Physiological constraints on geographic distributions of species

By

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Abstract

Understanding species' geographic distributions constitutes a major priority in biodiversity science, biogeography, conservation biology, and evolutionary biology. Species' geographic distribution are shaped by abiotic (climate) factors, biotic (e.g., resources for survival, competitors) factors, and dispersal factors. In this dissertation, I have used physiological parameters measured in the laboratory under controlled conditions to understand constraints on species' distributions.

In my first chapter, I explored how parameters documented in detailed physiological studies could be used to understand the constraints on the geographic distribution of Spansh moss (*Tillandsia usneoides*). I used four physiological parameters of Spanish moss that circumscribe optimal conditions for the species for survival and growth. Using high-temporal-resolution climate data, optimal and non-optimal areas in the species' geographic distribution could be identified. My results indicated that Spanish moss survives under suboptimal conditions for few days in many parts of its geographic distribution, although numbers of days differed for various physiological parameters. This chapter was published in *Global Ecology and Biogeography*.

Continuing from the first chapter's results, I investigated whether optimal physiological parameters are available for Spanish moss populations specifically during the flowering/fruiting season. Flowering/fruiting season is an important life stage for plant species, as it is during this period that the plant produces new recruits for maintaining populations. Results in this chapter indicated that flowering/fruiting period of Spanish moss frequently is under suboptimal

conditions, but that the flowering period tends to be tuned such that Spanish moss populations receive at least one optimal physiological parameter, and generally the parameter emphasized is that of minimum temperature. This chapter has been reviewed for publication at *AOB Plants*, has been revised to meet the reviewers' expectations, and is now again under consideration by the journal editors.

In the third and final chapter, I analyzed 33 anuran species for the critical maximum temperature parameter (CT_{max}). CT_{max} plays a crucial role in larval stages of anuran species. I evaluated whether any part of the species' distribution experiences CT_{max}, and whether this CT_{max} is being experienced more often in recent years as a consequence of warming climates. My analysis supported the idea that 70% of the anuran species experienced CT_{max} at some point over a 22-year time period. However, only a single species saw CT_{max} being experienced across its distribution more often through time. This manuscript is in preparation for submission for publication.

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I am grateful to Craig Martin, without his support my dissertation would not be complete. He helped me learn a lot about plant physiology. His special seminar class was a lot of fun with discussion on science as well as on black metal music. wish to thank Nathaniel Brunsell and Robert Moyle for being in my committee and helping me move forward in my research.

I would like to thank the KU niche modeling group, a vibrant and enthusiastic group, which improved my understanding about various modeling techniques. I would also like to thank the people in the Ornithology division in the Biodiversity Institute. I would like to thank Mark Robbins, who allowed us to use his office space during our last few days in our stay here at KU. A number of people have supported me during my PhD career in KU. I would like to thank specially, Alberto Jimenez Valvarde, Monica Papes, Arpad Nyari, Andres Lira, Yoshinori Nakazava, Lynnette Donark, and Lindsay Campbell.

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Introduction

Understanding geographic distributions of species represents a major priority in biodiversity science, biogeography, conservation biology, and evolutionary biology. Ecological niche modeling (ENM) and the related ideas termed “species distribution modeling” are techniques that have become popular in recent years, in light of their characterization of distributions and simplicity of implementation, as well as given broad availability of necessary data on species’ occurrences and environmental landscapes (Peterson *et al.*, 2011). With these correlative approaches, known occurrences of species are related to suites of environmental variables to estimate species’ ecological niches and identify corresponding potential geographic distributions. As these methods estimate niches based solely on environmental associations of known occurrences of species, however, they make no use of information that may be available regarding physiological tolerances of species.

A distinct set of approaches to understanding distributions of species makes explicit consideration of morphology, behavior, and physiological limits as they relate to distributional ecology. In these “biophysical” or “mechanistic” models, energy budgets and energy balance equations are developed as functions of characteristics of organisms under different conditions (Porter *et al*. 1973). These models are then related to maps of climate and other environmental features to identify areas as habitable or non-habitable under the assumptions of the models. For example, Niche Mapper™ (Porter & Mitchell 2006) incorporates aspects of behavior, morphology, and physiology, in relation to macro- and micro-scale environmental dimensions.

Heat-energy-balance equations are developed based on morphological, behavioral, and physiological traits of the species in question; through evaporation terms, water balance can be incorporated as well. Once these equations are established, available energy is calculated from microclimate models, and the potential distribution of the species can be estimated across the landscape (Porter *et al*. 1973; Porter *et al*. 2002; Kearney & Porter 2004; Porter & Mitchell 2006).

Both biophysical and correlative modeling approaches, however, have significant and substantive weaknesses. Biophysical models have been developed for relatively few species, are information-intensive, are highly parameterized owing to consideration of energy requirements, and require many assumptions, but represent a clear path to characterization of fundamental ecological niches of species (Peterson *et al.*, 2011). Correlative models, on the other hand, are simple, and may ignore biologically relevant facts, but are informative if placed within an improving conceptual framework (Peterson *et al.*, 2011). Model implementation in ENM is dependent on understanding configurations of relevant abiotic, biotic, and dispersal factors: using the conceptual framework referred as BAM (Soberón & Peterson, 2005; Soberón, 2007), model calibration is robust when the species' distribution is limited by abiotic factors and not by dispersal ability (Saupe et al., 2012). Hence, neither of the two dominant approaches is entirely satisfactory, which demands exploration of additional approaches and ideas that can enrich and educate research efforts.

This dissertation is in effect a broad overview and series of case studies of the role of physiological constraints in delimiting species' geographic distributions. The work centers on using detailed measurements of physiological parameters from other studies in tandem with high-temporal-resolution climate data. The result is an exploration of how physiological tolerances scale across many orders of magnitude to translate into limitations on geographic distributions of species.

In the first two chapters, I investigated distributional constraints on Spanish moss (*Tillandsia usneoides*) using detailed physiological measurements performed in the laboratory by Craig Martin, and 6 hourly weather/climate data covering the period 1989-2010. I explored the geographic distribution of Spanish moss using traditional correlative niche modeling approaches, and then compared the outputs to results of temporal scaling of optimal physiological conditions in the climate data. In the second chapter, I examined the timing of flowering and fruiting by Spanish moss populations across the species' broad geographic range, in relation to availability of optimal physiological conditions. I used herbarium specimen records of flowering and fruiting Spanish moss to identify population-specific flowering and fruiting periods, and tested detailed environmental data for associations with minimum temperature, maximum temperature, relative humidity, and rainless days requirements on a univariate basis.

Finally, in my third chapter, I analyzed 33 anuran species in relation to their critical maximum temperature (CT_{max}) values during the breeding period across each species' geographic range.

For anuran species, critical maximum temperature (CT_{max}) in larval stages represents an important constraint on life cycles. An individual experiencing conditions approaching CT_{max} has higher chances of death or abnormal larval development, which in turn is reflected in declining recruitment to reproductive populations. Little research has been done in regard to how often species experience CT_{max} temperatures in real life, or whether the frequency of exposure to CT_{max} is increasing over time as a consequence of climate change. Hence, this contribution, I used high-temporal-resolution climatic data to understand what parts of species' distributions experience conditions approaching CT_{max} and whether species have been experiencing CT_{max} increasingly frequently over the past two decades.

Chapter 1

The role of physiological optima in shaping the geographic distribution of Spanish moss[1]

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[1] Barve, N., Martin, C., Brunsell, N. A., & Peterson, A. T. (2014). The role of physiological optima in shaping the geographic distribution of Spanish moss. *Global Ecology and Biogeography*, *23*(6), 633–645. http://doi.org/10.1111/geb.12150

Abstract

To understand species' geographic distributions, ecological niche modeling is seeing broad application, in spite of challenges regarding estimation of fundamental niches that limit model transferability over time and space. Mechanistic models are an alternative, but can be difficult to implement, owing to the detailed knowledge that they require about the organism for full parameterization. In this paper, we explore the geographic projection of physiological measurements of optimal temperature, precipitation, and relative humidity requirements, as measured under controlled conditions, using high temporal resolution climate dataset as a case study for Spanish moss (*Tillandsia usneoides*), and compare scaling effects with correlative niche models calibrated in Maxent. We used high-temporal-resolution climate data to understand how often and where Spanish moss populations occur under optimal and sub-optimal conditions with respect to different environmental variables across their geographic range. We used higher-spatial-resolution weather station data for the United States to provide a finer-grained view. We also developed ecological niche model, to show how averaged climate data can present inaccurate views of physiological thresholds of the species. Few populations of Spanish moss are located at sites presenting sub-optimal conditions for more than two environmental parameters. The northern distributional limit of Spanish moss is set by minimum temperature requirements, whereas maximum temperatures are less limiting. However, when the same occurrences are analyzed with respect to averaged climate data, 95% of populations appear to fall within the optimal physiological intervals. Our analyses revealed that most Spanish moss populations do not experience optimal ecophysiological conditions for all environmental variables, even over long time scales. Physiological data may be of limited utility in delimiting suitable areas for populations of species, but offer unique perspectives on causes of range limitation.

Introduction

Understanding geographic distributions of species represents a major priority in biodiversity science, biogeography, conservation biology, and evolutionary biology. Ecological niche modeling (ENM) and the related species distribution modeling are techniques that have become quite popular in recent years, in light of their characterization of distributions and simplicity of implementation, as well as given broad availability of necessary data on species' occurrences and environmental landscapes (Peterson *et al.*, 2011). With these correlative approaches, known occurrences of species are related to suites of environmental variables to estimate species' ecological niches and identify corresponding potential geographic distributions. As these methods estimate niches based solely on environmental associations of known occurrences of species, however, they make no use of information that may be available regarding physiological tolerances of species.

A distinct set of approaches to understanding distributions of species makes explicit consideration of morphology, behavior, and physiological limits as they relate to distributional ecology. In these biophysical or mechanistic models, energy budgets and energy balance equations are developed as functions of characteristics of organisms under different conditions (Porter *et al*. 1973). These models are then related to maps of climate and other environmental features to identify areas as habitable or non-habitable under the assumptions of the models. For example, Niche Mapper™ (Porter & Mitchell 2006) incorporates aspects of behavior, morphology, and physiology, in relation to macro- and micro-scale environmental dimensions. Heat-energy-balance equations are developed based on morphological, behavioral, and physiological traits of the species in question; through evaporation terms, water balance can be incorporated as well. Once these equations are established, available energy is calculated from microclimate models, and the potential distribution of the species can be estimated across the landscape (Porter *et al*. 1973; Porter *et al*. 2002; Kearney & Porter 2004; Porter & Mitchell 2006).

Both biophysical and correlative models, however, have weaknesses. Biophysical models have been developed only for a relatively few species, are information-intensive, are highly parameterized due to consideration of energy requirements, and require many assumptions, but represent a clear path to characterization of fundamental ecological niches of species (Peterson *et al.*, 2011). Correlative models, on the other hand, are simple, and may ignore biologically relevant facts, but are informative if placed within an improving conceptual framework. Model implementation in ENM is dependent on understanding configurations of relevant abiotic, biotic, and dispersal factors: using the conceptual framework referred as BAM (Soberón & Peterson, 2005; Soberón, 2007), model calibration is robust when the species' distribution is limited by abiotic factors and not by dispersal ability (Saupe *et al.*, 2012).

In this paper, we examine the geographic distribution of Spanish moss (*Tillandsia usneoides*) using traditional correlative niche modeling approaches and then compare scaling effects with results from temporal scaling of optimal physiological conditions in the climate data. These physiological measurements were carried out under controlled environmental conditions;

implications of these measurements are explored over the species' entire geographic distribution. Our goal was to extend micro-scale, individual-plant-based physiological measurements to continent-wide resolutions and extents. Specific questions were (1) what periods of time outside optimal threshold values of temperature and precipitation must be withstood for persistence, and (2) can physiological measurements taken at a single site near a distributional extreme be relevant to illuminate distributional constraints across a species' entire geographic range. The result is a picture of what might be termed 'expected physiological distribution': a view of distributional constraints across multiple scales of time and space. Comparing results with ecological niche models developed using Maxent illustrates important scaling issues in climate data.

Methods

Study Organism

Spanish moss (*Tillandsia usneoides*) is an epiphytic flowering plant of the family Bromeliaceae, is distributed between approximately 38°N and 38°S latitude. It typically grows in warm and humid climates on trees or other supporting structures, such as telephone or power cables (Billings, 1904; Garth, 1964; Callaway *et al.*, 2002). Spanish moss occurs over a broad elevational range (about 100 to 3300 m), and associations with atmospheric moisture content and temperature vary significantly according to elevation (Gentry & Dodson, 1987; Kreft *et al.*, 2004). The species does not occur at very high elevations, which are apparently too cold for its persistence. No specific biotic interactions have been observed to affect Spanish moss distribution, except a possible association with a spider *Metaphidippus tillandsiae* in the

Mississippi Delta region (Young & Lockley, 1989). The general natural history of Spanish moss suggests that its distribution will prove to be highly constrained by climatic factors (Garth, 1964), more or less in line with the “Hutchinson’s dream” scenario of Saupe *et al.*, (2012).

Temperature, humidity, and drought conditions are known to affect growth and persistence of Spanish moss (Garth, 1964; Martin & Siedow, 1981; Martin *et al.*, 1981; Martin & Schmitt, 1989). A year-long field experiment (May 1978 to May 1979) was performed by Martin *et al*. (1981) near Elizabethtown, North Carolina (78.594ºW, 34.682ºN), and found that Spanish moss growth is concentrated in summer months, whereas winter growth is almost negligible. Martin *et al.* (1981) showed that CO_2 uptake is maximal when daytime temperature is 5–35ºC; CO_2 uptake was eliminated at or below 0ºC and at or above 40ºC. Kluge *et al*. (1973) also performed experiments on Spanish moss in the laboratory, with similar results regarding CO_2 uptake; however, they used greenhouse-grown Spanish moss, and their experiment was done in the laboratory under constant temperature and humidity. Martin *et al*. (1985, 1986) assessed North Carolina Spanish moss populations with respect to irradiance effects on morphology and physiology, finding that Spanish moss responds to irradiance by adjusting physiology more than morphology. Studying Spanish moss populations from Newton, Georgia, Garth (1964) showed that, without periodic rain, Spanish moss cannot survive, even when water is supplied externally; he found that Spanish moss has optimal performance only with $\leq$15 consecutive rainless days, and Martin *et al*. (1981) corroborated this result with additional information that CO_2 uptake is minimal when Spanish moss is wet by rain, suggesting that Spanish moss does not prefer locations where it rains every day, but rather needs some dry periods. Overall, then, results from these experiments suggest four parameters that could be analyzed at continental extents: minimum temperature ≥5ºC (Martin *et al.*, 1981), maximum temperature ≤35ºC (Martin & Siedow, 1981), nighttime humidity ≥50% (Martin *et al.*, 1981), and rainless days ≤15 (Garth, 1964).

Data

We examined how these thresholds are met (or not) for Spanish moss across the Americas over a 22 year period (Jan 1989 – Dec 2010). We used the ERA interim reanalysis climate data developed and supplied by the European Center for Medium-Range Weather Forecasts. These data are based on a combination of models and observations, with 3-hourly temporal resolution; every second datum is a forecast, whereas the other is a model result. We used only the model result data, thus reducing the 3-hourly dataset to a 6-hourly dataset. The dataset has a somewhat coarse native spatial resolution of 1.5° x 1.5°, or approximately 165 x 165 km at the Equator

ERA interim data were processed to generate optimal and sub-optimal areas with respect to each variable through time. Data were downloaded from http://data-portal.ecmwf.int/data/d/interim_daily/ for the following parameters: minimum temperature at 2 m, maximum temperature at 2 m, mean temperature at 2 m, dew point temperature at 2 m, and precipitation. The ERA interim data are stored in NetCDF format (http://www.unidata.ucar.edu/packages/netcdf/index.html; Rew & Davis 1990); these data were processed via the “ncdf” package in R (R Core Development Team 2008; Pierce 2011).

In the relatively coarse global climatic model data, details are averaged over broad areas and may be lost. For a finer-resolution view, we used data from the United States Historical Climate Network (USHCN, http://cdiac.ornl.gov/epubs/ndp/ushcn/ushcn.html), which are a subset of the Global Historical Climatology Network (GHCN) network. In total, weather station data were available from 1218 stations across the United States; these stations are also part of the Cooperative Observer (COOP) Network, which records precipitation details for the country. We buffered the United States portion of the Spanish moss range by 700 km, and data from the 608 weather stations within that area were downloaded for analysis. We extracted daily data from 1 Jan 1989 to 31 Dec 2010 for minimum temperature, maximum temperature, and precipitation.

To develop daily surfaces for temperature and precipitation using USHCN weather station data, we used elevation as a covariate in simple kriging model. Elevational data were downloaded from (http://topotools.cr.usgs.gov/GMTED_viewer/) at a spatial resolution of 30” (i.e., about 1 km at the equator), which were resampled to 5’ resolution (about 10 km) to match distances approximately among weather stations in the original data. Surfaces were fitted using variograms with R packages ncdf, raster, and geoR (Ribeiro & Diggle, 2001; Diggle *et al.*, 2003; Diggle & Ribeiro Jr., 2007; Bivand *et al.*, 2008; Hijmans & van Etten, 2012). These krigged surfaces for minimum temperature, maximum temperature, and precipitation were stored in NetCDF format (Pierce, 2011) for the entire time period. Root mean square (RMS) error was checked for interpolated data; RMS error measures error between observed values and values predicted by the model: 0 indicates perfect fit, while large values are considered bad model predictions. RMS errors were all ≤3, indicating robust predictions (Hyndman & Koehler, 2006).

For the development of ecological niche models, we used ERA interim climate data for generation of ‘bioclimatic’ variables using the “dismo” package in R (Hijmans *et al.*, 2012). To make niche models comparable with the physiological distribution model, we used only those variables used to analyze the physiological limits. We generated average relative humidity using dew point temperature and the mean temperature (Stull, 1988); we also generated maximum numbers of rainless days for each grid cell as a count from the data. Other bioclimatic variables used were annual mean temperature, maximum temperature of warmest month, minimum temperature of coldest month, mean temperature of warmest quarter, mean temperature of coldest quarter and annual precipitation. We employed Maxent (Phillips *et al.*, 2006) to develop niche models with default settings, except that we used 50% random subsetting with 100 bootstraps, and 2500 iterations for model calibration. The average of bootstrap models was thresholded by reclassifying the suitability of pixels to 0 below the highest suitability value that included 95% of occurrence points used in model calibration (Peterson *et al.*, 2011).

Data Analysis

An R script was developed using the raster, ncdf, and sp packages (Bivand *et al.*, 2008; Pierce, 2011; Hijmans & van Etten, 2012) to identify suitable and unsuitable areas for Spanish moss in terms of its physiological thresholds. For the ERA interim data, for minimum and maximum temperatures, the script checks the value of each variable across four daily observations; a grid square was marked as unsuitable for a day whenever two consecutive observations were outside the limit. For precipitation, whenever all four daily observations were 0 (i.e., no precipitation), it was considered as a day with no precipitation, and all consecutive sets of 15 days were checked; when any 15-day period had no precipitation, the grid square was considered as not suitable. For relative humidity, dew point temperature and mean air temperature at 2 m were used, and relative humidity was calculated as Rh = $e_s(T_d)/e_s(T_a)$, or the ratio of saturation vapor pressure, $e_{s,}$ at dew point (T_d) to that at air temperature (T_a), where e_s for any temperature T is given by $e_s(T) = 6.112*e^{(17.502*T\ /\ (240.97\ +T))}$ (Stull, 1988). We identified grid cells as unsuitable whenever two consecutive observations fell below the humidity limit.

For the USHCN data set, only single observations were available per day, and data were available only for minimum temperature, maximum temperature, and precipitation, so relative humidity data were not considered with this dataset. A grid cell was considered as non-suitable whenever a single temperature observation fell outside the limit in the given time period. For precipitation, any period of 15 consecutive days of no precipitation was considered as non-suitable.

Next, because optimal physiological measurements do not focus on population persistence, but rather on optimal individual performance, we explored relaxing temporal spans over which thresholds were applied. For example, initially, a grid cell was considered unsuitable for minimum temperature when two consecutive observations of the four daily observations fell below thresholds in ERA, or when a single daily observation fell below the threshold in USHCN.

We explored effects of increasing these time spans by 5-day intervals, and assessed at what point the key population in North Carolina fell in the suitable category: in both data sets, a grid cell was marked as unsuitable for minimum temperature over periods of 1, 5, 10, 15, 20, 25, and 30 days; for maximum temperature, time spans explored were 1, 5, 10, 15, 20, 25, 30, up to 135 days; for precipitation, time spans were 15, 20, 25, and 30 days; and for relative humidity, time spans were 1, 5, 10, 15,20,25,30, up to 70 days.

To compare the actual distribution of Spanish moss with the suitability maps developed, occurrence data were downloaded from GBIF (http://www.gbif.org) and *species*Link (http://splink.cria.org.br/): 1632 records from GBIF and 580 from *species*Link. Records were curated for inconsistencies like (a) wrong place names, where the place name and geographic coordinates did not match; (b) wrong geographic locations wherein geographic coordinates fell in the ocean or on a different continent; and (c) duplicate records; these data were either corrected or deleted (Chapman, 2005). After curation, the data set had 776 records remaining from GBIF and 381 from *species*Link, totaling 1157 records, of which 295 fell within United States, with highest spatial density. These occurrences were overlaid on the suitability maps for each variable to understand how occurrences relate to optimal parameter values for Spanish moss. We used cumulative binomial probability tests to evaluate whether coincidence between occurrences and mapped suitable areas was better than would be expected at random (Peterson *et al.*, 2011). Finally, we averaged temperature data over the 22 years for each occurrence site and for all pixels in the study area to understand effects of averaging climate data.

Results

Our results indicate that Spanish moss northern distributional limits are shaped by ability to tolerate low temperatures for long periods of time. In the south, however, relative humidity and rainless days appear more important in shaping the distribution (Figures 1, 2, 3). Central and northern South American populations always experience minimum temperatures of ≥5ºC, such that minimum temperatures do not constrain those populations. However, allowing a single day below minimum temperatures failed to identify known distributional areas at higher latitudes as suitable. Indeed, to include the location in North Carolina where plant was collected for the physiological measurements, it was necessary to allow up to ~30 days of sub-optimal minimum temperature. This calibrated temporal criterion (i.e., 30 days of minimum temperature) yielded a map that included almost all known populations of the species. Of the few occurrences that were omitted, the great majority fell along the edges of the coarse-resolution global ERA dataset. Similar approaches to the finer-resolution USHCN data suggested an appropriate temporal span for minimum temperature of ~45 days. However, averaging climate data over the 22-year period, temperatures at occurrence sites always fell within the optimal minimum temperature range (Figure 4a).

Maximum temperature depicts (Figure 5) an intriguing constraint: areas presenting optimal maximum temperatures under single-day criteria were very small, with optimal maximum temperatures available continuously only in western South America. High-latitude areas were not affected by sub-optimal maximum temperatures, but tropical and sub-tropical areas were severely constrained in this dimension. Indeed, increasing the number of days outside of the optimal range up to 135 days still did not include all occurrences (see, e.g., occurrences in Yucatan Peninsula and northern Brazil). The USHCN data similarly indicated that suitability for maximum temperature must “alleviate” sub-optimal conditions for longer periods (i.e., >30 days) to include most of the known occurrences of the species. However, 22-year average maximum temperatures almost always were below 35°C (Figure 4c, 4d, 6d).

For precipitation, again, strict temporal limits (15 days) had to be relaxed, such that occurrences are seen where rain occurs only at least every 30 days (Figure 2). Although the ERA data showed the western edge of the distribution in the United States and Mexico as unsuitable even when the temporal criterion was relaxed to 30 days, the finer-resolution USHCN data suggested that a criterion of ~30 days suffices to include all occurrences. A period of ~20 days was required to include the site where physiological measurements were taken.

For relative humidity (Figure 3), the initial 5-day temporal criterion identified few grid squares as suitable. The location where the physiological measurements were taken was within suitable conditions at 15 days. However, to cover almost all of the known occurrences, ~70 days were needed; even with a 70-day of temporal span, a few occurrences in Colombia, Peru, and Ecuador were not covered under suitable areas. This result suggests that the ERA data are at such a coarse resolution that the plants may encounter appropriate humidity microclimates.

To assess the result statistically, cumulative binomial probability tests assessing coincidence between single-variable climate suitability maps and known occurrences of the species were calculated in geographic space (Table 1). These probability values were significant for minimum temperature at all temporal scales in the ERA data, but only over broader temporal scales for the USHCN data. Probability values for rainless days were significant over all temporal scales for both climate datasets. For maximum temperature, probability values were significant only at single-day time scales in ERA, but also for longer scales in USHCN. Relative humidity showed significance only for temporal scales of 1 and 5 days.

Our results also indicate that when temperatures are averaged, over long periods, populations appear to experience optimal physiological thresholds, and the resultant picture appears much more acceptable than when optimal conditions are assessed without averaging. Bar plots of averaged data suggest that occurrence sites always experienced minimum temperature above 5ºC and maximum temperatures below 35ºC (Figure 4) even when the raw data make clear that such is frequently not the case.

Among seven variables which are used in niche model calibration, relative humidity was weighted most heavily in model calibration (See Figure S2 in supporting information). Viewing our niche models as responses to environments, we see that more than 95% of Spanish moss populations are always within optimal thresholds of temperature and relative humidity (Figure 6a-d, Figure S3). However, in the case of rainless days, many populations are not within optimal thresholds. This figure effectively shows the effect of averaged climate data used in niche models. Figure 4 (f) shows the potential distribution of Spanish moss populations throughout Americas, based on niche model results.

Discussion

In our simple univariate testing scheme, climate data were processed in various environmental dimensions to investigate temporal limits on Spanish moss occurrences. Physiological data are measured on very fine scales of time and space (e.g., 10^{-3}-10^{-2} m), whereas climatic data are by nature averaged broadly (~10^{5} m) and can be difficult to develop at such fine scales for hemispheric extents, owing to limited data availability and computational power (Potter *et al.*, 2013). Applying the physiological data across a hemisphere (as in this study) requires scaling at two levels: scaling physiological thresholds from individuals to populations to global distributions, and scaling from microclimates to macroclimates. Effects of physiological limits on individuals could include reproductive failure, loss of mobility or shortened life spans. Ribeiro *et al.* (2012) showed that effects of critical thermal limits on the recovery process in leaf-cutting ants (*Atta sexdens rubropilosa*) depended on the time over which critical temperatures were experienced, and how long and/or often those temperatures were experienced by the ants. At the scale of populations, one can see that extreme conditions outside physiological limits may affect population sizes, even causing local extirpation. At the species level, different environments manifested across geographic distribution of species may create distinct selective environments, under some circumstances leading to local physiological adaptation (Brady *et al.*, 2005). Hence scaling individual physiological tolerances to species entire distributional areas via simple assumptions is likely to introduce error (Addo-Bediako *et al.*, 2000).

Scaling from microclimate to macroclimate requires another set of assumptions, because processes affecting microclimates are different from those affecting macroclimates. For example, turbulence flux plays a role at fine scales that can be ignored at broader scales. Also, in development of equations for scaling, parameters at fine scales may be well defined and parameter relationships linear, but at broad scales relationships may be non-linear, owing to influx of heat, moisture, and momentum over heterogeneous areas. Interactions between land and atmosphere vary according to the scale of analysis, such that choosing the "right" scale is impossible. If data are gathered from different localities, finding a proper method for data aggregation is also a challenge (Brunsell & Gillies, 2003). Further, processes at different scales receive feedback from each other, which influences processes and in turn this complicates the system. Hence, while studies across diverse scales are essentials, integrating across these scales poses significant challenges (Wu & Li, 2009).

As mentioned earlier, although microclimatic conditions affect individual organisms on quite fine scales, many issues surround translation from microclimate and individual performance to macroclimate and population persistence. In biophysical models, which are based on first principles of growth and reproduction, physiological measurements are scaled to match available climatic data (Kearney *et al.*, 2008; Kearney & Porter, 2009). These approaches thus assume to some degree that local landscapes are homogeneous. In reality, however, local landscapes are highly heterogeneous, demanding approximations of parameters or broad assumptions regarding parameter values (Lhomme, 1992), such that spatial resolution subsumes a major set of assumptions of first-principles approaches to these issues. For Spanish moss, being an epiphyte in tree canopies, heterogeneity of the landscape is particularly difficult to parameterize. The effect of scaling is seen by contrasting our coarse (~165 km) ERA results with our fine-resolution (10 km) USHCN results (Figures 1-3,5): imagine if climate data were available at still-finer resolutions, such that we could capture these phenomena at biologically relevant resolutions. Generating fine resolution climate data is a challenge, requiring active collaboration among disciplines (Potter *et al.*, 2013).

In correlative niche model applications, environmental data are frequently averaged by month or year, and our results with such data indicated that modeled niches always tended to fall within measured physiological limits (Figure 6). That is, species did not encounter their physiological limits in correlative models owing to massive averaging of extremes in the climate data. On the other hand, with fine-temporal-resolution data, most populations encounter suboptimal conditions in at least one dimension. What is more, with these finer data, one can view the species' response against a broader range of conditions; in the averaged data, however, many of the extremes are never manifested (see Figure 6, Figure S3), such that the existing fundamental niche (i.e., the set of conditions manifested on geography) is notably smaller than the true fundamental niche (i.e., that determined by the species' physiology). Also, in many mechanistic models, climate data are used as monthly averages, thus ignoring temporal scale and allowing considerable information loss regarding temporal sequences, and thereby not taking fullest advantage of the mechanistic approach (Buckley *et al.*, 2010). The need for high temporal resolution in environmental data used in mechanistic models has been acknowledged previously (Kearney *et al.*, 2012).

Hence, in this analysis, we are making use of high temporal-resolution climate data, and we are not scaling physiological measurements in terms of particular environmental values, as any such values would be guesses at best. Rather, we scale them in temporal terms, to understand how long populations can persist under sub-optimal average conditions, at least in absence of local adaptation (Martin *et al.*, 1985), and then overlay available occurrence data from GBIF and *species*Link to understand whether most populations are within suitable limits or not. Available occurrence data suffice to outline major features of the species' distribution, even though not all populations are represented.

Martin *et al.* (1986) evaluated a few individuals of Spanish moss from sunny and shady locations in South Carolina with different irradiance levels. Their research showed that these plants respond physiologically to various irradiance levels, suggesting that Spanish moss adjusts physiologically to the microclimates it inhabits, posing still more difficulty in generalizing physiological responses from a few populations to entire distributions. In this paper, we emphasize that, even when climate data are scaled temporally, not all populations appear to exist under optimal conditions. Our result suggests (1) that optimal thresholds may be different in different places, (2) that tolerance limit may be quite broad, (3) that suitable microhabitats are not captured in the climate data, or (4) that temporal intervals of optimal conditions may need to be relaxed still further. Use of physiological parameters from a single population has caveats due to local adaptation or natural selection. To reduce the effect of local adaptation it is advisable to collect physiological data from populations from widely-scattered geographic locations that present distinct environmental conditions.

For example, for minimum temperature (Figure 1b), populations in North Carolina are within the area presenting suitable conditions, but some populations in the southern Peruvian Andes fall outside the limit. The degree to which local adaptation is involved in this model “failure” cannot be assessed without direct experimentation, and to develop a robust model, physiological data from populations in the unsuitable category would be very informative. Buckley *et al.* (2008) compared performance of biophysical and correlative approaches in anticipating range shifts under climate change scenarios, and concluded that projected range shifts were more pronounced in mechanistic models as compared to correlational models. However, to parameterize mechanistic models, many assumptions were required, such that comparisons of these two approaches are perhaps best considered as speculative. In applying measured physiological thresholds from one or a few locations to broad distributional areas in this study, we see that ecophysiological approaches have significant limitations as well. Because physiological measurements are taken at one or a few sites only, any local adaptation will not be taken into account in estimates of distributions, and projections of parameters may frequently fail to include all populations.

Our results also suggest an interesting contrast: Spanish moss populations can persist outside of optimal minimum temperature ranges for a few days, but can withstand sub-optimal thresholds for maximum temperatures for longer periods. Clearly, sub-optimal conditions in the growing or flowering season might have different implications than at other times. For example, the specific importance of conditions during particular life stages of insect populations has long been appreciated (Wellington, 1956). Clearly, if the species cannot reproduce in a place, it is not going to occupy that region, so, non-optimal conditions in the growing season may constitute a more specific constraint on the distribution of Spanish moss (See Figure S1 in Supporting Information). When the occurrence data are plotted against the average climate data (Figure 6), about 95% of the populations come within optimal physiological ranges. Our approach thus stresses the importance of using temporally fine-scale climate data for analysis, particularly when integrating physiology of species in the model.

The approach used here is not considering interaction of variables for a simple reason: as physiological limits data are usually assessed for different variables independently, these experiments assume other variables at constant value, although a few studies have attempted to assess physiological responses to multiple variables (Johnson *et al.*, 1997). The difficulty in capturing multi-variable responses lies in time investment required for experiments. Also, many times, particular combinations of variable values may not exist in the real world, yet interaction of variables may play important roles in limiting distributions of species. Smith (2013) examined the role of interactions of temperature and precipitation in constraining ranges of 67 mammalian species; their results indicated that interactions between the two climate dimensions play important roles in shaping the distributions of 85% of the species.

Ecophysiologists measure either optimal thresholds or tolerance limits for environmental parameters (Martin & Siedow, 1981; Martin *et al.*, 1981; Huey & Hertz, 1984; Angilletta *et al.*, 2010), which must be pondered if their results are to be used in these geographic views. Such studies cover only a few variables, and each variable is generally assessed independently of others, so using this information directly in biophysical models can be complex, because only a subset of key parameters is measured. Several studies have begun using biophysical data for estimating niches and distributions, yet physiological data are available from a relatively few species only. For example, Kearney *et al.* (2008), explored potential distributions of an invasive toad species in Australia based on biophysical parameters, but measurements were available only for adults, and only for invasive populations: characterizing these additional measurements would involve considerable time and resources. Hence, notwithstanding that biophysical measurements may be (in theory, at least) excellent ways to characterize fundamental niches, their application in practice is not straightforward.

Although this approach can be used to understand the physiological constraints on the populations, but cannot be used so readily to predict the distributions of species per se, the approach used here provides valuable insights into, and leads to new questions about, the biology and ecophysiology of the species under investigation. For example, when populations of the species occur well outside the physiologically optimal environmental conditions (e.g., Spanish moss populations in Brazil, Baja California, and central Mexico; Figures 4 and 5), are individuals in these populations under severe stress and growing poorly, or do these individuals possess unique ecophysiological features that prove adaptive in these putatively sub-optimal environments? Answers to such questions may well provide novel views of the physiology and natural history that may otherwise be impossible to obtain.

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Table 1: p-values of cumulative binomial probability tests used to assess coincidence between occurrences and suitable areas as per minimum temperature, maximum temperature, rainless days and relative humidity thresholds. – indicates no data availability, ERA = global interim reanalysis by European Center for Medium-Range Weather Forecast, USHCN = United States Historical Climate Network.

