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Description of *Penaincisalia biophot* with an emphasis on the optical properties of the wing dorsal surfaces
(Lepidoptera: Lycaenidae)

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ABSTRACT. A new eumaeine lycaenid species, *Penaincisalia biophot* sp. n. is described from Cordillera Blanca, Peru. Five characters used for the diagnosis are discussed. Spectral analyses of structural colours of forewings positioned at different angles were conducted.

Key words: New species, spectroscopy, structural colours, *Penaincisalia* species-groups.

INTRODUCTION

The genus *Penaincisalia* was proposed by JOHNSON with the type species *Thecla culminicola* STAUDINGER, 1894 for further 13 species (JOHNSON 1990). JOHNSON (1992) distinguished later in this assemblage the genera *Abloxurina*, *Penaincisalia*, *Pons* and *Thecloxurina*. Later ROBBINS (2004) lumped all these genera under the oldest available name, *Penaincisalia*.

In a recent revision, PRIETO (2008) applied the nomenclature and systematics suggested by ROBBINS. PRIETO argued, that for certain taxa, or groups of species that were discovered after JOHNSON's monograph was published (JOHNSON 1992), additional generic names should be erected, if the taxonomic division of JOHNSON is to be applied. According to PRIETO (2008), systematic position of the *culminicola* species group (= *Penaincisalia* s.str.) should be between the tailed and/or lobated *Penaincisalia*

species groups (cf. BÁLINT & WOJTUSIAK 2006, BÁLINT, BOYER & WOJTUSIAK 2006). The members of the *culminicola* group have no tail and no lobe in the anal corner of hindwings. They are distributed only in high altitude alpine habitats of the Andes from Central Colombia to northern Bolivia. In this group, as in other “Penaincisalia” clades (genera), the gradual or abrupt changes in structural colours can be observed (BÁLINT 2004).

The aim of the present paper is to describe a relatively well documented but hitherto misdiagnosed *Penaincisalia* taxon and to emphasize the importance of spectral differences in structural colours as a source of species specific visual signals that are used in visual communication between sexes.

MATERIALS AND METHODS

The data on butterfly behaviour were collected during the field observations conducted in the Peruvian Andes (BÁLINT 1997) by means of mark-recapture method. For comparative purposes the material stored in various natural history museums was also used. The detailed information on these specimens is given as comparative material examined (see below). Wing measurements were made with calibrated scale mounted in Olympus SZ60 stereomicroscope. The anatomical and morphological characters were examined with Olympus SZX12 stereomicroscope and recorded on Olympus DP70 digital camera. Standard method (WINTER 2000) was used for dissection of male genitalia.

Male individuals of each species have been measured to determine the variability of body size and spectral characteristics of the light reflected from dorsal wing surface. Three individuals of each species were measured. The label data of these specimens are also listed as comparative material examined (see below). The light reflectance spectra were always measured under the same angle of incidence in ultraviolet, visible and near-infrared light and recorded in Avantes 2048 fiber optic spectrometer. A diffuse white light was used to calibrate the spectrometer.

Abbreviations. – MTM = Magyar Természettudományi Múzeum, Budapest (Hungarian Natural History Museum); MZUJ = Muzeum Zoologiczne Uniwersytet Jagiellonski, Kraków (Zoological Museum of the Jagellonian University); NFMTA = Nanoszerkezetek Főosztály, Szilárdtest és Anyagtudományi Kutató Intézet, Magyar Tudományos Akadémia, Budapest (= Department of Nanostructures, Research Institute for Technical Physics and Material Science of the Hungarian Academy of Sciences); NHMW = Naturhistorisches Museum, Wien; ZSM = Zoologische Staatssammlung des Bayerischen Staates, München.

Comparative material examined

Penaincisalia alatus (DRUCE, 1909) JOHNSON, 1990 – ECUADOR: Pichincha, Calacali, Pela Gallo, 3100 m, 29.I.2002 (MZUJ: male), Pululahua, La Rinconada, 2600 m, 6.II.1990 (MTM: male, female).

