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Colour analysis for distinguishing closely related species of the subgenus *Mesembrynus* Hübner, [1819] *Zygaena* (*M.*) *minos* ([Denis & Schiffermüller], 1775) and *Zygaena* (*M.*) *purpuralis* (Brünnich, 1763) (Lepidoptera: Zygaenidae)

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Abstract

The wing colours and patterns of the genus *Zygaena* are limited and mainly red and black. This fact creates problems to distinguish closely related species by visual inspection. The aim of this study is to report differences in spectral reflectance of the wings between *Zygaena (Mesembrynus) minos* ([Denis & Schiffermüller], 1775) and *Zygaena (Mesembrynus) purpuralis* (Brünnich, 1763). The Pteridine Erythropterin is the pigment which creates the red wing colour of these species and its photo-sensitivity leads to its depletion during the lifetime of the moths. Both species show some different reflectance in the Transcaucasia but cannot be distinguished in the European region. It seems that a spatial vertical distribution can explain the findings rather than a horizontal one.
KEY WORDS: Lepidoptera, Zygaenidae, spectral light reflectance, pigments, pteridine, Georgia.

Análisis del color para distinguir las especies relativamente próximas del subgénero *Mesembrynus* Hübner, [1819] *Zygaena* (*M.*) *minos* ([Denis & Schiffermüller], 1775) y *Zygaena* (*M.*) *purpuralis* (Brünnich, 1763) (Lepidoptera: Zygaenidae)

Resumen

Principalmente los colores de las alas y de los dibujos del género *Zygaena* se limitan a los rojos y negros. Este hecho crea problemas para distinguir las especies relativamente próximas por inspección visual. El objetivo de este estudio es informar sobre la diferencia en el espectro reflectante de las alas entre *Zygaena (Mesembrynus) minos* ([Denis & Schiffermüller], 1775) y *Zygaena (Mesembrynus) purpuralis* (Brünnich, 1763). La pteridina eritropoyetina es el pigmento que crea el color rojo de las alas de estas especies y su fotosensibilidad se reduce durante la vida de los Zygaenidae. Ambas especies muestran alguna diferencia reflectante en la Transcaucasia pero no pueden distinguirse en la región europea. Vemos que una distribución vertical espacial puede explicar las conclusiones en vez de una horizontal.
PALABRAS CLAVE: Lepidoptera, Zygaenidae, reflectante luz espectral, pigmentos, pteridina, Georgia.

Introduction

Zygaena purpuralis complex has always been problematic. Wing pattern and wing colour are variable and thus altogether many subspecies have been described in Western Palaearctic where this complex is distributed. Species can be distinguished by their genitalia, larvae and hostplant. For most of the time a few species, i. e. *Zygaena (Mesembrynus) purpuralis* (Brünnich, 1763), and *Zygaena (Mesembrynus) minos* ([Denis & Schiffermüller], 1775), *Zygaena (Mesembrynus) diaphana*

Staudinger, 1887 and *Zygaena (Mesembrynus) pimpinellae* Guhn, [1913] have been recognized. Since NAUMANN *et al*. (1983) only the two species *Z. purpuralis* and *Z minos* were accepted. A few subspecies of *Z. minos* were raised to the species level again, i. e. *Zygaena (Mesembrynus) pseudorubicundus* Klír & Naumann, 2002 as reported by HOFMANN & TREMEWAN (2010) and just recently *Z. diaphana* and *Zygaena (Mesembrynus) smirnovi* Christoph, 1884 (NAHIRNIĆ, 2019). Nevertheless, unresolved relations remain in *Z. minos*, *Z. diaphana* and *Z. smirnovi* (NAHIRNIĆ, 2019). Similar problems occur with *Z. purpuralis*. HOFMANN & TREMEWAN (1996) consider 18 taxa as good subspecies. However, subdivision to subspecies is still not satisfactory. Some of the isolated taxa are *Zygaena purpuralis villosa* Burgeff, 1914 and *Zygaena purpuralis tirabzona* Sheljuzhko, 1936 which are similar to each other and have slightly different genitalia than all other taxa of *Z. purpuralis* and that difference is constant. Tips of the lobe of the uncus are flattened and in lamina dorsalis lower opening is smaller. Biology is unknown, neither larvae nor hostplants have ever been described which may contribute to clearing the status of these taxa and their relation within the *Z. purpuralis* complex. All Georgian *Z. purpuralis villosa* are collected at biotopes with abundant *Thymus* sp. at altitudes between 1000 and 2200 m. They are distributed in north-eastern Turkey and Georgia.