Days	Minimum temperature (5°C)		Maximum temperature (35°C)		Rainless days		Relative humidity (50%)
	ERA	USHCN	ERA	USHCN	ERA	USHCN	ERA
1	0.001	0.001	0.001	0.001	-	-	0.001
5	0.001	0.15	1.00	1.00	-	-	0.067
10	0.001	0.23	1.00	1.00	-	-	1.00
15	0.001	0.001	1.00	0.99	0.001	0.001	1.00
20	0.001	0.001	1.00	0.69	0.001	0.001	1.00
25	0.001	0.001	1.00	0.002	0.001	0.001	1.00
30	0.001	0.001	1.00	0.011	0.001	0.001	1.00
45	-	0.001	-	-	-	-	-
70	-	-	-	-	-	-	1.00
135	-	-	1.00	-	-	-	-

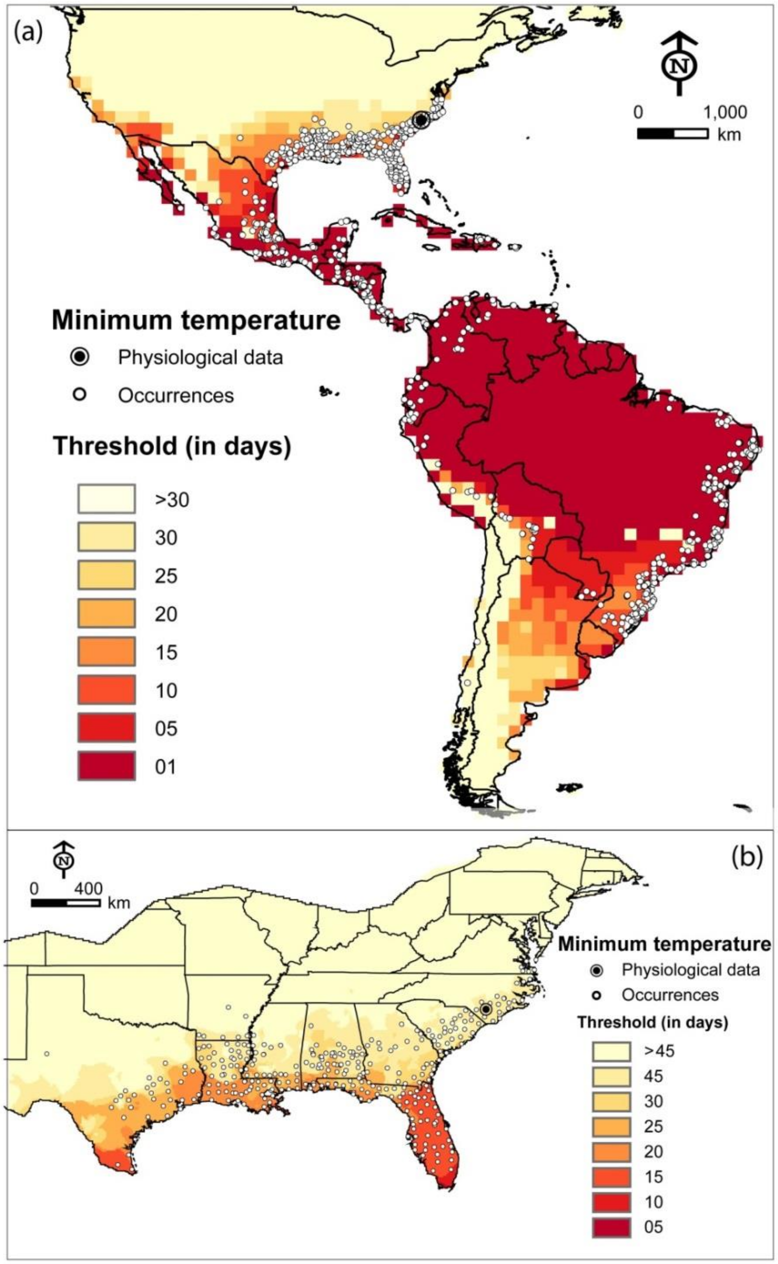

Figure 1. Map of effects of minimum temperature thresholds (≥5°C) over different temporal spans on Spanish moss distributions. (a) – hemispheric extent (ERA = global interim reanalysis by European Center for Medium-Range Weather Forecast). (b) – United States extent (USHCN = United State Historical Climate Network data)

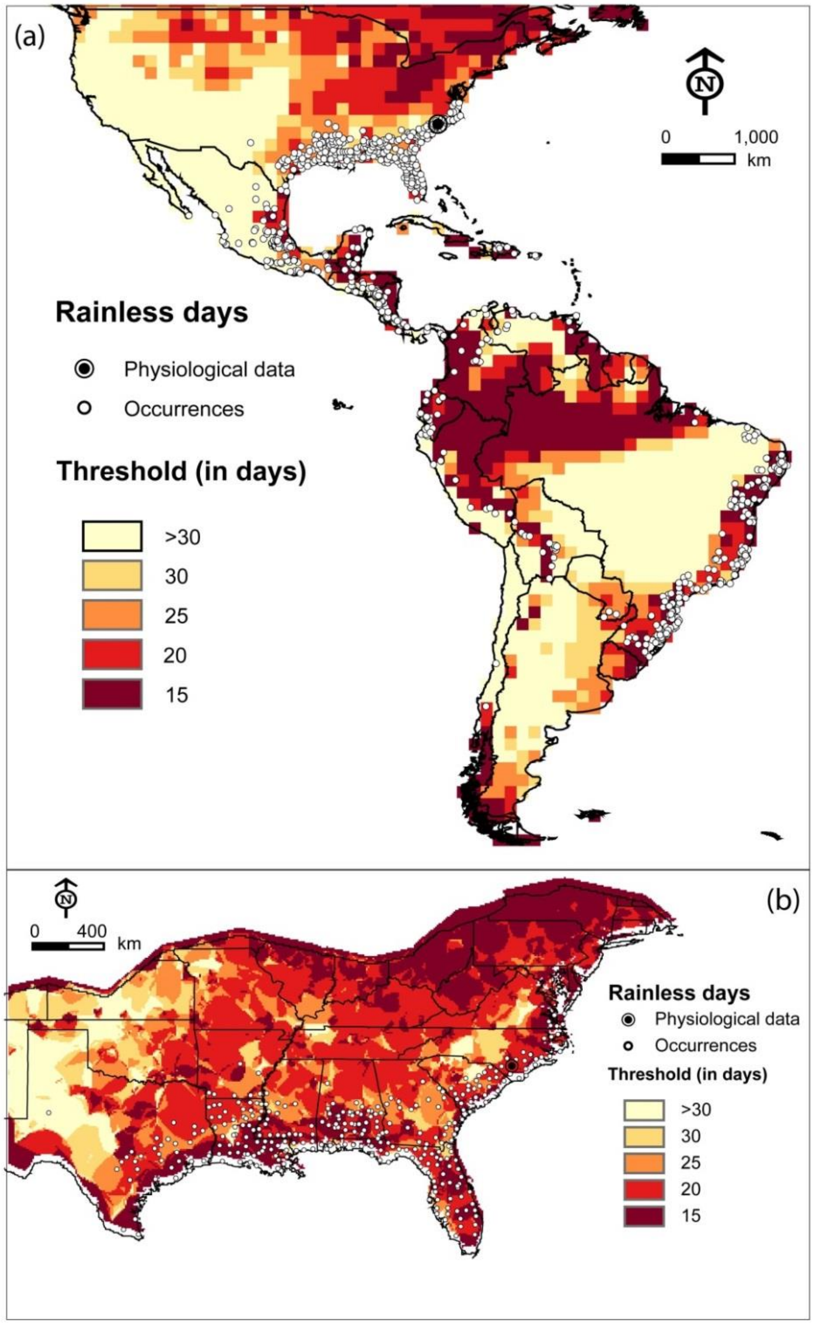

Figure 2. Map of effects of rainless days (≤15 days) over different temporal spans on Spanish moss distributions. (a) – hemispheric extent (ERA = global interim reanalysis by European Center for Medium-Range Weather Forecast). (b) – United States extent (USHCN = United States Historical Climate Network data).

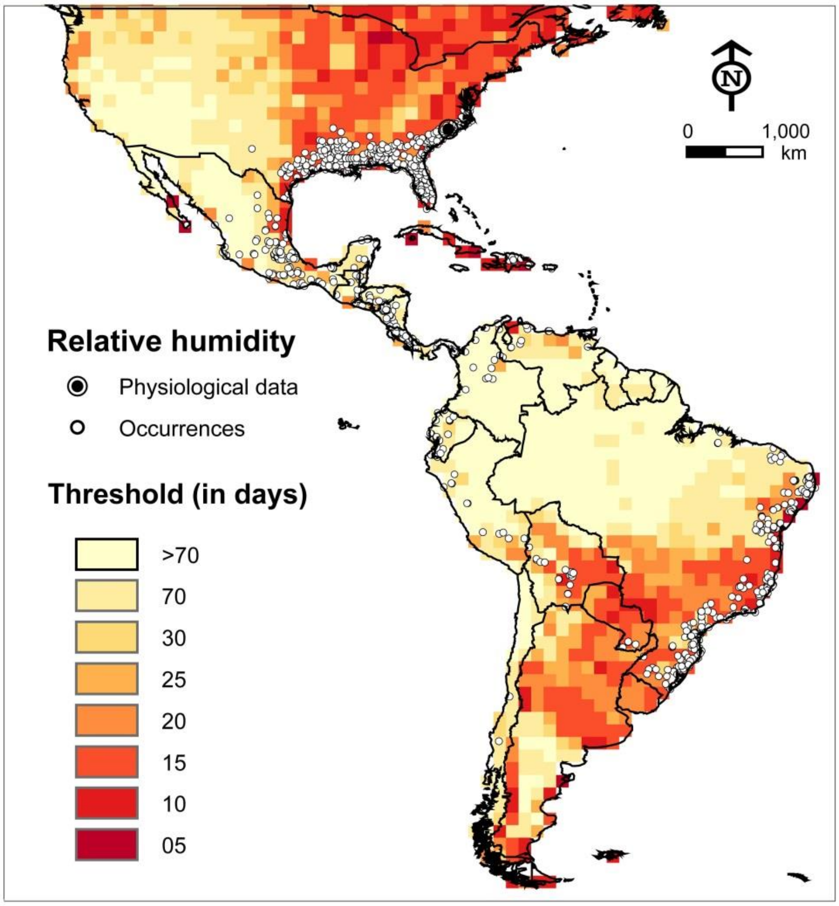

Figure 3. Map of effects of nighttime relative humidity (≥50%) over different temporal spans on Spanish moss distributions. (a) – hemispheric extent (ERA = global interim reanalysis by European Center for Medium-Range Weather Forecast). (b) – United States extent (USHCN = United States Historical Network data)

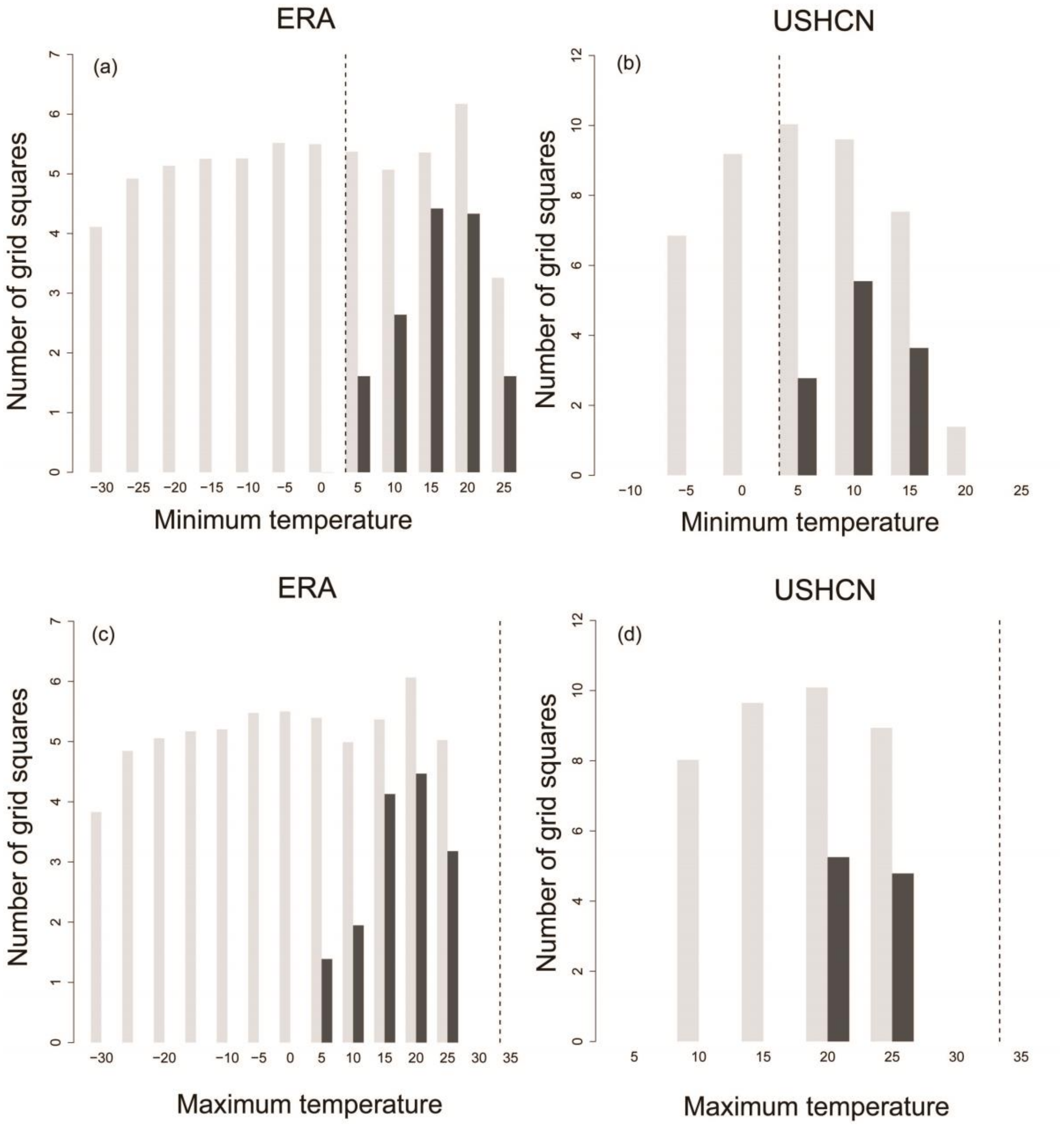

Figure 4. (a-d) Bar plot of average temperatures in the study area (gray bars) and occurrence sites (black bars). Number of grid squares is log transformed. Dashed lines indicate physiological thresholds to which climate data are compared.

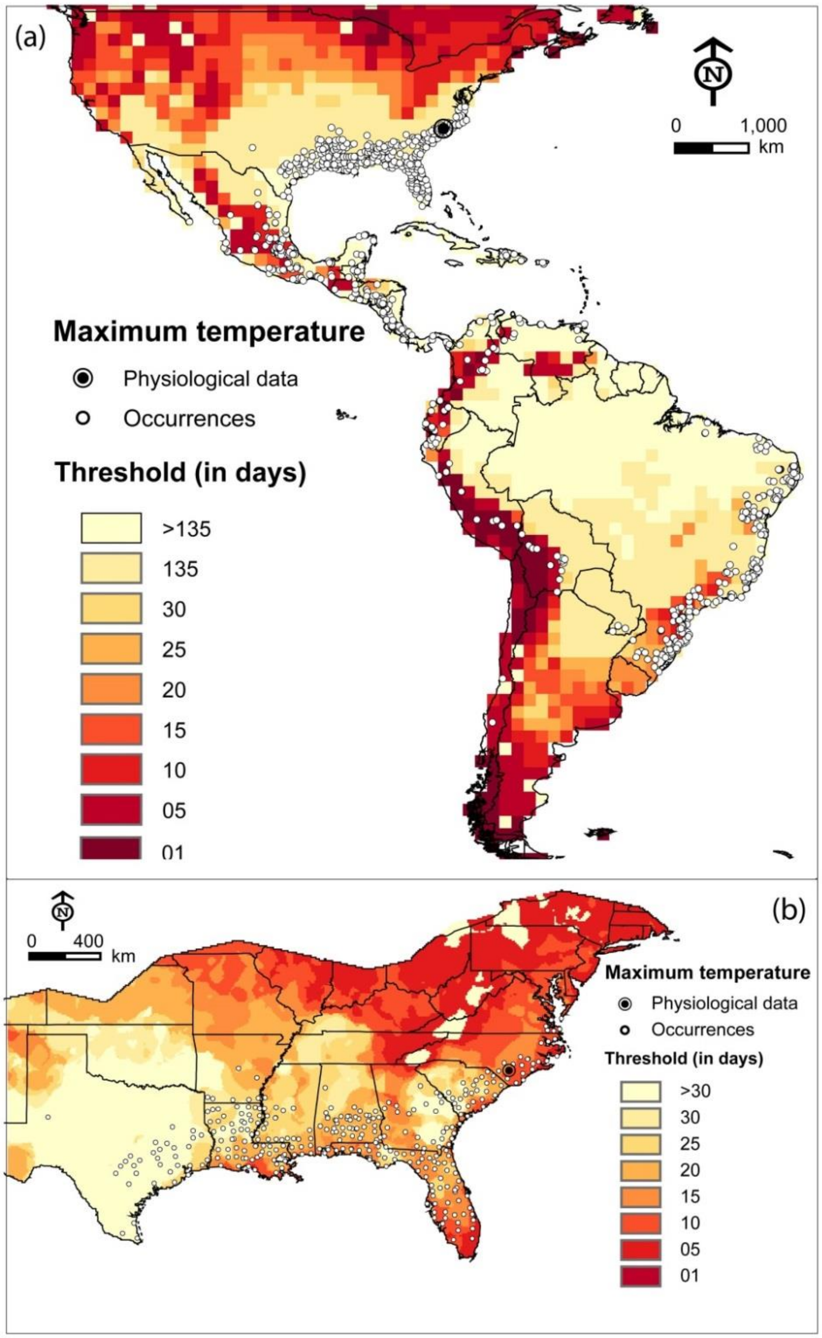

Figure 5. Map of effects of maximum temperature thresholds (≤35°C) over different temporal spans on Spanish moss distributions. (a) – hemispheric extent (ERA = global interim reanalysis by European Center for Medium-Range Weather Forecast). (b) – United States extent (USHCN = United States Historical Climate Network data)

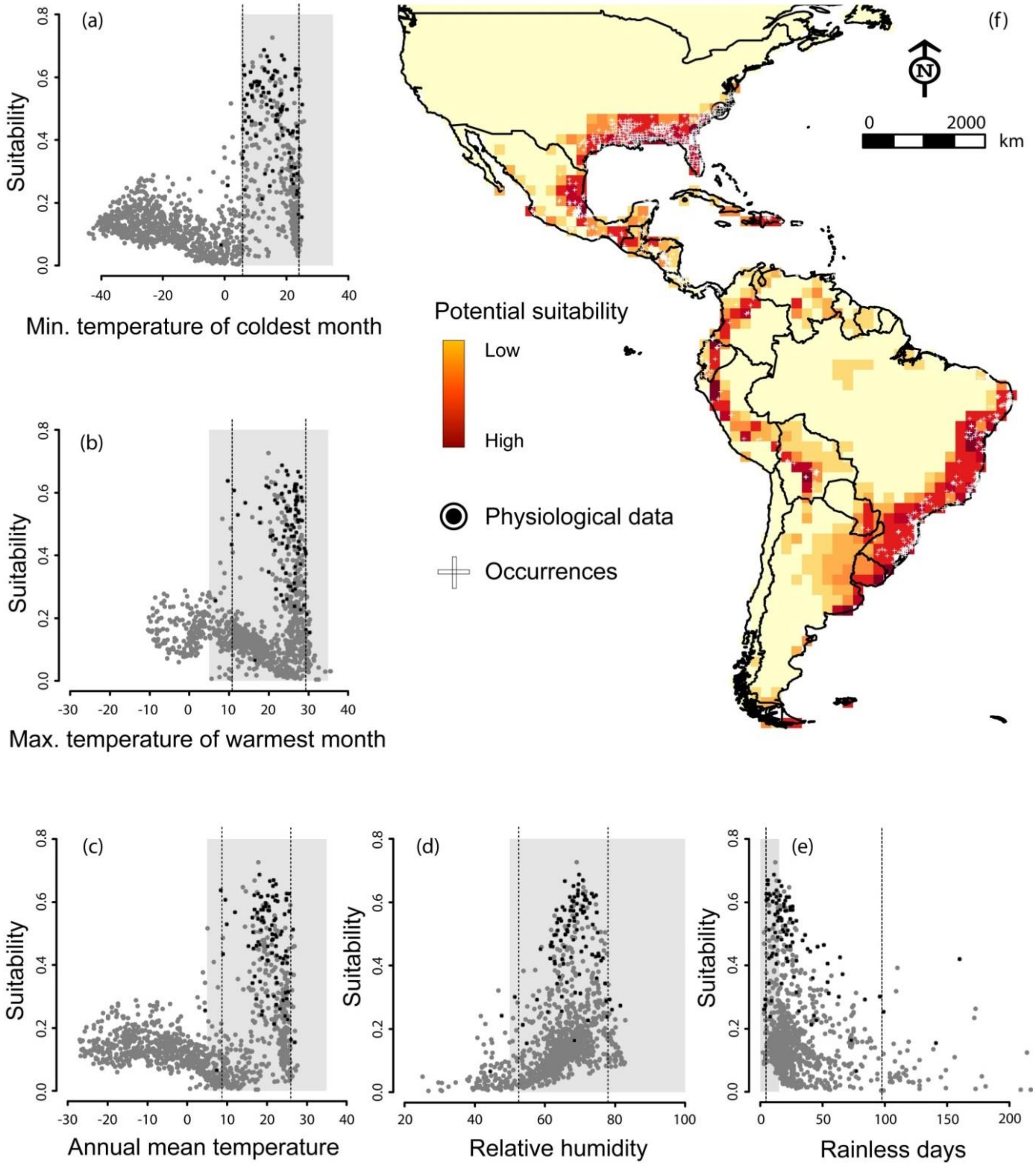

Figure 6. (a-e) Scatter plot of suitability and variables used in the correlative Maxent model. Gray dots show environments represented across the study area. Black dots show occurrences. The dotted lines show the location of 95% of occurrences. The gray box outlines optimal physiological limits. Panel (f) shows the potential suitability of Spanish moss using Maxent.

Supplimentary figures

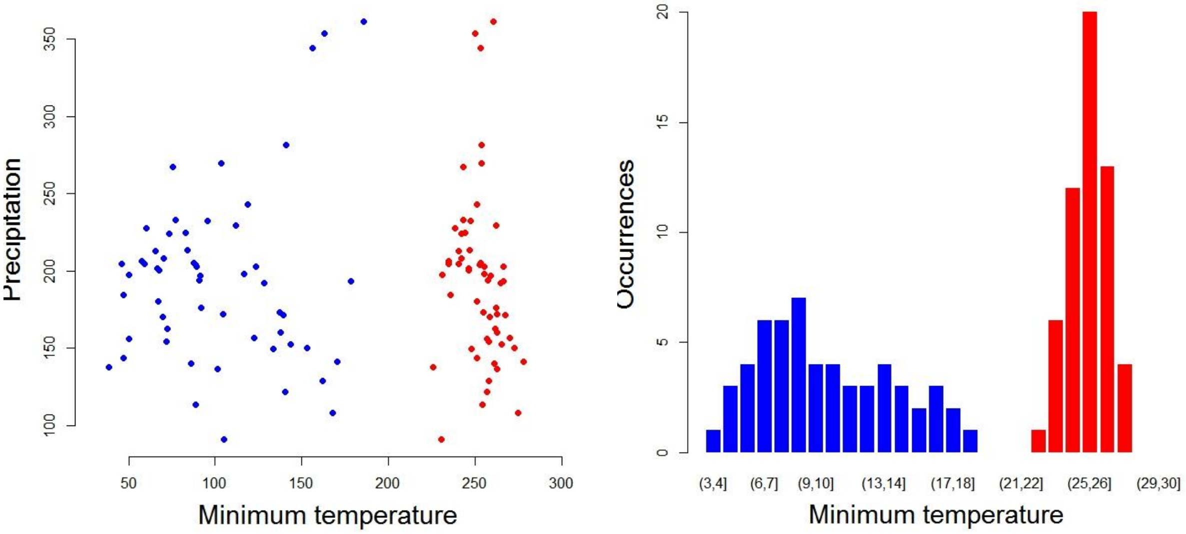

Figure S1: Average minimum temperature in two different months for United States populations of Spanish moss. Blue color shows temperature in January and red color shows temperature in July.

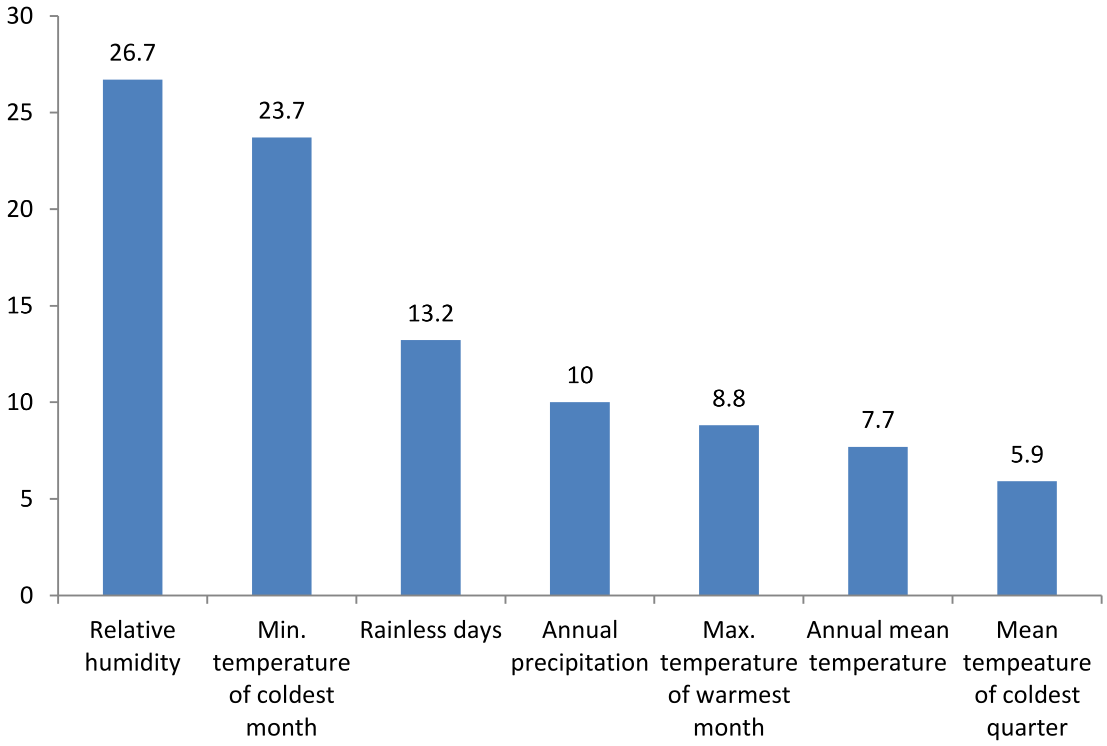

Figure S2 : Bar plot of variable contributions in the Maxent model.

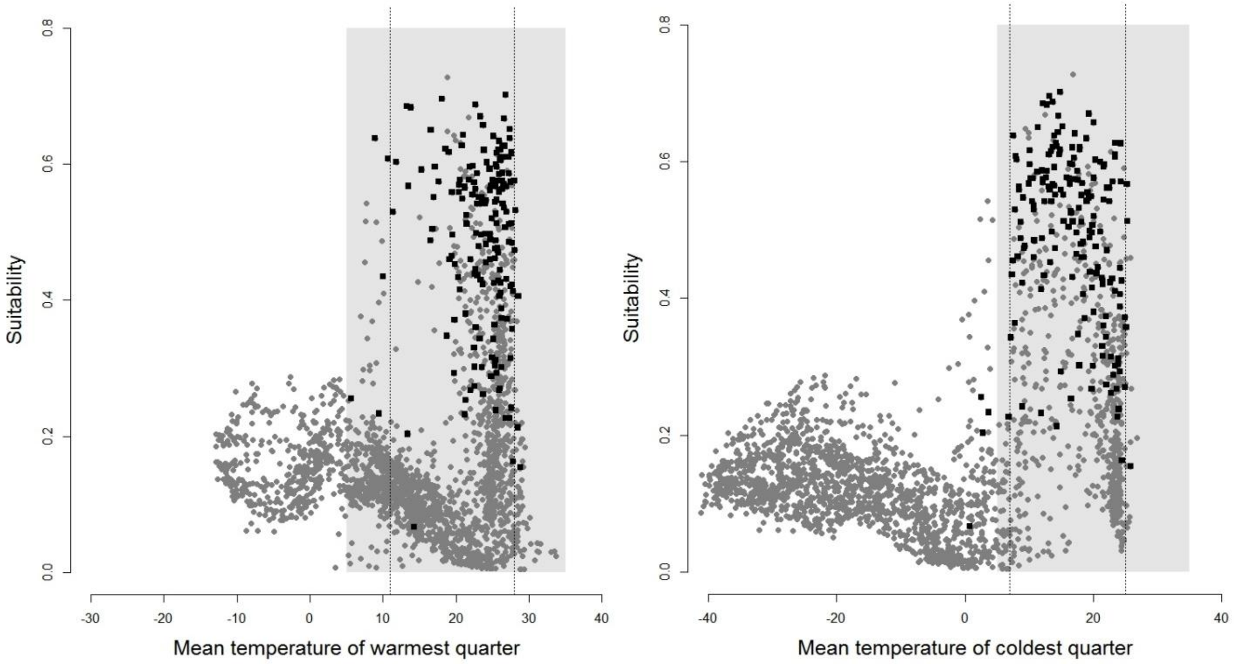

Figure S3. (a-b) Scatter plot of suitability and variables used in the correlative Maxent model. Gray dots show environments represented across the study area. Black dots show occurrences. The dotted lines show the location of 95% of occurrences. The gray box outlines optimal physiological limits.

Chapter 2

Climatic Niches and Flowering and Fruiting Phenology of Spanish moss (*Tillandsia usneoides*)

Abstract

Species have geographic distributions constrained by combinations of abiotic factors, biotic factors, and dispersal-related factors. Abiotic requirements vary across the life stages for a species; for plant species, a particularly important life stage is when the plant flowers and develops seeds. A previous year-long experiment showed that ambient temperature of 5-35°C, relative humidity of >50% and <15 consecutive rainless days are crucial abiotic conditions for Spanish moss (*Tillandsia usneoides*). Here, we explore whether these optimal physiological intervals relate to the timing of the flowering and fruiting period of Spanish moss across its range. As Spanish moss has a broad geographic range, we examined herbarium specimens to detect and characterize flowering/fruiting periods for the species across the Americas; we used high-temporal-resolution climatic data to assess the availability of optimal conditions for Spanish moss populations during each population's flowering period. We explored how long populations experience sub-optimal conditions, and found that most populations experience sub-optimal conditions in at least one environmental dimension. Flowering and fruiting periods of Spanish moss populations are either being optimized for one or a few parameters, or may be adjusted such that all parameters are sub-optimal. Spanish moss populations appear to be constrained most closely by minimum temperature during this period.

Introduction

Restricted geographic distributions of species are often a consequence of some set of constraints in terms of abiotic requirements, needs in terms of biotic interactions, and limitations to dispersal ability (Soberón, 2007). All species have a life cycle (be it simple or complex), and each stage in that cycle may have different requirements in terms of climate, soils, topography, other abiotic factors, and biotic requirements like food, competitors, or mutualisms. Grubb (1977) defined 4 components of ecological niches of plants: the habitat niche, life-form niche, phenological niche, and regeneration niche; much research has examined how regeneration niches may differ in different community assembly processes, and how these various niches act in different life stages (Fowler, 1988; Lavorel & Chesson, 1995; Miller-Rushing & Primack, 2008; Tilman, 2014). Although several studies have used the regeneration niche concept to explore competition and understand rarity of species at local scales (Engelhardt & Anderson, 2011; Ranieri *et al.*, 2012), few studies have used the regeneration niche idea to understand species' distributions in terms of their abiotic requirements at geographic scales (Pederson *et al.*, 2004; Sweeney *et al.*, 2006; Wellenreuther & Arson, 2012).

Phenological stages in plant life cycles comprise critical life stages, in which plants flower, produce seeds, grow, or remain dormant (Bond & Midgley, 2001; Silvertown, 2004). Plants have presumably evolved to flower in seasons and at intervals that ensure maximal reproductive success (Amasino, 2010). Considerable research has shown that plants sense and respond in complex ways to environmental cues (Garner, 1933; Lang, 1952; Bernier *et al.*, 1993; Dennis *et al.*, 1996). However, these factors have been investigated chiefly at local scales; at biogeographic scales, the question of whether phenology is optimized or not with respect to physiological responses to abiotic factors like temperature and precipitation remains little investigated (Engelhardt & Anderson, 2011; Ranieri *et al.*, 2012).

Here, we examine the timing of flowering and fruiting by Spanish moss (*Tillandsia usneoides*) populations across the species' broad geographic range in relation to availability of optimal physiological conditions (Barve *et al.*, 2014). Physiological measurements have been made in yearlong field experiments (Martin & Siedow, 1981; Martin *et al.*, 1981) to estimate ideal intervals of climate-related parameters. We used herbarium specimen records of flowering and fruiting Spanish moss to identify population-specific flowering and fruiting periods, and tested detailed environmental data for associations with minimum temperature, maximum temperature, relative humidity, and rainless days requirements on a univariate basis, building on our earlier analyses of physiological limits in relation to climate across the range of this species (Barve *et al.*, 2014). We use these analyses to test whether (1) all four parameters are at optimal physiological values as measured in previous studies during flowering periods, and (2) which physiological parameter(s) is (are) optimized during the flowering periods, if not all are optimized.

Methods

Study Organism

Spanish moss (*Tillandsia usneoides*) is an epiphytic flowering plant of the family Bromeliaceae, distributed approximately between 38°N and 38°S latitude. It typically grows in warm and humid climates on trees or other supporting structures, such as power cables (Billings, 1904; Garth, 1964; Callaway *et al.*, 2002). Spanish moss occurs over a broad elevational range (0–3300 m), and associations with atmospheric moisture content and temperature vary significantly according with elevation (Gentry & Dodson, 1987; Kreft *et al.*, 2004). The species does not occur at high elevations, which are apparently too cold for its persistence; indeed, its general natural history suggests that its distribution will prove to be highly constrained by climatic factors (Garth, 1964), more or less in line with the "Hutchinson's dream" scenario of Saupe *et al.* (2012).

Temperature, humidity, and drought are known to affect growth and persistence of Spanish moss (Garth, 1964; Martin & Siedow, 1981; Martin *et al.*, 1981; Martin & Schmitt, 1989). A year-long field experiment (May 1978 to May 1979) was performed by Martin *et al.* (1981) near Elizabethtown, North Carolina (78.594°W, 34.682°N); it found that Spanish moss growth is concentrated in summer months, with winter growth almost negligible. Martin *et al.* (1981) showed that CO_2 uptake was maximal when daytime temperature is 5–35ºC; CO_2 uptake was eliminated at or below 0ºC and at or above 40ºC. Kluge *et al*. (1973) also experimented on Spanish moss, with similar results regarding CO_2 uptake; however, they used greenhouse-grown Spanish moss, and their experiment was carried out in the laboratory under constant temperature and humidity. Martin *et al*. (1985, 1986) assessed North Carolina Spanish moss populations with respect to irradiance effects on morphology and physiology, finding that Spanish moss responds to irradiance by adjusting physiology more than morphology. Garth (1964) showed that Spanish moss cannot survive in Georgia without periodic rainfall, even when water is supplied externally; he found that Spanish moss achieves optimal performance in terms of growth only with ≤15 consecutive rainless days. Martin *et al*. (1981) corroborated this latter result, with the additional information that CO_2 uptake is minimal when Spanish moss is wet by rain, suggesting that

Spanish moss requires some dry periods for persistence. Overall, then, these experiments identified four parameters that can be analyzed at continental extents: minimum temperature ≥5ºC (Martin *et al.*, 1981), maximum temperature ≤35ºC (Martin & Siedow, 1981), nighttime humidity ≥50% (Martin *et al.*, 1981), and ≤15 rainless days (Garth, 1964).

Input Data

We collected information on flowering and fruiting periods of Spanish moss populations by examining herbarium specimens. We photographed 430 specimens in the collections of the Missouri Botanical Garden and 504 specimens from the New York Botanical Garden collections using a 16 megapixel Nikon P510 camera. We took 3–4 photographs per specimen to capture various details: one of the label to permit capture of associated data, one of the whole specimen, and 2–3 zoomed photographs of flowers or fruits. In addition, we reviewed published floras for flowering dates, although most floras either did not offer sufficient detail about flowering period, or do not provide precise locality information. Finally, we downloaded images from various herbaria listed on the *Index Herbariorum* site (http://sciweb.nybg.org/science2/IndexHerbariorum.asp) and others (http://herbarium.bio.fsu.edu, http://apps.kew.org/herbcat/navigator.do). Flowering and fruiting periods were assumed to be unimodal, so we filled temporal gaps for analyses of optimal physiological conditions. The temporal resolution of flowering and fruiting times was kept at months, so that imprecise date information (e.g., "April 1914") could be incorporated, and quantity of relevant data maximized.

Information from specimen labels was digitized and stored in a Microsoft Access database. Some labels had geolocations in terms of latitude-longitude coordinates, whereas others had only textual locality information at various administrative levels. In the latter case, geolocations were attached to each record via queries in Google Earth. Overall, we were able to obtain information for 361 sites where both flowering date and geolocations information was available, which we used to profile flowering/fruiting periods at sites across the range of the species.

We examined how physiological thresholds are met (or not) for Spanish moss across the Americas within empirically documented flowering intervals over a 22-year period (January 1989 – December 2010) following Barve *et al.* (2014). We used the ERA interim reanalysis climate data developed and supplied by the European Center for Medium-Range Weather Forecasts, which are based on a combination of models and observations, with 3-hourly temporal resolution: every second datum is a forecast, whereas the other is a model result. We used only the model result data, thus coarsening the data from 3-hourly to 6-hourly resolution, but retaining an impressively fine temporal resolution. The dataset has a somewhat coarse native spatial resolution of 1.5° x 1.5° or approximately 165 x 165 km grid square resolution at the Equator.

ERA Data were downloaded from http://apps.ecmwf.int/datasets/data/interim_full_daily/ for the following parameters: minimum temperature at 2 m, maximum temperature at 2 m, mean temperature at 2 m, dew point temperature at 2 m, and precipitation. The data are stored in NetCDF format (http://www.unidata.ucar.edu/packages/netcdf/index.html; Rew & Davis 1990); these data were manipulated and processed via the “ncdf” package in R (Pierce, 2011; R Core Development Team, 2012). ERA interim data were processed to identify optimal and sub-optimal areas and temporal duration of sub-optimal conditions with respect to each physiological variable through time.

