Geographical distribution, climatic variability and thermo-tolerance of Chagas disease vectors

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Understanding the relationship between geographic range limits and physiological traits of vector species is under increasing demand to predict the potential effects of global warming, not only in terms of geographic distribution of vector species but also in terms of the risk of disease transmission. Like in many other insect species, the geographical distribution of Chagas' disease vectors is affected by temperature. This study examines, for the first time, the relationship between the limits of geographic distribution and thermo-tolerance of the most important vectors of Chagas disease, *Triatoma infestans* in southern South America and *Rhodnius prolixus* in northern South America and Central America, to test the climatic variability hypothesis (CVH). We applied species distribution modeling (SDM) using bioclimatic variables and identified the most important limiting factors of the habitat suitability. Then, we measured and compared: the critical thermal maximum (CTmax) and the upper lethal temperature (ULT) (measured by thermo-limit respirometry), chilled coma recovery (i.e. the time to recovery from 4 h at 0°C) and the critical thermal minimum (CTmin). For both species the minimum temperature of the coldest month was the most important abiotic factor restricting their geographic distribution. By taking a correlative approach and testing predictions with thermal tolerance traits, it was possible to explain the southern limit distribution for both species in terms of physiological constraints. The greater temperature tolerance of *T. infestans* compared to *R. prolixus* supports the CVH.

From a macroecological perspective, the limits for geographic distribution of species may be understood as the result of the complex interactions among factors such as species-specific physiological and ecological traits, dispersal ability, and biotic interactions (intra- and inter-specific) (Spicer and Gaston 1999, Gaston et al. 2009). A comprehensive understanding of the underlying mechanisms that relate these factors is crucial to determine not only the current geographic distribution of a species, but also to predict potential distribution into new areas. Physiological traits have important roles to shape geographical range of species and help to predict species responses to environmental climatic changes (Spicer and Gaston 1999, Addo-Bediako et al. 2000). Understanding how climatic variables affect the size of the geographic distribution of species is one of the main goals of climate-based hypotheses. Macrophysiology uses these hypotheses as a framework, based on the assumption that species have evolved different optima and tolerance ranges, which are represented by their present geographic distribution limits (Chown et al. 2004, Bozinovic et al. 2011). Since no single mechanism has been proven to explain the relationship between

geographic distribution limits and climatic conditions across species (Gaston 2000, Gaston and Blackburn 2000), several climate-based hypotheses have been proposed. These include the climate variability hypothesis (CVH), which states that terrestrial organisms distributed in highly variable environments (e.g. high latitudes or altitudes) are adapted to withstand a broader range of climatic conditions than organisms in less variable environments (e.g. low latitudes or altitudes). Therefore, species at high latitudes (or altitudes) develop a broader range of tolerance to temperatures, hereinafter thermo-tolerance range, thus becoming more extensively distributed than species at low latitudes (or altitudes) (Snyder and Weathers 1975, Osovitz and Hofmann 2005, Compton et al. 2007).

The thermo-tolerance range is a critical trait that determines the physiological niche of insect species (Spicer and Gaston 1999) and it could be delimited by critical temperatures (minimum and maximum) within which the individuals are generally active (Chown and Nicolson 2004). The critical temperatures are defined as those beyond which the insects cannot respond to any further change in temperature, and therefore they will become vulnerable

to predation, catastrophes or further temperature excess or deficit (Byrne et al. 2004). Critical temperatures have a great value in ecological and evolutionary studies (Mitchell et al. 1993, Lighton and Turner 2004) and may be measured by different methods. For insect species, critical thermal minimum (CTmin) is the temperature resulting in the loss of coordinated muscle function, whereas critical thermal maximum (CTmax) is the temperature resulting in the onset of muscle spasms (Terblanche et al. 2006). Other relevant measures related to insect thermo-tolerance include lethal temperature and chill-coma recovery time (Chown and Nicolson 2004).

Previous studies suggest that global warming may affect insect physiology (e.g. by increasing metabolic and biting rate), thus affecting the transmission rate of vector-borne diseases (Rolandi and Schilman 2012). The knowledge of physiological tolerances such as thermo-tolerance range of insect vector species would not only contribute to the identification of the current active vectorial disease transmission areas, but also potential new areas under future global warming. In Central and South America, Chagas disease is one of the most important vector-borne diseases, which is transmitted by insects belonging to subfamily Triatominae (Hemiptera, Reduviidae) (Schofield 1994). The majority of the 141 species (Galvão et al. 2003, Schofield and Galvão 2009) are distributed within or close to the inter-tropical areas, though some species are found in temperate regions with cold winters like Patagonia, in Argentina, or Indiana and Maryland, in the USA (Moncayo 1992, Carcavallo et al. 1999). The two most important vector species are *Rhodnius prolixus* in Central America and northern South America and *Triatoma infestans* in southern South America (Schofield 1994, WHO 2000, Guhl 2009). The effects of heat and temperature on physiological or behavioral processes in *R. prolixus* and *T. infestans* have been relatively well studied, such as general aspects of thermo-preference (Lazzari 1991, Canals et al. 1997, Schilman and Lazzari 2004), as well as the effects of heat and temperature on host finding and feeding (Lazzari and Núñez 1989a, b, Ferreira et al. 2007, Fresquet and Lazzari 2011), egg production, hatching and immature development time (Clark 1935, Luz et al. 1999), cessation of moulting (Okasha 1964, 1968a), learning and memory (Vinauger et al. 2013), and changes on the metabolic rate (Okasha 1968b). However, thermal tolerance traits have not been characterized for these species nor any other Chagas disease vectors.

