Beetle Exoskeleton May Facilitate Body Heat Acting Differentially across the Electromagnetic Spectrum

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ABSTRACT

Exoskeletons of beetles and their associated morphological characteristics can serve many different functions, including thermoregulation. We study the thermal role of the exoskeleton in 13 Geotrupidae dung beetle species using heating experiments under controlled conditions. The main purpose was to measure the influence of heating sources (solar radiance vs. infrared), animal position (dorsal exposure vs. ventral exposure), species identity, and phylogenetic relationships on internal asymptotic temperatures and heating rates. The thermal response was significantly influenced by phylogenetic relatedness, although it was not affected by the apterous condition. The asymptotic internal temperature of specimens was not affected by the thoracic volume but was significantly higher under simulated sunlight conditions than under infrared radiation and when exposed dorsally as opposed to ventrally. There was thus a significant interaction between heating source and body position. Heating rate was negatively and significantly influenced by thoracic volume, and, although insignificantly slower under simulated sunlight, it was significantly affected by body position, being faster under dorsal exposure. The results constitute the first evidence supporting the hypothesis that the beetle exoskeleton acts differentially across the electromagnetic spectrum determining internal body temperatures. This interesting finding suggests the existence of a kind of passive physiology imposed by the exoskeleton and body size, where interspecific relationships play a minor role.

Keywords: body position, dung beetles, exoskeleton, heating rates, interspecific differences, radiation source.

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Introduction

Exoskeletons of arthropods are composed of chitin and various proteins and waxes as basic materials, being one of the most resistant, ancestral, functionally versatile, and abundant biomaterials (Gupta 2011). The evolution of the exoskeleton is key to the radiation of arthropods and the successful colonization of terrestrial environments by insects. Coleoptera constitutes the most diversified animal group on Earth (Chapman 2009), in which the presence of protective sheathed wings (elytra) has become their main identifiable morphological characteristic.

The cuticle of beetles and their associated morphological characteristics are a continuous source of inspiration in biomimetics (Gorb 2013), because they can serve many different functions (physical barrier for internal tissues, attachment for muscles, food grinding, body cleaning, adhesion, sound generation, friction, lubrication, filtering, crypsis, mimicry, substrate matching, aposematism, sexual and visual signaling, thermoregulation, etc.). For some authors, the external characteristics of the insect cuticle as carinae, reticulations, and macro- and microsculptures are not the consequence of specific selective environmental forces acting evolutionarily (Ball 1985) or related to habitat differences (Doberski and Walmesley 2007). By contrast, other studies suggest that reticulation changes (Drotz et al. 2010) or thin patches (Slifer 1953) can be considered adaptations capable of controlling body temperature and thus affecting environmental performance. Cuticle darkness has also been associated with body temperature (Gross et al. 2004; Clusella Trullas et al. 2007; Schweiger and Beierkuhnlein 2016), management of UV radiation (Mikhailov 2001), climatic changes (Davis et al. 2008; Zeuss et al. 2014), and immune response (Dubovskiy et al. 2013). Cold environmental conditions would promote the production of melanin in ectothermic species, being that this process is related to the key role of the enzyme phenoloxidase in both melanization and immune defense (the temperature-dependent immune physiological mechanism; see Fedorka et al. 2013). However, the association between immunity and cuticle darkness would not take place in Coleoptera because their color variations are not pigmentary (Kinoshita 2008) but structural (i.e., due to the electron excitation by the incident light). The available studies in Geotrupidae dung beetles indicate that color variations are due to simple multilayer reflectors (Pye 2010; Akamine et al. 2011), and, as in other insect groups (Digby 1955; Umbers et al. 2013; Välimäki et al. 2015), there is no empirical evidence that supports the role of color on heating differences and thermoregulation.

In this article we study the thermal response to heat of the exoskeleton of the so called earth-boring dung beetle species (Geotrupidae). We study 13 geotrupid species broadly distributed throughout the phylogeny of the family using heating experi-

