, H.A. (2016) Beyond Thermal Performance Curves: Modeling Time-Dependent Effects of Thermal Stress on Ectotherm Growth Rates. *The American Naturalist*, **187**, 283-294.

Kingsolver, J.G., Woods, H.A., Buckley, L.B., Potter, K.A., MacLean, H.J. & Higgins, J.K. (2011) Complex life cycles and the responses of insects to climate change. *Integr Comp Biol*, **51**, 719-32.

Klok, C.J. & Chown, S.L. (2003) Resistance to temperature extremes in sub-Antarctic weevils: interspecific variation, population differentiation and acclimation. *Biological Journal of the Linnean Society*, **78**, 401-414.

Knies, Jennifer L., Kingsolver, Joel G. & Burch, Christina L. (2009) Hotter Is Better and Broader: Thermal Sensitivity of Fitness in a Population of Bacteriophages. *The American Naturalist*, **173**, 419-430.

Körner, C. (2007) The use of 'altitude' in ecological research. *Trends in Ecology & Evolution*, **22**, 569-574.

Krenek, S., Petzoldt, T. & Berendonk, T.U. (2012) Coping with Temperature at the Warm Edge – Patterns of Thermal Adaptation in the Microbial Eukaryote Paramecium caudatum. *PLoS ONE*, **7**, e30598.

La Sorte, F.A. & Jetz, W. (2010) Projected range contractions of montane biodiversity under global warming. *Proceedings of the Royal Society B: Biological Sciences*,

Laurila, A., Lindgren, B. & Laugen, A.T. (2008) Antipredator defenses along a latitudinal gradient in *Rana temporaria*. *Ecology*, **89**, 1399-1413.

Lawler, J.J., Shafer, S.L., White, D., Kareiva, P., Maurer, E.P., Blaustein, A.R. & Bartlein, P.J. (2009) Projected climate-induced faunal change in the Western Hemisphere. *Ecology*, **90**, 588-597.

Lenoir, J. & Svenning, J.-C. (2013) Latitudinal and Elevational Range Shifts under Contemporary Climate Change. *Encyclopedia of Biodiversity* (ed. by S.A. Levin), pp. 599-611.

Lindgren, B. & Laurila, A. (2009) Physiological variation along a geographical gradient: is growth rate correlated with routine metabolic rate in Rana temporaria tadpoles? *Biological Journal of the Linnean Society*, **98**, 217-224.

Loarie, S.R., Carter, B.E., Hayhoe, K., McMahon, S., Moe, R., Knight, C.A. & Ackerly, D.D. (2008) Climate Change and the Future of California's Endemic Flora. *PLoS ONE*, **3**, e2502.

Logan, M.L., Cox, R.M. & Calsbeek, R. (2014) Natural selection on thermal performance in a novel thermal environment. *Proceedings of the National Academy of Sciences*, **111**, 14165-14169.

Lomolino, M.V. (2001) Elevation gradients of species-density: historical and prospective views. *Global Ecology and Biogeography*, **10**, 3-13.

Lüddecke, H. & Sánchez, O.R. (2002) Are Tropical Highland Frog Calls Cold-adapted? The Case of the Andean Frog Hyla labialis. *Biotropica*, **34**, 281-288.

Lutterschmidt, W.I. & Hutchison, V.H. (1997a) The critical thermal maximum: data to support the onset of spasms as the definitive end point. *Canadian Journal of Zoology*, **75**, 1553-1560.

Lutterschmidt, W.I. & Hutchison, V.H. (1997b) The critical thermal maximum: history and critique. *Canadian Journal of Zoology*, **75**, 1561-1574.

Lynch, J.D. & Duellman, W.E. (1997) Frogs of the genus *Eleutherodactylus* (Leptodactylidae) in western Ecuador: systematics, ecology and biogeography. *Univ. Kans. Nat. His. Mus. Spec. Publ.*, **23**, 1-236.

Lynch, M. & Gabriel, W. (1987) Environmental Tolerance. *The American Naturalist*, **129**, 283-303.

MacArthur, R.H. (1969) Patterns of communities in the tropics. *Biological Journal of the Linnean Society*, **1**, 19-30.

Martin, P.R., Bonier, F., Moore, I.T. & Tewksbury, J.J. (2009) Latitudinal variation in the asynchrony of seasons: implications for higher rates of population differentiation and speciation in the tropics. *Ideas in Ecology and Evolution*, **2**

Martin, Tara L. & Huey, Raymond B. (2008) Why "Suboptimal" Is Optimal: Jensen's Inequality and Ectotherm Thermal Preferences. *The American Naturalist*, **171**, E102-E118.

McCain, C.M., and J. A. Grytnes (2010) Elevational gradients in species richness *Encyclopedia of life sciences* (ed. by R. Jansson), pp. 1-10. John Wiley & Sons Ltd, Chichester, U.K.

McMenamin, S.K., Hadly, E.A. & Wright, C.K. (2008) Climatic change and wetland desiccation cause amphibian decline in Yellowstone National Park. *Proceedings of the National Academy of Sciences*, **105**, 16988-16993.

Mendenhall, C.D., Karp, D.S., Meyer, C.F.J., Hadly, E.A. & Daily, G.C. (2014) Predicting biodiversity change and averting collapse in agricultural landscapes. *Nature*, **509**, 213.

Menéndez-Guerrero, P.A. & Graham, C.H. (2013) Evaluating multiple causes of amphibian declines of Ecuador using geographical quantitative analyses. *Ecography*, no-no.

Menke, M.E. & Claussen, D.L. (1982) Thermal acclimation and hardening in tadpoles of the bullfrog, Rana catesbeiana. *Journal of Thermal Biology*, **7**, 215-219.

Meza-Joya, F.L. & Torres, M. (2016) Spatial diversity patterns of Pristimantis frogs in the Tropical Andes. *Ecology and Evolution*, 10.1002/ece3.1968.

Muir, A.P., Biek, R., Thomas, R. & Mable, B.K. (2014) Local adaptation with high gene flow: temperature parameters drive adaptation to altitude in the common frog (Rana temporaria). *Molecular Ecology*, **23**, 561-574.

Munch, S.B. & Conover, D.O. (2003) Rapid growth results in increased susceptibility to predation in *Menidia menidia*. *Evolution*, **57**, 2119-2127.

Muñoz, M.M., Langham, G.M., Brandley, M.C., Rosauer, D.F., Williams, S.E. & Moritz, C. (2016) Basking behavior predicts the evolution of heat tolerance in Australian rainforest lizards. *Evolution*, **70**, 2537-2549.

Muñoz, M.M., Stimola, M.A., Algar, A.C., Conover, A., Rodriguez, A.J., Landestoy, M.A., Bakken, G.S. & Losos, J.B. (2014) Evolutionary stasis and lability in thermal physiology in a group of tropical lizards. *Proceedings of the Royal Society of London B: Biological Sciences*, **281**

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B. & Kent, J. (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853.

Navas, C.A. (1996a) Implications of microhabitat selection and patterns of activity on the thermal ecology of high elevation neotropical anurans. *Oecologia*, **108**, 617-626.

Navas, C.A. (1996b) Metabolic physiology, locomotor performance, and thermal niche breadth in neotropical anurans. *Physiological Zoology*, **69**, 1481-1501.

Navas, C.A. (1996c) Thermal Dependency of Field Locomotor and Vocal Performance of High-Elevation Anurans in the Tropical Andes. *Journal of Herpetology*, **30**, 478-487.

Navas, C.A. (1997) Thermal extremes at high elevations in the Andes: Physiological ecology of frogs. *Journal of Thermal Biology*, **22**, 467-477.

Navas, C.A. (2002) Herpetological diversity along Andean elevational gradients: links with physiological ecology and evolutionary physiology. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, **133**, 469-485.

Navas, C.A. (2006) Patterns of distribution of anurans in high Andean tropical elevations: Insights from integrating biogeography and evolutionary physiology. *Integrative and Comparative Biology*, **46**, 82-91.

Navas, C.A., Carvajalino-Fernández, J.M., Saboyá-Acosta, L.P., Rueda-Solano, L.A. & Carvajalino-Fernández, M.A. (2013) The body temperature of active amphibians along a tropical elevation gradient: patterns of mean and variance and inference from environmental data. *Functional Ecology*, **27**, 1145-1154.

Niehaus, A.C., Angilletta, M.J., Sears, M.W., Franklin, C.E. & Wilson, R.S. (2012) Predicting the physiological performance of ectotherms in fluctuating thermal environments. *The Journal of Experimental Biology*, **215**, 694-701.

Nilsson-Örtman, V., Stoks, R., De Block, M. & Johansson, F. (2013) Latitudinal patterns of phenology and age-specific thermal performance across six Coenagrion damselfly species. *Ecological Monographs*, **83**, 491-510.

Nowakowski, A.J., Watling, J.I., Whitfield, S.M., Todd, B.D., Kurz, D.J. & Donnelly, M.A. (2017) Tropical amphibians in shifting thermal landscapes under land-use and climate change. *Conservation Biology*, **31**, 96-105.

O'Meara, B.C., Ané, C., Sanderson, M.J. & Wainwright, P.C. (2006) Testing for different rates of continuous trait evolution using likelihood. *Evolution*, **60**, 922-933.

Olalla-Tárraga, M.Á., McInnes, L., Bini, L.M., Diniz-Filho, J.A.F., Fritz, S.A., Hawkins, B.A., Hortal, J., Orme, C.D.L., Rahbek, C., Rodríguez, M.Á. & Purvis, A. (2011) Climatic niche conservatism and the evolutionary dynamics in species range boundaries: global congruence across mammals and amphibians. *Journal of Biogeography*, **38**, 2237-2247.

Orme, D. (2013) Caper: Comparative Analyses of Phylogenetics and Evolution in R. R package (version 0.5.2). In, https://cran.r-project.org/web/packages/caper/index.html.

Overgaard, J., Kearney, M.R. & Hoffmann, A.A. (2014) Sensitivity to thermal extremes in Australian Drosophila implies similar impacts of climate change on the distribution of widespread and tropical species. *Global Change Biology*, **20**, 1738-1750.

Oyamaguchi, H.M., Vo, P., Grewal, K., Do, R., Erwin, E., Jeong, N., Tse, K., Chen, C., Miyake, M., Lin, A. & Gridi-Papp, M. (2018) Thermal sensitivity of a Neotropical amphibian (Engystomops pustulosus) and its vulnerability to climate change. *Biotropica*, **50**, 326-337.

Pacifici, M., Foden, W.B., Visconti, P., Watson, J.E.M., Butchart, S.H.M., Kovacs, K.M., Scheffers, B.R., Hole, D.G., Martin, T.G., Akçakaya, H.R., Corlett, R.T., Huntley, B., Bickford, D., Carr, J.A., Hoffmann, A.A., Midgley, G.F., Pearce-Kelly, P., Pearson, R.G., Williams, S.E., Willis, S.G., Young, B. & Rondinini, C. (2015) Assessing species vulnerability to climate change. *Nature Climate Change*, **5**, 215.

Páez-Vacas, M.I. (2016) *Mechanisms of population divergence along elevational gradients in the tropics*. Colorado State University,

Pagel, M. (1999) Inferring the historical patterns of biological evolution. *Nature*, **401**, 877-884.

Paradis, E., Claude, J. & Strimmer, K. (2004) APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics*, **20**, 289-290.

Parmesan, C. (2006) Ecological and Evolutionary Responses to Recent Climate Change. *Annual Review of Ecology, Evolution, and Systematics*, **37**, 637-669.

Payne, N.L. & Smith, J.A. (2017) An alternative explanation for global trends in thermal tolerance. *Ecology Letters*, **20**, 70-77.

Payne, N.L., Smith, J.A., van der Meulen, D.E., Taylor, M.D., Watanabe, Y.Y., Takahashi, A., Marzullo, T.A., Gray, C.A., Cadiou, G. & Suthers, I.M. (2016) Temperature dependence of fish performance in the wild: links with species biogeography and physiological thermal tolerance. *Functional Ecology*, **30**, 903-912.

Pearman, P.B. (1995) Effects of pond size and consequent predator density on two species of tadpoles. *Oecologia*, **102**, 1-8.

Pearson, R.G. & Dawson, T.P. (2003) Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecology and Biogeography*, **12**, 361-371.

Perez, T.M., Stroud, J.T. & Feeley, K.J. (2016) Thermal trouble in the tropics. *Science*, **351**, 1392-1393.

Pincebourde, S., Murdock, C.C., Vickers, M. & Sears, M.W. (2016) Fine-Scale Microclimatic Variation Can Shape the Responses of Organisms to Global Change in Both Natural and Urban Environments. *Integrative and Comparative Biology*, **56**, 45-61.

Pinheiro, J., Bates, D., DebRoy, S. & Sarkar, D. (2018) nlme: Linear and Nonlinear Mixed Effect Models. In, https://CRAN.R-project.org/package=nlme.

Pintanel, P., Gutiérrez-Pesquera, L.M., Merino-Viteri, A. & Tejedo, M. (2017) La rana que no sabía que la estaban hirviendo. *Nuestra Ciencia*, **19**, 24-27.

Potter, K.A., Woods, H.A. & Pincebourde, S. (2013) Microclimatic challenges in global change biology. *Global Change Biology*, **19**, 2932-2939.

Quintero, I. & Jetz, W. (2018) Global elevational diversity and diversification of birds. *Nature*, **555**, 246.

R Core Team (2014) R: A Language and Environment for Statistical Computing. In: http://www.R-project.org/. R Foundation for Statistical Computing, Vienna, Austria.

Rahbek, C. (2005) The role of spatial scale and the perception of large-scale species-richness patterns. *Ecology Letters*, **8**, 224-239.

Randin, C.F., Engler, R., Normand, S., Zappa, M., Zimmermann, N.E., Pearman, P.B., Vittoz, P., Thuiller, W. & Guisan, A. (2009) Climate change and plant distribution: local models predict high-elevation persistence. *Global Change Biology*, **15**, 1557-1569.

Rapp, J.M. & Silman, M.R. (2012) Diurnal, seasonal, and altitudinal trends in microclimate across a tropical montane cloud forest. *Climate Research*, **55**, 17-32.

Rapp, J.M., Silman, M.R., Clark, J.S., Girardin, C.A.J., Galiano, D. & Tito, R. (2012) Intra- and interspecific tree growth across a long altitudinal gradient in the Peruvian Andes. *Ecology*, **93**, 2061-2072.

Raxworthy, C.J., Pearson, R.G., Rabibisoa, N., Rakotondrazafy, A.M., Ramanamanjato, J.-B., Raselimanana, A.P., Wu, S., Nussbaum, R.A. & Stone, D.A. (2008) Extinction vulnerability of tropical montane endemism from warming and upslope displacement: a preliminary appraisal for the highest massif in Madagascar. *Global Change Biology*, **14**, 1703-1720.

Revell, L.J. (2012) phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, **3**, 217-223.

Revell, L.J., González-Valenzuela, L.E., Alfonso, A., Castellanos-García, L.A., Guarnizo, C.E., Crawford, A.J. & Silvestro, D. (2018) Comparing evolutionary rates between trees, clades and traits. *Methods in Ecology and Evolution*, **9**, 994-1005.

Rice, W.R. (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223-225.

Ricklefs, R.E. (2006) Global variation in the diversification rate of passerine birds. *Ecology*, **87**, 2468-2478.

Richter-Boix, A., Tejedo, M. & Rezende, E.L. (2011) Evolution and plasticity of anuran larval development in response to desiccation. A comparative analysis. *Ecology and Evolution*, **1**, 15-25.

Richter-Boix, A., Katzenberger, M., Duarte, H., Quintela, M., Tejedo, M. & Laurila, A. (2015) Local divergence of thermal reaction norms among amphibian populations is affected by pond temperature variation. *Evolution*, **69**, 2210-26.

Ron, S.R. (2018) *Base de datos de la colección de anfibios del Museo de Zoología QCAZ. Versión 1.0*. Available at: (accessed February 26, 2018

Ron, S.R., Guayasamin, J.M. & Menéndez-Guerrero, P. (2011) Biodiversity and conservation status of Ecuadorian Amphibians. *Amphibian Biology* (ed. by H. Heatwole, C.B. Barrio-Amorós and H.W. Wilkinson), pp. 129–170. Surrey Beatty & Soons PTY Limited, Baulkham Hills, Australia.

Ron, S.R., Yanez-Muñoz, M.H., Merino-Viteri, A. & Ortiz, D.A. (2018) *BIOWEB: Anfibios del Ecuador. Version 2018.0.* Available at: (accessed September 12, 2018

Ruel, J.J. & Ayres, M.P. (1999) Jensen's inequality predicts effects of environmental variation. *Trends in Ecology & Evolution*, **14**, 361-366.

Sánchez-Fernández, D., Aragón, P., Bilton, D.T. & Lobo, J.M. (2012) Assessing the Congruence of Thermal Niche Estimations Derived from Distribution and Physiological Data. A Test Using Diving Beetles. *PLoS ONE*, **7**, e48163.

Santos, J.C., Coloma, L.A., Summers, K., Caldwell, J.P., Ree, R. & Cannatella, D.C. (2009) Amazonian Amphibian Diversity Is Primarily Derived from Late Miocene Andean Lineages. *PLoS Biol*, **7**, e1000056.

Sarmiento, G. (1986) Ecological features of climate in high tropical mountains. *High altitude tropical biogeography* (ed. by F. Vuilleumier and M. Monasterio), pp. 11-46. Oxford University Press, New York.

Savage, Van M., Gillooly, James F., Brown, James H., West, Geoffrey B. & Charnov, Eric L. (2004) Effects of Body Size and Temperature on Population Growth. *The American Naturalist*, **163**, 429-441.

Sayol, F., Maspons, J., Lapiedra, O., Iwaniuk, A.N., Székely, T. & Sol, D. (2016) Environmental variation and the evolution of large brains in birds. *Nature Communications*, **7**, 13971.

Schemske, D.W., Mittelbach, G.G., Cornell, H.V., Sobel, J.M. & Roy, K. (2009) Is There a Latitudinal Gradient in the Importance of Biotic Interactions? *Annual Review of Ecology, Evolution, and Systematics*, **40**, 245-269.

Scholander, P.F., Flagg, W., Walters, V. & Irving, L. (1953) Climatic Adaptation in Arctic and Tropical Poikilotherms. *Physiological Zoology*, **26**, 67-92.

Schou, M.F., Mouridsen, M.B., Sørensen, J.G. & Loeschcke, V. (2016) Linear reaction norms of thermal limits in Drosophila: predictable plasticity in cold but not in heat tolerance. *Functional Ecology*, n/a-n/a.

Schweiger, O., Heikkinen, R.K., Harpke, A., Hickler, T., Klotz, S., Kudrna, O., Kühn, I., Pöyry, J. & Settele, J. (2012) Increasing range mismatching of interacting species under global change is related to their ecological characteristics. *Global Ecology and Biogeography*, **21**, 88-99.

Seimon, T.A., Seimon, A., Daszak, P., Halloy, S.R.P., Schloegel, L.M., Aguilar, C.A., Sowell, P., Hyatt, A.D., Konecky, B. & Simmons, J.E. (2007) Upward range extension of Andean anurans and chytridiomycosis to extreme elevations in response to tropical deglaciation. *Global Change Biology*, **13**, 288-299.

Shah, A.A., Gill, B.A., Encalada, A.C., Flecker, A.S., Funk, W.C., Guayasamin, J.M., Kondratieff, B.C., Poff, N.L., Thomas, S.A., Zamudio, K.R. & Ghalambor, C.K. (2017) Climate variability predicts thermal limits of aquatic insects across elevation and latitude. *Functional Ecology*, n/a-n/a.

Sheldon, K.S., Yang, S. & Tewksbury, J.J. (2011) Climate change and community disassembly: impacts of warming on tropical and temperate montane community structure. *Ecology Letters*, **14**, 1191-1200.

Sheldon, K.S., Huey, R.B., Kaspari, M. & Sanders, N.J. (2018) Fifty Years of Mountain Passes: A Perspective on Dan Janzen's Classic Article. *The American Naturalist*, **191**, 553-565.

Sinclair, B.J., Marshall, K.E., Sewell, M.A., Levesque, D.L., Willett, C.S., Slotsbo, S., Dong, Y., Harley, C.D.G., Marshall, D.J., Helmuth, B.S. & Huey, R.B. (2016) Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecology Letters*, **19**, 1372-1385.

Sinervo, B., Méndez-de-la-Cruz, F., Miles, D.B., Heulin, B., Bastiaans, E., Villagrán-Santa Cruz, M., Lara-Resendiz, R., Martínez-Méndez, N., Calderón-Espinosa, M.L., Meza-Lázaro, R.N., Gadsden, H., Avila, L.J., Morando, M., De la Riva, I.J., Sepulveda, P.V., Rocha, C.F.D., Ibargüengoytía, N., Puntriano, C.A., Massot, M., Lepetz, V., Oksanen, T.A., Chapple, D.G., Bauer, A.M., Branch, W.R., Clobert, J. & Sites, J.W. (2010) Erosion of Lizard Diversity by Climate Change and Altered Thermal Niches. *Science*, **328**, 894-899.

