MICROMORPHOLOGICAL (SEM) ASPECTS OF WING SCALES OF SOME POLYOMMATINAE (LEPIDOPTERA: LYCAENIDAE) TAXA

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Abstract. The lycaenids, as well as other lepidopteran groups, present on the surface of the wings different types of scales, which perform various functions. Of these types, the scales producing structural colors generated a major interest, a inter- and intrageneric variability having been proved for these structures. The present paper analyses the shape and size of such scales with the aid of electronic microscopy in species of the *Cupido* (*C. argiades, C. decoloratus, C. alcetas*) and *Polyommatus* (*P. icarus, P. thersites*) genera. Qualitative (distal edge shape) and quantitative (scales width) differences were observed. The differences in sizes are statistically significant, proving the variability of investigated taxa at this level.

Keywords: wing scales, SEM, *Cupido, Polyommatus*.

Rezumat. Aspecte micromorfologice (SEM) la solzi de pe aripi ai unor taxoni ai subfamiliei Polyommatinae (Lepidoptera: Lycaenidae). În cadrul familiei Lycaenidae, ca şi la celelalte grupe de lepidoptere, pe suprafaţa aripilor sunt prezente diferite categorii de solzi, cu diverse funcţii. Dintre aceştia, solzii ce produc culori structurale au generat un interes deosebit, fiind demonstrată o variabilitate atât inter- cât şi intragenerică. Lucrarea de faţă analizează forma şi dimensiunile unor astfel de solzi, cu ajutorul microscopiei electronice, la specii ale genului *Cupido* (*C. argiades, C. decoloratus, C. alcetas*) şi *Polyommatus* (*P. icarus, P. thersites*). Se constată diferenţe calitative (forma marginii distale) şi cantitative (lăţimea solzilor). Diferenţele dimensiunilor sunt statistic semnificative, probând variabilitatea taxonilor studiaţi la acest nivel.

Cuvinte cheie: solzi, aripi, SEM, *Cupido, Polyommatus*.

Introduction

The lepidopterans, as well as most insects, feature a variety of epidermal products, playing diverse roles, genetic and environmental factors influencing the development of such structures (Weatherbee *et al*., 1999; Ghiradella & Butler, 2009). Among these structures, scales are prominently present, a single individual possessing several scale types on its wings. Scales serve different functions, such as thermoregulation, pheromone dispersal and color generation or predator escaping or cleaning (Kristensen & Simonsen, 2003; Reed, 2004). Scale arrangement is generally two-layered, with parallel orientation, however the positioning on the wing (peripheral, central, upper side or lower side of wing etc.) or the group to which the butterfly belongs to or even sexual polymorphism influences the pattern of scales (Kristensen & Simonsen, 2003; Kaaber *et al*., 2009). The size of scales is also variable within butterflies, with lengths between 40 and 500 μm, in correlation with wing size (Simonsen & Kristensen, 2003), typical dimensions being around 100 x 50 x 1 μm values (Pizster *et al*., 2011; Bálint *et al*., 2012).

In the Lycaenidae family, several scale types are known, with flat-type and androconial the most well described. Androconial scales in Polyommatinae are club-shaped, with participation in scent dispersal (Downey & Allyn, 1975). Another type of scales in Polyommatinae, as well as in Lycaenidae in general, is represented by those generating structural colors. This type of scales is classified into Morpho and Urania categories, with the general shape of a flattened sack, attached to the wing by a pedicle (Nijhout, 1985), with newer classifications based on tridimensional structuring existing (Prum *et al*., 2006). The scales consist of a lower and an upper surface, with numerous and complex structures (ribs, ridges, ridge lamellae etc.) present on the upper layer. Several layers present between the surfaces generate structural colors in Morpho type scales, the same kind of colors being generated by the arrangement of ribs in Urania type scales (Tilley & Eliot, 2002). In Lycaenidae, Urania type scales exist, with a "pepper-pot" structure occurring as a particular feature (Eliot, 1973).

The optical properties of structural color scales were shown to be distinct among several species of butterflies from different families, the group of Lycaenidae being a prominent example. Such species include *Polyommatus daphnis*, *P. marcidus* (Bálint *et al*., 2004), *P. icarus*, *P. coridon*, *P. dorylas*, *P. thersites* (Bálint *et al*., 2012), *Celastrina argiolus*, *Plebejus icarioides* (Wilts *et al*., 2009). Observed differences were correlated with the characteristics of microstructures of scales. The scales have been shown to be different among genera and species within Lycaenidae at the microstructures level, due to adaptive, ecological factors influence, thus with an evolutionary importance (Bálint *et al*., 2004; Bálint *et al*., 2007). Although above mentioned scales are well studied a from microstructural and physical properties point of view, we found no comparison of scales from related species concerning sizes or margin shape. The current paper analyses such aspects in scales possessing structural colors in three species of the *Cupido* genus and two species of the *Polyommatus* genus.

