Babes-Bolyai University, Cluj Napoca
Faculty of Biology and Geology
Taxonomy and Ecology Chair

- Doctoral Thesis Summary -

Description of Some Endemic Taxa at Insects in Romania, Using Techniques of Electron Microscopy and Molecular Biology

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KEY WORDS: Lepidoptera, endemic species, the red list , *Dahlica rakosyi*, *Pseudophilotes bavius hungarica*, *Euphydryas maturna opulenta*, *Erebia sudetica radnaensis*, structural colors, SEM, EDX, molecular biology, allozymes, DNA sequencing, *Erebia epiphron transylvanica*.

General considerations about studied bibliographical data

We conclude from the bibliographical data that the studies made on the endemic species of Lepidoptera from Romania up to present time, using as methods optical microscopy and scanning electron microscopy, didn't clarify many of the aspects regarding the structure and morphology of these species.

In the international scientific literature, the optical and electron microscopy technique has been used both in describing unknown evolution stages, through their morphologic description (egg, larva, pupa, adult); we also have the possibility of the comparative study of some homologue and non homologue morphological structures that furnish information that can be used in the traditional taxonomy; the morphological description of some ultra structures which explain the behavior and biology of some species; the SEM techniques can also be used in modern taxonomy studies through morphology studies where several supra taxa are involved, and their purpose is to show the morphological modifications produced along their evolution, creating cladograms on the basis of these morphological differences, highlighting new clades.

At the molecular level the richest information was obtained using the DNA sequency tehnique. The mitocondria genes are usually sequenced, but during the last years nuclear genes are also sequenced. A good synthesis of the DNA methods used in systematic and taxonomy is given by Hillis et al., 1996.

Other molecular markers used in the study of the population genetics are the DNA microsatellites, they are used to study metapopulations and in the philogeographic reconstruction of some populations. These are used because they have a high level of polymorphism. All these methods considerably increased the knowledge about the evolution and systematic of the insect populations. Lately the molecular methods divide into 2 tendencies: DNA sequence used in taxonomy studies/molecular phylogeny and evolution processes that will be shown by the apparition of a new markers system (Assman et al.,2007). The result of a phylogeny molecular analysis is expressed by constructing philogenetic trees. Their construction is made using philogenetic computational methods, which assume a working algorithm, a method and a soft of philogenetic interpretation. The purpose is the construction of the philogenetic trees which represent an evolution hypothesis of some genus, species or other taxa.

None of the studies made until now used the technique of scanning electron microscopy and the aspects of molecular biology (molecular phylogeny) at the same time in describing endemic Lepidoptera species from Romania.

6. Objectives

On the basis of our studies and considerations regarding our reseach subject up to present, we established the accomplishment of the following objectives within the doctoral thesis:

- morphology studies on *Dahlica rakosyi* Weidlich 2005,(Psychidae), using techniques of optical microscopy and electron microscopy

- morphology studies at *Pseudophilotes bavius hungarica* Dioszeghy 1913, (*Lycaenidae)*, using techniques of optical microscopy and electron microscopy

- morphology studies on the ultra structure of the *Pseudophilotes bavius hungarica* Dioszeghy 1913, (*Lycaenidae*) wing scale, compared to other Lepidoptera species that have structural colors.

- genetic polymorphism study within 19 populations of *Erebia sudetica radnaensis* Rebel 1915, (*Nymphalidae*), from Europe, using the allozymes technique.

- molecular validation of the subspecies *Erebia epiphron transylvanica* Rebel 1918, *(Nymphalidae)*, *Euphydryas (Hypodryas) maturna opulenta* Rakosy & Varga 2009, *(Nymphalidae*), and their own phyletic position.

- the evaluation of the phylogenetic relations between the subspecies sequenced by us and the genus of the *Nimphalinae* and *Satyrinae* subfamilies.

General discussions

The morphology studies on Dahlica rakosyi Weidlich, 2005 (Psychidae) and Pseudophilotes bavius hungarica Dioszeghy, 1913 (Lycaenidae) using optical microscopy and electron microscopy techniques.

The knowledge regarding the endemic taxa morphology Dahlica rakosyi previous to this study is resumed to a short description of the adult, without any references to the pre-imago stages. The description of the new taxa was based upon the taxa criteria used within this group, that is the morphology of the androconial male and female wing scale. The objective of our study was adding new data to the previous description, by adding a large amount of information both about the adult and especially about the pre-imago stages that had been unknown till the present time. As a scientific new data we show and describe for the first time the female of this species (figs. 1, 2).

Fig. 1. - *Dahlica rakosyi* Weidlich 2005, (*Psychidae*), female.

Fig. 2. - *Dahlica rakosyi* Weidlich 2005, (*Psychidae*). Female. Lateral view.

Besides the several morphological new data, our study represents a new model, much more precise and accurate in approaching the taxa descriptions in the future. The data referring to the endemic taxa Dahlica rakosyi are a little more detailed in the scientific literature, but they only talk about descriptions made with the naked eye or using the stereo microscope. By using the scanning electron microscopy technique we came to show various unknown structures until now, they exist especially at the egg, pupa and larval wormbag (figs. 3, 4, 5, 6)

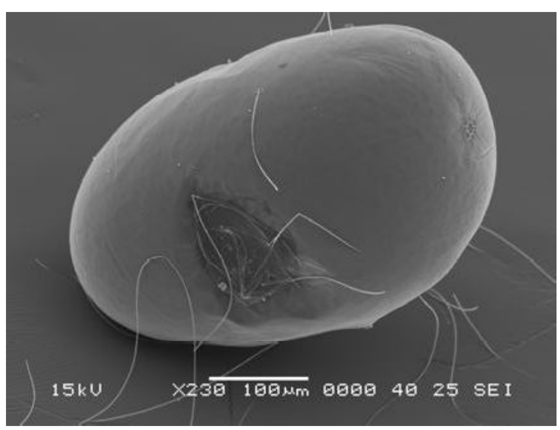

Fig. 3. – *Dahlica rakosyi* Weidlich 2005, (*Psychidae*). The egg.

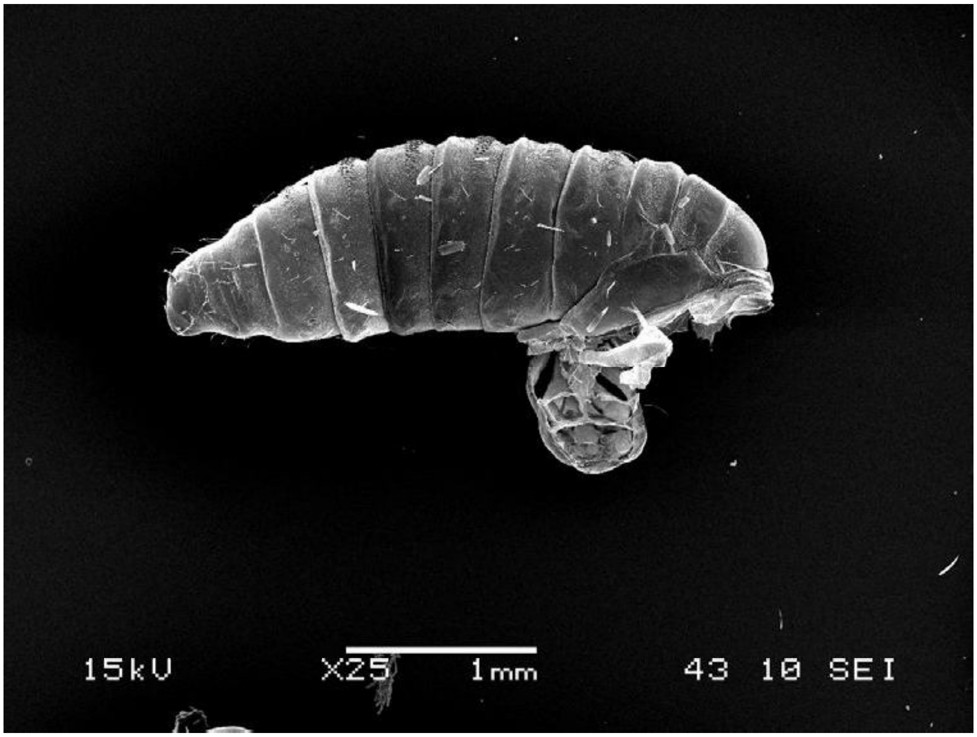

Fig. 4. - *Dahlica rakosyi* Weidlich 2005, (*Psychidae*). Female pupa.

