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Differential ecophysiological syndromes explain the partition of the thermal niche resource in coexisting Eucraniini dung beetles

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Abstract

1. The authors investigated the role of thermoregulation mechanisms and thermal limits on thermal niche differentiation in two hierarchical similar Eucraniini dung beetle assemblages inhabiting arid environments in north-western Argentina. Both dung beetle assemblages showed temporal and thermal niche segregation between all sympatric species, minimising overlap during their daily activity and soil temperature preferences.
2. Thermal tolerance of Eucraniini species was partially modulated by behavioural adaptations and complementary thermoregulatory mechanisms related to eliminating the temperature excess, increasing the metabolic rate promoting an effective regulation of the excess heat that permitted a decrease in their body temperature by evaporative cooling. Based on the physiological syndromes obtained, the authors found a greatest interspecific differentiation that allows us to differentiate each species using ecophysiological traits. Interspecific body size differences in sympatric Eucraniini contributed greatly to the metabolic capacity of individuals to thermoregulate.
3. *Eucranium* species, the largest species showed the lowest capacity to respond to thermal stress decreasing heat excess temperature, presenting the lowest thermal limit values coinciding with a ‘minithermy’ strategy that explains the preferred lower soil temperatures around 30°C. On the contrary, *Anomiopsoides* species showed a clear adaptation to being active during periods of the day in which the soil temperature reached over 50°C, adopting a strategy of ‘maxithermy’ by foraging when temperatures are closer to their thermal limits.
4. In conclusion, it can be determined that ecophysiological syndromes related to thermal stress regulation and thermal limits allowed a displacement in thermal niche dimension, minimising interspecific competition and so allowing species coexistence in Eucraniini dung beetles.

KEYWORDS
arid environments, ecophysiological traits, ectotherms, niche overlapping, Scarabaeidae, thermoregulation

INTRODUCTION

Temperature is a main factor that determines behavioural and physiological processes in most ectotherms (Angilletta et al., 2002; Bennett, 1980; Huey, 1982). Thermal resource partitioning has doubtless contributed to the evolution of breeding and food relocation behaviours, and thermoregulation strategies seem to favour intraspecific interactions over interspecific ones when sympatric species of dung beetles are studied (Caveney et al., 1995; Kawano, 1995; Verdú et al., 2004; Ybarrondo & Heinrich, 1996). However, when more dimensions of the ecological niche are involved (not only physiology), the interspecific competition can seem favoured (Feer & Pincebourde, 2005; Tokeshi, 1999). In highly diverse assemblages, dung beetle exhibit differences in several axes of the ecological niche due to the interspecific competition (Hanski & Cambefort, 1991a; Peck & Forsyth, 1982; Verdú et al., 2004), including endothermy, thermoregulation, thermal tolerance, metabolic rate (Giménez Gómez et al., 2020; Verdú et al., 2006, 2007; Verdú et al., 2019); nesting strategies (Chao et al., 2013; Halffter & Edmonds, 1982); trophic preferences (da Silva et al., 2012; Giménez Gómez et al., 2018, 2021; Hanski & Cambefort, 1991b; Lumaret & Iborra, 1996); resource size, time of colonisation, food relocation behaviour (Hanski & Cambefort, 1991b; Noriega & Vulinec, 2021); seasonal and daily activity (Hernández, 2012; Krell-Westerwalbesloh et al., 2004; Lobo & Cuesta, 2021). The study of the interaction among species (in different ecological axes) in a community allows understanding the mechanisms of coexistence under the same environmental conditions (Sexton et al., 2017).

Specifically, from a thermal niche viewpoint, the size of dung beetles is strongly related to daily activity (Hanski & Cambefort, 1991) and the thermal biology of species (e.g., Bartholomew & Heinrich, 1978; Verdú et al., 2004, 2006, 2007). Thermal tolerance limits and the control of the thermal stress (thermoregulation), in phylogenetically close-related dung beetle species, are correlated with the thermal conditions prevailing in the environment in which the species lives, both in ectotherms (species whose body temperature changes along with that of their environment) (Gallego et al., 2016, 2018) and in heterotherms species (endothermic species found some part of the day at environment temperature changing from a regulated to an unregulated state) (Addo-Bediako et al., 2000; Gaston & Chown, 1999; Verdú et al., 2012, 2019). Thermal resource partitioning has been observed in some sympatric heterothermic dung beetles, having observed physiological specialisation on thermal resources to limit interspecific competition (Verdú et al., 2012, 2019). However, no studies have examined the function of thermoregulatory behaviour on thermal niche differentiation for assemblages of closely related ectothermic dung beetle species. In dung beetles, ectothermy is relegated to a relatively small number of flightless species inhabiting deserts, high mountains or islands (Scholtz, 2000). Among the flightless dung beetles, the Eucraniini Tribe (Coleoptera: Scarabaeidae: Scarabaeinae), endemic to the arid and semi-arid areas of Argentina, constitutes a good example of phylogenetically related ectothermic dung beetles (except *Ennearabdus lobocephalus* (Harold, 1868), the only flight endothermic species; unpublished JRV data) characterised by highly specialised ecological adaptations (Ocampo & Hawks, 2006).

In general, both heterothermic and ectothermy dung beetles (Coleoptera: Scarabaeinae) are probably one of the most studied taxa among terrestrial arthropods (Raine & Slade, 2019; Spector, 2006) because they present important ecological functions that are essential to ecosystem functioning, both within natural and anthropic land-uses (Andresen & Feer, 2005; Nichols et al., 2008; Verdú et al., 2018). They are involved in the burial of organic matter, which leads to improving soil quality through nutrient recycling, the reduction of parasite incidence, the facilitation of seed germination through secondary seed dispersal, and to the reduction of greenhouse gas emissions associated with the global warming (Andresen, 2003; Davis, 1996; Gardner et al., 2008; Gittings et al., 1994; Penttilä et al., 2013; Slade et al., 2016; Verdú et al., 2020). All these ecological functions convert them into important bioindicators of sustainable forest management (Aguilar-Amuchastegui & Henebry, 2007; Maleque et al., 2009), of environmental changes in land-use gradient (Saleh et al., 2014), of forest restoration success (Audino et al., 2014; Noriega et al., 2021), of biodiversity, habitat transformation and pest control chemicals in agro-ecosystems (Davis et al., 2004), among other.

Considering the importance of the thermal niche to explain patterns observed in the nature, and the importance of dung beetles on the ecosystem, the main objective of this study is to determine differential ecophysiological syndromes capable of explaining the partition of the thermal niche resource, as a potential mechanism allowing the coexistence of sympatric Eucraniini dung beetle species. We expect that the species of ectotherm dung beetles studied present a significant thermal niche segregation given their a priori dependence on environmental temperatures and the difference in size observed between sympatric species. It is expected that the largest species present an activity concentrated on the hours of the day with more moderate temperatures, while the smaller ones would present an activity during periods of the day with higher temperatures. For this, we analyse the role of thermoregulation and metabolic mechanisms on thermal niche differentiation in two compositional similar Eucraniini dung beetle assemblages inhabiting arid environments at La Rioja and Mendoza Provinces (Argentina). Each beetle assemblage was composed of three flightless Eucranini species differing in body size: a species of *Eucranium* Brullé of large size (25–35 mm), a species of *Anomiopsoides* Blackwelder of medium size (15–20 mm) and a species of *Anomiopsoides* of small size (10–15 mm). All the studied species are endemic to the Argentina Monte ecoregion. First, the overlap in the thermal niche between the coexisting species of each assemblage was analysed in the field. Secondly, a set of physiological variables using thermolimit respirometry (TLR) combined with infrared thermography were determined in laboratory for all the considered species.