Material and Methods

The investigated taxa were represented by three species of the *Cupido* genus (*C. decoloratus*, *C. argiades*, *C. alcetas*) and two species of the *Polyommatus* genus (*P. icarus*, *P. thersites*). The material was collected between April and September 2012 from protected areas from Iasi county (Fânețele Seculare de la Valea lui David and Sărăturile de la Valea Ilenei). Butterflies were captured using a entomological net. Identification of taxa was done on the basis of external morphology and of male genitalia morphology.

Forewings were prepared for SEM analyses by placing on double-sided carbon tape and sputtering with Au layer. The microscope was operated at magnifications up to 50,000x. From each species, wings from five individuals were used for microscopic observations. Scales with structural colors from the center of the wings were identified by the presence of the pepper-pot structures under the superficial ridges and ribs.

The shapes of scales were observed with the unaided eye on SEM photographs. Scale sizes were measured using ImageJ software (ImageJ). The width of each analyzed scale (3 scales per each individual per species) was considered as the largest distance between scale margins in the upper third of the scale. The distances were statistically analyzed by calculating means and standard errors and by analysis of variance (ANOVA) for $p<0.05$.

Results and Discussion

In the current paper the shape of the margin and the width of the scales were analyzed. The shape of the apical margins was found to be variable among the two genera, with species of the *Polyommatus* genus having rounded margin while the species of the *Cupido* genus present scalloped margin (Fig. 1). The scales do not display obvious differences between the species of the same genus. Scales of *Polyommatus thersites* and *Polyommatus icarus* are both apically rounded (Fig. 1A, B), thus classifying as obtuse (Downey & Allyn, 1975). The scales of *Cupido argiades*, *Cupido decoloratus* and *C. alcetas* show variability in the number of tubercles, whose numbers can be 3, 4 or 5 on a single scale (Fig. 1C, D, E). Such a variability was noted for dentate scales by Downey and Allyn (1975), who consider that the region of the wing as well as environmental factors influence this character. All investigated scales presented the pepper-pot structure (Fig. 2) as this type of structure is characteristic for structural colors scales in Theclinae, Lycaeninae and Polyommatinae (Eliot, 1973). Figure 1(F) illustrates two scales from distinct layers, the scale from the upper layer (left side of image) with pepper-pot structure and the scale from the lower layer with empty cells.

The width of scales presents different values between congeneric species (Table 1). The scales of *P. icarus* are wider than those of *P. thersites*. In *Cupido* species, scales of *C. alcetas* are the largest, followed in order by scales of *C. argiades* and scales of *C. decoloratus*.

Table 1. Mean width of scales and statistical evaluation in Lycaenidae species.

Species	**Width (µm)**	**F/F crit. (n=15)**
Polyommatus thersites	45.634±7.30	62.708/4.195
Polyommatus icarus	53.724±7.14	
Cupido argiades	44.398±7.29	24.45/3.219
Cupido decoloratus	38.799±4.38	
Cupido alcetas	47.629±13.12	

Scale size is also distinct between different scale types, with scales in the upper layer larger than those in the lower layer. However, scale layering is variable to some extent with the region of the wing (Kristensen & Simonsen, 2003). Differences in scale sizes is considered to occur due to different sizes of trichogen forming cells as described for scale length for a broad selection of lepidopteran species (Simonsen & Kristensen, 2003; Kristensen & Simonsen, 2003). The size of scales is influenced by genetic factors, which regulate the type of scales also (Nijhout, 1985). Thus, a difference in scale characteristics can be expected between different taxonomic groups.

Conclusions

By analysis of scales’ morphology, the current paper reveals some differences among several taxa of Polyommatinae subfamily. The shape of the distal margin of scales with structural color is variable among two investigated genera, but is similar in species of the same genus. The width of structural color scales from the center of the wing is significantly variable among analyzed species. These results show a variability of Polyommatinae species at scales’ level, which complements other results, such as differences in optical properties of scales.