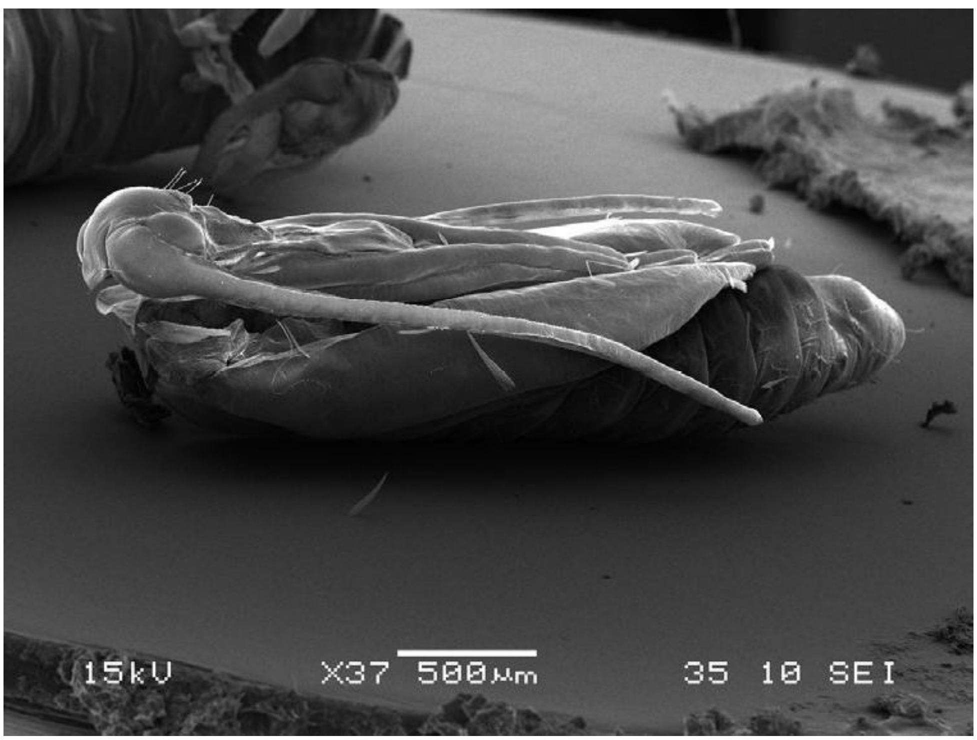

Fig. 5. - *Dahlica rakosyi* Weidlich 2005, (*Psychidae*). Male pupa.

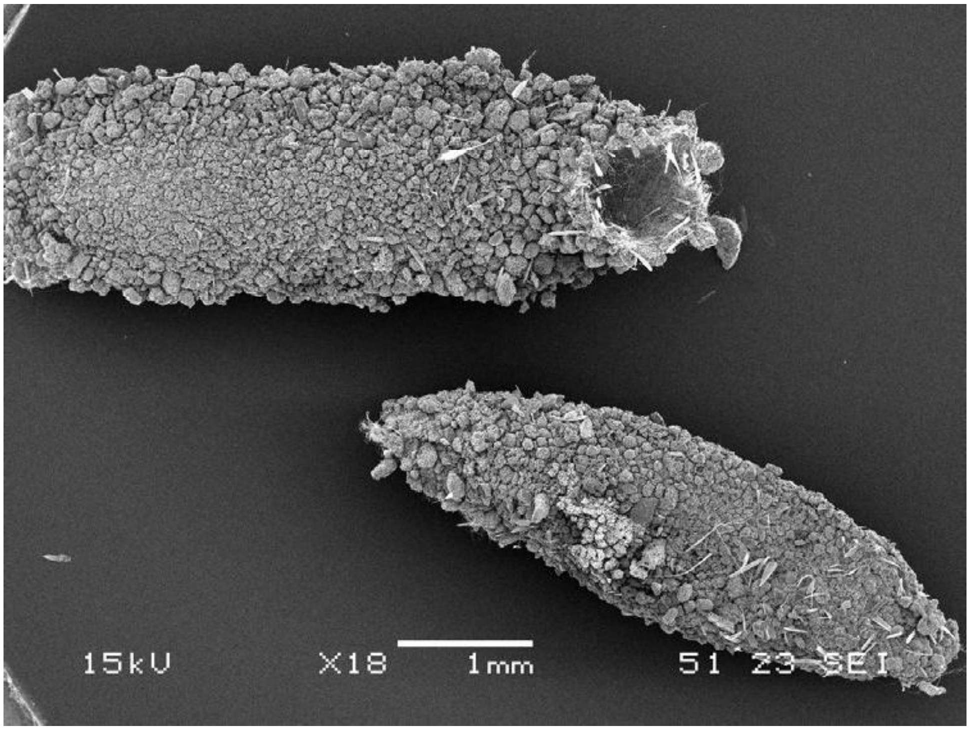

Fig. 6. - *Dahlica rakosyi* Weidlich 2005, (*Psychidae*). Larval wormbag covered with vegetal and solis particles.

We don't know the role and functionality of the shown structures *Pseudophilotes bavius hungarica* (fig. 7).

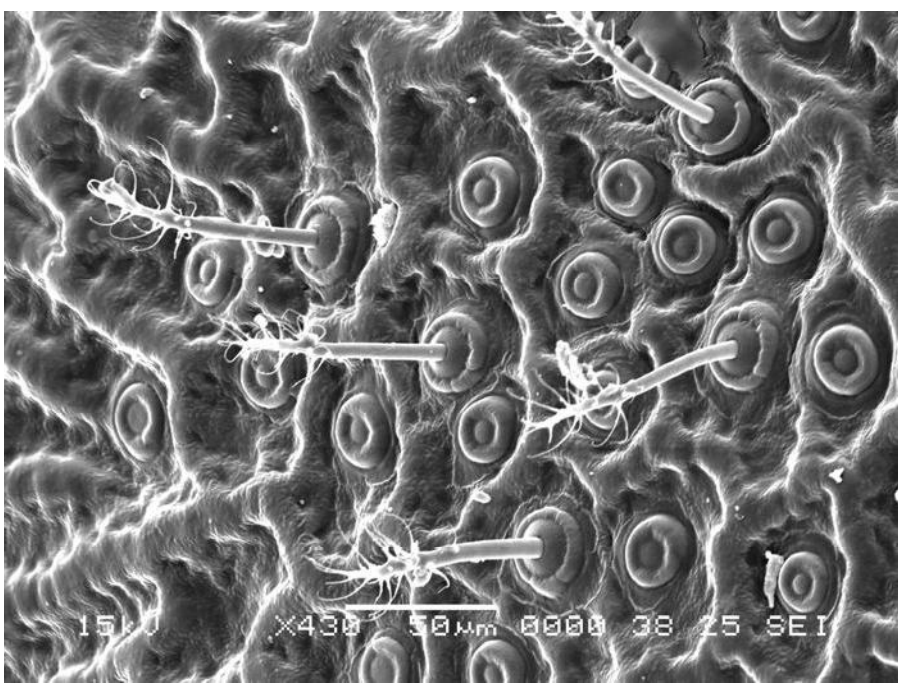

Fig. 7. - *Pseudophilotes bavius hungarica* Dioszeghy 1913, *(Lycaenidae)* – Pupa – detail sensilla.

. **Morphology study of the ultra structure of the *Pseudophilotes bavius hungarica* Dioszeghy 1913, (*Lycaenidae*) wing scale, compared to other Lepidoptera species that have structural colors.**

The methods we used are representative for this analysis (Goldstein J, 2003), we made the scanning in line, in dots, small areas on the ridges lamella and crossribs, the relative concentration in chemical elements passed through a scanning line through wing scale and also the presence and absence analysis of a chemical element with a known dispersion energy. The analyzed species are *Apatura ilia*, *Autographa bractea*, *Pseudophilotes bavius* (figs. 8, 9, 10, 11, 12). The comparison results in the study of the structural colors at butterflies using the EDX analysis, have shown the followings: the chemical elements are much better represented in the area between the crossribs and in a smaller amount in the ridge lamella. There are quality differences between the two studied areas, that is, at the crossribs the most important chemical elements are C and O and in very small amounts Na, Cu, Zn. In the area between the crossribs there is a higher concentration of Na, Cu, Zn.

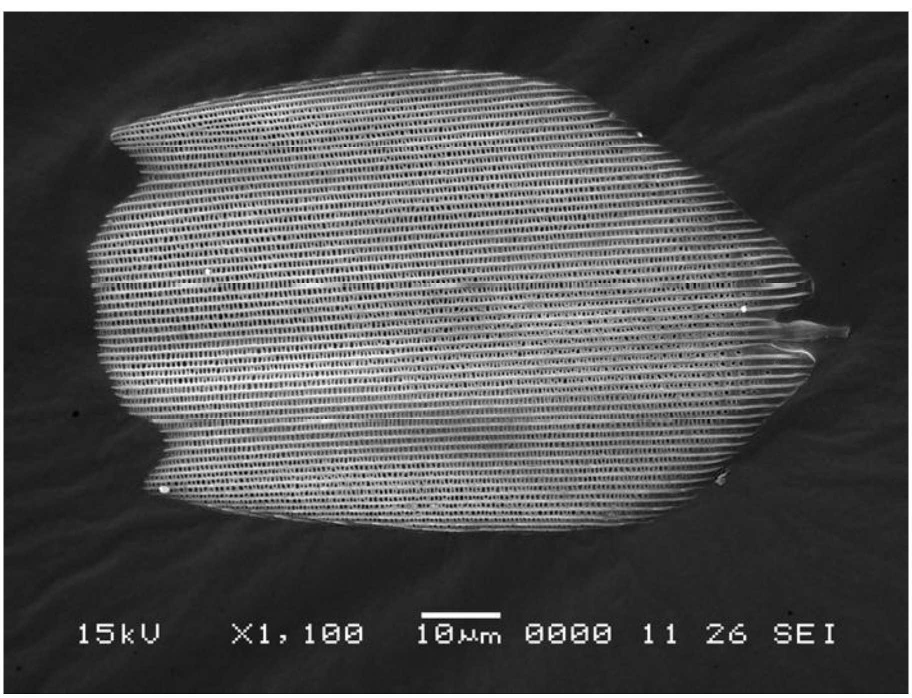

Fig. 8. – *Pseudophilotes bavius*, structural colours wing scale, general aspects.