MATERIAL AND METHODS

Study sites and dung beetle assemblages

The field study was carried out during January–February 2016 in two study sites (Lavalle and Aimogasta). The Lavalle locality (32°15′12″ S

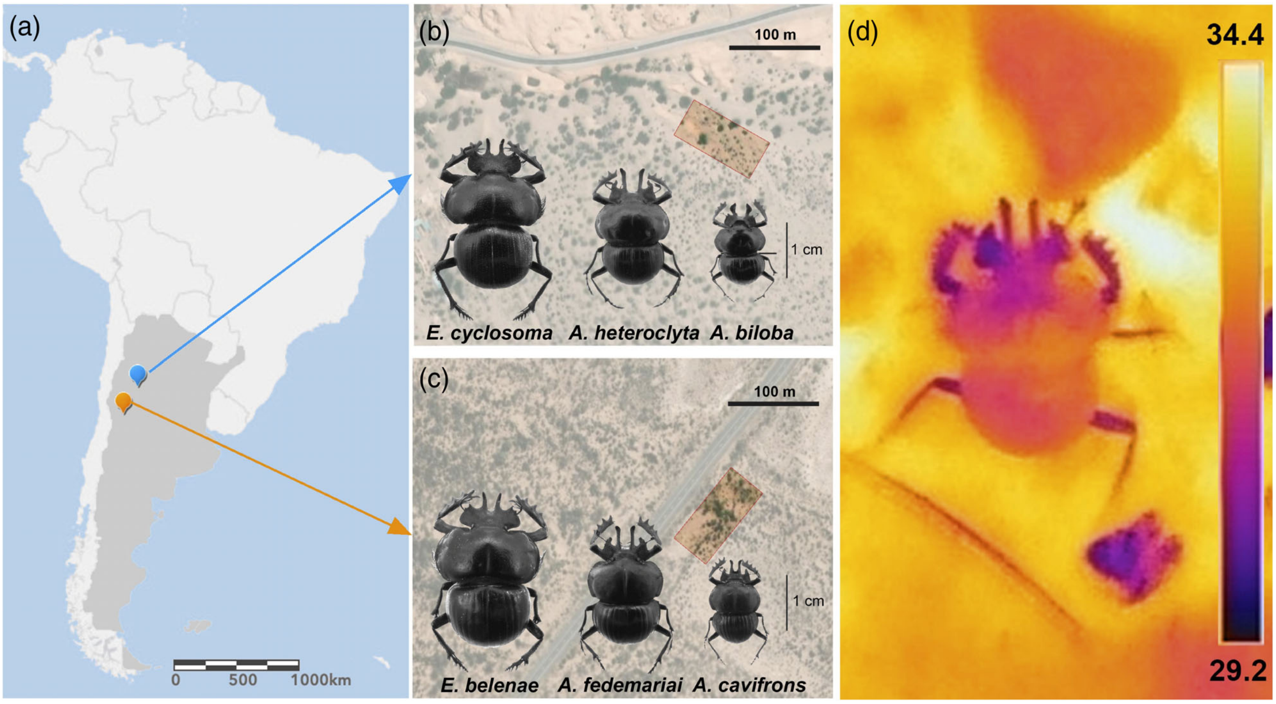

FIGURE 1 Study area and Eucraniini dung beetle assemblages studied. (a) Geographical location of the Aimogasta site (in blue) and the Lavalle site (in orange). (b) Dung beetle assemblage at the Aimogasta site composed of *Eucranium cyclosoma*, *Anomiopsoides heteroclyta* and *Anomiopsoides biloba*. (c) Dung beetle assemblage at the Lavalle site composed of *Eucranium belenae*, *Anomiopsoides fedemariai*, and *Anomiopsoides cavifrons*. For each site, the highlighted rectangular areas correspond to the selected study plots (0.58 ha for the Lavalle site; and 0.62 ha for the Aimogasta site). D) Infrared image showing an individual of *A. fedemariai* walking in search of dung.

– 67°49′13″ W; 750 masl; Mendoza Province, Argentina) has a mean annual rainfall of 210 mm, a mean maximum temperature of 38.6°C and a mean minimum temperature of 24.3°C during the study period. The Aimogasta locality (28°38′57″ S–66°36′4″ W; 830 masl; La Rioja Province, Argentina) has a mean annual rainfall of 190 mm, and mean maximum and minimum temperatures of 35.5 and 20.6°C, respectively. In both sites, the rainfall regime is monsoonal, with concentrated rains during the summer (between December and March). The two sites are separated by 460 km (Figure 1a). Both study areas are characterised by an arid climate and a xerothermic shrubby steppe with characteristic Monte Desert flora dominated by species of Zygophyllaceae, Fabaceae and Cactaceae (Abraham et al., 2009). At the Lavalle site, three species of dung beetles of the tribe Eucraniini were observed coexisting in the sand dunes: *Eucranium belenae* Ocampo, 2010, *Anomiopsoides fedemariai* Ocampo, 2007, and *A. cavifrons* (Burmeister, 1861). At the Aimogasta site, the three coexisting species were *E. cyclosoma* Burmeister, 1861, *Anomiopsoides heteroclyta* (Blanchard, 1845) and *A. biloba* (Burmeister, 1861) (Figure 1b,c). All Eucraniini species observed in this study feed on dry dung; however, in some cases, *E. belenae* feed *Prosopis* (Fabaceae; common name: Algarrobo) fruits and *A. biloba* and *A. cavifrons* feed small pieces of dry plant material (Gillet & Gillet, 2017; Giménez Gómez et al., 2017; Ocampo, 2005, 2007, 2010). Thus, in both sites, the coexisting Eucraniini species a priori share and compete for similar food sources.

Daily activity and temperature measurements

To estimate daily activity, we defined a plot of almost 0.6 ha in each site (0.58 ha for the Lavalle site; and 0.62 ha for the Aimogasta site). In each plot, three linear transects of 20 m (one per researcher) were carried out repeatedly during the entire activity period in search of the species of Eucraniini in each locality. Body and soil temperatures were obtained in the morning from before dawn (ca 06:00 h) to after dusk (ca 23:00 h). At the beginning of the sampling, we first verified that no species was active. From the beginning of the activity to the end of the activity, temperature measurements were carried out on all the specimens observed in the plot. The inactivity of the beetles was determined after 20 minutes passed without observing any specimen since the last observation. In each sample, an IR photograph was recorded (Figure 1d): the time and behaviour of the specimen were noted (walking: individual walking without food; foraging: individual walking carrying food). Body (T_b) and soil (T_s) temperatures were recorded with a FLIR ThermaCam P620 thermal infrared camera with a resolution of 640 × 480 pixels and a microbolometer focal plane array detector with a spectral range of 7.5–13 μm and thermal sensitivity of 0.06°C at 30°C. Each sampled specimen was then numbered in the pronotum using an acrylic paint pencil (0.5 fine tip) and kept acclimated (20°C) in a plastic container. At the same time, additional data on ambient temperature were obtained using Fluke1 52 (California) K/J thermometers (to the nearest 0.1°C). After each working day, the numbered specimens were weighed using a precision balance, sexed, and it was indicated if they were mature or immature individuals. This last was performed through the external age-grading method based on the hardness of the cuticle and the cuticular deterioration of the tibiae and clypeus (Tyndale-Biscoe, 1984; Verdú et al., 2010). After individuals were weighed, sexed, the acrylic paint pencil mark was removed, and, except for the specimens that would later be used for bioassays, the rest of the individuals were released, without any symptoms of stress or effect of marking, in the same collection site. The process was repeated 2 days at the Lavalle site and 3 days at the Aimogasta site. To represent the diel activity frequency of each species in function of time of activity and T_s variables, a kernel density analysis adjusted to the Gaussian function was performed for each species using PAST software (Hammer et al., 2001).

