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Starting with the volume 51, 2006, Revue Roumaine de Biologie, Série de Biologie Animale has changed its name into Romanian Journal of Biology-Zoology.

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#### **ROMANIAN JOURNAL OF BIOLOGY**

#### **ZOOLOGY**

VOLUME 51 Nos 1-2, 2006

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## ON THE SYSTEMATIC POSITION AND EVOLUTION OF THE BLACK-HEADED WAGTAIL

#### DAN MUNTEANU

The paper deals with the variation existing in the groups of grey-headed and black-headed wagtails belonging to the species *Motacilla flava*, and briefly analyses the hybrids appearing between these groups. In the author's view the black-headed wagtail *feldegg* has the rank of a real, valid, species and its name should be *Motacilla feldegg* Michachelles, 1830; it is a polytypical species and includes two subspecies, namely *Motacilla feldegg feldegg* and *Motacilla feldegg melanogrisea* Homeyer, 1878. On the other hand, the author agrees with the opinion that *Motacilla flava dombrowskii* Tschusi, 1903 is a valid, selfstanding subspecies, not a hybrid between *Motacilla flava flava* and *Motacilla feldegg*.

Key words: wagtail, hybrid population.

#### INTRODUCTION

Motacilla flava is one of the Palearctic bird species that has aroused numerous and contradictory discussions concerning its taxonomic and evolutionary aspects. All the debates have tried to explain the large geographical and individual variability recorded in this widespread species and the relationships between the different populations. The bibliographical list includes papers dealing with this matter, some of them by Romanian authors.

The reason for broaching this subject is the recent and present spread in Romania of the Black-headed Wagtail which entered our country in its south-eastern part about 150 years ago; afterwards it crossed the Carpathians and reached the central-western part of Transylvania during the 1970's. This paper will discuss some general problems concerning the systematics and evolution of this wagtail, taking into account the general principles elaborated in these fields.

#### DISCUSSIONS

According to contemporary taxonomists (Cramp, 1988), *Motacilla flava* includes several groups of subspecies, but the opinions are slightly different. A simple and realistic grouping of the races is the following: i) grey-headed subspecies – *flava, thunbergi, bema, simillissima, iberiae, cinereocapilla*, etc.; ii) yellow-headed subspecies – *lutea, flavissima, taivana*, which are considered by

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some authors as a separate species, *Motacilla lutea* (Gm.); iii) black- headed subspecies – *feldegg*, *melanogrisea*.

*Motacilla flava feldegg* was described by Michachelles in 1830 under the name *Motacilla feldegg\**. Later on, Hartert (1910) gave it the subspecific (trinominal) name *Motacilla flava feldegg*.

The Black-headed Wagtail *feldegg* has gradually spread its European range westwards and northwards during the last century, this process being noted and discussed by several authors (Cătuneanu *et al.*, 1962; Keve, 1978; Leisler, 1968). As for Romania, these authors asserted that *feldegg* is breeding in south Oltenia and Muntenia as well in the Danube Delta. In this latter place *feldegg* was recorded as back as 1897 by Dombrowski (1912), at a time when the northern boundary of its main range was situated in central Bulgaria. Consequently, for several decades, the Danube Delta represented a kind of *feldegg* "island" within the range of the grey-headed wagtails. This fact has been noted and emphasized by Radu (1975, 1979). However, the case is not unique since it has been ascertained that in other countries, too, *feldegg* had initially not produced hybrids, remaining for a while in a pure state (Leisler, 1968). Records of the black-headed wagtail in Romania were mentioned by other ornithologists too, Munteanu (1976, 1977), Radu (1979), Szabó (1966).

Another yellow-wagtail race interests us in connection with *feldegg: Motacilla flava dombrowskii*. It was described by Tschusi zu Schmidthoffen (1903) on the basis of the specimens collected by Dombrowski at Pantelimon, near Bucharest. The opinions of different authors about *dombrowskii* are contradictory. A group of ornithologists beginning with Dombrowski (1912) and Hartert (1910-1922) and continuing with Williamson (1955, 1956) or Voinstvenski (1953) admitted the validity of this subspecies, whereas other ornithologists like Gladkov (1954), Vaurie (1959) and Sammalisto (1968) regarded it as a synonym of the nominotypical race *flava flava*. These latter authors have the opinion that *dombrowskii* is a hybrid *flava* x *feldegg* (also Cramp) or *flava* x *thunbergi* (Voinstvenski, 1953). At the same time, for lack of an efficient geographic isolation, it could not sufficiently differentiate – Johansen (1946), quoted by Williamson (1956).

It is known that the range of *dombrowskii* (hybrid or subspecies) stretches from Serbia (Matvejev, 1976) and east Hungary (Magyar *et al.*, 1998) across Romania, south Poland, Republic of Moldova, the Ukraine and south European Russia eastwards to Voronezh or even to the Volga River (Cramp, 1988), (Gladkov, 1954), (Vaurie, 1959). The population from the Danube Delta arises a

peculiar systematic and zoogeographic question due to the overlapping, in this area, of the grey-headed (*flava/dombrowskii*) and black-headed (*feldegg*) wagtails. The first mention of this case was published by Voinstvenski (1953). He examined 90 specimens collected in the northern Delta and concluded that most of them were *feldegg* and *dombrowskii*, whilst a few belonged to the typical form, *M. flava flava*. It was not clear however, whether the latter specimens, collected at the end of April, were breeding birds or passage birds.

A detailed study of this question was carried out by Radu (1975). His main conclusions are the following: the wagtails *feldegg* and *flava* overlap in the whole Danube Delta (this is a case of sympatry of two conspecific subspecies); *feldegg* and *flava* hybridize, but the parental phenotypes remain more common than the hybrids; among the hybrids there are individuals similar to "dombrowskii" as well as to other subspecies of *Motacilla flava*; and, there is a partial reproductive isolation between the black-headed and grey-headed wagtails, caused by ecological and ethological factors.

The flava and *feldegg* hybrids have also been analysed by Sammalisto (1968). He came to the conclusion that these hybrids do not uniformly combine the parental characteristics. In most cases a serious decrease of the black head colour (*feldegg* feature) and of the white supercilium (from *flava flava*) is to be noted, so that the result is the appearance of relatively uniform grey-headed hybrids. That is the situation in the north of the former Yugoslavia (*i.e.* Voivodina and Serbia) considered by Sammalisto (1968) to be a successful and balanced hybridization. In the "unsuccessful" cases a different phenomenon takes place, the hybrids having much black and a visible white supercilium. However, all the hybrids acquire a new characteristic: an area of white on the chin and throat. In his opinion, the now existing population in the Danube Plain in Romania (not just in the Danube Delta) has resulted from the recent immigration of *feldegg* into the range of the old and intermediate population "dombrowskii".

If we accept Sammalisto's (1968) and Radu's (1975, 1979) opinions then it is clear that there are three distinct populations in the lower Danube Delta basin:

- 1. The hybrid population from the north "Yugoslavia", originating from a successful and balanced hybridization. It is distinguished by the uniformly grey head, reduced or absent superciliary stripe, white chin and throat.
- 2. The "dombrowskii" population which occupies a large area extending from the Tisza river to the Volga. Its main characteristics are the following: dark grey crown and nape, blackish lores and ear coverts, white supercilium (sometimes reduced), almost always white chin, under parts occasionally with an orange hue. Hence "dombrowskii" differs from the typical flava by the dark colour of the head and from thunbergi by the white supercilium. It has been recently modified ("altered") in Romania as a result of the recent feldegg immigration (range overlap).

<sup>\*</sup> It was dedicated to Cristoph F. F. von Feldegg (1779 or 1789-1845), an Austrian amateur ornithologist who collected the first specimen in south Dalmatia. The correct scientific name according to the Latin grammar would be *feldeggi* (in genitive case), but the original spelling has to be maintained by dint of the Article 32, paragraph 3, of the *International Code of Zoological Nomenclature* (1999).

3. The hybrid population which has occurred in the Romanian Plain (and more recently but rarely in Transylvania) during the second half of the 20th century as a result of the northwards expansion of *feldegg* over the ranges of *flava dombrowskii* (in south-east Romania) and *flava flava* (in Transylvania). It is characterized by a large variety of combinations of parental phenotypes, although a general tendency is to be observed for a blackish or black head with a white supercilium (rarely reduced). It follows that these two features are dominant in parental forms – the black colour of the head in *feldegg* and the white superciliary stripe in *flava flava*.

#### **MATERIAL AND METHODS**

During a long period, between the years 1976 and 2003 I found in the field 112 males belonging to the species *Motacilla flava*. The areas where our research was performed are the following: the Danube Delta; the surroundings of the Razim-Sinoe lagoon complex; south Dobrodja – Mangalia, Hagieni, Băneasa; the Râmnic Plain – Balta Albă, Amara, Jirlău; the Danube valley between Bistreț (south Oltenia) and Călărași (south Muntenia); the Olt valley between Drăgășani and Slatina; Banat – Sânnicolau Mare; Transylvanian Plateau – Luduș, Zau de Câmpie, Cluj-Dej; Crișana – Cefa, Salonta, Chișineu Criș. Occasional observations in other sites of Romania were also recorded. I have already published few comments about the habitats of the "yellow" wagtails (Munteanu, 1976, 1977).

The birds were examined in the field, watching them by a binocular. Their characteristics were noted in a special form where the plumage parts were registered: forehead, crown, nape; supercilium, chin, breast and belly. The colours (white, grey, black, yellow, orange) were conventionally divided into four categories (0, 1, 2, 3) according to their intensity, except for white. The forms written on sheets of paper (including copies) were grouped in different ways, according to different features of the examined individuals. Unfortunately, there are slight differences between the females belonging to the *Motacilla flava* races, so the study of the hybridization within this species is limited because it is impossible to be always sure how the pairs are constituted.

#### RESULTS

Analysing the available data, as they have been briefly summarized above, and my personal findings, I have come to some personal conclusions on the origin, taxonomic position and evolution of the *dombrowskii* and *feldegg* populations.

I first consider the populations listed under 1° and 2° above. They have already reached a certain degree of stability, as proved by the fact that their variability does not go far beyond the variability of the valid subspecies,

recognized by all researchers. As has been stated, the phenotypical likeness between the hybrid *dombrowskii* and *flava flava* would be explained by a backcrossing phenomenon, *i.e.* by interbreeding of F1 hybrids with *flava flava* partners. However, accepting the hybrid origin of both populations (1 and 2) I consider the idea highly unlikely that, in two different geographic areas, the crossing of the same parental forms led to different phenotypes (in particular the supercilium).

Moreover, I would note a very important fact: the hybrids coming into being nowadays in the Danube Plain and in Transylvania have phenotypical features of another type (just very variable types) than those characterizing *dombrowskii*. For these reasons I doubt the hybrid origin of *dombrowskii* (Table 1).

In view of the following characteristics of *dombrowskii* I deem it a valid subspecies: i) sufficiently clearly defined phenotypical features; ii) a coloration which partially differs from that of individuals with certain hybrid origin (those appearing today); iii) an amplitude of variability not going beyond the variability recorded in valid subspecies; iv) an absent (or rare) feature in its supposed parental forms *flava* and *feldegg* – the white chin; v) it has existed before the large spreading of *feldegg* to the west and the north of the Black Sea (a very important argument); vi) an extensive distribution ranging over an area 1,800 km long and up to 300 km wide.

 $\label{eq:Table 1} Table \ I$  The main phenotypical features of the  $\emph{Motacilla flava}$  populations in SE Europe

	M. flava flava	M. flava dombrowskii	M. flava feldegg	Present hybrids from Romania flava/dombrowskii x feldegg	Old hybrids from "Yugoslavia" flava x feldegg
Crown and nape	ashy-grey	dark grey	black	blackish or black, occasionally dark grey on nape	uniform grey
Lores and ear coverts	grey, occasio- nally with a dark hue	blackish	black	black	grey, sometimes dark grey
Supercilium	white	white (sometimes reduced)	(no supercilium)	white, occasionally reduced	reduced or absent
Chin	yellow, sometimes white	white (occasionally yellow)	intensive yellow (rare white)	yellow	white
Under parts	yellow	yellow, sometimes orange-yellow	yellow to orange- yellow	intensive yellow	yellow

Moreover, I think that the existence among this subspecies of birds close to flava flava or feldegg is not the result of the initial hybrid origin of dombrowskii, but of a later hybridization with the two neighbouring races: flava flava and (flava) feldegg.

The present hybrid population *flava/dombrowskii* x *feldegg* requires more discussion in addition to that by Sammalisto (1968) and Radu (1975). In fact, the general structure of the *Motacilla flava* population today in Romania, especially the maintenance in a pure state and high frequency of the grey-headed form *dombrowskii* (only recently, partially altered, as I already mentioned) and the black-headed *feldegg* leads us to other zoogeographical and taxonomic interpretation.

The way of formation of hybridization zones and the characteristics of hybrids that occur between two intergrading populations (subspecies or just species) are already well known. In a paper dealing with this phenomenon, Short (1969) shows that two cases may be distinguished: 1) hybrid zone, i.e. an area of hybridization where only hybrids occur, and 2) overlap and hybridization zone, populated by numerous hybrids and both parental forms as well (any way, this concept is generally accepted by taxonomists); (Ceapoiu, 1980; Mayr, 1971; Vuilleumier, 1980). These two zoogeographical situations have an important systematic significance. The presence of a hybrid zone means that the two populations/subspecies involved in contact are conspecific; the overlap and hybridization zones occur only between populations situated from a taxonomic point of view at a higher level than the subspecific rank and lower than the specific rank (semispecies). However, there are also known cases of hybridization between two valid bird species. Such a case that occurred in Romania in the last century was represented by the Syrian Woodpecker Dendrocopos syriacus (whose range extended across the Romanian territory) and the Great-spotted Woodpecker Dendrocopos major.

Several facts, as the examination in the field of the Yellow Wagtail population, *Motacilla flava sensu lato*, which lives today in the south, south-eastern and central Romania, the features of *feldegg* individuals, the way of *feldegg* spreading across Romania, and the relationships between these two "forms", led me to some particular conclusions concerning the examined species.

The "forms" *feldegg* and *dombrowskii* are characterized, as I have already mentioned, by: i) sympatry; ii) (partial) reproductive isolation; iii) coexistence of the pure parental phenotypes and of the hybrids, the latter being generally less frequent.

As to item ii), I point out that, according to different authors (Keve, 1978; Leisler, 1968; Radu, 1975, 1979) and from my own observations, the partial reproductive isolation is achieved by:

- a) later clutch laying by feldegg (phenological isolation);
- b) a certain difference between nuptial songs (behavioural isolation);

c) a slightly different habitat preference (ecological isolation).

Of particular note is the preference of *feldegg* for the low vegetation of marshes (including brackish marshes, as it can be seen in the area of the Razim - Sinoe lagoons (Munteanu, 1977), at Sânpaul/Harghita county (Szabó, 1966), at Someşeni – Cluj (pers. obs). Radu (1979) asserts that in the Dobrodja *feldegg* is breeding in drier habitats than those populated by grey-headed birds, but I everywhere saw exactly the contrary (Munteanu, 1977). (See also: Stresemann (1920) and Kleiner (1936), quoted by Leisler (1968)).

On the other hand, there are three main distinguishing features between flava/dombrowskii and feldegg which I should point out:

- a) the striking phenotypical difference in the head colour;
- b) a set of biological characteristics involving partial reproductive isolation (see above);
- c) difference in the location of the genetic center: the reliable origin of feldegg outside the contact with the flava range (despite it has been initially described from the Balkans) in a marginal or peripheral area, located in west Asia/Asia Minor (geographical isolate).

Considering all these elements, and taking into account the theoretical aspects of speciation (Helbig *et al.*, 2002; Mayr, 1971), in my opinion *Motacilla feldegg* (or the group of "black-headed wagtails") should be regarded as a separate species: *Motacilla feldegg* Michachelles, 1830.

Taking into account the opinion of Vaurie (1959) and other taxonomists about the subspecies *melanogrisea*, it may be concluded that *Motacilla feldegg* is a polytipical species including two subspecies, namely *Motacilla feldegg feldegg* Michachelles, 1830 and *Motacilla feldegg melanogrisea* (Homeyer, 1876).

It would be particularly interesting to monitor the future evolution of the "yellow" wagtails living in Romania, especially in the direction of possible changes in their genotypic and phenotypic structure.

As concerning the species that are able to interbreed, it is known (Ceapoiu, 1980; Mayr, 1971; Short, 1969) that in the case of a species A, the range of which spreads over the range of a closely related species B, hybrid frequency is higher along the distribution limit of species A (this species being more rare, the birds do not always find conspecific partners). This belt with a rather high frequency of hybrids A x B moves as the A species extends, while within the range of the two species, hybrids do no appear anymore (e.g. the case of *Parus cyanus-Parus caeruleus*, or *Dendrocopos syriacus — Dendrocopos major*, the later case just in Romania).

The situation of *Motacilla flava* and *Motacilla feldegg* is different, as the later one spread over the range of the first species many decades ago, but it remained for a long period, on large areas, a rare species, distributed in isolated sites. The number of hybrids has increased corresponding to the increasing of the birds' (*feldegg*) abundance within the area occupied by both species. I consider that a

future decrease in the same area/areas of the frequency of hybrids, due to a self-stabilization of the populations belonging to the two species, would be an additional evidence, and a very convincing one, to support the idea that *Motacilla feldegg* has a specific rank. Otherwise, the hybrids maintenance in their present form would prove to future researchers that they had/have not a hybrid origin, but appear as the result of mutations.

#### CONCLUSIONS

Analysing the plumage colour, the biological features and the zoogeographical aspects of the "yellow" wagtails that populate the south-eastern Europe (particularly Romania), and taking into account the previous researches, I support the thesis that *Motacilla flava dombrowskii* (Tschusi, 1903) is a valid and well defined subspecies, and I express the opinion that *Motacilla feldegg* Michachelles is a self-standing, valid, polytypical species.

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Received January 10, 2006.

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# CHRYSOMELIDS (COLEOPTERA: CHRYSOMELIDAE) FROM THE SYLVOSTEPPE FORESTS OF ROMANIA

#### SANDA MAICAN

The paper presents new data regarding the leaf-beetles fauna in some sylvosteppe forests from Romania. A total of 68 species belonging to 36 genera and 9 subfamilies have been recorded. For each species, the examined material and general distribution are given. In addition, there are presented plants where some chrysomelid species were found.

Key words: Coleoptera, Chrysomelidae, biodiversity, sylvosteppe forests, Romania.

#### INTRODUCTION

The information concerning Romanian leaf-beetles fauna is partial and incomplete. Only few data regarding the distribution of the chrysomelid species in the forests of the Romanian Plain have been mentioned until now in literature; the majority of these data is old, being published by many specialists such as: Fleck (1905) – material collected from Comana forest, Băneasa forest; Montandon (1906, 1908) – Comana forest, Bucharest, Mogoșoaia, Măgurele; Konnerth-Ionescu (1963, with older data) – Comana forest, Giurgiu, Ghimpați; Roșca (1973) – Comana, Cernica, Pasărea forests and Andronache forest (now destroyed), etc.

However, large areas from southern Romania are still unstudied. This study is trying to cover new sites in order to increase the knowledge of chrysomelids distribution in Romania.

#### MATERIAL AND METHODS

Most of the studied material was collected between 2002–2005, in the context of a broader research referring to the biodiversity knowledge in the sylvosteppe forests from the southern and South-eastern Romanian Plain. This material was collected by the author and the colleagues from the Ecology, Taxonomy and Nature Conservation Centre (from the Institute of Biology), using the sweeping method with an entomological net, by shaking the shrubs or directly from the vegetal substratum. The material collected by the author during the

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All samples were determined by the author, on the basis of external morphology and of the genitalia, according to the identification keys given by Warchałowski (1991, 1993, 2003).

Subfamilies are listed in systematic order and genera and species are alphabetically presented. The nomenclature and the general distribution are given according to Warchałowski (2003). The studied material is deposited in the Chrysomelids Collection of the Institute of Biology of the Romanian Academy.

The phytosociological characterization of the studied forests (in brackets are mentioned counties):

- Comana forest (GR): Quercus-forest, framed in the Fraxino pallisiae-angustifoliae-Quercetum roboris Popescu et al. 1979 association;
- Ciofliceanca forest (CL): consists of pure trees of Quercus pedunculiflora
   C. Koch, classified in the Quercetum pedunculiflorae Borza 1937 association;
- Spătaru and Frasinu Natural Reservations (BZ): depression ash trees forests, framed in the *Fraxino pallisiae-angustifoliae-Quercetum roboris* Popescu *et al.* 1979 association; in Spătaru forest the herbaceous layer is characterized by the presence of many hygrophilous species like: *Lythrum salicaria* L., *Iris pseudacorus* L., *Lycopus europaeus* L., *Carex riparia* Curtis, *Caltha palustris* L., *Phragmites australis* (Cav.) Steudel, etc.
- Sărata-Monteoru forest (BZ): Querco petraeae-Carpinetum Soó et Pócs 1957 association;
- Frecăței forest (BR): Salicetum albae-fragilis Soó (1933) 1958 association;
- Cobia forest (DB) and Gura-Foii forest (DB): the mesophilous pasture with
   Agrostis capillaris L., near the skirt forest of the Querco petraeae Carpinetum Soó et Pócs 1957 association;
- Călugăreni forest (GR) and Clinceanca forest (IF): alder depression forest with *Alnus glutinosa* (L.) Gaertner, characterized by the *Stellario nemori-Alnetum glutinosae* (Kästner 1938) Lohm. 1957 association;
- Ogarca forest (GR): thermophile Quercus-forest, included in the Quercetum cerris Georgescu 1941 association in which Quercus cerris L. is dominant;
- Greaca forest (GR): thermophile Quercus-forest, framed in the Ceraso mahaleb-Quercetum pubescentis Jakucs et Fekete 1957 association, characterized by the presence of some thermophile herbaceous species;
- Singureni forest (GR): ash forest, characterized by *Fraxino danubialis-Ulmetum* Sanda et Popescu 1999 association, with numerous mesophilous

species: Aegopodium podagraria L., Festuca gigantea (L.) Vill., Convallaria majalis L., Carex pilosa Scop., Scilla bifolia L., Rubus caesius L., Melica uniflora Retz. etc.;

- Căscioarele forest (GR): Querco robori-Carpinetum Soó et Pócs 1957 association;
- Cernica forest (IF): Quercetum cerris Georgescu 1941 association;
- Dobromir (CT), Dumbrăveni (CT) and Hagieni forests (CT): Cotino-Quercetum pubescentis Soó 1931 association;
- Băneasa forest (Bucharest) and Vedea forest (TR): *Querco robori- Carpinetum* Soó et Pócs 1957 association;
- Măriuța forest (CL).

#### Abbreviations:

Collectors' names: Dumitrescu Florentina – D.F.; Hoinic Cristina – H.C.; Maican Sanda – M.S.; Pârvu Corneliu – P.C.; Procheş Şerban – P.Ş.; Săftoiu Atanasie – S.A.; State Steluţa – S.S.; Şincu Daniela – Ş.D.

Other abbreviations: BZ – Buzău county; CL – Călărași county; CT – Constanța county; DB – Dâmbovița county; GR – Giurgiu county; IF – Ilfov county; TR – Teleorman county; spec./specs. – specimen/specimens; coll. – collection.

#### RESULTS

The specimens studied belong to 68 species from 36 genera and are included in the following subfamilies: Donaciinae (1 species), Criocerinae (8 species), Clythrinae (6 species), Cryptocephalinae (13 species), Eumolpinae (2 species), Chrysomelinae (14 species), Galerucinae (5 species), Alticinae (13 species) and Cassidinae (6 species).

For every species we mentioned: collecting date and place, the number of the studied specimens, collectors' names and the geographical distribution. For some of the taxa there are presented information about the plants where the adults were captured.

#### DONACIINAE Kirby, 1837

Genus: Donacia Fabricius, 1775

Donacia marginata Hoppe, 1795

Examined material: Călugăreni, 27.V.1995, (1  $\circlearrowleft$ ), H.C. (from the collections of the "Grigore Antipa" National Museum of Natural History, Bucharest); live on *Sparganium* sp. (Warchałowski, 2003).

Distribution: widely distributed in northern Africa, Europe and Middle Asia.