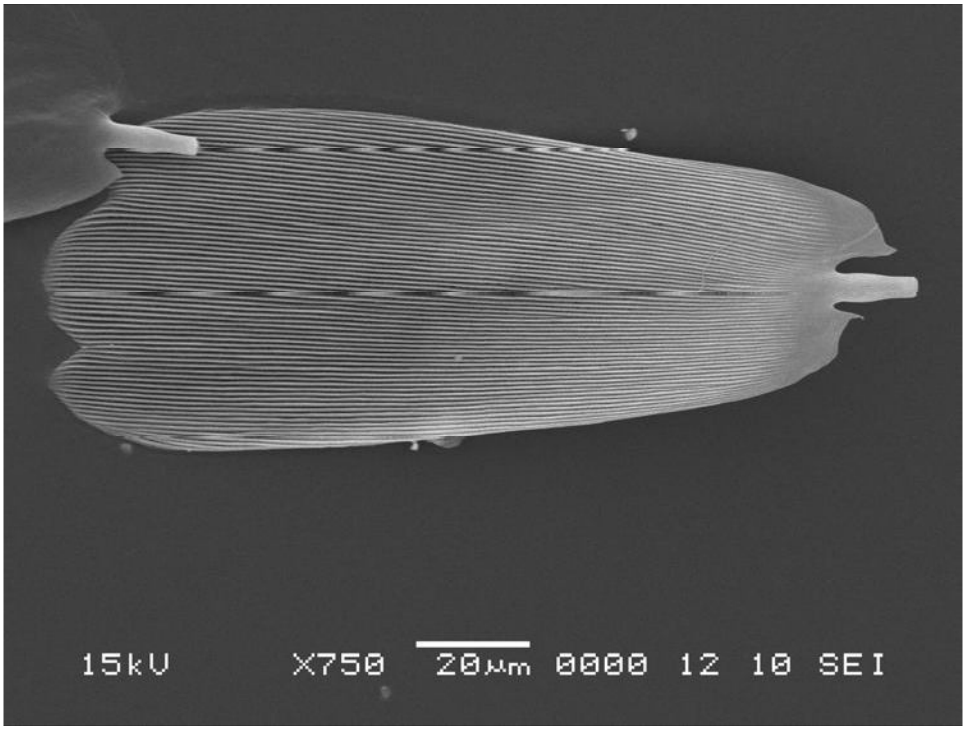

Fig. 9. - *Apatura ilia*, structural colours wing scale, general aspects.

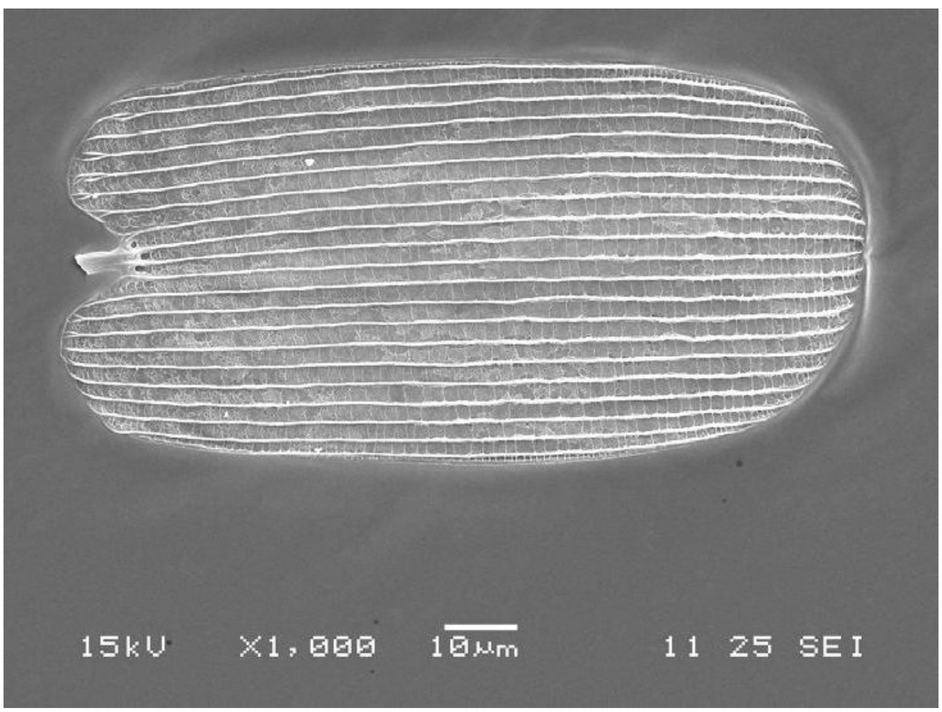

Fig. 10. – *Lycaena helle*, structural colours wing scale, general aspects.

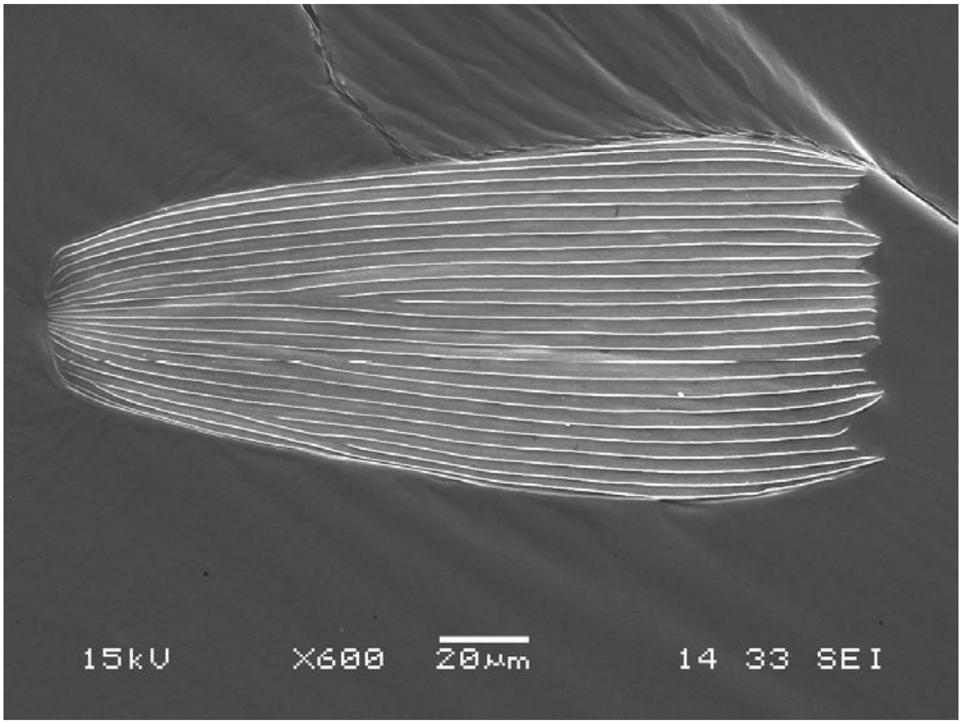

Fig. 11. – *Autographa bractea,* structural colours wing scale, general aspects.

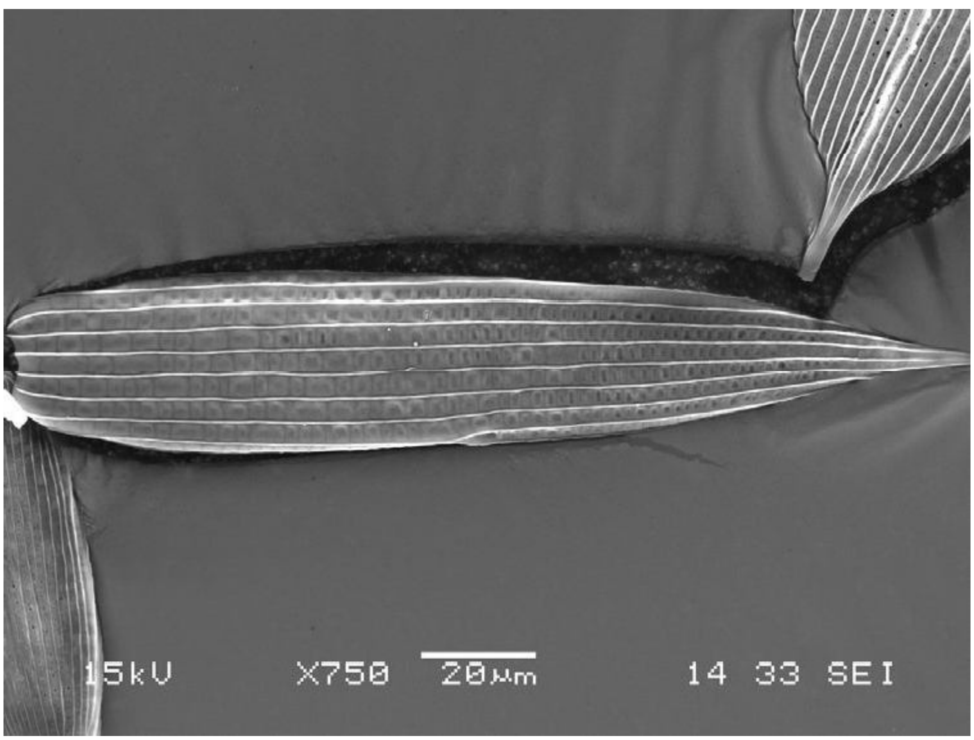

Fig. 12. – *Procris (Adscita) statices,* structural colours wing scale, general aspects.

