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Fine structure of wing scales of butterflies, *Euploea mulciber* and *Troides aeacus*

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ABSTRACT

Wing scales of male *Euploea mulciber* (*E. mulciber*) and *Troides aeacus* (*T. aeacus*) butterflies were investigated from interest in photonic crystal by scanning electron microscopy and optical reflectance measurement. On the basis of the structural observation, the colouration in different areas in their wings was discussed. It was particularly deduced that a violet-green iridescence characteristic of *E. mulciber*'s forewing is caused only in a wavelength range from ~380 to ~510 nm by multiple interference from a highly tilted, triple-layered cuticle arrangement on the brown scales. It was also found that *T. aeacus* does not produce a blue-green sheen such as observed by *Troides magellanus* because its scales have no multiple cuticle layers but microrib layers unable to produce any backscattering diffraction.

1. Introduction

Characteristic pattern and vivid colouration of the wing scales of butterflies have lately attracted considerable attention as photonic crystals in nature that can be seen in a series of optical microscopy (Mason, 1926, 1927a,b) and electron microscopy (Ghiradella, 1991). A Costa Rica male *Ancyluris meliboeus* (*A. meliboeus*)[1] butterfly, called a 'living jewel', exhibits a bright iridescence of broad wavelength range on its ventral wing scales and generates a strong flicker contrast from minimal wing movement, which are produced by the highly tilted, multilayered arrangement on the ridges (Vukusic et al., 2001). A *Troides magellanus* (*T. magellanus*)[2] butterfly (Magellan birdwing), inhabiting Philippine and Taiwan, exhibits a blue-green sheen on the hindwings when both illuminated and viewed at near-grazing incidence. Lawrence et al. (2002) showed that this effect is due to the presence of a constrained bigrating structure. At the time such a blue-green sheen had been known in only one other species of butterfly. It was the *A. meliboeus*. The *T. magellanus* uses pigmentary colouration at all but a narrow tailored range of angles to produce the characteristic effect by multilayered rib-like (or microrib) scales. This unique visual attraction of the *T. magellanus* was detailed by Vigneron et al. (2008), taking into account correlated diffraction and fluorescence in the backscattering iridescence. Recently, Matějková-Plšková et al. (2009, 2010) have observed a highly tilted, multilayered arrangement in vivid iridescent scales in a male *Sasakia charonda* (*S. charonda*)[3] butterfly (the great purple emperor). The structure of its scales is very similar to that found in the *A. meliboeus*. A kind of blazed diffraction grating of the scales has high efficiency in a shorter wavelength range of 200–450 nm. However, the details of colouration of these ridge-lamellar scales, in particular, limited in a certain region of wavelength need further investigation and discussion.

The structures of the photonic crystals of the wing scales would be applicable to fine light manipulators such as reflection elements in light-emitting devices. In fact, the photonic structures of butterfly scales have been prototyped using atomic layer deposition (Huang et al., 2006; Gaillot et al., 2008; Hsiao, 2009) and a biotemplate method (Zhang et al., 2009). Therefore, the structural

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[1] *Ancyluris meliboeus.*
[2] *Troides magellanus.*
[3] *Sasakia charonda.*

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investigations of the butterfly scales are still required for achieving tunable photonic properties in the artificial scales.

In the present paper, we investigate the microstructure and colouration of scales on different areas in the wings of male *Euploea mulciber* (*E. mulciber*)[4] and *Troides aeacus* (*T. aeacus*)[5] butterflies, by means of scanning electron microscopy (SEM)[6] and reflectance measurement as well as optical microscope observation. The violet-green iridescence of the ridge-lamellar scales in the *E. mulciber* and no blue-green sheen of the microrib scales in the *T. aeacus* are elucidated, comparing with the scale structures of *A. meliboeus, S. charonda* and *T. magellanus*.

2. Samples and methods

The male *E. mulciber* and the male *T. aeacus* used in the present experiment were reared from eggs. SEM observations were performed with a JEOL JSM-6335F equipped with a cold field-emission gun. The wings of the butterflies were dried at 40 °C for a few minutes and covered with a sputtered gold layer about 20 nm thick to avoid charging effects. Some specimens were cut to a thickness of about 3 μm with a Leica Ultracut UCT-GA-D/E-1/00 microtome to observe cross section. Reflectance of the wing scales was measured by using an opto-spectrometer (Perkin Elmer Lambda 900) with two light sources of variable wavelength ranges of 200–375 nm and 375–2500 nm. Two detectors for 200–860.8 nm and 860.8–2500 nm were used. To study the localized optical property of wing, the incident beam along the wing normal was focused to 2 mm^2. This small detected area led to degraded intensity of reflective light so that an integrating sphere was applied to collect the weak reflected light signals.

3. Results and discussion

3.1. Male E. mulciber butterfly

E. mulciber called 'the striped blue crow' is a common butterfly in Thailand, Malaysia, Singapore, Laos, Vietnam, South China, *etc*. It is in the subfamily Danainae of the family Nymphalidae. SEM observations and reflectance measurements were performed on several areas shown in Fig. 1a; vein **V**[7], iridescent blue background **B (1, 2)**[8], white spot **W**[9] and edge **EF**[10] in the forewing, and grey patch **B3**[11], pale **B4**[12] at the costal half, dark brown **B5**[13] and edge **EH**[14] in the hindwing.

