

Scale granules and colours: Sexual dimorphism in *Trichonis* (Lepidoptera: Lycaenidae, Theclinae)

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ABSTRACT

A large fraction of dorsal wing surface ground scales show an unusual granulated nature, composed of material apparently extruded from the scale lumen in male individuals of both *Trichonis* Hewitson, 1865 species in the tribe Eumaeini, a rare Guyanian–Amazonian genus. Only a few not-granulated male specimens are known, females are not granulated. The granulated scales are investigated by various microscopic (optical, scanning and transmission electron microscopy, focused ion beam lamella cutting) and spectroscopic (optical reflectance, energy-dispersive X-ray (EDS), Raman) techniques. The characteristic blue colour unique in the South American representatives of the tribe is documented and analysed. EDS spectra show that the granules contain additional calcium and oxygen as compared with the un-granulated regions of the same scale. Electron diffraction (inside the TEM) did not reveal any crystalline component in the granules. The granulated wing surfaces of the males exhibit a UV absorption band at 280 nm, characteristic for biogenic CaCO₃; therefore, the material of the granules is tentatively identified as CaCO₃. It is shown that the granules influence the optical properties of the dorsal wing surface resulting in a characteristic spectrum.

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1. Introduction

Papilionoid lepidopterans, especially members of the family Lycaenidae, are well known because of frequently encountered sexual dimorphism. This is expressed in various ways from wing shape and venation, via colouration and pattern, to the scale macro- and micromorphology (Vértesy et al., 2006). Regarding this last aspect, the most obvious one is the presence or absence of sexual secondary characters composed of highly specialised scales for thermal regulation and scent dissemination in the tribe Eumaeini (Faynel and Bálint, 2012).

The sexual dimorphism of the very rare Neotropical eumaeine lycaenid butterfly genus *Trichonis* Hewitson, 1865 (type species: *Papilio theanus* Cramer, 1777, by original designation) is expressed in all the above-mentioned ways. These are so remarkable that the opposite sexes of the *Trichonis* type species were described as

representing different species by the same classical worker Pieter Cramer (1721–1776) (Cramer, 1775, 1777). The male of the other species, discriminated later by Lathy (1930), was considered first as the female of *Trichonis*'s type species by William Chapman Hewitson (1806–1878), who established the genus (Hewitson, 1865). Later it was pointed out that this „female” was an undescribed species' male, which also belonged to the genus *Trichonis* (Lathy, 1930). Robbins (1986) monographing *Trichonis* pointed out and briefly described the remarkable male secondary sexual characters expressed by highly specialized scales found on the ventral wing surface of the forewings and dorsal wing surface of the hindwing as large scent pads. Robbins (1986) documented first the female of *Trichonis immaculata* Lathy (1930), a very rare jewel of the lowland Lycaenidae fauna in the Amazonian basin and the Guyana shield.

The male *Trichonis* has an unusual structural blue colour which is unique amongst the representatives of its Neotropical relatives, which was why it came to our attention (Bálint et al., 2008). Working on the scale nanomorphology and optics of *Trichonis* we discovered a remarkable trait on male wing scales: in their lumen

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under optical microscope they seemed to be „granulated”. Although there are extensive literature on Lycaenidae scale micro- and nanomorphology (Eliot, 1973; Tilley and Eliot, 2002; Wilts et al., 2008), a similar phenomenon has not yet been observed in this or the other butterfly families (Ingram and Parker, 2008). The aim of the present paper is to report on our findings via (1) documenting colour generating nanoarchitectures and the presence of granules and their properties by various microscopic techniques, (2) analysing the colour and the phenomenon by several materials science methods and (3) to discuss our results in the light of physiological and biological perspectives.

2. Materials and methods

2.1. Species investigated

Both *Trichonis* species, *Trichonis hyacinthus* (Cramer, 1775) and *T. immaculata* Lathy (1930), are rare lowland rain-forest species with Amazonian-Guyanese distribution (Robbins, 1986) (Fig. 1). The genus is classified as follows: section *Hypostrymon*: tribe Eumaeini: subfamily Theclinae: family Lycaenidae (Robbins, 2004).

2.2. Specimens

For non-destructive macroscopic and microscopic investigations 15 male and 5 female museum specimens of *T. hyacinthus* and 8 male and one female specimens of *T. immaculata* were used. For destructive investigations (optical spectroscopy, SEM, TEM and EDS, etc. see below) one male and one female specimen of *T. hyacinthus* were sacrificed as indicated (see Appendix 1).

2.3. Optical microscopy

Each specimen examined was mounted on a piece of Styrofoam beneath a Zeiss Stemi Apo II (Oberkochen, Germany) (at Natural History Museum, London) or an Olympus SZX12 (Shinyuku, Japan) (at Hungarian Natural History Museum, Budapest) microscope, in such manner that the left dorsal wing extended beyond the edge of the mount and could be illuminated, observed and documented in reflected (Fig. 2) and transmitted, or combined light (Fig. 3). Micrographs were taken using a digital camera attached to the microscope. Initially – when checking that the granule-density occurred on several specimens – observations were made of the entire left dorsal wing surface to reveal the presence or absence of granules. In those individuals with granulated scales, an average

granule frequency was calculated from all ground scales visible in 0.01 mm^2 . Based on these means, four categories of granule-density were established for comparison between individuals: (1) none: no granulated scales; (2) low: number of granulated scales 1–5; (3) medium: number of granulated scales 6–10; and (4) high: number of granulated scales 11 or more.

