

# Circularly polarised colour of the scarab beetle *Chalcothea smaragdina*: light scattering by a dual photonic structure

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November 30, 2016

## Abstract

Helicoidal architectures comprising various polysaccharides such as chitin and cellulose have been reported in biological systems. In some cases, these architectures exhibit stunning optical properties analogous to ordered cholesteric liquid crystal phases. In this work, we characterise the circularly polarised reflectance and optical scattering from the cuticle of the beetle *Chalcothea smaragdina* (Coleoptera: Scarabaeidae: Cetoniinae) using optical experiments, simulations and structural analysis. The selective reflection of left-handed circularly polarised light is attributed to a Bouligand-type helicoidal morphology within the beetle's exocuticle. Using electron microscopy to inform electromagnetic simulations of this anisotropic stratified medium, the inextricable connection between the colour appearance of *C. smaragdina* and the periodicity of its helicoidal rotation is shown. A close agreement between the model and the measured reflectance spectra is obtained. In addition, the elytral surface of *C. smaragdina* possesses a blazed diffraction grating-like surface structure, which affects the diffuse appearance of the beetle's reflected colour, and therefore potentially enhances crypsis amongst the dense foliage of its rainforest habitat.

**Keywords:** Coleoptera, Bouligand structure, circular polarisation, blazed grating

**Short title:** Circularly polarised colour of *Chalcothea smaragdina*

# 1 Background

Benefiting from millions of years of evolution, complex growth and formation mechanisms are utilised by numerous biological systems [1, 2, 3, 4, 5], often producing remarkable optical effects [6, 7, 8]. Animals, plants and minerals showcase an extraordinary diversity of biological photonic blueprints, illuminating the path towards designing dynamic and adaptive photonic devices [9, 10, 11]. The order Coleoptera is no exception [12], comprising approximately 400,000 species, many of which display interesting optical properties including: both, narrow- and broadband, metallic colours [13, 14], iridescent appearances [15, 16, 17], brilliant whiteness [18, 19], and distinct polarisation signatures [20, 21, 22].

Polarised light is exploited extensively throughout nature. Particularly in the animal kingdom, polarised light assists in predation [23, 24], navigation [25, 26] and intraspecific communication [27, 28] within a variety of ecosystems. In contrast to linear polarisation effects, which have been reported in numerous biological systems [29, 30, 31, 32], encountering circular polarisation in nature is, broadly speaking, far less common. The reflection of circularly polarised (CP) light is limited to a narrow range of organisms, primarily arthropods, with the highest concentration of species exhibiting CP structural colours belonging to the coleopteran family Scarabaeidae. Amongst scarabeid beetles, Michelson is attributed with the earliest observation of this phenomenon, described in his 1911 study “On metallic colouring in birds and insects” [33]. Early follow-up work showed that the optical properties of certain scarabeids were analogous to those exhibited by ordered cholesteric liquid crystal phases [34, 35]. The advent of electron microscopy facilitated significant advancements in the understanding of the similarities in structural organisation shared by liquid crystals and biological materials. Consequently, several investigations uncovered the precise physical origin of the optical activity displayed by these systems shed new light on the beetles’ photonic ultrastructure [36, 37, 38, 39, 21].

In beetle species reflecting CP light, an ultrastructure comprising clusters of parallel aligned chitin microfibrils woven into a lamellar architecture within the exocuticle was described. Upon transitioning adjacent microfibril planes, the microfibrils are rotated by small angles,  $\sim 7-8^\circ$  [40], thereby creating a helicoidal morphology. The rotation of this assembly through an angle of  $180^\circ$  forms the eponymous ‘Bouligand structure’ [39, 21, 41] typically associated with the exocuticle structures of many arthropods, including decapods [42, 43, 44] and stomatopods [45, 46] in addition to scarabeid beetle species. Beyond the animal kingdom, helicoidal morphologies assembled from cellulose have been reported in fruits [47, 48] and other plants [49, 50].

Overwhelmingly, investigations into the reflection of CP light by scarabeid beetles show that they exclusively reflect Left-handed Circularly Polarised (LCP) light in response to illumination at normal incidence [51]. Presently, the ‘jewel scarab’ *Chrysina resplendens*, coincidentally the subject of Michelson’s initial observations [33], is the sole example species reported to exhibit any deviation from this behaviour [52, 53]; a modification to the exocuticle ultrastructure of *C. resplendens* enables this species to reflect both left-handed and Right-handed Circularly Polarised (RCP) light simultaneously. In this beetle two spatially distinct left-handed helicoids are separated by a non-helicoidal layer that performs the function of a half-wave retarder; incident RCP light is reflected by the lower of these helicoids. Clockwise-rotating helicoidal structures that directly reflect RCP light are extremely rare, but are found alongside left-handed structures in the *Pollia condensata* fruit [47].

Known for their vibrant colours, cetoniine scarabs present a plenitude of candidate specimens for optical characterisation. Of particular relevance to this study, the reflection of CP light has been reported in several cetoniine species [54]. In this paper, we describe the CP colour appearance and light scattering properties of the cetoniine scarab species *Chalcothea smaragdina* through the use of a range of microscopy techniques, visible light spectroscopy, imaging scatterometry and theoretical modelling.

## 2 Methods

### 2.1 Beetle samples

*C. smaragdina* samples were obtained from a commercial insect retailer (<http://www.insect-sale.com>). Cuticle samples were examined from numerous exoskeleton sites across multiple specimen; the micrographic and optical analyses presented herein concentrate on samples removed from the specimens' dorsal regions.

### 2.2 Optical imaging

*C. smaragdina* specimens were photographed with a Canon EOS-1000D camera equipped with a Canon EF 12 II extension tube. Each specimen was photographed through an LCP analyser, an RCP analyser (Edmund Optics) and without any polariser between the camera and the specimen.