ments under controlled conditions. The main purpose of this study is to measure the influence of heating sources (solar radiance vs. infrared), animal position (dorsal exposure vs. ventral exposure), and species identity on heating rates and to quantify the proportion of the observed variability attributable to those effects (fig. 1). Seven of these species have fully developed wings, while six are apterous (no wings) with the two elytra fused together (table 1), an adaptation assumed to be directed to reduce water loss (but see Duncan 2002). The selected species also show slight differences in structural coloration (all are black) and surface reticulations and/or microsculptures. If interspecific differences in these traits are prominent for the acquisition of temperature in this group, we should expect a large proportion of variance in heating rates attributable to species identity under laboratory conditions. Moreover, these interspecific differences could be more notable under one of the two different sources of heat tested (simulated solar irradiance and infrared radiation) or considering the area of the animal being directly affected (dorsal surface or ventral surface),
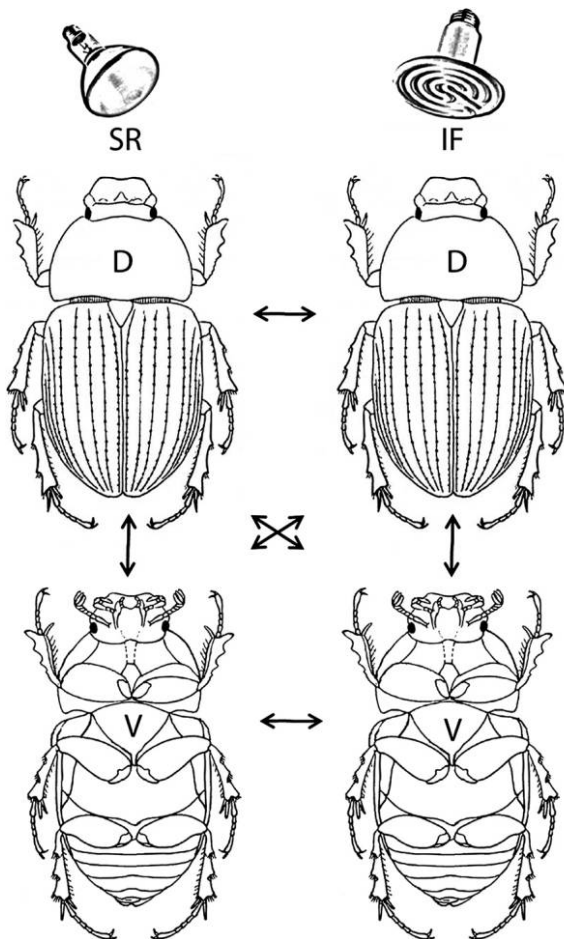

Figure 1. Representation of the factorial design and the effects tested (arrows) considering two sources of radiation (solar radiance [SR] and infrared [IF]) and two body positions (dorsal [D] and ventral [V]) on the heating response of 13 Geotrupidae dung beetle species. The trials were carried out in two different specimens belonging to each species.

illustrating potentially interesting interactions. It is reasonable to assume that the upper part of the beetle's exoskeleton mainly interacts with the range of wavelengths characterizing solar radiation, while the ventral part interacts primarily with the infrared radiation coming from the substrate. Thus, the comparison of the strikingly different smooth dorsal part and the complex and rough ventral texture (with setae and coxae, legs, etc.) would cast light on the importance of the exoskeleton surface in modulating heat rates. If present, a differential heat response between species would allow for further studies directed to identify the most probable characteristics of the exoskeleton responsible for these differences.

Material and Methods

Experimental Conditions

Thirteen species of Palearctic Geotrupidae were considered, all of them well distributed throughout the phylogenetic tree of this coleopteran family (Verdú et al. 2004; Cunha et al. 2011). For each one of these species, two specimens were selected from the insect collections available at the Museo Nacional de Ciencias Naturales (Madrid); specimens were dried from at least 1 yr before. The selection of the two specimens was carried out in order to maximize the body size differences and location of origin between them when possible (table 1).

Thorax length, width, and thickness were measured in each specimen using an Olympus SC100 CMOS camera associated with a SZX10 stereo microscope. The thoracic pseudovolume of each specimen was estimated as the corresponding one-fourth volume of the ellipsoid delimited by these three measurements and used to assess the influence of animal size on the measured heating parameters (i.e., a larger volume should require a longer time to reach the thermal asymptote, and smaller animals heat faster).

Each specimen was finely holed in the apex of the pygidium using an entomological pin, and a thin type-K thermocouple was inserted in this hole until its tip reached the middle of the abdomen. The thermocouple wire was welded to the abdomen using a small drop of hot thermoplastic glue. A Fluke 54 II B dual digital thermometer (0.05% accuracy) was used for recording the internal temperature of each specimen at 10-s intervals for 8 min, except the first record that was measured at 5 s after the beginning of the trial (49 temperature measurements in total).

Before each trial the specimen with the welded thermocouple was maintained in a freezer until its internal temperature reached $-5°C$. The specimen was then placed in the experimental arena, starting the recording of internal body temperatures (time 0) when the temperature reached $+5°C$. The so-obtained body temperatures were subsequently adjusted to the exponential function

$$\text{temperature } (T) = a \times (b - \exp(-c \times \text{time})).$$

The product of a and b represents the asymptotic value at the end of the heating process, while c measures the heating rate at the start of the recording process (i.e., heating trials).

Table 1: Flightless character and geographical distribution of the studied Geotrupidae species, as well as thoracic volume (mm³) of each one of the two treated individuals and the locality in which they were collected