The colourful wings of insects inspirit scientists since more than 100 years to understand the diversity in colour and patterns (HOPKINS, 1896; WIELAND & SCHÖPF, 1925; ILSE, 1928). A progress has been reported by DAUMER (1966) with a comparison of human and insect vision. LAND & NILSSON (2006) reviewed in detail the properties of the photoreceptors of insects. Whereas green and blue vision is common for insects and humans, the red colour cannot be seen by many insects and the humans cannot realize ultraviolet light with wavelengths shorter than 400 nm (deep indigo). This fact encouraged scientists to study the UV vision of butterflies and moths in detail. MEYER-ROCHOW & EGUCHI (1983) report about physically produced UV-reflection of scales which also can create eyespot elements not visible for humans (YANG *et al*., 2004). Wing patterns which are only visible in the UV-range can even be gender specific and depend also on the geographic latitude (MEYER-ROCHOW & JÄRVILEHTO, 1997).

Two different physical phenomena are to be considered. One group of colours is based on the reflectance of pigments, i. e. the property of an aggregated chemical compound. Another group of colours is based on structural elements, i. e. the property of thin layers resulting a metallic lustre as a consequence of the interference of light or a special structure of the surface of scales reflecting UV light only.

The wings of *Zygaena (Mesembrynus) minos* ([Denis & Schiffermüller], 1775) and *Zygaena (Mesembrynus) purpuralis* (Brünnich, 1763) are covered by red and black pigments without metallic lustre or reflectance of UV light of wavelengths shorter than 370 nm. The soluble red pigment coincides with the property of Erythropterin as first investigations on both species resulted (BUNTEBARTH, 2004, 2018). This group of chemical compounds which are called Pteridines was intensively studied by W. Pfleiderer and co-workers on several butterflies (PFLEIDERER, 1987). The black pigment is insoluble and belongs to the group of Melanins.

The goal of this study is to examine whether both species can be distinguished by their light reflectance and whether the reflectance can support a subdivision of populations at different regions.

Material and methods

The colour of wings is usually described applying a more or less detailed qualitative scale. The impression of a colour is, however, not a physical quantity and depends on the light source and the individual observer. In order to quantify the wing colour, the spectral analysis is applied. The colour of wings is determined at monochromatic light. The reflectance was measured in the range of ultraviolet (370 nm) to red (700 nm) at a wavelength width of 0.5 nm using the Shimadzu UV/VIS-spectrometer V260 with integrating sphere. The reflectance was measured separately at the upper and lower side of the hindwings. The size of the incident light beam was constant with 3x5.5 mm. The

investigations were done at 22 males and 13 females of *Z. purpuralis* and at 21 males and 9 females of *Z. minos* which are collected in the Transcaucasian region of Georgia and in Central Europe. Specimens of *Z. minos* from Transcaucasia belong to *Zygaena minos ingens* Burgeff, 1926, while those of *Z. purpuralis* from the same region belong to *Z. purpuralis villosa*. Their male adults and genitalia are illustrated on Fig. 1.

The locations in Georgia are given in BUNTEBARTH *et al*. (2011) and the European species are collected in Austria, Germany, Hungary, and Switzerland. Genitalia dissections were done according to ROBINSON (1976). Genitalia are mounted in Euparal on slides. Determination and nomenclature are done according to NAUMANN (1972), NAUMANN *et al*. (1983) and NAHIRNIĆ (2019).