Temporal and thermal niche partitioning

The Czekanowski index of niche overlap between species (Feinsinger et al., 1981) was calculated to quantify patterns of niche overlap between species. For the analyses of thermal niche overlap in the field, the resource states represent 10 different classes of T_s, ranging from 10 to 60°C. Thus, the index approaches zero for species that never coincide in activity at the same time of day or T_s, being one for pairs of species that are active in an identical time or T_s range. Afterwards, a cluster analysis was carried out using the Czekanowski index matrix as a measure of resemblance and the Ward method as linkage rule. To test the statistical significance of niche overlap, null model analyses were used by calculating the algorithm RA3, which randomly relocates observed data retaining niche breadth, and reshuffling the zero states of the matrix (Winemiller & Pianka, 1990). Ecosim 7.0 software (Acquired Intelligence Inc., and Kesey-Bear, Burlington, VT, U.S.A.) (Gotelli & Entsminger, 2003) was used for all these null model analyses.

Beetle maintenance and preparation for TLR bioassay

In both sites, 10–12 individuals of each species (with the exception of *E. cyclosoma* that only nine individuals were observed in total) were collected and transported from the collection site to the laboratory into plastic containers including moist paper (30 × 20 × 10 cm) and using a portable cooler provided with temperature control (20–25°C). In the laboratory, the individuals were fed with the resources they consume in their natural environment (dry dung and dry plant material, depending on the species). Prior to each experimental trial, individuals were acclimated for 72 h at 25°C and a photoperiod of LD 13:11 h without food supply to eliminate possible mistakes caused by the difference in the feeding states of individuals. Acclimation was performed using a refrigerated incubator (MIR-153, Sanyo Electric Co. Osaka, Japan). In each experimental trial, the ambient temperature of the laboratory (25°C) was controlled using a Fluke 152 K/J thermometer (Fluke Co. California, U.S.A.). To avoid bias due to possible stress due to maintenance in the laboratory, all specimens were analysed during the first 2 weeks after their collection in the field. For the same reason, physiological measurements were made by alternating individuals of each species. This work conforms to Argentina's legal requirements, including those relating to conservation and welfare. Additionally, beetle collection in the field and physiological analyses were made with relevant permissions (Flora and Fauna Certificate n° 002408; Legal certificate possession, collection and transport of the biological material n° 335/11).

TLR measurement

To determine physiological specialisations to thermal stress, carbon dioxide and water vapour production of individual beetles were measured using flow-through respirometry following previously described methods (Lighton & Turner, 2004; MacMillan et al., 2012; Verdú et al., 2019). Individuals of *E. belenae* ($n = 5$), *E. cyclosoma* ($n = 6$), *A. fedemariai* ($n = 9$), *A. cavifrons* ($n = 11$), *A. heteroclyta* ($n = 9$) and *A. biloba* ($n = 6$) were selected and placed individually into a flow-through respirometer measurement chamber made of methacrylate (15 × 5 × 5 cm). The chamber was placed above a precision digital hot plate (J. P. Selecta, Barcelona, Spain), and a temperature increase rate of 0.32°C min^{-1}, from 25 to 60°C, was applied inside the chamber. Dry, CO_2-free air was passed through the chamber at 150 ml min^{-1} using a gas pump Q-P103 (Qubit Systems Inc. Kingston, ON, Canada), and the flow rate was controlled 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). Prior to the entrance to the chamber, the humidity relative, dew point and water vapour were controlled using an RH-300 system (Sable Systems International, North Las Vegas, U.S.A.). The carbon dioxide and water vapour concentrations of air produced by beetles within the animal chamber were detected and measured with a Li7000 infrared gas analyser (LiCor, Lincoln, NE, U.S.A.). Carbon dioxide and water vapour data were recorded using a UI2 interface and Expedata software (Sable Systems International, North Las Vegas, U.S.A.). Carbon dioxide and water vapour data were converted into the rate of carbon dioxide emission (VCO_2, in ml h^{-1}) and the rate of water vapour loss (VH_2O, in ml h^{-1}) using Expedata software.

Body temperature and activity for TLR bioassay

Synchronised to TLR assays, temperatures and beetle movements were recorded with a FLIR ThermaCam P620 thermal infrared camera. Infrared sequences (IR) were recorded digitally at 10 frames s^{-1}. Body temperatures for each individual were measured using temperature profiles (ThermaCAM TM Researcher v 2.9 software). To adjust the cuticle emissivity of Eucraniini species, we measured the cuticle emissivity at different temperatures (40–70°C) using fresh cuticles of species, obtaining a similar value of 0.98. For the measurements, we use electrical tape (a reference method as described in ISO 18434-1) and black paint (NEXTEL-Velvet-Coating 811-21) as references.

TABLE 1 Description of explanatory variables derived from: (a) the heat response assay (t1, t2, t3, t4 and t5 correspond to the moments in which start of heat stress (*SHST*), heat regulation (*HRT*), closure of respiratory spiracles, critical thermal maximum (CT_{max}) and upper lethal (*ULT*) temperatures were obtained, respectively)

Acronym (units)	Expression
Body temperature	
rSHST (°C min^{-1})	$rSHST = \frac{HRT-SHST}{t2-t1}$
rHRT (°C min^{-1})	$rHRT = \frac{CST-HRT}{t3-t2}$
rTR (°C min^{-1})	$rTR = \frac{CT_{max}-HRT}{t4-t3}$
iTR (°C)	$iTR = \int_{t2}^{t4} T_c - \int_{t2}^{t4} T_b$
Carbon dioxide production	
$VCO_{2\ SSHT}$ (ml h^{-1})	$V\dot{CO}_{2\,SSHT} = \frac{V\dot{CO}_{2\,HRT} - V\dot{CO}_{2\,SHST}}{t2-t1}$
$VCO_{2\ HRT}$ (ml h^{-1})	$V\dot{CO}_{2\,HRT} = \frac{V\dot{CO}_{2\,CST} - V\dot{CO}_{2\,HRT}}{t3-t2}$
iCO_2 (ml)	$iCO_2 = \int_{t1}^{t5} V\dot{CO}_2$
Water vapour production	
$VH_2O_{\ SSHT}$ (ml^{-1} h^{-1})	$VH_2O_{SSHT} = \frac{V\dot{H}_2O_{HRT} - V\dot{H}_2O_{SHST}}{t2-t1}$
$VH_2O_{\ HRT}$ (ml^{-1} h^{-1})	$V\dot{H}_2O_{HRT} = \frac{V\dot{H}_2O_{CST} - V\dot{H}_2O_{HRT}}{t3-t2}$
iH_2O (ml)	$iH_2O = \int_{t1}^{t5} V\dot{H}_2O$

In each experiment, a living beetle was placed in the centre of the chamber on a strip of polyurethane to avoid contact between beetles, using a pin stuck to the prothorax disc with an innocuous and thermally resistant adhesive. A dead or 'control' individual of each species was used in all experiments to discriminate the active physiological thermal response of living individuals. This 'control' individual also allows us to obtain an unbiased measurement of the ambient temperature by means of the thermal infrared camera since both individuals (the living and the dead) have the same thermal emissivity. 'Control' individuals were previously killed by freezing and posteriorly dehydrated for 72 h at 80°C. The 'control' beetle was placed 3 cm apart from the living individual inside the chamber. The front of the respirometer chamber was covered hermetically with a thin polypropylene film (80 μm) transparent to infrared (IR) radiation but impermeable to carbon dioxide. Correction of temperature by partial polypropylene absorption was made using a reference plate coated with black paint (NEXTEL-Velvet-Coating 811–21), partially covered with the polypropylene film and by measuring the IR emission at different temperatures (20–60°C).