- Penaincisalia aurulenta* JOHNSON, 1990 – PERU: Ancash, PN Huascarán, Llanganuco, 3800 m, 9-13.II.1995 (MTM: 5 males, 4 females; NFMFTA: 1 male); (three males used for spectroscopy).
- Penaincisalia culminicola* (STAUDINGER, 1894) JOHNSON, 1990 – BOLIVIA: La Paz, 3250 m (ZSM: 2 males, female), Songotal, Cuticucho, 3700 m, 1.II.1954 (ZSM: female); PERU: Apurímac, Saywite, 3700 m, 11.II.2005. (MZUJ: 3 males, 2 females:); Ticlio, 4800 m, 16.X.1955 (NHMW: male); (MZUJ males used for spectroscopy)
- Penaincisalia descimoni* JOHNSON, 1990 – PERU: Junín, 4100-4400 m, Tartally exp. No. 8, 14.XI.2001 (MTM: female); Pasco, Cerro de Pasco, 4300 m, V. 2006 (MTM: male); Tishka, 3750 m, 3.VII.2005 (MZUJ: male).
- Penaincisalia lamasi* BÁLINT, 2001 – PERU: Ancash, PN Huascarán, Camino Portachuelo 4600 m, 10.II.2005 (MTM: male).
- Penaincisalia penai* JOHNSON, 1990 – ECUADOR: Saraguro, 24.VIII.2003 (MZUJ: male).
- Penaincisalia perezii* BÁLINT, 2001 – PERU: Ancash, PN Huascarán, Laguna Parón, 4300 m, 7.II.2005 (HNHM: male); Quebrada Demanda, 4400 m, 9.II.1995 (MTM: male); Quebrada Demanda, 4500 m, 10-11.II.1995 (MTM: 5 males, NFMFTA: male) (three males used for spectroscopy); Laguna Parón, 4300 m, 7.II.2005 (MTM: male).

RESULTS

***Penaincisalia biophot* BÁLINT & WOJTUSIAK, sp. n.**

(Figs 1-4)

“*Thecla*” *culminicola culmicola* (STAUDINGER) – LAMAS & PÉREZ 1983: 36, fig. 43 (male).

Penaincisalia sp. n. – BÁLINT 1996: 29, fig. 12.

Penaincisalia culminicola (STAUDINGER) JOHNSON – BÁLINT 1996: 28, fig. 1997: 15, fig. 4 (male); BÁLINT *et al.* 2005: 2938, fig. 1c (male), fig. 4 (scale micrographs).

DIAGNOSIS

Similar to *Penaincisalia culminicola* (STAUDINGER, 1894) JOHNSON, 1990, but smaller in size (all wing margins longer in *P. culminicola*) (character 1) (see Tables 1-2), dorsal forewing scent pad greyish black (grey in *P. culminicola*) (character 2). Dorsal wing surfaces with a deep blue structural colour of a wavelength peak at 380-390 nm and a very high (100%) reflectivity (lavender blue in *P. culminicola*) (character 3). Ventral surfaces of wings with identical ground colour and sharply marked pattern (forewing ground colour lighter with obsolete pattern in *P. culminicola*) (character 4). Valva in male genitalia longer than in *P. culminicola* with straight ventral edge (slightly convex in *P. culminicola*) (character 5).

DESCRIPTION

Male. Head, thorax and abdomen black on dorsal side; thorax and abdomen with greyish pubescence on ventral side; antennae 0,6 times the length of forewing costa, clubs black. Forewing length 11 mm (holotype), measured from the base of cubital vein to vein R3 terminus, triangular in shape. Ground colour on dorsal surface dark

iridescent blue depending on the direction of illuminating light; margins with very thin (< 1 mm); black marginal border, broadening in apical areas; fringes chequered, white between veins and black at vein termini; ventral surface of both wings warm grey, somewhat lighter in submedian areas. Forewing with delicate discoidal patch and black, sharp and slightly ruptured postmedian line; submarginal area darker than submedial but bluish grey between outer margin and antemarginal line; hindwing basal area darker than medial and postmedial areas; medial band black, ruptured and sharply