The wing colour is a property of pigments which are soluble in a buffer of pH = 10 (0,05 mol Na_2CO_3/$NaHCO_3$) and they are considered as Erythropterin (BUNTEBARTH, 2004). The depleted cyst of the scales appear light brown/orange coloured and remain on the hindwings from which their reflectance is also determined within the spectrum from 370 nm to 700 nm. Fig. 2a shows an untreated hind wing and a hindwing after extraction the red pigments (Fig. 2b). The same hindwings are shown at a magnification of 225x at Fig. 2c and Fig. 2d. The latter show the remaining cysts of the scales which appear light brown/orange coloured.

Results

The spectral distribution of the reflectance of one pair of hindwings is shown at Fig. 3. The qualitative trend is the same for both investigated species. Slight changes occur at the violet/indigo range where the maximum reflection varies between 380 and 440 nm. A further characteristic mark is the point of the steepest increase of the reflectance, i. e. the inflexion point, the maximum of the first derivative of the reflectance with respect to the wavelength. Fig. 3a illustrates the spectral distribution of a *Z. minos ingens* subspecies with the corresponding colour as an example.

The maximum reflectance at ca. 400 nm and the inflexion point are marked at Fig. 3b for illustrating their determination. The first derivative of the reflectance passes zero at the maximum and its maximum results the inflexion point. The gray monotonous increase of the reflectance with the wavelength (Fig. 3a) results the reflectance of the hindwings after extraction the red pigments. The slight steeper increase between orange and red explains the orange-coloured picture at Fig. 2b and Fig. 2d. The relation between the wavelength of the reflectance maximum with respect to the inflexion point varies at specimens of the same species as Fig. 5 demonstrates. The Transcaucasian subspecies *Z. minos ingens* and *Z. purpuralis villosa* can be distinguished (Fig. 5a). Specimens of *Z. minos ingens* show in mean a shift of the indigo/violet maximum to shorter wavelengths than that of *Z. purpuralis villosa*. Additionally, the cluster of the inflexion point is shifted to orange whereas that of *Z. purpuralis villosa* is orange-red. The linear trend at Fig. 5a shows the same slope of both species. The reason of the stretched data distribution instead of cluster is due to the fact that age and intensity of the colour vary. Old specimens are bleached by the sun or exposed to natural abrasion. Fig. 5c demonstrates with the imagines that the intensity of the wing pattern varies remarkably. The violet maximum of pale specimens is shifted to longer wavelengths and the inflexion point to shorter ones. The same characteristic can be realized at Fig. 5b which shows European specimens of *Z. minos* and *Z. purpuralis*. The trend calculated at Fig. 5a is copied to Fig. 5b and demonstrates that both species coincide with *Z. purpuralis villosa* and that the European specimens of *Z. minos* and *Z. purpuralis* cannot be distinguished by reflectance properties.

Discussion

The colour analysis is based on the human vision. Colour vision of insects is physically possible due to their three or more photoreceptors (LAND & NILSSON, 2006). However, some authors state that each photoreceptor has its own function (SCHERER & KOLB, 1987). These problems are not solved here. It is reported how the spectral distribution of the reflectance varies with the species and

with the condition of individual specimens. It is stated that the characteristic property which yields the colour is the concentration of the Pteridine Erythropterin on the wings of these species. Minor contribution of further Pteridines is possible, but not found yet. Additional pigments, first of all the black ones, are not soluble and belong to the group of Melanins (LINZEN, 1967). The spectral distribution allows the conclusion that the low value at ca. 500 nm and the steep increase at yellow/orange are properties of Pteridine. The high absorption at ca. 500 nm (blue green) is the complementary colour of orange and exaggerates the reflectance at orange, if exposed to sun light. The elevated absorption at blue green of the Pteridine is also measured at the dissolved pigments (BUNTEBARTH, 2004). Whereas the average of the inflexion point of *Z. minos ingens* has a value of ca. 595 nm, the mean of the European specimens of *Z. minos* is at 600 nm (Fig. 5). It agrees with the findings by (MEYER-ROCHOW & JÄRVILEHTO, 1997) that the colour appears to be brighter as the higher the geographic latitude is. However, *Z. purpuralis* does not show any difference between the investigated European and Transcaucasian subspecies. They neither can be distinguished from European *Z. minos*. The slight change of the reflectance is due to the change of the Pteridine composition. The reason can be manifold, e. g. variation of the mean annual temperature and its amplitude, humidity, length of winter and summer as well as accompanying vegetation change. The present climate in Georgia is subdivided into the maritime climate in Western Georgia and the continental one in Eastern Georgia. *Zygaena minos ingens* flies at altitudes below 1000 m in Eastern Georgia. Only a few old specimens were found within the Kolchis triangle (BUNTEBARTH *et al*., 2011). *Zygaena purpuralis villosa* is mainly abundant at altitudes above 1000 m. The investigated 26 specimens were collected mainly at altitudes above 1000 m (19:7), where *Z. minos ingens* has not been found.

