# Ultraviolet and near infrared radiation may be reflected or transmitted differently by beetle elytra according to habitat preference

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**Background:** The exoskeleton of insects could be an important factor in the success of its evolutionary process. This reaches its maximum expression in beetles which constituting the most diversified animal taxon. Its involvement in the management of environmental radiation could be one of the most important functions of the exoskeleton by passively contributing to the thermoregulation of body temperature. We study here whether the elytra of two sympatric and close 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). Potential adaptive variations to thermoregulation under these different ecological conditions were studied using responses of their exoskeletons to radiation of different wavelengths (ultraviolet, visible and near-infrared). For these two species, amounts of the three wavelengths, reflected, transmitted or absorbed by the exoskeleton were measured by use of a spectrophotometer. In addition, the darkness and thickness of the elytra were examined to determine if these two features influence the management of radiation by the exoskeleton. **Results:** Both species differ in the management of ultraviolet and near-infrared radiation. In agreement with habitat preferences, the species inhabiting shaded conditions would reflect less ultraviolet radiation than the sun-exposed one but would allow infrared radiation to penetrate the elytra more easily in order to heat internal body parts. 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 independently 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 found on the involvement of exoskeleton thickness and spots in thermoregulation of insects opens new research lines to a better understanding of the function of the exoskeleton as a passive thermoregulation

in Coleoptera.

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Running head: Elytra response to radiations

# ABSTRACT

**Background:** The exoskeleton of insects could be an important factor in the success of its evolutionary process. This reaches its maximum expression in beetles which constituting the most diversified animal taxon. Its involvement in the management of environmental radiation could be one of the most important functions of the exoskeleton by passively contributing to the thermoregulation of body temperature. We study here whether the elytra of two sympatric and close related beetle species respond differentially to the radiation of distinct wavelengths in agreement with their ecological preferences.

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**Results:** Both species differ in the management of ultraviolet and near-infrared radiation. In agreement with habitat preferences, the species inhabiting shaded conditions would reflect less ultraviolet radiation than the sun-exposed one but would allow infrared radiation to penetrate the elytra more easily in order to heat internal body parts. 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 independently 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 found on the involvement of exoskeleton thickness and spots in thermoregulation of insects opens new research lines to a better understanding of the function of the exoskeleton as a passive thermoregulation in Coleoptera.

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

**Keywords** Spectrophotometry, global radiation, thermoregulation**,** darkness, elytra thickness, *Onthophagus*.

# 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 propitiated by this radiation must have been a decisive force conditioning the behavioural, ecological, morphological, metabolic and physiological characteristics of living organisms (*Hessen, 2008*; *Angilletta, 2009*). This should be especially true for those animals that, like insects, depend basically on radiation and external temperature to warm their internal parts, thus enhancing metabolic processes and increasing evolutionary rates (*Brown et* al., *2004*). Body temperature optima of individuals may tend to match temperatures experienced in the environmental conditions where they occur (*Bozinovic et al*., 2011; *Deatherage et* al., *2017*). This correspondence can be conditioned, however, by morpho-functional characters originating in an evolutionary past that remain today as a consequence of constituting essential parts of successful body plans, thus acting as evolutionary constraints (*Riedl, 1977*; *Toman & Flegr, 2018*).

The exoskeleton of arthropods can be considered one of these essential structural features with limited evolvability and closely linked with the origin and the huge diversification experienced by this phylum. The development of an external skeleton protecting and supporting internal body parts reached its maximum expression in Coleoptera, the most diversified animal group on Earth (*Chapman, 2009*), which originated during the early Permian period (*Zhang et al., 2018*) in which even forewings are heavily sclerotized (elytra). 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 a "passive thermoregulation" without associated energetic costs has been 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 evidences obtained indicate 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*). Thus, this temperature increase probably results from the transmittance and/or absorbance by the dorsal cuticle of non-infrared wavelengths. The elytra of these species may be absorbing most of the ultraviolet and visible highly energetic radiation to convert it into body heat (*Alves et al., 2018*, *Pavlovic et al., 2018*). The capacity of the insect exoskeleton to propitiate "passive thermoregulation" may have positive implications for interpreting the ecological and biogeographical characteristics of these organisms, as well as for understanding their responses to climatic changes.

