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Physiological traits explain the response of dung beetles to land use at local and regional scales

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Physiological traits in insects are useful to understand their distribution at different spatial scales, their presence and abundance, and the use of different habitats. This study explored physiological parameters of dung beetles in two contrasting ecoregions of Argentina: Paranaense Forest and Dry Chaco. We capture dung beetles from both regions and habitats with different degrees of disturbance within each region, and performed lab experiments on metabolic rate and thermal tolerance. This study revealed that dung beetles inhabiting regions or habitats with higher temperatures (Dry Chaco and open pastures) showed greater temperature tolerance compared to those inhabiting regions or habitats with lower temperatures (Paranaense Forest and habitats preserving tree canopy). Furthermore, in the Dry Chaco, more species exploit open disturbed habitats, indicating less physiological sensitivity to temperature changes than dung beetles in the Paranaense Forest. This study is the first to compare dung beetle assemblages at both regional and local scales. The findings highlight the importance of preserving tree canopy in land use planning to mitigate microclimatic changes, especially in humid forests, to support dung beetle populations and their crucial ecosystem roles in the face of climate change.

Keywords Physiological response, Scarabaeinae, Global warming, Regional scale, Local scale

Species ecological, ethological, and physiological requirements determine, together with the dispersion abilities, their distribution among habitats within landscapes and among different regions[1,2]. Among these requirements, the physiological tolerance to environmental conditions, particularly temperature, is critical for explaining the presence and abundance of species. Whereas the majority of insects are not able to control their internal temperature, some species among different taxa (e.g., beetles, flies, bed bugs, bees) can physiologically regulate their metabolic rate and body temperature according to environmental conditions[2–9]. Specifically in the case of dung beetles, it has been observed that they exhibit exhibit different tolerance to changes in temperature at the local scale, ranging from species highly tolerant to a wide range of low and high temperatures to species with a restricted thermal niche[2,10,11]. This differential tolerance depends on the species' ability to actively regulate their body temperature (thermoregulation) and metabolic rate[7,8]. Previous studies suggest that forest dung beetles with high thermal tolerance, active thermoregulation, and low metabolic rate can occupy many habitats, including human land uses. In contrast, dung beetle species with lower thermal tolerance, poor thermoregulators, and higher metabolic rates tend to be restricted to habitats maintaining tree canopy and, consequently, microclimatic conditions[2,7,12].

Due to their role in dung burial, dung beetles have been extensively studied in cattle areas[13–21]. In forest ecosystems, the replacement of the native tree canopy by cattle pastures changes the richness, abundance, composition, and functional structure of dung beetle assemblages. However, the magnitude of these changes strongly depends on the regional context[19,20]. As an example, in humid forests, Gimenez Gómez et al.[2,12] showed that only a few dung beetles with active thermoregulation mechanisms persist in open areas (such as cattle pastures) after forest replacement. In contrast, in dry forests, cattle ranching has fewer (or no) effects on dung beetle assemblages[20]. While regional climate influences the response of dung beetles to cattle ranching[19,20,22], the mechanisms explaining these differences have been poorly explored.

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Based on the previous evidence, our objective was to explore the role of thermal tolerance (regulation of body temperature and metabolic rate) to explain the differential response of dung beetle assemblages' to human land uses at a local and regional scale. We focus on two contrasting subtropical ecoregions of Argentina: the Paranaense Forest and the Dry Chaco. Both ecoregions are located at similar latitudes but present differences in the seasonal thermal regimen and the annual precipitation regime[23,24]. Previous studies showed a contrasting response of dung beetles to similar land uses in both regions: the assemblage from the Paranaense Forest was highly susceptible to forest replacement, while in the Dry Chaco, dung assemblages were tolerant[19,20]. Based on this, we expect that: (1) species from the Dry Chaco would exhibit higher tolerance to high temperatures compared to the Paranaense Forest and, (2) species inhabiting open habitats (cattle pastures) in both ecoregions will exhibit higher tolerance than species inhabiting habitats with a tree canopy (native forest, agroforestry parklands, silvopastoral systems, and tree plantations).