Small elytral samples, typically measuring 1–2 mm<sup>2</sup>, were examined using a Zeiss Axiocam MRc5 USB camera connected to a Zeiss Axioskop 2 polarising optical microscope. The *C. smaragdina* samples were imaged using both bright-field and dark-field illumination, with CP analysers positioned in the illuminating and reflected light-paths. CP imaging was performed using custom-made Zeiss polarisers comprising a linear polariser in conjunction with a quarter-wave retarder and also employed in the microspectrophotometry and imaging scatterometry set-ups.

### 2.3 Atomic force microscopy (AFM)

The surface topology of the exterior exoskeleton was imaged using AFM for sample areas measuring 25 × 25 μm. For this analysis, cuticle samples were cut and mounted on AFM stubs. Measurements were performed using a Nanosurf NaioAFM instrument, equipped with a Nanosensors PPP-EFM silicon cantilever, in dynamic mode.

### 2.4 Scanning electron microscopy (SEM)

The ultrastructure of the surface of *C. smaragdina* was imaged using an FEI Nova 600 dual-beam field-emission SEM with an electron beam voltage of 10 kV and a 7.5 pA beam current. Exoskeleton samples were glued to an SEM stub using electrically conducting epoxy resin and subsequently sputter coated with ~ 6 nm of gold palladium.

### 2.5 Transmission Electron Microscopy (TEM)

Samples were prepared for TEM using a multi-stage process. The samples were first fixed 3% glutaraldehyde at 21°C for 2h, followed by rinsing in a sodium cacodylate buffer. Subsequent fixing in 1% osmic acid for 1h preceded: dehydration through an ethanol series (ending with 100% ethanol), bathing in propylene oxide for 30 minutes and embedding in Spurr resin. After ultrathin-sectioning using an ultra-microtome, the samples were stained using first 2% uranyl acetate and, second, lead citrate before being examined using a JEOL 100S TEM instrument.

### 2.6 Circularly polarised reflectance spectrophotometry

Spectral reflectance measurements of the specimen's CP response were made using an Ocean Optics HPX-2000 broadband fibre-coupled light source and an Ocean Optics USB2000+ spectrometer. CP light was produced using a rotatable polariser, followed by an achromatic Fresnel rhomb quarter-wave retarder, oriented at 45° azimuth. The Fresnel rhomb imposes a retardation of  $\pi/2$  via two instances of total internal reflection. The CP handedness was selected by setting the polariser

azimuth to either  $0^\circ$  or  $90^\circ$ . Incident light was focused on to the sample, normal to its surface, using an achromatic  $10\times$  objective lens, L, producing a beam spot with a diameter of  $\approx 30\ \mu\text{m}$ . The reflected light was collected by the same lens before being subsequently directed through a CP analyser comprising the Fresnel rhomb and a second rotatable polariser,  $P_2$ . The system was calibrated against a plane aluminium mirror of known reflectance.

## 2.7 Imaging scatterometry

The far-field spatial distribution of the light scattered from the beetles' elytra was visualised using an imaging scatterometer (ISM) [55]. The central component of the ISM is an ellipsoidal mirror. Small elytral samples are mounted to the tip of a micropipette and positioned in the mirror's first focal plane. Therefore, light scattered by the sample into the frontal hemisphere is focused by the mirror in its second focal plane and projected by a lens onto its back focal plane, thereby compressing the far-field scattering pattern into a an image that may be captured using a CCD camera. Two different illumination conditions are possible: (i) narrow-beam illumination through a central hole of the ellipsoidal mirror; (ii) wide-angle illumination via illumination through the ellipsoidal mirror by light focussed from the second focal-point [56]. Custom-written MATLAB programmes correct the resultant scatterograms for aberrations. A piece of MgO served as a white standard.