CRIOCERINAE Latreille, 1807

Genus: Crioceris Müller, 1764

Crioceris duodecimpunctata (Linnaeus, 1758)

Examined material: Spătaru, 28.VIII.2003, (2 specs.), M.S.; found on Asparagus officinalis L.

Distribution: Transpalaearctic species, distributed very widely from Portugal and England to Korea.

#### Crioceris quatuordecimpunctata (Scopoli, 1763)

Examined material: Spătaru, 25.VI.2003, (2 specs.), M.S.; Spătaru, 28.VIII.2003, (1 spec.), M.S.; found on Asparagus officinalis L.

Distribution: South-eastern Europe, Central Asia.

#### Crioceris quinquepunctata (Scopoli, 1763)

Examined material: Spătaru, 28.VIII.2003, (3 specs.), M.S.; found on Asparagus officinalis L.

Distribution: the basins of the Danube, Dnieper and Volga rivers.

## Genus: *Lema* Fabricius, 1798 *Lema cyanella* (Linnaeus, 1758)

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Examined material: Frasinu, 25.V.2003, (2 ♀♀), M.S.

Distribution: Transpalaearctic species, distributed from Spain and the British Isles to Korea.

Genus: Lilioceris Reitter, 1912

#### Lilioceris lilii (Scopoli, 1763)

Examined material: Cernica, 26.VI.1996, (1 spec.), P.Ş.; found on Lilium sp. Distribution: widely distributed from Marocco and England to eastern Asia.

#### Lilioceris merdigera (Linnaeus, 1758)

Examined material: Băneasa (Bucharest) 10.V.1992 (1 spec.), P.Ş.; Comana, 1.V.1996, (1 spec.), P.Ş.; found on *Polygonatum latifolium* (Jacq.) Desf. *Distribution*: Transpalaearctic species, distributed from Iberian Peninsula, France and southern Norway to Japan.

Genus: Oulema Gozis, 1886

#### Oulema gallaeciana (Heyden, 1870)

(Chrysomela lichenis Voet, 1806)

Examined material: Ciofliceanca, 10.IX.2002, (1 spec.), D.F.; found on Dactylis polygama Horvát.

Distribution: Europe, the basin of the Danube, European Russia, western Siberia, northern part of Central Asia.

#### Oulema melanopus (Linnaeus, 1758)

Examined material: Vedea, 30.V.1999, (1 spec.), M.S.; Comana, 3.VI.2002, (26 specs.), M.S.; Călugăreni, 7.VII.2005, (2 spec.), M.S.; found in large number on Carex pilosa Scop.

Distribution: western Palaearctic.

CLYTHRINAE Kirby, 1837

Genus: Clytra Laicharting, 1781

Clytra (s.str.) laeviuscula (Ratzeburg, 1837)

Examined material: Hagieni, 22.VI.1999, (1 spec.), M.S.; Vedea, 19.VII.1999, (3 specs.), M.S.; Frasinu, 25.V.2003, (1 spec.), M.S.; Gura-Foii, 5.VII.2005, (1 spec.), M.S.; Căscioarele, 5.VII.2005, (4 specs.), M.S.; found on Lythrum salicaria L., Cornus sanguinea L. and Erigeron annuus (L.) Pers.

Distribution: from France and England to Altai range.

Genus: Labidostomis Germar, 1822

#### Labidostomis (s.str.) longimana (Linnaeus, 1761)

Examined material: Greaca, 3.VI.2002, (1  $\circlearrowleft$ ), M.S.; Gura-Foii, 5.VII.2005, (1  $\circlearrowleft$ , 4  $\circlearrowleft$ ), M.S.; Gura-Foii, 5.VII.2005, (1 spec.), D.F.

Distribution: from southern Italy, northern Spain and southern Sweden to Central Russia and Mongolia.

Genus: Lachnaia Chevrolat, 1837

#### Lachnaia sexpunctata (Scopoli, 1763)

Examined material: Greaca, 8.VI.1997, (3 specs.); 3.VI.2002, (1  $\$ ), P.Ş, M.S.; found on *Gleditsia triacanthos* L. and *Robinia pseudoacacia* L.

Distribution: North-eastern France, southern Germany, basin of the Danube, Ukraine, Balkan Peninsula, Romania, Asia Minor.

Genus: Smaragdina Chevrolat, 1837

#### Smaragdina (Monrosia) affinis (Illiger, 1794)

Examined material: Călugăreni, 3.V.2004, (1 ♀), D.F.

Distribution: England, France, Italy, Central Europe, Romania, Bulgaria, southern Ukraine. Reported also from Finland.

#### Smaragdina (Monrosia) salicina (Scopoli, 1763)

(Cryptocephalus cyaneus Fabricius, 1775)

Distribution: Central and southern Europe, from northern Spain and Denmark to the basin of Volga and Caucasian countries.

#### Smaragdina (Monrosia) xanthaspis (Germar, 1824)

Examined material: Comana, 30.VI.1996, (2  $\circlearrowleft$ ), P.Ş; Greaca 3.VI.2002, (3  $\circlearrowleft$ ), M.S.; Călugăreni, 26.VI.2003, (3  $\circlearrowleft$ ), M.S.; Gura-Foii, 5.VII.2005, (2 specs.), M.S.; a common species, found especially on *Crataegus* sp.

Distribution: northern Italy, northern part of Balkan Peninsula, basin of the Danube, southern Ukraine, Asia Minor.

#### CRYPTOCEPHALINAE Gyllenhal, 1813

Genus: Cryptocephalus Müller, 1764

#### Cryptocephalus (s.str.) bipunctatus (Linnaeus, 1758)

Examined material: Hagieni 18.VI.1999, (1 spec.), M.S.; Dumbrăveni, 19.VI.1999, (1 spec.), M.S.; Gura-Foii, 5.VII.2005, (1 spec.), M.S.; Căscioarele, 5.VII.2005, (1 spec.), M.S.; found on *Crataegus monogyna* Jacq. and *Orlaya grandiflora* (L.) Hoffm.

Distribution: Transpalaearctic species, very widely distributed from Portugal and Ireland to Korea.

#### Cryptocephalus (Burlinius) connexus Olivier, 1808

Examined material: Singureni, 10.VII.1997, (1 spec.), P.Ş.

Distribution: southern Europe, Asia Minor, Central Asia.

#### Cryptocephalus (s.str.) flavipes Fabricius, 1781

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Examined material: Dumbrăveni, 19.VI.1999, (1 spec.), M.S; Gura-Foii, 5.VII.2005, (2 \(\phi\), 1 \(\preceq\), M.S., D.F.

Distribution: Europe (except Iberian Peninsula, British Isles and northern Scandinavia), Asia Minor, Russia, Caucasian countries and Central Asia.

#### Cryptocephalus (s.str.) frenatus Laicharting, 1781

Examined material: Măriuța, 29.VII.1985, (1 spec.), S.A.

Distribution: western France, Germany, Poland, basin of the Danube, northern Italy, northern part of Balkan Peninsula.

#### Cryptocephalus (s.str.) hypochoeridis (Linnaeus, 1758)

Examined material: Spătaru, 25.VI.2003, (3 specs.), M.S.; Frasinu, 25.V.2003, (2 ♀♀), M.S.; Gura-Foii, 5.VII.2005, (1 ♂), M.S.; Căscioarele, 5.VII.2005, (7 specs.), M.S.; found on *Crepis rhoeadifolia* (Bieb.) and *Inula germanica* L. *Distribution:* Europe, Caucasian countries and Central Asia.

#### Cryptocephalus (s.str.) janthinus Germar, 1824

Examined material: Comana, 16.IX.1994, (1 &), P.Ş.

Distribution: France, Italy, Central Europe, Romania, Bulgaria, Ukraine, southern Russia and Caucasian countries.

Remarks: a rare species, recorded in Romania from Valea Gârbăului (Marcu, 1964) and Caraorman (Ieniștea, 1974); host plants mentioned in literature: Phragmites communis Trin., Lythrum salicaria L. and Lysimachia vulgaris L.

#### Cryptocephalus (s.str.) moraei (Linnaeus, 1758)

Distribution: Europe (except northern part of Scandinavia), Asia Minor.

#### Cryptocephalus (Burlinius) ocellatus Drapiez, 1819

(Cryptocephalus geminus Gyllenhal, 1827)

Examined material: Căscioarele, 10.V.2005, (1 3), D.F.

Distribution: Palaearctic.

#### Cryptocephalus (s.str.) octacosmus Bedel, 1891

(Cryptocephalus sexpustulatus Rossi, 1790)

Examined material: Comana, 30.VI.1990, (2 specs.), P.Ş; Comana, 3.VI.2002, (3 specs.), M.S.; Singureni, 10.VII.1997, (1 spec.), P.Ş; Greaca, 3.VI.2002, (2 specs.), M.S.; Călugăreni, 26.VI.2003, (4 specs.), M.S.; Singureni,

26.VI.2003, (4 specs.), M.S.; Gura-Foii, 5.VII.2005, (1 3), M.S.; Căscioarele, 5.VII.2005, (4 specs.), Ş.D.

Distribution: southern and Central Europe, Asia Minor and the basin of Volga.

#### Cryptocephalus (s.str.) parvulus Müller, 1776

Examined material: Căscioarele, 10.V.2005, (1 3), D.F.

Distribution: from northern Spain, Ireland and southern Norway to Central Asia.

#### Cryptocephalus (s.str.) sericeus (Linnaeus, 1758)

Examined material: Gura-Foii, 5.VII.2005, (1  $\circlearrowleft$ ), M.S.; Căscioarele, 5.VII.2005, (1  $\circlearrowleft$ ), D.F.

Distribution: from northern Spain, Belgium and Denmark to North-western China.

#### Cryptocephalus (s.str.) violaceus Laicharting, 1781

Examined material: Dumbrăveni, 19.VI.1999, (2 33, 1 9), M.S.; found mainly on *Salix caprea* L.

Distribution: western, middle and South-eastern Europe.

Genus: Pachybrachis Chevrolat, 1837

#### Pachybrachis (s.str.) sinuatus (Mulsant & Rey, 1859)

(Pachybrachis haliciensis Miller, 1868)

Examined material: Căscioarele, 5.VII.2005, (10 specs.), M.S., D.F.; Gura-Foii, 5.VII.2005, (1 spec.), Ş.D.; found on Salix sp.

Distribution: southern France, Central Europe, Balkan Peninsula, Asia Minor. EUMOLPINAE Thomson, 1859

Genus: Eumolpus Illiger, 1798

#### Eumolpus asclepiadeus (Pallas, 1776)

Examined material: Frasinu, IX.2003, (1 3), D.F.; Frasinu, 25.V.2003, (1 spec.), M.S.; Spătaru, 25.VI.2003, (6 specs.), M.S.; found on *Acer campestre* L. *Distribution:* France, northern Italy, southern Germany, Poland, basins of the Danube and Volga rivers, Caucasian countries, Central Asia.

Genus: Pales Redtenbacher, 1858

#### Pales ulema (Germar, 1813)

Examined material: Greaca, 3.VI.2002, (1 ♀), M.S.; Cobia, 17.V.2005, (2 specs.), S.S.; Gura-Foii, 5.VII.2005, (1 spec.), M.S.; found on *Crataegus monogyna* Jacq.; thermophilous species.

Distribution: Balkan Peninsula, Hungary.

#### CHRYSOMELINAE Latreille, 1802

Genus: Chrysolina Motschulsky, 1860

#### Chrysolina (Sulcicollis) chalcites (Germar, 1824)

Examined material: Comana, 26.IX.1998, (1 3), P.Ş; Dumbrăveni, 19.VI.1999, (1 3), M.S.

Distribution: South-eastern Europe (Balkan Peninsula, basin of the Danube, southern Ukraine, southern Russia), Caucasus, Asia Minor, Central Asia.

Remarks: ponto-mediterranean species, rare in Romanian fauna; mainly in salted soils; host plants are not known.

Sanda Maican

#### Chrysolina (Fastuolina) fastuosa (Scopoli, 1763)

Examined material: Dobromir, 16.VI.1999, (1 spec.), M.S.; Ciofliceanca, 4.VI.2002, (47 specs.), M.S.; Ciofliceanca, 10.IX.2002, (11 specs.), D.F.; Ogarca, Schitu, 9.IX.2002, (13 specs.), D.F.; Greaca, 3.VI.2002, (19 specs.), M.S.; Greaca, 11.IX.2002, (3 specs.), D.F.; Călugăreni, V.2003, (1 spec.), D.F.; Călugăreni, 26.VI.2003, (1 spec.), M.S.; Singureni, 6.V.2003, (17 specs.), D.F.; Singureni, 27.VIII.2003, (10 specs.), M.S.; Frasinu, 25.V.2003, (11 specs.), M.S.; Singureni, 26.VI.2003, (28 specs., on), M.S.; Spătaru, 25.VI.2003, (18 specs.), M.S.; Spătaru, 28.VIII.2003, (10 specs.), M.S.; Clinceanca, 1.VII.2004 (17 specs.), D.F.; Călugăreni, VII.2004, (10 specs.), D.F.; Călugăreni, IX.2004, (8 specs.), D.F.; Clinceanca, 5.V.2004, (10 specs.), D.F.; Călugăreni, 7.VII.2005, (4 specs.), M.S.; a common species, found in a large number on Urtica dioica L., Ballota nigra L., in a small number on Leonurus cardiaca L. and Marrubium vulgare L.

Distribution: Europe, Asia Minor, Caucasian countries.

#### Chrysolina (Synerga) herbacea (Duftschmid, 1825)

Examined material: Singureni, 27.VIII.2003,  $(2 \ \ \ \ \ )$ , M.S.; Clinceanca, 5.V.2004,  $(1 \ \ \ \ )$ , D.F.; Căscioarele, 5.VII.2005,  $(5 \ \ \text{specs.})$ , M.S.; a common species, found on *Mentha longifolia* (L.) Hudson.

Distribution: Europe, Asia Minor, Caucasian countries, northern Iran, Central Asia.

#### Chrysolina (Erythrochrysa) polita (Linnaeus, 1758)

Examined material: Cernica, 7.VII.1995 (1 spec.), P.Ş; Comana, 3.VI.2002, (2 specs.), M.S.; Singureni, 26.VI.2003, (1 spec.), M.S.; Singureni, 27.VIII.2003, (2 specs.), M.S.; Căscioarele, 10.V.2005, (1 spec.), D.F.; found on Carex pilosa Scop. and Mentha sp.

Distribution: from northern Spain, Ireland and southern Norway to China.

#### Chrysolina (s.str.) staphylaea (Linnaeus, 1758)

Examined material: Căscioarele, 5.VII.2005, (3 specs.), M.S.; found on Mentha longifolia L. (Hudson).

Distribution: widely distributed from Ireland and Island to Kamchatka.

#### Chrysolina (Colaphosoma) sturmi (Westhoff, 1882)

#### (Chrysomela diversipes Bedel, 1892)

Examined material: Spătaru, 25.VI.2003, (1  $\circlearrowleft$ ), M.S.; Călugăreni, 26.VI.2003, (1  $\circlearrowleft$ , 5  $\circlearrowleft$  $\circlearrowleft$ ), M.S.; Clinceanca, 5.V.2004, (1  $\circlearrowleft$ ), D.F.

Distribution: from France and southern England, Central Europe, northern Italy and the basin of the Danube to Russia, Ukraine, Caucasian countries.

#### Chrysolina (Sphaeromela) varians (Schaller, 1783)

Examined material: Sărata-Monteoru, 16.V.2005, (1  $\circlearrowleft$ ), S.S.; found on Hypericum perforatum L.

Distribution: Europe, western Siberia. Introduced also in North America, for biological control of weeds.

Chrysomelids from the sylvosteppe forests of Romania

Genus: *Chrysomela* Linnaeus, 1758

### Chrysomela (s.str.) saliceti Suffrian, 1849

Examined material: Măriuța, 30.VI.1988, (1 spec.), S.A.; found on Salix sp.

Distribution: widely distributed from France to Mongolia.

#### Chrysomela (Strickerus) vigintipunctata (Scopoli, 1763)

Examined material: Măriuța, 30.VI.1988, (1 spec.), S.A.; Singureni, 3.IV.1999, (5 specs.), P.S.

Distribution: Palaearctic region, from eastern France to Japan.

Genus: Colaphus Dahl, 1823

#### Colaphus sophiae (Schaller, 1783)

Examined material: Comana, 26.IV.1995, (1 spec.), P.Ş; Dumbrăveni, 19.VI.1999, (1 spec.), M.S.

Distribution: Central and South-eastern Europe.

Genus: Gastrophysa Chevrolat, 1837

#### Gastrophysa (s.str.) polygoni (Linnaeus, 1758)

Examined material: Călugăreni, 26.VI.2003, (1 spec.), M.S.; Spătaru, 25.VI.2003, (1 spec.), M.S.; Sărata-Monteoru, 16.V.2005, (2 specs.), S.S.

Distribution: Europe, Asia Minor, Caucasian countries, Central Asia.

Genus: Gonioctena Chevrolat, 1837

#### Gonioctena (Spartomena) fornicata (Brüggemann, 1873)

Examined material: ab. conjuncta Endrödi: Gura-Foii, 5.VII.2005, (1 spec.), M.S.; typical form: Comana, 12.IV.1994, (1 spec.), P.Ş; Gura-Foii, 5.VII.2005, (3 specs.), M.S.; found on *Medicago sativa* L.

Distribution: the basin of the Danube, southern Poland, Balkan Peninsula, Ukraine, southern Russia, Caucasian countries, Asia Minor.

Genus: Plagiodera Chevrolat, 1837

#### Plagiodera versicolora (Laicharting, 1781)

Examined material: Singureni, 3.IV.1999, (3 specs.), P.Ş.; found on Salix sp. Distribution: Holarctic.

Genus: *Prasocuris* Latreille, 1802

#### Prasocuris phellandrii (Linnaeus, 1758)

Examined material: Călugăreni, 17.V.1997, (1 spec.), P.Ş.; found on Botrichium sp.

Distribution: Europe, northern part of Asia Minor.

GALERUCINAE Latreille, 1802

Genus: Calomicrus Stephens, 1831

#### Calomicrus circumfusus (Marsham, 1802)

(Luperus nigrofasciatus Weise, 1886)

Examined material: Gura-Foii, 5.VII.2005, (1 3), M.S.

Distribution: Iberian Peninsula, France, England, Germany, northern Italy, basin of the Danube, Romania.

Genus: Galeruca Müller, 1764

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Galeruca (s.str.) tanaceti (Linnaeus, 1758)

Examined material: Vedea, 30.V.1999, (11 specs.), M.S.; Comana, 16.IX.1994, (1 spec.), P.Ş; Spătaru, 25.VI.2003, (2 specs.), M.S.; Spătaru, 28.VIII.2003, (1 spec.), M.S.; Frasinu, 25.V.2003, (2 specs.), M.S.; found on Bromus tectorum L., Erysimum diffusum Ehrh., Marrubium vulgare L. and Cornus sanguinea L. Distribution: from Ireland and Portugal to Korea; introduced in North America.

Genus: *Phyllobrotica* Chevrolat, 1837 *Phyllobrotica adusta* (Creutzer, 1799)

Examined material: Ciofliceanca, 4.VI.2002, (30 specs.), M.S.; found especially on Urtica dioica L.

Distribution: Balkan Peninsula, basin of the Danube, Ukraine.

Phyllobrotica quadrimaculata (Linnaeus, 1758)

Examined material: Cernica, 15.V.1992, (1 spec.), P.Ş; Comana, 3.VI.2002, (1 spec.), M.S.; found on Carex pilosa Scop.

Distribution: Europe.

Genus: Xanthogaleruca Laboissière, 1934

Xanthogaleruca luteola (Müller, 1766)

Examined material: Comana, 3.VI.2002, (1 spec.), M.S.

Distribution: from Portugal and France to Caucasus and Central Asia.

HALTICINAE Newman, 1834

Genus: Altica Müller, 1764

Altica oleracea (Linnaeus, 1758)

Examined material: Singureni, 26.VI.2003, (4 specs.), M.S.; Singureni, 27.VIII.2003, (4 33), M.S.

Distribution: widely distributed, from Spain and Ireland to Japan.

Genus: Aphtona Chevrolat, 1837

Aphtona nonstriata (Goeze, 1777)

(Altica coerulea Geoffroy, 1785)

Examined material: Călugăreni, 17.V.1997,  $(1 \ \bigcirc, 1 \ \bigcirc)$ , P.Ş.

Distribution: Europe, Turkey, Caucasian countries, Iran, Kazakhstan.

Aphtona venustula (Kutschera, 1861)

Examined material: Călugăreni, 7.VII.2005, (2 33), D.F.

Distribution: from Pyrenees and Ireland to Ukraine, Asia Minor and Caucasus.

Genus: Crepidodera Chevrolat, 1837

Crepidodera aurata (Marsham, 1802)

(Haltica versicolor Kutschera, 1860)

Examined material: Singureni, 26.VI.2003, (1 3), M.S.

Distribution: Europe, Asia. Reported also from Marocco.

Crepidodera plutus (Latreille, 1804)

(Chalcoides chloris Foudras, 1860)

Examined material: Singureni, 26.VI.2003, (1  $\circlearrowleft$ ), M.S.

Distribution: Transpalaearctic species.

Genus: Epitrix Foudras, 1859

Epitrix pubescens (Koch, 1803)

Examined material: Comana, 1.V.1996, (2 33), P.Ş; Frecăței, 25.VIII.2005, (2

specs.), M.S.; found on *Dentaria* sp.

Distribution: from Azores to western Siberia.

Genus: Hermaeophaga Foudras, 1859

Hermaeophaga mercurialis (Fabricius, 1792)

Examined material: Comana, 3.V.1997, (1 3), P.Ş.; found on Mercurialis

perennis L.

Distribution: Europe, Central Italy, Ukraine, Asia Minor.

Genus: Neocrepidodera Heikertinger, 1911

Neocrepidodera transversa (Marsham, 1802)

Examined material: Comana, 18.IX.1994, (2 specs.), P.Ş; Singureni, 27.VIII.2003, (3 specs.), M.S.; Frasinu, 26.VIII.2003, (1 spec.), M.S.; found on Artemisia sp.

Distribution: Europe, from Portugal and Ireland to the Caspian Sea, in Asia Minor, Near East, Caucasian countries, Iran, Afghanistan.

Genus: Phyllotreta Chevrolat, 1837

Phyllotreta atra (Fabricius, 1775)

Examined material: Frasinu, IX. 2003, (1 3), D.F.

Distribution: Europe, western part of Asia to Mongolia.

Phyllotreta ochripes (Curtis, 1837)

Examined material: Călugăreni, 25.V.1995, (1 spec.), P.C.

Distribution: Europe, Asia Minor, Caucasian countries, Siberia, Russia, Iran.

Phyllotreta vittula (Redtenbacher, 1849)

*Examined material*: Singureni, 27.VIII.2003, (1  $\circlearrowleft$ ), M.S.; Clinceanca, IX.2004, (1  $\circlearrowleft$ ), D.F.; Frecăței, 25.VIII.2005, (1 spec.); found on *Raphanus* sp.

Distribution: from Spain and Ireland to Korea; introduced in North America.

Genus: Podagrica Chevrolat, 1837

Podagrica fuscicornis (Linnaeus, 1766)

Examined material: Singureni, 27.VIII.2003, (12 specs.), M.S.; found on Althaea officinalis L.

Distribution: Europe, Canary Islands, northern Africa, Asia Minor, Near East.

Genus: Sphaeroderma Stephens, 1831

Sphaeroderma testaceum (Fabricius, 1775)

Examined material: Spătaru, 28.VIII.2003, (1 3), M.S.

Distribution: Europe, Caucasian countries, Asia Minor. CASSIDINAE Gyllenhal, 1813

Genus: Cassida Linnaeus, 1758

#### Cassida (s.str.) lineola Creutzer, 1799

(Cassida signata Herbst, 1799)

Examined material: Comana, 30.VI.1996, (1 spec.), P.Ş.

Distribution: from Central Europe to Japan and Taiwan.

#### Cassida (Pseudocassida) murraea Linnaeus, 1767

Examined material: Măriuța, 20.VI.1982, (1 spec.) S.A.; Comana, 30.VI.1996, 3.V.1997, (2 specs.), P.Ş; Măriuța, 30.VI.1998 (1 spec.), S.A.

