Visible and near infrared radiation may be transmitted or absorbed differently by beetle elytra according to habitat preference

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Background: The exoskeleton of an insect could be an important factor in the success of its evolutionary process. This reaches its maximum expression in beetles, which constitute the most diversified animal taxon. The involvement in the management of environmental radiation could be one of the most important functions of the exoskeleton due to the passive contributions to the thermoregulation of body temperature. We study whether the elytra of two sympatric and closely related beetle species respond differentially to the radiation of distinct wavelengths in agreement with their ecological preferences.
Methods: *Onthophagus coenobita* (Herbst) and *O. medius* (Kugelaan) occupy different habitats and environmental conditions (shaded versus unshaded from solar radiation). The potential adaptive variations to thermoregulation under these different ecological conditions were studied using the responses of their exoskeletons to radiation of different wavelengths (ultraviolet, visible and near-infrared). For these two species, the amounts of the three wavelengths that were reflected, transmitted or absorbed by the exoskeleton were measured using of a spectrophotometer. In addition, the darkness and thickness of the elytra were examined to determine whether these two features influence the management of radiation by the exoskeleton. **Results:** Both species differ in the management of visible and near-infrared radiation. In agreement with habitat preferences, the species inhabiting shaded conditions would allow infrared and visible radiation to penetrate the elytra more easily to heat internal body parts, while the elytra of the heliophilous species would have increased absorbance of these same types of radiation. An increase in body size (and therefore in elytron thickness) and the quantity of dark spots may serve as barriers against exogenous heat gain. However, the maintenance of between-species differences independent of the effects of these two morphological features led us to suspect that an unconsidered elytron characteristic may also be affecting these differences. **Discussion:** The results of the involvement of the exoskeleton

thickness and spots in the thermoregulation of insects opens new research lines to obtain a better understanding of the function of the exoskeleton as a passive thermoregulation in Coleoptera.

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15 Running head: Elytra response to radiations

18 **ABSTRACT**

19 **Background:** The exoskeleton of an insect could be an important factor in the success of its

20 evolutionary process. This reaches its maximum expression in beetles, which constitute the most

21 diversified animal taxon. The involvement in the management of environmental radiation could

22 be one of the most important functions of the exoskeleton due to the passive contributions to the

23 thermoregulation of body temperature. We study whether the elytra of two sympatric and closely

24 related beetle species respond differentially to the radiation of distinct wavelengths in agreement

25 with their ecological preferences.

26 **Methods:** *Onthophagus coenobita* (Herbst) and *O. medius* (Kugelaan) occupy different habitats

27 and environmental conditions (shaded versus unshaded from solar radiation). The potential

28 adaptive variations to thermoregulation under these different ecological conditions were studied

29 using the responses of their exoskeletons to radiation of different wavelengths (ultraviolet,

30 visible and near-infrared). For these two species, the amounts of the three wavelengths that were

31 reflected, transmitted or absorbed by the exoskeleton were measured using of a

32 spectrophotometer. In addition, the darkness and thickness of the elytra were examined to

33 determine whether these two features influence the management of radiation by the exoskeleton.

34 **Results:** Both species differ in the management of visible and near-infrared radiation. In

35 agreement with habitat preferences, the species inhabiting shaded conditions would allow

36 infrared and visible radiation to penetrate the elytra more easily to heat internal body parts, while

37 the elytra of the heliophilous species would have increased absorbance of these same types of

38 radiation. An increase in body size (and therefore in elytron thickness) and the quantity of dark

39 spots may serve as barriers against exogenous heat gain. However, the maintenance of between-

40   species differences independent of the effects of these two morphological features led us to

41   suspect that an unconsidered elytron characteristic may also be affecting these differences.

42   **Discussion:** The results of the involvement of the exoskeleton thickness and spots in the

43   thermoregulation of insects opens new research lines to obtain a better understanding of the

44   function of the exoskeleton as a passive thermoregulation in Coleoptera.

45   **Subjects** Entomology, Thermal physiology, Ecology, Environmental Science.

46   **Keywords** Spectrophotometry, global radiation, thermoregulation**,** darkness, elytra thickness,

47   *Onthophagus*.

48

INTRODUCTION

The radiation emitted by the sun can be considered the ultimate cause of the functioning of biogeochemical cycles in nature, and the flux of energy created by this radiation is a decisive force that conditions the behavioural, ecological, morphological, metabolic and physiological characteristics of living organisms (*Hessen, 2008*; *Angilletta, 2009*). This phenomenon is especially true for the animals, such as insects, that depend on radiation and external temperatures to warm their internal parts, thus enhancing metabolic processes and increasing evolutionary rates (*Brown et al., 2004*).