2.4. Transmission electron microscopy (TEM)

After preparation according to standard techniques (Bálint et al., 2012) the 70 nm thin layers from wing cross-section and the

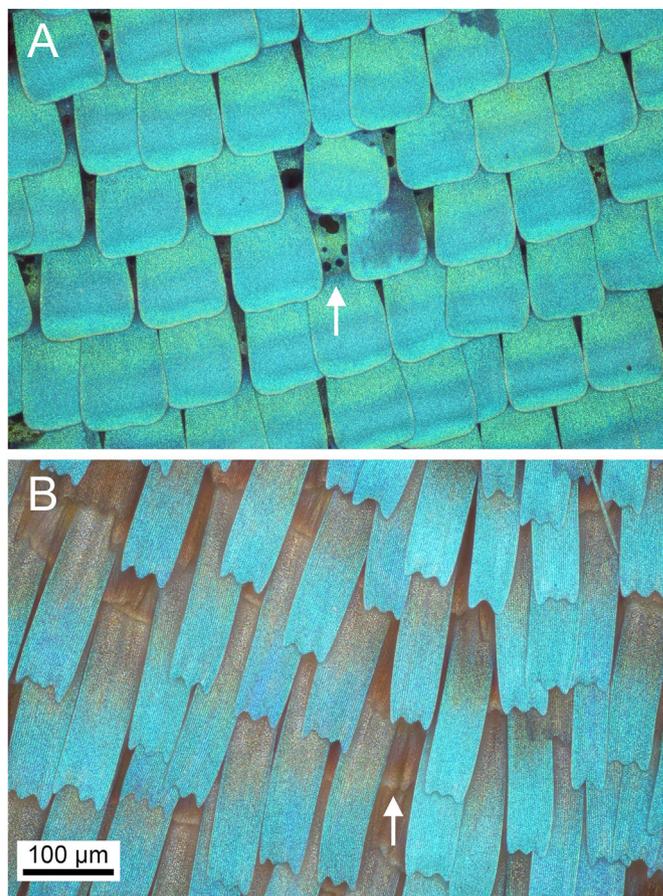


Fig. 2. Dorsal wing surface discal area of *Trichonis hyacinthus* (Hewitson, 1865) under optical microscope (arrows indicate ground scales). A = male, B = female.

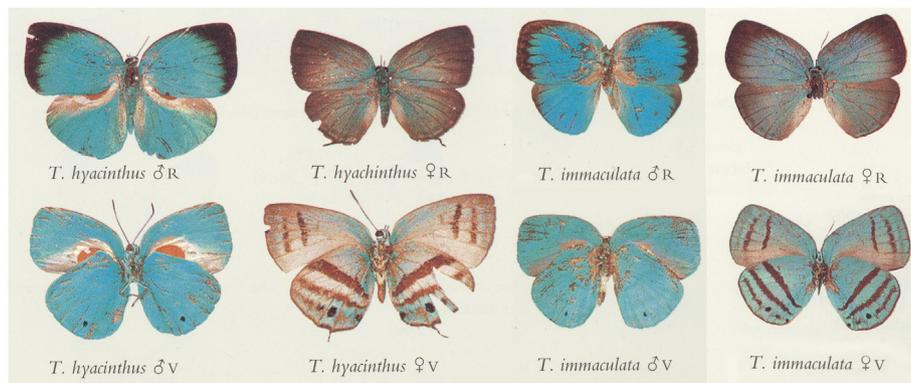


Fig. 1. *Trichonis* male and female phenotypes documented by D'Abreu (1995). The male secondary characters are especially well visible in *T. hyacinthus* having a large scent pad in the basal areas of the dorsal hindwing and the ventral forewing surfaces (all in same magnification: "*T. hyacinthus* ♂ R" forewing costa length = 18 mm).

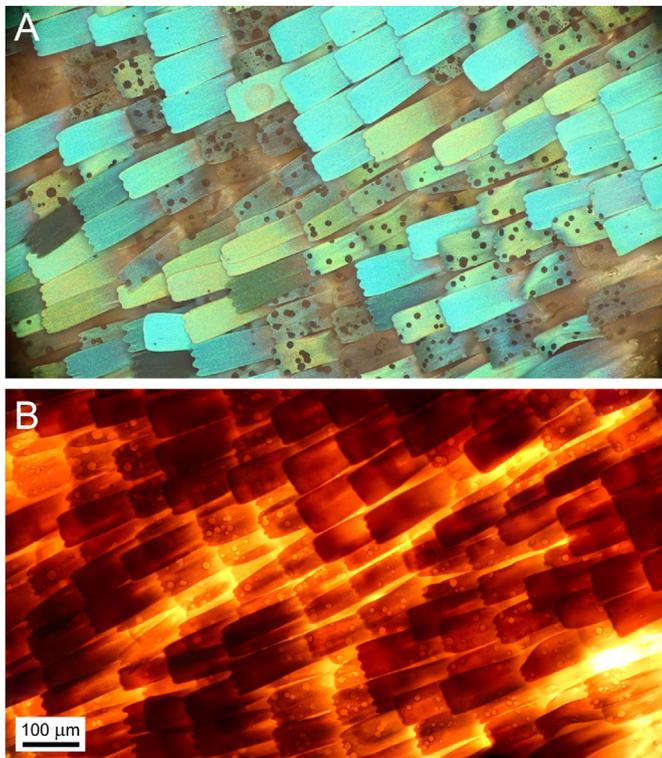


Fig. 3. A slightly worn area of male *Trichonis hyacinthus* (Hewitson, 1865) dorsal wing surface in the discal cell under various illuminations and magnifications. The granulated ground scales are obvious. A = in reflected light, B = in transmitted light.

lamella extracted using Focused ion Beam (FIB) sculpting from a granule located inside the combined SEM/FIB microscope (Zeiss LEO 1540 XB (Jena, Germany)) were inspected with a Philips CM20 (Eindhoven, The Netherlands) at 200 kV. After imaging, the electron-diffraction patterns were checked on the granules to decide if they were crystalline or amorphous.

2.5. Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS)

Scanning electron microscopy examination of wing scales was carried out as reported earlier (Bálint et al., 2012). Wing pieces and single scales were attached to stubs via conductive tape. In order to ensure the imaging of the original surface, no metallic coating was applied. All SEM images were captured by a Zeiss LEO 1540 XB (Jena, Germany) microscope. Inside this apparatus the EDS investigation and the preparation of TEM lamella were carried out using FIB. In FIB cutting after the deposition inside the SEM/FIB microscope of a protective layer of metallic Pt, a narrow beam of Ga ions was used to locally sputter away the sample, so that a TEM lamella of suitable thickness was cut from the sample. Then this lamella was broken away and placed on a conventional TEM grid. In this way it was ensured that the TEM lamella was cut precisely from the feature previously identified during the detailed SEM examination.

