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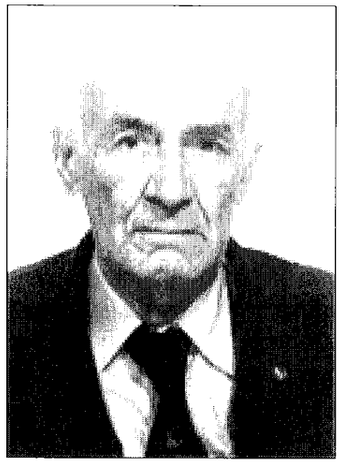

PETRU MIHAI BĂNĂRESCU

Zoologist-ichthyologist, biogeographer
Member of the Romanian Academy
Honorary Editor – *Romanian Journal of Biology-Zoology*

(15 September 1921, Craiova – 12 May 2009, Bucharest)

One of the most enthusiastic naturalists of our time and, at the same time, one of the most ardent researchers of waterworld, especially of freshwater fishes, **Dr. Petru Mihai Bănărescu** passed into eternity at the respectable age of 88.

We all mourn for his disappearance, but our feelings of grief are intertwined with the great pride we take in his achievements. He was a close and dear friend of rare modesty, a true man always striving for perfection and, I think, he was the best naturalist of the present generation, enthusiastic over the marvellous surrounding nature which he admired and loved and from which he acquired vast knowledge through profound study and research. Petru Mihai Bănărescu asserted himself not only in the national scientific community, but also in the international one, his perennial work contributing significantly to the Romanian and world science treasure. His papers deal with a wide range of researches into zoological taxonomy, systematics and the biology of freshwater and marine fishes in Romania, provide detailed studies of the systematics of Cyprinidae and Cobitidae families in Europe and Asia, tackle theoretical approaches to some biogeographical problems – especially the zoogeography of freshwater fauna and to problems concerning the principles of taxonomy, speciation and, equally important, of nature conservation.

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Petru Mihai Bănărescu was born in Craiova on September 15, of intellectual parentage. Since his childhood he showed special interest in plant and animal life, observed and took care of fishes and invertebrates in aquariums and grew autochthonous plants in his parents' garden; at the same time he procured and read biology books.

He was educated in Timişoara where he attended primary school, then "C. Diaconovici – Loga" secondary school and the Faculty of Sciences – Department of Natural Sciences of "King Ferdinand" University from Cluj, which moved to Timişoara for a while, during the war years (1940-1944).

As a student in the first university year, he started working at the Institute (Department) of Zoology of the faculty, vacation months included. Appreciated by Professor Vasile Radu, he was appointed junior assistant to this department on 1st April 1943, while still a student. In the summer of 1943 he worked for a month at the famous marine zoological station at Agigea, where he did find an elevated academic atmosphere and also modern literature (in those years) on ichthyology; thanks to the favourable conditions, he identified two new autochthonous fish species in the Bega and the Timiş rivers. In the winter of 1943-1944 he wrote his first scientific paper "*Les Poissons des environs de Timişoara*", published in 1946.

Petru Mihai Bănărescu graduated from university in 1944 when he defended his thesis on Romania's fish zoogeography. Highly appreciated, he worked as a junior assistant at the Zoology Department of the Faculty of Natural Sciences Cluj-Timişoara, then as a professor assistant at the Zoology Department of the University in Cluj (1946-1950) and a deputy lecturer of Biogeography (1948-1950).

The years spent in Cluj (1946-1950) were extremely fertile, he shaped his career as a researcher and prepared his Doctor of Science degree; under Prof. Vasile Radu's competent guidance, P.M. Bănărescu became a doctor in natural sciences in Cluj after defending his thesis on the compared anatomy of the encephalon at teleosts fishes (1949). Thus, he rose step by step, in the university hierarchy.

The intellectual activity and scientific successes of the young university graduated specialist were to trigger the envy of some people in those troubled times, when after World War II, Europe was divided by the Iron Curtain and Romania's former social and political order was completely replaced by the communist system; Petru Bănărescu was to suffer the consequences of evil and ignorance. Sympathizing with the historical parties, displaying an anti-communist attitude, Dr. Bănărescu was arrested in Cluj by "Securitate" (the Romanian Communist State Security Department) and sent, without any trial and conviction ("administrative punishment"), to the Danube – Black Sea Canal, where he worked for a year.

After he was set free, he was no longer employed at the university and, for almost two years, he could not find a job; meanwhile he wrote and managed to

publish several papers. He never complained about that bleak period in his life; moreover, on rare occasions, when he was asked about the Canal "faculty" he used to tell, with sincere, well-tempered optimism, about the wide range of fossils that the convicts brought to light by digging for the canal bed or about the rich diversity of plants and animals in that place called "the Romanian Gulag" by "the great tyrant" Stalin himself.

The "Canal" episode could not turn Dr. Bănărescu from the path of study, nor could the episode in June 1990, when Dr. Bănărescu was maltreated by the furious "miners" called up to defeat democracy; the senior scientist could not be annihilated and, once more, he overlooked this event, without hate or enmity, absorbed, as usual, in his study, reading, writing, and correcting his manuscripts.

In April 1953, he became a researcher at the Fishery Research Institute, where he worked for four years; he studied the fishes in the former ponds of the flood plain of the Danube and the Razelm Complex. Then he worked with the "Fauna" staff of the Academy. He was awarded the scientific title of Doctor Docent – 1962, and then he obtained the degrees of rank II senior researcher and chief of the scientific staff – 1970, then rank I senior researcher – 1990.

Among the most remarkable achievements of the illustrious researcher **Petru Mihai Bănărescu**, we can mention now, at random:

- Description of new taxa for the Romanian ichthyologic fauna;
- Publication of monograph papers in the thesaurus collection of "***The Fauna of Romania***", "***Pisces***", 1964 (a complete monograph on freshwater and marine ichthyofauna in Romania) and "***Cyclostoma Chondrichthyes***", 1969;
- Comparative studies of all species belonging to certain genera and subfamilies of Cyprinidae and Cobitidae families, based on his personal belief that the phyletic links between the freshwater fishes of the Romanian fauna cannot be understood without comparison to the related species in Romania and the world; he published over 60 papers on the topic, describing a new subfamily, 10 genera, 2 subgenera, 38 species and 26 new species (description of five Cyprinidae genera; revalidation of two Cyprinidae genera and four Cobitidae genera, as well as revision of a subfamily, etc.);
- Preparing the chapters about fauna in "***The geographic monograph of Romania***", "***The Geography Treatise of Romania***", vol I, 1983, then the chapters concerning aquatic fauna in "***Biogeography of Romania***", 1969, or the chapter about zoogeography in "***The Fresh Water Fishes of Europe***", vol. I, 1989;
- Training numerous disciples (over 30 students in biology working to obtain the Doctor's degree) and creating a favorable environment for team work (it is worth mentioning his excellent collaboration and the lifetime friendship with the talented ichthyologist Teodor Nalbant, or his collaboration with researchers in Pakistan, India, Italy, Turkey, Japan, etc. who provided with study materials).

Petru Mihai Bănărescu leaves us an impressive heritage, a monumental original scientific work consisting of nearly 350 papers, most of them published

(about one third in Romania and two thirds in English, French, German and Russian), out of which over 320 articles published in periodicals, collections or chapters of some volumes and 12 books. Out of the 12 published volumes we mention: two volumes from the series: "***Romania's Fauna***": "***Pisces***", 1964 and "***Cyclostoma/Chondrichthyes***", 1969, then "***Principles and problems of Zoogeography***", 1970 (translated into English), "***Biogeography***", 1973 (also translated into German), "***Principles and Methods of Systematic Zoology***", 1973, the monograph of Gobioninae subfamily, 1973, in the series "*Das Tierreich*" (in English) and 3 volumes, 1990, 1992, 1995 from "***Zoogeography of Fresh Waters***", at Aula-Verlag, Wiesbaden. "***Zoogeography of Fresh Waters***", written between 1966-1988, is the first zoogeography in world literature dealing with fresh waters, being the greatest in the biogeography field.

For over half of a century, **Dr. Petru Mihai Bănărescu** was held in great respect and high appreciation by the people who knew him; he was awarded honors and distinctions for his merits, among which:

- "Emil Racoviță" Prize of the Romanian Academy – 1964;
- Foreign honorary member of the American Society of Ichthyology and Herpetology – 1975;
- Foreign honorary member of the European Society of Ichthyology – 1988;
- Full member of the Romanian Academy – 2000.

Like an inextinguishable flame, **Dr. P. Bănărescu** was alight his whole life, keeping up the sacred fire of knowledge, everlasting fire whose mysteries he found out and offered.

Like an inextinguishable flame, **Dr. P. Bănărescu** left behind a trail of light to the infinite future to be followed in the times to come.

Like eternal ephemerality, Dr. P. Bănărescu left us, entering the absolute cosmic cycle of transformations and evolutions.

To honor him properly, let us follow the example of his life and may our mind take us in his footsteps; we will open his books for a long time and will sip from the truth of knowledge, proud of the flame we inherited from the illustrious scientist.

Now, let us have a moment's silence in the memory of **P. Bănărescu** and let our eyes shed a tear while we whisper the ritual words at the great departure: "Rest in peace"/Requiescat in Pace".

Professor M.-T. GOMOIU
Corresponding Member of the Romanian Academy
Editor-in-chief of the *Romanian Journal of Biology – Zoology*

DIVERSITY AND SPECIES DISTRIBUTION OF ORIBATID MITES (ACARI-ORIBATIDA) IN A GEOGRAPHICAL AND ECOLOGICAL UNIQUE AREA OF SOUTHERN SWEDEN

VIORICA HONCIUC*, LARS LUNDQVIST**

The researches were realized in 8 sites of Kullaberg Reservation, from north to south in different types of habitats of vegetation and microhabitats. A number of 115 species and 1.335 individuals were identified. 3 genera and 27 species were recorded for the first time in Sweden. 88 species were indicated before in different ecosystems in Sweden and on the continent, and by these 49 species were specified only in the central and south of Sweden. 22 species were found with large distribution in this reservation especially in the litter of forests and in moss. A large number of oribatid species identified in these habitats have the Palearctic distribution, with European and North-American specification. As a saprophagous mite, the panphytophages present the greatest share, followed by the makrophytophages and mikrophytophages. By its unique geographical position and high diversity of vegetation Kullaberg Reservation is an ideal terrestrial complex of ecosystems for oribatid mites. The present high taxonomic diversity of the area is probably a consequence of the area historically being part of a wider diversified forest area.

Key words: Acari, Oribatida, Kullaberg Reservation Sweden, new records.

INTRODUCTION

The Oribatida represents the most of the diverse Acari which inhabit a different type of habitats from the terrestrial ecosystems. The preferred habitats where they have a high number of species with a large number of individuals, are the forests ecosystem. One of the most favourable and interesting complexes of terrestrial ecosystem in Sweden is the Kullaberg Reservation. The geographical position in the extreme south west of Sweden, the mix of different types of vegetation, and the specific conditions which exist in this reservation made possible to be found here inhabiting most of the oribatid species, which are mentioned in different terrestrial ecosystems on the continent.

MATERIAL AND METHODS

The material is provided from the collection. The species were collected in different localities and type of habitats of the reservation from south to north, in

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1990-1991 (Table 1). The area was planted with *Pinus* sp., in the last century. Other tree species have since either been planted or spontaneously migrated into the area. Typical species are *Ulmus glabra, Fraxinus excelsior, Tilia cordata, Fagus sylvatica, Quercus* sp. Other species are *Sorbus aucuparia, Betula alba*, *Sambucus nigra*, and *Lonicera periclymenum* a widespread liana in the area. There are also typical stands of *Prunus spinosa*. Different dimensions of the samples were collected in polythene bags, and extracted in Berlese-Tullgren funnels. Mites were stored in Oudeman's fluid, sorted, fixed in lactic acid with distilled water and glycerine for determination. Every type of species was mounted in the Hoyer' medium on the slide (Krantz, 1978). The arrangement of species in systematical order followed the references: Balogh (1972), Gyliarov & Krivolutskii (1975), Marshall *et al*. (1987), Subias & Balogh (1989), Niedbała (1992).

The materials and examined specimens are deposited at the Zoological Museum of Lund University, and at the Institute of Biology in Bucharest.

The sites with the habitats and number of species and individuals are arranged in Table 1. The identified species are included in the list. In the list there were used:

The abbreviations for sites, habitats and species as follows:	
* = new record for genus;	letters in branches = habitats;
** = new record for species;	ordinary number = localities;
The abbreviations for biogeographical and ecological characteristics of the species were used too:	
A. = Alaska;	moss = live in moss;
arbo. = live in trees;	myrmec. = myrmecophyl;
arkt. = Arctic;	N.-Am. = North-American;
As. = Asian;	nearkt. = Nearctic;
Austr. = Australian;	necro. = necrophagous;
C-Am. = Central-American;	neotrop. = Neotropical;
Cauc. = Caucasian;	nidi. = nidicol;
Copro. = coprophagous;	ocean. = oceanic;
cosmop. = cosmopolitan;	orient. = oriental;
C-S-E-W-N-Euro = European;	pal. = Palaearctic;
eurosibir. = eurosiberian;	pan. = panphytophagous;
euryök. = eurioic;	Sc. = Scandinavian;
helio. = heliophile;	sibir. = Siberian;
hol. = Holarctic;	silv. = live in forests;
hygro. = hygrophilous;	subarkt. = underarctical;
macro. = makrophytophagous;	trop. = tropical;
magreb. = magrebien;	xeno. = xenophile;
mesohygro. = mesohygrophyle;	xerotherm. = xerothermophyle.
micro. = mikrophytophagous.	

RESULTS AND DISCUSSION

A total of 115 species with 1335 individuals (Table 1) were recorded from 8 analysed sites characterized by the 25 habitats from reservation. From the total identified species, 88 were mentioned in different ecosystems of Sweden, and from these species 49 were specified in the central and south of Sweden and in the Scandinavian area too (Dalenius, 1950; Lundqvist, 1987).

3 genera (*Liodes*, *Trichoribatulla*, *Astegistes*) and 27 species were recorded for the first time in Sweden. Most of the new recorded species were found in Denmark (Gjelstrup, 1978), Iceland (Gjelstrup and Solhøy, 1994), Finland (Niemi *et al*., 1997), in the north of Germany (Weigmann, 1971), and most of them in the north, central and south of Europe, in Austria (Schatz, 1983), Romania (Honciuc, 1993), and in the south of Siberia and in far east areas (Karppinen & Krivolutsky, 1982, 1983).

Table 1

Dates of collection from the sites and habitats, of Oribatida mites with their number of species and individuals from Kullaberg Reservation

Locality	Date	Habitat	Code of habitat	No. of sp.	No. of ind.
1. Ablahamn	1990				
	Aug-23	- mixed litter, close to sea	a	11	17
		- litter and moss under *Ulmus* sp., *Fraxinus* sp., *Alnus* sp.	b	10	21
	Oct-26	- moss	c	14	35
		- litter, *Erica tetralix*	d	22	55
2. Hjorthagen, moss	1990				
	Sept-13	- litter, *Fagus sylvatica*, wet	e	7	8
		- litter, *Carex* sp., wet	f	9	26
3. Vardshuset, p-plaster	1990				
	Oct-26	- litter, *Betulla* sp.	g	26	88
4. Ransvik	1990				
	Apr-28	- rotten log, *Ulmus glabra*	h	13	39
		- litter, *Quercus* sp.	i	16	31
	Aug-23	- litter, *Ulmus glabra*	j	11	19
	Sept-28	- litter, *Ulmus glabra*	j	11	27
		- litter inside log of *Fagus sylvatica*	k	27	38
		- litter, *Quercus* sp.	i	72	105
	1991				
	Apr-26	- litter, *Quercus* sp., wet mulm from rotten log	l	26	67

	Sept-13	- litter, *Ulmus glabra*	j	11	28
		- litter, *Quercus* sp.	i	16	31
5. Akersberg	1990				
	Sept-13	- mixed litter, rock	m	16	31
		- litter crack in rock, *ca* 4m.a.s.	n	6	11
		- mixed litter, close to sea	o	16	47
		- litter, meadow with *Erica tetralix, Sedum grass*	p	18	44
6. Lahibiagrottan	1990				
	Sept-13	- litter, *Pinus* sp.	r	14	27
	Oct-26	- litter, *Quercus* sp., *Lonicera* sp., *Rubus* sp	s	20	50
	1991				
	Apr-26	- litter *Sambucus nigra*	ss	19	30
		- litter *Juniperus communis*& *Prunus spinosa*	t	14	36
		- litter, *Quercus* sp.	i	21	43
	May-15	- litter *Prunus spinosa*	r	15	30
	Sept-13	- litter, mixed forest with *Quercus* sp.	u	21	31
7. Fyren, parkeringplats	1990				
	Aug-23	- rotten log of *Pinus* sp.	v	28	79
	Sept-13	- litter, *Quercus* sp., *Betula* sp., *Sorbus aucuparia*	x	35	70
	Oct-26	- litter, *Prunus spinosa*	r	21	58
	1991				
	Apr-26	- tree stump of *Quercus* sp.	y	11	21
	May-15	- litter, *Ouercus* sp.	i	21	37
8. Josefinelust	1990				
	Sept-28	- lichens on rock	z	11	15

The distribution and diversity of the oribatid mite species from the sites of reservation depend on the type of sites and the habitats diversity. As is observed from the table the most favourable sites for the diversity of species with a large number of individuals were in litter of forestry habitats formed by species of *Querqus* sp., *Ulmus glabra*, *Fagus sylvatica*, *Betula alba*, *Sorbus aucuparia*. From the total number of species, the greatest diversity with the largest number of individuals was found in the following sites: Ransvik (82 species and 152 individuals), Lahibiagrottan (49 species and 108 individuals), Fyren (51 species and 78 individuals), Åkersberg (45 species and 59 individuals) and Ablahamn (39 species and 53 individuals). In habitats formed by litter of shrubs such as *Erica tetralix*, *Sambucus nigra*, *Juniperus communis* and *Prunus spinosa* the species had a low diversity. In the other sites of reservation which are characterized by a less litter layer, the species diversity was equal to the number of individuals, and this

situation is in: Hjorthagen (14 species and individuals), Värdshuset (23 species and individuals) and Josefinelust (13 species and individuals). In these sites and even in whole reservation the following 22 species: *Tectocepheus velatus*, *Euphthiracarus cribrarius*, *Plathynothrus peltifer*, *Nanhermannia nannus*, *Spatiodamaeus verticilipes*, *Belba compta*, *Cepheus cepheiphormis*, *Adoristes ovatus*, *Xenillus tegeocranus*, *Liacarus coracinus*, *Quadroppia quadricarinata*, *Lauroppia neerlandica*, *Oribatulla tibialis*, *Scheloribates laevigatus*, *Chamobates spinosus*, *Euzetes globulus*, *Carabodes labirinthicus*, *Carabodes minusculus*, *Oribatella calcarata*, *Parachipteria willmanni*, *Phthiracarus anonnymus* and *Galumna lanceata* were found with a large distribution and a high number of individuals, especially in the litter of forests and in moss layers. Some of new recorded species had distribution in different habitats of reservation too, not only in the litter of trees where most of them were found. From this category the following species were found in the litter of meadow: *Euphthiracarus monodactylus*, *Ameronothrus schneideri*, *Eupelops subuliger*, *Protoribates longior*, *Ceratozetes fusiger*, in moss *Achipteria nitens* and *Pilogalumna alifera* in lichen on rock. Majority of the oribatid species identified in the reservation habitats have the Palearctic distribution, with European and North-American specification. As a saprophagous mite, the panphytophagous species present the greatest share (51 species); followed by the makrophytophagous (18 species) and mikrophytophagous (10 species). We supposed the rest of 36 species could be panphytophagous too. From the other points of view, such as ecological, trophical and behavioral characteristics, the following categories of species were identified: 10 arboricolous, 9 myrmecolous and 2 nidicolous as are presented in the list.

List of the oribatid mites species from the Kullaberg Reservation

1. *Hypochthonius rufulus* C.L.Koch, 1836, 1b, 3c, 4(i), 6(i, u), 7i, pal., hygro., arbo.
2. *Hypochthoniella pallidula* (C.L.Koch senses Willmann C., 1931), 4(j, l), 6(s, r), cosmop., trop., silv., micro.
3. ***Phthiracarus anonnymus* Grandjean F., 1933, 1b, 2e, 4(k, i, l, j), 6ss, pal., Ethiop., neotrop., silv., macro.
4. *Phthiracarus piger* (Scopoli J. A., 1763), 2e, 4j, Eur., cauc., magreb., N.-Am., silv., macro.
5. *Phthiracarus borealis* (Trägardh I., 1910), 3e, 4(i, j), 5m, 6i, 7i, Eur., cauc., sibir, macro.
6. ***Phthiracarus lignaeus* (C.L.Koch, 1841), 3g, 4j, 6s, C.-S.-Eur., N.-Am., silv., macro.
7. *Phthiracarus globosus* (C.L. Koch,1841), 3g, 4l, 6(r, s); Eur., orient., As., silv., macro.
8. *Steganacarus magnus* (Nicolet H., 1855), 2f, 4i,7i, Eur., cauc., N.-Am., silv., macro.
9. ***Steganacarus anomalus* (Berlese A., 1883), 4i, hol.

10. *Atropacarus striculum* (C.L. Koch, 1836), 1c, 4(i, k), 6s, hol, silv., macro.
11. *Euphthiracarus cribrarius* (Berlese A., 1904), 1b, 4(k, i, h, l, j), 6(r, ss, t, u), 7(x, y, i), Eur., N-Am.
12. ***Euphthiracarus monodactylus* (Willmann C., 1919), 4(h, l), 5p, 6r, 7g, C.-S.-Eur., silv., macro.
13. *Nothrus borussicus* Sellnick M., 1929, 4j., hol, euryök, myrmec., macro.
14. *Nothrus palustris* C.L.Koch, 1839, 4(j, k, i), 6(r, ss, t), 7x, Eur., N.-Am., mesohygro., macro., pan.
15. *Nothrus silvestris* Nicolet H., 1855; 3y 6s, 7(y, i), cosmop., trop., euryök., macro., pan.
16. *Heminothrus paolianus* (Berlese A., 1913), 1b, 4i, hol., pan.
17. *Platynothrus peltifer* C.L.Koch, 1839., 2e, 3g, 4(i, k, l, j), 5p, 6(r, s, ss, t, u, i, u), 7(x, r), pal., nearct., Austr., silv., hygro., nidicol., pan.
18. *Nanhermannia nannus* (Nicolet H., 1855), 2f, 4(j, i), 6(s, r, i), 7(v, x); pal., neotrop., nearct., subarct., hygro., pan.
19. *Hermannia gibba* (C.L.Koch, 1839), 5m, pal., N.-Am., nearct., silv., pan.
20. *Hermannia scabra* (C.L.Koch, 1879), 5(n, o), arkto-alp., N-Am., moss., pan.
21. *Hermannia reticulata* Thorell T., 1871, 1d, N-Eur.
22. **Liodes theleproctus* Hermann L van der., 1804, 6(ss, i), C.-S.-Eur., cauc., N-Am., ocean., xerophyl.
23. *Spatiodamaeus verticilipes* (Nicolet H., 1855), 1d, 2b, 4(i, k, h), 5p, 6(r, ss, t, u, i, u, r), 7(v, x, r, y, i), Eur.; hygro., micro.
24. *Belba compta* (Kulczynski 1902), 1(a, d), 3g, 4(a, f, o, k), 6(s, ss, t, i, r), 7(v, x, r, i); pal., hygro., micro.
25. *Metabelba pulverulenta* (C.L.Koch, 1840), 4k, 5p, 6r, 7x., pal., N.-Am., mesohygro., mirmec., micro.
26. *Cepheus cepheiphormis* (Nicolet H., 1855), 3g, 4(i, l), 5(o, p), 6(r, s, ss, i, r), 7(v, y), hol., euryök., macro.
27. ***Cepheus dentatus* (Michael A. D., 1888), 2f, 4i, 6u., pal., silv., arbo.
28. ***Cepheus latus* (C.L.Koch, 1836), 1b, 4i, 5m, hol, moss, arbo., macro.
29. ***Cepheus tuberculatus* Strenzke K., 1951, 4h, C-S-Eur.
30. *Ceratoppia bippilis* (Hermann J. F., 1804), 6(s, t), 7(y, r), pal., eury., silv., arbo., nidicol., pan., necro.
31. *Adoristes ovatus* (C.L.Koch, 1840), 1(a, c, d), 3g, 4(j, k, i), 5m, 6(r, s, t, u, i), 7(x, r, y), pal., N.-Am., pan.
32. ***Adoristes poppei* (Oudemans A. C., 1906), 6i, C-S-Eur., cauc., W-sibir.; macro.
33. *Xenillus clypeator* Robineau-Desvoidy ? 1839, 7x, pal., cauc., C-As.
34. *Xenillus tegeocranus* (Hermann J. F., 1804), 3g, 4(j, k, i, l), 5(b, p); 6(r, s, ss, i, u), 7(x, r, y, i), pal., magreb., As.; myrmec., euryök., macro., pan.

35. *Liacarus coracinus* (C.L.Koch, 1840), 3g, 4(j, i, h, l), 5(m, p), 6(r, s, t, u, r), pal., magreb., mesohygro., eury., pan.
36. *Liacarus vombi* Dalenius P., 1950, 4h, 6r; Eur., Sc.
37. *Furcoribulla furcillata* (Nordenskiöld E., 1901), 1c, C-N-Eur., myrmec., pan.
38. ***Cultroribulla bicultrata* Berlese A., 1908, 4k, C-Eur., sibir, N-Am.
39. *Carabodes coriaceus* C. L. Koch, 1836, 3g, 4(k, h), 7x., pal., N.-Am., silv., euryök., myrmec., pan.
40. *Carabodes femoralis* (Nicolet H., 1855), 4(i, h, l), 7x., pal., silv., euryök., pan.
41. *Carabodes forsslundi* Sellnick M., 1953, 4l, 8z; pal., silv., euryök., pan.
42. *Carabodes labirinthicus* (Michael A. D., 1879), 1c; 4j, 5(o, p), 7v, 8z., Eur., sibir., N-Am., silv., arbo., macro.
43. *Carabodes minusculus* Berlese A., 1923, 1(c, d), 4i, 3g, 5m, 7(x, r), pal., N.-Am., tropho., euryök., macro.
44. *Carabodes reticulatus* Berlese A., 1916, 4k, 7v., Eur.
45. *Odontocepheus elongatus* (Michael A. D., 1879), 1c, 5p, 7v, Eur., magreb., silv., xero., pan.
46. *Tectocepheus velatus* (Michael A. D., 1880), 1(a, d, c), 3g, 4(i, j, h) 5(n, o), 6(r, s, ss, u, r), 7(v, b, x, r, y, i), 8z.; pal., nearct., subarct., Austr.; euryök.; pan.
47. *Tectocepheus minor* Berlese A., 1903, 5m, C-S-Eur., E-Asia, mesohygro.
48. *Tectocepheus sarekensis* Trägårdh I., 1910, 3g, 4j, 6ss, hol., arkt., trop., cosmop., pan.
49. *Caleremaeus monilipes* (Michael A. D., 1882), 4l, 6u, Eur., N-Am., silv., arbo., macro.
50. *Oribella paoly* Oudemans A. C., 1913, 6u, eurosibir., macro., pan.
51. *Suctobelba trigona* (Michael A. D., 1888), 1(c, d), 7r, eurosibir., eur., cauc., W.-As., moss., pan.
52. *Suctobelba aliena* Moritz M., 1970, 3g, 4(k, l), 7x, C-S-Eur.
53. *Suctobelba acutidens* (Forsslund K.-H., 1941), 1d, 4i, 5n, 6(ss, r), 7(x, r), pal., nearct., hygro.
54. ***Suctobelbella baloghi* (Forsslund K.-H., 1958), 4i, 5o, 6(s, ss), pal; silv.
55. *Suctobelbella nasalis* (Forsslund K.-H.,1941), 4i., Eur., W-Sibir.
56. *Suctobelbella sarekensis* (Forsslund K.-H., 1941), 1(c, d), C-N-Eur., N-Am., mesohigro.
57. *Suctobelbella subcornigera* (Forsslund K.-H., 1941), 1a; 4(k, l, i), 5o, 7x, pal., N.-Am., euröyk., pan.
58. *Suctobelbella subtrigona* (Oudemans A. C., 1916), 3g, 4i, 6r; 7x, C-N-SE-Eur., pal., silv., euryök., pan.
59. *Suctobelba truncicola* (Forsslund K.-H., 1941), 4h, 6ss, 7y, C-N-Eur.

60. ****Suctobelbella forslundi*** (Strenzke K., 1950), 4i, C-SE-Eur., sibir., pal, hygro.
61. *Quadroppia quadricarinata* (Michael A. D., 1941), 1(c, d), 3g, 4(k, l), 5o, 7(v, x, i), pal., orient., Austr., neotrop., euryök., arbo., pan.
62. *Lauroppia falcata* (Paoli G., 1908), 1(c, d), 3g, 4i, 5o, 6s, 7v, 8a, pal., mesohygro., silv., pan.
63. *Lauroppia neerlandica* Oudemans A. C., 1900, 1c, 2e, 3g, 4(h, l), 6(i, t), 7(v, x), pal., moss, micro., pan.
64. *Medioppia obsoleta* (Paoli G., 1908), 2e, 4(k, i), 6r, 7r, Eur., euryök., pan.
65. *Mycroppia minus* (Paoli G., 1908), 4i, hol., arkt., xerothermo., pan.
66. *Dissorhina ornata* (Oudemans A. C., 1900), 1b, 4(i, l), 6(ss, u), pal., N-Am., silv., euryök., pan.
67. ***Berniniella sigma* Strenzke K., 1951, 3g, C-Eur., C-Asia., xero., pan.
68. ***Ramusella insculpta* (Paoli G., 1908), 4i, pal., xero.
69. *Oppia subpectinata* (Oudemans A.C., 1901), 4i, eurosibir., N-Am., euryök., micro.
70. *Oppia quadrimaculata* (Michael A. D., 1941), 5o, Eur
71. *Phauloppia lucorum* (C.L.Koch 1840), 5p, Eur.
72. ***Ameronothrus schneideri* (Oudemans A. C., 1903), 5p, Eur.
73. *Scutovertex minutus* (C.L. Koch, 1836), 8z, Eur., maghreb., moss., xero., micro.
74. *Cymbaeremaeus cymba* (Nicolet H., 1855), 4i, Eur., arbo., xero., micro.
75. *Oribatulla tibialis* Nicolet H., 1855, 1(a, d), 3g, 4(i, m), 5(m, n, o), 6(s, o), 7x, pal., nearct., mesohygro., euryök., micro., pan.
76. ***Oribatulla pannonicus* Willmann C., 1949, 6ss, C-Eur.
77. ***Trichoribatulla pilosa* (C.L. Koch, 1840), 4i, 8z, C-S-Eur.
78. ***Astegistes pilosus* (C. L. Koch, 1840), 2e, C-S-Eur.
79. *Licneremaeus licnophorus* (Michael A. D., 1888), 1d, 4h, 7v, eurosibir., silv., arbo., xero., micro.
80. *Zygoribatulla exilis* (Nicolet H., 1855), 1(a, d), 4i, 5(n, o), 6ss, 7v, pal., nearct., helio., xero., micro.
81. *Liebstsdia similis* (Michael A. D., 1888), 1a, 4(j, l, i), 5o, 8z, Eur., N-Am., helio., hygro., pan.
82. *Scheloribates laevigatus* (C.L.Koch, 1836), 1(b, d), 4(j, i), 5p, 7(v, x), pal., euryök., myrmec., pan., copro.
83. *Scheloribates confundatus* Sellnick M., 1928, 5m, 7r, Eur.
84. *Scheloribates pallidulus* (C.L.Koch,1840) 7v, Eur., N-S-Am., myrmec., mesohygro., pan.
85. *Protoribates badensis* Sellnick M., 1928, 4i, 5p, C-S-Eur., E-As., arbo.
86. ***Protoribates longior* Berlese A., 1908, 1c, Eur.
87. *Trichoribates trimaculatus* (C.L.Koch, 1836), 5m, pal., nearct., xero., moss., pan.