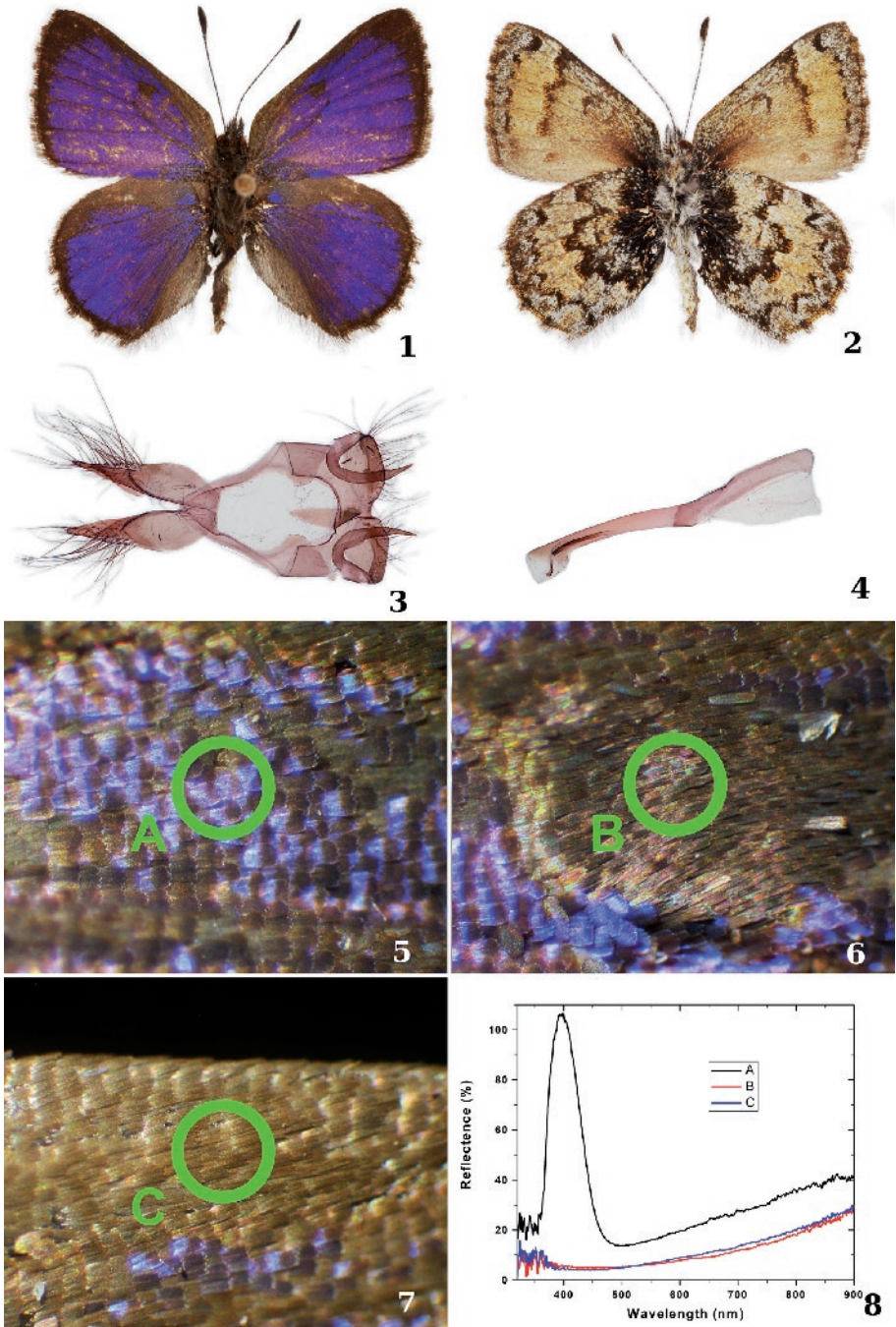
Table 1. *Penaincisalia biophot* forewing measurements. A = length measured from the vein Cubitus erection to vein Radius 3 terminus, B = length measured between vein R3 and vein 2V termini, C = length measured vein Cubitus erection to vein Vannal 2 terminus (below each columns sample quotients); ratios = $R_1 = A / B$, $R_2 = B / C$, $R_3 = A / C$; (data measured on paratype nos 1-5 = nos 1-5; see Type material).