2.6. Optical spectroscopy

Spectral measurements were carried out as reported earlier (Kertész et al., 2020) using an Avantes (Apeldoorn, The Netherlands) fibre-optic system consisting of an AvaSpec-HERO spectrometer, an AvaLight-DH-S-BAL light source, an integrating

sphere (AvaSphere-50-REFL) and a Avantes WS-2 diffuse reference tile. Opposite to the normal incidence probe, the integrating sphere picked up all light reflected under any angle in the upper hemisphere.

2.7. Raman spectroscopy

Raman measurements were carried out using a Witec 300RSA + confocal Raman system with 633 nm excitation laser. Laser power was set to 2 mW for the line cuts and 0.5 mW for single spectrum measurements.

3. Results

3.1. Optical microscopy

We observed that *Trichonis* is commonplace in respect of scale layers and scale shapes (Tilley and Eliot, 2002; Vértésy et al., 2006). On the wing surfaces of both sexes, there were two layers composed by the upper cover and the lower ground scales. The lumen of the cover scales contained amorphous gyroid photonic nanoarchitectures similar to *Pseudolycaena marsyas* (Linnaeus, 1758) (Vértésy et al., 2006; Seller et al., 2017) generating a characteristic blue colour, but the morphologies and the dimensions in the sexes were different. The typical length/width/thickness dimension of the male cover scales was 150/50/1 µm with straight or slightly undulated apices and outer margins slightly narrowing towards the wing membrane. In females the cover scale dimensions was 250/50/1 µm with heavily dentate apices and parallel outer margins. The ground scales were similar in both sexes with their wide shovel shape having the dimension 100/80/1 µm, but the male scales had the granulated nature in the dorsal wing surface (Fig. 2).

In the male dorsal wing surface in both *Trichonis* species around the main veins the ground scales were granulated, particularly in worn areas where there were no colour generating cover scales. Very rarely, the blue cover scales showed granules similar to the ground scales. There were no granulated scales in the ventral wing surfaces. None of the females investigated ($n = 6$) showed any granules. Three of the males investigated ($3/23 = 13\%$) also had no granulated scales. Fifteen specimens of *T. hyacinthus* males and eight specimens *T. immaculata* males were investigated. From these, low granule-density was recorded in the cases of four *T. hyacinthus* ($4/15 = 26.6\%$) and in four *T. immaculata* ($4/8 = 50\%$) specimens respectively. Medium granule-density was recorded in the cases of ten *T. hyacinthus* ($10/15 = 66.6\%$) and in three *T. immaculata* ($3/8 = 37.5\%$) specimens respectively. None of them were highly granulated (Fig. 3).

3.2. SEM and TEM

Based on the scanning and cross-sectional transmission electron microscopy, the lumen of the *Trichonis hyacinthus* cover scales of both sexes shows periodical quasiordered photonic nanoarchitecture (Fig. 4), which is responsible for the generation of the characteristic colour solely found in *Trichonis* amongst the representatives of the tribe presented by more than 1200 species in South America (D'Abrera, 1995; Robbins, 2004). This blue colour observable with the naked eye is very similar in the different sexes, but can be precisely characterized by spectral measurements. When comparing TEM images (Fig. 5) of the structure of the ground and the cover scales, one can clearly distinguish them. The ground scales contain in their lumen a dark granular filling which is disordered in distribution. The cover scales contain a quasiordered filling, showing some similarity with the photonic nanostructures of other blue lycaenids (Bálint, 2012; Kertész et al., 2017), however

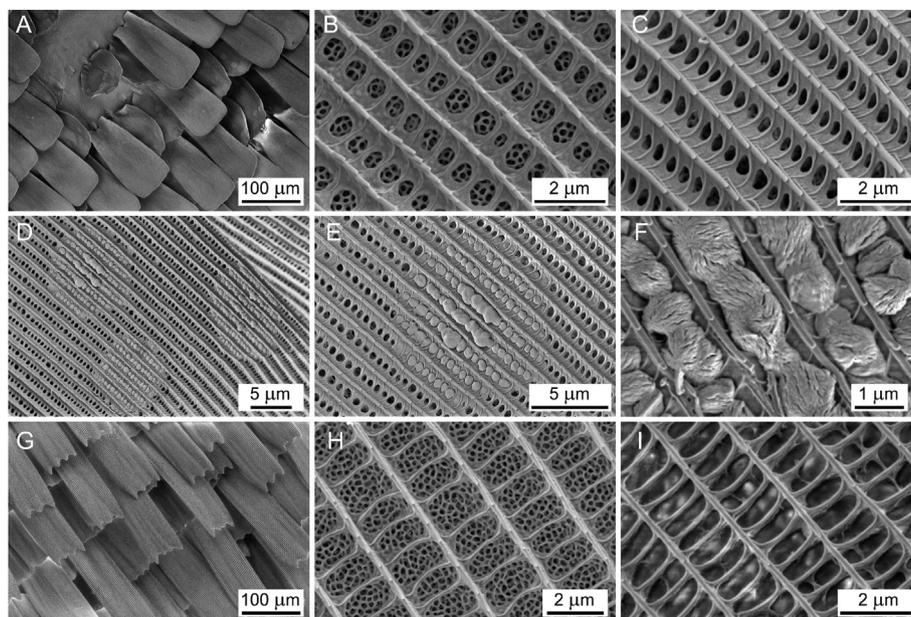


Fig. 4. Scanning electron micrographs of *Trichonis hyacinthus* (Hewitson, 1865), dorsal wing surface discal area, male (A–F) and female (G–H). A = showing small part where there are no cover scales, and the granulated ground scales can be seen. B = male cover scale surface with photonic nanoarchitecture in its lumen visible via windows. C = normal male ground scale surface without photonic nanoarchitecture and granule. D = male ground scale surface with three granules. E = one granule under larger magnification. F = pellet-like nanostructure of the granule extruded from the scale lumen via the windows. G = female cover scales with characteristic dentate morphology. H = female cover scale surface with photonic nanoarchitecture. I = female ground scale.

no continuous layers can be identified in the high-resolution TEM image in Fig. 5C. As the thickness of the ultramicrotomed TEM section is constant (70 nm), the layers exhibiting different contrast indicate changing pigment loading within the cover scales. The lower layer, closer to the wing membrane, is more pigmented than the region below the ridges.

