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6

7 **Title:** Multidimensionality of thermal niches and its implications to temperature changes  
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9

10 **Running Title:** Thermal niche multidimensionality

11

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35 **Abstract**

36 Approaching the consequences of climate change demands understanding how temperature  
37 controls species' responses across key biological aspects, as well as the coordination of  
38 thermal responses across these aspects. We study the role of temperature in determining  
39 the species' diel, seasonal, and geographical occurrence, using dung beetles as a model  
40 system. We found that temperature has relatively low –but not negligible– effects in the  
41 three studied species' aspects, once accounting for alternative factors. More importantly,  
42 the estimated thermal niches were largely incongruent across aspects. This shows that  
43 species have multidimensional thermal niches, entailing that adjustments to fulfil  
44 temperature requirements for one biological aspect, such as seasonal ontogenetic cycles,  
45 may result in detrimental effects on other aspects, like diel activity. Paradoxically, the  
46 relatively weak effects of temperature we found may have serious consequences for  
47 species' responses to warming if temperature regulates essential aspects of species' biology  
48 in divergent ways.

## 49 **Introduction**

50 Temperature is fundamental for the efficient capture and management of the energy that  
51 maintains living organisms (Brown et al. 2004). Temperature plays a critical role in  
52 controlling key aspects such as species' spatiotemporal distribution, physiological activity  
53 or individual growth rates (Somero 2005, Thackeray et al. 2016, Scranton & Amarasekare  
54 2017, Madrigal-González et al. 2018), among many other things. Here, the effects of  
55 temperature on species' geographic distributions and seasonal and diel activities are of  
56 particular interest since variation in these aspects can modify the spatial and temporal  
57 organization of biodiversity (Chapin III & Diaz 2020), which is leading to spatial and  
58 seasonal decouples of interacting species (Sheldon et al. 2011) and to the disruption of food  
59 webs and ecosystem services (Román-Palacios & Wiens 2020). Ecologists and  
60 climatologists have accumulated a large amount of evidence on these effects during recent  
61 decades, which are especially relevant for ectotherms (Paaijmans et al. 2013). Despite this,  
62 how temperature responses integrate across different species' aspects is still largely  
63 unknown.

64         Delimiting the actual effect of temperature on the distribution and abundance of  
65 species may become difficult when other variables that are either spatially or temporally  
66 correlated with temperature are considered. For instance, the latitudinal distribution of  
67 species in the Northern Hemisphere is associated with historical events and dispersal  
68 limitations, whose effects generate geographical patterns that can be confounded with those  
69 of temperature variations (Araújo et al. 2008, Hortal et al. 2011, Calatayud et al. 2016,  
70 2019). Similarly, the apparent relationships between temperature and either seasonal or diel  
71 activities may be indeed conditioned by several life-history constraints, including the time  
72 required to complete individual development, species' voltinism, the phase in which  
73 overwintering occurs, photoperiod limitations, light requirements, and the reliance on solar  
74 radiation independently on the environmental temperature (Bradshaw & Holzapfel 2007,  
75 2010, Teder 2020). Hence, assessing the predictive value of temperature in accounting for  
76 the species' spatial and temporal variations would require considering any alternative  
77 variables that could play a significant role.

78         Experimental setups can help unravel the “true” role of temperature in driving  
79 geographical, seasonal and diel patterns for some model organisms while controlling for  
80 other variables (Angilletta 2009). However, experiments based on artificial thermal  
81 gradients can subject individuals to new and unrealistic stress conditions, providing  
82 overestimated projections of species responses (Guo et al. 2020). Alternatively, one could

83 explore the contribution of temperature using observational data where the variations in  
84 temperature and other complementary predictors are decoupled. For example, the effects  
85 of temperature and solar radiation can be teased apart using diel activity from consecutive  
86 days with substantial temperature variations (*i.e.*, while presenting almost equal sunlight  
87 incomings). Similarly, the effects of temperature and day length can be teased apart using  
88 seasonal data along steep temperature gradients, with nearly equal day lengths (such as  
89 *e.g.*, elevational gradients). Finally, the role of temperature in determining the species'  
90 distribution can be assessed by comparing geographical areas with different temperature  
91 regimens. If temperature is an important variable, we should find similar responses under  
92 different background temperatures.

93         The relevance of temperature in accounting for the spatiotemporal variation in  
94 species occurrence and abundance may thus be estimated from observational data,  
95 comparing the results from including or not alternative predictors. Temperature will stand  
96 out as a relevant factor across different biological aspects if its association with several  
97 species' responses is high, but also if such responses are congruent across dimensions. The  
98 congruence in thermal responses to diel, seasonal and geographical gradients would  
99 support the universal and homogeneous role of temperature in delimiting the occurrence  
100 and abundance of species. Note here that expectations are that different mechanisms are  
101 behind the response to temperature variations associated with geography, seasonality and  
102 diel rhythms. For instance, daily temperature variations should also be related to changes  
103 in light or other environmental factors that can generate behavioural, endocrine, and  
104 physiological diel rhythms (Levy et al. 2019). In contrast, responses to seasonal  
105 temperatures should be associated with the annual rhythms and the need to synchronize life  
106 history phases with seasonal variations in climate (Saunders 2020). On the other hand,  
107 responses to geographical variations in temperature should relate to local adaptation  
108 processes acting at the population level, and likely involving the above-mentioned  
109 individual tolerances and ontogenetic timing, as well as other essential species attributes  
110 (Sunday et al. 2019).

111         Despite these differences, a certain level of congruence in the responses would  
112 indicate the consistent role of temperature as a holistic and predictable driver of key  
113 biological aspects. Such congruence would be evident, for example, if species occurring in  
114 colder regions are also active during colder periods of the year and at colder hours of the  
115 day in areas of milder climate. The hypothesized thermal congruence is fundamental to  
116 respond adequately to global warming, as decoupling responses across different

117 spatiotemporal gradients may expose local populations to critical temperatures, thus  
118 compromising their long-term persistence. For instance, if seasonal and diel responses to  
119 temperature are decoupled, species might not be able to adjust seasonal cycles as much as  
120 it would be necessary to prevent individuals from facing critical temperatures during diel  
121 activities. Following this line of evidence, studying the congruence of thermal responses  
122 across evolutionary lineages is also important because a marked phylogenetic signal in  
123 thermal niches would also point to the relevance of temperature changes. If thermal  
124 adaptations are evolutionarily conserved, species might present limited ability to modify  
125 their thermal responses, being unable to cope with climate warming and producing  
126 phylogenetic biases in the effects of climate warming. Despite the relevance of studying  
127 the consistency of thermal responses across biological aspects and evolutionary lineages,  
128 integrative studies are lacking.

129         Here we study the thermal responses associated with geographical, seasonal, and  
130 diel temperature variations using several temperate dung beetle species as a model system.  
131 Dung beetles are capable to self-regulate their body temperature and produce heat  
132 depending on their body size (Verdú & Lobo 2008, Verdú et al. 2012) a physiological  
133 adaptation directly linked to the need of a quick dispersal response to exploit an ephemeral  
134 resource. In addition, they feed on cattle from domestic and wild animals, participating in  
135 nutrient cycling and seed dispersion (Nervo et al. 2017, Milotić et al 2019), providing  
136 important ecosystem functions. These characteristics make dung beetles an ideal and  
137 important group to study thermal responses.

138         Specifically, we evaluated the responses of dung beetles to changes in temperature  
139 associated to: (i) diel rhythms across three consecutive days with contrasted temperatures;  
140 (ii) seasonal rhythms across six sites located at different elevations; and (iii) geographical  
141 ranges along five river basins in the Iberian Peninsula (Fig. 1). We hypothesized that if  
142 temperature is the main factor determining the activity and distribution of dung beetles, its  
143 effect should be observed along the three considered species aspects, and its relevance  
144 would be higher if the effects of other alternative and/or complementary factors are low.  
145 Furthermore, congruence in the different species' thermal responses to diel, seasonal and  
146 geographical changes would support a general and predictable role of temperature. On the  
147 contrary, a low explanatory capacity of temperature and a lack of congruence in its effects  
148 across the three spatiotemporal gradients would support a limited and dissimilar role of  
149 temperature depending on the biological aspect. Finally, if species are evolutionarily

150 limited to adapt to new thermal regimens, we expect thermal niches to be phylogenetically  
151 conserved.