Genetic polymorphism study on 19 populations of *Erebia sudetica radnaensis* Rebel 1915, (*Nymphalidae*) from Europe ,using the allozymes technique

From the 19 populations and the 20 studied allozymes loci, we identified four different groups. The used out group (*E. sudetica*) became an in group on the link between the two western (*E. melampus*) and eastern (*E. momos*) branches of the *E. sudetica – melampus* complex. Between the western and eastern branches we identified a distinct branch of a possible new species, *E. radnaensis*.

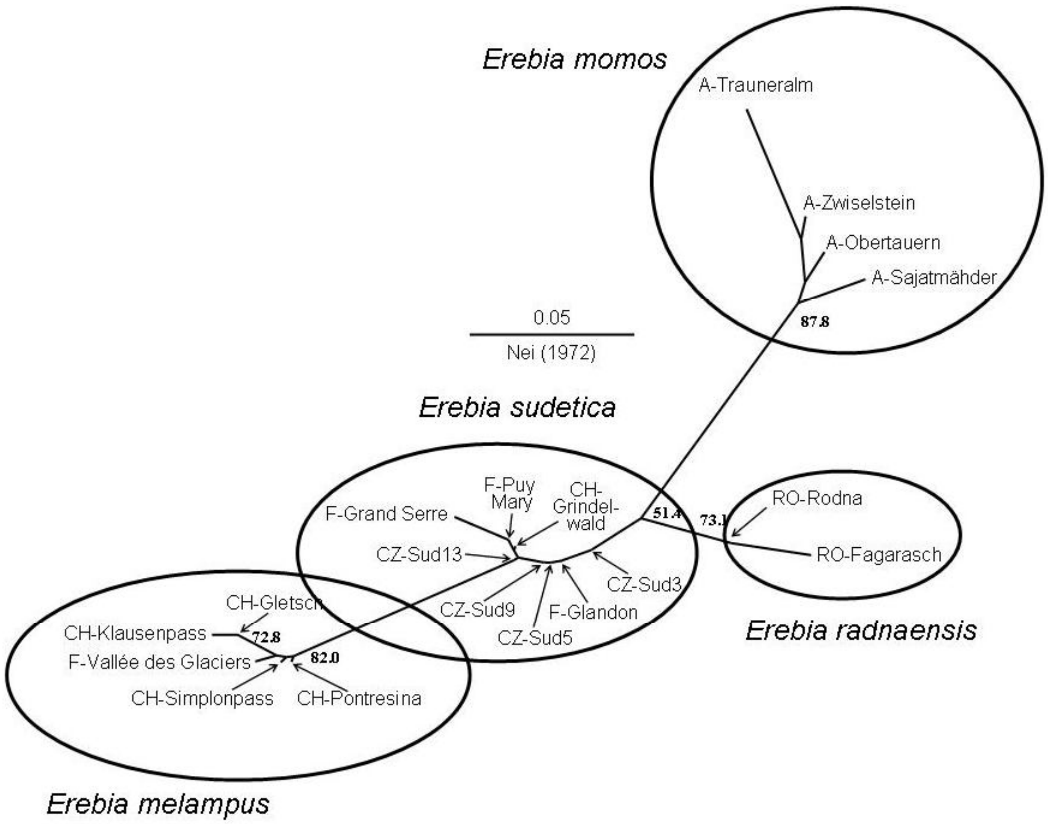

Fig. 13 – Neighbour-joining phenogram based on Nei's genetic distances (Nei 1972) of the 19 populations of *Erebia melampus/sudetica* representing at least 10 individuals. Bootstrap values over 50% are given at the nodes (only for the phenogram based on genetic distances). Abbreviations: car country signs.

DNA sequence and the construction of the phylogenetic trees

-the phylogenetic trees show the inter relational degree between different species on the basis of a molecular marker (in our case a fragment from COI). The molecular markers represent portions from the genom, usually very small portions compared to the whole genom of the organism, which are chosen as being representative for much larger portions of the DNA, or even for the entire organism (depending on the purpose of the analysis). In conclusion our sequences are a substitute of the species they represent and they are called OTUs-operational taxonomic units. The philogenetic hypothesis says that 2 sequences have a common ancestor (a closer or a further one). In the trees construction we actually calculate the common ancestors of the different sequences that form a tree. The common ancestors are inferred by calculation and are represented by internal nodes. The more similar 2 sequences are , they have a closer common ancestor, they are more related to each other, their divergent moment is very recent, and in the tree they will be the first ones that group 2 by 2 and they converge to the first internal node (which represents their most recent common ancestor). Ultimately the common ancestors of several sequences will group to a more internal node, which represents the common ancestor of all the sequences from its tree, and so on.

-if we evaluate the taxonomy through phylogeny, it's of great importance the covering percentage of the taxonomy groups over the phylogenetic ones, that is into what amount they correspond to the different branches of our tree. For the tree of the two genus taken into study *Euphydryas maturna opulenta* and *Erebia epiphron transylvanica* (figs. 14, 15), the subspecies group together as it was to be expected.

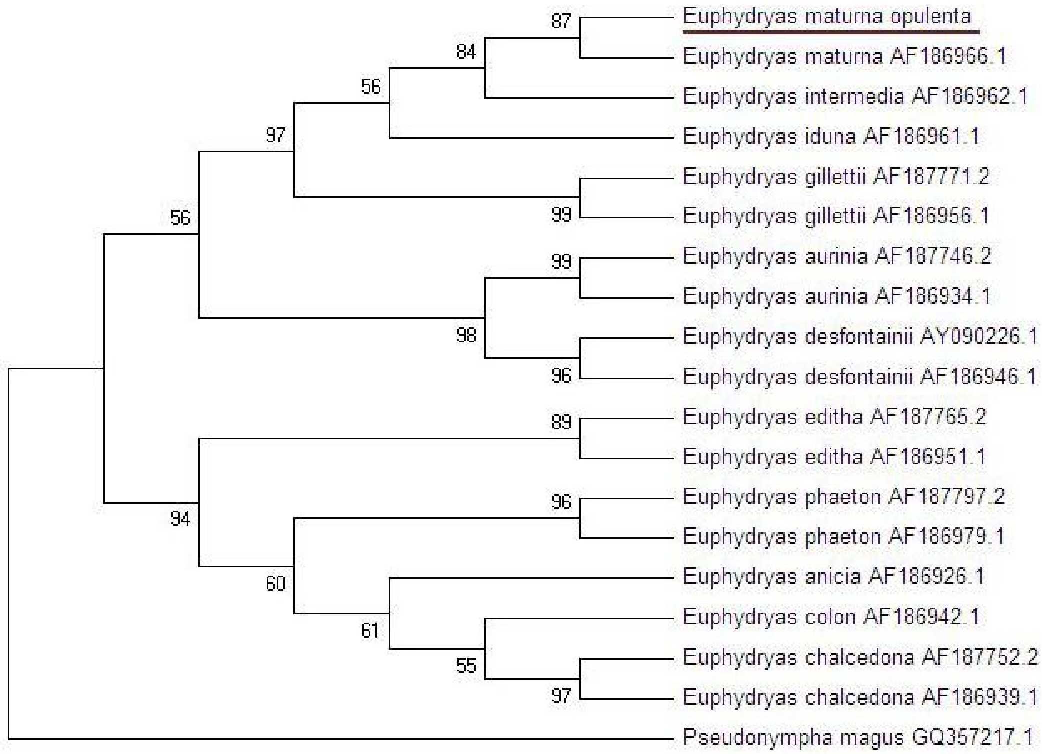

Fig. 14. - Phylogenetic tree of *Euphydryas* species, builted by using maximum parsimony on CO I subunit I, showing interrelations between *Euphydryas maturna opulenta* species (original sequence) with other representative species of genra.. The intern tree nodes indicate the bootstrap values for 1000 replicates.

