“ALEXANDRU IOAN CUZA” UNIVERSITY, IAŞI

THE FACULTY OF BIOLOGY

Diversity of Lycaenids (Insecta: Lepidoptera: Lycaenidae) in protected areas from Iaşi County and the imago-plants relation in some taxa

Ph.D. Thesis Summary

Supervisors:

Prof. Moglan Ioan, Ph.D.
Prof. Maria-Magdalena Zamfirache, Ph.D.

Ph.D. student:

Samson Odette (Lobiuc)

2014

Contents

Introduction

The current paper investigates the diversity of species in the Lycaenidae family in 11 natural protected areas in Iasi county (Fânețele Seculare de la Valea lui David, Sărăturile din Valea Ilenei, Poiana cu Schit, Dealul lui Dumnezeu, Pădurea Pietrosu, Pădurea Uricani, Pădurea Gheorghițoaia, Făgetul Secular Humosu, Pădurea Tătăruși, Pădurea şi pajiştile de la Mârzeşti, Lunca Mirceşti). The paper focuses on the study of the diversity of this species in the above mentioned areas, the study of external morphology and of some microstructural elements of various taxa, by means of both classical and modern methods, the investigation of the ecological features of some lycaenids communities, as well as the analysis of some ecological relationship between some lycaenids species and their host plants.

To achieve the purpose of this paper, the following objectives were set:

- the analysis of the external morphology and the male genitalia in the collected species;
- the analysis of the specific components of the communities of lycaenids in the observed areas;
- the analysis of the microstructure of the wing scales with structural colours in some lycaenids species;
- the analysis of the diversity and structure of the lycaenids communities in the observed areas;
- the analysis of the feeding preferences in the adult stage of some lycaenids species in some protected areas.

Ch. I. The history of research regarding the Lycaenidae family lepidopterans

I.1. The history of research in Europe

The term *Lycaenida* is introduced in 1815 by Leach. During the XIXth century, external morphology studies were done by Curtis, 1832; Boisduval, 1836; Duponchele, 1844 etc. In the XXth century there were numerous systematic reorganizations within the family, based mainly on morphological studies (Eliot, 1973; Eliot şi Kawazoé, 1983; Zhdanko, 2004).

I.2. The history of research in Romania

In Romania the study of this family began with Fuss (1850) who processess the material collected by Franzenau in Transilvania. Studies on the lepidoptera biodiversity, including the lycaenids in various areas of the country are conducted by Czekelius (1917), Székely (2004), Goia şi Dincă (2006), Rákosy (2009), Corduneanu (2011).

Ch. II. Morphology, biology and ecology of lycaenids

II.1. External morphology of lycaenids

The Lycaenidae family is part of the Papilionoidaea family. The wings have vivid colors on the back/lower side, blue, brown and orange being most frequent. The sexual dimorphism is present in many species, the males having different colors from the females on the back side of their wings, the ventral side being identical in both sexes (Korshunov şi Gorbunov, 1995).

II.2. Microstructural elements and wing colors in lycenids

The Lepidoptera scales with structural colours are composed of a lower, weakly structured layer and an upper layer with numerous structures in the shape of longitudinal ridges connected by transversal ribs (Ghiradella, 1991; Ghiradella şi Butler, 2009). These structures are chitinous, while the spaces between them consist of air, thus the scale is composed of substances with different light refraction indices. The scale is therefore reflecting the light in different ways, according to the disposition of microstructures (Biró et al., 2008; Tamáska et al., 2012). The structures can be presented as ridges, ribs, lamellae or perforated layers. According to the structure type, the scales are Morpho and Urania. Lycaenids belong to the Urania type (Tilley and Eliot, 2002).

II.3. Biology and ecology of lycaenids

Whereas the feeding preferences in the larval stage is well-documented in many lycaenids, the adults' preferences for certain species of plants is less known. Still, an obvious evolutionary correlation between various species of lepidoptera and certain species of plants has been noticed. To support this, references to the length of the proboscis and floral morphology are made, as well as the chronological overlap between the flight period and the blooming stage in some flower species (Dicks et al., 2002; Krenn, 2010). In various lepidoptera families, lycaenids included, preferences for certain plant characteristics have been demonstrated, such as flower morphology or color (Tiple et al., 2006).