In this study, the reflectance, transmittance and absorbance of elytra to different wavelengths of electromagnetic radiation is examined in two phylogenetically close Scarabaeinae species that are locally sympatric and diurnal but differ in their environmental preferences (shaded vs. open habitats). The main aim of this comparison is to verify whether there might be a correspondence between the general environmental preferences of these two species and their capacity to reflect, absorb or transmit radiation of different wavelengths. Specifically, it is hypothesized that inhabiting shaded conditions would be associated with a higher capacity for elytra transmittance mainly in the infrared range, while heliophilous species should have elytra able to cope with the high level of direct sunlight to minimize the risk of overheating. We additionally aim to discern if these possible interspecific differences in the management of radiation can be attributed to differences in body mass and darkness or if, alternatively, any other unknown characteristic of the exoskeleton must be sought as responsible.

## MATERIAL & METHODS

### Study species

The studied specimens are classically identified as belonging to *Onthophagus (Palaeonthophagus) coenobita* (Herbst, 1783) and *Onthophagus (Palaeonthophagus) vacca* (Linnaeus, 1767), two dung beetle species (Coleoptera; Scarabaeidae) widely distributed across the Palaearctic region (*Löbl et al. 2006*). *O. coenobita* has a geographical distribution ranging from Spain to Sweden and from Belgium to Turkmenistan. *O. vacca* (as classically described) is distributed in Europe from Spain to Finland and Russia, and from Great Britain to Kazakhstan, but also in Morocco and the Middle East. *O. vacca* was also intentionally introduced in Australia to improve dung burial and pasture productivity (*Doube, 2018*).

Recently, a long-recognized variation of *O. vacca* has been described as a sibling species (*Rössner et al., 2010*) named *Onthophagus (Palaeonthophagus) medius* (Kugelann, 1792). *O. vacca* and *O. medius* seem to overlap extensively in distribution, but *O. vacca* is absent in the northernmost localities of Europe, and *O. medius* does not inhabit North Africa. *O. vacca* and *O. medius* may co-occur in the same locality and even in the same cowpat, and the specimens can be accurately differentiated by using mitochondrial DNA sequences (*Rössner et al., 2010*; *Roy et al., 2016*) 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 are assigned to *O. medius* according to morphological characters.

Different studies agree on the phylogenetic closeness of *O. coenobita*, *O. vacca* and *O. medius* (*Villalba et al. 2002*; *Roggero et al., 2017*; *Rössner et al., 2010*; *Roy et al., 2016*), as well as on the ecological differences among the three species. *O. coenobita* is frequently reported as associated with forests and shaded localities eating human dung, corpses and mushrooms, in addition to herbivore dung (*Goljan, 1953*; *Jessop, 1986*; *Lumaret, 1990*; *Martín-Piera & López-Colón, 2000*). Instead, *O. vacca* is associated usually with open green pastures eating cow, horse or sheep dung. Despite the lack of reliable data about the environmental preferences of *O. medius*, the available information suggests that this species has a similar ecology to *O. vacca,* although with a seasonal activity mainly focused on the warmest spring months (*Rössner et al., 2010*; *Roy et al., 2016*). A yearly non-published survey carried out during 2017 and 2018 at "El Ventorrillo" biological station (Madrid, Spain, Lat: 40.75º, Long = -4.02, ≈1430 m a.s.l) clearly indicates that these species do not overlap environmentally but may partially coexist seasonally. While *O. medius* and *O. coenobita* do not differ in their general midday daily activity, *O. coenobita* shows a marked preference for woodland sites. Adults of *O. coenobita* are also active at higher air temperatures (around 21.8 ºC) than those of *O. medius* (around 16.8ºC), due basically to the early seasonal occurrence of *O. medius* (mean seasonal occurrence around 11 May) compared with *O. coenobita* (mean seasonal occurrence around 7 June).