As seen in Fig. 1c which is an optical microscope image of the scales around **B1** taken using transmitted white light, these scales have an intrinsic colour of dark brown due to melanin. They exhibit vivid blue-green iridescence only at parts where the incident light is reflected on the surfaces, as seen in Fig. 1d. In Fig. 2a is reproduced a SEM image of scales on the vein **V** together with the surrounding blue background. The iridescent blue background **B1** comprises two kinds of scales; broad and narrow scales, which are almost alternately arranged so that the spaces between the broad scales are covered with the narrow scales, as clearly seen in Fig. 2b. The scales on the **vein V** are almost the same in shape as the narrow scales in the background **B (B1** and **B2)**. Fig. 2c–f reveal a multilayered arrangement of cuticles in the scales in **B** (**B** scales), which is similar to that discovered in the *A. meliboeus* (Vukusic et al., 2001) and that observed in *Morpho peleides* (Huang et al., 2006) and *S. charonda* (Matějková-Plšková et al., 2009). The **B** scales form a three-dimensional optical diffraction grating. Fig. 2g illustrates schematic projections of the grating, which is composed of the grid of the ridges with the spacing d, the n multilayered arrangement of cuticles lapped on the ridge, and the surface arrangement of cuticles tilted at θ_B and spaced by D. The x-axis is defined along the ridges running the length of the scale from the root, the z-axis normal to the scale plane and the y-axis normal to the x–z plane. The width of the ridges d_1 and the width of the grooves d_2 as well as the spacings d and D were estimated to be $d_1 \approx 0.3$–0.4 μm, $d_2 \approx 0.6$–0.7 μm, $d \approx 0.9$–1.1 μm and $D \approx 0.5$ μm. The number of the piled cuticle layers is $n = 3$, which is smaller than 7 in the *S. charonda* and 4 in the *A. meliboeus*. From the cross-sectional SEM image in Fig. 2e, we estimated the thicknesses of the cuticle layers and air gaps to be $t_c = \sim 100$ nm and $t_a = \sim 100$ nm, respectively. From the image in Fig. 2f, we also estimated the angle of θ_B to be ~25o. The broad and narrow scales in **B** are almost the same in microstructure. The scales in **W** (**W** scales) resemble the broad scales in **B** in shape and structure but not in colour. They are transparent white or pearl due to little content of melanin pigment, as seen in Fig. 1e which is an optical microscope image taken using transmitted white light. The **W** scales also exhibit the iridescent hues when they reflect the incident light, as seen in Fig. 1f. The parameters of the grating of the **W** scales were measured and are shown in Table 1.

Half of the right forewing in Fig. 1a looks dark. This is caused by the highly tilted, multilayered arrangement of the blazed grating of the **B** scales. Vukusic et al. (2001) illustrated that the layer tilt of $\theta_B = 30°$ causes a 60° portion of the wing's 'observation hemisphere' not to appear iridescent 'dark zone' in the *A. meliboeus*. The angle of θ_B corresponds to the blaze angle for the blazed optical grating (Matějková-Plšková et al., 2010). For the *E. mulciber*, $\theta_B = \sim 25°$. The dark zone of $2\theta_B$ is schematically shown in Fig. 2h. The dark area of the right wing in Fig. 1a is surely in the dark zone. The butterfly can thereby generate a strong flicker contrast from minimal wing movement. Hence, the limited-view iridescence has been found in the wings of the *E. mulciber*, although it was previously known in the *A. meliboeus* (Vukusic et al., 2001), *T. magellanus* and *T. prattorum* (Lawrence et al., 2002; Vigneron et al., 2008), and *S. charonda* (Matějková-Plskova et al., 2010).

Fig. 3a and b shows bigger scales at the edge **EF**. The space between the ridges is wide as compared with the other scales as indicated in Table 1, and mono-layered cuticles are arranged on the ridge. The scales exhibit dark brown without iridescence. The ends of the peripheral scales are divided and have deep splits. Next is described the observation of the scales in the hindwing. Fig. 3c and d shows the scales in the area **B3**, which looks grey between the 7 and 4 veins using numerical notation of the wing venation. These scales are very strange; they are flat fibres as long as several 100 μm, looking like sea tangles grown from the seabed. The fibres widen at their ends (Fig. 3d), where hair-like objects grow on membrane, ventral side of scales. It still cannot be explained what is the function of the long scales and the hair-like objects. Such scales possibly play role in a kind of sense or they can be secretory organ. In any case, the hues of grey at **B3** are attributed to the diffused scattering of the light by these thin thread substances. Fig. 3e shows the scales in B4 in Fig. 1a. These scales are completely different from the scales in the neighbour area **B3**. Although their tails were not split, they rather resemble the dark brown scales around the edge **EF** in fine structure; the mono-layered arrangement of cuticle and the large spacing d_1 between ridges (Fig. 3f). The peripheral scales in the area **EH** have deep splits on the ends,

[4] *Euploea mulciber*.
[5] *Troides aeacus*.
[6] Scanning Electron Microscopy.
[7] Vein.
[8] Blue background.
[9] White spot.
[10] Edge in the forewing.
[11] Gray patch.
[12] Pale.
[13] Dark brown.
[14] Edge in the hindwing.