Species	Flightless	Distribution	Thoracic volume	Origin
Jekelius albarracinus	Yes	Iberian	325	Beceite, Teruel (Spain)
			284	Beceite, Teruel (Spain)
Jekelius hispanus	Yes	Iberian	287	Doñana National Park (Spain)
			254	Doñana National Park (Spain)
Thorectes armifrons	Yes	Moroccan	338	Middle Atlas (Morroco)
			246	Middle Atlas (Morroco)
Thorectes lusitanicus	Yes	Iberian	569	Los Acornocales, Cadiz (Spain)
			283	Sierra del Aljibe, Cadiz (Spain)
Typhaeus typhoeus	No	European	407	Pto Canencia, Madrid (Spain)
			374	El Pardo, Madrid (Spain)
Chelotrupes momus	Yes	Iberian	495	Doñana National Park (Spain)
			409	Tarifa, Cadiz (Spain)
Silphotrupes escorialensis	Yes	Iberian	246	El Escorial, Madrid (Spain)
			220	Sierra de Gredos, Avila (Spain)
Trypocopris vernalis	No	Euro-Caucasian	473	Rhodope Mountains (Bulgaria)
			431	La Viale Lozère (France)
Sericotrupes niger	No	Western Palearctic	672	Middle Atlas (Morroco)
			489	Villanueva del Fresno, Badajoz (Spain)
Geotrupes mutator	No	Euro-Caucasian	568	Sierra de Gredos, Avila (Spain)
			313	Pto. Cardoso, Madrid (Spain)
Geotrupes stercorarius	No	Holarctic	735	Pto. Quesera, Madrid (Spain)
			701	Somiedo, Asturias (Spain)
Anoplotrupes stercorosus	No	Western Palearctic	1,036	Puymorens (Andorra)
			400	Rhodope Mountains (Bulgaria)
Ceratophyus hoffmannssegi	No	Ibero-Maghrebian	807	Las Matas, Madrid (Spain)
			1,137	Doñana National Park (Spain)

Thus, the so-obtained asymptotic values were positively correlated with the temperature actually reached after 8 min ($r = 0.9995$, $n = 260$, $P < 0.001$; $T_{8\,min} = 0.02 + 0.999 \times [a \times b]$); the c parameter was positively correlated with the increase in body temperature 15 s after the beginning of the heating trial ($r = 0.882$, $n = 260$, $P < 0.001$).

Temperature variations were recorded by submitting each specimen to two different radiation sources (simulated solar radiance [SR] and infrared [IF]) and two different body positions (dorsal [D], or upside-up, and ventral [V], or upside-down). The experimental specimens were placed 10 cm over the lab table, not being kept in contact with the substrata. Recall that we were interested in the direct influence of radiation sources on heating rates, thus avoiding the potential influence of other heat sources (e.g., conduction, thermal boundary layer, etc.). A 100-W halogen neodymium lamp with a balanced daylight spectrum (from UVA to IF radiations) was used to simulate natural sunlight conditions. A ceramic heat emitter of 150 W was used to simulate long-wavelength IF emissions. These two radiation sources are frequently used in terrariophily to simulate natural sources of heat. The lamps were placed in the experimental arena at different heights above the experimental specimens, in order to match the air temperatures attained in the position of specimens subjected to heating. To control for subtle, random

variations in the air temperature near the specimens, one thermocouple was located 1 cm from the specimen and 10 cm over the lab table (T_{air}). This control temperature was used as a covariate to estimate and correct heating parameters independent of the temperature variations experienced during trials. The mean value ($\pm 95\%$ confidence interval) of T_{air} during the trials was $31.5° \pm 0.3°$C, being similar to those experienced by the species under Mediterranean natural conditions during summer at midday. In total, 260 assays were carried out (13 species × 2 specimens × 2 radiation sources × 2 body positions), repeating three times the temperature measurements of each specimen in dorsal position and twice that in ventral position, in order to obtain stabilized measurements (see table 2); all trial replicates were conducted on different days. This work conforms to the Spanish legal requirements including those relating to conservation and welfare.

Molecular Analyses

Total DNA was extracted from frozen tissue with a DNA Easy extraction kit (Qiagen). COI-Sca-Frevc (Lobo et al. 2015), HCO2198 (Folmer et al. 1994), C1-J-2183 (Simon et al. 1994), COI-Sca-R, COIIam-Sca, and COIIB-605-Sca (Villalba et al. 2002) were the primers used to amplify with polymerase chain

Table 2: Mean and standard errors (SE) of the increasing rate of temperature at the beginning of heating trials (heating rate) and body temperature finally reached in heating experiments (asymptote) for the 13 studied species under two sources of radiation (infrared [IF] vs. solar radiance [SR]) and two body positions (dorsal [D] vs. ventral [V])