## 2.8 Optical modelling

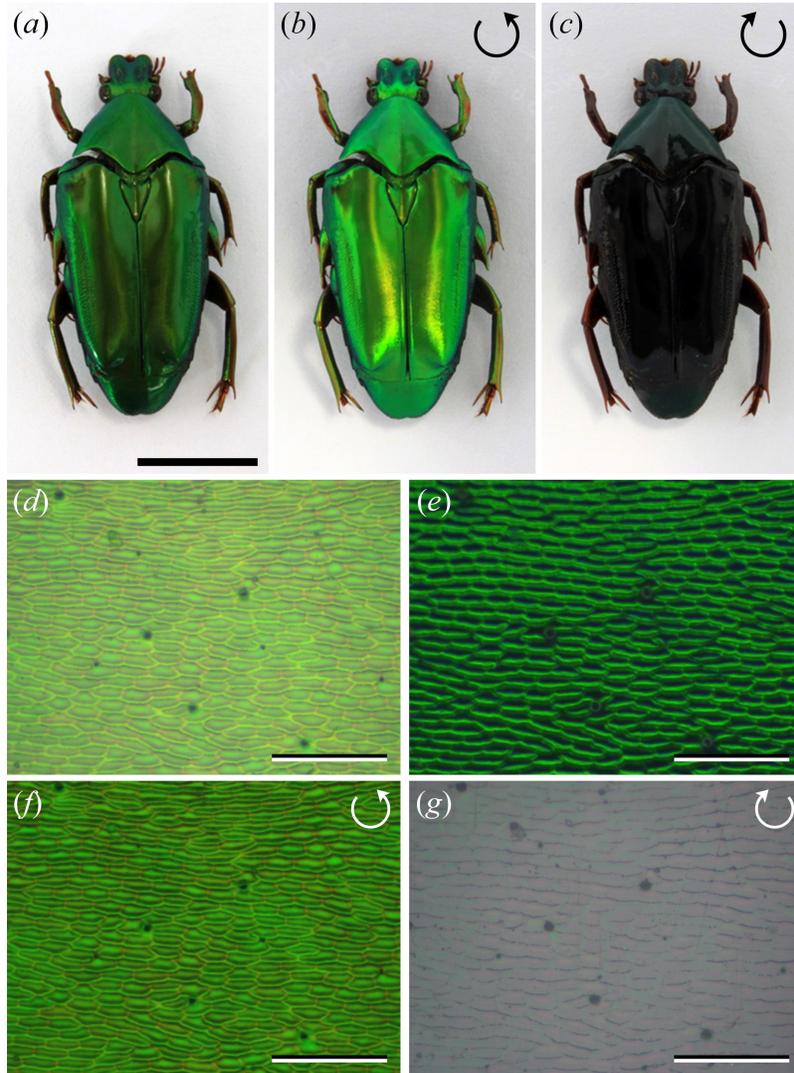
By adopting the  $4\times 4$  matrix methodologies for 1D anisotropic stratified media [57, 58, 59] and further incorporating the scattering matrix modification described by Ko and Sambles [60], a custom-written code compiled in MATLAB (<http://www.mathworks.com>) was used to model the CP reflectance arising due to the specimen's cuticle ultrastructure. The helicoidal pitch was assumed to vary continuously throughout the chiral exocuticle, with a resolution of 16 'sublayers' per helicoidal period. The software package ImageJ (<http://rsbweb.nih.gov/ij/>) was used to analyse TEM cross-sections of the ultrastructure; lamellar pitch lengths, equivalent to 8 'sublayers', were extracted to create profiles of the helicoidal pitch fluctuation, which subsequently informed the 'sublayer' thicknesses applied in the modelling. A wavelength-dependent average for the real component of the refractive index was used to describe the chitinous ultrastructure ( $\bar{n} = 1.595$  at  $\lambda = 525\ \text{nm}$ ), with a fixed extinction coefficient of  $k = 0.005$ , and a fixed birefringence of  $\Delta n = 0.018$ .

# 3 Results

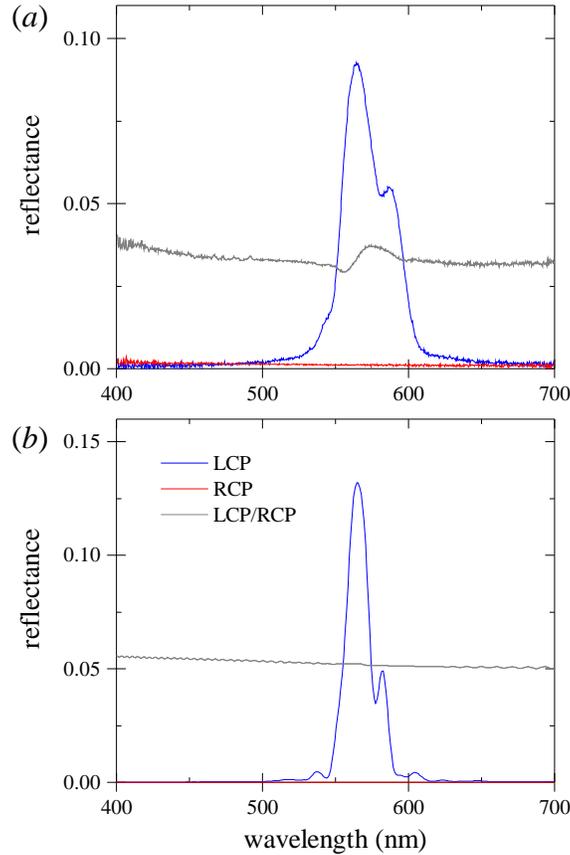
## 3.1 Optical appearance and spectral characteristics

The metallic green cetonine scarab species *Chalcothea smaragdina* (Coleoptera: Scarabaeidea; Gory & Percheron 1833), is indigenous to Indonesia. The body of this flower chafer reaches body lengths of up to 3 cm, with distinct green-metallic elytra. Contrary to many other scarabeid beetles, the body is prolong and features a pronounced neck-like feature between pronotum and head area.

Figures 1a– 1c show photographs of a beetle under different circular polarisers that provide a qualitative assessment of the specimen's CP properties. In common with the CP response typically associated with several genera of scarabeid beetles [51, 21, 22], the photographs illustrate that *C. smaragdina* exhibits the selective reflection of LCP light exclusively. Observing *C. smaragdina* through an LCP analyser thus accentuates the specimen's metallic green colour appearance; in contrast, repeating the observation using an RCP analyser leads to the specimen's colour being effectively extinguished over the entirety of its exoskeleton.



**Figure 1:** (a)–(c) Photographs of *C. smaragdina* capturing the beetle’s appearance for the following configurations: (a) without an analysing filter; (b) through an LCP analyser; (c) through an RCP analyser. The beetle selectively reflects LCP light. (d)–(g) Optical micrographs of the elytral surface corresponding to: (d) unpolarised bright-field illumination; (e) unpolarised dark-field illumination; (f) with the reflected light analysed using an LCP filter; (g) with the reflected light analysed using an RCP filter. Scale bars: (a) 1 cm; (d)–(g) 50  $\mu\text{m}$ .