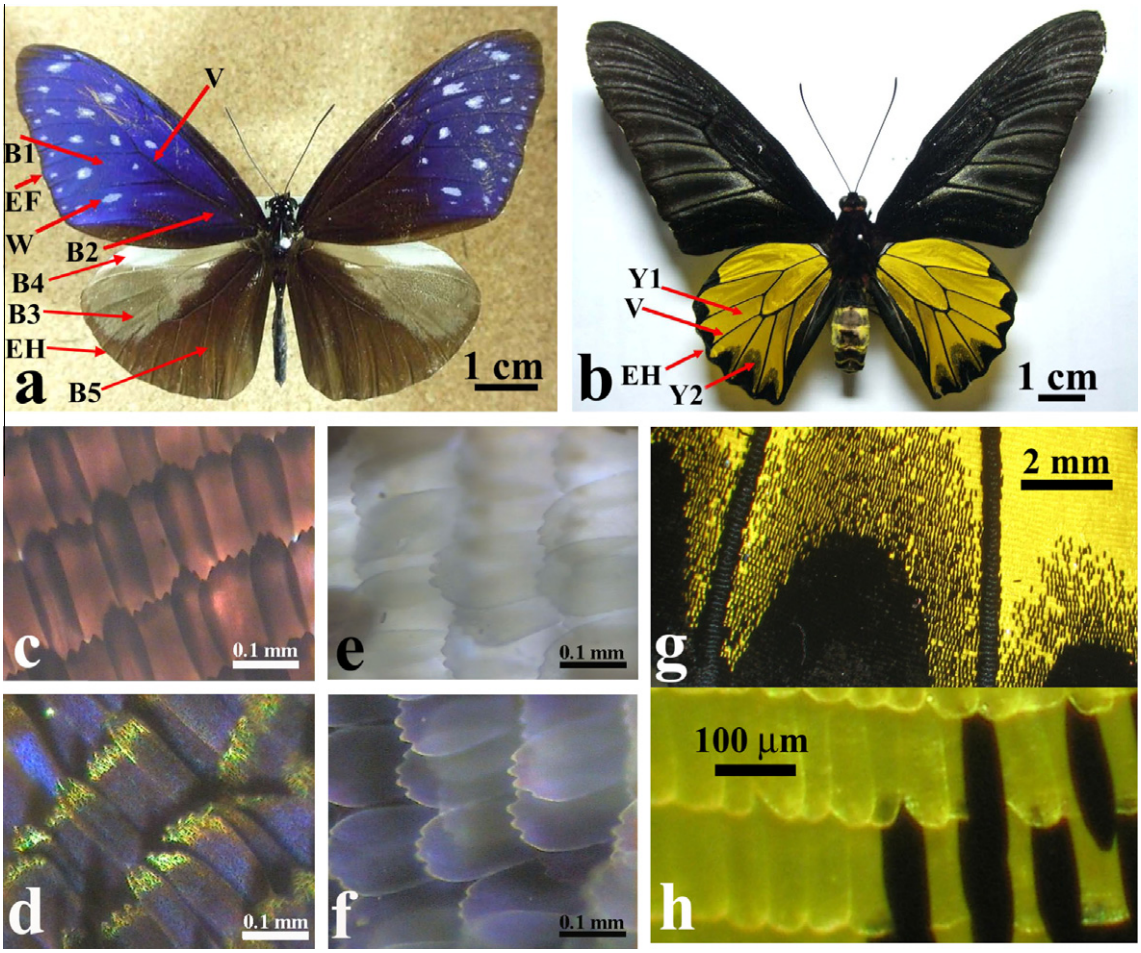

Fig. 1. Male butterflies (dorsal side). (a) *E. mulciber*. (b) *T. aeacus*. The arrowheads in (a) and (b) indicate the areas observed by SEM and opto-spectrometry. (c–h) Optical microscope images of the scales, taken at different incident angles of white light. Transmitted light image (c) and reflected light image (d) of the scales at **B1** in (a). Transmitted light image (e) and reflected light image (f) of the scales at **W** in (a). Transmitted light images (g, h) of the scales at **Y2** in (b).

which are very similar to the scales in the edge **EF** in the forewing. The scales in **B5** have a similar structure of that of the scale in **W**, **B1** and **B2** in the forewing. However, they are not exactly the same because of difference of the number of the piled cuticle layers. The scales in **W**, **B1** and **B2** have the triple-layered arrangement, while the scales in **B5** have a double-layered arrangement like the brown background scales of the *S. charonda*. The less interference from the piled layers must be one of the reasons why the area **B5** is not iridescent but dark brown. The observed SEM results for the scales in the male *E. mulciber* are summarised in Table 1.

Fig. 4 reproduces the reflectance in the UV (ultraviolet)[15] and visible region from the different areas. Fig. 4a shows a spectrum from **B1** with the dark brown scales exhibiting iridescent blue as seen in Fig. 1. The spectrum has a heap with a high reflectance of 4–6% in a range over UV(<380 nm) and violet (380–450 nm), and a valley with a lower reflectance below 4% in a range over blue (450–495 nm), green (495–570 nm), yellow (570–590 nm), and orange (590–620 nm). Small peaks can be seen at ~480 and ~240 nm in the spectrum. It was described above that the vivid blue colouration comes from the multiple cuticle layers on the ridges. For the cuticle-air multilayered arrangement, unless the layers are optically incoherent with each other (Matějková-Plšková et al., 2011), the optical multiple reflection occurs when the following interference condition is satisfied:

$$2(n_a t_a \cos\theta_a + n_c t_c \cos\theta_c) = m\lambda_p \quad (1)$$

where θ_a and θ_c are the angles of incidence and refraction of the rays to the cuticle layer normal, n_a and n_c are the relative refractive index of air and cuticles, respectively. An integer of m is the order of interference and λ_P is the wavelength of the reflected light. Since the incident rays are parallel to the scale plane normal (which is different from the cuticle layer normal as illustrated in Fig. 2h, $\theta_a = \theta_B$ in the reflectance measurement and then $\sin\theta_B/\sin\theta_c = n_c$. Taking $n_c = 1.55$ as an appropriate value for the cuticles (Yoshioka and Kinoshita, 2007; Yoshioka et al., 2008), $n_a = 1$, $t_c = \sim 100$ nm, $t_a = \sim 100$ nm, and $\theta_B = 25°$, we can obtain $\lambda_p = \sim 480$ nm for $m = 1$ and $\lambda_p = \sim 240$ nm for $m = 2$. These wavelengths correspond to the observed peaks in the spectrum shown in Fig. 4a. Resultingly, this supports the assumption of $n_c = 1.55$.

According to the dark zone mentioned above, the incident angle θ_a which causes the observed reflection is limited to $90° - \theta_B = 65° > \theta_a > -65° = -90° + \theta_B$. Since $\sin\theta_a/\sin\theta_c = n_c = 1.55$, the wave length of the reflected rays λ must be ~ 510 nm $> \lambda > \sim 335$ nm for $m = 1$ and ~ 255 nm $> \lambda > \sim 168$ nm for $m = 2$, because the wavelength calculated from Eq. (1) is $\lambda = 335$ nm at $\theta_a = \pm 65°$ and $\lambda = 510$ nm at $\theta_a = 0$ for $m = 1$, and $\lambda = 168$ nm at $\theta_a = \pm 65°$ and $\lambda = 255$ nm at $\theta_a = 0$ for $m = 2$. Hence, human eyes, which respond to wavelengths from about 380 to 790 nm, can see the iridescent reflected rays only in a range from about 510 nm (green) to 380 nm (violet), which is schematically illustrated in Fig. 2h.