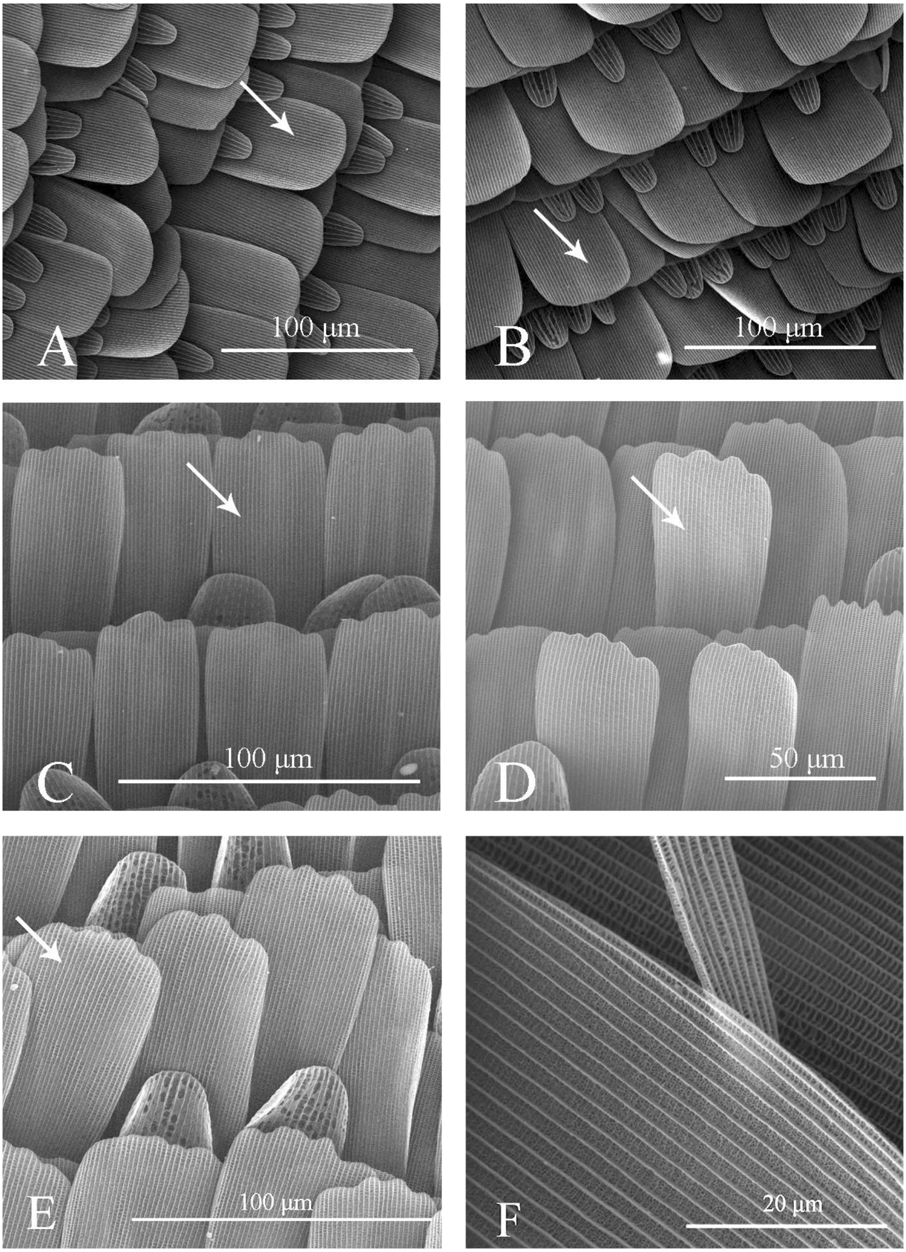

Figure 1. SEM images of wing scales - A. *Polyommatus thersites*; B. *Polyommatus icarus*; C. *Cupido argiades*; D. *Cupido decoloratus*; E. *Cupido alcetas*; F. *Polyommatus icarus* - detail (arrows indicate structural color scales selected for measurements).

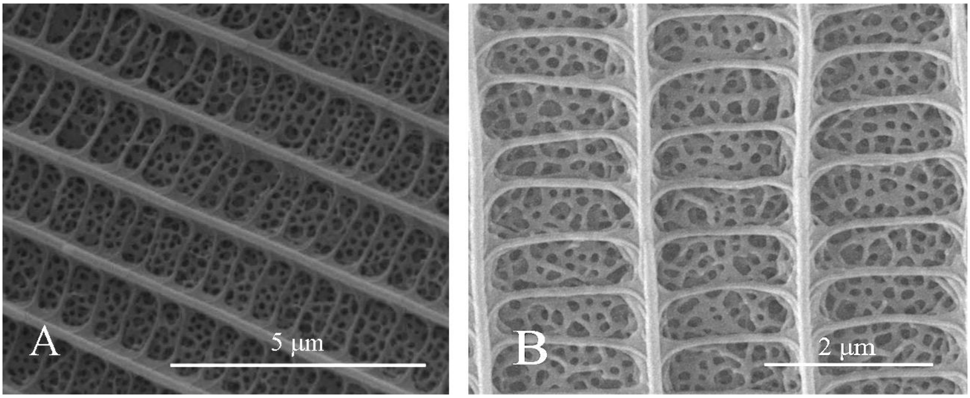

Figure 2. Pepper-pot structure in structural color scales - A. *Polyommatus thersites*; B. *Cupido argiades*.

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