Results

Three significant PLSR components were obtained in response to heat stress (see Table 1 for statistical details). The first component was strongly and positively influenced by variables associated with thermal limits: ULT, CT_{max}, and SCT, and thermal stress: HRT. The variability in this component was strongly explained by the *ecoregion factor*, as indicated by the weights of the predictors (Table 1; Fig. 1A). In summary, species from the Dry Chaco exhibited higher ULT, CT_{max}, SCT and HRT values than species from the Paranaense Forest.

The second component was also strongly and negatively influenced by variables associated with the thermal stress period (SHST, HRT) and thermal limits (SCT). In this case, the variability in this component is explained by the *habitat factor*, as indicated by the weights of the predictors (Table 1). Species with a preference for open pastures showed higher thermal stress temperatures (SHST and HRT) and limit temperatures (SCT) than species typical of habitats maintaining tree cover, especially dung beetle species typical from the native forests (Fig. 1B).

Finally, variables associated with thermal limits (CT_{max} and ULT) positively influenced the third component and related to the metabolic rate (iTR, iCO_2). The variability in this component is explained mainly by the *habitat factor* in the Paranaense Forest (Table 1). Within the Paranaense Forest, agroforestry parkland species have higher CT_{max}, ULT, iTR and iCO_2 than the rest of the habitats, mainly the pine plantations (Fig. 1C).

Discussion

Previous studies on neotropical dung beetles have focused on describing and comparing taxonomic and functional diversity among regions and habitats within regions, either natural or anthropogenic[19,20,25]. On the other hand, physiological studies exploring the mechanism behind these patterns were scarce and only conducted locally[2,12]. In this study, for the first time, we propose a mechanistic explanation of the differential regional responses of dung beetles to forest replacement by different land uses based on the thermal tolerance

	PLS1	R^2 (%)	PLS2	R^2 (%)	PLS3	R^2 (%)
Response variables						
SHST	0.14	5.67	**– 0.74**	104.41	0.09	1.22
HRT	**0.36**	38.65	**– 0.50**	47.18	0.00	0.00
SCT	**0.41**	49.69	**– 0.35**	23.54	0.20	5.54
CT_{max}	**0.55**	90.03	0.00	0.00	**0.49**	34.18
ULT	**0.57**	99.52	0.06	0.68	**0.45**	29.46
$\dot{V}CO_2$– SHST	– 0.02	0.13	0.03	0.17	0.29	11.84
$\dot{V}CO_2$– HRT	– 0.08	1.76	0.14	3.76	0.25	9.08
iCO_2	– 0.03	0.33	0.24	10.81	**0.35**	17.37
iTR	– 0.23	16.42	0.04	0.37	**0.49**	33.89
R^2 (%)	17.73		4.44		2.24	
Predictors						
Ecoregions						
Paranaense Forest	**– 0.64**		– 0.12		0.13	
Dry Chaco	**0.64**		0.12		– 0.13	
Habitats						
Agroforestry parkland	– 0.23		– 0.03		**0.64**	
Open pasture	0.18		**– 0.78**		– 0.05	
Native forest	0.20		**0.59**		0.02	
Pine plantation	**– 0.31**		– 0.04		**– 0.70**	
Silvopastoral system	0.12		0.18		– 0.27	
Eigenvalues	2.20		1.34		1.23	

Table 1. Results of the PLSR analysis summarizing the physiological traits associated with two contrasting ecoregions and habitats in Argentina. The variables whose square weights are larger than 1/number of variables, and therefore are statistically significant, are indicated in bold.