88. *Trichoribates novus* (Sellnick M., 1928), 6i, hol., helio., hygro., pan.
89. ***Ceratozetes fusiger* Mihelčič F., 1956, 1b, 4j, 8z, Eur.
90. *Ceratozetes gracilis* (Michael A. D., 1884), 1c, 2e, 3g, 4(j, k, i), 5p, pal., neotrop., Austr., euryök., pan.
91. *Ceratozetella thienemanni* Willmann C., 1943, 4(j, l), Eur., silv.
92. *Punctoribates punctum* (C.L.Koch, 1839), 4(k, l, j), hol., helio., pan.
93. *Punctoribates sellniki* Willmann C., 1928, 4k, pal., C-Eur., moss., hygro.
94. *Chamobates cuspidatus* (Michael A. D., 1884), 1d, 4(k, i, h), 5m, 7v, pal., nearct., silv., pan.
95. *Chamobates cuspidatiformis* (Trägårdh I., 1902), 5m, 7r, Eur.
96. *Chamobates spinosus* Sellnick M., 1928, 1(c, d), 4(i, l, j), 5(m; o), 6(ss, i), 7(v, r), 8z, Eur.; arbo.; pan.
97. *Euzetes globulus* (Nicolet H., 1955), 1b, 2d, 4(j, k, i, l), 5m, 7x, Eur., magreb., meso-hygro., silv., pan.
98. *Eupelops acromios* (Hermann L. van der1804), 5p, 6u, 7r, pal., silv., arbo., xerotrmo., pan.
99. *Eupelops torulosus* (C.L.Koch, 1840), 6t, C-W-S-Eur., pan.
100. *Eupelops occultus* (C.L.Koch, 1836), 7r, C-N-SE-Eur., W-Sibir., arbo., myrmec., pan.
101. ***Eupelops subuliger* (Berlese A., 1917), 4(j, i), 5(m, p), 6i, C-Eur.
102. ***Peloptulus phaenotus* C.L. Koch, 1844, 1a, 4k, 6s, pal., helio.
103. ***Ophidiotrichus vindobonensis* Piffl E., 1960, 4(i, k, l, i), C-Eur., xeroterm.
104. *Oribatella berlesei* (Michael A.D., 1898), 4k, Eur., silv.
105. *Oribatella calcarata* (C.L.Koch, 1836), 3g, 4(i, j, k), 6(r, u), 7r, 8z, pal., silv., pan.
106. ***Parachipteria bella* (Sellnick M., 1928), 5m, pal., silv.
107. *Parachipteria willmanni* Van der Hammen, 1952, 1a, 4(j, i), 5(m, n, p), 6(s, i, u, r), 7r, nearct., N.-Am., hygro., pan.
108. ***Achipteria nitens* (Nicolet, 1855), 1c, 4(j, i), 5m, 7v, pal., pan.
109. *Achipteria coleoptrata* (Linné, 1758), 4i, 5m, 8z, pal., nearct., euryök., myrmec., micro.; pan.
110. *Galumna elimata* (C.L.Koch, 1841), 4i, Eur., cosmo., helio., hygro., pan.
111. *Galumna lanceata* (Oudemans A. C., 1900), 1a, 2f, 4(j, k, h, l), 6i, 7(v, x), eurosibir., silv., myrmec., pan.
112. ***Galumna obvia* (Berlese A., 1915), 1a, 4(j, i), C-S-Eur.
113. *Pergalumna dorsalis* (C.L.Koch, 1836), 5o, C-S-SE-Eur., maghreb., S-Am., pan.
114. ***Pilogalumna alifera* (OudemansA. C., 1919), 7v, 8z., pal., xeroterm., myrmec., pan.
115. *Acrogalumna longiplumus* (Berlese A., 1904), 2f, 4(i, l), 5p, 7x, hol., euryök., myrmec., pan.

CONCLUSIONS

The presence of a large number of oribatid species in this reservation, most of them with a high number of individuals, is characteristic for diverse and mix forestry ecosystem.

One of the explanations for this situation is the very wide distribution of these mites, which appeared in general in ecosystems with good conditions for development, case of this reservation, which in our opinion is in a previous step of climax.

The other explanation could be the age of plantations of vegetation in this reservation, and its similarity and diversity with vegetation from the continent, referring to the mix habitat with trees, herbaceous layer, meadow, prairie, moss, rock with lichens, making possible the finding of a large diversity of oribatid species.

Another explanation could be the geographical localization of this reservation, between the continent and the Atlantic Ocean with Gulfstream influence, which determine good conditions for all habitats all over the seasons.

Not only the specified conditions above are responsible for this rich diversity, to them gathering others, determining the growing of vegetation (especially the primary producers-trees) and the production of an increased quantity of litter, which is the preferred habitat for oribatid mites.

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INSECTS SPECIES RICHNESS AND ABUNDANCE IN THE DANUBE DELTA (ROMANIA)

IRINA TEODORESCU

Insect species in Letea natural forest (areas with trees and grass-covered, small forest areas with herbaceous plants), in grasslands and sand dune zones near Letea forest, in Caraorman and Sfiştovca artificial forests, in ecotonal zones of Caraorman forest (sand dune zones, leaf litter, zones with a lot of herbaceous plants), sand dunes in C. A. Rosetti locality, Sărăturile sand bank, zones with herbaceous plants of Matiţa, Crişani, Sulina, Tulcea localities neighborhoods, and in aquatic basins were investigated. Many insects were detected also in stomach content of 39 bird species, and of 1191 individuals of *Rana ridibunda ridibunda* Pallas frog. A number of 495 identified insect species belonging to 382 genera, 172 families and 18 orders was found. In some cases only genus levels were established.

Key words: the Danube Delta, Romania, insects species.

INTRODUCTION

The Danube Delta (the territory between Sf. Gheorghe, Sulina and Chilia Danubian arms) together with the lacustrine complex Razim-Sinoe was declared a Biosphere Reserve in 1990. The Danube Delta includes over 30 ecosystem types: fresh, running and stagnant waters (arms, canals, lakes and marshes), wetland area, sea water, flooded zones, river and maritime levees, sand dunes, forests, towns, villages, agrosystems, fish farmings, and a high diversity of ecotone zones. The ecosystems and ecotones high diversity and favorable climate conditions create adequate conditions to a specific flora and fauna, with over 5,000 registered species, with approximately 3,500 fauna species, insects being dominant. As a result of high biodiversity registered a high productivity, this zone being an area which generates high biological resources and services.

MATERIAL AND METHODS

Semiquantitative methods of collection were: 10 pitfall traps placed with ethylene glycol, random sampling using 0.25 m^2 metrical frames placed on soil or on sand dunes, 500 sweeps with entomological net, stomach content of 1191 individuals of *Rana ridibunda* and of 56 individuals of 39 bird species analysed.

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Qualitative methods were: insects from plants, soil, moss hand collecting, leaves with cecidia, pseudocecidia collecting, visual observation, water samples analysis.

The paper represents both a review of anterior researches made by the author himself or in collaboration with others (Lăcătuşu *et al.*, 1971; Sin *et al.* 1975; Neacşu & Teodorescu, 1985; Teodorescu, 2004, 2008; Teodorescu *et al.*, 1984; 1999, 2003) and recent data, from Letea and Caraorman forests, Sfiştofca, Matiţa, C. A. Rosetti, Tulcea, Sulina, Crişani, Sărăturile, Jijila Fishing Complex neighbourhoods.

RESULTS AND DISCUSSION

A large diversity of insect taxa was revealed through the identification of 495 insect species, belonging to 382 genera, 172 families and 18 orders (Table 1). There are especially common species, easily identified, distributed in the whole Dobrogea and even in the whole country. The species identification was not allowed in all cases; therefore, especially in the cases of undigested pieces from birds and frog stomach contents, only genus level was identified.

Coleoptera (45 families with 201 species), Diptera (26 families with 75 species) and Hymenoptera (24 families with 59 species) were orders with high species and families richness. Thysanura, Blattaria, Megaloptera, Mecoptera and Dermaptera registered low species richness. Areas in forests (with trees, shrubs and herbaceous plants), ecotone zones of forests with many herbaceous plants and mixed forests leaf litter were habitats with high insect species richness. On sand dunes, as a result of low density or absence of primary producers (plants), there was the lowest insect species richness.

Some Orthoptera (*Gryllus* species, *Acrida hungarica*), Odonata, Heteroptera (*Dolycoris*, *Pyrrochoris*, *Anthocoris*, *Gerris*, *Notonecta*, *Naucoris*, *Plea* species), Homoptera (Pemphigidae, Aphididae), Neuroptera (Chrysopidae, Myrmeleonidae), Hymenoptera (Cynipidae, Formicidae, Myrmicidae), Coleoptera (Carabidae, Harpalidae, Coccinelidae, Rhynchitidae), Diptera (Culicidae, Tabanidae, Calliphoridae, Syrphidae, Muscidae) registered the highest numerical abundance.

In samples collected with pitfall traps (especially in underground Carabidae) and with metrical frames, in forests (especially in Letea natural forest) and in water samples, the highest insects abundance was registered.

On sand dunes, especially in zones without vegetation, low densities of insects, except of Myrmeleonidae larvae and their food (Formicidae and Myrmicidae) were registered. In the stomach content of *Rana ridibunda ridibunda*, Collembola, Homoptera (Aphididae), Hymenoptera (Formicidae, Myrmicidae, *Apis mellifera*) and Diptera species were abundant. In the case of birds, *Noterus* sp. (in stomach content of *Panurus biarmicus russicus*), *Donacia* larvae (in stomach content of *Remiz pendulinus*), Dolichopodidae larvae (in stomach content of *Larus*

minutus), *Messor* sp., and *Apis mellifera* (in stomach content of *Merops apiaster*) were abundant.

Many Coleoptera, Diptera, Heteroptera and Hymenoptera, some Odonata, Lepidoptera, Homoptera species were frequent.

Table 1

The list of insect species identified in the Danube Delta
(collected with classical entomological methods, detected in stomach contents of bird species and of *Rana ridibunda*)

Order /Family	Insect species	Classically collected	In stomach content	
			Birds	*Rana*
1	2	3	4	5
1. COLLEMBOLA				
Poduridae	*Podura aquatica*	+		
Onychiuridae	*Onychiurus armatus*	+		
Hypogastruridae	*Hypogastrura* sp.	+		
Entomobryiidae	*Entomobrya* sp.	+		+
	Orchesella sp.	+		+
Tomoceridae	*Tomocerus* sp.	+		
Sminthuridae	*Sminthurides* sp	+		
	Sminthurus viridis	+		+
Isotomidae	*Proisotoma* sp.			+
2. THYSANURA				
Machilidae	*Machilis* sp.	+		
3. EPHEMEROPTERA				
Polymitarcidae	*Polymitarcis virgo*		+	
Palingeniidae	*Palingenia longicauda*		+	
Caeniidae	*Caenis horraria*	+		
4. ORTHOPTERA				
Phaneropteridae	*Phaneroptera falcate*	+		
	Isophia longicauda	+		
	Isophia tenuicerca	+		
Tettigoniidae	*Tettigonia caudate*	+		
	Tettigonia viridissima	+		
Sagidae	*Saga pedo*	+		
Decticidae	*Metrioptera intermedia*	+		
	Decticus verrucivorus	+		
Gryllidae	*Gryllus campestris*	+ F, A	+	+
	Gryllulus desertus	+ F, A		
	Gryllus domesticus	+		
	Gryllus frontalis	+		
Gryllotalpidae	*Gryllotalpa gryllotalpa*	+		
Catantopidae	*Calliptamus italicus*	+		+

	Calliptamus barbarus	+	+	
	Podisma pedestris	+		
Mogoplistidae	*Arachnocephalus vestitus*		+	+
Tetrigidae	*Tetrix vittata*	+		
Acrididae	*Oedaleus decorus*	+		
	Oedipoda coerulescens	+	+	
	Acrida hungarica	+ F, A		+
	Dociostaurus maroccanus	+		
	Chorthipus brunneus	+		
	Aiolopus thalassimus	+		
	Celes variabilis subcoeruleipenis	+		
	Acrotylus longipes	+		
	Sphingonotus coerulans	+		
	Asiothmetis sp.		+	
Tridactylidae	*Tridactylus variegates*	+	+	
5. BLATTARIA				
Ectobiidae	*Ectobius erythronotus*	+		
6. DERMAPTERA				
Forficulidae	*Forficula auricularia*	+ F	+	+
	Forficula tomis	+		
	Apterygia media	+		
Labiduridae	*Labidura riparia*	+		
Labiidae	*Labia minor*	+		
7. ODONATA				
Lestidae	*Lestes viridis*	+ F, A		+
	Lestes virens	+		
	Lestes barbarus	+		
	Lestes dryas	+		
	Lestes macrostigma	+		
Calopterygidae	*Calopteryx splendens*	+ F, A		
	Agrion puella	+		
	Agrion pulchellum	+		+
	Ischnura elegans	+		
Aeschnidae	*Aeschna mixta*	+		
	Aeschna grandis	+ F	+	
	Aeschna isosceles	+		
	Anax imperator	+		
Libellulidae	*Libellula quadrimaculata*	+ F, A		
	Libellula depressa	+	+	
	Orthethrum coerulescens	+		
	Sympetrum meridionale	+		
	Sympetrum sanguineum	+ F		
	Sympetrum vulgatum	+	+	
	Crocothemis erythraea	+		
Gomphidae	*Gomphus flavipes*	+		
	Gomphus vulgatissimus	+		

8. HETEROPTERA				
Pentatomidae	*Aelia acuminate*	+		
	Aelia rostrata	+		
	Dolycoris baccarum	+ F, A		+
	Eurydema ornate	+		
	Eurydema oleracea	+		
	Carpocoris pudicus	+ F	+ F	
	Carpocoris fuscispinus	+		
Coreidae	*Syromastes marginatus*	+		
Pyrrochoridae	*Pyrrochoris apterus*	+ F, A		+
Scutelleridae	*Eurygaster integriceps*			+
	Graphosoma lineatum	+ F		
Miridae	*Adelphocoris lineolatus*	+ F		
	Lygus rugulipennis	+	+	
Lygaeidae	*Lygaeus equestris*	+		
Anthocoridae	*Anthocoris nemorum*	+ F, A		
	Orius niger	+		
Nabidae	*Nabis ferus*	+ F		
Reduviidae	*Reduvius personatus*	+		
Gerridae	*Gerris* sp.	+ F, A		
Hydrometridae	*Hydrometra* sp.	+		
Notonectidae	*Notonecta* sp.	+ F, A		
Nepidae	*Nepa rubra*	+ F	+	
	Ranatra linearis	+	+	+
Naucoridae	*Naucoris cimicoides*	+ F, A	+ F	+
Corixidae	*Corixa punctata*	+ F, A	+ F	+
Pleidae	*Plea leachi*	+ F, A		
9. HOMOPTERA				
Cercopidae	*Aphrophora salicina*	+		
	Philaenius spumarius	+		
Cicadellidae	*Cicadella viridis*	+ F		
	Macrosteles laevis	+ F		
	Macrosteles quadripunctulatus	+		
Membracidae	*Centrotus cornutus*			+
Psyllidae	*Psyllopsis fraxini*	+		
	Psylla sp.			+
Schizoneuridae	*Tetraneura ulmi*	+		
	Thecabius affinis	+		
Pemphigidae	*Pemphigus spirothecae*	+ F, A		
	Pemphigus bursarius	+		
Aphididae	*Brevicoryne brassicae*	+ F, A		
	Aphis fabae	+		
	Aphis evonymi	+		
	Cryptosiphum brevipilosum	+		
	Rhopalosiphum maidis	+		
	Myzus cerasi	+ A		
	Myzus persicae	+ F, A		
	Hyalopterus pruni	+		

	Therioaphis ononidis	+		
10. THYSANOPTERA				
Thripidae	*Limothrips denticornis*	+		
	Thrips tabaci	+		
Aeolothripidae	*Aeolothrips intermedius*	+		
11. MEGALOPTERA				
Sialidae	*Sialis* sp.		+	+
12. NEUROPTERA				
Coniopterygidae	*Coniopteryx borealis*	+		
Hemerobiidae	*Hemerobius* sp.	+		
Chrysopidae	*Chrysopa carnea*	+ F, A		
	Chrysopa abbreviate	+		
	Chrysopa commata	+		
	Chrysopa phyllocroma	+		
Myrmeleonidae	*Myrmeleon formicarius*	+ F, A		
	Dendroleon pantherinus	+		
	Myrmacaelurus trigrammus	+		
	Acanthaclisis baetica	+		
Ascalaphidae	*Ascalaphus meridionalis*	+		
13. MECOPTERA				
Panorpidae	*Panorpa communis*	+ F		
14. HYMENOPTERA				
Tenthredinidae	*Pontania viminalis*	+		
	Pontania vesicator	+		
	Athalia spinarum	+		+A
Cephidae	*Cephus* sp.	+		+
Ichneumonidae	*Diplazon laetatorius*	+		+
	Theronia atalantae	+		
Aphidiidae	*Lysiphlebus fabarum*	+ F, A		
	Diaretiella rapae	+		
	Praon volucre	+		
	Ophion sp.			+
Braconidae	*Apanteles porthetriae*	+		
	Apanteles spurious	+		
	Rhogas praetor	+		
	Rhogas geniculator			+
	Coeloides filiformis		+	
	Chelonella contracta		+	
	Sigalphus irroator		+	
	Dacnusa minuta			+
	Aspilota sp.			+
	Diospilus sp.			+
	Macrocentrus sp.			+
Megaspilidae	*Dendrocerus carpenteri*	+		
	Dendrocerus aphidum	+		
Serphidae	*Serphus gravidator*	+		+
Heloridae	*Helorus* sp.	+		
Diapriidae	*Trichopria cilipes*	+		

	Trichopria musciperda	+		
Scelionidae	*Telenomus nitidulus*	+		
	Telenomus laeviusculus	+		
Cynipidae	*Diastrophus rubi*	+		
	Andricus fecundatrix	+		
	Neuroterus lanuginosus	+ F, A		
	Neuroterus numismalis	+ F, A		
	Cynips quercus folii	+ F		
	Rhodites rosae	+		
Eupelmidae	*Anastatus disparis*	+		
	Anastatus bifasciatus	+		
Encyrtidae	*Ooencyrtus tardus*	+		
	Ooencyrtus kuwanae	+		
Torymidae	*Torymus bedeguaris*	+		
Trichogrammatidae	*Trichogramma* sp.	+		
Pteromalidae	*Pteromalus puparum*	+		
Aphelinidae	*Azotus celsus*	+		
	Aphelinus sp.	+		
Chrysididae	*Chrysis* sp.	+		
Vespidae	*Polystes gallicus*	+ F		+
	Vespa crabro	+		
	Vespa germanica	+ F		
	Vespa vulgaris	+ F, A		
Formicidae	*Messor* sp.	+	+F, A	
	Formica rufa	+ F, A		+A
	Tetramorium sp.	+ F, A	+ F	+
	Myrmecocystis cursor	+	+	
	Lasius sp.	+ F, A	+ A	+
Myrmicidae	*Myrmica* sp.	+		+A
Scoliidae	*Scolia flavifrons*	+		
Andrenidae	*Andrena* sp.	+		+
Apidae	*Apis mellifera*	+ A	+ F	+A
	Bombus sp.	+		
15. COLEOPTERA				
Cicindelidae	*Cicindela lunulata nemoralis*	+	+ F	
	Cicindela litterata	+		
Carabidae	*Carabus cancellatus sulinensis*	+ F, A		+
	Carabus clathratus stygius	+		
	Carabus granulatus	+ F, A		
	Carabus coriaceus	+		
	Calosoma inquisitor	+		
	Calosoma maderae auropunctatum	+		
Pterostichidae	*Platynus assimilis*	+		
	Pterostichus nigrita	+ F, A		
	Pterostichus niger	+		+
	Poecilus cupreus	+ F, A		
	Amara aenea	+		
	Amara apricaria	+		

	Amara similata	+		
	Agonum viridicupreum	+		
	Abax carinatus	+		
	Zabrus tenebrioides	+		
Broscidae	*Broscus cephalotes*	+		
Callistidae	*Chlaenius sulcicollis*	+		
	Chlaenius spoliatus	+	+	+
	Chlaenius vestitus	+ F		
Lebiidae	*Lebia* sp.	+		+
	Microlestes maurus	+	+	
Nebridae	*Nebria* sp.	+		
Bembidiidae	*Bembidion varium*	+	+	+
	Bembidion assimile	+		
Scaritidae	*Clivina fossor*	+		
	Scarites terricola	+		
	Scarites laevigatus	+		
	Dyschirius chalceus	+		
	Dyschirius politus	+		
Omophronidae	*Omophron limbatus*	+		
Panagaeidae	*Panageus crux-major*	+		
Harpalidae	*Harpalus azureus*	+		
	Harpalus rufipes	+ F, A		
	Harpalus distinguendus	+ F, A	+	+
	Harpalus griseus	+		
	Harpalus aeneus	+		
	Harpalus tardus	+		
	Dolichus halensis	+		
	Anisodactylus signatus	+		
	Anisodactylus binotatus	+		
	Acupalpus elegans	+		
	Dicheirotrichus obsoletus	+		
Brachinidae	*Brachinus psophia*	+		
	Brachinus crepitans	+		
Malachiidae	*Malachius bipustulatus*	+ F, A		
	Malachius aeneus	+		
Dytiscidae	*Dytiscus dimidiatus*	+		
	Dytiscus marginalis	+	+	
	Cybister lateralimarginalis	+	+	
	Hydaticus sp.	+		+
Noteridae	*Noterus* sp.	+	+ A	
	Copelatus sp.	+	+	
	Agabus sp.	+	+	
Hydrophilidae	*Hydrous picaeus*	+		
	Hydrous aterrimus	+		
	Hydrophilus sp.	+		
Gyrinidae	*Gyrinus natator*	+ F, A	+	+
Cantharidae	*Cantharis fusca*	+ F		+
	Cantharis livida	+		

Dermestidae	*Dermestes lardarius*	+ F		
	Dermestes laniarius	+		
	Dermestes erichsoni	+		
Silphidae	*Silpha carinata*	+		+
	Necrophorus germanicus	+		
	Necrophorus vespillo	+		
Staphylinidae	*Stenus* sp.		+	
	Aleochara bipustulatus	+		
	Paederus fuscipes	+		+
	Creophilus maxilosus	+		
	Microglossa gentiles	+		
	Oxytelus rugosus	+		
	Staphylinus olens	+		+
	Staphylinus caesareus	+		
Tenebrionidae	*Blaps letifera*	+		
	Opatrum sabulosum	+	+	+
	Tentyria frivaldschi	+		
Histeridae	*Platysoma* sp.	+ A		
	Hister sp.	+	+	
	Saprinus semistriatus	+		
Nitidulidae	*Meligethes maurus*	+		
	Meligethes aeneus	+		
Anthicidae	*Anthicus antherinus*	+ F		
	Anthicus humilis	+		
	Formicomus pedestris	+ F		
	Notoxus brachyuran	+		
Elateridae	*Agriotes ustulatus*	+ F, A		
	Agriotes lineatus	+ F		+
	Agriotes obscurus	+		+
	Ampedus elegantulus	+		
	Cardiophorus rufipes	+		
	Melanotus rufipes	+		
	Denticolis linearis	+		
	Corymbites sp.	+		
	Heteroderes sp.			+
Ostomidae	*Nemosoma elongatum*			+
Meloidae	*Mylabris variabilis*	+		
Buprestidae	*Eurytiraea aurata*	+		
	Dicerca aenea	+		
	Poecilonota variolosa	+		
	Agrilus salicis	+		
	Agrilus viridis		+	
Mordellidae	*Mordelistena parvula*	+		
Coccinellidae	*Coccinella septempunctata*	+ F, A		+
	Adalia bipunctata	+		
	Adalia decimpunctata	+		
	Tythaspis sedecimpunctata	+ F, A		+
	Propylaea quatuordecimpunctata	+		+

	Hypodamia tredecimpunctata	+		
	Adonia variegate	+ F, A		
	Coccinulla quatuordecimpustulata	+ F		
	Chilocorus bipustulatus	+		
	Exochomus quadripustulatus	+		
	Subcoccinella vigintiquatuorpunctata	+		+
	Thea vigintiduopunctatum	+		
	Syncharmonia conglobata	+		
Ptinidae	*Ptinus fur*			+
Chrysomelidae	*Chrysomela sanguinolenta*	+		
	Chrysomela polita	+	+ F	+
	Chrysomela herbacea	+ F, A	+	
	Agelastica alni	+		
	Coptocephala unifasciata	+		
	Galerucella luteola	+		
	Cryptocephalus sericeus	+		
	Plagiodera versicolora	+		
	Lema tristis	+		
	Lema melanopus	+		+
	Antipa macropus	+		
	Podagrica fuscicornis	+ F		
	Podagrica malvae	+		
	Donacia sp.		+ A	
	Leptinotarsa decemlineata	+		+
Halticidae	*Longitarsus tabidus*	+		
	Aphthona euphorbiae	+ F, A		
	Aphthona cyparissiae	+ F, A		
	Aphthona nigripes	+		
	Haltica tamaricis	+		
	Haltica oleracea	+		
	Phyllotreta atra	+		+
	Phyllotreta nemorum	+		+
	Phyllotreta undulata	+		
	Chaetocnema sp.	+		
	Haltica sp.			+
Cassididae	*Cassida rubiginosa*	+ F		
	Cassida viridis	+ F		
	Cassida nebulosa	+		+
Scarabaeidae	*Anisoplia austriaca*	+		
	Anisoplia deserticola	+		
	Anisoplia lata	+		
	Anisoplia segetum	+		+
	Anomala dubia	+		
	Epicometis hirta	+		
	Oxythirea funesta	+		
	Polyphylla fullo	+		
	Anoxia orientalis	+		

	Anoxia pillosa	+		
	Onthophagus sp.		+	
	Geotrupes sp.	+	+	
	Pentodon idiota	+		
	Pentodon bidens	+		
	Pentodon sulcifrons	+		
Aphodiidae	*Aphodius erraticus*	+	+	
Cetonidae	*Potosia aeruginosa*	+		
	Potosia cuprea	+	+	
Oedemeridae	*Oedemera virescens*	+	+	
Cerambycidae	*Agapantia* sp.		+	
	Megopis scabricorne	+		
	Xylothrechus pantherinus	+		
	Plagionotus floralis	+		
	Purpuricenus kaehleri	+		
	Rhopalopus clavipes	+		
	Chlorophorus varius	+		
	Oberea linearis	+		
	Oberea euphorbiae	+		
	Saperda charcharias	+		
	Saperda perforate	+		
	Saperda populnea	+		
	Exocentrus punctipennis signatus	+		
	Leptura steveni/unipunctata	+		
	Rhopalopus clavipes	+		
	Morimus funereus	+		
	Dinoptera collaris		+	
Curculionidae	*Stereonychus fraxini*	+		
	Phytonomus variabilis	+		
	Ceuthorrhynchus contractus	+ F		+
	Otiorrhynchus ovatus	+		+
	Polydrosus picus	+		
	Peritelus familiaris	+		
	Sibinia femoralis	+		
	Smyrconyx jungermannie	+		
	Tanymecus dilaticollis	+	+	+
	Tanymecus palliates	+		
	Sphaenophorus abbreviatus	+		
	Lixus albomarginatus	+		
Apionidae	*Apion* sp.	+		
Rhynchitidae	*Byctiscus populi*	+		
	Rhynchytes bacchus	+ F, A		
Ipidae	*Hylesinus fraxini*	+		
16. TRICHOPTERA				
Limnophilidae	*Limnophilus* sp.	+	+	
17. LEPIDOPTERA				
Tischeriidae	*Tischeria marginea*	+		

Yponomeutidae	*Yponomeuta cognatella*			+
Tortricidae	*Tortrix viridana*	+		
	Cacoecia sp.	+		
Nepticulidae	*Nepticula* sp.	+		
Pterophoridae	*Pterophorus* sp.	+		
Sphingidae	*Deilephila euphorbiae*	+		
Noctuidae	*Autographa gamma*	+ F		
	Mamestra brassicae	+		+
	Agrotis exclamationis	+		+
Arctiidae	*Arctia* sp.	+ F		+
	Hyphantria cunea	+ F, A		
Lymantriidae	*Lymantria dispar*	+		
	Leucoma salicis	+		+ A
	Hypogymna morio	+ F		
Lycaenidae	*Lycaena* sp.	+ F		
	Polyommatus sp.	+ F		
Nymphalidae	*Nymphalis polychloros*	+		
	Inachis io	+		
	Vanessa cardui	+		
	Argynnis lathonia	+		
	Melitaea sp.	+ F		
Pieridae	*Pieris rapae*	+ F		
	Pieris napi	+		
	Gonepteryx rhamni	+		
	Colias sp.	*		
Papilionidae	*Iphiclides podalirius*	+		
18. DIPTERA				
Culicidae	*Anopheles maculipennis*	+ F, A	+	+
	Culex pipiens	+ F, A	+	+ A
	Aedes vexans	+ F, A		
Chironomidae	*Chironomus plumosus*	+		
Cecidomyiidae	*Dasyneura fraxini*	+		
	Dasyneura fraxinea	+		
	Dasyneura trifolii	+		
	Dasyneura ignorata	+		
	Rhabdophaga rosaria	+		
	Rhabdophaga saliciperda	+		
	Contarinia medicaginis	+		
	Aphydolethes aphidimyza	+		
Ceratopogonidae	*Culicoides* sp.	+		
Limnobiidae	*Limnobia* sp.			+
Sciomyzidae	*Trypetoptera* sp.	+		
	Coremoneura sp.	+		
Tipulidae	*Tipula* sp.	+		+
Simuliidae	*Simulium* sp.		+	
Bibionidae	*Bibio hortulanus*			+
Sepsidae	*Sepsis* sp.			+
Scatopsidae	*Scatops* sp.			+

Pipunculidae	*Pipunculus sylvaticus*	+		+
Dolichopodidae	*Dolichopus* sp.	+		
Stratiomyidae	*Stratiomyia* sp.	+		
Tabanidae	*Tabanus bovines*	+ F, A	+ F	+
	Tabanus autumnalis	+ F, A	+	
	Tabanus acuminatus	+		
	Tabanus glaucopis cognatus	+		
	Tabanus solstitialis ciureai	+		
Syrphidae	*Episyrphus balteatus*	+		
	Metasyrphus luniger	+		
	Metasyrphus corollae	+ F, A		
	Sphaerophoria scripta	+ F		
	Sphaerophoria menthastri	+		
	Scaeva pyrastri	+		
	Syrphus ribesii	+ F, A		
	Syrphus vitripennis	+		
	Lasiopticus albomaculatus	+		
	Melanostoma melinum	+		
	Paragus quadrifasciatus	+		
	Eristalis arbustorum	+ F		
	Eristalis tenax	+		+ A
	Syritta pipiens	+		
Asilidae	*Satanas gigas*	+		
	Stenopogon sabaudus	+		
	Astochia caspica sienkiewiczi	+		
Chamaemyiidae	*Leucopis glyphinivora*	+ F		
	Leucopis rufithorax	+		
	Leucopis silesiaca	+		
	Parochthyphylla sp.	+		
	Chamaemyia sp.	+ F		
Drosophilidae	*Drosophila* sp.	+ F, A		
Ephydridae	*Ephydra riparia*			+
	Urophora sp.			+
	Euphrania sp.	+		
Psilidae	*Psila rosae*			+
Piophilidae	*Piophila casei*	+		+
	Phytagromyza heringi	+		
Cloropidae	*Chlorops pumilionis*	+ F		
	Oscinis fritt	+		
	Meromyza sp.	+		
Calliphoridae	*Calliphora vomitoria*	+ F, A		
	Calliphora vicina	+		
	Protocalliphora sp.	+		
	Lucilia caesar	+ F, A		+

	Phaenicia sericata	+ F, A		
Muscidae	*Musca domestica*	+ F, A		
	Musca vitripennis	+		+
	Stomoxys calcitrans	+ F, A		
	Euribia cardui	+		
	Muscina stabulans	+ A		+ A
	Pegomya bicolor	+		
	Lipara lucens			+
Tachinidae	*Compsilura concinnata*	+		
	Tachina sp.	+		+

CONCLUSIONS

A number of 495 terrestrial and aquatic insect species (adults and larvae) belonging to 382 genera, 172 families and 18 orders were collected or observed as a result of investigations in the Danube Delta zone, in many localities and years. From these, some species were detected in the stomach content of frog *Rana ridibunda ridibunda* and of 37 bird species.