[15] Ultraviolet.

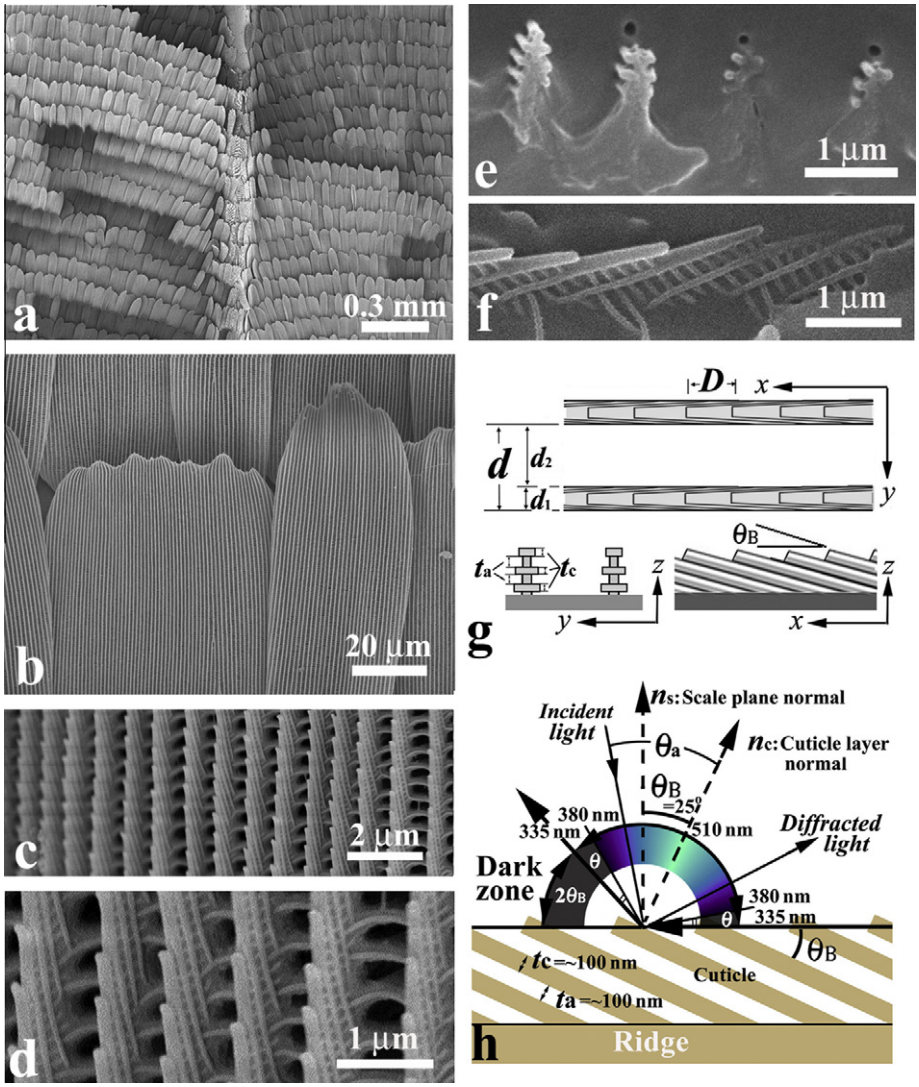

Fig. 2. SEM images of scales in the forewing of the male *E. mulciber*. (a) Scales on vein V **in** Fig. 1(a) and scales in the background around the vein. (b) Brown scales in area **B1**. (c and d) Almost the top views of ridges in a brown scale in area **B1**. (e) Cross section of the ridges on a *y*–*z* plane. (f) Cross section on an *x*–*z* plane. (g) Schematic of the piled cuticles. (h) Schematic illustration of the selective reflection from cuticle layers piled on the ridge.

The violet and green hues are observed in reflection from the scales in Fig. 1d and f. The incident (and reflection) angle θ_a corresponding to $\lambda = 380$ nm for $m = 1$ can be calculated to be ±54.5°. Therefore, besides the dark zone where no reflection geometrically occurs, human invisible zones due to ultraviolet reflection appear in an angle of $\theta = 10.5o(=90o\text{-}25o\text{-}54.5o)$ at both sides of the visible zone, as shown in Fig. 2h. As indicated in Fig. 2g, the tilting of the cuticles on the ridges forms a blazed grating. As well known, the commercial blazed diffraction gratings are designed to obtain high diffraction efficiency for a certain order m and wavelength. When the incident light and the m-th order diffracted light are related by mirror reflection with each other on the facet surfaces, most of the incident energy is concentrated into the m-th order diffracted light. This satisfies $\lambda = (2D/m)\ \sin\theta_B \cos(\alpha - \theta_B)$, where D is the spacing or the grating period and α is the incident light angle made with the normal to the grating. The angle θ_B is called blaze angle. The wavelength for $m = 1$ and $\alpha = \theta_B$, where the 1st-order diffracted light returns along the same path as the incident light, is called the blaze wavelength λ_B, and then $\lambda_B = 2D\sin\theta_B$. The blaze wavelength represents the blaze characteristics of the grating. For the **B1** scales $D = \sim 0.5$ μm and $\theta_B = \sim 25°$ so that the blaze wavelength is estimated to be $\lambda_B = \sim 400$ nm. The high diffraction efficiency from this multilayered grating hence would be obtained in low wavelength range of violet, which may be a reason of the heap of the spectrum in Fig. 4a. Thus, the reason why *E. mulciber* butterfly wings exhibit vivid iridescent violet hues has been completely elucidated.