Explanatory variables for TLR-IR bioassay

Variables related to heat stress and upper thermal limits have been selected due to their importance in the characterization of the thermal niche. Based on previous studies (Gallego et al., 2016; Verdú et al., 2019), three temperature ranges and a total of 15 variables were identified for the heat stress response by analysing the obtained IR video sequences and respirometry profiles (see Table 1 and Figure 2). The supraoptimal activity temperature range (SAR) comprises a range of temperatures in which the individual leaves his comfort temperature (optimal temperature range) and begins to increase his metabolic rate through breathing due to an increase in his mobility due to the thermal stress that in behaviour translates into the search for a place with lower temperatures (search for shade, burial, etc. under field conditions). During this period, the individual can potentially regulate their body temperature depending on the thermal stress they suffer. SAR can be divided into two phases differentiated by the VCO_2: (i) the start of heat stress temperature (*SHST*); and (ii) the heat regulation temperature (*HRT*, the setpoint at which the temperature of living individuals began to decrease from that of the control individual). In the SAR range, we defined eight explanatory variables (Table 1, Figure 1). During the first phase of SAR range, the *rSHST* was defined as the slope of the temperature increase (°C min^{-1}) of living individuals (from *SHST* to *HRT*). The *rHRT* was defined as the slope of the temperature increase (°C min^{-1}) of living individuals during the second phase of the SAR range (from *HRT* to *CST*, defined as the body temperature at which closure of respiratory spiracles occurs). During the start of heat stress phase, the $VCO_{2\ SHST}$ and $VH_2O_{\ SHST}$ were defined as the slopes of carbon dioxide (ml h^{-1}) and water vapour (ml^{-1} h^{-1}) produced by living individuals. In the following phase of the start of thermoregulation, we defined both $VCO_{2\ HRT}$ and $VH_2O_{\ HRT}$ calculated as the slopes of carbon dioxide (ml h^{-1}) and water vapour (ml h^{-1}) produced by individuals. To measure the total capacity of CO_2 and H_2O emission during the heat stress response, we defined both iCO_2 and iH_2O as the area under VCO_2 and VH_2O curves, respectively, from the start of the SAR phase to the upper lethal temperature (*ULT*, described below). All metabolic explanatory variables were normalised by the body weight of each individual.

During the critical temperature range, the individual must lose excess body heat to avoid heat shock and death; When it loses its thermoregulation capacity and heat shock occurs, its metabolism paralyses, entering a critical phase that causes death. In this phase we defined three variables (Table 1, Figure 2). The *rTR* corresponds to the cooling rate (i.e., the slope of the body temperature per unit of time) in which living individuals decrease their temperature if metabolic cooling by respiration is effective. To measure the total capacity of regulation of the excess heat (*iTR*), we calculated the differences in the area under the response curves (according to the differences between integrals) of the control and living individuals from the start of thermoregulation to the critical temperature maximum (CT_{max}) (Figure 2). The CT_{max} was defined via observation of activity (i.e., the stop of voluntary movements of legs) (Hazell et al., 2008; Hazell & Bale, 2011; Klok & Chown, 1997; Lighton & Turner, 2004; Lutterschmidt & Hutchison, 1997; Verdú et al., 2019), and via TLR (i.e., cease of tracheal gas exchange visualising by CO_2 release) (Lighton & Turner, 2004). Finally, in the lethal temperature range, we measured the *ULT*, which is defined as the temperature at which the individual becomes completely paralysed and dies across 50–52°C. The post-mortem peak of carbon dioxide derived from aerobic metabolism of mitochondria (E. C. Heinrich et al., 2017) was not included as an explanatory variable. For all individuals, the difference in body

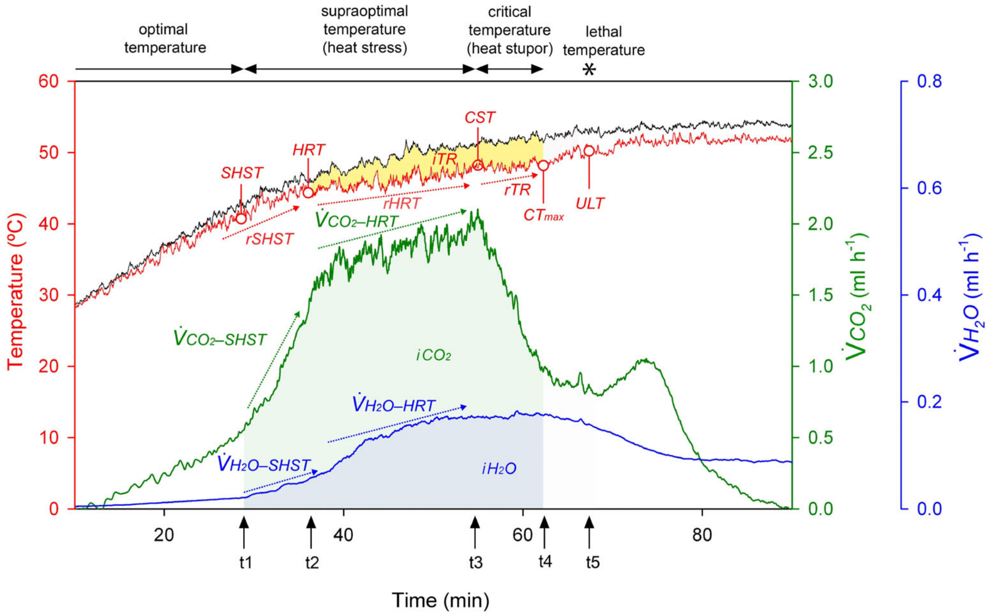

FIGURE 2 Representative recordings of a thermolimit respirometry trial showing all explanatory variables measured in the bioassays. TLR of an individual of *Anomiopsoides heteroclyta* (Ah8, male, 0.51 g body weight) during the heat stress response showing VCO_2 (in green), VH_2O (in blue) and body temperature profiles (living individual is red and control is grey). The upper border describes (with arrows) the different temperature ranges described in each case. The arrows (t1–t5) in the x-axis correspond to the moments used in the expressions described in Table 1.

weight between the beginning and the end of the experiment was measured using a precision balance with a measurement resolution of the weighing of 0.0001 g (AG104 Analytic Balance; Mettler Toledo, Columbus, OH, U.S.A.).

TLR-IR statistical data analyses

We selected partial least regression analysis (PLSR) as the appropriate multivariate technique to analyse the large number of potential explanatory variables, together with their use for those species with a small number of individuals available. PLSR finds latent components that are linear combinations of many multicollinearity predictors and can maximise the explained variance in several correlated response variables as a statistical technique highly suitable when thermal physiological data are used (Carrascal et al., 2009; Gallego et al., 2016, 2017; Verdú et al., 2019). Thus, considered physiological response variables are grouped into syndromes composed of a reduced number of new orthogonal components derived from the linear combination of predictors (in this case, the species identity and fresh body weight). The link between syndromes and response variables allows for the identification of the most relevant parameters that are capable of discriminating between the examined species. This property of PLSR, as well as its ability to work with a small number of sample units, maximises the likelihood of obtaining significant differences in the thermal responses of species when the available data are scarce (Gallego et al., 2016; Verdú et al., 2019).