Skelly, D.K. & Freidenburg, L.K. (2001) Evolutionary Responses to Climate Change. *eLS*. John Wiley & Sons, Ltd.

Smith-Gill, S.J. & Berven, K.A. (1979) Predicting Amphibian Metamorphosis. *The American Naturalist*, **113**, 563-585.

Smith, M.A. & Green, D.M. (2005) Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography*, **28**, 110-128.

Smith, S.A., Stephens, P.R. & Wiens, J.J. (2005) Replicate patterns of species richness, historical biogeography, and phylogeny in Holartic treefrogs. *Evolution*, **59**, 2433-2450.

Snyder, G.K. & Weathers, W.W. (1975) Temperature Adaptations in Amphibians. *The American Naturalist*, **109**, 93-101.

Soberón, J. & Peterson, A.T. (2005) Interpretation of Models of Fundamental Ecological Niches and Species' Distributional Areas. *2005*, **2**

Sørensen, J.G., Norry, F.M., Scannapieco, A.C. & Loeschcke, V. (2005) Altitudinal variation for stress resistance traits and thermal adaptation in adult Drosophila buzzatii from the New World. *Journal of Evolutionary Biology*, **18**, 829-837.

Spotila, J.R., O'Connor, M.P. & Bakken, G.S. (1992) Biophysics of heat and mass transfer. *Environmental physiology of the amphibians* (ed. by M.E. Feder and W.W. Burggren), pp. 59-80. The University of Chicago Press, Chicago.

Stefansson, T.S., McDonald, B.A. & Willi, Y. (2013) Local adaptation and evolutionary potential along a temperature gradient in the fungal pathogen Rhynchosporium commune. *Evolutionary Applications*, **6**, 524-534.

Stevens, G.C. (1989) The Latitudinal Gradient in Geographical Range: How so Many Species Coexist in the Tropics. *The American Naturalist*, **133**, 240-256.

Stevens, G.C. (1992) The elevational gradient in altitudinal range: an extension of Rapoport's latitudinal rule to altitude. *The American Naturalist*, **140**, 893-911.

Stevenson, L.A., Alford, R.A., Bell, S.C., Roznik, E.A., Berger, L. & Pike, D.A. (2013) Variation in Thermal Performance of a Widespread Pathogen, the Amphibian Chytrid Fungus *Batrachochytrium dendrobatidis*. *PLoS ONE*, **8**, e73830.

Stuart, S.N., Hoffmann, M., Chanson, J.S., Cox, N.A., Berridge, R.J., Ramani, P. & Young, B.E. (2008) *Threatened Amphibians of the World*. Lynx Edicions, Barcelona, Spain.

Suggitt, A.J., Wilson, R.J., Isaac, N.J.B., Beale, C.M., Auffret, A.G., August, T., Bennie, J.J., Crick, H.Q.P., Duffield, S., Fox, R., Hopkins, J.J., Macgregor, N.A., Morecroft, M.D., Walker, K.J. & Maclean, I.M.D. (2018) Extinction risk from climate change is reduced by microclimatic buffering. *Nature Climate Change*, **8**, 713-717.

Sugiura, N. (1978) Further analysts of the data by akaike' s information criterion and the finite corrections. *Communications in Statistics - Theory and Methods*, **7**, 13-26.

Sunday, J.M., Bates, A.E. & Dulvy, N.K. (2011) Global analysis of thermal tolerance and latitude in ectotherms. *Proceedings of the Royal Society B: Biological Sciences*, **278**, 1823-1830.

Sunday, J.M., Bates, A.E. & Dulvy, N.K. (2012) Thermal tolerance and the global redistribution of animals. *Nature Clim. Change*, **2**, 686-690.

Sunday, J.M., Bates, A.E., Kearney, M.R., Colwell, R.K., Dulvy, N.K., Longino, J.T. & Huey, R.B. (2014) Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. *Proceedings of the National Academy of Sciences*, **111**, 5610-5615.

Tewksbury, J.J., Huey, R.B. & Deutsch, C.A. (2008) Putting the Heat on Tropical Animals. *Science*, **320**, 1296-1297.

Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C., Erasmus, B.F.N., de Siqueira, M.F., Grainger, A., Hannah, L., Hughes, L., Huntley, B., van Jaarsveld, A.S., Midgley, G.F., Miles, L., Ortega-Huerta, M.A., Townsend Peterson, A., Phillips, O.L. & Williams, S.E. (2004) Extinction risk from climate change. *Nature*, **427**, 145.

Thomas, G.H., Freckleton, R.P. & Székely, T. (2006) Comparative analyses of the influence of developmental mode on phenotypic diversification rates in shorebirds. *Proceedings of the Royal Society B: Biological Sciences*, **273**, 1619-1624.

Thomas, M.K., Kremer, C.T., Klausmeier, C.A. & Litchman, E. (2012) A Global Pattern of Thermal Adaptation in Marine Phytoplankton. *Science*, **338**, 1085-1088.

Torres, V., Hooghiemstra, H., Lourens, L. & Tzedakis, P.C. (2013) Astronomical tuning of long pollen records reveals the dynamic history of montane biomes and lake levels in the tropical high Andes during the Quaternary. *Quaternary Science Reviews*, **63**, 59-72.

Tracy, C.R. (1976) A Model of the Dynamic Exchanges of Water and Energy between a Terrestrial Amphibian and Its Environment. *Ecological Monographs*, **46**, 293-326.

Travis, J. (1980) Growth variation for larval specific growth rate in the frog *Hyla gratiosa*. *Growth*, **44**, 167-181.

Tuff, K.T., Tuff, T. & Davies, K.F. (2016) A framework for integrating thermal biology into fragmentation research. *Ecology Letters*, **19**, 361-374.

Tylianakis, J.M., Didham, R.K., Bascompte, J. & Wardle, D.A. (2008) Global change and species interactions in terrestrial ecosystems. *Ecology Letters*, **11**, 1351-1363.

van Berkum, F.H. (1986) Evolutionary patterns of the thermal sensitivity of sprint speed in *Anolis* lizards. *Evolution*, **40**, 594-604.

van Berkum, F.H. (1988) Latitudinal Patterns of the Thermal Sensitivity of Sprint Speed in Lizards. *The American Naturalist*, **132**, 327-343.

van der Hammen, T. (1974) The Pleistocene Changes of Vegetation and Climate in Tropical South America. *Journal of Biogeography*, **1**, 3-26.

Veloz, S.D., Williams, J.W., Blois, J.L., He, F., Otto-Bliesner, B. & Liu, Z. (2012) No-analog climates and shifting realized niches during the late quaternary: implications for 21st-century predictions by species distribution models. *Global Change Biology*, **18**, 1698-1713.

Visser, M.E. & Both, C. (2005) Shifts in phenology due to global climate change: the need for a yardstick. *Proceedings of the Royal Society B: Biological Sciences*, **272**, 2561-2569.

von May, R., Catenazzi, A., Corl, A., Santa-Cruz, R., Carnaval, A.C. & Moritz, C. (2017) Divergence of thermal physiological traits in terrestrial breeding frogs along a tropical elevational gradient. *Ecology and Evolution*, **7**, 3257–3267.

Wellborn, G.A., Skelly, D.K. & Werner, E.E. (1996) Mechanisms creating community structure across a freshwater habitat gradient. *Annual Review of Ecology and Systematics*, **27**, 337-363.

Wells, K.D. (2007) *The ecology and behavior of amphibians*. The University Chicago Press, Chicago, United States of America.

Whittaker, R.H., Levin, S.A. & Root, R.B. (1973) Niche, Habitat, and Ecotope. *The American Naturalist*, **107**, 321-338.

Wiens, J., xa, J, Graham, C., H, Moen, D., S, Smith, S., A, Reeder, T., W, Associate Editor: Michael, E.H. & Editor: Jonathan, B.L. (2006) Evolutionary and Ecological Causes of the Latitudinal Diversity Gradient in Hylid Frogs: Treefrog Trees Unearth the Roots of High Tropical Diversity. *The American Naturalist*, **168**, 579-596.

Wiens, J.J., Kuczynski, C.A., Duellman, W.E. & Reeder, T.W. (2007) Loss and re-evolution of complex life cycles in marsupial frogs: Does ancestral trait reconstruction mislead? *Evolution*, **61**, 1886-1899.

Williams, S.E., Shoo, L.P., Isaac, J.L., Hoffmann, A.A. & Langham, G. (2008) Towards an Integrated Framework for Assessing the Vulnerability of Species to Climate Change. *PLOS Biology*, **6**, e325.

Wisz, M.S., Pottier, J., Kissling, W.D., Pellissier, L., Lenoir, J., Damgaard, C.F., Dormann, C.F., Forchhammer, M.C., Grytnes, J.-A., Guisan, A., Heikkinen, R.K., Høye, T.T., Kühn, I., Luoto, M., Maiorano, L., Nilsson, M.-C., Normand, S., Öckinger, E., Schmidt, N.M., Termansen, M., Timmermann, A., Wardle, D.A., Aastrup, P. & Svenning, J.-C. (2013) The role of biotic interactions in shaping distributions and realised assemblages of species: implications for species distribution modelling. *Biological Reviews*, **88**, 15-30.

Woodin, S.A., Hilbish, T.J., Helmuth, B., Jones, S.J. & Wethey, D.S. (2013) Climate change, species distribution models, and physiological performance metrics: predicting when biogeographic models are likely to fail. *Ecology and Evolution*, **3**, 3334-3346.

Woods, H.A., Dillon, M.E. & Pincebourde, S. (2015) The roles of microclimatic diversity and of behavior in mediating the responses of ectotherms to climate change. *Journal of Thermal Biology*, **54**, 86-97.

Yamahira, K., Kawajiri, M., Takeshi, K. & Irie, T. (2007) Inter- and intrapopulation variation in thermal reaction norms for growth rate: Evolution of latitudinal compensation in ectotherms with a genetic constraint. *Evolution*, **61**, 1577-1589.

Zani, P.A., Swanson, S.E.T., Corbin, D., Cohnstaedt, L.W., Agotsch, M.D., Bradshaw, W.E. & Holzapfel, C.M. (2005) Geographic variation in tolerance of transient thermal stress in the mosquito *Wyeomyia smithii*. *Ecology*, **86**, 1206-1211.

Zuloaga, J. & Kerr, J.T. (2017) Over the top: do thermal barriers along elevation gradients limit biotic similarity? *Ecography*, **40**, 478-486.

Zwickl, D.J. (2006) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation,*. The University of Texas at Austin.

Supplementary Information for Chapter 1

Table S1.1: Summary of the geographical location (the coordinates are in decimal degrees) and microclimatic data for each datalogger: tmax (absolute maximum temperature); tmin (absolute minimum temperature); tmean (mean temperature. Additionally, macroclimatic measurements for the same coordinates where the loggers were located are summarized (WorlClim): TMAX (maximum of the average monthly maximum temperature); TMIN (minimum of the average monthly minimum temperature) and TMEAN (mean of the average monthly temperature).

Microclimate datalogger		Locality information			Datalogger (ºC)				WorldClim (ºC)		
	Alt (m)	Habitat	latitude	longitude	tmax	tmin	tmean	days	TMAX	TMIN	TMEAN
DUR_CACAO	23	Open	1.036	-78.620	37.38	21.47	28.50	116	29.7	20.1	24.7
BALZAR	32	Open	-1.170	-79.970	41.69	22.53	27.94	77	31.4	19.4	25.6
PASAJE	38	Forest	-3.319	-79.803	31.17	24.84	27.07	59	31.5	19.9	25
YAS	220	Forest	-0.674	-76.398	26.78	23.97	25.06	4	31	19.8	25.2
DUR_AEL	242	Forest	1.034	-78.624	25.22	22.53	23.85	35	29.7	20.1	24.7
DUR_TAXI	242	Open	1.034	-78.624	34.16	20.81	24.71	241	29.7	20.1	24.7
ZJ_RIVER	926	Forest	-1.357	-77.862	23.48	16.81	19.95	59	27.1	15.8	21.4
ZJ_DMIN	937	Open	-1.371	-77.854	28.85	20.33	22.43	47	27.1	15.8	21.3
PUYO_CAIMAN	1070	Forest	-1.412	-77.705	31.37	20.23	23.19	45	27.8	16.5	22.1
LLANG_PRIST1	1500	Forest	-2.096	-78.159	21.47	15.76	17.74	13	26.1	13.9	19.7
LLANG_BRO	1700	Forest	-2.096	-78.159	19.76	14.23	16.31	5	26.1	13.9	19.7
LLANG_HOJ	1700	Open	-2.096	-78.159	24.64	13.75	16.22	6	26.1	13.9	19.7
ZURUCUCHU	2800	Open	-2.843	-79.126	20.23	8.68	13.02	235	16.4	5.5	10.7
MOLINUCO	2830	Forest	-0.410	-78.407	18.05	3.58	11.61	510	18.8	5.8	12.4
ZULETA	2900	Open	0.200	-78.083	19.38	4.21	10.16	56	17.4	5.6	11.5
GUARANDA	2970	Open	-0.187	-78.464	25.32	7.88	16.22	123	19.6	6.4	13
VIRGEN	4130	Open	-0.322	-78.200	25.81	0.78	6.94	542	8.9	-0.7	3.7

Table S1.2: Summary of the physiological traits and geographical sampling location for the 24 populations (see Figure S1.1 and Table S1.8) ordered altitudinally. The coordinates of the sampling point are in decimal degrees. In habitat; GEN: Generalist-species or Open habitat exploiters and FOR: Forest restricted species (see Suppl. Material 1.1). For more information of the climate information used for each species see Table S1.1.

Specie	**Sample point**				**CTmin (ºC)**			**CTmax (ºC)**			**Weight (g)**			**Climate info**
	Habitat	**Alt (m)**	**Lon**	**Lat**	**X**	**SE**	**N**	**X**	**SE**	**N**	**X**	**SE**	**N**	
Craugastor_longirostris	GEN	60	-79.7683	-3.3286	6.00		1	40.70		1	2.87		1	DUR_CACAO
P._achatinus_POP_1	GEN	272	-79.2749	-0.0879	6.20		1	39.50		1	2.13	0.32	2	DUR_CACAO
P._latidiscus	FOR	296	-78.5730	1.0252	9.50		1	37.20		1	1.08	0.63	2	DUR_AEL
P._laticlavius_POP_1	FOR	296	-78.5730	1.0252	7.85	0.15	2	33.90	0.10	2	0.58	0.32	2	DUR_AEL
P._matidiktyo	GEN	940	-77.8646	-1.3548	5.80		1	38.00		1	0.93	0.16	2	ZJ_DMIN
P._incomptus	GEN	1318	-78.0522	-1.3619				34.55	0.35	2	0.42	0.18	2	PUYO_CAIMAN
P._quaquaversus	FOR	1318	-78.0522	-1.3619				34.70		1	0.55		1	LLANG_BRO
P._achatinus_POP_2	GEN	1323	-78.7811	0.3097	6.08	0.06	9	37.26	0.26	9	2.42	0.31	18	PUYO_CAIMAN
P._crenunguis	FOR	1323	-78.7811	0.3097	5.45	0.05	2	33.83	0.12	3	1.36	0.41	5	LLANG_PRIST1
P._crucifer	FOR	1323	-78.7811	0.3097	5.70		1				0.65		1	LLANG_PRIST1
P._parvillus	FOR	1323	-78.7811	0.3097	4.70		1				0.14		1	LLANG_BRO
P._trachyblepharis	FOR	1543	-78.9726	-4.1120	4.98	0.11	4	32.57	0.21	5	0.57	0.08	9	LLANG_BRO
P._laticlavius_POP_2	FOR	1608	-78.2384	0.9530	4.18	0.27	5	35.37	0.19	6	2.60	0.50	11	LLANG_BRO
P._bicantus_POP_1	FOR	1850	-78.2198	-2.0693	4.40	0.28	5	32.12	0.25	5	0.41	0.13	9	LLANG_BRO
P._bicantus_POP_2	FOR	1994	-77.9518	-0.4496	4.25	0.05	2	31.07	0.18	3	0.47	0.17	2	LLANG_BRO
P._actites	GEN	2258	-78.9987	-0.9713	4.90	0.60	2	33.25	0.25	2	1.14	0.25	3	LLANG_HOJ
P._unistrigatus_POP_1	GEN	2258	-78.9987	-0.9713	-1.84	0.11	10	34.66	0.17	8	1.57	0.19	18	LLANG_HOJ
P._vertebralis	FOR	2644	-78.9880	-0.9364	1.67	0.35	6	30.46	0.17	5	0.31	0.02	11	MOLINUCO
P._riveti	GEN	2800	-79.1266	-2.8431	-2.09	0.06	7	34.84	0.21	7	1.60	0.28	11	ZURUCUCHU
P._phoxocephalus	GEN	2820	-78.9899	-0.9618	0.35	0.15	2	31.00		1	0.28	0.03	3	ZURUCUCHU
P._unistrigatus_POP_2	GEN	2969	-78.4639	-0.1873	-1.92	0.24	3	36.43	0.15	3	2.29	0.39	6	GUARANDA
P._pycnodermis	GEN	3509	-78.5065	-2.1796	-2.05	0.05	2	34.18	0.10	3	1.35	0.83	5	VIRGEN
P._curtipes	GEN	4100	-78.2017	-0.3270	-3.00	0.00	3	34.45	0.05	2	3.22	1.80	5	VIRGEN
P._festae	GEN	4236	-78.1999	-0.3221	-3.00	0.00	3	32.80	0.98	4	0.61	0.12	6	VIRGEN

Table S1.3: Selection of the best evolutionary model and values of phylogenetic signal for the physiological traits. We selected the simplest model (i.e. BM) using the AICc criteria. According to Burnham & Anderson (2002) best models are those with a ΔAICc < 4.

Traits	K	P	λ	P	AICc			ΔAICc		
					OU	BM	EB	OU	BM	EB
Ctmax	0.9111	0.009	0.807	0.059	110.567	110.808	113.51	0	0.241	2.943
Ctmin	1.032	0.005	1	<0.001	120.465	118.021	120.722	2.444	0	2.701
TR	0.586	0.129	0.554	0.679						
Weight	0.547	0.224	0	1						

Table S1.4: Physiological traits and thermal stress risk in relation to environmental variables and trade-off between both thermal limits.

PGLS	Estimate ± SE	Slope ± SE	F value	p-value
CTmax ~ altitude (*N=22; R2=0.079; λ=0.584*)	36.615 ±1.4	-0.001 ±0.0004	2.797	0.11
CTmin ~ altitude (*N=22; R2=0.806; λ=0.736*)	9.198 ±1.05	-0.003 ±0.0003	88.24	**<0.001**
TR ~ altitude (*N=20; R2=0.491; λ=1*)	23.357 ±2.733	0.003 ±0.001	19.31	**<0.001**
CTmax ~ weight (*N=22; R2=0.358; λ=0.962*)	31.929 ±1.542	1.242 ±0.376	12.69	**0.004**
CTmin ~ weight (*N=22; R2=0.287; λ=0.987*)	6.117 ±2.498	-1.325 ±0.54	5.805	**0.026**
wt ~ altitude (*N=22; R2=0.015; λ=1*)	10.25 ±2.987	0.001 ±0.001	1.313	0.265
WT ~ altitude (*N=22; R2=0.67; λ=0.462*)	4.944 ±1.855	0.004 ±0.001	43.67	**<0.001**
ct ~ altitude (*N=22; R2=0.592; λ=0.397*)	-15.31 ±1.341	0.003 ±0.0005	31.42	**<0.001**
CT ~ altitude (*N=22; R2=0.453; λ=0.979*)	-7.896 ±1.996	0.002 ±0.0005	18.41	**<0.001**
CTmax ~ CTmin (*N=20; R2=-0.051; λ=0.860*)	34.563 ±1.77	0.043 ±0.167	0.077	0.785

Table S1.5: Physiological variables and warming and cooling tolerances (dependent variables) in relation to weight, elevation of the population sample point and habitat (open versus forest) in direct-developing frogs using a PGLS approach.

Physiological traits		Sum Sq	Df	Fvalue	Pr (>F)
1. CTmax	**Altitude**	60.079	1	48.365	**<0.001**
(R^2 =0.826, λ= 0)	**Habitat**	18.721	1	15.071	**0.001**
	Weight	30.436	1	24.501	**<0.001**
	Residuals	22.36	18		
2. CTmin	**Altitude**	262.833	1	104.123	**<0.001**
(R^2 =0.834, λ= 0.460)	**Habitat**	9.32	1	3.692	0.071
	Weight	2.155	1	0.8535	0.368
	Residuals	45.437	18		
3. TR	**Altitude**	34.711	1	8.79	**0.0091**
(R^2 =0.761, λ= 0)	**Habitat**	49.386	1	12.506	**0.0027**
	Weight	40.221	1	10.185	**0.0057**
	Residuals	63.185	16		

Table S1.6: PGLS results for CTmax in relation to elevation of the sample point, habitat and maximum microenvironmental temperature (tmax).