Overall, 136 1.5° grid squares held at least one Spanish moss record with flowering and fruiting information. As numbers of flowering records were not numerous with respect to so many grid squares, to improve data density, we coarsened the 1.5° grid to 3° grids only to characterize flowering periods, but climate data were kept at the original 1.5° resolution. We generated flowering and fruiting month ranges for each 3° grid square; we assumed single flowering/fruiting months in grid squares in which only single specimens were available, which may be a restrictive assumption in our analyses. We also generated non-flowering month datasets for each grid square for comparison; for example, for a grid square with a flowering/fruiting range of March-May, we generated the remaining 11 possible three-month sequences for comparison. We identified the average flowering/fruiting month, flowering/fruiting season start, and flowering/fruiting season end for each grid square. Average flowering/fruiting month was calculated as a weighted average based on number of flowering or fruiting specimens in each month.

Data Analysis

An R script was developed using the raster, ncdf, and sp packages (Bivand *et al.*, 2008; Pierce, 2011; Hijmans & van Etten, 2012) to calculate the percentage of time over the 22-year span of the data set that Spanish moss populations experienced optimal conditions with respect to the physiological thresholds described above. For minimum and maximum temperatures, the script checks the value of each variable across four daily observations; a grid square was marked as unsuitable for a day whenever two consecutive observations were outside the limit. For precipitation, whenever all four daily observations were 0 (i.e., no precipitation), it was considered as a day with no precipitation, and all consecutive sets of 15 days were checked; when any 15-day period had no precipitation, the grid square was considered as not suitable. For relative humidity, dew point temperature (T_d) and mean air temperature at 2 m (T_a) were used, and relative humidity was calculated as Rh = $e_s(T_d)/e_s(T_a)$, or the ratio of saturation vapor pressure at dew point to that at air temperature, where e_s for any temperature T is given by $e_s(T) = 6.112*e^{(17.502*T / (240.97 + T))}$ (Stull, 1988). We identified grid cells as unsuitable whenever two consecutive observations fell below the humidity threshold. Likewise, we calculated the percentage of time that the grid square spent outside its optimal physiological thresholds within the flowering period for that grid square across the 22-year time span; for comparison, we also generated these percentages for all possible non-flowering periods of similar duration.

We ranked each grid square based on the percentage of time spent outside optimal values in flowering and fruiting periods and each other possible non-flowering period of similar duration. We calculated the rank of each of the observed flowering periods with respect to all other possible periods of the same duration as the number of time periods of non-flowering months that are more suitable. We used Kolmogorov-Smirnov test to compare distributions of the four variable ranks.

Based on ranks of each grid square for each of the variables, we compared the actual flowering period with the optimum flowering and fruiting period with respect to those variables. This distance was calculated as a Euclidian distance from an optimal rank of 1 for each of the variables, such that small distances indicate optimal flowering and fruiting periods for a population, whereas large distances suggest that the population flowers during suboptimal periods. We averaged this distance across all 4 physiological parameters, and mapped these deviations from optimum. We tested for effects of number of herbarium specimen records in each grid square to these optimum distances.

Results

We were able to assemble 361 flowering or fruiting records for Spanish moss across the species' range. Although records concentrated in the US portion of the species' range (159 records, or 44%), the remaining 202 (56%) records came from Latin America. Although densities of Latin American points were low at finer spatial resolutions (i.e., most grid squares had single or no flowering-period records), 3° spatial resolution was sufficient to create 83 grid squares, within which we had 1–28 flowering/fruiting records.

Average flowering and fruiting month of Spanish moss populations across the species' range is shown in Figure 1. The flowering and fruiting period in eastern Brazil was November to April, while the flowering and fruiting period in western South America was June to September, with a few exceptions extending to October-November. The flowering period in the US and Mexico was May to September, with a few exceptions in November-December. Because our identification of flowering and fruiting month(s) was in some sense dependent on numbers of specimens available, we suspect that insufficient data density may be driving the exceptions.

Flowering periods invariably fell in time periods in which least one physiological parameter was optimal in a grid square. The "optimal" parameter was generally minimum temperature: that is, in 212 of 262 cases, flowering and fruiting period coincided with months in which minimum temperature was within optimal ranges in at least 70% of grid square-month combinations. Very few populations experienced minimum temperatures below the 5°C criterion during their respective flowering and fruiting periods (Figure 2a). The remainder of Figure 2 suggests that flowering and fruiting period depends less critically on parameters like maximum temperature, rainless periods, or relative humidity. Ranking months by their optimality for each parameter, a Kolmogorov-Smirnov test revealed that distributions of ranks for minimum temperature were significantly lower than those for the other three factors ($P < 0.0001$). The distribution of ranks among grid squares did not differ between maximum temperature and rainless days, whereas ranks of rainless days *versus* relative humidity showed the latter as significantly more optimal ($P < 0.001$).

We identified the optimal month for each pixel across the Americas in terms of each dimension of Spanish moss physiology. Figure 3a shows the optimal flowering and fruiting month for minimum temperature, which centered on July at the northern limit of the distribution, but in January-April at the southwestern distributional limit. However, for maximum temperature, the average expected flowering/fruiting month was February-April at the northern limit of distribution, and April-August at the southwestern limit. Similar variation can be seen for relative humidity and rainless days: in short, no pixel had any period in which all four physiological parameters were in optimal states for flowering and fruiting to occur.

To explore how far observed flowering and fruiting months departed from optimal months, we calculated average Euclidean distance in four-dimensional parameter space, ranking months by their suitability, standardizing each dimension to a range of 0–1 (thus creating an index of distance that has rather unclear units but that is useful for visualization), and counting ranks as greater distance from optimal conditions (Figure 4). Most populations (46%) showed flowering and fruiting periods with Euclidean distances of $\leq$0.5. Only a few pixels were under extremely bad conditions and these higher-distance populations were arrayed at the extremes of the distribution (Figure 4). We tested whether number of available flowering/fruiting specimens affected these latter results (see scatterplot inset in Figure 4), but found no effect of sample size on distance to optimal month.

Discussion

In overview, we found that Spanish moss populations appear to 'tune' their phenological niches such that they experience optimum minimum temperatures for most of their respective flowering and fruiting periods. Among populations analyzed, flowering and fruiting periods of about 76% of Spanish moss populations experienced optimal minimum temperatures when compared with other time periods through the year. Conversely, Spanish moss populations appear to flower and fruit without much consideration of optimality of maximum temperature or relative humidity optimality, though rainless days do have some importance.

Numerous recent studies have documented shifts in flowering and fruiting season as a consequence of climate change (Telemeco *et al.*, 2013; Molau *et al.*, 2005; Miller-Rushing & Primack, 2008). Veriankaite *et al*. (2010) explored optimum temperatures for flowering and fruiting by comparing air temperatures in climate models with long-term flowering data. However, for this study, we took advantage of known optimum physiological parameters (Martin *et al.*, 1981; Martin & Siedow, 1981; Martin & Schmitt, 1989), so we could explore the degree to which Spanish moss flowering and fruiting periods coincide with months presenting optimal physiological conditions for growth.

Phenological differences are well documented as functions of elevation and latitude (Ruml *et al.*, 2011; CaraDonna *et al.*, 2014). However, we generated our phenological information from herbarium specimens: few had elevation information, so effects of elevation on flowering phenology cannot be examined particularly in light of the coarse spatial resolution of our weather data. Clearly, as the climate data are coarsened and averaged over broader extents, such details average out in the climate and become invisible to our analyses, as was noted in our previous analyses (Barve *et al.*, 2014). Our analyses may also be compromised by our rather coarse characterization of flowering and fruiting periods (i.e., to month), and by our filling of temporal gaps in flowering periods under the assumption of a single, continuous flowering and fruiting period for each population.

Hadley *et al.*, (1984) observed that elevated maximum temperatures can delay flowering, in effect slowing down the reproduction in soybeans (*Glycine max*). However, for Spanish moss, we observed that flowering phenology does not generally depend much on maximum temperature. Rather, minimum temperature appears to play a major role (Figure 2). Comparisons with every other period of similar length in the year for each location suggested that Spanish moss flowering and fruiting periods are molded such that flowering populations experience optimal minimum temperatures. Hence, an interesting challenge for long-term studies would be to test whether Spanish moss flowering and fruiting advances temporally in relation to rising minimum temperatures, rather than other climate characteristics of warming climates.

In our trade-off maps (Figure 2, 4), we see that most Spanish moss populations show trade-off distances of 0.5 or less; nonetheless, some populations showed more substantial trade-off distances. Spanish moss populations under such sub-optimal conditions likely face challenges to long-term persistence, suggesting that optimality of conditions in flowering period represents a constraint on Spanish moss geographic distributions. Although it is hard to say whether or to what degree climate change will change the geographic distributional potential of Spanish moss, Spanish moss may not flower and produce seeds successfully if climate change takes populations too far from optimal conditions. Even under present-day conditions, our approach can be used to locate where populations of the species will be under particular physiological stress.

Conclusions

We analyzed high-temporal-resolution (6-hour resolution) climate data over a 22 year span to assess the availability of optimal conditions during flowering and fruiting periods of Spanish moss populations. Our results indicate that Spanish moss populations appear to flower and produce fruit seasonally such that populations experience optimum minimum temperatures. Our finding also shows that the least optimal conditions are experienced by populations along the fringes of the species' distribution. This research is novel in that we used herbarium specimens to assign flowering period to populations, that actual physiological measurements were used to assess optimality of conditions, and that high-temporal-resolution weather data were used to provide a near-real-time view of the environmental conditions experienced by the species.

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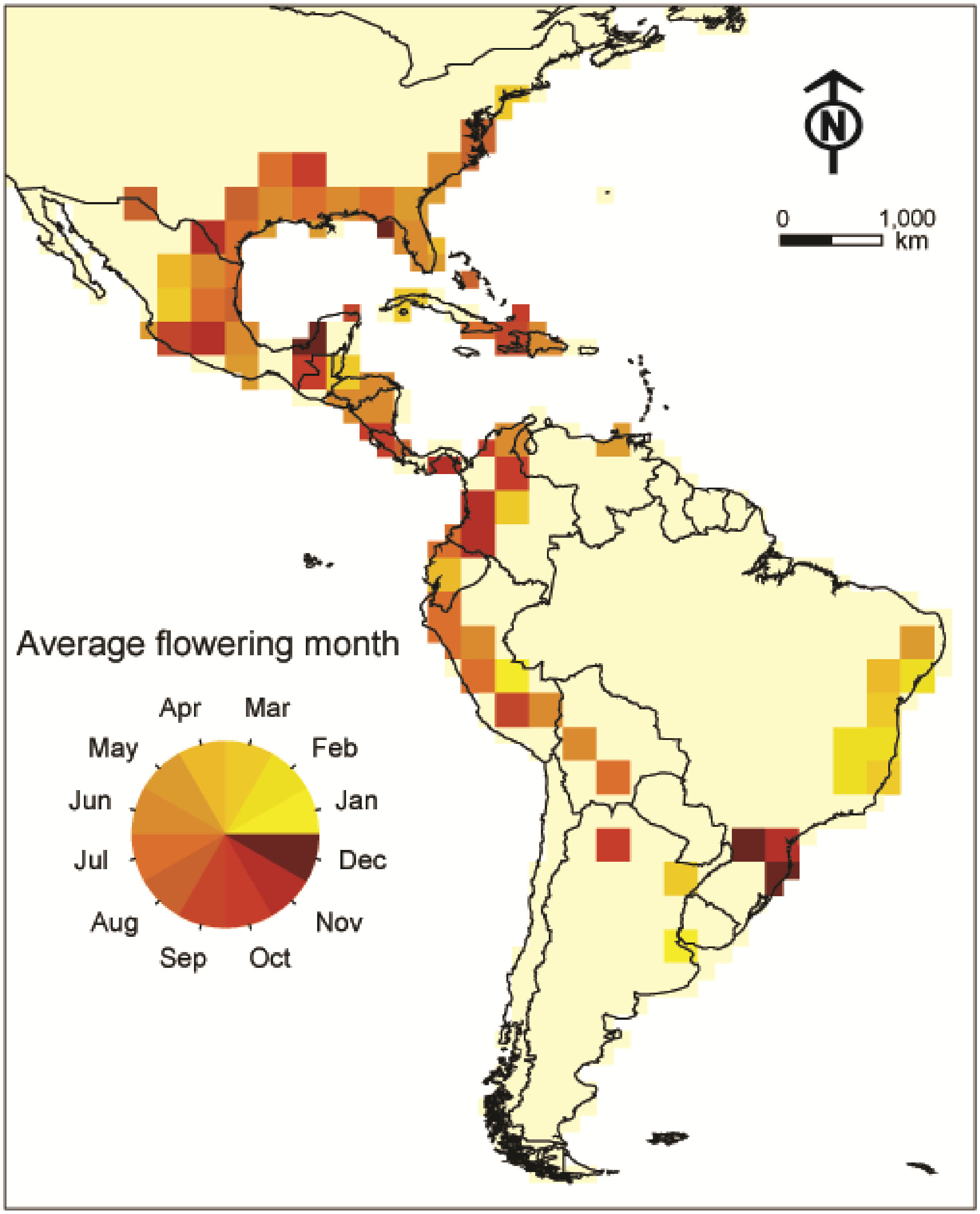

Figure 1. Average flowering month of Spanish moss populations across the Americas calculated as weighted average of flowering or fruiting specimens recorded from each grid square.

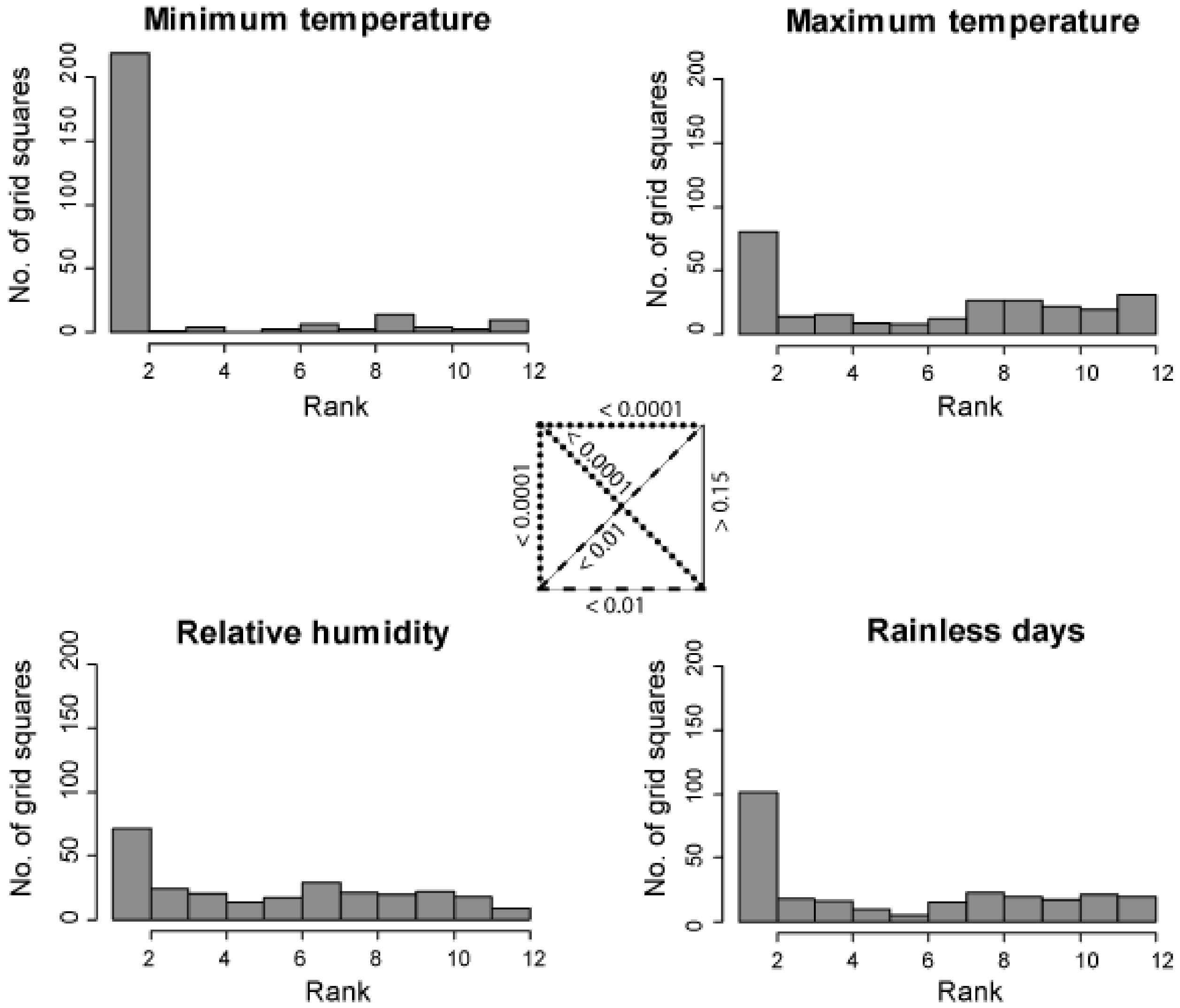

Figure 2. Histogram of ranks based on how long populations in each grid square are outside optimal conditions for each of the four parameters during their flowering and fruiting periods. The box at the center shows results of Kolmogorov-Smirnov tests results for comparison of distributions. Dotted line indicates highly significant difference; dashed lines significant differences, and continuous lines for non-significant difference.

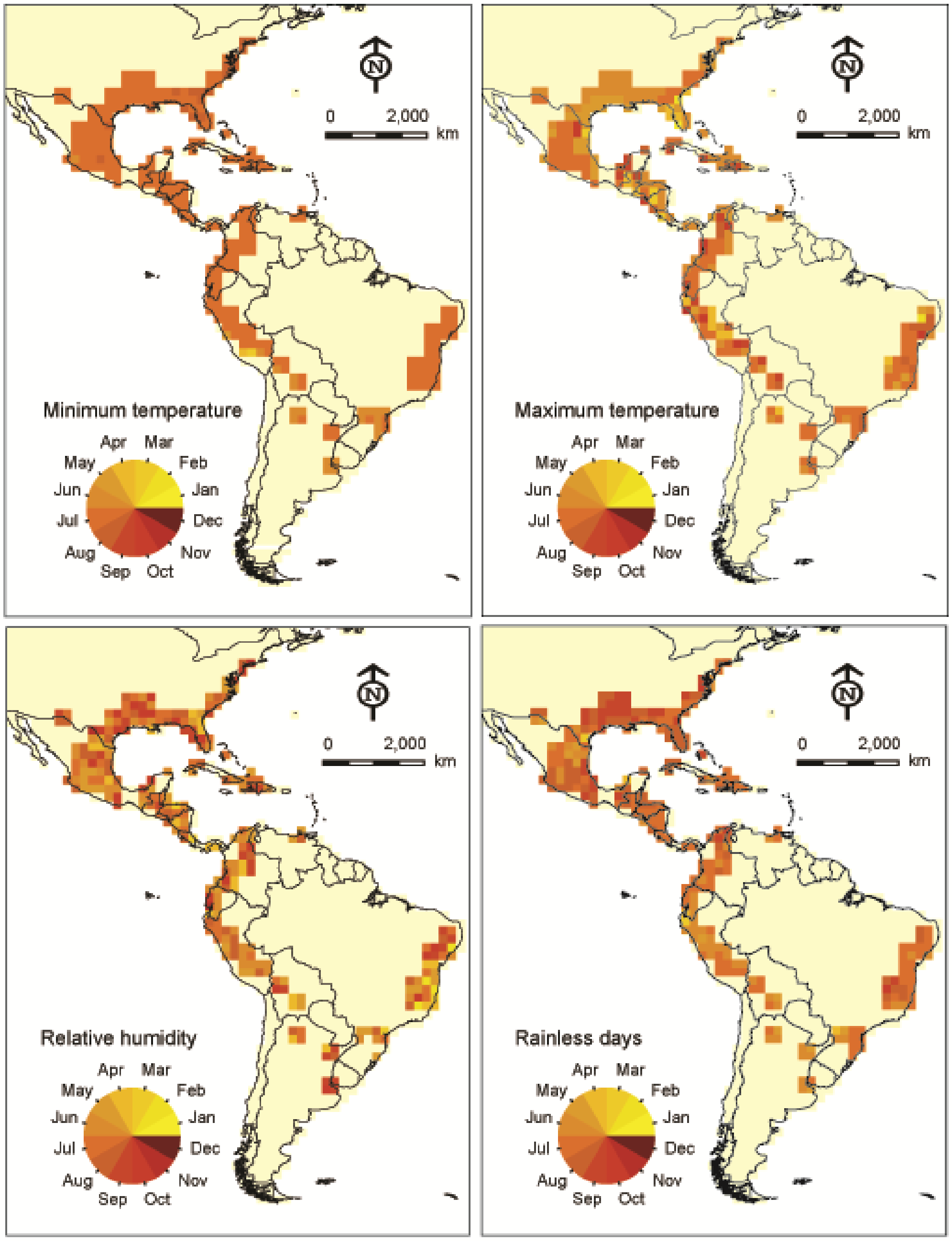

Figure 3. Optimal flowering and fruiting month for Spanish moss populations based on each physiological parameter in isolation.

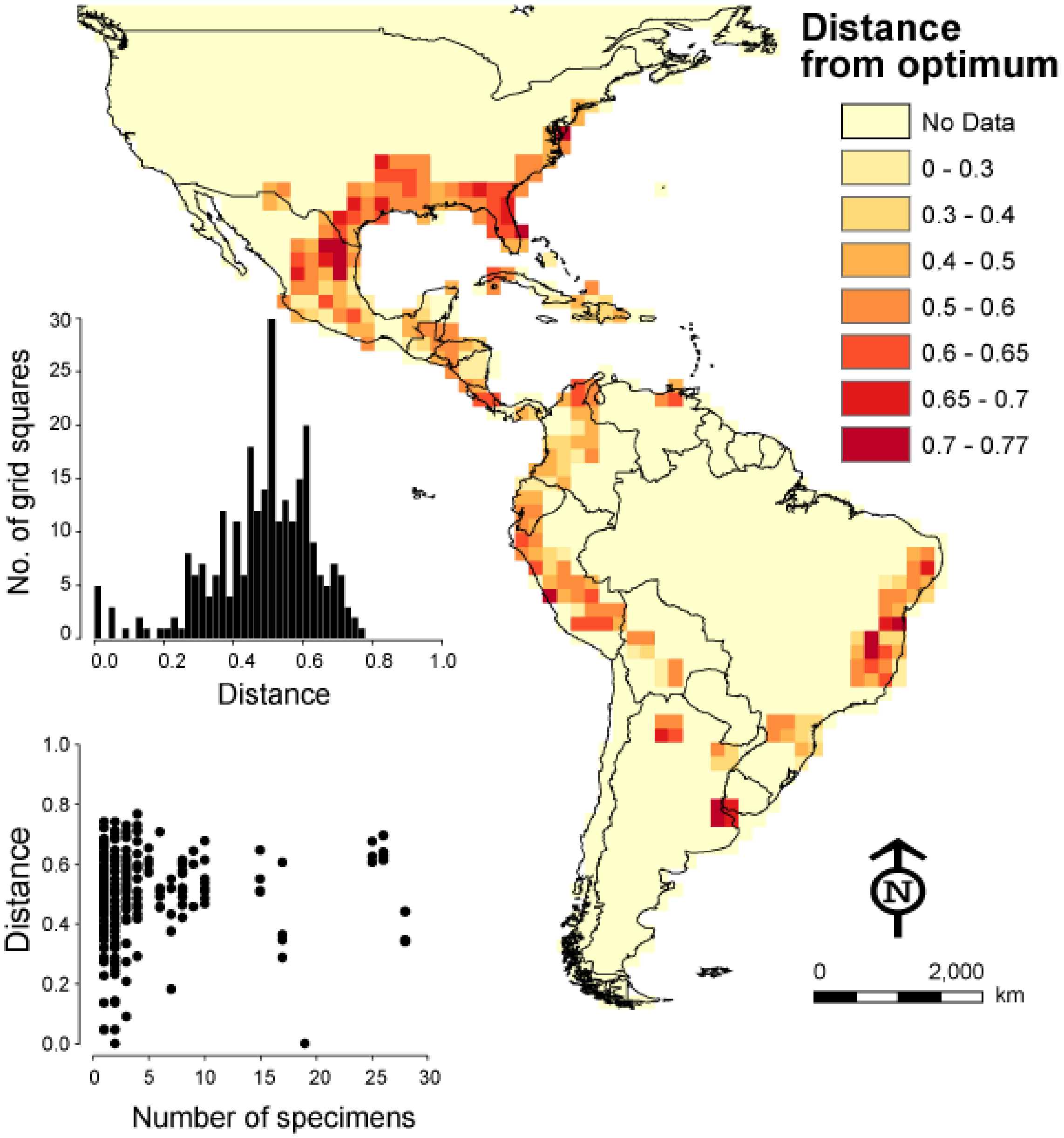

Figure 4. Map of Euclidean distances from observed conditions for Spanish moss populations to the best available across the species' distribution. Inset shows a frequency histogram of distances in grid squares (top) and relationship to numbers of specimens on which distance calculations were based (bottom).

Chapter 3

Physiological thermal maxima and impact of warming climates across the geographic distributions of 33 anuran species

Abstract

Species populations are affected by diverse aspects of weather and climate when variation takes conditions out of normal tolerance ranges; particularly of concern may be increases in temperature. Projected mean temperature increases may induce increases in extreme values which can be detrimental for many populations, as it will often not be possible to adapt to these changes. Amphibians are particularly affected by climate change owing to their small body size and ectothermic physiology: critical maximum temperature CT_{max} is an important element at the larval stage. We use experimentally measured CT_{max} values for 33 anuran species and 6-hourly resolution ERA weather data from 1989-2010 to understand in which populations of each species may experience CT_{max} and be under physiological stress. We also explored how frequently CT_{max} has been reached, and whether that frequency is increasing. Our results indicate that about 70% of the species have experienced CT_{max} at least for a single day during the last 22-year period somewhere in their ranges, but only one species experienced CT_{max} often during the 22-year time period.

Introduction

Global warming and climate change more generally are causing significant changes in species' geographic distributions. Many studies have demonstrated that species' ranges are shifting polewards owing to effects of warming temperatures (Root *et al.* 2003; Burrows *et al.* 2011; Chen *et al.* 2011). The temperature increase of 0.6°C to date has not only already affected species' distributions, but has also affected parameters of ecosystem function and timing of biological processes (Visser & Both 2005; Garrett *et al.* 2006). The projected change of temperature increase of 2.5°C over the next 100 years is anticipated to manifest significant changes in frequency of extreme climatic events, which will further affect species, and also may even cause extinctions (Wake & Vredenburg 2008; Moritz & Agudo 2013). Numerous research efforts have attempted to anticipate changes in distributions under future climatic scenarios using correlative, niche-based approaches (Pearson & Dawson 2003; Zurell *et al.* 2009; Poulos *et al.* 2012). In the few studies to date, correlative and mechanistic approaches have yielded similar predictions for species' distributions under future climatic conditions (Buckley 2008; Kearney *et al.* 2009, 2010). In this regard, it is essential to link geographic patterns of species' distributions to known physiological tolerances of species to evaluate the impacts of the rapidly changing climate.

Even though most species are experiencing climate change amphibians are particularly affected as a whole, and are experiencing a large-scale declines, which are at least partly climate-driven (Stuart *et al.* 2003; Wake 2006). An estimated 43% of amphibian species are declining (Stuart *et al.* 2003); indeed, 32% of the species are threatened with extinction, and this period could be considered a mass extinction event (Wake & Vredenburg 2008). Four major factors affecting amphibian populations include climate change, environmental pollutants, habitat modification, and invasive species and pathogens (Hayes *et al.* 2010). Considering climate warming, amphibian populations may frequently be exposed to conditions close to critical maximum temperatures tolerance limits, and their ability to adapt to temperature increases may be limited (Huey & Berrigan 2001; Hayes *et al.* 2010; Burrows *et al.* 2011). In this regard, identifying species and populations living at sites manifesting conditions close to critical maximum temperatures and potentially causing physiological stress becomes crucial.

For anuran species, critical maximum temperature (CT_{max}) in larval stages represents an important constraint in life cycles. An individual experiencing conditions approaching CT_{max} has higher chances of death or abnormal larval development, which in turn is reflected in declining recruitment to reproductive populations (Benard 2015). Little research has been done in regard to how often species experience CT_{max} temperatures in real life, or whether the frequency of exposure to CT_{max} is increasing over time as a consequence of climate change. Hence, this contribution, we use high-temporal-resolution climatic data to understand what parts of species' distributions experience conditions approaching CT_{max} and whether species have been experiencing CT_{max} increasingly frequently over the past two decades.

Methods

Species Data

Durate *et al.* (2012) studied 47 amphibian species at 3 locations presenting varied environmental conditions; we focus on the 41 anuran species included in that study. Study sites included (1) the Gran Chaco region of northern Argentina (23.8°–27.5°S), which has a warm subtropical climate;

(2) the Atlantic Forest biome of Misiones Province in northeastern Argentina (27.5°–27.1°S), with cooler subtropical environmental conditions; and (c) the Iberian Peninsula of Europe (36.9°–60.5°N), with cool temperate conditions. Duarte *et al.* (2012) derived critical maximum temperatures for each species following Hutchinson's dynamic method. In this method, the endpoint is determined by onset of muscular spasms after heating the individual at a constant heating rate of 1°C min^{-1} (Hutchison 1961). (Details of parameters and procedures are in (Duarte *et al*. 2012).

Of the initial 41 species, we discarded those species with areas of <10 1.5° grid squares, (about 1650 x 1650 km^2), leaving 33 species for analysis (Table 1), for each of which the original study had measured CT_{max}. We collected breeding season information for each species from the literature and from AmphibiaWeb (2015 (www.amphibiaweb.org; Table 1). In two cases, lacking breeding season information, we used the collection periods given in Duarte *et al*. (2012) as breeding period, in absence of more detailed information.

We downloaded crude summaries of geographic distributions of each species from the Global Amphibian Assessment (http://www.iucnredlist.org/initiatives/amphibians; IUCN, (2009) This dataset was developed by herpetologists from their expert knowledge and provides a rough distributional summary for each species. We buffered the IUCN distributional polygon by 80 km, to identify the set of grid squares that cover the entire distributional area for each species. We also downloaded occurrence data for each species from GBIF (www.gbif.org) and HerpNET (www.herpnet.org). Records of each species were assessed for inconsistencies such as (1) place name and geographic coordinates do not match; (2) geographic coordinates fall in the ocean or on a different continent; and (3) duplicate records. These data were corrected where possible, and otherwise deleted from analysis (Chapman 2005).

Climate Data

We examined how the maximum thermal temperature (CT_{max}) was or was not met for each species across its geographic distribution during the breeding season over a 22-year period (January 1989 – December 2010). To this end, we used the ERA Interim Reanalysis Climate Data developed and supplied by the European Center for Medium-Range Weather Forecasts. These data are based on a combination of models and observations, with 3-hourly temporal resolution: every second datum is a forecast, whereas the other is a model result. We used only the model result data, thus coarsening the data from 3-hourly to 6-hourly temporal resolution. The dataset has a somewhat coarse native spatial resolution of 1.5° x 1.5°, or approximately 165 x 165 km grid square resolution at the Equator.

ERA data were downloaded from http://apps.ecmwf.int/datasets/data/interim_full_daily/ for maximum temperature at 2 m above the ground. The ERA data are stored in NetCDF format (http://www.unidata.ucar.edu/packages/netcdf/index.html; Rew & Davis 1990); these data were manipulated and processed via the "ncdf" package in R (Pierce 2011; R Core Development Team, 2012

Data Analysis

An R script was developed using the raster, ncdf, and sp packages (Bivand *et al.* 2008; Pierce 2011; Hijmans & van Etten 2012) to calculate the number of days above CT_{max} in each breeding season for each species over the 22-year span of the climate data set. The script checked the value of maximum temperature across four daily observations; a grid square was marked as "unsuitable" for a day whenever two consecutive observations were at or above the temperature limit. It also calculated maximum number of days in sequence outside physiological tolerance limits for each grid square in the distribution of each species. Maximum number of days outside the physiological niche for each grid square was stored for every breeding season.

Using this information, we developed maps in which each grid square across the distribution is marked as unsuitable whenever a grid square is outside the physiological tolerance limit on at least one day, during any breeding season over the 22-year period. We calculated the proportion of the distributional area of each species outside the physiological niche. We overlaid the curated occurrence data (see above) on distributional maps and calculated proportions of occurrence points falling outside species' distribution areas (i.e data problem), and proportion of occurrences falling outside the physiological niche but within the distributional area.

We calculated, for each species in each year, the maximum temperature within the distribution area, maximum number of days in the distributional area above CT_{max}, and maximum number of grid squares above CT_{max}. For each species, we developed a linear regression model assessing the number of days outside the physiological niche, and the number of grid squares outside the physiological niche as a function of time.

Results

Our analysis indicates that $\mathrm{CT}_{\max}$ conditions have been experienced in at least one grid square during the breeding season by 23 (70%) of the 33 anuran species. For nine species, the number of grid squares going out of the physiological niche increased during the 22 years of this study. Among the 33 species analyzed, species' ranges covered 16–800 grid squares (Figure 1a). Figure 1(b) shows a summary of relationships between extent of distributional area and the proportion of the distribution experiencing $\mathrm{CT}_{\max}$ for each of 33 anuran species during the 22 years. The maximum proportion of distribution area outside the physiological niche is for the species *Ceratophrys cranwelli,* which does not have particularly large distribution. The species with the largest distribution was *Bufo bufo*; 90% of the distributional area of this species was within the physiological niche limits for the entire study period. For ten species, none of the distributional area manifested temperatures was outside the physiological niche during the study period.

Distributional areas within and outside the physiological niche are shown for four example species in Figure 2 (see also Supplementary Figure 2). For example, *Bufo bufo* (Figure 2c) has a broad distribution, and only a few peripheral grid squares were outside of the physiological niche. For *Alytes cisternasii*, no part of the distribution experienced critical maximum temperature on any day during the 22-year study period. In contrast, major parts of the distribution of *Ceratophrys cranwelli* experienced $\mathrm{CT}_{\max}$ on at least one day in the 22-year period. Distributional summaries were coarse in nature, but in many cases the actual occurrence data fell outside tolerance limits. Occurrence data at sites manifesting conditions outside the physiological niche limits ranged 1-42% (Figure 3). For *Rhinella ornata*, no data were available on GBIF or HerpNet, as this species is vulnerable; none of the species had all occurrence data falling within the IUCN distribution outline. For *Phyllomedusa sauvagii*, most of the occurrences fell under low–suitability physiological conditions. However, for *Leptodactylus bufonius* and *Lepidobatrachus llanensis*, even though the distributional area contained grid squares with low physiological suitability, known occurrences did not fell in those areas.

Temporal trends in frequency of experiencing CT_{max} from year to year are summarized in Figure 4. For *Bufo bufo*, the number of grid squares experiencing CT_{max} increased significantly over the study period ($R^2 = 0.26$, $P < 0.02$). For *Scinax fuscovarius*, the number of consecutive days above CT_{max} and number of grid squares experiencing CT_{max} increased within the distribution (Figure 4b, Supplementary Figure 1). Overall, however, of the 33 anuran species analyzed, only *Bufo bufo* showed significant ($P < 0.05$) temporal trends (Table 2).

Discussion

Physiological tolerance limits can be informative in understanding geographic distributions and range limits of species, and may be particularly relevant in climate change scenarios (Porter *et al.* 2000; Pörtner & Farrell 2008). To understand how species may shift their geographic distributions, the frequency of exceptions to physiological tolerances can be projected on to geography. This approach is potentially important but has been implemented only for few ectothermic species (Kleidon & Harold 2000; Kearney & Porter 2004; Kearney *et al.* 2008; Buckley 2008; Helmuth 2009; Buckley *et al.* 2010). The correlational analog technique is to infer physiological limits from the occupied geographic distribution (Peterson 2001; Soberón & Peterson 2005; Elith & Leathwick 2009; La Sorte & Jetz 2010; Poulos *et al.* 2012; Bentlage *et* *al.* 2013); this latter technique has become much more popular owing to ease of operation and convenient data availability.

Physiological parameters are not available for most species, owing to time and cost constraints involved with obtaining estimates of key parameter values. However, when such data are available, they can be highly informative about how climate change may affect species' distributions. We used CT_{max} estimates for 33 anuran species to understand which species will prove more vulnerable in light of warming climates, and how much of each species' distribution is under unsuitable conditions in terms of maximum temperature. In overview, about 70% of the species experienced CT_{max} conditions during 22 years in at least part of their geographic distributions (Figures 1, 4). For only one species, the number of days with CT_{max} and proportion of the distribution experiencing CT_{max} increased dramatically and significantly over the study period.

Duarte *et al* (2012) explored differences between the CT_{max} and maximum pond temperatures (T_{max}) at three different localities with different environmental conditions. Their result suggests that the difference between CT_{max} and T_{max} is less in subtropical warm locations, such that species in subtropical warm locations may have more narrow tolerances. Our results also supported this idea: species in subtropical locations appeared to experience CT_{max} more frequently than species in temperate regions. For some temperate species, the peripheral parts of the distribution were frequently outside of the physiological niche, such that populations in those regions may be more physiologically stressed.