Orders with high species, genera and family richness, high values of abundance and frequency were Coleoptera, Diptera and Hymenoptera and those with low species richness and abundance were especially Thysanura, Blattaria, Megaloptera, Mecoptera, and Dermaptera. Caraboidea, Chrysomelidae, Cerambycidae, Coccinellidae, Halticidae, Elateridae, Curculionidae, Scarabaeidae, Syrphidae, Muscidae and Cecidomyiidae, Braconidae, Cynipidae and Formicidae were families with high species richness.

For some Orthoptera (*Gryllus* species, *Acrida hungarica*), Odonata adults, Heteroptera (*Dolycoris*, *Pyrrochoris*, *Anthocoris*, *Gerris*, *Notonecta*, *Naucoris*, *Plea* species), Homoptera (Pemphigidae, Aphididae), Neuroptera (Chrysopidae, Myrmeleonidae), Hymenoptera (Cynipidae, Formicidae, Myrmicidae), Coleoptera (Carabidae, Harpalidae, Coccinelidae, Rhynchitidae), Diptera (Culicidae, Tabanidae, Calliphoridae, Syrphidae, Muscidae) the highest numerical abundances were registered. In the case of species collected with pitfall traps (especially underground Carabidae) and with metrical frames, in forests and in water samples, abundance values were the highest.

On sand dunes, especially in zones without vegetation, except for Myrmeleonidae larvae and their Formicidae and Myrmicidae food, there were low densities of insects.

Presence and abundance of different insect species in *Rana* and bird species stomach content is a result of their trophic preference and of local abundance of these insects.

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DATA ON FEEDING AND NESTING OF SOME BEE SPECIES (HYMENOPTERA: MEGACHILIDAE, ANTHOPHORIDAE) IN ROMANIA

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The present paper comprises a synthesis regarding the biology of some Megachilidae and Anthophoridae species from Romania, based on the data from literature, from the collections found in "Grigore Antipa" National Museum of Natural History and in "Bruckenthal" National Museum, and from our own samplings from different areas between the years 1995-1998 and 2003-2008. Among the 128 species identified, 60% of Megachilidae and 70% of Anthophoridae species are polilectics, representing the dominant category. In the paper are presented data on the nest and cells' type, as well as on the building material.

Key words: Megachilidae, Anthophoridae, trophic resources, nest types, Romania.

INTRODUCTION

Along time mankind was strongly attracted by bees. They were among the first insects represented by men in paintings. The literature on Apoidae is vast. The role played by bees in nature and man's life is known since ancient times.

Most species of shrubs and herbaceous plants from the temperate region are pollinated by bees. Thus, the conservation of many habitats depends on the conservation of bee populations. If these populations disappear, the reproduction of some major elements of flora can be severely limited.

In Romania, data regarding the biology of Apoidea were published by: Iuga (1954, 1958, 1960, 1961, 1968), Iuga and Scobiola-Palade (1959), Varga and Ciurdărescu (1967), Ciurdărescu (1969, 1970, 1971, 1973), Ciurdărescu and Varga (1972), Goagă and Tomozei (1999). These authors show that, although domestic bees that visit cultivated plants are prevailing in number, they are less efficient pollinators than wild bees, opening only 3-10% of the visited flowers. These papers highlight the need for protection of wild bees.

Varga (1961) emphasizes the relation between the abiotic factors and the presence of pollinating insects on the flowering lucerne. Ciurdărescu *et al.* (1975) in the paper "Problems of plant protection" present a more complex study, comprising data on the species' distribution, numerical variation of lucerne pollinators, the influence of abiotic, biotic and anthropic factors. The authors

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highlight the necessity of controlled pollination in order to increase the lucerne seed production.

Aftene (1995) mentions for the first time in Romania pollinating species of Megachilidae, and Matache (2006) shows that the main role in the pollination is played by females, which are adapted to the harvest and transport of pollen.

MATERIAL AND METHODS

The biological material that was used for this synthesis comes from the samplings made by us and the specialists from the "Grigore Antipa" National Museum of Natural History (Bucharest), in different areas of Romania, between 1995-1998 and 2003-2008, namely: Maramureş (1995-1998 and 2003, 2004), Crişana: Bihor county (2008), Oltenia: Gorj county (2004) and Mehedinţi county (2007), Dobrogea (2005, 2006), Transilvania: Piatra Craiului National Park (2004, 2005) and Făgăraş Mountains Area (2005-2008), Muntenia: Bucureşti and its surroundings (2006, 2007, 2008).

Besides, we also examined the material from the entomological collections from "Grigore Antipa" National Museum of Natural History (Bucureşti) and Bruckenthal National Museum (Sibiu), as well as the data from literature.

The apoids species were collected either from the visited plants, or from the nest's vicinity. The places preferred for nesting are clayey or sandy hills, house walls, plant stems, representing an essential condition for the bees development and distribution.

Nomenclature and biology data are given according to Iuga (1958), Osychnyuk *et al.* (1978), Michener (2000) and Banaszak and Romasenko (2001).

Abbreviations: p-polilectics; o-oligolectics; --- – unknown trophic resource or unknown nest.

RESULTS AND DISCUSSION

There were identified 128 species of Apoidea, belonging to 29 genera of two families: Megachilidae (63 species, 16 genera) and Anthophoridae (65 species, 13 genera).

For each species are presented aspects regarding the feeding and nest building technique (in case of nest building species) or the host species (in case of cleptoparasite species) (Tables 1-2).

The identified Megachilidae are grouped, based on their food, into two groups: oligolectics and polilectics. The oligolectic species represent 37% of the total number of identified species. These are grouped according to their preferences for plants from different families: 34% feed on plants from Asteraceae family, 21%

on Fabaceae and 17% on Campanulaceae. 8% from the identified Megachilidae feed on Boraginaceae, the rest of the species collect nectar and pollen from Ranunculaceae, Brassicaceae, Rosaceae, Oleaceae and Moraceae. The polilectic species represent 60% of the total identified species, being thus the dominant category. For 3% of the species the trophic resource is unknown (Fig. 1).

Table 1

Data on Megachilidae biology

Taxon	Trophic base	Nest type
Lithurgus chrysurus	o Asteraceae	in soft rotten wood, linear or linear-branched type, having a main cell and one or more lateral, with walls between them
Lithurgus cornutus	o Asteraceae	nest similar to that belonging to *L. chrysurus*, with the difference that the cells are not separated
Trachusa byssina	o Fabaceae	in cavities in the ground, the cells are made of pieces of leaves
Rhodanthidium septemdentatum	p	---
Paraanthidiellum lituratum	o Asteraceae	in dry stems, carving galleries in the soft marrow or using the pre-existent neighbouring galleries, made either by other bees or by xylophagous beetles
Anthidium florentinum	p	in preexistent cavities from different substrata: nests abandoned by other bees, galleries of other insects in the soil, in dry hollow stems, in clayey walls
Anthidium manicatum	p	in preexistent cavities from different substrata
Anthidium punctatum	p	in preexistent cavities from sandy soil
Proanthidium oblongatum	o Fabaceae	in preexistent cavities from different substrata
Anthidiellum strigatum	o Fabaceae	on stones' surface or fixed on the shrubs' branches
Stelis minuta	p	nest parasite of *Hoplitis claviventris*
Stelis ornatula	p	cleptoparasite of *Hoplitis claviventris, Ceratina cucurbitina*
Stelis phaeoptera	p	cleptoparasite of *Hoplitis anthocopoides, Osmia emarginata, O. fulviventris, O. rufa, Megachile rotundata*
Chelostoma campanularum	o Campanulaceae	in preexistent cavities from wood walls, dry hollow stems
Chelostoma distinctum	o Campanulaceae	in dry hollow stems and old cinipid galls on *Rosa canina*
Chelostoma florisomne	o Ranunculaceae	in dry hollow stems or in preexistent cavities in wooden buildings, trees branches and stems
Chelostoma rapunculi	o Campanulaceae	in preexistent cavities from hollow stems and wooden buildings

Heriades crenulatus	o Asteraceae	in preexistent cavities from wooden buildings and reed roofs
Heriades truncorum	o Asteraceae	in preexistent cavities from wooden buildings and reed roofs
Hoplitis anthocopoides	o Boraginaceae	on stones' and rocks' surface
Hoplitis claviventris	p	in dry stems' marrow, in shrub branches, carving galleries inside
Hoplitis leucomelana	p	---
Hoplitis manicata	o Oleaceae, Moraceae	---
Hoplitis praestans	o Campanulaceae	in dry stems' marrow, carving linear galleries, with cells separated by transversal walls, made of leaves
Hoplitis ravouxi	o Fabaceae	---
Anthocopa papaveris	p	in preexistent cavities in the soil females often excavate their own tunnels; the nest contains a cell with all its walls made of petals
Osmia aurulenta	p	in empty snail shells, in hollow dry stems and rock crevices
Osmia bicolor	p	in empty snail shells
Osmia brevicornis	o Brassicaceae	in dead wood and hollow stems of plants; often the transversal walls between the cells are absent
Osmia caerulescens	p	in preexistent cavities from different substrata; linear nest with cells separated by transversal walls
Osmia cerinthidis	o Boraginaceae, Rosaceae	in preexistent cavities from different substrata, preferring vegetal substratum; linear nest, with 2-9 cells separated by transversal walls
Osmia cornuta	p	in different substrata; linear nest comprising 2-10 cells, separated by transversal cells
Osmia emarginata	p	in preexistent cavities from stones or rocks; the cells have an unregulated form, being covered by a common stratum
Osmia fulviventris	o Asteraceae	linear nest, built in preexistent cavities from dead wood or hollow stems
Osmia leaiana	o Asteraceae	in preexistent cavities in dead wood
Osmia rufa	p	in preexistent cavities: in wooden buildings, dry hollow stems, reed roofs, snail shells; nest is linear or linear-branched, containing 2-10 cells, separated by transversal walls
Chalicodoma ericetorum	o Fabaceae	in preexistent cavities from wood and clayey walls or hollow dry stems
Chalicodoma parietina	p	on stones and rocks, cells being made of sand and little stones glued with saliva

Megachile alpicola	p	in preexistent cavities from different substrata
Megachile centuncularis	p	in preexistent cavities from different substrata
Megachile lagopoda	p	in preexistent cavities from soil
Megachile lapponica	p	in preexistent cavities from dead wood
Megachile leucomalla	p	in preexistent cavities from soil
Megachile ligniseca	p	in preexistent cavities from dead wood
Megachile melanopyga	p	---
Megachile nigriventris	p	in preexistent cavities from soil or dead wood
Megachile octosignata	p	in preexistent cavities from dead wood
Megachile pilicrus	o Asteraceae	in preexistent cavities from dead wood or hollow dry stems
Megachile pilidens	p	in preexistent cavities from soil
Megachile rotundata	p	in preexistent cavities from different substrata
Megachile versicolor	p	in preexistent cavities from dead wood or hollow dry stems
Megachile willughbiella	p	in preexistent cavities from dead wood or hollow dry stems
Coelioxys afra	p	cleptoparasite of *Megachile leachella, M. pilidens*
Coelioxys aurolimbata	o	cleptoparasite of *Chalicodoma ericetorum*
Coelioxys caudata	---	cleptoparasite; unknown host.
Coelioxys elongata	p	cleptoparasite of *Megachile centuncularis, M. ligniseca, M. pilidens, M. willughbiella.*
Coelioxys haemorrhoa	---	cleptoparasite; unknown host.
Coelioxys inermis	p	cleptoparasite of *Megachile centuncularis, M. versicolor, Anthocopa papaveris*
Coelioxys mandibularis	p	cleptoparasite of *M. centuncularis, M. leachella, M. versicolor, Anthocopa papaveris*
Coelioxys polycentris	p	cleptoparasite of *Tetralonia nana*
Coelioxys quadridentata	p	cleptoparasite of *Megachile willughbiella, Trachussa byssina, Anthophora parietina*
Coelioxys rufescens	p	cleptoparasite of *Megachile, Anthophora quadrimaculata, A. bimaculata*
Coelioxys rufocaudata	p	cleptoparasite of *Megachile rotundata*

Table 2

Data on Anthophoridae biology

Taxon	Trophic resource	Nest type
Habropoda zonatula	o Fabaceae, Lamiaceae	along clayey roads
Anthophora aestivalis	p	the female digs galleries in the ground
Anthophora bimaculata	p	the female digs galleries in the ground

Anthophora crassipes	o Boraginaceae	---
Anthophora crinipes	p	---
Anthophora furcata	o Lamiaceae	nest carved in dead wood
Anthophora plagiata	p	in vertical clayey walls
Anthophora plumipes	p	in clay walls of human dwellings, in clayey ravines
Anthophora quadrimaculata	p	in walls, in human dwellings where the plaster is coming off
Anthophora retusa	p	---
Anthophora robusta	---	---
Amegilla magnilabris	o Boraginaceae	---
Amegilla quadrifasciata	p	the nest is parasitized by the cuckoo-bee: *Crocisa major*
Amegilla salviae	o Fabaceae, Lamiaceae	---
Eucera cinerea	p	---
Eucera clypeata	p	in hills exposed to the sun and covered with grasses, in clayey terrains with much sand
Eucera dalmatica	o Boraginaceae	---
Eucera helvola	o Fabaceae	---
Eucera interrupta	p	in sunny hills, covered by rare grasses
Eucera longicornis	p	in sunny hills, covered by rare grasses
Eucera nigrescens	p	in clayey-sandy terrains
Eucera nigrilabris	o Lamiaceae	---
Eucera nitidiventris	p	---
Eucera parvicornis	p	---
Eucera pollinosa	p	in clayey-sandy terrains
Eucera taurica	p	---
Tetralonia armeniaca	o Lamiaceae	---
Tetralonia dentata	p	nests piled in compact clayey terrains
Tetralonia hungarica	p	---
Tetralonia lyncea	o Asteraceae	---
Tetralonia salicariae	p	---
Tetralonia scabiosae	o Asteraceae	---
Tetralonia tricincta	p	nests piled in compact clayey terrains
Melecta albifrons	p	cleptoparasite of *Anthophora plumipes, A. parietina, A. crinipes*
Melecta luctuosa	p	cleptoparasite of *Anthophora aestivalis, A. plagiata, A. retusa*
Thyreus ramosus	p	cleptoparasite of *Amegilla albigena*

Thyreus scutellaris	p	cleptoparasite of *Anthophora quadrimaculata*
Pasites maculatus	o Asteraceae, Lamiaceae	cleptoparasite of *Nomia ruficornis*
Biastes brevicornis	o Boraginaceae, Dipsacaceae	cleptoparasite of *Systropha curvicornis*
Biastes emarginatus	o Lamiaceae	cleptoparasite of *Rophites qiunquespinosus*
Epeolus variegatus	p	cleptoparasite of *Colletes daviesanus*
Nomada armata	o Dipsacaceae	cleptoparasite of *Andrena hattorfiana*
Nomada cruenta	---	cleptoparasite of *Andrena scita*
Nomada fabriciana	p	cleptoparasite of *Andrena bicolor*
Nomada ferruginata	p	cleptoparasite of *Andrena sp*
Nomada fucata	p	cleptoparasite of *Andrena flavipes*
Nomada fulvicornis	p	cleptoparasite of *Andrena carbonaria, A. bimaculata*
Nomada goodeniana	p	cleptoparasite of *Andrena cineraria, A. nigroaenea, A. nitida, A. thoracica,*
Nomada mutica	---	cleptoparasite of *Andrena*
Nomada nobilis	o Lamiaceae, Rosaceae	cleptoparasite of *Andrena nasuta*
Nomada obtusifrons	p	cleptoparasite of *Andrena*
Nomada ruficornis	p	cleptoparasite of *Andrena haemorrhoa*
Nomada sexfasciata	p	cleptoparasite of *Eucera longicornis, E. nigrescens*
Nomada stigma	p	cleptoparasite of *Andrena humilis, A. labialis, A. schencki, A. taraxaci*
Nomada zonata	p	cleptoparasite of *Andrena dorsata*
Ceratina acuta	p	in dry plants stems with soft marrow (*Rubus idaeus*)
Ceratina callosa	p	in dry plants stems with soft marrow (*Rubus idaeus*)
Ceratina chrysomalla	p	---
Ceratina cucurbitina	p	---
Ceratina cyanea	p	in stems of dry plants (*Rubus, Euphorbia*).
Ceratina gravidula	p	nests in *Rubus* stems
Ceratina nigroaenea	p	---
Ceratina nigrolabiata	p	nests in *Rubus* stems
Xylocopa valga	p	the female carves vertical galleries in dead trees' trunks, in natural state or worked (linden, willow, fir; oak, beech)
Xylocopa violacea	p	the female carves vertical galleries of about 25 cm length in the rotten trunks of trees; in these galleries food reserves (nectar and pollen) chambers are built.

25% of the identified Anthophoridae species is represented by oligolectic species. These are grouped as follows: 38% feed on Lamiaceae, 19% on Boraginaceae, 14% on Asteraceae and the same percent on Fabaceae. The rest of the species feed on Dipsacaceae and Rosaceae. Similarly to Megachilidae, the polilectic species of Anthophoridae represent the dominant category, comprising 70% of the species. The Anthophoridae with unknown trophic base represent 5% (Fig. 2).

Depending on the nesting habit, the identified Megachilidae can be split into 3 groups: 3 genera with free nests, 11 genera with nests closed in the substratum and 2 genera of nest parasites (Figs. 3-4).

The species with free nests belong to *Anthidiellum, Proanthidium* and *Chalicodoma* genera. While the *Anthidiellum* females build nests of resin, the *Proanthidium* and *Chalicodoma* females build nests of non resin materials, such as: mix of fiber and pubescence from leaves and stems (*Proanthidium*) or mix of different mineral substances, sand, soil and little stones glued with saliva (*Chalicodoma*).

Most Megachilidae build their nests closed in the substratum. *Lithurgus* females build wooden nests, with or without separating walls. The other identified genera use different substrata: soil, wood, dry hollow stems, empty snail shells. Some have completely separated cells (*Anthidium, Paranthidiellum, Trachusa, Anthocopa, Megachile*), other have partly separated cells (*Heriades, Rhodanthidium, Chelostoma, Osmia, Hoplitis*).

Two of the identified Megachilidae genera are cleptoparasite.

Anthophoridae species build their nests in clayey, loamy, sandy soils (*Habropoda, Amegilla, Eucera, Tetralonia,* representing 36%), in dry wood (*Xylocopa,* 14%) and in hollow stems (*Ceratina,* 7%). The species belonging to *Anthophora* genus use a large variety of substrata for nests, namely soils, dry wood and human dwelling walls (Fig. 5). Unlike Megachilidae, a high number of genera are nest parasites: 43% of the identified Anthophoridae species (Fig. 6).

CONCLUSIONS

Based on the observation made in 1995-1998 and 2003-2008 periods, we found that the daily activity of Megachilidae and Anthophoridae is strongly influenced, on the one hand, by the variation of abiotic factors and on the other hand, by the presence of some competitive Apoidae species, as well as by the anthropic factor. As they are thermophille and heliophille species, most of them are distributed in the lowlands and only a few are present also at higher altitudes, up to 900 m. Out of the 128 identified species 60% of Megachilidae and 70% of Anthophoridae species are polilectics, representing the dominant category, the rest being oligolectics (37%, respectively 25%), and a small part is represented of species with an unknown trophic base (3%, respectively 5%).

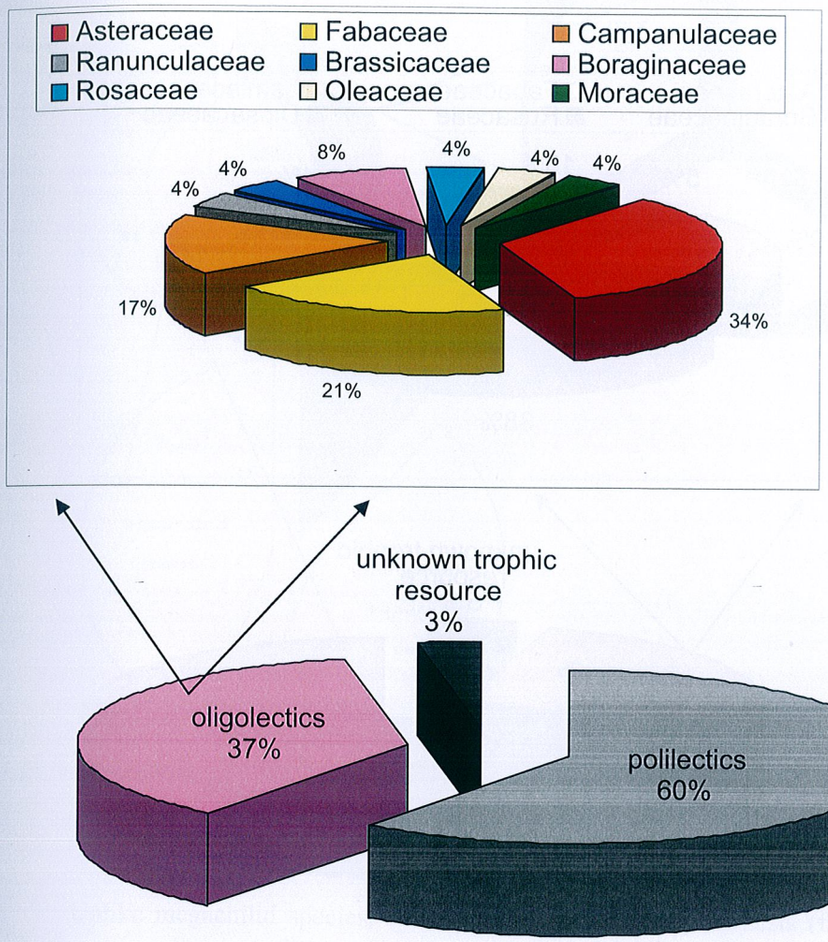

Fig. 1 – The trophic base of identified Megachilidae.

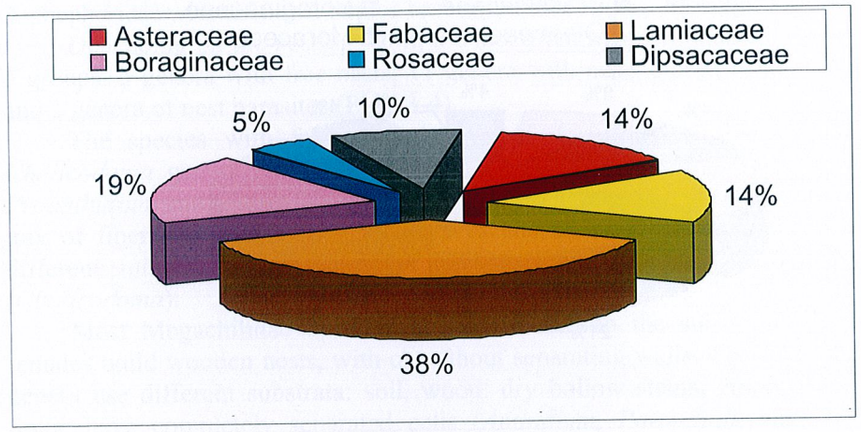

Fig. 2 – The trophic base of identified Anthophoridae.

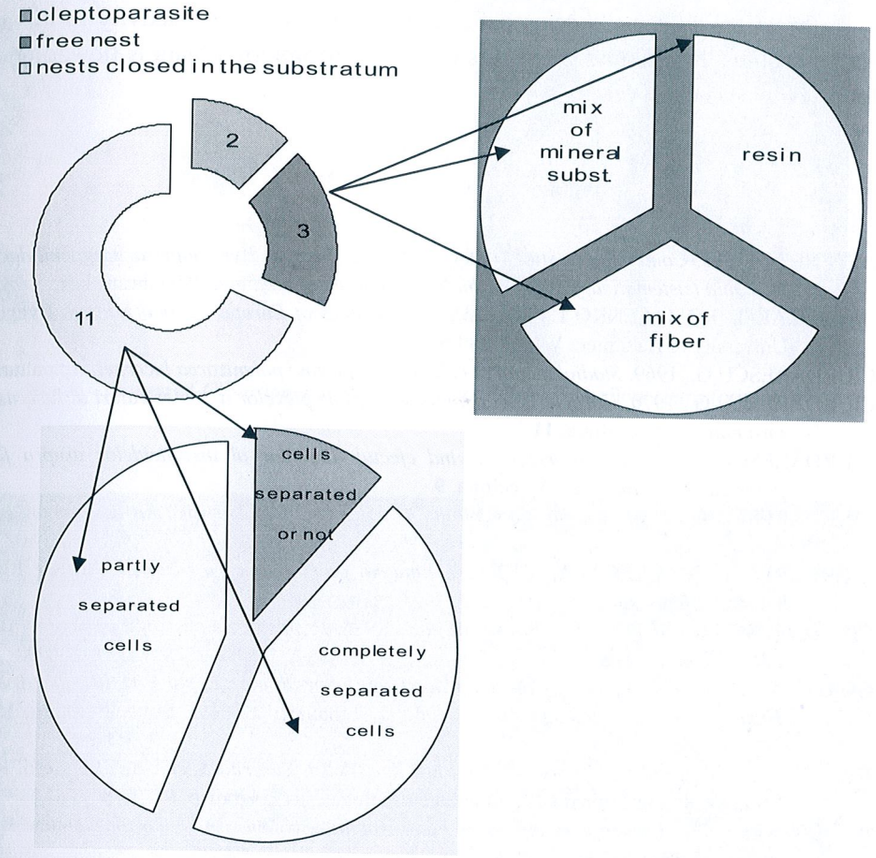

Fig. 3 – Types of nests used by Megachilidae.

Unlike megachilid species, which build a wide variety of nests (free nests glued with saliva to different substrata or nests closed in the substratum), anthophorid species build only nests closed in different substrata. Two of the Megachilidae and six of the Anthophoridae genera are nest parasites.

The efficiency of a bee as pollinator depends upon many factors, respectively the flower structure and the bee's movements. These differ in each bee, as they are partly learned, and are different for different bee species, as they are partly characteristic for the species. Many bee species are specialists in the pollen of a certain plant species and even among generalists, different bee species have

frequently strong preferences regarding the plants from which they collect.

Most solitary bee species have short seasons of flying activity in the adult stage and thus they can be specialists even if their preferred plant is flowering only for a few weeks each year.

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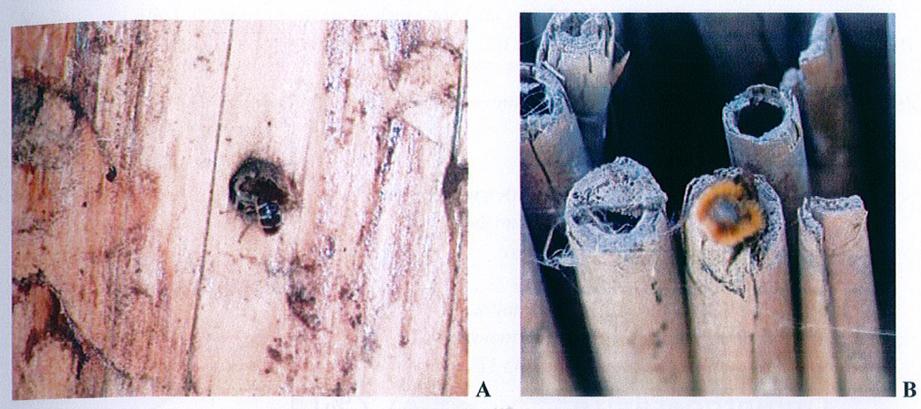

A B

Fig. 4 – A: nest of *Chelostoma* in wood; B: nest of *Osmia* in hollow stems (original).

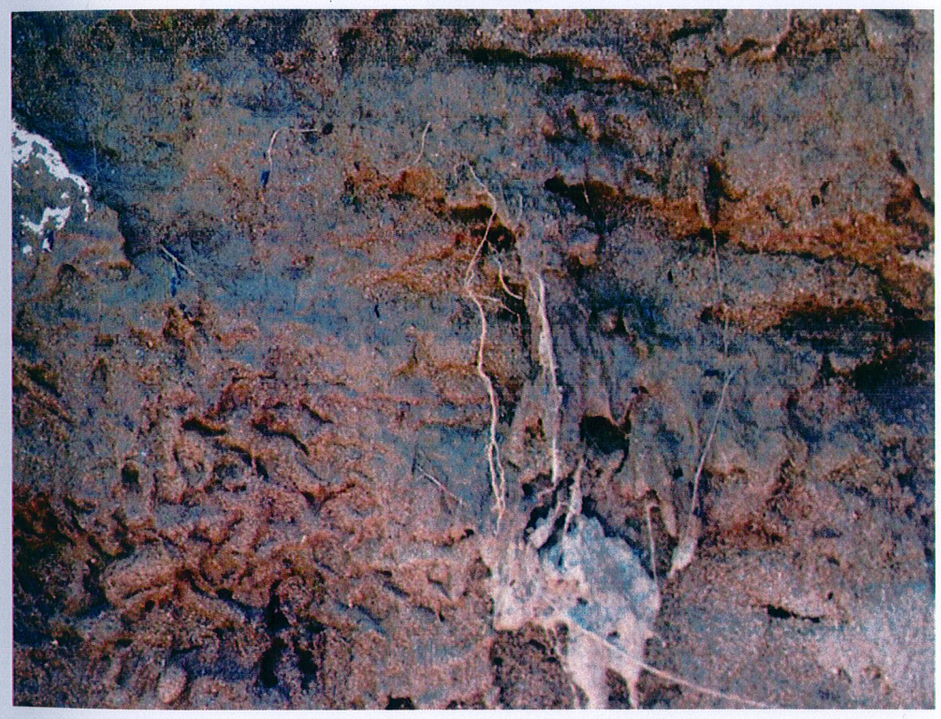

Fig. 5 – Nests of *Anthophora* in soil (original).

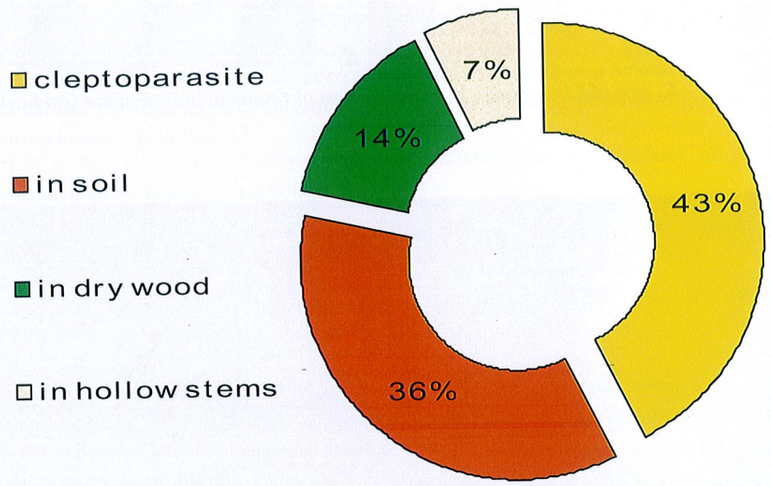

Fig. 6 – Types of nests used by Anthophoridae.