The lumen of the ground scales does not contain any regular or quasiregular nanoarchitectures, only the discontinuous traces of such a nanoarchitecture can be seen both in the SEM and the TEM images. Therefore the homogenous, pale blue colour may be generated by the scale lamina, which is a widely distributed phenomenon, also in lycaenids (Bálint et al., 2018, but other butterflies see e.g., Stavenga, 2014; Thayer et al., 2020) (see later, Fig. 7A). It is worth pointing out that, as seen for example in Fig. 5, the ground scales give a much stronger contrast in the TEM when compared with the cover scales. This is an indication that the pigment loading of the ground scales is higher. When granules are present within the TEM section, they can be observed easily on the TEM images of the scale as large voids, because the material building the granules falls out during sample preparation (Fig. 5). The TEM sections are 70 nm thick, and the cutting is done by a very sharp diamond knife.

If the mechanical properties of the scale and of the material building up the granules are different, due to the high forces during the cutting, the thin section of the granule can be fractured and will fall out of the TEM lamella. As can be seen on the SEM images the material of the granule completely fills the lumen of the ground scales, protruding through the top (Fig. 4D–F). The arrangement and the size of the protrusions clearly show that the pressure causing the protruding was highest in the central regions of the granules (Fig. 4F).

3.3. Optical spectroscopy

The reflectance measurements carried out using the integrating sphere reveal that the blue colour generated by the cover scales is almost identical in both male fore and hind wing surfaces (Fig. 6A). The dorsal blue colour of the males and the females is similar, with the highest reflectivity around 510 nm, however, fine differences can be revealed (Fig. 6B). The blue colour generated by the dorsal wing surface of the males measured with the integrating sphere is different in the near-UV wavelength region as the reflectance spectrum has a different shape between 250 and 400 nm. This may

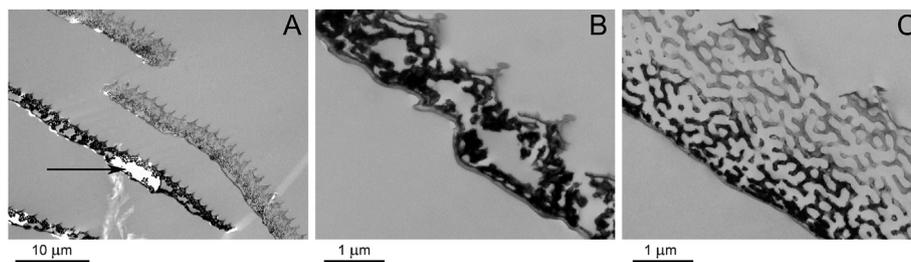


Fig. 5. Transmission electron micrographs of male *Trichonis hyacinthus* (Hewitson, 1865) scales. A = ground and cover scales (arrow indicates the place of the granule in one ground scale); B = ground scale in higher magnification with pigment granules. C = cover scale in higher magnification with colour generating nanoarchitectures, the lower stratum contains pigments.

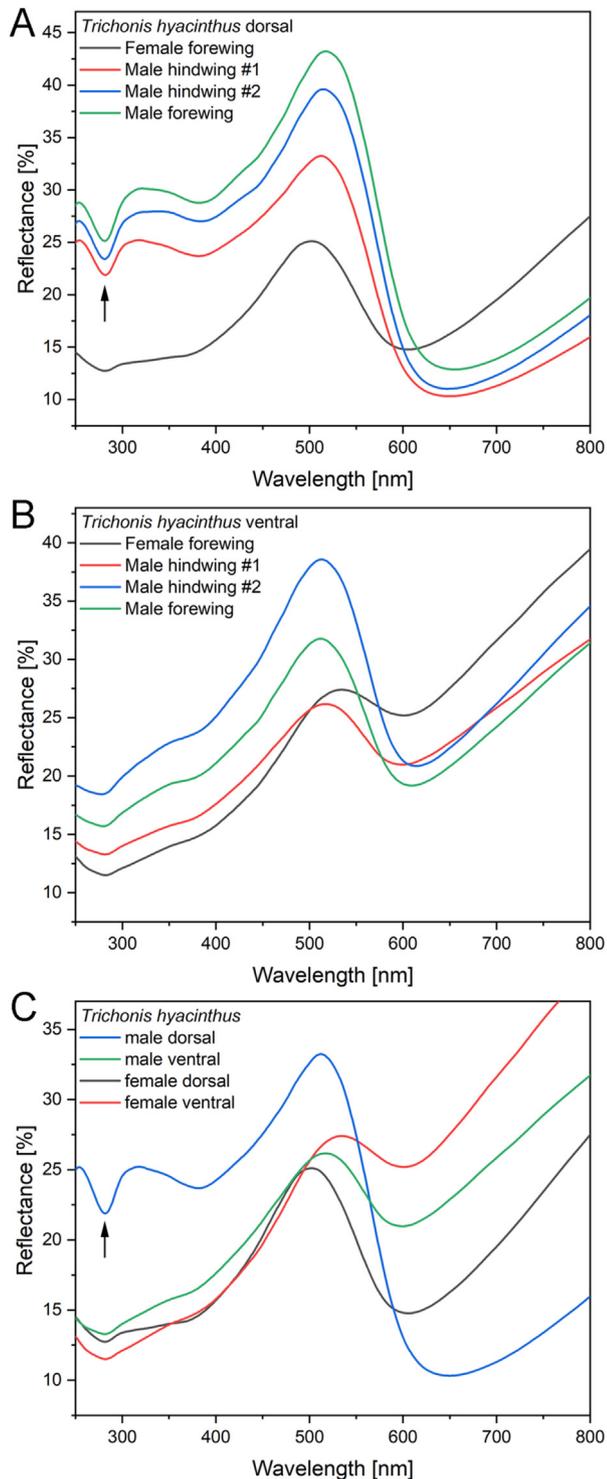


Fig. 6. Reflectance spectra of male and female *Trichonis hyacinthus* (Hewitson, 1865) measured with the integrating sphere. Spectra of (A) dorsal and (B) ventral wing surfaces are shown. In (C), the measured reflectance of male and female specimen can be compared. In (A) and (C) arrow indicates the difference caused by granules.