Species	Radiation of source	Body position	Heating rate		Asymptote	
			Mean	SE	Mean	SE
Anoplotrupes stercorosus	IF	D	.029	.0077	49.49	2.63
A. stercorosus	IF	V	.025	.0059	43.46	2.01
A. stercorosus	SR	D	.024	.0071	48.24	2.16
A. stercorosus	SR	V	.027	.0083	49.02	3.96
Ceratophyus hoffmannseggi	IF	D	.023	.0020	53.38	8.21
C. hoffmannseggi	IF	V	.014	.0068	44.43	.93
C. hoffmannseggi	SR	D	.023	.0051	50.79	2.09
C. hoffmannseggi	SR	V	.016	.0083	48.63	.65
Geotrupes mutator	IF	D	.033	.0045	46.42	5.62
G. mutator	IF	V	.025	.0059	43.02	.93
G. mutator	SR	D	.027	.0080	47.14	3.59
G. mutator	SR	V	.028	.0085	42.53	.43
Geotrupes stercorarius	IF	D	.020	.0029	50.70	5.95
G. stercorarius	IF	V	.017	.0011	44.63	.29
G. stercorarius	SR	D	.016	.0000	51.80	3.81
G. stercorarius	SR	V	.017	.0017	46.94	3.73
Jekelius albarracinus	IF	D	.017	.0062	42.43	2.69
J. albarracinus	IF	V	.021	.0011	39.89	2.28
J. albarracinus	SR	D	.021	.0024	50.37	2.65
J. albarracinus	SR	V	.019	.0049	44.16	.24
Jekelius hispanus	IF	D	.022	.0030	40.32	3.29
J. hispanus	IF	V	.024	.0001	41.20	4.09
J. hispanus	SR	D	.024	.0003	48.74	1.65
J. hispanus	SR	V	.023	.0015	42.44	.01
Silphotrupes escorialensis	IF	D	.030	.0030	43.33	5.01
S. escorialensis	IF	V	.027	.0038	40.57	4.21
S. escorialensis	SR	D	.027	.0014	49.82	2.38
S. escorialensis	SR	V	.023	.0057	41.85	1.20
Sericotrupes niger	IF	D	.023	.0068	48.84	.06
S. niger	IF	V	.022	.0041	47.45	5.50
S. niger	SR	D	.019	.0026	50.39	.70
S. niger	SR	V	.020	.0029	40.06	3.14
Thorectes armifrons	IF	D	.022	.0092	40.04	3.37
T. armifrons	IF	V	.024	.0050	42.96	4.23
T. armifrons	SR	D	.024	.0045	45.57	2.18
T. armifrons	SR	V	.021	.0064	43.27	.21
Thorectes lusitanicus	IF	D	.022	.0101	40.24	4.33
T. lusitanicus	IF	V	.021	.0113	37.96	1.54
T. lusitanicus	SR	D	.023	.0143	49.56	3.79
T. lusitanicus	SR	V	.020	.0119	38.14	2.12
Chelotrupes momus	IF	D	.030	.0030	44.17	.47
C. momus	IF	V	.025	.0050	39.18	2.57
C. momus	SR	D	.029	.0055	47.08	3.85
C. momus	SR	V	.025	.0033	45.93	2.05
Typhaeus typhoeus	IF	D	.022	.0020	37.91	1.77
T. typhoeus	IF	V	.018	.0014	39.21	.41
T. typhoeus	SR	D	.017	.0020	44.08	.37
T. typhoeus	SR	V	.019	.0007	40.00	4.43
Trypocopris vernalis	IF	D	.021	.0051	40.54	.52
T. vernalis	IF	V	.021	.0041	43.42	7.22
T. vernalis	SR	D	.021	.0042	43.81	1.50
T. vernalis	SR	V	.019	.0024	45.23	1.25

reaction (PCR) three overlapping fragments comprising the 3′ end of mitochondrial cytochrome oxidase I (COI), the adjacent complete tRNA-Leu (UUR), and the 5′ end of cytochrome oxidase II (COII) genes. A total of 40 PCR cycles (denaturing at 94°C for 30 s, annealing at 42°C for 30 s, and extension at 72°C for 60 s) with an initial denaturing step at 94°C for 5 min, and a final extension step at 72°C for 5 min were performed to amplify the mitochondrial fragments. All PCR amplifications were conducted in 25-μL reactions containing 3 mM MgCl$_2$, 0.4 mM of each dNTP, 0.2 μM of each primer, template DNA (10–100 ng), and DNA polymerase (1 unit; Biotools). After PCR purification, samples were directly sequenced using the corresponding PCR primers. Samples were sequenced in an automated DNA sequencer (ABI PRISM 3700) using the Big-Dye Deoxy Terminator cycle-sequencing kit (Applied Biosystems) following the manufacturer's instructions. Sequences were deposited in European Molecular Biology Laboratory under the accession numbers provided in table 3.

DNA sequences were aligned with BioEdit v7.2.5 (Hall 1999) using the default options. Both the Akaike and Bayesian information criteria, as implemented in jModelTest v2.1.7 (Darriba et al. 2012), selected GTR + I + G as the evolutionary model that best fitted the data. The selected evolutionary model and model parameters (GTR + I + G; I = 0.503; G = 0.681) were used in the maximum likelihood analysis performed with PhyML v.3.1 (Guindon et al. 2010). The robustness of the inferred trees was tested by nonparametric bootstrapping using 1,000 pseudoreplicates. Bayesian inference was also performed with MrBayes v.3.2.5 (Ronquist et al. 2012), running for 1×10^7 generations (four simultaneous Markov chains; sample frequency 100). Four independent partitioned analyses were performed and checked for stationarity and convergence of the chains with Tracer v1.6 (Rambaut et al. 2013). Seven data partitions were analyzed: mitochondrial COI gene (first, second, and third codon positions), mitochondrial COII gene (first, second, and third codon positions), and mitochondrial tRNA-Leu (UUR) gene. Model parameters were estimated independently for each one of the respective data partitions. Burn-in was set to the first 1,000,000 generations. The robustness of the inferred Bayesian trees was determined using Bayesian posterior probabilities (as obtained from majority-rule consensus trees of the post-burn-in trees). Two species of *Lethrus* (*Lethrus elephas* and *Lethrus perun*; table 3) were selected as outgroups, based on a previous study (Cunha et al. 2011).