	A	B	C	R_1	R_2	R_3
1.	110	75	95	1,47	0,79	1,16
2.	113	75	94	1,51	0,8	1,2
3.	121	73	90	1,66	0,81	1,34
4.	110	71	86	1,55	0,83	1,22
5.	111	70	90	1,59	0,78	1,23
	113	74,8	91			

Table 2. *Penaincisalia culminicola* forewing measurements (in mm). A = length measured from the vein Cubitus erection to vein Radius 3 terminus, B = length measured between vein R3 and vein 2V termini, c = length measured from vein Cubitus erection to vein Vannal 2 terminus (below each columns sample quotients); ratios = $R_1 = A / B$, $R_2 = B / C$, $R_3 = A / C$; data measured on MZUJ and ZSM male specimens (nos 1-3: Aparimac, nos 4-5: La Paz; see Type material).

	A	B	C	R_1	R_2	R_3
1.	131	82	101	1,6	0,81	1,3
2.	130	85	100	1,53	0,85	1,3
3.	123	77	94	1,6	0,82	1,31
4.	131	83	96	1,58	0,86	1,36
5.	125	80	99	1,56	0,81	1,26
	128	81,4	99,8			

1–8 (see next page). *Penaincisalia biophot* BÁLINT & WOJTUSIAK, sp. n.: 1 - holotype, dorsal view; 2 - holotype, ventral view; 3 - paratype, male genitalia, ventral view, aedeagus removed; 4 - male aedeagus, dorsal view; 5–7 - small areas on forewings encircled in green are points at which measurements were taken: 5 - blue area on forewing left to the scent patch with generated structural blue colour (A); 6 - scent patch (B); 7 - forewing costal margin above the scent patch with black (non iridescent) cover scales (C); 8 - graph of reflectance spectra



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marked; antemarginal line as continuous intercellular black arrowhead mark bordered distally by bluish grey crescent pattern; vein termini with long black and cellular spaces with drab, or warm brown fringes. Androconia greyish black, scent pad trapezoid in shape, scent patch minute (Figs 1-2). In male genitalia tegumen and uncus large when seen from lateral and dorsal sides; gnathos straight when seen from lateral but curved when seen from ventral side, with pointed apex; valve slender, as long as tegumen, with straight ventral and convex dorsal margins. Length of narrow apical part one third the length of the valva; vinculum slender but well sclerotized, saccus membranous; aedeagus slender, three times longer than the valva with cornuti straight dorsally and bent ventrally (Figs 3-4).

Female. As male, but with paler blue dorsal forewing ground colour. Genitalia not examined.

ETYMOLOGY

Species was named after scientific program BioPhot, developed partly to conduct spectral analysis of structural colours of various organisms, including lycaenid species (BÁLINT *et al.* 2005, 2007, 2008).