We observed that these phenomenon of distributional areas outside of the physiological niche was particularly frequent at lower latitudes are from lower latitudes (Figure 2, Supplementary Figure 2) which may have particularly serious implications for these species' geographic ranges. Besides anurans, many marine invertebrates and lizards are believed threatened by increases in temperatures, as they appear to live close to their thermal tolerance limits (Sinervo *et al.* 2010; Somero 2010). A few case studies suggest that species cannot adapt fast enough to keep up with warming climate (Hoffmann & Sgrò 2011), a few recent studies (Logan *et al.* 2014; Ferri-Yáñez & Araújo 2015) have demonstrated temperature adaptation in species of lizards with strong natural selection favoring individuals who could run faster in warmer environments. Whether similar process is happening or could happen in anuran species, or how targets of selection may differ, is not yet known.

One concern about this study's design is that the distributional areas used in this study (IUCN 2009) are rather crude, such that our estimates or physiological stress across distribution areas may be rather noisy. For almost all (95%) of the species, we found that many populations occur outside the IUCN distribution region (and we checked taxanomic concepts carefully to avoid artifacts). It is important to validate these occurrences or update the distributional summaries, to make estimates of threat under climate change scenario more accurate. For example, for species *Pseudis platensis* more than 50% of available occurrences fell outside the IUCN distributional border. We could not validate the IUCN distributional area for species *Rhinella ornata* as no occurrences are available; as this species is considered vulnerable, geo-locations have not been made public.

Overall, our analysis revealed that, among 33 species of anurans, some part of the distributional areas for 23 of the species experienced CT_{max} during a 22-year time period. However, our analysis of temporal trends during the 22-year time span showed only a single significant result. Only *Bufo bufo* showed that number of days experiencing CT_{max} , and number of pixels experiencing CT_{max} had increased. Using our approach, we could not conclude that climate change has already been affecting these species' distributional areas significantly.

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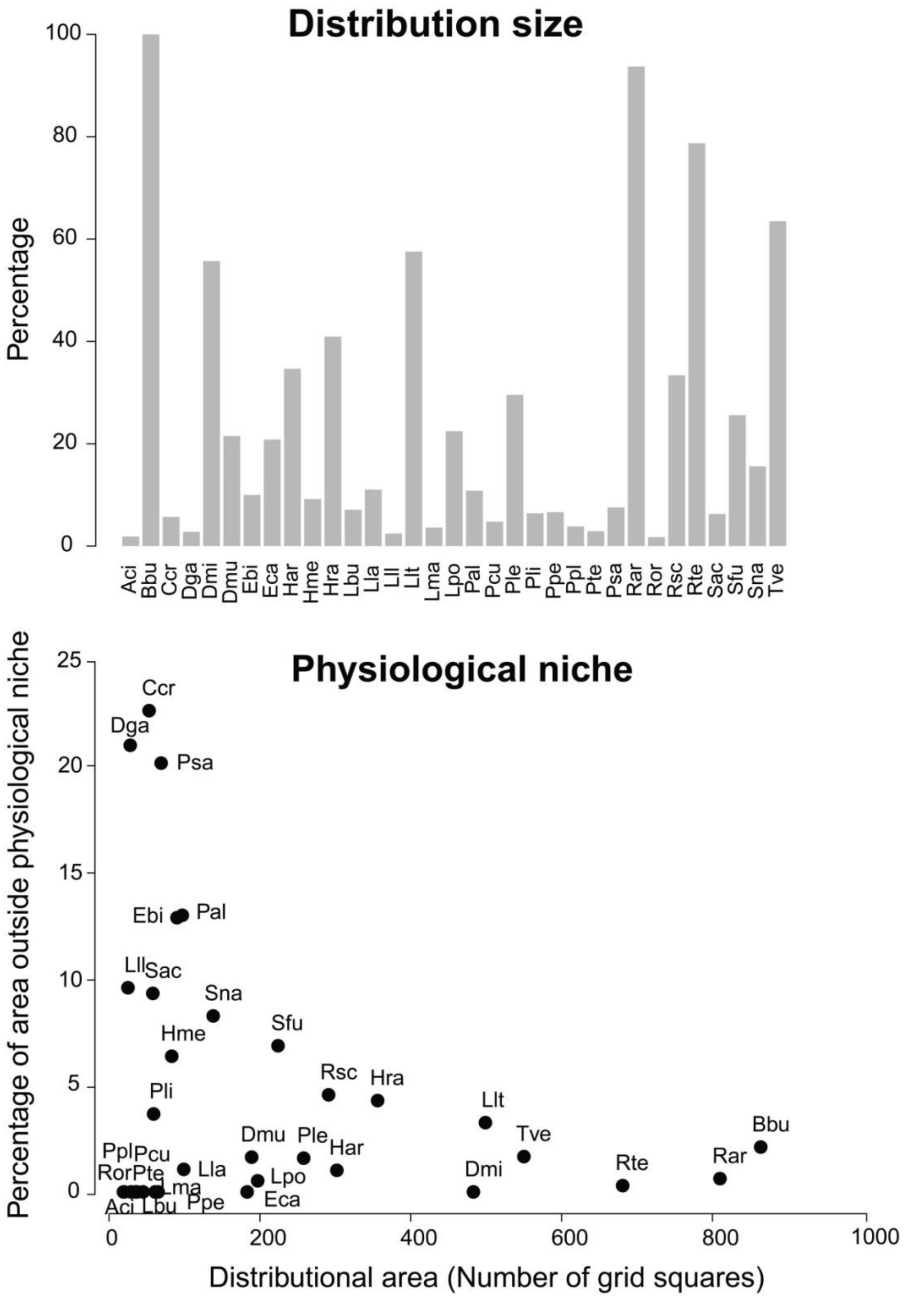


Figure 1. (Top) Distributional areas from IUCN (2009) for 33 anuran species. (Bottom) Summary of relationships between extent of distributional area expressing conditions of temperature within CT_{max} for each of 33 anuran species.

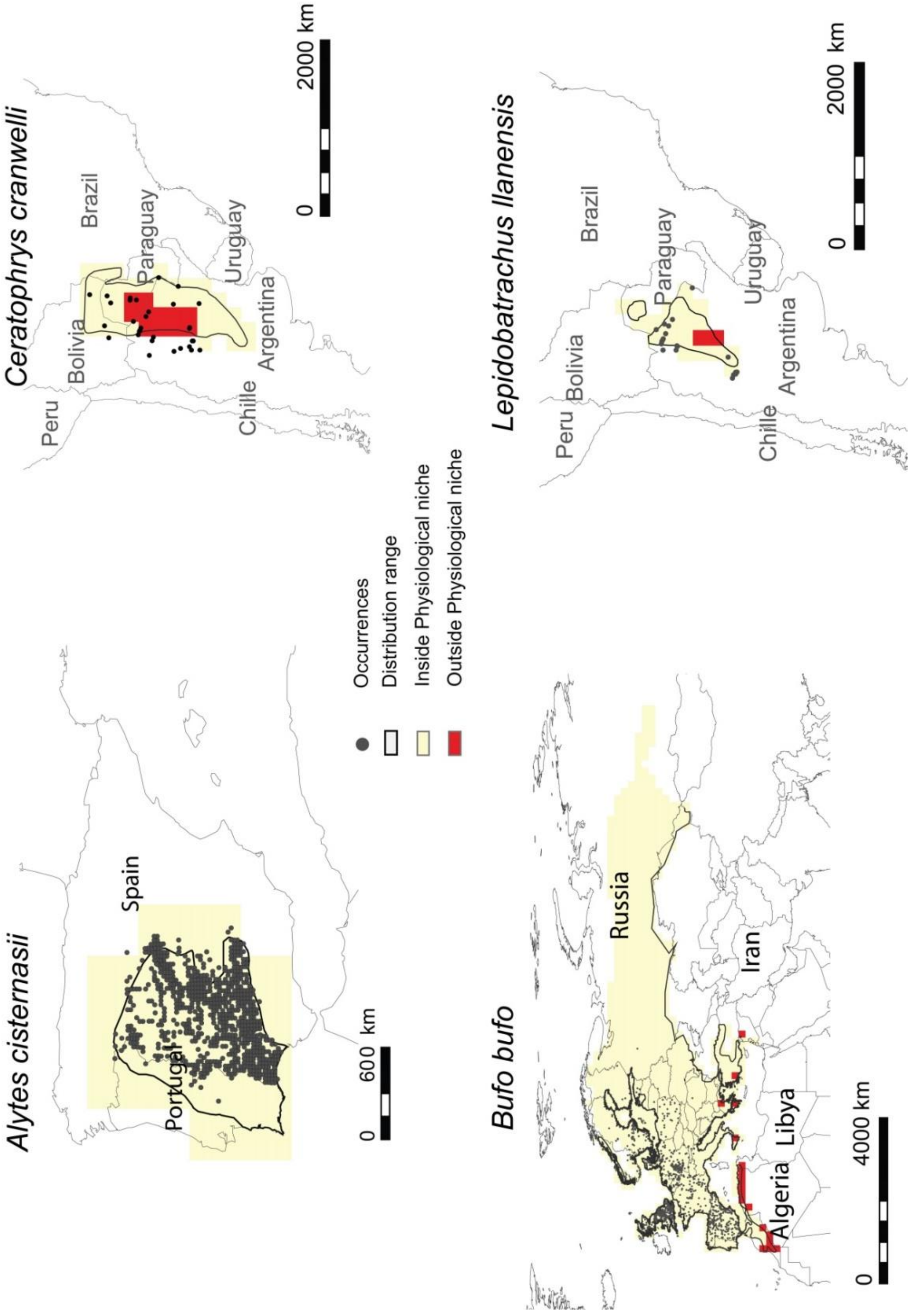


Figure 2. Four examples of species' distributional areas within and outside physiological niches. Yellow shading indicates conditions inside physiological niche; and red shading represents conditions outside of the physiological niche

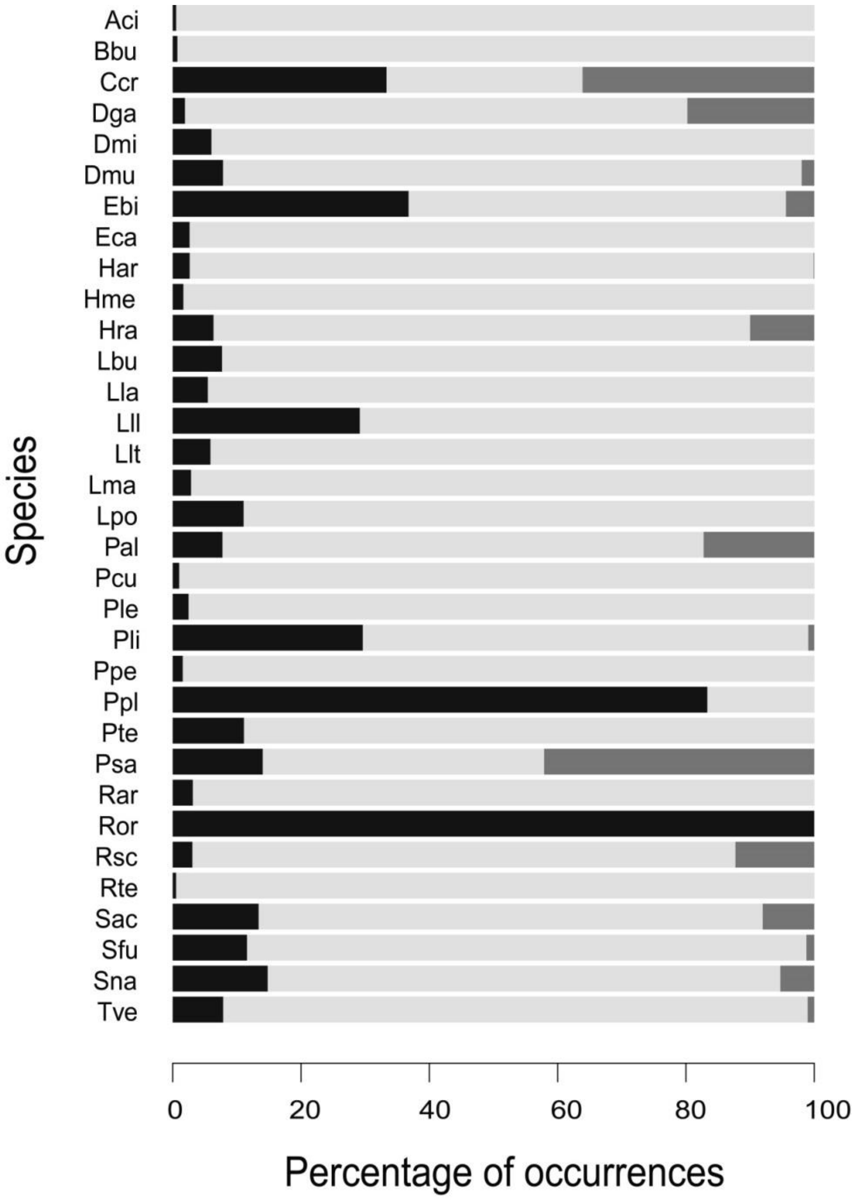


Figure 3. Summary of anuran species' occurrence data in relation to IUCN (2009) range extent polygons and physiological tolerances. Black indicates occurrences falling outside distributional areas defined in the IUCN polygons; Dark gray summarizes proportion of occurrence points falling in areas with conditions outside of physiological niche. Light gray summarizes proportion of occurrence points falling in areas with conditions inside of physiological niche.

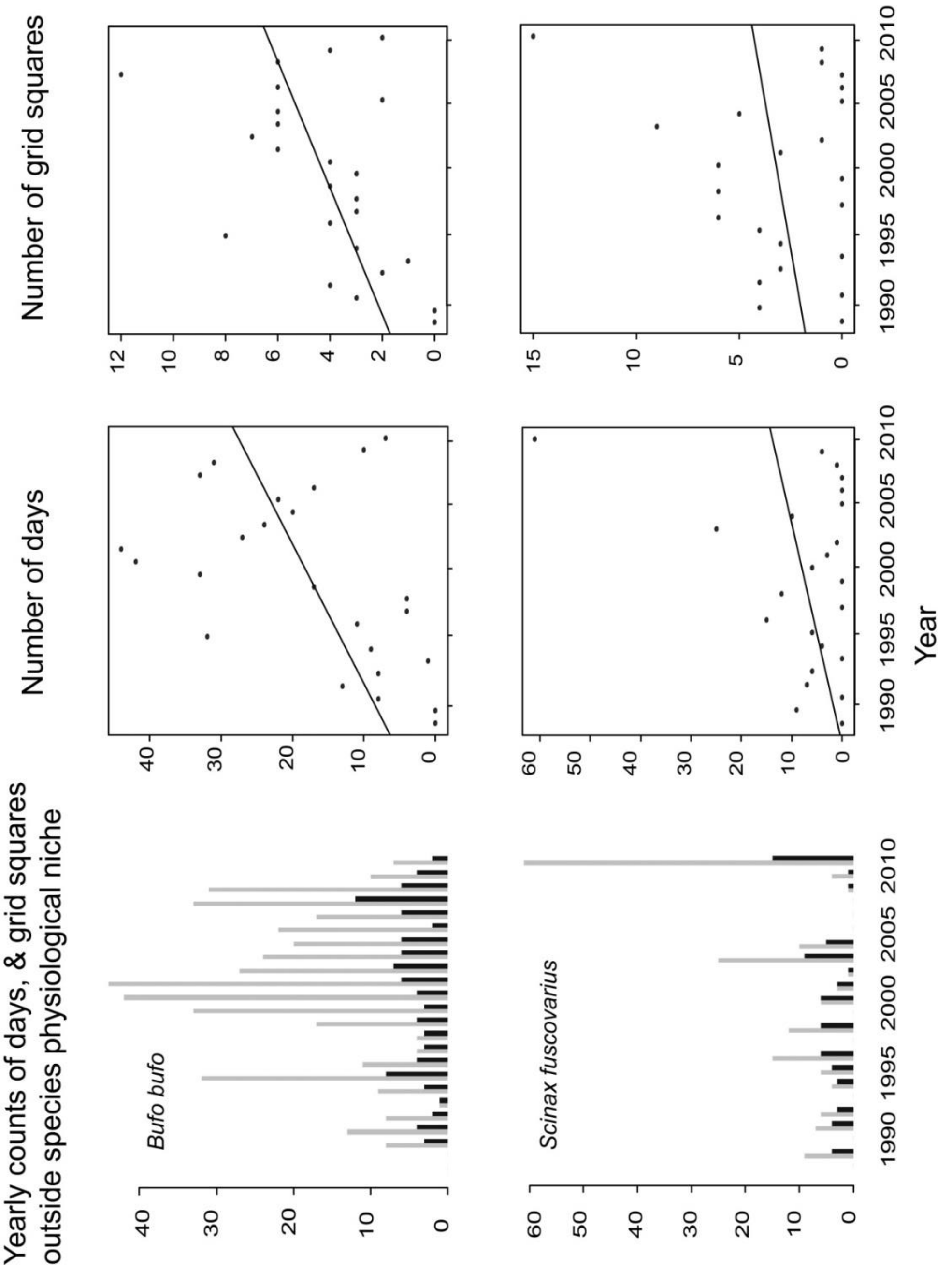


Figure 4. Summary of temporal trends for two example species. (Left): Number of days outside physiological niche (dark gray), number of grid squares outside physiological niche (light gray) (Middle): Relationship of number of days outside physiological niche and breeding year. (Right): Relationship of number of grid squares outside physiological niche in distributional areas and breeding year.

Table 1: List of anuran species with its respective CT$_{max}$ and breeding seasons

Name	Code	CT$_{max}$ in (C)	Community	Breeding season
Alytes cisternasii	Aci	38.2 ± 0.2	Temperate	September – March (AmphibiaWeb, 2015)
Bufo bufo	Bbu	38.3 ± 0.1	Temperate	March – June (AmphibiaWeb, 2015)
Ceratophrys cranwelli	Ccr	42.0 ± 0.1	Subtropical warm	November – February (Duarte *et al.* 2012)
Discoglossus galganoi	Dga	38.4 ± 0.1	Temperate	October – July (AmphibiaWeb, 2015)
Dendropsophus minutus	Dmi	40.6 ± 0.1	Subtropical cool	September – February (AmphibiaWeb, 2015)
Dermatonotus muelleri	Dmu	43.6 ± 0.3	Subtropical warm	September – February (AmphibiaWeb, 2015)
Elachistocleis bicolor	Ebi	41.7 ± 0.2	Subtropical warm	September – March (Rodrigues *et al.* 2003)
Epidalea calamita	Eca	39.7 ± 0.1	Temperate	September – May (“Froglife”)
Hyla arborea	Har	40.0 ± 0.1	Temperate	April – July (Friedl & Klump 1997)
Hyla meridionalis	Hme	39.8 ± 0.1	Temperate	March –June (Diaz-Paniagua 1988)
Hypsiboas raniceps	Hra	41.2 ± 0.2	Subtropical warm	September – March (Haddad *et al.* 2005)
Leptodactylus bufonius	Lbu	43.3 ± 0.1	Subtropical warm	December (Heyer & Bellin 1973)
Leptodactylus latinasus	Lla	44.7 ± 0.2	Subtropical warm	September – May (Diaz-Paniagua 1988)
Lepidobatrachus llanensis	Lll	44.7 ± 0.2	Subtropical warm	October – February (Cei 1968)
Leptodactylus latrans	Llt	41.4 ± 0.2	Subtropical warm	September – February (AmphibiaWeb, 2015)
Limnomedusa macroglossa	Lma	39.9 ± 0.2	Subtropical cool	November – January (Both *et al.* 2008)
Leptodactylus podicipinus	Lpo	43.3 ± 0.3	Subtropical cool	November – March (Almeida *et al.* 2015)
Physalaemus albonotatus	Pal	41.1 ± 0.2	Subtropical warm	September – March(Rodrigues *et al.* 2004)
Pelobates cultripes	Pcu	39.4 ± 0.1	Temperate	October – May (Tejedo 1993)
Pelophylax lessonae	Ple	38.6 ± 0.2	Temperate	May – July (AmphibiaWeb, 2015)
Pseudis limellum	Pli	41.9 ± 0.1	Subtropical warm	August – March
Pelophylax perezi	Ppe	39.6 ± 0.2	Temperate	February – June(Gómez-Rodríguez *et al.* 2009)

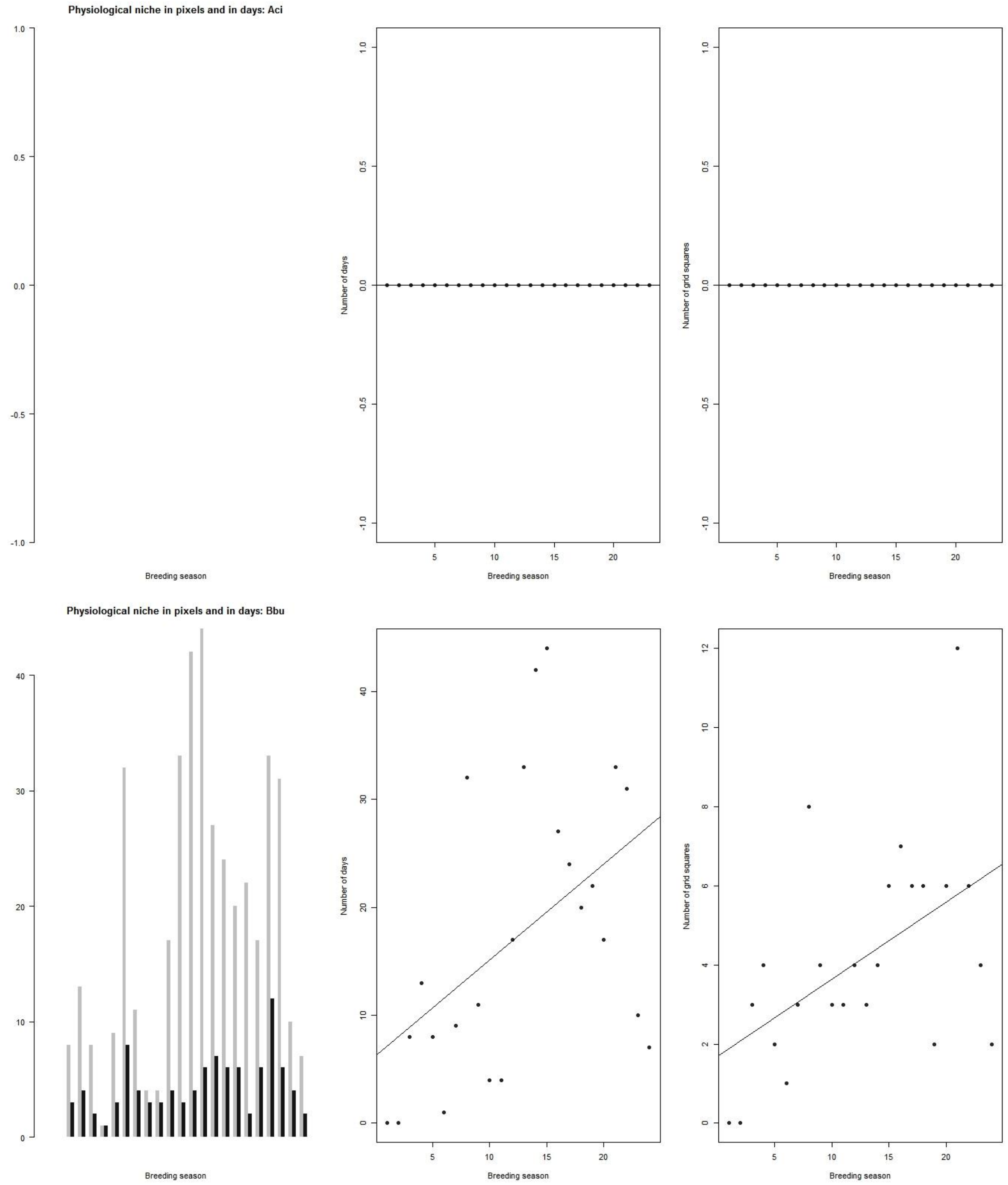
Pseudis platensis	Ppl	42.3 ± 0.1	Subtropical warm	November – April (South & Journal 2011)
Phyllomedusa sauvagii	Psa	42.1 ± 0.3	Subtropical warm	October – February (Wogel *et al.* 2005)
Phyllomedusa tetraploidea	Pte	41.6 ± 0.2	Subtropical cool	October – December (Dias *et al.* 2013)
Rana arvalis	Rar	35.8 ± 0.1	Temperate	March – June (AmphibiaWeb, 2015)
Rhinella ornata	Ror	40.7 ± 0.1	subtropical	August – February (Toledo *et al.* 2012)
Rhinella schneideri	Rsc	42.5 ± 0.1	Subtropical warm	October – April (AmphibiaWeb, 2015)
Rana temporaria	Rte	37.2 ± 0.1	Temperate	March – June (AmphibiaWeb, 2015)
Scinax acuminatus	Sac	43.0 ± 0.2	Subtropical warm	October – March (Journal 2003)
Scinax fuscovarius	Sfu	41.0 ± 0.3	Subtropical	October – February (AmphibiaWeb, 2015))
Scinax nasicus	Sna	42.6 ± 0.2	Subtropical warm	November – February (Peltzer *et al.* 2008)
Trachycephalus venulosus	Tve	41.9 ± 0.1	Subtropical warm	November – February (AmphibiaWeb, 2015))

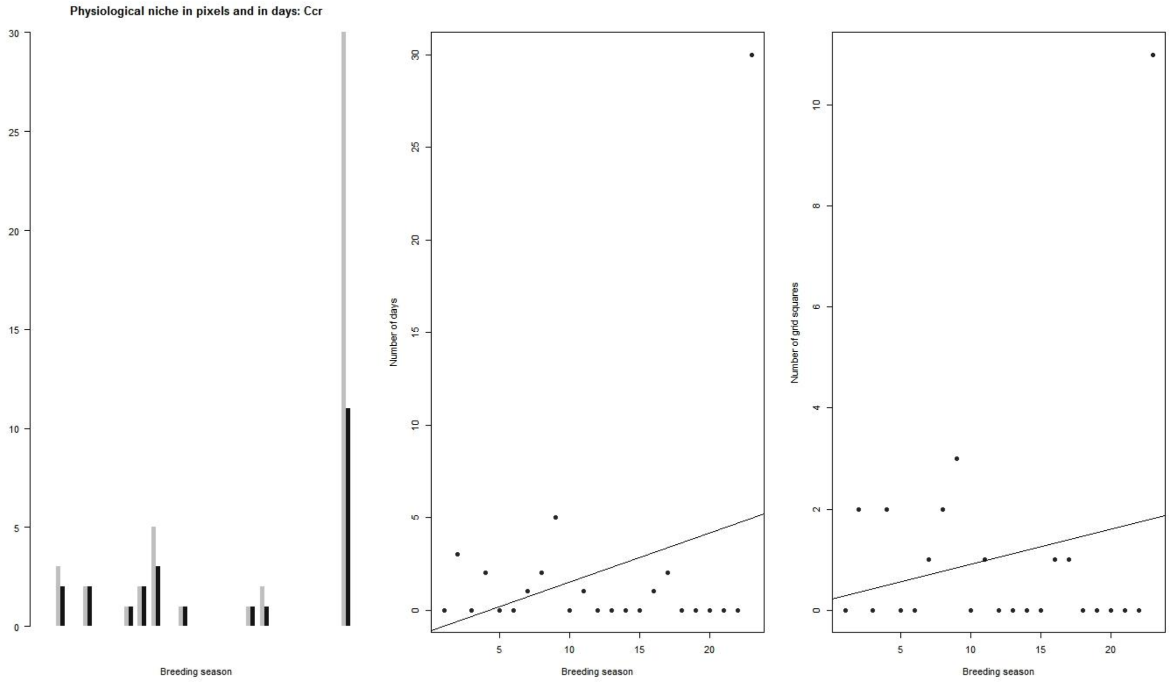
Table 2: Summary of linear regression models assessing trends in maximum temperature as a function of time across species' distributions

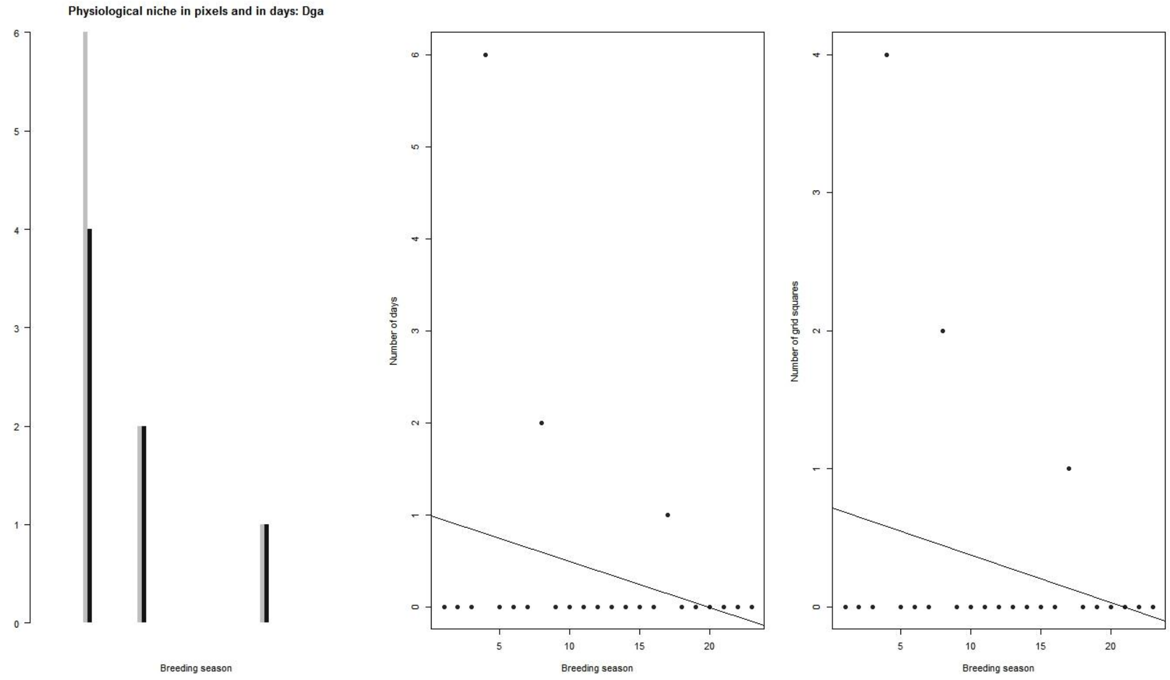
	P	
Species	Number of days	Number of grid squares
Alytes cisternasii	-	-
Bufo bufo	**0.0195**	**0.0104**
Ceratophrys cranwelli	0.1802	0.3531
Discoglossus galganoi	0.2274	0.2436
Dendropsophus minutus	-	-
Dermatonotus muelleri	0.0979	0.0979
Elachistocleis bicolor	0.1675	0.4123
Epidalea calamita	-	-
Hyla arborea	0.2185	0.2721
Hyla meridionalis	0.1047	0.2056
Hypsiboas raniceps	0.1875	0.4053
Leptodactylus bufonius	-	-
Leptodactylus latinasus	0.3517	0.7616
Lepidobatrachus llanensis	0.0979	0.0979
Leptodactylus latrans	0.1777	0.3183
Limnomedusa macroglossa	-	-
Leptodactylus podicipinus	0.0979	0.0979
Physalaemus albonotatus	0.1384	0.2548
Pelobates cultripes	-	-
Pelophylax lessonae	0.2272	0.2272
Pseudis limellum	0.0979	0.0979
Pelophylax perezi	-	-
Pseudis platensis	-	-
Phyllomedusa sauvagii	0.1542	0.2107
Phyllomedusa tetraploidea	-	-
Rana arvalis	0.9277	0.8067
Rhinella ornata	-	-
Rhinella schneideri	0.1513	0.1889
Rana temporaria	0.2272	0.2272
Scinax acuminatus	0.2091	0.2222
Scinax fuscovarius	0.1608	0.3505
Scinax nasicus	0.1731	0.212
Trachycephalus	0.1923	0.4625

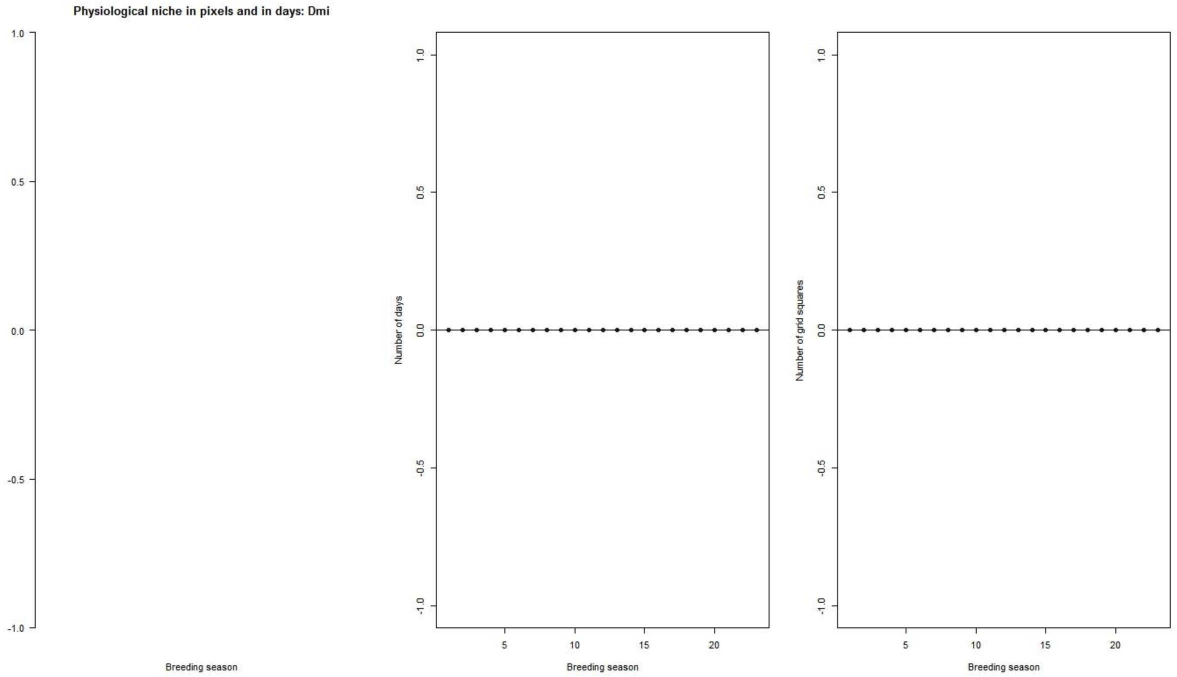
venulosus

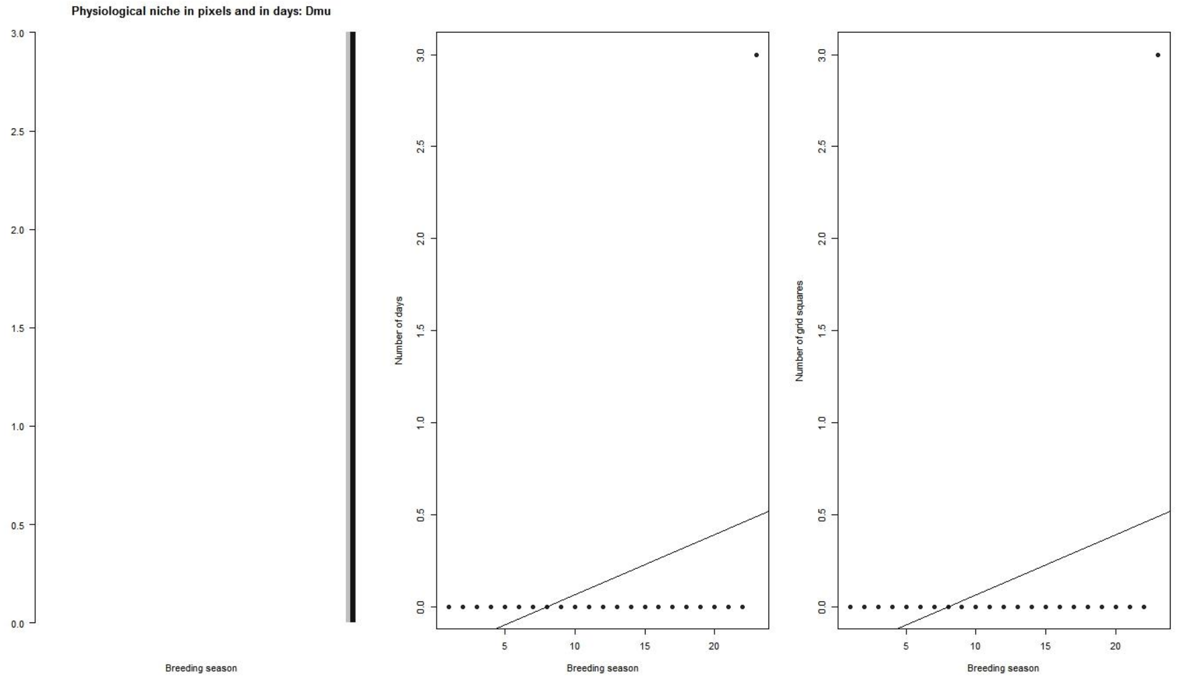
Supplimentary figure 1 Summary of temporal trends for two example species. (Left): Number of days outside physiological niche (dark gray), number of grid squares outside physiological niche (light gray) (Middle): Relationship of number of days outside physiological niche and breeding year. (Right): Relationship of number of grid squares outside physiological niche in distributional areas and breeding year.

