Climatic niche at physiological and macroecological scales:
thermal tolerance–geographic range interface and niche dimensionality

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**ABSTRACT**

**Aim** Under the Hutchinsonian concept of realized niche, biotic interactions and dispersal limitation may prevent species from fully occupying areas that they could tolerate physiologically. This can hamper transferring physiological limits into climatically-defined range limits and distorts inferences of evolutionary changes of adaptive limits (i.e. niche conservatism). In contrast, heritable physiological limits should conform more closely to the position of the niche in the climatic hyperspace. Here we hypothesize that a measure of niche position in the climatic hyperspace is more reliable than niche boundaries to capture the variability and evolutionary pattern of physiological tolerance.

**Location** Neotropic and Palaeartic.

**Methods** We used non-phylogenetic and phylogenetic regressions to test relationships between physiological requirements and macroecological niche features (i.e. based on known species distributions) among anurans. We measure physiological responses through larval critical thermal maximum ($CT_{\text{max}}$), and realized niche in the geographical space through maximum temperature ($T_{\text{max}}$) temperature variability ($T_{\text{var}}$), and the position and breadth of niche at the climatic hyperspace. We also compare evolutionary rates among these parameters using the Phylogenetic Signal-Representation curve.

**Results** $CT_{\text{max}}$ is better related to niche position ($R^2 = 0.414$) than to $T_{\text{var}}$. Further, $CT_{\text{max}}$ is unrelated to both $T_{\text{max}}$ and niche breadth. $CT_{\text{max}}$ and macroecological niche position also show similar, high evolutionary rates, i.e. faster than Brownian motion, whereas $T_{\text{max}}$ and $T_{\text{var}}$ evolve slower, and niche breadth evolves at random.

**Main conclusions** Transferability between thermal tolerance and realized climatic niche limits is weak. Only macroecological niche position in the multivariate climatic
hyperspace correlates with physiological tolerance. It thus appears to be more suitable to
describe the variability and evolutionary pattern of the species’ adaptive limits. We link
these results to ‘niche dimensionality’, as manifold, interacting factors outweigh single
ones in demarcating the species’ realized climatic niche, thereby determining the
conserved upper thermal limits of the species.

**Keywords:** Anuran larvae, $CT_{\text{max}}$, macrophysiology, phylogenetic comparative methods,
Phylogenetic Signal-Representation curve, thermal tolerance.

**INTRODUCTION**

George E. Hutchinson (1957) formalized the modern idea of the ecological niche,
defining it as an $n$-dimensional hypervolume that encompasses all environmental
conditions experienced and all the relationships played by a species. Hutchinson also
distinguished fundamental from realized niches, to demarcate the conditions that species
could live from that they do live on, respectively. He further viewed the species realized
niche reflected into the geographical space (Hutchinson, 1978), a property that would
allow addressing major questions in the interface between the ecological requirements
and broad-scales patterns of species distributions (Colwell & Rangel, 2009; Soberón &
Nakamura, 2009). These questions include patterns of species distribution, diversity
gradients, the assembly of ecological communities, trait evolution and speciation, species
invasiveness and response to global climate changes (see e.g. Wiens & Donoghue, 2004;

The niche–space duality also enabled the development of the field of ecological
niche modelling (ENM; aka. species distribution modelling), which uses environmental
variable and occurrence data to estimate, reconstruct, and forecast the geographic
distribution of the realized species niches at distinct spatial and temporal frames (Guisan & Zimmermann, 2000; but see Hortal et al., 2012). More recently, approaches based on the niche–space duality have resorted to direct measures of physiological data as a means to account for real constraints to the occurrence-based estimates of the realized niche (Kearney & Porter, 2009). These techniques have been referred to as mechanistic niche modelling, to distinguish from the occurrence-based, correlative niche modelling (Kearney & Porter, 2009). Another vein to account for how fundamental niche features are reflected into space is by assessing physiological correlates of the geographical distributions of species, particularly their position and limits (Chown & Gaston, 1999; Calosi et al., 2010; Sunday et al., 2010, 2012; Bozinovic et al., 2011). This bridging between macroecology and physiology has been termed ‘macrophysiology’ (Chown et al., 2004), a field that aims to describe general properties of the interface between individuals’ endurance to environmental conditions and the patterns of geographical distribution of their species (see also Gaston et al., 2009). To avoid the misleading implication that macrophysiology is an effective measure of physiological tolerance, which we demonstrate it is not, herein we will use ‘macroecological niche’ to refer to measures of the realized niche of the species obtained from the relationship between their geographic distributions and current climatic conditions.