Statistical Analyses

Nonlinear regressions between temperature and time in each trial were carried out using CurveExpert 1.4 for Windows. The average explanatory capacity of the exponential regressions previously mentioned was 99.77% (95% confidence interval of R^2 = 99.73%–99.80%).

Within-individual variation in heating parameters across trials was low (three repetitions in the upside-up position and two in the upside-down position), accounting for a very small percentage of variance: 3.5% for the asymptote and 4.9% for the increasing rate at the beginning of the heating experiments (nested ANOVA analyzing the residual variation of models that include the individuals nested within radiation sources and positions, divided by the total sum of squares). Therefore, the results obtained for the trials under the different experimental circumstances were very repeatable.

We used generalized linear mixed models (GLMM) to analyze the variation in the asymptote and the increasing rate at the beginning of the heating experiments (canonical distribution of the response variables: Gaussian; link functions: identity).

Table 3: List of species used in this study, collection locality, and accession number for mitochondrial gene fragments

Species	Locality	Accession number COI-COII	
Jekelius albarracinus (Wagner, 1928)	Albacete, Spain: El Ballestero	GU984604[a]	KP657665[b]
Jekelius hispanus (Reitter, 1893)	Huelva, Spain: Doñana	GU984628[a]	KP657670[b]
Thorectes armifrons (Reitter, 1892)	Morroco: Ifrane		KP657673[b]
Thorectes lusitanicus (Jekel, 1866)	Cádiz, Spain: Los Alcornocales		KP657674[b]
Typhaeus typhoeus (Linnaeus, 1758)	Ávila, Spain: El Tiemblo	GU984619[a]	LT560390[c]
Chelotrupes momus (Olivier, 1789)	Huelva, Spain: Doñana	GU984620[a]	LT560391[c]
Silphotrupes escorialensis (Jekel, 1866)	Ávila, Spain: El Barraco		LT560384[c]
Trypocopris vernalis (Linnaeus, 1758)	Bulgaria: Vitosha Mountains		LT560385[c]
Sericotrupes niger (Marsham, 1802)	Morroco: Moyen Atlas	GU984609[a]	LT560392[c]
Geotrupes mutator (Marsham, 1802)	Ávila, Spain: El Tiemblo	GU984606[a]	LT560393[c]
Geotrupes stercorarius (Linnaeus, 1758)	León, Spain: Puerto Ancares	GU984634[a]	LT560394[c]
Anoplotrupes stercorosus (Scriba, 1791)	Bulgaria: Belasitsa Mountains		LT560386[c]
Ceratophyus hoffmannseggi (Fairmaire, 1856)	Huelva, Spain: Doñana		LT560387[c]
Lethrus elephas (Reitter, 1890)	Greece: Tsotili Village		LT560388[c]
Lethrus perun (Král & Hillert, 2013)	Bulgaria: Drangovo Village		LT560389[c]

[a]Cunha et al. 2011.
[b]Lobo et al. 2015.
[c]This study.

The subtle variations in the temperature experienced during each heating trial (T_{air}) and the thoracic volume of the studied specimens were treated as fixed covariates, while the source of radiation (SR vs. IF), the body position (D vs. V), and species identity were considered fixed factors. Specimen identity (two different individuals per species) was considered as a random factor. The GLMM also included all possible interactions among species, source, and position (three two-way and one three-way). The mean square and the degrees of freedom of the error terms were estimated following the Kenward and Roger method, which finds the linear combinations of sources of random variation that serve as appropriate error terms for testing the significance of the respective effect of interest (Kenward and Roger 1997). We used the R packages *lme4* and *lmerTest* (Kuznetsova et al. 2014; Bates et al. 2015) run under R, version 3.1.2 (R Core Team 2014). The nested ANOVAs of the within-individual variation in heating parameters were carried out using the R command *aov{stats}*. In order to partition the variance according to the partial effects attributable to species, source of radiation, animal position, and the two covariates, we built new mixed models deleting the interaction terms and used the sum of squares of the marginal model.