II.4. Distribution of lycaenids

The Lycaenidae family representatives are found in all major biogeographical regions in the temperate and tropical areas. In Romania, the species we identified are present in most areas, few species being absent in some regions.

Ch. III. Material and Research Methods

III.1. Collecting and preparing the biological material

The collection of lycaenids is done using an entomological net. The butterflies were placed in special pressing devices with entomological needles to spread their wings and antennas.

III.2. Assessing the diversity of the lycaenids species

To asses the diversity of species in this family, studies were conducted in five natural protected areas in Iasi county, classified in I.U.C.N. IV category, for which the data regarding the biodiversity of this group are relatively few. The chosen areas were selected for the different habitat types present and due to the lack of data regarding the diversity of lycaenids for some of them. The material was collected between 2012 and 2014, over an area of 500 m length and 10 m width. In each protected area there were 5 observations/sessions.

III.3. Methods of analysis on a micromorphologic level

III.3.1. Male genitalia

For male genitalia observation, physical and chemical methods were used. The prepared item was analysed under the microscope and the result was drawn using a clear chamber .

III.3.2. Scale shape and width

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 shapes of scales were observed with the unaided eye on SEM photographs. Scale sizes were measured using ImageJ software (ImageJ). The distances were statistically analyzed by calculating means and standard errors and by analysis of variance (ANOVA) for $p<0.05$.

III.3.3. Microstructural parameters of scales

In order to assess interspecific differences at the level of scales, measurements of chitinous structures were performed. The distances between longitudinal ridges and between transversal ribs were calculated on three scales from each individual of the three species. Statistical analyses were performed in MS Excel by obtaining means and standard errors and by analysis of variance (ANOVA).The number of holes contained within scale microcells was calculated using ImageJ software. The microcells are considered delimited by two consecutive ridges and two consecutive ribs. The same software was used to calculate the "filling factor" of the cells.

III.4. The sinecological analysis and biodiversity of Lycaenids

The characterization of investigated areas using the lycaenids populations in these reservations was realised through calculation of some ecological indexes that indicate the number of species and the structure of the lycaenids communities. The calculated parameters are: Abundance (A), Constancy (C), Dominance (D), The ecological significance index (W), The Shannon-Wiener (H) diversity index, The Simpson (1/D) index.

III.5 Assessing the food preferences in adults

The material consisted in adults of the *Plebejus argus*, *Lycaena thersamon*, *Satyrium acaciae*,species, both males and females. The observations were conducted in the protected areas Valea lui David, Sărăturile din Valea Ilenei, Pădurea şi pajiştile de la Mârzerşti and Pădurea Uricani, over and area of aproximately 5 m width and 180 m length, in each area.

Ch. IV. Diversity of Lycaenids in protected areas investigated in Iaşi County

IV.1 Morphological characteristics of identified species

As part of the external morphology, seven features have been investigated (eyes, antennae, the color of the proboscis, labial palpi, wing venation, pterygodes, legs). Some differences can be noticed, especially on an intergeneric and intrafamilial level. For the investigated species there are qualitative and quantitative differences regarding the male and female genitalia, on an interspecific, intergeneric and intrafamilial level.

IV.2. Lycaenids species identified and their distribution in investigated areas

The collection of lycaenids was conducted in 11 protected areas in Iasi County, 21 species were identified of 9 types and 3 subfamilies: Theclinae, Lycaeninae and Polyommatinae. The greatest diversity was determined in Valea lui David where 15 species were identified, followed by Poiana cu Schit, 13 species and Făgetul Secular Humosu and Pădurea Tătăruşi, 2 species each.

Ch. V. Micromorphology of scales with structural colors

V.1. Scale shape and width

The shape of the apical margins was found to be variable among the two genera, (*Polyommatus* şi *Cupido*). The species of the *Polyommatus* genus have rounded margin while the species of the *Cupido* genus present scalloped margin (Fig. 1). The width of scales presents different values between congeneric species (Tab. 1).