Zygaena minos ingens has the same genitalia, larva, and biology like most populations of *Z. minos* from Europe. *Zygaena minos ingens* and *Z. minos dagestana* Sheljuzhko, 1936 are geographically isolated from other populations of *Z. minos* by the Black Sea and the Caucasus. Possible scenario is that during the Pleistocene *Z. minos* migrated to the south and one of the regions it reached was Transcaucasia which was possible by low-land connection when the level of Black Sea was lower. For example, during the Last Glacial Maximum, ca. 25 000 - 18 000 BP, the surface of the Black Sea was lower for at least 100 m (YANKO, 1990). *Z. minos ingens* have probably been isolated in Transcaucasian refugia since at least the last Pleistocene interglacial period. The past climate within the geographic latitudes between 40ºN and 50ºN passed an optimum with temperatures of 1.2 K to 2.5 K above the present-day values between 7,000 B.P. to 5,500 B.P. (FRENZEL *et al*., 1992). During this climate optimum the humidity decreased within the mentioned range of latitudes and had also consequences in the phytocenosis. After a long stable level of the Black Sea at 40 m below the present level, the level of the Mediterranean Sea increased at about 7,200 B.P. and the dry valleys of the Dardanelles and the Bosporus were flooded (VAN ANDEL & SHACKLETON, 1982) which limited the migration of the mentioned *Zygaena* species at least.

As demonstrated at Fig. 5c, the condition of the specimens can vary remarkably and they are gradually bleached by the sunlight during their lifetime. This bleaching results from the deterioration of Erythropterin. Fig. 5 supports the assumption.

It can be concluded that the light reflectance can be helpful in separation subspecies or species from each other. It can be used as a supplementary method which can indicate direction of taxonomic research, especially when the biology of the taxa is not known. Reasons of regional variations, however, are not detectable. Speculations on the effect of climate, elevation of mountainous regions or latitude of populations fail, because all cases can be proven with counter-examples.

A more detailed feature is shown at Fig. 4 as an example. The reflectance of the upper wing (blue) approaches closer to the reflectance of the depleted wing (green) than that of the lower wing (red). The lower side of the wings is less exposed to the sunlight. Therefore, the Erythropterin concentration is higher at the lower side than at the upper side. The depletion of colour is also accompanied by a shift of the inflexion point to shorter wavelengths and a shift of the violet maximum to longer wavelengths. Both effects coincide with spectral distribution of the wings which

are pale and at least without red pigments, so that he reflection of the cyst of the wings is going to dominate the spectral distribution displaying its own properties.