The spectrum from **V** (Vein) shown in Fig. 4b resembles that from **B1** in profile but not in intensity. The reflectance on the **V** is weak, being consistent with its dark looks seen in Fig. 1a. The reflectance of **W** in Fig. 4c is greater than that of **B1**, and exceeds 10%, corresponding to its white hues. Heaps appear with peaks at ~500 and ~250 nm. The iridescent colouring (Fig. 1f) can be explained using these peaks as made for the spectrum from **B1**. The valley of the reflectance spectrum from green to red region is ascribed to the nonreflective, transmitted light, most of which is absorbed by melanin pigments in the scales, because the valley in the **B1** spectrum is deeper than that in the **W** spectrum. **B1** and **W** scales have almost the same microstructure and exhibit similar peaks in the reflectance spectra. Therefore it may be considered that the vivid blue colouration of **B1** is caused by the absorption of shorter wavelength rays by melanin in the cuticle layers. According to Ou-Yang et al. (2004), the typical absorbance spectrum of soluble eumelanin includes a linear in-

Table 1
Characteristics of the scales in different areas shown in Fig. 1.

E. mulciber	B1	Two kinds of dark brown scales, broad and narrow, reflecting iridescent blue hues in the background. Triple-layered arrangement of cuticles. $d_1 \approx 0.3$–0.4
	B2	μm, $d_2 \approx 0.6$–0.7 μm, $d \approx 0.9$–1.1 μm, D ≈ 0.5 μm. $t_c \approx 100$ nm, $t_a \approx 100$ nm, $\theta_B \approx 25°$
Forewing	W	Iridescent white scales in white spots. The shape and structure are almost the same as the broad scales in B1 and B2, with triple layered arrangement of cuticles. $d_1 \approx 0.25$ μm, $d_2 \approx 0.8$–0.9 μm, $d \approx 1.0$–1.2 μm, $D \approx 0.5$–0.8 μm
	V	On the vein. Almost the same with narrow dark brown scales in B1
	EF	At edge. Dark brown scale with deep splits on the end. No iridescence. Mono- layer cuticle arrangement. $d_1 \approx 0.2$ μm, $d_2 \approx \sim 1.3$ μm, $d \approx 1.5$ μm, $D \approx 0.6$–1.7 μm
Hindwing	B3	Scales in long flat fibre like sea tangles, having wider end where hair cuticles grow on the ridge
	B4	Scales in the pale costal area. The structure resembles that of the dark brown scale in EF
	B5	Dark brown scales, similar to W, B1 and B2 scales in the forewing but not the same because of double-layers arrangement of cuticles
	EH	Very similar to the scales at EF
T. aeacus	V	Black scales on the upper branch of the 1st cubitus. They are narrow with splits, and are almost the same in structure as the scales in V of the *E. mulciber*
Hindwing	Y1	Yellow scales in the cell. The cuticles are not piled on the ridges but microribs are on the sides of triangular ridges, perpendicularly to the scale plane. $d_1 \approx 0.5$ μm, $d_2 \approx 1.2$ μm, $d \approx 1.8$ μm, $D^* \approx 0.2$ μm, $\theta_B^* \approx 90°$
	Y2	Comprises the yellow and black scales in the submarginal spot
	EH	Black scales in the margin. They are almost the same as the scales in EF and EH of the *E. mulciber*. The interior structure of the scale is disclosed, which has bigger columns than the scale in Y2 and mechanically stronger. $d_1 \approx 0.6$ μm, $d_2 \approx 1.4$ μm, $d \approx 2$ μm, $D \approx 0.6$–1.2 μm, $\theta_B \approx 0$

d_1, d_2, d, D, and θ_B are the grating parameters indicated in Fig. 2f. D^* and θ_B^* are for microribs on **Y1**.