Distribution: Transpalaearctic species, distributed from the British Isles to Japan.

#### Cassida (s.str.) rubiginosa Müller, 1776

Examined material: Comana, 3.VI.2002, (13), M.S.; Căscioarele, 5.VII.2005, (1 spec.), M.S.

Distribution: Palaearctic region. Introduced also in North America.

#### Cassida (Odontionycha) viridis Linnaeus, 1758

Examined material: Călugăreni, 17.V.1997, (1 spec.), Ş.P.; Căscioarele, 10.V.2005, (1 spec.), D.F.; a common species, found on *Mentha longifolia* (L.) Hudson.

Distribution: Transpalaearctic species, distributed from the British Isles to Japan.

Genus: Hypocassida Weise, 1893

#### Hypocassida subferruginea (Schrank, 1776)

Examined material: Călugăreni, 17.V.1997, (1 spec.), P.Ş. Distribution: from Marocco and the British Isles to Korea.

Genus: Pilemostoma Desbrochers Des Loges, 1891

#### Pilemostoma fastuosa (Schaller, 1783)

Examined material: Comana, 18.IV.1994, (1 spec.), P.Ş; Căscioarele, 5.VII.2005, (1 spec.), M.S.; found on *Inula* sp.

Distribution: from the British Isles and France to the Baikal Lake.

#### CONCLUSIONS

This paper presents preliminary data concerning the distribution of leaf-beetles fauna in the sylvosteppe forests from southern and South-Eastern Romania. A total of 68 species belonging to 36 genera and 9 subfamilies have been recorded, being in concordance with vegetation varieties of the studied biotopes. We consider that the list of chrysomelid species recorded in these areas cannot be ended here, its completion remaining as subject for future studies.

Acknowledgements. I would like to express my sincere thanks to Prof. Dr. Andrzej Warchałowski (Zoological Institute, University of Wrocław, Poland), for his helpful comments to improve the manuscript and to Vasile Sanda PhD (from the Institute of Biology, Romanian Academy) for the information regarding the phytosociological characterization of the studied forests.

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Received July, 2006

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# A SURVEY OF THE COLLEMBOLAN FAUNA OF SOME AGROECOSYSTEMS IN A DIKE ISLAND OF THE LOWER DANUBE FLOODPLAIN (INSULA MARE A BRĂILEI)

#### CRISTINA FIERA

The aims of the present study are: to quantify the collembolan richness diversity of various types of agroecosystems (meadows, arable land with wheat, corn and soybean and a forest plantation with *Salix* sp.); to determine the differences between collembolan communities in these types of ecosystems; to discover the environmental factors responsible for these differences. *Hemisotoma orientalis* (Stach, 1947) is reported for the first time in the Romanian fauna.

Key words: Collembola, Insula Mare a Brăilei, agroecosystems, flooding area, dike island.

#### INTRODUCTION

Insula Mare a Brăilei (IMB) is the largest island of the Danube floodplain. It was largely drained during the previous political era. Regional management plans in the past have tended toward intensive agriculture and aquaculture, but more recently sustainability has emerged as an objective. Currently IMB is dyked and used as agricultural land, but it is planned to be partly restored into a multifunctional complex of terrestrial and wetland ecosystems (Bodescu and Iordache, 2005). Now there is a large degree of forests fragmentation, the presence of a rather large area with natural ("unproductive") ecosystems, as well as of seminatural forests, a trees population structure shifted towards young individuals, indicating overexploitation, and a low productivity of the forests. Current managerial measures are directed towards wood production and hydrological services, neglecting the biodiversity services. Changes in the managerial regimen are recommended in order to enhance the potential role of the forests from this microlandscape in the recolonisation process occurring envisaged to contribute to the IMB restoration.

River floodplains are disturbance-dominated landscapes characterized by high levels of habitat diversity and the presence of biota adapted to exploit the spatial and temporal heterogeneity (Ward *et al.*, 1999). Collembola are frequent inhabitants of floodplains. Their life cycle depends on both aquatic and terrestrial

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habitats, and collembolan species richness in floodplains can vary according to levels of disturbance induced by flood events. Even after inundation, Collembola retain high densities in the drying forest floor. It is thus apparent that they possess strategies for recolonization of the forest floor immediately after flooding (Gauer, 1997).

This study of the Collembola communities inhabiting a dike island of the Lower Danube Floodplain was done in 2005. Biological and ecological processes on the island are determined by periodic floods on the Danube River (Botnariuc, 1967) that can submerge most of it and pose a threat to animal survival.

This group was not subject to extensive and faunistical studies in Insula Mare a Brăilei. In one study, in the lower Danube floodplain, Cogălniceanu *et al.* (1998) analyzed the food of a population of *Pelobates fuscus* and revealed the low occurrence of Collembola, which is possible due to their small dimensions that make them difficult to locate and no energetical rewarding to pursue. But, they do not determine at the species level.

#### MATERIAL AND METHODS

The study site, Insula Mare a Brăilei, is an island situated in the lower Danube River floodplain south of the town of Brăila, Romania. Covering 81,766,195 hectares and having shallow lakes (25% of the surface), forests (35%) and agricultural fields (40%) IMB is still under a natural inundation regime. Beside this, IMB was completely diked and drained in the early 1960, adding new pressure on the remaining wetlands by increasing the inundation level.

The landscape is in constant change, depending on the water level. In late spring some parts of the island can be almost entirely covered by water and in late summer and autumn the inner lakes and channels can be entirely dry. There are at least two major flood events each year, in spring and in autumn, sometimes lasting 3 months. Apart from the naturally occurring changes in water level there are unpredictable changes due to variable rates of hydroelectric-power exploitation in reservoirs along the main river and its tributaries. Changes in water level are rapid, since most of the floodplain upstream was dammed and drained and its buffering capacity lost (Cogălniceanu & Miaud, 2003).

The studies on the collembolan fauna, inhabiting the island from the lower Danube floodplain were carried out in 2005, in June and August, in various types of agroecosystems:

SI. meadow (*Hordeetum murini* Libbert 1932 em. Pass. 1964 – *Agropyretum pectiniformae* (Prodan, 1939) Dihoru 1970

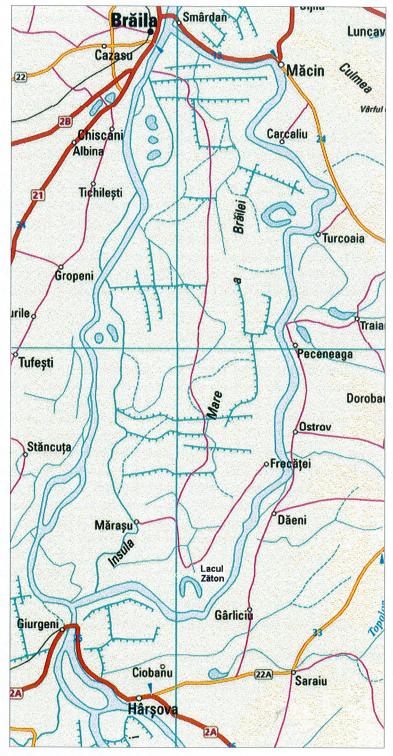


Fig. 1. Insula Mare a Brăilei - map.

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SII. meadow (*Xeranthemo cylindracei-Brometum arvensis* Popescu Gh. 1992 [Syn.: *Brometum arvensis* (Şerbănescu, 1957 n.n.) Kiss 1964] — *Typhetum laxmannii* Nedelcu 1969)

SIII. meadow (*Conietum maculati* I. Pop 1968 – *Arctio-Ballotetum nigrae* (Felföldy 1942) Morariu 1943

SIV. arable land (*Triticum aestivum* – wheat)

SV. arable land (*Glycine mas* – soybean)

SVI. arable land (*Zea mays* – corn)

SVII. forest ecosystem – plantation (Salicetum albae Issler 1924 s.l.)

The last site is located in Frecăței locality (Fig. 1). It was completely flooded in spring of 2005.

Five random soil samples were collected in each plot. All soil samples were transported in plastic bags to the laboratory. Extraction was done with modified Berlese-Tullgren funnels at a temperature of 18–23 degrees C for 7 days, Edwards (1991). The individuals were collected and preserved in ethyl alcohol 96%. The material was determined under a phase-contrast microscope. Collembola were identified to species level, using the most recent keys: Zimdars & Dunger (1994), Pomorski (1998), Bretfeld (1999), Potapov (2001), Thibaud *et al.* (2004).

#### RESULTS AND DISCUSSIONS

31 species of Collembola were found in the studied area (Table 1). The number of species on the area under study reached values from 4 and 11 species of Collembola. Higher species richness was revealed in the second site, which is a meadow with *Xeranthemum*. 558 individuals were obtained from 55 soil samples taken in two occasion sampling.

Hemisotoma orientalis (Stach, 1947) is recorded for the first time in the Romanian fauna. There are 3 further species with uncertain taxonomic position and they might be new to the Romanian fauna: Ceratophysella cf. gibbosa (Bagnall, 1940), Protaphorura cf. glebata Gisin, 1952, Lepidocyrtus cf. arrabonicus Traser, 2000. The Collembola richness species at particular sites is lower than it has been reported in other studies from agroecosystems: Clemen & Pedigo (1970), Shams et al. (1981), Kovac (1994), Kovac et al. (2001), probably due to unfavorable ecological factors.

Table 1

The species of Collembola reported from Insula Mare a Brăilei

No.	Species	St. 1	St. 2	St. 3	St. 4	St. 5	St. 6	St. 7
1	Ceratophysella succinea			х		х		
2	Ceratophysella denticulata					х		<u> </u>

Table 1 (continued)

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No.	Species	St. 1	St. 2	St. 3	St. 4	St. 5	St. 6	St. 7
3	Ceratophysella cf. gibbosa					x		
4	Schoettella ununguiculata							Х
5	Axenyllodes bayeri					X		
6	Pseudachorutes subcrassus					X		
7	Friesea mirabilis							Х
8	Mesaphorura macrochaeta	х						
9	Protaphorura cf. glebata					X	ļ	
10	Folsomia quadrioculata						Х	
11	Folsomia manolachei		L				Х	
12	Parisotoma notabilis	х		Х		х	х	
13	Hemisotoma thermophyla	x	X		х	X		ļ
14	Hemisotoma orientalis*		X			ļ	<u> </u>	
15	Isotoma anglicana*	x	x			<u> </u>	X	
16	Isotoma viridis		х		<u></u>	ļ	<u> </u>	
17	Isotomiella minor	x			х			X
18	Desoria sp. j.		х			-		
19	Seira domestica		X	X	ļ			
20	Lepidocyrtus sp.				X	ļ		ļ
21	Lepidocyrtus cf. arrabonicus		Х	х	<u> </u>		-	<u> </u>
22	Pseudosinella picta	х				ļ	ļ	ļ
23	Pseudosinella sexoculata	<u> </u>				<b>_</b>	X	<u> </u>
24	Entomobrya sp. j.		x					ļ
25	Entomobrya sp.				<u> </u>	-	ļ	X
26	Orchesella sp.			ļ			<u> </u>	X
27	Tomocerus vulgaris	ļ	1		X	ļ	<u> </u>	<u> </u>
28	Lipothrix lubbocki		↓	<b> </b>	ļ	<del> </del>	<del> </del>	X
29	Lipothrix sp. n.			1	ļ	х	<u> </u>	
30	Sminthurinus elegans		x			<del> </del>		
31	Sminthurinus sp. j.		x	<u> </u>			⊥	

<sup>\*</sup> new species for Romanian fauna

The distribution and number of soil Collembola are more stable on meadow than on arable soil. The forests ecosystems and meadows bear a vegetative covering through a period when little, if any, vegetation exists on arable land. Consequently, food material is always present for the large majority of species. At the same time land is not cultivated and the hibernation of species or period of low activity proceeds normally.

On arable land, the opposite is the case, the winter plugging and working of the soil brings the fauna to the surface and exposes the animal life, not only to the harsh climatic condition, but also to predator attack. There is a corresponding increase in the collembolan fauna on arable land, forests and meadow as vegetative growth increase. The circumstance is to be expected since conditions favorable to plants are those most suited to animal life. It may be said that the collembolan fauna of arable land consists of: a) species which

have existed from the previous passing the winter in the soil; b) species which are introduced or migrate in the soil in the growing season.

Meadow can be a source of the influx of fauna on arable land. Of course, certain species will be attracted to the particular crop and may form the dominant ones for that year. With regard to their common occurrence it does not seem that their limited numbers on meadow or forests are sufficient to cause a strenuous competition for food and consequent migration of members of species to less populated areas, such as arable land. At the same time the invading species will have to face the more unsuitable environmental conditions found on arable soil. Accidental migration will take place, but it will hardly be followed by an adequate explanation. The predominant species on arable land are those commonly found on the meadow. So, there is no characteristic collembolan fauna of arable soil.

The fauna of the inundation forest (Frecăței) comprises terricolous and arboricolous animals. For Collembola inhabiting the inundation forest, the ability to migrate can serve as a preadaptation for a quick use of optimal conditions in the habitat after flooding. Flood resistant eggs, subject to inundation for up to several months in oxygen free water, can be considered to be a direct adaptation to the conditions in the inundation forest (Gauer, 1997).

#### CONCLUSIONS

All species of Collembola reported from Insula Mare a Brăilei are new records for this area.

There are great differences in community characteristics between all types of agroecosystems. It is necessary to get more information about collembolan communities of other sites within the same type of vegetative association to compare the results.

Since our results include only some preliminary data about communities of Collembola in some agroecosystems of Insula Mare a Brăilei, data from this area for a long period of study will be necessary to obtain a complete list of collembolan fauna.

Acknowledgements. We thank Dr. Kovac L., Dr. Babenko A. and Dr. Weiner W. for helping in determination and confirmation of some species of Collembola and Avramescu Claudiu for collecting the biological material. This study was supported by AGRAL RES-NAT Project: "Amenajarea Luncii Dunării" (801/2005).

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Received November 6, 2006.

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#### TAXONOMICAL STRUCTURE OF THE EPICRIINA AND GAMASINA MITES (ACARI-MESOSTIGMATA: EPICRIINA, GAMASINA) IN FORESTRY ECOSYSTEMS FROM THE BUCEGI MASSIF

#### MINODORA STĂNESCU, VIORICA HONCIUC

This paper presents the structure of the Mesostigmata-Epicriina, Gamasina mites from three ecosystems with *Picea abies, Abies alba* and *Fagus sylvatica*. Species composition of edaphic mites showed the presence of the two suborders: Epicriina with one family (Epicriidae) and three species and Gamasina with 10 families (Parasitidae, Veigaidae, Ameroseiidae, Aceosejidae, Rhodacaridae, Macrochelidae, Pachylaelaptidae, Laelaptidae, Eviphididae and Zerconidae) with 39 genera and 96 species. The temporal dynamics shows the presence of two periods of study. In the first period were recorded 89 species and in the second one 81 species. The identified species were classified in the following trophical categories: predators, omnivorous, polyphagous and phythophagous.

Key words: mites, Gamasina, taxonomy, ecosystem, the Bucegi Massif.

#### INTRODUCTION

Mites (Arachnida, Acari) are microarthropods that live in various ecological habitats: plants, animals, on soil and in its levels. Their presence in every type of habitat is due to the complex of evolutional, ecological and stochastic factors.

The study of their biodiversity is not developed, but the number of the taxonomical described mites is high (about 40.000–50.000 individuals). The recent estimations showed the existence of 1.000.000 species (Walter & Proctor, 1999). One of the causes of this taxonomical diversity is the trophic preferences. In general, species from the Mesostigmata order are detritivorous, predators and endoparasites or ectoparasites. Most of these mites are secondary and tertiary consumers in terrestrial ecosystems (Evans, 1992).

#### MATERIALS AND METHODS

The researches were made in 2001–2003, in two periods (2001–2002; 2002–2003), in forests with *Picea abies, Abies alba* and *Fagus sylvatica* from the Bucegi Massif.

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The ecosystem with *Picea abies* is situated on a slope of 35°, on the east side of the mountain, at 1 350 m altitude. The vegetal association is *Leucanthemo waldsteinii–Piceetum* Krajina 1933 (Sanda, 2002).

The ecosystem with *Abies alba* is situated on a slope of 10°-15°, on the north west side of the mountain, at 950-1 000 m altitude. The vegetal association is *Oxalis pleurozium-Abietetum* Beldie 1967 (Beldie, 1967).

The ecosystems with Fagus sylvatica is situated on a slope of 10°, on the south side of the mountain, at 1 200 m altitude. The vegetal association is Symphyto cordati–Fagetum Vida (59) 63 (Sanda, 2002).

Fauna of mites from these areas was sampled (14 samples) with MacFadyen soil core. The identification of the mites from the Mesostigmata order was made till species level: Athias Henriot (1968); Blaszak (1974); Hyatt (1980); Juvara (1976); Karg (1993); Giliarov and Bregetova (1977). The extraction was performed with a modified Berlese-Tullgren extractor. After taxonomical identification, numerical abundance was obtained that constituted a database for the characterization of the population dynamics of mites.

#### RESULTS AND DISCUSSIONS

Species composition of edaphic mites from Mesostigmata showed the presence of the two suborders: Epicriina with one family (Epicriidae) and 3 species and Gamasina with 10 families (Parasitidae, Veigaidae, Ameroseiidae, Aceosejidae, Rhodacaridae, Macrochelidae, Pachylaelaptidae, Laelaptidae, Eviphididae și Zerconidae) with 39 genera and 96 species (Table 1).

From all identified taxa, 19 are signalized for the first time in Romania: Leptogamasus parvulus, Leptogamasus obesus, Paragamasus similis, Lysigamasus neoruncatellus, Lysigamasus truncus, Pergamasus alpinus, Epicriopsis rivus, Cheroseius sp., Zerconopsis remiger, Dendrolaelaps rotundus, Dendrolaelaps foveolatus, Dendrolaelaps willmanni, Dendrolaelaps samsinaki, Rhodacarellus kreuzi, Protogamasellus sp., Macrocheles insignitus, Zercon arcuatus, Zercon athiasi and Zercon tatrensis. 45 species are common for these three ecosystems, 6 have preferences for spruce forest, 10 for fir forest and 9 for beech ecosystem.

Table 1

Species composition of Mesostigmata mites in forest ecosystems from the Bucegi Massif

Species	Picea abies	Abies alba	Fagus sylvatica
EPICRIINA			
Epicriidae			
Epicrius bureschi Balogh, 1959	+	+	+
Epicrius resinae Karg, 1871	+	+	+

Table 1
(continued)

Species	Picea abies	Abies alba	Fagus sylvatica
Epicrius mollis Lramer, 1976	+	+	+
GAMASINA			
Parasitidae			
Amblygamasus mirabilis Willmann, 1951	+	+	+
Holoparasitus excipuliger Karg, 1971	+	+	+
Holoparasitus rotulifer Willmann, 1940		+	
Holoparasitus minimus Holzmann, 1969		+	+
Holoparasitus excisus Berlese, 1905	+		
Leptogamasus parvulus Berlese, 1903	+	+	+
Leptogamasus obesus Holzmann, 1969		+	
Leptogamasus sp.		+	
Leptogamasus variabilis Juvara-Balş, 1981		+	
Leptogamasus doinae Juvara-Balş, 1981	+		
Leptogamasus tectegynellus Athias- Henriot, 1967	+	+	+
Paragamasus similis Willmann, 1953	+	+	+
Paragamasus (Aclerogamasus) motasi Juvara-Balş, 1977		+	+
Paragamasus (Anidogamasus) vagabundus Karg, 1968			+
Paragamasus (Adinogamasus) sp.		+	. +
Lysigamasus neoruncatellus Schweizer, 1961	+	+	+
Lysigamasus truncus Schweizer, 1961	+	+	+
Pergamasus barbarus Berlese, 1904	+	+	+
Pergamasus quisquiliarum R. & G. Canestrini, 1882	+	+	+
Pergamasus alpinus Berlese, 1903	+		
Pergamasus laetus Juvara-Balş, 1970	+	+	+
Pergamasus athiasae Juvara-Balş, 1970	+	+	+
Eugamasus monticolus Berlese, 1905	+		+
Eugamasus magnus Kramer, 1876	+	+	+
Gamasodes spiniger Tragardh, 1910	+	+	
Parasitus furcatus G. & R. Canestrini, 1882	+	+	+
Vulgarogamasus kraepelini Berlese, 1905	+	+	+

Table 1 (continued)

Species	Picea abies	Abies alba	Fagus sylvatica
Vulgarogamasus oudemansi Berlese, 1903	+	+	+
Vulgarogamasus zschokkei Schwiezer, 1922	+	+	+
Vulgarogamasus remberti Oudemans, 1912	+	+	+
Porrhostaspis lunulata Muller, 1859	+	+	+
Veigaidae			
Veigaia nemorensis C.L. Koch, 1939	+	+	+
Veigaia exigua Berlese, 1917	+	+	+
Veigaia cerva Kramer, 1876	+	+	+
Veigaia propinqua Willmann, 1936	+	+	+
Veigaia paradoxa Willmann, 1951		+	+
Veigaia transisalae Oudemans,1902	+	+	+
Veigaia kochi Tragardh, 1901			+
Gamasolaelaps multidentatus Karg, 1965			+
Gamasolaelaps excisus C.L.Koch, 1879			+
Ameroseiidae			
Epicriopsis rivus Karg, 1971		+	+
Aceosejidae			
Cheroseius sp.			+
Iphidozercon venustulus Berlese, 1917	+		+
Zerconopsis remiger Kramer, 1876	+	+	+
Lasioseius lawrencei Evans, 1958	+	+	
Leioseius magnanalis Evans, 1958	+	+	+
Arctoseius brevichelis Karg, 1969		+	
Arctoseius semiscissus Berlese, 1892	+	+	+
Arctoseius cetratus Sellnick, 1940	+	+	+
Arctoseius eremitus Berlese, 1918	+	+	+
Proctolaelaps pomorum Oudemans, 1929	+	+	+
Melichares juradeus Schweizer, 1949	+	+	+
Amblyseius sp.		+	
Rhodacaridae			
Leitneria granulata Halbert, 1923		+	+
Asca bicornis Canestrini & Fanzago, 1887		+	
Dendrolaelaps rotundus Hirschmann, 1963	+	+	+

Table 1 (continued)

(continued)					
Species	Picea abies	Abies alba	Fagus sylvatica		
Dendrolaelaps foveolatus Leitner, 1949	+	+			
Dendrolaelaps willmanni Hirschmann, 1960		+			
Dendrolaelaps samsinaki Hirschmann şi Wisniewski, 1982			+		
Rhodacarellus kreuzi Karg, 1965	+	+	+		
Rhodacarellus silesiacus Willmann, 1936			+		
Protogamasellus sp.			+		
Macrochelidae					
Neopodocinum mrciaki Sellnick, 1968	+	+	+		
Geholaspis mandibularis Berlese, 1904		+			
Geholaspis longispinosus Kramer, 1876	+	+	+		
Macrocheles decoloratus C.L.Koch, 1839		+	+		
Macrocheles montanus Willmann, 1951	+	+	+		
Macrocheles insignitus Berlese, 1918			+		
Pachylaelaptidae					
Pachylalaeps latior Berlese, 1920	+		+		
Pachylaelaps pectinifer G. și R. Canestrini	+	+			
Pachylaelaps furcifer Oudemans, 1903	+	+	+		
Pachylaelaps magnus Halbert, 1915		+	+		
Olopachys scutatus Berlese, 1910		+			
Olopachys vysotskajae Koroleva, 1976		+	+		
Laelaptidae					
Pachyseius humeralis Berlese, 1910	+	+	+		
Hypoaspis aculeifer Canestrini, 1883	+	+	+		
Hypospis nolli Karg, 1962	+	+	+		
Hypoaspis oblonga Evans & Till, 1966	+	+	+		
Hypoaspis montana Berlese, 1904	+				
Eviphididae					
Eviphis ostrinus C.L.Koch, 1836	+	+ ;	+		
Zerconidae					
Zercon fageticola Halaskova, 1969	+	+	+		
Zercon peltatus C.L.Koch, 1836	+	+			
Zercon peltadoides Halaskova, 1970	+	+			
Zercon triangularis C.L.Koch, 1836	+	+	Ę		

Table 1 (continued)

Species	Picea abies	Abies alba	Fagus sylvatica
Zercon romagniolus Sellnick, 1944	+	+	+
Zercon arcuatus Tragardh, 1931	+		+
Zercon pinicola Halaskova, 1970	+	+	
Zercon carpathicus Sellnick, 1958	+	+	
Zercon athiasi Vincze, 1965	+		
Zercon tatrensis Blaszak, 1974	+		
Prozercon kochi Sellnick, 1943	+	+	+
Prozercon traegardhi Halbert, 1923	+	+	+
Prozercon fimbriatus C.L.Koch, 1839		+	
Prozercon sellnicki Halaskova, 1963	+	+	+

The spatial dynamics shows a maximum number of species in *Abies alba* ecosystem (78), followed by the *Fagus sylvatica* ecosystem (71 species) and the minimum was recorded in the *Picea abies* forest (67 species) (Fig. 1).