The development of an external skeleton that protects and supports internal body parts is an essential feature of Coleoptera, which is the most diversified animal group on Earth (*Chapman, 2009*) and originated during the early Permian period (*Zhang et al., 2018*). Among the many functions attributed to the exoskeleton of Coleoptera (*Vincent & Wegst, 2004; Gorb, 2013*), some authors suggest that its structure and colour may help in controlling temperature (*Mikhailov, 2001*; *Ishay et al., 2003*; *Gross et al., 2004*; *Clusella-Trullas et al., 2007*; *Davis et al., 2008*; *Drotz et al. 2010*; *Roulin, 2014*; *Schweiger & Beirkuhnlein, 2016*). In a series of recent studies carried out on specimens of different species belonging to the Geotrupinae and Scarabaeinae subfamilies (Coleoptera, Scarabaeoidea), the existence of "passive thermoregulation" without associated energetic costs was proposed as a consequence of the interaction of the exoskeleton with different types of electromagnetic radiation (*Carrascal et al., 2017*; *Amore et al., 2017*; *Alves et al., 2018*). The obtained evidence indicates that there are interspecific differences in internal body temperatures when dry specimens are exposed dorsally to simulated sunlight (*Amore et al., 2017*), and that these internal temperatures are lower when these specimens are exposed to infrared radiation (*Carrascal et al., 2017*; *Amore et al., 2017*).

72   Thus, this temperature increase probably results from the transmittance and/or absorbance of

73   non-infrared wavelengths by the dorsal cuticle. The elytra of these species may absorb most of

74   the highly energetic radiation from the ultraviolet and visible parts of the spectrum and convert it

75   into body heat (*Alves et al., 2018, Pavlovic et al., 2018*). All these results suggest that the beetle

76   exoskeleton may allow for the "passive thermoregulation" of body temperatures. As the optima

77   body temperatures of individuals may tend to match the temperatures experienced in the

78   environmental conditions where they occur (*Bozinovic et al*., 2011; *Deatherage et* al., *2017*), it

79   can be hypothesized that differences in the structures and colours of exoskeletons may help

80   explain the ecological and biogeographical characteristics of these organisms, as well as to

81   understand their responses to climatic changes.

82   In this study, the reflectance, transmittance and absorbance of elytra to different

83   wavelengths of electromagnetic radiation are examined in two phylogenetically close

84   Scarabaeinae species that are locally sympatric and diurnal but differ in their environmental

85   preferences (shaded vs. open habitats). The main aim of this comparison is to verify whether

86   there might be a correspondence between the general environmental preferences of these two

87   species and their capacity to reflect, absorb or transmit radiation of different wavelengths.

88   Specifically, it is hypothesized that species inhabiting shaded conditions would be associated

89   with a higher capacity for elytra transmittance mainly in the infrared range, while heliophilous

90   species should have elytra that are able to cope with a high level of direct sunlight to minimize

91   the risk of overheating. We additionally aim to discern whether these possible interspecific

92   differences in the management of radiation can be attributed solely to differences in body mass

93   and darkness or if, alternatively, any other exoskeletal characteristic could be involved in the

94   thermoregulation mechanism.

95

MATERIAL & METHODS

Studied species

Individuals of *Onthophagus (Palaeonthophagus) coenobita* (Herbst, 1783) and *Onthophagus*

(Palaeonthophagus) medius (Kugelann, 1792) were used in this study. The used specimens of

these two species were collected within the El Ventorrillo field station with the required

permissions. The used specimens *O. medius* is a recently proposed taxa (*Rössner et al., 2010*)

that is very similar to *O. vacca* (Linnaeus, 1767) and can be accurately differentiated by using

mitochondrial DNA sequences and, to a lesser extent, by some subtle and overlapping

morphological characters among which elytra darkness stands out (*Roy et al., 2016*). In our case,

all the studied specimens were carefully selected according to non-overlapping morphological

character states (*Roy et al., 2016*), thus the specimens were unambiguously assigned to *O.*

medius. These two dung beetle species (Coleoptera; Scarabaeidae) are widely distributed across

the Palaearctic region. *O. coenobita* has a geographical distribution ranging from Spain to

Sweden and from Belgium to Turkmenistan. Although the knowledge of the geographic

distribution of *O. medius* is limited, the available data suggest that *O. vacca* and *O. medius*

overlap extensively in their distributions, although *O. medius* is assumed to be absent in North

Africa and present from Spain to Finland and Russia and from Great Britain to Kazakhstan

(*Rössner et al., 2010*; *Roy et al., 2016*).