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	

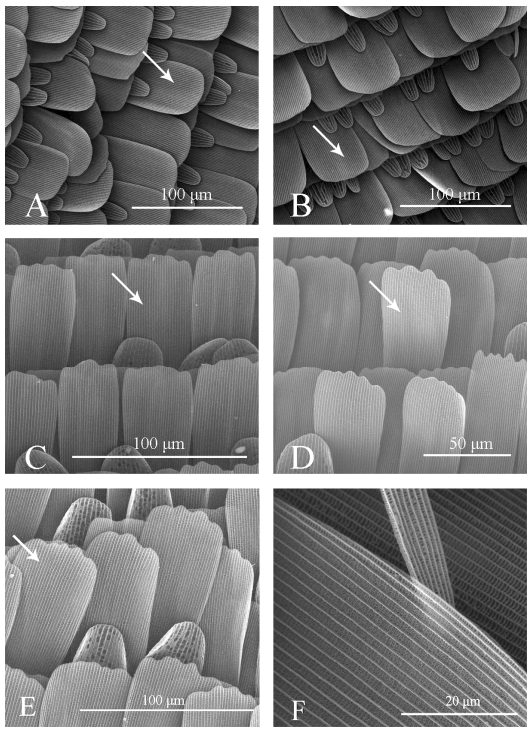

Figure 1. SEM images of wing scales - A. *Polyommatus thersites*; B. *Polyommatus icarus*; C. *Cupido argiades*; D. *Cupido decoloratus*; E. *Cupido alcetas*; F. *Polyommatus icarus* - detail (arrows indicate structural color scales selected for measurements)

V.2. Quantitative analysis of microstructural parameters

The species investigated by us, *Cupido argiades*, *C. decoloratus* and *C. alcetas* (Fig. 2), show specific values for the analysed parameters. The distances between ridges present higher values in *C. argiades* and *C. alcetas*, with *C. decoloratus* showing lower values than the other two species. The distances between ribs follow a slightly different pattern, with closer values in *C. argiades* and *C. decoloratus*, while *C. alcetas* records the highest values.(Tab. 2). *C. alcetas* records the highest number of holes (Tab. 3) Regarding the "filling factor", representing the ratio of chitin to holes, the three species record a degree of variability, *C. decoloratus* shows the highest values of the filling factor.

The Principal Component Analysis of the four above investigated parameters (Tab. 4) revealed correlations between variables. The percentage of variability comprised in computed factors is highest for F1 and F2 (Tab.4). The placement of the species in relation with the chosen factors suggests the possible taxonomic value of the analyzed parameters due to the disjunct values of the calculated scores (Fig. 3).