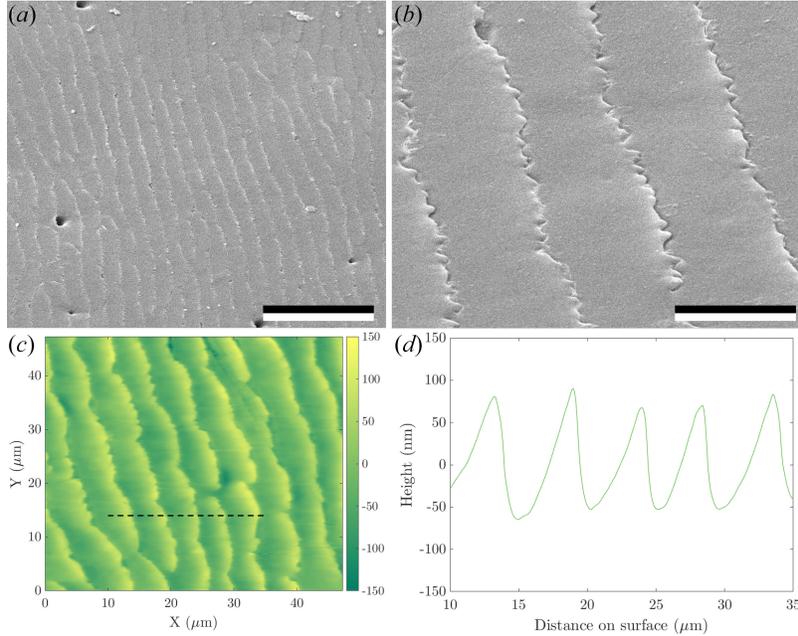


**Figure 2:** (a) Spectrophotometry measurements showing both the co-polarised (LCP and RCP) and cross-polarised (LCP/RCP) CP reflectance spectra for *C. smaragdina*. (b) Optical modelling of the co-polarised and cross-polarised reflectance from the specimen.

Closer inspection of the exoskeleton surface using optical microscopy is shown in figures 1d–g. The images reveal the elytral surface to possess a reticulated structure comprising elongated, irregularly shaped domains that run approximately latitudinally across the whole breadth of the elytra. This morphology is most starkly represented in the dark-field micrograph (figure 1e), leading to the inference that this reticulated surface patterning dominates the beetle’s colour at oblique angles; the domain interiors predominantly reflect light specularly. As shown in figures 1b and 1c respectively, the photographs using a LCP and RCP filter show the strong accentuation and depletion of the specimen’s green colour appearance. To quantify this, we measured the circular-polarisation-dependent reflectance of the elytral surface, shown in figure 2a. For LCP light, a pronounced optical bandgap is observed, with a principal reflection peak centred at  $\sim 565$  nm and a less intense feature at  $\sim 585$  nm, which extends the long-wavelength band-edge. These spectral features correspond to the saturated green-orange colour observed in figures 1b and 1f; the RCP analysis shows an absence of distinguishable spectral features.

### 3.2 Integument ultrastructure

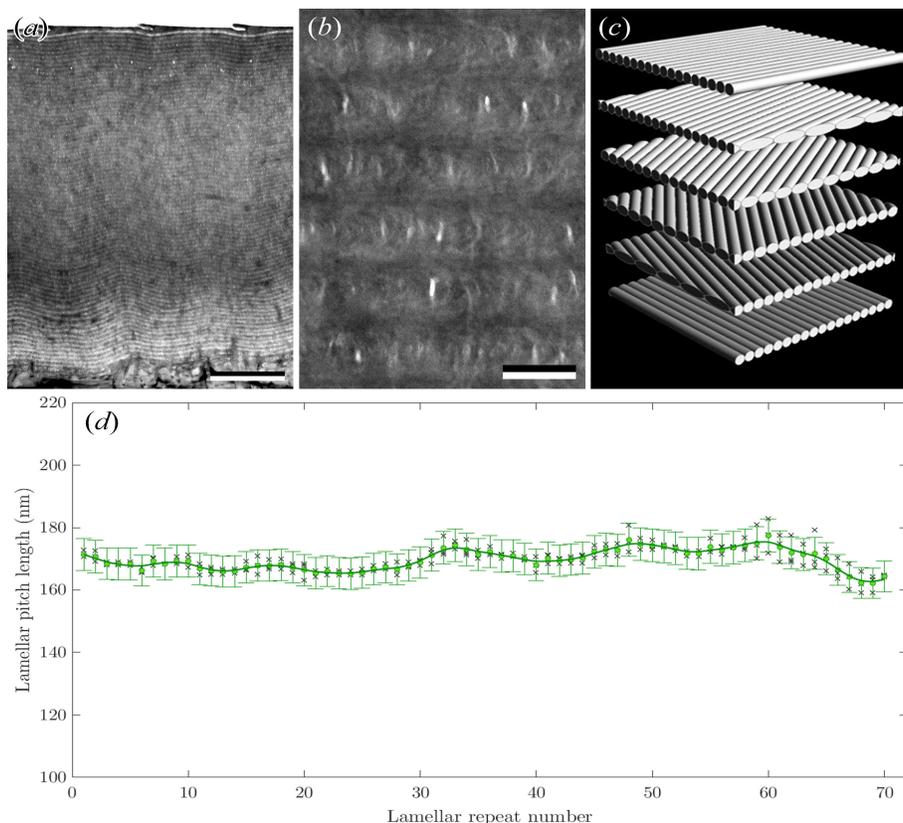
To further explore the reticulated surface morphology identified in the optical micrographs, elytral samples were examined using both SEM and AFM. Elucidating the exterior features using SEM



**Figure 3:** (a)–(b): SEM images detailing the reticulated elytral surface structure for *C. smaragdina*. (c)–(d) AFM measurements reveal the form of the blazed grating-like surface topography. Scale bars (a) 30  $\mu\text{m}$ ; (b) 5  $\mu\text{m}$ ).