Different studies (*Villalba et al. 2002*; *Roggero et al., 2017*; *Rössner et al., 2010*; *Roy et*

al., 2016) agree on the phylogenetic closeness of *O. coenobita* and *O. vacca* and therefore

between *O. coenobita* and *O. medius*. Both species also show clear ecological differences; *O.*

coenobita is frequently reported to be associated with forests and shaded localities and consemes

118   human dung, corpses and mushrooms, in addition to herbivore dung (*Goljan, 1953*; *Jessop,*

119   *1986*; *Lumaret, 1990*; *Martín-Piera & López-Colón, 2000*). Despite the lack of reliable data on

120   the environmental preferences of *O. medius*, the available information suggests that this species

121   has a similar ecology to *O. vacca*, which is associated with open green pastures and the

122   consumption of cow, horse or sheep dung, but with the seasonal activity mainly focused on the

123   warmest spring months (*Rössner et al., 2010*; *Roy et al., 2016*). A yearly non-published survey

124   conducted during 2017 and 2018 at the "El Ventorrillo" biological station (Madrid, Spain,

125   Lat: 40.75º, Long = -4.02º, ≈1430 m a.s.l) clearly indicates that these species do not overlap

126   environmentally but may partially coexist seasonally. While *O. medius* and *O. coenobita* do not

127   differ in their general midday daily activity, *O. coenobita* shows a marked preference for

128   woodland sites. Adults of *O. coenobita* are also active at higher air temperatures (approximately

129   21.8 ºC) than those of *O. medius* (approximately 16.8ºC), which is basically due to the early

130   seasonal occurrence of *O. medius* (mean seasonal occurrence around 11 May) compared with *O.*

131   *coenobita* (mean seasonal occurrence around 7 June).

132

133   **Body measurements and spectrophotometric analysis**

134   Ten individuals of each of the two taxa preserved in 70% ethanol were randomly selected from a

135   collection of 4,502 dung beetles belonging to 53 species collected at the "El Ventorrillo"

136   biological station during 2012-2013 (collection deposited in the Museo Nacional de Ciencias

137   Naturales of Madrid). After drying, each specimen was weighed using a Tx423L Shimadzu®

138   balance with a precision of 0.001 g. Subsequently, the left elytron of each specimen was

139   carefully removed with tweezers (Fig. 1) and mounted on black vinyl to estimate their

140   reflectance. The total area of each elytron and the proportion with dark spots were calculated

141   using ImageJ 1.52i software (Schneider et al., 2012; see Fig. 1). The area of the

142   spectrophotometer light beam falling perpendicular to the surface of the elytron was 10.89 mm^2,

143   thus the beam practically covered the entire area of the elytron (see supplementary data).

144   However, when the area of the elytron was slightly smaller than the area of the light beam, the

145   obtained reflectance measurements were corrected to subtract the part of the light that fell

146   outside of the elytron by using the following equation: $RE = RT-(Rv*Av)$, where RE is the

147   elytron reflectance, RT is the total obtained reflectance, Rv is the reflectance of the vinyl per mm^2

148   for the different wavelengths, and Av is the vinyl area not covered by the elytron (10.89- elytron

149   area). In the case of transmittance, such correction is unnecessary because the elytron is mounted

150   on an opaque metal plate with a hole smaller than the minor size of an elytron (3.301 mm^2).

151   The convexity of the elytra can be considered negligible in both species. The thickness of the left

152   edge of the elytron was also measured with a Nikon Measurescope 10 monocular stereo

153   equipped with a Nikon Digital Counter CM-6S (all measurements in mm). Each elytron was

154   measured on three different occasions by two researchers, and their data were averaged.

155   Reflectance (R; the return of the electromagnetic radiation from the surface of the elytra)

156   and transmittance (T; the passage of the electromagnetic radiation through the elytra) of the

157   external part of the left elytron (dorsal) were measured with a Shimadzu® UV-2600

158   spectrophotometer in the wavelength spectrum from 185 to 1400 nm (at 5-nm intervals). This

159   spectrophotometer is equipped with an integrating sphere (ISR-2600Plus) that is able to measure

160   the diffuse and specular reflectance of solid samples. In our case, the measurement conditions of

161   the optical system were adjusted to those needed to measure diffuse reflectance due to the

162   slightly rough characteristics of the elytral surface. Before each spectrophotometer measurement,

163   a white plate of barium sulfate was used to correct the baseline. The obtained data covering the

164   complete wavelength spectrum from 185 to 1400 nm were divided into three bands; ultraviolet

165   (UV; 185-385 nm), visible (VIS; 390-745 nm) and near-infrared (NIR; 750-1400 nm).

166   Absorbance (A; the transformation of the electromagnetic radiation received by the elytra into

167   internal energy) was estimated as A = 100-(T+R) (see *Kinoshita, 2008*). Thus, the values of T, R

168   and A were averaged to obtain only one value for each of the three bands as the response

169   variable to determine whether there was variation among elytra in response to the different

170   wavelengths emitted by the sun. The reflectance and transmittance of the internal sides of each

171   elytron (ventral) were also measured but in only the near-infrared range to estimate the possible

172   capacity of the elytra to reflect or transmit body heat generated by beetles. Each measurement

173   was repeated three times by two researchers (2 species x 10 individuals x 2 sides x 3

174   measurements = 120 measurements for transmittance and reflectance). The three repeated

175   measurements of transmittance and reflectance for each individual were averaged to obtain more

176   stable data that were not dependent on the position of the elytra or the sector sampled by the

177   spectrophotometer. As the immersion of the elytra in alcohol can modify spectrophotometer