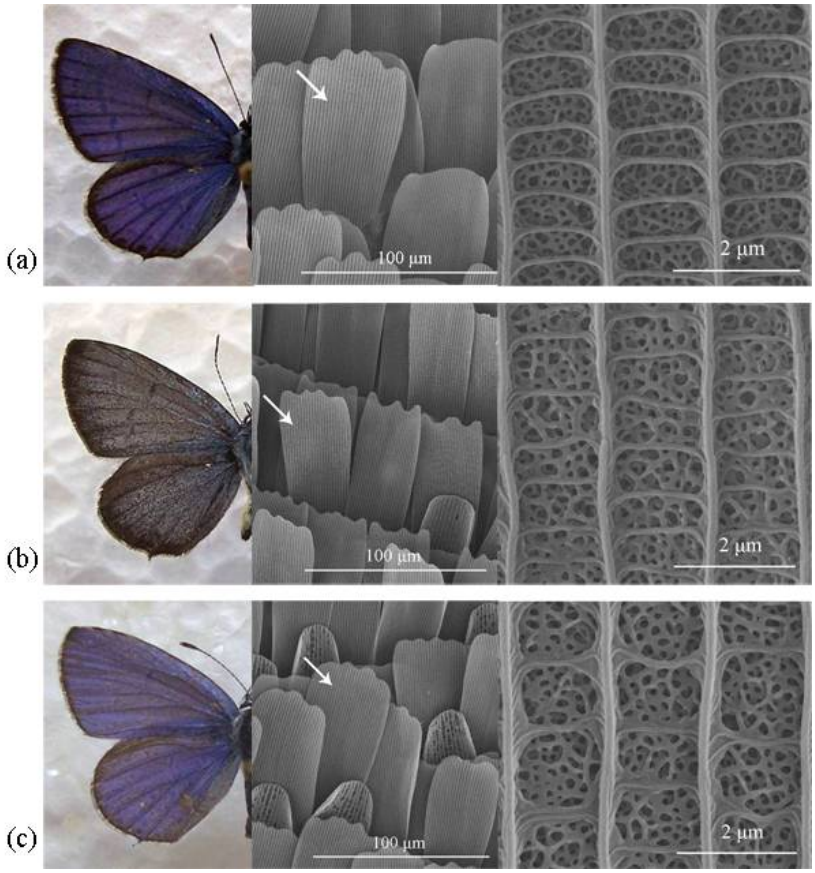

Figure 2. Wings and wing scales aspects of three *Cupido* species (a - *C. argiades*; b - *C. decoloratus*; c - *C. alcetas*)

Table 2. Dimensions of scale distances in investigated species (Drid - distance between ridges; Drib - distance between ribs; values in the same column are significantly different as shown by ANOVA for $p<0.01$)

Species	Drid (N=75)	Drib (N=210)	Drid/Drib ratio
Cupido argiades	1.71 ± 0.018	0.73 ± 0.007	2.33
Cupido decoloratus	1.40 ± 0.017	0.60 ± 0.006	2.32
Cupido alcetas	1.63 ± 0.015	1.10 ± 0.020	1.48

Table 3. Cells number of holes and filling factor values in investigated species (values in the same column are significantly different as shown by ANOVA for $p<0.01$)

Species	No. of holes (N=30)	Filling factor (N=30)
Cupido argiades	22.66 ± 0.682	2.11 ± 0.073
Cupido decoloratus	18.70 ± 0.867	3.24 ± 0.185
Cupido alcetas	33.30 ± 1.495	2.54 ± 0.102

Table 4. Correlation matrix and Principal Component Analysis results

Correlation matrix (Pearson (n)):				
Variables	Drid	Drib	Fill. fact.	Holes nr.
Drid	**1**	**0.394**	**-0.322**	**0.225**
Drib	**0.394**	**1**	-0.128	**0.459**
Fill. fact.	**-0.322**	-0.128	**1**	-0.189
Holes nr.	**0.225**	**0.459**	-0.189	**1**
Values in bold are different from 0 with a significance level alpha=0.05				
Principal Component Analysis:				
Eigenvalues:				
	F1	F2	F3	F4
Eigenvalue	1.876	0.952	0.714	0.458
Variability (%)	46.912	23.792	17.843	11.453
Cumulative %	46.912	70.704	88.547	100.000