It is commonly acknowledged that species are evolutionarily related throughout a phylogenetic scheme, and, therefore, they should not be treated as independent sample units in comparative analyses (Harvey and Purvis 1991; Rezende and Diniz-Filho 2012). Although this article mainly deals with the biophysical response of beetle heating to sources of radiation and body position in 13 Geotrupidae species, we have also estimated the phylogenetic signal (Blomberg and Garland 2002) in interspecific differences of the heating rate at the beginning of the experiments and the asymptotic internal temperature. The residuals of the linear mixed models (GLMM) were analyzed onto the phylogenetic hypothesis (fig. 2). We quantified the association with phylogeny for each trait by means of Blomberg's K statistic (Blomberg et al. 2003), to assess whether traits conserved a phylogenetic signal; when K approaches 1, trait evolution follows a mode of evolution that is consistent with Brownian motion; if $K > 1$, close relatives are more similar than expected under Brownian motion; while if $K < 1$, closely related species are less similar than expected (Blomberg et al. 2003). K has a high type II error rate and a low power to detect a significant phylogenetic signal in small phylogenies (less than 20 species; Blomberg et al. 2003; Münkemüller et al. 2012). Thus, we complemented the phylogenetic signal analysis with Abouheif's test (Abouheif 1999; Pavoine et al. 2008). We used the R packages *phytools* (Revell 2012; command "phylosig" with method = "K") and *adephylo* (Jombart et al. 2010; command "abouheif.moran" with method = "oriAbouheif" and specifying the alternative hypothesis as "greater") run under R, version 3.1.2 (R Core Team 2014).

Results

Variation in the temperature finally reached in the heating experiments (i.e., asymptote) was significantly explained by a mixed ANCOVA model (table 4). The asymptotic internal temperature of the specimens was positively related to subtle variations in the
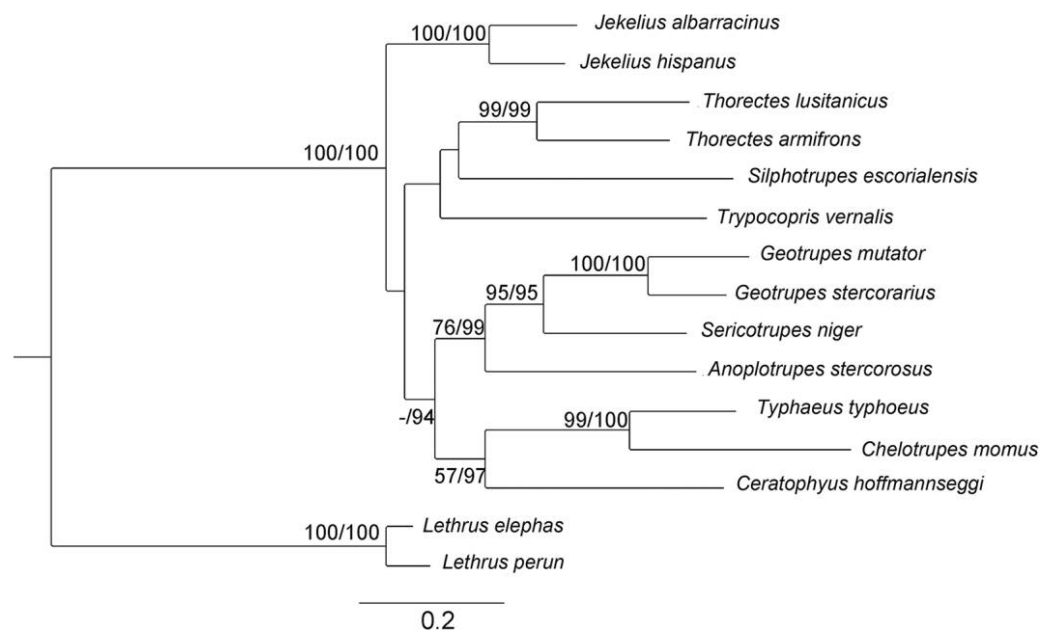

Figure 2. Phylogenetic relationships of the studied Geotrupidae species based on maximum likelihood (ML) and Bayesian inference analysis (both procedures generate similar trees) using mitochondrial (COI, COII, tRNA-Leu) genes and the GTR + I + G evolutionary model. Numbers at nodes correspond to ML bootstrap proportions (BP; first number) and Bayesian posterior probabilities (BPP; second number). Only BP and BPP values above 50% and 90%, respectively, are represented.

Table 4: Results of general mixed ANCOVA GLMM models analyzing the internal body temperature finally reached in heating experiments (asymptote) by the studied beetles and the increasing rate of temperature at the beginning of heating trials

	Asymptote			Starting heating rate		
	F	df	P	F	df	P
Thoracic volume	.05	1, 13	.829	12.42	1, 16	.003
Temperature	23.49	1, 164	$\ll$.001	.1	1, 50	.75
Species	1.17	12, 12	.392	.94	12, 12	.543
Source of radiation	29.86	1, 21	$\ll$.001	2.11	1, 35	.155
Body position	10.65	1, 13	.006	17.54	1, 13	<.001
Species × source	.55	12, 13	.847	.57	12, 13	.828
Species × position	.51	12, 13	.872	1.79	12, 12	.161
Source × position	5.02	1, 13	.043	.19	1, 13	.674
Full interaction	1.59	12, 13	.208	1.29	12, 13	.327

Note. The models analyze two different individuals of the 13 studied species under two sources of radiation (infrared vs. solar radiation) and two body positions (dorsal vs. ventral). Denominator degrees of freedom follow the Kenward-Roger approach to generalized linear mixed model (GLMM) and have been rounded to the nearest unit. For more details see "Material and Methods."