178   measurements (e.g., by eliminating cuticular hydrocarbons), the UV, VIS and NIR reflectance

179   and transmittance values of five fresh elytra of *O. medius* were estimated before and after being

180   subjected to an immersion in 96º alcohol for sixteen days. Only the dorsal reflectance in the UV

181   band suggested an effect of alcohol soaking (*t* test = 2.81, df = 8; P = 0.02), although the

182   statistical significance of this relationship disappeared when a Bonferroni correction was applied

183   (mean UV reflectance ± SD; fresh elytra = 2.44 ± 0.18; alcohol elytra = 2.70 ± 0.11). If there was

184   a potential effect of the immersion in alcohol on elytra reflectance, we assume here that it was

185   relatively small and similar in the two considered species.

186

187   **Statistical analyses**

188   Between-taxa differences in elytron darkness (percentage of the elytron area that was dark) and

189   biometric variables (body mass, elytron area, and elytron thickness) were tested by means of

190   Student's *t*-tests considering that these variables follow a normal distribution (n = 10 for each

191   species), and the probability levels were corrected for unequal variances, if applicable. Darkness

192   was considered in these analyses because the melanic compounds responsible for darkness are

193   associated with the absorbance of shortwave radiation and the regulation of body heat (*Pinkert &*

194   *Zeuss, 2018*). Because the correlations between the three biometric variables were always

195   positive and high (Pearson *r* values oscillating from 0.83 to 0.96; P< 0.0001 in all cases), elytron

196   thickness was selected for further analyses assuming that a greater elytron thickness could

197   negatively affect the transmittance of radiation towards the interior of the body.

198   The variation between species (*O. coenobita* vs. *O. medius*) and between elytron sides

199   (internal vs. external; in the case of only NIR) in reflectance, transmittance and absorbance

200   (response variables) was examined by ANCOVAs using elytron thickness and elytron darkness

201   as covariates. In these analyses, the explanation of the additive main effects was obviated when

202   two-way interactions showed a relevant effect. Type III sums of squares were used to estimate

203   the partial effect of each explanatory variable once the effects of the other variables were

204   controlled for. The obtained standardized partial regression coefficients can be considered

205   unbiased estimates of the relative importance of predictors, even when they are highly correlated

206   (*Smith et al. 2009*). The effects of the species identity factor, including and excluding covariates,

207   were compared because their change in magnitude and/or sign may indicate the existence of

208   influential confounding or suppressor variables able to overestimate or underestimate the effect

209   of species identity (*Legendre & Legendre, 1998; Smith et al. 2009*).

210   The use of P-values as thresholds to discriminate significant and non-significant results is

211   increasingly questioned (*Halsey, 2019*), which is mainly due to their inability to inform about the

212   rate of false positives (*Colquhom, 2017*). As a consequence, we have abandoned here the use of

213   the terms "statistically significant" and "statistically non-significant", considering P-values as

214   indicators of the strength of the evidence of the studied relationships. Thus, Bonferroni corrected

215   P-values for multiple comparisons (3 wavelength ranges x 3 response variable; $0.05/9 = 0.006$)

216   were considered to identify "strong evidence" of relationships, while relationships with P-values

217   from 0.05 to 0.006 were considered "weak evidence". We checked for homoscedasticity and

218   normality in the residuals of these models. StatSoft's STATISTICA v12.0 was used for these

219   analyses

220

221   **RESULTS**

222   **Biometric and colour differences**

223   The average body mass (mg), elytron area (mm^2) and elytron thickness (μm) differed between *O.*

224   *coenobita* and *O. medius*, with higher values for the latter taxon (P<0.001, Table 1). The area of

225   dark pigmentation was also lower for *O. coenobita* than for *O. medius* (Table 1).

226

227   **General responses of elytra to wavelength spectrum**

228   The average values of reflectance, transmittance and absorbance across the examined wavelength

229   spectrum for both species and elytron sides (internal and external) are shown in Figure 2. On

230   average, the reflectance values were lower than the absorbance and transmittance values

231   throughout the complete wavelength spectrum, while absorbance was very high in the ultraviolet

232   and visible wavelength ranges.

233

The effect of elytron side

235   The interaction between species identity and the elytron side factor was highly unlikely to

236   explain the NIR reflectance, transmittance or absorbance (probabilities higher than 0.30 in all

237   cases), indicating that the effect of the elytra side was similar in the two species (Table 2). The

238   dorsal or ventral position of the elytra did not seem to influence the NIR reflectance values, but

239   strong evidence of the influence of elytra position on transmittance and absorbance existed. The

240   ventral NIR transmittance in *O. medius* (56.4 %; adjusted means) was higher than that in *O.*

241   *coenobita* (45.1%) and lower than those measured from the external side of the elytra (52.5%

242   and 37.5%, respectively). Elytra NIR absorbance also seems to be influenced by the elytron side

243   factor (Table 2) as it was lower in *O. medius* than in *O. coenobita* for both the internal side (30.3

244   % vs 45.8%) and the dorsal side (34.7% vs 53.5%). Interestingly, the addition of covariates in

245   the regression analyses changed the comparative transmittance and absorbance values of the two

246   species (Table 2). *O. medius* had lower percentages of NIR transmittance and higher percentages

247   of NIR absorbance, both dorsally and ventrally, than *O. coenobita* when the raw data were

248   considered. However, this comparative situation was reversed when the effect of elytron

249   thickness and darkness was considered (Table 2).