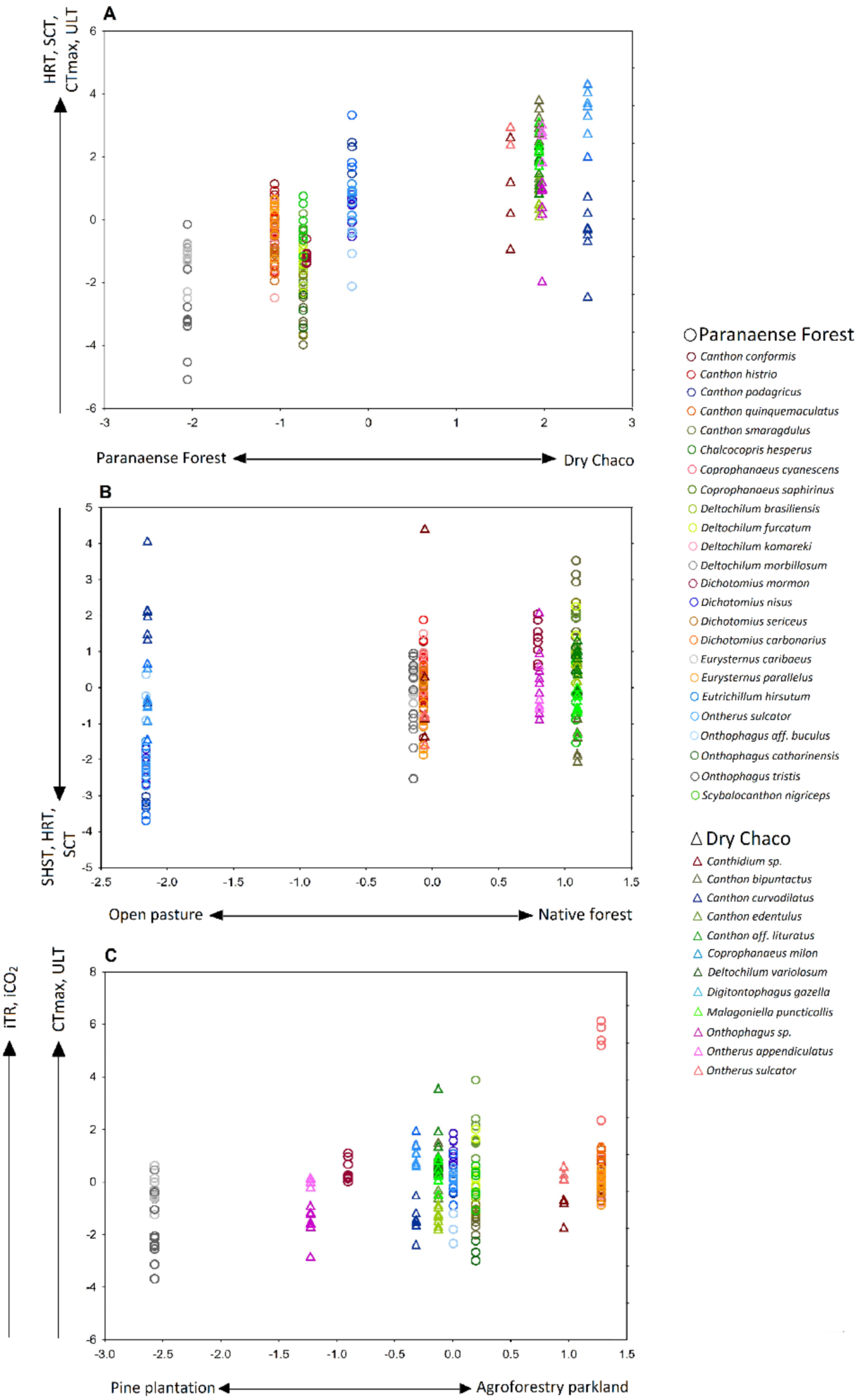

Fig. 1. PLSR analysis summarizing dung beetles' thermal response in different land uses in two ecoregions of Argentina. Arrows represent the sign of the relationships. Only statistically significant response variables are shown. The y-axis represents the response variables, and the x-axis represents the predictor variables.

of species and assemblages. Supporting our hypothesis, species from the most extreme and seasonal climatic region (Dry Chaco) exhibited higher physiological tolerance to temperature than species inhabiting the less seasonal forest (Paranaense Forest). Moreover, irrespective of the region, species inhabiting open land uses (open pastures) showed higher thermal tolerance than species inhabiting habitats preserving tree canopy (native forests, agroforestry parklands, silvopastoral systems, and pine plantations).

The Paranaense Forest and the Dry Chaco constitute two of South America's most biologically diverse and threatened subtropical forests[26]. Our results show that dung beetle species from both regions are separated by physiological mechanisms associated with heat stress and thermal limits, explained by their differences in HRT, SCT, CT_{max}, and ULT. Dung beetles from the Dry Chaco begin to eliminate excess heat and reach very high thermal stress (based on the closure of the respiratory spiracles; see SCT) at higher temperatures, tolerating higher temperatures than those of the Paranaense Forest. On the other hand, no differences were observed associated with matabolic rate (iCO_2) and excess heat regulation (iTR) between communities at regional scale. However, there were differences in these last two variables (iCO_2 and iTR) between closed environments of the Paranaense Forest (Pine Plantation and Agroforestry Parklands). These results suggest that the species of the Dry Chaco would be adapted to high temperature conditions during their periods of activity, being able to colonize environments with high ambient temperature (open or semi-enclosed environments) without additional energy expenditure. However, the dung beetles species of the Paranaense Forest are not adapted to extreme temperature conditions in open environments; when subjected to high temperatures, they reduce their body temperature and increase their respiration rate, which implies a higher energy expenditure (higher metabolic rate). These results explain why, in this ecoregion, the forest species are not able to colonize open areas exposed to strong solar radiation[2].