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LEAF BEETLES (COLEOPTERA: CHRYSOMELIDAE) FROM PROTECTED NATURAL AREAS OF ROMANIA

SANDA MAICAN

The paper represents a new contribution to the knowledge of the leaf beetles fauna in nine natural reservations of Romania, situated in the Southern Carpathians, Eastern Carpathians and in the Danube floodplain area. A total of 64 species, from 32 genera, belonging to 9 subfamilies are reported. The presence of some mountainous species with distribution within the Alpine-Carpathian chain is recorded: *Chrysolina hemisphaerica* (Germar, 1817), *C. marcasitica* (Germar, 1824), *C. umbratilis* (Weise, 1887), *Oreina virgulata* (Germar, 1824), *O. alpestris* (Schumell, 1844), *O. cacaliae* (Schrank), *O. intricata* (Germar, 1824), *Sclerophaedon carniolicus* (Germar, 1824), *Neocrepidodera corpulenta* (Kutschera, 1860). The collecting sites are new records for the leaf beetles presence.

Key words: Coleoptera, Chrysomelidae, protected natural areas, Romania.

INTRODUCTION

At present, in Romania there are about 960 protected natural areas (Smaranda, 2007). These are represented by: national parks, natural parks, monuments of nature, natural reservations, special protected areas, special areas of conservation, wetlands of international importance, scientific reservations, biosphere reservations, natural sites of universal natural patrimony, covering about 18% of the country area.

The leaf beetles family (Chrysomelidae) is one of the most numerous within the Coleoptera Order. In Romanian fauna, there are about 570 recorded species, most of them being cited from Transylvania region (Maican, 2005).

Studies concerning the diversity of chrysomelids from different protected areas of Romania were published by: Crişan (1994, 1995, 1996), Maican (2007), Ungureanu *et al.* (2008), etc.

MATERIAL AND METHODS

The material was collected from nine protected areas in Romania, situated in the Southern Carpathians, Eastern Carpathians and in the Danube floodplain region:

- the Sinaia alder forest, Cumpătu (Prahova county);

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- Tâmpa Mountain (Braşov county);
- Stejărişul Mare (Braşov county);
- Postăvaru Peak, Postăvaru Mountain (Braşov county);
- Bogăţii forest, Măieruş-Hoghiz (Braşov county);
- Piatra Mare Mountain (Braşov county);
- Glodeasa forest, Trăisteni, Doftana Valley (Prahova county);
- Giumalău forest, Valea Putnei (Suceava county);
- Gura Vedei-Şaica-Slobozia, including Danube floodplain area, km 518-452 (Giurgiu county), Cama islet and also dam area from around Malu, Găujani, Cetăţuia, Vedea and Pietroşani localities.

The collecting methods used were: sweeping of the herbaceous vegetation using the entomological net, shaking the shrubs and collection directly from plants or soil. The most part of material was collected by the author.

Data on the chrysomelids from the Danube floodplain area were based on the study of the material collected in 2004 by the specialists from "Grigore Antipa" National Museum of Natural History (Bucharest), during the project *Protection of wetlands of the Danube – a pilot project for Cama Dinu islets area (Romania)*.

The specimens were determined on the basis of external morphology and on the study of genitalia, using the following taxonomic studies and monographs: Mohr (1966), Warchałowski (1994, 1995, 2003).

The species are presented in alphabetical order, according to subfamilies (Seeno & Wilcox, 1982).

The examined material is part of the collections from the Institute of Biology (Bucharest) and "Grigore Antipa" National Museum of Natural History.

RESULTS

Sixty four leaf beetles species were identified for the investigated areas, these representing about 10% of the total species known in the Romanian fauna until now. They belong to nine subfamilies, as follows: Donacinae – two species, Criocerinae – five species, Clythrinae – three species, Cryptocephalinae – four species, Chrysomelinae – twenty nine species, Galerucinae – five species, Alticinae – ten species, Hispinae – one species and Cassidinae – five species (Table 1).

It is mentioned, for every species, the collecting site and date, the number of examined specimens and their general distribution.

The occurrence of some mountainous species distributed within the Alpine-Carpathian chain is recorded: *Sclerophaedon carniolicus* (Germar), *Chrysolina hemisphaerica* (Germar), *C. marcasitica* (Germar), *C. umbratilis* (Weise), *Oreina virgulata* (Germar), *O. alpestris* (Schumell), *O. cacaliae* (Schrank), *O. intricata* (Germar) and *Neocrepidodera corpulenta* (Kutschera).

We mention here the presence of *Neocrepidodera femorata* – a boreo-alpine species, distributed in the alpine and subalpine regions (the Alps, Carpathians, Dinaric Alps) and also in the northern part of Scandinavia.

Table 1

The occurrence of the Chrysomelidae in the studied areas

Nr. crt.	Subfamily/species	Material Collecting site/ date/ number of specimens	General distribution
	Subfamily DONACIINAE		
1.	*Plateumaris consimilis* (Schrank, 1781)	Piatra Mare Mountain, Timişu de Sus, 16.05.2006, 1 ♂; Piatra Mare Mountain, Valea pârâului 7 scări, 17.05. 2006, 1 ♂	Palaearctic Region
2.	*Plateumaris sericea* (Linnaeus, 1761)	Piatra Mare Mountain, Dâmbu Morii, 16.05.2006, 1 spec.	Palaearctic Region, from northern Spain to Japan
	Subfamily CRIOCERINAE		
3.	*Crioceris quatuordecimpunctata* (Scopoli, 1763)	Cetăţuia (Găujani), dike km 516, 26.04.2004, 1 spec.	South-eastern Europe, southern part of South Europe, Central Asia
4.	*Crioceris quinquepunctata* (Scopoli, 1763)	Cetăţuia (Găujani), dike km 516, 26.04.2004, 1 spec.; Cetăţuia, 26.04.2004, 6 specs; Malu, km 502, 27.04.2004, 3 specs	Basins of the Danube, Dniepr and Volga
5	*Oulema erichsonii* (Suffrian, 1841)	Bogăţii Forest, Valea cu Stejari, 07.09.2006, 1 ♂, Valea Măieruşului, 07.09.2006, 1 ♂	North and Central Europe from North Spain to Finland, North Russia, Volga basin
6.	*Oulema gallaeciana (*Heyden, 1870)	Piatra Mare Mountain, Dâmbu Morii, 16.05.2006, 6 specs	West Palaearctic Region, from Spain to western Siberia
7.	*Oulema melanopus* (Linnaeus, 1758)	Malu, dike km 502, 27.04.2004, 1 ♀; Piatra Mare Mountain, Dâmbu Morii, 16.05.2006, 1 ♂; Bogăţii Forest, Valea Măieruşului, 07.09.2006, 2 specs; Doftana Valley, Glodeasa Forest, 27.06.2007, 1 spec.	North and Central Europe, from northern Spain to Finland, Russia, Volga basin

	Subfamily CLYTHRINAE		
8.	*Labidostomis longimana* (Linnaeus, 1761)	Bogății Forest, Valea Rădăcini, 24.08.2005, 1 spec.	West Palaearctic region, from France to Mongolia
9.	*Smaragdina affinis* (Illiger, 1794)	Cetățuia (Găujani), dike km 518, 26.04.2004, 3 specs; Pietroşani, km 521, 28.04.2004, 2 specs; Cetățuia, Raif canton, 26.04.2004, 8 specs	Europe
10.	*Smaragdina salicina* (Scopoli, 1763)	Doftana Valley, Glodeasa Forest, 27.06.2007, 1 ♀	Europe, Caucasus
	Subfamily CRYPTOCEPHALINAE		
11.	*Cryptocephalus bipunctatus* (Linnaeus, 1758)	Doftana Valley, Glodeasa Forest, 27.06.2007, 2 specs	Palaearctic Region, from Portugal to Korea
12.	*Cryptocephalus elegantulus* Gravenhorst, 1807	Tâmpa Mountain, Braşov, 19.08.2005, 2 specs (1 ♀, 1 ♂)	Palaearctic Region, from France to Korea
13.	*Cryptocephalus ocellatus* Drapiez, 1819	Cumpătu, Sinaia alder forest, 14.07.2004, 1 ♀	Europe and Kazakhstan
14.	*Pachybrachis sinuatus* (Mulsant & Rey, 1859)	Cumpătu, Sinaia alder forest, 14.07.2004, 1 ♂; Doftana Valley, Glodeasa Forest, 27.06.2007, 1 spec.	South France, Central Europe, Balkans, Turkey
	Subfamily CHRYSOMELINAE		
15.	*Chrysolina fastuosa* (Scopoli, 1763)	Pietroşani, dike km 524, 28.04.2004, 3 specs; Braşov, Stejărişul Mare, 23.08.2005, 1 spec.; Bogății Forest, Valea cu Stejari, 24.08.2005, 23 specs; Bogății Forest, Valea Măieruşului, 07.09.2006, 1 spec.; Piatra Mare Mountain, Timişu de Sus, 16.05.2006, 2 specs; Valea pârâului 7 scări, 17.05. 2006, 2 specs; Piatra Mare Mountain, Dâmbu Morii, 24.07.2006, 2 specs; Tâmpa Mountain, Braşov, 05.09.2006, 1 spec.	Europe, North Turkey, Caucasus
16.	*Chrysolina hemisphaerica* (Germar, 1817)	Piatra Mare Mountain, Valea pârâului Chiva, 17.05.2006, 2 specs. (1 ♂, 1 ♀)	Alps, Sudetes, Carpathians, North Balkans
17.	*Chrysolina herbacea* (Duftschmid, 1825)	Cumpătu, Sinaia alder forest, 14.07.2004, 6 specs; Bogății Forest, Valea Rădăcini, 24.08.2005, 1 spec.; Bogății Forest, Valea Molde, 24.08.2005, 4 specs, Valea cu Stejari, 24.08.2005, 21 specs; Piatra Mare Mountain, Dâmbu Morii, 16.05.2006, 1 spec.; Bogății Forest, Valea Măieruşului, 07.09.2006, 2 specs; Doftana Valley, Glodeasa Forest, 27.06.2007, 1♀	
18.	*Chrysolina marcasitica* (Germar, 1824)	Piatra Mare Mountain, Dâmbu Morii, 16.05.2006, 1 ♂; Piatra Mare Mountain, Timişu de Sus, 16.05.2006, 1 ♂	Alps, Sudetes, Carpathians
19.	*Chrysolina polita* (Linnaeus, 1758)	Cetățuia (Găujani), dike km 516, 26.04.2004, 1 spec.; Bogății Forest, Valea Rădăcini, 24.08.2005, 2 specs, Valea cu Stejari, 24.08.2005, 3 specs; Piatra Mare Mountain, Dâmbu Morii, 16.05.2006, 24.07.2006, 6 specs; Timişu de Sus, 16.05.2006, 1 ♂	From North Spain to China
20.	*Chrysolina sturmi* (Westhoff, 1882)	Cetățuia (Găujani), dike km 516, 26.04.2004, 3 specs; Vedea, km 510, 27.04.2004, 2 specs; Cama islet, 29.04.2004, 1 spec.	From France to Caspian area
21.	*Chrysolina umbratilis* (Weise, 1887)	Piatra Mare Mountain, Timişu de Sus, 16.05.2006, 2 specs	Erzgebirge, Sudetes, Carpathians, ? Alps
22.	*Chrysolina varians* (Schaller, 1783)	Bogății Forest, Valea cu Stejari, 07.09.2006, 2 specs (1 ♀, 1 ♂), Valea Măieruşului, 07.09.2006, 2 ♂♂	From Spain to western Siberia
23.	*Oreina alpestris* (Schumell, 1844)	Postăvaru Peak, 1700 m alt., 25.08.2005, 2 ♂♂	European mountains (excepting Fennoscandia)
24.	*Oreina cacaliae* (Schrank, 1785)	Piatra Mare Mountain, Dâmbu Morii, 16.05.2006, 1 ♂; Valea pârâului 7 scări, 17.05. 2006, 4 ♂♂	European mountains
25.	*Oreina intricata* (Germar, 1824)	Piatra Mare Mountain, Valea pârâului Chiva, 17.05.2006, 1 ♂	Alps, Sudetes, Carpathians, Balkans
26.	*Oreina virgulata* (Germar, 1824)	Piatra Mare Mountain, Timişu de Sus, 16.05.2006, 2 ♂♂; Postăvaru Peak, 1780 m alt., 06.09.2006, 1 ♂	Alps, Apennines, Sudetes, Carpathians, Balkans

27.	*Gonioctena fornicata* (Brüggemann, 1873)	Cetățuia (Găujani), dike km 516, 26.04.2004, 1 spec.; Vedea, km 510, 27.04.2004, 1 spec.	Danube basin, South Poland, Balkan Peninsula, Ukraine, South Russia, Turkey, Caucasus
28.	*Gonioctena linnaeana* (Schrank, 1781)	Piatra Mare Mountain, Dâmbu Morii, 16.05.2006, 2 specs	
29.	*Gonioctena viminalis* (Linnaeus, 1758)	Piatra Mare Mountain, Timişu de Sus, 16.05.2006, 3 specs	From Ireland to Korea
30.	*Gonioctena pallida* (Linnaeus, 1758)	Piatra Mare Mountain, Timişu de Sus, 16.05.2006, 1 ♂	From France to Central Siberia
31.	*Gastrophysa polygoni* (Linnaeus, 1758)	Cetățuia (Găujani), dike km 518, 26.04.2004, 6 specs; Pietroşani, km 524, 28.04.2004, 2 specs; Cama islet, 29.04.2004, 1 spec.; Braşov, Stejărişul Mare, 06.09.2006, 6 specs; Postăvaru Peak, 1780 m alt., 06.09.2006, 1 spec.	Europe, Turkey, Caucasus, Central Asia
32.	*Gastrophysa viridula* (Degeer, 1775)	Postăvaru Peak, 1780 m alt., 25.08.2005, 06.09.2006, 10 specs; Piatra Mare Mountain, Dâmbu Morii, 16.05.2006, 3 specs; Piatra Mare Mountain, Valea pârâului 7 scări, 17.05. 2006, 1 spec.; Doftana Valley, Glodeasa Forest, 27.06.2007, 2 specs	Europe, Turkey, Caucasus, Central Asia, East Siberia, Korea, U.S.A
33.	*Hydrothassa marginella* (Linnaeus, 1758)	Giumalău Forest, 11.09.2006, 1 spec.	North, North-western and Central Europe
34.	*Sclerophaedon carniolicus* (Germar, 1824)	Piatra Mare Mountain, Valea pârâului Chiva, 17.05.2006, 20 specs	Alps, Sudetes, Carpathians
35.	*Chrysomela populi* Linnaeus, 1758	Pietroşani, km 519, 28.04.2004, 1 spec.	From Ireland to Japan, Pakistan
36.	*Chrysomela saliceti* Suffrian, 1849	Piatra Mare Mountain, Timişu de Sus, 16.05.2006, 1 ♂	From France to Mongolia
37.	*Chrysomela vigintipunctata* (Scopoli, 1763)	Piatra Mare Mountain, Dâmbu Morii, 16.05.2006, 2 specs; Timişu de Sus, 16.05.2006, 14 specs	From eastern France to Japan
38.	*Colaphus sophiae* (Schaller, 1783)	Cama islet, 28.-29.04.2004, 5 specs; Pietroşani, km 521, 28.04.2004, 14 specs	Central and South-eastern Europe, Turkey
39.	*Linaeidea aenea* (Linnaeus, 1758)	Piatra Mare Mountain, Dâmbu Morii, 16.05.2006, 13 specs; Timişu de Sus, 16.05.2006, 12 specs; Piatra Mare Mountain, Valea pârâului 7 scări, 17.05. 2006, 1 spec.; Cumpătu, Sinaia alder forest, 18.05.2006, 9 specs.; Doftana Valley, Glodeasa Forest, 27.06.2007, 3 specs	From Ireland to Japan
40.	*Phratora tibialis* (Suffrian, 1851)	Cumpătu, Sinaia alder forest, 14.07.2004, 2 specs; Piatra Mare Mountain, Dâmbu Morii, 16.05.2006, 12 specs	Europe, Turkey
41.	*Phratora vitellinae* (Linnaeus, 1758)	Piatra Mare Mountain, Timişu de Sus, 16.05.2006, 2 ♂♂	From Ireland to Kamchatka
42.	*Plagiodera versicolora* (Laicharting, 1781)	Cumpătu, Sinaia alder forest, 14.07.2004, 1 spec.	Holarctic Region, Pakistan, Taiwan
43.	*Prasocuris phellandrii* (Linnaeus, 1758)	Cetățuia (Găujani), dike km 516, Raif canton, 26.04.2004, 1 spec.	Europe, Turkey
	Subfamily GALERUCINAE		
44.	*Agelastica alni* (Linnaeus, 1758)	Cumpătu, Sinaia alder forest, 14.07.2004, 1 spec.	Palaearctic Region, from Pyrenees to Korea
45.	*Lochmaea caprea* (Linnaeus, 1758)	Bogății Forest, Valea cu Stejari, 07.09.2006, 6 specs	Palaearctic Region, from Spain to Japan
46.	*Galeruca tanaceti* (Linnaeus, 1758)	Bogății Forest, Valea cu Stejari, 07.09.2006, 1 ♀; Giumalău Forest, 11.09.2006, 1 spec.	Palaearctic Region, from Portugal to Japan, introduced in North America
47.	*Galerucella lineola* (Fabricius, 1781)	Cumpătu, Sinaia alder forest, 14.07.2004, 1 ♀	Palaearctic Region, from Ireland to Japan
48.	*Galerucella pusilla* (Duftschmid, 1825)	Malu, dike km 502, 27.04.2004, 1 ♂	West Palaearctic Region, from Catalonia to Mongolia
	Subfamily ALTICINAE		
49.	*Crepidodera aurata* (Marsham, 1802)	Cumpătu, Sinaia alder forest, 14.07.2004, 2 specs (1♀, 1 ♂)	Palaearctic Region, east to Korea
50.	*Chaetocnema coyei* (Allard, 1863)	Cetățuia (Găujani), dike km 516, 26.04.2004, 1 ♂	Romania, Balkan Peninsula, Turkey, Near East, Caucasus, Iran
51.	*Hermaeophaga mercurialis* (Fabricius, 1792)	Cumpătu, Sinaia alder forest, 18.05.2006, 3 specs.	Europe (except Iberian Peninsula), Turkey, Caucasus, Jordan
52.	*Hippuriphila modeeri* (Linnaeus, 1761)	Piatra Mare Mountain, Dâmbu Morii, 16.05.2006, 1 spec.	West Palaearctic Region, to Mongolia
53.	*Phyllotreta armoraciae* (Koch, 1803)	Cumpătu, Sinaia alder forest, 18.05.2006, 1	From East France and South Sweden to

		spec.	eastern Siberia; introduced also in North America
54.	*Phyllotreta tetrastigma* (Comolli, 1837)	Cumpătu, Sinaia alder forest, 18.05.2006, 2 specs	West Palaearctic Region, east to Yakutia, except North Africa and Mediterranean coasts
55.	*Podagrica menetriesii* (Faldermann, 1837)	Pietroşani, dike km 521, 28.04.2004, 1 spec.	southern parts of Central Europe, Turkey, Caucasus, Central Asia, western China
56.	*Neocrepidodera corpulenta* (Kutschera, 1860)	Piatra Mare Mountain, Dâmbu Morii, 16.05.2006, 1 ♂; Timişu de Sus, 16.05.2006, 1 ♂	West Alps, Apennines, Dinaric Alps, mountains of Bulgaria, Carpathians
57.	*Neocrepidodera femorata* (Gyllenhal, 1813)	Piatra Mare Mountain, Valea pârâului Chiva, 17.05.2006, 2 specs (1 ♂, 1 ♀)	North Scandinavia, Alps, Carpathians, Dinaric Alps
58.	*Neocrepidodera ferruginea* (Scopoli, 1763)	Braşov, Stejărişul Mare, 06.09.2006, 1 ♂; Doftana Valley, Glodeasa Forest, 27.06.2007, 3 specs	Azores, Europe, Turkey, Caucasus, Iran
	Subfamily HISPINAE		
59.	*Hispa atra* (Linnaeus, 1767)	Cetăţuia (Găujani), dike km 516, 26.04.2004, 5 specs	Europe, Mediterranean subregion, Caucasus, Central Asia, Mongolia, northern China
	Subfamily CASSIDINAE		
60.	*Cassida murraea* Linnaeus, 1767	Bogăţii Forest, Valea Măieruşului, 07.09.2006, 1 spec.	
61.	*Cassida nebulosa* Linnaeus, 1758	Pietroşani, km 521, 28.04.2004, 1 spec.	Palaearctic Region
62.	*Cassida vibex* Linnaeus, 1767	Malu, dike km 502, 27.04.2004, 2 specs	Palaearctic Region
63.	*Cassida viridis* Linnaeus, 1758	Bogăţii Forest, Valea cu Stejari, 24.08.2005, 1 spec.; Piatra Mare Mountain, Dâmbu Morii, 16.05.2006, 1 spec; Piatra Mare Mountain, Valea Pârâului 7 scări, 17.05. 2006, 1♂; Doftana Valley, Glodeasa Forest, 27.06.2007, 2 specs	Palaearctic Region
64.	*Cassida rubiginosa* Müller, 1776	Cetăţuia (Găujani), km 516, Raif canton, 26.04.2004, 1 spec.; Cama islet, 29.04.2004, 1 spec.	Palaearctic Region; introduced also to Canada

Abbreviations:
spec. (s) – specimen (s); alt. – altitude.

CONCLUSIONS

This study presents new data about the presence of leaf beetles species in nine natural protected areas of Romania, situated in Southern Carpathians, Eastern Carpathians and Danube floodplain area (Giurgiu sector). On the basis of the study of the material collected between 2004 and 2007, a total of sixty four species, of thirty two genera and nine subfamilies, are reported. All the collecting sites are new records for the presence of the leaf beetles species. We consider that there are necessary further researches in order to complete the leaf beetles species inventory.

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RHOPALOCERA (LEPIDOPTERA) OF THE NATURAL RESERVE "THE LIMESTONES OF MĂGURA HILL" (METALIFERI MOUNTAINS, WESTERN CARPATHIANS, ROMANIA)

SILVIA BURNAZ*, CORNEL ALEXANDRU**

During 1985-2007, the author undertook lepidopterological researches in one of the most spectacular natural reserves of Hunedoara County (Romania). This protected area, situated in the southern part of the Metaliferi Mountains, is known as "The limestones of Măgura Hill" and represents only a part of Crăciuneşti Gorges. At present, this area is included in the Site Natura 2000 known as "Băiţa Hills". 88 species of Rhopalocera (Ord. Lepidoptera) have been recorded from this natural protected area. The checklist of the species is given. For each species, data about the preferred habitat, larval and adult food are given. *Zerynthia polyxena*, *Euphydryas aurinia*, *Euphydryas maturna partiens*, *Maculinea arion*, *Maculinea alcon*, *Lycaena dispar rutila*, *Lycaena thersamon*, *Lycaena alciphron,* considered as vulnerable or endangered species according to IUCN criteria, find here optimal conditions to live.

Key words: Rhopalocera, checklist, the limestones of Măgura Hill, Hunedoara County, Romania.

INTRODUCTION

In the southern part of the Metaliferi Mountains (the Western Carpathians), the affluents of Mureş River cross limestone areas and form spectacular gorges. One of these gorges, known as Crăciuneşti Gorges, is situated on the territory of Băiţa locality (Hunedoara County). Here, in 1988 it was limited, on a surface of 120 ha, a protected area named "The Limestones of Măgura Hill". The purpose of the foundation of this reserve was to stop the exploitation of the limestone and protect the landscape, the flora and fauna. At present, the protected area is a part of the Site Nature 2000 known as "Băiţa Hills".

The relief is represented by small calcareous hills (400-500 m altitude) that border the Valley of Căianu River. This area is characterized, from the geological point of view, by the presence of Mesozoic limestone formations.

The importance of the flora and vegetation of this calcareous area was, at first, pointed out by Pop & Hodişan (1964). Rare species like *Fritillaria montana*, *Stippa pennata ssp. eriocaulis*, *Centaurea atropurpurea* var. *crassifolia* f. *integrifolia*, were recorded from the habitats of the Crăciuneşti Gorges. Later, Balazs (1999, 2002) described the saxicolous and arborescent associations. Fauna

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of this natural reserve was less studied. They are not published studies concerning the vertebrate fauna even if this zone is rich especially in reptiles and birds. *Vipera ammodytes* finds here excellent habitats for living and has a great population.

Preliminary data about Macrolepidoptera fauna (Ord. Lepidoptera, S. Ord. Rhopalocera) of this area were published by Burnaz (1992, 2002).

MATERIAL AND METHODS

The investigations on Rhopalocera fauna were carried out in 1985-2007. The lepidopterological material was collected in April-October of each year. The collected specimens are kept in the collection of the Museum of Dacian and Roman Civilisation (Deva, Hunedoara County, Romania).

The following habitats have been selected for sampling:

1. Xerophytic grasslands (As. *Thymetum comosi* Pop & Hodişan 1963 subass. *teucrietosum montani* (Csüros 1958), Coldea 1991));

2. Mesophytic grasslands (As. *Festuco rubrae-Agrostietum capillaris* Horv. (51) 52));

3. Wet meadous (mesohygrophilous and hygrophilous phytocoenoses), spread in Căianu Valley and edified by As. *Epilobio palustri-Juncetum effusi* Obend. 1953, 1957, As. *Agrostetum stoloniferae* (Ujv. 1941) Burduja *et al.*, 1956, As. *Festucetum pratensis* Soó (1938), 1955, 1966 and As. *Ranunculetum repentis* Knapp. 1946 em. Oberd. 1957.

4. Forest edge and shrubs (As. *Corylletum avellanae* Soó 1927, As. *Pruno spinosae-Crataegetum monogynae* (Soó 1927) Hueck 1931) and As. *Sambucetum racemosae* Oberd. 1973).

The frequency of the species was calculated according to their occurrence on the samples. Five stages of frequency were applied according to Rákosy & Viehmann (1991) classification: Frequent species – 6-15 specimens /day; Very frequent species – over 16 specimens/day; Relative frequent species – 1-5 specimens/day; Rare species – 5-10 specimens /generation; Very rare species – 1-4 specimens/generation.

The identification of species, systematics, nomenclature and larval food are based on the following papers: Niculescu (1961, 1963, 1965), Still (1996), Feltwell (2001), Rákosy (2002, 2005), Tolman & Lewington (2007).

RESULTS AND DISCUSSION

88 species were recorded from the Natural Reserve "The limestones of Măgura Hill" (Metaliferi Mountains), that represent 41.50 % of butterflies species of Romania. The check list of the species accompanied by data about the preferred habitats, larval and adult food are given (Table 1).