CTmax	Sum Sq	Df	F value	Pr (>|t|)
Altitude	7.193	1	3.08	0.096
Habitat	1.578	1	0.676	0.422
tmax	10.759	1	4.607	**0.0457**
Residuals	42.036	18		

Table S1.7: The coefficient of variation (CV) was obtained following the conversion of the temperature values to Kelvin (ºC + 273,15) to make all variables positive, so avoiding problems associated with a CV that includes zero or negative values.

		n	mean	SD	min	max	Range	CV	
CTmax	All	22	34.67	2.67	30.46	40.7	10.24	0.867	
	Forest	9	33.47	2.14	30.46	37.20	6.74	0.699	$F_{1,20}$ = 3.47, P=0.077
	Open	13	35.51	2.75	31.00	40.70	9.70	0.891	Levene’s $F_{1,20}$ = 0.71 , P= 0.409
CTmin	All	22	3.09	3.84	-3.00	9.50	12.50	1.392	
	Forest	10	5.27	2.14	1.67	9.50	7.83	0.768	$F_{1,20}$ = 7.72, P=0.012
	Open	12	1.29	4.08	-3.00	6.20	9.20	1.486	Levene’s $F_{1,20}$= 17.31, P=0.00048
Levene´s test	All sample	$F_{1,42}$ = 6,43, P=0.015							
	Forest	$F_{1,17}$ = 0.12, P=0.728							
	Open	$F_{1,23}$ = 8.45, P=0.008							
tmax	All	22	25.05	5.43	18.04	37.38	19.33	1.820	
	Forest	9	21.35	2.46	18.04	25.22	7.18	0.836	$F_{1,22}$ = 10.11, P=0.005
	Open	13	27.60	5.49	20.23	37.38	17.15	1.827	Levene’s $F_{1,20}$ = 5.18, P=0.034
tmin	All	22	13.32	7.12	0.78	22.52	21.74	2.486	
	Forest	10	15.44	5.23	3.58	22.52	18.95	1.811	$F_{1,20}$ = 1.68, P=0.210
	Open	12	11.55	8.18	0.78	21.47	20.69	2.875	Levene’s $F_{1,22}$= 5.28 P=0.032
Levene´s test	All sample	$F_{1,46}$ = 1.71, P=0.197							
	Forest	$F_{1,20}$ = 1.10, P=0.307							
	Open	$F_{1,23}$ = 3.54, P=0.072							
TMAX	All	22	22.23	6.40	8.90	31.40	22.50	2.166	
	Forest	9	25.30	3.58	17.90	29.20	11.30	1.201	$F_{1,20}$ = 4.00, P=0.059
	Open	13	20.11	7.15	8.90	31.40	22.50	2.438	Levene’s $F_{1,20}$ = 5.15, P=0.034

TMIN	All	22	10.33	6.77	-7.00	19.70	26.70	2.388	
	Forest	10	13.24	4.04	6.50	19.60	13.10	1.410	$F_{1,20}$ = 3,84, P=0.064
	Open	12	7.91	7.75	-7.00	19.70	26.70	2.757	Levene's $F_{1,20}$= 2.97, P=0.100
Levene´s test	All sample Forest Open	$F_{1,42}$ = 0.0001, P=0.988 $F_{1,17}$ = 0.087, P=0.771 $F_{1,23}$ = 0.0001, P=0.991							
Thermal range (CTmax-CTmin)	All	20	31.79	4.06	26.05	38.35	12.30	1.332	
	Forest	8	28.03	1.53	26.05	31.19	5.14	0.509	$F_{1,18}$ = 27.29, P=0.00006
	Open	12	34.30	3.13	28.35	38.35	10.00	**1.020**	Levene's $F_{1,18}$ = 6.7,1 P=0.018

		n	mean	SD	min	Max	Range	CV	
Absolute range (tmax-tmin)	All	20	11.84	7.14	2.70	25.02	22.32	2.504	
	Forest	8	5.98	3.67	2.70	14.47	11.77	1.315	$F_{1,18}$ = 16.09, P=0.00008
	Open	12	15.74	6.15	8.52	25.02	16.50	2.130	Levene's $F_{1,18}$ = 3.98, P=0.061
Levene´s test	All sample Forest Open	$F_{1,38}$ = 3.97, P=0.05 $F_{1,14}$ = 0.94, P=0.347 $F_{1,22}$ = 5.04, P=0.035							
ct	All	22	10.22	4.12	1.91	15.59	13.68	1.456	
	Forest	10	10.17	3.30	1.91	14.67	12.76	1.165	$F_{1,20}$ = 0.003, P=0.958
	Open	12	10.26	4.85	2.83	15.59	12.76	1.713	Levene's $F_{1,20}$= 4.192, P=0.054
wt	All	22	9.63	3.61	2.12	15.61	13.49	1.277	
	Forest	9	12.11	1.84	8.68	15.61	6.93	0.646	$F_{1,20}$ = 10.49, P=0.004
	Open	13	7.91	3.56	2.12	14.61	12.4910	1.268	Levene's $F_{1,20}$ = 3,96 , P= 0.060

Thermal risk (ct vs wt) Levene´s test	**All sample** **Forest** **Open**	**$F_{1,42}$ = 0.26, P=0.615 / $F_{1,42}$ = 0.05, P=0.817$F_{1,17}$ = 0.37, P=0.553 / $F_{1,17}$ = 0.37, P=0.553** **$F_{1,17}$ = 2.44, P=0.136 / $F_{1,17}$ = 0.37, P=0.553** **$F_{1,23}$ = 1.94, P=0.177 / $F_{1,23}$ = 1.95, P=0.175**							
CT	All	22	7.24	3.85	-4.00	13.70	17.70	1.372	
	Forest	10	7.97	2.23	4.83	11.75	6.92	0.793	$F_{1,20}$ = 0.66 P=0.425
	Open	12	6.62	4.83	-4.00	13.70	17.70	1.725	Levene's $F_{1,20}$= 3.13, P=0.092
WT	All	22	12.44	5.68	3.37	24.75	21.38	1.988	
	Forest	9	8.17	2.89	3.37	12.56	9.19	1.026	$F_{1,20}$ = 13.97, P=0.001
	Open	13	15.40	5.26	9.05	24.75	15.70	1.822	Levene's $F_{1,20}$ = 3.54 , P= 0.074
Thermal risk (CT vs WT) Levene´s test	All sample Forest Open	$F_{1,42}$ = 12.68, P=0.0009 / $F_{1,42}$ = 3.79, P=0.058 $F_{1,17}$ = 0.03, P=0.869 / $F_{1,17}$ = 0.37, P=0.551 $F_{1,23}$ = 18.82, P=0.0002 / $F_{1,23}$ = 0.28, P=0.603							

Table S1.8: Microenvironmental (datalogger) extreme temperatures and absolute range in relation to elevation.

Microenvironmental temperatures	**Estimate ± SE**	**Slope ± SE**	**F value**	**p-value**
tmax ~ altitude (*N=17; R^2=0.389*)	31.559 ±1.91	-0.003 ±0.001	11.2	**0.004**
tmin ~ altitude (*N=17; R^2=0.942*)	23.791 ±0.685	-0.006 ±0.0003	263	**<0.001**
ar ~ altitude (*N=17; R^2=0.224*)	7.768 ±1.989	0.002 ±0.001	5.63	0.31

Table S1.9: Localities and voucher information of the individuals sequenced in this study. QCAZ: Museo de Zoología de la Pontificia Universidad Católica de Ecuador. Quito. Ecuador. All species were collected in Ecuador. Pop: number of population sampled.

QCAZ	Species	Pop	lon	lat	Province	Locality
62884	*Pristimantis achatinus*	1	-79.2749	-0.0879	Sto Domingo	Hostería “Valle Hermoso“. cerca de una pequeña cascada.
62885	*Pristimantis achatinus*	1	-79.2749	-0.0879	Sto Domingo	Hostería “Valle Hermoso“. cerca de una pequeña cascada.
63227	*Pristimantis achatinus*	2	-78.7795	0.3084	Imbabura	Reserva Biológica Los Cedros
63237	*Pristimantis achatinus*	2	-78.7795	0.3084	Imbabura	Reserva Biológica Los Cedros
59202	*Pristimantis bicantus*	1	-78.2192	-2.0713	Morona Stgo	Parque Nacional Sangay. Sardinayacu. alrededores Laguna Cormorán
59211	*Pristimantis bicantus*	1	-78.2198	-2.0693	Morona Stgo	Parque Nacional Sangay. Sardinayacu. alrededores Laguna Cormorán
58592	*Pristimantis bicantus*	2	-77.9518	-0.4496	Napo	Sector San Isidro. aprox. 12 km SE Cuyuja. en la vía a Baeza.
58597	*Pristimantis bicantus*	2	-77.9518	-0.4496	Napo	Sector San Isidro. aprox. 12 km SE Cuyuja. en la vía a Baeza.
49701	*Pristimantis aff. curtipes*	-	-78.2019	-0.3336	Napo	Guamaní. en la Virgen por el carretero antiguo hacia Papallacta
49704	*Pristimantis aff. curtipes*	-	-78.2019	-0.3336	Napo	Guamaní. en la Virgen por el carretero antiguo hacia Papallacta
67550	*Pristimantis festae*	-	-78.1993	-0.3221	Pichincha	Páramo de Papallacta. Virgen
67551	*Pristimantis festae*	-	-78.1993	-0.3221	Pichincha	Páramo de Papallacta. Virgen
59041	*Pristimantis incomptus*	-	-78.0522	-1.3619	Pastaza	Parque Nacional Llanganates. Comunidad de Pumaloma
62608	*Pristimantis laticlavius*	1	-78.573	1.0252	Esmeraldas	Durango
62609	*Pristimantis laticlavius*	1	-78.573	1.0252	Esmeraldas	Durango
64666	*Pristimantis laticlavius*	2	-78.2384	0.953	Carchi	Chical; Esperanza; Río Pailón
64667	*Pristimantis laticlavius*	2	-78.2384	0.953	Carchi	Chical; Esperanza; Río Pailón
58429	*Pristimantis unistrigatus*	1	-78.988	-0.9364	Cotopaxi	Pilaló y alrededores
58474	*Pristimantis unistrigatus*	1	-78.9987	-0.9713	Cotopaxi	Pilaló y alrededores
67878	*Pristimantis unistrigatus*	2	-78.461	-0.1763	Pichincha	Parque Metropolitano Guanguiltagua
67879	*Pristimantis unistrigatus*	2	-78.461	-0.1763	Pichincha	Parque Metropolitano Guanguiltagua

Figure S1.1: Phylogenetic hypothesis for the populations analysed for Ecuadorian species in this work.

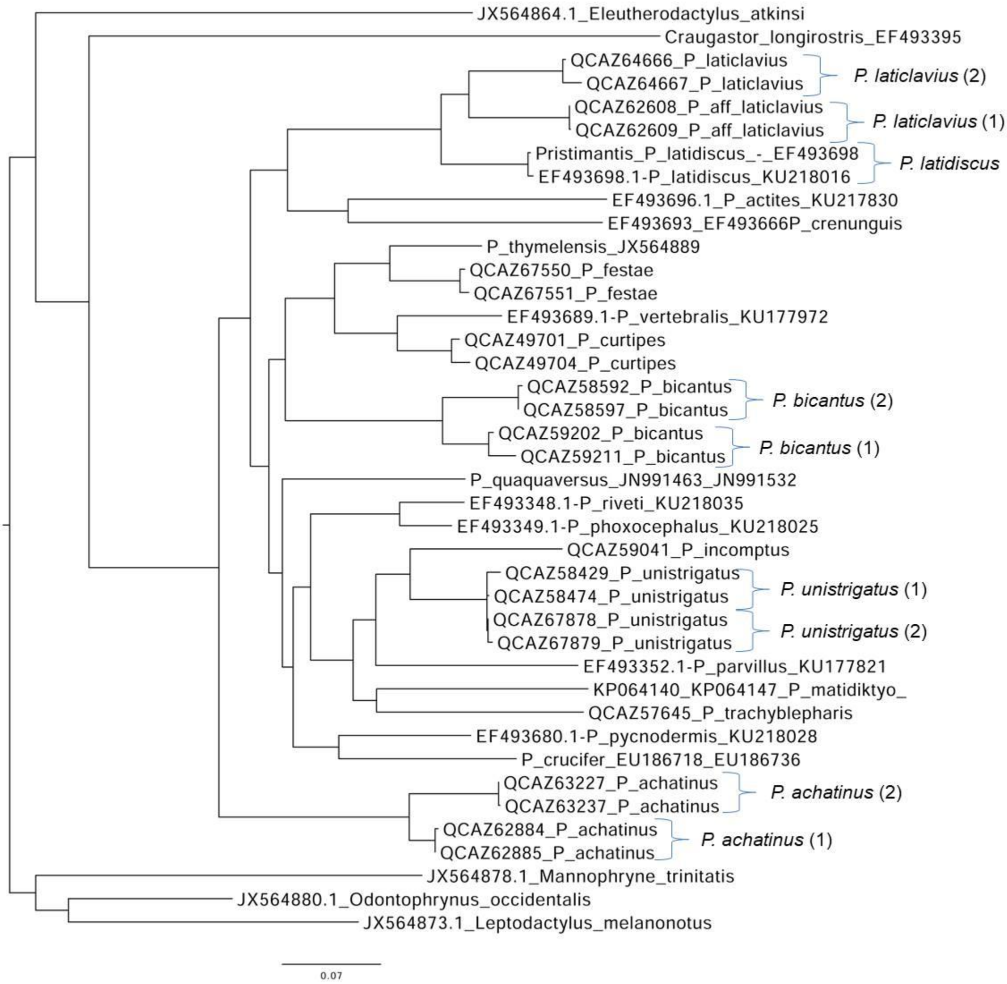


Figure S1.2: Phylogenetic generalized least squares for CTmax and CTmin with habitat (open versus forest) included in the model (see Table S1.4).

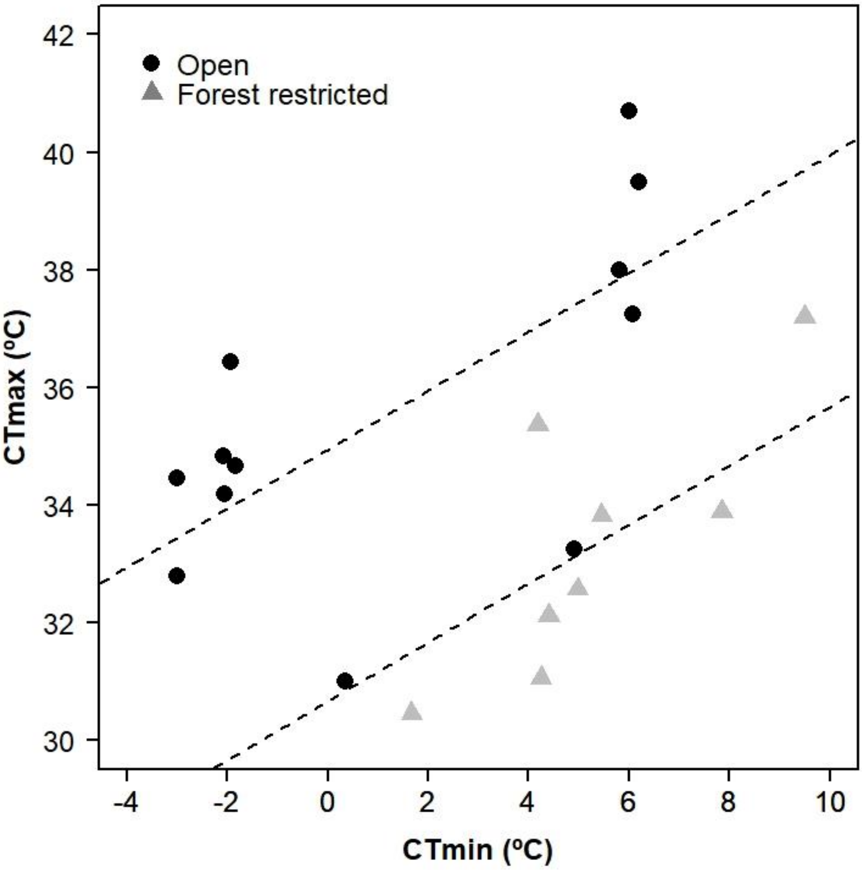


Fig. S1.3: Altitudinal variation of CTmin, tmin and TMIN.

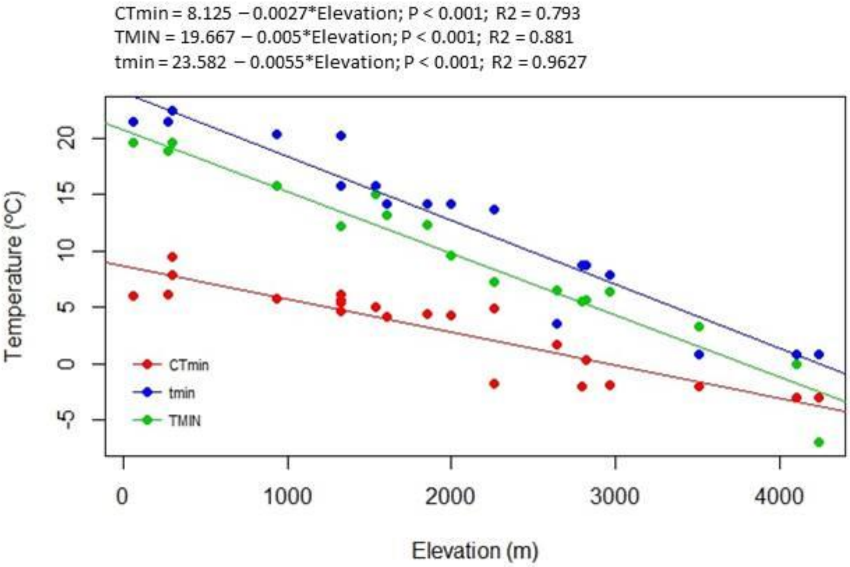
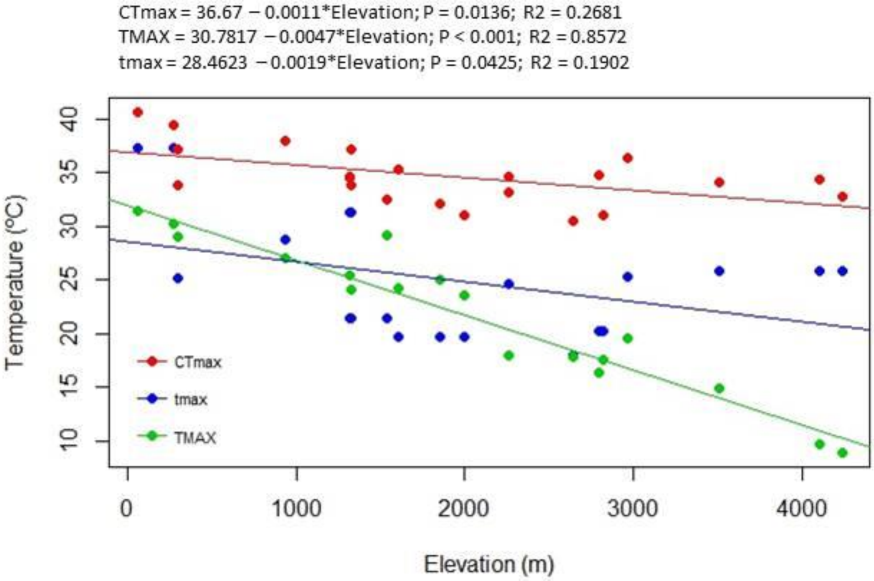


Fig. S1.4: Altitudinal variation of CTmax, tmax and TMAX.



Supplementary Material 1.1: Information for species grouped as forest-restricted species (FR) or habitat generalist species (HG) according to literature, field surveys and museums information.

Craugastor longirostris (HG)
Our individuals were found during daytime in cleared areas in banana plantations, but according to the literature it is usually found near rivers or forests, both secondary and primary and rarely on open areas (Lynch & Myers, 1983; Lynch & Duellman, 1997; MECN, 2010; Ortega-Andrade et al., 2010; Ron et al., 2017).

Pristimantis achatinus (HG)
Found in opened areas and secondary forests (Lynch & Burrowes, 1990; Lynch & Duellman, 1997; MECN, 2010; Ron et al., 2017).

Pristimantis actites (HG)
Rarely observed in the forest (Lynch & Duellman, 1997). Found on disturbed areas such as pastures and secondary forests (Lynch, 1979a; Stuart et al., 2008; Ron et al., 2017).

Pristimantis bicantus (FR)
Mostly found in primary forests although it has also been found in secondary forests (Guayasamín & Funk, 2009; Reyes-Puig et al., 2013; Brito et al., 2017; Ron et al., 2017).

Pristimantis crenunguis (FR)
Found in primary and secondary forests (Lynch & Duellman, 1997; Stuart et al., 2008; Ron et al., 2017).

Pristimantis crucifer (FR)
Found in forests and near rivers. Not known in perturbed areas (Lynch, 1976; Lynch & Duellman, 1997; Stuart et al., 2008; Ron et al., 2017).