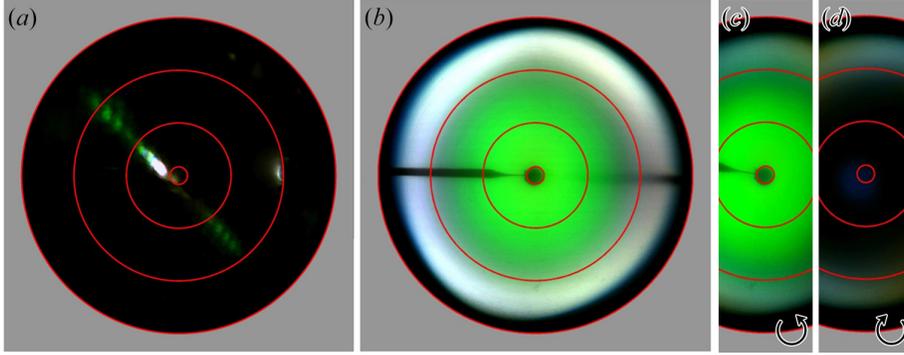
imaging resolved the precise topology of the reticulated surface structure. Typically, the elongated domains observed in the optical micrographs were not fully enclosed, with the mesh-like appearance arising due to deviations in the direction followed by the otherwise approximately parallel surface striations (figure 3a). In addition, the edges of these striations were shown to possess a fine irregular sawtooth-like profile parallel to the surface plane (figure 3b). The surface topography was revealed using dynamic mode AFM. Indeed, the surface is highly irregular showing pronounced acclivities and declivities at the edges of the striations (figure 3c). A line scan traversing the surface of *C. smaragdina* reveals that its surface topography closely resembles a blazed grating structure (figure 3d). Although the spacing between individual striations exhibits some variation due to the irregularities in this surface patterning, we found the grating structure to have a mean periodicity of  $5.07 \pm 0.15 \mu\text{m}$ . Analysis of AFM measurements from several elytral locations revealed that the blaze profile typically extends from the surface to a mean height of  $118 \pm 10 \text{ nm}$ . These dimensions correspond to a blaze angle of  $\sim 3.1^\circ$ . The wavelength of light for which scattering is optimised by this configuration is  $\lambda_b = 2a \sin \theta_b$ , where  $a$  is the grating periodicity and  $\theta_b$  is the blaze angle; substituting the values measured for *C. smaragdina* results in  $\lambda_b \sim 554 \text{ nm}$ .

To determine the origin of the specimen’s LCP colour appearance, the underlying photonic ultrastructure of *C. smaragdina* was investigated using TEM. The TEM cross-section image shown in figure 4a displays the exocuticle ultrastructure, responsible for producing the specimen’s colour, in its entirety. A lamellar arrangement is apparent throughout the full depth of the exocuticle, which was measured to have a total thickness ranging from 10–11  $\mu\text{m}$  across several sample locations. The inter-lamellar contrast is consistent with TEM studies of other CP reflectors identified in scarabeid species [21, 61, 20], as opposed to the starkly contrasted alternating layers commonly observed in traditional coleopteran multilayer reflectors such as those found in buprestid beetles [62, 32, 63]. The lamellar appearance indicates that the *C. smaragdina* exocuticle comprises a helicoidal morphology (figure 4c), where the individual lamellae represent the rotation of the constituent chitin microfibrils



**Figure 4:** (a) TEM cross-section showing the full extent of the photonic ultrastructure within the specimen's pygidial cuticle. (b) High-magnification TEM cross-section showing the Bouligand structure within the beetle's elytral exocuticle. (c) Schematic of the helicoidal morphology within the *C. smaragdina* exocuticle. The helicoid is formed by a lamellar stack comprising successively rotated chitin microfibril layers. (d) Lamellar pitch profile describing the distribution of helicoidal periods in *C. smaragdina*. Each lamellar pitch measurement (circles) represents the distance for which the chitin microfibrils comprising the helicoid rotate through  $180^\circ$ . Scale bars: (a)  $3\ \mu\text{m}$ ; (b)  $200\ \text{nm}$ .

through an angle of  $180^\circ$  and, therefore, the parallel alignment of the microfibrils. Thus, each lamellar pitch length corresponds to half the helicoidal period. This assembly is displayed clearly in the magnified TEM image shown in figure 4b. The inter-lamellar distances were measured to ascertain the distribution of lamellar pitch lengths throughout the depth of the exocuticle. The lamellar pitch profile for the *C. smaragdina* helicoid is presented in figure 4d. It shows that although the lamellar pitch length undulates continuously throughout the exocuticle, the amplitude of these undulations is small ( $\sim 5\text{--}10\ \text{nm}$ ). The profile shows that the lamellar pitch length varies between  $160\text{--}180\ \text{nm}$ , with the mean lamellar pitch length  $169.7 \pm 5.1\ \text{nm}$ . Consequently, the mean pitch of the helicoid is  $\sim 340\ \text{nm}$ . Lamellar pitch profiles were constructed relating to several measurement locations from elytral samples, with the total number of lamellae measured showing high consistency, ranging from 68–70.



**Figure 5:** ISM results obtained for *C. smaragdina*, showing the far-field scattering arising following (a) narrow-aperture illumination and (b-d) wide-aperture illumination using the imaging scatterometer. (c,d) Angle-dependent scattering pattern upon insertion of a left- and right-handed circular polariser in the detection pathway. The images are cropped around the central axis due to polarisation accuracy.