Table 1

Macrolepidoptera species (Ord. Lepidoptera, S. Ord. Rhopalocera) recorded from "The Limestones of Măgura Hill" Natural Reserve (Metaliferi Mountains, Western Carpathians, Romania)

Taxa	H				L.F.	AD.F.	FR
	XG	M	W	F			
1	2	3	4	5	6	7	8
HESPERIIDAE							
Erynnis tages (Linnaeus, 1758)	-	+	+	+	Fabaceae, Umbelliferae	*Medicago lupulina, Melilothus officinalis, Trifolium montanum, Hypericum perforatum, Leucanthemum vulgare, Dianthus carthusianorum*	F
Carcharodus alceae (Esper, 1780)	-	-	-	+	Rosaceae	*Rosa canina, Rubus caesius*	VR
Carcharodus floccifera (Zeller, 1847)	-	-	-	+	Rosaceae	*Rosa canina, Rubus caesius*	VR
Pyrgus malvae (Linnaeus, 1758)	-	-	-	+	Rosaceae	*Hypericum perforatum, Linum catharticum, Potentilla reptans, Salvia nemorosa, Galium verum, Senecio vulgaris*	VF
Carterocephalus palaemon (Pallas, 1771)	+	+	-	+	Poaceae	*Salvia nemorosa, Potentilla reptans, Viola tricolor*	RF
Thymelicus sylvestris (Poda, 1761)	-	-	-	+	Poaceae	*Hypericum perforatum, Inula hirta, Senecio vulgaris, Leucanthemum vulgare, Salvia nemorosa, Galium verum, Viola tricolor, Potentilla reptans*	RF
Hesperia comma (Linnaeus, 1758)	+	+	+	+	Poaceae Fabaceae	*Viola tricolor, Galium verum, Hypericum perforatum, Leucanthemum vulgare*	VF
Ochlodes venatus faunus Turati, 1905	+	+	+	+	Poaceae	*Lotus corniculatus, Leucanthemum vulgare, Galium*	VF

						verum, Trifolium montanum	
PAPILIONIDAE							
Zerynthia polyxena (Denis & Schiffermüller, 1775)	+	-	-	+	*Aristolochia pallida*	*Knautia arvensis, Trifolium montanum*	R
Parnassius mnemosyne distincta Bryk & Eisner, 1930	-	-	-	+	Papaveraceae: *Corydalis*	*Menta aquatica, Epilobium angustifolium, Eupatorium cannabinum*	RF
Iphiclides podalirius (Scopoli, 1763)	-	+	+	+	Rosaceae	*Sambucus racemosa, Rosa canina, Epilobium angustifolium, Leucanthemum vulgare*	RF
Papilio machaon (Linnaeus, 1758)	+	+	+	+	Umbelliferae	*Eupatorium cannabinum, Epilobium angustifolium, Sambucus nigra, Leucanthemum vulgare, Tanacetum vulgare*	RF
PIERIDAE							
Leptidea sinapis (Linnaeus, 1758)	+	+	+	+	Fabaceae	*Lotus corniculatus, Salvia pratensis, Trifolium pratense, Aster amellus, Scabiosa columbaria, Eupatorium cannabinum*	VF
Anthocharis cardamines Verity, 1908	-	-	+	+	Brassicaceae	*Viola canina, Viola tricolor, Vinca minor*	VF
Pieris brassicae (Linnaeus, 1758)	-	-	-	+	Brassicaceae	*Silene vulgaris, Leucanthemum vulgare, Telekia speciosa, Tanacetum vulgare, Dianthus carthusianorum, Origanum vulgare, Scabiosa columbaria*	R
Pieris rapae (Linnaeus, 1758)	+	+	+	+	Brassicaceae	*Leucanthemum vulgare, Lotus corniculatus,*	VF

						Dianthus carthusianorum, Trifolium montanum	
Pieris napi napi (Linnaeus, 1758)	+	+	+	+	Brassicaceae	*Trifolium campestre, Lotus corniculatus, Dianthus carthusianorum, Epilobium hirsutum, Mentha arvensis, Mentha longifolia, Telekia speciosa*	VF
Colias hyale (Linnaeus, 1758)	+	+	+	+	Fabaceae	*Knautia arvensis, Telekia speciosa, Leucanthemum vulgare, Dianthus carthusianorum, Trifolium, montanum, Sanguisorba officinalis, Lotus corniculatus, Vicia faba, Tanacetum vulgare*	VF
Colias croceus (Fourcroy, 1785)	+	+	+	+	Fabaceae	*Lotus corniculatus, Genista sagitalis, Trifolium pratense, Leucanthemum vulgare, Tanacetum vulgare, Dianthus carthusianorum, Telekia speciosa, Digitalis grandiflora*	RF
Gonepteryx rhamni rhamni (Linnaeus, 1758)	-	-	-	+	Rhamnaceae	*Carduus nutans, Origanum vulgare, Solidago virgaurea, Scabiosa columbaria, Centaurea cyanus, Sambucus racemosa racemosa, Rosa canina*	RF
LYCAENIDAE							
Hamearis lucina (Linnaeus, 1758)	-	-	+	+	Primulaceae	*Trifolium montanum, Knautia arvesis, Solidago virgaurea, Scabiosa columbaria, Centaurea cyanus, Sambucus nigra,*	VF

Lycaena phlaeas (Linnaeus, 1761)	-	-	+	+	Polygonaceae	*Salvia pratensis, Trifolium montanum, Leucanthemum vulgare*	VF
Lycaena dispar rutila (Werneburg, 1864)	-	-	+	+	*Rumex* sp.	*Epilobium angustifolium, Mentha longifolia*	VF
Lycaena virgaureae (Linnaeus, 1758)	-	+	+	+	*Rumex* sp.	*Eupatorium cannabinum, Epilobium angustifolium, Mentha longifolia, Galium verum*	VF
Lycaena tityrus tityrus (Poda, 1761)	-	-	-	+	*Rumex* sp.	*Galium verum*	R
Lycaena alciphron alciphron (Rottemburg, 1775)	-	+	+	+	*Rumex acetosa*	*Galium verum, Leucanthemum vulgare*	R
Lycaena thersamon (Esper, 1784)	+	-	-	+	*Polygonum aviculare*	*Epilobium angustifolium*	R
Thecla betulae (Linnaeus, 1758)	-	-	-	+	Rosaceae	*Sambucus nigra* (fruits)	R
Neozephyrus quercus quercus (Linnaeus, 1758)	-	-	-	+	*Quercus* sp.	*Sambucus racemosa*	R
Callophrys rubi virgatus Verity, 1913	-	-	-	+	Polyphagous (Fabaceae, Cistaceae, Rhamnaceae)	*Leucanthemum vulgare*	RF
Satyrium w-album (Knoch, 1782)	-	-	-	+	*Ulmus glabra*	*Sambucus nigra* (fruits), *Sambucus racemosa (*fruits)	RF
Satyrium spini (Denis & Schiffermüller, 1775)	-	-	-	+	*Rhamnus catharticus*	*Sambucus racemosa*	R
Cupido minimus (Fuessly, 1775)	+	+	-	+	Fabaceae	*Viola tricolor, Tanacetum vulgare, Trifolium pratense, Lotus corniculatus, Genista sagitalis, Astragalus sp.*	F
Cupido argiades (Pallas, 1771)	-	-	-	+	Fabaceae	*Origanum vulgare, Potentilla reptans, Trifolium montanum*	F
Celastrina argiolus (Linnaeus, 1758)	+	+	+	+	Fabaceae	*Lotus corniculatus, Lathyrus sylvestris, Vicia cracca, Scabiosa columbaria, Knauria arvensis*	F

Scoliantides orion lariana Fruhstorfer, 1910	+	-	-	-	*Sedum album, Sedum hispanicum*	*Trifolium montana, Knautia arvensis, Sedum hispanicum*	F
Glaucopsyche alexis (Poda, 1761)	+	+	-	+	Fabaceae	*Lotus corniculatus, Medicago sativa, Potentilla reptans, Hypericum perforatum*	RF
Maculinea alcon (Denis & Schiffermüller, 1775)	-	-	+	-	*Gentiana pneumonanthe, G. asclepiadea*	*Filipendula ulmaria, Agrimonia eupatoria, Leucanthemum vulgare, Linum flavum, Thymus comosus*	R
Maculinea arion (Linnaeus, 1758)	-	+	+	-	Labiatae: *Thymus* sp.	*Solidago virgaurea and different Poaceae*	R
Plebejus argus (Linnaeus, 1758)	+	+	-	-	Fabaceae	*Lotus corniculatus, Potentilla recta, Viola tricolor, Medicago lupulina, Mentha longifolia*	F
Aricia agestis (Denis & Schiffermüller, 1775)	-	+	-	+	*Helianthemum Geranium*	*Lotus corniculatus, Medicago sativa, Trifolium pratense, Mentha arvensis*	F
Polyommatus semiargus (Rottemburg, 1775)	-	+	-	+	Fabaceae	*Medicago sativa, Hypericum perforatum, Lotus corniculatus, Potentilla reptans, Leucanthemum vulgare, Solidago virgaurea, Senecio vulgaris, Aster amellus*	F
Polyommatus icarus (Rottemburg, 1775)	+	+	+	+	Fabaceae	*Genista tinctoria, Aster amellus, Viola tricolor, Potentilla recta, Leucanthemum vulgare*	VF
Polyommatus daphnis (Denis & Schiffer-muller, 1775)	+	-	-	-	Fabaceae	*Hypericum perforatum, Leucanthemum vulgare, Aster amellus, Inula hirta*	F
Polyommatus bellargus (Rottemburg, 1775)	+	-	-	-	Fabaceae	*Dianthus carthusianorum, Leucanthemum*	RF

						vulgare, Aster amellus	
Polyommatus coridon (Poda, 1761)	+	-	-	-	Fabaceae	*Dianthus carthusianorum, Sedum hispanicum*	F
NYMPHALIDAE							
Argynnis paphia (Linnaeus, 1758)	-	+	+	+	Violaceae	*Carduus nutans, Cirsium arvense, Tanacetum vulgare, Leucanthemum vulgare, Centaurea atropurpurea, Cychorium intybus*	VF
Argynnis aglaja (Linnaeus, 1758)	+	+	+	+	Violaceae	*Leucanthemum vulgare, Telekia speciosa, Aster amellus, Solidago virgaurea, Mentha longifolia, Origanum vulgare, Scabiosa ochroleuca, Thymus comosus*	VF
Argynnis adippe (Denis & Schiffermüller, 1775)	+	+	-	+	Violaceae	*Leucanthemum vulgare, Telekia speciosa, Aster amellus, Senecio nemorensis, Solidago virgaurea, Mentha longifolia*	VF
Argynnis niobe niobe (Linnaeus, 1758)	+	+	-	+	Violaceae	*Solidago virgaurea, Leucanthemum vulgare, Origanum vulgare, Thymus comosus, Senecio vulgare, Knautia arvensis, Scabiosa ochroleuca*	VF
Issoria lathonia (Linnaeus, 1758)	+	+	-	+	Violaceae	*Leucanthemum vulgare, Aster amellus, Senecio vulgare, Solidago virgaurea, Centaurea atropurpurea, Dianthus carthusianorum*	F
Brenthis daphne (Denis & Schiffermüller,	+	+	-	+	Rosaceae	*Aster amellus Leucanthemum vulgare, Dianthus*	R

1775)						*carthusianorum, Tanacetum vulgare, Linum tenuifolium*	
Brenthis hecate (Denis & Schiffermüller, 1775)	+	+	-	+	*Filipendula ulmaria*	*Leucanthemum vulgare, Dianthus carthusianorum, Origanum vulgare, Knautia arvensis*	R
Clossiana selene (Denis & Schiffermüller, 1775)	+	+	+	+	Violaceae	*Lotus corniculatus, Medicago sativa, Dianthus carthusianorum, Hypericum perforatum, Leucanthemum vulgare*	VF
Clossiana euphrosyne (Linnaeus, 1758)	+	+	+	+	Violaceae	*Lotus corniculatus, Medicago sativa, Dianthus carthusianorum, Hypericum perforatum, Leucanthemum vulgare, Origanum vulgare*	VF
Clossiana dia (Linnaeus, 1767)	+	+	+	+	Violaceae	*Leucanthemum vulgare, Senecio vernalis, Lamium purpureum, Galium verum, Achillea millefolium, Solidago virgaurea, Silene vulgaris, Stellaria holostea*	VF
Vanessa atalanta (Linnaeus, 1758)	-	-	-	+	Urticaceae	*Rarely on Telekia speciosa, frequently on rotting fruits, tree sap*	VF
Vanessa cardui (Linnaeus, 1758)	-	-	-	+	Urticaceae	*Carduus nutans, Carduus candicans, Centaurea cyanus, Cirsium arvense, Telekia speciosa*	VF
Inachis io (Linnaeus, 1758)	-	-	-	+	Urticaceae	*Rarely on Telekia speciosa, Leucanthemum vulgare, rotting fruits*	F
Aglais urticae (Linnaeus, 1758)	-	-	-	+	*Urtica dioica*	*Aster amellus, Senecio vulgare, Leucanthemum*	RF

						vulgare, Telekia speciosa, Eupatorium cannabinum	
Polygonia c-album (Linnaeus, 1758)	-	-	-	+	*Urtica dioica, Salix, Corylus, Ulmus*	*Urtica dioica, Leucanthemum vulgare, Telekia speciosa,Dipsacus fullonum, Succisa pratensis*	RF
Araschnia levana (Linnaeus, 1758)	-	-	-	+	Urticaceae	*Telekia speciosa, Aster amellus, Urtica dioica, Hypericum perforatum*	F
Nymphalis antiopa (Linnaeus, 1758)	-	-	-	+	Salicaceae	*Rarely on Sambucus racemosa*	RF
Euphydryas maturna partiensis Varga, 1973	-	-	+	+	*Fraxinus excelsior, Salix caprea, Plantago lanceolata, Veronica chamaedrys, Succisa pratensis*	*Fragaria vesca, Chamaecytisus leiocarpus, Anthyllis vulneraria, Primula veris, P. elatior*	VR
Euphydryas aurinia (Rottemburg, 1775)	-	-	+	+	*Succisa pratensis, Scabiosa columbaria, Knautia arvensis*	*Taraxacum officinale, Ranunculus repens, Primula elatior, Potentilla recta, Danthonia provincialis*	F
Melitaea cinxia (Linnaeus, 1758)	+	+	+	+	Polyphagous on different herbaceous plants	*Lotus corniculatus, Medicago sativa, Hypericum perforatum, Leucanthemum vulgare, Tanacetum vulgare*	VF
Melitaea phoebe (Denis & Schiffermüller, 1775)	+	+	+	+	Polyphagous on different herbaceous plants	*Lotus corniculatus, Medicago sativa, Hypericum perforatum, Leucanthemum vulgare, Tanacetum vulgare*	VF
Melitaea didyma (Esper, 1779)	+	+	-	+	Polyphagous on different herbaceous	*Lotus corniculatus, Medicago sativa, Hypericum*	VF

					plants	*perforatum, Leucanthemum vulgare, Tanacetum vulgare, Origanum vulgare*	
Melitaea athalia (Rottemburg, 1775)	+	+	+	+	Polyphagous on different herbaceous plants	*Lotus corniculatus, Medicago sativa, Hypericum perforatum, Leucanthemum vulgare, Origanum vulgare*	VF
Neptis hylas (Linnaeus, 1758)	-	-	-	+	Fabaceae: *Lathyrus* sp.	*Sambucus racemosa* leaves	RF
Neptis rivularis (Scopoli, 1763)	-	-	-	+	*Spiraea chamaedryfolia, Filipendula ulmaria*	*Sambucus racemosa* (leaves)	R
Apatura ilia ilia (Denis & Schiffermüller, 1775)	-	-	-	+	Salicaceae	Dung, carrion	VF
Apatura iris (Linnaeus, 1758)	-	-	-	+	Salicaceae	Dung, carrion	VF
Pararge aegeria tircis (Butler, 1867)	-	-	-	+	Poaceae	*Telekia speciosa, Tanacetum vulgare, Leucanthemum vulgare*	VF
Lasiommata megera (Linnaeus, 1767)	-	-	-	+	Poaceae	*Rarely on Urtica dioica, Leucanthemum vulgare, Tanacetum vulgare*	F
Lasiommata maera (Linnaeus, 1758)	-	-	-	+	Poaceae	*Rarely on Urtica dioica, Leucanthemum vulgare, Taraxacum officinale, Ranunculus repens*	RF
Coenonympha arcania (Linnaeus, 1761)	+	+	-	+	Poaceae	*Achillea millefolium, Trifolium pratense, Trifolium repens, Centaurea cyanus, Medicago lupulina, Lotus corniculatus, Veronica spicata, Digitalis grandiflora, Vicia faba, Filipendula hexapetala*	VF

Coenonympha glycerion (Borkhausen, 1788)	+	+	-	+	Poaceae	*Trifolium repens, Centaurea cyanus, Medicago lupulina, Lotus corniculatus, Veronica spicata, Digitalis grandiflora*	RF
Coenonympha pamphilus (Linnaeus, 1758)	+	+	+	+	Poaceae	*Leucanthemum vulgare, Dianthus carthusianorum, Hypericum perforatum, Scabiosa columbaria, Origanum vulgare, Lathyrus sylvestris, Vicia cracca*	VF
Pyronia tithonus tithonus (Linnaeus, 1758)	+	-	-	-	Poaceae	*Dianthus carthusianorum, Aster amellus*	R
Aphantopus hyperanthus (Linnaeus, 1758)	-	+	-	+	Poaceae	*Leucanthemum vulgare, Dianthus carthusianorum, Aster amellus, Cirsium arvense, Carduus candicans, Lotus corniculatus*	VF
Maniola jurtina (Linnaeus, 1758)	+	+	+	+	Poaceae	*Telekia speciosa, Knautia arvensis, Centaurea atropurpurea, Cirsium arvense, Origanum vulgare, Thymmus comosus, Achillea millefolium Galium verum*	VF
Erebia aethiops aethiops (Esper, 1777)	-	+	-	+	Poaceae	*Aster amellus, Knautia arvensis, Origanum vulgare, Scabiosa columbaria, Centaurea atropurpurea*	RF
Melanargia galathea (Linnaeus, 1758)	+	+	+	+	Poaceae	*Centaurea atropurpurea, Leucanthemum vulgare, Aster amellus, Origanum vulgare, Dianthus carthusianorum, Salvia pratensis,*	VF

						Lotus corniculatus, Scabiosa columbaria	
Minois dryas (Scopoli, 1763)	-	+	-	+	Poaceae	Rarely on *Telekia speciosa*	F
Hipparchia fagi (Scopoli, 1763)	-	-	-	+	Poaceae	Rarely on *Telekia speciosa*	F
Hipparchia semele semele (Linnaeus, 1758)	-	-	-	+	Poaceae	Rarely on *Telekia speciosa*	F
Brinthesia circe pannonica Fruhstorfer, 1911	-	-	-	+	Poaceae	Rarely on *Telekia speciosa*	R

Abbreviations: Frequency: R – Rare species (5-10 individuals/generation); VR – Very rare species (1-4 individuals/generation); F – Frequent species (6-15 individuals/day); RF – Relatively frequent species (1-5 individuals/day); FF – Very frequent species (>16 individuals/day); H – Habitats: XG – Xerothermophilous grasslands; M – Mesophilous grasslands; W – Wet meadows; F – Forest edge and shrubs.

The most of the species are belonging to Nymphalidae family – 42 species, followed by Lycaenidae – 26 species. Hesperiidae is represented by 8 species, Papilionidae – 4 species and Pieridae by 8 species (Fig. 1).

The diversity of species varies among the studied habitats (Fig. 2). 79 species, that represent the majority of the identified species, were collected at the edge of the deciduous forests. Some of them are specific to shrub associations such as *Thecla betulae*, *Neozephyrus quercus* and *Satyrium spini*. 40 species were collected in the limestone area, especially on mesoxerophytic grasslands. *Polyommatus coridon* and *Polyommatus daphnis* are specific to limestone habitats. *Zerynthia polyxena*, *Lycaena thersamon*, *Polyommatus bellargus* and *Scoliantides orion* were also identified in the habitats of the limestone area. 33 species were identified in mesohygrophilous and hygrophilous meadows. Especially, *Euphydryas aurinia* and *Euphydryas maturna partiensis* prefer wet meadows at Căianu Valley. 43 species were collected in mesophilous grasslands that covered the western part of the protected area.

The species with a high frequency are: *Pieris rapae*, *Pieris napi*, *Melitaea athalia*, *Melitaea cinxia*, *Coenonympha pamphilus*, *Coenonympha arcania*, etc. A low frequency (1-4 individuals/generation) have *Neozephyrus quercus*, *Carcharodus alceae*, *Carcharodus floccifera*, *Euphydryas maturna partiensis*.

The trophic spectrum of the larvae was studied (Fig. 3).

In the protected area of Măgura Hill, 71 species are oligophagous and feed on several plants of the same family. For example, Nymphalidae, especially Satyrinae

species and some Hesperiidae species feed on Monocotyledonata plants as Poaceae. Other Nymphalids feed on Violaceae and Urticaceae. Rosaceae are preferred by *Carcharodus alceae*, *Carcharodus floccifera*, *Pyrgus malvae*, *Iphichides podalirius* and *Brenthis daphne*. The species belonging to Pieridae family prefer Brassicaceae and Fabaceae. Polyphagous species exploit a variety of resources. Most polyphagous species are nymphalids, including *Euphydryas aurinia*, *Polygonia c-album*, *Melitatea phoebe*, *Melitaea didyma*, *Melitaea cinxia*, *Melitaea athalia*, *Euphydryas maturna partiensis*. *Zerynthia polyxena*, *Lycaena alciphron*, *Lycaena thersamon*, *Satyrium spini*, *Brenthis hecate* and *Aglais urticae* are monophagous and limited to a particular habitat where their host plants occur.

The adult resources, especially the flowering plants are also studied. The most visited plants are *Leucanthemum vulgare*, *Dianthus carthusianorum*, *Galium verum*, *Origanum vulgare*, *Thymus comosus*, *Scabiosa columbaria*, *Telekia speciosa*, *Aster amellus*, *Knautia arvensis*, *Trifolium montanum*, *Centaurea atropurpurea*, *Hypericum perforatum* and *Salvia nemorosa*. Other species such as *Apatura iris* and *Apatura ilia* feed on nonfloral food like carrion or dung.

Some species with a local distribution in the fauna of Romania were recorded from this area. Flora-Fauna and Habitats Directive as well as the list of the national and communitary interest species which was recently published (Government Urgency Ruling no. 57/2007) also emphasize the importance of the species recorded from this area. The protection of the Lepidoptera species of Romania according to the national legislation and Flora-Fauna-Habitats Directive was also emphasized by Rákosy (2005).

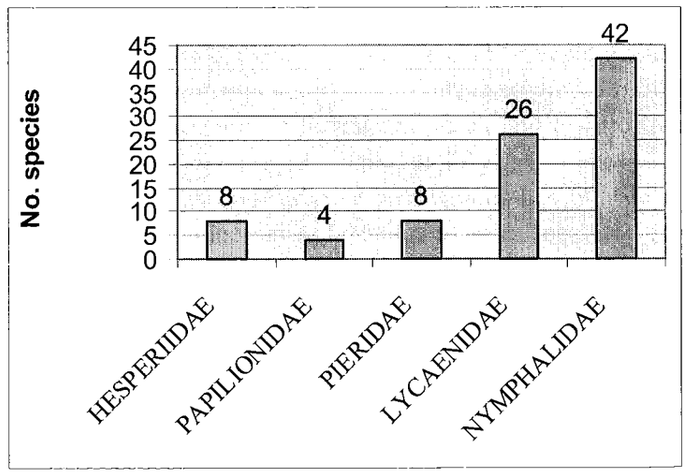

Fig. 1 – Macrolepidoptera families and the number of the collected species.

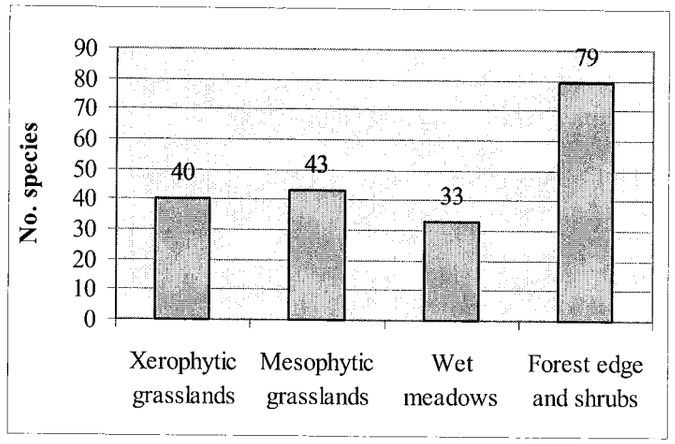

Fig. 2 – Distribution of Macrolepidoptera species among different types of habitats.

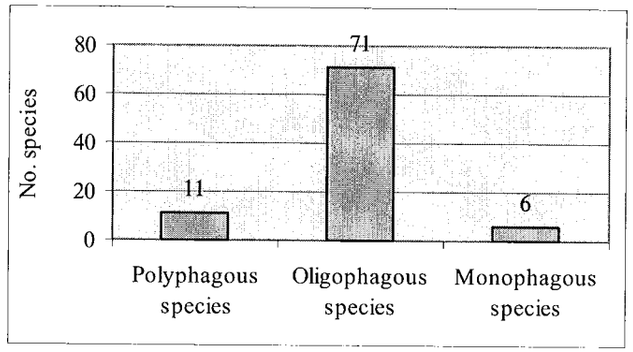

Fig. 3 – The trophic spectrum of the larvae of Macrolepidoptera species.

Zerynthia polyxena polyxena (Denis & Schiffermüller, 1775) (Fig. 4). This species has a wide distribution and it was recorded from Central and Eastern Europe, Western Caucasus, Turkey, southern part of Urals, Kazakhstan and eastern part of Russia. It prefers the hot dry places and the edge of the forests. The adults fly in May. Larvae breed on *Aristolochia pallida*. Adults visit especially *Knautia arvensis* and *Trifolium montanum*. It is listed as an endangered species in "The Red List of Romanian butterflies" (Rákosy, 2002).

Lycaena dispar rutila (Werneburg, 1864) – Euroasiatic species. It is a very common species, found especially in mesohygrophilous meadows (Fig. 5). Adult plant resources are especially *Epilobium angustifolium* and *Menta longifolia*. This species is listed as a vulnerable species in "The Red List of Romanian butterflies" (Rákosy, 2002).

Euphydryas maturna partiensis Varga, 1973 – Euroasiatic species. In the studied area it was found 3♂♂ at 25.05.2006, in a meadow near Căianu Valley. The adults fly in May and prefer wet meadows (Fig. 6). Larvae, polyphagous, breed on *Fraxinus excelsior*, *Populus tremula*, *Salix caprea*, *Plantago lanceolata*, *Veronica chamaedrys*, *Succisa pratensis*. This species is listed as a vulnerable species in "The Red List of Romanian butterflies" and also included in the annex (Rákosy, 2002).

Euphydryas aurinia aurinia (Rottemburg, 1775) – It was recorded especially in the wet meadows situated in Căianu Valley (Fig. 7). The adults fly in May and rarely visit flowery plants such as: *Taraxacum officinale*, *Ranunculus repens*, *Primula elatior*, *Potentilla recta*, etc. Adults prefer to rest on various Poaceae. Larvae breed on *Succisa pratensis*, *Scabiosa ochroleuca*, *Knautia arvensis*, *Digitalis* sp., *Plantago* sp. (Niculescu, 1965). This species is listed as an endangered species in "The Red List of Romanian butterflies" (Rákosy, 2002).

Brenthis hecate (Denis & Schiffermuller, 1775) – It was recorded from Southern Europe, Turkey, Iran, Central Asia and Altai (Tolman & Lewington, 2007). The adults fly in May-July and prefer grasslands and the edge of the deciduous forests (Fig. 8). Larvae, monophagous, breed on *Filipendula ulmaria*. This is considered as a vulnerable species according to IUCN criteria of endangerment (Rákosy, 2002).

Maculinea alcon (Denis & Schiffermuller, 1775) – 1♂, 20.06.2006. This species is spread in Europe and Asia. In the studied area it is a very rare species and occurs in wet meadows, forest margins and sunny rocks. The adults fly in July-August. The flowers selected by adults are *Dianthus carthusianorum*, *Leucanthemum vulgare*, *Origanum vulgare*, etc. Larvae start by feeding on their host plant: *Gentiana pneumonanthe* and then switch to being looked after by ants of Myrmica. The larvae emit surface chemicals (allomone) causing the ants to carry the larvae into their nests. The species is listed as endangered taxa in "The Red List of Romanian butterflies" (Rákosy, 2002). It is also included in the 4B annexe of the Government Urgency Ruling no. 57/2007 as a species of national interest that requires a strict protection.

Maculinea arion (Linnaeus, 1758) – Euroasiatic species. It occurs in dry grasslands, especially with *Thymus serpyllum*, the host plant of its larvae (Fig. 9). The young larvae feed on the leaves of Thymus. Later, at the third larval instar, larvae are attended by *Myrmica sabuleti* or *M. scabrinodis*. This species is also listed as near threatened taxa in "The Red List of Romanian butterflies" (Rákosy, 2002).

According to 4A annex of the Government Urgency Ruling no. 57/2007, *Euphydryas aurinia aurinia*, *Euphydryas maturna partiensis*, *Lycaena dispar rutila*, *Maculinea arion*, *Parnassius mnemosyne distincta* and *Zerynthia polyxena* have a communitary interest and they need a strict protection. *Maculinea alcon* and *Neptis hylas* are included in the annex 4B that contains species of national interest

Fig. 4 – *Zerynthia polyxena.*

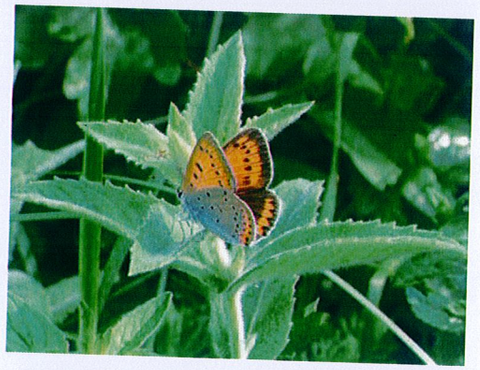

Fig. 5 – *Lycaena dispar rutila.*

Fig. 6 – *Euphydryas maturna partiensis.*

Fig. 7 – *Euphydryas aurinia.*

Fig. 8 – *Brenthis hecate.*

Fig. 9 – *Maculinea arion.*

that need a strict protection. These species are also included in the annexes of the Flora-Fauna-Habitats Directive of the European Union.

CONCLUSIONS

88 species of Butterflies (Ord. Lepidoptera, S. Ord. Rhopalocera) were recorded from the habitats of the protected area named "The Limestones of Măgura Hills" (Metaliferi Mountains, Western Carpathians).

The most of the species belong to Nymphalidae and Lycaenidae families.

In the studied area, the butterflies' communities prefer open habitats, especially the edge of the deciduous forests, shrub associations, mesoxerophilous grasslands and mesophilous grasslands.

Regarding the trophic spectrum of larvae, the majority of the species are oligophagous, followed by polyphagous and monophagous species.

The majority of adult butterflies visit numerous flowering plants for searching nectar. 55 species of flowering plants, visited by butterflies, were noticed in this protected area. *Leucanthemum vulgare*, *Dianthus carthusianorum*, *Galium verum*, *Origanum vulgare*, *Thymus comosus*, *Scabiosa columbaria*, *Telekia speciosa*, *Aster amellus*, *Knautia arvensis*, *Trifolium montanum*, *Centaurea atropurpurea*, *Hypericum perforatum* and *Salvia nemorosa* were the most visited species by butterflies.

Some species like *Zerynthia polyxena*, *Euphydryas maturna partiensis*, *Euphydryas maturna partiensis*, *Maculinea alcon* and *Maculinea arion* are considered vulnerable, endangered or near threatened taxa according to "The Red List of Romanian Butterflies".

Zerynthia polyxena, *Euphydryas maturna partiensis*, *Euphydryas aurinia*, *Lycaena dispar rutila*, *Maculinea arion* and *Parnassius mnemosyne distincta* have a communitary interest and therefore they need a strict protection.

Maculinea alcon and *Neptis hylas* have a national importance and therefore they must be strictly protected.

It seems that *Euphydryas aurinia* has the greatest population in the protected area of Măgura Hill, comparing with other calcareous zones of Metaliferi Mountains and therefore it should be especially protected.

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CORRELATION BETWEEN TYPE OF PEST POPULATION CONTROL (CHEMICAL, BIOLOGICAL, NATURAL) AND DEFOLIATOR LEPIDOPTEROUS POPULATION DYNAMICS

NELA MIAUTĂ*, IRINA TEODORESCU **

Our investigations, in 7 deciduous forests in Romania, 3 infested by *Lymantria dispar* L. and 4 by *Tortrix viridana* L., during 1997-2005, established a correlation between type of pest population control and defoliator population dynamics. The comparison between six forests, chemically (by inhibitory of chitin-synthesis and pyrethrin pesticides) or biologically (by viral and bacterial treatment) controlled, against one forest with natural control (by parasitoids, predators, pathogens) enables to assess the impact of control methods on the pests and entomophagous populations. Previous to chemical or biological treatments, the Lepidoptera defoliator populations were in a progradation, but after these interventions, the defoliator passed into the retrogradation. Bacterial and viral treatments was benefic, favoring the parasitoids and predators action. In forest without human intervention, defoliator populations were in latency, with innocuous effectives, missing defoliation action and with high parasitism degree. Our results underline the necessity to use the biological control methods.

Key words: defoliator, oak forest, *Lymantria dispar*, *Tortrix viridana*.

INTRODUCTION

The defoliator Lepidoptera represented by Lymantriidae, Tortricidae and Geometridae are present in most Romania's deciduous forests and frequently reached outbreak levels. Especially in the Southern part, the majority of oak woods are often heavily infested by defoliator Lepidoptera (Constantineanu & Constantineanu, 1994). Over 76 % of the *Lymantria dispar* attacks occurred in the southern area of the country, especially in the forests of the plains zone. *Tortrix viridana* infested areas were also widespread in the south, but with a more balanced distribution between the hills and plains (Teodorescu & Simionescu, 1994). These insects can cause considerable damage by affecting the trees survival or wood production.

The *Lymantria dispar* larvae are wide polyphagous (over 658 host species), in forests particularly with preference for *Quercus* species. As an olygophagous species, the *Tortrix viridana* larvae attack only *Quercus* species.

In Romania, before pesticide use, some Lepidoptera population dynamics had cyclic gradations, with increases from innocuous to outbreak levels (every 7 years in *Lymantria dispar*). The gradation contains two periods: progradation, with

incipient, numerical increase, eruption or outbreak phases, and retrogradation or population decline, with crisis phase. The outbreak phase initially includes high pest density and defoliation activity, very low ecosystem stability. Soon after, the population collapses registered, with decrease of density level as a result of overpopulation mechanism, by intervention of diseases, parasites, predators, food quality, and reduced fecundity. Between gradations, the population levels are very low, characteristic to latency period (innocuous level), with high ecosystem stability.

The major problem in forests protection is that, after an outbreak, which is very dangerous, to recovery back to latency period. Therefore, we analyzed the possibility to predict gradation phase and degree of defoliation through parasite activity and control measures type (chemical, biological and natural).

MATERIAL AND METHODS

The researches were carried out in 7 deciduous forests, 3 of them being infested by *Lymantria dispar* L. (Lepidoptera, Lymantriidae) and 4 by *Tortrix viridana* L. (Lepidoptera, Tortricidae), located in 5 forest units (Bucharest, Hanu Conachi, Focşani, Tecuci, Grivița), belonging to Bucharest, Vrancea and Galați forest districts.