Permeating the niche-space transferability is the fact that environment changes in space and time, altering the geography of species, and sometimes forcing them to modify their Hutchinsonian niches (Pearman et al., 2008; Colwell & Rangel, 2009). Whether (and to what extent) niches change has become a key topic in current ecology (Pearman et al., 2008; Losos, 2008; Wiens et al., 2010), which is centred around the ‘niche conservatism hypothesis’, or the tendency of closely related species to share more niche similarities among each other than with less related species (Wiens & Donoghue, 2004).
Under a phylogenetic framework (Losos, 2008; Cooper et al., 2010), this balance between niche evolution and niche conservatism has provided a powerful analytical tool to link evolutionary theory, ecology and biogeography. Most often, studies on this topic have used the distributional limits to infer patterns of macroecological niche conservatism of species’ tolerance to climate across large spatial and temporal scales (e.g. Roy et al., 2009; Olalla-Tárraga et al., 2011).

However, real limits of climatic tolerance may be loosely defined by – or even divorced from – range limits, particularly for terrestrial organisms (Sexton et al., 2009; Buckley et al., 2010 Sunday et al., 2012; but see Calosi et al., 2010). Multiple factors govern the species’ distributional range, but how they combine to define range boundaries are still poorly understood (Pulliam, 2000; Gaston, 2003; Sexton et al., 2009). Soberón & Peterson’s (2005) BAM diagram (from biotic, abiotic, and movement) summarizes in part how different factors affect the species distribution at large spatial scales. Besides tolerance limits (depicted by the abiotic factor), species distributions are also affected by a number of biotic interactions and movement constraints, which modify the geographical response of the species. If one factor falls short of others, the species will fail to accomplish its potential distribution, and the characterization of any of these factors from the observed distribution of the species will be distorted (see Soberón, 2007; Soberón & Nakamura, 2009; Hortal et al., 2012).

Multiple interactions of factors, rather than abiotic constraints alone, set the limits of species ranges, and hence their realized niche (Soberón, 2007). However, no species populations can persist for long outside its tolerance limits (which outline the species fundamental niche in the first place) (see Soberón & Nakamura, 2009). Due to this, the parameters of climatic tolerance of each species remain, to some extent, close to their distribution in the environmental space (Hoffmann et al., 2012). In addition, being a
heritable trait, biophysical tolerance of individuals should evolve precisely in response to the species’ overall position in the climatic space after a spatial and/or temporal change takes place (Huey & Steverson, 1979; Huey & Kingsolver, 1993). This may suggest that a given measure of the species’ modelled niche that weights the position of its centroid in the climatic hyperspace (hereafter niche position) over its limits should be less affected by the biotic and movement constraints that affect its boundaries, thus remaining spatially and phylogenetically related to the physiological features of that species. Consequently, this measure of niche position should outperform climatic boundaries in summarizing both the variation and the evolutionary pattern of the species’ biophysical tolerance (Soberón & Nakamura, 2009).

In the absence of true data on species physiology, Hof et al., (2010) recently used a macroecological measure of niche position to assess the broad-scale patterns of niche conservatism among amphibians. Here, we resort to data on amphibian thermal tolerance to test the hypothesis that a measure of niche position in the multidimensional climatic hyperspace describes the variation in species tolerance at the level of individuals better than climatic niche features drawn from either species’ niche boundaries. To do this, we compare physiological limits characterizing individuals within species to some macroecological metrics of species’ niche, in terms of both explanatory ability and evolutionary rate. This latter analysis describes how traits have evolved along the phylogeny, thus enabling a model-based estimation of niche conservatism/evolution. We use anurans to test which macroecological niche measure (maximum air temperature, temperature variability of the species’ range, multivariate niche position, or niche breadth at the climatic hyperspace) best explains the variation in a true measure of an individual’s physiological performance, the larval critical thermal maximum ($CT_{\text{max}}$). By doing this, we show the divergence between physiological and geographical (i.e. macroecological)
thermal limits and suggest a finer approach to describe the variability and the evolutionary pattern of thermal tolerance that takes into account the multi-dimensionality of the species’ climatic niches.

**METHODS**

**Species Data**

We gathered data about the upper thermal limit of physiological tolerance ($CT_{\text{max}}$) for 47 species of anurans (information on 42 species comes from Duarte et al. [2012], and data for other five species were gathered by H. Duarte, M. Tejedo, and collaborators following the same protocol; see Duarte et al., 2012). The five species of Caudata analysed by Duarte’s et al. (2012) study were excluded. The data employed here comprises species from three communities located at distinct environmental conditions: the subtropical warm Gran Chaco region, the subtropical Atlantic Forest, and Temperate Europe and northern Africa (see Duarte et al., 2012 for further details). However, each of these species is distributed at varying geographical positions and climatic conditions, including most of Neotropical and Palearctic realms (Figure S1). We should anticipate, however, that the low resolution of the climatic data prevents us from refining conclusions on the species’ fine-tuning to particular microhabitats and local conditions. Nevertheless, our aim here is to provide a broad description of how specific thermal features are expressed at coarse scales, after multiple interactions with other niche dimensions have taken place.