Pristimantis curtipes (HG)
Our individuals were found under rocks during daytime in Papallacta's paramo and according to literature and QCAZ museum is generally found in pastures and prairie-like paramos (Lynch, 1981; Almendáriz & Orcés, 2004; Brito et al., 2017; Ron et al., 2017).

Pristimantis festae (HG)
Our individuals were found under rocks during daytime in Papallacta's paramo and according to literature and QCAZ museum is generally found in prairie-like paramos (Lynch & Duellman, 1980; Stuart et al., 2008; Ron et al., 2017).

Pristimantis incomptus (HG)
Found in forest clearings or margins of primary and secondary forests (Lynch & Duellman, 1980; Stuart et al., 2008; Ron et al., 2017).

Pristimantis laticlavius (FR)
Found in primary and secondary forests or near rivers (Lynch & Burrowes, 1990; Lynch & Duellman, 1997; MECN, 2009; Ron et al., 2017).

Pristimantis latidiscus (FR)
In undisturbed forests and forests margins but never found in opened areas (Lynch et al., 1994; Lynch & Duellman, 1997; MECN, 2009, 2010; Ron et al., 2017).

Pristimantis matidiktyo (HG)
Our individuals were found on leaves on scrubs near a road and according to literature individuals have been found both in forests and natural opened areas (Ortega-Andrade & Valencia, 2012; Ron et al., 2017).

Pristimantis parvillus (FR)
In primary and secondary forests and forests margins but never found in opened areas (Lynch & Duellman, 1997; MECN, 2010; Ron et al., 2017).

Pristimantis phoxocephalus (HG)
Found in subparamos and forests, generally found on forest margins and are scarce in the interior of the forests (Lynch, 1979b; Lynch & Duellman, 1997; Almendáriz & Orcés, 2004; MECN, 2009; Ron et al., 2017).

Pristimantis pycnodermis (HG)
Found in opened areas as paramos, meadows and pastures (Lynch, 1979b; Lynch & Duellman, 1980; Stuart et al., 2008; Ron et al., 2017).

Pristimantis quaquaversus (FR)
Found in primary and secondary forests (Duellman & Pramuk, 1999; Brito et al., 2017; Ron et al., 2017).

Pristimantis riveti (HG)
Our individuals were found in forest remnants and open pastures, it's also found in paramos and subparamos (Almendáriz & Orcés, 2004; Coloma et al., 2004; Stuart et al., 2008; Ron et al., 2017).

Pristimantis trachyblepharis (FR)
Found in primary and secondary forests (Lynch & Duellman, 1980; Brito et al., 2017; Ron et al., 2017).

Pristimantis unistrigatus (HG)
Found in open habitats as pastures and urban areas (Lynch & Duellman, 1980; Lynch, 1981; Lynch & Duellman, 1997; Almendáriz & Orcés, 2004; Ron et al., 2017).

Pristimantis vertebralis (FR)
Found in primary and secondary forests (Lynch & Duellman, 1997; Stuart et al., 2008; Ron et al., 2017). Lynch and Duellman (1997) suggest that can only be found in well conserved forests.

References for Supplementary Material 1.1

Almendáriz, A. & Orcés, G. (2004) Distribución de algunas especies de la herpetofauna de los pisos: altoandino, temperado y subtropical. *Politécnica*. 25. 97-150.

Brito, J., Batallas, D. & Yánez-Muñoz, M.H. (2017) Ranas terrestres Pristimantis (Anura: Craugastoridae) de los bosques montanos del río Upano. Ecuador: Lista anotada, patrones de diversidad y descripción de cuatro especies nuevas. *Neotropical Biodiversity*. 3. 125-156.

Burnham, K.P. & Anderson, D.R. (2002) *Model selection and multimode inference: a practical information theoretic approach*. Springer. New York.

Coloma, L., Ron, S., Yánez-Muñoz, M. & Almeida, D. (2004) Pristimantis riveti. *The IUCN Red List of Threatened Species*. 2004. e. T56919A11553288.

Duellman, W.E. & Pramuk, J.B. (1999) *Frogs of the genus Eleutherodactylus (Anura: Leptodactylidae) in the Andes of northern Peru / by William E. Duellman and Jennifer B. Pramuk*. Natural History Museum. University of Kansas. Lawrence. Kansas.

Guayasamín, J.M. & Funk, W.C. (2009) The amphibian community at Yanayacu Biological Station, Ecuador, with a comparison of vertical microhabitat use among Pristimantis species and the description of a new species of the Pristimantis myersi group. *Zootaxa*. 2220. 41-66.

Lynch, J.D. (1976) *New species of frogs (Leptodactylidae, Eleutherodactylus) from the Pacific versant of Ecuador.* Museum of Natural History. University of Kansas. Lawrence.

Lynch, J.D. (1979a) A New Frog Species of the Eleutherodactylus fitzingeri Group from the Pacific Andean Versant in Ecuador*. Herpetologica*. 35. 228-233.

Lynch, J.D. (1979b) The frogs of the genus Eleutherodactylus in the Andes of southern Ecuador. Misc. *Publ. Nat. Hist. Univ. Kansas*. 66. 1-62.

Lynch, J.D. (1981) *Leptodactylid frogs of the genus Eleutherodactylus in the Andes of northern Ecuador and adjacent Colombia*. University of Kansas. Lawrence.

Lynch, J.D. & Duellman, W.E. (1980) The Eleutherodactylus of the Amazonian slopes of the Ecuadorian Andes (Anura: Leptodactylidae). *The University of Kansas. Museum of Natural History Miscellaneous*. 69. 1-86.

Lynch, J.D. & Myers, C.W. (1983) Frogs of the fitzingeri group of Eleutherodactylus in eastern Panama and Chocoan South America (Leptodactylidae). *Bulletin of the American Museum of Natural History*: 481-572. 175. 481-572.

Lynch, J.D. & Burrowes, P.A. (1990) The frogs of the genus Eleutherodactylus (family Leptodactylidae) at the La Planada Reserve in southwestern Colombia with descriptions of eight new species. *Occasional papers of the Museum of Natural History. the University of Kansas*. 136. 1-31.

Lynch, J.D. & Duellman, W.E. (1997) Frogs of the genus Eleutherodactylus (Leptodactylidae) in western Ecuador: systematics, ecology and biogeography. *Univ. Kans. Nat. His. Mus. Spec. Publ.*. 23. 1-236.

Lynch, J.D., Ruíz-Carranza, P.M. & Ardila-Robayo, M.C. (1994) The identities of the Colombian frogs confused with Eleutherodactylus latidiscus (Boulenger) (Amphibia: Anura: Leptodactylidae). *Occasional papers of the Natural History Museum. the University of Kansas*, Lawrence, Kansas. 170. 1-42.

MECN (2009) *Guía de campo de los pequeños vertebrados del Distrito Metropolitano de Quito (DMQ)*. Publicación Miscelánea N° 5. Serie de Publicaciones del Museo Ecuatoriano de Ciencias Naturales (MECN) - Fondo Ambiental del MDMQ. Imprenta Nuevo Arte. Quito, Ecuador.

MECN (2010) *Serie Herpetofauna del Ecuador: El Choco Esmeraldeño*. Museo Ecuatoriano de Ciencias Naturales. Quito, Ecuador.

Ortega-Andrade, H., Aulestia, C., Bermingham, J. & Paucar, C. (2010) Herpetofauna of the Bilsa Biological Station, province of Esmeraldas, Ecuador. *Check List*. 6

Ortega-Andrade, H.M. & Valencia, J.H. (2012) A New Species of the Pristimantis frater Group (Anura: Strabomantidae) from the Eastern Evergreen Lowland Forests Of Ecuador. *Herpetologica*. 68. 244-255.

Reyes-Puig, M.M., Reyes-Puig, J.P. & Yánez-Muñoz, M.H. (2013) Ranas terrestres del género Pristimantis (Anura: Craugastoridae) de la Reserva Ecológica Río Zúñag, Tungurahua, Ecuador: Lista anotada y descripción de una especie nueva. *ACI Avances en Ciencias e Ingenierías*. 5

Ron, S.R., Yanez-Muñoz, M.H., Merino-Viteri, A., Ortiz, D.A. & Nicolalde, D.A. (2017) *AmphibiaWebEcuador. Version 2017.0.* Available at: http://zoologia.puce.edu.ec/Vertebrados/anfibios (accessed june 26, 2017).

Stuart, S.N., Hoffmann, M., Chanson, J.S., Cox, N.A., Berridge, R.J., Ramani, P. & Young, B.E. (2008) *Threatened Amphibians of the World*. Lynx Edicions. Barcelona, Spain.

Supplementary Information for Chapter 2

Table S2.1: Summary of the physiological traits, geographical sampling and thermal data of the location of the 75 studied species. The coordinates of the sample point are in decimal degrees, altitude in meters and temperature in Celcius.

			sample point		CTmin			CTmax			thermal data			
Species	**altitude**	**microenv**	**long**	**lat**	**X**	**SE**	**N**	**X**	**SE**	**N**	**tmax**	**tmin**	**tmean**	**dr**
Agalychnis hulli	937	POND	-77,85	-1,37	7,19	0,07	18	40,49	0,11	19	26,39	20,90	22,53	1,02
Agalychnis spurrelli	227	POND	-78,62	1,04	7,51	0,12	11	41,53	0,07	11	26,20	23,97	24,51	0,75
Allobates insperatus	672	POND	-77,74	-0,93	6,77	0,07	7	38,63	0,13	7	26,49	21,86	23,97	0,55
Allobates zaparo	672	POND	-77,74	-0,93	7,34	0,04	29	38,27	0,10	29	26,49	21,86	23,97	0,55
Ameerega sp.	1081	POND	-77,73	-1,41	7,55	0,45	2	38,90		1	26,98	19,38	21,82	0,52
Atelopus elegans	243	RIVER	-78,62	1,04	5,73	0,09	9	35,99	0,05	9	25,81	23,39	24,18	0,99
Atelopus spumarius gr. (limón)	840	RIVER	-78,43	-3,22	7,07	0,07	15	36,87	0,05	15				
Boana almendarizae	1223	POND	-78,06	-1,37	5,90	0,07	32	40,70	0,12	31	33,54	17,19	20,53	8,96
Boana boans	361	POND	-79,85	-3,69	5,43	0,08	16	41,29	0,05	16				
Boana cinerascens	949	POND	-77,86	-1,35	6,40	0,10	18	39,73	0,05	19	23,68	21,09	22,17	1,02
Boana fasciata	1217	POND	-78,94	-4,16	6,10	0,05	16	40,46	0,04	16	30,26	18,33	22,74	2,81
Boana geographica	672	POND	-77,74	-0,93	5,53	0,14	9	40,60	0,06	8	32,98	19,57	22,21	1,08
Boana lanciformis	949	POND	-77,86	-1,35	5,59	0,09	18	41,16	0,07	18	23,68	21,09	22,17	1,02
Boana pellucens	600	POND	-79,05	-0,29	7,22	0,07	16	42,87	0,10	16	32,70	18,43	23,79	4,21
Boana rosenbergi	23	POND	-78,75	1,17	7,44	0,13	8	44,07	0,06	9	36,84	24,64	28,51	6,38
Chiasmocleis ventrimaculata	937	POND	-77,85	-1,37	7,88	0,06	5	37,94	0,09	5	26,39	20,90	22,53	1,02
Chimerella mariaelenae	1540	RIVER	-78,19	-1,39	6,47	0,08	20	37,76	0,05	20	17,95	17,48	17,69	0,05
Cruziohyla calcarifer	242	POND	-78,62	1,03	6,72	0,09	11	40,82	0,08	13	26,20	23,97	24,51	0,75
Dendropsophus bifurcus	1223	POND	-78,06	-1,37	6,31	0,09	8	41,60	0,16	7	26,98	18,52	21,96	3,12
Dendropsophus bokermanni	937	POND	-77,85	-1,37	5,46	0,09	16	39,62	0,08	17	26,39	20,90	22,53	1,02
Dendropsophus brevifrons	1540	POND	-78,19	-1,39	5,45	0,15	4	39,43	0,10	4	17,95	17,48	17,69	0,05
Dendropsophus carnifex	1200	POND	-78,79	-0,04	4,31	0,21	18	39,97	0,03	18	30,15	18,81	22,01	3,58
Dendropsophus ebraccatus	227	POND	-78,62	1,04	7,51	0,12	11	41,53	0,07	11	26,20	23,97	24,51	0,75
Dendropsophus marmoratus	949	POND	-77,86	-1,35	6,01	0,15	7	42,71	0,08	7	23,68	21,09	22,17	1,02
Dendropsophus minutus	1326	POND	-78,05	-1,36	6,96	0,24	13	39,45	0,13	11	26,98	18,52	19,47	3,12

			sample point		CTmin			CTmax			thermal data			
Species	**altitude**	**microenv**	**long**	**lat**	**X**	**SE**	**N**	**X**	**SE**	**N**	**tmax**	**tmin**	**tmean**	**dr**
Dendropsophus parviceps	1081	POND	-77,73	-1,41	5,28	0,08	16	39,46	0,05	16	31,98	19,57	22,21	1,08
Dendropsophus sarayacuensis	1217	POND	-78,94	-4,16	5,07	0,08	16	40,51	0,04	14	30,26	18,33	22,74	2,81
Dendropsophus triangulum	672	POND	-77,74	-0,93	5,55	0,13	4	42,47	0,18	3	26,49	20,90	23,97	1,02
Engystomops coloradorum	600	POND	-79,05	-0,30	8,15	0,15	2	40,50		1	30,15	18,81	22,01	3,58
Engystomops guayaco	100	POND	-80,02	-2,21	8,00	0,31	8	39,68	0,07	16				
Engystomops petersi	949	POND	-77,86	-1,35	5,82	0,08	16	39,21	0,07	19	23,68	21,09	22,17	1,02
Engystomops cf. randi	47	POND	-79,97	-1,18	7,46	0,20	9	41,98	0,09	9	38,49	24,16	27,52	2,46
Epipedobates anthonyi	737	POND	-79,81	-3,77	7,14	0,11	17	38,41	0,05	16	29,35	21,57	24,89	2,21
Epipedobates boulengeri	227	POND	-78,62	1,04	7,90	0,09	17	38,76	0,10	18	26,20	23,97	24,51	0,75
Epipedobates darwinwallacei	1066	POND	-78,81	0,02	7,57	0,23	10	38,31	0,07	11	30,15	18,81	22,01	3,58
Epipedobates espinosai	600	POND	-79,05	-0,30	8,10	0,20	3	38,48	0,11	3	30,15	18,81	22,01	3,58
Epipedobates machalilla	50	POND	-80,07	-0,07	8,02	0,12	19	38,60	0,11	18	38,49	24,16	27,52	2,46
Epipedobates tricolor	1300	POND	-79,13	-1,42	7,29	0,08	16	38,26	0,07	16				
Espadarana callistomma	243	RIVER	-78,62	1,04	7,41	0,09	12	37,24	0,09	12	25,81	23,39	24,18	0,99
Espadarana prosoblepon	331	RIVER	-79,37	-2,57	7,41	0,12	16	37,09	0,06	15	25,81	23,39	24,18	0,99
Gastrotheca lateonota	3254	POND	-79,44	-4,71	-0,31	0,45	6	37,93	0,08	6				
Gastrotheca litonedis	2634	POND	-79,04	-2,99	-1,34	0,07	16	38,95	0,05	16	16,81	11,33	14,64	0,40
Gastrotheca lojana	2265	POND	-79,23	-3,61	0,07	0,09	6	39,53	0,11	7	17,00	16,14	16,44	0,70
Gastrotheca pseustes	3631	POND	-78,76	-1,34	-3,58	0,08	19	37,93	0,07	19	19,66	6,17	12,20	4,23
Gastrotheca riobambae	2969	POND	-78,46	-0,19	-1,03	0,10	16	38,61	0,09	16	22,43	7,88	14,78	4,55
Hyloscirtus alytolylax	1200	RIVER	-78,80	-0,07	5,76	0,09	17	37,51	0,05	18	20,90	19,19	19,92	0,43
Hyloscirtus lindae	2650	RIVER	-78,06	-0,39	1,51	0,08	15	36,54	0,08	18	15,38	11,33	12,70	1,21
Hyloscirtus phyllognathus gr.	926	RIVER	-77,86	-1,36	6,28	0,08	16	38,22	0,05	17	22,14	19,85	20,93	0,63
Hyloxalus bocagei	1820	POND	-77,60	-0,10	6,74	0,07	16	37,96	0,07	16	21,09	15,19	17,30	0,99
Hyloxalus elachyhistus	737	POND	-79,81	-3,77	5,64	0,10	9	37,14	0,07	13	23,39	14,13	16,66	2,40

			sample point		CTmin			CTmax			thermal data			
Species	**altitude**	**microenv**	**long**	**lat**	**X**	**SE**	**N**	**X**	**SE**	**N**	**tmax**	**tmin**	**tmean**	**dr**
Hyloxalus italoi	926	RIVER	-77,86	-1,36	7,33	0,20	4	37,90	0,26	3	22,14	19,85	20,93	0,63
Hyloxalus jacobuspetersi	2830	RIVER	-78,41	-0,41	0,30	0,15	3	32,83	0,07	3	13,46	7,28	12,46	0,93
Hyloxalus maculosus	1139	POND	-78,13	-1,45	7,40	0,08	22	37,78	0,08	23	21,19	18,24	19,25	0,52
Hyloxalus nexipus	391	POND	-77,81	-2,99	7,44	0,08	20	38,10	0,07	18				
Hyloxalus pulchellus	1900	POND	-77,90	-0,47	1,67	0,10	15	34,59	0,16	15	22,33	13,85	16,89	1,86
Hyloxalus toachi	242	POND	-78,62	1,03	7,30	0,06	6	38,03	0,12	10	26,20	23,97	24,51	0,75
Hyloxalus vertebralis	2500	POND	-78,99	-2,90	-0,73	0,09	13	35,72	0,04	14	19,57	17,28	18,31	0,46
Leptodactylus labrosus	38	POND	-79,80	-3,32	8,93	0,09	8	40,18	0,06	8	29,35	21,57	24,89	2,21
Leptodactylus rhodomystax	1326	POND	-78,05	-1,36	8,50		1	39,30		1	26,98	18,52	19,47	3,12
Leptodactylus ventrimaculatus	1066	POND	-78,81	0,02	8,45	0,12	15	39,39	0,09	16	32,39	18,14	23,79	7,11
Leptodactylus wagneri	1217	POND	-78,94	-4,16	9,46	0,09	17	39,17	0,07	17	30,26	18,33	22,74	2,81
Osteocephalus mutabor	1223	POND	-78,06	-1,37	7,85		1	39,28	0,17	2	26,98	18,52	21,96	3,12
Phyllomedusa cf.coelestis	937	POND	-77,85	-1,37	7,40	0,13	9	41,74	0,07	10	26,39	20,90	22,53	1,02
Phyllomedusa vaillantii	1081	POND	-77,73	-1,41	6,98	0,09	16	40,12	0,10	16	31,98	19,57	22,21	1,08
Rana bwana	31	POND	-79,96	-3,53	3,86	0,13	16	39,37	0,07	16	38,49	24,16	27,52	2,46
Rana vaillanti	32	POND	-79,97	-1,17	3,14	0,07	11	40,28	0,06	12	38,49	24,16	27,52	2,46
Rhinella horribilis	737	POND	-79,81	-3,77	5,21	0,11	16	42,47	0,05	16	32,50	18,43	21,93	4,21
Rhinella margaritifera	1638	POND	-78,20	-1,35	4,65	0,10	8	38,45	0,11	8	21,28	14,23	18,22	1,26
Rhinella marina	1081	POND	-77,73	-1,41	4,75	0,08	16	42,97	0,05	16	32,50	18,33	22,79	1,80
Scinax elaeochroa	1200	POND	-78,82	-0,03	6,28	0,12	18	40,71	0,08	13	32,39	18,14	23,79	7,11
Scinax garbei	949	POND	-77,86	-1,35	6,37	0,09	13	40,15	0,14	12	23,68	21,09	22,17	1,02
Scinax quinquefasciatus	23	POND	-78,75	1,17	7,87	0,15	10	41,57	0,14	11	36,84	24,64	28,51	6,38
Scinax ruber	672	POND	-77,74	-0,93	5,99	0,17	17	41,96	0,08	18	32,50	19,85	22,79	1,80
Smilisca phaeota	242	POND	-78,62	1,03	5,14	0,10	5	42,50	0,10	2	25,61	24,64	24,99	0,44
Trachycephalus cunauaru	207	POND	-75,89	-0,92	5,47	0,09	3	41,33	0,03	3	26,59	24,45	25,62	1,20

Table S2.2: Minimum (min) and maximum (max) elevation of the distribution of each species. Mid-elevation for each species was calculated as [min + (max-min)/2]. For the species with no data, we used field locality as mid-elevational distribution.