To analyse the statistical differences in the physiological variation between species, a weight proportional to their explanatory capacity (eigenvalues obtained in PLSR analysis) was given to each PLSR component in order to generate a triangular similarity matrix by means of the Euclidean distance able to better reflect the physiological similarity among all the considered species. Between species, differences were analysed using a Permutational multivariate analysis of variance (PERMANOVA) test on these new orthogonal components. Post-hoc pairwise comparisons among sites were obtained by calculating a pseudo-F statistic for each site and *p* values were estimated by using a permutation procedure (9999 iterations in this study) followed by a Bonferroni correction to the *p* values. Finally, non-metric multidimensional scaling was used to provide a bi-dimensional visual representation of the physiological metabolic similarity of the species according to their formerly mentioned syndromic components. Data were analysed using StatSoft's Statistica 12 (StatSoft Inc., Tulsa, Oklahoma, U.S.A.) and PRIMER v.6 software (Anderson et al., 2008).

RESULTS

Temporal and thermal niche partitioning

We observed a total of 308 Eucraniini dung beetles at both sites. In the Lavalle site (Figure 3a,b), *E. belenae* ($n = 20$) was active mainly during morning and evening, when the soil temperatures were around 28.3 ± 6.0°C (mean ± 95% CI); during its activity, the recorded body temperatures were about 27.6 ± 5.3°C. *A. cavifrons* ($n = 129$) was active after midday when the soil temperatures were around 34.0 ± 7.0°C, and showed body temperatures over 32.6 ± 5.5°C. The

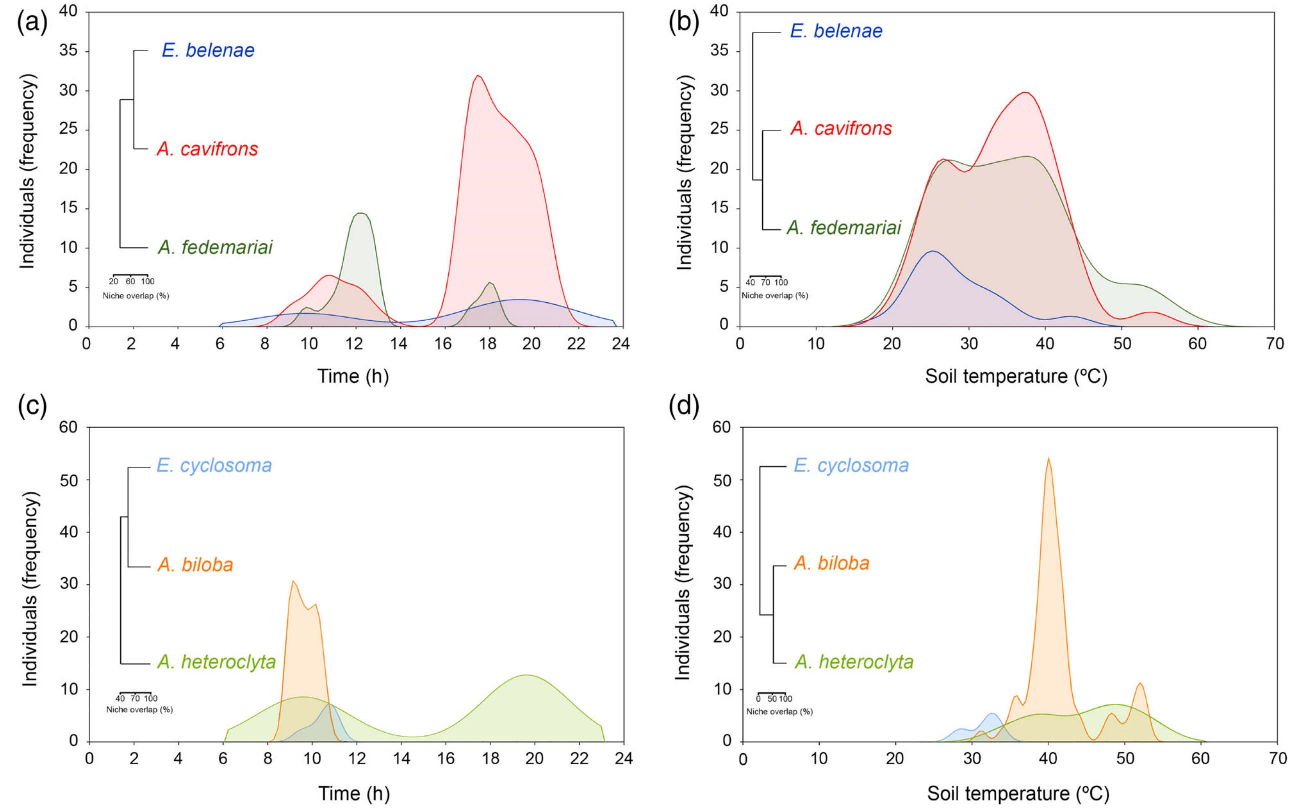

FIGURE 3 Temporal (daily time) and thermal (soil temperature) niche partitioning of Eucraniini species at Lavalle site (a and b) and at the Aimogasta site (c and d). Dendrograms show niche overlap based on Czekanowski index (in percentage) between species in both temporal and thermal axis.

activity of *A. fedemariai* ($n = 32$) was well-defined just before midday when the soil temperature was over 42.2 ± 8.2°C, and the recorded body temperatures was around 37.8 ± 5.1°C. In the Aimogasta site (Figure 3c,d), *E. cyclosoma* ($n = 8$) was observed during morning, when the soil temperatures were around 31.4 ± 0.8°C, and the body temperature was about 32.2 ± 2.0°C. *Anomiopsoides biloba* ($n = 34$) was active from morning until around midday, when the soil temperatures reached 44.9 ± 6.4°C, and the body temperature of the beetles was 42.6 ± 3.6°C. The activity of *A. heteroclyta* ($n = 70$) was well-defined just before the midday when the soil temperature was over 41.5 ± 4.6°C, and the body temperatures achieved 40.3 ± 2.7°C.

Significant niche differentiation appears between all pairs of sympatric species when diel activity was used as the focal niche axis. However, a partial but non-significant temporal overlap was observed between *E. belenae* and *A. cavifrons* ($O = 0.68$; $p = 0.09$), at Lavalle site (Figure 3a,b); and between *E. cyclosoma* and *A. biloba* ($O = 0.55$; $p = 0.148$), at Aimogasta site (Figure 3c,d). Considering the soil temperature niche axis, a clear thermal niche differentiation was observed between sympatric species. As in the case of the temporal axis, non-significant cases of thermal niche overlap were detected only between *A. fedemariai* and *A. cavifrons* ($O = 0.63$; $p = 0.07$) in the Lavalle site (Figure 3a,b); and between *A. heteroclyta* and *A. biloba* ($O = 0.54$; $p = 0.06$) in the Aimogasta site (Figure 3c,d).

Physiological response to heat stress

Three significant PLSR components were obtained in response to heat stress (see Table 2, for statistical details). The first component was strongly explained by the loss of body weight during the bioassays (Table 2 and Figure 4a). Furthermore, the variables associated with the thermal limits (CT_{max} and *ULT*), the phase of higher heat stress (*HRT*, *rHRT*, *iTR* and iH_2O), and the slope of the temperature increase during the start of heat stress (*rSHST*) were also statistically significant in explaining the variability of this component. This component was positively associated with the loss of body weight as well as with a higher production of water vapour during the phase of heat regulation, the ULT and critical temperature maximum (CT_{max}) experienced by the individuals. The variability in this component is mainly explained by body weight and the species identity factor (see Table 2). Furthermore, this component showed that the smallest species, *A. cavifrons* and *A. biloba*, were species correlated with high heat regulation temperatures (*iTR*, *rHRT*, *HRT*) and higher thermal limits (CT_{max} and *ULT*), which was associated with increased body weight loss and water vapour emission (iH_2O). On the contrary, the bigger species, such as *E. belenae* and *E. cyclosoma*, showed a negative significant correlation with the loss of body weight and the variables associated with the high heat stress regulation and thermal limits (*iTR*, *rHRT*, *HRT*, iH_2O, CT_{max} and *ULT*) (Table 2 and Figure 4a).