Also following Duarte et al. (2012), we used a phylogenetic hypothesis for the 47 anurans according to Frost et al. (2006), including branch length estimation based on three nuclear and two mitochondrial genes. Species missing from the phylogeny had their branch lengths inferred from sister-taxa, which have, by definition, the same age of the
target species (see details in Duarte et al., 2012, including their electronic Supporting Information).

**Physiological data**

$CT_{\text{max}}$ was estimated from anuran larvae under controlled trials in laboratory, following Hutchison’s dynamic method (Hutchison, 1961). Tadpoles were heated until individuals reach the onset of muscular spasms, which was assumed as maximum thermal limit for species tolerance (see details of laboratorial procedure and parameterization in Duarte et al., 2012). Fully aquatic, small anuran larvae can be considered isothermal with the environment (Lutterschmidt & Hutchison, 1997a), so they are expected to mirror the environmental tolerance at the species geographical limits better than adults. In addition, the tadpoles of the species studied develop in temporary, shallow ponds, where thermoclines are virtually absent and individuals are fully subject to the actual thermal variation. Ponds temperatures, in turn, are ruled by the outer climate, which is related to the macroclimatic dominion. Adults, in contrast, are capable to circumvent thermal stress by actively searching for more suitable microhabitats outside the ponds – including fossorial and nocturnal activity, thus their thermal tolerance limits may not match the environmental limits, as those of larvae. Therefore, we can assume that anuran larvae can reasonably represent the susceptibility to thermal variability of the species.

Threshold temperature limits such as $CT_{\text{max}}$ are important parameters for describing the Hutchinsonian fundamental niche as they set hard boundaries for animal survivorship (Huey & Stevenson, 1979; Lutterschmidt & Hutchison, 1997b). Besides, there is a correspondence between upper thermal resistance and optimal temperature of performance in lizards (Huey et al. 2009) and in tadpoles (Katzenberger, M & Tejedo, M, unpublished results). So, variation in $CT_{\text{max}}$, not only may provide insights on species’
fundamental niche position through thermal tolerance itself but also can be a proxy of optimal performance and then explaining sub-lethal viability of species. However, according to its earlier definition, $CT_{\text{max}}$ is “the thermal point at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death” (Cowles & Bogert, 1944). Therefore, it remains challenging to determine the ecological meaning of the $CT_{\text{max}}$ of the larval stage for the anuran species as a whole, and out of controlled laboratorial experiences. Indeed, this is a frequent problem with other organisms used as physiological models (e.g. Castañeda et al. 2012; Ribeiro et al., 2012; also reviewed in Terblanche et al. 2011). Probably even lower temperatures could cause other sub-lethal distresses in nature, including developmental disorders or decrease the ability to forage or to evade from predators, thus undermining the viability of the populations (Huey & Stevenson, 1979). In addition, $CT_{\text{max}}$ is a complex function of experimental heating rates, and usually information of field heating rates is absent (Ribeiro et al., 2012). In this sense, we should assume that, whichever is the outdoor ecological meaning of acute measures of $CT_{\text{max}}$, it should be linearly correlated with an actual measure of thermal tolerance to higher temperatures.

**Macrolecological data**

We gathered data on the geographic distribution of all species from the ‘Global Amphibian Assessment’ database (IUCN, 2009). Maximum air temperature of species distribution ($T_{\text{max}}$) was calculated as the mean of the maxima among grid cells within each species’ range (see below). Temperature variability ($T_{\text{var}}$) was characterized by the range ($T_{\text{max}}$ minus $T_{\text{min}}$) in temperature. We used average measures instead of, say, the maximum of the cells maxima, to circumvent errors in climatic measurements within some species’ range (particularly larger ones).
For the multivariate macroecological niche measurement (see below), we assembled a set of seven environmental descriptors widely recognized as direct or indirect limiting factors constraining the climatic niche of amphibians (mean actual evapotranspiration – AET, mean potential evapotranspiration – PET, maximum temperature of the warmest month, minimum temperature of the coldest month, annual range of temperature, mean annual precipitation, and annual range in precipitation). These environmental variables were drawn from interpolated surfaces of time data series (AET and PET from Willmott & Matsuura, 2001; and the others from Hijmans et al., 2005). Both the species’ geographical range and the environmental variables were projected onto a grid cell system of 1° × 1° resolution covering the geographic region outlined by the distribution of all 47 species together, i.e. parts of the Neotropics and the Palaearctic. Each grid cell defined a sample unit for estimating the environmental maximum temperature and the multivariate niche features.