be attributed to the granulated nature of the ground scales as all the other spectra of the wing surfaces without granules do not contain this type of feature. The three measurements carried out on the male wing surface in different points all show the typical features attributed to the granules, while neither the ventral surface on the

male wing nor the dorsal, or ventral surface of the female wings showed this type of feature (Fig. 6C).

The spectra measured in the range of 600–800 nm show different reflectance between male and female and fore and hindwing. This is due to the less pigmented nature of the ground scales which results in a brighter colour of the ventral wing surface. Male dorsal colour is outstanding because a higher reflectance peak associated with also higher pigment concentration yielding the lowest reflectance in the range of 600–800 nm.

3.4. Materials composition of the granules

To reveal the nature and properties of the granules found in the lumen of the dorsal wing surface ground scales of males, we carried out a series of experiments, including Raman spectroscopy, FIB sectioning, and energy-dispersive X-ray spectroscopy (EDS).

Using the light source of the scanning Raman microscope (Witec 300RSA+), a 2 mW red (633 nm) laser beam was scanned in a line across the object on a ground scale. We produced a cut easily discernible in the optical or SEM image (Fig. 7). The laser created a clear separation of the scale ridges and cross ribs, but there was no difference in the granule area, suggesting higher heat capacitance and material compactness in that circular area. According to our earlier studies, the pepper-pot type nanoarchitecture, or any other solid chitinous material in the scale has 100–150 nm thickness, which was cut through with laser. The Raman spectroscopy did not find any characteristic differences between the investigated areas of the ground scales due to the high photoluminescence of this biological sample.

Transmission electron microscopy, and electron beam diffraction on the edges of a single granule was carried out in the TEM. For this purpose, a single detached scale was fixed between two copper TEM grids with square windows. Placing this sandwich of grid-scale-grid in the microscope, we obtained an image (Fig. 7C) where it was easy to observe the parallel ridges, cross-ribs and the structure in the lumen of the ground scale. The granule appeared black in this image, meaning high opacity for the electron beam of 200 kV. We were able to obtain a diffraction image only near the edge of the circular area, but there were no signs of any crystalline component.

Focused ion beam was used to cut a thin section from the ground scale by eliminating the material under two rectangular areas, leaving only a 50 nm thick slice (Fig. 7). After being sculpted, the lamella was broken away and placed on a standard TEM grid and examined. It was possible to observe (Fig. 8) the homogeneous chitinous material of the scale and a spongy material filling the air voids. In this volume, a section of the granule can be found which gives a clear contrast respective to the chitin and air, but again, we were not able to detect any crystalline component with electron beam diffraction.

The material composition of the granules was investigated with EDS. A single wing scale was placed on conductive carbon tape to ensure the imaging without charging of the sample. The false colour mapping of the elements (Fig. 9) shows contrast in the granulated area and the rest of the scale. The granules contained calcium (Ca) and show excess in Oxygen (O), as compared to the non-granulated region of the scale. As a reference, in top right corner of the image the carbon tape is visible, delineated in broken red lines. In the carbon map the granules appear darker, this means that the compact body of the granules transmits a smaller fraction of the signal coming from the carbon tape on which the scale is fixed than the holey, not filled regions of the chitinous scale. But this does not mean that the granules do not contain carbon. It only means that the carbon content of the granules is below the combined C signal from the carbon tape on which the scale was fixed

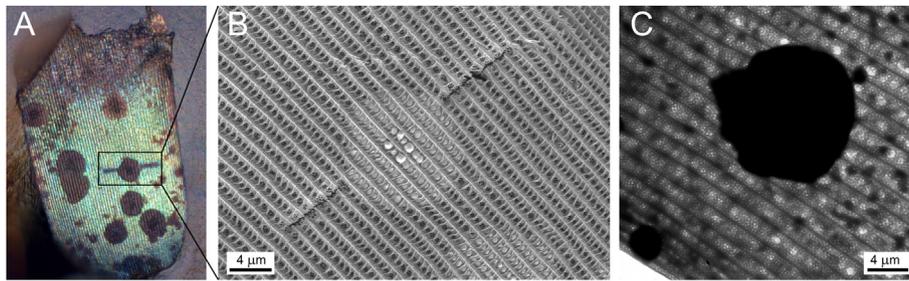


Fig. 7. Experiments on *Trichonis hyacinthus* (Hewitson, 1865) ground scale. (A) Optical and (B) SEM images showing the effect of a linear scan with the laser beam of the Raman microscope carries on sample. (C) TEM image of a single (another) ground scale near the granule area. The electron beam was perpendicular to the plane of the scale in this measurement.

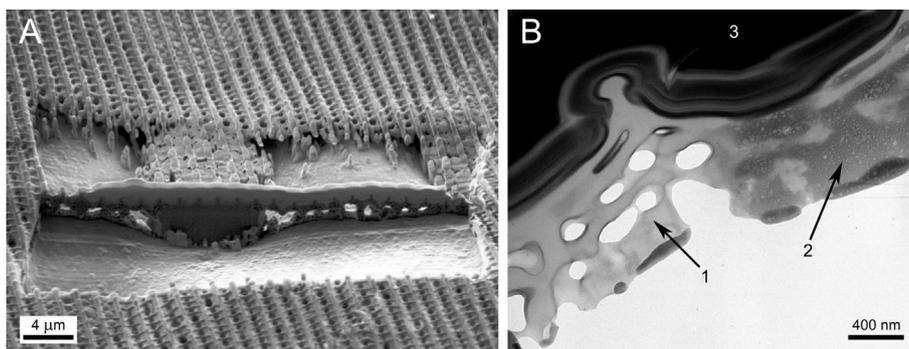


Fig. 8. SEM image showing from one side the lamella cut crossing *Trichonis hyacinthus* (Hewitson, 1865) wing scale containing a granule (A) and TEM image of the same section (B). The numbers indicate the chitin of the scale (1), the granule substance (2) and the protective layer (3).