The aim of this study was to explore the possible relationships between the thermo-tolerance and the geographic distribution limits of *R. prolixus* and *T. infestans*. To this end, we combined two methodological approaches, species distribution modeling (SDM) and physiological measurements. We first modeled the habitat suitability of each species using bioclimatic variables and identified the most important limiting factor that predicts habitat suitability. Then we evaluated the thermal-tolerance limits by measuring CTmax, CTmin, upper lethal temperature (ULT) and chill-coma recovery time in both species. Finally, we analyzed the relationship between the most important limiting factors of habitat suitability and the thermo-tolerance range to test the CVH for *R. prolixus* and *T. infestans*.

Material and methods

Geographic analyses

Triatomine geographic distribution and ecological niche modeling

The geographic distribution of *R. prolixus* and *T. infestans* was based on an Atlas with maps depicting the range of 115 triatomine species (Carcavallo et al. 1999). Based on these maps, a 0.1° (~10 km) resolution grid was generated, the information digitized and a database built. From this database, which was previously used by Diniz-Filho et al. (2013) and Fergnani et al. (2013), and can be found in the public domain site (doi: http://dx.doi.org/10.6084/m9.figshare.653959), a 1% random subsample was taken for each species, resulting in 422 pseudo-presence data points for *R. prolixus* and 470 pseudo-presence data points for *T. infestans*. This selection included data points at central and border areas of the complete species distribution range assuming that species ranges are essentially the expression of a species' ecological niche in space (Sexton et al. 2009). In order to be more conservative, we did not take pseudo-absences due to the potential errors that can be introduced (Mackenzie and Royle 2005). A similar approach was used in Richmond et al. (2010). Using random pseudo-presence data has the advantage that there is no bias problem with sampling, although it may lead to over-predictions in characterizing climatic suitability, mainly because large-scale 'extent-of-occurrence' in geographical ranges may include some unsuitable areas, and thus tend to exaggerate actual occurrences (Graham and Hijmans 2006, Hurlbert and Jetz 2007, Jetz et al. 2008). However, a recent study concluded that some SDMs, including presence-only models like MaxEnt, are to some degree robust to locational errors in occurrence data (Graham et al. 2008).

The environmental variables used to model habitat suitability and identify the potentially limiting factor of the distribution were the bioclimatic variables related to temperature and precipitation at 5 arc-minutes resolution (~10 km) available from WorldClim (<www.worldclim.org>). We used all the 19 bioclimatic variables (plus altitude) defined by WorldClim, which will be referred to their standard WorldClim acronym. The ecological relevant variables bio5 and bio6 were selected to compare our physiological analyses. In order to avoid collinearity among the variables, an analysis that computes a matrix of Pearson's rank correlation coefficients for all possible pairs of variables was carried out for both species using function rcorr from R software, package 'Hmisc' (R Core Team). Variables that correlated ($r \geq 0.7$) with bio5 or bio6 were excluded from further analyses. With the remaining explanatory bioclimatic variables, we assessed the extent of any remaining multicollinearity by the variance inflation factor (VIF) for both species models using function vif of package 'car' from R software (R Core Team). To calculate VIF, we fit a generalized linear model (glm) with binomial response ('presence/absence') containing the explanatory bioclimatic variables (10 000 pseudo-absences points were generated and used by randomly assigning unoccupied grid cells, VanDerWal et al. 2009, Barbet-Massin et al. 2012). Any variable with VIF > 10 was excluded (Zuur et al. 2009). The remaining variables were used to fit a maximum entropy (MaxEnt) SDM with standard settings for the maxent

function from the 'dismo' package in R software (Hijmans and Elith 2013). MaxEnt is a presence-only method that works with background points (Phillips et al. 2006). This model yields a continuous presence probability from 0 to 1, which represents the relative habitat suitability index of a given grid cell (Hijmans and Graham 2006, Phillips et al. 2006).

In order to investigate the limiting factors for geographic distribution, within MaxEnt we used the limiting factor analysis and map (Elith et al. 2010, Hill et al. 2012). At each pixel, the limiting factor analysis replaces each variable predictor by its average value at occupied sites. If a change results in the highest probability of occurrence, therefore the variable changed is viewed as a limiting factor (see Elith et al. 2010 and Hill et al. 2012, as well as its Supporting information, for more details).

Final models were run with 10-fold cross-validation and the goodness of fit of the predictions were evaluated by the AUC (area under the receiver operating characteristic (ROC) curve) criterion, which varies from 0 to 1, with 0.5 for a model that performs not better than random, to 1.0 for a model with perfect ability to predict presence versus absence. Although, the use of AUC for testing ecological niche models obtained from presence-only data has been criticized (Lobo et al. 2007), it is very useful in SDMs for evaluating predictor contribution across consistent modeling conditions (e.g. background) (Elith et al. 2006, Terribile et al. 2010). MaxEnt predictions were visualized and mapped with Quantum-GIS ver. 2.0 software (<www.qgis.org>).