### 3.3 Modelling of the beetle’s CP reflectance

The ultrastructural results allowed us to perform electromagnetic simulations. For this, we implemented the ultrastructure of the equivalent components and calculated the total CP response, the results of which are presented in figure 2b. The helicoidal morphology comprising the exocuticle of *C. smaragdina* was treated as an anisotropic stratified medium with the helicoidal period defined using the TEM-based analysis presented above. The modelled CP reflectance closely matches the experimental CP reflectance measurements shown in figure 2a with regards to the shape and position of the spectral features, particularly so for the co-polarised CP spectra. The small oscillatory feature recorded in the experimental measurement of the cross-polarised response, spanning the breadth of the LCP bandgap, was not replicated in the model. Additionally, the simulation predicts a slightly increased intensity in the LCP reflectance ( $\sim 4\%$ ). These differences are discussed below.

### 3.4 Angle-dependency and scattering of CP colour

The effect of both the blazed surface topography and the helicoidal ultrastructure on the reflection properties of *C. smaragdina* was further investigated using imaging scatterometry. Upon illumination of the elytron with an unpolarised, narrow-aperture light beam, the captured scattering pattern demonstrates highly directional reflection from the sample surface; a series of diffracted orders is observed, extending radially outwards from the image centre along the direction perpendicular to the surface striations (figure 5a). The green colour of the scattered light shows good agreement with the blaze wavelength anticipated from the analysis of the surface ultrastructure. We note that the central reflection, upon shorter illumination times, is also strongly green, in full accordance with the observations from optical microscopy (figures 1, 2).

When the sample is illuminated with a wide-aperture beam *via* the ellipsoidal mirror at the core of the scatterometer, the angle-dependency of the photonic structure in *C. smaragdina*’s elytron can be assessed [32, 56]. Figure 5b presents the scatterogram of the specimen’s elytron using unpolarised light. The sample displays relatively weak iridescence, with the reflected green colour remaining essentially unaltered for scattering angles extending from normal incidence to approximately  $50^\circ$ . At this point a slight blue-shift in the reflected hue is observed and an annulus of blue-green colour can be seen that extends to the circular line denoting a scattering angle of  $60^\circ$ . For larger scattering angles ( $\gtrsim 60^\circ$ ), the surface reflectance becomes dominant and both polarisations show a

strong whitish reflectance.

We repeated this measurement using a LCP and RCP analyser, respectively, positioned such that they acted to impart their CP handedness to the incident beam and also analyse the CP state of the reflected light. The LCP-LCP and RCP-RCP co-polarised scatterograms are shown in figures 5c and 5d, respectively. These scatterograms display close agreement with the optical images and MSP spectra presented above. For scattering angles  $\lesssim 60^\circ$  the specimen’s LCP green reflected colour is more saturated compared to the unpolarised scatterogram (figure 5b), whilst the reflected colour is extinguished for the RCP-RCP scatterogram. The blue and brown-green fringes observed for scattering angles greater than  $60^\circ$  are artefacts that arise when investigating chiral samples; following oblique angle illumination with a CP light source, the handedness of the reflected light loses its purity.

## 4 Discussion

### 4.1 Optical effect of the helicoidal morphology

In selectively reflecting LCP light, *C. smaragdina* displays the archetypal optical response associated with Bouligand-type structures found in biological systems. The cardinal factor that determines the handedness of the reflected light is the direction of rotation in which the helicoidal ultrastructure assembles prior to sclerotisation. The reflection of LCP light is manifested by an anti-clockwise rotation of the helicoidal architecture. From the TEM-constructed lamellar pitch profile (figure 4d) we were able to establish the average pitch of the helicoid ( $\sim 340 \pm 10$  nm). At normal incidence, periodic helicoidal structures exhibit Bragg reflection, with a peak wavelength,  $\lambda_0$ , given by  $\lambda_0 = 2d\bar{n}$ , where  $\bar{n}$  represents the average refractive index and  $d$  is the lamellar pitch length (or helicoidal half-pitch) [64, 21]. It should be noted that over many repeat measurements of the CP reflectance, small variations in the LCP spectral profile were observed in response to localised changes to the area of illumination, with the greatest variability occurring for the long-wavelength band-edge. This can be attributed to the undulations observed in the lamellar pitch distribution profile; where more prominent peaks in the pitch occur closer to the proximal edge of the exocuticle, an increase in the longer-wavelength component of the reflection is observed.

Typically, spectral measurements of *C. smaragdina* showed peak reflectance at  $\sim 560$ – $570$  nm; a Bragg response at these wavelengths is satisfied by  $\bar{n} \sim 1.647$ – $1.676$ . The existing literature provides a broad range for the refractive index of chitinous material encountered in the ultrastructure of arthropods [52, 65, 66, 63, 67, 68]; here, simulations of the CP reflection observed for *C. smaragdina* were informed using Caveney’s measurement of the average refractive index for the cetoniine specimen *Protaetia* (formerly *Potosia*) *speciosissima* ( $\bar{n} = 1.595$ ) [52] and, also, wavelength-dependent refractive index data published by Vargas et al. [69]. Although smaller, Caveney’s value is comparable to that anticipated by the spectral measurements, however its use does lead to the modelled LCP bandgap being slightly blue-shifted. Further considering the applicability of this refractive index value, it is noted that concurrent investigations into the optical characteristics of broadband reflecting scarabeids indicated that the cuticle incurs a small degree of shrinkage ( $\sim 5$ – $10$  %) during the TEM preparation process. This artefact represents a historical challenge faced when preparing biological samples for TEM [70]. To facilitate a direct comparison between measured and modelled spectra, close agreement with the experimentally measured LCP spectra was achieved by applying a scaling factor of 1.05 to the sublayer thicknesses defining the modelled structure (figure 2).