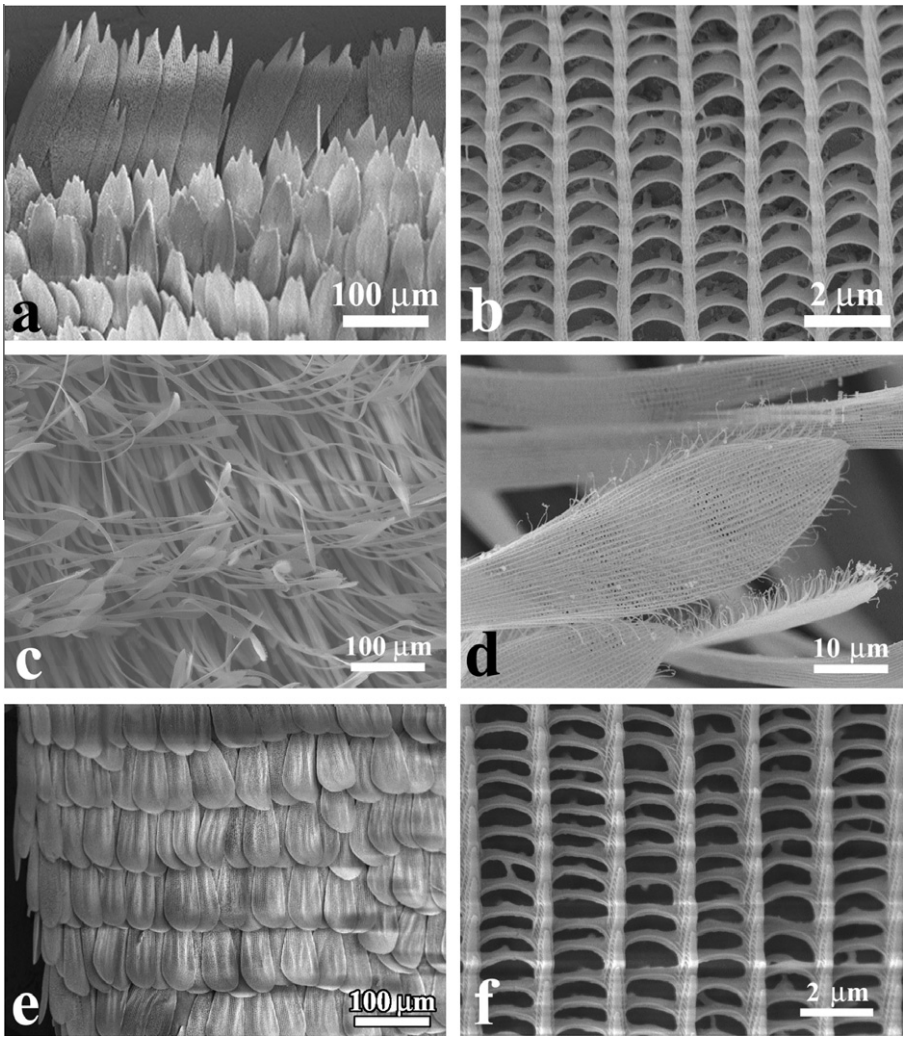

Fig. 3. SEM images of scales in the male *E. mulciber*. (a and b) Dark brown scales in area **EF** in Fig. 1a and the top view of the ridges in a scale. (c and d) Scales in grey area **B3** in Fig. 1a, and ends of the scales. Hair-like objects grow on membrane, ventral side of the scales. (e, f) Scales in the costal pale brown area **B4** in Fig. 1a and almost the top view of rides in a scale. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

crease of absorbance from 800 to 600 nm and an exponential increase of absorbance from 600 to 300 nm. In any case the strong reflection is caused from areas satisfying the interference condition. The parts being out of the interference condition look dark brown due to absorption of shorter wavelength rays in the incident rays (see Fig. 1d).

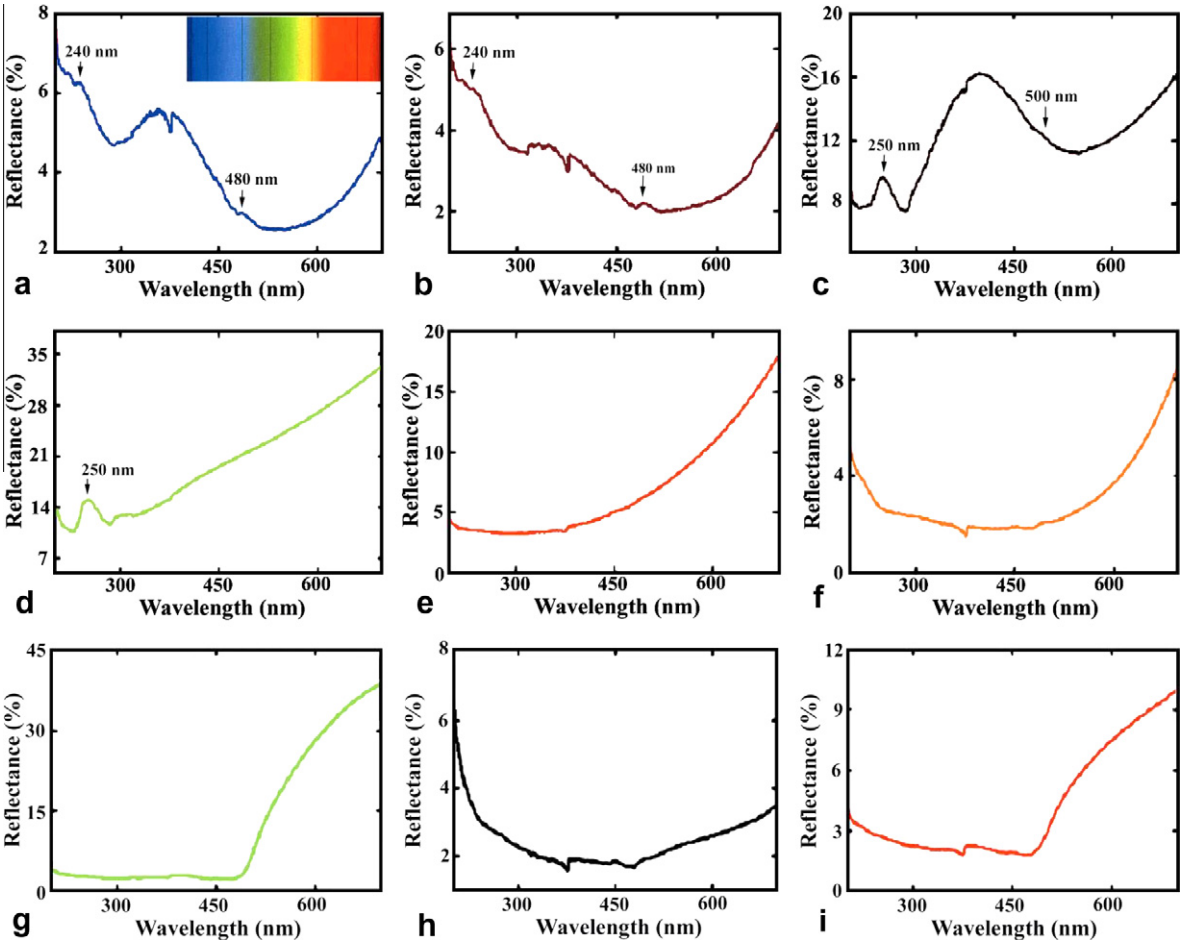

Fig. 4. Reflectance spectrum in UV and visible region from different areas in the dorsal wings of the male *E. mulciber* (a–f) and the male *T. aeacus* (g–i). (a): **B1**. (b): **V**. (c): **W**. (d): **B4**. (e): **B3**. (f): **B5**. (g): **Y1**. (h): **EH**. (i): **Y2**. Small caves at 375 nm were caused by change of the incident light source so that they should be neglected. Inset in (a) is the spectrum of the sunlight.