152

## 153 **Material and methods**

### 154 *Data origin*

155 We use data on 16 Iberian dung beetle species of the family Scarabaeidae. These species  
156 were selected because they occurred in at least 10% of the samples of the three datasets  
157 considered (see below). All considered species (Table 1) are of small body size, with body  
158 weights far smaller than 1.9 g (0.2 g at most), the threshold from which endothermy is  
159 thought to appear in this group of beetles (Verdú et al. 2006). Temperature–distribution  
160 associations for all these species were examined along: (i) five geographical areas of  
161 similar extent but different temperature regimes within the Iberian Peninsula (geographical  
162 dataset or GD); (ii) six sites placed across a steep elevational range in Central Iberia, and  
163 sampled during the same dates but differing in their environmental temperatures (seasonal  
164 dataset or SD); and (iii) three consecutive days with similar daily variations but different  
165 weather conditions in a single locality near the centre of the same elevational range (diel  
166 dataset or DD).

167

168 *Geographical Dataset.* The GD is divided in five study areas, corresponding to the major  
169 river basins of the Iberian Peninsula (Ebro, Duero, Tajo, Guadiana and Guadalquivir; limits  
170 extracted from HydroBASINS available at [www.hydrosheds.org](http://www.hydrosheds.org), Lehner & Grill 2013,  
171 Fig. 1a). We used these natural areas since they are relatively similar in extent and show  
172 contrasting environmental temperatures (Fig. 1b). In each basin, we collected all  
173 georeferenced occurrences of the selected species from GBIF ([www.gbif.org](http://www.gbif.org), accessed  
174 May 2020) and additional published sources (Hortal & Lobo 2011). We pooled the  
175 occurrences within UTM grid cells of 10 x 10 km to avoid uneven sampling efforts (Lobo  
176 et al. 2018, Appendix S1). We computed the frequency of each species' occurrence in  
177 temperature bins of 1°C (ranging from -3 to 20°C, n=24) for each river basin (n = 24 x 5 =  
178 120), and these figures were used as dependent variables in the subsequent regression  
179 analyses.

180

181 *Seasonal dataset.* We sampled six sites along an elevational gradient located in the Sierra  
182 de Guadarrama (Central Spain) (Fig. 1a, Espinoza 2016) to explore the effect of

183 temperature variations in SD. Elevations range from 755 to 1900 m a.s.l., separating sites  
184 approximately 200 m a.s.l.. Each survey site was sampled every three weeks, totalling 14  
185 times from May 2012 to June 2013. The sites show considerable variations in temperature  
186 during the whole period of the surveys (Fig. 1b). We obtained an estimation of each species'  
187 abundance per elevation site and date ( $n = 6 \times 14 = 84$ , see Appendix S1 for details), which  
188 were used as response variables in subsequent statistical analyses.

189

190 *Diel Dataset.* We sampled a grassland located in the Sierra de Guadarrama at an  
191 approximate elevation of 1500 m a.s.l. (Fig. 1a) to assess temperature effects on dung beetle  
192 diel activity. We chose this locality for its high diversity of dung beetles (between 30 and  
193 40 species belonging to the considered subfamilies; Cuesta & Lobo 2019). We sampled  
194 three consecutive days (April 28<sup>th</sup>–30<sup>th</sup> 2015) from dawn to dusk (approximately from 7:30  
195 am to 7:00 pm). These days showed contrasting temperatures, with around 8 °C of  
196 difference between the mean temperatures of the coldest and the hottest days (Fig. 1b). We  
197 obtained an estimation of the abundance of active individuals from each species each 30  
198 min. ( $n = 23 \times 3 = 69$ , see Appendix S1 for details), which were further used as dependent  
199 variables.

200

### 201 *Temperature measures and alternative correlates*

202 Temperature measures were obtained from different standardized methods for each dataset.  
203 For the GD, we obtained mean annual temperatures at a 30 sec resolution from WorldClim  
204 (Hijmans et al. 2005). For SD, we set up a temperature data logger in the shadow and at  
205 one meter from the ground in each elevational point during the whole period of the study.  
206 Finally, for DD, we used temperature measurements from a data logger placed on the  
207 ground and in the sun as they were best correlated with the species' diel activity among  
208 temperature measurements from data loggers placed to recover different microclimatic  
209 conditions available for dung beetles (see Appendix 1 for details).

210 The effects of temperature measurements might be overestimated due to its  
211 collinearity with other factors with which it shares spatial or temporal structure. We  
212 quantified this potential overestimation by using different “contrast variables”: alternative  
213 predictors which are often partly correlated with temperature but are measures or proxies  
214 of other potential causal factors for dung beetle spatial and temporal variations. These  
215 alternative predictors were temperature availability in the case of GD, day of the year for  
216 SD (included as date sine and cosine), and hour of the day in the case of DD (see Appendix

217 1 for details). The effect of temperature on the frequency of occurrence (GD) or abundance  
218 (SD and DD) that is independent of these contrast variables was assessed as the “pure”  
219 effect of temperature that is independent of the range of temperatures available (GD), the  
220 period of the year (SD), and the hour of the day (DD) as explained below.

221

## 222 *Statistical analyses*

### 223 *Explanatory capacity of temperature*

224 We explored the independent capacity of temperature to explain variations in dung beetle  
225 data in GD, SD, and DD. For each dataset, we conducted Generalized Linear Regression  
226 Models (GLMs) of the species’ frequency or abundance as a function of temperature. Data  
227 coming from the five basins (in GD), the six elevational sites (SD), and the three days (in  
228 DD) were considered at the same time in each case. A curvilinear quadratic function of  
229 temperature was included to account for the typical unimodal performance curves of  
230 ectotherms (Huey & Kingsolver 1989). A negative binomial error distribution for the  
231 dependent variable was assumed to avoid overdispersion issues associated with the Poisson  
232 error distribution (Blasco-Moreno et al. 2019), and it was related to the set of predictors  
233 via a logarithmic link function. We, then, fitted three supplementary GLMs representing  
234 different hypotheses regarding the importance of temperature: (i) a full model where  
235 temperature and contrast variables are included, (ii) a model including only contrast  
236 variables, and (iii) a null model where only the intercept was included. We assumed a linear  
237 relationship between the density of occurrence and temperature availability (GD); whereas  
238 in SD and DD, we assumed curvilinear relationships between abundance and contrast  
239 variables by including a quadratic term of both the number of minutes from dawn, and date  
240 sine and cosine. It is important to note that we did not include a term in the models to  
241 account for the different spatial (*i.e.*, basins and elevations) and temporal (*i.e.*, days) units.  
242 By doing so, we were ignoring other factors that may affect the distribution and activity of  
243 dung beetles, besides temperature and the contrast variables analysed. However, this allows  
244 us to tease apart the effects of temperature and contrast variables while avoiding model  
245 overparameterization. We used a deviance partition approach (Legendre 1993, see also  
246 Calatayud et al. 2019) to calculate the deviance explained by each set of variables alone  
247 (*i.e.*, temperature *vs.* contrast variables; herein, total pseudo  $R^2$ ) and once accounting for  
248 the collinearity with other variables (herein, partial pseudo  $R^2$ ). Model performance was  
249 assessed using the Akaike Information Criterion corrected for small sample size (AICc).

250

251 *Thermal niche attributes*

252 Deriving thermal niches from occurrence data typically provides a partial description of  
253 the whole potential response of the species (Sánchez-Fernández et al. 2012, Saupe et al.  
254 2018). Still, occurrence-based thermal niches may be characterized by different attributes  
255 such as the optimum temperature and niche breadth (Gouveia et al. 2014, Löffler & Pape  
256 2020, Fig. 2). The temperature optimum of each species was assessed by fitting quadratic  
257 curves in a GLM and calculating the maxima as their inflection point (see Villén-Pérez &  
258 Carrascal 2015 for a similar procedure). Thermal niche breadth was also obtained as the  
259 area under the curve of these fitted curves. Fitted values were normalized to reach a  
260 maximum value of one to make calculations comparable among datasets and species.