Physiological analyses

Insects
For all the experiments we used unfed fifth-instar nymphs of *R. prolixus* and *T. infestans* that had entered that instar one week before the start of the experiments. Insects were reared at 28°C under a 12:12 light/dark photoperiod (light on 08:00 am). Both species had been reared for over 10 generations under the same controlled laboratory conditions.

Critical thermal maxima and upper lethal temperature
We used thermo-limit respirometry to determine CTmax (Lighton and Turner 2004). Within a temperature controlled cabinet, we set-up a flow-through respirometry system with a CO_2 IRGA attached to an activity detector to measure real time CO_2 production and motor activity in an unrestrained individual triatomine. The analog outputs from the analyzers measuring CO_2, the insect's activity, the temperature of the chamber, and the air flow rate were connected to an A/D converter and stored in a computer at 1 Hz by ExpeData data acquisition software (Sable Systems International, SSI). A protocol similar to that of Lighton and Turner (2004) was used. After recording an empty chamber as a baseline, an individual triatomine was placed in the respirometry chamber and the recording re-started with a temperature profile beginning with 15 min at 35°C, and followed by a ramp at a rate of 0.25°C min⁻¹. CTmax was defined by two criteria: motor activity and respiratory breakdown. To determine the respiratory CTmax more accurately, Lighton and Turner (2004) proposed the identification of the inflection point in the absolute difference sum (ADS), which is the cumulative

sum of the absolute differences between adjacent VCO_2 data points. This inflection point is suggested to be an objective method for identifying the point at which short-term variability in the VCO_2 data declines abruptly, thus indicating the cessation of spiracular activity by the insect. This technique has the advantage of being highly repeatable and as objective as may be possible with such measurements. After CTmax occurred, we distinguished the ULT in the same recording (see Supplementary material Appendix 1, 5 for full details). Each insect was weighed before and after the assay to the nearest 0.1 mg on an analytical balance (Mettler AJ100, OH, USA).

Critical thermal minimum
To measure CTmin, triatomines were tested on a temperature plate (Pelt-plate, Sable Systems International (SSI), Las Vegas, NV, USA) connected to a SSI's Pelt-5 temperature controller, which generated a temperature profile that began with a baseline of 15 min at 20°C followed by a ramp at a rate of –0.25°C min⁻¹ for 100 min. Final temperature was maintained for 10 min, and finally the program resets the temperature plate to 20°C. Insects were placed over the plate individually and confined by a 5 cm diameter acrylic container with plastic mesh on top. Temperatures were measured by a thermocouple (type T) attached to a SSI TC-2000 thermocouple meter placed as close as possible to the container. CTmin was defined as loss of coordinated muscle function (Terblanche et al. 2006) and it was determined when insect did not respond to a soft touch by a thin cupper filament through the mesh. The temperature at which this occurred was recorded manually by a mark superimposed on the temperature profile recorded with data acquisition software (ExpeData; SSI, Las Vegas, NV, USA).

Chill-coma recovery time
The time required to recover from cold exposure (ca 0°C), usually referred as chill-coma recovery, was measured as another way to assess cold resistance. Insects were individually placed for 4 h in small containers in melting ice, and then transferred to Petri dishes (70 × 10 mm) with the bottom covered by filter paper in a controlled temperature cabinet at 28°C (i.e. same temperature at which insects were reared). Using forceps kept under cold conditions, each insect was positioned on its back on the center of a Petri dish. Recovery time was recorded when insects assumed an upright position (visually observed through a glass), or recorded as non-recovered if insects did not assumed an upright position after one hour. Each individual was weighed to the nearest 0.1 mg on an analytical balance (Mettler AJ100, OH, USA) before the assay.

In all cases, means are accompanied by sample sizes and standard deviations, except when noted (for a detailed explanation of methods see Supplementary material Appendix 1, 5).

Results

Geographic analyses

Geographic distribution and ecological niche modeling
After correlation analysis among bioclimatic variables, six variables for *T. infestans* and seven variables for *R. prolixus*

Table 1. Relative contribution for bioclimatic variables and AUC values.

Variable	R. prolixus (percentage)	T. infestans (percentage)
Annual precipitation (bio12)	11	19
Isothermality (bio3)	N/A	28
Maximum temperature of the warmest month (bio5)	3	7
Mean diurnal range (bio2)	11	N/A
Minimum temperature of the coldest month (bio6)	50	34
Precipitation (bio15)	2	6
Precipitation of coldest quarter (bio19)	21	5
AUC (mean ± SD)	0.838 ± 0.032	0.839 ± 0.018

AUC = area under the receiver operating characteristic (ROC) curve. N/A = data not applicable.

were selected to fit glm models. For *T. infestans*, all variance inflation factors (VIFs) were below 10, suggesting that collinearity was no longer a major issue (Zuur et al. 2009). For *R. prolixus*, however, the VIFs of bio2, bio3, bio5 and bio6 were larger than 10, so it was necessary to remove one variable. Since bio3 is a byproduct of bio2, bio5 and bio6 (bio3 = bio2 × (bio5 – bio6)$^{-1}$ × 100), we decided to exclude this variable and after this exclusion all VIFs for *R. prolixus* were below 10 (see Table 1 for selected variables).