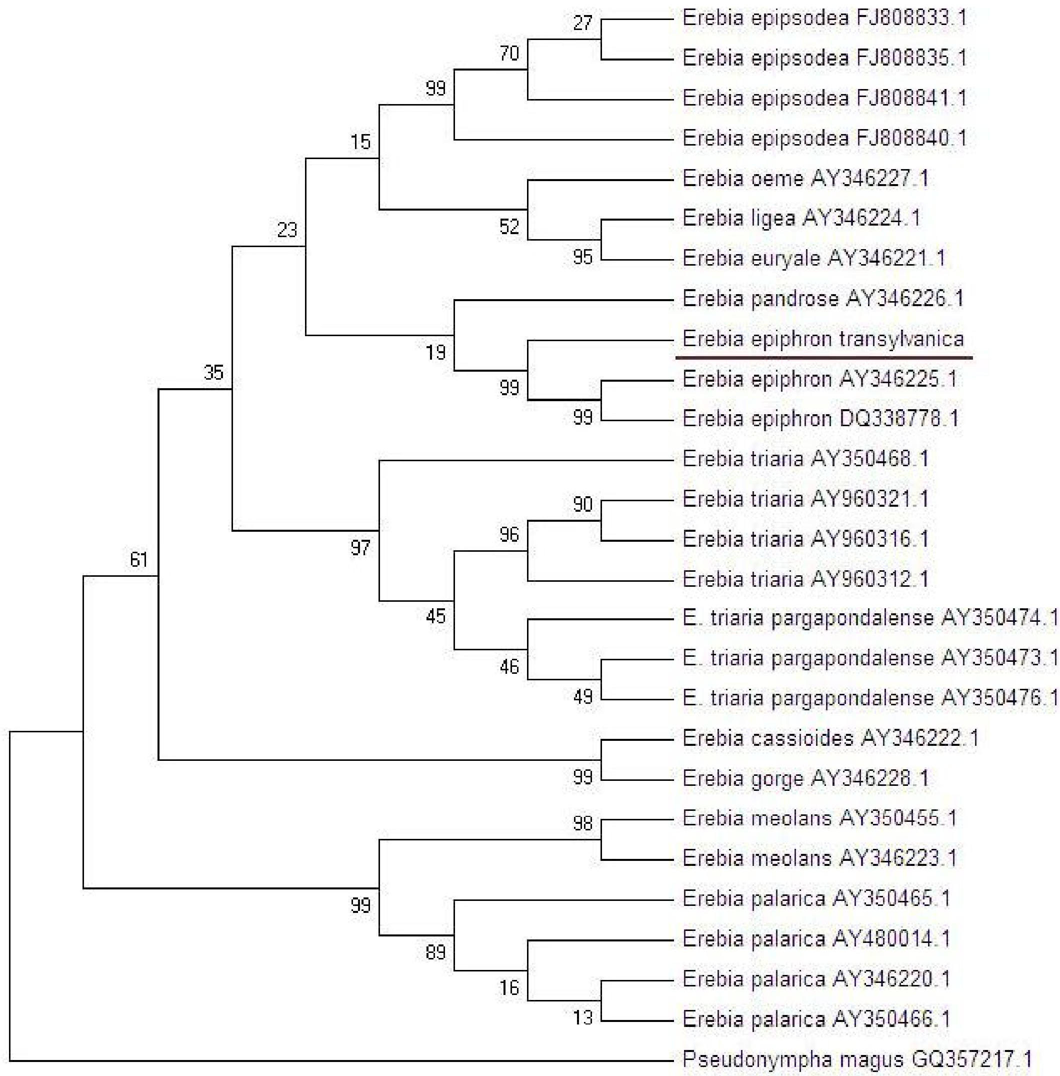

Fig. 15. – Phylogenetic tree of *Erebia* species, builted by using maximum parsimony on CO I subunit I, showing interrelations between *Erebia epiphron transylvanica* species (original sequence) with other representative species of genra.. The intern tree nodes indicate the bootstrap values for 1000 replicates.

Populations of different species diverge from the genetic point of view when there is a physical barrier (insular populations, mountain arches) or an apparent one (isolation by distance-for instance populations on different continents) in the genetic flux. No matter the nature of the barrier, the genetic diverging rate between populations is the balance resulting between the genetic drift that promotes the genetic diverging and flux which tends to homogenize the genetic variability.

-for the tree of the subfamilies Nymphalinae and Satyrinae, it perfectly shows the 2 subfamilies that are split into 2 distinct branches (fig. 16).

-the bootstrap test must also be taken into analysis. The further we go from our sequences to the out group species, the smaller the bootstrap values are, the algorithm is less sure of the common inferred ancestor.

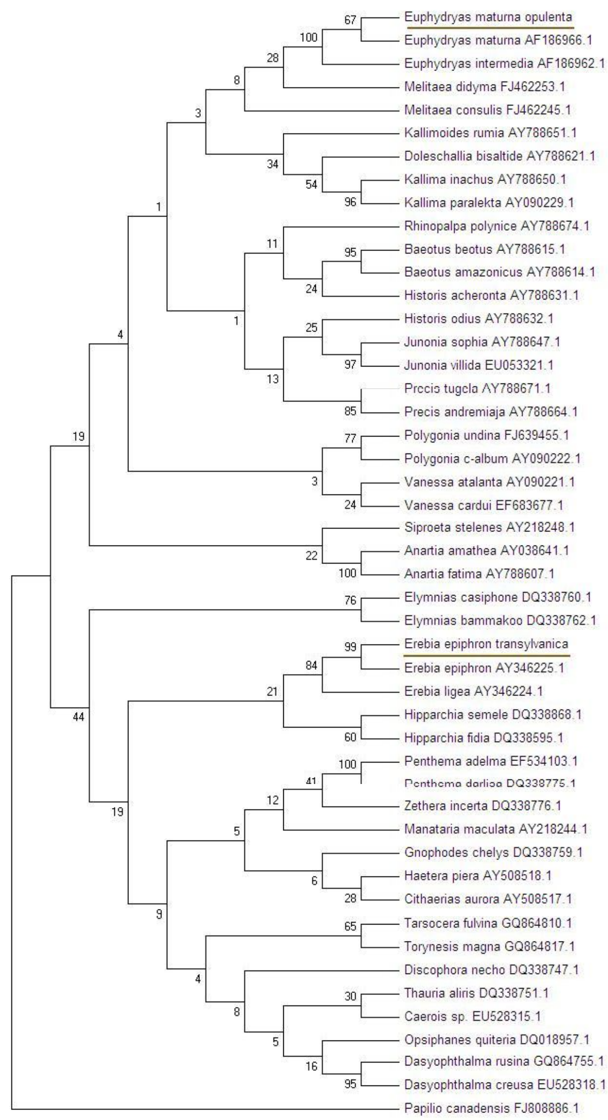

Fig. nr. 16 –*Nymphalinae and Satyrinae* subfamilies consesus tree, builted by using maximum parsimony on CO I subunit I, showing interrelations between *Erebia epiphron transylvanica* and *Euphydryas maturna opulenta* species species (original sequences) with other representative species of genra.. The intern tree nodes indicate the bootstrap values for 1000 replicates.

8. Conclusions

1. Using the scanning electron microscopy technique we succeeded showing several morphologic structures that allow the description and the exact individualization of Lepidoptera species and subspecies both at an adult and at the pre-imago stages. We don't know the role and the functionality of some of the shown structures.

2. In order to know the intrinsic mechanism of the structural colors apparition we have shown several types of wing scales having differenced nanostructure, through which iridescent colors are produced. We analyzed the shown nanostructures using EDX technique through quantitative and quality measurements. There are quality differences between the two sites of interest, that is, at the lamella ridges the important chemical elements are C and O and in very small amounts Na, Cu, Zn. The following chemical elements: Na, Cu, Zn are in a larger amount in the area between the cross ribs.

3. From the 19 populations and the 20 studied allozymes loci, we identified four different groups. The used out group (*E. sudetica*) became an in group on the link between the two western (*E. melampus*) and eastern (*E. momos*) branches of the *E. sudetica – melampus* complex. Between the western and eastern branches we identified a distinct branch of a possible new species, *E. radnaensis*.

4. We made the molecular validation of the subspecies *Erebia epiphron transylvanica (Nymphalidae), Euphydryas (Hypodryas) maturna opulenta (Nymphalidae*), and their own phyletic position.

5. We established the exact position of the following taxa: *Erebia epiphron transylvanica (Nymphalidae)* and *Euphydryas (Hypodryas) maturna opulenta (Nymphalidae)* and we have shown the phylogenetic relationships within the subfamilies *Nimphalinae* and *Satyrinae*.

9. References

Aagaard K., Hindar K., Pullin A. S., James C. H., Hammarstedt O., Balstad T., Hanssen O., (2001): Phylogenetic relationships in brown argus butterflies (Lepidoptera: Lycaenidae: Aricia) from north-western Europe. Biol. J. Linn. Soc. Lond. 75, pp. 27-37.

Ahlen J., (1999): Evaluating the incidence function model of metapopulation dynamics on the butterfly Euphydryas maturna in Sweden, Exammensarbete I entomologi – Sveriges lantbruksuniversitet, pp. 1-26.

Altschul S. F., Madden T. L.,Schsaer A. A., Zhang J., Zhang Z., Miller W., Lipman D. J., (1997):Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, Nucleic acids research 25(17), pp. 3389-3402

Arthur V. E, Rosser W. G., Schlager N., Torrado-Caputo V., (2004): Grzimek`s Animal Life Encyclopedia, Second Edition, Volume: 3, Insects, Schlager Group Inc., pp. 383.