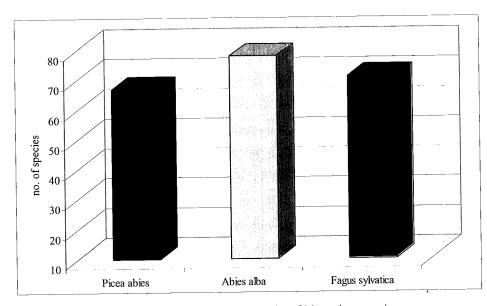


Fig. 1 – Spatial dynamics of number of species of Mesostigmata mites in forestry ecosystems from Bucegi Massif.

Taxonomical analysis shows the presence of the 24 predator genera (Amblygamasus, Holoparasitus, Leptogamasus, Paragamasus, Lysigamasus, Pergamasus, Eugamasus, Gamasodes, Parasitus, Vulgarogamasus, Porrhostaspis,

Veigaia, Gamasolaelaps, Leitneria, Asca, Dendrolaelaps, Rhodacarellus, Protogamasellus, Neopodocinum, Geholaspis, Macrocheles, Pachylaelaps, Olopachys, Eviphis), 4 omnivorous genera (Lasioseius, Proctolaelaps, Zercon, Prozercon), 9 polyphagous genera (Cheroseius, Iphidozercon, Leioseius, Pachyseius, Amblyseius, Melichares, Arctoseius, Hypoaspis, Zerconopsis) and one phythophagous genus (Epicriopsis). Trophical characterization of the Epicrius genus is not being made yet, because there are no informations about the food preferences (Karg, 1993; Walter & Proctor, 1999).

Analysing the trophical spectrum of the gamasid mites from the studied ecosystems dominant are the predators, followed by the polyphagous and omnivorous species and the most slightly represented are the phythophagous species.

The dynamics of the trophical structure was constant, with little fluctuations in the *Picea abies* ecosystems, where 38 predators were recorded from a total number of identified species, 49. The same situation was in *Fagus sylvatica* ecosystems where 6 omnivorous species were recorded from a total of 14 (Fig. 2).

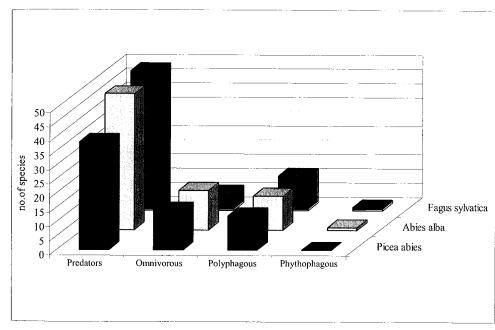


Fig. 2 – Dynamics of the trophical structure of the gamasid mites in forestry ecosystems from the Bucegi Massif.

The most abundant species are: Paragamasus similis, Veigaia nemorensis, Neopodocinum mrciaki (predators), Zercon triangularis and Zercon fageticola (omnivorous). Although these species represent only 5.28% from the total number, their trophical characterization is very important for the structure and functions

(especially productivity), participating indirectly to the flow of energy and matter through regulation of structure of other groups of invertebrates (springtails, nematodes, oribatids, etc.): Honciuc and Stănescu (2000); Stănescu & Gwiazdowicz (2004 a); Stănescu & Gwiazdowicz (2004 b).

The temporal dynamics showed the presence of two distinct periods in structural characterization of gamasids: 2001–2002 – the first one and 2002–2003 – the second one.

In forestry ecosystems studied the number of identified species decreased in the second period (from 89 to 81 species), due to the climatic changes (fluctuation of the humidity and temperature) and to the human influences. The most important decrease of the total number of species in these two periods was recorded in the *Picea abies* ecosystems. This process is caused by the presence of a big slope (35°) and the powerful washing of the litter and fermentation layer (Fig. 3).

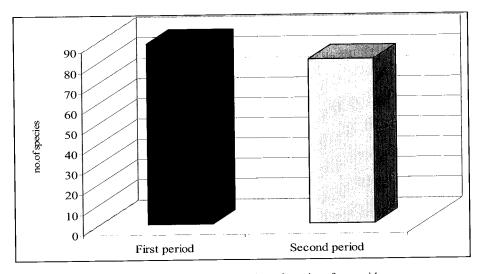


Fig. 3 – Temporal dynamics of the number of species of gamasids, in the two periods, in forestry ecosystems in the Bucegi Massif.

The evolution in these two periods of the taxonomical structure of the gamasids is constant. The first period of research recorded the biggest number of identified species. From 89 identified species, 67% are predators, 17.55% omnivorous, 14.89% polyphagous and only 0.53% phythophagous.

In the second period, the taxonomical spectrum decreases with 8.33%, in comparison with the first one. Predators represent 66% from the total number of identified species (81), the omnivorous 14.54%, polyphagous 17.57% and phythophagous 1.06% (Fig. 4).

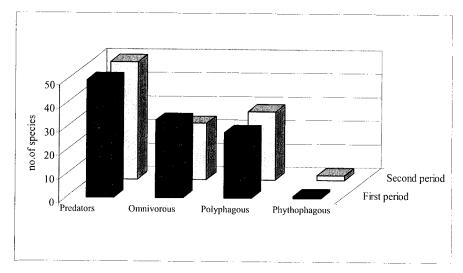


Fig. 4 – Dynamics of the taxonomical structure of the gamasids in forestry ecosystems from the Bucegi Massif.

#### CONCLUSIONS

Species composition of edaphic mites from Mesostigmata in these forestry ecosystems showed the presence of the two suborders: Epicriina with 3 species and Gamasina with 96 species.

In these three ecosystems 45 species are common, 6 have preferences for the *Picea abies* ecosystem, 10 for the *Abies alba* ecosystem and 9 for the *Fagus sylvatica* one.

In the first period of study the highest numerical abundance was recorded (89 species), in comparison with the second period (81 species).

In trophical structure of the gamasid mites dominant are predators, followed by the polyphagous and omnivorous species and the most weakly represented are the phythophagous species.

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Received November 20, 2006.

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## THE MAIN ALIEN/INVASIVE INSECTS SPECIES IN ROMANIA

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The data refer to 98 insect species belonging to Orthoptera, Blattaria, Thysanoptera, Homoptera, Heteroptera, Hymenoptera, Coleoptera, Lepidoptera and Diptera orders, from which, 43 can be considered invasive species. A number of 85 species are pests, 5 are vector and household species and 8 are useful species (parasitoid, predator, pollinator, silk worm).

Key words: alien species, alien invasive species, non-native species.

#### INTRODUCTION

Alien species are species, subspecies or lower taxons, introduced from outside its natural past or present distribution; it includes any part, gametes, seeds, eggs, or propagules of such species that might survive and subsequently reproduce. Invasive alien species is an alien species whose introduction and/or spread threatens biological diversity. Alien species include all taxonomic groups, including viruses, prions, bacteria, mycorrhiza and feral animals of domestic species (European Strategy on Invasive Alien Species). Alien species problem is a complex, double-edged problem. These species interfere with any local species, destroy, but in some cases enrich local biodiversity. After the introduction of most alien species, ineluctable dispersion and inevitable damages were registered, which may have an adverse effect upon autochthonous biodiversity. From Fakir (1998) "the alien organisms easily colonized the country's natural habitats to compete with indigenous biota and, in many cases, caused the extinction of irreplaceable endemic species". But not all alien species become invasive in a new area and many of these bring considerable benefits in biological control, as natural enemies of different animal or vegetal pests.

In the classical and modern period of biological control, thousands of parasitoid, predator and phytophagous were intentionally introduced in new territories with a view to exert the biological control of many alien invasive pests (phytophagous, vectors, intermediate host animals, weeds). Having this idea in view, Waage (1998) asks and answers "Can the solution to an alien species problem really be to introduce another alien species? The answer is yes, if that alien predator or parasite is specific to the target species and not a risk, directly or indirectly, to native species".

ROM. J. BIOL. – ZOOL., VOLUME **51**, N<sup>os</sup> 1–2, P. 43–61, BUCHAREST, 2006

Insects are able to disperse into new habitats through a variety of means: flying, dissemination through air currents, water, human intentional or unintentional introduction (import and exceeding of international trading with grains, nuts, fruits, vegetables, tobacco and other merchandises; import of ornamental plants or vegetative organs for seeding and breeding). Alien insect species that become invasive can have a significant environmental, economic and upon public health impact as pests in agriculture, horticulture, forestry, as vectors for viral, bacterial, fungal or protozoan human or plant diseases, or as etiological agents for certain diseases.

There are three possible approaches to the problem of alien species: (prevention of introduction, eradication as soon as possible after introduction, and population density control after establishment, to maintain its impact at an acceptable level from the economic point of view and of the public health). In the most cases, the only or at least the best solution is control, especially the biological control methods in front of the integrated management techniques. Insects have some characteristics, which explain impossibility of alien species prevention and eradication. There are flying ability, small dimensions, possibility to be clandestine passengers on airplanes, ships, cars, impossibility to detect their initial entry in a new area, high reproductive capacity and adaptability to new environmental conditions, the capacity to switch the trophic range (from monophagous to oligophagous and polyphagous).

The problem of the invasive alien species is not only a local or national issue; it is a very important global matter, which must be approached through transnational, regional and international co-operation. The problem of the invasive alien species is in attention of many institutions. The Global Invasive Species Program has been developed which has elaborated a Global strategy on Invasive Alien Species and a Toolkit of Best Prevention and Management Practices. This Strategy promotes the development and implementation of coordinated measures and cooperative efforts throughout Europe to prevent and minimize adverse impact of invasive alien species on Europe's biodiversity (as well as its economy and human health and well-being).

#### MATERIAL AND METHODS

The paper is the result of authors' observations and researches: Manole (1997), Manole, Iamandei (1996), Teodorescu (1988, 1994, 1998), Teodorescu et al. (1997, 2001, 2004), Tudor, Teodorescu, Ciurdărescu (1980) and also of Balachowsky (1963), Borror et al. (1989), Capinera (2001), Gherasim (1973), Manolache (1978), Perju et al. (2001), with an elaborate study concerning the alien/invasive species introduced in Europe and Romania.

#### RESULTS AND DISCUSSIONS

Personal investigations and literature reviews point out the introduction of a number of 98 alien species in Romania (Tables 1–3), between 1864 (*Viteus vitifolii* Fitch.) and 1996 (*Diabrotica virgifera virgifera* LeConte), belonging to 9 insect orders. A number of 85 species are pests, 5 are vector and household species and 8 are useful species (parasitoid, predator, pollinator, silk worm). A number of 43 can be considered invasive. From literature data, these species were introduced mostly unintentionally, but some of these (predators, parasitoids, silk worm and pollinator) were intentionally introduced. The most species are cosmopolitan, or with tropical and subtropical origin. The ways of introduction are mainly attributed to the human vector (transport of people and goods, trading activities).

Table 1
The main alien/invasive insect species

Orders, Family, Species	Hosts trophic range	Native area, year of introduction, actual status in Romania
1	2	3
Orthoptera  Dociostaurus  maroccanus  Acrididae	Polyphagous: cereal crops, Medicago, Trifolium, Helianthus anuus, Vitis, Beta, Solanum, Nicotiana, wild plants	Central Asia. In Romania, it is a common species in the whole country, especially in pastures; in the last years increased densities were registered and some damages. Alien invasive crop pest.
Calliptamus italicus Catantopidae	Polyphagous: cereals, Solanum, Beta vulgaris, Medicago, Helianthus, Vitis, etc.	Central Asia. In Romania, it is common species, in the whole country, especially in pastures.  Alien invasive crop pest.
Thysanoptera		
Haplothrips niger Phloeothripidae	Trifolium pratense	North America. In Europe, it was mentioned in 1952. In Romania it was detected in 1937, on Piatra Arsă (Bucegi Massif); now it is present in the whole country on the red clover crops. Alien crop pest.
<i>Thrips tabaci</i> Thripidae	Polyphagous: over 300 plant species from 25 families (Liliaceae, Leguminosae, Cruciferae, Solanaceae Cucurbitaceae, Compositae)	Easter Mediterranean zone or India. It is transported on plant material. In Romania it is present in the whole country; the most serious problems occur on Nicotiana, Allium, Gossypium and in the greenhouse. Alien invasive crop pest.

Orders, Family, Species	Hosts trophic range	Native area, year of introduction, actual status in Romania
Heliothrips haemorrhoidalis Thripidae	Citrus, Coffea, Mangifera, Theobroma, Persea, Palmae	Tropical America. In Romania, it is present only in some greenhouses. Invasive species in greenhouses.
Frankliniella occidentalis Thripidae	Polyphagous: ornamental and vegetable plants species (Allium, Solanum, Lactuca, Raphanus, Lycopersicum, Cucurbita, Chenopodium)	North America. In Europe, it was mentioned in 1970, in France. In Romania it was detected in 1990, in Dolj district (Işalniţa greenhouses); it is very dangerous. Invasive species in greenhouses.
Homoptera		
Ceresa bubalus Membracidae	Malus, Pyrus, Prunus, Armeniaca, Persica, Cerasus, Amygdalus vulgaris, Vitis	North America. In Europe, it was introduced in 1912, in Hungary. In Romania it is dispersed in all regions. Alien orchard pest.
Trialeurodes vaporariorum Aleyrodidae	Polyphagous: more than 300 plant species from 18 families of ornamental and vegetable plants	The origin is not clear (Mexico or the southwestern United States). In Europe it was mentioned in 1941. In Romania, it was signalled in 1949, in Bucharest. It is present and very dangerous in all greenhouses and in the field, only during the summer. Alien invasive greenhouse pest.
<i>Bemisia tabaci</i> Aleyrodidae	High polyphagous: over 500 plant species (Solanaceae, Leguminosae, Euphorbiaceae, Cucurbitaceae, Compositae, Cruciferae, etc.)	Tropical and subtropical regions. In Europe it was mentioned in 1889 in Greece. In Romania, it was detected in 1969; now, sporadically attacks registered in the tobacco, bean and cotton crops. Alien invasive crop pest.
Aleyrodes proletella Aleyrodidae	Polyphagous: Cruciferae, Euphorbiaceae, Papaveraceae, Compositae	Africa. In Europe, it was mentioned in 1938. In Romania it was cited in 1949, in Bucharest; some damages were registered in greenhouses and in the field, on <i>Brassica oleracea</i> v. capitata, B. oleracea v. botrytis and Lycopersicum esculentum

Orders, Family, Species	Hosts trophic range	Native area, year of introduction, actual status in Romania
Bulgarialeurodes cotesii (syn. B. rosae) Aleyrodidae	Rosa sp. (cultivated and spontaneous)	In Romania, it was signalled in 1969; it is dispersed especially in south regions. Alien crop pest.
<i>Rhopalosiphum</i> <i>maidis</i> Aphididae	Polyphagous: over 67 plant species, especially Poaceae (Zea mays, Hordeum vulgare, Avena sativa, Secale cereale, Sorghum halepense, Triticum, Echinochloa crusgalli, Setaria)	Asia. Now, it has a world distribution. In Romania, it is one of the most important maize crop pests, in the whole country.  Alien invasive crop pest.
Brevicoryne brassicae Aphididae	Cruciferae, especially <i>Brassica</i> and <i>Capsella bursa-pastoris</i>	United States and Canada. In Europe, it was introduced back to the late 1700. In Romania it is a very common species in the whole country. Alien invasive crop pest.
<i>Myzus persicae</i> Aphididae	Highly polyphagous: over 40 plant families (Compositae, Cruciferae, Chenopodiaceae, Solanaceae, Cucurbitaceae)	Central Asia. At present, it is found all over the world. In Romania, it is very common, present in the whole country, in the field and greenhouses; damages on peach and in greenhouse on tomato, eggplant, pepper. Alien invasive pest.
<i>Macrosiphum</i> euphorbiae Aphididae	Polyphagous: Solanaceae, Chenopodiaceae, Amaranthaceae, Malvaceae, Cruciferae, Cucurbitaceae, Leguminosae, etc.	North America. At present, it is found over the world. In Romania, it was signalled in 1942 in greenhouses, as <i>M. koehleri</i> ; now it is present in whole country, being one of the most important potato crop pest.  Alien invasive crop pest.
Eriosoma lanigerum Eriosomatidae	Malus species, very rare on Pyrus and Cydonia	North America. In 1787 it was introduced in Europe (England). In Romania it is dispersed in the whole country; initially it was a common and important apple pest, but at present, population density and attack intensity decreased. Alien orchard pest.

Orders, Family, Species	Hosts trophic range	Native area, year of introduction, actual status in Romania
Viteus vitifolii (syn. Phylloxera vastatrix) Phylloxeridae	Vitis vinifera, other Vitis species	North America (Allegany district). In 1863, it was introduced in Europe (in England, in greenhouses Hammersmith, near London, and in France, near Pujault and Bordeaux). In France, it destroyed initially over 1,500,000 hectares. In Romania it was mentioned in 1864, in Prahova district (Dealu Mare vineyard). Now it is distributed in all vineyard regions. Initially, were registered very serious damages, but at present the attack is low. Alien invasive pest.
Aspidiotus hederae Diaspididae	Polyphagous (over 200 plant species): Citrus, Olea europaea, Mangifera indica, Asparagus, Palmae, other ornamental plants	Mexico. In Europe, it was mentioned in 1935 and, in Romania, it was detected in 1944, in Bucharest; severe damage was registered especially on Asparagus.  Invasive species.
<i>Diaspis echinocacti</i> Diaspididae	Exclusively on Cactaceae species, especially <i>Echinocactus</i> , <i>Opuntia</i> , <i>Zygocactus</i> , <i>Erdisia</i>	Central America. In Europe, it was mentioned in 1912. In Romania, it was detected in 1949, in Bucharest; it is present only in greenhouses, without damages. Alien greenhouses pest.
<i>Diaspis boisduvali</i> Diaspididae	Palmae, Cactaceae, Bromeliaceae, Orchidaceae	Central America. In Europe, it was mentioned in 1935 and, in Romania, in 1949, in Bucharest; in greenhouses, without significant damages. Alien greenhouses pest.
Chrysomphalus dictyospermi Diaspididae	Polyphagous: Citrus, Persea, Musa, Mangifera, Camellia sinensis, Palmae	Mexico. In Europe, it was mentioned in 1935. In Romania, it was detected in 1956, in Bucharest; now, it is frequent in greenhouses and registered some damages on ornamental plants.  Alien invasive greenhouses pest.

Orders, Family, Species	Hosts trophic range	Native area, year of introduction, actual status in Romania
<i>Quadraspidiotus</i> <i>perniciosus</i> Diaspididae	Polyphagous: over 200 trees species (including orchard trees), ornamental shrub, herbaceous plants	North of China. In the U.S.A., it was identified in Carolina, San José Valley, in 1873, and in Europe, in 1928 in Hungary (Seghed locality). In Romania, it was mentioned in 1933, in Bihor, Arad, Timiş districts. Between 1933 and 1948 it destroyed about 27 % of the orchards trees. Now it is one of the most important orchards (especially <i>Malus</i> , <i>Pyrus</i> , <i>Persica</i> , <i>Cerasus</i> ) pest.  Alien invasive orchard pest.
<i>Saissetia oleae</i> Lecanidae	Polyphagous: over 100 genera of spontaneous and cultivated plants	Africa. In Europe, it was mentioned in 1911. In Romania, it was signalled in 1944, in Bucharest; significant damages registered in greenhouses, especially on <i>Chrysanthemum</i> .  Alien greenhouses pest.
Saissetia hemisphaerica Lecanidae	Polyphagous: Cycas, Asparagus, Anthurium, Coffea, Citrus, Philodendron, Asplenium	Asia (India). In Europe, it was mentioned in 1936. In Romania it was found in 1955, in Bucharest; sporadical attack on ornamental plants observed.  Alien invasive greenhouses pest.
Lecanium hesperidum Lecanidae	Polyphagous: Citrus, Ficus, Agave, Camellia, Nerium, Cycas, Hibiscus, Thea, Cyclamen	Asia. In Europe, it was mentioned in 1912. In Romania, it was detected in 1940; Alien invasive and very dangerous greenhouses pest.
Pulvinaria floccifera Lecanidae	Anthurium, Camelia, Mahonia	Japan. In Romania, it is present only in greenhouses.
Pulvinaria mesembryanthemi Lecanidae	Mesembryanthemum	Mediterranean regions. In Romania, it is present only in greenhouses.
Pseudococcus citri Pseudococcidae	Polyphagous: Citrus, Coffea, Musa, Ficus, Mangifera, Liliaceae, Orchidaceae	Tropical and subtropical regions. In Europe, it was mentioned in 1915. In Romania, it was signalled in 1960, in Bucharest; it is very dangerous in greenhouses and on houseplants. Alien invasive greenhouses pest.

Orders, Family, Species	Hosts trophic range	Native area, year of introduction, actual status in Romania
Pseudococcus adonidum Pseudococcidae	Polyphagous: <i>Cycas</i> , <i>Citrus</i> , <i>Musa</i> , <i>Ficus</i> , <i>Dracaena</i> , Cactaceae, Liliaceae, Orchidaceae	Subtropical and Mediterranean regions. In Europe, it was mentioned in 1922. In Romania, it was detected in 1976, in Bucharest; it is very dangerous in greenhouses and on houseplants. Alien invasive greenhouses pest.
Pseudococcus mammilariae Pseudococcidae	Cactaceae	Subtropical and Mediterranean regions. In Romania, it is present only on Cactaceae, in greenhouses. Alien invasive greenhouses pest.
Heteroptera		Central Asia. In Romania,
Eurygaster integriceps Scutelleridae	Triticum aestivum, Avena sativa, Hordeum vulgare, Secale cereale, wild Poaceae (Bromus, Astragalus, Poa)	central Asia. In Romania, it is an important pest, in some conditions with high damages, not only as a result of feeding activity, but because its saliva is highly toxic. Alien invasive crop pest.
Hymenoptera		
<i>Trachelus tabidus</i> Cephidae	Triticum, Secale cereale, Avena sativa, Hordeum vulgare	Mediterranean region. In Romania, it was detected in 1961, in south districts. Alien invasive crop pest.
Bruchophagus roddi Eurytomidae	Trifolium pratense, T. repens, Medicago sativa	North America. In Europe, it was mentioned in 1912. In Romania it was detected in 1950, in Braşov and Bucharest regions; now it is present in the whole country, with significant damages in the clover crops.
Bruchophagus platypterus Eurytomidae	Lotus corniculatus, L. tenuis, L. uliginosus	North America. In Europe, it was mentioned in 1914. In Romania it was signalled in 1960, in Transylvania with some damages in <i>Lotus</i> crops.
Bruchophagus gibbus Eurytomidae	Cultured and spontaneous species of genus <i>Trifolium</i>	North and South America. In Europe, it was mentioned in 1932 (in France) and in Romania in 1960, in Transylvania region. At present, the species is spread in Transylvania and North Moldova. Alien crop <i>Trifolium</i> pest.