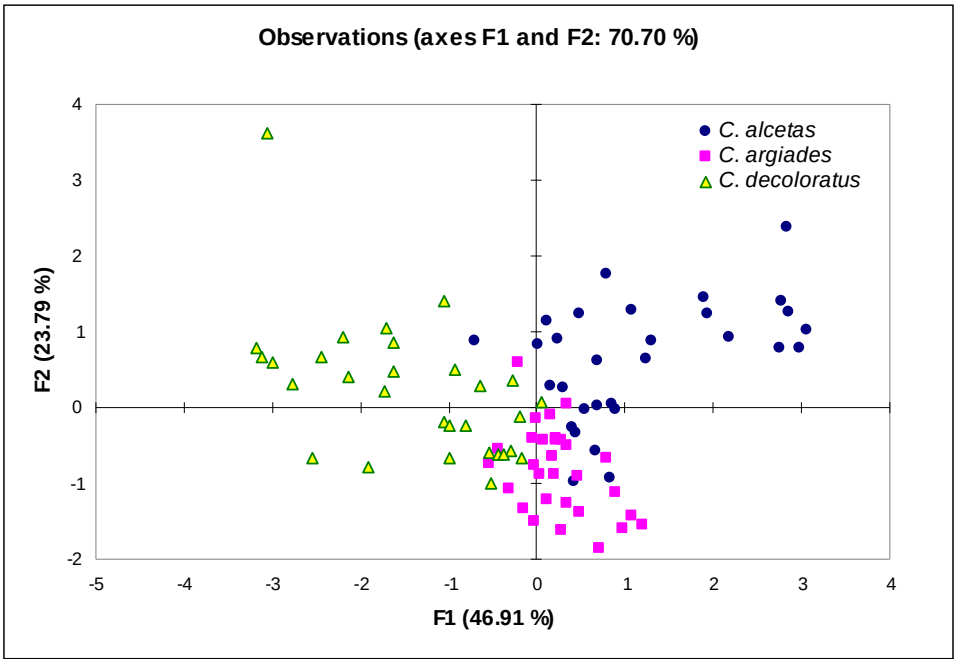

Figure 3. PCA observations biplot

Ch. VI. The ecological analysis of Lycaenids identified in the protected areas investigated

VI.1 The sinecological analysis of Lycaenids in protected areas investigated

Eudominant and characteristic species in floristic areas:

Fânețele Seculare de la Valea lui David: *Plebejus argus, Polyommatus icarus, Cupido argiades, Lycaena dispar, Plebejus argyrognomon, Polyommatus thersites.* **Poiana cu Schit:** *P. argus, P. icarus, Cupido argiades, Lycaena dispar, Cupido decoloratus, Satyrium w-album.*

Sărăturile de la Valea Ilenei: Plebejus *argus, P. icarus, C. argiades, Lycaena thersamon, Cupido decoloratus.* **Dealul lui Dumnezeu:** *P. argus, P. icarus.*

Eudominant and characteristic species in forest areas:

Pădurea Uricani*: Cupido argiades, C. decoloratus, Celastrina argiolus, Satyrium acaciae*. **Pădurea Pietrosu***: C. argiades, C. decoloratus, Lycaena dispar, Satyrium w-album.* **Rezervația Pădurea Gheorghițoaia***: C. argiades, Polyommatus icarus, Plebejus argus.* **Pădurea şi pajiştile de la Mârzeşti***: P. icarus, P. argus, Lycaena dispar, L. thersamon, L. phlaeas*. **Lunca Mirceşti***: P. icarus, Celastrina argiolus, Lycaena dispar*

VI.2. The analysis of the diversity of Lycaenids in the protected areas investigated

The Shannon-Wiener indices of investigated areas show that the highest diversity is present in Fânețele Seculare de la Valea lui David and Poiana cu Schit areas, indicating the highest diversity. The species equitability shows the species are equaly distributed in Valea lui David and Poiana cu Schit and unequaly in Dealul lui Dumnezeu, Pădurea Gheorghițoaia and Lunca Mirceşti.