Assman T., Buse J., Drees C., Habel J., Hardtle W., Matern A., von Oheimb Goddert, Schuldt A., Wrase D. W., (2007): From Latreille to DNA systematics – towards a modern synthesis for carabidology, Proceedings of the XIII European Carabidologists meeting, pp. 41-76.

Astrid K. B., Sally C. J., (2002): Ultraviolet reflectance patterns of male guppies enhance their attractiveness to females, The Assoc. for the stud of Anim.Beh., pp. 391-396.

Aubert J., Barascud B., Descimon H., Michel F., (1996): Systématique moléculaire des Argynnes (Lepidoptera: Nymphalidae). Comptes Rendus de l'Academie des Sciences Serie III. Sciences de la Vie 319, pp. 647-651.

Aubert J., Barascud B., Descimon H., Michel F., (1997): Ecology and genetics of interspecific hybridization in the swallowtails, Papilio hospiton Géné and P. machaon L., in Corsica (Lepidoptera: Papilionidae). Biological Journal of the Linnean Society 60, pp. 467-492.

Aubert J., Legal L., Descimon H., Michel F., (1999): Molecular phylogeny of swallowtail butterflies of the tribe Papilionini (Papilionidae, Lepidoptera). Molecular Phylogenetics and Evolution 12, pp. 156-167.

Balint Zs., Vertesy Z., Kertesz K., Biro L. P., (2004): Scanning Electron Microscopic Investigations in Butterfly wings: detecting scale micro- and nanomorphology and understanding their functions, Current Issues on Multidisciplinary microscopy research and education, Formatex, pp. 87-92.

Beltran M. S., Jiggins C. D., Bull V., McMillan O., Bermingham E., Mallet J., Linares M., (2001): Phylogenetic discordance at the species boundary: comparative gene genealogies in Heliconius butterflies. GenBank., http://www.ncbi.nlm.nih.gov (13.02.2009).

Blum M. J., Bermingham E., Dasmahapatra K., (2001): A molecular phylogeny of the neotropical butterfly genus Anartia (Lepidoptera: Nymphalidae). GenBank. http://www.ncbi.nlm.nih.gov (12.04.2010).

Boggs C. L., Watt W. B., Ehrlich P. R., (2003): Butterfly Molecular Systematics: From Species Definitions to Higher-Level Phylogenies, Evolution Taking Flight, pp. 431-458.

Brookes M. I., Graneau Y. A., King P., Rose O. C., Thomas C. D., Mallet J. L. B., (1997): Genetic analysis of founder bottlenecks in the rare British butterfly Plebejus argus. Conservation Biology 11, pp.48-661.

Brower A. V. Z., (1994a): Phylogeny of Heliconius butterflies inferred from mitochondrial DNA sequences (Lepidoptera: Nymphalidae). Molecular Phylogenetics and Evolution 3 , pp. 159-174.

Brower A. V. Z., (1994b): Rapid morphological radiation and convergence among races of the butterfly Heliconius erato inferred from patterns of mitochondrial DNA evolution. Proceedings of the National Academy of Sciences, U.S.A. 91, pp. 6491-6495.

Brower A.V.Z., DeSalle R., (1998): Patterns of mitochondrial versus nuclear DNA sequence divergence among nymphalid butterflies: the utility of wingless as a source of characters for phylogenetic inference. Insect Molecular Biology 7, pp. 73-82.

Brower A. V. Z., Egan M. G., (1997): Cladistics of Heliconius butterflies and relatives (Nymphalidae: Heliconiiti): the phylogenetic position of Eueides based on sequences from mtDNA and a nuclear gene. Proceedings of the Royal Society of London, B 264, pp. 969-977.

Brunton C. F. A., (1998): The evolution of ultraviolet patterns in European Colias butterflies (Lepidoptera, Pieridae): a phylogeny using mitochondrial DNA. Heredity 80, pp. 611-616.

Brunton C. F. A., Hurst G. D. D., (1998): Mitochondrial DNA phylogeny of brimstone butterflies (genus Gonepteryx) from the Canary Islands and Madeira. Biological Journal of the Linnean Society 63, pp. 69-79.

Caterino M. S., Reed R. D., Kuo M. M., Sperling F. A. H., (2001): A partitioned maximum likelihood analysis of swallowtail butterfly phylogeny (Lepidoptera: Papilionidae). Systematic Biology 50, pp. 106-127.

Caterino M. S., Sperling F. A. H., (1999): Papilio phylogeny based on mitochondrial cytochrome oxidase I and II genes. Molecular Phylogenetics and Evolution 11, pp. 122-

137.

Clary D. O., Wolstenholme D. R., (1985): The mitochondrial DNA molecular of Drosophila yakuba: nucleotide sequence, gene organization, and genetic code, 22(3), pp. 252-271.

Cupedo F., (1995): Die morphologische Gliederung des Erebia melampus-Komplexes, nebst Beschreibung zweier neuer Unterarten: Erebia melampus semisudetica ssp. n. und Erebia sudetica belladonnae ssp. n. (Lepidoptera, Satyridae), Nota lepid. 18(2), pp. 95-125.

Davis S. R., (2006): Description of a new lepidopteran structure, the abdominal tubercles, Journal of the Lepidopterists Society, 60 (40), pp. 194-202.

Dincă V., Goia M., (2006): Contribuţii la cunoaşterea faunei lepidopterologice a Munţilor Rodnei / Contributions to the knowledge of the Lepidoptera fauna of Rodna Mountains. Bul. inf. entom., 16/2005, pp. 125-164.

Dioszeghy L., (1912): Adatok Magyarorszag lepkefaunajahoz. Rov. Lapok, Budapest, 19, pp. 114.

Donald R. D., David L. W., (2005): Biology and systematics of the neotropical leafminer genus Eucosmophora (Lepidoptera: Gracillariidae), Tropical Lepidoptera, 13 (1-2), pp. 1-40.

Doniţă N., Paucă-Comănescu M., Popescu A., Mihăilescu S., Biriş I. A., (2005): Habitatele din Romania, Editura Tehnică Silvică, Bucureşti, , pp.29.

Dresp B., Jouventin P., Langley K., (2005): Ultraviolet reflecting photonic microstructures in the King Penguin beak, Biology letters, pp. 310-313.

Efron B., (1982): The Jackknife, the Bootstrap and Other Resampling Plans. CBMS-NSF Regional Conference Series in Applied Mathematics, Monograph 38, SIAM, Philadelphia.

Egerton R. F., (2005): Physical Principles of Electron Microscopy: An Introduction to TEM, SEM, and AEM, Springer, pp. 216.

Fanger H., (2010): Comparative morphological study of pretarsal structures of ditrysian Lepidoptera, with some phylogenetic implications, 120 (1), pp. 15-23.

Faucheux M. J., (1999): Biodiversite et unite des organs sensoriels des insectes lepidopteres, Supp. Hors Serie, Bulletin de la Societe des Sciences Naturelles de l'Ouest de la France, pp. 288.

Felsenstein J., (1993): Phylogeny Inference Package (PHYLIP). Version 3.5. University of Washington, Seattle.

Felsenstein J (1985): Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39, pp.783-791.

Freese A., Benes J., Bolz R., Cizek O., Dolek M., Geyer A., Gros P., Konvicka M., Leigl A., Stettmer C., (2006): Habitat use of the endangered butterfly Euphydryas maturna and forestry in central Europe, Animal conservation, 9, pp. 388-397.

Goldstein J., Newbury D., E., David C. J., Lyman C., Echlin P., Lifshin E., Sawyer L., (2003): Scanning Electron Microscopy and X-ray Microanalysis, Third Edition, Springer , pp.689.

Hall T. A., (1999): BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT'. Nucleic acids symposium series 41, pp. 95-98.

Hebert P. D. N., Beaton M. J., (1993): Methodologies for allozyme analysis using cellulose acetate electrophoresis: a practical handbook. in Helena Laboratories, Beaumont, Texas.

Hebert, P. D. N., A. Cywinska, S. L. Ball, and J. R. deWaard. (2003): Biological identifications through DNA barcodes. Proceedings of the Royal Society of London B Biological Sciences 270, pp.313-321.

Hedrick P. W., (2005): Genetics of populations, Third Edition, Jones and Bartlett Publisher, Inc., pp 737.

Helsdingen P. J., (2006): Background information on invertebrates of the habitats directive and the Bern convention, Part I – Crustacea, Coleoptera and Lepidoptera, Nature and Environment, no. 79, pp. 113.

Hillis D. M., Craig M., MableB. K., (1996): Molecular Systematics, Second Edition, Sinauer Associates Inc., Publishers Sunderland, pp. 6-13.

Iohara K., Yoshimura M., Tabata H., Shimizu S., (2000): Chem. Fibers Int., 50, pp. 38-39.

Ingolf P. R., Modarressie R., Bakker T.C.M., (2004): Male three-spined sticklebacks reflect in ultraviolet light, Behaviour 141, pp. 1531-1541.