From a behavioural viewpoint, CT_{max} is the temperature at which an individual begins to exhibit uncoordinated movements of their legs, followed by spasms and, eventually, paralysis in one of the extremities[27–30]. ULT is reached when the individual ceases all movement and dies[8]. The higher values of these two thermal limits in the Dry Chaco species are also probably an evolutionary response to this region's extreme temperatures and seasonality. At the biogeographical scale, although other factors such as dispersion, behaviour, feeding requirements, and competition may have an influence, it is probable that physiology, particularly the tolerance to high temperatures, plays a central role in explaining both the local and regional distribution of dung beetles[12]. Among insects, CT_{max} has only been used in ants to explore the regional distribution of species[31]; ants from regions with higher temperatures show higher CT_{max}. Regarding other animals, it has also been used in crustaceans[32], finding similar results. CT_{max} and ULT, have been used to explain both local (e.g. spatial segregation) and biogeographical (altitudinal and latitudinal) scales, and temporal scale distributions in dung beetles[8,9,12,33–35], as well as in other insects such as triatomines[36], rolled-leaf beetles[37], flies[38,39], butterflies[40], bees[41,42], wasps[41], and dragonflies[43]. However, the other physiological parameters that showed differences, HRT and SCT, have only explained patterns at a more local scale (niche overlap, daily activity, etc.)[8,9].

In the Atlantic Forest, only a few native species could colonise and persist in deforested areas, such as open pastures. These species have lower endothermy, higher thermal tolerance, greater ability to regulate excess heat, and lower metabolic rates than species restricted to native forest[2,12]. In a general revision of South America, Guerra et al.[19,20,44] showed that the dung beetles assemblages from more extreme and seasonal ecosystems (such as dry forests) were less affected by forest conversion than assemblages from more stable ecosystems (tropical and subtropical wet forests). Our results explain this general pattern mechanistically; species from more extreme ecosystems probably exhibit physiological mechanisms to tolerate drastic temperature changes, whereas species from more stable ecosystems cannot tolerate these changes.

This is the first time that the physiological response of dung beetle on similar land use but different ecoregions have been explored. Despite regional differences, dung beetles exhibited similar physiological responses to forest conversion by open pastures. In both regions, species inhabiting open pastures showed higher SHST, HRT, and SCT values. In other words, species in open pastures experience stress, thermoregulate and close their spiracles at higher temperatures than forest species. However, the difference between ecoregions is the proportion of species able to tolerate the extreme conditions in open pastures; in the Paranaense Forest only a small proportion of native species were tolerant, whereas in the Dry Chaco, a large proportion of species are tolerant to these conditions.

In dung beetles of the Paranaense Forest, the results obtained for SHST and HRT were reported in a previous study, considering the same habitats[12], while the result for SCT is novel. On the other hand, the results of the three physiological variables (SHST, HRT, SCT) are novel for the species of the Dry Chaco. In general and according to what was explained above for the Dry Chaco and Selva Paranaense, the results would be showing that those species from open environments (open pastures) would be adapted to high temperature conditions, tolerating stress at higher temperatures, thermoregulating excess heat, and without expending excess energy. While Native Forest species are not adapted to extreme temperature conditions; when subjected to high temperatures they reduce their stress limit and their thermoregulation tends to be poor. The latter would be associated with the thermoregulatory mechanisms found in a previous work for the Native Forest dung beetles species under study in Paranaense Forest, where it was observed that most species do not exhibit well-defined temperature regulation mechanisms[2].