250

The role of thickness and darkness

252   Our analyses support the existence of strong evidence of the role of elytron thickness in dorsal

253   NIR transmittance and absorbance and weak evidence of the effect of this covariate on NIR

254   reflectance and visible transmittance (Table 2). Thus, the transmittance of the NIR radiation

255   decreased and the NIR absorbance increased when the elytron thickness was higher (according to

256   the signs of the standardized coefficients); a thicker elytron thickness obstructed the penetration

257   of infrared radiation but facilitated its absorbance. The effect of thicker elytra in increasing NIR

258   reflectance and diminishing the transmission of visible radiation should be viewed with caution.

259   Darkness seems to be the most influential covariate (*i.e.*, highest absolute values of the

260   standardized regression coefficients), showing strong evidence of being an influential variable in

261   explaining the variation in NIR and visible transmittance and absorbance (Table 2). Additionally,

262   the possibility should not be discounted that darker elytra reduce the reflectance of visible

263   radiations. Thus, darker elytra block the passage of infrared and visible radiation, but they favour

264   the absorption of these types of radiation.

265

266   **Interspecific differences**

267   Our results provide strong evidence that both species differ in the dorsal transmission and

268   absorbance of NIR and visible radiations and weak evidence of interspecific differences in the

269   dorsal reflectance of these radiation types (Table 2). The elytra of *O. medius* seem to have a

270   higher capacity to prevent the passage of these two types of radiation, while the elytra of *O.*

271   *coenobita* would better absorb these same types of radiation when the effect of the studied

272   covariates is considered. Again, the addition of elytron thickness and elytron darkness in the

273   regression analyses reversed the comparative transmittance and absorbance values of the two

274   species (Table 2).

275

276   **DISCUSSION**

277   This research aims to assess whether the characteristics of the elytral exoskeleton may contribute

278   to facilitating the thermoregulation of beetles by differentially transmitting, absorbing or

279   reflecting radiation of distinct wavelengths in correspondence with the environmental

280   preferences of the species. The results obtained in this study support this assumption, although

281   more evidence will be needed to clearly discern the extent of the passive role of the beetle

282   exoskeleton in thermal performance. Thus, although the observed disparities can be associated

283   with biometric and darkness differences, the thermal performance of elytra is consistent with the

284   expectations.

285   Our results agree with those of previous studies (*Carrascal et al., 2017*; *Amore et al.,*

286   *2017*; *Alves et al., 2018*) in that elytron reflectance is minimal, transmittance of infrared radiation

287   is very high, and most of the ultraviolet and visible radiation is absorbed by the elytra. All these

288   exoskeletal characteristics are consistent with the requirements of an ectothermic organism that

289   spends a good deal of time in the soil and would need to obtain body heat from the surrounding

290   infrared and visible radiation (see also *Pavlovic et al., 2018*). Thus, elytra seem to be highly

291   transparent to the heat coming from the sun or the environment but opaque to the most energetic

292   wavelengths capable of causing harmful effects (*Beresford et al., 2013*). Of course, this pattern

293   may vary in those insects exposed to the extreme temperature conditions of deserts in which

294   large parts of visible and near-infrared radiation are reflected (*Shi et al., 2015*). On the other

295   hand, and in agreement with previous results (*Alves et al., 2018*), elytral transparency to infrared

296   radiation seems to be slightly higher on the inside part of the elytron than on the outside part,

297   suggesting that the elytra can be slightly more effective at facilitating the removal of body heat in

298   these dung beetle species. In a recent paper, Pavlovic *et al.* (2018) demonstrated that short (1400-

299   3000 nm) and mid (3000-8000) infrared wavelengths, which are mostly absorbed by atmospheric

300   gasses (*Eltbaakh et al., 2011*), can be used to dissipate body heat. Further studies are needed to

301   assess whether the transmittance of these infrared wavelengths is especially high from the

302   internal side of the elytra.