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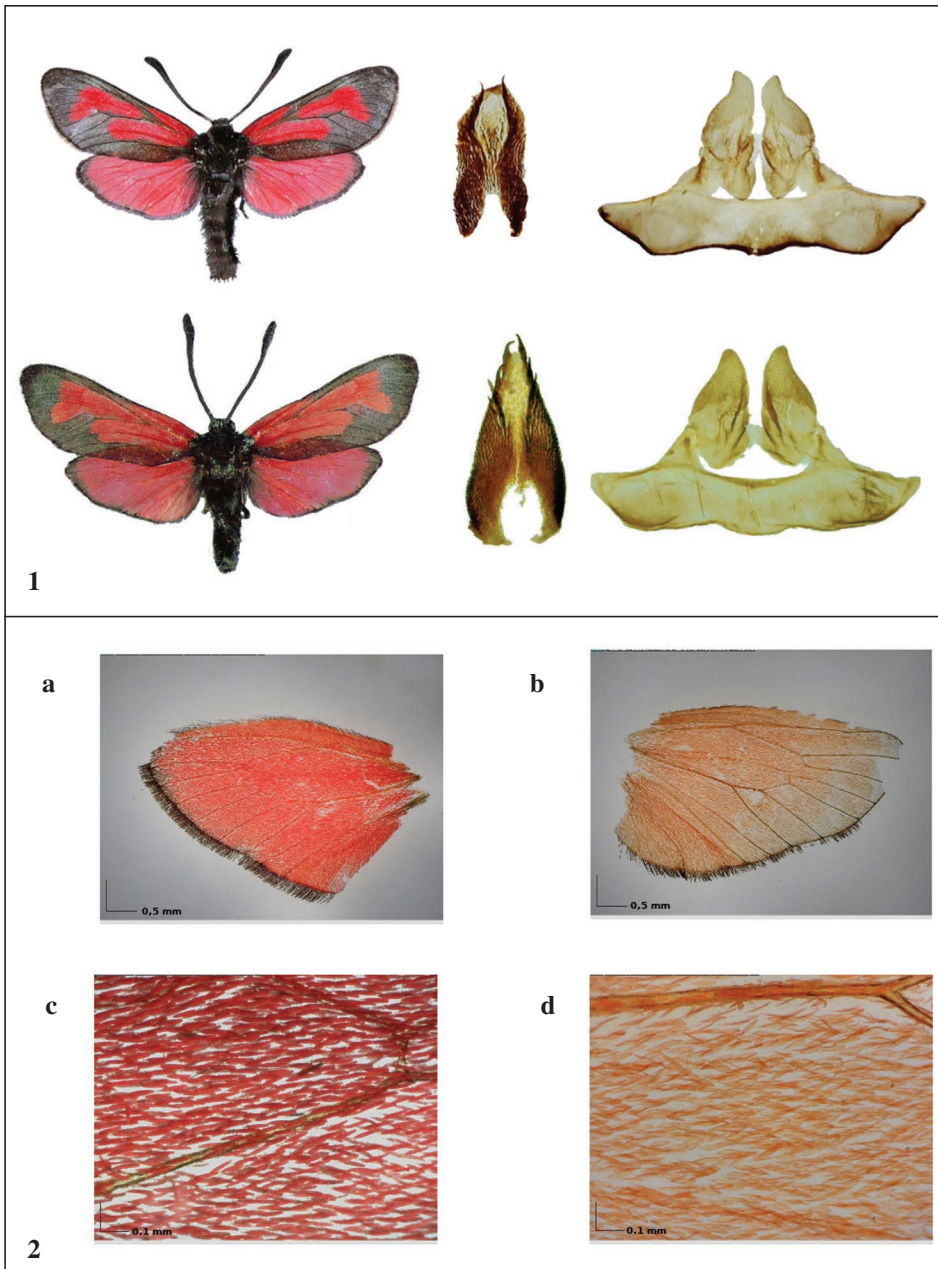