Ecological niche modeling in MaxEnt found the minimum temperature of the coldest month (bio6) to be the most influential abiotic factor for the distribution of both species (Table 1). For *R. prolixus*, the jackknife analysis also determined bio6 to be the most important factor for the final model, while for *T. infestans* isothermality (bio3) had a greater contribution to the final model (Supplementary material Appendix 3). The maximum temperature of the warmest month (bio5) showed only a minor contribution (Table 1). The suitability index revealed a decreasing gradient with higher latitudes for *R. prolixus* (Fig. 1, left map), but an increasing suitability gradient for *T. infestans* (i.e. the unsuitable areas were located at low latitudes; Fig. 1, right map).

The goodness of fit of MaxEnt predictions was high for both species, with an AUC value (mean ± SD) for the test group of 0.838 ± 0.032 and 0.839 ± 0.018 for *R. prolixus* and *T. infestans*, respectively (Table 1).

Physiological analyses

CTmax for both species showed a temporal response to ramped temperatures typical of the ones found in other insects. Figure 2 illustrates the seven distinct phases described by Lighton and Turner (2004) in two *Pogonomyrmex* ant species: 1) the equilibration phase during which VCO$_2$ is constant, 2) beginning of the temperature ramp, with VCO$_2$ increasing exponentially, 3) a 'pre-mortal plateau' phase, during which VCO$_2$ does not increase with temperature, 4) a steep decline in VCO$_2$ during which a 'mortal fall' takes place because spiracular control (as measured by VCO$_2$ ADS) and activity (as measured by activity ADS) abruptly cease, 5) a 'post-mortal valley', indicated by a low point in VCO$_2$, 6) a new rise in VCO$_2$ (the 'post-mortal peak'), followed by a slow decline in VCO$_2$, and 7) a classic exponential decay which progresses (if the recording is allowed to continue for long enough) back to baseline levels. CTmax occurred during phase 4 (Fig. 2) and comparative statistics for both species are summarized in Table 2. All the parameters showed significant differences between species except for VCO$_2$ time constant decay progress; eleven out of twelve thermorespirometry and size variables were significantly larger for *T. infestans* than for *R. prolixus* (Table 2). However, the live body mass differed significant between species (t$_{19}$ = –7.77, p < 0.0001; Table 2). Thus, the mass-independent metabolic rate was calculated by dividing catabolic flux rates in µW by live mass in mg raised to the 0.856 power, which is the inter-specific mass scaling exponent for tracheate arthropods (Lighton et al. 2001). After live body mass and Bonferroni corrections were performed, no significant differences in the mass-independent metabolic rate during equilibration phase (phase 1; t$_{19}$ = 1.35; p = 0.19) and plateau phases (phase 3; t$_{19}$ = 2.48; p = 0.02) were found between species. In the ramping phase (phase 2), the regressions of mass-independent metabolic rate vs temperature for *R. prolixus* (slope: 1.89 ± 0.44; intercept: –63.99 ± 17.58) and for *T. infestans* (slope: 0.99 ± 0.20; intercept: –34.34 ± 8.01) revealed that
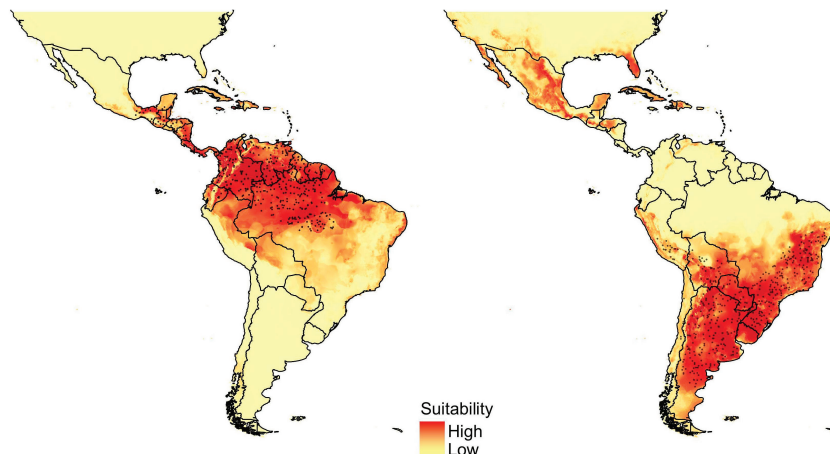