The chemical pesticides used for *Lymantria dispar* and *Tortrix viridana* populations control were Dimilin SC-48 (1.5 litres/ha), applied in Creața Cernichii forest (1998) and Karate ULV 0.8 ULV (1 litre/ha, a pyrethroid with an insecticide contact and ingestion action), applied in Fundeanu forest (2000).

The microbiological preparations used for forest pest populations control were: viral preparation Inf-Ld (20 g/ha), applied in Leuca forest (1999) against *Lymantria dispar* and bacterial preparation Dipel 8L (1.5 litres/ha, mixed with 1.5 litres of water/ha), applied in Corbu Vechi forest (1999) against *Lymantria dispar* and in Arhipoaia (2003) and Torceşti forests (2003), against *Tortrix viridana.*

In order to determine *Lymantria dispar* gradation's phase, 50 egg masses from each of all three infested forests were collected. Female usually deposit eggs in a single egg mass, covered by hairs sloughed from the abdomen and found on the trunks of trees, near the ground. The eggs were separated of the rests of rhytidome and other impurities, were weighed together at an electronic balance, and the average weight (g) was calculated. The fecundity (F) or average number of eggs per egg mass was determined using formula: $F = 1204.56 \times g + 40.89$; in which, F = average fecundity and g = average weight for one egg mass. Overwintering eggs mortality was determined in October-November by counting the eggs present in 5 egg masses, collected from every control area. After 24 hours keeping in 10% KOH solution, the sterile eggs (having white or orange content) and those with endoparasites (with small, yellow or dark brown larva) were

separated from the fertile eggs (with a hairy *Lymantria* larva). The eggs sterility rate was estimated as ratio of sterile eggs number against total number of eggs (fertile, with parasites and sterile). The real values of fecundity were calculated by subtracting the sterility and mortality values. The eggs parasitism's degree was estimated as ratio of number of eggs with parasites inside to 100 analyzed eggs, from each area. The defoliation percent (Dp) was calculated using the formula: Dp (%) = the average number of fertile eggs / critic number × 100. The critic numbers were established taking into account the species and age of trees, and the defoliator's gradation phase.

Because *Tortrix viridana* female deposits the eggs isolated on the buds, in order to collect its eggs we used a special procedure. Five control trees with uniform distribution in forest were selected, and six branches from each were collected, in September-October interval. The developed buds and the *Tortrix* eggs were counted on each two branches taken from lower, middle and top treetop. The trees infestation with *Tortrix viridana* eggs was assessed by the ratio between the number of eggs and the number of *Quercus* buds. The attack's intensity was calculated as the ratio between the fertile eggs number to the number of buds.

The gradation phase for both defoliator species was established by comparing the values of the average fecundity with the qualitative parameters existing in the forestry literature.

RESULTS AND DISCUSSION

The investigations were carried out in 1997-2005 interval, in order to know the phytosanitary status of the forests attacked by the main defoliators of Romanian deciduous forests, *Lymantria dispar* L. (Lepidoptera: Lymantriidae) and *Tortrix viridana* L. (Lepidoptera: Tortricidae) in correlation to the main control methods of their populations (Table 1). In one forest it was registered an associated attack produced by *Tortrix viridana* and other Lepidoptera species, represented by Geometridae, especially *Operophtera brumata* L., *Erranis aurantiaria* Hb. and *Colotois pennaria* L.

Regarding the populations dynamics of two defoliator species we observed that a latency period, with very low pests effectives, without economic importance was in Broşteni forest, during 1998-2005.

A natural control of pest's populations was present in Broşteni forest, where no treatment was applied.

In the other 6 forests, bacterial or viral treatments, or with inhibitors of insect's chitin or pyretroid pesticide, were applied.

Table 1

The characteristics of forests infested by the main species of Lepidoptera defoliators

Forest/ District	Forest area (ha)	Altitude	Type of station and the composition of trees	Trees age
Creaţa Cernichii/ Bucureşti	158.5	85 – 90	Oak forest (*Quercus cerris*) and *Populus, Salix* species	55 – 60
Leuca/ Galaţi	631.6	7 – 8	Forest steppe, marsh, riverside coppice with *Populus alba, P. canadensis, Salix alba*	20 – 25
Corbu Vechi/ Galaţi	293.8	10	Riverside coppice with *Populus canadensis, P. alba, Salix alba*	15 – 20
Arhipoaia/ Galaţi	57.4	20	Middle forest steppe with *Quercus pedunculiflora*, on sandy deposits with tops of dunes	25 – 68
Fundeanu/ Galaţi	791.7	240	Forest xerophile steppe with *Quercus* species	80
Torceşti/ Galaţi	490	20	Oak forest (*Quercus* sp.)	65 – 80
Broşteni/ Vrancea	334	210 – 570	*Quercus petraea, Fagus sylvatica, Tilia cordata, Acer pseudoplatanus, Fraxinus angustifolia*	20 – 140

Before treatment with chemical or biological pesticides, both defoliator species were in progradation period, within incipient phase, in Arhipoaia forest, and numerical increase phase, in Creaţa Cernichii, Leuca, Corbu Vechi, Fundeanu and Torceşti forests. After the treatment applications, the defoliator populations decreased, passed in retrogradation or in latency periods, with low or very low effectives (Table 2).

The defoliations produced by Lepidoptera larvae had the highest values in 1997 (100 % in Creaţa Cernichii forest), 1998 (80.90 % in Corbu Vechi forest), 1999 (56.96 % in Fundeanu forest) and 2002 (77.31 % in Arhipoaia forest and 56.7 % in Torceşti forest).

In the next two years after Dimilin treatment, the defoliations produced by *Lymantria dispar* in Creaţa Cernichii forest were reduced 12 times, but soon after the defoliation degree they increased again (about five times during 2000-2002).

Table 2

The effects of populations control type on the defoliators gradations

Forest	The main defoliator species	Population control / year of application	The defoliator gradation phase	
			Before treatment	After treatment
Chemical control with the inhibitory of chitin- synthesis and pyrethroid pesticides				
Creaţa Cernichii	*Lymantria dispar*	Dimilin SC-48 (1998)	**Progradation** (1998-1999) Numerical increase phase	**Retrogradation** (1999-2002) Crisis phase
Fundeanu	*Tortrix viridana*	Karate ULV 0.8 ULV 2000	**Progradation** (1998-1999) Numerical increase phase	**Retrogradation** (1999-2003) Crisis phase
Biological control with viral or bacterian treatments				
Leuca	*Lymantria dispar*	Viral treatment Inf-Ld (1999)	**Progradation** (1998-1999) Numerical increase phase	**Retrogradation** (1999-2002) Crisis phase
Corbu Vechi	*Lymantria dispar*	Dipel 8 L 1999	**Progradation** (1998-1999) Numerical increase phase	**Retrogradation** (1999-2003) Crisis phase
Arhipoaia	*Tortrix viridiana*	Dipel 8 L (2003)	**Progradaţion** (Incipient phase)	Latency period
Torceşti	*Tortrix viridana Operophtera brumata Erranis aurantiaria Colotois pennaria*	Dipel 8 L (2003)	**Progradation** (2000-2002) Numerical increase phase	Latency period
Natural control with parasitoids, predators, pathogens				
Broşteni	*Tortrix viridiana*	Natural control	Latency period	

By applying microbial treatment with Dipel, in Corbu Vechi forest, the *Lymantria dispar* defoliation decreased about nine times and these low values maintained during the next four years, and in Arhipoaia forest, the *Tortrix viridana* defoliation reduced more than eight times. The interventions in Fundeanu forest (with pyrethroid Karate ULV) and Torceşti forest (with Dipel) had also good results (Table 3).

The attack intensity was estimated to be very strong in Creaţa Cernichii forest (in 1997) and strong in the other 5 forests (in 1999 in Leuca, Corbu Vechi, Fundeanu and in 2002 in Arhipoaia and Torceşti). After treatment application, the defoliators effective decreased, the populations being in a retrogradation period, crisis phase in four forests (Creaţa Cernichii, Leuca, Corbu Vechi, Fundeanu) and even in latency period, in two forests (Arhipoaia and Torceşti) (Table 4).

Table 3

Dynamics of defoliation degree produced by Lepidoptera larvae

Forest	Year of treatment application	Defoliations produced by Lepidoptera larvae (%)	
		Before treatment	After treatment
Creaţa Cernichii	1998	**1997 = 100.00**	1998-1999 = 6.42-9.17 2000-2002 = 34.41-46.15
Leuca	1999	1998 = 33.76	1999-2002 = 3.28-4.59
Corbu Vechi	1999	**1998 = 80.90**	1999-2003 = 8.88-9.21
Arhipoaia	2003	1996-2001 = 8.94- 19.86 **2002 = 77.31**	2003-2004 = 8.90-9.55
Fundeanu	2000	**1999 = 56.96**	2000-2004 = 7.84-19.90

Torceşti	2003	1998-2001 = 6-12 **2002 = 56.7**	2003-2004 = 3.5-8.8
Broşteni	**1998-2005 = 4.10-9.73**		

The parasitism degree registered in the next years after treatment reflected effect of chemical, viral or bacterial preparation, on the parasitoid populations.

In Creaţa Cernichii forest where Dimilin was applied, the parasitism degree increased only 3.5-4.25 times, because this pesticide was a chitin synthesis inhibitor for both pests and their natural enemies.

In Corbu Vechi forest, after microbial treatment with Dipel, the parasitism degree increased about 10 times, comparatively to the values recorded before treatment application. This microbiological preparation destroys the pests and has no negative effect on the natural enemies (parasitoids, predators), which increased their effectives and contributed to decrease of pests densities.

The Fundeanu forest, where Karate ULV was applied, is an example which demonstrates the negative action of chemical pesticides. In the next three years after treatment application, the parasitism degree increased only 1.7-1.8 times that represents an insignificant action in *Tortrix viridana* population's control.

In Broşteni forest, without human intervention, a high parasitism degree was registered a long period of time, all components of natural control (parasitoids, predators and pathogens) being unaffected by pesticides toxicity, maintained the pest populations at low levels.

Table 4

The attack intensity of defoliators and the average parasitism percentage before and after the treatment application

Forest	**The main defoliator**	**The attack's intensity of defoliators**		**The middle percentage of parasitism (%)**	
		Before treatment	After treatment	Before treatment	After treatment
Creaţa Cernichii	*Lymantria dispar*	Very strong (1997)	Low (1999-2002)	2.40 (1997)	8.60-10.20 (1998-1999)
Leuca	*Lymantria dispar*	Strong (1999)	Very low (2001)	3.2	10-15 (1999-2003)
Corbu Vechi	*Lymantria dispar*	Strong (1999)	Very low (2000-2003)	2.6 (1998)	12.60-26.00 (1999-2002)
Fundeanu	*Tortrix viridana*	Strong (1999)	Low and very low (2000-2004)	7.12-8.28 % (1998-2000)	12.24-15.72 (2000-2002)
Arhipoaia	*Tortrix viridana*	Low (1998-2001) Strong (2002)	Very low (2002-2005)	6.12-7.18 % (1998-2000)	11.14-16.02 (2000-2002)
Torceşti	*Tortrix viridana* *Operophtera brumata* *Erranis aurantiaria* *Colotois pennaria*	Strong (2002)	Very low (2002-2005)	35.13- 48.10 (2002-2005)	36.18- 47.80 (2002-2005)
Broşteni	*Tortrix viridana*	Very low			

In Broşteni forest, the particularity of the attack produced by *Tortrix viridana* was due not only to absence of human intervention, but also to variety of tree species (*Quercus, Fagus*, *Tilia, Fraxinus*, *Acer*), as well as to their different ages (20-140 years old). As a result, in this forest there was a special situation from all points of view (the attack intensity, the defoliations degree, the gradation phase of defoliator populations, and the parasitism degree). The defoliations produced by *Tortrix viridana* had low values (4.10-9.73 %) during 1998-2005 and the attack of other Lepidoptera defoliator species was insignificant. In Broşteni forest all pest populations were maintained at very low densities and intensities of attacks.

The results are in concordance with anterior investigations referring to the role played by biotic factors in regulating, restoring and maintaining factors of the ecological balance (disturbed by man's activities, mainly by the use of pesticides), and to necessity of a correlated management of density level of the two components of the host-natural enemy system (Mihalache & Simionescu, 1990; Simionescu & Teodorescu, 1990; Mihalache *et al.*, 1994; Teodorescu & Simionescu, 1988, 1989, 1994; Teodorescu *et al.*, 2001).

CONCLUSIONS

Long time investigations (1997-2005) in seven deciduous forests in Romania, infested by *Lymantria dispar* and *Tortrix viridana*, established a correlation between these defoliator population dynamics and population control type (chemical, biological, natural).

In six forests, chemical or biological interventions to reduce the defoliator effectives caused a perturbation of the gradation phase, with an interruption of the prograding period (incipient or numerical increase phases) and passing to the retrogradation (crisis phase) or latency periods.

Before treatments, the defoliations produced by Lepidoptera larvae had the highest values in Creaţa Cernichii forest (100 %, in 1997), Corbu Vechi (80.90 %, in 1998) and Arhipoaia (77.31 % in 2002).

The attack intensity produced by *Lymantria dispar* was very strong (in 1997, in Creaţa Cernichii forest) and strong too, in other two forests (Leuca and Corbu Vechi). For *Tortrix viridana*, the strong attack intensity was recorded at the beginning (in Fundeanu forest, in 1999) and in the middle (in Arhipoaia and Torceşti forests, in 2002) of the 1997-2005 intervals.

A reduction of the defoliators attack intensity, from strong (in Corbu Vechi, Leuca, Fundeanu, Arhipoaia, Torceşti forests) or very strong (in Creaţa Cernichii forest) to low or very low, correlated to changes of the gradation phase, due to the treatments applied in the other forests, was registered.

The impact of intervention measures was also reflected in the change of the parasitism degree of *Lymantria dispar* eggs and *Tortrix viridana* pupae. If in the

case of pyrethroid Karate ULV, an increase of the parasitism degree of about 2 times was recorded, in the case of Dimilin and Inf-Ld, it was of about 3-4 times. In the case of bacterial substance Dipel, such values increased about 10 times, an argument to recommend the use with priority of the microbiological substances.

The particular situation of Broşteni forest, with a high diversity of forest species (*Quercus, Fagus, Tilia, Acer*, *Fraxinus*, etc.) and its tree ages, Tortricidae and many Geometridae defoliator species (especially *Operophtera*, *Erranis*, *Colotois*), the absence of pesticide use and the existence of an efficient natural control of populations, represents an argument that a strong multiplication of pests does not exist in the forests which are preserved in natural or semi natural conditions.

For a sustainable management of forests, the foresters in Romania used preponderantly the biological methods, complying with the environment and not affecting the biological diversity. The microbial and viral methods are environmentally acceptable and fully compatible with the natural enemy activity, maintaining the pests at densities that do not damage the forest ecosystems.

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BIOLOGICAL FEASIBILITY OF LABORATORY MAINTENANCE OF THE DEEP SEA HYDROTHERMAL MUSSEL *BATHYMODIOLUS AZORICUS* FOR POST-CAPTURE PHYSIOLOGICAL INVESTIGATIONS AT ATMOSPHERIC PRESSURE

ENIKO KADAR

Harbouring endosymbiotic chemoautotroph bacteria is a typical strategy developed by macro-invertebrates at a variety of reducing marine habitats including deep sea hydrothermal vents, as an adaptation to the harsh conditions. Consequently, a prerequisite of their laboratory maintenance is having control on their endosymbiosis. The methodology used in post-capture manipulations on vent bivalves from various vents with special focus on *Bathymodiolus azoricus* from the Menez Gwen vent site of the Mid Atlantic Ridge (MAR) is reviewed. Evidence is provided for the continuing growth of endosymbionts in bacteriocytes, provided that inorganic nutrients, *i.e.* H_2S for sulphur oxidizers and CH_4 for methanotrophs, are supplied. Lack of hydrothermal gases, however, resulted in gradual disappearance of bacteria from bacteriocytes. Experimental conditions to maintain the hydrothermal vent bivalve for prolonged periods of time, using a range of discriminatory diets to support specific types of endosymbiotic bacteria are described. The potential of using this species as animal model in further physiological studies of the adaptations to extreme environments is discussed.

Key words: hydrothermal vent, *Bathymodiolus azoricus*, post-capture experiment, endosymbiosis.

INTRODUCTION

Over the past 15 years deep sea vent research, especially that involving experimental approach, was limited by sample availability. Biological studies on live specimens were restricted to shipboard studies which were mainly carried out during periods when the weather permitted access to underwater locations (usually summer months). Thus a complete assessment of the life cycle and general physiology of these species was not possible. Laboratory experimentation became possible on various vent species since pressurized flow-through systems became available (Shillito *et al.*, 2001), but were still limited to short post-capture, episodic studies following scientific cruises to vent locations. However, novel technologies, that involved the use of acoustically retrievable mussel cages to supply permanent, land-based laboratories (Kadar *et al.*, 2006 a), considerably extended access to live

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animal samples as well as duration of experimentation and enlargement of the scientific community involved.

The purpose of this review is to present a synthesis on available data concerning the laboratory experimentation on vent bivalves, and to provide supporting evidence for results obtained from in situ experiments. More specifically, we aimed at showing that endo-symbiont prevalence reflects environmental condition, *i.e.* levels of exposure to hydrothermal activity, as reported by Raulfs *et al.* (2004). Emphasis is on physiological adaptations to the vent environment in the hope that readers find these studies motivating for their involvement.

Bathymodiolus azoricus (Von Cosel, 1999) is proposed as the suitable model organism in post-capture investigations for various reasons. Firstly, because it is biomass-dominant species at Mid Atlantic Ridge (MAR) vent sites and thus it is representative to study distinct exposure conditions within these geochemically distinct natural pollution laboratories (Kadar *et al.*, 2007). Secondly, it is euribarofilic being adapted to a baric gradient along the MAR ranging from 850 m at Menez Gwen (37°35'N – 38°N) to 1700 m at Lucky Strike (37°00'N – 37°35'N) (Charlou *et al.*, 2000) and 2300 at Rainbow (36°14'N) (Douville *et al.*, 2002) or even below 3000 m depth at Broken Spur (29°10'N) (Langmuir *et al.*, 1997). Consequently, the hydrothermal mussel is clearly suitable for hydrostatic pressure simulation studies. In addition, its nutritional flexibility to rely on a whole range of food supplies from endosymbiosis (Kadar *et al.*, 2005 a; Fiala-Medioni *et al.*, 2002; Le Pennec & Bejaoui, 2001; Dubilier *et al.*, 1998; Fiala-Medioni *et al.*, 1986) to filter-feeding (Le Pennec & Bejaoui, 2001). The preponderance of the autotrophic vs heterotrophic nutritional processes to total nutrition is suggested to be dependent on the ecological conditions to which the organisms are subjected. Such flexible strategy enables discriminatory studies on selective nutritional reliance on either of the supplies. Finally, being a mytilid bivalve, a widely accepted pollution biomonitor, metal uptake and detoxification can be studied and the results are compared with abundant data available from the literature on its shore analogues from polluted sites (Kadar *et al.*, 2006 b).

Despite the intense international effort over the past two decades to understand functioning of hydrothermal vent ecosystems, there are still critical areas of uncertainty. These include the evolutionary histories of hydrothermal sites; the dispersal mechanisms of hydrothermal vent species; the contributions of the venting systems to thermal and chemical fluxes to the ocean; or what are the genetically imprinted mechanisms, if any, that enable vent species to survive extreme conditions at deep sea hydrothermal vents, to name but a few. LabHorta is the laboratory setup specifically designed for long term maintenance of hydrothermal invertebrates from the sites on the Azores Triple Junction (ATJ) of the MAR (for more details see http://www.horta.uac.pt/projectos/fisiovent/labhorta.htm). It consists of a refrigerated unit within which seawater is supplied with the typical hydrothermal

gases, methane and sulphide, to maintain endosymbiosis in mussels. It provides a variety of feeding regimes under controlled conditions, and has been successful in maintaining the vent mussel for over 12 months in captivity (Kadar *et al.*, 2006 a, b, c).

Four types of distinct diets were developed as follows: 1) the sulphide-feeding that consisted in supplying the aquarium with inorganic S; 2) the methane-feeding regime obtained by diffusion of the gas through the water column; 3) the mixed-feeding was supplying both hydrothermal gases; 4) the filter-feeding regime that was intended to starve out endosymbionts rendering muco-ciliary nutrition.

Using acoustically retrievable mussel cages (Kadar *et al.*, 2006 a) LabHorta can be supplied continuously with live specimens allowing not only the involvement of a larger scientific community, but also most needed seasonal studies on the biology of vent species.

In this review we present a synthesis of the available data concerning the functionality of a laboratory set-up for post-capture experimental investigations with an emphasis on endosymbiont development and subsequent changes in gill ultra structure.

MATERIAL AND METHODS

Sample collection

Mussels are placed into acoustically retrievable cages using the robot arm of Victor6000 of the R/V Atalante (Fig. 1.A). These cages are fitted with floats (Fig. 1 B) with transponders to signal their exact position and also with acoustic release mechanisms for later recovery (Fig 1.D&E). A temperature probe indicates penetration of the vent fluid. They are housing approximately 500 mussels (Fig. 1.C) and provide a major improvement that it enables sampling at times when submersible operation is impossible, and thus it allows investigation of seasonal patterns.

The cages (Sonardyne International Ltd., UK), of 1.25 m^2 size consist of a frame constructed of glass-reinforced plastic, covered in 2 cm wide plastic mesh, and surrounded by a weighted rubber skirt around the cage base to divert sulphide- and methane-laden fluid through the bottom.

Fitness of mussels is indicated by their gill appearance and by foot waving when left undisturbed. During shipment, mussels are held in plastic cool boxes and then are relocated into 40-l volume tanks housed within a refrigerated unit (ambient temperature 8-11°C, water temperature 8.5°C) at atmospheric pressure.

Animal maintenance and experimental conditions

Mussels were placed in 40-l volume aquaria supplied with sand-filtered/UV-treated seawater from an unpolluted bay in Horta, Azores (38.5° N 28.7° W). Aeration was maintained using ordinary aquarium air diffusers and oxygen levels

measured twice a day using portable sensors (WTW Oxical and pH). Four types of specific diets were elaborated targeting different populations of endosymbionts: sulphur oxidizers were maintained on H_2S formed in the water column following administration of a concentrated Na_2S solution (Kadar *et al.*, 2005 a); methanotrophs were supplied with CH_4 dissolved by diffusion of commercially available gas; mussels to harbor both types of endosymbionts were supplied with both gases; and finally the mussels that were not supplied any gas but natural seawater to filter-feed on particles. Water parameters in these four aquaria were listed in Table 1.

A detailed description of the experimental design, the nutrient supplying regime, the equipment used, the analytical monitoring, and long-term data are given elsewhere (Kadar *et al.*, 2005 a on the sulphide-feeding regime and Kadar *et al.*, 2006 c on the methane-feeding regime).

Table 1

Water quality parameters in aquaria providing distinct feeding regimes

Diet	O_2 (% of saturation)	pH	T (°C)	ΣS (μM)	CH_4 (μM)
Sulphide feeding	50 ± 12	7.65 ± 0.15	7.5 ± 0.5	26± 5	
Methane feeding	57 ± 2	7.65 ± 0.15	7.5 ± 0.5	–	**50 ± 9**
Mixed feeding	47 ±12	7.65 ± 0.15	7.5 ± 0.5	20±3	**50±13**
Filter feeding	67 ± 4	7.65 ± 0.15	7.5 ± 0.5	–	–

Tissue preparation to study gill ultra structure

Small (1 mm^3) tissue pieces were fixed in modified Trump's fixative (3% glutaraldehyde and 3% paraformaldehyde made up with a fixation buffer containing: 0.15 M Na-cacodylate, 0.3 M sucrose, 0.2 M NaCl and 0.008 M CaCl. Following primary fixation, samples were washed in 0.1 M cacodylate buffer (pH 7.8), post-fixed in 1% osmium tetroxide in cacodylate buffer for 1 hour, dehydrated in ethanol and embedded in Spurr resin (Sigma). Semi-thin (2 μm) sections were obtained using diamond knife on a LKB-BROMMA ultramicrotome and stained with methylene blue. Ultra-thin sections were mounted on copper grids and were double stained with uranyl acetate and lead citrate.

Five individuals from each experimental group together with a control group, *i.e.* freshly dissected upon collection from the vent, were investigated (3 blocks per individual) and observations were made on filaments detached from the mid portion of the external demibranches.

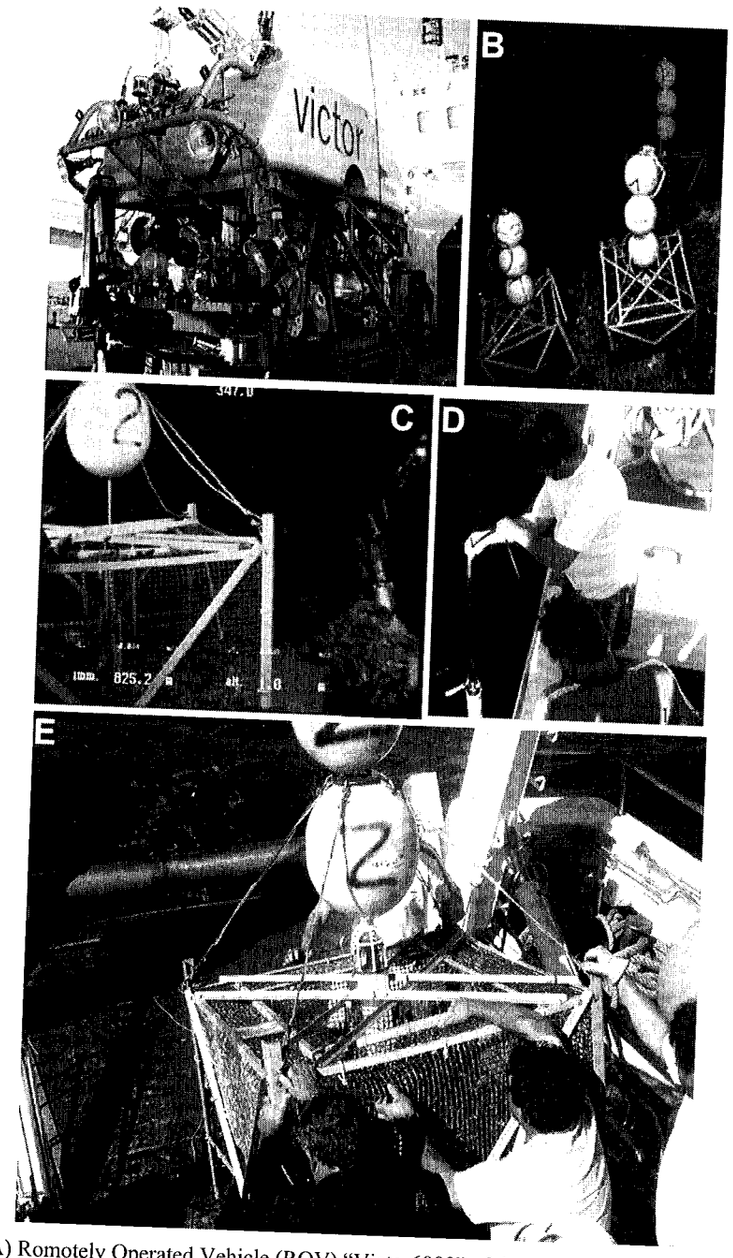
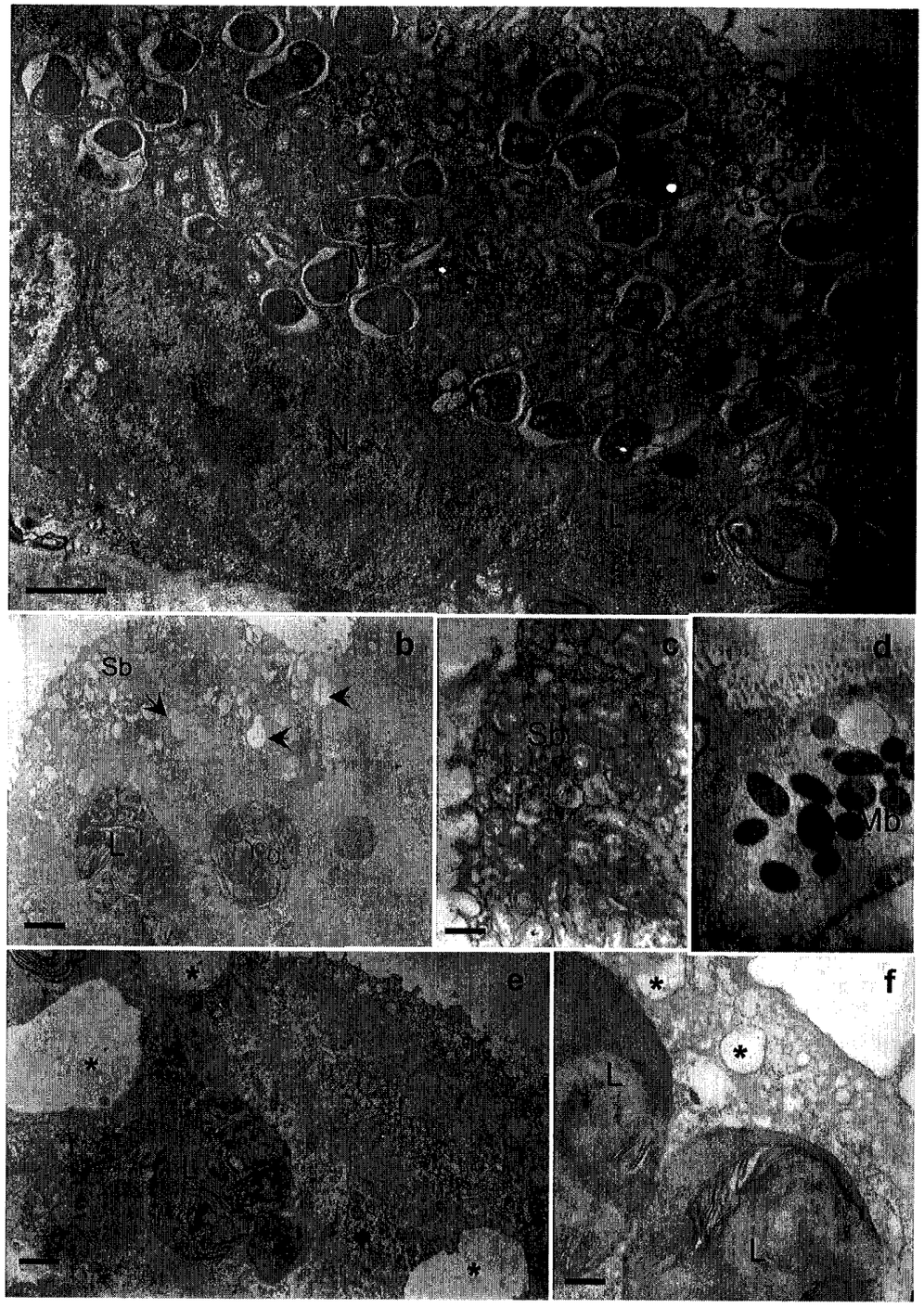


Fig. 1 – A) Romotely Operated Vehicle (ROV) "Victor6000" of the French R/V Atalante used for placing and filling the acoustically retrievable mussel cages (B); cages are filled using the robot arm of Victor 6000 (C) and are fitted with floats with transponders to signal their exact position and also with acoustic release mechanisms for later recovery (D&E).