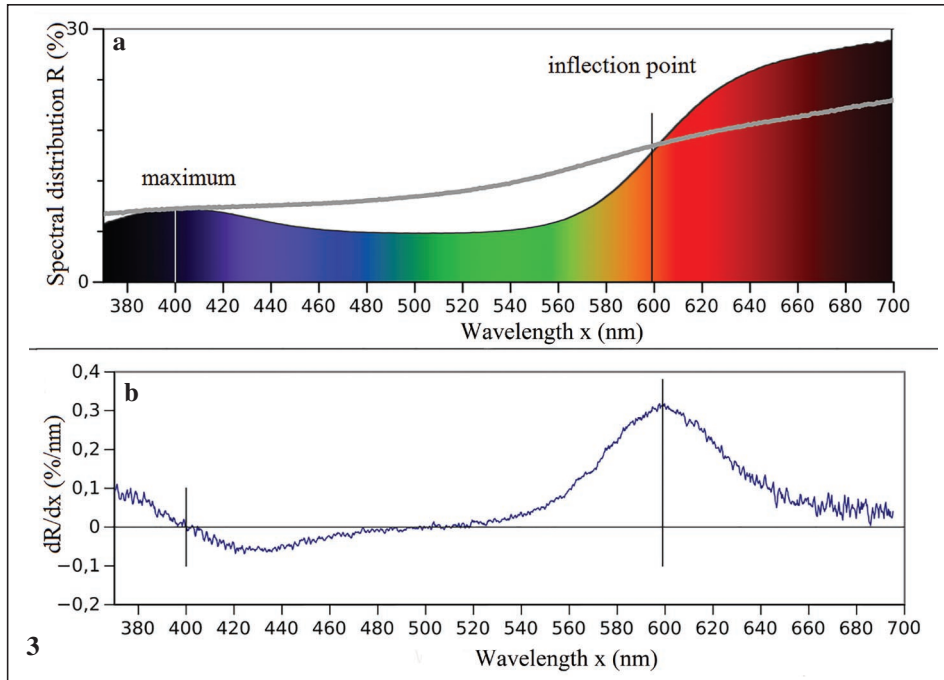
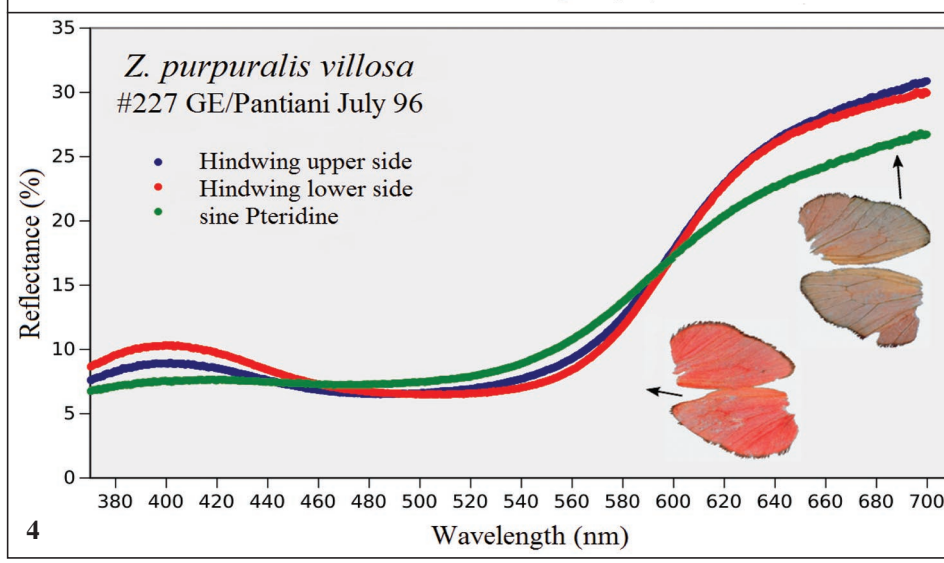