We used the Outlying Mean Index (OMI; Doledec et al., 2000) to obtain macroecological measures of niche position and breadth in the multivariate climatic hyperspace. OMI is a multivariate ordination technique that calculates the hypervolumetric space of species niche (i.e. a subset of the Hutchinsonian niche in the multidimensional space) according to the selected factors (e.g. environmental variables). OMI characterizes niche breadth, ‘niche marginality’, ‘inertia’ – which provides an estimate of niche overlap – and ‘residual’ variation – which describes the variation in the niche breadth unrelated to the variables of the model. Niche breadth is measured as the dispersion of the sampling units of each species at the multivariate climatic hyperspace, whereas niche marginality describes the amount of differentiation of the species niche relative to a theoretical, average niche that is drawn from the environmental data inputted (Doledec et al., 2000), so it can be interpreted as a measure of niche position in the
climatic hyperspace (see also Hof et al., 2010). Because niche marginality measures the ecological distance of each species relative to an average, theoretical niche, species similarly distant to this mid-point but at opposite points of a niche axis will have similar marginality values. We thus used the species scores along the first axis of the OMI ordination (which encompassed 92.35% of the variation among all axes) as a measure of niche position.

The macroecological and physiological data we use involve measures at two very different scales and levels of biological organization. Therefore, some unavoidable assumptions are required. For instance, that the $CT_{max}$ of the individuals are representative of the entire species; that the geographical range of each species is assumed to describe the distribution of its breeding populations; and that the climatic variables are good enough to reflect suitable conditions for the studied species. Although hard for the data at hand, these assumptions are nonetheless common for virtually all broad-scale studies, especially for our case. This is because we are precisely focused on the possibility of identifying macroscale correlates of the species variability in a physiological property that is shared by all individuals of the species.

**Phylogenetic Comparative Analysis**

We first ran ordinary least square (OLS) regression of $CT_{max}$ against each macroecological niche feature ($T_{max}, T_{var}$ position, and breadth) independently to test if physiological tolerance and climatic niche parameters at the macroscale are capable to explain each other in a direct way. This could help determining, for example, if $CT_{max}$ could systematically approximate the environmental maximum temperature of the species or other niche description. We do not expect, however, $CT_{max}$ and $T_{max}$ to coincide
because $T_{\text{max}}$ may underestimate the maximum body temperature of individual amphibians. Instead, we question whether $CT_{\text{max}}$ and $T_{\text{max}}$ are in some way correlated.

Next, we evaluated if these features are related to each other while accounting for phylogenetic autocorrelation, which can bias significance tests of standard statistical techniques such as OLS, when applied to cross-species data. We analysed the phylogenetic signal using Phylogenetic Signal-Representation (PSR) curves (Diniz-Filho et al., 2012) for each trait, as also a means to access their intrinsic evolutionary rate through the phylogeny. PSR curve is built upon the eigenvectors from the phylogenetic eigenvector regression (PVR; Diniz-Filho et al., 1998), in which the models fit ($R^2$) of successive PVRs of accumulated eigenvectors are plotted against the phylogenetic representation that is given by the accumulated percentage of the corresponding eigenvalues ($\lambda$%). The shape of the curve describes the model of evolution of the trait across the phylogeny. A PSR curve near the reference $45^\circ$ line indicates an evolutionary pattern equivalent to the Brownian motion of trait evolution (Fig. 1), whereas a curve bending below the reference line implies a stronger phylogenetic signal, which can be described by an Ornstein-Uhlenbeck (O-U) process, or by a low lambda model (Freckleton et al., 2002). In contrast, models of accelerated divergence should generate PSR curves traced above the reference line (see Diniz-Filho et al., 2012 for further details). In comparative terms, the trait evolution either slower or faster than an assumed model can be indicative of niche conservatism or niche evolution, respectively (Wiens et al., 2010; Cooper et al., 2010). We used permutations to test the evolutionary model of each trait against a null (random) and a neutral (Brownian motion) model of trait evolution. Departures from these models denote accelerated (PSR<sub>area</sub> > 0.0) or O-U process (PSR<sub>area</sub> < 0.0).
Finally, we tested the associations among traits using a Phylogenetic Generalized Linear Models (PGLS), with maximum likelihood estimation for $\lambda$ (Freckleton et al., 2002). Analyses were run using the PVR and caper packages in R 2.14, R Core Development Team 2010.

RESULTS

Species’ $C_{\text{max}}$ varied from 35.42 to 44.73°C ($\bar{T} \pm SD = 40.29 \pm 2.20$), whereas geographically-measured $T_{\text{max}}$ was considerably lower, as expected, varying between 18.76 and 35.63°C (29.94 ± 4.31). $T_{\text{var}}$ varied from 12.45° to 40.54°C. The multivariate macroecological niche breadth and position (in terms of departure from the theoretical average niche) were slightly higher for some Neotropical species (e.g. _Dendropsophus minutus_, _Hypsiboas raniceps_ and _Trachycephalus venulosus_) than they were for other species (Fig. S2).