**Body measurements and spectrophotometric analysis**

Ten individuals of each of the two taxa preserved in 70% ethanol were randomly selected from a collection of 4,502 dung beetles belonging to 53 species collected at "El Ventorrillo" biological station during 2012-2013 (collection deposited in the Museo Nacional de Ciencias Naturales of

Madrid). After weighting each specimen soaked in alcohol, the left elytron of was carefully removed with tweezers (Fig. 1) and allowed to dry. The total area of each elytron was calculated as well as the proportion of it with dark spots using the Image J software (Fig. 1). The convexity of the elytra can be considered negligible in both species. The thickness of the left edge of the elytron was also measured with a Nikon Measurescope 10 monocular stereo equipped with a Nikon Digital Counter CM-6S (all measurements in mm). Each elytron was measured on three different occasions by two researchers and their data averaged.

Reflectance (R; the return of the electromagnetic radiation in the surface of the elytra) and transmittance (T; the passage of the electromagnetic radiation through the elytra) of the external part of the left elytron (dorsal) were measured with a Shimadzu® UV-2600 spectrophotometer in the wavelength spectrum from 185 to 1400 nm (at 5-nm intervals). This spectrophotometer is equipped with an integrating sphere (ISR-2600Plus) able to measure diffuse and specular reflectance of solid samples. In our case, measurement conditions of the optical system were adjusted to those needed to measure diffuse reflectance due to the slightly rough character of the elytral surface. Before each spectrophotometer measurement a white plate of barium sulfate was used to correct the baseline. The so obtained data covering the complete wavelength spectrum from 185 to 1400 nm was divided into three bands, namely, ultraviolet (UV; 185-385 nm), visible (VIS; 390-745 nm) and near-infrared (NIR; 750-1400 nm). Absorbance (A; the transformation into internal energy of the electromagnetic radiation received by the elytra) was estimated as A = 100-(T+R) (see *Kinoshita, 2008*). Thus, the values of T, R and A were averaged in order to obtain only one value for each on the three bands as the response variable to determine whether there is variation among elytra in response to the different wavelengths emitted by the sun. Reflectance and transmittance of the internal sides of each elytron (ventral) were also measured but only in the near-infrared range in order to estimate the possible capacity of the elytra to reflect or transmit body heat generated by beetles. Each measurement was repeated three times by two researchers (2 species x 10 individuals x 2 sides x 3 measurements = 120 measurements for transmittance and reflectance). The three repeated measurements of each individual, for transmittance and reflectance, were averaged in order to obtain more stable data not dependent on the position of the elytra or the sector sampled by the spectrophotometer (see Supplementary Material). Taking into account the possibility that the immersion in alcohol of the elytra can modify spectrophotometer measurements (e.g. by eliminating cuticular hydrocarbons) UV, VIS and NIR reflectance and transmittance values of five fresh elytra of *O. medius* were estimated before and after being subjected to an immersion in 96º alcohol during sixteen days. Only the dorsal reflectance in the UV band could suggest an effect of the alcohol soaking ($t$ test = 2.81, df = 8; P = 0.02), although this statistical significance disappeared when a Bonferroni correction is applied (mean UV reflectance ± SD; fresh elytra = 2.44 ± 0.18; alcohol elytra = 2.70 ± 0.11). If there were a potential effect of the immersion in alcohol on elytra reflectance we assume here that it would be relatively small and similar in the two considered species.

**Statistical analyses**

Between-taxa differences in darkness (percentage of the elytron area that was dark) and biometric variables (body mass, elytron area, and elytron thickness) were tested by means of Student's *t*-tests considering that these variables follow a normal distribution (n = 10 for each species), with significance levels corrected for unequal variances, if applicable. Darkness was considered in these analyses because the melanic compounds responsible are associated with the absorbance of shortwave radiation and the regulation of body heat (*Pinkert & Zeuss, 2018*). As the correlations between the three biometrical variables are always positive and highly significant (Pearson *r* values oscillating from 0.83 to 0.96; $P < 0.0001$ in all cases), elytron thickness was selected in further analyses assuming that a greater elytron thickness could negatively affect the transmittance of radiation towards the interior of the body.

