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.

1   **Visible and near-infrared radiation may be transmitted or absorbed differently by beetle**

2   **elytra according to habitat preference**

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15   Running head: Elytra response to radiations

16

17

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*). First, heating experiments under controlled conditions on dried specimens of 13 species of Palaearctic Geotrupidae (*Carrascal et al.,* 2017) and seven Neotropical Scarabaeinae species (*Amore et al., 2017*) were carried out. The evidence obtained in these studies suggested that there are interspecific differences in internal body

72   temperatures when these specimens are exposed dorsally to simulated sunlight (*Amore et al.,*

73   *2017*), and also that these internal temperatures are lower when these specimens are exposed to

74   infrared radiation (*Carrascal et al., 2017*; *Amore et al., 2017*). In a subsequent step (*Alves et al.,*

75   *2018*), a spectrophotometric analysis was used to examine if the reflectance, transmittance and

76   absorbance of the elytra exoskeleton to different wavelengths (ultraviolet, visible and near-

77   infrared) could help to explain the obtained patterns in the internal body heat of the beetles (what

78   can be the main source of body heat?). Thus, analysing the elytra of five Neotropical

79   Scarabaeinae species of the genus *Canthon* a similar spectrophotometric pattern was obtained:

80   the light from shorter wavelengths is almost entirely absorbed by the elytra, while radiation from

81   longer wavelengths can mostly pass through the elytra. Consequently, this temperature increase

82   probably results from the transmittance and/or absorbance of non-infrared wavelengths by the

83   dorsal cuticle. The elytra of these species could absorb most of the highly energetic radiation

84   from the ultraviolet and visible parts of the spectrum and convert it into body heat (*Alves et al.,*

85   *2018, Pavlovic et al., 2018*). All these results suggest that the beetle exoskeleton may allow for

86   the "passive thermoregulation" of body temperatures. As the optima body temperatures of

87   individuals may tend to match the temperatures experienced in the environmental conditions

88   where they occur (*Bozinovic et al*., 2011; *Deatherage et* al., *2017*), it can be hypothesized that

89   differences in the structures and colours of exoskeletons may help explain the ecological and

90   biogeographical characteristics of these organisms, as well as to understand their responses to

91   climatic changes.

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

93   wavelengths of electromagnetic radiation are examined in two phylogenetically close

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

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

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

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

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

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

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

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

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

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

104 thermoregulation mechanism.

105

106 **MATERIAL & METHODS**

107 **Studied species**

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

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

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

111 permissions provided by the Consejería de Medio Ambiente y Ordenación de Territorio of the

112 Comunidad de Madrid (approval number 10/069528.9/18). The used specimens *O. medius* is a

113 recently proposed taxa (*Rössner et al., 2010*) that is very similar to *O. vacca* (Linnaeus, 1767)

114 and can be accurately differentiated by using mitochondrial DNA sequences and, to a lesser

115 extent, by some subtle and overlapping morphological characters among which elytra darkness

116 stands out (*Roy et al., 2016*). In our case, all the studied specimens were carefully selected

117 according to non-overlapping morphological character states (*Roy et al., 2016*), thus the

118   specimens were unambiguously assigned to *O. medius*. These two dung beetle species

119   (Coleoptera; Scarabaeidae) are widely distributed across the Palaearctic region. *O. coenobita* has

120   a geographical distribution ranging from Spain to Sweden and from Belgium to Turkmenistan.

121   Although the knowledge of the geographic distribution of *O. medius* is limited, the available data

122   suggest that *O. vacca* and *O. medius* overlap extensively in their distributions, although *O.*

123   *medius* is assumed to be absent in North Africa and present from Spain to Finland and Russia

124   and from Great Britain to Kazakhstan (*Rössner et al., 2010*; *Roy et al., 2016*).

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

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

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

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

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

130   *1986*; *Lumaret, 1990*; *Martín-Piera & López-Colón, 2000*). Despite the lack of reliable data on

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

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

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

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

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

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

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

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

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

140   21.8 ºC) than those of *O. medius* (approximately 16.8ºC), which is basically due to the early

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

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

143

144   **Body measurements and spectrophotometric analysis**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

196   relatively small and similar in the two considered species.

197

198   **Statistical analyses**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

230   analyses

231

RESULTS

Biometric and colour differences

The average body mass (mg), elytron area (mm^2) and elytron thickness (μm) differed between *O. coenobita* and *O. medius*, with higher values for the latter taxon (P<0.001, Table 1). The area of dark pigmentation was also lower for *O. coenobita* than for *O. medius* (Table 1).

General responses of elytra to wavelength spectrum

The average values of reflectance, transmittance and absorbance across the examined wavelength spectrum for both species and elytron sides (internal and external) are shown in Figure 2. On average, the reflectance values were lower than the absorbance and transmittance values throughout the complete wavelength spectrum, while absorbance was very high in the ultraviolet and visible wavelength ranges.