According to OLS regressions, $C_{\text{max}}$ was unrelated to $T_{\text{max}}$ ($R^2 = 0.0008; F = 0.0385; P = 0.845$), though it ability in predicting $T_{\text{var}}$ was significant, but weak ($R^2 = 0.105; F = 6.394; P = 0.0150$). In contrast, a significant and substantial amount of variation in macroecological niche position within the climatic hyperspace was explained by $C_{\text{max}}$ ($R^2 = 0.414; F = 31.840; P << 0.001$), although not by niche breadth ($R^2 = 0.013; F = 0.604; P = 0.441$). However, these results may be affected by phylogenetic signal in data.

The PSR curves showed that $C_{\text{max}}$ and macroecological niche position had evolutionary rates slightly, but significantly faster than Brownian motion ($C_{\text{max}}$, PSR\text{area} = 0.037; $p < 0.001$; niche position, PSR\text{area} = 0.026, $p < 0.001$). In contrast, $T_{\text{max}}$ and $T_{\text{var}}$ showed slower rates. Both patterns were described by the O-U processes ($T_{\text{max}}$, PSR\text{area} =
-0.196, \(p < 0.001\); \(T_{\text{var}}\), \(\text{PSR}_{\text{area}} = -0.070, p < 0.001\), whereas the evolutionary pattern of realized niche breadth did not differ from random (\(\text{PSR}_{\text{area}} = -0.237, p = 0.10\)) (Fig. 1).

Because of the phylogenetic signal in data, it is worthwhile to apply comparative analyses to test for relationships among variables. Despite this signal, however, PGLS analyses provided similar results to OLS. These included a non-significant between \(CT_{\text{max}}\) and the \(T_{\text{max}}\) (\(\beta = -0.064 \pm 0.038; p = 0.095\)), a weak, though significant, relationship of \(CT_{\text{max}}\) to \(T_{\text{var}}\) (\(\beta = -0.079 \pm 0.039; p = 0.023\)), and a non-significant relationship between \(CT_{\text{max}}\) and niche breadth (\(\beta = 0.067 \pm 0.739; p = 0.992\)). On the other hand, we found a positive, highly significant explanation of macroecological niche position by \(CT_{\text{max}}\) (\(\beta = 1.965 \pm 0.073; P << 0.001\)) (Table 1; Fig. 2).

**DISCUSSION**

The ability of physiological tolerance limits (e.g. \(CT_{\text{max}}\)) to describe geographical range limits (e.g. \(T_{\text{max}}\)) – and *vice versa* – has been the “holy grail” of ecophysiology (and, more recently, of macrophysiology). Physiological tolerance limits are informative on the susceptibility of species to rapid climatic changes in terms of the maximum amount of heat they can withstand (Duarte *et al.*, 2012). Thus, knowing the relationship between tolerance and range limits would allow both (i) using individuals physiological parameters to infer species distributional shifts during climatic changes (reviewed in Bozinovic *et al.*, 2011), and (ii) inferring species tolerances based on their geographical distributions. The latter is in fact a fairly common practice (e.g. Roy *et al.*, 2009; Olalla-Tárraga *et al.*, 2011), despite the lack of knowledge about how physiological tolerance relates with current species distributions. Strikingly, our results evidence that upper physiological limits alone may fail in characterizing macroecological (i.e. geographical) climatic boundaries of species’ distributions, such as maximum air temperature or a
multivariate measure of niche breadth within the climatic hyperspace. Conversely, a
climatic parameter that reflects realized variability ($T_{\text{var}}$) can be better described by $CT_{\text{max}}$
than a single climatic limit (Clusella-Trullas et al. 2011), although this explanatory ability
was weak in our case.