Our results show that the distribution of dung beetle species among regions and habitats (both natural and anthropogenic) can be partially explained by their physiological response to temperature. At a regional scale, thermal limits and heat stress temperatures mainly explain dung beetles species distribution, while at a local scale, habitat use can be explained by thermal limits, heat stress temperatures and metabolic rate. These results provide a mechanistic explanation for a general pattern of response previously described in dung beetles. At regional scale, dung beetle species in regions with higher temperatures, exhibit high thermal limits, high heat stress temperatures, and there are capable of releasing excess heat (thermoregulation) at higher temperatures without additional energy expenditure; while dung beetle species of regions with lower temperatures, when subjected to high temperatures, reduce their body temperature (low thermal limits and stress temperature) by increasing their respiration rate, which implies a higher energy expenditure. At local scale, the response is similar, dung beetles species that make use of higher temperature environments tend to present high heat stress temperatures and there are capable of releasing excess heat (thermoregulation) at higher temperatures without additional energy expenditure; while, on the other hand, species adapted to live in lower temperature environments, in the face of rising temperatures, present lower heat stress temperature, poor heat thermoregulation, increase in its respiration rate and thus a higher energy expenditure. Furthermore, the fact that more species can use open habitats in the Dry Chaco compared to the Paranaense Forest suggests that the replacement of native forests by open pastures has less impact on the taxonomic and functional diversity of dung beetle assemblages in dry ecosystems than humid ones. Having demonstrated that physiological variables play a fundamental role in dung beetles' response to environmental changes (both at regional and local scales), within the context of the global environmental crisis and considering the central role of dung beetles in nutrient cycling, greenhouse gas reduction and parasite control in livestock areas, our results strongly support the idea that livestock areas should consider partial retention of the native canopy to enhance soil microclimatic conditions, particularly in humid and less seasonal forests.

Methods

Study area, dung beetle collection and sampling design

We conducted fieldwork in two subtropical ecoregions of Argentina: the Paranaense Forest and the Dry Chaco, during October-February 2016–2018 and November 2019 respectively. The Paranaense Forest (our southern Atlantic Forest) is among the most diverse and threatened forests worldwide[45]. In Argentina, the Paranaense Forest is a continuous forest with three to five vegetation strata and a diverse understory of ferns, bamboo, and herbs. The landscape in the study area includes continuous forest in protected areas, large tracts of exotic tree monocultures (mainly *Pinus taeda* L.) for the production of cellulose and wood, small properties dedicated to subsistence agriculture with crops such as corn (*Zea mays* L.), tobacco (*Nicotiana tabacum* L.), and yerba mate (*Ilex paraguariensis* Augustin Saint-Hilaire), as well as small pastures for cattle raising[46,47]. The Paranaense Forest experiences distinct temperature changes throughout the year, with summer temperatures typically ranging from 17 to 36 °C and winter temperatures from 7 to 19 °C. However, precipitation remains consistent across seasons, generally falling between 1000 and 2000 mm annually[23,24,48].

The Dry Chaco ecoregion is characterized by xerophytic species associated with closed to open broadleaf forests, thorn forests and shrub savannas. The most representative tree species are Quebracho Colorado (*Schinopsis balansae* Engler), Mistol (*Ziziphus mistol* Griseb), and Palo Cruz (*Tabebuia nodosa* Griseb), along with other trees and shrubs that play an essential role in forestry and livestock activities. In the shrub savannas, species up to 2.5 m in height are observed, with the most common being Pichanilla (*Senna aphylla* Cavanilles), Piquillín (*Condalia microphylla* Cavanilles), Cardon (*Cereus aethiops* Hawort), and Retamo (*Bulnesia retama* Griseb)[49]. The landscape includes continuous forest in protected areas, extensive cattle raising, forestry exploitation, and properties devoted to annual crops, such as soya (*Glycine max* L.), cotton (*Gossypium barbadense* L.), sunflower (*Helianthus annuus* L.), and corn (*Zea mays* L.). The Dry Chaco exhibits distinct seasonal patterns in both temperature and precipitation. Summer temperatures typically range from 4 to 47 °C, accompanied by precipitation between 50 and 350 mm. In winter, temperatures range from −6 to 24 °C, with precipitation ranging from 10 to 80 mm[23,24,44].