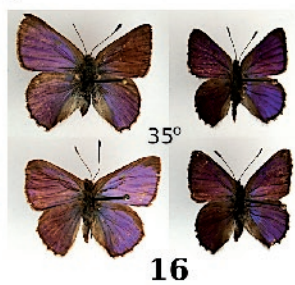
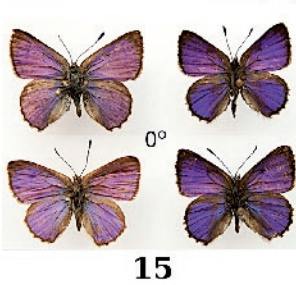
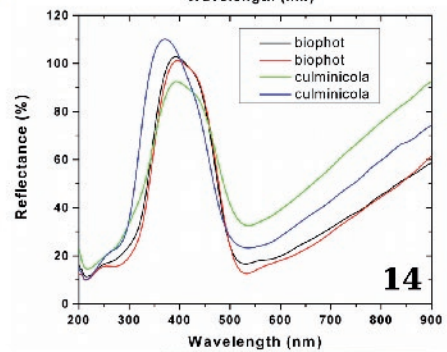
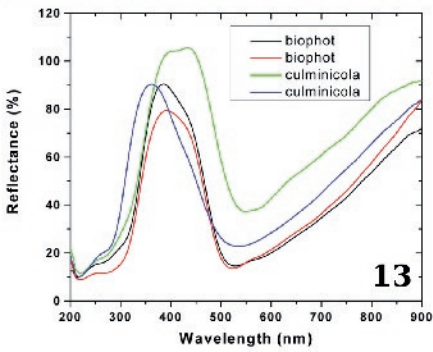
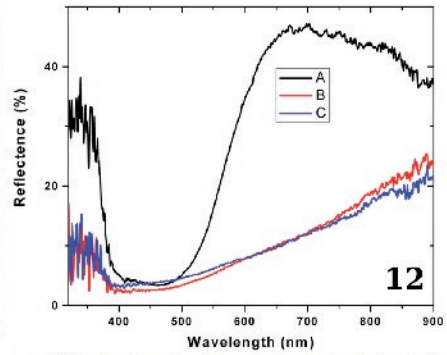
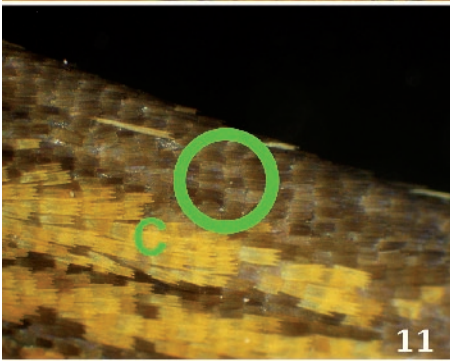
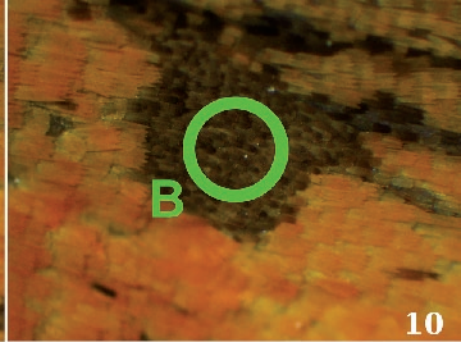
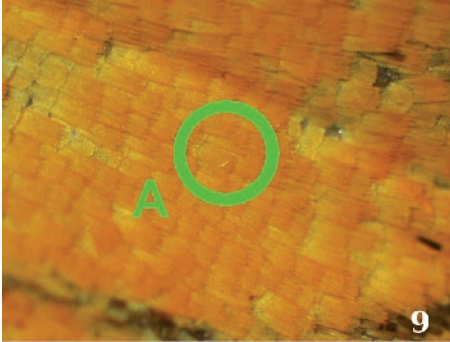
TYPE MATERIAL

Holotype. Male labelled “PERU, Dept. Ancash, PN Huascarán, Llanganuco, 3800 m, 9-13.II.1995, leg. Zs. Bálint”, at present in Magyar Természettudományi Múzeum (will be deposited in Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru).

Paratypes – Male nos 1-18, female paratype no. 20, all with holotype data, paratype male nos 1-17 and paratype female are deposited in MTM. Paratype male no. 18 is in the special Lepidoptera collection of NFMTA and was used in scanning electron microscopy, transmission electron microscopy and also for spectroscopic measurements, as a specimen no. 32 which was recorded in the BioPhot database with all the data and resulting micrographs (see <http://www.softadmin.ro/biophot/index.php>). Paratype male 19: “Lago Conococha, M. Peru, 11.X.1966” (NHMW KÖNIG collection no. 104).