261 We evaluated the intraspecific dissimilarity in the thermal niches across different  
262 spatial and temporal scales, herein called “thermal lability”, using data from the different  
263 study units used in each dataset; that is, between river basins, elevation sites, and days (Fig.  
264 2). Thermal lability between pairs of units was measured using the Simpson index as  
265 follows:

266 
$$S = \frac{\min(b,c)}{a + \min(b,c)},$$

267 where  $a$  represents the area under the curves where both curves overlap, and  $b$  and  $c$   
268 represent the independent areas under the curves in study units (see Fig. 2). The larger the  
269 overlap between the curves obtained at different scales, the smaller the thermal lability will  
270 be. We computed this index for all pairs of units in each dataset (*i.e.*, for each pair of basins,  
271 each pair of elevations, and each pair of days) and then considered the maximum  
272 dissimilarity among all pairs from the same dataset, as this measure will provide a more  
273 realistic estimate of the potential thermal lability of each species.

274

275 *Congruencies in thermal niches*

276 The congruence in the thermal niches of the different species derived from the three  
277 datasets (*i.e.*, GD, SD, and DD) was assessed using Spearman rank correlations between  
278 the deviance explained by temperature (*i.e.*, both for the total and partial pseudo  $R^2$ s), as  
279 well as the obtained temperature optima, thermal niche breadths and thermal labilities for  
280 each pair of datasets. We also explored the overlap in the thermal niches estimated from  
281 different datasets. For this, we examined whether interspecific thermal niche dissimilarities  
282 were correlated between the different datasets. We computed dissimilarities between the  
283 models' normalized fitted values where the temperature was the only explanatory variable

284 using the Simpson index as previously explained, but in this case between pairs of species  
285 (see also Fig. 2). Thus, we created a thermal niche pairwise dissimilarity matrix for each  
286 dataset. We conducted Mantel tests based on Spearman's  $\rho$  coefficient to assess the  
287 relationship between dissimilarity matrices obtained from the different datasets.  
288 Significance was evaluated by comparing observed  $\rho$  coefficients with 999 null values  
289 obtained by permuting the dissimilarity matrices.

290

### 291 *Phylogenetic signal*

292 The potential lability of thermal niches shall be also assessed from an evolutionary point  
293 of view. A marked phylogenetic signal would indicate potential evolutionary constraints for  
294 temperature responses, and phylogenetically-structured effects of global warming. We  
295 reconstructed a Bayesian phylogeny for the species present in our datasets based on two  
296 mitochondrial (COI and COII) and one nuclear markers (28S RNA, see Appendix S2 for  
297 details). DNA markers were sequenced for this study and retrieved from Genbank (Table  
298 S1, accessed in June 2016). Pagel's  $\lambda$  test (Pagel, 1999) and Blomberg's K statistics  
299 (Blomberg et al. 2003) were used to explore the phylogenetic signal in the five variables  
300 considered (total and partial deviance explained by temperature, temperature optimum,  
301 thermal niche breadth, and thermal lability). Significance for Pagel's  $\lambda$  was assessed with  
302 a likelihood ratio test comparing the negative log likelihood obtained from the original tree  
303 topology with the negative log likelihood from a topology transformed to remove the signal  
304 (*i.e.*,  $\lambda = 0$ ). In the case of Blomberg's K, we tested for significance by randomizing the  
305 labels of the phylogenetic tips and comparing observed and random K values. Finally, we  
306 also investigated for phylogenetic signal in the thermal niche dissimilarities for each  
307 dataset. To do so, Spearman correlations between thermal dissimilarities and phylogenetic  
308 distances were used, assessing significance by comparing observed correlations with null  
309 values where the tip labels of the phylogeny were randomized. In all cases where tip labels  
310 were randomized, p-values were calculated as the proportion of null values being equal or  
311 higher than observed values.

312 All analyses were conducted in R environment (R Core Team 2020), using the  
313 *AICcmodavg* package (Mazerolle 2019) to calculate AICc values, the function "sintegral"  
314 as implemented in the *Bolstad2* package (Curran 2013) to assess areas under the curves, the  
315 *vegan* package (Oksanen et al. 2019) for the Mantel tests, and the *phytools* package (Revell  
316 2012) to calculate Pagel's  $\lambda$  and Blomberg's K.

317

## 318 **Results**

319 There is an evident gradient in the explanatory relevance of temperature towards higher  
320 relevance at progressively larger scales (*i.e.*, geographical > seasonal > diel). Model  
321 selection revealed that the full model, including temperature and contrast variables, was  
322 the most parsimonious for most species in most datasets (Table 1). As exceptions to this  
323 general pattern, in the geographical dataset, the model only including temperature was  
324 equivalent to the full model (according to AICc) for one species, and it was also the best  
325 supported for another species. In the seasonal dataset, the model only including temperature  
326 was the best supported for four species, whereas the model only including contrast  
327 variables was equivalent to the full model for just one species. Finally, the model including  
328 minute from dawn in DD data was equivalent to the full model for only two species and  
329 even better for one species (Table 1). In general, the total deviance explained by the models  
330 including temperature and contrast variables was considerably high (mean pseudo-R<sup>2</sup>s =  
331 0.62, 0.63, and 0.77; ranges = 0.51-0.75, 0.38-0.86, and 0.64-0.86, respectively for GD,  
332 SD, and DD; see Fig. 3). Partial regressions revealed that the effects of temperature and  
333 contrast variables largely overlap, being the deviance independently explained by  
334 temperature considerably low (see Fig. 3). Interestingly, the percentage of deviance  
335 explained by temperature decreased from the geographical (mean pseudo-R<sup>2</sup>s = 0.33; range  
336 0.13–0.48), to the seasonal (0.19; 0.05–0.36) and diel datasets (0.08; 0.01–0.20) (see Fig.  
337 3).

338 Thermal niche attributes derived from the different datasets showed little  
339 congruence. Neither the pseudo R<sup>2</sup> explained by temperature alone nor the total pseudo R<sup>2</sup>  
340 were positively and significantly correlated between any pair of datasets, and none of the  
341 thermal niche attributes were significantly correlated between the three considered datasets  
342 (Table 2). Moreover, Mantel tests showed that interspecific niche dissimilarities were not  
343 correlated among the three studied spatiotemporal scales (Table 2). Finally, we did not find  
344 phylogenetic signal for any of these variables in any of the datasets, except in the case of  
345 niche breadth for the diel dataset (Table 3).

346

## 347 **Discussion**

348 Our results show that the spatial and temporal responses of the studied species show large  
349 associations to contrast variables besides temperature, but also that temperature controls to  
350 dung beetle occurrence may increase towards larger temporal and spatial scales. This

351 contrasts with our preliminary expectations of a high importance of temperature for dung  
352 beetle occurrence and activity based on the known basal ectothermic physiology of the  
353 considered species. Further, thermal niches were incongruent across scales for the studied  
354 species and lacked phylogenetic signal, indicating that thermal adaptations are highly  
355 variable both between and within species.