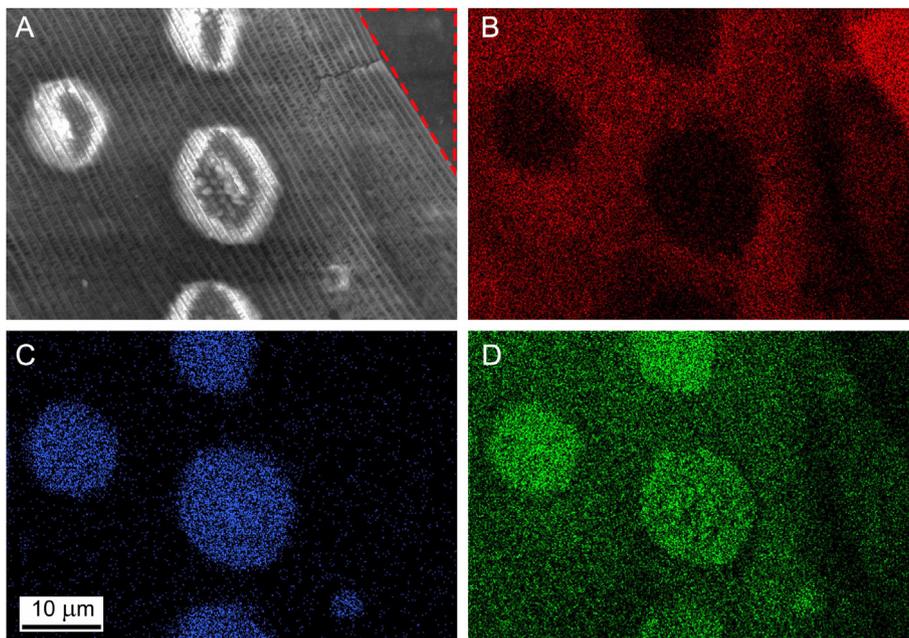


Fig. 9. Analysis of *Trichonis hyacinthus* (Hewitson, 1865) ground scale granules. SEM and EDS false colour maps of the elemental composition of ground scale with granules. A = normal (in the left upper corner a region of the carbon tape is seen delineated in red broken lines. This gives the highest Carbon signal seen in the carbon map). B = Carbon, C = Calcium, D = Oxygen. The granules being more compact than the holey chitin scale are seen as dark spots on the Carbon map, while they appear brighter on the Calcium and Oxygen maps. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

and the signal generated by the scale itself. As the scale material is mostly chitin, it must contain carbon, too. Therefore, the quantitative evaluation of the carbon content of the scale and of the granules is made difficult by conductive carbon tape.

4. Discussion

4.1. *Androconia*

In general, it is a consensus that Lycaenidae wing surfaces have two layers of scales responsible of the characteristic colouration and pattern (Schwanwitsch, 1949; Eliot, 1973; Tilley and Eliot, 2002). The scales of the lower layer lack photonic nanoarchitectures. The cover scale lumen of the upper layer contains the photonic nanoarchitecture responsible for colour generation. However it is a rather simplified view as there are many cases when beside these two kinds of scales further specialised scales occur, primarily on males. Therefore they are called androconia (Eliot, 1973). Although many of these scales are often arranged in eumaeine lycaenids as patches or pads (Faynel and Bálint, 2012), there are examples where androconia are dispersed amongst the normal colour-generating scales, the most remarkable example is the tribe Polyommataini (Courvoisier, 1916).

In *Trichonis*, there is a complex system of scent patch and scent pad (see Fig. 1) that can be observed in many papilionoid families, as well as in representatives of eumaeines. But in the tribe Eumaeini, it is also recorded that in certain areas of the wing androconia may occur mixed with the colour-generating scales (Bálint, 2009; Faynel and Bálint, 2012; Martins et al., 2019). In the bordering areas of scent patches, the androconia are often dispersed further and have been gradually replaced by normal colour generating scales. Scent patches containing androconia are mostly present in wing areas bordering by the principal veins, radius and cubitus. This is obligatory from a physiological point of view, as the wing membrane itself is not dead matter as the cells in the veins are living and used to sense the environment (Salcedo and Socha, 2020). Most recently, it was experimentally shown that not only in the tube-like veins was there circulating haemolymph but also in the wing membrane discal area bordered by the principal veins (Tsai et al., 2020). Hence, we can presume that living cells in the veins and wing membrane are responsible for scent dissemination.

The granulated areas in *Trichonis* males are restricted to the dorsal wing surfaces around the main veins. In the ventral wing surface there are no granules. This may suggest that the dorsal wing surface ground scales may function as scent disseminators, somehow homologous with the polyommataine androconia. However the material composition of the granules may contradict this hypothesis, knowing that Lycaenidae scent aphrodites are composed of very different chemical compounds (Ömara et al., 2013). Other contradicting evidence is, that *Trichonis* males have a sophisticated system of androconia, as mentioned above in the Introduction.

4.2. *The granules*

Ground scales with a granulated nature were not recorded anywhere, although we checked representatives of genera *Balintus* d'Abrera, 2001; *Celmia* Johnson, 1991; *Dicya* Johnson, 1991; *Erora* Scudder, 1872 and *Iaspis* Kaye, 1904 placed in the *Hypostrymon* section of Robbins in his phylogenetically oriented checklist, indicated as closely related groups (Robbins, 2004). We did not find granulated scales in specimens representing further relatives of the same or other families randomly selected on the basis of a superficially similar androconial system: *Antirrhoea* Hübner, 1822; (Morphinae, Nymphalidae; Neotropical); *Aslauga* Kirby,

1890 (Miletinae, Lycaenidae; Afrotropical); *Dismorphia* Hübner, 1816 (Dismorphiinae, Pieridae; Neotropical), *Euploea* Fabricius, 1807 (Danaina, Nymphalidae; Austral and Oriental). Hence according to our best knowledge granulated scales occur exclusively in male *Trichonis*.