Joyce D. A., Pullin A. S., (2000): Phylogeography of the Marsh Fritillary Euphydryas aurinia (Lepidoptera: Nymphalidae) in the U.K. GenBank. http://www.ncbi.nlm.nih.gov, (14.05.2010).

Jutzeler D., Rakosy L., Bros E., (1997) : Observation et elevage de *Pseudophilotes bavius* (Eversmann, 1832) des environs de Cluj; distribution de cette espece en Roumanie. Une nouvelle plante nourriciere de *Colias alfacariensis* (Ribbe, 1905), Bull. Soc. Ent. Mulhouse, vol. 53, pp. 23-30.

Konig F., (1986): Date morfologice si ecologice referitoare la Philotes bavius hungarica Dioszeghy 1913 (Lepidoptera, Lycaenidae) Lucrarile celei de a IV-a Conferinte Nationale de entomologie, Cluj-Napoca, 29-31 mai, pp. 175-182.

Kristensen N. P., (2003): Handbook of Zoology (Handbuch Der Zoologie/Handbook of Zoology, Volume IV : Arthropoda : Insecta), Lepidoptera, Moths and Butterflies, Volume2: Morphology, Physiology, and Development, Publishers Walter de Gruyter GmbH & Co. KG, pp. 427.

Kurachi M., Takaku Y., Komiya Y., Haryiama T., (2002): Naturwissenschaften, 89, pp. 295-298.

Lord Rayleigh, (1917): On the reflection of light from a regularly stratified medium, Roy. Soc. Proc., A93,, pp. 565-577.

Lushai, G., Smith, D. A. S., Allen, J. A., Goulson, D. and Maclean, N., (2002): The origin and evolution of Monarch and Queen butterflies, with special references to the African Queen, Danaus chrysippus (L.). GenBank. http://www.ncbi.nlm.nih.gov (12.10.2008).

Lythgoe J. N., Julia S., (1989): The structural basis for iridescent colour changes in dermal corneal iridophores in fish, J. exp. Biol., 141, pp. 313-325.

Makita H., Shinkawa T., Nakazawa T., (1999): GenBank. http://www.ncbi.nlm.nih.gov. (13.07.2009).

Makita H., Shinkawa T., Ohta K., Kondo A., Nakazawa T., (2000): Phylogeny of Luehdorfia Butterflies Inferred from Mitochondrial ND5 Gene Sequences. Entomological Science 3: 321-329.

Makita H., Shinkawa T., Kondo K., Nakazawa T., (2001): Tribe Graphiini mitochondrial ND5 genes. GenBank. http://www.ncbi.nlm.nih.gov (12.09.2008).

Martin J., Gilles A. Descimon H., (2000): Molecular phylogeny and evolutionary patterns of the European satyrids (Lepidoptera: Satyridae) as revealed by mitochondrial gene sequences. Mol. Phylogenet. Evol. 15 pp. 70-82.

Martin J. F., Gilles A., Descimon H., (2001): Species concepts and sibling species: the case of Leptidea sinapis and L. reali (Lepidoptera: Pieridae). In C. Boggs, W. Watt, and P. Ehrlich, eds. Ecology and evolution taking flight: butterflies as model study systems. University of Chicago Press, Chicago.

Mihut S., Dinca V., (2006): Important areas for Lepidoptera in Romania, Preinventory for a draft list of Natura 2000 sites for Lepidoptera species, Final Report.

Monteiro A., Pierce N., (2001): Phylogeny of Bicyclus (Lepidoptera: Nymphalidae) inferred from COI, COII, and EF-1a gene sequences. Molecular Phylogenetics and Evolution 18, pp. 264-281.

Morinaka S., Minaka N., Sekiguchi M., Erniwati D., Prijono S. N., Ida G. K., Miyata T., Hidaka T., (2000): Molecular phylogeny of birdwing butterflies of the tribe Troidini (Lepidoptera: Papilionidae). Biogeography 2, pp. 103-111.

Morinaka S., Maeyama T., Maekawa K., Erniwati D., Prijono S. N., Ginarsa I. K., Nakazawa T., Hidaka T., (1999): Molecular phylogeny of birdwing butterflies based on the representatives in most genera of the tribe Troidini (Lepidoptera: Papilionidae). Entomological Science 2, pp. 347-358.

Moss M. O., Gibbs G., (1995): The wing scales of the peacock butterfly, Quekett Journal of Microscopy, 37, pp.392-395.

Moss M. O., Gibbs G., (2000): On the nature of the hairs of the wings of the Trichoptera, Quekett Journal of Microscopy, 38, pp. 511-517.

Moss M. O., Gibbs G., (2001): The wing scales of the swallowtail butterfly, Papilio palinurus, Quekett Journal of Microscopy, 39, pp. 133-138.

Myers P., Espinosa R., Parr C. S., Jones T., Hammond G. S., Dewey T. A., 2008, http://animaldiversity.ummz.umich.edu/site/accounts/classification/butterfly/ (08.02.2010).

Newton I., Opticks, (1952): Reprinted by Dover Publications, Inc., New-York, pp. 252.

Nice C. C., Shapiro A. M., (1999a): Molecular and morphological divergence in the butterfly genus Lycaeides (Lepidoptera: Lycaenidae) in North America: evidence of recent speciation. Journal of Evolutionary Biology 12, pp. 936-950.

Nice C. C., and Shapiro A. M., (1999b): Patterns of morphological, biochemical and molecular evolution in the Oeneis chryxus complex (Lepidoptera: Satyridae): a test of historical biogeographic hypotheses. GenBank.

Nice C. C., Shapiro, A. M., (2001): Patterns of Morphological, Biochemical, and Molecular Evolution in the Oeneis chryxus Complex (Lepidoptera: Satyridae): a test of historical biogeographical hypotheses. Mol. Phylogenet. Evol. 20, pp. 111-123.

Niculescu E.V., Koenig F., (1970) : Lepidoptera, parten generala, Fauna Republicii Socialiste Romania, Insecta: Vol. 11, Fasc. 10, 303 pg.

Noel G. R., Henk C. M., (2004): News on eggs and first instar larvae of the species of Speyeria (Nymphalidae), News of the Lepidopterists Society, Vol. 46, (2), pp. 53-58.

Nylin S., Nyblom K., Ronquist F., Janz N., Belicek J., Kallersjo M., (2001): Phylogeny of Polygonia, Nymphalis and related butterflies (Lepidoptera: Nymphalidae): a total-evidence analysis. Zool. J. Linn. Soc. 132, pp. 441-468.

Otakar K., (2002): The Distribution Atlas of European Butterflies, nr.20., Apollo Books.

Page R. D. M., Holmes, E. (1998): Molecular evolution: a phylogenetic approach. Blackwell Science, Oxford.

Pollock D. D., Watt W. B., Rashbrook V. K., Iyengar E. V., (1998): Molecular phylogeny for Colias butterflies and their relatives (Lepidoptera: Pieridae). Annals of the Entomological Society of America 91, pp. 524-531.

Prum R. O, Torres R. H., (2003): A fourier tool for the analysis of coherent light scattering by bio-optical nanostructures, Integr. Comp., Biol., 43, pp. 591-602.

Prum R. O, Quinn T., Torres R. H, (2006): Anatomically diverse butterfly scales all produce structural colours by coherent scattering, The J. of Exp. Biol. 209, pp. 748-765.

Rakosy L., (1997): Die endemischen Lepidopteren Rumaniens, Entomologica romanica, Vol. 2., pp. 59 – 81.

Rakosy L., (2002): Lista rosie pentru fluturii diurni din Romania – Bul. Inf. Soc. Lepid. Rom. 13 (1-4), pp. 9-26.

Rakosy L., Goia M., Kovacs Z., (2003): Catalogul Lepidopterelor Romaniei /Verzeichnis der Schmetterlinge Rumaniens, S.L.R., pp. 5-7.

Rakosy L., Goia M., (2007): Catalogul Lepidopterelor Romaniei /Verzeichnis der Schmetterlinge Rumaniens, S.L.R.

Rakosy L., Goia M., (2006): Addenda und Corrigenda zu dm Verzeichnis der Schmetterlinge Rumäniens / Addenda et corrigenda la Catalogul Lepidopterelor României, Entomol.rom.,11, pp. 69-79.

Rakosy L., Pecsenye K., Mihali C., Toth A., Varga Z., (2010): Taxonomic review of Euphydryas (Hypodryas) maturna (Linnaeus, 1758) (Lepidoptera, Nymphalidae) with description of a new subspecies from Dobrogea (Romania) and notes on conservation biology, Acta Zoologica Academiae Scientiarum Hungaricae 56 (2), 25-29 pp.

Rakosy L., (2005): U.E. si legislatia pentru protectia lepidopterelor din Romania, Bul. Inf. Entomol., 16, pp.89-96.

Rand D. B., Heath A., Suderman T., Pierce, N. E., (2000): Phylogeny and Life History Evolution of the Genus Chrysoritis within the Aphnaeini (Lepidoptera: Lycaenidae), Inferred from mitochondrial cytochrome oxidase I sequences. Mol. Phylogenet. Evol. 17, pp. 85-96.