356         The generally low partial effects of temperature lead to two important  
357 conclusions: (i) the distribution and activity of dung beetles are controlled by other factors  
358 different from temperature, which are at least partially represented by the *ad hoc* contrast  
359 variables used here; and (ii) dung beetle species must have biological mechanisms that  
360 allow them to cope with the temperature variations associated to each spatiotemporal  
361 context. Thermoregulation and body heat gain are intimately linked to solar radiation in  
362 ectotherms (Angilletta 2009). Indeed, empirical evidence suggest that solar radiation is  
363 associated with dung beetles' body temperatures (Bartholomew & Heinrich 1978) and  
364 temporal variations in their abundance and species richness (Lobo et al. 1998). Hence, it is  
365 likely that this factor is a key environmental control of the diel activity of dung beetles.  
366 Regarding annual rhythms, photoperiod seems to be a crucial environmental cue regulating  
367 insects' seasonality (Nijhout 1994, Bradshaw & Holzapfel 2007). This is likely the case for  
368 dung beetles, given the relatively weak effects of temperature on their phenology we found.  
369 Also, the different life-history phases of an insect need to be synchronized seasonally, and  
370 these require a minimum amount of time to complete. The development of a dung beetle  
371 individual requires from 30 to 80 days depending on the species (Christensen & Dobson  
372 1977, Romero-Samper & Martín-Piera 1995, 2007, Arellano et al. 2017), a time that  
373 determines key life-history characteristics such as the number of generations per year or  
374 the overwintering phase. These developmental constraints are therefore hard to modify  
375 without major evolutionary changes (Teder 2020), limiting the effects of temperature on  
376 the seasonal abundance and occurrence of dung beetle species. Finally, many factors  
377 contribute to shaping the geographical distribution of dung beetle species, including  
378 dispersal limitations (Lobo et al. 2006), historical events (Hortal et al. 2011), or the  
379 response to other environmental variables such as precipitation, soil, habitat, or trophic  
380 preferences (Hanski et al. 1991, Hortal et al. 2001, Lobo & Martín-Piera 2002). It is  
381 important to note that we have not quantified the effects of these variables explicitly, so  
382 their inclusion could further weaken the pure effect of temperature.

383         Regardless of alternative factors, it seems evident that dung beetles have  
384 mechanisms to withstand marked temperature variations, especially those associated with

385 diel and seasonal rhythms. Given the nature of our data and analyses, these mechanisms  
386 can be operating either at the population level, at the individual level, or both. At the  
387 population level, a large phenotypic variability linked to a high genetic diversity can  
388 produce the apparently labile thermal responses. As individuals are sorted in time and/or  
389 space according to their environmental adaptations, population(s) formed by individuals  
390 with different thermal preferences would show a certain level of thermal independency.  
391 This mechanism seems more plausible to explain results in the geographical datasets,  
392 where river basins can act as dispersal barriers, limiting gene flow and enhancing local  
393 adaptation to different temperature regimens (Lenormand 2002). However, it seems less  
394 likely that this phenotypic variability alone is responsible for the responses to diel and  
395 seasonal temperature variations, where a high gene flow is expected between the  
396 populations that are active at different elevations or days. Physiologically plastic responses  
397 allowing individuals to be active at different temperatures seem a more plausible  
398 mechanism in this case (Crispo 2008). In any case, these two potential mechanisms  
399 (population phenotypic variability and individual plasticity) are in agreement with the  
400 observed lack of phylogenetic signal on species responses to temperature across scales,  
401 which indeed suggests a lack of thermal niche conservatism (Gilbert & Miles 2019). The  
402 relative contribution of these mechanisms remains elusive, calling for further studies  
403 directed to unravel the detailed mechanisms behind the diverse responses to temperature  
404 we found.

405         Be that as it may, the effects of temperature were significant and not negligible,  
406 being larger for species distribution than for seasonal activity, and even smaller for diel  
407 activity. The increasing importance towards larger scales may be related to the fact that the  
408 effects of temperature on the studied biological aspects are nested. That is, the occurrence  
409 in a given location would entail that a species holds the adaptations required to maintain a  
410 stable population there, which include physiological and/or behavioural adaptations to cope  
411 with the seasonal temperature variations that occur in that locality. In the same way, a  
412 population with adults active during a given period of the year should present adaptations  
413 to handle the daily temperature variations happening during the days when adults are  
414 active. Hence, the hierarchically cumulative effects of temperature across these biological  
415 scales may explain why temperature becomes more important for geographic distributions  
416 than for temporal activities. Ascertaining the plausibility of this idea requires further  
417 investigation of intraspecific responses to daily temperature variations across seasons and

418 seasonal temperature responses throughout different populations placed across the species'  
419 geographic distribution.

420         Perhaps the most interesting of our results is the lack of congruence in the realized  
421 thermal niches across the studied biological aspects. This means that, for instance, species  
422 occurring in colder regions do not appear in colder months nor at colder hours of the day  
423 in other regions. This somehow counterintuitive result could be related to the uneven  
424 relevance of the alternative variables for the different biological aspects, which facilitates  
425 the decoupling of the thermal responses. It is likely that the processes involved in adult  
426 movements, life-history cycles, and population maintenance are differently regulated by  
427 temperature, despite of their nested nature. In other words, our results suggest that species  
428 have multidimensional thermal niches, where each critical biological aspect responds to  
429 temperature along a different dimension. Therefore, rather than exerting a universal effect,  
430 temperature plays multiple roles in a species' biology. This lack of congruence, together  
431 with the low independent effects of temperature found, indicates that estimates of thermal  
432 niches will be, in general, inaccurate and context-dependent. This calls from being  
433 particularly cautious when using responses measured at different scales as proxies for  
434 future responses to climate change. Overall, our results show the difficulties in estimating  
435 general thermal niches of species, challenging forecasts of species future dynamics under  
436 climate warming based on unidimensional thermal niches (Gvoždik, 2018).

437         The partial control of temperature on the activity and distribution of dung beetles  
438 may be both a blessing and a curse regarding the effects of climate warming. On the one  
439 hand, the apparent thermal lability suggests that temperature increases should not strongly  
440 modify neither diel and seasonal activities nor the geographic distribution of dung beetles,  
441 likely preventing mismatches with interacting species and the subsequent food chain  
442 perturbations. This assumption would contradict the results of studies suggesting moderate  
443 or even large effects of climate change on dung beetle distributions (Dortel et al. 2013,  
444 Menéndez et al. 2013, Holley & Andrew 2019). On the other hand, the diel, seasonal, or  
445 geographical adjustments are among the fastest responses to climate warming (Levy et al.  
446 2019, Duchenne et al. 2020). However, our results suggest that the response towards  
447 temperature variations is relatively independent at each spatiotemporal context. This entails  
448 that adjustments to temperature requirements may not be coordinated across key biological  
449 aspects. Hence, adjustments to fulfil the temperature requirements for one biological aspect  
450 may result in detrimental effects on other aspects, thereby reducing individual and  
451 population performance as, *e.g.*, seasonal adjustments may expose individuals to

452 inadequate temperatures during diel activity. In the worst-case scenario, the incapacity of  
453 species to adjust their temperature requirements by modifying diel, seasonal, and  
454 geographical patterns at convenience can increase the likelihood of local extinctions when  
455 the individuals are exposed to critical temperatures in their daily or yearly periods of  
456 activity. Paradoxically, the partially weak effects of temperature we found may have  
457 serious consequences for climate warming if temperature regulates important aspects of  
458 species' biology in divergent ways (Tsai et al. 2020).

459 Overall, our results show that temperature may be less important than other factors  
460 in determining dung beetle activity and distribution. Further, the incongruences in thermal  
461 niches estimated from the geographic distribution and seasonal and diel activities show the  
462 complex effects of temperature on key species aspects, pointing to a truly multidimensional  
463 nature of thermal niches. Together with the partially weak control of temperature on species  
464 activity and distribution, these incongruences may difficult fast responses to climate  
465 warming, potentially exposing individuals to critical, or at least inadequate, temperatures  
466 and reducing individual and population's fitness.

467

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**Table 1.** AICc values for the models of each species in each dataset. In all cases, we conducted a complete model (Full) including temperature and the corresponding contrast variables, a model only including temperature (Temp), a model only including contrast variables (Cont), and a null model where no predictor variable was included (Null). Contrast variables were minutes from dawn and its quadratic term for the diel data set; date sine and cosine and their quadratic terms for the seasonal dataset; and temperature availability for the geographic data set. The best models in terms of AICc and the equivalent ones ( $\Delta AICc < 2$ ) are highlighted in bold.