Species	Min	Reference (min)	Max	Reference (max)
Agalychnis hulli	270	(Ron et al., 2018)	950	(this study; Ron et al. 2018)
Agalychnis spurrelli	70	(Frost, 2018; Ron et al., 2018)	1999	(Frost, 2018)
Allobates insperatus	250	(IUCN, 2018; Ron et al., 2018)	570	(Ron et al., 2018)
Allobates zaparo	200	(Ron et al., 2018)	1000	(this study; Ron et al. 2018)
Ameerega sp.	NA	NA	NA	NA
Atelopus elegans	300	(Frost, 2018; IUCN, 2018; Ron et al., 2018)	1140	(Frost, 2018; IUCN, 2018; Ron et al., 2018)
Atelopus sp.(limon)	NA	NA	NA	NA
Chiasmocleis ventrimaculata	0	(Ron et al., 2018)	950	(this study)
Chimerella mariaelenae	1400	(Frost, 2018; IUCN, 2018; Ron et al., 2018)	1820	(Frost, 2018; IUCN, 2018; Ron et al., 2018)
Cruziohyla calcarifer	0	(Ron et al., 2018)	750	(IUCN, 2018)
Dendropsophus bifurcus	200	(IUCN, 2018; Ron et al., 2018)	1200	(IUCN, 2018; Ron et al., 2018)
Dendropsophus bokermanni	100	(IUCN, 2018)	950	(this study; but see IUCN, 2018)
Dendropsophus brevifrons	0	(Ron et al., 2018)	1300	(Ron et al., 2018)
Dendropsophus carnifex	1000	(IUCN, 2018)	2456	(Frost, 2018)
Dendropsophus ebraccatus	0	(this study; Frost, 2018; IUCN, 2018)	1600	(Frost, 2018; IUCN, 2018)
Dendropsophus marmoratus	0	(IUCN, 2018)	1000	(IUCN, 2018)
Dendropsophus minutus	0	(Ron et al., 2018)	1800	(IUCN, 2018; Ron et al., 2018)
Dendropsophus parviceps	186	(Ron et al., 2018)	1600	(Ron et al., 2018)
Dendropsophus sarayacuensis	0	(Ron et al., 2018)	1500	(Ron et al., 2018)
Dendropsophus triangulum	34	(Frost, 2018; Ron et al., 2018)	900	(this study; also see IUCN, 2018)
Engystomops coloradorum	100	(IUCN, 2018)	1000	(IUCN, 2018; Ron et al., 2018)
Engystomops guayaco	32	(IUCN, 2018)	92	(IUCN, 2018)
Engystomops petersi	89	(IUCN, 2018)	1200	(IUCN, 2018)
Engystomops cf. randi	NA	NA	NA	NA
Epipedobates anthonyi	153	(Frost, 2018; IUCN, 2018)	1800	(IUCN, 2018; Ron et al., 2018)
Epipedobates boulengeri	10	(Ron et al., 2018)	1500	(this study; Ron et al., 2018)
Epipedobates darwinwallacei	858	(Frost, 2018)	1719	(Frost, 2018)
Epipedobates espinosai	305	(Ron et al., 2018)	600	(this study; but see IUCN 2018)
Epipedobates machalilla	10	(Frost, 2018; IUCN, 2018; Ron et al., 2018)	515	(Frost, 2018; IUCN, 2018; Ron et al., 2018)
Epipedobates tricolor	750	(Ron et al., 2018)	1769	(Frost, 2018; IUCN, 2018; Ron et al., 2018)

Species	**Min**	**Reference (min)**	**Max**	**Reference (max)**
Espadarana callistomma	0	(aproximate; Ron et al., 2018)	800	(Ron et al., 2018)
Espadarana prosoblepon	0	(IUCN, 2018; Ron et al., 2018)	1500	(IUCN, 2018; but see Frost et al., 2018)
Gastrotheca lateonota	2770	(IUCN, 2018)	3260	(this study)
Gastrotheca litonedis	2630	(this study; but see Frost, 2018; Ron et al., 2018)	3600	(IUCN, 2018)
Gastrotheca lojana	1700	(Frost, 2018; Ron et al., 2018)	3020	(Frost, 2018; Ron et al., 2018)
Gastrotheca pseustes	2200	(Frost, 2018; IUCN)	4080	(Ron et al., 2018, see also Frost, 2018)
Gastrotheca riobambae	1800	(Ron et al., 2018)	3500	(IUCN, 2018)
Hyloscirtus alytolylax	500	(Frost, 2018)	2159	(Frost, 2018)
Hyloscirtus lindae	2000	(IUCN, 2018; Ron et al., 2018)	2650	(this study, also see Frost, 2018; IUCN, 2018)
Hyloscirtus phyllognathus gr.	NA	NA	NA	NA
Hyloxalus jacobuspetersi	1500	(Frost, 2018; IUCN, 2018; Ron et al., 2018)	3800	(Frost, 2018; IUCN, 2018; Ron et al., 2018)
Hyloxalus bocagei	1420	(Frost, 2018; Ron et al., 2018)	1750	(Frost, 2018; Ron et al., 2018)
Hyloxalus elachyhistus	600	(IUCN, 2018)	2000	(Ron et al., 2018)
Hyloxalus italoi	200	(Frost, 2018; Ron et al., 2018)	1000	(Frost, 2018; Ron et al., 2018)
Hyloxalus maculosus	460	(Frost, 2018; Ron et al., 2018)	1150	(Frost, 2018; Ron et al., 2018)
Hyloxalus nexipus	325	(IUCN, 2018)	1550	(Frost, 2018; IUCN, 2018)
Hyloxalus pulchellus	1590	(Frost, 2018; IUCN, 2018; Ron et al., 2018)	2970	(Frost, 2018; IUCN, 2018; Ron et al., 2018)
Hyloxalus toachi	200	(Frost, 2018; IUCN, 2018; Ron et al., 2018)	1410	(Frost, 2018; IUCN, 2018; Ron et al., 2018)
Hyloxalus vertebralis	1770	(Frost, 2018; IUCN, 2018; Ron et al., 2018)	3500	(Frost, 2018; IUCN, 2018; Ron et al., 2018)
Boana almendarizae	500	(Frost, 2018; Ron et al., 2018)	1950	(Frost, 2018; Ron et al., 2018)
Boana boans	0	(IUCN, 2018)	1000	(IUCN, 2018)
Boana cinerascens	0	(Ron et al., 2018)	1200	(Ron et al., 2018)
Boana fasciata	700	(Ron et al., 2018; also see Frost, 2018)	1600	(Ron et al., 2018; also see Frost, 2018)
Boana geographica	0	(aproximate; IUCN, 2018)	1200	(IUCN, 2018)
Boana lanciformis	0	(aproximate; IUCN, 2018)	1500	(IUCN, 2018)
Boana pellucens	20	(Frost, 2018; IUCN, 2018)	1500	(Ron et al., 2018)
Boana rosenbergi	10	(Frost, 2018; IUCN, 2018)	966	(Frost, 2018; IUCN, 2018; Ron et al., 2018)
Leptodactylus labrosus	0	(IUCN, 2018; Ron et al., 2018)	1300	(Ron et al., 2018)
Leptodactylus rhodomystax	0	(IUCN, 2018)	1100	(this study; but see Frost, 2018; IUCN, 2018)
Leptodactylus ventrimaculatus	0	(Frost, 2018, IUCN, 2018)	1760	(Ron et al., 2018)

Species	Min	Reference (min)	Max	Reference (max)
Leptodactylus wagneri	200	(IUCN, 2018)	1800	(IUCN, 2018)
Osteocephalus mutabor	200	(IUCN, 2018)	1200	(IUCN, 2018)
Phyllomedusa cf. coelestis	NA	NA	NA	NA
Phyllomedusa vaillantii	0	(IUCN, 2018, Ron et al., 2018)	900	(Ron et al., 2018)
Rana bwana	0	(this study)	700	(IUCN, 2018, Ron et al., 2018)
Rana vaillanti	0	(IUCN, 2018)	880	(IUCN, 2018)
Rhinella horribilis	0	(as R. marina; IUCN, 2018; also see Ron et al., 2018)	3000	(as R. marina; IUCN, 2018; also see Ron et al., 2018)
Rhinella margaritifera	0	(Frost, 2018; IUCN, 2018)	2400	(Frost, 2018; IUCN, 2018)
Rhinella marina	0	(IUCN, 2018)	3000	(IUCN, 2018)
Scinax elaeochroa	0	(IUCN, 2018)	1200	(this study; IUCN, 2018)
Scinax garbei	0	(aproximate; Ron et al., 2018)	1260	(Ron et al., 2018)
Scinax quinquefasciatus	0	(Frost, 2018; IUCN, 2018; Ron et al., 2018)	600	(Ron et al., 2018)
Scinax ruber	0	(IUCN, 2018)	2600	(IUCN, 2018)
Smilisca phaeota	0	(IUCN, 2018, Ron et al., 2018)	1600	(IUCN, 2018, Ron et al., 2018)
Trachycephalus cunauaru	0	(Ron et al., 2018)	800	(Ron et al., 2018)

References for Table S2.2:

Frost, D. R. 2018. *Amphibian Species of the World: an online* reference. Version 6. Electronic Database accessible at http://research.amnh.org/herpetology/amphibia/index.html. American Museum of Natural History, New York, USA. Access on 12th June, 2018.

UICN. 2018. *The IUCN Red List of Threatened Species 2008*. www.iucnredlist.org. Access on 12th June, 2018.

Ron, S. R., Yanez-Muñoz, M. H., Merino-Viteri, A. Ortiz, D. A. 2018. *Anfibios del Ecuador. Version 2018.0*. Museo de Zoología, Pontificia Universidad Católica del Ecuador. https://bioweb.bio/faunaweb/amphibiaweb. Access on 12th June, 2018.

Table S2.3: Summary of the geographical location (the coordinates are in decimal degrees) and microclimatic data for each datalogger: tmax (absolute maximum temperature); tmin (absolute minimum temperature); tmean (mean temperature); dr (mean daily range) (ºC) and total number of days. Microe: microenvironment; OPEN (Open pond); FOREST (Canopy covered pond); PERM (Permanent pond); RIVER (River). Elev: elevation (in metres)

Name	microenv	longitude	latitude	elev	tmax	tmin	tmean	dr	days
DUR_CACAO	OPEN	-78,75272	1,16527	23	36,84	24,64	28,5	6,37	179
BALZAR	OPEN	-79,9699	-1,17017	32	38,49	24,16	27,52	2,46	158
PASAJE_W	FOREST	-79,80289	-3,31886	38	29,35	21,57	24,89	2,21	177
YASUNI_1	FOREST	-76,39785	-0,67359	220	26,59	24,45	25,62	1,2	4
YASUNI_4	FOREST	-76,39785	-0,67359	220	27,86	25,22	26,49	2,25	2
DUR_NIÑAS	PERM	-78,62322	1,041265	227	26,2	23,97	24,51	0,75	115
DUR6_agua	RIVER	-78,62405	1,04186	242	25,81	23,39	24,18	0,99	35
DUR5_W	PERM	-78,62361	1,034484	268	25,61	24,64	24,99	0,44	26
TENA1	PERM	-77,7388	-0,93493	665	26,49	21,86	23,97	0,55	223
ZJ_RIO.AGUA	RIVER	-77,86219	-1,35675	926	22,14	19,85	20,96	0,63	72
ZJ_DMIN	OPEN	-77,85368	-1,37135	937	26,39	20,9	22,53	1,02	85
ZJ_CASA	PERM	-77,86462	-1,35477	949	23,68	21,09	22,17	0,35	132
PUY3	PERM	-77,82062	-1,44436	1034	23,1	18,71	20,62	1,03	453
MIN2	RIVER	-78,8076	0,01823	1066	20,9	19,19	19,92	0,43	78
MIN3	OPEN	-78,8076	0,01823	1066	30,15	18,81	22,01	3,58	78
CHARCA ARRIBA	PERM	-77,72948	-1,40557	1070	31,98	19,57	22,21	1,08	316
CHARCA CAIMAN	FOREST	-77,72948	-1,40557	1070	32,5	19,85	22,79	1,8	275
CHARCA CASA	PERM	-77,72948	-1,40557	1070	27	19,38	21,82	1,62	419
H.MACU	PERM	-78,13326	-1,44838	1139	21,19	18,24	19,25	0,52	435
MINDO_DEP2r	OPEN	-78,78783	-0,04797	1206	32,7	18,43	21,93	4,21	92
MINDO_DEP2s	OPEN	-78,78785	-0,04785	1207	32,39	18,14	23,79	7,11	92
ROMERILLOS1	OPEN	-78,94445	-4,16417	1217	30,26	18,33	22,74	2,81	302
PL1	OPEN	-78,05594	-1,367396	1326	27,27	15,19	18,82	6,27	12
PL2	OPEN	-78,05594	-1,367396	1326	25,42	18,52	20,21	2,64	13
PL3	OPEN	-78,05594	-1,367396	1326	22,05	19,47	20,38	0,71	12
PL4	OPEN	-78,05594	-1,367396	1326	26,98	19,47	21,96	3,12	15
PL5	PERM	-78,05224	-1,36196	1326	23	18,62	20,09	1,59	12
MACAS_1	OPEN	-78,19222	-2,27393	1415	33,54	17,19	20,52	8,96	37
LLANG_FRANCY2	OPEN	-78,15941	-2,09560	1500	19,38	16,9	18,07	1,72	13
LLANG_FRANCY3	OPEN	-78,15941	-2,09560	1500	18,43	17,76	18,04	0,2	13

Name	microenv	longitude	latitude	elev	tmax	tmin	tmean	dr	days
TOPO_1	RIVER	-78,19208	-1,39031	1540	17,95	17,48	17,69	0,05	121
LLANG_JAMB	PERM	-78,20374	-2,07467	1700	17,09	15,19	16,35	1,1	6
RioTOPO	RIVER	-78,19774	-1,37419	1736	21,28	14,23	18,22	1,26	224
REV1	FOREST	-77,59621	-0,09704	1820	21,09	15,19	17,3	0,99	213
REV2	OPEN	-77,59453	-0,09779	1820	22,33	17,48	18,82	1,06	213
PACCHA-PASAJE	OPEN	-79,68916	-3,52819	1827	23,39	14,13	16,66	2,39	78
BAEZA	FOREST	-77,895	-0,466	1900	22,33	13,85	16,89	1,86	124
URDANETA2	OPEN	-79,22644	-3,6138	2265	17	16,14	16,44	0,7	3
Hvertebralis	PERM	-78,98978	-2,9028	2500	19,57	17,28	18,31	0,46	310
CUENCA_1	PERM	-79,03556	-2,98873	2634	16,81	11,33	14,64	0,4	231
PAPALL1	RIVER	-78,06188	-0,38767	2800	15,38	11,33	12,7	1,21	156
POZA.AZOLA	PERM	-78,49152	-0,21054	2812	17,38	11,24	14,2	0,81	301
PITA_MOL	RIVER	-78,40729	-0,41041	2830	13,46	7,28	12,46	0,93	546
PITA_CASC	RIVER	-78,41056	-0,429875	2930	12,69	12,4	12,62	0,14	33
GAS_RIO	OPEN	-78,46394	-0,187319	2970	22,43	7,88	14,77	4,55	123
GAS2_PSE	OPEN	-78,75931	-1,33659	3631	19,66	6,17	12,2	4,23	133

Table S2.4: Summary of the coefficient of determination (R^2) between the different WorldClim and the microenvironmental variables. BIO1 = Annual Mean Temperature; BIO5 = Max Temperature of Warmest Month; BIO6 = Min Temperature of Coldest Month. WorldClim variables were extracted of climatic information from the coordinates of the pond.

	tmax	tmin	tmean	BIO5	BIO6	BIO1
tmin	0,550					
tmean	0,786	**0,918**				
BIO5 (TMAX)	0,648	**0,877**	**0,880**			
BIO6 (TMIN)	0,625	**0,923**	**0,902**	**0,957**		
BIO1 (TMEAN)	0,644	**0,914**	**0,902**	**0,985**	**0,991**	
daily range (dr)	0,536	-0,137	0,146	-0,042	-0,014	-0,025

Table S2.5. Microclimatic data (depenent variables) in relation to elevation.

LM (n = 46)	Intercept ± SE	Slope ± SE	F value	p-value
tmax ~ elevation	30.897 ±1.212	-0.005 ±0.0007	42.15	**<0.001**
tmin ~ elevation	24.682 ±0.419	-0.005 ±0.0003	379.9	**<0.001**
tmean ~ elevation	26.246 ±0.379	-0.004 ±0.0002	370.2	**<0.001**
ar ~ elevation	6.215 ±1.336	0.0002 ±0.0008	0.0484	0.827
dr ~ elevation	2.143 ±0.565	-0.0001 ±0.0003	0.126	0.725

Table S2.6; Microclimatic data (depenent variables) in relation to altitude and microenvironment.

Factor	Df	Sum Sq	Mean Sq	Fvalue	P (>F)
Model 1. tmax					
Altitude	1	787,8	787,8	60,097	**<0,001**
Microenvironment	3	284,9	95,0	7,243	**<0,001**
Residuals	41	537,5	13,1		
Model 2. tmin					
Altitude	1	847,81	847,81	371,549	**<0,001**
Microenvironment	3	4,63	1,54	0,6767	0,571
Residuals	41	93,55	2,28		
Model 3. tmean					
Altitude	1	678,24	678,24	433,623	**<0,001**
Microenvironment	3	16,47	5,49	3,511	**0,023**
Residuals	41	64,13	1,56		
Model 4. ar (tmax-tmin)					
Altitude	1	1,1	1,1	0,064	0,802
Microenvironment	3	293,4	97,79	5,677	**0,002**
Residuals	41	706,3	17,23		
Model 5. Dr					
Altitude	1	0,51	0,511	0,189	0,666
Microenvironment	3	67,84	22,612	8,376	**<0,001**
Residuals	41	110,69	2,7		

Table S2.7: Tukey's post-hoc test for ANCOVA between tmax and elevation and microenvironment (Open = Open temporal pond; Forest = canopy covered pond; Perm = Permanent pond and River).

		diff	**lwr**	**upr**	**p adj**
OPEN -	**FOREST**	-0.0366	-4.687	4.6132	0.9999965
PERM -	**FOREST**	-3.6147	-8.515	1.286	0.2128183
RIVER -	**FOREST**	-7.9189	-13.281	-2.5564	**0.0017021**
PERM -	**OPEN**	-3.5781	-7.1520	-0.0041	**0.0496455**
RIVER -	**OPEN**	-7.8822	-12.067	-3.6974	**0.0000631**
RIVER -	**PERM**	-4.3042	-8.766	0.1577	0.0620767

Table S2.8: Simple PGLS regressions to test the relationship between physiological traits (CTmax, CTmin and TR) with altitude and altitudinal range; weight effect on thermal tolerance limits and tradeoffs between both thermal tolerance limits. pop_altitude = elevation of the population analized; centroid_elevation = species' midpoint elevational distribution. We used logarithm of weight in order to normalize data.

PGLS		**Intercept ± SE**	**Slope ± SE**	**F value**	**p-value**
CTmax ~ pop_altitude	*(N=75; R^2=0.304; λ=0.977)*	40.412 ±0.765	-0.001 ±0.0002	33.31	**<0.001**
CTmax ~ centroid_altitude	*(N=75; R^2=0.138; λ=0.968)*	40.227 ±0.867	-0.0011 ±0.0003	12.87	**<0.001**
CTmin ~ pop_altitude	*(N=75; R^2=0.382; λ=0.908)*	6.828 ±0.788	-0.0017 ±0.0003	46.8	**<0.001**
CTmin ~ centroid_altitude	*(N=75; R^2=0.544; λ=0.837)*	7.751 ±0.672	-0.0025 ±0.0003	86.94	**<0.001**
TR ~ pop_altitude	*(N=75; R^2=0.006; λ=1)*	33.797 ±1.14	0.0002 ±0.0003	0.569	0.453
TR ~ centroid_altitude	*(N=75; R^2=0.102; λ=1)*	32.744 ±1.11	0.0012 ±0.0004	9.446	**0.003**
TR ~ altitudinal range	*(N=70; R^2=0.039; λ=1)*	33.3 ±1.13	0.0007 ±0.0004	3.811	0.055
CTmax ~ log(weight)	*(N=73; R^2=0.014; λ=0.988)*	38.955 ±0.933	0.0017 ±0.171	0.0001	0.992
CTmin ~ log(weight)	*(N=73; R^2=0.082; λ=1)*	4.096 ±1.037	-0.496 ±0.182	7.393	**0.008**
CTmax ~ CTmin	*(N=75; R^2=0.139; λ=1)*	37.325 ±0.953	0.336 ±0.093	12.92	**<0.001**
wt ~ pop_altitude	*(N=69; R^2=0.147; λ=0)*	8.875 ±0.801	0.0037 ±0.0006	33.83	**<0.001**
wt ~ centroid_altitude	*(N=69; R^2=0.243; λ=0.077)*	8.73 ±1.077	0.0035 ±0.0008	21.5	**<0.001**
ct ~ pop_altitude	*(N=69; R^2=0.415; λ=0.826)*	-17.347 ±1.129	0.0029 ±0.0004	49.33	**<0.001**
ct ~ centroid_altitude	*(N=69; R^2=0.148; λ=0)*	-15.351 ±0.588	0.0017 ±0.0005	18.81	**<0.001**

Table S2.9. Physiological traits (dependent variables) in relation to microenvironmental temperatures.