The variation between species (*coenobita* vs. *medius*) and between elytron sides (internal vs. external; only in the case of NIR) in reflectance, transmittance and absorbance was first examined using Student's *t*-tests. When these between-species comparisons are statistically significant ($P \leq 0.01$), elytron thickness and darkness were subsequently included as covariates in two-way ANCOVAs to assess if these differences are maintained. The loss of statistical significance for the species factor when these covariates are included would mean that these morphological features are relevant in explaining the observed interspecific differences in the management of solar radiation by the exoskeleton. On the contrary, the maintenance of the between-species differences when these covariates are included in the model would suggest that an unconsidered factor may be responsible for the detected interspecific differences. Only statistical relationships significant at $P \leq 0.01$ were retained and considered. We checked for homoscedasticity and normality in the residuals of these models. StatSoft's STATISTICA v12.0 was used for these analyses.

# RESULTS

## Biometric and colour differences

Average body mass (mg), elytron area ($mm^2$) and elytron thickness (µm) differed significantly between *O. coenobita* and *O. medius*, with significantly higher values for the latter taxon

(P<0.001, Table 1). The area of dark pigmentation was also significantly lower for *O. coenobita* than for *O. medius* (Table 1).

**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, reflectance figures were lower than those for absorbance and transmittance throughout the complete wavelength spectrum, while absorbance was very high in the ultraviolet and visible wavelength ranges.

Ultraviolet reflectance values differed significantly between the two taxa ($t$=2.98, P=0.008), but values for NIR and visible wavelengths did not ($t$=2.44, P=0.02 and $t$=1.09, P=0.28, respectively). UV reflectance values are slightly higher in the specimens of *O. medius* than in *O. coenobita* (0.53% vs. 0.27%). Average transmittance values in the ultraviolet and visible ranges did not vary between taxa ($t$=1.21, P=0.24 and $t$= 1.55, P=0.14, respectively), but they tended to vary in the case of NIR ($t$=2.56, P=0.01). Elytron position also seemed to influence NIR transmittance values ($t$=3.12, P=0.003) but independently of the species (*i.e.*, the interaction species x side was non-significant). Thus, transmittance of NIR radiation by the internal side of the elytra seems to be higher than those experienced dorsally, and the NIR transmittance values of *O. coenobita* (54.0% for the internal part and 46.4% dorsally) are higher than those of *O. medius* (47.5% and 43.5%, respectively). Finally, absorbance in the ultraviolet, visible and NIR wavelengths did not significantly differ between the two taxa ($t$=1.52, P=0.15; $t$=1.26, P=0.22, and $t$=1.54, P=0.13, respectively), although the NIR absorbance was higher for the dorsal than for the internal sides of the elytra (46.2% vs. 40.2%; $t$=3.02, P=0.004).

### The role of thickness and darkness

Reflectance in the ultraviolet spectrum is significantly accounted for by a complete model including the two covariates and the species factor ($F_{3,16}$=7.90, P=0.002, $R^2$=59.71%), and the standardized regression coefficients obtained in the regression analyses showed that elytron thickness positively influences the reflectance in the ultraviolet spectrum (Table 2) and that darkness is irrelevant. The species identity factor is marginally significant (P=0.02), despite considering the two covariates.

NIR transmittance was significantly explained by the model including the species factor and the two covariates ($F_{5,34}$=18.03, P<0.001, $R^2$=72.61%). The transmittance of the NIR radiation significantly decreased when elytron darkness and thickness were higher, obstructing the penetration of infrared radiation. Darkness seems to be the most influential parameter in this case (*i.e.*, highest absolute values of the standardized regression coefficients). Transmittances were again higher for the internal part of the elytra. Interestingly, the relevance of the species factor is maintained when these two covariates are included in the model (Table 2). However, between-taxon differences are reversed so that *O. medius* specimens have higher corrected mean values of NIR transmittance both in the external (52.5% vs. 37.5%) and in the internal sides of the elytra (56.4% vs. 45.1%).