The effect of elytron side

The interaction between species identity and the elytron side factor was highly unlikely to explain the NIR reflectance, transmittance or absorbance (probabilities higher than 0.30 in all cases), indicating that the effect of the elytra side was similar in the two species (Table 2). The dorsal or ventral position of the elytra did not seem to influence the NIR reflectance values, but strong evidence of the influence of elytra position on transmittance and absorbance existed. The ventral NIR transmittance in *O. medius* (56.4 %; adjusted means) was higher than that in *O. coenobita* (45.1%) and lower than those measured from the external side of the elytra (52.5% and 37.5%, respectively). Elytra NIR absorbance also seems to be influenced by the elytron side factor (Table 2) as it was lower in *O. medius* than in *O. coenobita* for both the internal side (30.3

255 % vs 45.8%) and the dorsal side (34.7% vs 53.5%). Interestingly, the addition of covariates in

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

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

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

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

260 thickness and darkness was considered (Table 2).

261

262 **The role of thickness and darkness**

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

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

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

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

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

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

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

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

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

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

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

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

275 the absorption of these types of radiation.

276

277 **Interspecific differences**

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

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

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

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

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

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

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

285 species (Table 2).

286

287 **DISCUSSION**

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

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

290 reflecting radiation of distinct wavelengths in correspondence with the environmental

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

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

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

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

295 expectations.

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

297 *2017*; *Alves et al., 2018*) in that elytron reflectance is minimal, transmittance of infrared radiation

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

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

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

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

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

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

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

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

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

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

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

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

310   3000 nm) and mid (3000-8000) infrared wavelengths, which are mostly absorbed by atmospheric

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

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

313   internal side of the elytra.

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

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

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

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

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

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

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

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

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

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

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

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

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

327   the infrared radiation to attain thermal equilibrium.

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

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

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

331   *1975*). In biological and environmental data, the use of non-independent explanatory variables is

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

358   patches in thermal balance.

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

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

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

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

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

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

365   *Fox et al., 2017*). However, *Pantelić et al. (2017)* highlight the low capacity of melanin to absorb

366   the infrared radiation in a dusk moth species, which could indicate that the structural component

367   in which the pigment is embedded could influence this response. Additional experiments are

368   needed to better estimate whether the elytra of different beetle species differ in their capacities to

369   manage distinct wavelengths and discern the comparative roles of body size and darkness in the

370   thermoregulation of beetles.

371

CONCLUSIONS

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

374   will manage environmental radiation differentially in agreement with their contrasting

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

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

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

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

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

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

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

382

391

392 **Competing Interests**

393 The authors declare there are no competing interests.

394

395 **Ethical approval**

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

397 Madrid (Dirección General de Medio Ambiente; approval number 10/069528.9/18), considering

398 all applicable international and national guidelines for the care and use of animals.

399

400 **Author Contributions**

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

402 participated in data analysis, and drafted the manuscript.

403

404

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Table 1(on next page)

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

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Table 2 (on next page)

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).

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

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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.
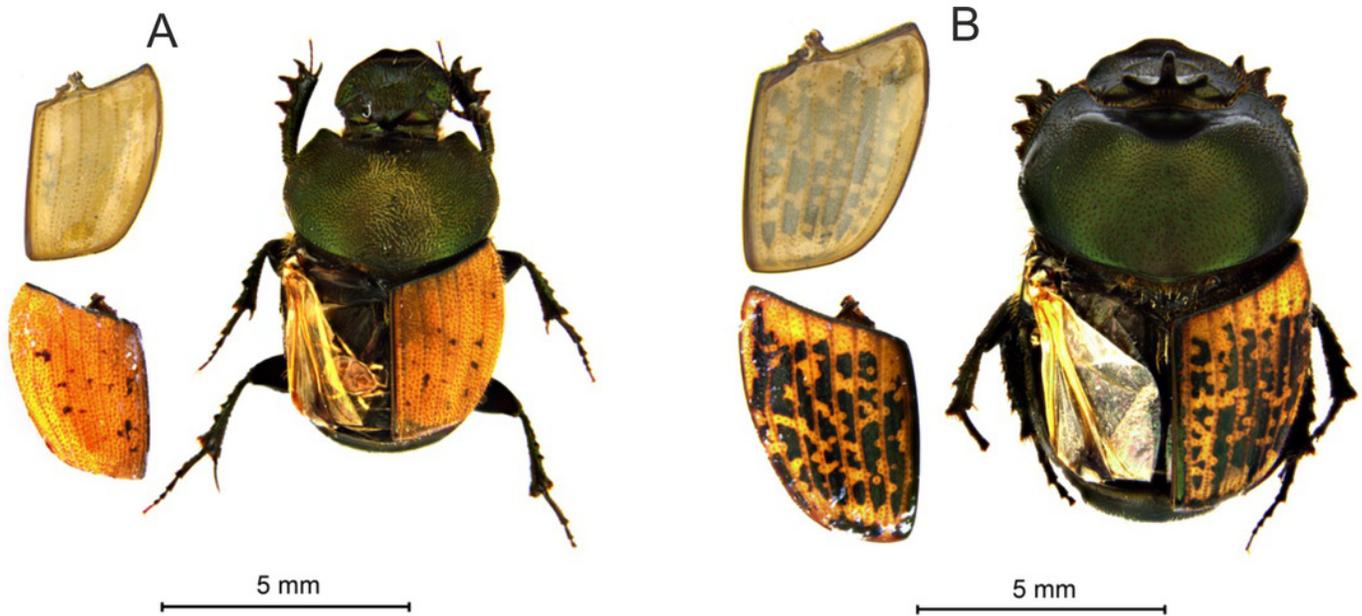

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Figure 2

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).