It can be argued that the lack of or low predictability of $T_{\text{max}}$ and $T_{\text{var}}$ is due to
other climatic parameters being more important for defining the thermal limits of the
studied species in the geographical space (e.g. Addo-Bediako et al., 2000; Sunday et al.,
2010). However, the rationale of the physiological–geographical transferability of
climatic tolerance, as has been applied, builds upon the assumption that tolerance limits
define some boundaries of the species fundamental niche and, as species ranges reflect
their niche at the geographical space, tolerance and range boundaries should mirror one
another (Calosi et al., 2010). However, there is more in a species’ distribution than
climatic requirements. Besides various sources of noise in tolerance estimates that can be
anticipated (Terblanche et al., 2011; Castañeda et al., 2012; Ribeiro et al., 2012),
geographical ranges reflect the limits of the macroecological niche after it interacts with a
series of other recent and historical factors, particularly biotic interactions and constraints
to movement (i.e. biogeographical processes and occupancy dynamics; Hortal et al.,
2010), that conform the realized niche (i.e. the BAM diagram of Soberón & Peterson,
2005; see Soberón, 2007; Soberón & Nakamura, 2009; Godsoe, 2010). When the
geographical projection of these three dimensions (biotic, abiotic and movement) fails to
fully overlap with each other, the species will inhabit only a subset of its potentially
suitable area, and hence the response to a single dimension will fail to predict the whole
species’ distribution (see discussion in Hortal et al., 2012). As a consequence, the
physiological–geographical transferability would only be possible in the particular case in
which these three dimensions fully coincide in the geographic space.
The same reasoning made for $T_{\text{max}}$ and $T_{\text{var}}$ applies to niche breadth. This latter measure summarizes the range of environmental conditions that are experienced by each species. Thus, it is also related to the conditions in the boundaries of the regions where the species are distributed. Our results also show that $T_{\text{max}}$, $T_{\text{var}}$, and niche breadth may differ from $CT_{\text{max}}$ in their evolutionary rates. In fact, there is evidence of both faster and slower rates of evolution for either physiological (Huey & Kingsolver, 1993; Angilletta et al., 2002; Kellermann et al., 2012) or macroecological (Pearman et al., 2008) niche features. Within a clade, different features in the same clade may also evolve at specific rates, or yet have varying rates through evolutionary time (Pearman et al., 2008). Therefore, the observed patterns of trait evolution are contingent to the trait and the clade involved. However, different rates – or amounts – of trait change can yield different conclusions on patterns of niche conservatism/evolution for these traits, which in turn can indicate different evolutionary processes (Cooper et al., 2010). Flawed inferences of trait change may therefore misguide conclusions on the evolutionary process of that trait. Since the evolutionary pattern observed in $T_{\text{max}}$, $T_{\text{var}}$, and niche breadth should incorporate other factors affecting distributional range, then these macroecological niche parameters – that depict the boundaries of the response of the species to climate – may lead to inaccurate conclusions on the actual evolutionary pattern of thermal tolerance, if taken as a measure of such aspect of the fundamental niche.

A caveat of our results comes from the quality of the macroecological data used. It is possible, for example, that coarse range maps are poor descriptors of both the geographical (Hurlbert & White, 2005) and climatic limits of the species (which are also coarse). In such case, the poor ability of climatic limits measured in the geographical space (e.g. $T_{\text{max}}$ or $T_{\text{var}}$) to describe physiological limits could be an artefact caused by deficiencies in the data. The same problem would affect the estimation of the
evolutionary rate through the PSR curve. That is, because most species share part of their
distribution, it would be possible that an overall low ability in discriminating their
climatic boundaries make their climatic limits to be most similar than expected by chance
(i.e. Brownian motion), thus resulting in a more conserved pattern of trait evolution. In
fact, some of these caveats are related to the scaling issue referred before, for we are
dealing with variables that stand at contrasting spatial scales (pond vs. continent) and
levels of organization (individuals vs. species). In addition, our physiological data comes
from tadpoles, whereas macroecological data characterize terrestrial environments of
adults. Although the broad-scale distributions of both larvae and adults should coincide,
we overlook possible, particular developmental modifications in the physiological
machinery of each species.

From the physiological standpoint, some important information needed to discuss
species tolerance limits is also missing. Species’ physiological limits are defined by the
pool of physiological tolerance limits of the individuals, including acclimation and inter-
individual plasticity in critical temperatures, and are expected to vary according to the
conditions of their position in the species’ range (Huey & Stevenson, 1979; Hoffmann et
al., 2012). However, we only have individual limits under acute change, i.e. a subset of
the range of thermal tolerances that characterize the entire species.

From a macroecological point of view, there is still the challenge of identifying
the species’ range limits accurately, even for well-known species. Many factors besides
those summarized in the Soberón & Peterson’s (2005) BAM scheme are known to cause
the species’ range to behave dynamically. They include, for example, population’s
source-sink dynamics (Pulliam, 2000), adaptation at peripheral distributions, Allee effect,
among many others (see e.g. Sexton et al., 2009). The dynamic nature of range limits is
common even during environmentally stable periods, and may often hamper their clear
demarcation (Gaston, 2003), especially in the context of the realized niche (i.e. presupposing non-negative population growth rates). In addition, species abundance tends to clump around the centroid of the environmental space, thus making suboptimal sites placed farther from this environmental optimum to represent the species inherited niches poorly (Martinez-Meyer et al., 2013). Therefore, discriminating between niche conservatism and niche evolution from the species distributional limits is, at least, problematic because of the coarseness of range maps, the dynamic nature of geographic ranges, and the decay of niche optimality towards its boundaries, particularly when inference about niche conservatism comes from single variables. This is expected because these inferences may take into account the sort of factors involved in range determination that prevents the species to occupy suitable regions.