Orders, Family, Species	Hosts trophic range	Native area, year of introduction, actual status in Romania
Eurytoma schreineri Eurytomidae	Prunus domestica, P. spinosa, P. cerasifera, Cerasus vulgaris	Asia (Astrakhan). In Europe, it was mentioned in 1910 and in Romania in 1979, in Jasy district; it is now a plums orchards pest, with significant damages.
Monomorium pharaonis Formicidae  Coleoptera	Stored foods	Central Asia. In Europe, it was detected in 1970 and in Romania it was introduced in stored foods. Alien storage pest.
Trogoderma granarium Dermestidae	Stored grains (especially wheat), cereal products, peanuts	Asia (India). In Romania it was introduced with stored food products; it is an important pest of stored grains.
Rhizopertha dominica Bostrychidae	Stored grains	Tropical and subtropical regions. In Romania it was mentioned in 1975; it is a pest of stored products, especially grains.
Lasioderma serricorne Anobiidae	Tobacco, wheat flour, peanuts	Tropical and subtropical regions. In Romania it was introduced with stored products; it is an important pest of tobacco, wheat flour, cereals, peanuts and bean.
Tenebrioides mauritanicus, Ostomidae	Stored grains	Africa. In Romania, it was introduced with stored food products; it is an important pest of stored grains.
Oryzaephilus surinamensis, Cucujidae	Wheat, rice seeds and other cereals, flour, dry fruits and vegetables, tobacco	Asia. The introduction in Europe and Romania is not clear. In Romania, it is present in the whole country, in stored food products. Alien invasive storage pest.
<i>Oryzaephilus</i> <i>mercator</i> Cucujidae	Oil plant seeds, nuts and wheat and maize flour, stored rice	Asia. The introduction in Europe and Romania is not clear. In Romania it is present in the whole country, in stored food products. Alien invasive storage pest.
Tribolium castaneum Tenebrionidae	Wheat flour, cornmeal, chocolate, nuts, bran, dried fruits	Subtropical regions. In Romania, it was introduced with stored food products being an important pest of stored grains, in the whole country. Invasive species.

	<del> </del>	Native area, year
Orders, Family,	Hosts trophic range	of introduction, actual status
Species	Hosts tropine range	in Romania
		Africa. In Romania it was
		introduced with stored food
Tribolium confusum	Wheat flour, cornmeal, chocolate, nuts,	products, being an important
Tenebrionidae	bran, snuff	pest of stored grains, in whole
		country. Invasive species.
		Africa (Tunisia). In Europe, it
		was mentioned in 1903. In
Phyllopertha lineata	Vitis vinifera, ornamental trees	Romania it was detected only in
Scarabaeidae	, this ringer of a second	Dobroudja region, on the sandy
		soils. Alien vineyard pests.
		Middle Asia. In Europe, it was
		mentioned in 1890, and in
	Polyphagous: Vitis vinifera, Phaseolus	Romania, in 1955, in the South
Anoxia orientalis	vulgaris, Beta vulgaris, Allium cepa,	regions. It is one of the most
Scarabaeidae	Dahlia, Fragaria, Phlox, Althaea,	important pests of vineyard,
	Rosa	especially on the sandy soils.
		Alien vineyard pest.
		Perhaps the mountainous
		middle-eastern region from
Bruchus pisorum		Ethiopia to Afghanistan.
Bruchidae	Pisum sativum	In Romania, it is an alien storage
Brucilidae		pest species, with significant
		damages.
		Africa. In Europe, it was
		mentioned in 1926 (Wahl,
Bruchus lentis		Austria) and in Romania in
Bruchidae	Lens culinaris	1944; now it is present in whole
Diucinaac		country. Unimportant storage
		pest.
		Africa (Egypt). In Europe, it was
		mentioned in 1932 (Crebert,
Bruchus rufimanus		Germany) and in Romania in
Bruchidae	Vicia faba, Phaseolus vulgaris	1949; now it is present in the
Dracmade		whole country.
		Alien storage pest.
		North of Africa (Morocco).
		In Europe, it is present from
	Vicia villosa, V. sativa, V. pannonica,	1876. In Romania, the date of
Bruchus brachialis	V. cracca, Pisum sativum, Lens	introduction is not so clear.
Bruchidae	esculenta	It is spread in the whole country,
		but with unimportant damages.
		Alien storage pest.
		Middle Asia. In Europe, it was
	Lens esculenta, Lathyrus latifolius	mentioned in 1957 and in
Bruchus ervi Bruchidae		Romania in 1970; sporadically
		damage on Lens was registered.
		Alien storage pest.

Orders, Family, Species	Hosts trophic range	Native area, year of introduction, actual status in Romania
Bruchus tristiculus Bruchidae	Lathyrus annuus, L. odoratus, L. cicera L. latifolius, L. tingitonus, Cicer, Pisum, Lens	North of Africa. In Europe, it was present from 1943, in France and in Romania the presence was cited from 1980.  Alien storage pest.
Bruchus marginatus Bruchidae	Vicia, Lathyrus, Pisum, Cicer arietinum	Middle Asia. In Europe, it was mentioned in 1944 and in Romania, in 1966.  Alien storage pest.
Acanthoscelides obtectus Bruchidae	Phaseolus vulgaris, Lens culinaris, Vicia faba, Soja hispida	Central Asia. In Europe, it was mentioned in 1874 (Spain) and in Romania in 1946 (introduced probably from France); now it is a common species, dispersed in the whole country.  Alien invasive storage pest.
Leptinotarsa decemlineata Chrysomelidae	Solanum tuberosum, S. melongena, S. niger, Lycopersicum esculentum, Hyosciamus niger, Nicotiana rustica, Datura stramonium	Probably Mexico. Potato crop damages registered beginning about 1859 in Nebraska, North America (Colorado region). In Europe, it was introduced in 1876 in Germany (Bremen) and in 1922 in France (near Bordeaux). In Romania, it was signalled in 1952, in Maramureş district, Săpânţa locality; now it is the most important pest of potato crops, present in the whole country.  Alien invasive crop pest.
Diabrotica virgifera virgifera Chrysomelidae	Especially <i>Zea mays</i> , and other cultivated ( <i>Soya</i> ) and spontaneous plants	North America, first maize attacks noticed in Colorado, in 1909. In Europe, it was introduced in 1992 in Serbia. In Romania, it was detected in 1996, in Nădlac locality, Arad district; now it is present in many west and south districts. Dispersed rate is high.  Alien invasive crop pest.

Orders, Family, Species	Hosts trophic range	Native area, year of introduction, actual status in Romania
Anarsia lineatella Gelechiidae	Persica vulgaris, Prunus domestica, Armeniaca vulgaris	Eurasia origin. In Romania, it was mentioned in 1961, in west (Bihor district); it is a serious peach orchards pest.  Invasive species.
Phthorimaea operculella Gelechiidae	Solanum, Lycopersicum, Nicotiana, Datura stramonium, Hyoscyamus, Lycium europaeum	North America. In Europe, it was mentioned in 1906. In Romania it is dispersed in the whole country, being a pest of potato and related plants.  Alien invasive crop pest.
Sitotroga cerealella Gelechiidae	Maize and wheat grains	USA. In Romania it was signalled in 1914, in Bucharest; it is a common species, in whole country. Damages both in field and warehouses.  Invasive species.
Etiella zinckenella Phycitidae	Leguminosae species: Soya hispida, Vicia faba, V. sativa, Phaseolus vulgaris, Pisum sativum, Lupinus, Astragalus	It was first observed in the South of U.S.A (California) in 1885, but its origin is unknown. In Romania it was introduced in 1941, with some damages in soybean crops.  Alien invasive crop pest.
<i>Plutella xylostella</i> Plutellidae	Only wild and cultivated crucifers	Tropical and subtropical regions. In Romania, it is one of the main cabbage and other cruciferous plants pests. Alien invasive crop pest.
Plodia interpunctella Pyralidae	Dried fruits and vegetables, cereals (especially maize, wheat), nuts, sweets drug	Tropical and subtropical regions (India). In Romania it was introduced with stored products; it is an important pest in granaries, warehouses, markets and homes, in the whole country. Invasive species.
Margaritia (syn. Loxostege) sticticalis Pyralidae	Poyphagous (over 80 plant species): Cruciferae, Liliaceae, Leguminosae, Solanaceae, Cucurbitaceae, Chenopodiaceae, Compositae, Amaranthaceae, Vitaceae, Quercus, Robinia	Central Asia. Now it is present in Europe, Asia and North America. In Romania it was signalled in 1898 in Moldova region and was registered sporadically density and damages increases, especially in south and eastern parts of country.  Alien invasive crop pest.

Invasive species.

in orchard.

Invasive species.

invasive crop pest.

with alfalfa.

Hosts trophic range

All types of grain products

Initial mulberry tree (Morus sp.) pest. In present it is very polyphagous (feed

over 620 plant species, orchard and

ornamental trees, shrubs, vineyard,

herbaceous plants)

Persica vulgaris, Armeniaca vulgaris,

Prunus domestica, sometimes Malus

domestica,

Pyrus sativa, Cydonia vulgaris

Poyphagous (over 120 plant species):

Compositae

Cucurbitaceae, Leguminosae,

Solanaceae, Linaceae, Euphorbiaceae,

Convolvulaceae etc.

Trifolium species

Medicago sativa

Orders, Family,

Species

Ephestia kuehniella

Pyralidae

Hyphantria cunea

Arctiidae

Cydia (syn

Grapholitha)

molesta

Tortricidae

Heliothis armigera

Noctuidae

Diptera

Dasyneura

legumicola

Cecidomyiidae

Dasyneura ignorata

Cecidomyiidae

Orders, Family, Species	Hosts trophic range	Native area, year of introduction, actual status in Romania
Haplodiplosis marginata (= H. equestris) Cecidomyiidae	Cultured and spontaneous Poaceae species	Europe. In Romania, it was mentioned in 1969, in Argeş district; yield losses in wheat and barley crops, registered in west and southwest of the country. Alien crop pest.
<i>Liriomyza</i> huidobrensis Agromyzidae	Highly polyphagous: 14 plants families (Chenopodiaceae, Compositae, Liliaceae, Cruciferae, Cucurbitaceae, Leguminosae, Linaceae, Solanaceae)	South America. In Europe, it was mentioned in 1987. In Romania, it was detected in 1989, on <i>Lactuca sativa</i> ; sometimes damages area registered.
<i>Liriomyza trifolii</i> Agromyzidae	Polyphagous: over 22 plant species, especially Compositae (Chrysanthemum, Lycopersicum, Capsicum, Phaseolus, Daucus, Cucurbita, Solanum, Lactuca, Cucumis, Allium, Pisum)	Eastern U.S.A. and Canada, Northern of South America, Caribbean. In Europe it was mentioned in 1977. In Romania, it was signalled in 1981, near Bucharest; significant damages on Chrysanthemum, Lycopersicum, Capsicum were registered in greenhouses. Invasive species.
Eumerus strigatus Syrphidae	Allium cepa, Narcisus, Iris, Beta, Daucus	Asia. In Europe, it was detected in 1822 and, in Romania, it was signalled in 1988 in Bucharest; significant yield losses on onion crops in the south regions.  Alien invasive crop pest.
Eumerus tuberculatus Syrphidae	Allium cepa, Narcisus, Iris, Beta, Daucus	Asia. In Europe, it was detected in 1857 and in Romania it was signalled in 1988, in Bucharest; reduced damages registered in onion crops.
Rhagoletis alternata Tephritidae	Rosa canina, Lonicera xylosteana	Asia. In Europe, it was signalled in 1820. In Romania, it was detected in 1900, in Sibiu district; at present it is distributed in the whole country, but without economic importance.

Species, Orders, Families	Hosts trophic range	Native area, year of introduction, actual status in Romania
1	2	3
Blatta orientalis Blattaria, Blattidae	Omnivorous	Tropical regions. In Romania it is a very common species, in the whole country, in markets also homes but and outside, during the summer.
Periplaneta americana Blattaria, Blattidae	Omnivorous	Cosmopolitan. In Romania it was recently introduced (approximately in 1990 in Bucharest and perhaps 1990 in Constanța locality); it is present in markets and homes but also outside during the summer.
Blatella germanica Blattaria, Blattelidae	Omnivorous	Cosmopolitan. In Romania it is a very common species, in the whole country, in markets and homes but also outside during the summer.
Cimex hemipterus Heteroptera, Cimicidae	Haematophagous	Africa. In Romania it was introduced by migratory birds.
Anopheles sacharovi Diptera, Culicidae	Haematophagous	In Romania it was mentioned in 1933, in Dobroudja region, as a very important vector of malaria. It was eradicated in 1962, due chemical control measures.

Table 3

Alien useful species: intentional introduction

Species, Orders, Families	Hosts trophic range	Native area, year of introduction, actual status in Romania
1	2	3
Bombyx mori Lepidoptera, Bombycidae	Monophagous species (Morus)	Asia. Morus silkworm. In Romania, rearing with good results.
Phylosamia ricini Lepidoptera, Attacidae	Monophagous (Ricinus communis)	Asia. Ricinus silkworm. In Romania, rearing with good results.
Antheraea pernyi Lepidoptera, Attacidae	Quercus species	Asia. Oak silkworm. In Romania, experimental rearing, on <i>Salicis</i> leaves, with good results.

Species, Orders, Families	Hosts trophic range	Native area, year of introduction, actual status in Romania			
Aphelinus mali Hymenoptera Aphelinidae	Monophagous: parasitoid on Eriosoma lanigerum	From Canada and East U.S.A., it was introduced in 1918 in France, and in 1921 in Romania. Very efficacious parasitoid; it first determined a high pest populations but consequently his own depression, with pest populations increases.			
Prospaltella perniciosi Hymenoptera, Aphelinidae	Monophagous: parasitoid on Quadraspidiotus perniciosus	East Asia. It was introduced in 1950 in Germany and in 1951 in France. In Romania it was introduced in 1954 from Russia, in 1971 from France and in 1974 from Switzerland. Very efficacious in <i>Quadraspidiotus perniciosus</i> biological control.			
Aphytis proclia Hymenoptera, Aphelinidae	Monophagous: parasitoid on Quadraspidiotus perniciosus	Central Asia. In Romania it was introduced in 1971.			
Megachile centuncularis Hymenoptera, Megachilidae	<i>Rosa</i> sp.	U.S.A. In Romania it was introduced in 1972 to pollinate <i>Trifolium</i> species, but it is not established yet.			
Podisus maculiventris Heteroptera, Pentatomidae	Polyphagous predators, with a special tactism for all stages  Leptinotarsa decemlineata	Mexico, Central America. In Europe (inclusively Romania) it was introduced in 1990. Mass released in Muntenia, Moldavia, Dobroudja, Transylvania regions, for Leptinotarsa decemlineata populations control.			

As compared to the first mentioned cases in Europe, the first date of alien species detection in Romania was more or less close in time. In many cases, the small size of species can explain the long period between their date of detection in Europe and in Romania. The very long time period between the two mentions of *Eumerus* species (131–166 years) is due to the lack of taxonomic studies. In contrast, *Diabrotica virgifera virgifera* was detected in Romania, after only four years from the date when they were first mentioned near Belgrade airport.

One mention must be made about the change and enlargement of the trophic range in some alien species. The best example is *Hyphantria cunea*, which eats a very large number of plant species, although during its first years in Romania its food was only mulberry leaves.

#### CONCLUSIONS

The data is referring to 98 alien/invasive insect species belonging to Orthoptera, Blattaria, Thysanoptera, Homoptera, Heteroptera, Hymenoptera, Coleoptera, Lepidoptera and Diptera orders. A number of 43 can be considered invasive species. Among the listed species, 85 are phytophagous and pests (in vineyard, field crops, orchards, trees, greenhouses, and food products storage). Five species are vectorial and household species, being haematophagous or omnivorous. The piercing-sucking mouthparts and household species are biological or mechanical vectors from viral, bacterial, fungal and protozoan diseases. Eight species are beneficial (parasitoids, predators, introduced for biological pest control, pollinator or natural silk producers).

The most numerous alien species introduced in Romania are from Coleoptera (25), Homoptera (24) and Lepidoptera (18) orders, many of them being dangerous pest species.

The most numerous species were introduced in field crops (30), warehouses (23) and greenhouses (19).

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Received October, 2006

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# DYNAMICS OF THE *EURYGASTER* NUMERICAL DENSITY, IN HIBERNATION AND IN WHEAT CROPS (BRĂILA COUNTY, ROMANIA, 1996–2003)

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Some investigations extended in time (1996–2003) and in space (crops and forests from Brăila County), on the most important pest for the cereals in Romania (*Eurygaster integriceps*) revealed the main factors, which determined its population's dynamics. In the forests, differences between the adult's densities registered in each autumn, and those in the next spring, in the hibernation places, were caused mainly by the non-biotic factors (floods, high temperature intervals in early spring, followed by sudden and sharp decreases of temperatures). More efficient, mortality in the forests was caused by the entomo-pathogenic fungi (during the warm and wet periods) and by the predators, in the warm autumns and springs. In the crops, mortality of the adults, larvae and eggs was caused mainly by the biotic factors (Scelionidae oophagous parasitoid), in comparison with non-biotic (strong wind, rain downpour, long drought).

Key words: Eurygaster species, sunn pest, cereals bugs.

#### INTRODUCTION

The species of *Eurygaster* genus (family Scutelleridae) and *Aelia* genus (family Pentatomidae) called by common name "the cereals bugs" are ones of the most important pests, which affect the wheat, barley, oat, crops on million hectare areas, from Near East, towards North Africa and South Europe.

Only 4 of 14 species of *Eurygaster* genus, known all over the world, were found in Romania: *E. maura* L., 1758; *E. austriaca* Schr., 1778; *E. testudinaria* Geoff., 1975; *E. integriceps* Put., 1881. The first mention in Romania regarding the *E. integriceps* (originating from high Asian plateau) was registered in 1938, location in a restricted Southeast area. Over the years, relatively significant increases of its populations, mainly in the Southeast areas, as well as its spreading towards Northwest, were observed.

In the latest years this species has been present in almost the whole country and it is in the attention of many authors, especially of Popov (1974, 1975, 1980, 1982, 1983, 1989, 1992, 1996, 1998) and Teodorescu (1988), Teodorescu *et al.* (1983 a, b, 2001, 2005), Vădineanu *et al.* (1987).

ROM. J. BIOL. – ZOOL., VOLUME **51**, N<sup>os</sup> 1–2, P. 63–70, BUCHAREST, 2006

The competition with *Eurygaster integriceps* caused the change of the numerical relations with other cereal bug species present in Romania, whose effectives diminished, while the *Eurygaster integriceps* effectives had compensatory increases.

Eurygaster integriceps is in the permanent process of enlarging its northwest area. In Romania this alien species is considered now the main specific and dangerous pest for the cereals crops.

The adult's damages are caused both by those coming from forests and which feed on the wheat small plants (for reproducing organs maturation), and those of the new generation, which, before harvesting, feed on the mature plants, wheat grains, and after harvesting, continue to feed on the spontaneous Graminaea plant species. The most important attack is upon the grains, which are emptied of their contents (with quantitative losses of grain production), or without modifying their shapes or weighs, suffer content alteration (with qualitative damages of grain and of the resulted flour). The larvae feed on all wheat aerial organs.

#### MATERIALS AND METHODS

The investigations were carried out during the 1996-2003 interval, in Brăila County, both in the litter of 4 Quercineae forests (Camniţa, Corbu, Tichileşti and Viişoara), and in the wheat fields from 4 localities (Ianca, Suţeşti, Tichileşti, Insurăţei). In the forests, the adults retire for wintering (hibernal diapause), and in the wheat fields adults and larvae feed.

**Our investigations aim** was to realise the *Eurygaster* attack prognosis, by correcting the long term prognosis, based on the autumn adults density in the hibernation places, with a short term prognosis, based on the spring adult and larvae densities, in forests and in crops, and to establish the main factors responsible for density dynamics.

#### The main investigation objectives were:

- The *Eurygaster* adult's numerical density and the death rate assessment, in the forests litter, in autumn (September-October months) and in spring (March-April months).
- The *Eurygaster* adults, eggs, larvae numerical density establish, in wheat field, in spring and summer.
  - The factors involved in *Eurygaster* population's dynamics establish.

#### The main activities to realise these objectives were:

- To assess the live and dead hibernant *Eurygaster* adults numerical density.
- To assess the numerical density of the *Eurygaster* adults coming out from wintering and located in the wheat fields, for feeding and eggs lying.
  - To assess the numerical density of the *Eurygaster* eggs, on wheat leaves.

- To establish the parasitism degree of  $\it Eurygaster$  eggs by Scelionidae oophagous species.
- To assess the new generation of *Eurygaster* larvae and adults numerical density, during feeding in the wheat crops.

For investigations, the metric frame was used both in the forests (in autumn and spring, for the alive and dead hibernating adults number registered) and in the wheat fields (for density assessment of the adults coming out of the wintering, of the larvae and of the next generation adults).

In order to have a complete spectrum of *Eurygaster* mortality determined in every winter by non-biotic and biotic factors, the mortality was established by sampling two times in the forest ecosystems (in autumn of each year and in spring of the next year). The summing up of the death rates of these two moments was made by the alive adults which are those populations effectively moving towards the cultivated fields. In the years 1996 and 1997, the samplings in the forests were carried out only in autumn.

To permanently notice the bug's biological development, the sampling performed in the wheat fields was carried out to estimate the numerical density of all stages (eggs, larvae and adults).

#### RESULTS AND DISCUSSION

There were established the dynamics of the *Eurygaster* adults numerical density during hibernation, the dynamics of the numerical density of the *Eurygaster* adults coming out from wintering, of the larvae and of the adults from the new generation, in the wheat crops, and the factors involved in the *Eurygaster* populations dynamics.

### THE DYNAMICS OF THE *EURYGASTER* ADULT'S NUMERICAL DENSITY DURING HIBERNATION

The density of the hibernating adults in the forests had the highest values in the autumn of 1996, in Camniţa (57.7 adults/ sq. m.) and Corbu forests (43.2 adults/sq. m) (Tables 1 and 2).

Favourable conditions of hibernation were recorded in the winters 1999-2000, 2000-2001 and in the autumns of 1996 and 2003. Extremely favourable conditions for the hibernating *Eurygaster* adults were registered in the winter 2000-2001 when in Corbu forest, no dead adults were found in autumn and spring.

The mortality during hibernation had the highest values in forests, in winters of 1997-1998, in Tichileşti, Corbu and Camniţa. This fact favoured the pest effective decreasing in nearby crops in the following year (1998) spring. The explanation for the high values of the mortality rate lies in the weather conditions.

During 19-29 February 1998 intervals, there were registered high temperatures (above  $+ 11,5^{\circ}$ C and even  $+ 20^{\circ}$ C on 23 February), followed in March by negative ones (-5,1°C). The high temperatures determined the wintering adults to become active, to leave the foliage, being numerically reduced by the frost, which soon followed. The warming up of the weather during spring determined the flooding of more than 52 % of the Tichileşti forest area, and the pest mass mortality was registered. In the 1999 spring, the Tichileşti forest was again flooded.

Table 1

The dynamics of the numerical density and death rate of *Eurygaster* hibernating adults (1996-1999)

D	1996	1997	1998		1999				
Parameters	autumn	autumn	spring	autumn	spring	autumn			
Camnița forest									
Density of all adults in litter	57.7	4.9	4.7	13.6	11.5	9.7			
Density of dead adults	1.2	0,2	2.7	2.1	0.1	0.2			
Death rate	2.08	4.08	57.45	15.44	0.87	2.06			
Death rate (autumn + spring)	2.08	61.53	16	5.31		2.06			
	C	orbu for	est						
Density of all adults in litter	43.2	3.7	37.0	2.2	1.7	6.9			
Density of dead adults	0.9	0.9	1.8	0.5	0.1	0.1			
Death rate	2.78	19.56	48.65	22.73	5.89	1.45			
Death rate (autumn + spring)	2.78	68.21	28.62		2.90				
	Ticl	hileşti fo	rest						
Density of all adults in litter	6.9	16.0	12.3	4.6	-	1.4			
Density of dead adults	0.3	3.7	7.2	0.2	-	0.3			
Death rate	4.3	23.12	58.54	4.35	-	21.43			
Death rate (autumn + spring)	4.3	81.66	4.35		21.43				
Viișoara forest									
Density of all adults in litter	99.6	28.2	27.7	8.6	7.4	24.1			
Density of dead adults	3.6	0.5	14.0	1.2	0.3	0.5			
Death rate	3.61	1.77	50.54	13.95	4.05	2.07			
Death rate (autumn + spring)	3.61	52.31	18.00 2.07		2.07				

The analysis of the death rate dynamics recorded in autumn, in forests, pointed out the existence of low values (between zero and 5 %, except for the years 1997 (in Corbu and Tichileşti forests), 1998 (in Camniţa, Corbu and Viişoara forests), 1999 and 2003 (in Tichileşti forest). The lowest values were recorded in the 2000-2001 period, in all the forests. The highest values (above 20 %) were recorded in Tichileşti forest (in 1997 and 1999) and in Corbu forest (in 1998).