Genital dissections – paratype no 11 were dissected by C. PRIETO; paratype no 14: gen. prep. no. 653 by BÁLINT (in glycerion vial); Paratype no 15: gen. prep. no. 655 by

9–17 (see next page). *Penaincisalia* optical properties. 9-11: *Penaincisalia aurulenta* JOHNSON, small areas on forewings encircled in green are points at which measurements were taken; 9 - forewing orange area left to the scent patch with scales coloured by pigment (A); 10 - scent patch (B); 11 - forewing costal margin above the scent patch with black cover scales (C); 12 - graph of reflectance spectra measured at areas A, B and C; 13 - graph of spectrographic measurements of *P. biophot* and *P. culminicola* forewing; 14 - graph of hindwing spectrographic measurements of *P. biophot* and *P. culminicola*; 15–17: wings surface of two specimens of *P. culminicola* (left) and two specimens of *P. biophot* (right) photographed under different angles, revealing spectral differences in light reflection, 15 - view at 0°, 16 - view at 35°, 17 - view at 70°. Specimens of *P. biophot* and *P. culminicola* have similar wing colour when seen by the naked eye, but they differ as the angle of observation increases from 0° to 70°. Specimens of *P. biophot* have weaker colouration that disappears at 35° and 70°, while specimens of *P. culminicola* exhibit almost unchanged colour intensity on the right hand side wings, irrespectively of the angle of observation



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BÁLINT (in glycerion vial); paratype no 16: gen. prep. no. 2008 by BÁLINT (mounted on slide); paratype 17: gen. prep. no. 2009 by BÁLINT (mounted on microscope slide).

DISTRIBUTION

Known only from the valley of Llanganuco, Cordillera Blanca, Peruvian Andes from elevations between 3800-4100 m. According to LAMAS and PÉREZ (1983) the species flies around the year with its main flight period during the dry season.

BIOLOGY

Male specimens were found in two small “quebradas” north to Chinancocha at 4000–4100 m. They were exhibiting territorial behaviour between 10:30 a.m and 14:00 p.m. (BÁLINT 1997: 12, nos 10-11). Males were always choosing their observation sites above the ground on rocks, or twigs of bushes from which they checked and attacked bypassing conspecifics. Females were flying close to the ground when visiting flowers. Two pairs in copula were observed at the bottom of a rocky wall close to the ground in early afternoon.

DISCUSSION

The species was recorded first as *Thecla culminicola culminicola* by LAMAS and PÉREZ (1983) and their nomenclature was followed by BÁLINT (1997). Subsequently, after lycaenid generic names became available, the genus name *Penaincisalia* was applied. The taxonomic status of specimens from Llanganuco valley was revised when topotypical specimens of *Penaincisalia culminicola* became available for study, as well as other, recently collected *Penaincisalia* specimens (see comparative material examined). The characters were evaluated to find out whether Llanganuco specimens represent a distinct taxon, or not. Five characters have been used for the diagnosis.

Two of these characters are the wing shape and its dimension (character 1) and ventral wing pattern colouration (character 2). These characters are often used to discriminate taxa but because they are under strong environmental pressure, they cannot fully reflect interspecific differences. Individuals of the same species living at higher elevations may have smaller wings and body size than those living at lower elevations where environmental conditions are different. However, according to our experience, wing shape is often used as a useful character to detect “cryptic” species, especially, when long series of specimens from various museum collections are also used for comparative study (cf. Tables 1-2). Similarly, differences in colouration and pattern on ventral side of wings may correspond to different colouration of soil and light illumination at higher and at lower elevations.

Therefore, the characteristics of androconia (character 3) may be useful, because the perception of species specific pheromones is one of the major factors responsible for maintaining the sexual isolation. The role of chemical signals in sexual behaviour of butterflies have been demonstrated in many cases. However, the role of the androconial scales in eumaeine lycaenids is still not well understood. Nothing is known about the

mechanism of the pheromone release and the relation between the scent pad shape and colour, and the ultrastructure of scales. In the *Penaincisalia* group there is a strong evidence that both, the scent pad shape and its colour, can serve as good characters enabling the separation of otherwise very similar looking species. An example can be a pair of species *Pons browni* (JOHNSON, 1992) and *Pons saraha* JOHNSON, 1992.

It has to be mentioned that genital structures in lycaenids are qualitatively similar and can be used by taxonomist only when they are carefully measured and statistically evaluated (character 5). The male genitalia of *P. biophot* and the related species *P. aurulenta* (JOHNSON, 1990) looks very similar but they differ in length and width of certain genital structures (cf. the figures in JOHNSON 1990 and 1992). This is in concordance with other observations made on another *Penaincisalia* species group in which the shape of male valva can be accepted as a good character enabling the identification of particular species (PRIETO *et al.*, in prep.).