As seen in Fig. 4d, the area **B4** has higher reflectance over the visible region than the other areas. The spectrum explains the prominent pale of the **B4** area and indicates that the structure, with no multilayered arrangement of cuticle and the large spacing holes between the ridges, makes the incident light diffuse as frosted glass does. Fig. 4e is from **B3** having long flat fibre scales like sea tangles, and Fig. 4f is from **B5** having dark brown scales with double-layer arrangement of cuticles on the ridges. The reflectance increases monotonously with increasing wavelength through a visible and near-infrared range. The bright grey scales in **B3** exhibit higher reflectance, while dark brown scales in **B5** exhibit lower reflectance. No peak in the **B3** spectrum is understandable from no cuticle layer arrangement of the scales. The spectrum from **B5** indicates that the double-layer arrangement is not enough to reflect observable interference light.

3.2. Male T. aeacus butterfly

T. aeacus, called 'the golden birdwing', is in the subfamily Papilioninae of the family Papilionidae. It is also a common butterfly distributed in Thailand, Nepal, India, Myanmar, Laos, Cambodia, Vietnam, and west China. We observed the yellow scales and the black scales in the areas V (upper branch of the first cubitus), Y1[16], Y2[17](submarginal spot) and EH (margin), indicated in the hindwing in Fig. 1b. The term *cubitus* means 'vein along lower edge of the cell' (Ek-Amnuay, 2006). Fig. 1g shows the black scales on the vein V and surrounding yellow scales. The black scales on V are narrow with splits and are almost the same in microstructure as the scales in V of the *E. mulciber*. The shape and structure of the scales on the veins seem common among the butterflies, at least in the *S. charonda*, *E. mulciber* and *T. aeacus*.

The yellow scales look macroscopically similar in shape to the brown scales of the *E. mulciber* and the *S. charonda*. However, Fig. 5a and b reveal that they are completely different in microstructure from these brown scales. The scale looks like a construction arranging with triangle bars, as seen in Fig. 5c which is a cross-sectional SEM image of the three ridges. The main difference is that any cuticles are not piled on the ridges but protrusions called "microribs" (Ghiradella, 1991, 1998; Vigneron et al., 2008) can be seen on the sides of triangular ridges as seen in Fig. 5b. There are also seen irregular gratings closer to the scale membrane. The microribs stand perpendicularly to the scale plane (as ignoring their bent tops). Fig. 5c cannot distinguish the microribs from the sides of ridges. The bases of the triangle bars are as long as $d \sim 1.8$ μm and the spacing of the microribs D^* is about 0.2 μm. No cuticles on the ridges do not produce such an iridescence as observed in the *E. mulciber*. It is known that the *T. magellanus* (and also *T. Prattorum*) which closely resembles the *T. aeacus*, however, exhibits a blue-green sheen when it is observed at near-grazing incidence. The sheen is a result of the correlated diffraction and fluorescence in the backscattering iridescence which is caused by steeply-set multilayered microribs (Vigneron et al., 2008). The *T.*

[16] Yellow scales.
[17] Yellow and black scales in the submarginal spot.

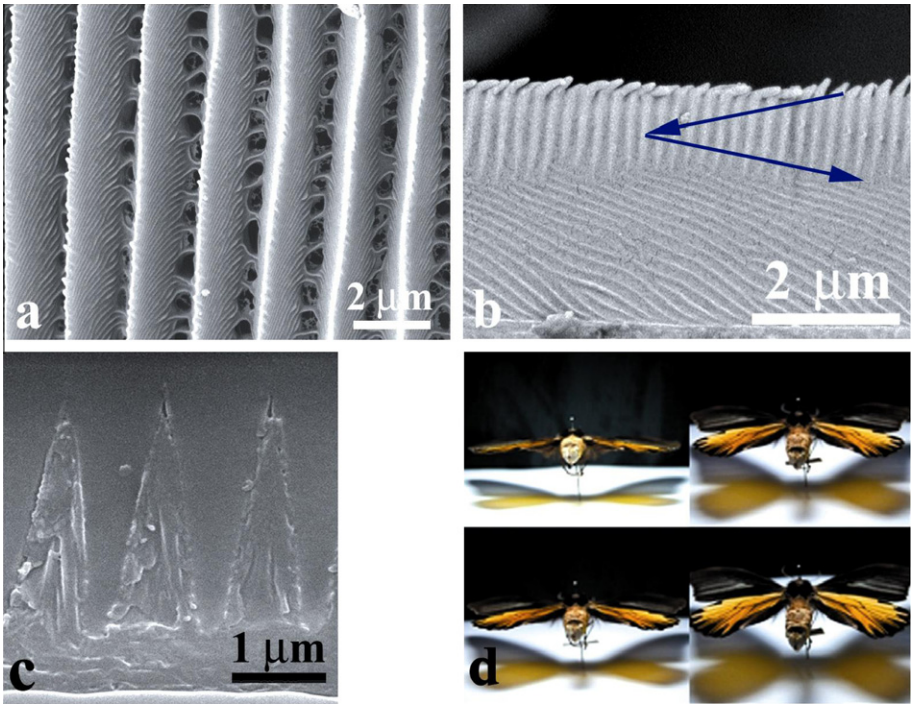

Fig. 5. (a–c) SEM images of yellow scales in the hindwing of the male *T. aeacus*. (a) Top view of ridges. (b) Side-view of a ridge. (c) Cross section of three ridges. (d) Photographs of the male *T. aeacus*, taken at different near-grazing incidences. At any near-grazing incidence cannot be observed a blue-green iridescence, which is observed in *T. magellanus* that belongs to the same genus, *Troides*.