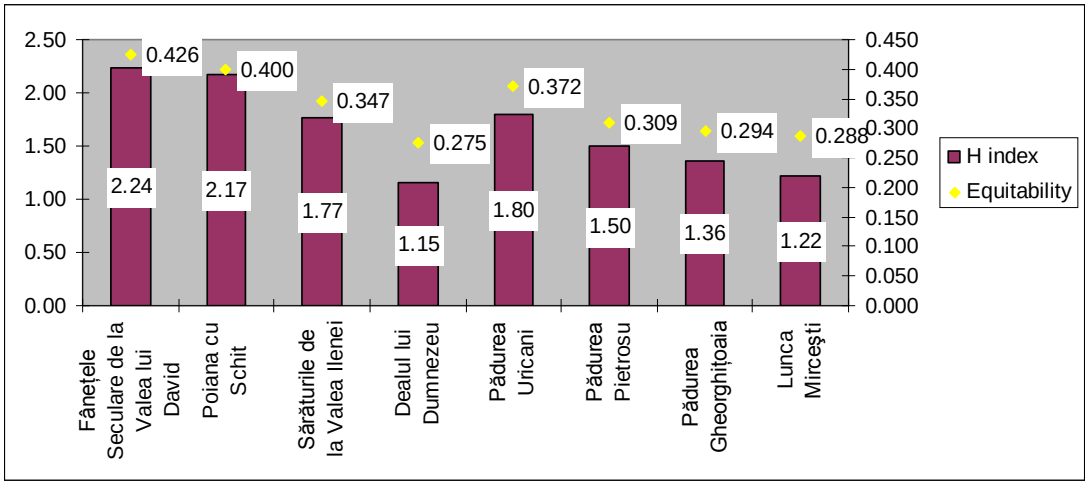

Figure 4. The Shannon-Wiener index and equitability of Lycaenids communities in the investigated areas

The Simpson indices of the investigated areas is highest in Poiana cu Schit and Fânețele Seculare de la Valea lui David areas. The equitability of the species shows a high uniformity in Lunca Mirceşti area and a lower uniformity in Sărăturile de la Valea Ilenei.

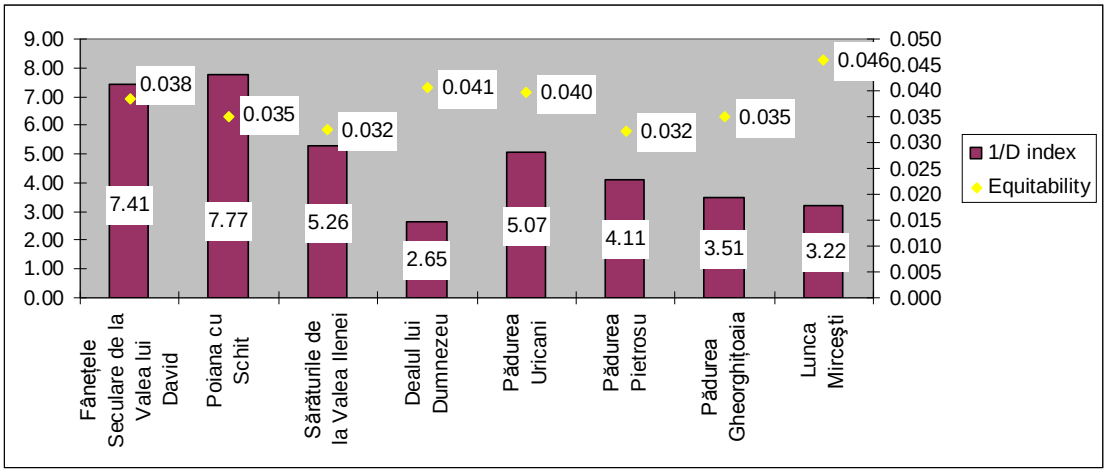

Figure 5. The Simpson index and equitability of Lycaenids communities in the investigated areas

VI.3. Food preferences of adults of the *Plebejus argus, Lycaena thersamon* şi *Satyrium acaciae* species

For Valea lui David protected area, individuals of the *Plebejus argus* species made a total of 150 visits to a number of 15 plant taxa, belonging to 8 families. The highest number of visits, 67, was recorded for *Salvia nemorosa*, and the highest average time of visits, 52.33 sec., was calculated for the *Cynoglosum officinale* species. The total time of visits recorded was the highest for the *Salvia nemorosa* species.

In Sărăturile din Valea Ilenei protected area, the highest number of adult visits of the *Plebejua argus* species was recorded for *Salvia nemorosa* species, of a total of 83 visits to 12 plant taxa of 6 families. The highest average time of visits was recorded for the *Filipendula vulgaris* species, 93 sec., followed by *Trifolium montanum*, 89 sec. The highest total feeding time for adults was recorded for *Salvia nemorosa.*

In Mârzeşti forest and meadows protected area, individuals of the *Lycaena thersamon* species made a total of 105 visits, to a number of 3 plant taxa, belonging to 2 families (Asteraceae and Dipsacaceae). The highest number of visits, 67, was recorded for the *Inula salicina* species and the highest average time of visits, 20,76 sec., was calculated for the *Scabiosa ochroleuca* species. The total time of visits recorded was the highest for the *Inula salicina* species.