We have shown, however, that a multivariate description of the species climatic niche – its position in the climatic hyperspace – may provide a reasonable characterization of both among-species variability and the evolutionary rate of physiological tolerance. Although this measure of niche position also derives from the climatic domain defined by the species’ distribution (thus being subject to the same sources of error of both range maps and climatic data layers), species range boundaries seem to be better described by combined rather than single climatic parameters (Kellermann et al., 2012; Smith, 2012). Accordingly, combinations of factors (e.g. temperature and humidity) and properties of these factors (e.g. total amounts, variability, range) impose direct and indirect limits to the species niche, thus outperforming single parameters in demarcating their position in this climatic hyperspace.

What our findings emphasize beyond any doubts is the importance of taking into account the multiple dimensions of the modelled niche while studying niche conservatism or niche evolution. Although dimensionality is a central part of Hutchinson’s (1978)
concept of the niche, it is often seen as a caveat to understand the conservatism/evolution of particular niche dimensions (e.g. Peterson, 2011). Of course, pooling in as many factors as possible to describe the niche may be of little help to understand its evolutionary dynamics, particularly because of data collinearity. But we provide empirical evidence that relying on a single dimension may not only be insufficient, but rather misleading (see Godsoe, 2010 for an in-depth discussion on the caveats of identifying niche features from incomplete environmental measurements). Taking into account dimensionality in studies of niche dynamics may improve our understanding on the variability and evolution of fundamental attributes (e.g. physiological) of the species, which ultimately determine their endurance across temporally and spatially changing conditions. In addition, this approach may circumvent the problem of dealing with macroecological variables that are more subject to external constraints, such as climatic boundaries or niche breadth drawn from the species’ geographical distribution. This leaves the question on the number of niche dimensions that should be included in the macroecological niche description (Godsoe, 2010), which depends on the context and the taxon involved. If our approach proves useful, defining the modelled niche dimensions to be studied would be a necessary step for any investigations of niche evolution. Here, making available additional data on the fundamental properties of physiological endurance of species, together with a proper manner to handle them, is of critical importance.

Our results may also foster discussion on the differences between correlative and mechanistic niche models (Kearney & Porter, 2009; Buckley et al., 2010). On the one hand, physiological limits of species may fail in predicting their climatic limits, either currently or after potential range shifts, thus supporting previous reservations about the accuracy of mechanistic models in estimating realized niches (Buckley, 2010; Buckley et
On the other hand, the link of a physiological feature (i.e. $CT_{\text{max}}$) to the macroecological climatic niche position of species reinforces the importance of considering studies on species’ fundamental traits to advance the field of species distribution modelling (Kearney & Porter, 2009; Buckley, 2010; Buckley et al., 2010). Nonetheless, and despite the problems of using correlative models of species distributions to describe adaptations to climate (see Hortal et al., 2012), our results point out that multivariate descriptions of climatic niche are needed to address questions on the conservatism or evolution of upper adaptive limits (Peterson et al., 1999; Araújo & Peterson, 2012).

Hof’s et al. (2010) proposition on broad-scale evolutionary pattern of species’ climatic tolerance drawn from a similar macroecological measure of niche position finds empirical support in our study. However, the differences in taxonomic resolution and comprehensiveness impair a direct comparison between their results and ours. In fact, it is possible that our findings are benefited by particular features of amphibians. In general, among ectotherms, upper thermal limits (e.g. $CT_{\text{max}}$) are less spatially variable and more phylogenetically constrained than other physiological responses, such as lower thermal limits (reviewed in Hoffmann et al., 2012). If this is the case of amphibians, Hof’s et al. (2010) results on the general tendency for retaining the realized climatic niche in the geographical space, together with ours, suggest an explanation to the parallelism between $CT_{\text{max}}$ and macroecological niche position. Accordingly, the ecological and evolutionary ‘hardness’ of the upper boundary of the tolerance to temperature makes it more closely related to the climatic hyperspace where the species’ multidimensional niche is centred.

A final issue that is critical for the interpretation of our results is phylogenetic scale. Depending on the scale investigated, one can draw distinct conclusions on the species adaptability to changing climates and inferences of niche conservatism/evolution...
Our dataset covers species with varied phylogenetic distances, from deep temporal distances (than 200 Myr, between Alytidae/Pelobatidae and the remaining clades) to relatively close ones (such as the species within the *Leptodactylus* genera, separated ca. 5 Myr) (Wiens, 2011; Figure S3).

It is likely that our results on evolutionary rates reflect average large phylogenetic distances among clades (e.g. families or genera), and for this scale, larger amounts of fundamental niche change may be the standard expectation. In this case, the term “faster” as employed here is only relative to the other traits investigated and to the evolutionary model of reference, i.e. the Brownian motion model. It does not imply that anurans are capable to track rapid climatic changes, in shorter time scales (e.g. decades or hundreds of years).