The analysis of the death rate dynamics recorded in spring, in forests, evidenced very high values in 1998 (between 48 % and 58 %), due to the high temperatures recorded in the 19-29 February interval, followed in March by low

temperatures. The moderate values of the death rate (9 %-11 %) were recorded in all forests, in 2002, and in Tichileşti forest, in 2003. In 1999, 2001 and 2003 (except for Tichileşti forest), the values were low.

Table 2

The dynamics of the numerical density and death rate of *Eurygaster* hibernating adults (2000-2003)

Parameters	2000		2001		2002		2003		
	spring	autumn	spring	autumn	spring	autumn	spring	autumn	
Camniţa forest									
Density of all adults in litter	9.5	8.8	8.7	18.0	17.7	14.6	14.5	13.0	
Density of dead adults	0.1	0.1	0.0	0.3	1.7	0.1	0.7	3.0	
Death rate	1.05	0.14	0.0	16.7	9.60	0.68	4.83	2.31	
Death rate (autumn + spring)	0.14		26.3		5.51		2.31		
Corbu forest									
Density of all adults in litter	6.9	7.9	7.9	17.6	17.6	15.4	15.0	5.5	
Density of dead adults	0.1	0.0	0.0	0.1	2.3	0.4	0.6	1.5	
Death rate	1.45	0.0	0.0	0.57	13.09	2.60	4.0	27,27	
Death rate (autumn + spring)	0.0		13.66		6.60		27.27		
Tichileşti forest									
Density of all adults in litter	1.1	3.6	3.6	4.2	4.2	5.4	4.9	2.9	
Density of dead adults	0.0	0.0	0.1	0.0	0.5	0.3	0.5	1.5	
Death rate	0.0	0.0	2.78	0.0	11.9	5.55	10.2	51.72	
Death rate (autumn + spring)	2.78		11.9		15.75		51.72		
Viișoara forest									
Density of all adults in litter	23.5	25.6	25.2	26.8	26.1	79.0	77.0	13.9	
Density of dead adults	0.3	0.4	0.0	0.7	3.0	2.0	6.8	3.6	
Death rate	1.27	1.56	0.0	2.61	11.49	2.53	8.83	2.58	
Death rate (autumn + spring)	1.56		14.10		11.36		2.58		

The mortality rate of entire wintering period, ranging between 23.07 % at Camniţa and 27.27 % at Corbu, had an economic significance only at Camniţa and Viişoara, where the densities of the dead adults were quite high.

THE DYNAMICS OF THE NUMERICAL DENSITY OF THE *EURYGASTER* ADULTS COMING OUT FROM WINTERING, OF THE LARVAE AND OF THE NEW ADULTS, IN THE WHEAT CROPS

For adults, a special attention was focused both on those coming from forests and which feed on the wheat small plants, and on those of the new generation, which feed especially on the wheat grains, before harvesting and eventually continue to feed on the spontaneous Graminaea plant species, after harvesting. The adults from the new generation are most damaging their attack being not only quantitative but also with significant qualitative consequences (Table 3).

Table 3

The dynamics of the Eurygaster adults and larvae density in the wheat crops (1998-2003)

Density (ind /sq. m.)	1996	1997	1998	1999	2000	2001	2002	2003	
Wheat crops in lanca locality									
Density of the <i>Eurygaster</i> adults installed in the wheat fields	4.3	1.6	1.1	2.1	1.9	0.6	2.1	2.9	
Density of the <i>Eurygaster</i> larvae in the wheat fields	16.9	4.5	4.1	3.6	5.4	5.1	3.8	6.8	
Density of the new generation adults, during their feeding time, to prepare for diapause	9.2	0.2	0.3	0.4	0.5	0.9	1.1	1.5	
Wi	eat cro	ps in Ticl	ileşti lo	cality					
Density of the <i>Eurygaster</i> adults installed in the wheat fields	3.1	1.1	1.2	2.2	1.9	1.8	2.1	7.0	
Density of the <i>Eurygaster</i> larvae in the wheat fields	14.7	-	-	3.8	5.4	5.2	4.3	7.4	
Density of the new generation adults, during their feeding time, to prepare for diapause	1.2	1	0.4	0.3	0.3	1.1	2.3	2.4	
W	heat cr	ops in Şu	țești loc	ality		_	_		
Density of the <i>Eurygaster</i> adults installed in the wheat fields	12.3	2.6	0.7	2.1	2.0	1.5	1.6	2.3	
Density of the <i>Eurygaster</i> larvae in the wheat fields	19/7	5.5	3.8	2.9	5.2	4.9	3.3	2.8	
Density of the new generation adults, during their feeding time, to prepare for diapause	4.4	0.2	0.3	-	0.4	1.1	2.1	1.7	
Wheat crops in Insurăței locality									
Density of the <i>Eurygaster</i> adults installed in the wheat fields	5.6	2.8	2.7	3.2	2.7	2.4	2.9	2.9	
Density of the <i>Eurygaster</i> larvae in the wheat fields	21.3	8.2	4.9	4.3	5.4	5.5	6.2	6.6	
Density of the new generation adults, during their feeding time, to prepare for diapause	3.4	0.8	0.6	0.3	0.6	1.3	1.4	4.4	

- In the wheat crop from Ianca locality, in the vicinity of Camnita forest, the adult's density registered low values (between 0.6 and 4.3 ind/sq. m) except for 1996, when they registered higher values, but still below the level of the economic damaging threshold. The larvae coming out of the eggs laid by these adults and new generation adults had generally low densities, under the economic threshold or somewhere around it, but in 1966 the economic threshold was surpassed.
- In the wheat crop from Suţeşti locality, near Camniţa forest, only in 1996 there was recorded a higher density of the hibernating adults. The larvae density was also higher in 1996. The densities of the new generation adults had the lowest values in all years, sometimes, under the unit.

- In the wheat crops from Tichileşti locality, near the Tichileşti forest, the density of all stages was low, under the economic damaging threshold, in most of the years. Some high values, but not much under the damaging threshold, were registered, in 2003. An exception registered in 1996, when larvae density was high.
- In the wheat crops from Insuratei locality, in the vicinity of Viisoara forest, the larvae densities registered high values in 1996, four times higher as compared to the economic damaging threshold. Values somewhere near the damaging threshold were noticed in the case of the larvae, during the 2000-2003 interval. The adult's density was low, under the damaging threshold.

The low densities of adults from the new generation are very important from the point of view of wheat crop protection and flour quality.

#### FACTORS INVOLVED IN EURYGASTER POPULATIONS DYNAMICS

The death rate recorded in the forests, in autumn, was due especially to the exogenous factors of the populations (predators and pathogens, mainly fungous). In spring, mortality had as causes both exogenous factors (low temperatures, floods, predators and pathogens) and endogenous ones (the restrictive physiological conditions of the adults, which could not take up enough quantities of fat reserves, enabling them to survive during the hibernation).

The non-biotic factors (the low temperatures in wintering interval and the spring floods in forests) are especially implied in the numerical density dynamics of the wintering *Eurygaster* adults, causing adult mass mortality. Sudden increases of temperatures, and warm spring period (when adults stop hibernation and move towards the wheat fields), followed by very cold intervals, especially during the nights, with negative temperatures, have as a consequence, the high mortality rate.

The biotic factors (especially Scelionidae oophagous parasitoid) were important in the field dynamics of the numerical density of the adults coming out from wintering, of the eggs, larvae and new generation adults, in the wheat crops, than the commonly, non-biotic factors (strong wind, rain downpour, long drought).

#### **CONCLUSIONS**

The investigations, on the most important cereals pest in Romania, the alien invasive species *Eurygaster integriceps*, established the main factors, which determined their population's dynamics. The differences between the adults densities registered in each year, autumn, and those in the next spring, in the hibernation places, were caused mainly by the non-biotic factors (floods, high temperature intervals in early spring, followed by sudden and sharp decreases of values). The mortality in the forests caused by the entomo-pathogenic fungi, was

more efficient during the warm and wet periods. The predators are active in the litter, in the warm autumns and springs and determine a lower mortality.

The differences between the wheat crops densities of adults coming out from wintering, and the new generation of adults before harvesting, were caused mainly by the biotic factors, in comparison with non-biotic ones. Some favourable weather conditions in the crops caused the increase of the pest effective, the economic damaging threshold surpassing, and the feeding process extension, followed by the quantitative and qualitative damages of crop production.

The long term prognosis regarding the attack in the wheat fields, based on the values of autumn adults density at the hibernation place, in the forests, must be corrected in spring, with a short term prognosis in forests and crops. For the control methods application the *Eurygaster* attack correct prognosis implies the supervision of the density of adults during the hibernation, the density after their installation in the crops, the densities of larvae and adults from the new generation, in the crops, and the supervision of the various biotic and non-biotic factors. The low *Eurygaster* effectives arriving in the forest from the cultivated fields are favourable for the wheat crops of the next year and the high ones are an alarm signal for the next year crops.

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Received October, 2006

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# THE BIOMASS OF ZOOBENTHOS IN THE IRON GATES I DAMLAKE (ROMANIAN STRETCH) IN 2002

## VIRGINIA POPESCU-MARINESCU

We present in this paper the biomass of the invertebrate benthos, found in the Iron Gates I damlake. In comparison with biomass value found before the accumulation construction, the 2002 value is higher. The highest biomass values belonged especially to the species, primarily of fresh water, among the lamellibranchiate and gastropods. The Ponto-Caspian elements (gammarids, corophiids, isopods, polychaets) (secondarily of fresh water) had very rarely a significant contribution to the biomass of benthos invertebrates. In the two analyzed 2002 seasons, there were observed (at the level of benthos populations) wide spatial and seasonal variations. From the spatial variation point of view, the numbers corresponding to the lake middle navigable zone situated on the first place. Seasonally, the highest biomass values were found on all analyzed profiles, usually in summer.

Key words: zoobenthos, biomass, damlake, the Danube, Iron Gates.

### INTRODUCTION

Numerous researches were performed within the area of the present Iron Gates I damlake, both before by Bacesco (1948), Popescu & Prunescu-Arion (1961), Popescu-Marinescu (1970), and after the construction of the respective accumulation by Cure *et al.* (1975), Popescu-Marinescu (1986, 1987, 2002, 2004); Popescu-Marinescu *et al.* (1996, 2005). But, the major studies made in certain intervals were related to restricted river sectors or lake parts. The complex hydrological, chemical, biological researches (including our studies) concomitantly performed in 2002, within a contract with the "Iron Gates Hydroelectric station Branch", extended from 1072.4 km to 950 km, thus covering the entire lake area.

In this paper we present the results regarding the zoobenthos biomass in the Iron Gates I accumulation, obtained in 2002. The names of the profiles and stations executed by us, as well as the results of some observations and field measurements (the facies nature, water depth, etc.) are shown in Table 1.

We precise that the data regarding the taxonomic composition and numerical density of the zoobenthos, in the mentioned lake, in 2002, were already published.

ROM. J. BIOL. – ZOOL., VOLUME **51**, Nos *1–2*, P. 73–90, BUCHAREST, 2006

#### MATERIALS AND METHODS

The material and method were already published by us. Namely, the benthos samples were collected on three longitudinal profiles located between Baziaş and Bahna, while 14 transversal profiles included the zones near the left riparian (Romanian one), the lake middle navigable zone and the intermediary portion between the riparian and the lake middle navigable zone (Table 1). The samples were collected by a quantitative method, using a bodengreiffer of 400 cm<sup>2</sup> surface, on muddy, sandy, stony or mosaic facies.

 $Table \ 1$  Profiles and stations of zoobenthos sample collection, in the damlake Iron Gates I, in 2002

Profile name	Danube km	Stations	Substratum nature <u>June</u> September	Water depth, m <u>June</u> September
0	11	2	3	4
BAZIAŞ	1072.4	Left riparian Intermediary zone Middle navigable zone	Muddy, gravel Sandy, muddy <u>Muddy, sandy</u> Sandy, muddy <u>Sandy, muddy</u>	1.10 4.00 9.50 10.54 14.00
		Tittadie navigasie zone	Sandy	9.90
OSTROV	1062.0	Middle navigable zone	Sandy, stony, muddy Sandy, muddy	8.40 9.06
MOLDOVA VECHE	1048.7	Intermediary zone  Middle navigable zone	Sandy Sandy, stony, muddy Sandy, stony, gravel	9.10 17.40 19.50
SIRINA	1012.3	Intermediary zone	Sandy, stony, graver  Sandy, muddy  Stony	11.30 9.00
ELIȘEVA	1007.0	Intermediary zone  Middle navigable zone	<u>Sandy</u> Muddy <u>Muddy</u> Stony	9.64 12.00 17.00 17.50
GREBEN	998.0	Left riparian Middle navigable zone	<u>Stony</u> Muddy <u>Sandy</u> Sandy, stony	3.64 6.50 17.00 18.30
SVINIȚA	995.0	Left riparian Middle navigable zone	<u>Muddy</u> Muddy <u>Muddy, sandy</u> Muddy	4.60 7.00 14.00 13.15

Table 1 (continued)

0	1	2	3	1 4
PLAVIȘEVIȚA	976.0	Intermediary zone	Muddy Muddy	9.00 10.00
DUBOVA	970.0	Gulf middle Middle navigable zone	<u>Muddy</u> Muddy <u>Muddy</u> Muddy	13.40 15.00 16.40 17.00
MRACONIA	967.0	Middle navigable zone	<u>Muddy</u> Stony, sandy	12.40 20.00
IEȘELNIȚA	960.0	Middle navigable zone	<u>Muddy</u> Muddy	17.00 15.80
ORȘOVA VECHE	957.0	Left riparian  Middle navigable zone	<u>Muddy</u> Muddy <u>Muddy</u> Muddy	7.00 7.00 16.70 24.00
CERNA	954.0	Gulf tail Gulf middle Middle navigable zone	<u>Muddy</u> Muddy, sandy <u>Muddy, sandy</u> Muddy <u>Muddy, sandy</u> Muddy	8.00 4.20 10.50 18.00 17.00 24.00
BAHNA	950.0	Intermediary zone	<u>Muddy</u> Muddy	14.50 20.00

The collected material was firstly washed and then fixed with formol 4%, in the field. The labeled and well packaged samples were transferred in the laboratory and processed as follows: a new washing, invertebrates distribution on taxonomic groups, their determining at genus or species level, and storing in 70° alcohol. The organisms were well dried on filter paper and weighed by an electronic balance, in order to assess their biomass. The zoobenthos species were determined using a binocular magnifying glass and an optic microscope. The obtained quantitative data were referred to 1 m² substratum surface.

## RESULTS AND DISCUSSION

As regards the biomass of different zoobenthos taxonomic groups present in Iron Gates I damlake, in 2002 (Table 2), we observed that the highest values belonged to the mollusks, most of them being species primarily of fresh water, especially the lamellibranchiate, followed by gastropods. This state was peculiar between Baziaş and Dubova. Thus, the 3,115,100 mg/m² maximum value of lamellibranchiata, through the dominance of *Sphaerium* sp., was found at

Moldova Veche, in autumn, on the lake middle navigable zone; in that place, at water depth of 19.50 m existed a mosaic facies composed of sand, stone, gravel. Another lamellibranchiata high biomass of 2,570,000 mg/m<sup>2</sup> was determined at Dubova, also on the lake middle navigable zone, but in summer; the components being two big size species, Unio tumidus and Anodonta piscinalis. The muddy facies and water depths of 16.40 m were favorable for the development of the respective taxa. The values of the lamellibranchiata biomass determined at Bazias, in summer, are also high enough, with 1,601,175 mg/m<sup>2</sup>, on the lake middle navigable zone, while 1,194,625 mg/m<sup>2</sup>, in the intermediary zone between the left riparian and lake middle navigable zone. On the lake middle navigable zone prevailed *Dreissena polymorpha* (Ponto-Caspian), in conditions of a sandy-muddy facies, at depth of 14 m, while in the intermediary zone Sphaerium sp. on a muddysandy facies, at 9.5 m water depth. Also the lamellibranchiata, on the lake middle navigable zone at Greben, where prevailed the sandy facies, at water depth of 17 m, in summer, through the Sphaerium sp. juvenile exemplars, reached only 25 mg/m<sup>2</sup>; a number which represented the minimum biomass of the respective taxonomic group, in the Iron Gates I damlake, in 2002. The second important group of mollusks, namely the gastropods, were poorer represented as biomass (against the lamellibranchiate) almost on whole accumulation area. Although, high values were remarked, like those at Bazias (the upper part of the lake), especially in the intermediary zone, 930,000 mg/m<sup>2</sup>, in summer (representing the maximum value of the group) and 450,940 mg/m<sup>2</sup> in autumn. The respective gastropods developed on muddy-sandy or sandy-muddy facies, at 9.50 m or 10.54 m water depth. But, relatively high biomass values of these organisms were obtained at Bazias, too, on the lake middle navigable zone and left riparian. For the lower part of the lake, at Ieşelniţa, were remarked 277,700 mg/m<sup>2</sup>, in autumn. In all cases, Viviparus acerosus, a species with the biggest size among gastropods in the area, was dominant.

Table 2

Variation of biomass (mg/m²) of the zoobenthos organisms on the taxonomic groups, in the damlake Iron Gates I, in June / September, 2002

Profiles and	Taxonomic group						
stations	Coelenterata	Turbellaria	Nematoda	Oligochaeta	Oligochaeta cocoons		
0	1	2	3	4	5		
BAZIAŞ Left riparian Intermediary zone Middle navigable zone	2.00 100.00 4,303.00 840.00 986.25 19.14	10.25 94.50 6,750.00 4.00 9.75 0.15	 0.43 	1,282.50 150.00 221,383.75 3,900.00 6,500.00 253.00	15.00  525.00 97.50		

Profiles and			Taxonomic gro		
stations	Coelenterata	Turbellaria	T	Oligochaeta	Oligochaeta cocoons
0	1	2	3	4	5
OSTROV Middle navigable zone	<u>257.00</u> 21.00	<u>47.50</u>	0.25	<u>52.00</u> 186.00	_
MOLDOVA VECHE	<u>In Jun</u>	e sampl	es were no	tcollected	
Intermediary zone			_	10.00	_
Middle navigable zone	198.00 50.00	50.00 8.75	<u>0.05</u> 0.21	380.00 5,422.50	24.00
SIRINA Intermediary zone	29.00	1.67 1.25	_	50.00	_
ELIŞEVA Intermediary zone	-		_	250.00 387.00	<u>50.00</u>
Middle navigable zone	2.50	_	_	27,945.00 2.50	1,000.00
GREBEN Left riparian Midde navigable	<u>74.70</u> 	33.20	_	373.50 180.00	_
zone	_	_	-	_	
SVINIȚA Left riparian Middle navigable		_		76,500.00 34,800.00	3,500.00 87.50
zone	_	<del></del>		18,662.50 5,817.50	292.50 9.10
PLAVIŞEVIŢA Intermediary zone	_	_	0.04	46,682.00 25,050.00	925.00 997.50
DUBOVA Gulf middle	-	0.25	1.26 0.07	150,000.00 17,500.00	72.50 2,445.00
Middle navigable zone	-		12.75 5.74	6,150.50 40,675.00	900.00
MRACONIA Middle navigable zone		1.00	0.05	29,937.50 927.50	22.50

Profiles and	Taxonomic group						
stations	Coelenterata	Turbellaria	Nematoda	Oligochaeta	Oligochaeta cocoons		
0	1	2	3	4	5		
IEŞELNIŢA Middle navigable zone	_	-	<u>0.05</u> 1.38	<u>5,025.00</u> 11,250.00	800.00 500.00		
ORȘOVA VECHE		-			1		
Left riparian		_	0.85	38,075.00 8,250.00	400,00 150.00		
Middle navigable zone	_		1.30	40,000.00 22,830.00	5,925.00 81.50		
CERNA							
Gulf tail		_	2.97 2.00	38,463.75 19,315.00	532.50 240.00		
Gulf middle	_	_	<u>2.50</u>	5,118.00 2,579.50	<u></u> 67.50		
Middle navigable zone	_	<u>—</u>	<u>0.50</u>	1,767.50 1,710.00	50.00		
BAHNA					<del></del>		
Intermediary zone	_		1.05	58,980.00 28,125.00	355.00 5,300.00		

## Table 2 (continued)

Profiles and	Taxonomic group				
stations	Polychaeta	Hirudinea	Lamellibranchia x)	Gastropoda x)	
0	6	7	8	9	
BAZIAŞ				*	
Left riparian	<u>740.00</u>	<u>75.00</u>	<u>1,925.00</u>	<u>257,</u> 600.00	
•			406.00	12,562.50	
Intermediary zone	158,269.50 495.00	2,850.00	1,194,625.00 93,500.00	930,000.00 450,940.00	
Middle navigable zone	4,410.00 186.40	1,250.00 13.90	1,601,175.00 36,500.00	188,150.00 113,750.00	
OSTROV Middle navigable zone	10,040.00 258.00	77.50 125.00	100,012.50 21,250.00	12,512.50 2,300.00	

Profiles and		Taxonomic group				
stations	Polychaeta	Hirudinea	Lamellibranchia x)	Gastropoda x)		
0	6	7	8	9		
MOLDOVA VECHE Intermediary	In June samples were not collected					
zone				10.00		
Middle navigable zone	5,037.00 705.00		250.00 3,115,100.00	<u>7,625.00</u> 		
SIRINA Intermediary zone	<u>44.15</u> 62.50	_	14,994.00 325.00	216.25 125.00		
ELIŞEVA Intermediary zone	725.00 62.50		26,250.00 270,000.00	1,298.00		
Middle navigable zone GREBEN	<u>53,250.00</u> 25.00	_	3,975.00 1,975.00	3,250.00 250.00		
Left riparian	<u>747.00</u> 	_	249.00 170.00	5,810.00 2,506.00		
Middle navigable zone	30.00	_	25.00 13,125.00			
SVINIȚA Left riparian	1,200.00 		400.00 750.00			
Middle navigable zone	<u>41,299.50</u> 	_	175.00 187.50	_		
PLAVIŞEVIŢA Intermediary zone	<u>150.00</u> 	<u>300.00</u>	1,025.00 750.00	67,550.00 72,500.00		
DUBOVA Gulf middle Middle	75.00 		12,500.00 200.00	<u>2,500.00</u> 375.00		
navigable zone	<u>25.00</u> 12.50		2,570,000.00 485,000.00	<u>95,000.00</u> 		
MRACONIA Navigable zone	3,255.00 675.00	 125.00	- <u></u> 167,562.00	1,875.00		

Profiles and		Taxonomic group					
stations	Polychaeta	Hirudinea	Lamellibranchia x)	Gastropoda x)			
0	6	7	8	9			
IEȘELNIȚA							
Middle							
navigable zone	<u>725.00</u> 		1,000.00	277,700.00			
ORŞOVA							
VECHE	<u>27.50</u>		<u>150.00</u>				
Left riparian		_					
	25.00	_	<u>1,300.00</u>				
Middle			425.00				
navigable zone							
CERNA							
Gulf tail	<u>772.50</u>	225.00	340,000.00	<del></del>			
		225.00	200.00				
Gulf middle	1,690.00		33,098.50	_			
	20.00		263,025.00				
Middle	340.00	_	<u>35.00</u>	_			
navigable zone	135.00		1,350.00				
BAHNA							
Intermediary	<u>400.00</u>		22,500.00				
zone			75.00				

## Table 2 (continued)

Profiles and		Tax	conomic group	
stations	Isopoda	Gammaridae	Corophiidae	Diptera
0	10	11	12	13
BAZIAŞ				
Left riparian	<u>6.00</u>	<u>120.00</u>	<u>165.00</u>	<u>135.00</u>
Intermediary zone		400.00	260.00	147.50
Middle navigable	930.50 24.00	1,962.50	115.00 3,892.50	<u>25.00</u> 150.00
zone	165.00 6.00	40,090.00 1,937.50	90.00 22.70	
OSTROV				
Middle navigable zone	144.00 95.00	40,875.00 17,500.00	<u>23,000.00</u> 950.00	_
MOLDOVA				**
VECHE	<u>In Ju</u>	ne sample	s were not col	lected
Intermediary zone	26.16	2,355.00	8,355.00	2.00
Middle navigable zone	<u>690.00</u> 3.00	765.00 1,926.25	17.50 100.00	

Profiles and		Ta	xonomic group	
stations	Isopoda	Gammaridae	Corophiidae	Dinton
0	10	11	12	Diptera 13
SIRINA Intermediary zone	3.17	249.90	212.41	
	12.50	112.50	155.00	5.00
ELIŞEVA Intermediary zone	4.00	130.00	100.00	
Middle navigable	6.00	322.50	273.75	_
zone	11.25	4,105.00 68.00	212.50	
GREBEN		+		<del> </del>
Left riparian	<u>166.00</u>	<u>290.50</u>	_	207.50
Middle navigable		125.00	<u>30.00</u>	
zone		58.88		
SVINIŢA Left riparian				
Middle navigable				50.00
zone	<del></del>	1,100.00 	_	75.00 12.50
PLAVIŞEVIŢA Intermediary zone				225.00
DUBOVA				50.00
Gulf middle				12.50
Middle navigable				150.00
zone			_	<u>50.00</u> 20.00
MRACONIA Middle navigable				
zone	1.00	78.50		
IEȘELNIȚA	1.00	13,775.00	5,557.50	
Middle navigable				
zone	_	-	_	1.925.00 100.00
ORȘOVA VECHE				
Left riparian			_	550.00 675.00
Middle navigable		_		175.00
zone				0.25
CERNA				242.50
Gulf tail	_	87.50		242.50 3,900.00
Gulf middle		-	_	<u>397.50</u>
Middle navigable		_		3,850.00
zone				50.00

x) The values of Lamellibranchia and Gastropoda biomass include the organism shell.