PGLS (n = 69)	Intercept ± SE	Slope ± SE	F value	p-value
CTmax ~ tmax *(R^2=0.282; λ=0.659)*	34.477 ±1.025	0.169 ±0.032	27.75	**<0.001**
CTmin ~ tmin *(R^2=0.278; λ=0.947)*	-0.602 ±1.344	0.285 ±0.055	27.21	**<0.001**
TR ~ dr *(R^2=-0.014; λ=1)*	34.126 ±1.129	0.0195 ±0.115	0.029	0.866
TR ~ ar *(R^2=-0.003; λ=1)*	33.856 ±1.156	0.039 ±0.044	0.775	0.382

Table S2.10. Physiological traits (dependent variables) in relation to elevation to the population georeferenced point and habitat (river or pond).

Physiological traits		Estimate (±SE)	tvalue	Pr (>|t|)
1. CTmax	**Intercept**	40.479 (±0.56)	72.282	**<0.001**
	Elevation	-0.0011 (±0.0002)	-5.608	**0.001**
	Habitat (river)	-2.526 (±0.462)	-5.461	**<0.001**
(N=75; Pvalue <0.001; R2=0.493; λ=0.823)				
2. CTmin	**Intercept**	6.827 (±0.796)	8.576	**<0.001**
	Elevation	-0.002 (±0.0003)	-6.6	**<0.001**
	Habitat (river)	-0.296 (±0.615)	-0.481	0.632
(N=75; Pvalue < 0.001; R2=0.374; λ=0.912)				
3. TR	**Intercept**	33.856 (±1.107)	30.594	**<0.001**
	Elevation	0.0004 (±0.0003)	1.136	0.260
	Habitat (river)	-1.86 (±0.786)	-2.358	**0.021**
(N=75; Pvalue < 0.052; R2=0.053; λ=1)				

Table S2.11: Physiological traits (depenent variables) in relation to and logarithm of mean weight of the population and midpoint elevation of the population (model1) and habitat (river or pond; model2).

	CTmin	Estimate (±SE)	tvalue	Pr (>|t|)
Model 1.	Intercept	7.199 (±0.724)	9.948	**<0.001**
	Elevation	-0.0024 (±0.0003)	-8.682	**<0.001**
	log(weight)	-0.268 (±0.151)	-1.774	0.08
	(N = 73; Pvalue < 0.001; R^2 = 0.55; λ=0.807)			
Model 2.	Intercept	7.186 (±0.732)	9.814	**<0.001**
	Elevation	-0.0024 (±0.0003)	-8.404	**<0.001**
	Habitat (River)	-0.162 (±0.532)	-0.304	0.762
	log(weight)	-0.271 (±0.152)	-1.777	0.08
	(N = 73; Pvalue < 0.001; R^2 = 0.542; λ=0.814)			

Table S2.12: Results of phylogenetic signal for the physiological traits for Pagel's lambda (λ) and Blomberg's K (K) for 1000 phylogenetic trees (median and the 2.5 and 97.5% confidence intervals). P-value was calculated from 1000 pylogenetic results as $n_{(p\text{-value} > 0.05)}$ / n.

	K	p-value	λ	p-value
Ctmax	0.7778 (0.5738 – 0.8897)	<0.001	0.9999 (0.969 – 0.9999)	<0.001
Ctmin	0.6605 (0.3339 – 0.8331)	0.013	0.9572 (0.9405 – 0.9886)	<0.001

Table S2.13: Support for best model (Brownian Motion, BM; Orstein-Uhlebeck, OU and Early Bust, EB) for CTmax and CTmin using the corrected Akaike information criteria (AICc). Models' AICc (median and the 2.5 and 97.5% confidence intervals) and p-value (evolutionary models with AICc > BM model) are based on 1000 phylogenies. We use the simplest model (i.e. BM) if evolutionary models (i.e. EB and OU) are not significantly better.

N = 1000	**BM**	**OU**	**p-value**	**EB**	**p-value**
Ctmax	261.9615	263.7956	0.938	264.1328	1
	(255.417-283.714)	(257.451 – 279.97)		(257.462 – 285.886)	
Ctmin	310.254	309.3782	0.408	316.4816	1
	(293.978 – 360.033)	(295.965 – 339.541)		(296.15 – 362.204)	

Table S2.14: Comparison of evolutionary rates of CTmax and CTmin using 1000 phylogenetic trees. LRT = likelihood ratio test. (median and the 2.5 and 97.5% confidence intervals).

σ^2 (CTmax)	**σ^2 (CTmin)**	**LRT**	**p-value ($n_{(p\text{-value} > 0.05)}$ / n)**
4.372 (3.674 – 5.999)	8.353 (6.504 – 16)	7.453 (2.316 – 29.551)	0.108

Table S2.15: Support for best model of CTmax and CTmin evolution on elevation (two Brownian Motion models, BM and two Orstein-Uhlebeck, OU) using the mean absolute value of corrected Akaike information criteria (AICc).

	BM1	**BMS**	**OU1**	**OUM**
CTmax	**263.327**	265.38	264.493	265.376
CTmin	314.138	**242.753**	310.994	255.631

Figure S2.1: Differences between maximum micro-environmental and macro-environmental temperature (tmax - BIO5) on different habitats (shadow-temporal, open-temporal, permanent and river) of tadpoles.

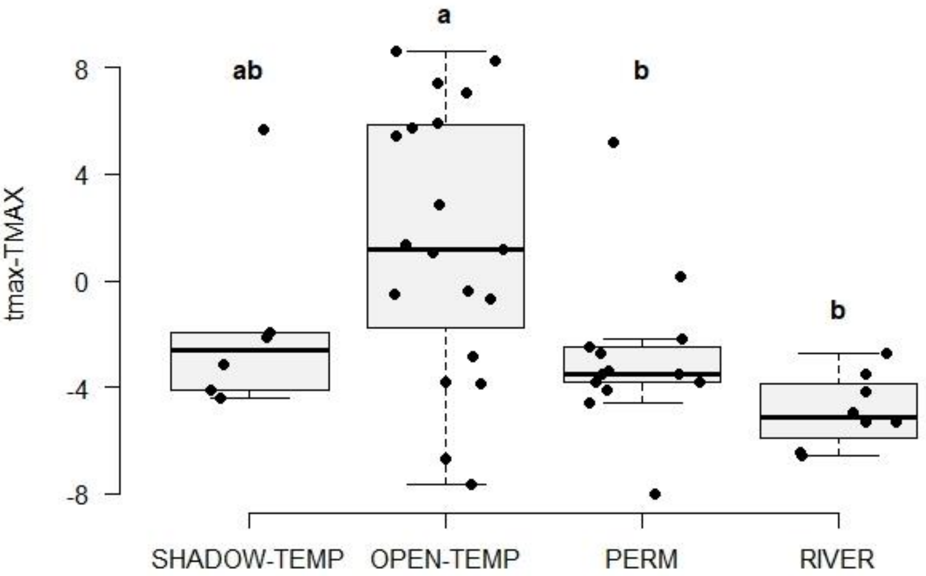


Figure S2.2: Distribution of p-values of PGLS models using 100 different phylogenetic trees linking CTmax with elevation (**a**) and habitat (**b**), CTmin with elevation (**c**) and habitat (**d**) and TR with elevation (**e**) and habitat (**f**).

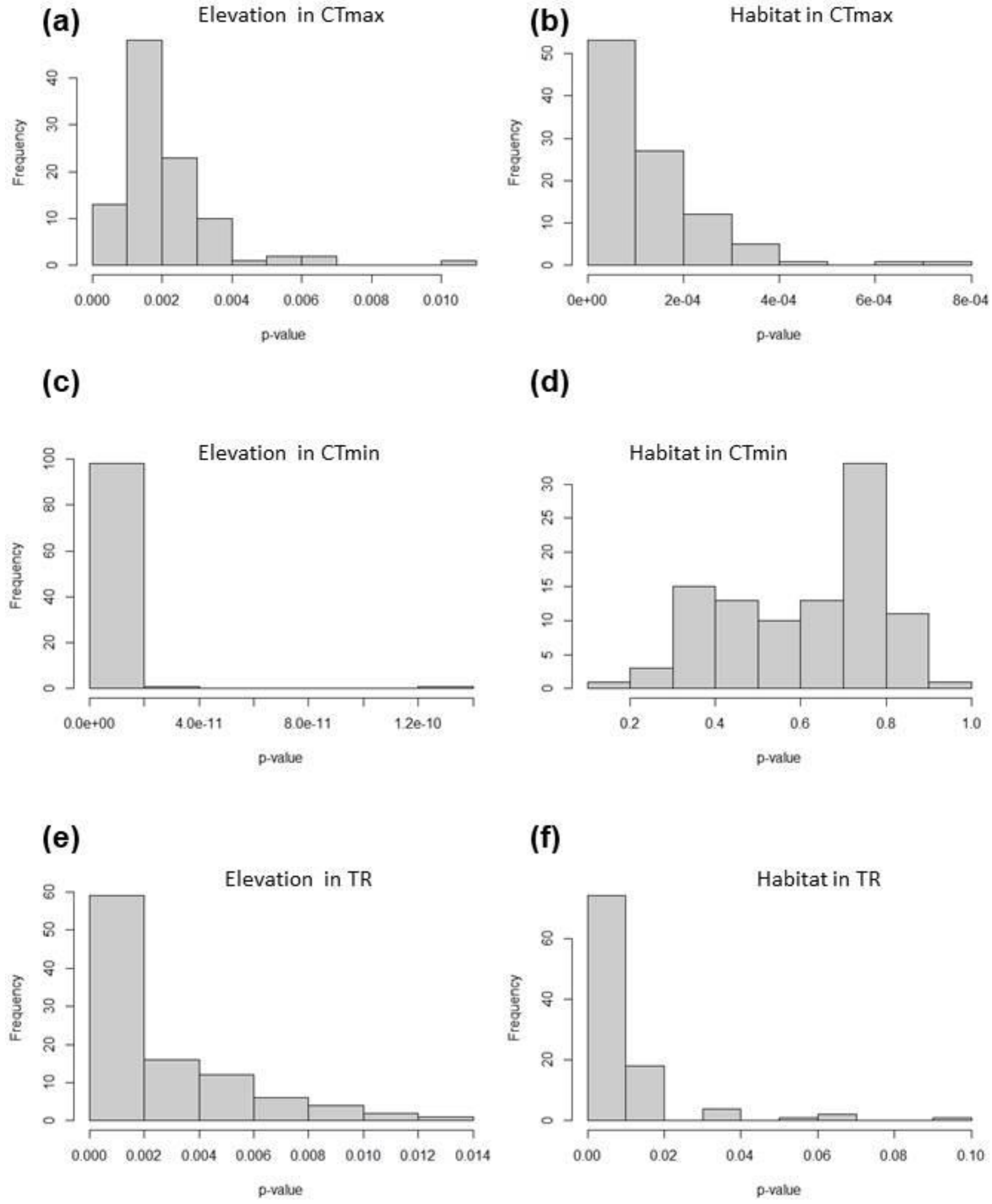
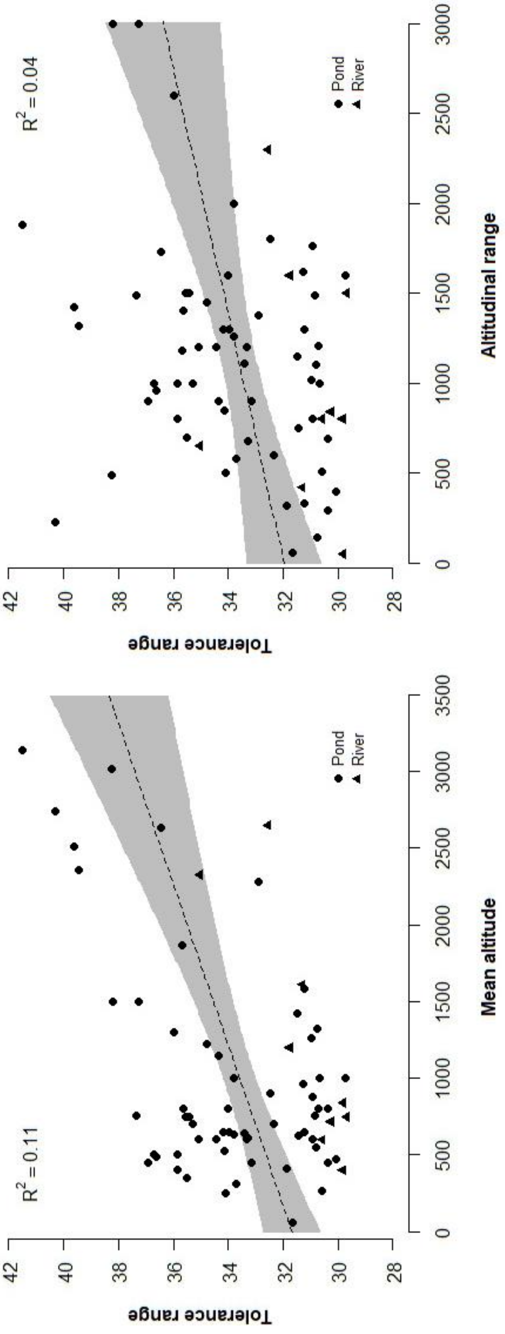


Figure S2.3: Tolerance range in relation to elevational parameters in tadpoles: (**a**) mean altitude of the distribution (PGLS: Slope = 0.001 ±0.0003, N = 70, p < 0.004) and (**b**) altitudinal range (PGLS: Slope = 0.001 ±0.0003, N = 70, p = 0.06). The fitted lines and standard errors in the figure are derived from the raw data.



Supplementary Information for Chapter 3

Tabla S3.1: Summary of the geographical location (the coordinates are in decimal degrees) and estimated physiological traits. Mic: microenvironment (st, stream and po, pond). Elev, elevation (in metres). Zmax, maximum performance (g g^{-1} day^{-1}). T50min/max; minimum and maximum temperature in which performance is above 50%. Topt, optimum temperature. DTS, daily thermal stress (more info in Table S2.1).

Especie	longitude	latititude	elev	mic	Zmax	T50min	T50max	Topt	DTS
Ameerega_sp.	-77,8646	-1,3548	949	po	0,233	20,899	29,52	25,451	0
Atelopus_elegans	-78,6241	1,0419	243	st	0,0705	20,273	26,89	23,582	95,36
Chimerella_mariaelenae	-78,1921	-1,3903	1540	st	0,147	20,001	28,684	25,66	0
Dendropsophus_carnifex	-78,7917	-0,0411	1066	po	0,239	19,792	31,151	28,942	4,12
Dendropsophus_minutus	-77,8537	-1,3713	937	po	0,2	22,698	31,251	27,932	0
Epipedobates_anthonyi	-79,8029	-3,3189	38	po	0,58	24,325	31,627	28,869	0,2
Epipedobates_boulengeri	-78,6236	1,0345	242	po	0,222	20,975	30,082	26,346	0
Epipedobates_darwinwallacei	-78,8076	0,01823	1066	po	0,063	20,523	27,836	24,18	7,95
Epipedobates_machalilla	-80,0726	-0,0728	50	po	0,144	20,586	30,663	27,41	44,41
Epipedobates_tricolor	-79,125	-1,4197	1300	po	0,235	19,073	30,092	24,947	
Gastrotheca_pseustes	-78,7593	-1,3366	3631	po	0,21	15,677	29,925	23,0243	0
Gastrotheca_riobambae	-78,4639	-0,1873	2969	po	0,503	19,135	32,054	27,708	0
Hyloscirtus_phyllognatus gr.	-78,1922	-2,274	1495	st	0,065	17,11	28,897	23,423	
Hyloxalus_bocagei	-77,5962	-0,0970	1820	po	0,098	18,842	28,15	24,201	0
Hyloxalus_maculosus	-78,1333	-1,4484	1139	po	0,151	17,452	28,384	24,09	0
Hyloxalus_nexipus	-77,8078	-2,9898	391	po	0,243	19,659	30,406	27,719	
Hyloxalus_pulchellus	-77,895	-0,466	1900	po	0,12	16,629	24,838	20,733	0,29
Hypsiboas_almendarizae	-78,0559	-1,3674	1223	po	0,356	22,587	34,362	28,475	0
Hypsiboas_cinerascens	-77,8646	-1,3548	949	po	0,158	20,852	31,817	26,918	0
Hypsiboas_pellucens	-78,7879	-0,0479	1066	po	0,31	21,088	30,362	25,725	22,83
Hypsiboas_rosenbergi	-78,7527	1,16527	23	po	0,482	22,995	34,921	28,958	32,99
Leptodactylusventrimaculatus	-78,8076	0,01823	1066	po	0,217	19,812	30,616	27,278	10,73
Leptodactylus_wagneri	-78,94445	-4,1642	1217	po	0,774	25,526	30,211	28,545	0
Phyllomedusa_sp.	-77,85368	-1,3714	937	po	0,12	23,49	30,842	27,609	0
Rhinella_horribilis	-78,7879	-0,0479	1066	po	1,692	23,414	37,532	30,473	1,2
Rhinella_margaritifera	-78,1966	-1,34679	1638	po	0,171	16,897	29,422	23,16	0
Scinax_quinquefasciatus	-78,7527	1,16527	23	po	0,863	22,662	34,251	29,702	26,04
Smilisca_phaeota	-78,6236	1,03448	242	po	0,933	21,432	32,909	29,552	0

Table S3.2: Comparisons of models fit to the growth rates of larval anurans. For each model, we report both the log likelihood, AIC and the R-squared (R2). The best-fitting model for each developmental stage is denoted with bold font. G-g: Gaussian-Gompertz adjusts.

Species	**Adjust**	**Log likelihood**	**AIC**	**R^2**
Ameerega sp.	**G-G**	**183,426**	**-358,185**	**64,929**
	Gaussian	181,108	-355,822	62,336
	Quadratic	172,285	-338,177	50,589
	Cubic	183,054	-357,442	64,526
Atelopus elegans	**Gaussian**	**111,365**	**-215,841**	**36,855**
	Quadratic	108,504	-210,119	24,054
	Cubic	110,872	-212,206	34,816
	G-G	111,451	-213,363	37,204
Chimerella mariaelenae	**G-G**	**193,153**	**-377,536**	**70,623**
	Gaussian	186,97	-367,488	63,507
	Quadratic	180,894	-355,335	54,834
	Cubic	186,026	-363,283	62,278
Dendropsophus minutus	**G-G**	**243,912**	**-479,276**	**69,969**
	Gaussian	237,725	-469,127	64,806
	Quadratic	221,931	-437,538	47,235
	Cubic	243,055	-477,562	69,301
Dendrosophus carnifex	**G-G**	**201,604**	**-394,759**	**35,123**
	Gaussian	198,734	-391,201	31,037
	Quadratic	192,955	-379,642	22,014
	Cubic	200,829	-393,209	34,045
Epipedobates anthonyi	**G-G**	**161,916**	**-315,327**	**66,449**
	Gaussian	155,384	-304,469	60,803
	Quadratic	137,379	-268,459	39,823
	Cubic	156,689	-304,872	62,002
Epipedobates boulengeri	**G-G**	**232,091**	**-455,682**	**56,872**
	Gaussian	229,12	-451,943	53,749
	Quadratic	225,011	-443,725	49,054
	Cubic	230,517	-452,534	55,244
Epipedobates darwinwallacei	**Gaussian**	**88,095**	**-168,928**	**47,135**
	Quadratic	86,346	-165,429	38,448
	Cubic	86,838	-163,454	41,028
	G-G	88,205	-166,188	47,637
Epipedobates machalilla	**G-G**	**112,434**	**-215,618**	**53,165**
	Gaussian	110,241	-213,755	47,271
	Quadratic	108,501	-210,276	42,072
	Cubic	111,744	-214,238	51,386
Epipedobates tricolor	**G-G**	**220,506**	**-432,518**	**50,473**
	Gaussian	219,06	-431,827	48,78
	Quadratic	217,602	-428,912	47,014
	Cubic	219,152	-429,81	48,89