Within each region, we sampled the native forest and three land uses: agroforestry parkland, silvopastoral systems, and open pastures. Additionally, in the Paranaense Forest, we sampled pine plantations (this land use was not available in the Dry Chaco). The selected habitats represent different degrees of disturbance of native forest within each region, with agroforestry parklands being the most preserved and open pastures the most disturbed habitats. Silvopastoral systems and pine plantations represent intermediate situations. A detailed description of each habitat including their temperature can be found in Table 2. Temperature was taken during fieldwork using temperature sensors (HOBOS ProV 2) located at ground level. Each datalogger stored a value of both variables every five minutes. Within each habitat, we selected three replicates. We placed ten pitfall traps on each replicate to collect live individuals for physiological experiments (150 traps for Paranaense Forest and 120 for Dry Chaco). We baited five with rotten meat and five with human faeces to capture necrophagous and coprophagous species. We included leaf litter inside each trap to reduce the mortality of falling individuals and a plastic cover on the plastic cup with a 3–4 cm open triangle to allow dung beetles to enter but prevent them from escaping. Upon arriving at the laboratory, we placed the collected individual in a terrarium consisting of circular plastic containers 15 cm in diameter and 10 cm in height and filled with moist paper. These terrariums were maintained in a refrigerated incubator (MIR-153, Sanyo Electric Co. Osaka, Japan) at 25 °C and a photoperiod of 13:11 (light: dark)[7,54]. To avoid bias due to possible stress due to maintenance in the laboratory, all specimens were analyzed during the first two weeks after their collection in the field. For the same reason, physiological measurements were made by alternating individuals of each species. During this time, we provided cow dung to coprophagous species and fish to necrophagous species. Prior to each experimental trial, individuals were fasted for 72 h to eliminate possible mistakes caused by the difference in the feeding states of individuals. At the end of the experiments, the individuals were discarded. Finally, we associated species to specific habitats based on the frequency of capture and previous studies in the same study area[2,12,19]. The number of individuals per species used for the experiments is detailed in SI Appendix, Table S1.

Thermolimit respirometry and infrared thermography (TLR-IR)

TLR measurement

To determine physiological specializations to thermal stress, we measured carbon dioxide production using flow-through respirometry following previously described methods[8,55,56]. We placed each living individual near a control individual (a dead and dry individual of the same species) to discriminate the active physiological thermal response. We placed both individuals into a flow-through respirometer measurement chamber made of methacrylate. We put the chamber above a precision digital hot plate (J. P. Selecta, Barcelona, Spain) and applied a temperature increase rate of 0.32 °C min^{-1}, from 25 to 60 °C, inside the chamber. Dry, CO_2-free air

		NATIVE FORESTS	AGROFORESTRY PARKLANDS	SILVOPASTORAL SYSTEMS	OPEN PASTURES	PINE PLANTATIONS
Paranaense forest	General characteristics	A diverse canopy and understory, conforming 3–5 strata, with vines, shrubs and bamboo[50]	Devoted to cattle raise. Open native forests are mainly composed of native trees and vines, and the understory is composed of shrubs, grasses, and bamboo[51]	Devoted to cattle raising and wood production. A 9–10 m tree canopy composed of *Pinus taeda*, with an average density of 400 plants/ha. The understory was comprised mainly of grasses and shrubs[51]	Devoted to cattle raising. Open areas with exotic grasses and scattered native trees[17]	Devoted to wood production. Characterized by a dense canopy composed of 7–9 m-tall pines (Pinus taeda), with an average of 1200 plants/ha. Plantations showed a low understory cover[52,53]
	Temperature	*Absolute maximum T°*: 27.9 *Average maximum T°*: 25.7 *Absolute minimum T°*: 16.1 *Average minimum T°*: 18.5	*Absolute maximum T°*: 29.9 *Average maximum T°*: 28.1 *Absolute minimum T°*: 17.4 *Average minimum T°*: 18.9	*Absolute maximum T°*: 30.2 *Average maximum T°*: 29.2 *Absolute minimum T°*: 18.2 *Average minimum T°*: 20.4	*Absolute maximum T°*: 36.2 *Average maximum T°*: 34.5 *Absolute minimum T°*: 14.6 *Average minimum T°*: 17	*Absolute maximum T°*: 29.6 *Average maximum T°*: 25.8 *Absolute minimum T°*: 14.8 *Average minimum T°*: 20.9
Dry chaco	General characteristics	A xerophytic forest with a high cover of shrubs and emergent trees of *Aspidosperma* spp. [49]. Patches of grasslands located inside a forest	Devoted to cattle raise. Sparced native forest with xerophytic trees, shrubs, and grasses (*Megathyrsus maximus*) in the understory. Mostly uncovered soil[18,19]	Devoted to cattle raised with sparse grasses in the understory. Regular planted individuals of *Prosopis* sp. of 4–6 m height, with a density of 400–600 individuals/ha	Devoted to cattle pastures with a high cover of the exotic grass *Megathyrsus maximus.*	Pine plantations are not available in the Dry Chaco
	Temperature	*Absolute maximum T°*: 37.1 *Average maximum T°*: 35.4 *Absolute minimum T°*: 8.5 *Average minimum T°*: 8.7	*Absolute maximum T°*: 34.8 *Average maximum T°*: 32.1 *Absolute minimum T°*: 8,9 *Average minimum T°*: 9	*Absolute maximum T°*: 33.8 *Average maximum T°*: 33.8 *Absolute minimum T°*: 10.3 *Average minimum T°*: 10.3	*Absolute maximum T°*: 39.6 *Average maximum T°*: 38.6 *Absolute minimum T°*: 4.3 *Average minimum T°*: 6.2	–