## DISCUSSION

This research aims to assess if the characteristics of the elytral exoskeleton may contribute to facilitating the thermoregulation of beetles by differentially transmitting, absorbing or reflecting radiation of distinct wavelengths in correspondence with the environmental preferences of the species. The results obtained support this assumption, although more evidence will be needed to clearly discern the extent of the passive role of the beetle exoskeleton in thermal performance. Thus, although the observed disparities can be associated with biometric and darkness differences, the point is that the thermal performance of elytra is consistent with expectations; the species inhabiting shaded conditions (*O. coenobita*) would reflect less ultraviolet radiation than the sun-exposed one (*O. medius*) but would allow infrared radiation to penetrate the elytra more easily in order to heat internal body parts. Our results also agree with those of previous studies (*Carrascal et al., 2017*; *Amore et al., 2017*; *Alves et al., 2018*) in that elytron reflectance is minimal, that transmittance of infrared radiation is very high, and that most of the ultraviolet and visible radiation is absorbed by the elytra. All these exoskeletal characteristics are consistent with the requirements of an ectothermic organism that spends a good deal of time in the soil and that would need to obtain body heat from the infrared radiation around it. Thus, elytra seem to be highly transparent to the heat coming from the sun or the environment but opaque to the most energetic wavelengths capable of causing harmful effects (*Beresford et al., 2013*). On the other hand, and in agreement with previous results (*Alves et al., 2018*), elytral transparency to the infrared radiation seems to be slightly higher from the inside part of the elytron, suggesting that the elytra can be slightly more effective at facilitating the removal of body heat.

Interspecific differences in the thermal role of exoskeletons are mediated by biometric and colour characteristics, as exemplified in our analyses of elytron thickness and area of dark pigmentation. Elytron thickness seems to be especially effective for reflecting UV radiation, while elytron darkness seems to be especially relevant in preventing the entry of NIR radiation into the beetle body. The effects of these physical or physicochemical attributes become so important that they may even reverse the sign of the factor representing species identity, as in the case of NIR transmittance. Therefore, it cannot be excluded that some additional and unknown morpho-structural difference may also be relevant to explaining the detected interspecific differences in the capacity of the elytra to manage radiation. Notwithstanding the above, caution is required when discriminating the comparative roles of these two features because elytron thickness and darkness are in our case associated; the largest species is also the one that has a more extensive dark area. Additional studies are thus needed to cover a broad range of species with different degrees of darkening and elytron thicknesses to better discriminate the comparative roles of biometric and colour characteristics on the thermal performance of the beetle cuticle. Nevertheless, elytron thickness seems to positively affect ultraviolet reflectance but negatively affect the transmittance of infrared radiation. Similarly, the area of dark pigmentation is negatively associated with infrared transmittance.

Maintaining the strength of the exoskeleton with the increase in body size may imply increasing thickness both allometrically and isometrically (*Evans & Sanson, 2005*; *Lease & Wolf, 2010*). Thus, an increase in the body size of dung beetles can provide extra advantages in open habitats by avoiding the internal overheating of the body under sunny conditions. In a similar way, the darkening of the exoskeleton could be considered an evolutionary strategy to diminish the heat transmission into the body. This supposition would contradict the thermal melanism hypothesis, which predicts that darker colour may be advantageous under colder environments (*Kalmus, 1941*; *Schweiger and Beierkuhnlein, 2016*, *Galván et al. 2018*), but could explain why desert beetles are often dark (Turner & Lombard, 1990). In our case, the darkest elytra seem to make the access of infrared radiation into the body more difficult but do not seem to influence the absorbance of visible and UV radiation, unlike other situations that have been studied (see *Pavlovic et al., 2018*). As in the case of body size, the lower transmittance of near-infrared radiation by the dark specimens of our two considered species may be a strategy to avoid overheating under some circumstances. Considering that more than 50% of the total sunlight incident energy corresponds to this wavelength spectrum (*Stuart-Fox et al., 2017*), the management of near-infrared radiation by the beetle exoskeleton should be an option for consideration. In this case, darkness can affect thermal performance due to its effect on long-wave radiation invisible to the human eye (*Stuart-Fox et al., 2017*). Additional experiments are needed to better estimate if the elytra of different beetle species differ in their capacities to manage distinct wavelengths and to discern the comparative roles of body size and darkness in the thermoregulation of beetles.