Figure 1. Habitat suitability maps for the two triatomines. Habitats predicted with MaxEnt overlapped with data distribution for *R. prolixus* (left; black dots represent presence data used) and *T. infestans* (right; black dots are presence data used).
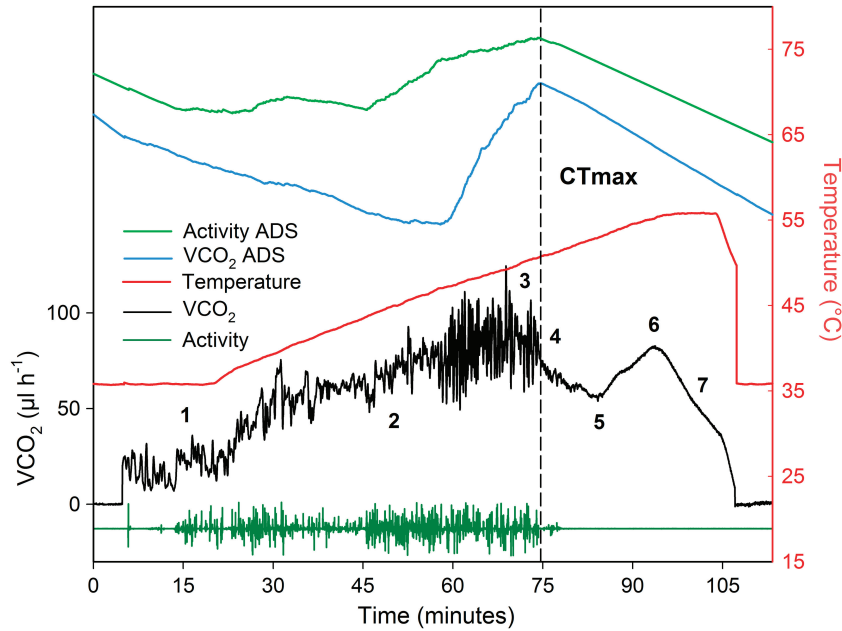
Figure 2. Thermo-limit respirometry on a triatomine. An unfed fifth-instars of *R. prolixus* (mass: 29.9 mg). Clearly visible on the VCO_2 trace (black) are the seven stages of the response (phases 1 to 7; see text for details and definitions). The equilibration temperature (right red scale) was 35.79°C. The ramping rate was 0.25°C min⁻¹. CTmax was 50.60°C (spiracular CTmax) and 50.61°C (activity CTmax). Bottom trace in green show activity (arbitrary units). On top, activity ADS and CO_2 residuals lines used to determine objectively CTmax (light green and blue respectively; scales not shown). The VCO_2 trace begins and ends with a baseline. Abscissa shows time in minutes.

R. prolixus responds significantly faster to temperature than does *T. infestans* ($T_{19} = -5.93$; $p < 0.0001$). Results are summarized in Table 3.

CTmax for *R. prolixus* determined by loss of spiracular control (spiracular CTmax) was 50.43 ± 0.01°C and determined by loss of muscular control (activity CTmax) was 50.53 ± 0.02°C. There were no significant differences between them ($t_{20} = 0.35$; $p = 0.72$). The same occurred for *T. infestans*, where the spiracular CTmax (53.19 ± 0.03°C) and activity CTmax (53.37 ± 0.02°C) were not significantly different ($t_{18} = 0.45$; $p = 0.65$). However, the activity CTmax differed between *R. prolixus* and *T. infestans* by 2.84°C ($t_{19} = -8.52$, $p < 0.0001$; Table 2), even after

taking into account the effects of body mass (ANCOVA, $F_{1, 18} = 16.71$; $p < 0.001$).

The ULT was significantly higher for *T. infestans* (53.80 ± 0.87°C) than for *R. prolixus* (51.76 ± 1.10°C) ($t_{19} = -4.68$; $p < 0.001$); the difference between the spiracular CTmax and the ULT was also statistically significant in *R. prolixus* but not in *T. infestans* ($t_{20} = -3.68$, $p = 0.001$ and $t_{18} = -1.62$, $p = 0.122$, respectively).

There were also significant differences between the species for CTmin: 0.61 ± 1.26°C (*T. infestans,* n = 25) and 6.10 ± 1.48°C (*R. prolixus,* n = 25), even after controlling for the effect of body mass ($t_{48} = -14.07$; $p < 0.001$), which was not significant ($F_{1, 47} = 0.26$; $p = 0.61$). The

Table 2. Thermo-limit respirometry parameters and CTmax of *Rhodnius prolixus* and *Triatoma infestans*. Degrees of freedom = 19.

Parameters (units)	*R. prolixus* (mean ± SD)	*T. infestans* (mean ± SD)	t-value	p
Sample size (n)	11	10		
Live body mass (mg)	26.80 ± 7.64	62.55 ± 12.21	−7.77	< 0.0001
Equilibration VCO_2 (μl h⁻¹)	18.38 ± 7.96	28.86 ± 6.81	−3.01	< 0.007
Plateau VCO_2 (μl h⁻¹)	82.64 ± 10.54	145 ± 15.61	−10.65	< 0.0001
Plateau entry (°C)	46.69 ± 1.12	52.50 ± 0.90	−12.53	< 0.0001
Plateau exit (°C)	49.69 ± 1.08	52.56 ± 1.40	−5.31	< 0.0001
Ramping duration (min)	37.46 ± 2.13	64.56 ± 3.67	20.95	< 0.0001
CTmax Activity ADS (°C)	50.23 ± 1.06	53.22 ± 0.88	−8.52	< 0.0001
CTmax VCO_2 ADS (°C)	50.13 ± 0.97	53.14 ± 0.95	−8.09	< 0.0001
LOG VCO_2 at CTmax (μl h⁻¹)	1.89 ± 0.05	2.17 ± 1.3	−10.36	< 0.0001
LOG Valley VCO_2 (μl h⁻¹)	1.73 ± 0.07	2.11 ± 0.9	−10.60	< 0.0001
PM Peak VCO_2 (μl h⁻¹)	82.17 ± 7.42	145.71 ± 18.19	−5.90	< 0.0001
Decay TC VCO_2 (min⁻¹)	−0.05 ± 0.02	−0.05 ± 0.04	0.08	0.93

VCO_2 = rate of CO_2 production per insect. Equilibration VCO_2 was at 35°C. ADS = absolute difference sum (see Material and methods and Supplementary material Appendix 1). PM = post-mortal. TC = time constant.