Table 2. Description of sampled habitats in two ecoregions of Argentina: Paranaense forest and dry Chaco.

was passed through the chamber at 150 ml min^{-1} using a gas pump Q-P103 (Qubit Systems Inc. Kingston, ON, Canada). We controlled the flow rate by a gas pressure blow-off valve (Qubit Systems Inc. Kingston, ON, Canada) connected to a G-265 gas controller and monitor (Qubit Systems Inc. Kingston, ON, Canada). Before entering the chamber, we controlled the relative humidity, dew point, and water vapour using an RH-300 system (Sable Systems International, North Las Vegas, U.S.A.). We measured the carbon dioxide concentrations of air produced by beetles within the chamber with a Li7000 infrared gas analyzer (LiCor, Lincoln, NE, U.S.A.). We recorded carbon dioxide data using a UI2 interface and Expedata software (Sable Systems International, North Las Vegas, U.S.A.). Finally, we converted carbon dioxide into the carbon dioxide emission rate ($\dot{V}CO_2$, in ml h^{-1}) using Expedata software.

Body temperature and activity for TLR bioassay

We recorded synchronized to TLR assays, temperatures, and beetle movements with a FLIR ThermaCam P620 thermal infrared camera with a resolution of 640 × 480 pixels, a microbolometer focal plane array detector with a spectral range of 7.5–13 µm, and a thermal sensitivity of 0.06 °C at 30 °C. We measured body temperatures for each individual using temperature profiles (ThermaCAM TM Researcher v 2.9 software). To adjust the cuticle emissivity of dung beetle species, we measured the cuticle emissivity at different temperatures (40–80 °C) using fresh cuticles of each species[7,57].

Explanatory variables for TLR-IR bioassay

We selected thermal and metabolic variables related to heat stress due to their importance in characterizing the thermal niche. Based on previous studies[8,9], we identified a total of nine variables for the heat stress response and heat thermal limits by analyzing the obtained IR video sequences and respirometry profiles (Fig. 2): SHST (the start of heat stress temperature), HRT (the setpoint at which the temperature of living individuals began to decrease from that of the control individual), SCT (body temperature at which closure of respiratory spiracles occurs), CTmax (critical temperature maximum), ULT (upper lethal temperature), $\dot{V}$CO2-SHST (slopes of CO_2 produced by living individuals in SHST, normalized by their body weight (g)), VCO_2-HRT (slopes of CO_2 produced by living individuals in HRT, normalized by their body weight (g)), iCO_2 (total capacity of CO_2 production during heat stress response, area under the respirometry curve from the start of heat stress temperature to the upper lethal temperature), and iTR (total capacity of regulation of the excess heat, differences in the area under the response curves of the control and living individuals from the start of thermoregulation to the critical temperature maximum). These variables are explained in more detail in Verdú et al.[8]. The reason why these variables were selected is because they have been observed to be the ones that explain to a greater extent the response of the species to environmental changes[8,12].