◆ Fig. 2 – Electron micrographs showing bacteriocyte cells from gill filaments of *B. azoricus* a) specimen dissected freshly upon collection (control) containing both types of symbionts at the apical region; the smaller rod shaped ones are sulfur-oxidizer bacteria (Sb) and the larger are methanotrophic bacteria (Mb), basal nucleus (N) and several lysosomes (L) with membranous content are also visible. Scale bar: 1.6 μm; b) mussels placed in sulphide-supplied seawater showing that methanotrophs are no longer present but several empty vacuoles (arrowheads) and sulfur-oxidizer bacteria (Sb) are observed in bacteriocytes. Scale bar: 2 μm; c) sulfur-oxidizer bacteria (Sb) do not show morphological alterations. Scale bar: 0.5 μm; d) bacteriocytes from animals kept in H_2S-free/CH_4 supplied seawater showing the presence of methanotrophs (Mb) and symbiont-free vesicles. Scale bars: 1 μm; e) bacteriocytes from animals kept in seawater supplied with both H_2S and CH_4 showing the presence of both types of symbionts in the apical region. Note that cells seem damaged by detaching from each other forming large holes (asterisks). Scale bar: 1 μm; f) mussels kept in seawater not supplied with H_2S nor with CH_4 showing disappearance of both types of symbionts leaving behind empty vesicles (asterisks) that confer a spongy aspect to the cell. Note the presence of large lysosmes (L) with membraneous content. Scale bar: 0.5 μm.

RESULTS

Bacteriocytes from freshly collected animals were composed of membrane bound symbionts that occupied the apical region, a nucleus at basal region and several small lysosomes (Fig. 2a). The symbiont-bearing vesicles contained two distinctive types of bacteria: the smaller, rod shaped and more abundant, sulphur oxidizers, and the larger, oval shaped, methanotrophic bacteria with rich membranous content, probably, type I methanotrophs.

The two types of bacteria did not show signs of aggregation within the same vesicle. They both had double membranes (gram negative) with DNA strands found in the centre of an electron-translucent area. The larger methanotrophs also contained folded membranous material (Fig. 2a).

Mussels from the sulphide-feeding regime have lost their methane-oxidising symbionts within one month, but not the sulphur oxidizers that were abundant even after 90 days (Fig. 2b). The apical region of bacteriocytes presented "holes" of empty vacuoles with the size of methanotroph bacteria and large lysosomes with membraneous content on the basal region. The smaller, sulphur oxidizers were present in copious number at the apical region of cells (Fig. 2c).

Ultrastructural changes that occurred as a result of keeping animals in sulphide-free, but methane supplied seawater, *i.e.* the methane-feeding regime, consisted of a reduced volume of bacteriocyte cells due to the disappearance of sulphur-oxidisers. Methanotroph bacteria were still present, but in reduced numbers (Fig. 2d).

The mixed feeding regime rendered damage in the gill filament: bacteriocytes were seemingly in a process of detaching from each other as suggested by the large space between two cells (Fig. 2e). However, few endosymbiont bacteria of both types were still present.

The feeding regime designed to starve endosymbionts by not providing inorganic C supply and thus impose the host to filter-feed on particles from natural seawater, resulted in the most obvious changes in bacteriocytes, where the apical zone appeared spongy due to the loss of both types of bacteria from vesicles (Fig. 2f). The general morphological changes observed on bacteriocytes were an increased incidence of lysosomes, and also, an increase in their size as compared to those in control animals. In addition, the lysosomal content appeared as being in a more advanced degradation stage with unfolded and more heterogeneous membranous material.

DISCUSSION AND CONCLUSIONS

Relatively frequent (3-6 monthly) animal supply is achieved using the acoustically retrievable cage technique that significantly reduced the costs involved and increased flexibility/frequency of sampling (Fig. 1). It is dependent on the ROV only for deployment and filling of cages, recovery can be pursued any season using less expensive vessels at any time of the year. Moreover, our experience also shows that cage-recovery dramatically improved animal fitness as compared to those collected using the classical slurp gun technique.

The main purpose of post-capture experimental studies using the deep-sea hydrothermal mussel *B. azoricus* was to test some hypotheses proposed in other hydrothermal bivalves following in-situ experiments (Raulfs *et al.*, 2004; Fisher *et al.*, 1988) and also to test the suitability of this organism to be used in toxicological investigations. Although improvements will be necessary in the future, the present experiment not only provided evidence for the potential of long term laboratory-maintenance of the vent mussel, but also enabled development of groups with distinct nutritional reliance.

Relatively high survival rate (data not shown) in all experimental aquaria (over 60%) attested the previously suggested highly flexible feeding strategy adopted by *B. azoricus* based on both mixotrophy (filter feeding and symbiosis) and a dual symbiosis (methanotrophic and thiotrophic) (Pond *et al.*, 1998). Such flexibility enabled the mussel to tolerate our discriminatory diets providing energy support for selected endosymbiont bacteria or even non-symbiotic nutrition. Surprisingly, best surviving rates (over 80%, according to Colaço, *pers. comm.*) were reached in the aquarium not supplied with hydrothermal gases in order to allow muco-ciliary nutrition of mussels. Unfortunately, the lack of replication of the experimental treatments, due to both infrastructure and sample limitation associated to deep sea vent research, prevented detection of statistically significant differences between rates of mortality under various diets.

Consequently, making comparison between these diets as nutritional support is highly speculative at this stage, and is beyond the scope of the present study.

However, future physiological condition assessments are being developed to enable detection of such differences, if there is any. More importantly, reasonable survival rate over 90 days in all experimental aquaria attests that the hydrothermal mussel collected from depth of 850m is able to survive at atmospheric pressure in the laboratory, and may be used for a whole range of post-capture ecotoxicological investigations. For instance, specific type of experimentation such as biomechanical recordings on mussel behavior was possible (Kadar *et al.*, 2005 b), which would otherwise be impeded within a hydrostatic pressure chamber owing to instrumental limitations to date.

Moreover, this experiment was valuable in investigating the mechanisms underlying nutritional responses in relation to environmental variations under controlled laboratory conditions. It was successful in maintaining endosymbiosis (both types: methane and/or sulphide oxidisers) in the host vent bivalve. This opens the possibility to use endosymbiont bacteria, otherwise unculturable under laboratory conditions, as a new experimental tool in vent research.

Ultrastructural evidence presented here for the loss of those bacterial symbionts that were deprived from their energy source, unlike those that were supplied with inorganic nutrients. In spite of presenting signs of stress, mussels were able to survive for duration over 3 months either without their endosymbionts, or relying on a single type of bacteria. Whether these signs of stress are nutritionally determined or hydrostatic pressure-related, or both at the same time, remains to be confirmed by future pressure simulation experiments that are under development in our laboratory.

Here we confirm that both types of endosymbionts are naturally present in *B. azoricus* from Menez Gwen and they continue to grow in bacteriocytes, provided that inorganic nutrients, *i.e.* H_2S for sulphur oxidizers and CH_4 for methanotrophs are supplied. Lack of hydrothermal gases, however, resulted in gradual disappearance of bacteria from bacteriocytes. Methanotrophs are likely to be released massively to the blood space. In natural conditions, intra-gill symbiont population seems to be regulated via digestion within the gill lysosomes, while nutrient transfer is more likely to be accomplished through leaking of metabolites from the symbiont to the host, not excluding lysosomal resorption of dead bacteria as an auxiliary strategy for organic molecule transfer (Kadar *et al.*, 2008).

Curiously, supplying the mixture of the hydrothermal gases (H_2S and CH_4) did not support endosymbiont population at a pre-experimental abundance, and also resulted in disrupted cellular organization of the filament indicating that animals were under a stress that may not necessarily be of nutritional origin. The toxic effect of this gas mixture in terms of their ratio as well as chemical behavior under various hydrostatic pressures remains to be investigated. Mussels kept in seawater lacking any hydrothermal gas supply did not show any ultrastructural indication of muco-ciliary feeding. In spite of the total loss of both types of endosymbionts, resulting in thinned bacteriocytes with spongy appearance, these

mussels exhibited highest survival rates among experimental treatments indicating that such minimal experimental requirements may be sufficient in some types of post-capture investigations.

Water quality parameters such as O saturation, temperature and pH and above all hydrostatic pressure are all important factors influencing the physiological processes of the vent mussel. Under anoxic conditions anaerobic sulphide production takes place, as reported by Arndt *et al.* (2001) for several sulphur-storing symbioses, and also confirmed in lab-maintained mussels by Kadar *et al.* (2005 a), concluding an optimal range of O around 30% of saturation. Thus, for a functional experimental set-up, rigorously controlled conditions are essential.

Laboratory maintenance allows for specific investigations to be conducted with a great advantage over the costly and both time- and human-resource-consuming *in situ* observations. By developing a functional experimental set-up for the maintenance of *B. azoricus*, and consequently enabling the preservation and manipulation of endosymbiosis, we provide a basis for a more elaborated ecophysiological research, in order to understand the general principles that govern adaptations to the hydrothermal environment.

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EPITHELIAL-STROMAL INTERACTIONS DURING TUMORIGENESIS AND INVASION PROCESS OF BASOCELLULAR AND SQUAMOUS CELL CARCINOMAS AT THE TUMOR-PERITUMORAL STROMA INTERFACE

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In this paper we focus our interest on the dynamics changes in the carcinomas phenotypes (basocellular carcinoma and squamous cell carcinoma), especially at the tumor-stroma interface corelated with the desmosomal and hemidesmosomal junctions as well as the basement membrane aspects during degradation in the process of tumor cells invasion. Our results showed that tumor cells from both carcinoma types are severely altered phenotypes of the cells they are originated from, but still keeping infrastructures which remember their epithelial origin. Moreover, peritumoral stroma also showed significant alterations. Basal tumor keratinocytes extend numerous and polymorphic cell protrusions which sometimes contain lysosome-like infrastructures which probably facilitate migration of basal keratinocytes by lytic degradation of precarious basement membrane. A drastic reduction of desmosomal junctions of basal tumor keratinocytes with adjacent cells as well as loss of hemidesmosomes increase the freedom of tumor epithelial cells which tend to grow invasively into the host tissue.

Key words: basal cell carcinoma, squamous cell carcinoma, tumor-stroma interface, hemidesmosomal junction, invadopodia.

INTRODUCTION

A tumor is a complex ecosystem formed by neoplastic genetic altered cells and peritumoral stroma represented by cells and extracellular matrix. During the process of malignant tumor formation, genetic alterations are necessary (Sahai, 2005) but this is not a sufficient condition. In order to behave as a real malignat tumor, the existence of a permissive peritumoral stroma is a stringent request. The continuous growth of a neoplastic tumor involves the initiation of tumor angiogenesis able to support respiratory gases exchange and non-innterrupted supply with nutrients required by the tumor-organ (Mueller & Fusenig, 2004; Bissell & LaBarge, 2005).

The large majority of human cancers derive from the epithelial tissues. Essentially, neoplastic epithelial tissues are represented by (1) tumor cells and (2) peritumoral stroma. Epithelial–stroma interactions play a major role during initiation and tumor development. The importance of stromal tissue in regulating

embryo development and the physiological processes of the body is undeniable (Condeelis *et al.,* 2005). Likewise, the role of stromal tissue in supporting the tumorigenic process is also demonstrated (Tlsty & Hein, 2001). For many years, most studies of neoplastic transformation have focused on the unit of the malignant cell. Interactions between neoplastic cells and host tissue (cells and extracellular matrix components) play a major role in the carcinoma architecture and invasive process of malignant epithelial cells.

The step by step evolution of malignant cell phenotype to invasive tumorigenic behaviour involved a cascade of gradually cellular and molecular events which could be very well investigated at high resolution by transmission electron microscopy and immune electron microscopy. Invasion is a phenotypic behaviour of a malignant cell status and a previous process before to metastasis. Tumor progression requires drastic changes in cell-cell and cell-extracellular interactions leading to increased motility and invasiveness. Only a restraint number of malignant cells from primary tumor mass have ability to disseminate and to be located in an ectopic place into organism (often in preferential tissue types, and so far from the primary tumour where these are originated from). There, malignant cells are able to proliferate and to form secondary tumors, the major cause of cancer mortality in humans (Yamaguchi *et al.*, 2005 a). The key to answer the major question of what determines tumor cells remain in one place, retaining their associations with their neighbour cell (tight and desmosomal junctions) and extracellular matrix (hemidesmosomal junctions) or dissociate and move elsewhere to arrest and grow at the ectopic site of metastatic lesion should arise from complex investigations at the ultrastructural and molecular level of changes in cell adhesion properties.

The proteins encoded by the *ras* proto-oncogenes play critical roles in normal cellular growth, differentiation and development in addition to their potential for malignant transformation. Three closely related *ras* oncogenes are known: Ha-, Ki-, and N-*ras* which are capable of transforming mammalian cells when activated by point mutation in several positions (Basset-Seguin *et al.*, 1992; Downward, 1995). Point mutation in the cellular *ras* genes at the amino acids 12, 13 or 61 confer on p21 the ability to transform cell type in culture and, these mutations may be closely linked to the onset of some types of human tumors (Bar-Sagi & Gomperts, 1988).

Spontaneously immortalized human skin keratinocytes (HaCaT cell line) were transfected with the c-Ha-*ras* oncogene *via* a plasmid construct (Boukamp P. *et al.*, 1990).The selected clones with stable integrants of Ha-*ras*-oncogene fell into three classes of c-Ha-*ras* transfected HaCaT cells with respect to postgrafting tumorigenicity: class I clones non-tumourigenic, class II clones which differentiated benign tumors and class III clones which developed highly differentiated, locally invasive squamous cell carcinoma (Fusenig *et al.*, 1990).

In order to get relevant information about malignant behaviour of HaCaT II-4 tumorigenic invasive clone (which belongs to above mentioned class III clones)

and the basement membrane formation and degradation *in vitro* and postgrafting, electronmicroscopic investigations are required.

In previous studies, we showed ultrastructural evidences that isolated normal human skin keratinocytes as precultured *in vitro* on type I collagen gel incorporating dermal fibroblasts, after transplantation onto athymic nude mice express a similar pattern of *in situ* differentiation of epidermal cells including basement membrane ontogenesis related with mature hemidesmosomes (Stark *et al.*, 2004; Boehnke *et al.*, 2007).

Here, we describe the ultrastructure of *in situ* developed basal cell carcinoma and HaCaT-*ras* transfected human keratinocytes malignant clone after transplantation onto athymic nude mice which behave as squamous cell carcinoma or subcutaneously injected HaCaT A5-RT3. We focus our interest on the dynamics of changes in carcinomas phenotype at the tumor-stroma interface in the process of malignant invasion.

MATERIALS AND METHODS

Basal cell carcinoma tissue

Small fragments of tumor skin resulted from a surgical therapy from a patient suffering from basal cell carcinoma (the surgeon got patient consent) were processed for electron microscopic investigation.

Transplantation of HaCaT II-4 cultures onto athymic nude mice and subcutaneously injected HaCaT A5-RT3 cells into athymic nude mice

The use of animal models of human cancers has proved useful in the elucidation of cellular and molecular events which occur during tumour development (Brown & Balmain, 1995; Harris, 1991; Noel *et al.*, 1995; Taghian & Huang, 1995). HaCaT-*ras* clones II-4 (malignant), (transfection procedure was described in detail by Boukamp *et al.*, 1990). Briefly, HaCaT II-4 cells were transplanted onto congenitally athymic nude mice as organotypic culture grown on type I collagen gels (Willhauck *et al.*, 2007). Isolated A5-RT3 cells were subcutaneously injected to athymic nude mice (Vosseler *et al.*, 2005). Transplants as well as subcutaneously developed tumors were excised at 3 weeks and processed for electron microscopic investigation.

Electronmicroscopy

Specimens for electronmicroscopy were pre-fixed in 4% glutaraldehyde in 0.05 M sodium cacodylate buffer pH 7.2 at 4° C for minimum 2h and post-fixed in 2% osmium tetroxide in 0.1 M cacodylate buffer for 2h at room temperature. The specimens were then over night stained in block with aqueous 0.5% uranyl acetate and dehydrated in a graded series ethanol, then infiltrated with propylene oxide followed by embedding in Glycidether 100 (Epon 812 equivalent). Ultrathin sections of 70–90 nm were obtained with a Reichert OM U3 ultramicrotome with a

diamond knife, double counterstained with uranyl acetate and lead citrate, and investigated in an electron microscope operated at 80 kV. 1 μm as semithin sections were stained with toluidine blue for light microscopic examination.

RESULTS AND DISCUSSION

Basal cell carcinoma (BCC)

Quite often, at the interface with associated peritumoral stroma, the basement membrane of epithelial tumor skin is missing or some amorphous material can be seen attached to the plasma membrane facing peritumoral stroma. Some tumor cells exhibited shedding vesicles process. Inside of another tumor cell numerous lysosome-like infrastructures can be detected (Fig. 1, enlarged view in Fig. 2). Keratin filaments do not abut the basal pole because of missing inner plaque of hemidesmosomes (Fig. 2). Moreover, desmosomal junctions between adjacent tumor cells are scarcely organized and an amorphous material can be seen inside of intercellular space (Fig. 3).

Sometimes, basal pole of the tumor cell is filled with a plethora of lysosome-like and apparently empty vesicles (Fig. 4 and Fig. 5). Another characteristic of the tumor basal carcinoma is the process of emitting cell protrusions. Such kind of tumor cell protrusions (termed also invadopodia) penetrate inside of the tumor associated stroma (Fig. 5).

Inside of the stromal tissue located in close vicinity to the tumor mass, impaired blood vessel can be detected (not shown) and extravasated cells penetrate between tumor cells or, even inside of some broken tumor cells (Figs. 6–8).

Squamous cell carcinoma (SCC)

Chimeric neoplastic new-epidermis reconstituted postgrafting from Ha-*ras* transfected HaCaT cells shows deficiencies in differentiation among which we remark: high cell proliferation and no normal cell layered distribution. An overview of a SCC tumor-stroma interface showed that while tumor mass strands penetrate inside of the stroma, strands of the stroma penetrate inside of the tumor mass (Fig. 9).

Between adjacent tumor cells affronted with stroma, most desmosomal junctions are lost or they are impaired and intercellular spaces are filled with numerous thin cell extensions (Fig. 10 and Fig. 11, frame C). Connection of desmosomes with tonofilaments is missing. Sometimes, to some extent, affronted plasma membranes of two adjacent tumor cells are showing recombinant membrane figures associated with clathrin coated vesicles (Fig. 11, frame A, detailed in Fig. 12).

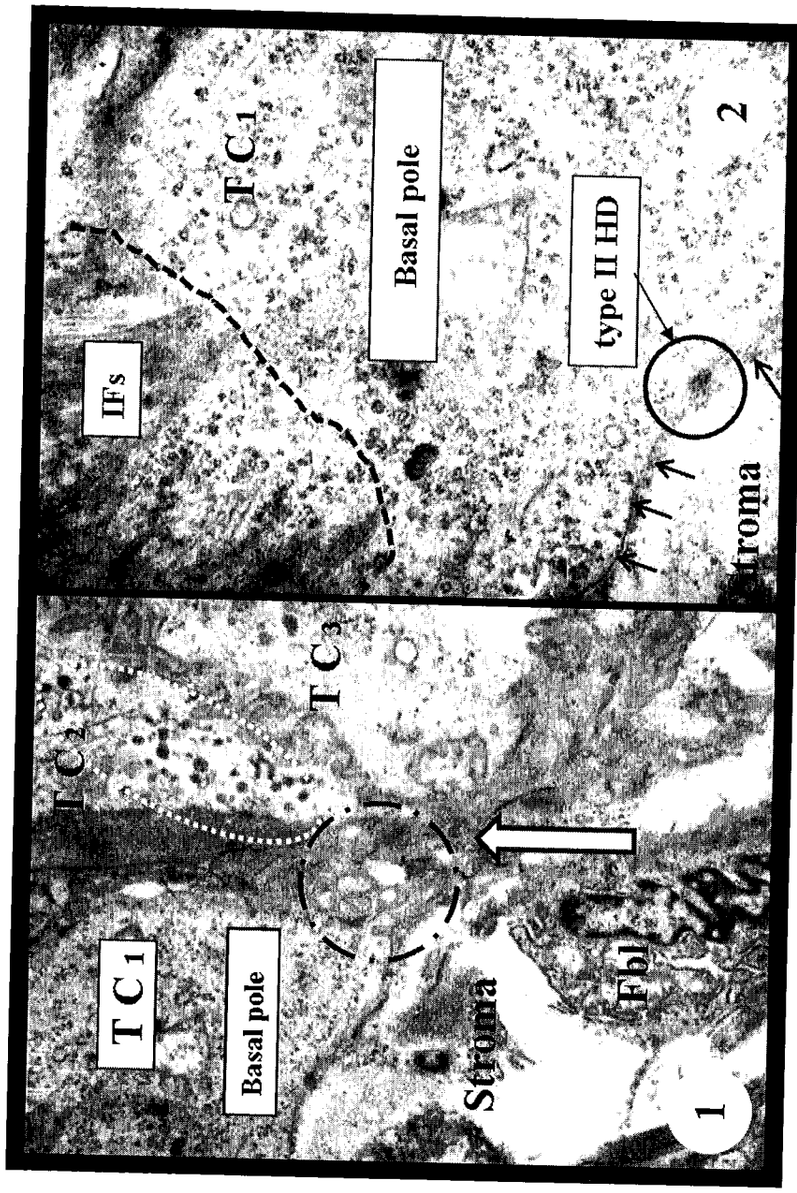

Fig. 1 – Basal pole of few basocellular epithelioma tumor cells (TC1-TC3) at the interface with associated stroma. Encircled area by black circular lines and dots depicts an altered sector of basal pole of tumor cell TC1 by shedding vesicles. Inside of another tumor cell (TC2) numerous lysosome-like infrastructures can be detected (white ellipsoidal depicted area). Fbl = fibroblast. (× 10,500).

Fig. 2 – Enlarged view from the tumor cell TC1 in Fig. 1. Keratin filaments (KFs) do not abut the basal pole because of missing inner plaque of hemidesmosome (encircled area). (× 10,500).

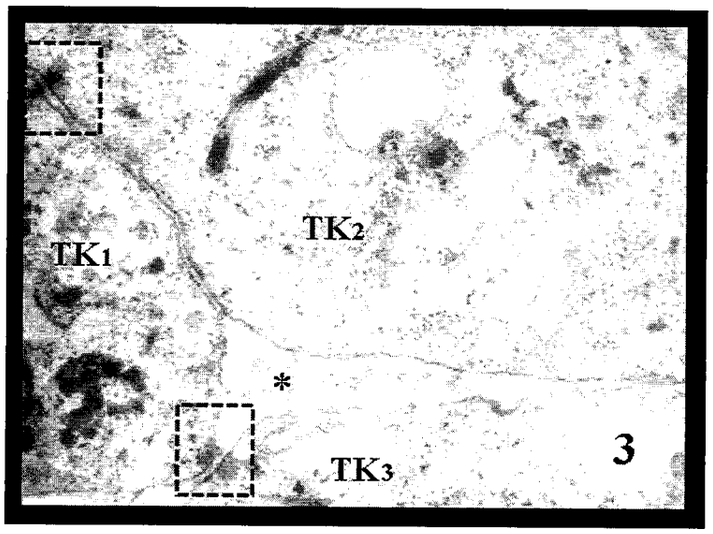

Fig. 3 – Desmosomal junctions between tumor cells are scarcely organized and an intercellular space filled by an amorphous material can be seen. (× 34,000).

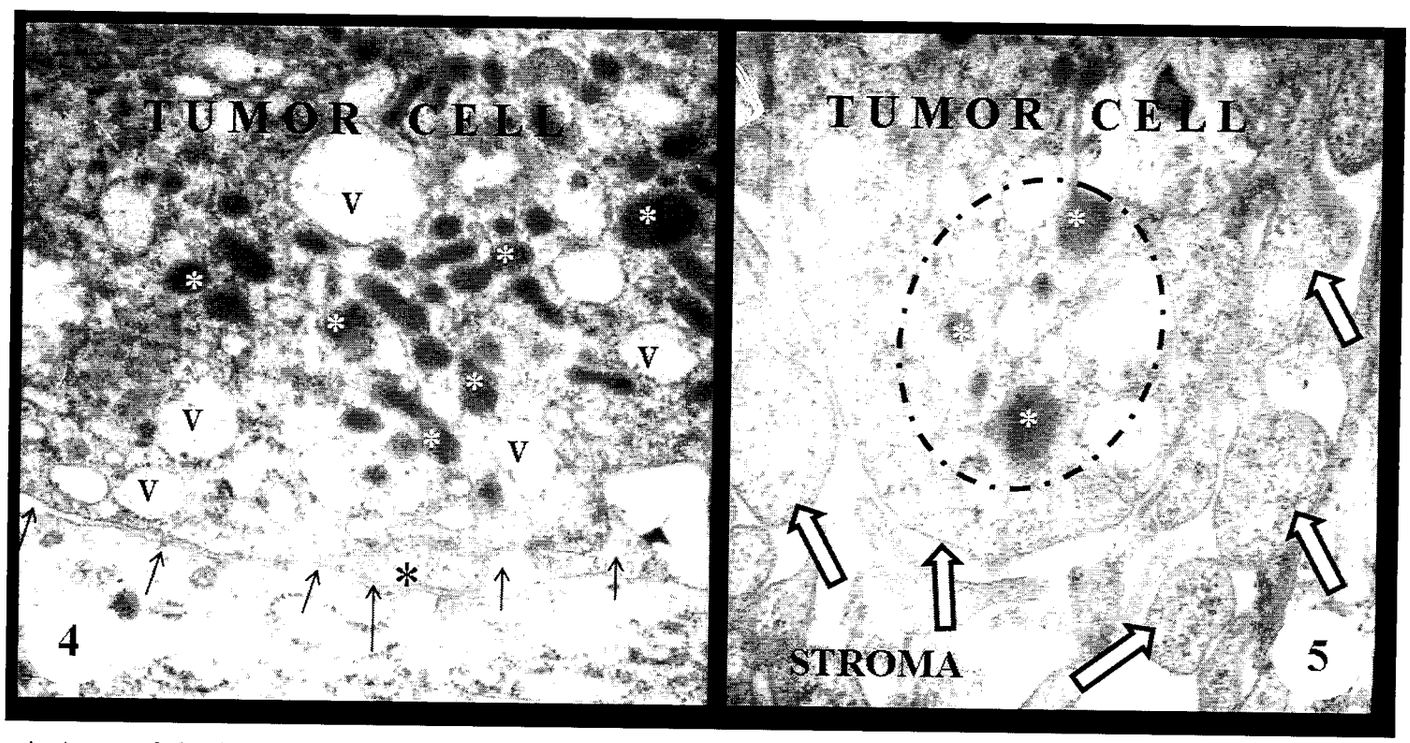

Fig. 4 – A sector of a basal pole in a tumor cell showing a plethora of lysosome-like (white asterisks) at the tumor-stroma interface but no basement membrane can be detected. Black asterisk marks a tumor cell protrusion. (× 29,000).

Fig. 5 – Numerous invadopodia (white arrows) from a tumor cell penetrate inside of the peritumoral stroma. Inside of a large invadopodium lysosomes (white asterisks) can be seen. (× 30,000).

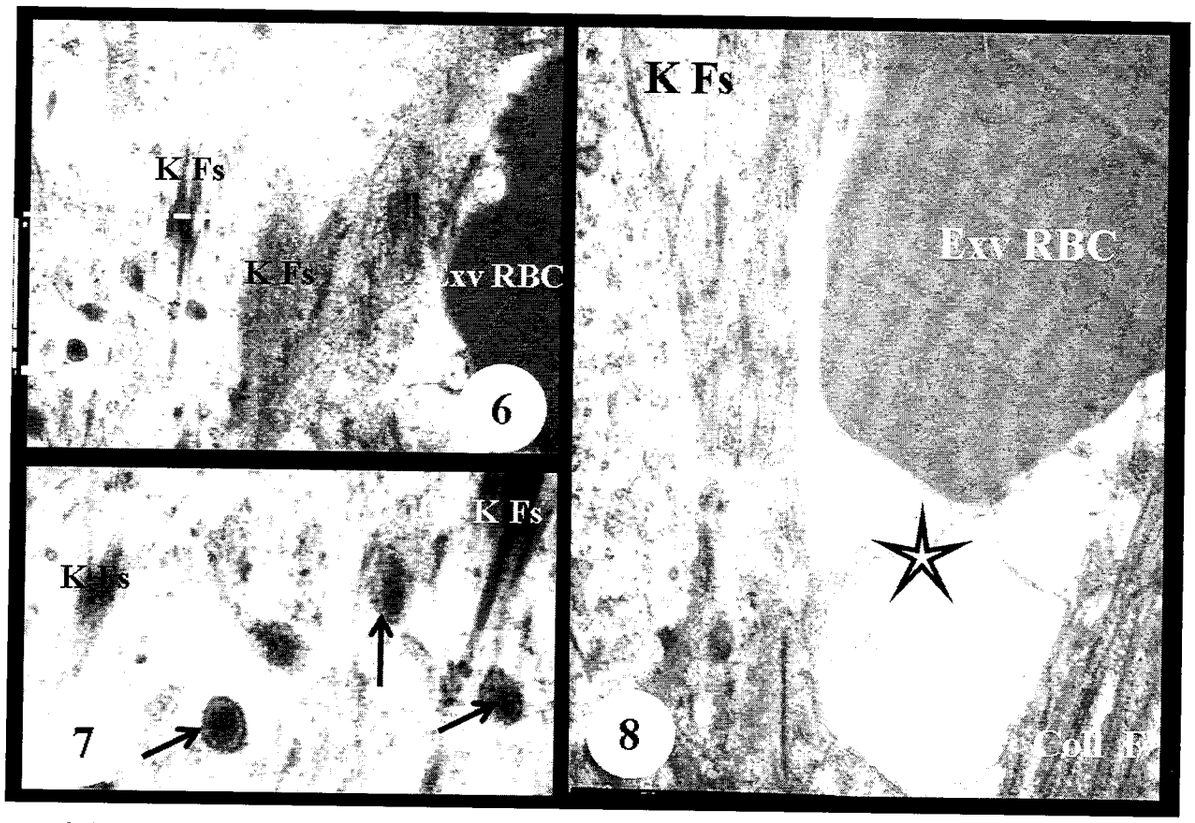

Fig. 6 – A sector of a broken tumor cell. In close vicinity an extravasated red blood cell (Exv RBC) can be seen. White delineated area marks the presence of lysosomes detailed in Fig. 7. KFs = keratin filaments. (× 19,000).
Fig. 7 – Enlarged area framed in Fig. 6. Arrows mark individual lysosomes. KFs = keratin filaments. (× 40,000).
Fig. 8 – Inside of a broken tumor cell (star) penetrates an extravasated red blood cell (Exv RBC). (× 34,000).