Subfamily	Species	Diel				Seasonal				Geographic			
		Full	Temp	Cont	Null	Full	Temp	Cont	Null	Full	Temp	Cont	Null
Aphodiinae	<i>Acrossus depressus</i> (Kugelann, 1792)	<b>176.53</b>	245.53	187.59	264.43	<b>120.25</b>	126.26	<b>120.67</b>	149.35	<b>172.98</b>	<b>173.17</b>	209.50	209.95
Aphodiinae	<i>Agriolus constans</i> (Duftschmid, 1805)	<b>140.21</b>	197.94	145.50	214.29	<b>200.92</b>	210.56	206.92	216.62	<b>211.85</b>	218.76	269.52	289.54
Aphodiinae	<i>Aphodius fimetarius</i> (Linnaeus, 1758)	<b>116.91</b>	162.51	121.10	177.61	201.68	<b>199.58</b>	217.13	214.00	<b>380.68</b>	404.55	419.41	455.12
Aphodiinae	<i>Aphodius foetidus</i> (Herbst, 1783)	<b>42.72</b>	56.56	<b>44.51</b>	64.09	138.39	<b>133.18</b>	146.06	144.19	<b>634.53</b>	679.39	753.81	861.04
Aphodiinae	<i>Colobopterus erraticus</i> (Linnaeus, 1758)	<b>128.81</b>	150.10	136.82	164.96	<b>208.49</b>	251.21	217.59	272.82	<b>343.34</b>	366.05	372.06	410.67
Aphodiinae	<i>Esymus pusillus</i> (Herbst, 1789)	175.62	227.70	<b>171.67</b>	241.45	<b>151.68</b>	163.52	217.18	191.76	<b>147.93</b>	153.05	177.47	180.48
Aphodiinae	<i>Melinopterus sphaelatus</i> (Panzer, 1798)	<b>471.34</b>	534.10	493.63	577.91	<b>307.12</b>	322.42	321.94	343.35	<b>289.63</b>	304.26	354.89	390.33
Aphodiinae	<i>Teuchestes fossor</i> (Linnaeus, 1758)	<b>194.71</b>	256.95	208.75	280.58	<b>89.06</b>	98.58	96.18	116.19	<b>258.00</b>	268.42	304.76	318.01
Aphodiinae	<i>Trichonotulus scrofa</i> (Fabricius, 1787)	<b>144.87</b>	185.24	160.35	207.64	<b>204.82</b>	226.12	216.37	261.94	<b>182.51</b>	186.38	221.98	242.86
Aphodiinae	<i>Volinus sticticus</i> (Panzer, 1798)	<b>305.09</b>	342.35	310.59	371.12	<b>116.05</b>	407.35	121.57	122.05	<b>133.88</b>	<b>132.11</b>	167.53	169.92
Scarabaeinae	<i>Euoniticellus fulvus</i> (Goeze, 1777)	<b>39.24</b>	59.86	49.17	67.31	<b>446.74</b>	473.23	454.04	519.71	<b>285.43</b>	306.31	306.07	352.08
Scarabaeinae	<i>Onthophagus fracticornis</i> (Preyssler, 1790)	<b>255.26</b>	325.6	266.11	350.04	188.32	<b>184.52</b>	200.87	201.75	<b>274.14</b>	279.86	317.51	329.60
Scarabaeinae	<i>Onthophagus lemur</i> (Fabricius, 1781)	<b>117.11</b>	153.81	124.15	170.30	200.57	<b>158.52</b>	205.75	174.36	<b>231.64</b>	238.74	290.70	313.01
Scarabaeinae	<i>Onthophagus opacicollis</i> Reitter, 1892	<b>71.03</b>	80.84	76.13	90.69	<b>343.31</b>	355.11	350.70	356.49	<b>207.58</b>	215.60	218.29	251.59
Scarabaeinae	<i>Onthophagus similis</i> (Scriba, 1790)	<b>256.91</b>	342.23	260.48	359.42	<b>612.48</b>	617.79	646.30	658.81	<b>312.48</b>	328.99	363.43	400.08
Scarabaeinae	<i>Onthophagus vacca</i> (Linnaeus, 1767)	<b>248.03</b>	300.37	<b>248.30</b>	318.63	<b>285.06</b>	296.59	299.48	318.99	<b>315.14</b>	337.44	352.21	409.05

**Table 2.** Spearman’s  $\rho$  correlation coefficients and P-values between the considered thermal niche attributes measured by the three studied datasets are detailed.. DD: Diel dataset. SD: Seasonal dataset. GD: Geographical dataset. \* Results based on Mantel test.

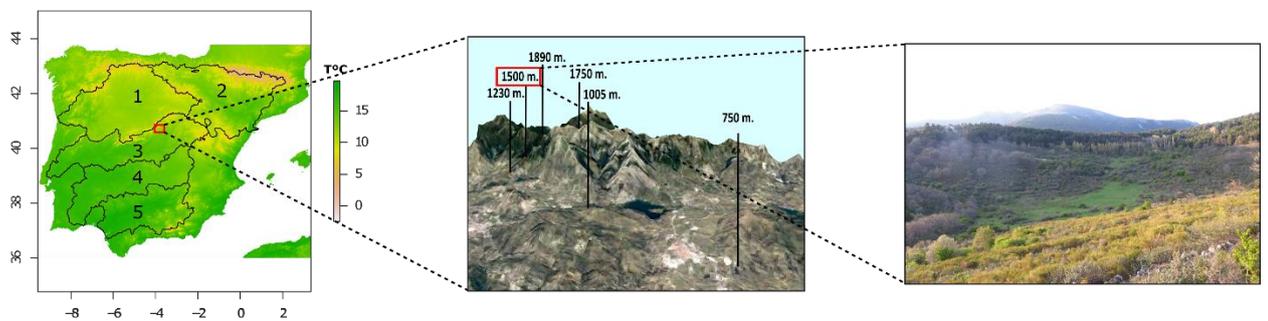
	DD vs SD		DD vs GD		SD vs GD	
	$\rho$	<i>P</i>	$\rho$	<i>P</i>	$\rho$	<i>P</i>
<b>Breadth</b>	-0.074	0.788	-0.385	0.141	0.100	0.713
<b>Optimum</b>	-0.262	0.326	-0.179	0.505	0.394	0.132
<b>Thermal lability</b>	-0.261	0.347	-0.339	0.216	0.132	0.625
<b>Total pseudo R<sup>2</sup></b>	0.029	0.914	-0.016	0.953	-0.200	0.456
<b>Partial pseudo R<sup>2</sup></b>	-0.561	0.024	-0.440	0.088	0.053	0.848
<b>Niche dissimilarity *</b>	0.260	0.056	0.242	0.051	0.120	0.153

**Table 3.** Phylogenetic signal in thermal niches attributes for the three studied datasets (*i.e.*, geographical, seasonal and diel). Significant variables are highlighted in bold. \* Results based on Mantel test.

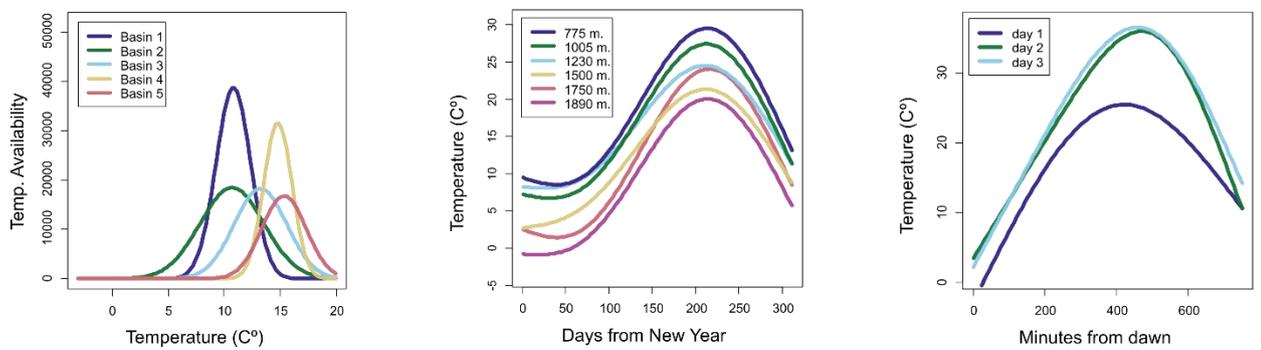
Variable	Dataset	K	P	$\lambda$	P	$\rho$	P
<b>Breadth</b>	Geographical	0.480	0.150	0	1	-	-
	Seasonal	0.285	0.798	0	1	-	-
	<b>Diel</b>	<b>0.998</b>	<b>0.001</b>	<b>1.096</b>	<b>0.002</b>	-	-
<b>Optimum</b>	Geographical	0.292	0.811	0.002	0.990	-	-
	Seasonal	0.477	0.188	0.361	0.160	-	-
	Diel	0.292	0.710	0	1	-	-
<b>Thermal lability</b>	Geographical	0.475	0.171	0	1	-	-
	Seasonal	0.295	0.900	0	1	-	-
	Diel	0.467	0.228	0.007	0.970	-	-
<b>Total pseudo R<sup>2</sup></b>	Geographical	0.287	0.857	0	1	-	-
	Seasonal	0.324	0.710	0	1	-	-
	Diel	0.313	0.726	0	1	-	-
<b>Partial pseudo R<sup>2</sup></b>	Geographical	0.367	0.476	0	1	-	-
	Seasonal	0.388	0.398	0	1	-	-
	Diel	0.425	0.350	0	1	-	-
<b>Niche dissimilarity *</b>	Geographical	-	-	-	-	-0.100	0.089
	Seasonal	-	-	-	-	0.200	0.980
	Diel	-	-	-	-	-0.080	0.139