On the basis of our EDS analysis the granules seem to contain excess calcium and oxygen. Calcium oxide is usually made by the thermal decomposition of materials, that contain calcium carbonate (CaCO_3). Theoretically this is accomplished by heating the material to above 825 °C, a process called calcination or lime-burning, to liberate a molecule of carbon dioxide (CO_2). This is certainly impossible for a living organism, so there must be another mechanism during the biosynthesis process. Furthermore, CaO is unstable in air and under humid conditions. On the other hand, CaCO_3 is frequently synthesized on biogenic route, for example in pearls, and nacre and coral (Reef et al., 2009; Yan et al., 2017, 2021). As the EDS elemental maps show that the granules contain carbon too, although less carbon than the carbon tape on which the scale was fixed, it is reasonable to investigate if the presence of CaCO_3 can be ruled out or supported.

The characteristic of this biogenic CaCO_3 is an UV absorption band at 280 nm (Reef et al., 2009; Yan et al., 2017, 2021) (see also in Fig. 6), which is associated with the biotemplated growth of CaCO_3 . This characteristic band can be identified only on the optical spectra of the dorsal wing surface of the male (Fig. 6). Calcium is known to have an important role in the scale synthesis process during metamorphosis (Otaki, 2012). The careful examination of the fine structure of the granules as revealed by SEM images (Fig. 4C and E) indicates that the system of ridges and cross-ribs must be in place before the formation of the granules, as the material of the granules is “extruded” through the grating formed by the ridges and cross-ribs. The FIB sectioning and the examination of the sculpted lamella in the TEM shows that the material of the scale and the material of the granule are interpenetrated.

The curious feature in the reflectance spectra of the male dorsal wing surfaces in the range of 250–400 nm taken by the integration sphere can be attributed to the increased light scattering due to the presence of the granules and the contribution of the UV absorption of the biogenic CaCO_3 . This is supported by the 280 nm UV absorption band characteristic for biogenic CaCO_3 .

4.3. *Colouration and pattern*

The colouration and pattern of male and the female *Trichonis* are basically different; only the characteristic blue colour of the male is shared by some female wing areas. The male and female *Trichonis* phenotypes express distinct behaviour and lifestyle. We hypothesize that *Trichonis* males are lekking in sunny forest glades or in the canopy. Presumably, males are territorial as most of the forest dwelling theclines, and their flight is rapid and furious, which for the human eye is difficult to follow (Takeuchi and Imafuku, 2005; Prieto and Dahners, 2006). What is curious in the *Trichonis* male phenotype is the presence of the same structural colour in both of the almost patternless wing surfaces (see Fig. 1).

Female *Trichonis* adopt a phenotype which is well presented amongst the Neotropical papilionoid butterflies. Not only genera of the tribe Eumaeini but other families apply this pattern of transverse bands displayed by the hindwing ventral wing surfaces. This phenotype mainly typifies butterfly imagines which live deep under the forest canopy or forest understory which reveals a rhythmic pattern of dappled light. Most probably in these circumstances it is difficult for sexes to locate each other and females depend first on optical signals provided by males.

According to our knowledge regarding male structural colouration, the only tribes possessing a comparable appearance in the

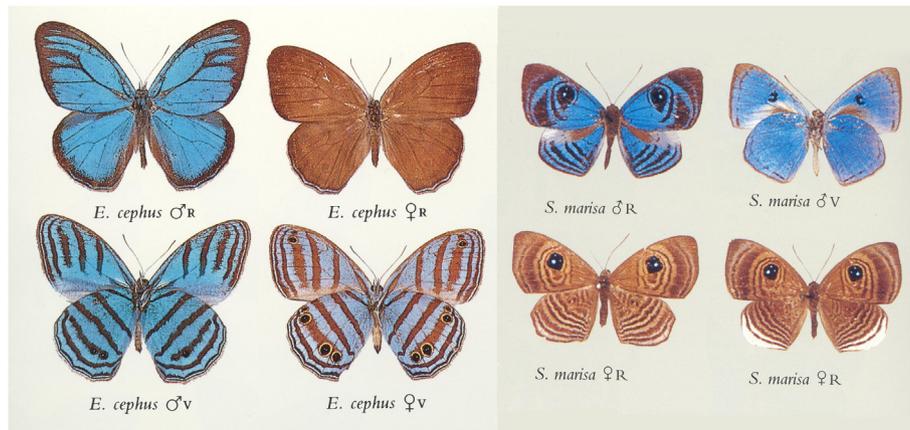


Fig. 10. Male and female satyrid and rioidinid butterfly phenotypes from the Amazonian fauna superficially resembling *Trichonis* documented by D'Abbrera (1988 and 1994). The satyrid *Cepheutyhia cephus* (Fabricius, 1775) male has a striped wingsurface verso. The rioidinid *Semomesia marisa* (Hewitson, 1858) male wingsurface recto is striped but has androconial system somewhat similar to *Trichonis*. The female wingsurface versos are striped (all in same magnification: “*E. cephus* ♂R” forewing costa length = 21 mm).

Neotropical region are the satyrid Euphythyciini (cf. D'Abbrera, 1988: 766–769; Nakahara et al., 2018) and the rioidinid Mesosemiini (cf. D'Abbrera, 1994: 901–902). Both are also primarily Amazonian. Males of some species of these enigmatic butterflies have wing surfaces with identical structural colouration (Fig. 10). But in contrast to *Trichonis*, both male and female phenotype ventral wing surfaces are striped and granules were not found in scale lumina. This phenotypic appearance may indicate that both sexes of these rioidinid and satyrid butterflies spend their lives in an environment optically similar to that of *Trichonis* females. However because male *Trichonis* are characteristically patternless, they may produce a unique signal that can be easily spotted by their females.