303   As we expected, interspecific differences in the thermal role of the exoskeleton are

304   clearly mediated by biometric and colour characteristics, as exemplified by the effects of elytron

305   thickness and area of dark pigmentation in our analyses. Elytron thickness and especially elytron

306   darkness seem to be particularly relevant in preventing the entry of NIR and visible radiation into

307   the beetle body but also in absorbing these types of radiation. The species with lighter and

308   thinner elytra, which inhabit areas with shaded conditions (*O. coenobita*), would allow these

309   types of radiation to penetrate the elytra more easily to heat their internal body parts. Quite the

310   contrary, the species with darker and thicker elytra (*O. medius*), which inhabited sunny areas,

311   appears to be better able to absorb infrared and visible radiation. These results are in agreement

312   with those of a recently published study on the thermal capacity of the elytra of the saproxylic

313   beetle *Rosalia alpina* (Linnaeus, 1758) (*Pavlovic et al., 2018*), which inhabits the sun-exposed

314   forest along the Euro-Caucasian region. The black patches present in the elytra of this species are

315   also able to absorb visible radiation to heat its body, but the elytra also serve to quickly transmit

316   the infrared radiation to attain thermal equilibrium.

317   The effects of these physical or physicochemical attributes become so important that they

318   may even reverse the sign of the factor representing species identity. This statistical result has

319   been long recognised and is especially frequent when dealing with correlated predictors (*Leamer,*

320   *1975*). In biological and environmental data, the use of non-independent explanatory variables is

321   the norm rather than the exception and not including a valuable predictor because it is correlated

322   with others may imply under or overestimations of the effects of the considered predictors (*Smith*

323   *et al., 2009*). In our case, the inclusion of covariates in the models reversed the comparative

324   transmittance and absorbance values that could be obtained for the two considered species.

325   Therefore, as the species identity factor continued to be relevant when elytron darkness and

326   thickness were considered, it could not be excluded that some additional and unknown morpho-

327   structural differences may also be relevant in explaining the detected interspecific differences in

328   the capacity of the elytra to manage radiations. Notwithstanding the above, caution is required

329   when determining the comparative roles of correlated features such as elytron thickness and

330   darkness. Additional studies are thus needed to cover a broad range of species with different

331   degrees of darkening and elytron thicknesses to better discriminate the comparative roles of

332   biometric and colour characteristics on the thermal performance of the beetle elytral cuticle.

333   The maintenance of the strength of the exoskeleton with the increase in body size may

334   imply increasing thickness both allometrically and isometrically (*Evans & Sanson, 2005*; *Lease*

335   *& Wolf, 2010*). Thus, an increase in the body size of dung beetles can provide extra advantages

336   in open habitats by avoiding the internal overheating of the body under sunny conditions.

337   Similarly, the darkening of the exoskeleton could be partially considered an evolutionary

338   strategy to diminish heat transmission into the body. This supposition collides with the thermal

339   melanism hypothesis, which predicts that a darker colour may be advantageous in colder

340   environments (*Kalmus, 1941*; *Schweiger and Beierkuhnlein, 2016*, *Galván et al. 2018*) but could

341   explain why desert beetles are often dark (Turner & Lombard, 1990). In our case, the darkest

342   elytra seem to make the access of infrared and visible radiations into the body more difficult,

343   also facilitating the absorbance of these types of radiation; however, the elytra do not influence

344   the management of UV radiation. As the transmittance and absorbance of solar radiation may

345   vary between the elytral parts with different colours and structures (*Pavlovic et al., 2018*), the

346 future use of microspectrometry will be recommended to further assess the specific role of black

347 patches in thermal balance.

348   As in the case of body size, the reduced transmittance of near-infrared radiation by the

349 dark specimens of our two considered species may be a strategy to avoid overheating under some

350 circumstances. Considering that more than 50% of the total sunlight incident energy corresponds

351 to this wavelength spectrum (*Stuart-Fox et al., 2017*), the management of near-infrared radiation

352 by the beetle exoskeleton should be considered. In this case, darkness can affect thermal

353 performance due to its effect on longwave radiation, which is invisible to the human eye (*Stuart-*

354 *Fox et al., 2017*). Additional experiments are needed to better estimate whether the elytra of

355 different beetle species differ in their capacities to manage distinct wavelengths and discern the

356 comparative roles of body size and darkness in the thermoregulation of beetles.

357

358 **CONCLUSIONS**

359 The main hypothesis of this research has been that the elytra of two closely related beetle species

360 will manage environmental radiation differentially in agreement with their contrasting

361 environmental preferences. Thus, the results are in line with what was expected because the

362 elytra of the species inhabiting areas under shaded conditions (*O. coenobita*) allow the entry of

363 infrared and visible radiation more easily, while the heliophilous species (*O. medius*) would

364 better absorb these same types of radiation. These differences are determined largely by the

365 thickness and darkness of the elytra, but we cannot rule out the role that other unknown factors

366 could play in these differences. Further and more comprehensive studies are needed to

367 corroborate the role of the elytral exoskeleton as a mechanism of "passive thermoregulation".

368

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371

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376

377   **Competing Interests**

378   The authors declare there are no competing interests.

379

380   **Ethical approval**

381   Beetle collection was conducted with relevant permissions provided by the Comunidad de

382   Madrid (Dirección General de Medio Ambiente), considering all applicable international and

383   national guidelines for the care and use of animals.

384

385   **Author Contributions**

386   Both authors participated in the design of the study, carried out spectrophotometric analyses,

387   participated in data analysis, and drafted the manuscript.