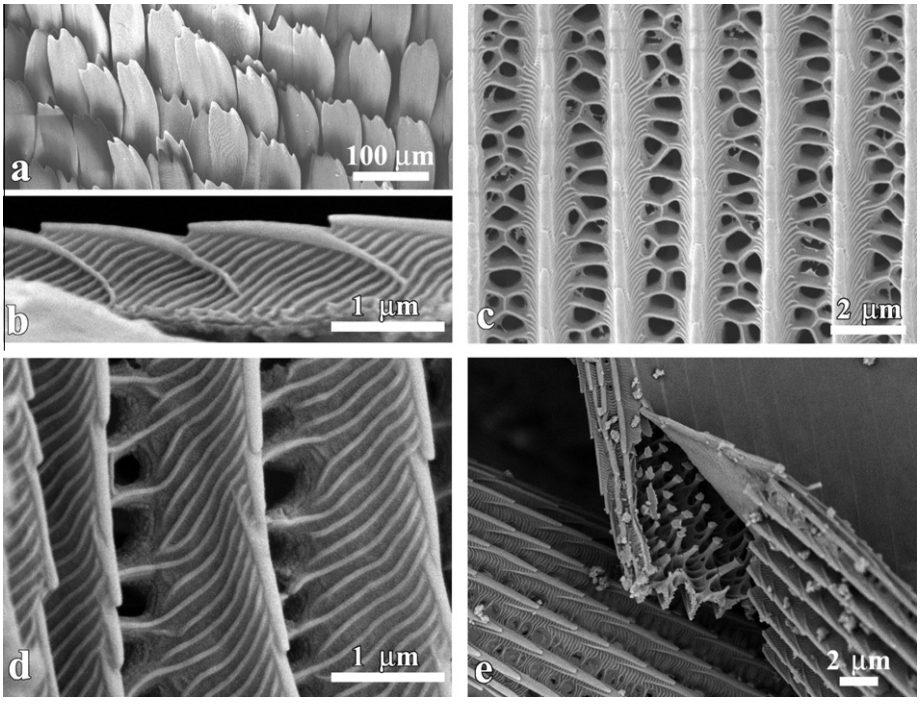

Fig. 6. SEM images of scales in the hindwing of the male *T. aeacus*. (a) Black scales in area EH in Fig. 1b. (b) Side view of the ridge of a black scale. (c) Top view of another black scale. (d) Almost top view of the black scale. (e) Two black scales. The right scale was broken during preparation and consequently displays the interior.

aeacus did not display a blue-green sheen such as demonstrated in Fig. 1 in the paper by Vigneron et al. (2008) when we viewed it from oblique angles (Fig. 5d). In the *T. magellanus* the repeat period of the microribs D^* is ~0.26 μm, and the slant angle of microribs θ_B^* is ~54° with respect to the scale surface (Lawrence et al., 2002), or ~53°, exactly which is ~61° with respect to the ridge crest that is tilted to ~8° to the scale surface (Vigneron et al., 2008). The slant of the multilayered microribs is the requirement for the backscattering iridescence. The *T. aeacus* has the microrib layers perpendicular to the scale plane so that the backscattering diffraction hardly occurs from light with any incidence angle as shown in Fig. 5b. That is a reason why *T. aeacus* does not exhibit iridescence on the yellow

scales unlike *T. magellanus*. As seen in Fig. 1g and h these scales are intrinsically yellow. This is confirmed by the yellow shadows of the wings, which are transmitted images, in Fig. 5d. The hues of the yellow colour may be ascribed to the absorption and scattering of the incident rays by papiliochrome in the scales (Lawrence et al., 2002).

Fig. 6a shows black scales in the marginal area **EH**. The scales have splits. Fig. 6b–d shows a side view of a ridge, a top view and an incline view of the ridges, respectively. The black scales are almost the same in structure as the dark brown scales at the edge of *E. mulciber*'s wing shown in Fig. 3a and b, although the parameters, shown in Table 1, are a little different between these butterflies. The difference in colour is perhaps ascribed to difference of the content of pigment melanin. In Fig. 6e are seen two black scales. One shows an incline view of its ridges similar to Fig. 6d, and another was broken during the specimen preparation so that it discloses the interior of the scale. The heavier columns and the bigger tendons than those in other scales show that the scales at the edges are mechanically strong.

As seen in Fig. 4g, the **Y1** area comprising yellow scales exhibits strong reflectance which increases with wavelength from 480 nm and has a plateau (750–1000 nm). Any peak is not detected in the spectrum, which confirms that any interference reflection is not expected. As well-known, the photopic luminosity function has the maximum at 555 nm, where the reflectance of **Y1** is as high as 16%. Since the reflectance increases with wavelength, the wings could be seen in yellow (570–590 nm) hues by human eyes. Fig. 4h shows the spectrum from the **EH** margin with black scales. The scales have a structure as shown in Fig. 6. In any case the reflectance is less than a few% due to the containing pigments. The spectrum in Fig. 4i is from **Y2** where the yellow scales and the black scales are mixed (see Fig. 1b and g), and it is also mixed with the spectra in Fig. 4g and h.

4. Conclusion

Wing scales of male *E. mulciber* and *T. aeacus* butterflies have been investigated by SEM and optical reflectance measurement. Blue backgrounds of the *E. mulciber*'s forewing have brown scales. It has been elucidated that a highly tilted, triple-layered arrangement in these scales produces a violet-green iridescence in a wavelength range from ~380 to ~510 nm due to multiple interference from the cuticle-air layers. Besides the dark zone where no reflection geometrically occurs, human invisible zones due to ultraviolet reflection appear so that the visible reflection is limited within an angle range of 10.5–119.5°. No iridescence in dark brown area in the hindwing indicates that the double-layer arrangement is not enough to reflect observable interference light. The patch between the 7 and 4 veins in the hindwing is covered with strange scales, which have a form of flat fibre as long as several hundred μm, having wider end with long hair-like objects. The *T. aeacus* does not produce a blue-green sheen such as observed by *T. magellanus* at near-grazing incidence. Yellow scales in the *T. aeacus*'s hindwing do not have any multilayered cuticle arrangement but they have microribs on the sides of triangle ridges. The microrib layers are perpendicular to the scale plane so that they do not produce any backscattering diffraction. That is a reason why they do not produce the iridescent sheen, unlike the slant microribs in the *T. magellanus*. The yellow hues of the scales may be ascribed to absorption and scattering of the incident rays by papiliochrome.

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