air temperature during heating trials ($P \ll 0.001$), but it was not affected by the thoracic volume of dung beetles. Radiation source and animal position also significantly affected the asymptote ($P \ll 0.001$ and $P = 0.006$, respectively), with the magnitude effect of heating source larger than that attributable to position (see fig. 3 for the partition of variance in the main effects mixed model). The heat finally reached by the specimens was higher under simulated sunlight conditions than under IF radiation and was higher when exposed dorsally than ventrally (fig. 4). The source-by-position interaction term attained significance ($P = 0.043$), showing that the influence of animal position on the internal temperature finally reached was comparatively higher under sunlight conditions. The temperature reached by dung beetles when exposed dorsally was approximately 4°C higher in the case of simulated sun radiation than when they were heated with IF radiation, this difference being only ca. 1.5°C higher when

exposed ventrally to the two sources of radiation. Interspecific differences did not reach significance and accounted for a low proportion of variance (fig. 3). The interaction terms including the species did not reach statistical significance, indicating that the above-mentioned patterns were generalizable across species.

Variation in the increasing rate at the beginning of the heating experiments was also significantly explained by a mixed ANCOVA model (table 4). Interspecific differences did not reach significant values, accounting for a low proportion of variance (fig. 3). The starting heating rate was negatively related to the thoracic volume of beetles ($P = 0.003$; large beetles heated more slowly) and was not affected by temperature variations during trials. Only animal body position had a significant influence on the heating rate ($P \ll 0.001$), while the source of radiation did not affect this parameter (fig. 4): beetles heated faster when exposed dorsally, independent of the source of radiation. None
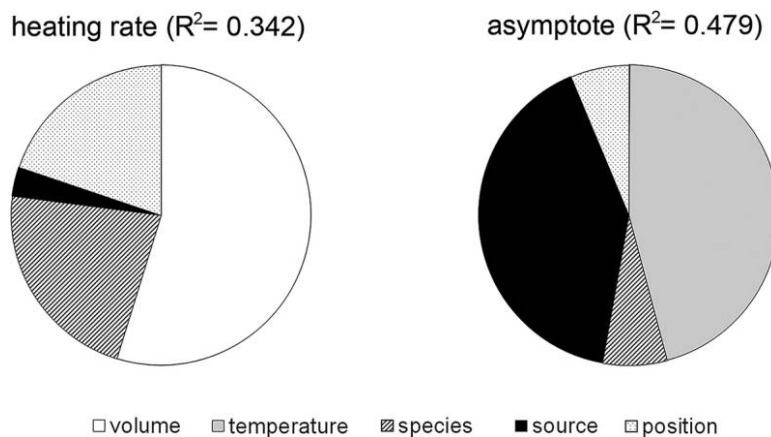

Figure 3. Partition of variance in asymptote of the temperature finally reached by the studied specimens and heating rate at the beginning of the trials among five different effects: size of individual beetles (thoracic pseudovolume), subtle random variations in air temperature during heating trials (temperature), source of radiation (infrared vs. solar radiation), body position (dorsal vs. ventral), and species identity (13 Geotrupidae dung beetle species). R^2 = marginal proportion of the variance explained by the fixed factors in the generalized linear mixed model without interaction terms.
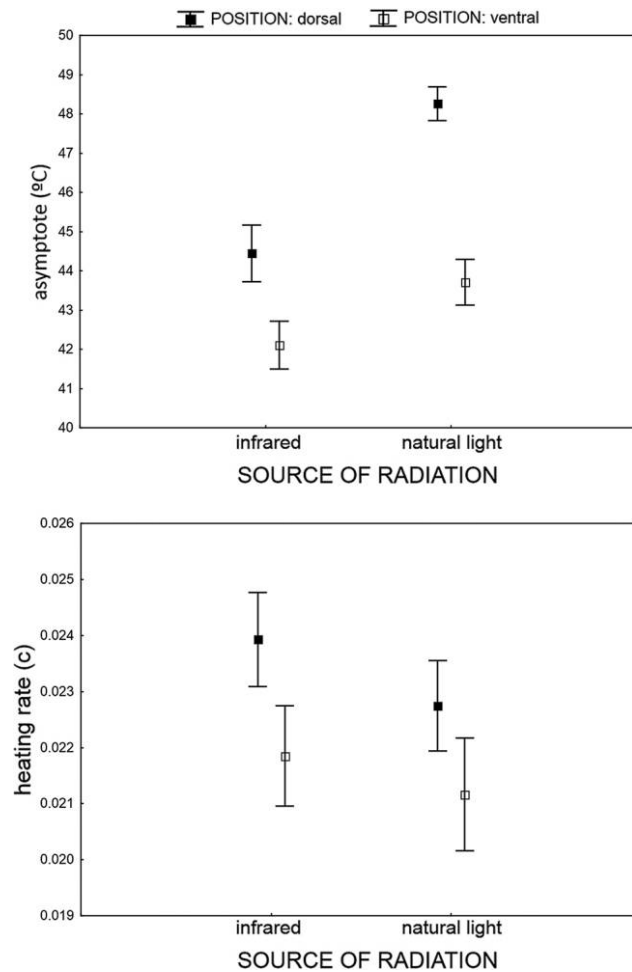
Figure 4. Effect of the source of radiation and the body position of the studied specimens on the asymptote of the temperature finally reached and the heating rate at the beginning of the heating trials. Values are adjusted means (±1 SE) controlling for the effect of interindividual differences in the thoracic volume of dung beetles and subtle, random variations in temperature during trials. Sample size is two different individuals in 13 species of Geotrupidae.