388

389

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505

506

Morphometric values of *Onthophagus coenobita* and *O. medius*.

Mean and standard deviation (sd) of the considered darkness and biometrical variables among *O. coenobita* (N=10) and *O. medius* (N=10) specimens. Student's *t*-tests, corrected for unequal variances, were used to establish statistical differences in these parameters between the two species.

1 **Table 1**:

2 **Morphometric values of *Onthophagus coenobita* and *O. medius*.** Mean and standard deviation

3 (sd) of the considered darkness and biometrical variables among *O. coenobita* (N=10) and *O.*

4 *medius* (N=10) specimens. Student's *t*-tests, corrected for unequal variances, were used to

5 establish statistical differences in these parameters between the two species.

	O. coenobita		*O. medius*			
	mean	sd	mean	sd	*t*	P
Body mass (mg)	52.70	12.68	104.90	33.43	4.62	<0.001
Elytral area (mm^2)	8.79	1.35	13.11	1.94	5.78	<0.001
Elytral thickness (µm)	82.10	7.52	100.10	9.90	4.58	<0.001
% Darkness	3.68	1.83	24.59	5.20	12.00	<0.001

ANCOVAs results using species identity (*Onthophagus coenobita* and *O. medius*) and elytron side as factors and elytron thickness and elytron darkness as covariates to estimate its effects on reflectance (R), transmittance (T) and absorbance (A)

β are the standardized regression coefficients obtained in the regression analyses representing the comparative magnitude and sign of the predictor variables. Results including elytron side and the interaction species x side are only estimated in the case of NIR (*β* is negative if the average of the internal side is higher than that for the external side). In the case of the species factor *β* is negative if the average of *O. medius* is higher than that for *O. coenobita*. Those relationships showing P-values equal or lower than a Bonferroni corrected P-value for multiple comparisons (0.05/9=0.006) are considered as "strong evidences" (in underlined bold), while relationships with P-values from 0.05 to 0.006 are considered as "weak evidences" (in bold). The two first columns represent average dorsal R, T or A percentages for each species taking into account raw data (first figure) and adjusted means taking into account the effect of the covariates estimated considering that the effect of the covariates is zero in the two species (second figure).

1   **Table 2**:

2   **ANCOVAs results using species identity (*Onthophagus coenobita* and *O. medius*) and**

3   **elytron side as factors and elytron thickness and elytron darkness as covariates to estimate**

4   **its effects on reflectance (R), transmittance (T) and absorbance (A) for three different**

5   **wavelength ranges (NIR = near infrared from 750 to 1400 nm; VIS= visible from 390 to 745**

6   **nm; and UV = ultraviolet from 185 to 385 nm).** β are the standardized regression coefficients

7   obtained in the regression analyses representing the comparative magnitude and sign of the

8   predictor variables. Results including elytron side and the interaction species x side are only

9   estimated in the case of NIR (β is negative if the average of the internal side is higher than that

10   for the external side). In the case of the species factor β is negative if the average of *O. medius* is

11   higher than that for *O. coenobita*. Those relationships showing P-values equal or lower than a

12   Bonferroni corrected P-value for multiple comparisons (0.05/9=0.006) are considered as "strong

13   evidences" (in underlined bold), while relationships with P-values from 0.05 to 0.006 are

14   considered as "weak evidences" (in bold). The two first columns represent average dorsal R, T or

15   A percentages for each species taking into account raw data (first figure) and adjusted means

16   taking into account the effect of the covariates estimated considering that the effect of the

17   covariates is zero in the two species (second figure).