**Figure 1.** a) The areas of study for the geographical, seasonal and diel datasets (from left to right). Red squares show the position of the following down-scaled study site. b) Temperature variations in study sites. Lines correspond with predictions of general additive models (GAM) of: (i) temperature availability (measured as the number of 10 km<sup>2</sup> grid cells whose temperature fell within predefined temperature bins) as function of temperature for the geographic dataset (left); (ii) temperature as a function of days from New Year and minutes from dawn for the seasonal and diel datasets respectively. Analyses were computed independently for each basin, for each elevational site and for each day. GAMs explained an average of 0.90 of deviance across all analyses (median = 0.92, ranging from 0.79 to 0.97).

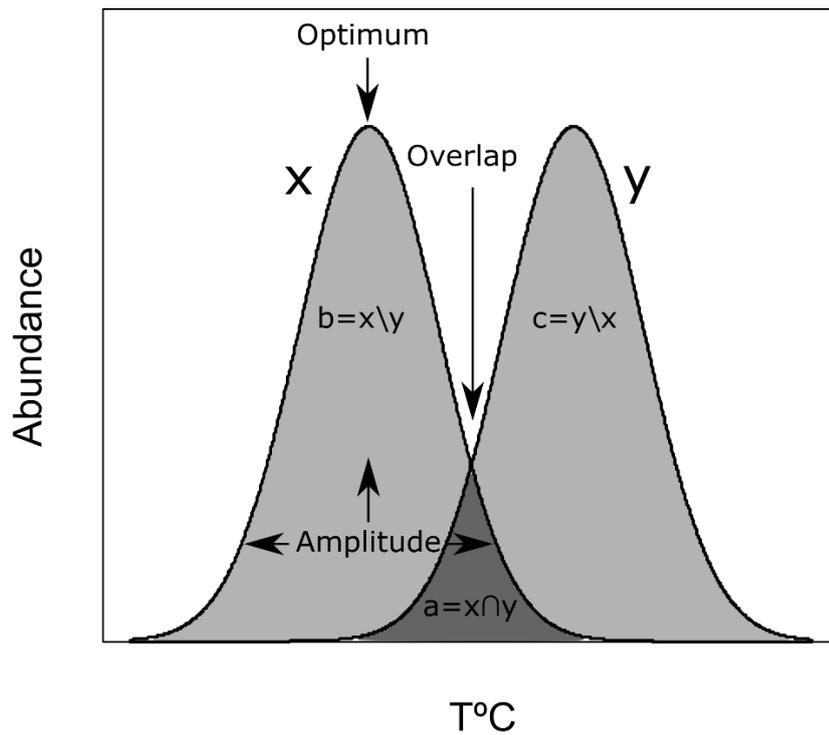
a) Study site



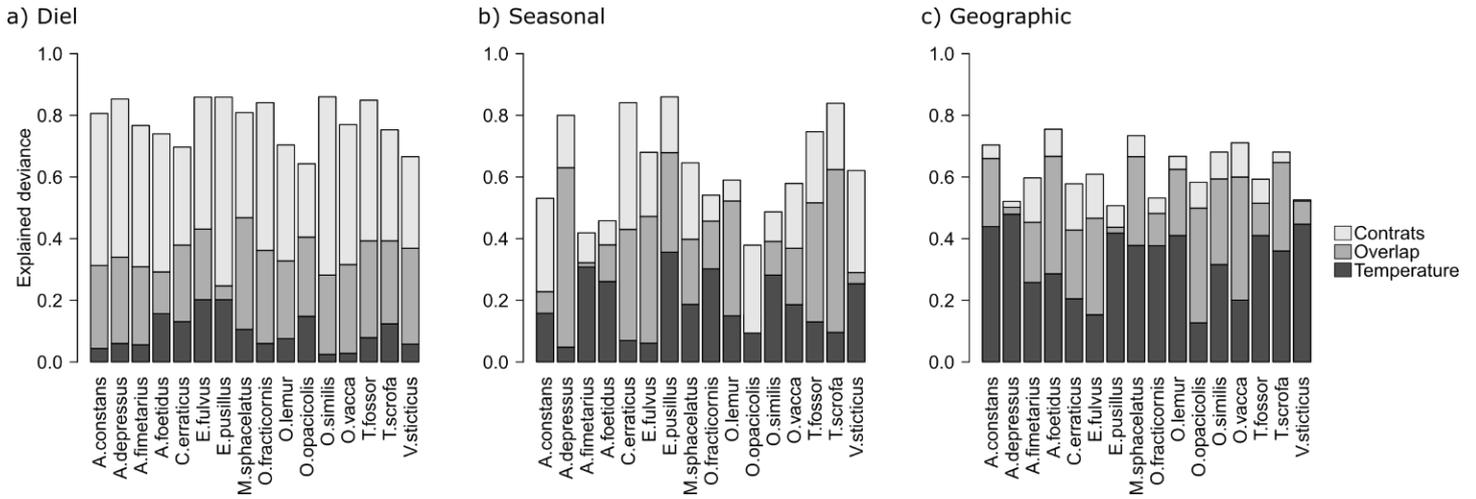
b) Temperature variations



**Figure 2. Thermal niche attributes and overlap measure.**  $x$  and  $y$  represent thermal response curves of two species or of a single species in two different study units (*i.e.*, days, elevation or river basins). From this curve we obtained the optimum temperature and the niche amplitude. Further, we used the overlap between them (a) and the two independent areas (b and c) to calculate the Simpson's dissimilarity index, as a measure of the congruence between the responses to temperature of the same species at different scales, and of different species within the same scale.



**Figure 3. Partial regression results.** The deviance explained by temperature alone, the contrast variables alone, and the overlap between them is shown. The contrast variables were minutes from dawn and its quadratic term for the diel data set (a); date sine and cosine and their quadratic terms for the seasonal data set (b), and temperature availability for the geographic data set (c).



## Supporting information

### Appendix S1. Extended data origin

We use data on 16 Iberian dung beetle species of the family Scarabaeidae (ten from Aphodiinae and six from Scarabaeinae subfamilies). These species were selected because they occurred in at least 10% of the samples of the three datasets considered (see below). All considered species (Table 1) are of small body size, with body weights far smaller than 1.9 g (0.2 g at most), the threshold from which endothermy is thought to appear in this group of beetles (Verdú et al. 2006). Temperature–distribution associations for all these species were examined along: (i) five geographical areas of similar extent but different temperature regimes within the Iberian Peninsula (geographical dataset or GD); (ii) six sites placed across a steep elevational range in Central Iberia, and sampled during the same dates but differing in their environmental temperatures (seasonal dataset or SD); and (iii) three consecutive days with similar daily variations but different weather conditions in a single locality near the centre of the same elevational range (diel dataset or DD).