The electromagnetic simulations of the CP response further predicted a greater LCP reflectance than measured in experiments. A number of previous studies concerning the reflection of CP light by coleopteran species have focused upon rutelinid scarabs belonging to the *Chrysina* genus [20, 21, 71, 72, 73]. Several of these beetles display silver and gold hues owing the chirped distribution of pitch lengths within their helicoids [61, 71, 72, 74]. Additionally, the intensity of LCP light reflected

by these brilliantly coloured specimens, in response to illumination at normal incidence, exceeds that observed herein for *C. smaragdina* [71]. This result requires consideration since the number of full rotations of the *C. smaragdina* helicoid ( $\sim 35$ ) is only slightly fewer than typically observed in broadband *Chrysina* specimens ( $\sim 40$ ). Furthermore, the lamellar pitch lengths measured for *C. smaragdina*'s narrowband reflecting cuticle are concentrated over a far narrower range of values, which should increase the reflected intensity.

Two likely factors are considered as contributing to the lower luminance of *C. smaragdina*. Firstly, Caveney demonstrated that the coruscating precious metal-like colours of species such as *Chrysina resplendens* are reliant on the presence of uric acid within the ultrastructure, which enhances its birefringence significantly [52]. Indeed, the birefringence in species such as *Chrysina aurigans* has been reported to be as high as 0.19 [75]. In contrast, Caveney reported an absence of uric acid in the exocuticle of *P. speciosissima*, subsequently measuring the birefringence to be 0.018 [52]. As mentioned previously, although guided principally by Caveney's measurement of the refractive index for *P. speciosissima*, we also considered the wavelength-dependent description of the refractive indices of chitin and uric acid reported by Vargas et al. [69]. In this case, the authors performed measurements on fresh shrimp shells. By applying an effective medium approximation, the effect of altering the concentration of uric acid can be explored. Following Caveney's conclusion that *P. speciosissima* contains no uric acid component, the data presented by Vargas et al. indicates that a structure comprised purely of chitin has an average refractive index of  $\sim 1.55$  at 525 nm. Of course, the hierarchical structures formed by scarab beetles and crustaceans likely exhibit differences in how they incorporate chitin crystallites into a protein matrix. Subsequently, by adopting the measurements published by Vargas et al., we found that a uric acid fraction of 0.2 yielded Caveney's value of 1.595 at 525 nm whilst also accounting for material dispersion. The average value of the real component of the refractive index in *C. smaragdina* was thus described by the Sellmeier equation:

$$\bar{n}^2 = 1 + \frac{1.447776\lambda^2}{\lambda^2 - 0.017085}. \quad (1)$$

Thus, the reduced specular CP reflection from *C. smaragdina* may be principally attributed to the absence, or at least low concentration, of uric acid in the structure since this limits the birefringence achievable within the exocuticle structure. We note that the scattering from the surface topography will also play an important role, as is discussed in the following section.

## 4.2 Optical effect of the surface topography

The reticulated surface topography exhibited by *C. smaragdina* was elucidated initially through optical microscopy (figure 1) and in more detail via SEM and AFM measurements (figure 3). As illustrated in figure 5a, the diffractive scattering that results due to this component of the specimen's structure reflects a proportion of incident light away from the direction normal to the sample surface. Consequently, the amount of incident light that interacts with the underlying helicoid is modulated.

The optical scattering behaviour resulting from the blazed grating-like surface topography encountered for *C. smaragdina* is elucidated in the imaging scatterogram shown in figure 5a. Diffracted orders are observed perpendicular to the direction in which the specimen's surface striations are oriented. Clear resolution of the individual diffracted modes captured in figure 5a is only accessible via narrow-beam illumination and requires a small beam spot. The quasi-periodicity resulting from the imperfect geometry of the blazed architecture leads to increased interference between the elements of the diffraction pattern as the size of the illumination spot increases. Overall, the optical characteristics arising from blazed grating-like structures have received little attention in beetle species, and are more typically associated with photonic systems in Lepidoptera [76, 77]. Other lepidopteran species, notably *Morpho* butterflies [78] and pierids [79], have also been reported to exhibit highly directional scattering. Diffraction gratings enabling spectral iridescence are by no means uncommon amongst coleopteran species, however, having been identified in one of

the earliest electron microscope studies of structurally coloured insects [80]. Across several papers, Hinton and Gibbs described diffraction gratings in phalacrid [81], carabid [82] and gyrid beetle [83].

One report describing similar blazed grating structures in cetonine scarabs was made by Xu et al. [84]. The authors examined three species of cetonine scarab beetles, all of which possess topographical surface features remarkably similar to those observed here for *C. smaragdina*. SEM images of the specimens investigated by Xu et al., which belong to the *Mycterophallus* and *Lomaptera* genera, revealed striations with a finer periodicity than that observed for *C. smaragdina* in two of the three specimens examined. These species exhibited a grating periodicity between 1–2  $\mu\text{m}$ . Moreover, both these species displayed the sawtooth-like profile along the striation edges with greater regularity relative to *C. smaragdina*. The third beetle appeared to show grating dimensions closer to those of *C. smaragdina* ( $\sim 4\text{--}5 \mu\text{m}$ ), although the relatively small surface area displayed in the image limited the extent to which comparisons could be made. Qualitative analysis of the beetles appeared to show each beetle exhibiting spectral iridescence under “bright” illumination [84].

Beyond confirming the reflection of LCP light in two of the investigated specimens, the authors offered no additional insight into the CP behaviour exhibited by the beetles. The observation of CP reflected light for species belonging to the *Lomaptera* genus has been reported elsewhere, first by Neville and Caveney [37], and, more recently, by Carter et al. [85], although, neither makes reference to the beetles’ surface topography. Xu et al. [84] presented a simplified model for the origin of the reflectance of the exocuticle structures revealed via TEM cross-sections, in which traditional multilayer reflectors, formed from alternating layers comprising distinct materials of high and low refractive index, were assumed. Whilst such reflectors are common amongst coleopteran species, and indeed many other 1D biological photonic systems, this scalar approximation neglects the chiral aspect of the cuticle and the resulting CP component of the observed reflectance.