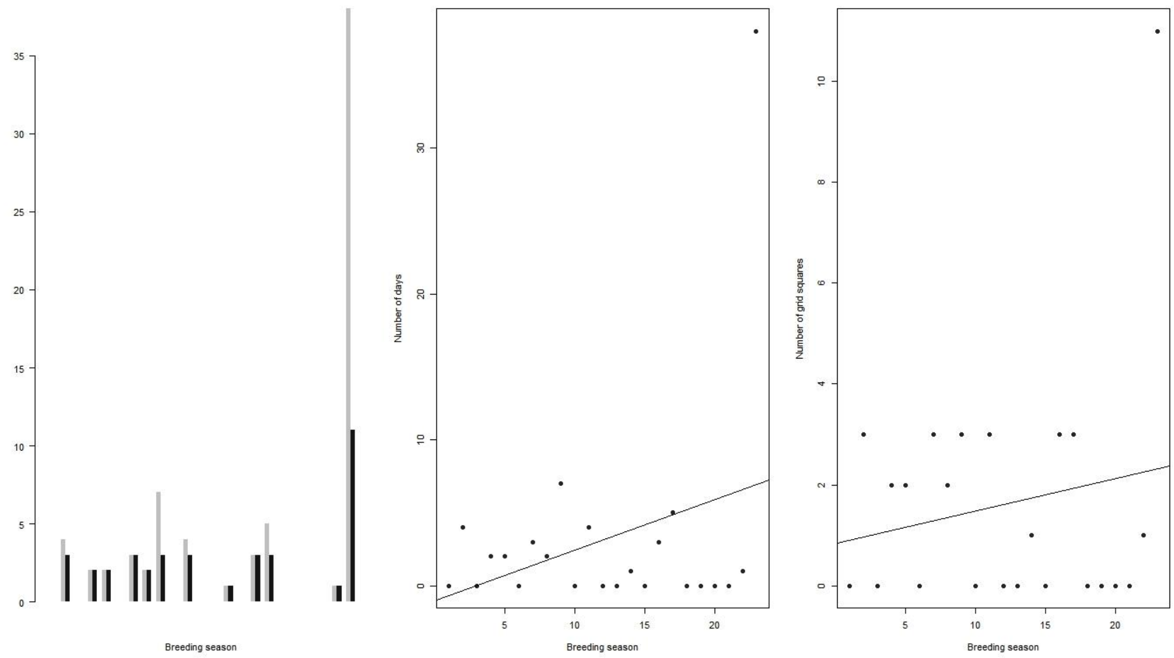


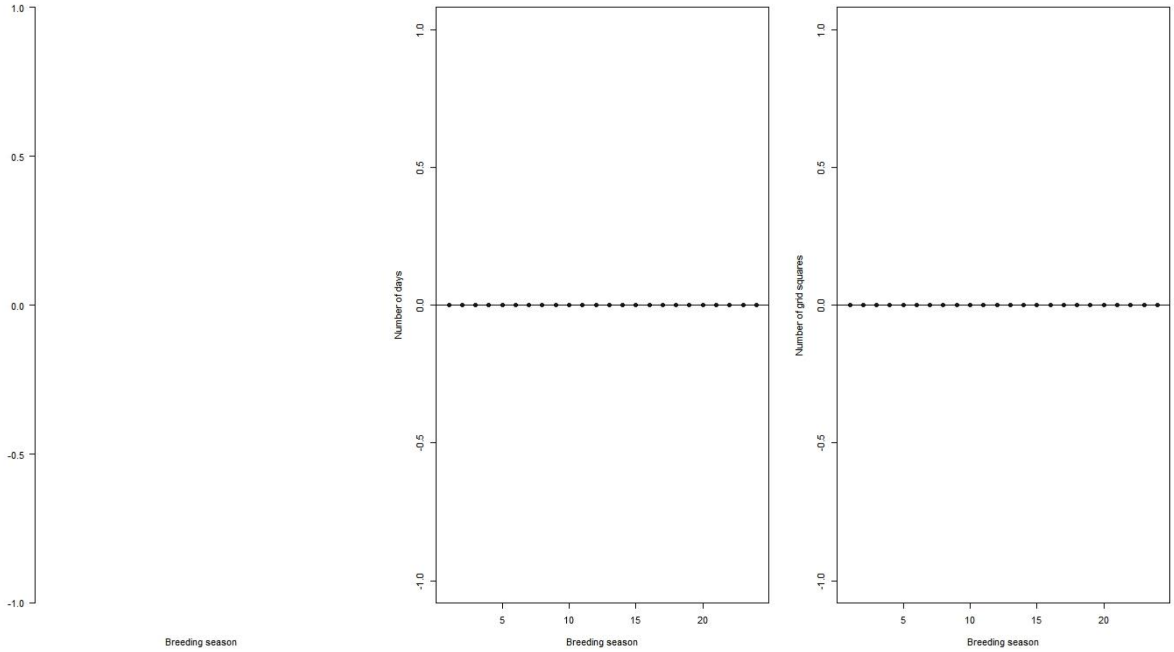

Physiological niche in pixels and in days: Ccr
0
5
10
15
20
25
30
Breeding season
Number of days
Breeding season
Number of grid squares
Breeding season

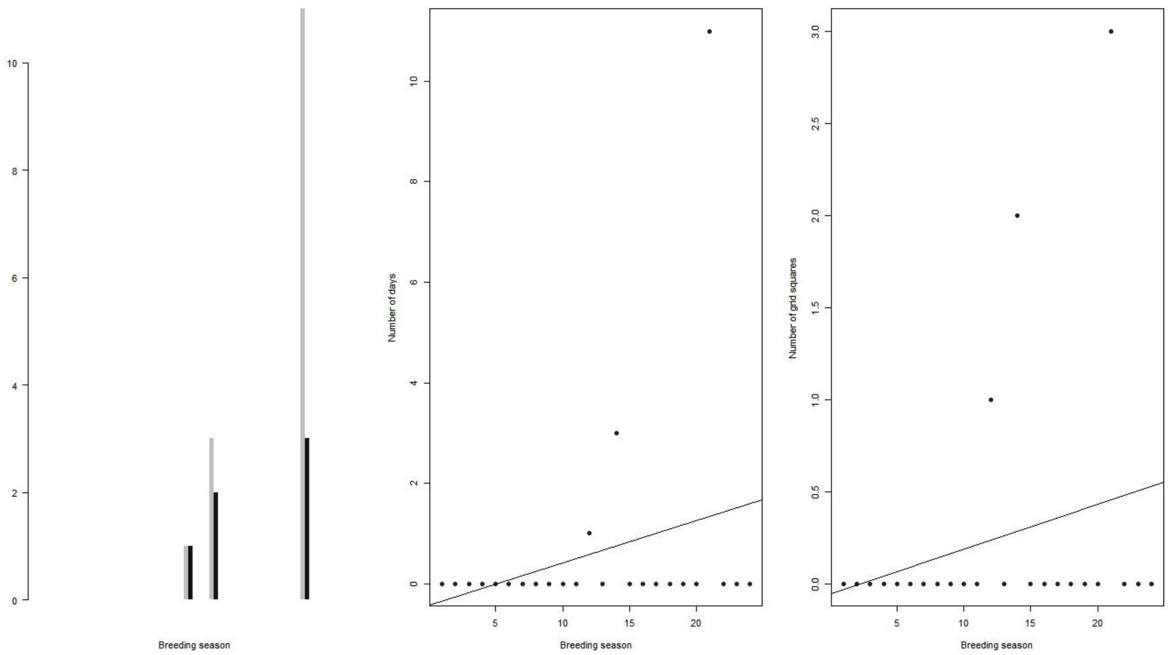

Physiological niche in pixels and in days: Dga
0
1
2
3
4
5
6
Breeding season
Number of days
Breeding season
Number of grid squares
Breeding season

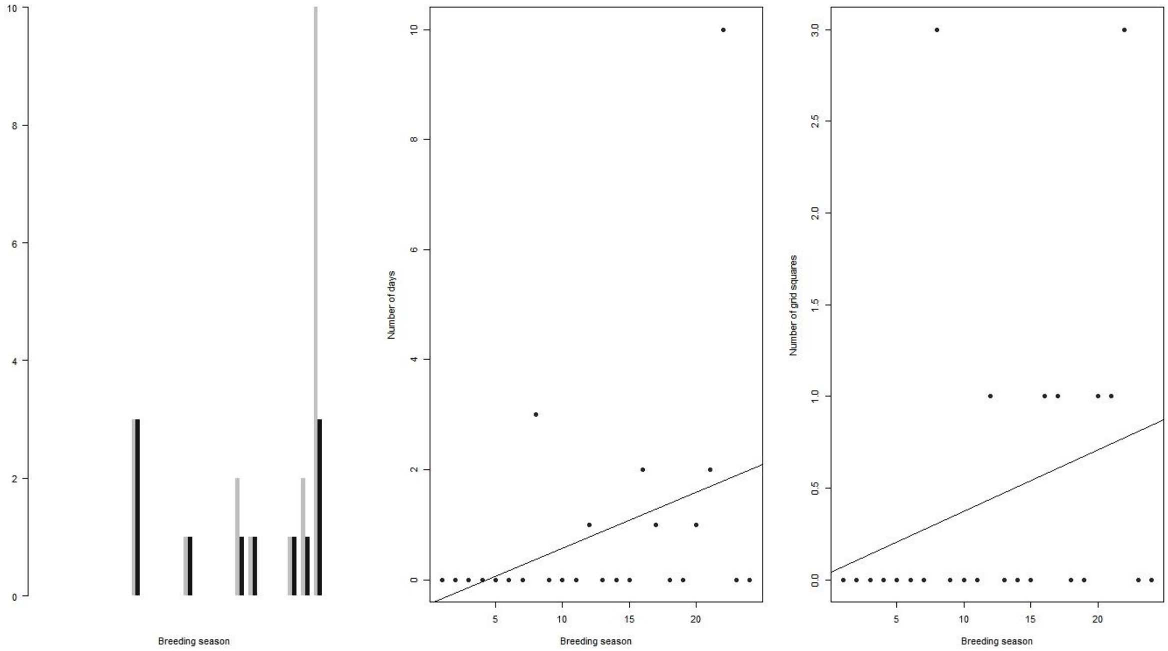

Physiological niche in pixels and in days: Dmi
1.0
0.5
0.0
-0.5
-1.0
Breeding season
Number of days
5
10
15
20
Breeding season
Number of grid squares
5
10
15
20
Breeding season


Physiological niche in pixels and in days: Dmu
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Breeding season
Number of days
5
10
15
20
Breeding season
Number of grid squares
5
10
15
20
Breeding season

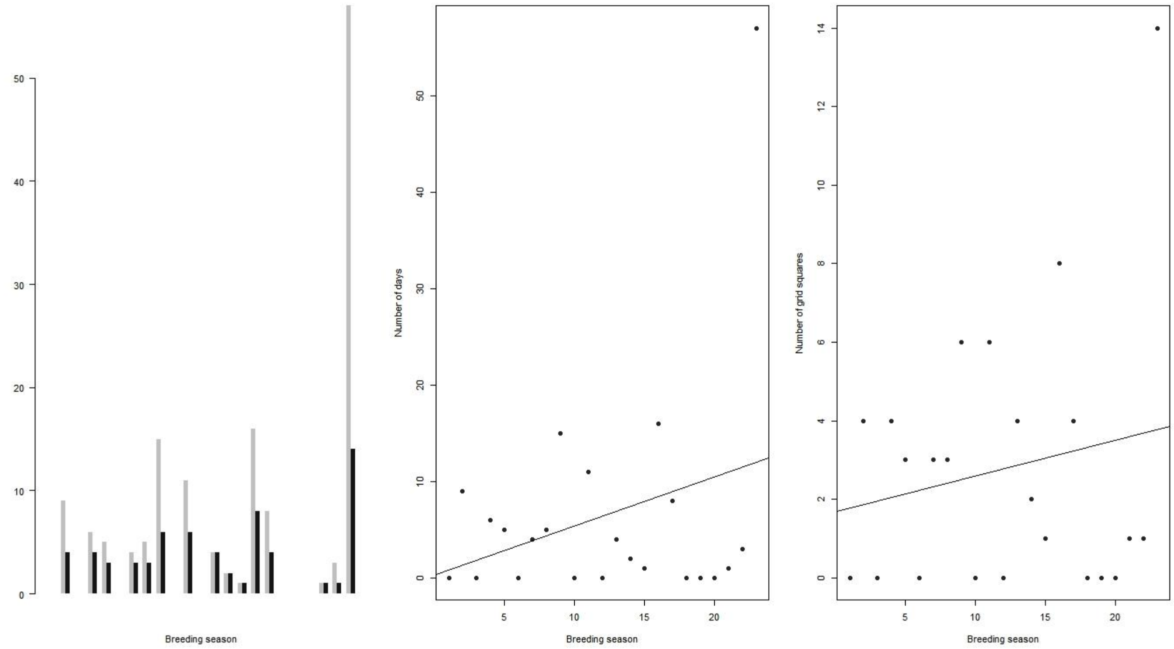

Physiological niche in pixels and in days: Ebi


Physiological niche in pixels and in days: Eca

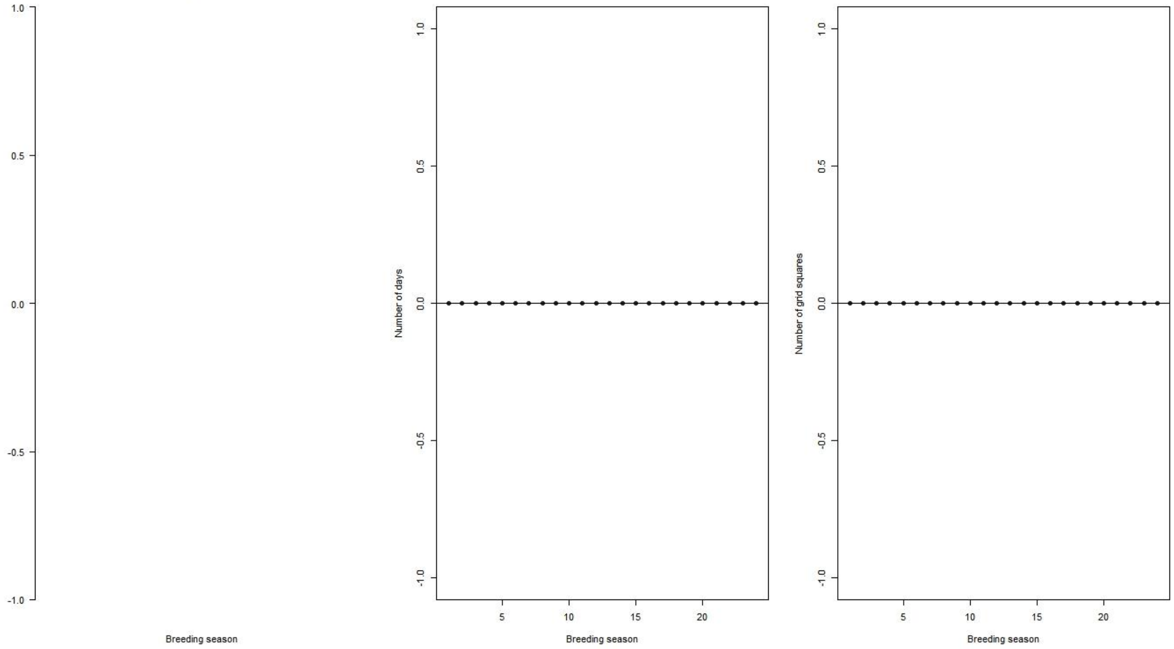

Physiological niche in pixels and in days: Har

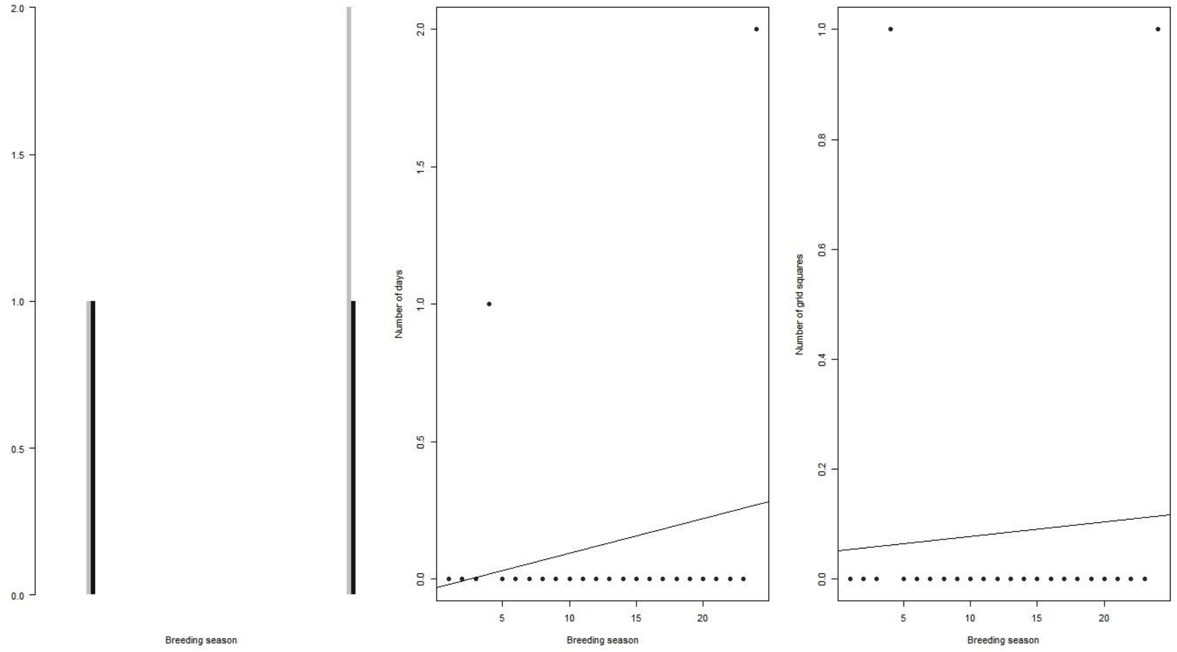

Physiological niche in pixels and in days: Hme

Physiological niche in pixels and in days: Hra

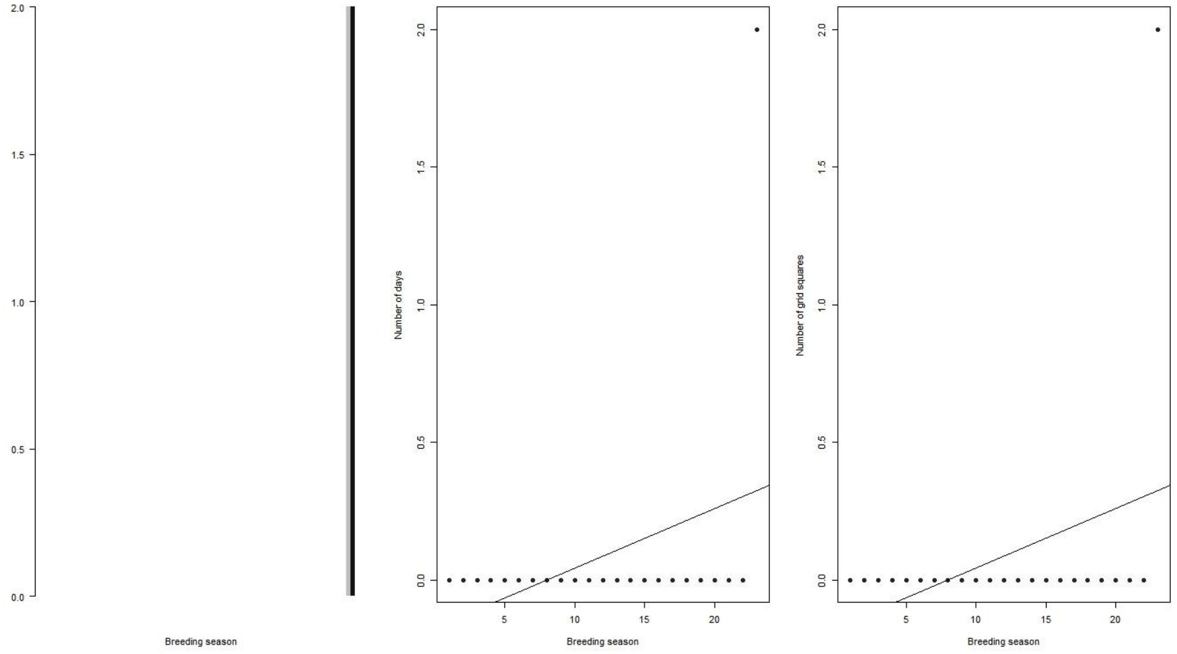


Physiological niche in pixels and in days: Lbu

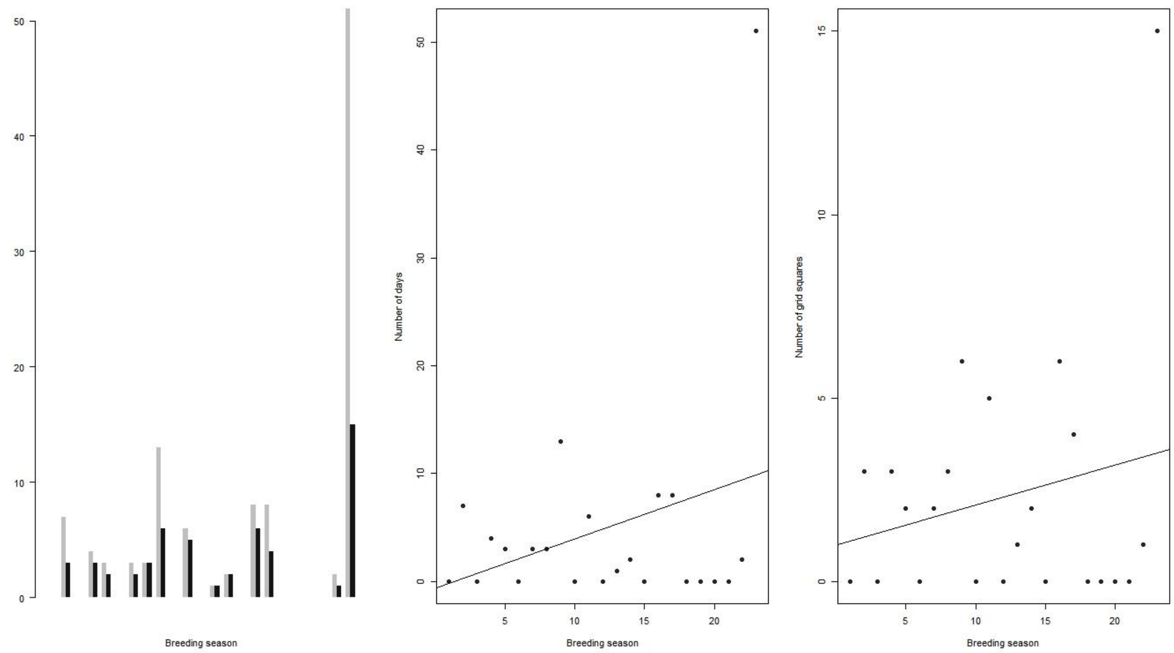




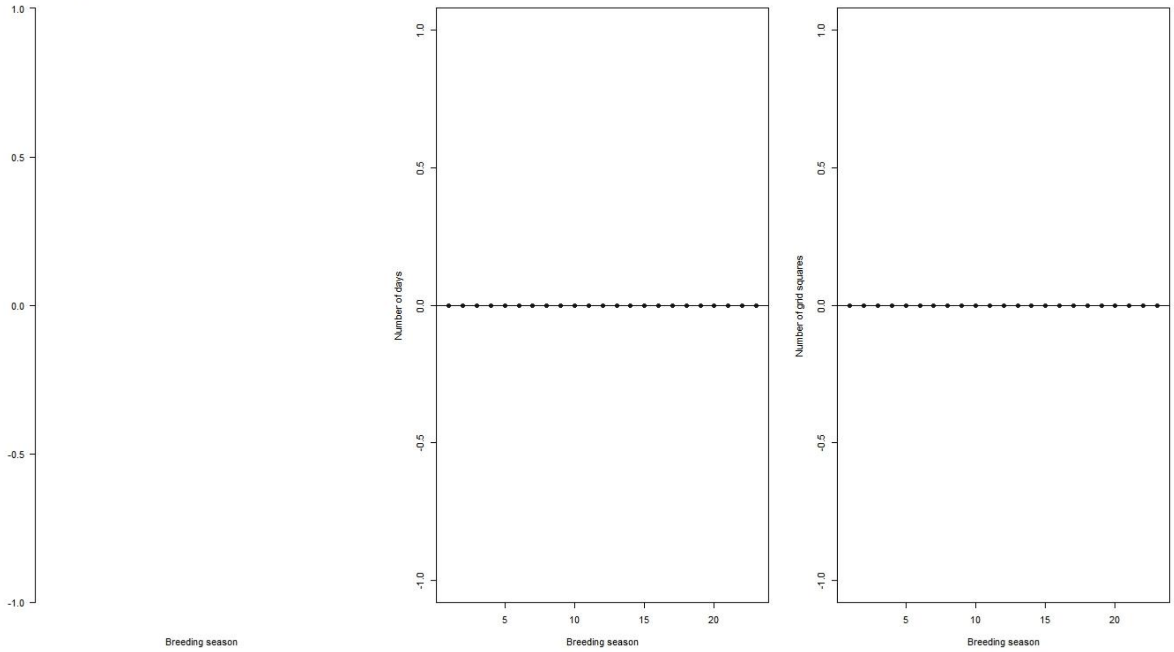
Physiological niche in pixels and in days: LIa



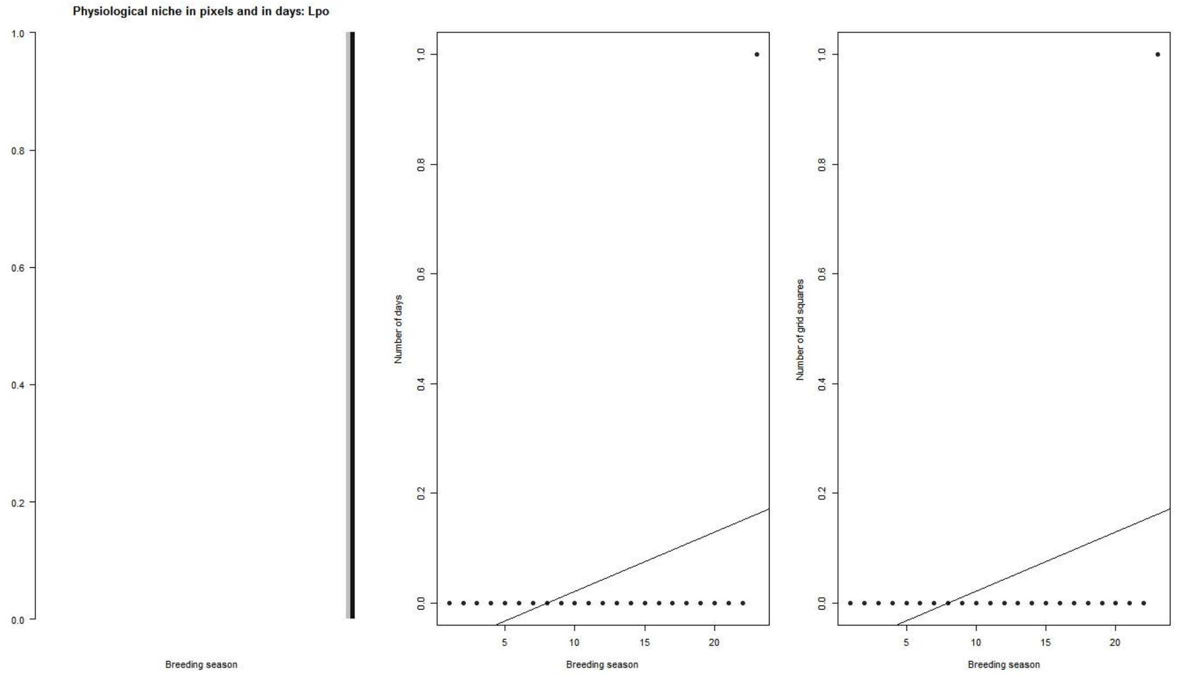
Physiological niche in pixels and in days: LII

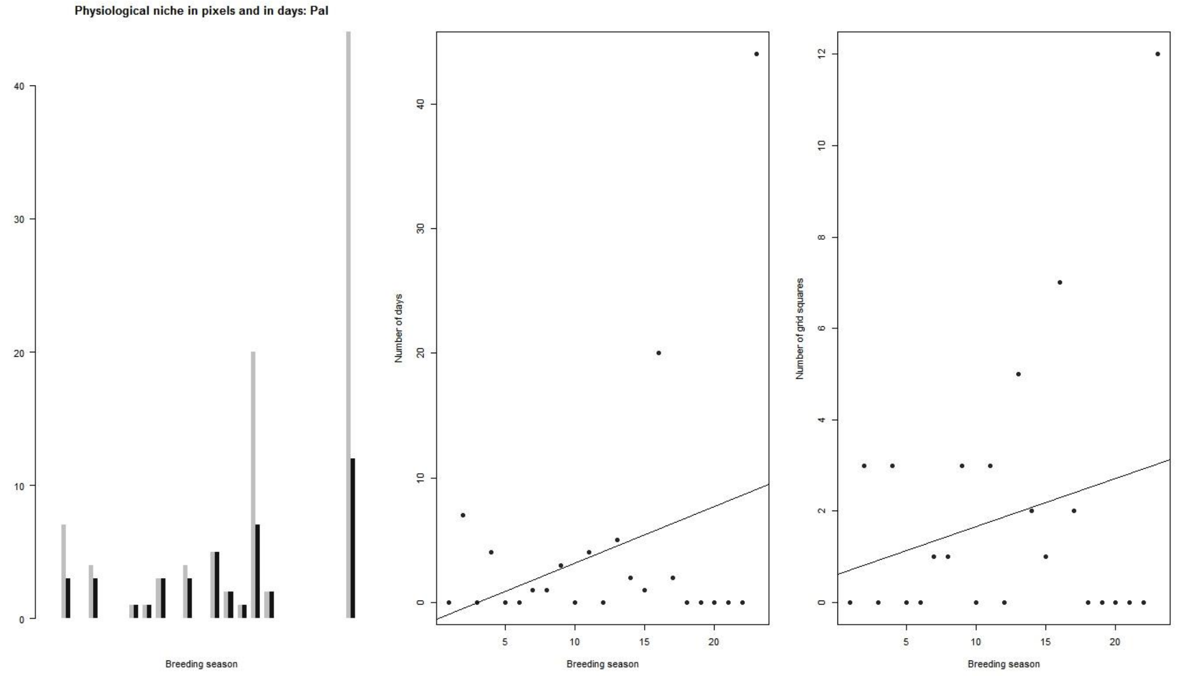


Physiological niche in pixels and in days: Llt

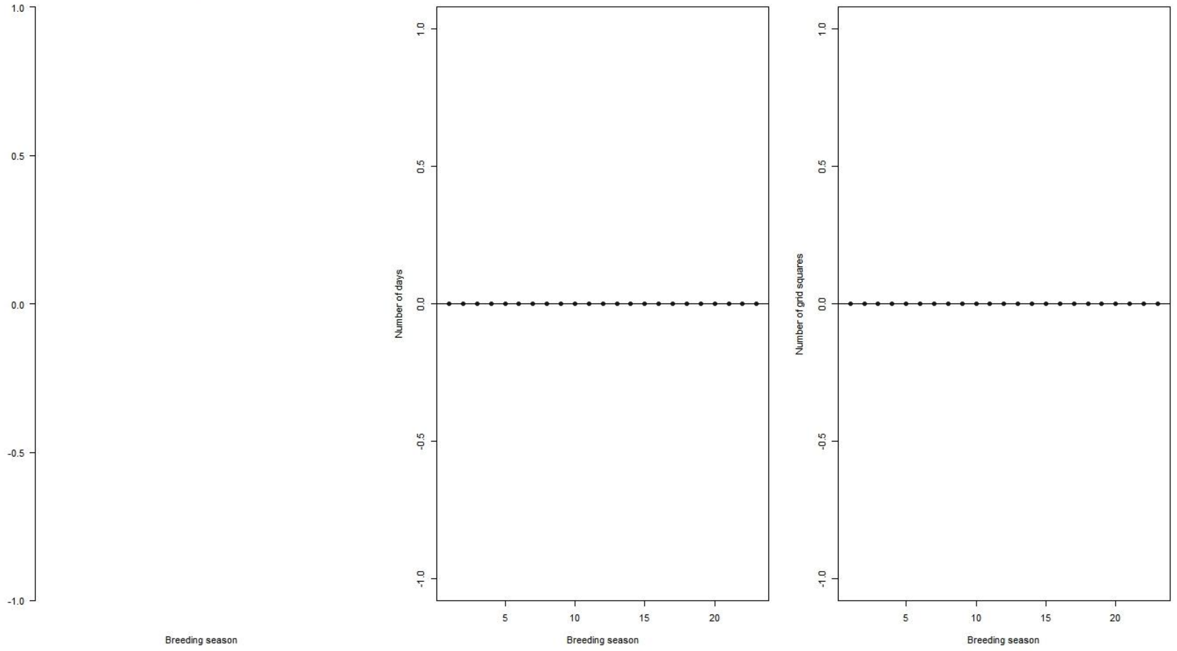


Physiological niche in pixels and in days: Lma

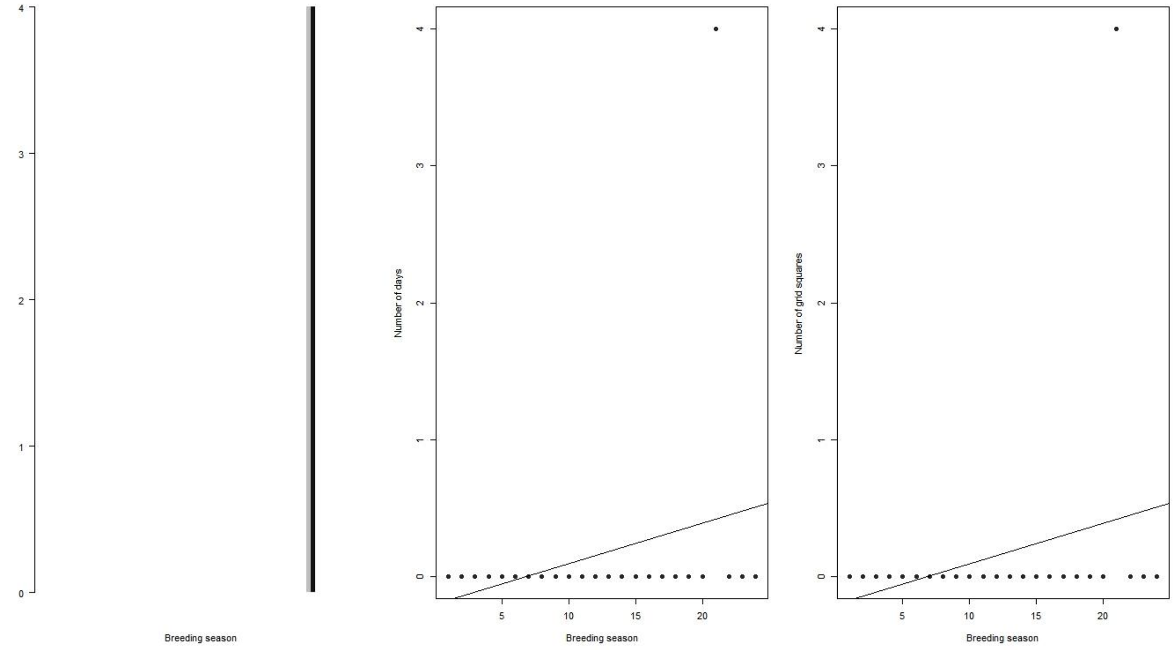

Physiological niche in pixels and in days: Lpo

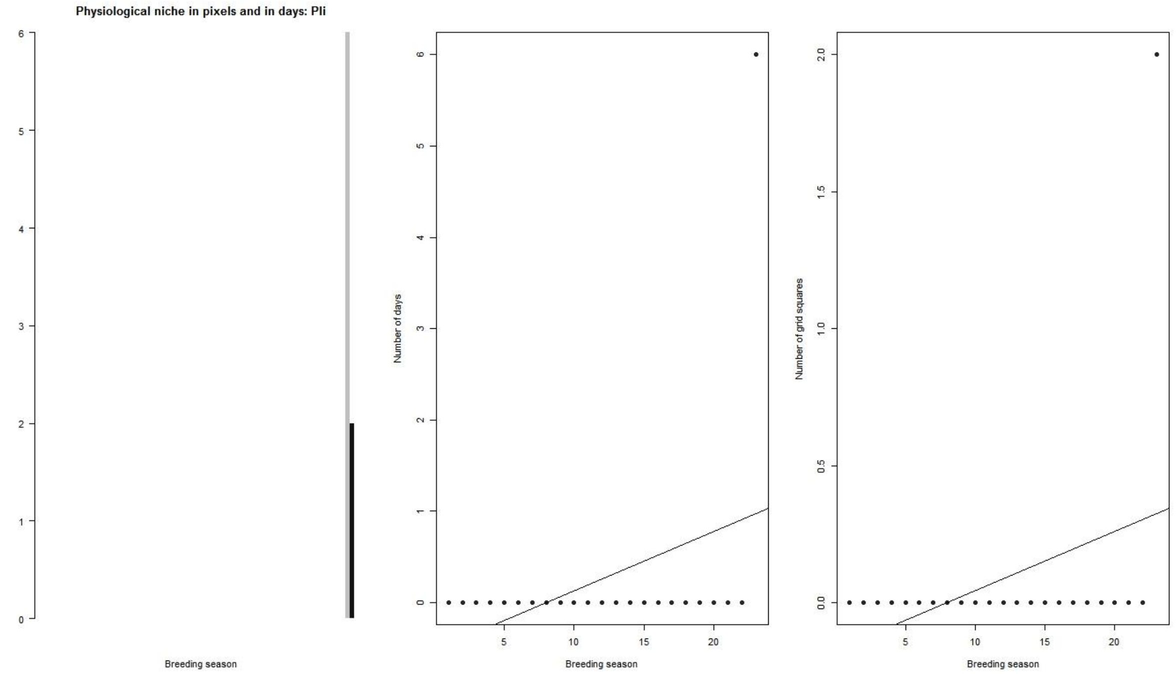

Physiological niche in pixels and in days: Pal

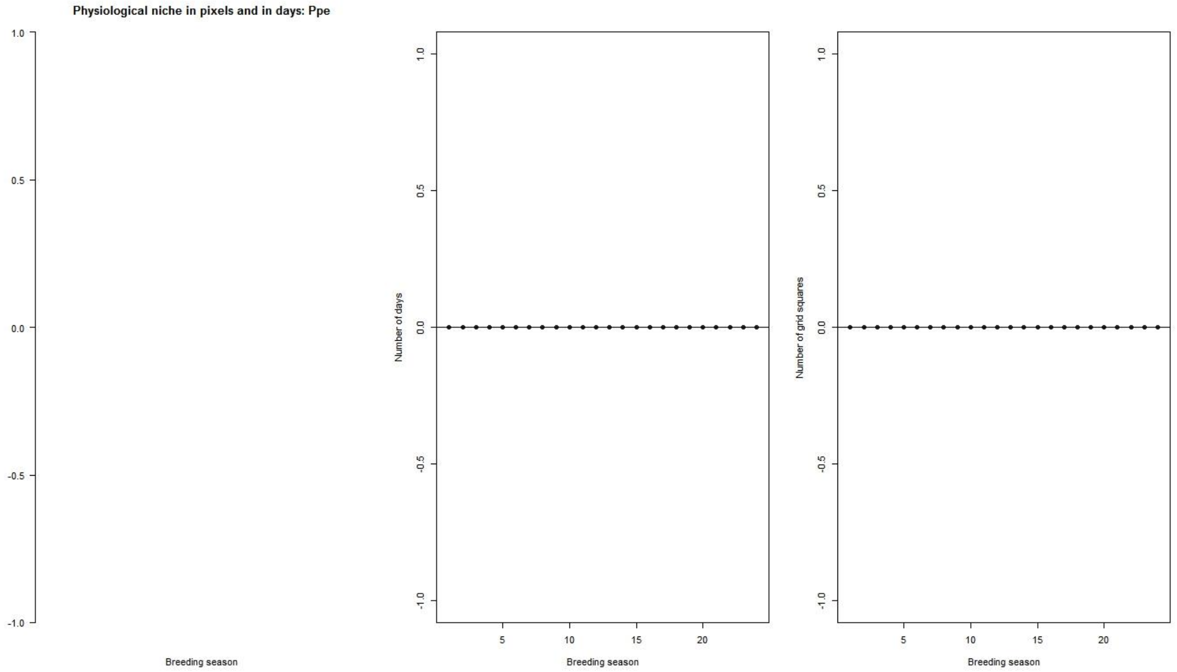
Physiological niche in pixels and in days: Pcu