*Geographical Dataset.* The GD is divided in five study areas, corresponding to the major river basins of the Iberian Peninsula (Ebro, Duero, Tajo, Guadiana and Guadalquivir; limits extracted from HydroBASINS data available at [www.hydrosheds.org](http://www.hydrosheds.org), Lehner & Grill 2013, Fig. 1a). These natural areas were used since their borders correspond with marked geographical accidents, which are expected to act as dispersal barriers. Furthermore, they are relatively similar in extent (areas ranging from  $5.6 \times 10^4$  to  $9.7 \times 10^4$  km<sup>2</sup>) and almost follow a latitudinal gradient, showing contrasting environmental temperatures (Fig. 1b). In each of these basins, we collected all georeferenced occurrences of the selected species from GBIF ([www.gbif.org](http://www.gbif.org), accessed May 2020) and additional published sources (Hortal & Lobo 2011). As this kind of data is biased due to historically uneven sampling effort (Lobo et al. 2018), the occurrences were pooled within UTM grid cells of 10 x 10 km spatial resolution. This grain was selected because it corresponds to the effective resolution of most of the occurrence information in the dataset, and it is appropriate to avoid the effects of oversampled localities while retaining a reasonable climatic detail. The frequency of each species' occurrence data in temperature bins of 1°C (ranging from -3 to 20°C, n=24) was calculated for each river basin ( $24 \times 5 = 120$ ), and these figures were used as dependent variables in the subsequent regression analyses.

*Seasonal dataset.* Six sites along an elevational gradient located in the Sierra de Guadarrama (Central Spain) (Fig. 1a, Espinoza 2016) were used to explore the effect of temperature variations in SD. Elevations range from 755 to 1900 m a.s.l., separating sites approximately 200 m a.s.l. Each survey site was sampled approximately every three weeks, totalling fourteen times from May 2012 to June 2013. We choose this elevation gradient because these sites show considerable variations in temperature during the whole period of the surveys (Fig. 1b). The sampling protocol in each periodical sample consisted of five pitfall-traps baited with fresh cattle dung and separated around 30 m from each other. Traps were placed in open habitats to avoid potential habitat and shadow effects and were active during 48 h. The individuals of these traps were pooled together, obtaining an estimation of each species' abundance per elevation site and date (6 x 14 = 84), which were used as response variables in subsequent statistical analyses.

*Diel Dataset.* Temperature effects on diel activity were assessed using dung beetle data from a grassland located next to El Ventorrillo MNCN field station, placed in the Sierra de Guadarrama at an approximate elevation of 1500 m a.s.l. (Fig. 1a). This locality was chosen as it shows a high diversity of dung beetles (between 30 and 40 species belonging to the considered subfamilies; Cuesta & Lobo 2019). We sampled three consecutive days (April 28<sup>th</sup>–30<sup>th</sup> 2015) that showed contrasting temperatures, with around 8 °C of difference between the mean temperatures of the coldest and the hottest days (Fig. 1b). Each day, ten pitfall traps baited with fresh cattle dung were distributed around a circumference of approximately 50 m. of radius (*i.e.*, traps were at least 30 m apart from each other). Since we intended to measure the flight activity during short periods, the bait was introduced into a nylon stocking piece to avoid the stagnancy of beetle individuals within the dung bait along different sampling events. We checked all traps every 30 min. from dawn to dusk (approximately from 7:30 am to 7:00 pm, n=23), collecting all individuals to subsequently identify them in the laboratory. Traps were also checked during the night to discard nocturnal activity. Individuals from the ten traps were pooled together, obtaining an estimation of the abundance of active individuals from each species each 30 min (23 x 3 = 69), which were further used as dependent variables.

#### Temperature measures and alternative correlates

Temperature measures were obtained from different standardized methods for each one of the different spatio-temporal scales considered, but trying to maintain a considerable

degree of congruence among them. For the *Geographical Dataset*, we obtained mean annual temperatures at a 30 sec resolution from the WorldClim database (see [www.worldclim.org](http://www.worldclim.org), Hijmans et al. 2005). We preferred mean annual temperatures over monthly average figures since the precise seasonal activity over the complete study area was unknown for most of the species. Nevertheless, spring and autumn temperatures (the seasons when phenological peaks occur for most species) were positively correlated with mean annual temperatures (Pearson's  $r = 0.99$  and  $0.97$ , respectively), so we assume that mean annual temperature is a reasonable proxy for both of them.

For the *Seasonal Dataset*, we set up a temperature data logger in each of the elevational points during the whole period of the study. This device was placed in the shadow at one meter from the ground to escape from extreme temperatures due to insolation, mimicking the meteorological stations on which WorldClim data are based on. Temperature was recorded each 10 min. and we used the mean daily temperature when pitfall-traps were active.

In the case of the *Diel Dataset*, temperature measurements were taken using five data loggers placed in the study site just in the centre of the circumference formed by the traps. Data loggers were placed to recover temperature measurements from the different microclimatic conditions available for dung beetles: two at one meter over the ground, in the sun and shadow; another two directly on the ground, also both in the sun and shadow; and one buried at 10 cm depth. Preliminary results showed that the mean temperatures from the data logger placed on the ground in the sun were those that best correlated with the species' diel activity, so we used these measurements in subsequent analyses. Temperature was recorded each minute, and average temperatures during the 30 min before traps were checked were used as predictors.

As previously stated, the effects of temperature measurements might be overestimated due to its collinearity with other factors with which it shares spatial (in the case of GD) or temporal structure (in the case of SD and DD). We quantified this potential overestimation effect by using different “contrast variables”, alternative predictors which are often partly correlated with temperature but are either measures or proxies of other potential causal factors for dung beetle spatial and temporal responses. These alternative predictors were temperature availability and survey effort in the case of GD, day of the year for SD, and hour of the day in the case of DD. The effect of temperature on the frequency of occurrence (GD) or abundance (SD and DD) that is independent of these contrast variables was assessed as the “pure” effect of temperature variations that is

independent of the range of temperatures available (GD), the period of the year (SD), and the hour of the day (DD) (see analytical methods below).

Temperature availability for each basin is the relative frequency of 10 x 10 km UTM cells in each 1°C temperature bin. This variable aims to represent the thermal spectrum available in each basin. Hence, a high explanatory capacity of this variable on the frequency of occurrence of a species would imply that the apparent thermal preference of this species can be simply because its spatial pattern of occurrence mimics the distribution of temperatures in the analyzed basin. Further, the typical correlation between the observed pattern of occurrence of a species and the spatial distribution of survey effort can also generate spurious correlations between species' frequency and temperature in each basin. This potential source of error was considered here by calculating the relative frequency for each 1°C temperature bin of all dung beetle records included in the formerly mentioned databases and pooled within the 10 x 10 km UTM cells. Nevertheless, we found that this estimation of survey effort and temperature availability were highly and positively correlated in all basins (Pearson's  $r$  ranging from 0.97 to 0.99), since the most frequent temperatures have been also surveyed more often, which implies that the surveys are randomly allocated within the available temperatures. Consequently, we discarded using survey bias as contrast variable, considering that the effect of temperature availability also includes differences in survey effort. In the case of SD data, the day of the year was obtained by first ordering the available dates from the day corresponding to the summer solstice (June 21<sup>th</sup> = 0 or 360), to subsequently convert these figures into radians and obtaining two circular variables by calculating their cosine and sine values. Thus, the summer-winter oscillation is represented by the cosine of the date and oscillates from 1 to -1, whereas the spring-autumn transition is represented by the sine of the date scale 1 to -1. Finally, the hour of the day (DD data) is simply codified as the number of minutes from dawn.