Table 3. Thermo-limit respirometry parameters with mass-independence correction for *Rhodnius prolixus* and *Triatoma infestans*. Degrees of freedom = 19.

Parameters (units)	*R. prolixus* (mean ± SD)	*T. infestans* (mean ± SD)	t-value	p
Equilibration VCO_2 ($\mu W \times mg^{-0.856}$)	0.72 ± 0.20	0.63 ± 0.10	1.39	0.19
Plateau VCO_2 ($\mu W \times mg^{-0.856}$)	1.42 ± 0.06	1.34 ± 0.06	2.48	0.02
Ramping phase ($\mu W \times mg^{-0.856} \times °C^{-1}$)	1.89 ± 0.44	0.99 ± 0.20	−5.93	<0.0001

VCO_2: rate of CO_2 production per insect. Equilibration VCO_2 was at 35°C.

interspecific difference for CTmin (5.49°C) was larger than that for CTmax (2.84°C).

When insects were 4 hours exposed to a stressful cold temperature of ca 0°C, *T. infestans* had an average chill-coma recovery time of 15.63 ± 3.63 min with 100% recovery (n = 25), while none of *R. prolixus* (0%) recovered (n = 25).

Link between geographical and physiological analyses

Figure 3 shows the temperature range of the presence data points used for each species and their physiological thermo-tolerance range obtained experimentally. In terms of the minimum temperature of the coldest month, the geographic presence enclosed by the 95% ellipse suggests broader limits for *T. infestans* (range ca 24°C) than for *R. prolixus* (range ca 8°C) (Fig. 3). Moreover, *T. infestans* was found up to ca 4°C below of its CTmin and 10.3°C when considering all points, in contrast to *R. prolixus,* which was not found below its CTmin (6.1°C). The upper limit of the maximum temperature of the warmest month was similar for both species (around 36°C), which was approximately 14°C and 17°C below their respective CTmax for *R. prolixus* and *T. infestans*, respectively.

Discussion

This study represents the first attempt to link geographical distribution and physiological limitations for *T. infestans* and *R. prolixus*, the two most important vectors of Chagas disease with very different latitudinal distribution. The macrophysiology approach used combining SDMs with physiological experiments allows us to test the CVH on these insects that could explain their pattern of distribution in agreement with Rapoport's rule. Ecological niche modeling corroborated the link between climatic parameters of the distribution of these species with their thermo-tolerance physiology.

Ecological implications

Results of the ecological niche modeling (Fig. 1) with minimum temperature of the coldest month as the dominant bioclimatic variable indicate that the habitat suitability index has a complementary pattern of geographic distribution between *R. prolixus* and *T. infestans*: the non-suitable area for *R. prolixus* seems to be a suitable one for *T. infestans* and vice-versa. However, there is a geographic area between the two species distributions that is not predicted to hold a suitable climate space for either species: the Andes and
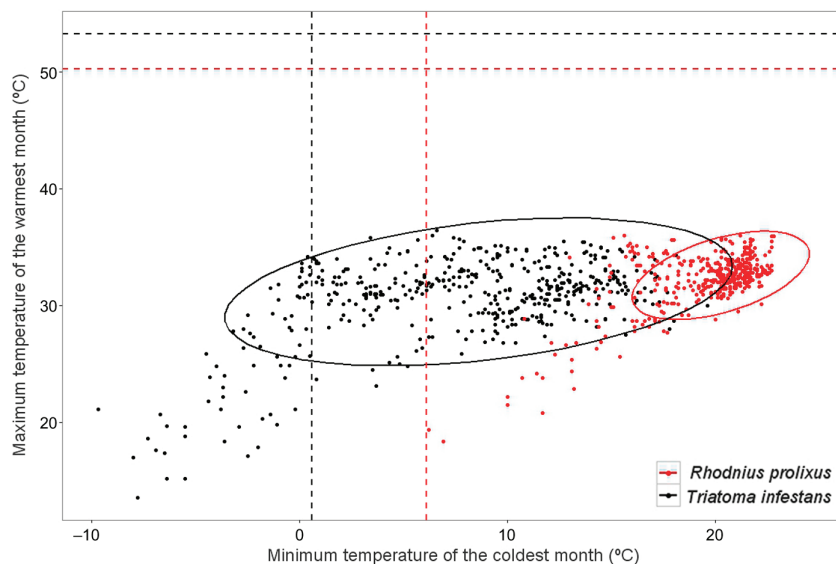

Figure 3. Extreme temperatures for the two triatomines distributions. The maximum temperature (°C) of the warmest month and the minimum temperature (°C) of the coldest month are depicted for each coordinate with triatomine presence for *R. prolixus* (red) and *T. infestans* (black). The upper and lower physiological thermal limits (CTmax = horizontal dashed lines; CTmin = vertical dashed lines for each species) define the potential maximum thermo-tolerance range: 44.13°C (CTmax = 50.23°C; CTmin = 6.10°C) for *R. prolixus*, and 52.61°C (CTmax = 53.22°C; CTmin = 0.61°C) for *T. infestans*. Ellipses enclose 95% of the presence data for each species.