TABLE 2 Results of the PLSR analysis summarising the heating response trials.

	PLS1	R^2 (%)	PLS2	R^2 (%)	PLS3	R^2 (%)
Response variables						
Loss of body weight	0.37*	45.57	−0.25	13.23	−0.05	0.33
SHST	0.17	9.56	0.31*	20.37	−0.17	4.71
rSHST	0.33*	36.65	−0.21	9.55	0.10	1.69
HRT	0.33*	36.65	0.07	1.11	−0.14	3.12
rHRT	0.30*	30.57	−0.26*	13.82	0.23	8.02
rTR	0.22	15.95	−0.23	11.13	−0.27*	11.69
iTR	0.35*	42.18	−0.17	5.99	0.11	1.91
CT_{max}	0.27*	24.63	−0.11	2.33	−0.03	0.16
ULT	0.28*	25.86	0.05	0.63	0.17	4.50
$VCO_{2\ \mathrm{SHST}}$	0.20	13.25	0.07	1.02	0.29*	12.96
$VCO_{2\ \mathrm{HRT}}$	0.12	5.25	0.48*	48.29	−0.36*	19.80
iCO_2	0.15	7.62	0.59*	73.12	0.08	1.08
$VH_2O_{\ \mathrm{HRT}}$	0.19	11.92	−0.11	2.46	−0.54*	45.45
iH_2O	0.28*	25.82	0.17	6.44	0.49*	37.54
R^2 (%)	39.85		9.67		4.09	
Predictors						
Body weight	−0.67*		−0.10		−0.01	
Species						
Anomiopsoides biloba	0.28*		−0.24*		0.73*	
Anomiopsoides cavifrons	0.41*		−0.37*		−0.60*	
Anomiopsoides fedemariai	−0.06		0.60*		−0.23	
Anomiopsoides heteroclyta	0.00		0.50*		0.24*	
Eucranium belenae	−0.44*		−0.30*		−0.02	
Eucranium cyclosoma	−0.33*		−0.31*		0.04	
Eigenvalues	2.08		1.23		1.21	

Note: The variables whose square weights are larger than 1/number of variables (15 response and 2 predictor variables; i.e., magnitude effect larger than that expected by chance), and therefore are statistically significant, are indicated with asterisks. Response variables as defined in Table 1 and Figure 4.

The second component was strongly explained by the metabolic variables associated with the higher heat stress phase ($VCO_{2\ \mathrm{HRT}}$ and iCO_2), the temperature excess regulation capacity (*rHRT*), and the temperature at the start of the heat stress phase (*SHST*) and (Table 2 and Figure 4b). Thus, the start of heat stress and the thermoregulation capacity of individuals during the higher thermal stress phase were positively associated with a higher production of carbon dioxide. The variability in this component is only explained by the species identity factor according to the weights of the predictors (see Table 2). This syndrome showed that the medium-sized species, *A. fedemariai* and *A. heteroclyta*, showed a strong positive correlation with high volume of carbon dioxide exhaled (iCO_2) and thermoregulation capacity, while the larger and smaller species showed a lower temperature excess regulation capacity (high *rHRT*) (Table 2 and Figure 4b).

The third component was explained by variables related to heat regulation phases (*rTR*, iH_2O, $VH_2O_{\ \mathrm{HRT}}$, and $VCO_{2\ \mathrm{HRT}}$) and by variables related to the start of heat stress phase ($VCO_{2\ \mathrm{SHST}}$) (Table 2 and Figure 4c). The variability in this component strongly influenced by thermoregulation variables is also explained by the species identity factor according to the weights of the predictors (see Table 2). This component of PLSR showed that *A. biloba* and *A. heteroclyta* were the most associated with the variables related to the regulation of body temperature (*rSHST* and *rTR*). Furthermore, *A. cavifrons* showed a highest correlation with the rate of water vapour and carbon dioxide production ($VH_2O_{\ \mathrm{HRT}}$, $VCO_{2\ \mathrm{HRT}}$) and the thermoregulation rate (*rTR*) during the heat regulation phase (Table 2 and Figure 4c).

Interspecific ecophysiological variation

We observed notable differences in the physiological metabolic traits related to heat thermoregulation and heat stress tolerance between the studied Eucraniini species (*pseudo-F* $= 47.01$, permutation $N = 9999$, $p = 0.0001$; Figure 5). At the Lavalle site, all sympatric species showed statistically significant physiological metabolic differences (*E. belenae* vs. *A. fedemariai*: *Bonferroni corrected* $p < 0.001$; *E. belenae* vs. *A. cavifrons*: *Bonferroni corrected* $p < 0.001$; *A. fedemariai* vs. *A. cavifrons*: *Bonferroni corrected* $p < 0.001$). At the Aimogasta site, similar significant physiological metabolic differences were obtained among all the species of this assemblage (*E. cyclosoma* vs. *A. heteroclyta*: *Bonferroni corrected p* < 0.001; *E. cyclosoma* vs. *A. biloba*: *Bonferroni corrected p* < 0.01; *A. heteroclyta* vs. *A. biloba*: *Bonferroni corrected p* < 0.001). The only pair of species that did not show significant differences in physiological metabolic traits is the