the two traits examined using Abouheif's test, with a positive autocorrelation describing the relation of cross-taxonomic trait variation to phylogeny; heating rate: $C_{mean} = 0.37$, $P = 0.024$; asymptotic temperature: $C_{mean} = 0.36$, $P = 0.033$.

Discussion

The interspecific variability in exoskeleton characteristics of the selected Geotrupidae species does not seem to be enough to generate contrasted thermal responses to heating independently of the radiation source. Neither species identity nor flightless condition significantly affected the thermal response of the exoskeleton. Although it is not possible to discard the existence of interspecific significant differences when a higher number of species and individuals are considered, our results suggest that the amount of these differences should be in any case very low, especially in the case of asymptotic temperatures. For example, the contribution of species identity per se to heating rate was approximately one-third of that attributable to body size per se (see fig. 3) and was similar to the amount related to the dorsal position versus the ventral position. The amount of variance related to interspecific differences in the asymptote of body temperature was one-seventh to one-eighth of that accounted for by radiation source or animal position. Nevertheless, Abouheif's tests show significant results, indicating that related species resemble each other more than they resemble species drawn at random from the phylogenetic hypothesis in figure 2 (Blomberg and Garland 2002). Interspecific differences among the 13 studied Geotrupidae species appear to follow a mode of evolution that is not easily distinguishable from Brownian motion (Blomberg et al.'s K approaches 1), in which the amount of differentiation among species increases monotonically with the time since they diverged. Brownian motion is what should be expected if selection is not acting on the trait (see Blomberg et al. 2003). However, to infer a process from such phylogenetic patterns is risky (Revell et al. 2008). Thus, the cuticle characteristics of different species belonging to lineages that diverged around 80 million years ago (Cunha et al. 2011) have not changed substantially such that they promote important changes in the measured heating parameters. Hence, it is highly probable that any observable variation in exoskeleton characteristics should be evolutionarily examined taking into account functional properties other than temperature control (Gorb 2013).

Beetle exoskeleton exerted a differential influence on asymptotic internal temperature and heating rate depending on whether the specimens were dorsally or ventrally exposed to the radiation sources. The smooth dorsal cuticle seems to facilitate acquiring higher internal temperatures and at higher rates than the ventral intricate surface. Interestingly, the dorsal cuticle also allowed a final higher internal temperature when exposed to simulated sun irradiance than to IF radiation. These results suggest that the higher internal temperature reached when the dorsal surface of the beetle is submitted to simulated natural sunlight is probably due to the transmittance and/or absorbance across the cuticle of non-IF wavelengths. Thus, sun

of the interaction terms reached significance, indicating that the above-mentioned patterns were generalizable across species and experimental conditions.

There were no significant differences among species regarding the flightless condition: $P = 0.296$ in the t-test for the heating rate and $P = 0.860$ for the asymptote (considering the species as the sample unit because the flightless condition is a constant within species; df $= 11$ in both t-tests,).

The interspecific residual variation of the mixed ANCOVA model in the heating rate (i.e., after controlling for temperature experienced during each heating trial, the thoracic volume of beetles, and the two radiation sources and two body positions) shows a Blomberg's $K = 0.99$ ($P = 0.126$). A similar result was obtained when analyzing the residual variation in the temperature finally reached in the heating experiments: Blomberg's $K = 0.90$ ($P = 0.235$). Phylogenetic signal was significant in

radiation should be the main heating source when beetles are dorsally exposed. Acquiring heat dorsally from natural sunlight should be a valuable adaptation considering that most of the ca. 350 Geotrupidae species of dung beetles inhabit temperate-cold environments (Hanski and Cambefort 1991). On the other hand, the lower difference between ventral and dorsal cuticle in the asymptotic internal temperatures when beetles are exposed to IF radiation suggests that IF wavelengths have a comparatively higher importance in the acquisition of heat from the ventral cuticle. This is to be expected in animals that spend much of their life buried, acquiring heat from the soil or from dung pats to be active.

These results are in agreement with the importance conferred many years ago to shorter wavelengths in explaining body temperature excess of insect species (Digby 1955; Tracy 1979), as well as with the mentioned relevance of IF radiation in explaining insect thermal responses (Porter and Kearney 2009). The results provided by this study would constitute the first evidence supporting that the exoskeleton of beetles acts differentially across the electromagnetic spectrum determining the internal body temperatures. This interesting finding suggests the existence of a kind of passive physiology imposed by the exoskeleton, where interspecific differences not related to mere body size play a very minor role.

Acknowledgments

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