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The minimum value of the gastropod biomass in the Iron Gates I damlake, in 2002, was of 10 mg/m², at Moldova Veche, on a sandy facies. But, frequently, the representatives of such group were even absent, at Sviniţa, Orsova Veche, Cerna, both in summer and autumn. We must emphasize that the two group mollusk organism biomass includes also their shells, as it is shown in Table 2. The adult mollusks, due to the shell hardness, are neither an easily accessible trophic basis for most fishes, nor a biomass totally productive.

In a descendant order, the oligochaets and polychaets worms had lower biomass values, in the zone. They constituted a main trophic base for the fishes, being a productive biomass almost 100% (excepting only the chaeta). The oligochaets and polychaets were present, like lamellibranchiata, almost constantly in the entire accumulation perimeter. Both worm groups reached the maximum biomass values of 221,385.75 mg/m<sup>2</sup> (oligochaets) and 158,269.50 mg/m<sup>2</sup> (polychaets) at Bazias in the intermediary zone between the lake middle navigable zone and left riparian, in summer; namely in places, where prevailed the muddy facies (otherwise, their preferred substratum) with depth water of 9.50 m. The next weight high value of the oligochaet worms, namely 150,000 mg/m<sup>2</sup>, was obtained at Dubova in the middle zone of the gulf, which developed on a muddy facies, too, while that of the polychaets, of 53,250 mg/m<sup>2</sup>, at Eliseva, on the lake middle navigable zone, also on a muddy facies, in summer. In autumn, generally, the low biomass values were due to conditions less favorable for the development of these organisms, generated by the floods which extended towards autumn, which dragged the mobile facies. The species which realized these biomass values were Limnodrilus hoffmeisteri and Tubifex tubifex among oligochaets, and Hypania invalida, among polychaets. The minimum biomass values of chaetopods were found in autumn, namely 2.5 mg/m<sup>2</sup> (oligochaets) and 25 mg/m<sup>2</sup> (polychaets), on the lake middle navigable zone, at Eliseva, where the stony facies prevailed.

Besides the mentioned two worm groups, the amphipod crustaceans, through the gammarid and corophiid groups were important in the lake, in 2002, from the trophic point of view. The maximum biomass value of gammarids, namely 108,240 mg/m², was found at Baziaş, in the zone between the lake middle navigable zone and left riparian, on a muddy-sandy facies, at water depth of 9.50 m, in summer. The dominant taxa were *Obesogammarus obesus*, *Dikerogammarus haemobaphes fluviatilis* and *D. villosus bispinosus*. *O. obesus* and *D. v. bispinosus* prevailing at Mraconia, realizing a biomass of 13,775 mg/m², on the lake middle navigable zone, in autumn. The corophiids, generally, with a poorer development (owing to the limiting of the preferred by them rocky facies, after the accumulation construction) reached the maximum biomass value of 23,000 mg/m² at Ostrov, on the lake middle navigable zone, in summer, where the

stony facies remained. The *Corophium chelicorne* and *C. curvispinum* were found as dominant forms. Both amphipod groups had a better development in the upper zone of the Iron Gates I damlake. Downstream of Sviniţa, in major stations performed by us, the gammarids and corophiids were absent, like isopods, all comprising Ponto-Caspian elements. Referring to this organism group, we must underline that the Ponto-Caspian elements present in Iron Gates I damlake, in 2002, were not dominant in the respective conditions, from the biomass point of view, excepting some isolated cases. The respective organisms were more exigent in respect of the environment conditions, but also had smaller sizes in comparison with those of the main representatives of lamellibranchiata and gastropods. After the Danube blocking the mollusks found favorable conditions for their development in the newly created accumulation.

Other zoobenthos groups, like Isopoda, Diptera, Turbellaria, Coelenterata, Hirudinea, Nematoda (as it is observed in Table 2) represented a reduced contribution to total biomass realized by benthos invertebrates in Iron Gates I damlake, in 2002.

The spatial and seasonal variations of the total biomass of the benthos zoocoenoses organisms, in stations located at left riparian along Iron Gates I damlake, are represented by the values of the two curves in Figure 1.

The curve aspects are somehow similar. But, in summer, the curve showed two peaks, corresponding to the stations located at the accumulation extremities, namely Baziaș and Cerna. In autumn, the maximum value was at Svinița. The numbers represented in Figure 1 showed biomass values much higher in June than in September. The already mentioned cause was the much prolonged floods in 2002. By point of dominant organism view, the maximum value of June, at Cerna gulf tail (the highest curve peak), had the lamellibranchiata as main component. The value of 340,000 mg/m<sup>2</sup> was realized especially by Unio tumidus, which developed on muddy facies, at water depth of 8 m, in conditions of a very slow water flow. At Baziaş, where corresponded the second curve peak of summer biomass values, the gastropods were dominant with 257,600 mg/m<sup>2</sup> by Viviparus acerosus, which was found on a muddy facies with gravel, at low water depths of 1.1 m. As regards the autumn maximum value, corresponding to Sviniţa, which was much lower, the main contribution belonged to oligochaets of the tubificide group, by Limnodrilus hoffmeisteri and Tubifex tubifex. These worms totalized 34,800 mg/m<sup>2</sup>, on a muddy facies, at a water depth of 13.15 m. The minimum values of the zoobenthos organism biomass, in both seasons, were found at Greben. In June, the minimum biomass belonged mainly to gastropods, with 5,810 mg/m<sup>2</sup>, Theodoxus danubialis being dominant. The same species represented the minimum value in September having 2,506 mg/m<sup>2</sup> of total biomass in the respective station.

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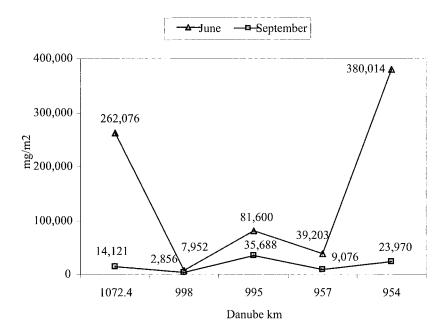


Fig. 1. Spatial and seasonal variations of the total biomass (mg/m²) of the zoobenthos organisms in the left riparian zone of the damlake Iron Gates I, in 2002.

The spatial and seasonal variations of the total biomass of the zoobenthos organisms in stations of longitudinal profile in the intermediary zone between the left riparian and lake middle navigable zone are presented in Figure 2. The two curves, representing the biomass values in summer and autumn, had a similar aspect solely as regards the maximum and minimum development of the benthos invertebrates (in Iron Gates I damlake, in 2002). Thus, the maximum value of the biomass of all organisms during both analyzed seasons was found at Bazias, in summer, its major part being constituted by lamellibranchiata and gastropods (Table 2). The representative taxa, namely Sphaerium sp. and Viviparus acerosus, found in that place favorable conditions for their development, on a muddy-sandy facies, at a water depth of 9.50 m. In autumn, the maximum value of zoobenthos organism biomass, much lower than in summer, was especially due to the gastropods, through Viviparus acerosus, with 350,000 mg/m<sup>2</sup> and Bithynia tentaculata, with 99,000 mg/m<sup>2</sup>. The two species were found on sandy-muddy facies, at water depth of 10.54 m, having an enough strong water flow. The minimum biomass values of total zoobenthos organisms, on the intermediary zone of the lake, were determined at Sirina, in both seasons. In summer, the major contribution belonged to the lamellibranchiate Dreissena polymorpha with 14,994 mg/m<sup>2</sup> which did not have favorable conditions for their development on washed muddy facies, at 11.70 m depth and a water flow higher than 2 m/sec. In autumn,

the same species prevailed, but, with only 325 mg/m², in more unfavorable conditions than in summer. In the rest of the stations (analyzed by us in the respective accumulation) the two curves of the biomass of all benthos invertebrates presented variations, without being synchronized in the studied seasons. To note that, although in major stations the biomass values were higher in summer, an inverse situation was observed at Elişeva and gulf tail Cerna, in both cases, due to the lamellibranchiata development.

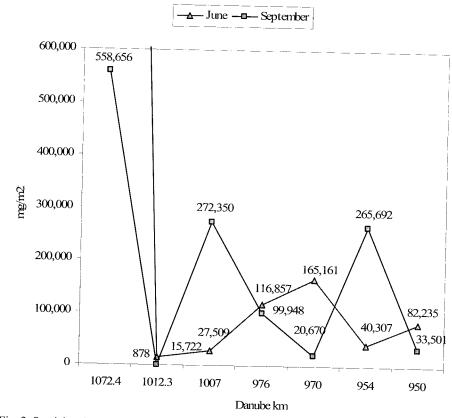


Fig. 2. Spatial and seasonal variation of the total biomass (mg/m²) of the zoobenthos organisms in the intermediary zone from among left riparian and middle navigable zone of the damlake Iron Gates I, in 2002.

The spatial and seasonal variations of the total biomass of the zoobenthos organisms on lake middle navigable zone, in Iron Gates I damlake, shown in Figure 3, presented a more interesting aspect, for this longitudinal profile, than the case of the other two profiles along the accumulation. This state was, in part, due to the fact that on the lake middle navigable zone we have the possibility to collect samples in several stations, but mainly, due to the different geo-morpho-

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hydrological conditions, even in near points. In this sense, the presence of two peaks, on each curve of the total biomass of zoobenthos organisms, was revealed in summer and in autumn.

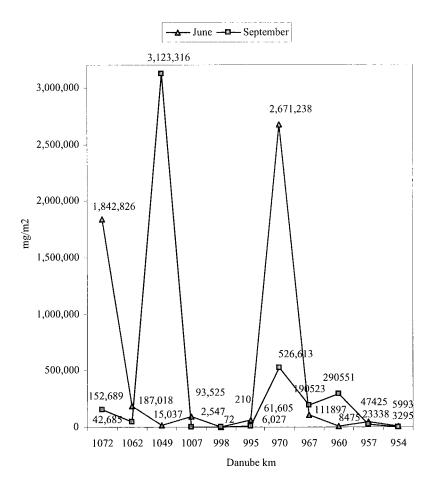


Fig. 3. Spatial and seasonal variations of the total biomass (mg/m²) of the zoobenthos organisms in the middle navigable zone of the damlake Iron Gates I, in 2002.

Thus, the June maximum value was found at Dubova, prevailing the *Unio tumidus* and *Anodonta piscinalis* lamellibranchiata (as we already mentioned) which amounted 2,570,000 mg/m² (Table 2). The second enough high peak of the curve of summer total biomass values was located at Baziaş, where the majority was constituted by the *Dreissena polymorpha* lamellibranchiate, which realized 1,395,900 mg/m². The autumn maximum value on the lake middle navigable zone,

determined at Moldova Veche, represented the highest number of the total biomass of zoobenthos, in lake, in 2002, respectively 3,123,315.71 mg/m<sup>2</sup>, which were dominated by Spherium sp. with 3,115,100 mg/m2. The second peak was determined at Dubova, in September, where, the first place in the zoocoenosis, as biomass, also belonged to the lamellibranchiata, through Unio tumidus with 485,000 mg/m<sup>2</sup> (on a muddy facies). The biomass minimum value on the lake middle navigable zone was located at Greben, in summer, where the hard-rocky facies and depths of 17 m allowed the development, merely of populations dominated by gammarids with 125 mg/m<sup>2</sup>. In autumn, the biomass minimum value, located some upstream, at Elişeva, had as main component the Dreissena polymorpha lamellibranchiate, with 1975 mg/m<sup>2</sup>, on a rocky facies, at water depth of 17.50 m. Also on the lake middle navigable zone (as in the intermediary zone between the lake middle navigable zone and left riparian) in some stations, like Moldova Veche and Ieşelniţa, the total biomass values in autumn were higher than in summer. The increase of the individuals of the dominant populations (mollusks and oligochaets), which are organisms with rapid growth, may be one side explanation. On the other side, it is possible that the organisms were brought from the adjacent zones agglomerated on the lake middle navigable zone, as effect of the floods. But, values of the total biomass of the benthos invertebrates were higher in summer than in autumn in major stations on the lake middle navigable zone.

The spatial and seasonal variations of averages of the total biomass of the zoobenthos organisms in the transversal profiles in Iron Gates I damlake, studied in 2002, are presented in Figure 4. The two curves of data from the 14 transversal profiles, in summer and autumn, showed clearly large variations from very high to very low values, even in near zones. The differences from one profile to another were in close correlation with the geo-morphohydrologic conditions, which were favorable or not, for the development of certain aquatic organisms. In 2002, a peculiar year, from the hydrological point of view, great disturbances took place at the level of benthos zoocoenoses components. More affected were those on the moving facies. We must recognize that the muddy and sandy facies frequently dominated in 2002; on the profiles we collected the samples. Analyzing the numbers in Table 2 and Figure 4 it may be concluded that in many transversal profiles the averages of the zoobenthos organisms biomass were higher in June than in September. The main cause was the prolonged floods, as we already mentioned. The high values of the benthos invertebrates were observed at Baziaș and Dubova, in summer while in autumn, at Moldova Veche. The minimum values were determined at Greben, in summer and at Sirina, in autumn.

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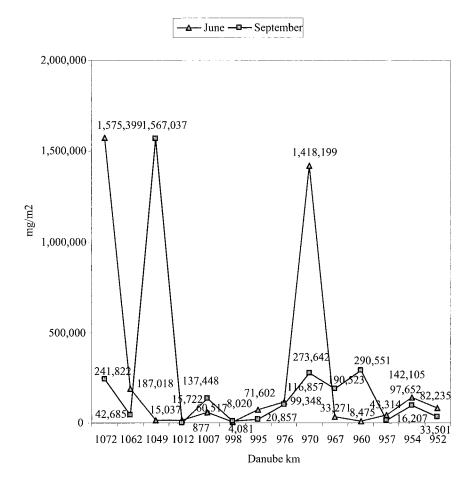


Fig. 4 – Spatial and seasonal variations of the total biomass (mg/m²) of the zoobenthos organisms averages on transversal profiles in the damlake Iron Gates I, in 2002.

Some specifications must be made regarding the benthos invertebrate biomass in the Iron Gates I accumulation. Firstly, in major cases, the high biomass values in the belonged lake, we repeat, to the mollusks, their weight including the shells. But, it must be also underlined that these organisms, due to their shells, are not accessible for many fishes in the zone. Therefore, we should not confound the presence of a high remnant biomass with a good productivity of the ecosystem. At the same time, the abundance of the trophic base may also indicate the consumer absence.

A retrospective view upon the state before the Danube blocking at Iron Gates, and the state after the construction of the first accumulation lake in the zone, shows that significant changes occurred in the structure of the zoobenthos (closely

correlated to the important changes of the environment conditions) determined the clear dominance (as density and biomass) of the pelophilic species, prevailing those primarily of fresh water. As regards the Ponto-Caspian elements from the river (of fresh water), namely all gammarids, corophilds, the *Jaera istri* isopod and *Hypania invalida* polychaete (although, as numerical density they reached high values in certain zones, especially in the upper part of Iron Gates I damlake), they had, from the biomass point of view, a small enough contribution in most cases, in 2002. These species had a higher percent of the total biomass of the zoobenthos organisms at Baziaş and Ostrov. In the last 50 km of the lake, downstream of Sviniţa, these Ponto-Caspian elements almost disappeared.

## **CONCLUSIONS**

The invertebrate biomass study in the benthos biocoenoses in Iron Gates I damlake, in 2002, allowed us to reveal interesting conclusions.

- 1. We may assess that the biomass of the zoobenthos organisms was high, in comparison, especially, with that existed before the construction of the respective accumulation.
- 2. The zoobenthos organisms which contributed to high biomass values were mainly the mollusks, lamellibranchiata and gastropods, in majority, the species which were primarily of fresh water.
- 3. The organism groups comprising the Ponto-Caspian elements (the species which were secondarily of fresh water), namely the gammarids, corophiids, isopods, polychaets, very seldom had a significant contribution to the total biomass of the benthos invertebrates.
- 4. In the two analyzed seasons, at the level of the zoobenthos biomass, important spatial and seasonal variations were observed, both on longitudinal and transversal profiles:
- usually the biomass values determined on the lake middle navigable zone were higher than in other lake zones;
  - in major cases, higher biomasses were revealed in summer than in autumn.

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Received November, 2006

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## STRUCTURAL CHANGES CAUSED BY COPPER ACTION ON THE GILLS OF *CYPRINUS CARPIO* (PISCES-TELEOSTEI) JUVENILE FISHES

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Our investigations on the action of various copper concentrations (0.1; 0.5; 1 mg/l) during various intoxication times (from 24 h to 7 days) upon *Cyprimus carpio* L. young fish gills revealed a number of important cell and tissue structural changes. Gill disorganization was such that it resulted in gill lamellae destruction by: cell hypertrophy, nuclei deformation and pycnosis, dilatation, even breaking of blood vessels endothelia and extravasations of blood from affected vessels. The found changes are, generally, directly related to toxicant concentration in water and to intoxication time.

Key words: Pisces, Cyprinus carpio, gills, structural change, copper.

#### INTRODUCTION

The heavy metals are among the most important pollutants in the environment, implicitly in the aquatic media, where they persist long time and are able to cumulate in aquatic organisms.

The copper is among the essential heavy metals, having an important role in different biological processes, like the oxidative phosphorylation, gene regulation and homeostasis of free radicals. As a consequence, when the environment concentration of this metal is high, some disturbances of the organism functions occur, such as: inhibition of some enzymes, disorders in metabolic paths, neuronal transmission breakage (Syed, 2002).

In the present paper we analyzed the structural changes produced upon the gills of *Cyprinus carpio* juvenile fishes, under the action of various copper concentrations.

## MATERIALS AND METHODS

The fishes, a selected material originated from known genitors, were purchased from the Research Development Centre for Pisciculture, Nucet. After transport, the *Cyprinus carpio* juvenile fishes were maintained in aquaria with

ROM. J. BIOL. – ZOOL., VOLUME **51**, N<sup>os</sup> *1*–2, P. 91–97, BUCHAREST, 2006

water free of copper, 8 days, in order to adapt to the laboratory conditions. Then, lots of 10 exemplars were subjected to intoxication with copper (in CuSO<sub>4</sub>), in concentrations of 0.1 mg/l; 0.5 mg/l and 1 mg/l. The experiment lasted between 24 h and 34 days. At certain time intervals, the fishes were sacrificed and their gills pieces were fixed in Bouin and subjected to the classical histological technique. The microphotographs were realized after hematoxyline-erythrosine staining and examining at Olympus optical microscope.

The fluctuations of water temperature, oxygen concentration and pH were registered during the experiments, as well as the fish behavior and survival. These data helped us to understand the histopathological changes caused by the copper action upon the gills.

#### **RESULTS**

The microscopic observations regarding the influence of various copper concentrations upon the gills were performed in comparison to control structure at *Cyprinus carpio*, described by Dornescu & Mişcalencu (1964).

## Effect of 0.1 mg/l Cu action during 24 h-48 h

The gills lamellae maintained their normal aspect in some areas, but cells detached from the epithelium were also observed. In other areas, the lamellae appeared fully disorganized. In the last case, the epithelial cells were fully destroyed, and the lamellae broken. Some disorganized portions showed the lamellae fusion. The pilaster cells appeared dispersed. In some lamella areas the cell hypertrophy was also observed. The gill lamella having multilayer epithelium seemed structurally not affected. However, nuclei with pycnosis were observed here and there, in some cells. The conjunctive tissue within the gills lamella skeleton appeared not affected (Fig. 1).

## Effect of 0.1 mg/l Cu action during 72 h-96 h

The gill lamellae appeared more damaged, with cells detached from the epithelium, dispersed and degraded. We found hypertrophy of the gills lamellae especially at 96 h exposure of the juvenile fish. The gill lamellae maintained their structure (similar to former periods of fish exposure) almost normal, and sometimes the alterations remarked in some cells showed the nucleus pycnosis (Fig. 2).

## Effect of 0.1 mg/l Cu action during 7 days

The gill lamellae were more degraded compared to previous concentrations, the cells being destroyed and many of them detached from the epithelium. In certain zones the cell hypertrophy was remarked, in others, the lamellae became even thinner. The blood vessels within the gill axis had the endothelium damaged

and the figurate elements dispersed. Sometimes, the cartilaginous tissue also appeared affected, with damaged nuclei (Fig. 3).

## Effect of 0.1 mg/l Cu action during 10 days

After this treatment interval a pronounced damage of the gills lamellae was observed. These appeared, either with entirely hypertrophied cells at their free edges, or dispersed among the lamellae. The integral remained cells had nucleus pycnosis. Similar damages of blood vessels and conjunctive tissue were observed in the gills axis (Fig. 4).

## Effect of 0.1 mg/l Cu action during 34 days

At this long interval of exposure, the juvenile fishes of *Cyprinus carpio* survived (probably owing to the adaptation to the toxic environment). However, much less pronounced changes occurred in some areas of the gills lamella epithelium. Through a careful analysis of the structures, either damaged lamellae with dispersed cells, or cell hypertrophy were seen, in some parts.

We emphasize that this concentration of 0.1 mg/l revealed to be sublethal in our experiments (Popescu-Marinescu *et al.*, 2005).

## Effect of 0.5 mg/l Cu action during 24 h

The gill lamellae appeared sometimes damaged, especially to their free ends, and when they kept the epithelium integrity, the cells appeared hypertrophied. The gill lamella capillaries were destroyed, too, and dispersed like the lamella cells. The multilayer epithelium of the gills lamella appeared unaffected, in some cases. Sometimes, the cells were degraded in the area of lamella fixing on its axis. The cartilaginous tissue was unaffected, as well as the musculature within the gills axis (Fig. 5).

## Effect of 0.5 mg/l Cu action during 48 h

The disorganization of gills lamella epithelium was more pronounced than in the previous situation. Generally, the great blood vessels of gills axis presented a destroyed epithelium, also the blood invading the space between lamellae (Fig. 6).

## Effect of 0.5 mg/l Cu action during 72 h - 96 h

The structural changes of the gills lamellae, to this exposure duration, were generally stronger. The epithelium cells appeared usually dispersed. When the epithelium organization was maintained, the hypertrophy of the lamellae, towards their ends, was remarked. Some pilaster cells had nucleus pycnosis. The multilayer epithelium of the gills lamella was also destroyed in some areas. The cell limits were not distinguished and thus, the cells appeared dispersed (Figs. 7, 8).