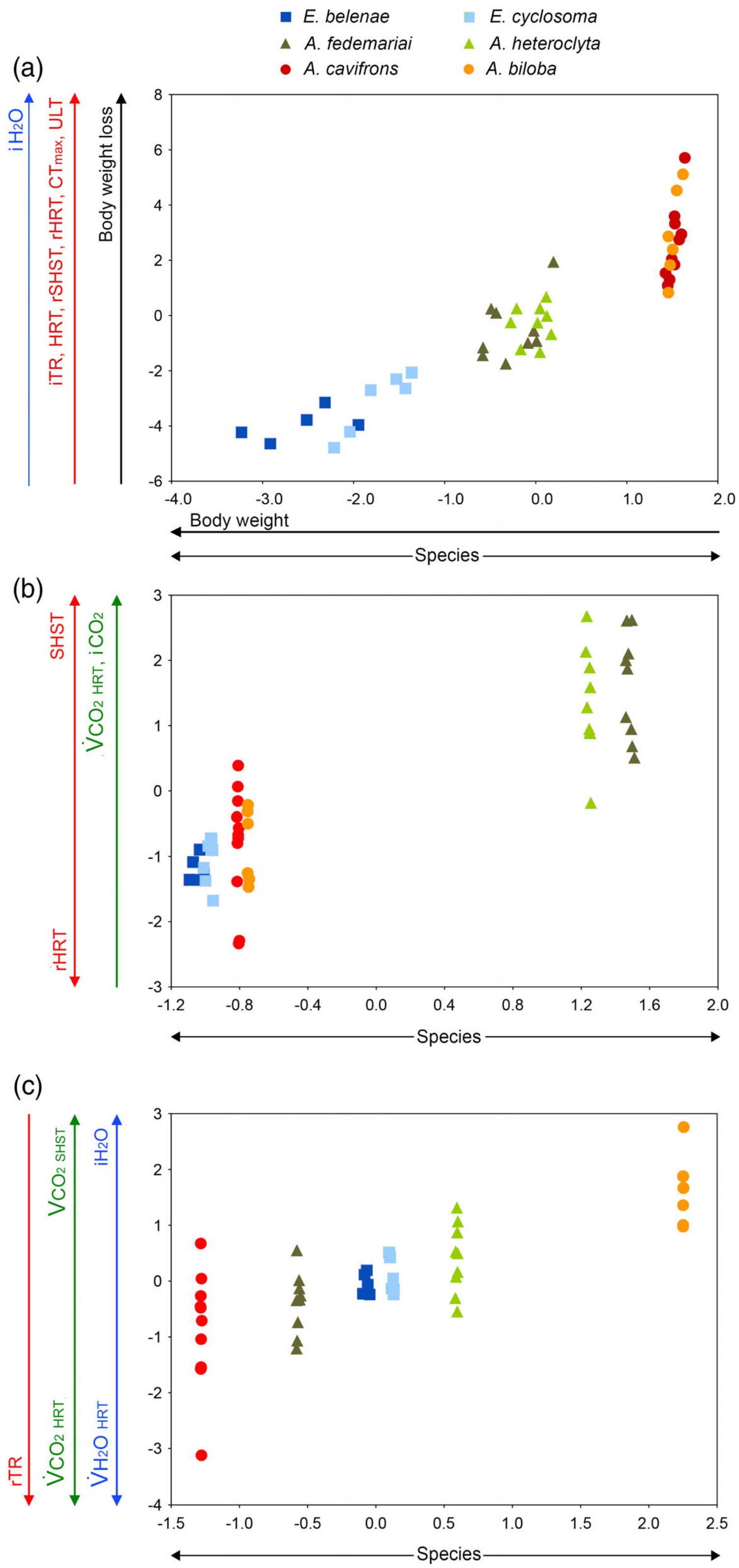

FIGURE 4 Legend on next page.

one composed of the two *Eucranium* species (*Bonferroni corrected* $p = 0.07$).

DISCUSSION

Thermal niche segregation in Eucraniini assemblages

Both Eucraniini assemblages showed similar thermal niche segregation patterns displaying different temperature preferences during the course of the daily activity (Figure 3). The two *Eucranium* species preferred lower soil temperatures around 30°C, whereas *Anomiopsoides* species generally selected higher temperatures around 40°C. Thus, *Eucranium* species are active mainly during morning and evening, adopting a strategy of 'minithermy' by foraging when body temperatures are much lower than their thermal limits (CT_{max}, *ULT*; see Figure 4a and Table S1), while *Anomiopsoides* species preferred to be active before midday or in the afternoon, adopting a strategy of 'maxithermy' by foraging when body temperatures are closer to their thermal limits (CT_{max}, *ULT*; see Figure 4a and Table S1). This strategy of 'maxithermy' (Angilletta et al., 2010; Hamilton, 1971, 1973) was clearly adopted by *Anomiopsoides* species, and as has been suggested for other poikilotherm insect species that inhabit arid zones, it could constitute a strategy modulated by the metabolic rate and with significant advantages in their fitness (Henwood, 1975). Both assemblages showed thermal niche segregation between all sympatric species minimising overlap during their daily activity and soil temperature preferences. In the few cases in which partial time of day overlap was observed, soil and body temperatures between species were very different, suggesting possible differences in microclimatic preferences during their activity. Results on daily activity segregation agree with observations described by Giménez Gómez et al. (2017) and Gillet and Gillet (2017) for the Eucraniini assemblage composed of *E. belenae*, *A. cavifrons* and *A. fedemariai*. Although our results are consistent with what is observed in nature, we do not exclude that there are other variables that are also involved in the reduction of niche overlap among the species studied, for example, food preference and availability, seasonality, and possible anti-predatory strategies (Giménez Gómez et al., 2018; Hanski & Cambefort, 1991; Paine et al., 1981).

Our results showed that thermal tolerance of flightless Eucraniini species was partially modulated by behavioural adaptations to exploit in the daily range of temperature different microhabitats existing in the same local area. However, the differences between T_s and T_b obtained during diel activity suggest the existence of complementary thermoregulatory mechanisms related to eliminate the temperature excess. This behaviour is common in homeothermic dung beetles where different mechanisms exist to eliminate excess heat produced during flight (Bartholomew & Heinrich, 1978; Caveney et al., 1995; Chown et al., 1995; Gallego et al., 2018; Verdú et al., 2004, 2006, 2012; Ybarrondo & Heinrich, 1996). However, in ectothermic dung beetles, as the studied Eucraniini species, there are few observations, especially since ectotherm in medium and large dung beetles is relegated to a relatively small number of flightless species inhabiting deserts, high mountains or islands (Scholtz, 2000). From the studies carried out on flightless dung beetles, we can highlight that despite not requiring mechanisms for the elimination of excess heat during flight, the studied species have high respiration rates that may be associated with eventual thermoregulation at elevated environmental temperatures (Duncan & Byrne, 2005; Gallego et al., 2018). In order to clarify the possible adaptive mechanisms of thermoregulation that help to explain with greater accuracy the niche segregation in Eucraniini, it is necessary to explore these mechanisms from a physiological point of view.

Ecophysiological adaptations

When Eucraniini dung beetles were exposed to supraoptimal temperatures (SAR range), the increase of the metabolic rate measured from carbon dioxide production promoted an effective regulation of the excess heat that permitted a decrease in their body temperature by evaporative cooling. Within the studied ectothermic Eucraniini, there are significant divergences in temporal and environmental preferences, implying that a general ecophysiological pattern to manage heat stress is not plausible. Although based on the ecophysiological syndromes obtained, we could differentiate three groups corresponding to each pair of species with relatively similar size (Figure 4). We also found a greatest interspecific differentiation that allows us to differentiate each species ecophysiologically (Figure 5).

FIGURE 4 Relationship between the PLSR components for the response variables and predictors obtained in heat stress response of Eucraniini species. a) First ecophysiological syndrome obtained, which is positively associated with body weight loss, the total H_2O emission (iH_2O) and with variables of thermal stress regulation (*HRT*, *rHRT*, *iTR*) and thermal limits (CT_{max}, *ULT*). Body weight and species identity were the predictors that significantly influenced this component derived from the response variables. b) the second thermoregulatory syndrome obtained was positively associated with the higher heat stress phase ($VCO_{2\ HRT}$ and iCO_2), the start of heat stress temperature (*SHST*) and with the temperature excess regulation capacity (*rHRT*; high values indicate low thermoregulation capacity). Species identity was the only predictor that significantly influenced this component derived from the response variables. c) the third thermoregulatory syndrome obtained was negatively associated with the rates of CO_2 and H_2O production during the thermoregulation phase of the supraoptimal temperature range ($VCO_{2\ HRT}$, $VH_2O_{\ HRT}$) and the total capacity of regulation of the excess heat (*iTR*), and positively associated to the rate of CO_2 emission during the phase of start of thermal stress ($VCO_{2\ SHST}$) and the total water vapour emission (iH_2O). Species identity was the only predictor that significantly influenced this component derived from the response variables. Arrows represent the sign of the relationships. Only statistically significant response variables were represented.