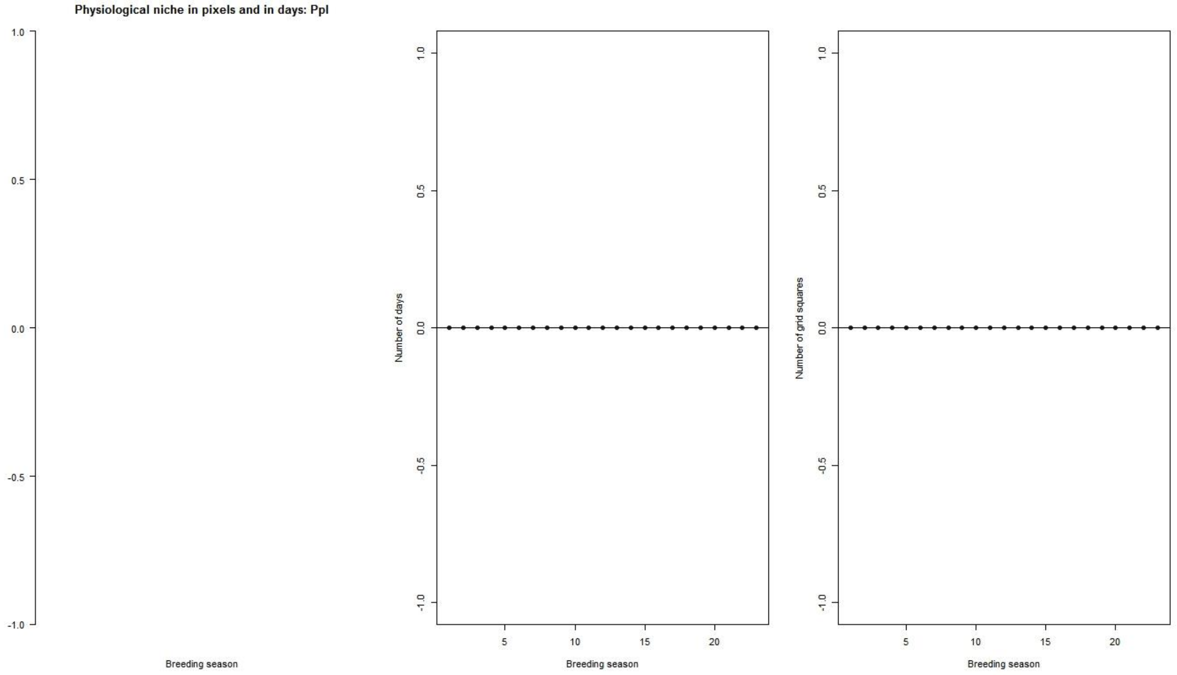


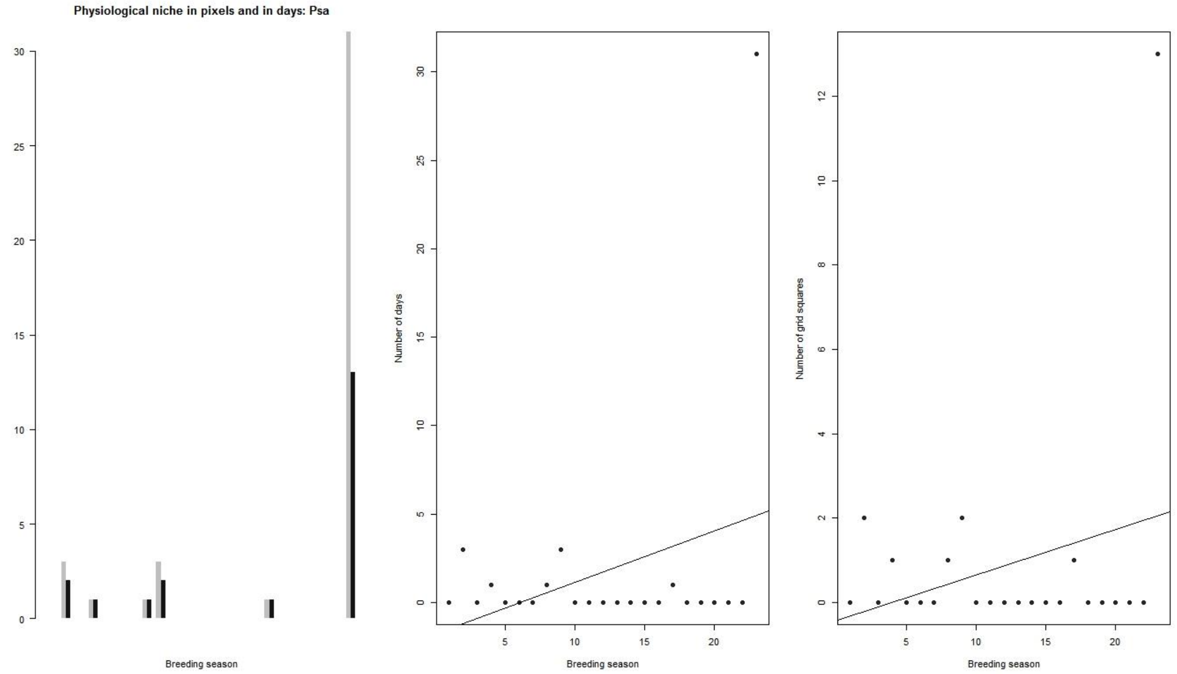
Physiological niche in pixels and in days: Ple

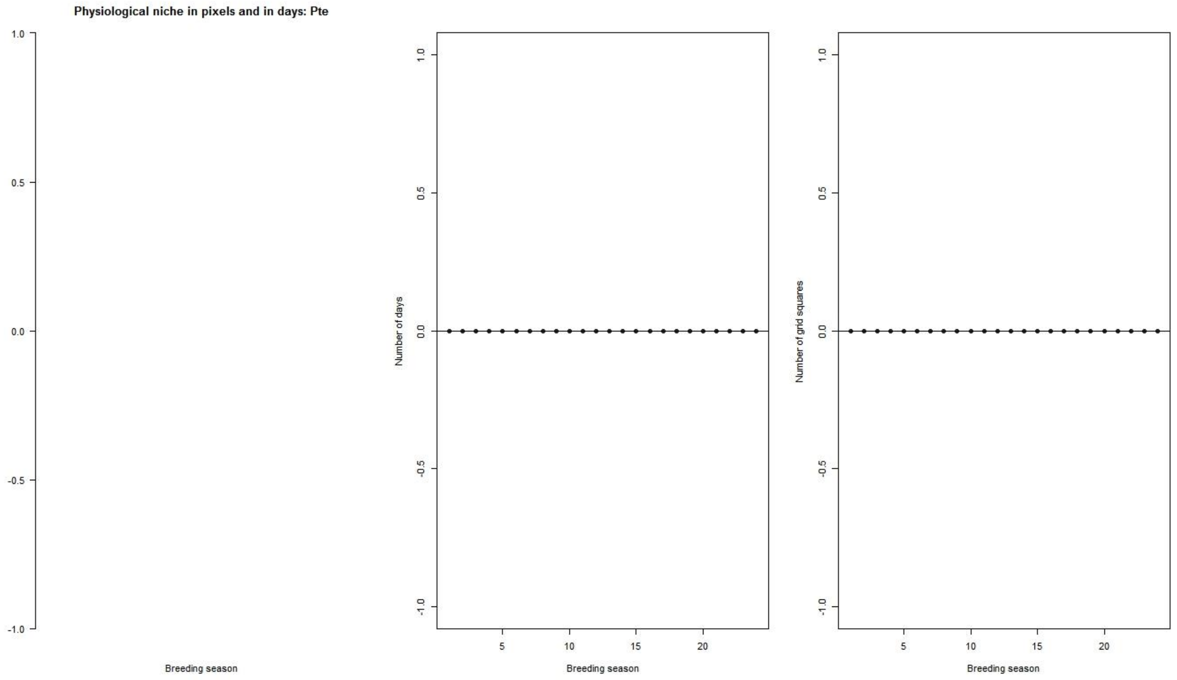


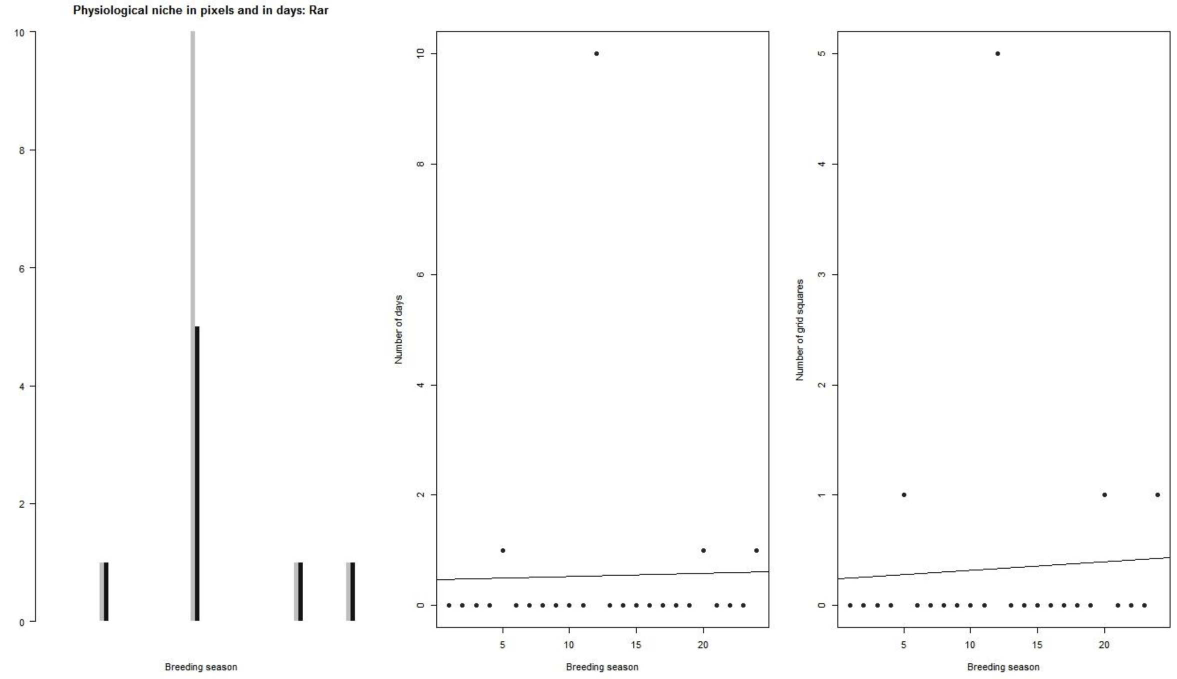

Physiological niche in pixels and in days: Pli
0
1
2
3
4
5
6
Breeding season
Number of days
5
10
15
20
Breeding season
Number of grid squares
0.0
0.5
1.0
1.5
2.0
5
10
15
20
Breeding season

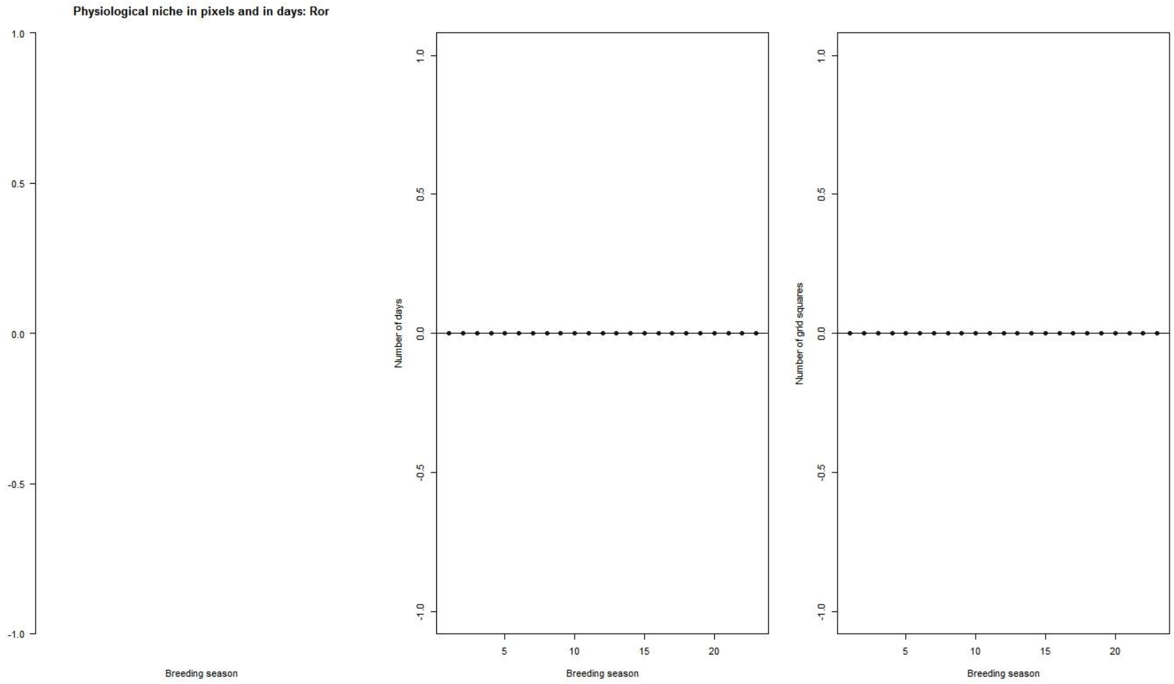

Physiological niche in pixels and in days: Ppe
1.0
0.5
0.0
-0.5
-1.0
Breeding season
Number of days
5
10
15
20
Breeding season
Number of grid squares
5
10
15
20
Breeding season


Physiological niche in pixels and in days: Ppl
1.0
0.5
0.0
-0.5
-1.0
Breeding season
Number of days
5
10
15
20
Breeding season
Number of grid squares
Breeding season

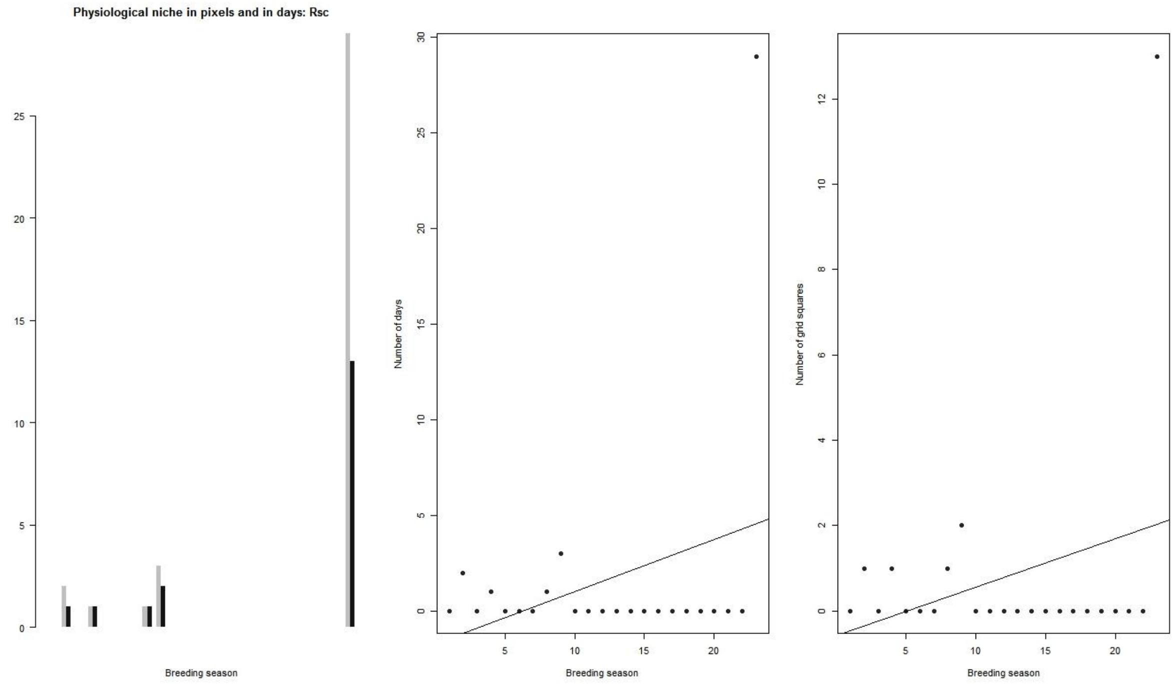

Physiological niche in pixels and in days: Psa
30
25
20
15
10
5
0
Breeding season
Number of days
Breeding season
12
10
8
6
4
2
0
Number of grid squares
5
10
15
20
Breeding season


Physiological niche in pixels and in days: Pte

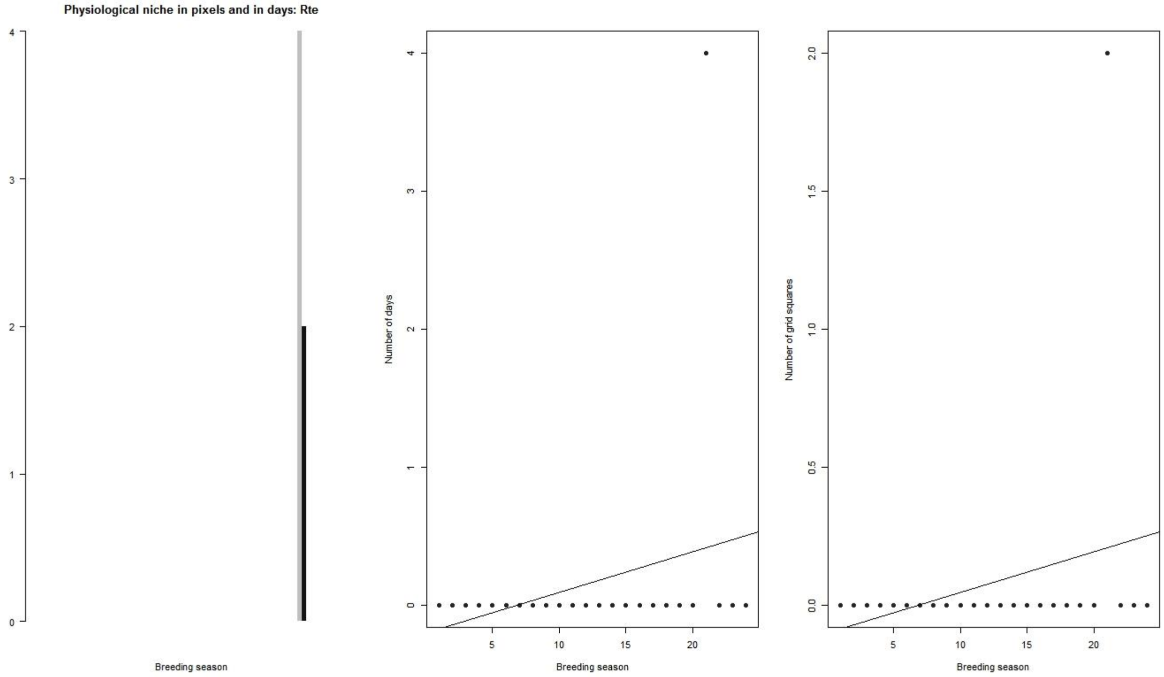

Physiological niche in pixels and in days: Rar



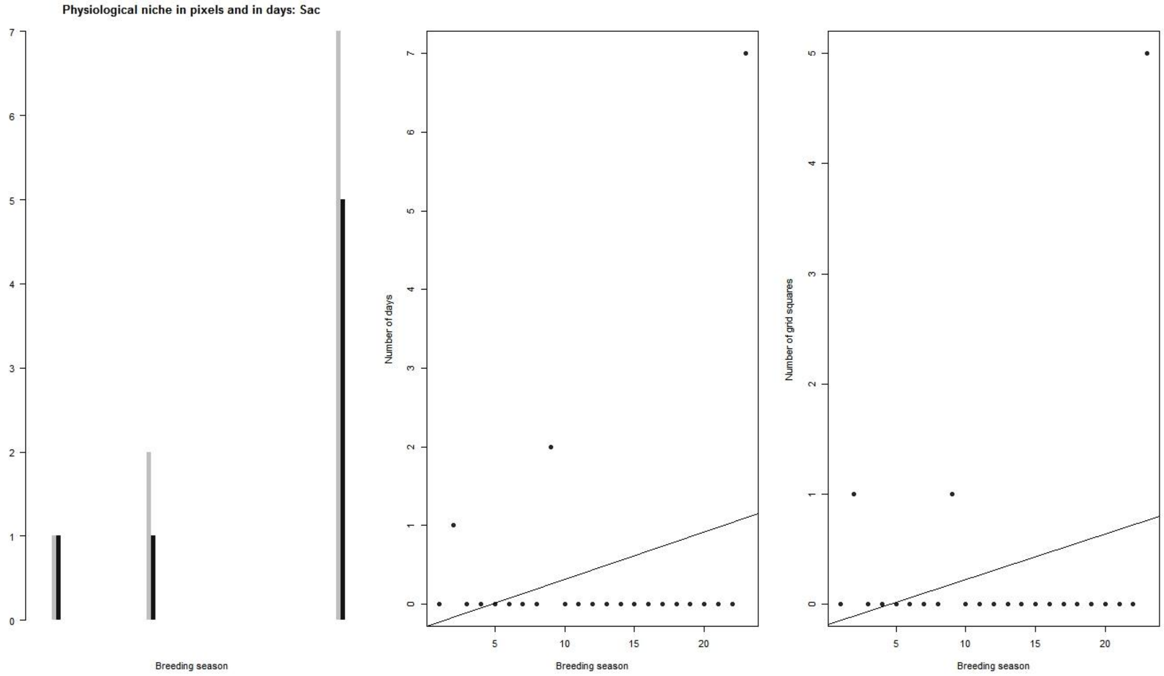
Physiological niche in pixels and in days: Ror



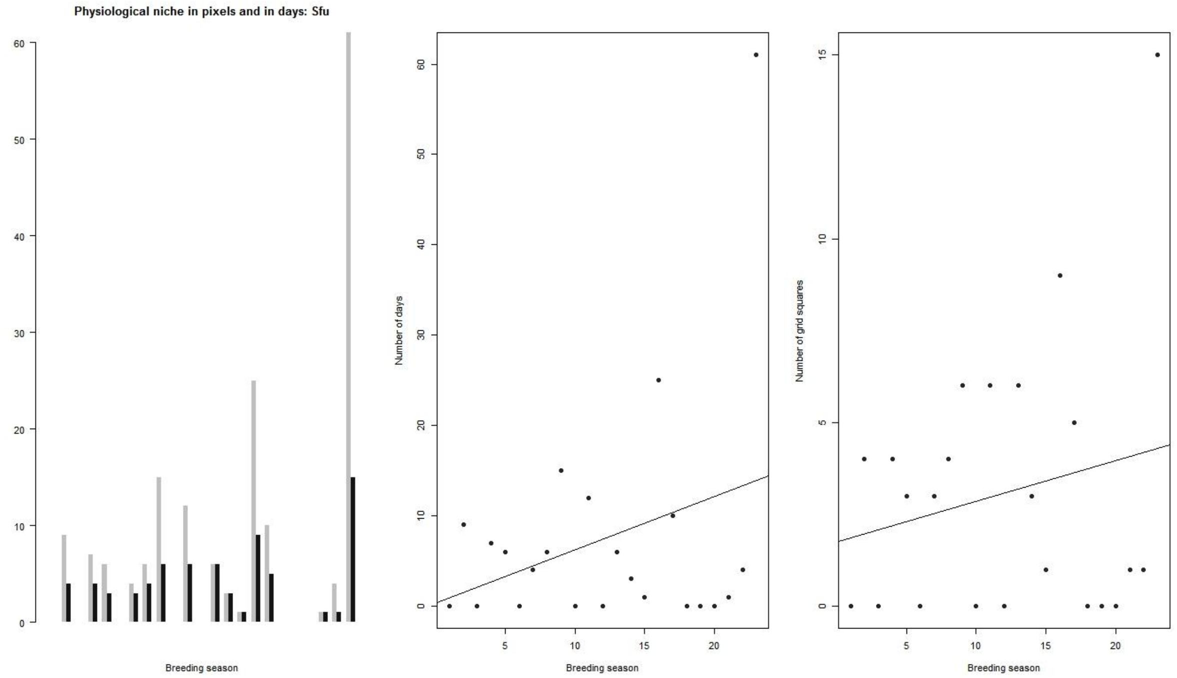
Physiological niche in pixels and in days: Rsc

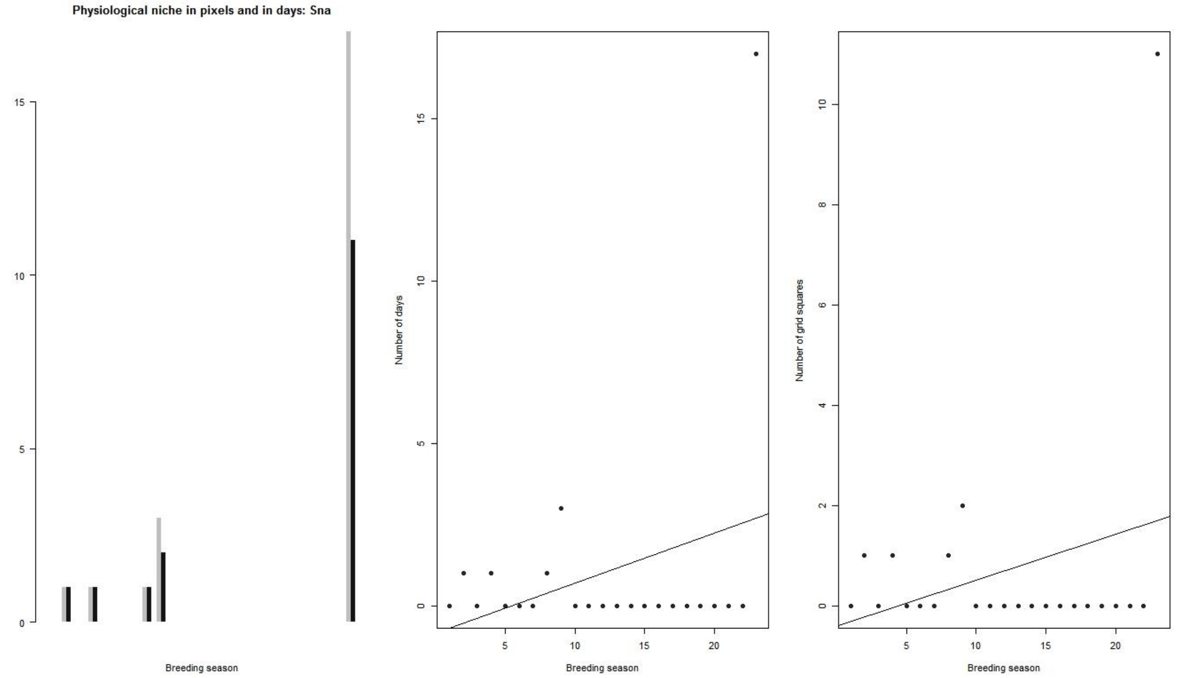


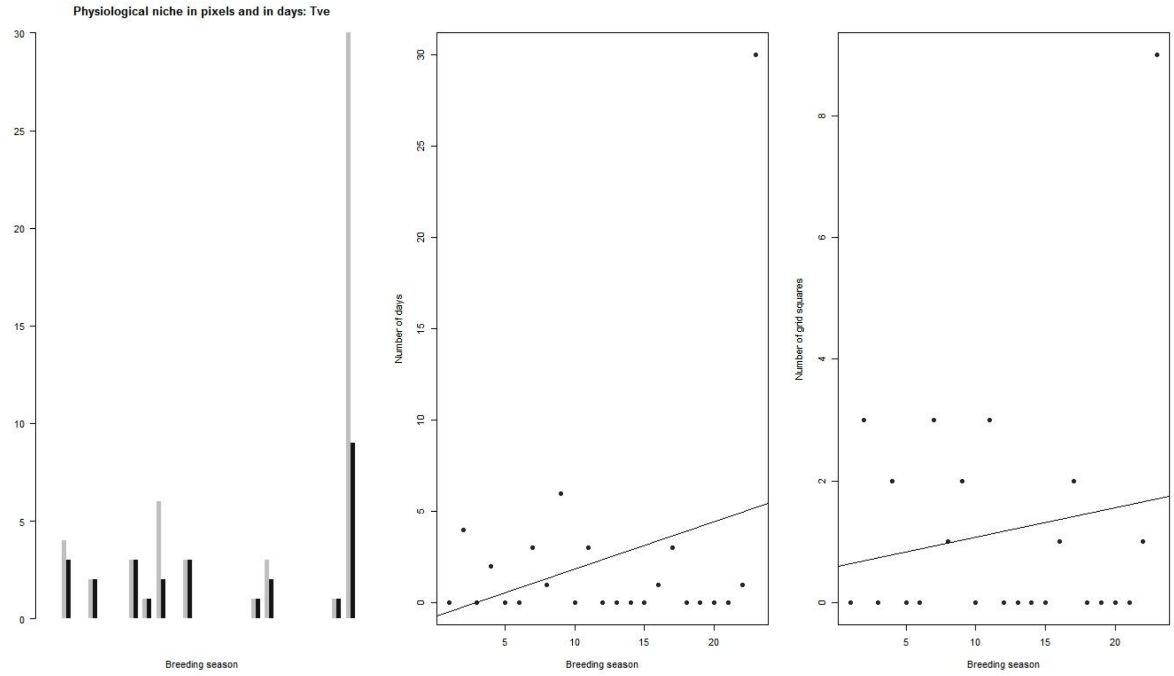
Physiological niche in pixels and in days: Rte



Physiological niche in pixels and in days: Sac

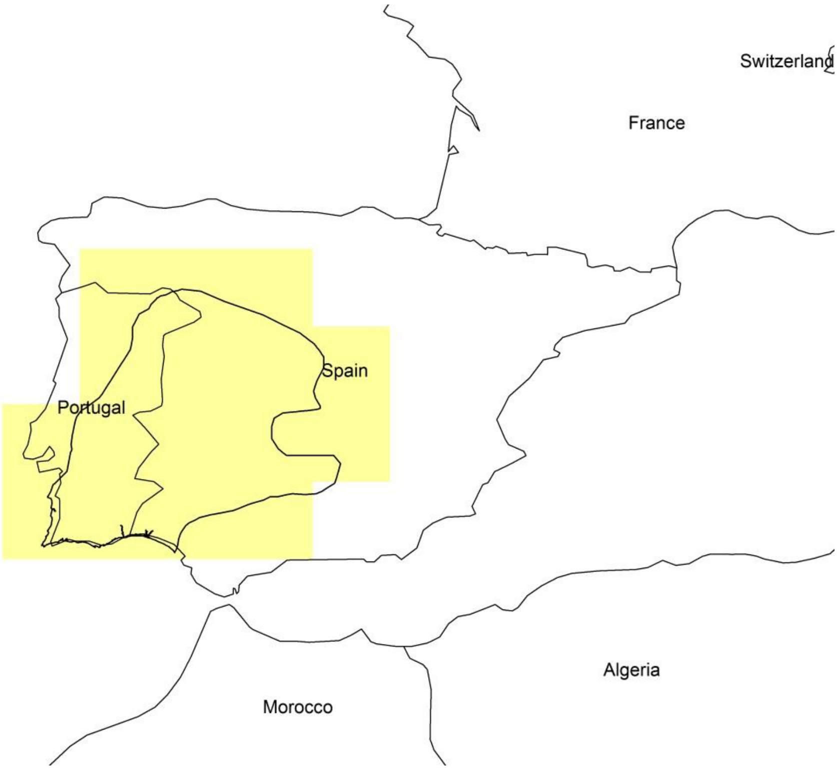

Physiological niche in pixels and in days: Sfu
0
10
20
30
40
50
60
Breeding season
Number of days
5
10
15
20
Breeding season
Number of grid squares
0
5
10
15
Breeding season


Physiological niche in pixels and in days: Sna
0
5
10
15
Breeding season
Number of days
5
10
15
20
Breeding season
Number of grid squares
0
2
4
6
8
10
Breeding season