In the Llanganuco valley, four *Penaincisalia* species were observed in the same period of the year (BÁLINT 1997 and 2001). According to the field observations, individuals of *P. aurulenta* were not attacked by *P. biophot* males, but interactions between the males of the two species were numerous and sometimes virulent. We may therefore speculate that the differences in forewing structural colours are sufficiently distinctive, so that males can easily discriminate visual signals of other species from those of conspecifics. By the contrast, spectral properties of scent pads examined in selected species of the *Penaincisalia* genus group (BÁLINT *et al.*, in prep.) are not good characters that can be used to discriminate particular species. (Figs 5-12).

Results obtained in the present study indicate that scent pad scales do not generate structural colours, so they cannot affect the spectral properties of light reflected from dorsal surface of wings covered by structural scales.

There is no need to compare light spectra of *P. aurulenta* and *P. biophot* as their colours are significantly different, what can be easily seen even by the naked eye. (Figs 5-12). However, in the case of *P. culminicola* and *P. biophot* it is necessary to measure any subtle optical characteristics to decide whether the differences between individuals are reflecting intraspecific variations within the population of the same species, or indicate a distinct species. The measurements of spectral properties of dorsal surfaces of fore- and hindwings were conducted on specimens of *P. biophot* and *P. culminicola* (Figs 13-14).

The intensity of the reflectance peak of the light spectra from *P. biophot* wings is the highest within the range of 380 - 390 nm for both wings, hence they were spectrally harmonized. By the contrast, spectral characteristic of light reflected from wings of *P. culminicola* was different for forewing and hindwing. Moreover, the spectra of two different specimens of *P. culminicola* show significant differences between each other. One of them had reflectance maxima for both wings in the range of 350 - 360 nm, while the other had the maximum of forewing reflectance at 420 nm (the shape of the graph suggest that in fact there are two closely lying, overlapping maxima). The hindwing spectral maximum was coincident in position with that of *P. biophot*, but slightly broader.

The maximum of reflectivity measured perpendicularly to the wing surface was almost the same in *P. biophot* for its forewing and hindwing, whilst in *P. culminicola* it was positioned on a graph more randomly. Indeed, the wing surfaces of the two species reflect different shade of blue. In *P. culminicola* reflectivity is much wider and less directed than in *P. biophot* in which it decreases when observed under a narrow angle, smaller than 45 degrees (Figs 15-17). The results indicate, that the two species generate different visual signals when in flight.

As can be seen on figs 15-17, the angular light reflectance pattern between the two *P. culminicola* individuals is different, with different spectral characteristics (Figs 13 and 14). By the contrast, in both individuals of *P. biophot* it is identical. The illustrated, upper *P. culminicola* individual, reflects very little light when it falls under the angles of 35 and 70 degrees, whilst the lower one (the right hand wings) has similar colouration at 0 degrees (normal) observation angle. This suggests that the angular jet of the blue-violet light reflected from the wings of the two specimens of *P. culminicola* is significantly different. This observation rise the question, whether the measured specimens of *P. culminicola* are representing the same species or not?

We suggest that the two specimens (in Figs 15-17) represent the same taxon *P. culminicola*, which is distributed along the entire puna belt of the Andes. Puna individuals may probably have wider ecological tolerance than *P. biophot*, which seems to be restricted in its distribution to specific, local environments of glacial valleys of Cordillera Blanca and therefore is endemic for this area. This is reflected by different optical characteristics of *P. biophot* wings. As it was reported earlier, in the case of Panamerican eumacine lycaenid *Theritas mavors* HÜBNER, 1808, certain specimens characterize by broader spectral amplitude. Similar phenomenon is reported in widely distributed, transpaleartic *Polyommatus icarus* (ROTTEMBURG, 1775) (BÁLINT *et al.*, in prep.) which displays a wide array of phenotypes with distinctive blue structural colours throughout the geographic range of its distribution. More studies are needed to estimate the range of variation in spectral characteristics within populations of a species that have wide ranges of geographical distribution and analyse the results from the point of view of speciation.

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