central part of Brazil. For the Andean region, a possible abiotic cause for the absence of both species could be the temperature decrease due to altitude, which is consistent with the fact that most triatomine species and populations are found between sea level and 1500 m a.s.l. (Carcavallo et al. 1999). For the central part of Brazil, the limiting factor maps suggests that bio6 and precipitation variables (bio12 and bio19) are limiting factors for the southern distribution of *R. prolixus* and the northern distribution of *T. infestans* (Supplementary material Appendix 4, Fig. A3). In addition, competitive biotic interactions among triatomines should not be disregarded, since most triatomines species in South America are found in this region (Rodriguero and Gorla 2004, Diniz-Filho et al. 2013).

Our results confirm the importance of temperature in the distribution of *T. infestans,* as was determined by Gorla (2002), who showed by stepwise linear discriminant analysis a function with six statistical descriptors (four of them associated with air temperature, one associated with medium infrared radiation and one with the vegetation index) that identified correctly 90% of presence and absence sites of *T. infestans*. In a more recent study, de Souza et al. (2010) showed that annual rainfall and annual rainfall of the most humid trimester apparently limit the spatial distribution of *T. vitticeps* in Brazil. On the other hand, Diniz-Filho et al. (2013) found that temperature seasonality plays a key role in richness and distribution of triatomines species.

It would be of interest to extend our results to estimate the impact of extreme temperatures on the population dynamics and demographic processes (e.g. mortality, birth rate, dispersion) of these two triatomine species and the subsequent consequences on the transmission risk of Chagas disease. However to do so, one would have to use an approach similar to that of Gorla et al. (1997), but such development would depend on the conversion of the upper and lower critical and lethal temperatures to the conditions of the microhabitat (domestic and sylvatic), their duration, and an estimation of the probability of reaching those critical and lethal values. We strongly suggest that such a line of research be pursued because understanding the force of transmission in epidemiology as it relates to future climate change scenarios is important (Randolph 2009), and may represent the most robust basis for predicting future geographic changes in the transmission risk of Chagas disease within each of the affected countries.

Physiological implications

The CO_2 emission profiles of *R. prolixus* and *T. infestans* show the same seven distinct phases that have been found in all insect species studied by thermo-limit respirometry so far (Klok et al. 2004, Lighton and Turner 2004). This would suggest that the mechanisms responsible for the changes in CO_2 release could be conserved across insects. Addo-Bediako and collaborators found that, after controlling for trial temperature and body mass, environmental temperature significantly influences inter-specific variation in insect metabolic rate, with insects from colder environments tending to have higher metabolic rates (Addo-Bediako et al. 2002). However, after Bonferroni correction our results do

not show any significant differences between species in their mass-independence metabolic rates during the equilibration and plateau phases (Table 3). Thus, our results do not support the 'metabolic cold adaptation' hypothesis, which predicts populations or species from cooler environments (high altitude or latitude) will have either a higher metabolic rate at a common temperature or greater sensitivity of aerobic metabolism to temperature (Clarke 1991, Terblanche et al. 2009). Instead, the sensitivity of metabolic rate to temperature of *R. prolixus* (inhabiting lower latitudes) was higher than the sensitivity of *T. infestans* (inhabiting higher latitudes). A possible explanation could be that although *T. infestans* is geographically distributed at lower latitudes than *R. prolixus*, the latter experience higher mean temperatures, but not necessary higher maximal ones. During the temperature increasing ramp (phase 2 from Fig. 2), the regression of mass-independence metabolic rate vs temperature revealed that *R. prolixus* has a significantly higher temperature sensitivity (higher slope) than *T. infestans*. These results, together with differences between species in the temperature at which they enter the plateau (higher for *T. infestans*) and the duration of the ramping phase (longer for *T. infestans*; Table 2) would explain the species differences in CTmax.

Interestingly, the significant differences in the upper and lower thermal limits found between *T. infestans* and *R. prolixus* have persisted even after more than 10 generations under the same controlled laboratory conditions (they were reared in the same laboratory). Thus, even if there has been no differential acclimation, we have found intrinsic differences between the two species. It was demonstrated that acclimation account for most of the phenotypic plasticity in tsetse fly *Glossina pallidipes* (Terblanche et al. 2006). The latter together with previous results of other hematophagous insects (Lyons et al. 2012) indicate that it is reasonable to expect similar results when compared with wild populations of triatomines. This is evident for two malaria vector species (*Anopheles arabiensis* and *A. funestus*), where the thermal tolerance from long-standing laboratory colonies and field-collected animals were sufficiently similar to provide reasonable surrogates when making inferences about wild population responses (Lyons et al. 2012). In fact, preliminary results for *T. infestans* suggest a similar CTmin measured from a field population and our laboratory colony.