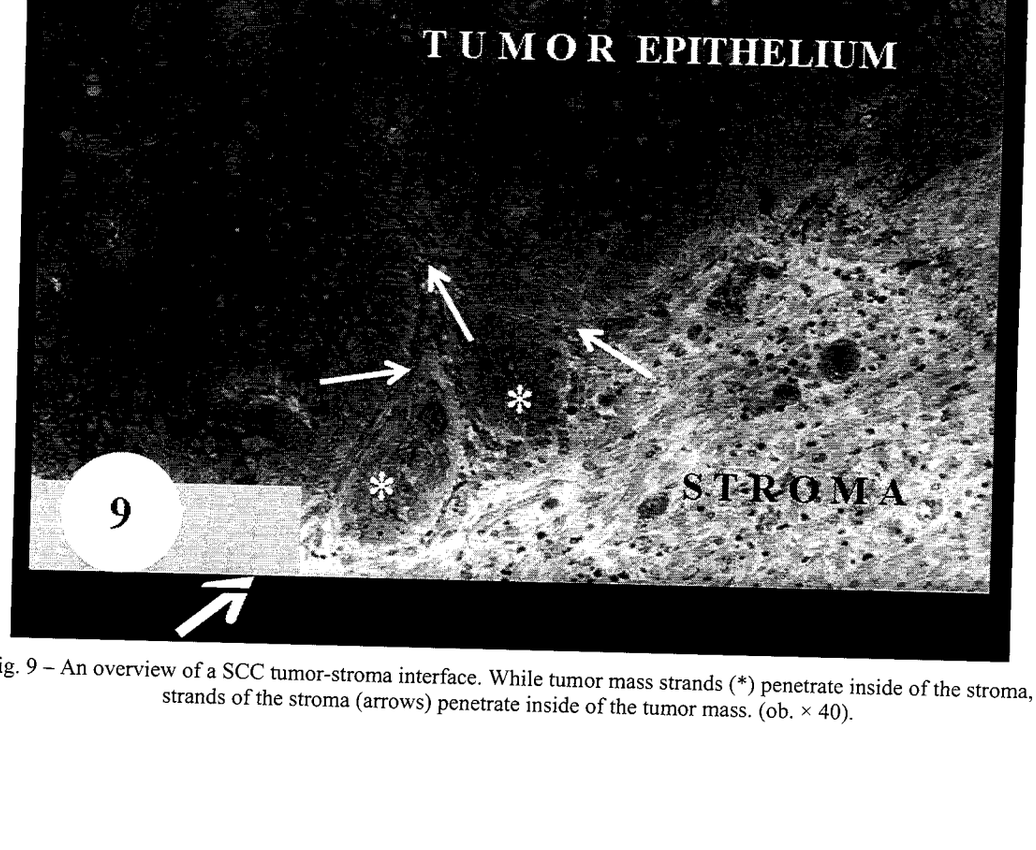

Fig. 9 – An overview of a SCC tumor-stroma interface. While tumor mass strands (*) penetrate inside of the stroma, strands of the stroma (arrows) penetrate inside of the tumor mass. (ob. × 40).

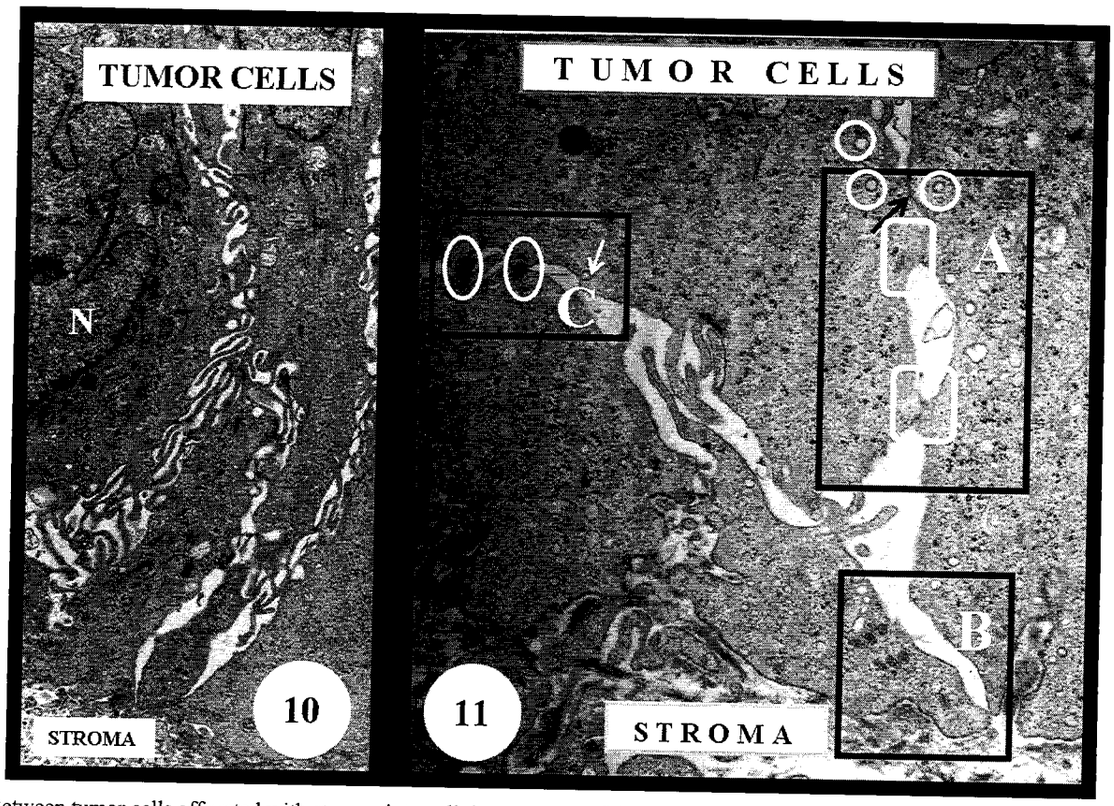

Fig. 10 – Between tumor cells affronted with stroma, intercellular spaces are filled with numerous thin cell extensions. N = nucleus. (× 12,700).
Fig. 11 – An overview from the basal pole of few tumor cells facing each other or peritumoral stroma. Intercellular spaces are large. Desmosomal junctions are almost missing, but where they are present (elliptic areas in C), they are impaired and their connection with tonofilaments is missing. Sometimes, to some extent, affronted plasma membranes of two adjacent tumor cells are showing recombinant membrane figures (white frames in A). Inside of the edge of some invadopodia there are lysosomes; hemidesmosomes are missing (frame B). (× 19,000).

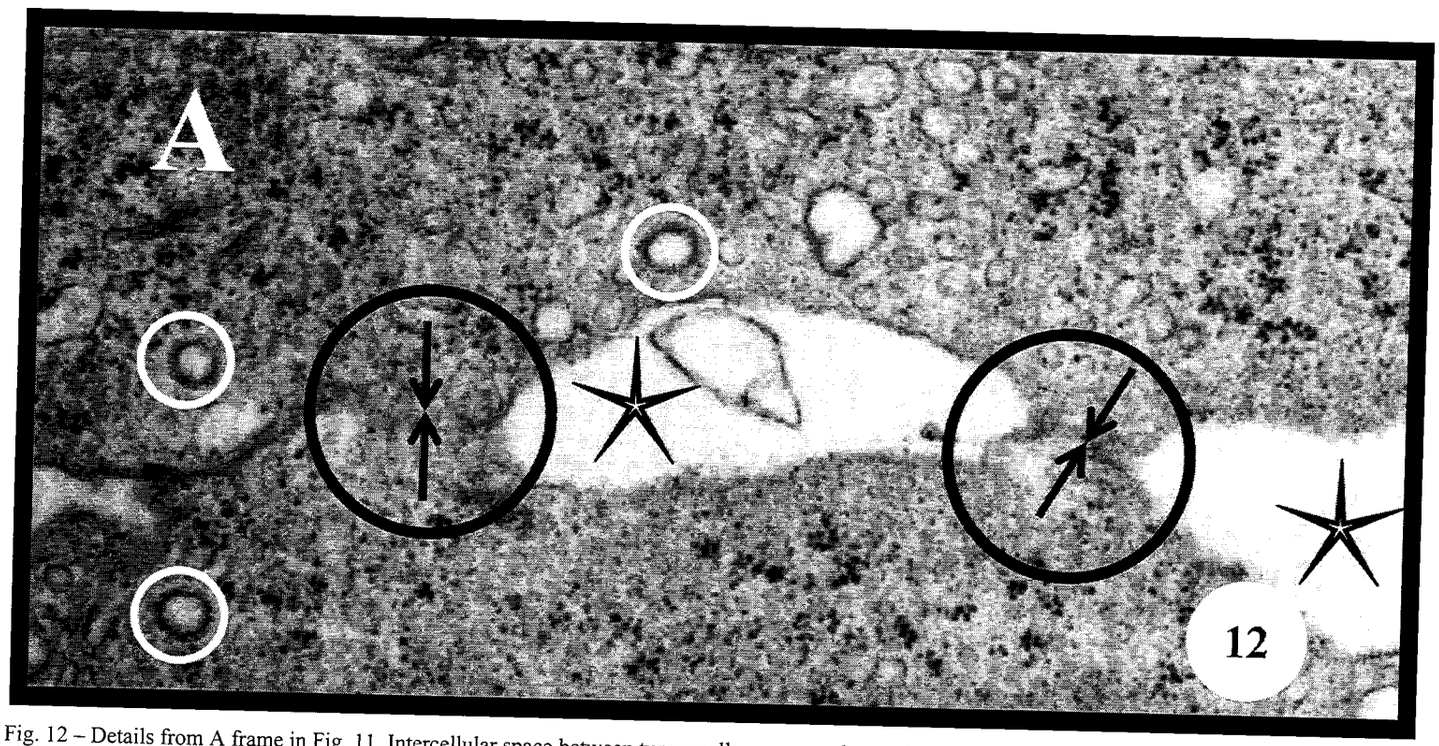

Fig. 12 – Details from A frame in Fig. 11. Intercellular space between tumor cells appears enlarged (stars). Affronted arrows in encircled black area depicted recombinant plasma membranes belonging to two adjacent tumor cells. In close vicinity to plasma membrane, clathrin coated vesicles. (× 65,700).

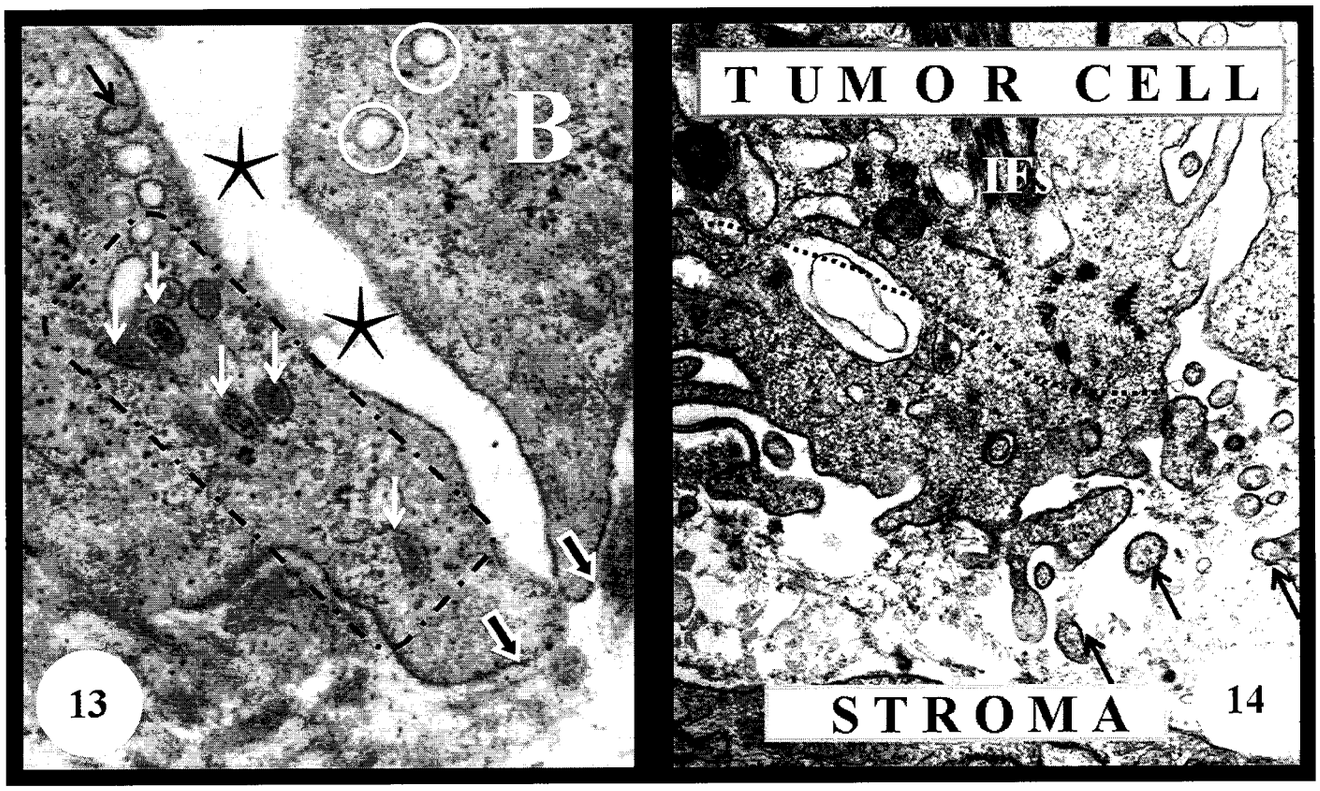

Fig. 13 – Detail for B frame in Fig. 11. Large intercellular space between tumor cells (stars). No hemidesmosomal junction can be detected at the invadopodia edges or very scanty deposits on the inner face of plasma membrane mimic defective hemidesmosomes (filled arrows). Black arrow indicates a clathrin coated pit, and white arrows mark lysosomes. Encircled infrastructures are clathrin coated vesicles. (× 58,000).

Fig. 14 – A sector of a tumor cell at the interface with stroma showing numerous invadopodia (black arrows). Hemidesmosomes are absent, so that intermediate filaments (IFs) do not abut basal plasma membrane (dotted line). (× 17,000).

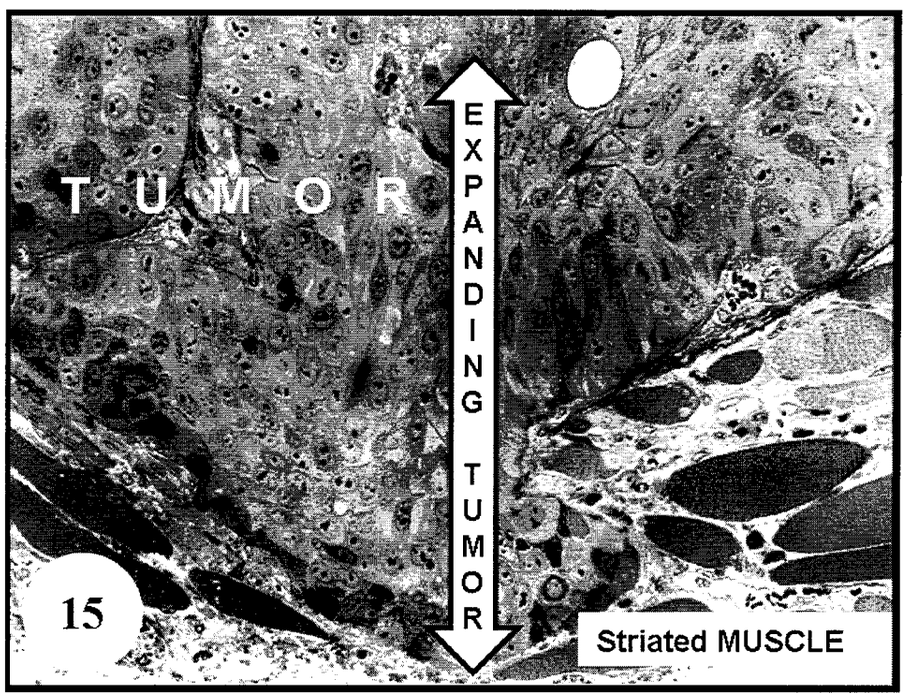

Fig. 15 – A tumor mass invades a striated muscle. (ob. × 60).

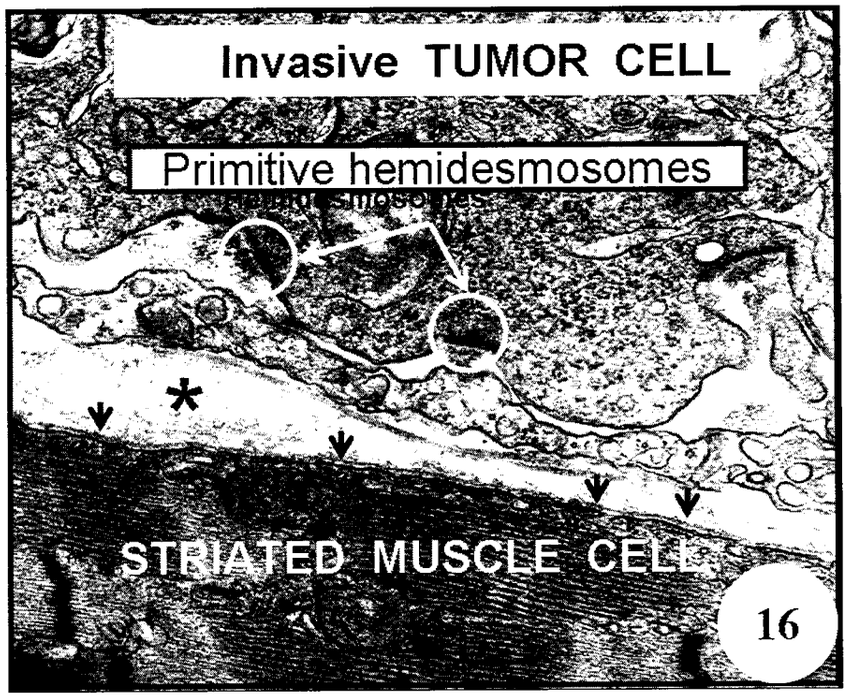

Fig. 16 – A sector of an invasive tumor cell showing defective hemidesmosomes (white circles) in close vicinity with a striated muscle cell. Between tumor cell and striated muscle cell, there is some interstitial material represented by fibrilar collagen (asterisk). Striated muscle cell has an intact basement membrane (arrows). (× 23,000).

Fig. 17 – At the invasive cell-striated muscle cell impact, severe alterations are visible (encircled area):basement membrane of striated muscle is missing and myofibrils are disorganized. Invadopodium (arrow). (× 68,000).

At the edge of the basal pole of tumor cells with peritumoral stroma, the basement membrane is missing; very seldom, only small patches of amorphous material basal lamina-like can be detected (Fig. 11, frame B). Inside of the edge of some invadopodia there are lysosomes (Fig. 11, frame B, detailed in Fig. 13). Moreover, hemidesmosomal junctions are lost and when are detected, they are defective for inner plaque, so that their connection with keratin intermedium filaments is abolished (Fig. 11 and Fig. 14). Occasionally, precarious and ectopic located hemidesmosomes (in baso-lateral position) can be detected (not shown). Some invadopodia tend to detach from the tumor cell to form free vesicles (Fig. 14).

Reactive dermal tissue, including active fibroblasts coming from the host tissue can be detected in the vicinity of basal keratinocytes. Fibroblasts are rich in rough endoplasmic reticulum and a lot of amorphous substances, pre-collagen and collagen fibres can be observed connected with the peripheral cytoplasm of fibroblasts (new synthesised collagen fibres).

When subcutaneously injected, HaCaT A5-RT3 cells have grown and developed tumor masses which tend to grow invasively. At 3 weeks post-injection, a huge tumor mass was formed and the front of tumor invaded and disorganized the histo-architecture of the striated muscle (Fig. 15). Fig. 16 depicted a tumor cell in a very close position to a striated muscle cell still keeping an intact basement membrane. At the place where tumor cell struggled a striated muscle cell, the proper basement membrane is absent and tumor invadopodia penetrated inside of the striated muscle cell and myofibrils are disorganized, so that the cyto-architecture of highly organized striated muscle cell will be compromised both structurally and functionally (Fig. 17).

Invasive growth of cell aggregates or individual cells plays a major role during early embryo development as well as in adult organism in tissue remodelling during wound healing or tumor invasion (Gentle & Comoglio, 2004; Yamaguchi, 2005 a).

Invasive growth of malignant tumors is often associated with loss both of differentiation and cell polarization as well as decrease or absence of basement membrane and, finally, invasive growing of malignant cells into peritumoral stroma. In order to study the significance of these parameters, here we focus our investigations at the high resolution microscopy on the *in situ* developed basal cell carcinoma as well as on an experimental induced squamous cell carcinoma. Our results showed that tumor cells from both basal carcinoma and squamous cell carcinoma are severely altered phenotypes of the cells they are originated from, but still keeping infrastructures which remember their epithelial origin. Moreover, peritumoral stroma appears also significantly altered.

Previous studies demonstrate that the HaCaT cell line provides an appropriate model to study tumorigenic conversion by activated cellular Ha-*ras* oncogene (Fusenig *et al.*, 1990; Boukamp *et al.*, 1990; Breitkreutz *et al.*, 1991). Here, we

electronmicroscopically investigated the ultrastructural aspects of Ha-*ras* transfected tumorigenic HaCaT II-4 and HaCaT A5-RT3 clones.

It is tempting to speculate that abnormal ultrastructural aspects issued by our electronmicroscopic investigations demonstrate that inserted Ha-*ras* oncogene disturbs normal genetic expression programme and induces dramatic changes in phenotypical behaviour of malignant HaCaT clones.

The extracellular matrix influences many aspects of cell behaviour including proliferation, polarized cytoarchitecture, three dimensional multicellular organization, terminal differentiation, migration, invasion, apoptosis (Stadler & Dziadek, 1996).

Invasion is a phenotypic hallmark of malignancy and a prerequisite for their ability to give rise to secondary metastatic spread, as selective process in distant organs (Rougon *et al.*, 1992; Rusciano & Burger, 1992). Cells that disperse lose cell-cell adhesion receptors (cadherins) and acquire cell-matrix receptors (integrins and others) which then mediate the selection of tumor cell subpopulation that have acquired the capacity to invade and disseminate in response to extracellular matrix components (Rougon *et al.*, 1992; Grant *et al.*, 1991). Malignant epithelial tumors are composed of heterogeneous cell populations which differ in their abilities to invade the peritumoral stroma and eventually to perform the metastatic location in ectopic sites (Remy *et al.*, 1993). It seems that local extracellular microenvironment induces a selection of tumor cell subpopulation with intrinsic ability to invade. This behaviour leads to the formation of invading strands (Mueller & Fusenig, 2004).

Normal, but especially tumoral cells shed cell surface material as molecules or as membrane fragments. Activation of the *ras* oncogene induces ruffling of plasma membranes (the oncogene product p21 has been detected in pseudopodia, the locomotory infrastructure of a cell), enhances cell locomotion and invasive behaviour (Noble *et al.*, 1993). Bar-Sagi and Feramisco (1986, cited by Bar-Sagi et Gomperts, 1988) showed that *ras* proteins stimulate membrane ruffling and pinocytosis shortly after microinjection into fibroblasts. Bar-Sagi & Gomperts (1988) reported that microinjection of the *ras* oncogenic protein into mast cells induces exocytotic degranulation. These demonstrate the ability of *ras* proteins to stimulate membrane traffic (for ex. control of exocytosis). Dolo *et al.*, (1995) suggested that shedded membrane vesicles derived from tumor cells could in principle present antigens to the immune system. Moreover, Boyse & Stockert (1965, cited by Evans, 1991), Dolo *et al.*, (1995) and Neuner *et al.*, (1996) demonstrated that the loss of a tumor cell surface antigen (by shedding membrane vesicles) may lead to the avoidance of the host's immunosurveillance system, facilitaing tumor escape.

At the tumor-stroma interface, all kind of tumor epithelial cells investigated in this paper extended numerous and polymorphic cell protrusions (Fig. 5, Fig. 11, Fig. 14 and Fig. 17). Pseudopodial protrusions at the leading edge of the migrating

malignant cells are involved in the process of propels of tumor cell across the basement membrane and adjacent stroma (Yamaguchi *et al.*, 2006 b). Yu *et al.*, (1996) termed the tip of the projections of invading cells "invadopodia". Inside of some invasive cell extentions of tumor cells (invadopodia) as well as inside of shedding vesicles, lysosome-like infrastructures, actin microfilaments and proteases can be detected, what probably facilitates migration of tumor cells by lytic degradation of precarious basement membrane (Ayala *et al.*, 2006; Gimona & Buccione, 2006). One considers that cell-surface receptors – *via* cytoskeletal elements – induce a vectorial distribution of the pseudopodial protrusions (Liotta & Stetler-Stevenson, 1991). Artym *et al.*, (2006) and Weaver (2006) define the invadopodia as membrane protrusions that localize enzymes required for extracellular matrix degradation.

Before crossing into tissue where tumor epidermal cells do not belong, they lose cell-cell adhesion and cell adhesion with basement membrane, initially partially, then totally (Fig. 3, Figs. 10-14, Fig. 16). Our ultrastructural findings concerning the reduced number of desmosomal junctions and dramatical reduction or absence of hemidesmosomes during both *in situ* basal cell carcinoma development and *in vivo* neoplastic new-epidermis formation from isolated c-Ha-*ras* transfected human keratinocytes (postgrafting) are in line with the observation that downregulation of both desmosomal glycoproteins (as members of cadherin family) and hemidesmosome molecular components may promote an invasive and metastatic behaviour of the tumor cells (Legan *et al.*, 1992; Guo *et al*, 1995).

Hemidesmosomes are specialized integrin mediated adherent junctions (Jones *et al.*, 1994; Guo *et al.*, 1995; Borradori & Sonnenberg, 1996; Borradori *et al.*, 1997; Green & Jones, 1996; Mirancea *et al.*, 2001). There are two important components of the hemidesmosome which have transmembrane domain: dimer $\alpha 6\beta 4$ integrin with his $\beta 4$ subunit long intracytoplasmic domain (Green & Jones, 1996) making connection with intermedium filaments. Another hemidesmosomal component is bullous pemphigoid antigen 1 (BPAG 1 of 230 kDa) which is located intracytoplasmically and similar to $\beta 4$ integrin subunit, connects keratin intermedium filaments (Mirancea & Mirancea, 2007-2008).

Ultrastructurally, a normal hemidesmosome exhibits a hemidesmosomal attachment plaque (electrondense membrane plaque) located on cytoplasmic face of basal plasma membrane and adjacent to this (to hemidesmosomal attachment plaque), but more cytoplasmic there is also an electrondense structure termed inner plate of hemidesmosome which connects intermedium filaments (Borradori & Sonnenberg, 1996; Breitkreutz *et al.*, 1997; Mirancea & Mirancea, 1997). When HaCaT II-4 basal keratinocytes occasionally exhibit hemidesmosomes, ultrastructurally they seem to be immature: hemidesmosomes displayed the electrondense membrane plaque (hemidesmosomal atttachment plaque) and subbasal dense plaque, but the inner plate is missing which made hemidesmosomes defective for keratin filaments connexion. These data strongly correlate with the

report of Guo *et al.*, (l995) using immunofluorescence and electronmicroscopic investigations, they deduced that different from mouse wild type, constructed mutant (-/-) BPAG 1 (without BPAG 1) were lacking inner plate and hemidesmosomes fall to connect with tonofilaments.

It is remarkable that ultrastructurally, defective hemidesmosomes for inner plaque expressed by tumor skin basal carcinoma and malignant HaCaT II-4 which grow invasively, strongly remember of the immature hemidesmosomes during normal human and golden hamster (*Mesocricetus auratus*) epidermal morphogenesis (McMillan & Eady, 1996; Mirancea & Mirancea, 1997; Mirancea & Mirancea, 2007-2008).

Because basement membrane is the first barrier encountered for malignant epithelial cells, that plays a peculiar role in the behaviour of carcinomatous tumors related with progression from *in situ* lesion to an invasive neoplasm into surrounding tissues or to move away -*via* sanguine fluid, to distant ectopic (abnormal) location where they do not belong as metastatic proliferation (secondary tumor). In these circumstances, we focus our attention on the ultrastructural aspects of the basement membrane.

Basement membrane is an extracelluar matrix component which interposes and separates epithelial cells from adjacent connective tissue. The basal cells of the epithelium, muscle cells, blood vessels and the nervous system had no direct contact with the mesenchyme. These are surrounded by a complete basement membrane.

Taking into consideration that basement membrane is a dense meshwork of type IV collagen, laminin, glycoproteins, proteoglycans which are not porous for passive tumor cell transversal (Liotta *et al.*, 1991; McMillan *et al.*, 2003; Kalluri, 2003), invasion of the basement membrane must be an active process.

Usually, a continuous basement membrane separates the benign epithelial tumor. By contrast, if a malignant tumorigenic invasive proliferative disorder takes place, the basement membrane is degraded by aggressive tumor cells. These directly secrete enzymes or induce the host tissue to elaborate proteinases to degrade the basement membrane and cell-surface receptors of the integrins and non-integrin-variety (Liotta & Stetler-Stevenson, 1991; Mueller & Fusenig, 2004). Dolo *et al.*, (1995) reported the fact that these vesicles are rich in gelatinolytic activities and the presence in membrane vesicles of some integrins suggest a possible role of these structures in the invasive and metastatic behaviour of carcinoma cells.

Our electronmicroscopic study demonstrates that malignant HaCaT II-4 cells are still able to synthesize the basement membrane components *in vivo* so that, ultrastructurally, a relative continuous but weakly basement membrane is formed 1 week postgrafting (not shown). Interestingly, within 2-3 weeks postgrafting, the basement membrane is almost missing. The basement membrane is also

precariously developed or is totally absent in case of *in situ* developed basal cell carcinoma analyzed in this paper.

There is a body of evidence that increasing pressure created by tumor cell proliferation and expansion of resulted tumor mass results in a direct mechanical compression of the peritumoral stroma (Gabbert *et al.*, 1987; Paku *et al.*, 1990).

Tumor cells are bad neighbours. There is a struggle between tumor cells and stroma cells. When tumoral mass of subcutaneously injected RT3 cells becomes in close vicinity of striated muscle cells, then malignant cells exhibit their ugly behaviour! First, they attach to the basement membrane of striated muscle cell, so that at one moment, we can see a kind of mutual tolerance: malignant cell becomes focally attached to the basement membrane of striated muscle cell- the places where malignant keratinocytes express defective hemidesmosomes, also disconnected by cytokeratins (Fig. 16).

Subcutaneously injected A5-RT3 tumor cells were able to destroy basement membrane of striated muscle cells. Moreover, by their invadopodial cell extentions, tumor cells destroyed plasma membrane of muscle cell, disorganized the myofilaments and penetrate inside of the striated muscle cell, provoking severe alterations of the muscle highly organized architecture, clearly detectable at the ultrastructural level (Fig. 17). For sure, all together lead inexorably to disabilitation or failure of the striated muscle cell functions.

Invasive growth of malignant tumours is often associated with loss of differentiation and cell polarization as well as decrease or absence of basement membrane and finally, invasive growing of malignant cells into peritumoral stroma.

Hornung *et al.* (1987) reported that six malignant C3H mouse epidermal cell lines, with different capacities for epidermal differentiation, was never observed to invade into the collagen gel *in vitro* but, in contrast, within the first week after transplantation the organotypic culture invaded the collagen layer still interposed between malignant epidermis and syngeneic mouse host mesenchymal tissue. The authors concluded that altered epithelial-mesenchymal interactions are of importance for the invasive behaviour mutual activation of proteases, chemotactic attraction of tumor and stroma cells and degradation of surrounding tissue. Wang *et al.* (2005) proposed a model of invasion called "tumor microenvironment invasion model." According with this hypothesis, sequential stable genetic changes during tumor progression can give rise to a tumor microenvironment that elicits the transient gene expression patterns that support invasion. Tumor invasion appears similar to morphogenesis, whereby the transient expression of genes leads to a change in the position and proliferation status of cells (Wang *et al.*, 2005; Condeelis *et al.*, 2005).

CONCLUSIONS

When comparing the ultrastructure of the tumor epithelia *in situ* developed as basal cell carcinoma and experimentally induced squamous cell carcinoma (grafted HaCaT II-4 malignant cells) as well as subcutaneously injected HaCaT A-5 RT3 malignant cells, some similarities are evident: rudimentary hemidesmosomes (type II) and invadopodia formation lead to cell depolarization and signalize severe alteration of tumor cell-matrix interactions. In all kinds of tumors, loss of basement membrane, decreased cell-cell contacts by desmosomes reduction together with the absence of hemidesmosomal junctions (or defective hemidesmosome for inner plaque formation) and consecutive abortion of keratin filaments with basal pole of tumor keratinocytes increase the freedom of tumor cells which tend to grow invasively into the host tissue.

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