Species	**Adjust**	**Log likelihood**	**AIC**	**R^2**
Gastrotheca pseustes	**G-G**	**450,914**	**-893,579**	**49,988**
	Gaussian	449,21	-892,272	48,951
	Quadratic	439,71	-873,272	42,761
	Cubic	447,804	-887,359	48,079
Gastrotheca riobambae	**G-G**	**129,045**	**-249,706**	**27,818**
	Gaussian	127,036	-247,844	25,107
	Quadratic	123,132	-240,036	19,545
	Cubic	128,131	-247,877	26,596
Hyloscirtus sp.	**G-G**	**240,209**	**-471,703**	**59,081**
	Gaussian	238,098	-469,775	56,149
	Quadratic	231,44	-456,458	45,45
	Cubic	240,009	-471,303	58,811
Hyloxalus bocagei	**G-G**	**274,409**	**-540,193**	**80,219**
	Gaussian	265,772	-525,175	74,591
	Quadratic	240,7	-475,031	47,448
	Cubic	262,367	-516,108	71,955
Hyloxalus maculosus	**G-G**	**141,402**	**-273,971**	**35,071**
	Gaussian	139,723	-272,957	30,823
	Quadratic	140,07	-273,649	31,722
	Cubic	140,442	-272,05	32,674
Hyloxalus nexipus	**G-G**	**97,992**	**-187,115**	**27,119**
	Gaussian	95,582	-184,653	19,893
	Quadratic	95,057	-183,603	18,226
	Cubic	97,044	-185,219	24,358
Hyloxalus pulchellus	**Gaussian**	**164,1**	**-321,729**	**43,332**
	Quadratic	158,954	-311,438	31,672
	Cubic	164,642	-320,484	44,439
	G-G	164,783	-320,765	44,722
Boana almendarizii	**Gaussian**	**184,178**	**-362,023**	**64,592**
	Quadratic	174,326	-342,319	54,112
	Cubic	183,634	-358,705	64,081
	G-G	184,178	-359,793	64,592
Boana cinerascens	**G-G**	**208,317**	**-408,078**	**39,897**
	Gaussian	207,09	-407,852	37,952
	Quadratic	203,563	-400,798	31,999
	Cubic	208,201	-407,847	39,717
Boana pellucens	**Gaussian**	**86,63**	**-166,661**	**35,146**
	Quadratic	85,053	-163,506	30,324
	Cubic	86,731	-164,437	35,443
	G-G	86,949	-164,873	36,079
Boana rosenbergi	**Gaussian**	**92,783**	**-179,208**	**28,387**
	Quadratic	90,374	-174,39	23,358
	Cubic	93,507	-178,407	29,831
	G-G	93,455	-178,303	29,729

Species	Adjust	Log likelihood	AIC	R^2
Leptodactylus ventrimarmoratus	**G-G**	**159,336**	**-310,028**	**42,806**
	Gaussian	155,665	-304,95	36,182
	Quadratic	153,861	-301,341	32,65
	Cubic	156,863	-305,081	38,423
Leptodactylus wagneri	**G-G**	**125,054**	**-241,601**	**56,796**
	Gaussian	111,928	-217,555	40,945
	Quadratic	102,274	-198,249	25,686
	Cubic	112,241	-215,976	41,385
Phyllomedusa sp.	**G-G**	**345,639**	**-682,843**	**69,83**
	Gaussian	343,973	-681,687	68,776
	Quadratic	314,345	-622,431	42,483
	Cubic	342,421	-676,408	67,761
Rhinella horribilis	**Gaussian**	**86,585**	**-167,002**	**51,927**
	Quadratic	78,348	-150,528	46,227
	Cubic	85,741	-163,199	51,372
	G-G	86,596	-164,91	51,934
Rhinella margaritifera	**Gaussian**	**136,132**	**-265,719**	**44,71**
	Quadratic	133,001	-259,457	37,006
	Cubic	135,605	-262,281	43,484
	G-G	136,288	-263,646	45,068
Scinax quinquefasciatus	**G-G**	**125,796**	**-243,012**	**74,953**
	Gaussian	118,88	-231,417	69,805
	Quadratic	106,604	-206,865	57,925
	Cubic	124,295	-240,01	73,916
Smilisca phaeota	**G-G**	**111,034**	**-213,672**	**50,316**
	Gaussian	106,109	-205,983	45,478
	Quadratic	91,739	-177,243	28,498
	Cubic	109,431	-210,466	48,791

Table S3.3: Phylogenetic signal of parameters obtained from TPC (Topt, log-transformed Zmax and B_{50}). We used the most common indices: Bloomberg's K and Pagel's λ. We used the consensus tree and repeated for phylogenetic uncertainty using 1000 randomly selected trees.

	λ	**p-value**	**$\lambda_{1000\ trees}$**	**K**	**p-value**	**$K_{1000\ trees}$**
Topt	0.081	0.717	0.06	0.242	0.478	0.231
log(Zmax)	0	1	0	0.228	0.547	0.219
B_{50}	0.356	0.454	0.362	0.255	0.453	0.262

Table S3.4: Simple regression models (PGLS) between physiological variables, estimated from TPCs, and environmental variables of the aquatic breeding-site (n = 26; tmax = maximum temperature; tmin = minimum temperature; tmean = mean temperature; dr = mean daily thermal range and ar = absolute range, tmax-tmin).

Formula	Lambda	AIC	ΔAIC	BIC	wi
Topt ~ tmean	0.191	111.421	0	113.937	0.602
Topt ~ tmax	0.055	112.593	1.172	115.109	0.335
Topt ~ tmin	0.203	116.23	4.809	118.747	0.054
Topt ~ dr	0.057	120.709	9.288	123.225	0.006
Topt ~ ar	0.145	122.558	11.137	125.075	0.003
log (Zmax) ~ dr	0	60.998	0	63.514	0.532
log (Zmax) ~ tmax	0.013	62.829	1.832	65.345	0.213
log (Zmax) ~ ar	0	64.191	3.193	66.707	0.108
log (Zmax) ~ tmean	0.046	64.26	3.262	66.776	0.104
log (Zmax) ~ tmin	0.019	66.008	5.01	68.524	0.043
B50 ~ dr	0.125	117.161	0	119.677	0.402
B50 ~ tmin	0.074	118.63	1.469	121.146	0.193
B50 ~ ar	0.446	118.631	1.471	121.148	0.193
B50 ~ tmean	0.227	119.778	2.617	122.294	0.109
B50 ~ tmax	0.719	119.879	2.718	122.395	0.103

Figure S3.1: Correlations between parameters of the TPC (i.e. Topt (**a-b**), log-transformed Zmax (**c-d**) and B_{50} (**e-f**)) and environmental parameters (tmax = maximum temperature, tmean = mean temperature, tmin = minimum temperature, dr = daily thermal range and ar (tmax-tmin) = absolute thermal range). Dashed lines represent equal values for dependent and independent variables. Only the two best models for each TPC parameters are presented here (see **Table S3.4**).

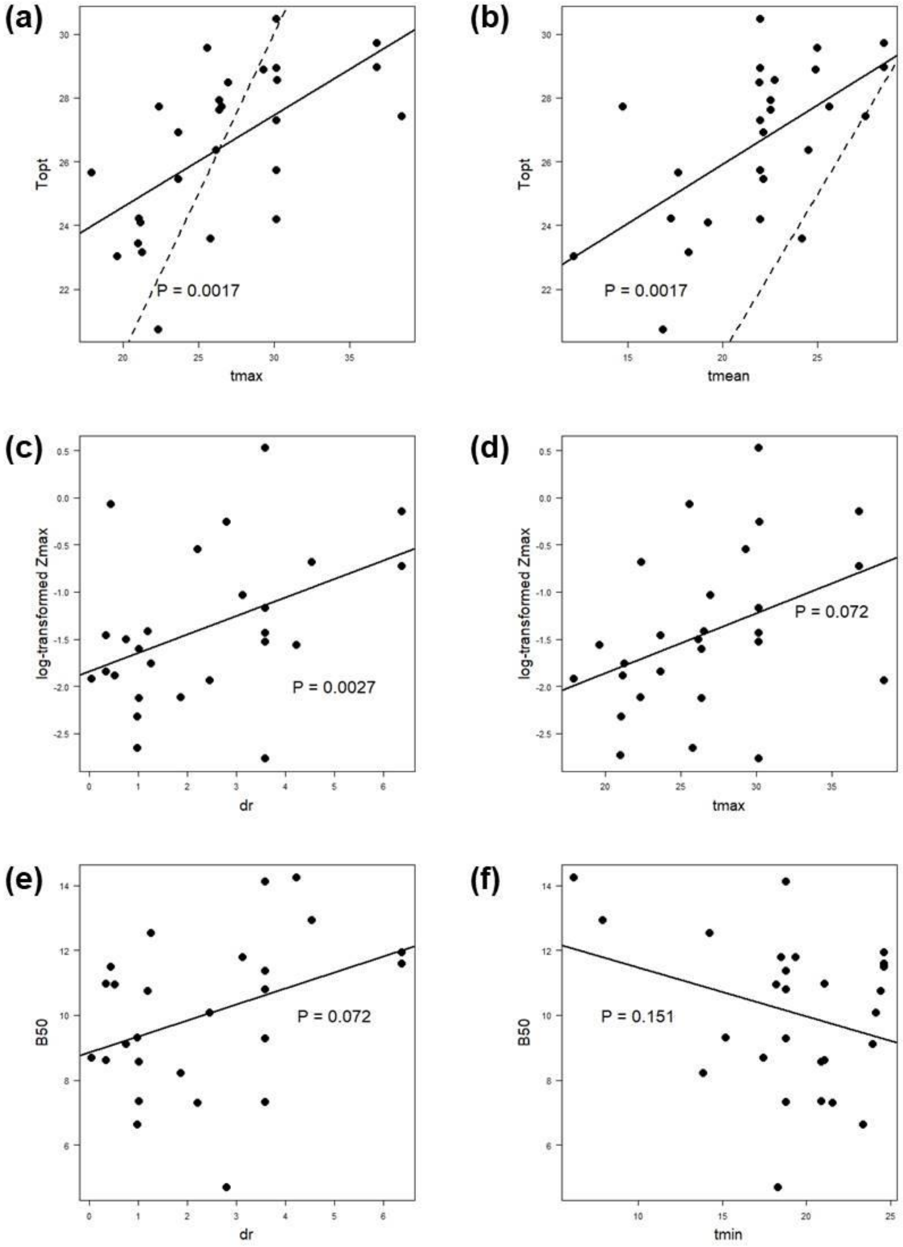
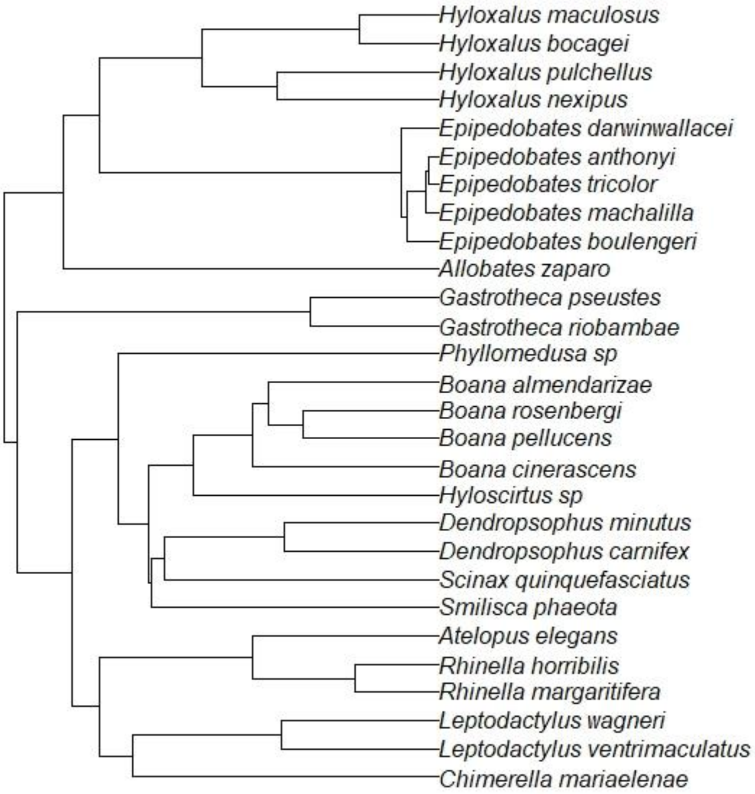


Figure S3.2. Phylogenetic tree of the species included in this study from Jetz & Pyron (2018).



Supplementary Information for Chapter 4

Supplementary Material 4.1

Climate data

We estimated maximum and minimum mean temperatures for *low* (river and permanent deep ponds), *medium* (forested ponds and permanent opened ponds) and *high* (open temporal ponds) microclimates. This categorization was based on daily thermal range (dr = tmax - tmin). First, we performed different models with *in situ* microclimate information obtained from 38 aquatic environments and their WorldClim estimates. As explanatory variables we used BIO1 (annual mean temperature), BIO5 (maximum temperature of the warmest month), BIO6 (minimum temperature of the coldest month), their squared values and microclimate. The best models was selected based on the Akaike information criterion (AIC; Burnham & Anderson, 2002) with the structure shown below:

$$\text{tmax} \sim \text{BIO1} + \text{BIO5} + \text{microclimate}$$

$$\text{tmin} \sim \text{BIO1}$$

Then, we extracted the coefficients of the intercept and each explanatory variable and interpolated this data across the Ecuadorian *Epipedobates* and *Hyloxalus* distributional points. We considered that tmin was invariant across microenvironments because our analysis showed that minimum mean temperature (datalogger) is not related to microclimate when elevation is included as a covariate (see **Table S4.3a**).

To incorporate present and future daily thermal variation in our data, we interpolated estimated mean maximum and minimum microenvironmental temperature to the three current microclimates with contrasting temperatures in Ecuador (1) river in Mindo, Pichincha, (2) forested pond in Baeza, Napo and (3) opened pond in a cacao plantation in Durango, Esmeraldas. We used the function 'spline' in basic R. We added two extra points between the temperatures at a proportional distance for a better fit (see **Fig. S4.1**).

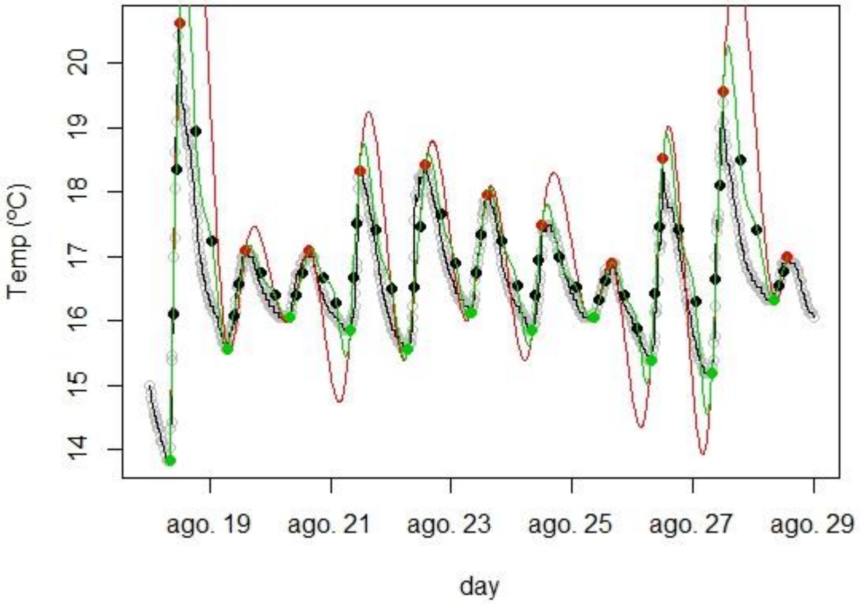


Figure S4.1: Observed (black line and grey dots) and predicted temperature using 'spline' only with extreme temperatures (red line) (red dots: tmax, green dots: tmin) and with extreme temperatures including two extra middle points (black dots) (green line).

We considered that temperature is constant throughout the year. Our temperature measurements (datalogger) showed that variation across microclimates on the same geographic area is much higher than possible annual thermal fluctuations (see **Fig. S4.2**). Also, to validate our results, we compared our predictions for *low*, *mean* and *high* daily variation with other climate data obtained from dataloggers from other localities (see **Fig. S4.3**).

We repeated the same procedures to estimate future microhabitat temperatures. We used future Tmax and Tmean for the CCSM4 global circulation models at two different emission escenarios (RCP 4.5 and 8.5) from WorlClim (Hijmans et al., 2005).

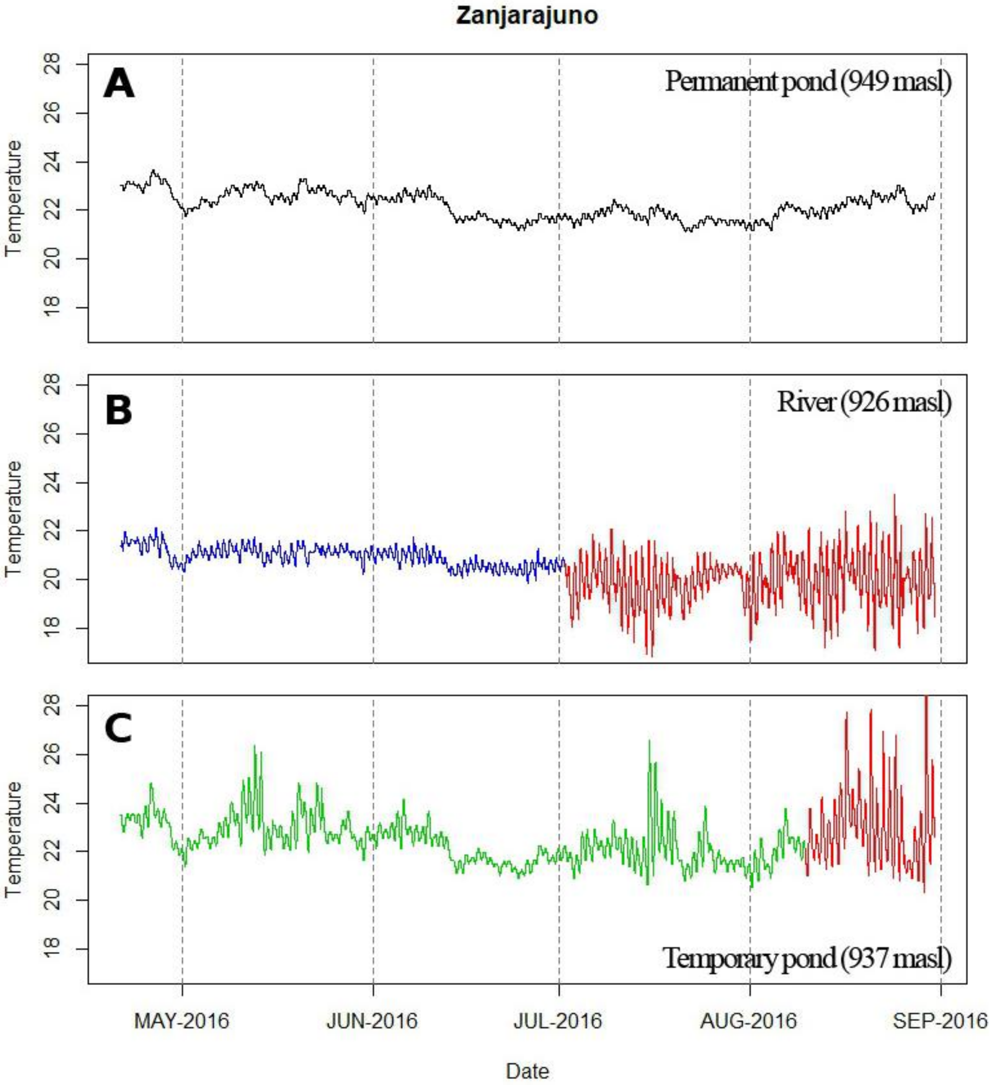


Figure S4.2: Temperature variation (ºC) on three different aquatic environments in Zanjarajuno ecological center (77º51'52.6"S, 1º21'17.2"W), (A) permanent pond, (B) River and (C) temporary pond. Red line for figures B and C represent temperature values for air and mud respectively. In B, the current dragged the logger out of the water and in C the pond dried out.