TLR-IR statistical data analyses

We utilized partial least squares regression analysis (PLSR) as the appropriate multivariate technique to analyze numerous potential explanatory variables for species with a limited number of individuals. PLSR identifies components that are linear combinations of many correlated predictors and can maximize the explained

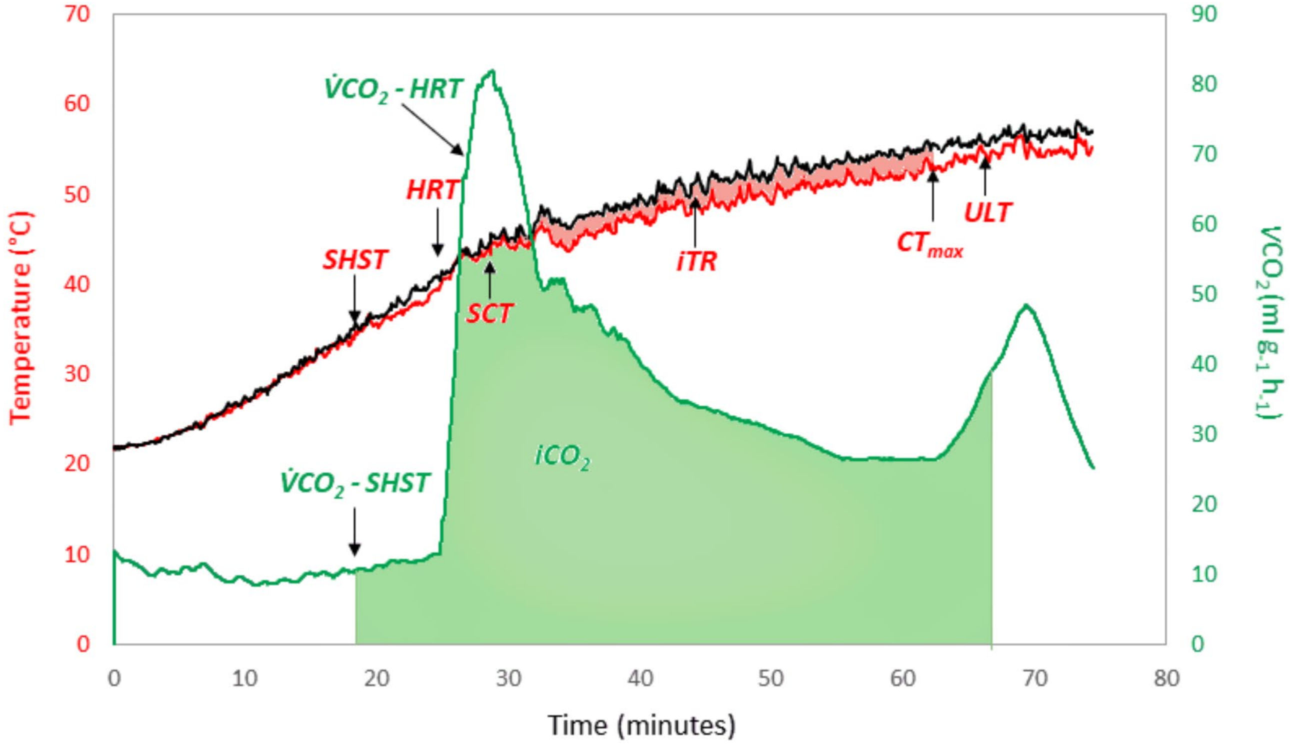

Fig. 2. Representative recordings of a thermolimit respirometry trial showing all explanatory variables measured in the bioassays. TLR of an individual of *Canthon conformis* during the heat stress response showing $\dot{V}CO_2$ (in green) and body temperature profiles (living individual is red and control is black).

variance in several related response variables. This statistical approach is particularly suitable for analyzing thermal physiological data[8,33,34,58]. Thus, we grouped physiological variables into syndromes composed of fewer new orthogonal components derived from the linear combination of predictors (ecoregion and habitat). The relationship between syndromes and response variables enables the identification of the most relevant parameters that can distinguish among the examined species. This characteristic of PLSR, along with its ability to handle a small number of sample units, increases the likelihood of detecting significant differences in the thermal responses of species even when data are limited[8,33].

Data availability

Data is provided within the supplementary information files.

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Author contributions

V.C.G.G., J.R.V., and G.A.Z. conceived the ideas and designed the methodology. V.C.G.G. and J.R.V. collected the data. J.R.V. analyzed the data. V.C.G.G. wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Declarations

Competing interests

The authors declare no competing interests.

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