**CONCLUDING REMARKS**

By combining physiological experimental data, macroecological and phylogenetic data coupled with evolutionary models, we have shown that both the variability and the evolutionary pattern of physiological limits, such as $CT_{\text{max}}$, may be loosely described by the variables that characterize the realized limits of species distributions, such as $T_{\text{max}}$, $T_{\text{var}}$, or niche breadth. These findings challenge the transferability of physiological data into the geographical space, warning for the usage of macroecological environmental limits measured from species distribution ranges as indicators of tolerance in studies on both the effects of climatic shifts on species distributions and niche conservatism/evolution. Supporting our claim is the fact that species range limits, and hence their realized niche, are also determined by other factors different from climate (e.g. abiotic, biotic, movement, population dynamic and intraspecific variability).
In contrast, we show that the among-species variability and evolutionary pattern of $CT_{\text{max}}$ can be better described by a multivariate measure of the macroecological niche position in the climatic hyperspace. We attribute this result to the lower lability of both upper thermal limits and species niche as a whole, which may be linked to the interaction of multiple environmental factors in exerting direct and indirect constraints on the species distribution and realized niche, a property that permeates the definition of niche since Hutchinson (1957), i.e. the multi-dimensionality of the niche. Our results also warn for some applications of mechanistic and correlative species distribution modelling (i.e., niche modelling), regarding inferences of realized niches and patterns of niche conservatism, respectively. Further studies involving closely related species – for which fundamental attributes of the Hutchinsonian niche (e.g. physiology, interaction, and dispersal limitations) are known – are of primer importance to understand their effect on the evolutionary and spatial dynamics of the niche.

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Sidney F. Gouveia, Joaquín Hortal, Fernanda A. S. Cassemirom and José Alexandre F. Diniz-Filho work on different aspects of biogeography, macroecology and macroevolution.

Miguel Tejedo, Helder Duarte and Carlos A. Navas work on ecophysiology and evolutionary physiology.
**Table 1.** Phylogenetic Generalized Least Squares (PGLS) models between $CT_{\text{max}}$ and macroecological niche traits. $\lambda$ is the index that transforms the trait phylogeny to fit a Brownian motion model. $\beta =$ models slope; $\varepsilon =$ standard error.

<table>
<thead>
<tr>
<th>$CT_{\text{max}}$ vs.</th>
<th>$\lambda$</th>
<th>$\beta$</th>
<th>$\pm \varepsilon$</th>
<th>$F$-value</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{\text{max}}$</td>
<td>0.980</td>
<td>0.064</td>
<td>0.038</td>
<td>2.9</td>
<td>0.09</td>
</tr>
<tr>
<td>$T_{\text{var}}$</td>
<td>0.964</td>
<td>-0.079</td>
<td>0.039</td>
<td>4.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Niche position</td>
<td>1.00</td>
<td>1.965</td>
<td>0.073</td>
<td>717.6</td>
<td>$2.2 \times 10^{-16}$</td>
</tr>
<tr>
<td>Niche breadth</td>
<td>0.969</td>
<td>0.067</td>
<td>0.739</td>
<td>0.0</td>
<td>0.99</td>
</tr>
</tbody>
</table>
**Figure 1.** Phylogenetic Signal Representation (PSR) curves showing the evolutionary rates of critical thermal maximum ($CT_{\text{max}}$), geographical maximum temperature ($T_{\text{max}}$), temperature variability ($T_{\text{var}}$), niche marginality (the 1st axis of OMI – Outlying Mean Index) and niche breadth for 47 anurans. Lighter and darker grey bands are the confidence intervals for the neutral (Brownian motion) and null (random) expectations, respectively (Diniz-Filho et al., 2012). Note that $CT_{\text{max}}$ and OMI 1st axis have very similar patterns of evolution (i.e., slightly faster than Brownian motion).

**Figure 2.** Phylogenetic Generalized models between the anuran larvae $CT_{\text{max}}$ and macroecological climatic niche features: a = niche position (the 1st axis of OMI – Outlying Mean Index); b = $T_{\text{max}}$; c = $T_{\text{var}}$; d = niche breadth.
FIGURES

Figure 1
Figure 2

(a) Observed values vs. fitted values.
(b) Observed values vs. fitted values.
(c) Observed values vs. fitted values.
(d) Observed values vs. fitted values.
SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Spatial distribution and local richness after overlapping the extent of distribution of all 47 anurans onto a cells grid of 1° × 1° resolution.

Figure S2. Interspecific variability of climatic niche traits ($CT_{max}$; $T_{max}$; $T_{var}$; niche position and niche breadth) among 47 anurans. Species are ordered alphabetically.

Figure S3. Non-ultrametric phylogeny for 47 anurans, after Frost (2006). Different colours denote different families.
Figure S1

Figure S2
Figure S3