	O.coenobita	*O. medius*	Species	Elytron thickness	Elytron darkness	Elytron side	Species x side	R^2
R-NIR	10.0/9.0	11.8/12.8	$F_{1,34}$ = 4.41, P=0.04 β = -0.883	$F_{1,34}$ = 5.42, P=0.03 β = 0.400	$F_{1,34}$ = 2.95, P=0.10 β = -0.722	$F_{1,34}$ = 0.21, P=0.65 β = -0.063	$F_{1,34}$ = 0.13, β = 0.049	34.12%
T-NIR	46.4/37.5	43.5/52.5	$F_{1,34}$ = 12.85, β = -0.973	$F_{1,34}$ = 29.76, β = -0.605	$F_{1,34}$ = 14.53, β = -1.031	$F_{1,34}$ = 22.74, β = -0.428	$F_{1,34}$ = 2.31, β = -0.136	72.61%
A-NIR	43.6/53.5	44.7/34.7	$F_{1,34}$ = 15.23, β = 1.261	$F_{1,34}$ = 12.36, β = 0.465	$F_{1,34}$ = 15.33, β = 1.264	$F_{1,34}$ = 17.35, β = 0.445	$F_{1,34}$ = 1.23, β = 0.118	61.14%
R-VIS	4.2/3.1	4.7/5.8	$F_{1,16}$ = 6.10, P=0.03 β = -1.519	$F_{1,16}$ = 0.99, P=0.33 β = 0.250	$F_{1,16}$ = 5.63, P=0.03 β = -1.458			33.78%
T-VIS	17.0/9.0	14.0/22.0	$F_{1,16}$ = 9.69, P=0.007 β = -1.470	$F_{1,16}$ = 5.85, P=0.03 β = -0.467	$F_{1,16}$ = 12.01, β = -1.636			60.94%
A-VIS	78.8/87.9	81.3/72.2	$F_{1,16}$ = 11.93, β = 1.674	$F_{1,16}$ = 3.92, P=0.06 β = 0.393	$F_{1,16}$ = 14.10, β = 1.819			58.85%
R-UV	1.8/1.9	2.5/2.5	$F_{1,16}$ = 1.63, P=0.22 β = -0.611	$F_{1,16}$ = 0.51, P=0.49 β = 0.139	$F_{1,16}$ = 0.03, P=0.87 β = 0.078			59.94%
T-UV	1.1/0.4	2.2/2.9	$F_{1,16}$ = 0.83, P=0.38 β = -0.652	$F_{1,16}$ = 0.17, P=0.68 β = -0.122	$F_{1,16}$ = 0.22, P=0.66 β = -0.324			10.02%
A-UV	97.0/97.7	95.3/94.7	$F_{1,16}$ = 1.24, P=0.28 β = 0.752	$F_{1,16}$ = 0.09, P=0.76 β = 0.085	$F_{1,16}$ = 0.18, P=0.67 β = 0.290			20.01%

Figure 1

Habitus and elytra of *Onthophagus coenobita* (A) and *O. medius* (B).

The left elytra was removed, showing coloured external and pale internal sides.
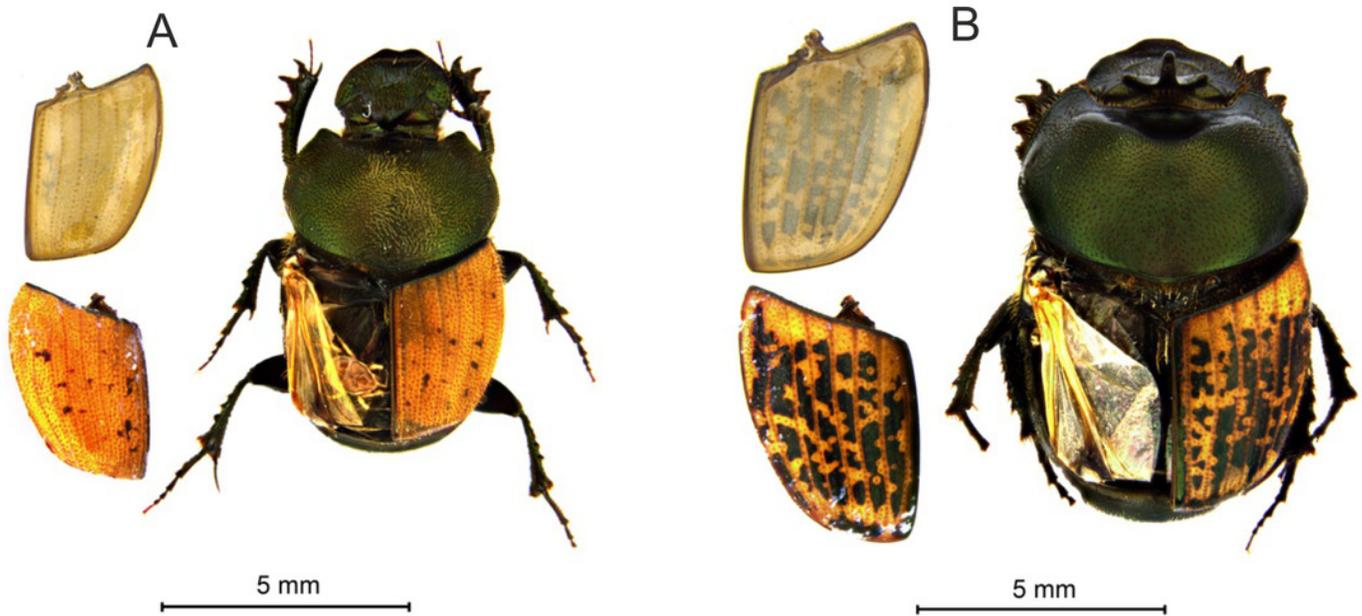
Figure 2

Spectrophotometric graphs.

Mean absorbance (ABS), transmittance (TRA) and reflectance (REF) from 185 to 1400 nm of ten individuals of *O. coenobita* (A, B) and *O. medius* (C, D), both for the external (A, C) and the internal sides of the elytra (B, D). The comparison between the two species was facilitated by including a thin broken line representing the transmittance pattern of *O. medius* in the plot of *O. coenobita*. The peak observed at 830 nm is due to the automatic detector change wavelength (the photomultiplier and the InGaAs detector).