In the Uricani forest pretected area, individuals of the *Satyrium acaciae* species made a total of 129 visits to a number of 2 plant taxa belonging to 2 families (Asteraceae şi Caprifoliaceae). The highest average time of visits was recorded for the *Erigeron annuus* species, 24,98 sec., followed by *Sambucus ebulus, 23,73 sec.* The highest total feeding time for adults was recorded for *Erigeron annuus*

Conclusions

1. As part of the external morphology, seven features have been investigated (eyes, antennae, the color of the proboscis, labial palpi, wing venation, pterygodes, legs).

2. For the investigated species there are qualitative and quantitative differences regarding the male and female genitalia, on an interspecific, intergeneric and intrafamilial level.

3. Regarding the diversity of the Lycaenids, 11 protected areas in Iasi County were investigated between 2012-2014, where 21 species were identified belonging to a total number of 9 genera and 3 subfamilies: Theclinae, Lycaeninae and Polyommatinae.

4. The highest number of species was recorded in Valea lui David protected area (15 species) followed by Poiana cu Schit (13 species), and the lowest number in Făgetul Secular Humosu (2 species) and Pădurea Tătăruşi (2 species).

5. The morphological analysis of the wing scales with structural colors revealed some differences between various taxa of the Polyommatinae family (*Polyommatus icarus, P. thersites, Cupido argiades, C. decoloratus, C. alcetas*). The shape of the distal margins was found to be variable among the two genera. The width of wing scales with structural colors varies statistically between congeneric species.

6. The investigated species (*Cupido argiades, C. decoloratus* and *C. alcetas)* show specific values for the analysed parameters (distance between ridges, distance between ribs, cells number of holes and filling factor), of the wing scale microstructure with structural colors, differences which have been statistically measured and demonstrated.

7. The Principal Component Analysis of the four above investigated parameters revealed correlations between variables. The placement of the species in relation with the chosen factors suggests the possible taxonomic value of the analyzed parameters due to the disjunct values of the calculated scores.

8. The investigated floristic reservations have a similar structure regarding the Lycaenids communities, a series of species (*Plebejus argus, Polyommatus icarus*) having the same degree of dominance in the respective areas. In forest type reservations eudominant species common to all the investigated areas have not been identified, instead these species vary depending on the existing flora in the respective areas.

9. The Shannon-Wiener indices of investigated areas show that the highest diversity is present in Valea lui David., while the lowest is Dealul lui Dumnezeu protected area. The Simpson indices of the investigated areas is highest in Poiana cu Schit and lowest in Dealul lui Dumnezeu protected area.

10. In Valea lui David protected area, the adults of the *Plebejus argus* species recorded the highest number of visits to *Salvia nemorosa* and *Vicia cracca* species, and in Saraturile din Valea Ilenei, the preferences were *Salvia nemorosa* and *Achillea setacea.* In Mârzeşti forest and meadows, the adults of the *Lycaena thersamon* species recorded the highest number of visits to *Inula salicina.* In Padurea Uricani, the adults of the *Satyrium acaciae* species preferred 2 plant species, *Erigeron annuus* and *Sambucus ebulus,* the highest number of visits being recorded for the *Erigeron annuus* species.

Bibliography

Biró, L.P., Kertész, K., Vértesy, Zofia, Bálint, Zs., 2008. *Photonic nanoarchitectures occurring in butterfly scales as selective gas/vapor sensors*. In The Nature of Light: Light in Nature II, Katherine Creath (ed.), Proc. of SPIE Vol. 7057: 705706-1 - 705706-8.

Boisduval, J.A., 1836. *Histoire naturelle des insectes; spécies général des lépidoptères*. Vol. I, Paris.

Corduneanu, C., 2011. *Protected Lepidopteran species (Insecta: Lepidoptera) in north-east of Romania*. Travaux du Muséum National d'Histoire Naturelle «Grigore Antipa», LIV (1): 115–123.