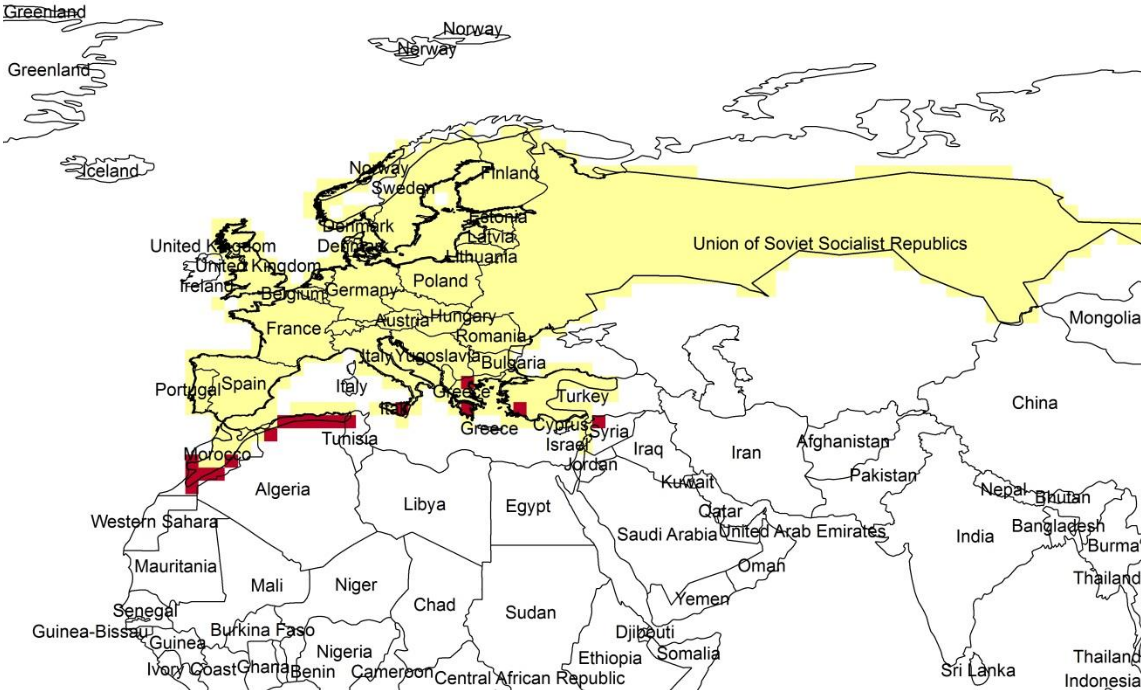

Physiological niche in pixels and in days: Tve
30
25
20
15
10
5
0
Breeding season
Number of days
30
25
20
15
10
5
0
5
10
15
20
Breeding season
Number of grid squares
8
6
4
2
0
5
10
15
20
Breeding season

Supplimentary figure 2 **S**pecies' distributional areas within and outside physiological niches. Yellow shading indicates conditions inside physiological niche; and red shading represents conditions outside of the physiological niche

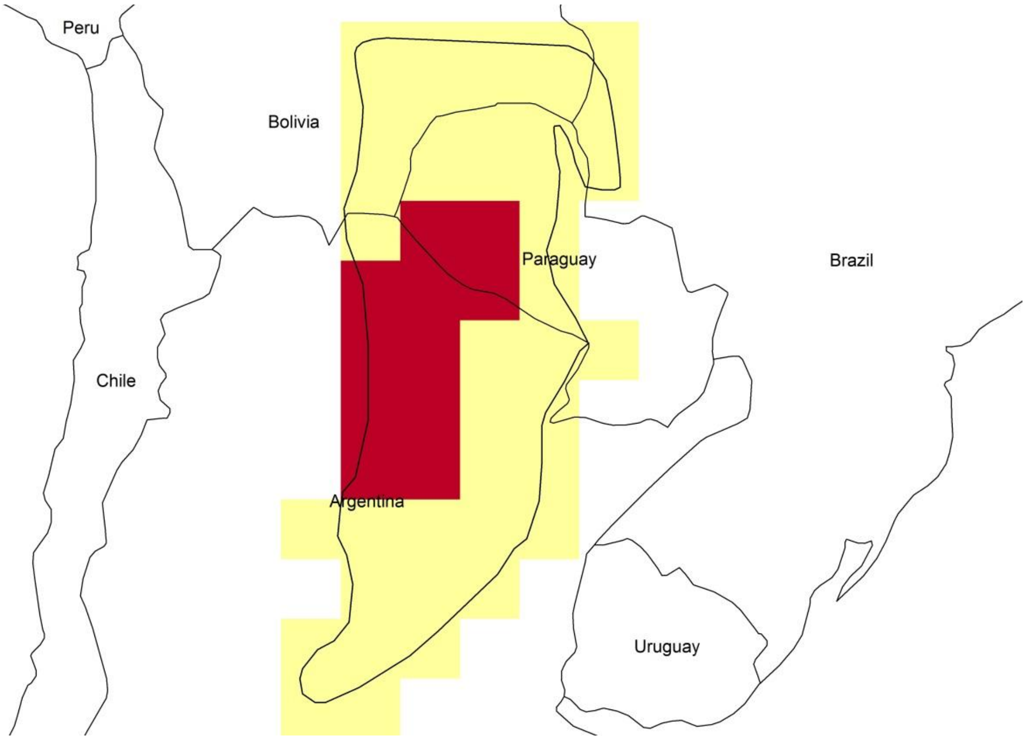
Alytes cisternasii



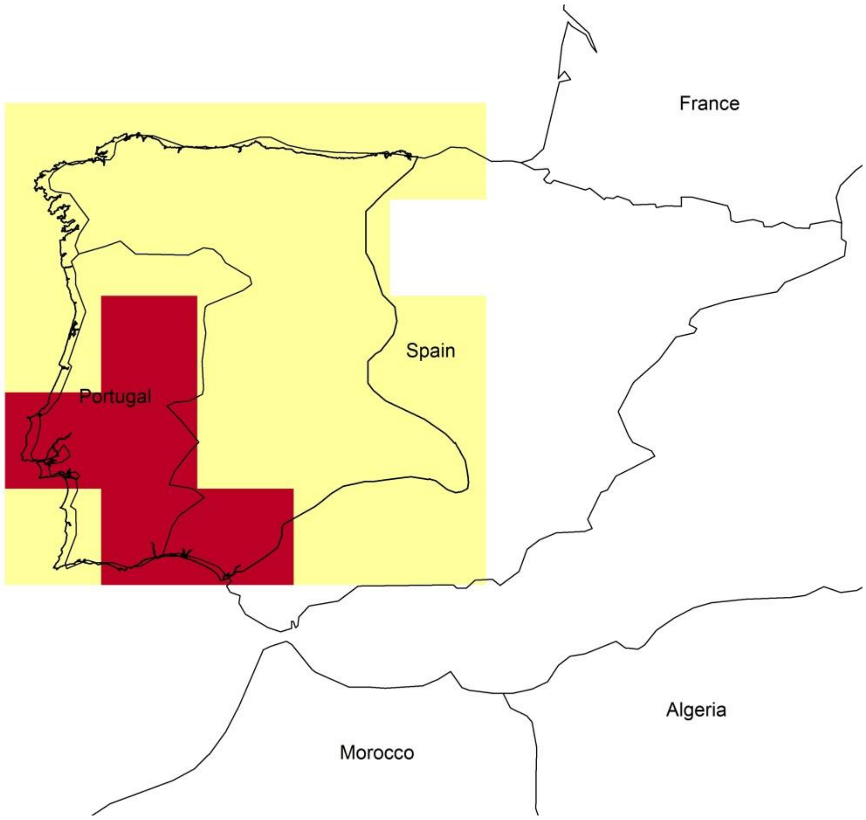
Bufo bufo


Greenland
Greenland
Norway
Norway
Iceland
Norway
Sweden
Finland
Denmark
Estonia
Latvia
United Kingdom
Denmark
Lithuania
United Kingdom
Union of Soviet Socialist Republics
Ireland
Belgium
Germany
Poland
Austria
Hungary
Mongolia
France
Romania
Italy
Yugoslavia
Bulgaria
Portugal
Spain
Italy
Greece
Turkey
China
Italy
Greece
Cyprus
Syria
Tunisia
Israel
Iraq
Iran
Afghanistan
Morocco
Jordan
Kuwait
Pakistan
Algeria
Libya
Egypt
Nepal
Bhutan
Western Sahara
Qatar
Saudi Arabia
United Arab Emirates
India
Bangladesh
Burma
Mauritania
Oman
Mali
Niger
Thailand
Chad
Yemen
Senegal
Sudan
Guinea-Bissau
Burkina Faso
Djibouti
Guinea
Nigeria
Ethiopia
Somalia
Thailand
Ivory Coast
Ghana
Benin
Cameroon
Central African Republic
Sri Lanka
Indonesia