## **Appendix S2. Extended phylogenetic reconstruction**

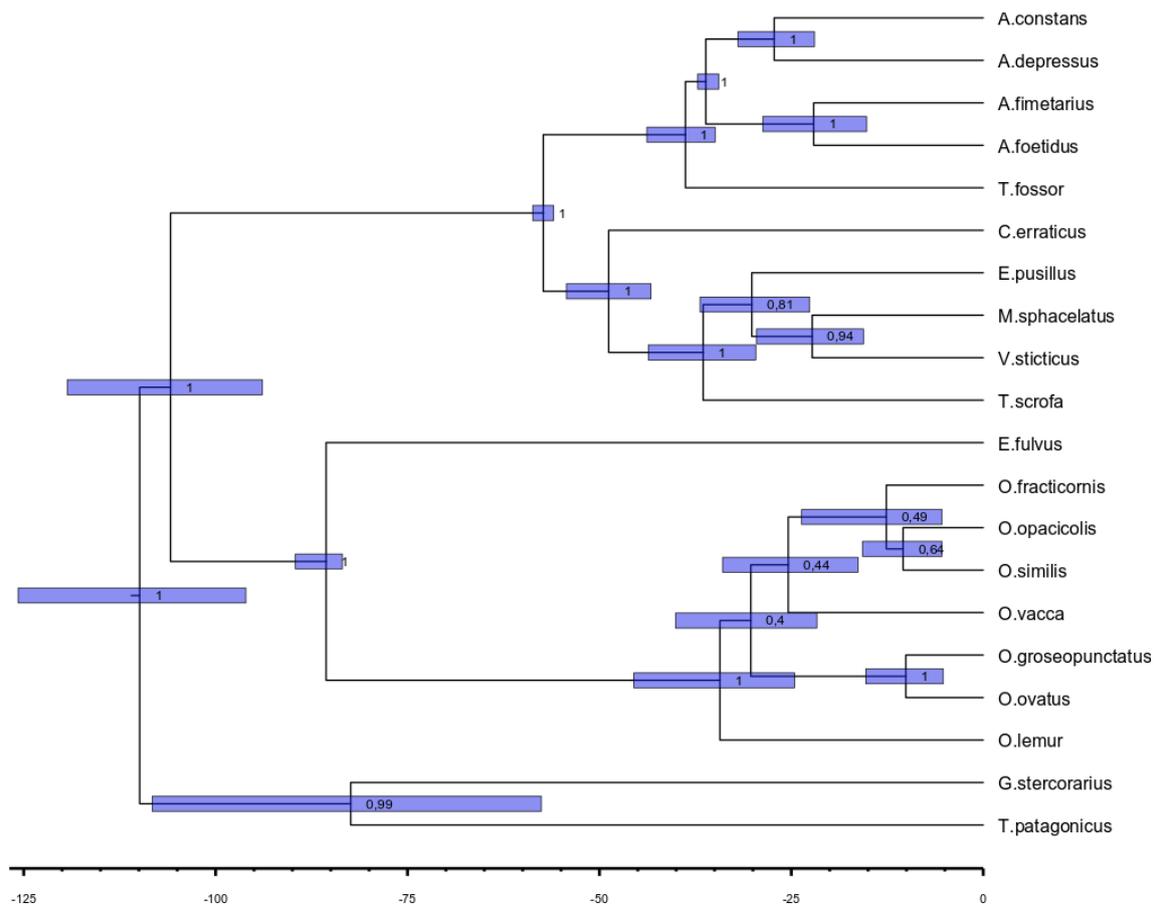
Genomic DNA was extracted from each individual using the BIOSPRINT 15 DNA Kit (Qiagen), following standard manufacturer's protocols for blood, and resuspended in 100 µl of buffer AE. We used COI Sca F, COI Sca R, COII am Sca and COII B 605 Sea (Villalba et al. 2002) and the universal 28S a y 28S 5b primers to amplify fragments of the mitochondrial cytochrome oxidase I (COI), the cytochrome oxidase II (COII) and the 28S genes. Amplifications for all gene fragments were performed in a 50 µl reaction

containing 39.7  $\mu\text{l}$  of  $\text{H}_2\text{O}$ , 5  $\mu\text{l}$  of 10x PCR buffer, 1  $\mu\text{l}$  of dNTP mix (10 mM), 0.5  $\mu\text{l}$  of each primer (10  $\mu\text{M}$ ), 0.3  $\mu\text{l}$  of AmpliTaq® DNA polymerase (Applied Biosystems) and 3  $\mu\text{l}$  of DNA template. Thermocycling conditions consisted of an initial denaturing step at 94 °C for 4 min, followed by two cycles: (i) a precycle of 5 amplification cycles of 94 °C for 45 sec, 40 °C for 1 min and 72 °C for 1 min, and (ii) a cycle of 35 amplification cycles of 94 °C for 45 sec, 44 °C for 1 min and 72 °C for 1 min, followed by a final elongation step at 72 °C for 10 min and a rapid thermal ramp down to 4 °C. For all reactions, the presence of amplicons of the expected sizes was checked by electrophoresis on a 0.8 % agarose gel. PCR products were purified with the ethanol-precipitation method (Sambrook et al., 1989). Sequencing was performed by Secugen S.L. (Madrid, Spain), using BigDye® and the automated ABI PRISM 3730xl DNA Analyzer. Sequence chromatograms were read and contigs assembled using Sequencher version 4.7 (Gene Codes Corporation, Ann Arbor, MI). All new sequences were deposited in GenBank (see accession numbers in Table S1).

Sequences were aligned in CLUSTALW and MUSCLE, followed by visual inspection using BioEdit (Hall, 1999). Prior to phylogenetic analysis, jModeltest 2.1.1 (Darriba et al., 2012) was used to choose the best-fit model of nucleotide substitution for each of the four genes, and for combined matrices under the corrected Akaike information criterion (AICc). For the COI and COII, HKY was obtained, while Jukes Cantor for 28S. Phylogenetic analyses were performed in a Bayesian framework using BEAST v 2.4 (Drummond and Rambaut, 2007). We established 3 calibrations points based on Ahrens et. al (2014), setting uniform priors with lower and upper boundaries. The calibrations represent the basal split of the following taxa: Aphodiinae (58.7 – 55.8 Million years ago), *Aphodius* (37.2 – 33.9 Mya) and Scarabaeinae (92 – 83.5 Mya). For the age of the rest of the nodes, we set a LogNormal relaxed molecular clock for each gene and let the software estimate the rate from the priors. The MCMC chain ran for 100.000.000 steps, sampled every 10.000 steps. Posterior distribution of all the parameters were checked using Tracer, as well as all ESS values being above 200. We built the tree using Tree Annotator, using the Maximum Clade Credibility implemented method after discarding the first 25% samples as a burn-in.

<b>Species</b>	<b>28</b>	<b>COI</b>	<b>COII</b>
<i>Aphodius constans</i>	-	AY039372	AY039372
<i>Aphodius depressus</i>	ABXXXX	ABXXXX	ABXXXX
<i>Aphodius erraticus</i>	ABXXXX	ABXXXX	ABXXXX
<i>Aphodius fimetarius</i>	ABXXXX	ABXXXX	ABXXXX
<i>Aphodius foetidus</i>	-	ABXXXX	ABXXXX
<i>Aphodius fossor</i>	ABXXXX	ABXXXX	ABXXXX
<i>Aphodius pusillus</i>	ABXXXX	ABXXXX	ABXXXX
<i>Aphodius scrofa</i>	ABXXXX	ABXXXX	ABXXXX
<i>Aphodius sphacelatus</i>	ABXXXX	ABXXXX	ABXXXX
<i>Aphodius sticticus</i>	-	ABXXXX	-
<i>Euoniticellus fulvus</i>	ABXXXX	ABXXXX	ABXXXX
<i>Geotrupes stercorarius (OUT)</i>	KP419463	AY039377	AY039377
<i>Onthophagus fracticornis</i>	ABXXXX	-	-
<i>Onthophagus grossepunctatus</i>	ABXXXX	AY039347	AY039347
<i>Onthophagus lemur</i>	ABXXXX	AY039353	AY039353
<i>Onthophagus opacicollis</i>	-	ABXXXX	ABXXXX
<i>Onthophagus ovatus</i>	ABXXXX	AY039351	AY039351
<i>Onthophagus similis</i>	ABXXXX	ABXXXX	ABXXXX
<i>Onthophagus vacca</i>	ABXXXX	AY039359	AY039359
<i>Taurocerastes patagonicus (OUT)</i>	KP419662	GU984611	GU984611

**Table S1.** GenBank accession numbers of the used sequences. Outgroup species are indicated.



**Fig. S1.** Bayesian phylogenetic hypothesis for the studied species. Posterior probabilities are provided. Blue bars represent the 95% credible interval around node ages.

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