Razmerita I., (1971): Rezervatia botanica de la Suatu, Ocrot. Nat., Bucuresti , 5 (2), pp. 129-138.

Rennwald E., Pro Natura, (1997): Schmetterlinge und ihre Lebensräume. Arten Gefährdung-Schutz Bd.2. Lepidopterologen-Arbeitsgruppe, Schweizerischer Bund für Naturschutz, Egg.

Richardson B. J., Baverstock P. R., Adams M., (1986): Allozyme electro- phoresis. A handbook for animal systematics and population studies, Academic Press, San Diego, pp.410.

Saitou N, Nei M., (1987): The neighbor-joining method: a new method for reconstructing phylogenetic trees, Mol Biol Evol. Jul;4(4), pp.406-25.

SBN, (1987): Tagfalter und ihre Lebensraume, Arten – Gefahrdung-Schutz. – Basel, XI, p. 515.

Schmitt T., Habel, J. C., Zimmermann, M., Müller, P., (2006): Genetic differentiation of the Marbled White butterfly, *Melanargia galathea*, accounts for glacial distribution patterns and postglacial range expansion in southeastern Europe. — *Molecular Ecology* 15, pp.1889-1901.

Schmitt T., Rákosy L., Abadjiev S., Müller P., (2007): Multiple differentiation centres of a non-Mediterranean butterfly species in south-eastern Europe. — *Journal of Biogeography* 34: pp. 939-950.

Schneider S., Excoffier L., Laval, Arlequin G., (2000):Arlequin, (version 3.0): An integrated software package for population genetics data analysis, Computational and Molecular Population Genetics Lab, Zoological Institute, University of Berne, Baltzerstrasse 6, 3012 Berne, Switzerland.

Scott J. A., (1986): The Butterflies of north America, A natural history and field guide, Stanford University Press, pp. 15.

Shuichi K., Shinya Y., (2005): Structural colors in nature: the role of regularity and irregularity in the structure, ChemPhysChem, 6, pp. 1442-1459.

Siegismund H. R., (1993): G-STAT, Version 3, Genetical Statistical Programs for the Analysis of Population Data. The Arboretum, Royal Veterinary and Agricultural University, Horsholm, Denmark.

Simon C., Frati F., Beckenbach A., Crespi B., Liu H., Flook P., (1994): Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers, Annals of the Entomological Society of America 87(6), pp. 651-701.

Sutherland R. M., Axton J. M., (2000): PCR-RFLP and sequence identification of insect species in bird diet. GenBank., http://www.ncbi.nlm.nih.gov (05.07.2009).

Tada H., Seth E., Mann I., Miaoulis N., Peter Y. W., (1999): Effects of a butterfly scale microstructure on the iridescent color observed at different angles, Applied Optics, Vol.5, No.4, pp. 1579-1584.

Tamura K., Dudley J., Nei M., Kumar S.,(2007): MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0, Molecular biology and evolution 24(8), pp.1596-1599.

Taylor M. F. J., McKechnie S. W., Pierce N., Kreitman M., (1993): The lepidopteran mitochondrial control region: structure and evolution. Molecular Biology and Evolution 10, pp. 1259-1272.

Tolman T., Lewington R., (1997) : Guide des papillons d'Europe et d'Afrique du nord, Delachaux et Niestle, Paris, 320 pp.

Trevor B.,Graham R., (2003): Molecular Ecology, Oxford University Press, pp.123-142.

van Swaay Chris, Cuttelod A., Collins S., Maes D., Munguira M. L., Šašić M., Settele J., Verovnik R., Verstrael T., Warren M., Wiemers M., Wynhoff I., (1999: European Red List of Butterflies, Published by IUCN, pp. 191-192.

Vane-Wright R. I., Raheem D. C., Cieslak A., Vogler A. P., (1999): Evolution of the mimetic African swallowtail butterfly Papilio dardanus: molecular data confirm relationships with P. phorcas and P. constantinus. Biological Journal of the Linnean Society 66, pp. 215-229.

Vertesy Z., Balint Zs., Kertesz K., Mark I. G., Vigneron J. P., Lousse V., Biro L. P., (2003): Nanostructures in Butterfly scales: Photonic crystals generating blue colors.

Vukusic P., Sambles J. R., (2003): Photonic structures in biology, Nature, Vol.424, pp. 852-855.

Vukusic P., Sambles J. R., Lawrence C. R., Wootton R. J., (2002): Limited-view iridescence in the butterfly Ancyluris meliboeus, Proc. R. Soc. Lond. B, 269, pp. 7-14.

Wagner D. L., Hossler W. E., Hossler F. E., (2003): The larva and pupa of Lytrosis permagnaria pack. (Geometridae), Journal of the lepidopterists society, 57(2), pp. 107-112.

Wahlberg N., (2001): On the status of the scarce fritillary Euphydryas maturna (Lepidoptera: Nymphalidae) in Finland, Entomol. Fennica, pp. 244-250.

Wahlberg N., Zimmermann M., (2000): Pattern of phylogenetic relationships among members of the tribe Melitaeini (Lepidoptera: Nymphalidae) inferred from mtDNA sequences. Cladistics 16, pp. 347-363.

Walter B., (1895): Die Oberflachen-oder Schillerfarben, Braunschweig.

Warren B., Somerville C., (1936): Monograph of the genus Erebia. British Museum of Natural History, London.

Watanabe K., Hoshino T., Kanda K., Haruyama Y., Matsui J., (2005): Jpn. J. Apl. Phys. 44, pp.L48-L50.

Weidlich M., (2005): Ein neuer Sacktrager aus den Muntii Apuseni in Siebenburgen (Rumanien) und ein Beitrag zur Schmetterlingsfauna des Natur-schutzgebietes "Thorenburger Schlucht" (Lepidoptera: Psychidae), Entomologische Zeitschrift, 115(6), pp. 261-266.

Weller S. J., Pashley D. P., Martin J. A., Constable J. L., (1994): Phylogeny of noctuoid moths and the utility of combining independent nuclear and mitochondrial genes. Systematic Biology 43, pp. 194-211.

Weller S. J., Pashley D. P., (1995): In search of butterfly origins. Molecular Phylogenetics and Evolution 4, pp. 235-246.

Weller S. J., Pashley D. P., Martin J. A., (1996): Reassessment of butterfly family relationships using independent genes and morphology.
Annals of the Entomological Society of America 89, pp. 184-192.

Wickham S., Large M. C. J., Poladian L., Jermiin L. S., (2006): Exaggeration and suppression of iridescence: the evolution of two-dimensional butterfly structural colour, J. R. Soc., 3, pp. 99-109.

Wink M., von Nickisch-Rosenegk E., (1997): Sequence data of mitochondrial 16s rDNA of Arctiidae and Nymphalidae: evidence for a convergent evolution of pyrrolizidine alkaloid and cardiac glycoside sequestration. Journal of Chemical Ecology 23, 1549-1568

Wink M., Legal L., von Nickisch-Rosenegk E., (1998): Response by Micheal Wink, Eva von Nickisch-Rosenegk, and Luc Legal. Journal of Chemical Ecology 24, 1285-1291.

Yagi T., Katoh T., Chichvarkhin A., Shinkawa T., Omoto K., (2001): Molecular phylogeny of butterflies Parnassius glacialis and P. stubbendorfii. Genes and Genetic systems 76, pp. 229-234.

Yagi T., Sasaki G., Takebe H., (1999): Phylogeny of Japanese papilionid butterflies inferred from nucleotide sequences of mitochondrial ND5 gene. Journal of Molecular Evolution 48, pp. 42-48.

Yack J. E., Timbers T. A., Conner W. E., Annette Aiello, Frank C. Schroeder, (2004): Journal of the Lepidopterists Society, 58(3), pp.173-177.

Zhang, D. X., Hewitt G. M., (1997a):Assessment of the universality and utility of a set of conserved mitochondrial COI primers in insects'. Insect molecular biology 6(2), pp. 143-150.

Zhang, D.-X. and G. M. Hewitt: (1997b): Insect mitochondrial control region: a review of its structure, evolution and usefulness in evolutionary studies, Biochemical systematics and ecology 25(2), pp.99-120.

Zi J., Xindi Y., Li Y., Xinhua H., Chun X., Xingjun W., Xiaohan L., Rongtang F., (2003): Coloration strategies in peacock feathers, PNAS, vol.100, no.22, pp. 12576-12578.

Zimmermann M., Aubert J., Descimon H., (1999) : Systématique moléculaire des Mélitées (Lepidoptera: Nymphalidae). Comptes Rendus de l'Academie des Sciences Serie III. Sciences de la Vie 322, pp. 429-439.

Zimmermann M., Wahlberg N., Descimon H., (2000) : Phylogeny of Euphydryas Checkerspot Butterflies (Lepidoptera: Nymphalidae) Based on Mitochondrial DNA sequence data. Ann. Entomol. Soc. Am. 93, pp. 347-355.

Zuckerkandle, L., Pauling, L. (1965): Molecules as documents of evolutionary history. Journal of Theoretical Biology, 8, pp.357-366.

- http://taxonomicon.taxonomy.nl (18.03.2010).