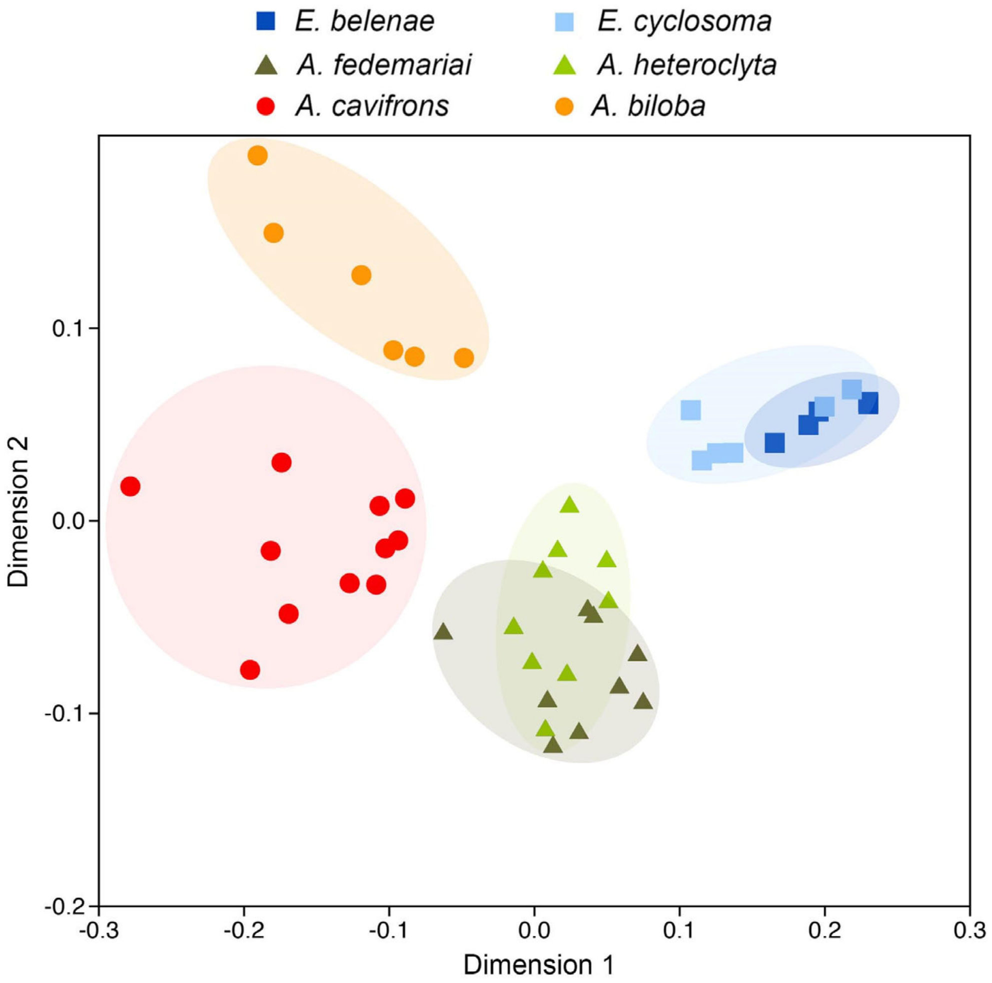

FIGURE 5 Multidimensional scaling (MDS) bi-dimensional representation of the matrix of ecophysiological similarity according to the three syndromes obtained. Only the overlapping ellipses did not show significant differences in physiological metabolic traits (see text for PERMANOVA statistical results)

This wide range of ecophysiological niches may be one reason why Eucraniini tribe is so relatively diverse in arid environments of Argentina, explaining the low niche overlap observed, and so the high sympatry observed in this group (Figure 3). Surprisingly, both assemblages have turned out to be similar from an ecological but also a physiological point of view, reinforcing the idea of an ecophysiological diversity that determines the configuration of these assemblages with a similar hierarchical structure.

Our study revealed that interspecific body size differences in sympatric Eucraniini contributed greatly to the metabolic capacity of individuals to thermoregulate (Figure 4). In general, ectotherms with a larger surface area lose and gain heat faster, and with larger volumes although more heat can be stored, more temperature is also needed to generate a change in body temperature (Ray, 1960). Here, *Eucranium* species, the largest species, in terms of loss of body weight and carbon dioxide production, they had the lowest values, and so the lowest capacity to respond to thermal stress decreasing heat excess temperature, presenting the lowest thermal limit values (CT_{max} and *ULT*; see Figure 4a and Table S1). Coinciding with the 'minithermy' strategy, this limited ability to decrease excess temperature (cf. *rHRT* and *iTR*) and have relatively low thermal limits may be the reason why the daily activity of *E. belenae* and *E. cyclosoma* was relegated to colder hours of the day, maintaining a lower metabolic rate and avoiding the hottest periods of the day as observed in the field (Figure 3). This alternative strategy to 'maxithermy' has been observed in tenebrionid beetles in northern Namib where two large species of *Onymacris* Allard are confined to the coldest hours of the day while the small *Stenocara desertica* Koch, 1952 present their activity in the hottest hours of the day (Henwood, 1975).

On the contrary, the species that recorded higher body temperatures, the medium-sized species *A. fedemariai* and *A. heteroclyta*, produced more carbon dioxide ($VCO_{2\ HRT}$, iCO_2) than the other species, and so thermoregulated more efficiently, decreasing the slope of the body temperature increase (*rHRT*) (Figure 4). In general, *Anomiopsoides* species showed a clear adaptation to being active during periods of the day in which the soil temperature reached over 50°C (Figure 3). This adaptation was strongly supported by the physiological syndromes that describe the resistance to heat stress and an efficient decrease of the heat temperature excess by thermoregulation (cf. *SHST*, *HRT*, *iTR*; Figure 4 and Table S1), presenting for these species values very close to thermal shock described for heterothermic dung beetles in flight (∼42°C, see Verdú et al., 2006). Thus, we could verify a clear trend of these species towards the 'maxithermy' hypothesis, which has been proposed as a mechanism directed to maintaining high T_b because they lead to higher rates of many metabolic processes and are therefore advantageous. However, this mechanism was not similar in all *Anomiopsoides* species, since while in *A. heteroclyta* and *A. fedemariai*, the elimination of temperature body excess had a clearly metabolic component represented by the high rates of carbon dioxide emission. In the smaller species *A. biloba* and *A. cavifrons*, the thermoregulation of their body temperature was not accompanied by a significant metabolic response, so it seems that in these small species, as they show T_b closely related to the T_s, convection cooling may be the most frequent mechanism, coinciding with thermodynamic models found in terrestrial ectotherms (Porter & Gates, 1969; Stevenson, 1985).

In conclusion, based on the results of this study, we suggest that the thermal resource partitioning in ectotherms of arid environments is very widespread considering the importance of body temperature for their performance and fitness, taking into account the great spatial and temporal heterogeneity in these environments, and considering the great diversity of assemblages of many ectotherms in terrestrial ecosystems. Here, empirical evidence in both behavioural and ecophysiological traits invokes thermal resource partitioning as a mechanism for minimising interspecific competition and so allowing species coexistence in Eucraniini dung beetles. Future studies on the trophic preference in sympatric species of Eucraniini will be able to complement the knowledge of the niche segregation in this group of species that, due to their endemicity and wingless character, are undoubtedly an ideal group to explore displacements in the different niche dimensions.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

José R. Verdú and Daniela Oliva conceived the ideas and designed field methodology. José R. Verdú, Daniela Oliva and Victoria C. Giménez Gómez collected the data in the field. José R. Verdú, Vieyle Cortez and Victoria C. Giménez Gómez collected the data in the laboratory. José R. Verdú analysed the data. All authors contributed to the writing of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in RUA at http://hdl.handle.net/10045/119016.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Table S1. Values of the statistically significant explanatory variables (mean ± SD) from PLSR analysis and average of body weight of species. Asterisk indicates variables normalised by body weight.

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