Curtis, J., 1832. *British entomology; being illustrations and descriptions of the genera of insects found in Great Britain and Ireland: containing coloured figures from nature of the most rare and beautiful species, and in many instances of the plants upon which they are found*. Vol. V, Lepidoptera, Partea I, London, 66-80.

Czekelius, D., 1917. *Beiträge zur Schmmetterlingsfauna Siebenbürgens. Verhandlungen und Mitteilungen des siebenbürgischen Vereins für Naturwissenschaften zu Hermannstadt*. 67 (1-6): 1-56.

Dicks, L.V., Corbet, S.A., Pywell, R.F., 2002. *Compartmentalization in plant–insect flower visitor webs*. Journal of Animal Ecology, 71: 32–43.

Duponchele, M.P.A.J., 1844. *Catalogue méthodique des lépidoptères d' Europe*. Méquignon-Marvis Fils, Paris, 28-33.

Eliot, J.N., 1973. *The higher classification of the Lycaenidae (Lepidoptera): a tentative arrangement* în Bulletin of the British Museum (Natural History) Entomology, 28 (6): 371-505.

Eliot, J.N., Kawazoé, A., 1983. *Blue butterflies of the Lycaenopsis group*. British Museum (Natural History).

Fuss, C., 1850. *Verzeichniss der bis jetzt Siebenbürgen aufgefundenen Lepidopteren*. Verhandlungen und Mitteilungen des Siebenbürgischen Vereins für Naturwissenschaften, Hermannstadt, 1: 54–64.

Ghiradella, H.T., 1991. *Light and color on the wing: structural colors in butterflies and moths*. Applied Optics, 30(24): 3492-3500.

Ghiradella, H.T., Butler, M.W., 2009. *Many variations on a few themes: a broader look at development of iridescent scales (and feathers)*. Journal of the Royal Society Interface, 6: 243–251.

Goia, M., Dincă, V., 2006. *Structura şi răspândirea faunei de lepidoptere diurne (Hesperioidea & Papilionoidea) în împrejurimile municipiului Cluj-Napoca şi aspecte actuale ale influenței antropozoogene asupra mediului de viață al acestora.* Bul. inf. Soc. lepid. rom., 17: 139-197.

ImageJ. http://imagej.nih.gov/ij/index.html

Korshunov, Y., Gorbunov, P., 1995. *Butterflies of the Asian part of Russia. A handbook*. Ural University Press, Ekaterinburg.

Krenn, H.W., 2010. *Feeding Mechanisms of Adult Lepidoptera: Structure, Function, and Evolution of the Mouthparts*. Annu. Rev. Entomol., 55:307–27.

Leach, W.E., 1815. *Entomology* în Brewster D. (Ed.) (1808-1830), *The Edinburgh Encyclopaedia*. Vol. IX, 57-172.

Rákosy, L., 2009. *Macrolepidoptere din perimetrul comunei Şinca Nouă* (jud. Braşov). Bul. inf. Soc. lepid. rom., 20: 5- 26.

Székely, L., 2004. *Noutăți lepidopterologice din sud-estul Transilvaniei (Județul Braşov, România)*. Bul. Inf. Entomol., 14-15: 41-56.

Tamáska, I., Kertész, K., Vértesy, Zofia, Bálint, Z., Kun, A., Yen, S.-H., Biró, L. P., 2012. *Colour changes upon cooling of Lepidoptera scales containing photonic nanoarchitectures*. arXiv:1206.2749v1 [physics.bio-ph]

Tilley, R.J.D., Eliot, J.N., 2002. *Scale microstructure and its phylogenetic implications in lycaenid butterflies (Lepidoptera, Lycaenidae).* Transactions of the Lepidopterological Society of Japan, 53(3): 153-180.

Tiple, A.D., Deshmukh, V.P., Dennis, R.L.H., 2006. *Factors influencing nectar plant resource visits by butterfl ies on a university campus: implications for conservation*. Nota lepidopterologica 28 (3/4): 213-224.

Zhdanko, A.B., 2004. *A revision of the supraspecific taxa of the Lycaenid Tribe Polyommatini (Lepidoptera, Lycaenidae).* Entomological Review, 82 (7): 782-796.