For wide-beam illumination, the scattering imparted by the surface topography is less obvious since the structure is being simultaneously illuminated over all incident angles. In optics, stratified structures exhibiting appropriate periodicity tend to behave as 1D photonic crystals. For specularly reflecting systems, illumination at oblique angles results in iridescence manifested as a blue-shift in the reflected colour; the wavelength of the first-order Bragg reflection peak is given by  $\lambda = 2d\bar{n} \cos(\theta)$ , where  $\theta$  is the angle between the propagation direction and the axis of the helicoid [86]. However, as indicated by the scatterograms corresponding to the unpolarised and LCP-LCP copolarised configurations (figures 5b and 5c), *C. smaragdina*’s blazed surface profile may suppress the iridescent response from the underlying helicoidal reflector. The ensemble colour is attributed to the sum of the scattered reflections, with additional interference attributed to the irregularities observed in the surface topography. Indeed, the beetle’s green colour is expected to extend to high scattering angles as evidenced by dark-field optical microscopy observations (figure ??e). Similar effects, albeit from different structural configurations, have previously been observed in wide-beam illumination scatterometry of weevil scales [17] and of the dorsal wing scales of the butterfly *Parides sesostris* [87]. In the weevils’ case, their elytral scales comprise many differently oriented crystal domains. The interference of reflected light from the individual domains creates an additive effect that gives rise to an angle-independent colour appearance [17]. *P. sesostris*, on the other hand, utilises an entirely different mechanism; a pigmented honeycomb structure situated in the upper lamina of the elytra inhibits the iridescent behaviour arising from its gyroid photonic crystal structure [87].

### 4.3 Biological significance

The question of whether this dual structural colour producing system is designed to fulfil a precise ecological function cannot be answered conclusively. Cetoninae primarily feed from the entomophilous flowers of tropical rainforest flora and are diurnally active, therefore, the green colour appearance of *C. smaragdina* is anticipated to aid its camouflage against potential predators. Ad-

ditional optical functionality may be provided by the beetle’s surface topography; by acting to both reduce the intensity of the specular reflection from the beetle’s surface and suppressing iridescence, the grating structure may further assist crypsis amongst the Indonesian rainforest. This represents a deviation from grating structures that produce spectral iridescence, which have been hypothesised to have either aposematic functionality, or to confuse depth- and/or colour-perception in predators [12].

On the other hand, since the three flower chafers investigated by Xu et al. were predominantly red or black in colour [84], any visual benefit may be secondary; it is equally possible that non-visual functionality may have driven the evolution of grating structures in certain Coleoptera. It has been suggested that diffraction gratings provide friction-reducing and water-repellent capabilities to certain beetles and that blazed gratings, in particular, allow burrowing species movement in compressed environments [12]. However, this behaviour is not typically associated with cetonine species.

Whilst Bouligand-type structures are extremely common in crustaceans, significantly enhancing the mechanical strength of their exoskeletal armours [44], it is not immediately obvious why this morphology would be adopted in some coleopteran species but not others unless it serves an alternative purpose. At present the visual systems of Coleoptera represents a gap in the general breadth of knowledge concerning arthropod vision. For example, it is known that fluorescent signalling is utilised by birds [88], spiders [89], fish [91] and mantis shrimp [92]. More pertinently, stomatopods can detect CP stimuli [93] and, further, actively rotate their eyes to align particular photoreceptors to optimise their detection of polarised signals [94]. Some debate remains as to whether scarabeid beetles are capable of detecting CP signals. Brady and Cummings reported that the jewel scarab *Chrysina gloriosa* is capable of differentiating between light sources of equal intensity based upon their polarisation signatures alone [95]. In contrast, another jewel scarab, *C. woodi*, exhibited no phototactic discrimination. Moreover, Blaho et al. [96] investigated four scarab beetles including two cetonine species, *Cetonia aurata* and *Potosia cuprea*, finding no evidence of a behavioural response to CP light. Clearly, further behavioural experiments have to resolve this enigma.

## 5 Conclusion

The flower chafer *C. smaragdina* displays a metallic green colour appearance. The colour of *C. smaragdina* arises due to the helicoidal ultrastructure of its exocuticle, which performs as a Bragg reflector of LCP light, as previously described for beetles belonging to several other subfamilies of Scarabaeidae. Unlike the majority of beetles exhibiting CP reflection properties, however, *C. smaragdina* possesses a quasi-irregular blazed grating surface structure. As a consequence, *C. smaragdina* exhibits the angle-independent suppression of the reflection of RCP light, whilst the scattering of light by the beetle’s surface topography enhances the angle-independence of its green colour. In this way, the blazed surface topography may assist in modulating the metallic appearance of *C. smaragdina* sufficiently that it is afforded more complete camouflage when feeding on the surface of rainforest flora.

## Competing Interests

We have no competing interests.

## Author contributions

LTM performed experiments, analysed experiments, participated in the design of the study and drafted the manuscript; EDF performed experiments, analysed experiments, participated in the design of the study and drafted the manuscript; BDW performed experiments, analysed experiments,

participated in the design of the study and drafted the manuscript; PV designed the study, coordinated the study and helped draft the manuscript. All authors gave final approval for publication.

## Acknowledgements

The authors would also like to thank P. Splatt for assisting in preparing samples for TEM.

## Funding

This work was financially supported by the National Centre of Competence in Research Bio-Inspired Materials and the Ambizione program of the Swiss National Science Foundation SNSF (PZ00P2\_168223, to BDW).

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