Linking geographical distribution to physiological traits

Both species, *R. prolixus* and *T. infestans*, are geographically distributed along areas where maximum temperatures of the warmest month are considerably lower than their respective CTmax (Fig. 3). This could be considered as an adaptive strategy to prevent individuals from exposure to temperatures near their CTmax, which is extremely close to their ULT (ca 1 degree), providing a large safety buffer to extreme conditions. With respect to the minimum temperatures of the coldest month (bio6), the more tropical species, *R. prolixus*, inhabits areas where this variable is higher than its CTmin (Fig. 3, red ellipse), whereas *T. infestans* inhabits areas where the bio6 is up to ca 4°C lower than its CTmin (Fig. 3, blue ellipse). Since *T. infestans* could be found in

colder areas than their CTmin, the effect of microclimatic conditions on the geographic distribution of this species needs to be studied. There is a seasonal variation on the domesticated populations of *T. infestans,* with a marked decrease of the population associated with low temperatures during winter (Gorla and Schofield 1989). This population drop implies that reduced encounter rates between vectors and hosts during this season and that transmission of *T. cruzi* would be at its lowest rate during the cold months. Even more important is that the lower lethal temperature (LLT) of *T. infestans* is ca 12°C below CTmin (estimated to be –10 to –12°C by Blaksley and Carcavallo 1968), which is lower than the minimum temperature of the coldest month of its geographical distribution. Thus, the LLT could be a limiting factor for the distribution of this species. In concordance with our chill-coma recovery results (Fig. 3 and Supplementary material Appendix 2), the stressful cold temperature of ca 0°C is ecologically relevant for *R. prolixus*, but would have no ecological consequences nor constraints for *T. infestans*'s distribution.

Our results conform well with the macroecological principle that at higher latitudes (or altitudes) species experience lower temperatures and greater seasonal variation, and consequently are expected to withstand greater temperature extremes (Gaston and Chown 1999). A meta-analysis by Sunday et al. (2011) tested for relationships between thermal tolerance breadth in ectothermic animals and the latitude of the specimen's location, while accounting for habitat, hemisphere, methodological differences and taxonomic affinity; they found that thermal tolerance breadth generally increased with latitude. Our findings of a broader thermal tolerance in *T. infestans* (higher latitudinal distribution) with respect to the narrower thermal tolerance of *R. prolixus* (lower latitudinal distribution) provide further support for this relationship between thermo-tolerance and latitude.

Many important predictions of the CVH based on other ectothermic species are consistent with our thermo-tolerance results. For example, similar responses were obtained with reptiles: species living in higher latitudes have lower CTmin values than species living in lower ones (Cruz et al. 2005). In insects, the upper lethal limits show much less variation than do the lower lethal limits (Addo-Bediako et al. 2000). Same occurs for plants, terrestrial endotherms and other ectotherms (Araújo et al. 2013).

Maximum temperatures of the warmest month are more similar between the geographical distributions of *R. prolixus* and *T. infestans,* than minimum temperatures of the coldest month (Supplementary material Appendix 2). Therefore, the lower thermo-limit tolerance could be limiting the latitudinal distribution of this two species. In addition, species with low CTmin and LLT are able to occupy high, relatively cooler latitudes as well as warmer latitudes, while species with high CTmin could occupy only warmer places, such that the thermal component of climatic niches across species overlaps more than expected (Araújo et al. 2013). This idea, together with MaxEnt results of the more important abiotic factors for *R. prolixus* and *T. infestans* distribution (Table 1) conforms well, at least for *T. infestans*, with findings of Gorla and Schofield (1989) who determined that *T. infestans* populations densities are determined by winter low temperatures. Similarly, winter temperature was found as the key factor limiting the northwest expansion of leafcutter ant *Atta texana* populations in North America (Mueller et al. 2011) and northern populations of the pitcher plant mosquito *Wyeomyia smithii* where northern populations were more cold tolerant than those from southern ones, a consequence that the authors attributed largely to the degree of the acclimation response (Ragland and Kingsolver 2008). Physiological limits (particularly those that are heritable) should conform more closely to the position of the niche in the climatic hyperspace, being more reliable than niche boundaries to capture the variability and evolutionary pattern of physiological tolerance (Gouveia et al. 2014).

Conclusions

We present the first measurements of critical thermal limits, ULT and chill-coma recovery times in vectors of Chagas disease. Data presented here for the geographical distribution of *R. prolixus* with a low latitude distribution and *T. infestans* with a high one, support the climatic variability hypothesis (CVH) in both species that explains Rapoport's rule in physiological terms. The larger geographical distribution of *T. infestans* compared to *R. prolixus* could be explained in physiological terms by a greater tolerance to thermal variation, i.e. the CVH (Stevens 1989). The larger amplitude of temperature tolerance in *T. infestans* compared with that of *R. prolixus* could be explained by a significant higher CTmax and simultaneously a lower CTmin of *T. infestans* compared to those of *R. prolixus*. Differences between species in CTmin were more marked than differences in CTmax. Ecological niche modeling and limiting factor analysis determined that the minimum temperature of the coldest month is the key range-limiting factor of these two vector species of Chagas disease. Physiological differences in thermal relationships across species should be taken into account in future predictions of the geographical distribution and spread of diseases' vectors under the effects of global climate change.

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Supplementary material (Appendix ECOG-01028 at <www.ecography.org/readers/appendix>). Appendix 1–5.