Figures 1-2.– 1. Example of *Zygaena purpuralis villosa* (upper row) and *Zygaena minos ingens* (lower row) with their lamina dorsalis and uncus. **2.** Hindwing of *Zygaena minos*; **a**) untreated; **c**) magnified (225x); **b**) without Pteridin; **d**) magnified (225x).





Figures 3-4.– 3. a) Spectral reflectance of *Zygaena minos ingens* with the spectral distribution of the wings and the reflectance without the Pteridin pigments as gray function; **b**) the first derivation of the spectral distribution with respect to the wavelength and the mark at the maximum reflectance at 400 nm and at the inflexion point. **4.** Spectral reflectance of hindwings of a male specimen *Z. purpuralis villosa* at the upper and lower side and the reflectance after extraction the Pteridines.

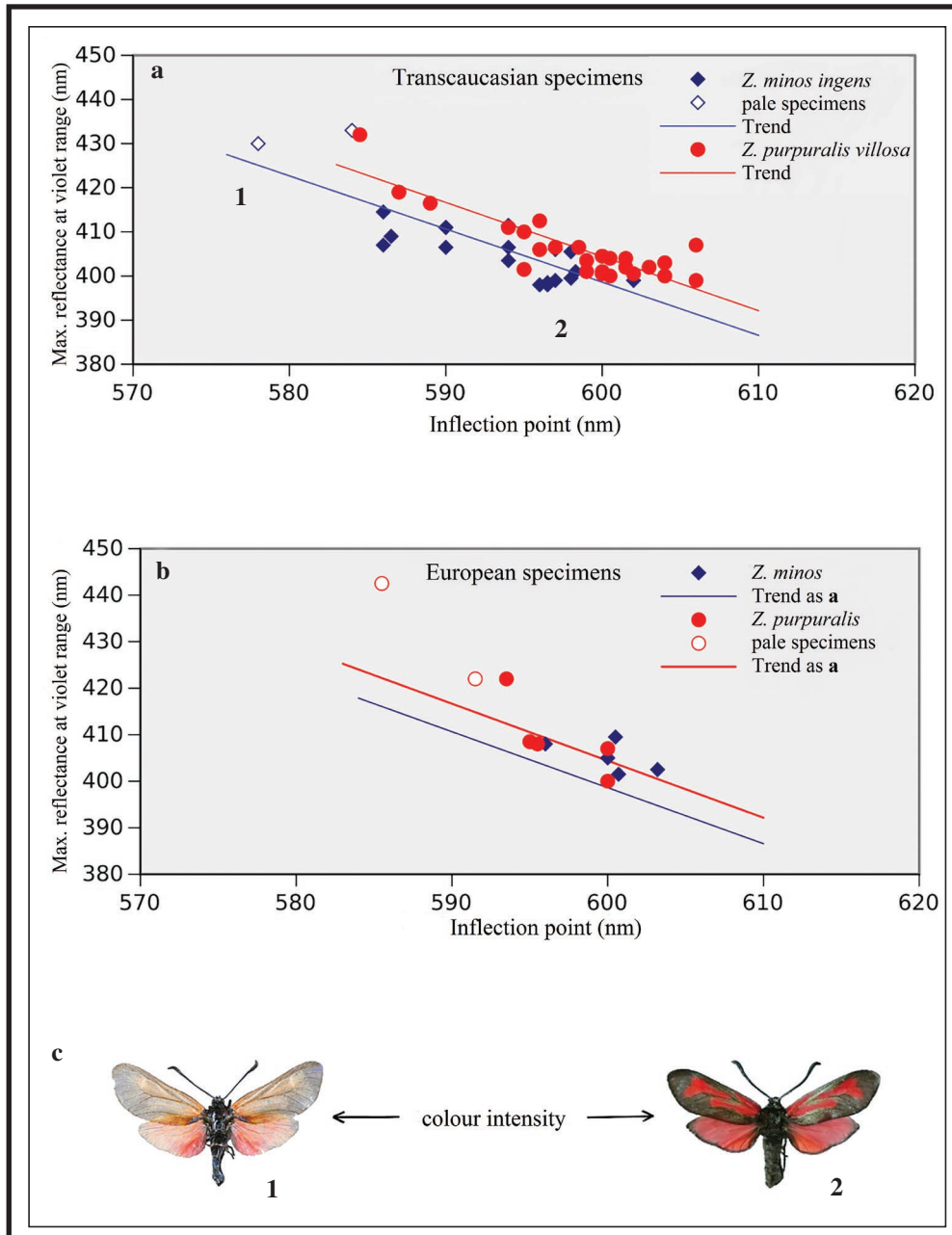


Figure 5.– Colour properties of *Zygaena minos* and *Zygaena purpuralis;* **a**) the maximum reflectance at ca. 400 nm with respect to the wavelength of the inflexion point of Transcaucasian subspecies; the straight lines show the trend; **b**) the same display format of European species is applied; the trends calculated at panel **a**) are copied to panel **b**); **c**) demonstrates the effect of colour depletion with marks in panel **a**).