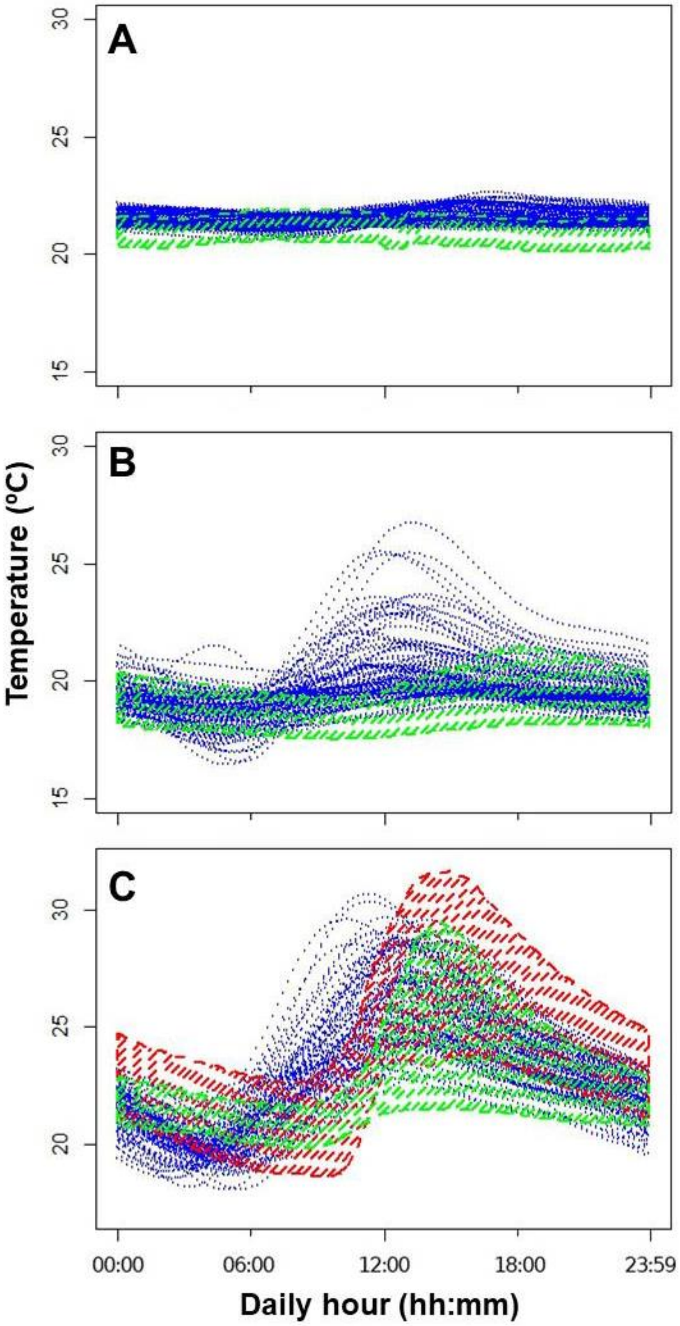


Figure S4.3: Predicted (blue dots) and 95% interval of observed temperatures (green) in a (**A**) river in Zanjarajuno, Pastaza (926 m.a.s.l.); (**B**) pond near the Reventador volcano, Napo (1850 m.a.s.l.) and (**C**) two different temporal ponds in Mindo, Ecuador (1200 m.a.s.l.).

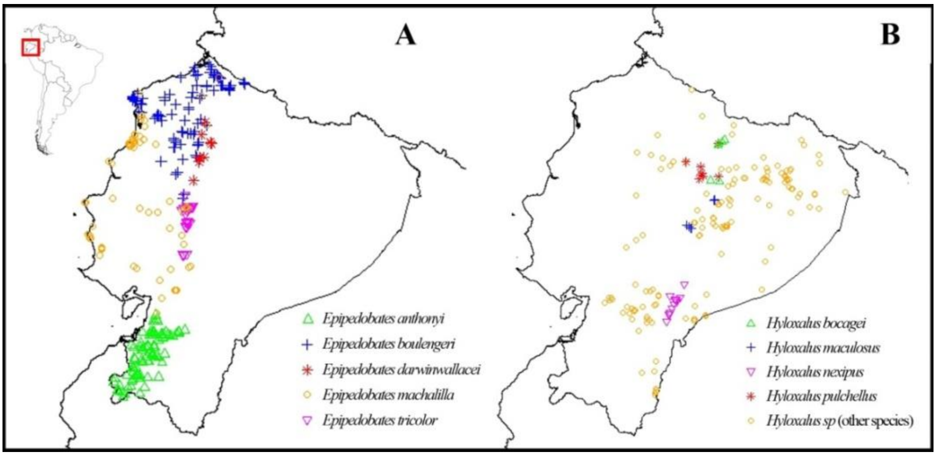


Figure S4.4: Distributional points used in this study for (A) *Epipedobates* and for (B) *Hyloxalus*. For *Hyloxalus* we included all the Amazonian-side species and high elevation species (such as *H. vertebralis*) to increase the elevational georeferenced records.

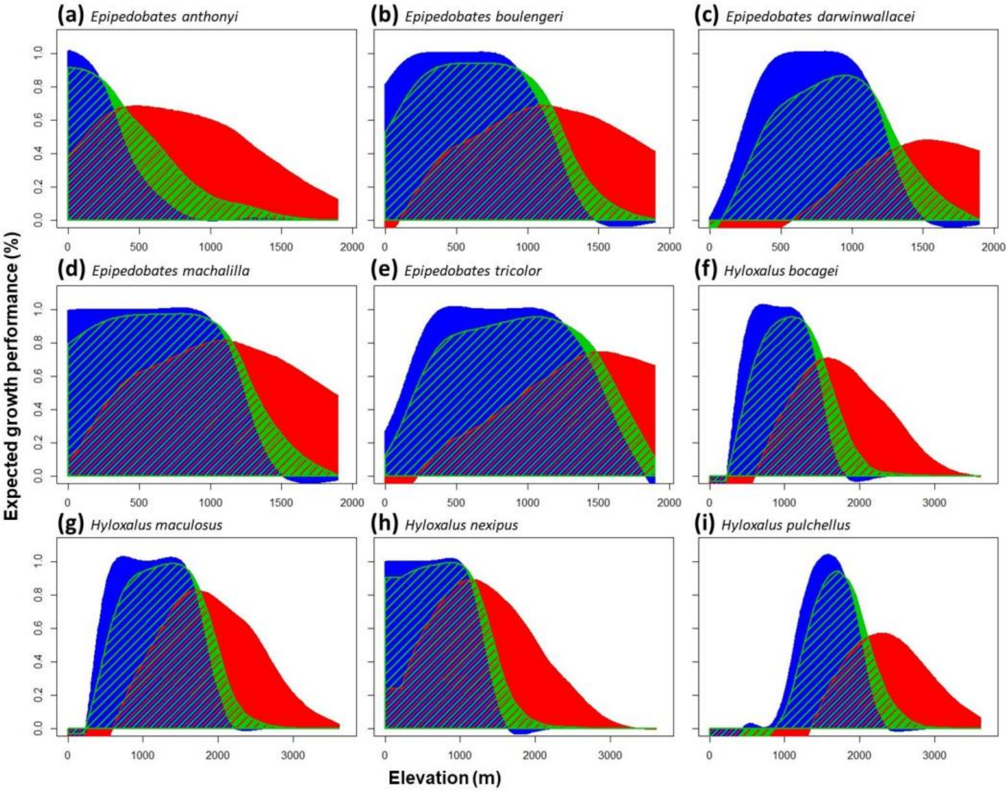


Figure S4.5: Expected maximum performance for growth for the *Epipedobates* (**a-e**) and Hyloxalus species (**f-i**) in *low* (blue), *medium* (green) and *high* (red) variation microclimates.

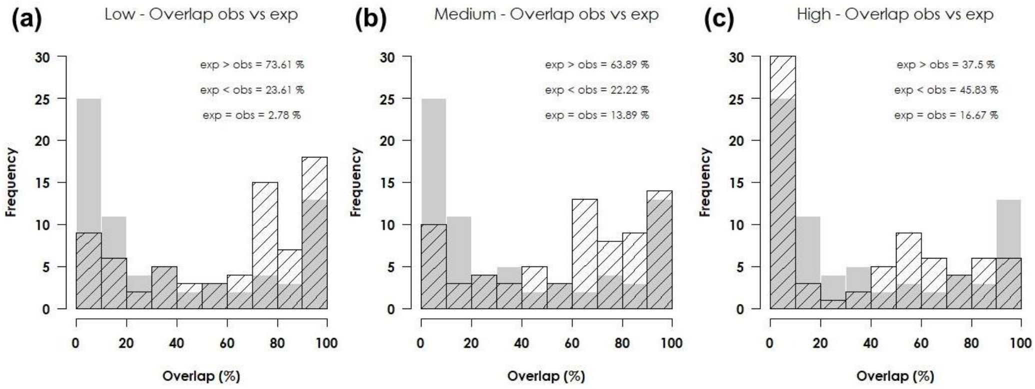


Figure S4.6: Observed overlap (grey) versus expected overlap (stripped) of elevational distribution in Dendrobatidae frogs for (**a**) low variation, (**b**) medium variation and (**c**) high variation.

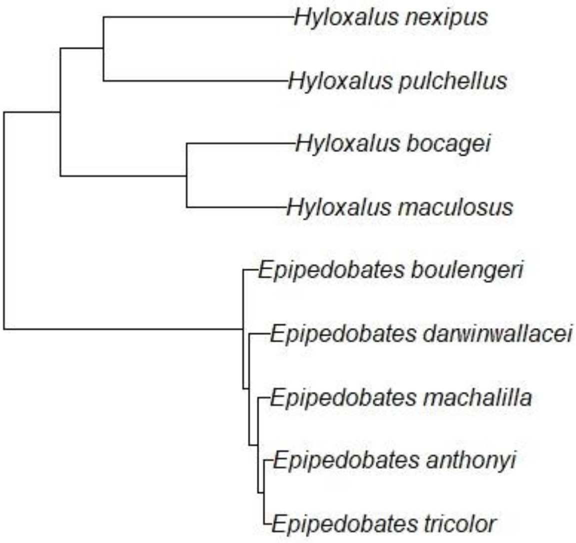


Figure S4.7. Phylogenetic tree of the species included in this study from Jetz & Pyron (2018).

Table S4.1: Summary of the geographical sampling points (The coordinates are in decimal degrees) and physiological traits (ºC) of the species used in this study. The physiological variables were estimated using the fitted curves (see Fig. 4. 1 and Table S3.2).

Specie	Altitude (m)	Longitude	Latitude	Topt	B_{50}_min	B_{50}_max
Epipedobates anthonyi	38	-79.80289	-3.31886	28.72	23.42	32.16
Epipedobates boulengeri	242	-78,62361	1.034484	26.35	20.98	30.08
Epipedobates darwinwallacei	1066	-78.8076	0.01823	24.18	20.52	27.84
Epipedobates machalilla	50	-80.07259	-0.07277	27.41	20.59	30.66
Epipedobates tricolor	1300	-79.125	-1.4197	24.95	19.07	30.09
Hyloxalus bocagei	1820	-77.59620	-0.097042	24.2	18.84	28.15
Hyloxalus maculosus	1139	-78.13326	-1.44838	24.09	17.45	28.38
Hyloxalus nexipus	391	-77.80784	-2.98982	27.72	19.66	30.41
Hyloxalus pulchellus	1900	-77.895	-0.466	20.73	16.63	24.84

Table S4.2: Summary of the geographical location (the coordinates are in decimal degrees) and microclimatic data for each datalogger: tmax (mean maximum temperature); tmin (mean minimum temperature); tmean (mean temperature); dr (daily range = tmax - tmin). Additionally, macroclimatic measurements for the same coordinates where the loggers were located are summarized (WorlClim): TMAX (maximum temperature); TMIN (minimum temperature); TMEAN (mean temperature). In factor, water bodies are categorized in three factors according to its dr (low, med = medium and high thermal variation). In Microenv is summarized the description of the aquatic environment: River, Permanent (permanent pond), Forest temp (canopy covered temporal pond) and Open temp (open temporal pond).

	Sample site information					Datalogger (ºC)					WorldClim (ºC)		
Datalogger	**Factor**	**Microenv**	**longitude**	**latitude**	**Alt (m)**	**tmax**	**tmin**	**tmean**	**dr**	**N**	**TMAX**	**TMIN**	**TMEAN**
TOPO_1	low	River	-78,1921	-1,3903	1540	17,71	17,66	17,69	0,05	121	24,4	12,8	18,5
PITA_GC	low	River	-78,4106	-0,4299	2930	12,69	12,55	12,62	0,14	33	19,3	6	12,7
ZJ_CASA	low	Permanent	-77,8646	-1,3548	949	22,37	22,02	22,17	0,35	132	27,1	15,8	21,4
CUENCA1	low	Permanent	-79,0356	-2,9887	2634	14,87	14,47	14,64	0,40	231	20,3	7,5	13,7
MIN2	low	River	-78,8076	0,0182	1066	20,14	19,71	19,92	0,43	78	24,4	13,4	19,1
DUR5_W	low	Permanent	-78,6236	1,0345	268	25,28	24,85	24,99	0,44	26	29,7	20,1	24,7
HVER	low	Permanent	-78,9898	-2,9028	2500	18,57	18,11	18,31	0,46	310	22,3	8,4	15,2
HMACU	low	Permanent	-78,1333	-1,4484	1139	19,58	19,06	19,25	0,52	435	25,8	14,6	20
TENA1	low	Permanent	-77,7388	-0,9349	665	24,27	23,72	23,97	0,55	223	28,7	17,4	22,9
ZJ_RIO	low	River	-77,8622	-1,3568	926	21,27	20,64	20,96	0,63	72	27,1	15,8	21,4
DUR_NINAS	low	Permanent	-78,6232	1,0413	227	24,95	24,21	24,51	0,75	115	29,7	20,1	24,7
AZOLA	low	Permanent	-78,4915	-0,2105	2812	14,60	13,79	14,20	0,81	301	21,2	6,6	14
PITA_MOL	low	River	-78,4073	-0,4104	2830	12,89	11,97	12,46	0,93	546	18,8	5,8	12,4

	Sample site information					Datalogger (ºC)					WorldClim (ºC)		
Datalogger	**Factor**	**Microenv**	**longitude**	**latitude**	**Alt (m)**	**tmax**	**tmin**	**tmean**	**dr**	**N**	**TMAX**	**TMIN**	**TMEAN**
DUR6_W	low	River	-78,6241	1,0419	242	24,74	23,75	24,18	0,99	35	30	20,4	25
REV_1	low	Permanent	-77,5962	-0,0970	1820	17,78	16,80	17,30	0,99	213	25,2	12,7	19
ZJ_DMIN	med	Permanent	-77,8537	-1,3714	937	23,09	22,07	22,53	1,02	85	27,1	15,8	21,3
PUY3	med	Permanent	-77,8206	-1,4444	1034	21,15	20,13	20,62	1,03	453	26,9	15,4	21
REV2	med	Permanent	-77,5945	-0,0978	1820	19,41	18,35	18,82	1,06	213	25,2	12,7	19
PUY_ARR	med	Permanent	-77,7295	-1,4056	1070	22,84	21,76	22,21	1,08	316	26,8	15,2	20,9
LLANG_LAG	med	Permanent	-78,2037	-2,0747	1700	16,84	15,74	16,35	1,10	6	25,1	12,3	18,3
YAS1	med	Forest temp	-76,3979	-0,6736	220	26,29	24,93	25,62	1,20	4	31	19,8	25,2
TOPO3	med	Forest temp	-78,1977	-1,3742	1736	18,90	17,64	18,22	1,26	224	24	12	17,8
PUY_CASA	med	Permanent	-77,7295	-1,4056	1070	22,77	21,15	21,82	1,62	419	26,8	15,2	20,9
PUY_CAIMAN	med	Forest temp	-77,7295	-1,4056	1070	23,83	22,02	22,79	1,80	275	26,8	15,2	20,9
BAEZA	med	Forest temp	-77,8950	-0,4660	1900	18,00	16,14	16,89	1,86	124	24,3	10,9	17,7
PASAJE_W	med	Forest temp	-79,8029	-3,3189	38	26,17	23,97	24,89	2,21	177	31,5	19,9	25

	Sample site information					**Datalogger (ºC)**					**WorldClim (ºC)**		
Datalogger	**Factor**	**Microenv**	**longitude**	**latitude**	**Alt (m)**	**tmax**	**tmin**	**tmean**	**dr**	**N**	**TMAX**	**TMIN**	**TMEAN**
YAS4	med	Forest temp	-76,3979	-0,6736	220	27,52	25,42	26,49	2,25	2	31	19,8	25,2
PACCHA	high	Open temp	-79,6892	-3,5282	1827	18,13	15,74	16,66	2,39	78	23,8	11,5	17,4
BALZAR	high	Open temp	-79,9699	-1,1702	32	28,95	26,50	27,52	2,46	158	31,4	19,4	25,6
ROM1	high	Open temp	-78,9445	-4,1642	1217	24,24	21,44	22,74	2,81	302	29,1	14,9	21,4
MIN3	high	Open temp	-78,8076	0,0182	1066	24,34	20,76	22,01	3,58	78	24,4	13,4	19,1
MIN_DEP.R	high	Open temp	-78,7878	-0,0480	1206	24,52	20,31	21,93	4,21	92	24,1	12,9	18,7
GAS_PSE	high	Open temp	-78,7593	-1,3366	3631	14,48	10,25	12,20	4,23	133	14,2	2,7	8,2
GAS_RIO	high	Open temp	-78,4639	-0,1873	2970	17,33	12,79	14,77	4,55	123	19,6	6,4	13
PL1	high	Open temp	-78,0559	-1,3674	1326	23,03	16,76	18,82	6,27	12	25,9	14,9	20,3
DUR_CACAO	high	Open temp	-78,7527	1,1653	23	32,67	26,30	28,50	6,37	179	30,9	21,4	25,9
MIN_DEP.S	high	Open temp	-78,7879	-0,0479	1207	27,98	20,87	23,79	7,11	92	24,1	12,9	18,7
AT_PUYO	high	Open temp	-78,1922	-2,2739	1415	27,17	18,21	20,52	8,96	37	26,1	13,5	19,4

Table S4.3: Environmental temperature (depenent variable; (**a**) tmin, (**b**) tmax and (**c**) dr) in relation to elevation (Elev) and microenvironment (Microenv; low, medium and high).

(**a**) tmin

```
          Sum Sq Df  F value Pr(>F)    
Elev      581.82  1 366.4862 <2e-16 ***
Microenv    0.02  2   0.0048 0.9953    
Residuals  53.98 34
```

(**b**) tmax

```
          Sum Sq Df F value    Pr(>F)    
Elev      609.92  1  243.48 < 2.2e-16 ***
Microenv  118.94  2   23.74 3.526e-07 ***
Residuals  85.17 34
```

(**c**) daily range (dr = tmax - tmin)

```
          Sum Sq Df F value    Pr(>F)    
Elev        0.33  1  0.2335     0.632    
Microenv  121.64  2 43.0897 4.765e-10 ***
Residuals  47.99 34
```

Table S4.4: Tukey's post-hoc analyses for survival time at 9 ºC. Ean: *E. anthonyi*; Ebo: *E. boulengeri*; Eda: *E. darwinwallacei*; Ema: *E. machalilla*; Etr: *E. tricolor*; Hbo: *H. bocagei*; Hma: *H. maculosus*; Hne: *H. nexipus*; Hpu: *H. pulchellus*.

```
                 Estimate Std. Error z value Pr(>|z|)    
Ebo - Ean      -5.442e-16  4.688e-01   0.000   1.0000    
Eda - Ean       5.878e-01  4.336e-01   1.356   0.8945    
Ema - Ean       1.504e-16  7.596e-01   0.000   1.0000    
Etr - Ean       1.431e-01  3.789e-01   0.378   1.0000    
Hbo - Ean       1.435e+00  3.174e-01   4.522    <0.01 ***
Hma - Ean      -9.900e-16  4.494e-01   0.000   1.0000    
Hne - Ean      -7.785e-16  6.405e-01   0.000   1.0000    
Hpu - Ean       2.303e+00  2.933e-01   7.851    <0.01 ***
Eda - Ebo       5.878e-01  5.040e-01   1.166   0.9536    
Ema - Ebo       6.946e-16  8.018e-01   0.000   1.0000    
Etr - Ebo       1.431e-01  4.577e-01   0.313   1.0000    
Hbo - Ebo       1.435e+00  4.082e-01   3.515   0.0108 *  
Hma - Ebo      -4.458e-16  5.175e-01   0.000   1.0000    
Hne - Ebo      -2.343e-16  6.901e-01   0.000   1.0000    
Hpu - Ebo       2.303e+00  3.898e-01   5.907    <0.01 ***
Ema - Eda      -5.878e-01  7.817e-01  -0.752   0.9973    
Etr - Eda      -4.447e-01  4.216e-01  -1.055   0.9745    
Hbo - Eda       8.473e-01  3.673e-01   2.307   0.2967    
Hma - Eda      -5.878e-01  4.859e-01  -1.210   0.9429    
Hne - Eda      -5.878e-01  6.667e-01  -0.882   0.9919    
Hpu - Eda       1.715e+00  3.467e-01   4.946    <0.01 ***
Etr - Ema       1.431e-01  7.528e-01   0.190   1.0000    
Hbo - Ema       1.435e+00  7.237e-01   1.983   0.5076    
Hma - Ema      -1.141e-15  7.906e-01   0.000   1.0000    
Hne - Ema      -9.289e-16  9.129e-01   0.000   1.0000    
Hpu - Ema       2.303e+00  7.135e-01   3.227   0.0279 *  
Hbo - Etr       1.292e+00  3.008e-01   4.295    <0.01 ***
Hma - Etr      -1.431e-01  4.378e-01  -0.327   1.0000    
Hne - Etr      -1.431e-01  6.325e-01  -0.226   1.0000    
Hpu - Etr       2.159e+00  2.752e-01   7.846    <0.01 ***
Hma - Hbo      -1.435e+00  3.858e-01  -3.720    <0.01 ** 
Hne - Hbo      -1.435e+00  5.976e-01  -2.401   0.2459    
Hpu - Hbo       8.675e-01  1.814e-01   4.783    <0.01 ***
Hne - Hma       2.115e-16  6.770e-01   0.000   1.0000    
Hpu - Hma       2.303e+00  3.662e-01   6.288    <0.01 ***
Hpu - Hne       2.303e+00  5.852e-01   3.935    <0.01 ** 
```