Ceratophrys cranwelli



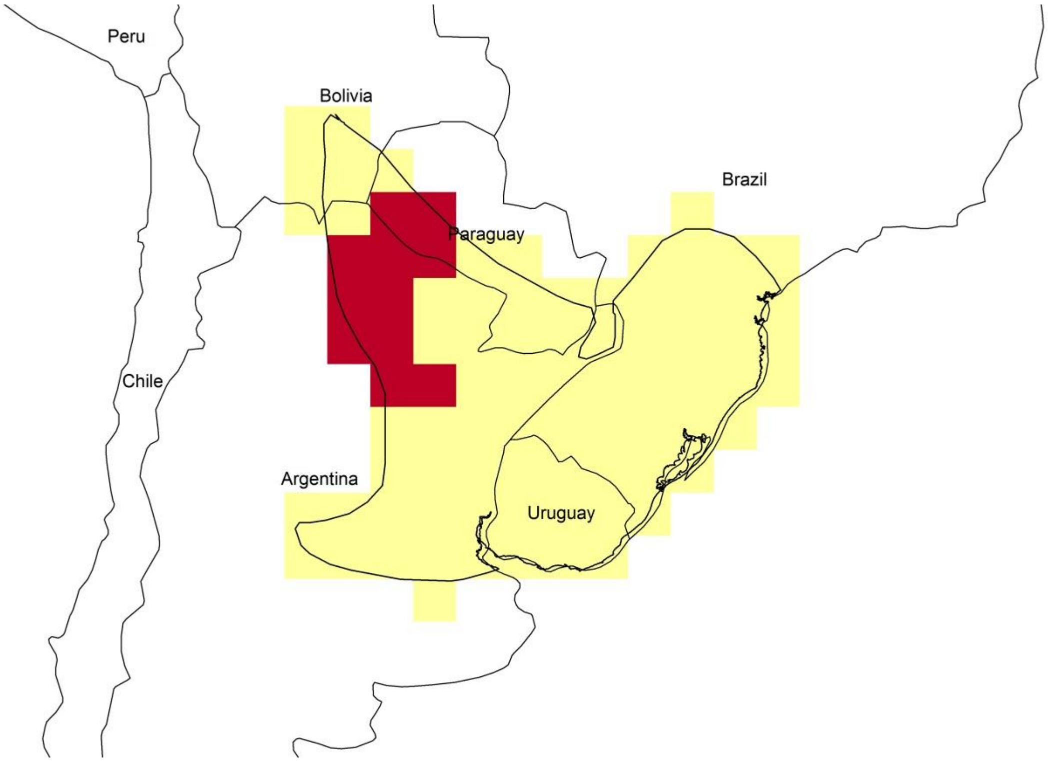
Discoglossus galganoi



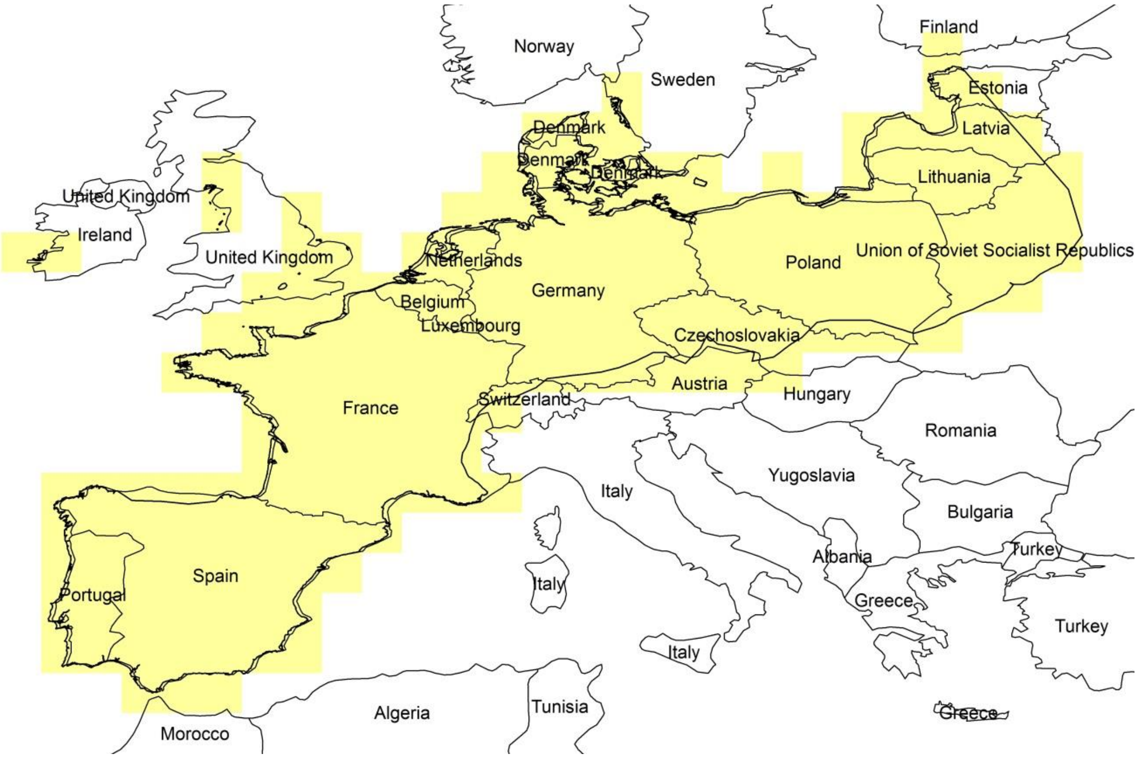
Dendropsophus minutus

Dermatonotus muelleri

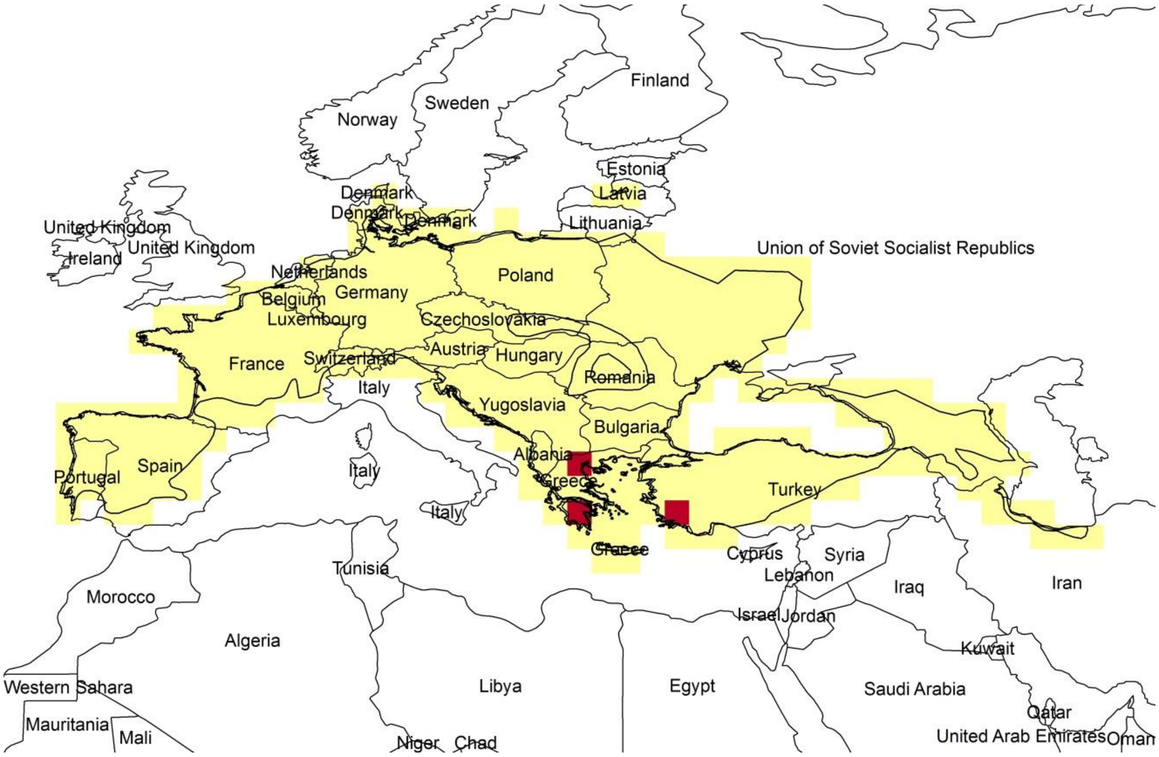
Elachistocleis bicolor



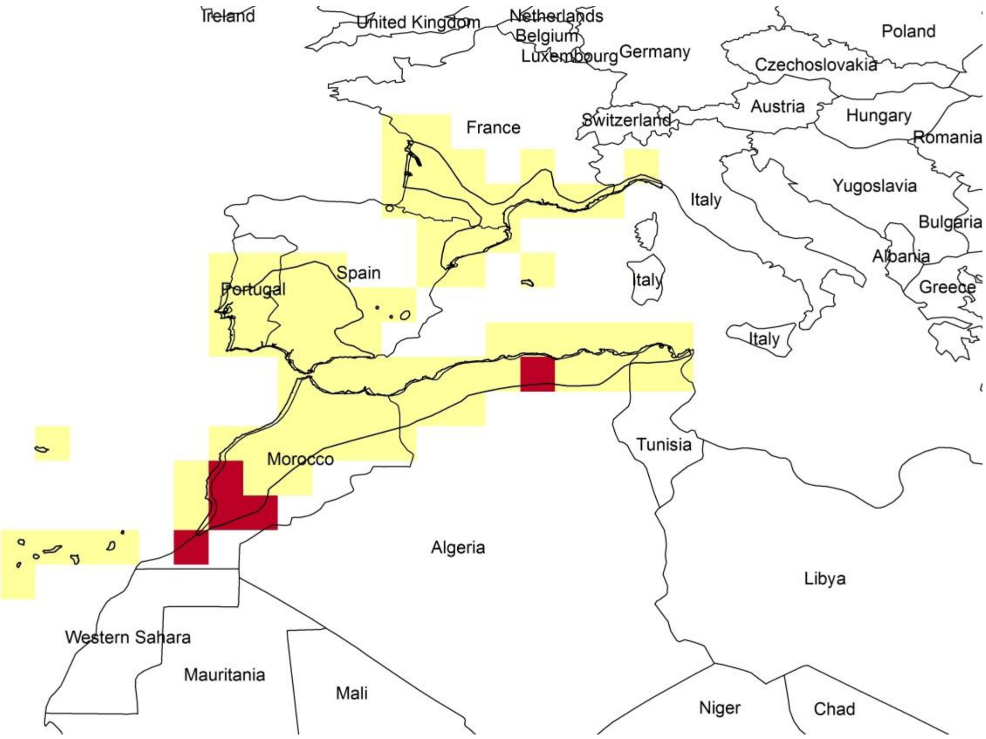
Epidalea calamita



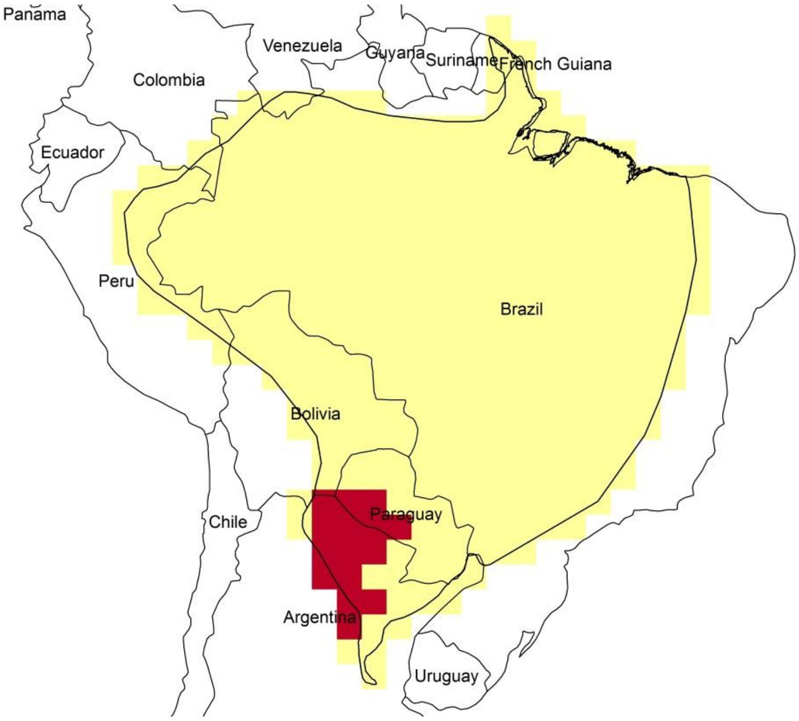
Hyla arborea



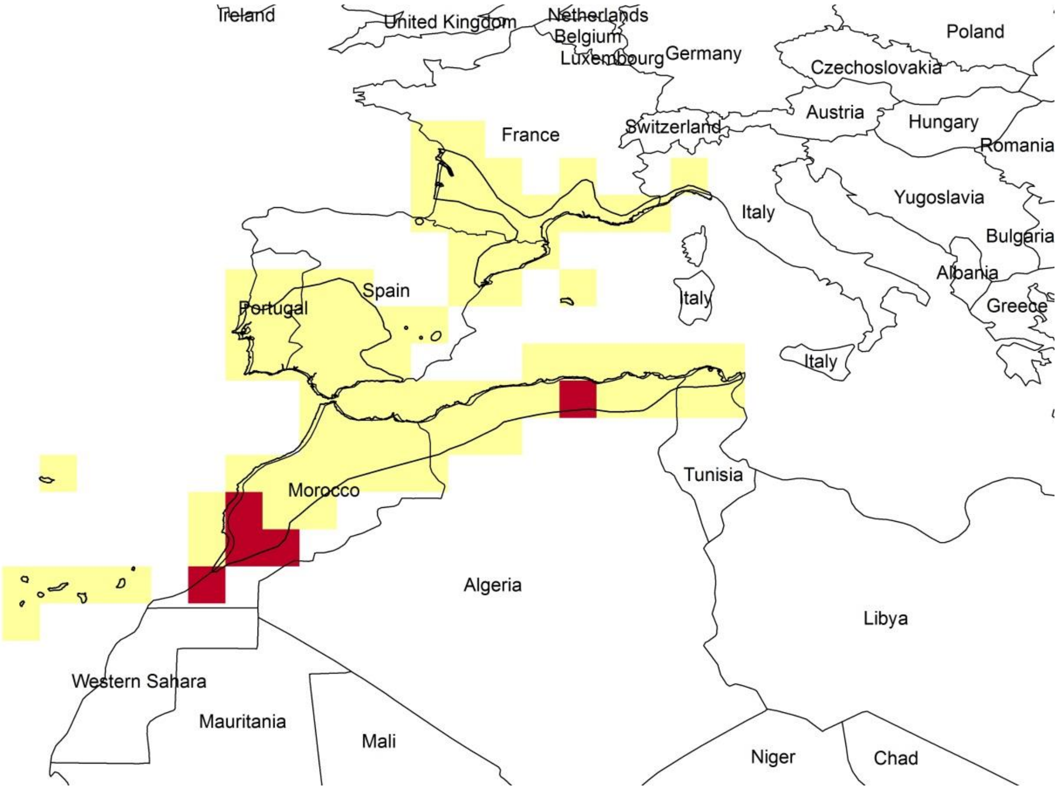
Hyla meridionalis



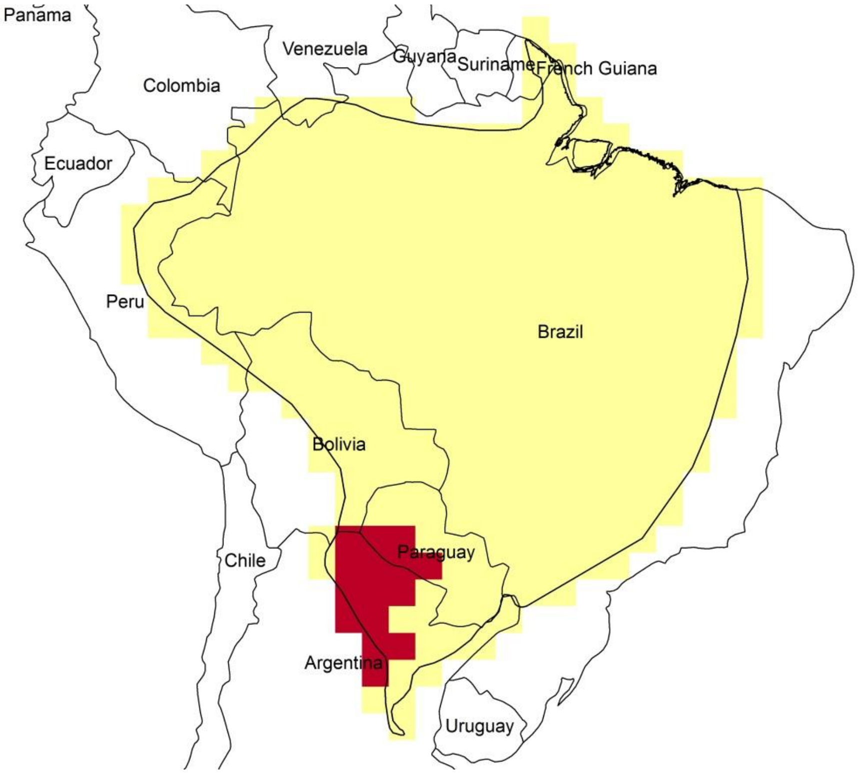
Hypsiboas raniceps



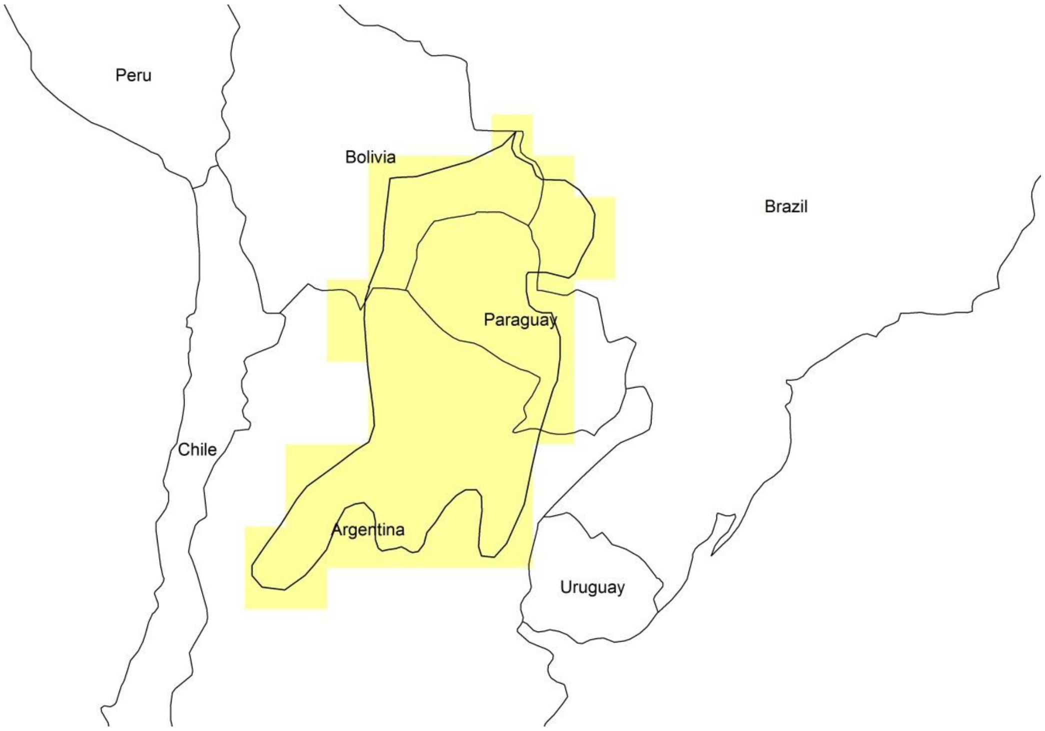
Hyla meridionalis



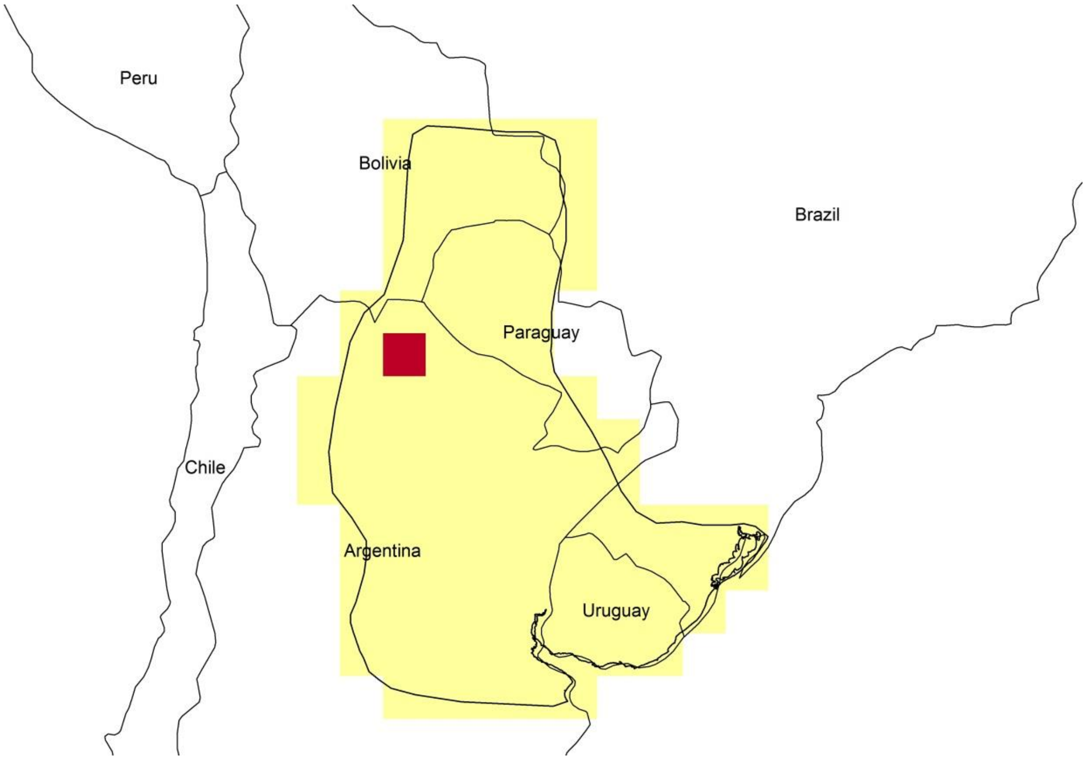
Hypsiboas raniceps



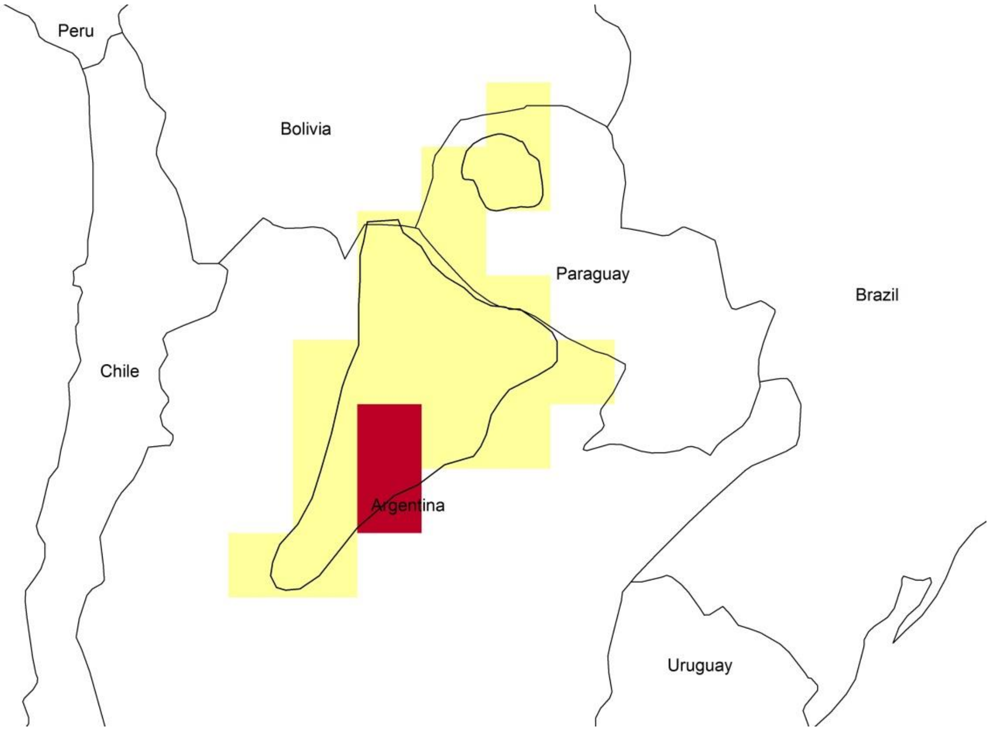
Leptodactylus bufonius



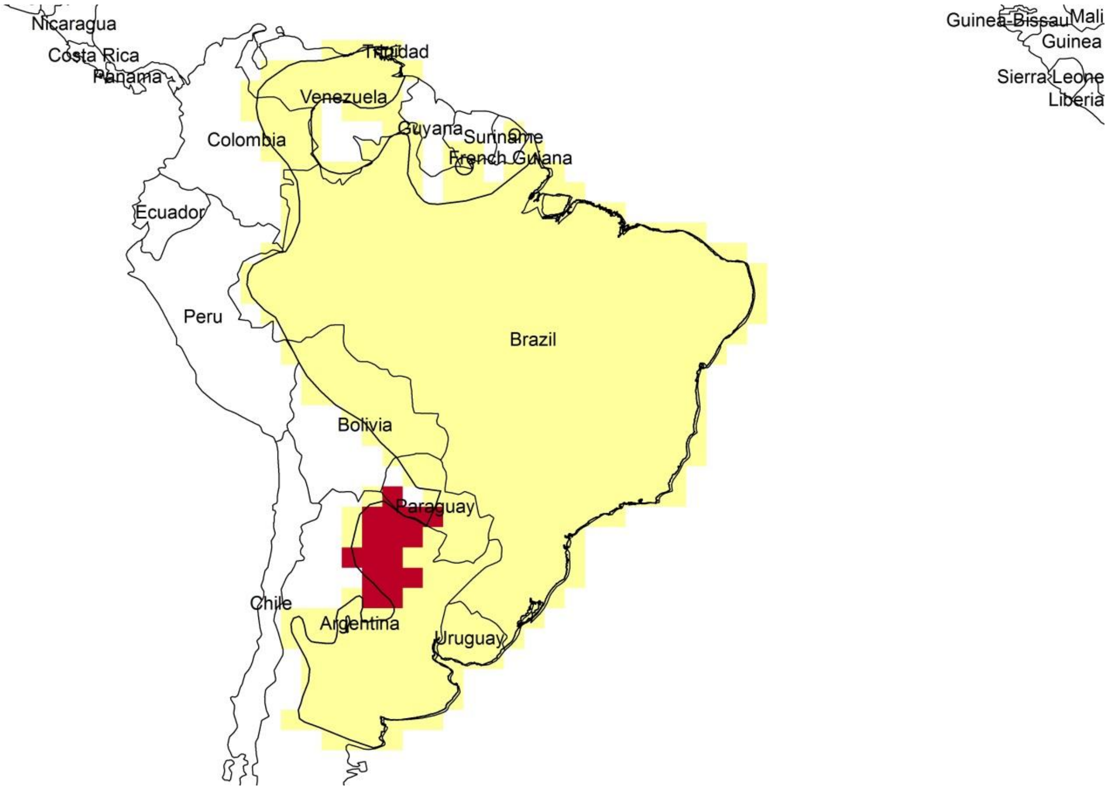
Leptodactylus latinasus



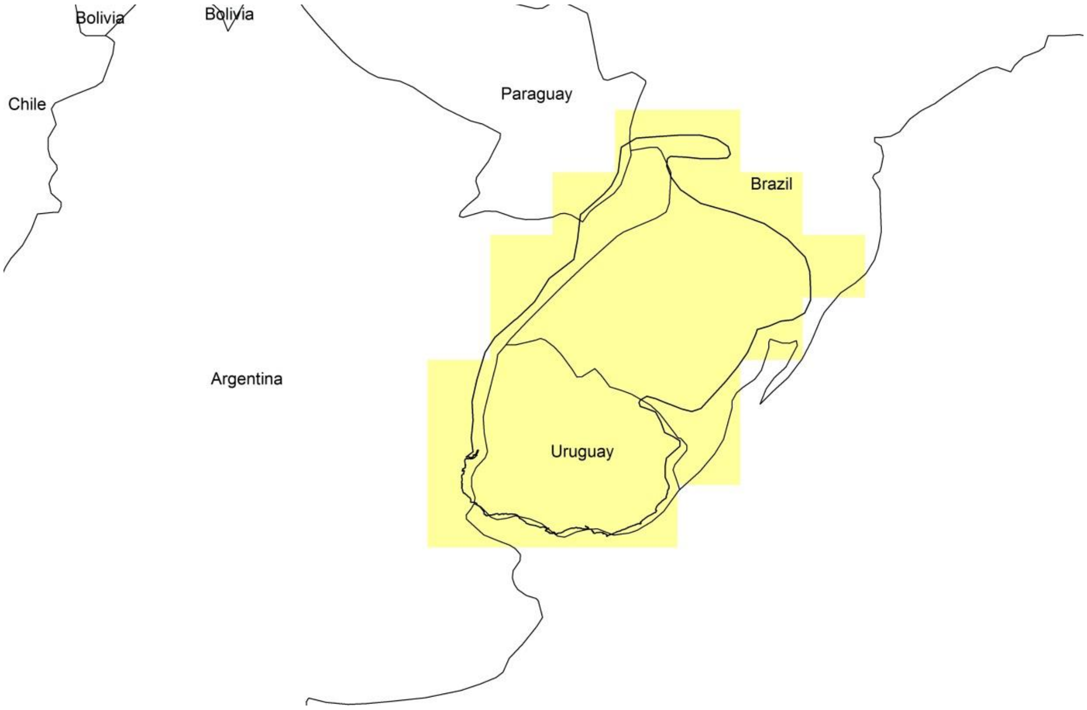
Lepidobatrachus llanensis



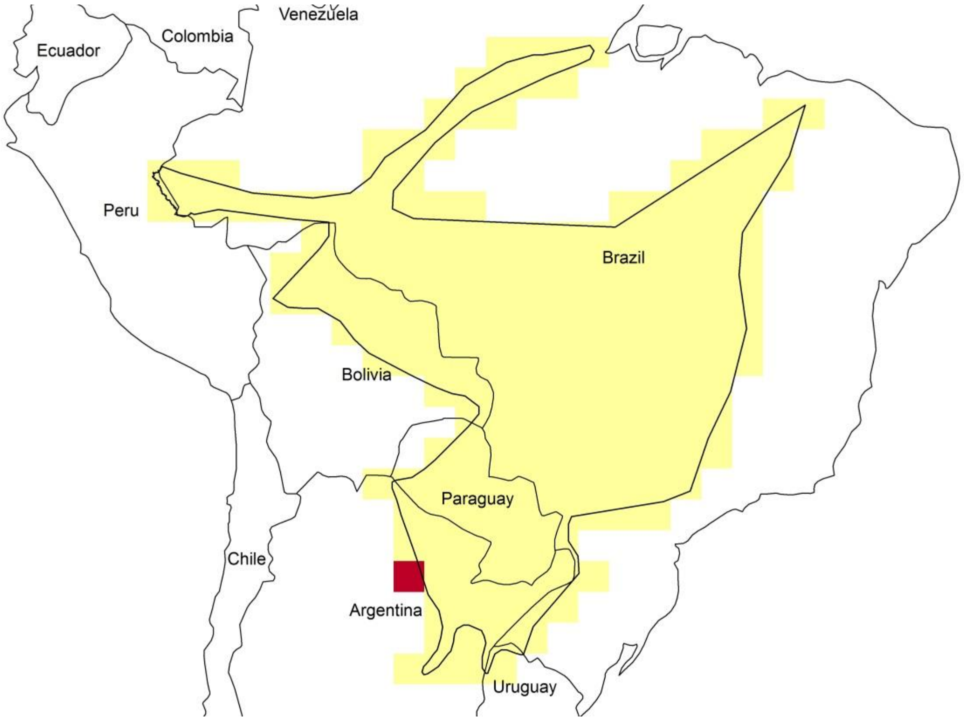
Leptodactylus latrans



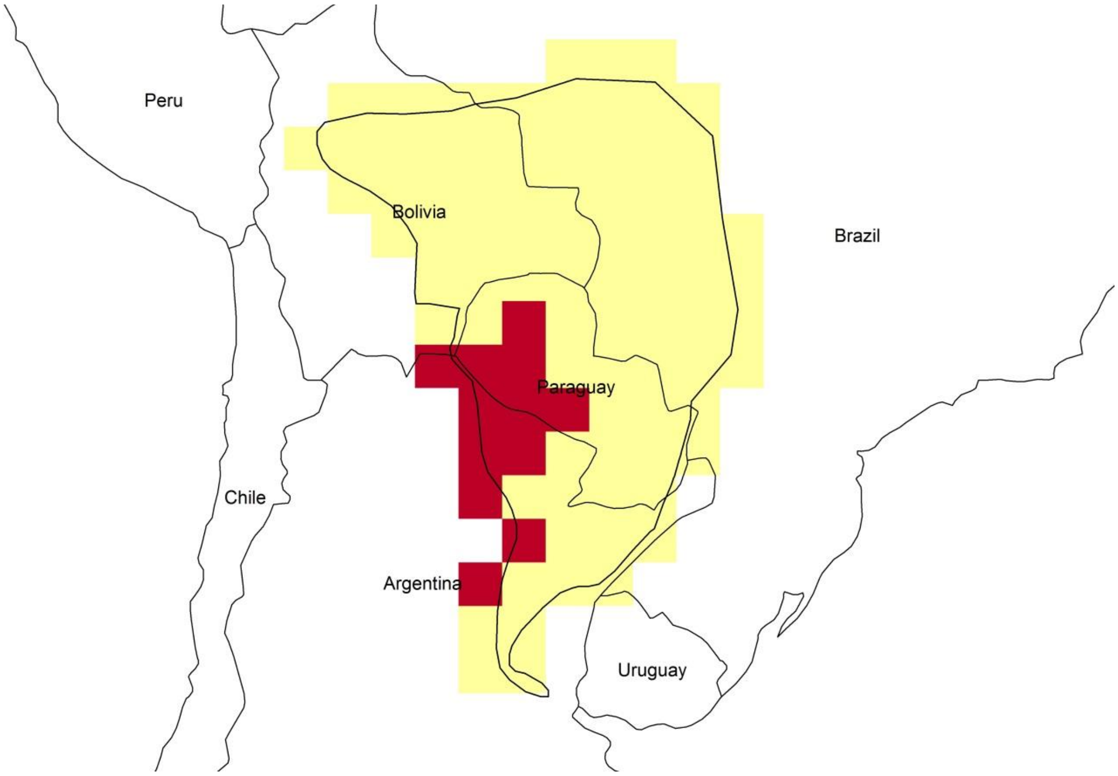
Limnomedusa macroglossa



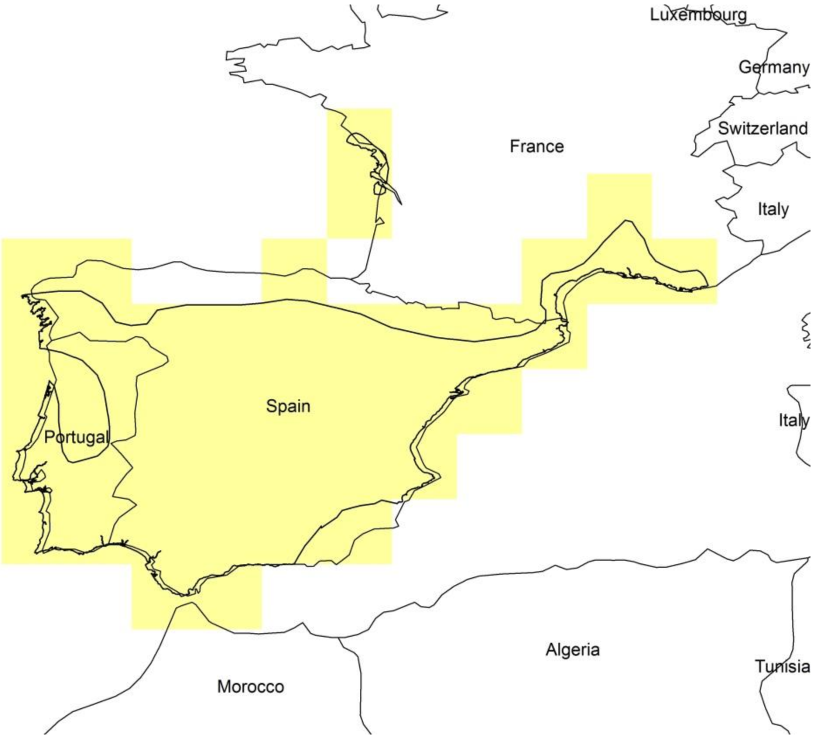
Leptodactylus podicipinus



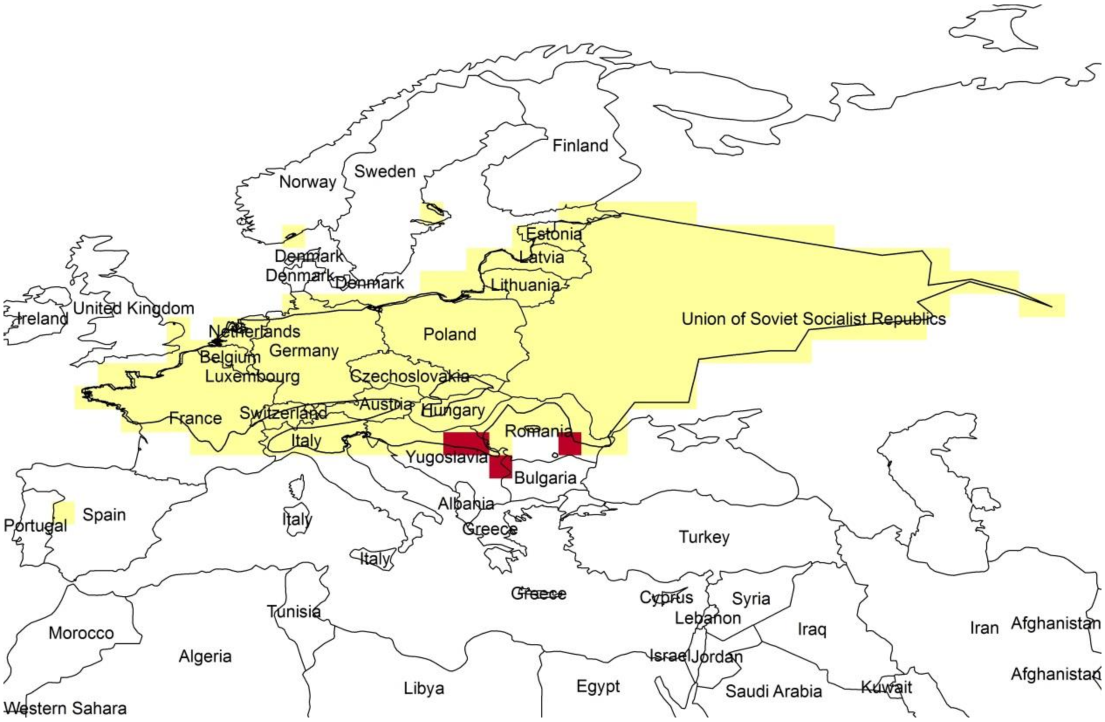
Physalaemus albonotatus



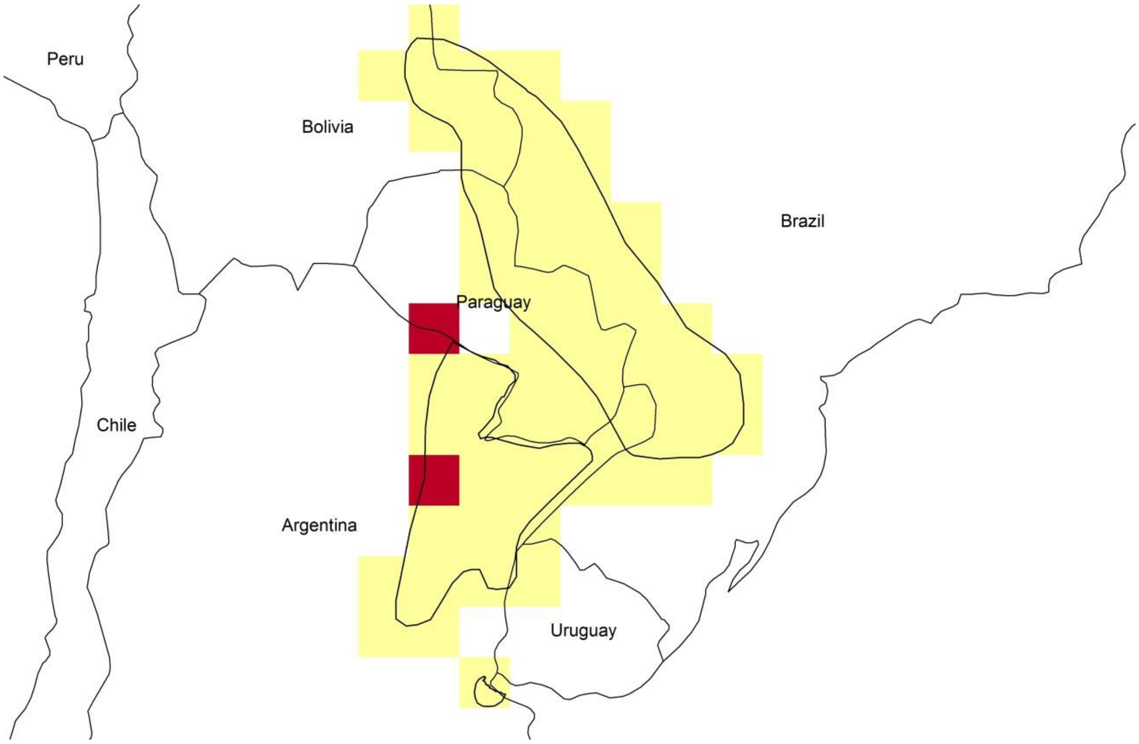
Pelobates cultripes



Pelophylax lessonae

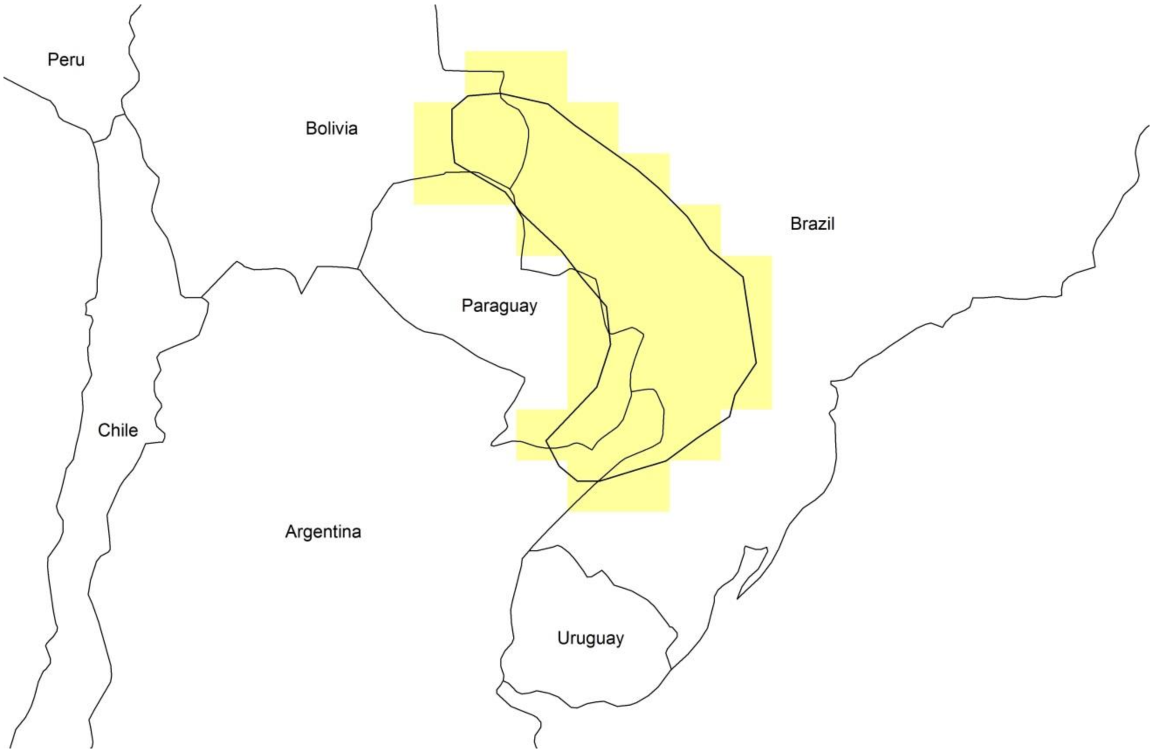


Pseudis limellum

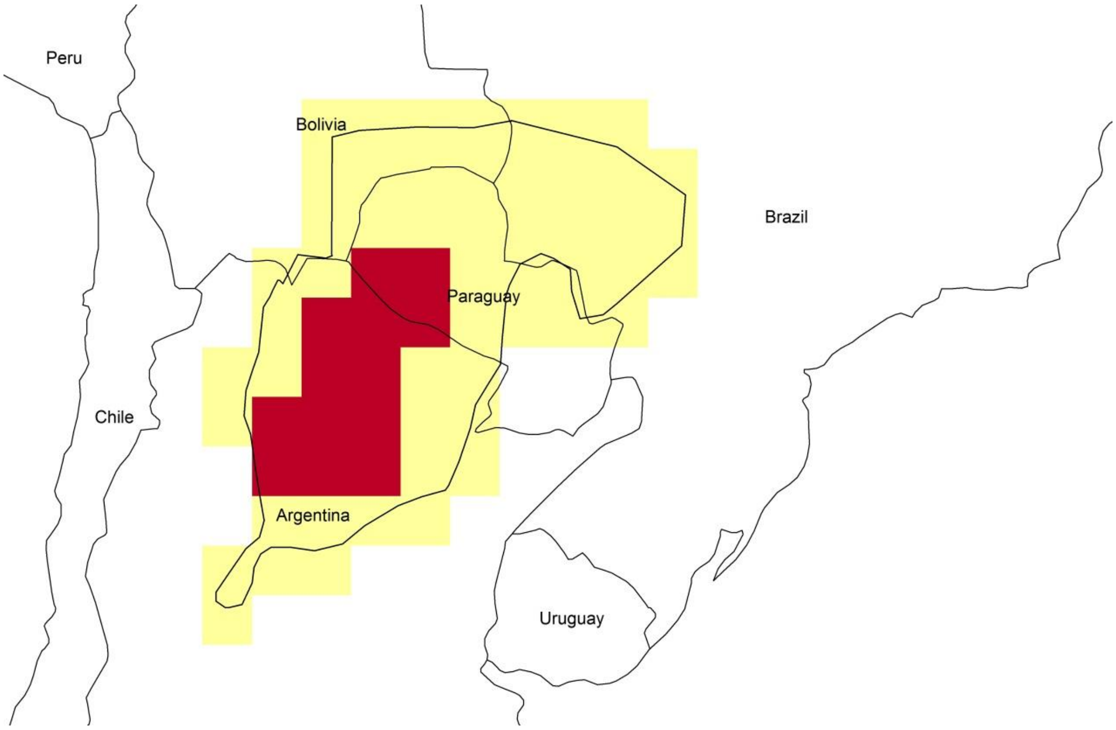


Pelophylax perezi

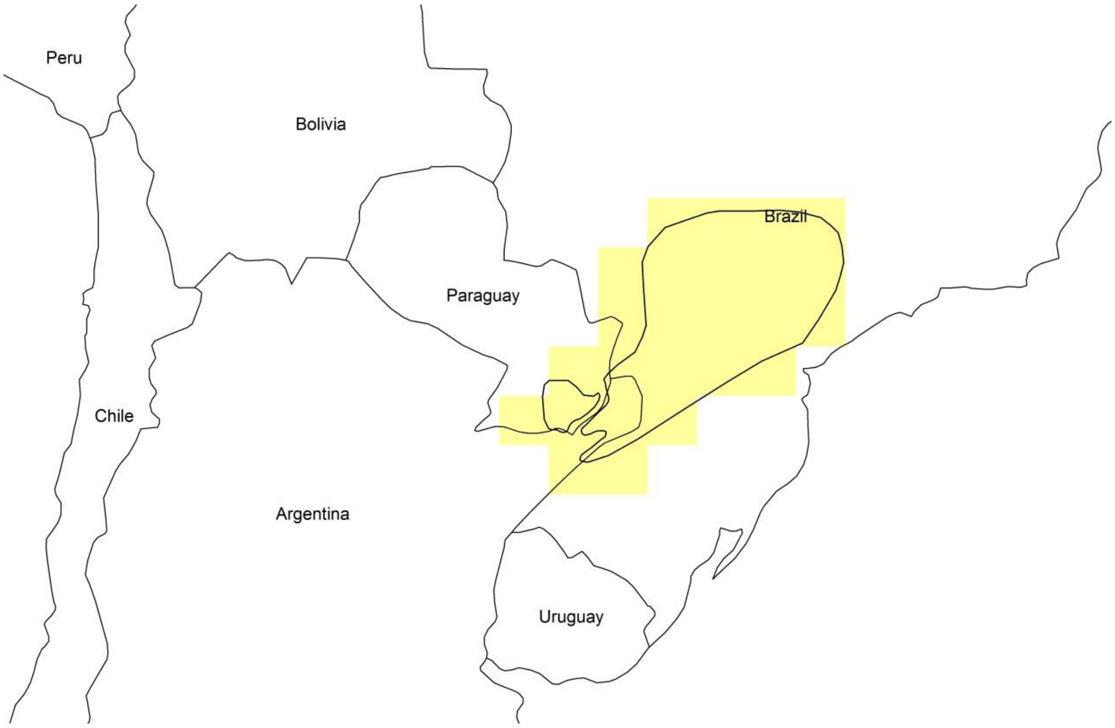
Pseudis platensis



Phyllomedusa sauvagii

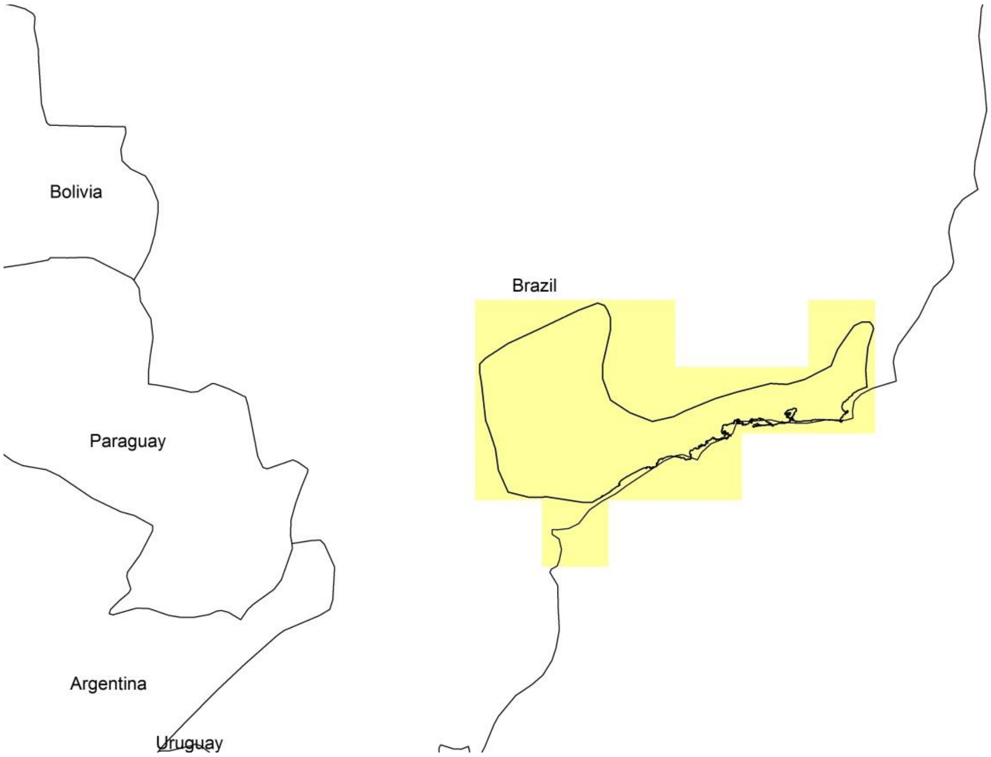


Phyllomedusa tetraploidea

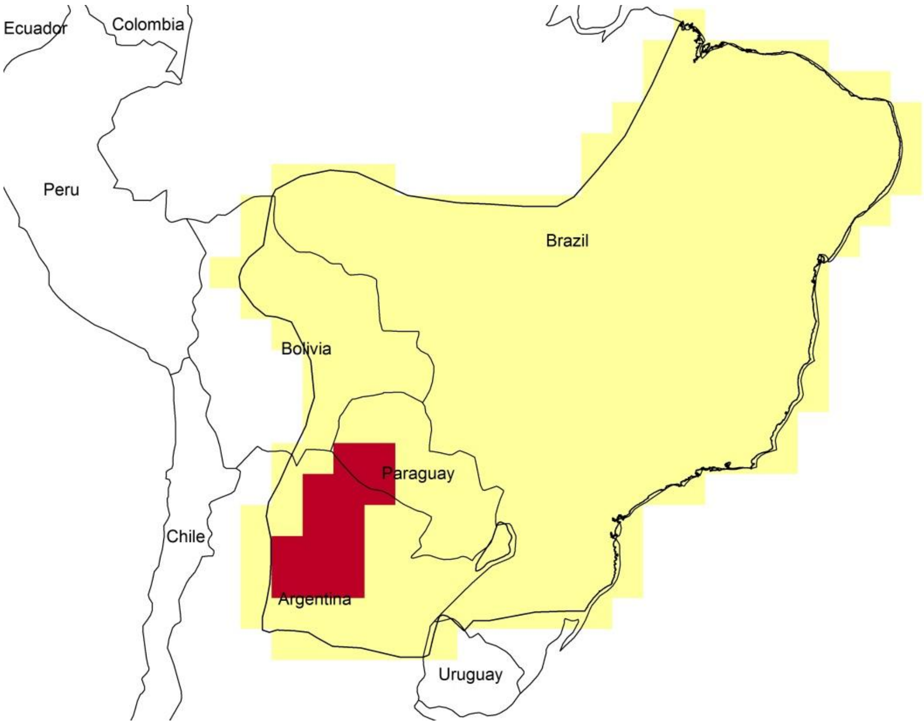


Rana arvalis

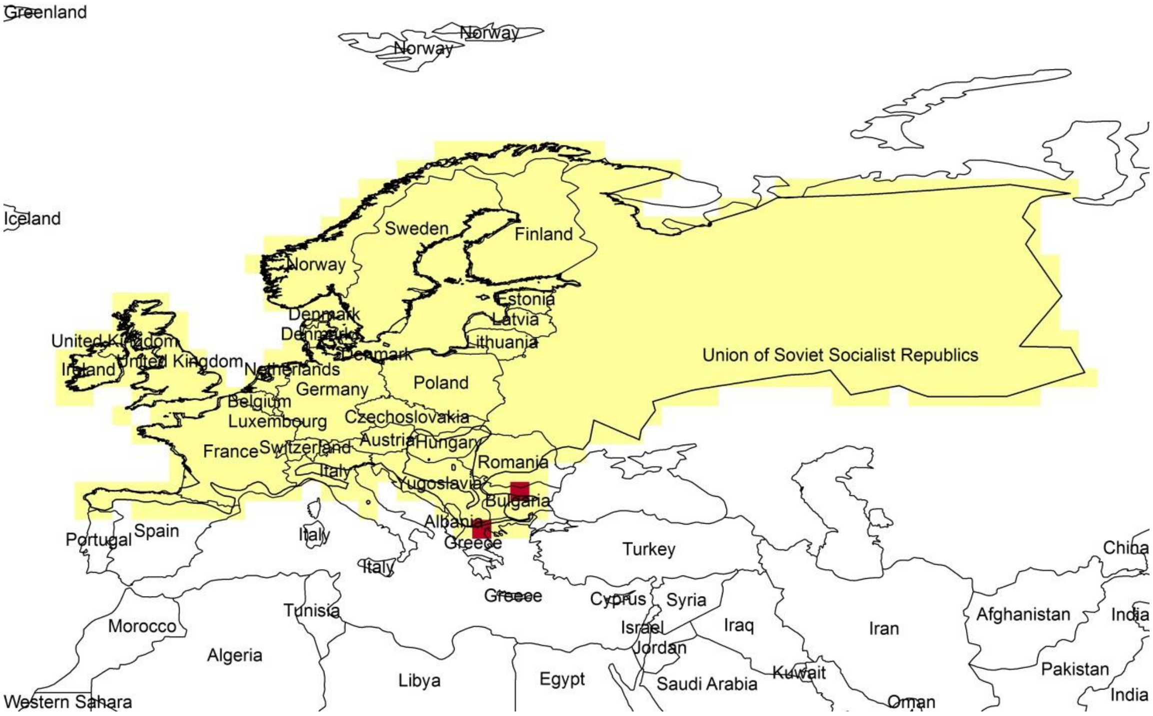
Rhinella ornata



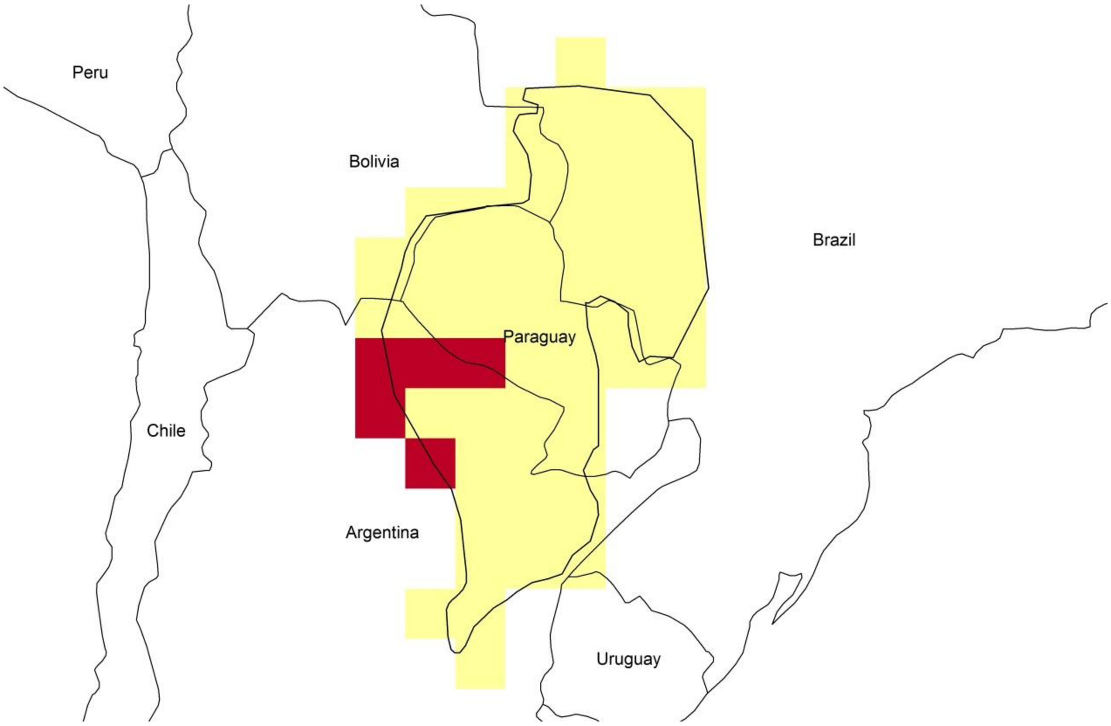
Rhinella schneideri



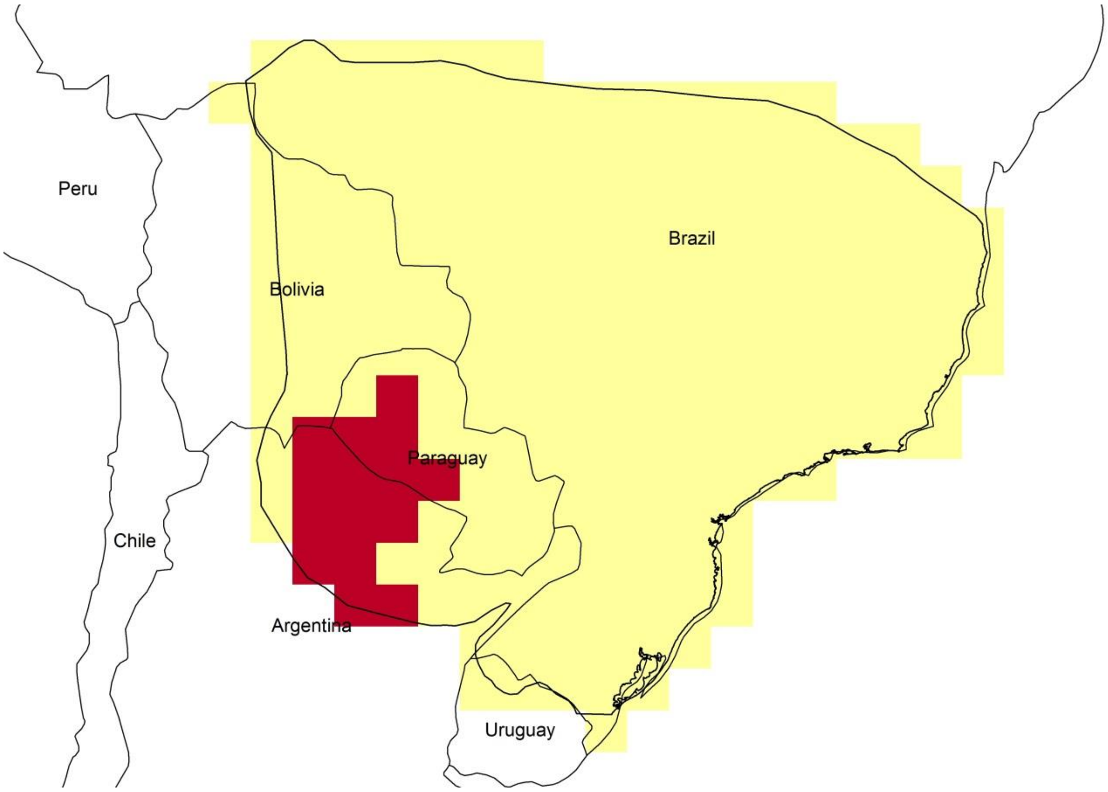
Rana temporaria



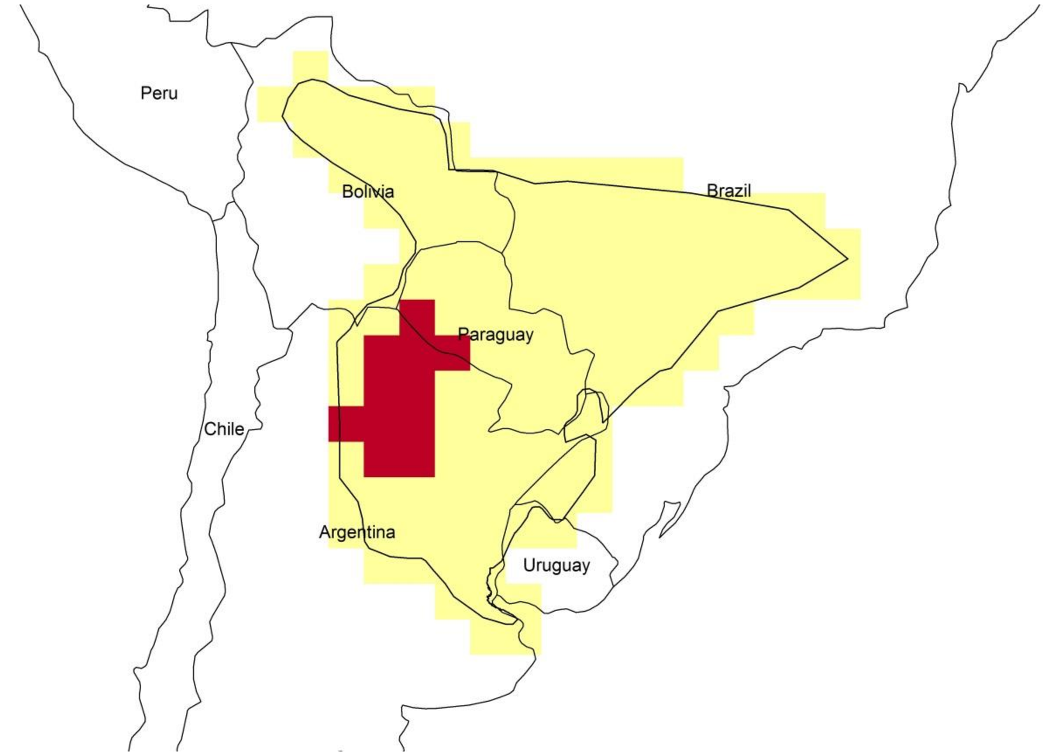
Scinax acuminatus



Scinax fuscovarius



Scinax nasicus



Trachycephalus venulosus


United States
Cuba
Haiti
Dominican Republic
Puerto Rico
Jamaica
Mexico
Belize
Guatemala
Honduras
El Salvador
Nicaragua
Costa Rica
Panama
Trinidad
Venezuela
Colombia
Guyana
Suriname
French Guiana
Ecuador
Peru
Brazil
Bolivia
Paraguay
Chile
Argentina
Uruguay