5. Concluding notes

We showed that the sexual dimorphism of *Trichonis* is remarkable, as beside the commonplace grossly-differing phenotype expressed by wing shape, pattern, colouration and male secondary characters (androconia), the sexes differ in an additional character: the layer of ground scales with granulated physiology. Although we elaborated on the morphology and material composition of the granules, its development remains unknown; only various hypotheses could be formulated: (1) the granules may start to develop sometime after the hatching of the male individual proceeding interactions with the environment or (2) it is a male individual aberration. Further, the function of the granules is not understood, but we have shown that it has an influence on the spectral properties of the wing surfaces, and as such may have an effect on mate selection. It would be interesting to determine whether the female is able to distinguish between the male's colouration with and without granules.

It is interesting to note that physical changes of butterfly imagines usually caused by external physical factors are becoming more and more intensive with time. This in general may result in the loss of scales, which cause reduced attractiveness in males (Kemp, 2007), but in females it may bring benefits (Pyrz et al., 2018). If it has been confirmed that the male *Trichonis* individuals produce the granules after hatching in the scales that would be supporting evidence for the idea that the external physiology of butterfly imagines is sometimes incomplete.

In the future, it would be interesting to study in the field how mesosemine rioidinids, euptychine satyrids and *Trichonis* males and females share their habitat in temporal, spatial and

microgeographical terms. Although such questions are worth pursuing, the rarity of *Trichonis* and the deforestation taking place in the Amazonian ecosystem together suggest that these questions will remain unanswered.

Author contributions

Conceptualization: Zs.B.; Methodology: Zs.B., L.P.B., K.K., G.P.; Investigation: A.I., Zs.B., K.K., G.P.; Z.E.H.; L.I.; Resources: Zs.B.; Data curation: Zs.B., A.I.; Writing – original draft: Zs.B.; Writing – review and editing: Zs.B., L.P.B., A. I., K.K., A.P., P.G.; Visualization: K.K., G.P.; Funding acquisition: Zs.B., L.P.B., K.K., G.P.

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Appendix

Museum specimens of *Trichonis* examined. A – serial number; B – scientific binomen; C – sex (* = specimen examined by SEM and TEM); D – collecting locality; collector or collection; E – degree of granule-density (0 = none, 1 = low, 2 = medium); F – condition of the specimen; (*) = documented in D'Abbrera (1995, see Fig. 1); specimen examined by SEM and TEM; G – depositor (BMNH = Natural History Museum, London (formerly: British Museum of Natural History); HNHM = Hungarian Natural History Museum, Budapest).

A	B	C	D	E	F	G
1	<i>Trichonis hyacinthus</i>	♂	French Guyana: St Jean du Maroni; Le Moul	2	poor	BMNH
2	<i>Trichonis hyacinthus</i>	♂	French Guyana; Bar	0	good	BMNH
3	<i>Trichonis hyacinthus</i>	♂	British Guyana; Joicey	2	fair	BMNH
4	<i>Trichonis hyacinthus</i>	♂	British Guyana; Kaden	1	fair	BMNH
5	<i>Trichonis hyacinthus</i>	♂	British Guyana; Kaden	1	good	BMNH
6	<i>Trichonis hyacinthus</i>	♂	French Guyana: Cayenne; Hewitson	2	good	BMNH
7	<i>Trichonis hyacinthus</i>	♂	French Guyana: Cayenne; Hewitson	1	fair	BMNH
8	<i>Trichonis hyacinthus</i>	♂	British Guyana	1	fair (*)	BMNH
9	<i>Trichonis hyacinthus</i>	♂	British Guyana; Druce	0	poor	BMNH
10	<i>Trichonis hyacinthus</i>	♂	French Guyana: Cayenne; Oberthür	1	good	BMNH
11	<i>Trichonis hyacinthus</i>	♂	French Guyana: St. Laurent, Maroni; Joicey	2	poor	BMNH
12	<i>Trichonis hyacinthus</i>	♂	Brazil: Para; Moss	2	poor	BMNH
13	<i>Trichonis hyacinthus</i>	♂	Brazil: Para; Moss	2	fair (*)	BMNH
14	<i>Trichonis hyacinthus</i>	♀	French Guyana; Oberthür	0	good	BMNH
15	<i>Trichonis hyacinthus</i>	♀	Brazil: Maranhão; Belt	0	poor (*)	BMNH
16	<i>Trichonis hyacinthus</i>	♀	French Guyana: Maroni; Joicey	0	fair (*)	BMNH
17	<i>Trichonis hyacinthus</i>	♀	French Guyana; Bar	0	good	BMNH
18	<i>Trichonis immaculata</i>	♀	Peru: Iquitos, Rio Cachiyaca; Stuart	0	good (*)	BMNH
18	<i>Trichonis immaculata</i>	♂	French Guyana: Gourdonville; Le Moul	1	poor	BMNH
19	<i>Trichonis immaculata</i>	♂	Surinam; Fruh	1	fair (*)	BMNH
20	<i>Trichonis immaculata</i>	♂	Peru: Iquitos, Rio Cachiyacu; Stuart	1	good	BMNH
21	<i>Trichonis immaculata</i>	♂	No patria; Boisduval	0	poor	BMNH
22	<i>Trichonis immaculata</i>	♂	No patria; Felder	2	poor	BMNH
23	<i>Trichonis immaculata</i>	♂	No patria; Felder	1	poor	BMNH
24	<i>Trichonis immaculata</i>	♂	Surinam; Fruhstorfer	2	fair	BMNH
25	<i>Trichonis immaculata</i>	♂	Surinam; Fruhstorfer	2	fair	BMNH
27	<i>Trichonis hyacinthus</i>	♂	French Guyana: Cacao; Faynel	2	good	HNHM
28	<i>Trichonis hyacinthus</i>	♂ (*)	French Guyana: Cacao; Faynel	2	good	HNHM
29	<i>Trichonis hyacinthus</i>	♀ (*)	French Guyana: Matouri; Faynel	0	fair	HNHM

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