# CONCLUSIONS

The main hypothesis of this research has been that the elytra of two evolutionary close beetle species will manage environmental radiation differentially in agreement with their contrasting environmental preferences. Thus, results are in line with what was expected since the elytra of the species inhabiting under shaded conditions (*O. coenobita*) allow the entry of infrared radiation more easily while the heliophilous species (*O. medius*) would reflect more the ultraviolet radiation. These differences are determined largely by the thickness and darkness of the elytra, but we cannot rule out the role that other unknown factors could play in these differences. Further and more comprehensive studies are needed to corroborate the role of the elytral exoskeleton as a mechanism of “passive thermoregulation”.

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## ADDITIONAL INFORMATION AND DECLARATIONS


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### Competing Interests

The authors declare there are no competing interests.

### Ethical approval

Beetle collection was conducted with relevant permissions provided by the Comunidad de Madrid (Dirección General de Medio Ambiente), considering all applicable international and national guidelines for the care and use of animals.

### Author Contributions

Both authors participated in the design of the study, carried out spectrophotometric analyses, participated in data analysis, and drafted the manuscript.

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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.

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| | *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 |

## Table 2 (on next page)

Two ways ANCOVAs results of Ultraviolet Reflectance (RefUV; 185-385 nm) and Near Infrared transmittance (TransNIR; 750-1400 nm) in *Onthophagus coenobita* and *O. medius*.

Results including elytron thickness and elytron darkness as covariates (degrees of freedom in brackets). $\beta$ are the standardized regression coefficients obtained in the regression analyses representing the comparative magnitude effects of the predictor variables. In the case of NIR transmittance, elytron side is tested ordering the levels of the factor from internal to external ($\beta$ is negative if the average of the internal side is higher than that for the external side). Species factor is tested ordering its levels from *O. medius* to *O. coenobita* ($\beta$ is negative if the average of *O. medius* is higher than that for *O. coenobita*). Only statistical relationships significant at $P \leq 0.01$ are retained and considered (in bold).

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| | ***F*** | ***P*** | $R^2$ | ***β*** | ***P*** | |
|---|---|---|---|---|---|---|
| RefUV | 7.90 (3, 16) | 0.002 | 59.71% | **0.534** | **0.01** | Elytron thickness |
| | | | | -1.004 | 0.05 | Elytron darkness |
| | | | | -1.212 | 0.02 | Species |
| TransNIR | 18.03 (5, 34) | <0.001 | 72.61% | **-0.605** | **<0.0001** | Elytron thickness |
| | | | | **-1.031** | **0.0006** | Elytron darkness |
| | | | | **-0.428** | **<0.0001** | Elytron side |
| | | | | **-0.973** | **0.001** | Species |
| | | | | -0.136 | 0.14 | Species*Side |

## Figure 1(on next page)

Habitus and elytra of Onthophagus coenobita (left) and O. medius (right).

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

Figure 1: **Habitus and elytra of *Onthophagus coenobita* (left) and *O. medius* (right).** The left elytra was removed showing coloured external and pale internal sides.

## Figure 2 (on next page)

Spectrophotometric graphs

Mean absorbance (ABS), transmittance (TRA) and reflectance (REF) from 185 to 1400 nm of ten individuals of O. coenobita (above) and O. medius (below), both for the external (left column) and the internal sides of the elytra (right column). 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).

Figure 2: **Spectrophotometric graphs.** Mean absorbance (ABS), transmittance (TRA) and reflectance (REF) from 185 to 1400 nm of ten individuals of *O. coenobita* (above) and *O. medius* (below), both for the external (left column) and the internal sides of the elytra (right column). 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).