## Effect of 0.5 mg/l Cu action during 7 days

The gills lamellae appeared, sometimes, on their whole length, either hypertrophied, or thinner in other parts. Other times, the hypertrophy was seen, till

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the free end of the lamella, the epithelium cells being disintegrated and dispersed. The epithelial multilayer of the gills lamellae had sometimes cells with nucleus pycnosis. The damaged blood vessels allowed the blood infiltration in the surrounding space between lamellae.

## Effect of 0.5 mg/l Cu action during 10 days

After 10 days of exposure, much more pronounced structural changes were remarked. The most gill lamellae had a destroyed epithelium, the cells being dispersed among them. When the integrity of the lamella epithelium was maintained, the change of the pilaster cells was observed. The epithelium of gills lamella was much disorganized, so that its large portions appeared displaced towards the gills axis. In some parts, the cartilaginous tissue of gills axis was also damaged and the nuclei had pycnosis (Fig. 9).

The 0.5 mg/l concentration was lethal for the juvenile fishes treated by us. The 100% mortality occurred after 576 h, the minimum lethal interval being very close to it, namely 504 h, Popescu-Marinescu *et al.* (2005).

## Effect of 1 mg/l Cu action during 24 h

This concentration caused a high mortality of fishes, 60% of lot exemplars having 20 h lethal time. The surviving fishes had the maxim lethal time of 29 h., Popescu-Marinescu *et al.* (2005).

At fishes sacrificed after 24 h of toxic substance action a severely disorganized lamella structure and gills lamella was remarked. Also, the conjunctive tissue of gills axis was damaged, and the figurate elements originated from the damaged blood vessels were dispersed within it.

## **DISCUSSIONS**

Our observations regarding the action of various copper concentrations during certain time intervals, causing structural changes directly proportional to it and with the interval of the fish's exposure to the toxic substance action, agreed with the statements of Salih *et al.* (1980), as well as of Olojo *et al.* (2005).

We emphasize that the histopathological changes observed by us, caused by the copper action upon the *Cyprinus carpio* juvenile fish, were more pronounced in gills than other fish organs. This fact is in accordance with the mention of Rajbanski *et al.* (1980) according to which the gills cumulate the greatest amount of copper, in comparison to the liver, followed by the kidney. Thus, from these authors' studies, performed on *Heteropneustes fossilis* and *Channa punctatus* species individuals, exposed to the copper action, resulted a sequential accumulation of the respective ions in the tissues. In same respect, Gravi *et al.* 

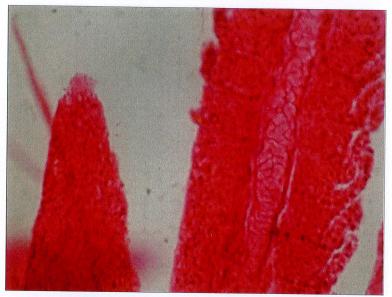


Fig. 1. Effect of 0.1 mg/l Cu action during 24 h - 48 h  $(40 \times 0.65)$ .



Fig. 2. Effect of 0.1 mg/l Cu action during 72 h ( $40 \times 0.65$ ).

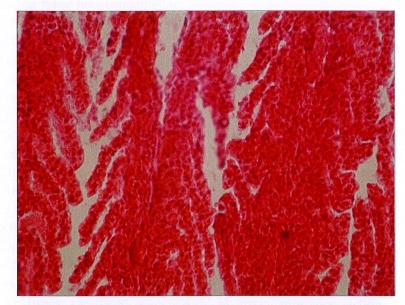


Fig. 3. Effect of 0.1 mg/l Cu action during 7 days ( $40 \times 0.65$ ).

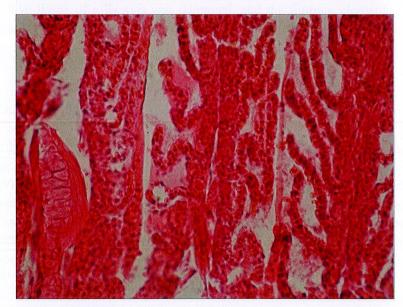


Fig. 4. Effect of 0.1 mg/l Cu action during 10 days ( $40 \times 0.65$ ).

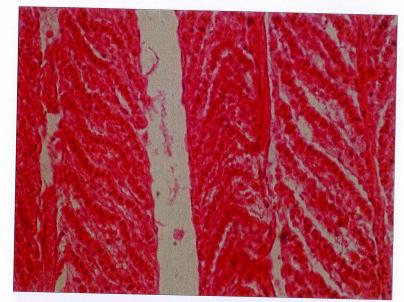


Fig. 5. Effect of 0.5 mg/l Cu action during 24 h ( $40 \times 0.65$ ).

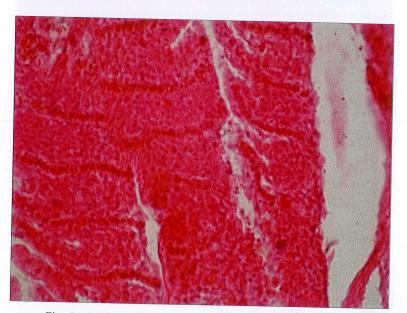


Fig. 6. Effect of 0.5 mg/l Cu action during 48 h ( $40 \times 0.65$ ).



Fig. 7. Effect of 0.5 mg/l Cu action during 72 h (40  $\times$  0.65).



Fig. 8. Effect of 0.5 mg/l Cu action during 96 h ( $40 \times 0.65$ ).



Fig. 9. Effect of 0.5 mg/l Cu action during 10 days ( $40 \times 0.65$ ).

(2004) concluded that the assimilation of the toxic components by the fresh water fishes is carried out mainly at the gills surface, the first target organ of all pollutants.

On the other side, the structural changes at the level of fish gills, observed by us, consisting mainly in the epithelium damage especially of the gills lamellae, their partial or total hypertrophy, damage of the blood vessels endothelium and the figurate elements extravasation, were also remarked by other researchers, both in the carp and various fish species, being determined by copper or other heavy metal intoxications (Iankovic, 1980; Labat *et al.*, 1974).

Thus, Labat *et al.* (1974), observed in *Cyprinus carpio* especially the gills lamellae damage, their hypertrophy and hyperplasia induced by the toxic copper concentrations. Some of the gills structural changes reported by Jankovic (1980), provoked to some fishes living in the Rek river (a Danube affluent), polluted with copper within the waste waters from the "Majdanpek" mine, correspond to those shown by us in the present work.

Also, Gravi *et al.* (2004) described the histopathological changes, which occurred within gills at *Prochilodus scrofa* exposed to 96 h copper concentration of 29 μg Cu L<sup>+</sup>1. The respective authors established the hypertrophy and hyperplasia of mucous cells, chloride cells, lamellar epithelium, epithelium filaments, as well as the incomplete fusion of several lamellae and the cell damage. The gills were affected in proportion of 25%. Mourad & Wahby (1999) analyzing the copper influence (discharged in effluents by the Egyptian copper factories) at sublethal concentrations, upon the *Tilapia zillii* species found physiological disturbances and histological changes in fish gills, liver and kidney. The histological examination of the individuals exposed to 2.5 cm³/dm³ concentration established the hyperplasia of the epithelium cells of the primary lamella determining the partial reduction of the inter lamella space. The 5.0 cm³/dm³ concentration (sublethal, too) also induced the cell hyperplasia of the primary epithelium, the inter lamella space being more limited. These changes blocked the water passing and the reduction of the respiratory surface of the gills.

Popescu-Marinescu *et al.* (2000, 2005) described several changes at *Cyprinus carpio* juvenile fishes of summer age, under the lead action, in laboratory experiments (static tests). The most pronounced changes were induced by the 1 ml/l concentration during 7 days. Among these, similar to, but more severe (Pb being more toxic) than those caused by the copper, we mention: the disorganization and damage of the gills lamellae, cell hypertrophy, disappearance of the epithelium and pilaster cell limits, dispersion and mixing of different type cells, nucleus pycnosis, dilatation of blood vessels, extravasation of the blood cells, affecting the

conjunctive tissue within the gill axis. Parashar & Banerjee (2002), experimenting in the laboratory on *Heteropneustes fossilis* with lethal concentrations (constant on whole experiment) of lead nitrate found profound changes at level of fish gills, after 96 h. On the other side Olojo *et al.* (2005), also in the laboratory, studied the lead (contained in lead nitrate) effect, but of sublethal concentrations (0.006  $\mu$ g./l and 0.008  $\mu$ g./l, also being constant during the experiment) a maximum interval of 3 weeks on *Clarias gariepinus* species observed much more pronounced histopathological changes.

## **CONCLUSIONS**

- The produced damages of the structure, especially of the gill lamella, were more pronounced, as the concentration and period of exposure to the copper action increased.
- The damages consisted of epithelium disorganization and cell dispersion or by the total or partial hypertrophy of the gill lamellae.
- The structure of both the gills lamellae, and the conjunctive tissue within the gills axis was lesser affected.

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Received November, 2006

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# BIPHASIC DYNAMICS OF THE "IN VIVO" GLUCOSE-INDUCED INSULIN SECRETION IN MICE AND HAMSTERS

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A biphasic pattern of insulin secretion stimulated with glucose, similar to those recorded in perfused rat and human pancreas and in the perfused rat and mice pancreatic islets, was described in hamsters after i.p.injection of a single hexose dose. In the two rodents studied (mice and hamsters), the dose of 3.3 g glucose/kg body weight has induced biphasic variations of the amounts of secretory granules produced by the B-cells within a 90 min time- interval. In its turn, the B-cell response to glucose has evoked in the next few minutes a similar biphasic dynamics of glycemia in both species. The histochemical observations on the pancreatic islets, corroborated with the glycemia values at 90 min after hexose administration, supported notable differences between mice and hamsters as regards the recovery rate of B-cells from the second phase of hormone secretion. The results are discussed in connection with the findings of other authors.

*Key words:* "in vivo" biphasic time-course, insulin secretion and glycemia, pancreatic B-cells, mice and hamsters.

#### INTRODUCTION

Glucose, like other nutrients, induces a biphasic pattern of insulin secretion as result of the successive hormone release from two distinct pools of the B cells (Bratanova-Tochkova et al., 2002; Nesher & Cerasi, 2002; Rorsman et al., 2000; Straub & Sharp, 2002, 2004). The transient stimulation of hormone release, known also as "the first phase" of secretion, was associated with the immediate exocytosis of the secretory granules already docked under the cell membrane, whereas "the second phase", implying a prolonged and energy-dependent process, was assigned to the increased mobilization of granules from the so-called "reserve pool" (Barg et al., 2002; Bratanova-Tochkova et al., 2002; Henquin et al., 2002; Olofsson et al., 2002; Straub & Sharp, 2002). Under the conditions in which the cellular and molecular mechanisms controlling both phases of the hormone release have separate rate-limiting steps Straub and Sharp (Straub et al., 2004; Straub & Sharp, 2004) have ascribed an important role to the rate at which the readily releasable (RR) insulin granules docked in the reserve pool are converted to an immediately releasable (IR) state or pool. According to this hypothesis, the rate of granules conversion from RR to the IR state and of their priming for exocytosis in both

ROM. J. BIOL. – ZOOL., VOLUME **51**, Nos 1–2, P. 99–104, BUCHAREST, 2006

states determines the magnitude of the first and second phase of glucose-stimulated hormone release.

Most data concerning the biphasic time-course of glucose-induced insulin secretion and the selective loss of the first phase of secretion in type II diabetes, derived from the studies "in vitro" performed on rat and mouse pancreas and B cells (Brenner *et al.*, 2003; Elliasson *et al.*, 1997; Henquin, 2000; Khan *et al.*, 1990; Rorsman *et al.*, 2000; Tsuura *et al.*, 1993; Zawalich *et al.*, 2001). Considering the above, we undertook "in vivo" the present comparative investigations in mice and hamsters in an attempt to correlate the dynamics of hormone release, revealed in histochemically stained sections, with that of glycemia induced by the sole administration of glucose.

#### MATERIALS AND METHODS

25 young adult males of *Mus musculus* mice and the same number of *Mesocricetus auratus* golden hamsters, housed in the animal care facilities of the Institute of Biology, were used. Their free access to water and food was ceased 24 hours prior to initiation of the experiments.

Except for the control animals (5 mice and 5 hamsters), the others received, by simultaneous i.p. injections, a sole dose of 3.3 g glucose/kg body weight. They were shared in 4 groups, each of them of 5 individuals and were sacrificed by decapitation at periods of 10, 30, 45 and 90 min. Blood samples and pancreas pieces from all the control and treated specimens were collected immediately and used for glycemic determinations, respectively for histological processing.

Blood glucose concentrations were determined with the aid of an Optimum Xceed glucometer (Abbott Labs., USA) and expressed as means  $\pm$  standard deviations (M $\pm$ SD).

For estimations of the B-cell insulin loadings, Bouin-fixed and paraffin embedded pancreas pieces were sectioned at  $6\mu$ -thickness with a sledge microtome and then mounted on poly-L-lysine (Sigma, USA) coated slides. The serial sections were stained with pseudoisocyanine (Serva, Germany) according to the procedure of Coalson (1966) and finally examined in 580 nm monochromatic light (Epple, 1966).

## RESULTS AND DISCUSSIONS

The glycemia oscillations recorded during the first 90 min following injection of a single glucose dose were considered as indirect proofs for the dynamics of insulin release both in mice and hamsters. These fluctuations, which complied with the biphasic pattern of hormone secretion "in vitro", showed two peaks at 10 and 45 min after hexose administration (Fig. 1) (Bratanova-Tochkova *et al.*, 2002;

Henquin et al., 2002; Olofsson et al., 2002; Rorsman et al., 2000). The high amplitudes of the first hyperglycemic peak, exceeding two times the normal glycemic levels of the two rodents, represented undoubtedly the consequence of a rapid depletion of the insulin already docked under B cell membrane (Fig. 1) (Barg et al., 2002; Henquin et al., 2002; Olofsson et al., 2002). On the other hand, the second and more attenuated hyperglycemic peak was ascribed to a rate of hormone biosynthesis lower than that of its exocytosis (Straub & Sharp, 2002, 2004). As regards the blood glucose levels recorded around the nadir (at 30 min) between the two phases, slightly increased (in hamster) or comparable (in mice) to those of control animals, we assigned them to the equal low rates of both hormone biosynthesis and releasing mechanisms (Fig.1). The single striking difference in the parallel profiles of glycemia dynamics occurred at the 90 min interval, when the mice displayed slight hypoglycemic, whereas the hamsters still displayed moderate hyperglycemic values (Fig. 1).

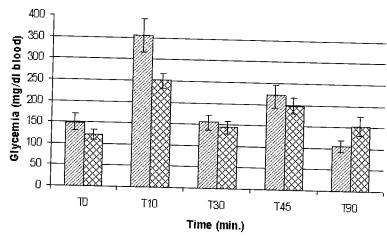


Fig. 1. Biphasic time-course of glycemia induced by the "in vivo" administration of a single glucose dose (3.3 g /kg body weight) in mice and hamsters. The columns represent mean values ± standard deviations (M±SD). T0 – control animals; T10, T30, T45, T90 – minutes following glucose i.p. injection.

The above dynamics of glycemia was induced in both rodent species by a biphasic secretory response of the B-cells (Fig. 2 a-j). Thus, the two hyperglycemic peaks recorded at 10 and 45 min. after glucose administration corresponded to various degranulation degrees of the B-cells, as a rule more pronounced at 45 min interval. If the histochemical picture of pancreatic islets at 10 min interval expressed the discharge of the insulin already docked in the B-cells, that observed at 45 min pleaded for an intense biosynthetic process, exceeded only by the rate of hormone release in the blood (Fig.2 c, d; g, h). In such an interpretation, which

confirms at least partly the hypothesis advanced by Straub and Sharp (2004), the insulin depleted islets detected at 30 min after the hexose injection, were considered as illustrating the consequence of a rate of hormone release equal to the low forward rate for the conversion of readily releasable (RR) to immediate releasable (IR) secretory granules (Fig. 2 e, f). Finally, the dissimilar histochemical pictures of mice and hamsters pancreatic islets encountered at the interval of 90 min proved in our opinion a faster recovery of insulin biosynthesis capability in the first species than in the second one (Fig. 2 i, j).

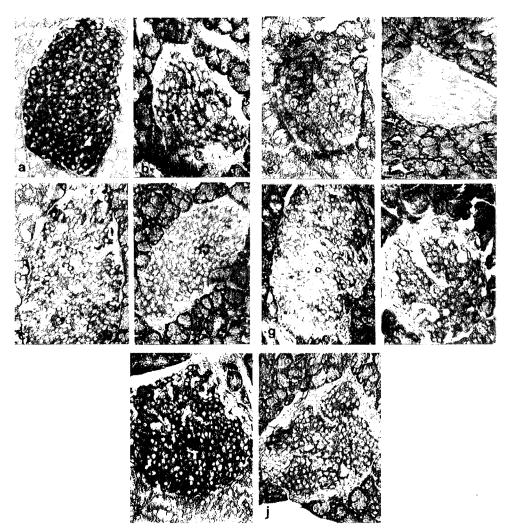


Fig. 2 a-j. The biphasic secretory response of mice and hamsters B-cells showing to peaks of insulin release at 10 (c, d) and 45 (g, h) min. after the glucose administration. Magnifications between  $\times$  641 (e) - 650 (i).

The insulin biphasic release in the blood after the glucose administration preceded undoubtedly, at least with several minutes, the development of the biphasic pattern of glycemia (Fig. 1). In the absence of correlative information regarding the time-course of insulinemia and glycemia "in vivo", our observations on histochemically stained sections supported rather a narrow, spaced out display of their biphasic profiles than one entirely successive.

Special attention should be paid to the lack of synchronization of exocytosis among the B-cells of an islet, expressed through their uneven degranulations. Reported also "in vitro" in humans and several rodent species (Rorsman *et al.*, 2000; Straub & Sharp, 2004), the cellular and insular desynchronizations of the hormone release could explain, at least partly, the individual variations of glycemia induced by a single dose of glucose.

Finally, the differences concerning the histochemical expressions of the B-cells and the glycemia of mice and hamsters 90 min. after glucose administration, both might be ascribed to a genetic species – specific substrate (Zawalich & Zawalich, 1996; Zawalich *et al.*, 2001).

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Received August 10, 2006

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## THE SEQUENCE OF INFRASTRUCTURAL EVENTS OF THE HAIR FOLLICLE MORPHOGENESIS DURING GOLDEN HAMSTER (Mesocricetus auratus) EMBRYO DEVELOPMENT

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In this paper we reported on hair follicle morphogenesis during embryo development of the golden hamster (*Mesocricetus auratus*). Hair follicle morphogenesis starts when epidermal basal cells proliferate acceleratedly to form an epithelial cell conglomerate. Subsequently, the epithelial cells of such a cell conglomerate will migrate profoundly into the subjacent dermal tissue, but remaining cohesive each other by desmosomal junctions keeping the continuity with the epidermis from which this formed, the whole ensemble taking the form of an epithelial column (cylinder). The lowermost part of the epithelial column becomes dilated (bulbar aspect) and an invagination is formed – a virtual cavity – within the dermal mesenchymal cells will immigrate to form a dermal papilla and together with the surrounding cells will form the hair. Some infrastructural aspects of the golden hamster hair follicle will be compared to that of mouse hair follicle. While generally the golden hamster morphogenesis of hair follicle follows the mammalian pattern, some ultrastructural particularities are significant.

Key words: hair follicle, dermal papilla, epithelial-mesenchymal interactions, morphogenesis.

#### INTRODUCTION

About 60% of the differentiated tissue types in a mammalian body are epithelia (Slack, 2000). Epithelial cells are widely distributed in the animal body either forming linings or surface structures (Byrne, 1997).

Epithelial cell differentiation as well as epithelia morphogenesis and homeostasis are very dependent on epithelial-mesenchymal interactions. Development and homeostasis of skin and its appendages as pilosebaceous system depend on epithelial-mesenchymal interactions (Mirancea & Mirancea, 1997). Mammalian epidermis is a pluristratified epithelium that is formed by keratinocytes in a tightly regulated differentiation process (Alibardi *et al.*, 2005). Proliferation of keratinocytes is restricted to the basal layer where stem cells undergo an asymmetric division to yield one stem cell daughter which remains in the basal layer and one daughter transit-amplifying cell destined to differentiate (Slack, 2000; Bickenbach & Grinnel, 2004). Transit-amplifying cells are the only cells in the interfollicular epidermis that are actively proliferating (Koster & Roop, 2004).

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On detachment from the basement membrane, cells move upwards into the spinous layer and later into the granular and transitional layers and finally into the external dead corneous layer (Alibardi *et al.*, 2005; Eckert *et al.*, 2005).

The skin is a vital organ at the body-environment interface. To serve its functions, the skin must maintain its integrity by continuously renewing the epidermis, while also maintaining associated appendages such as hair. Hair is produced and maintained by the pilosebaceous unit, which includes a hair producing follicle and a pilosebaceous gland (Whitney, 1963). Hairs are epidermal appendages characteristic of mammalian skin, made of concentric layers of corneous materials (Langbein & Schwizer, 2005). Like interfollicular epidermis, hair follicles have a similar organization in all mammals (Alibardi et al., 2005). The hair follicle as an appendage of the mammalian tegument is composed of (a) an upper portion which extends from the epidermis to the opening of the sebaceous gland and (b) a lower cycling portion. The lower portion modifies its structure throughout the phases of active hair shaft growth (anagen), apoptosis - driven regression (catagen) (involution) and relative resting (telogen) (Selleri et al., 2005; Zarach et al., 2004; Botchkareva et al., 2006) hair shedding (exogen). During its anagen phase, the hair follicle is a solid cylindrical structure composed of concentric tubes of cells opening on the epidermal surface. The deepest portion (its lower end), a bulbous expansion (the hair bulb) with a concave lower surface contains a specialized area of a finger-like projection of dermis called dermal papilla (Breatchnach, 1971).

Hair follicle morphogenesis is a complex process of the neuroectodermal-mesodermal interactions (Paus & Foitzik, 2004).

The aim of this paper is to investigate and to get knowledge about some ultrastructural aspects of the golden hamster hair follicle morphogenesis during embryo development.

### MATERIALS AND METHODS

Fresh tissue samples as small fragments of golden hamster (*Mesocricetus auratus*) tegument from the cervico-dorsal region taken at different stages of development (6 mm, 10 mm cranial-caudal axis length and perinatal time), as well as mouse (*Mus musculus*) tegument (perinatal or postpartum stages) were prefixed in ice-cold 2.5% glutaraldehyde in 0.05 M sodium cacodylate, pH 7.4 and postfixed in 2% osmium tetraoxide in 0.1 M sodium cacodylate pH 7.4 for 2½ hours, 4°C. Following dehydration through an increasing concentration of ethanol and infiltration with propylene oxide, specimens were embedded in Epon 812, equivalent (glycide ether 100, Serva, Heidelberg, Germany) and polymerised at +60°C for 48 hours. Semithin sections of 1 µm were stained with 0.1% toluidine blue for light microscopy. Ultrathin sections, 60–100 nm in thickness, were cut by

an ultramicrotome, conterstained with uranyl acetate and subsequently with lead citrate and examined using an electronmicroscope operated at 60 kV.

## RESULTS AND DISCUSSIONS

When cranio-caudal axis length of the golden hamster reaches approximately 6 mm, interfollicular dorsal epidermis consists of 2–3 cell layers. The uppermost epithelial cell layer is represented by the peridermal cells (Fig. 1). The periderm overlaps the developing epidermis in embryonic skin and functions as a protective layer for embryonic skin. The periderm sloughs off during late development of the epidermis. The periderm disappears when epidermal keratinisation is completed (Akyama *et al.*, 1990; Morita *et al.*, 2002). Scarce desmosomal junctions connect nucleated epidermal cells to the suprajacent periderm as well as each other but their connection with cytokeratine filaments is reduced. Apparently wide intercellular spaces are visible. A discontinuous basement membrane separates the epithelium from the dermal stroma.

At the stage of 10 mm cranio-caudal axis length of the golden hamster (3–7 cell layers epidermis), the basal lamina shows a distinct lamina lucida and a continuous lamina densa (Fig. 2). Immature and mature hemidesmosomes are coexistent. Different from the immature hemidesmosomes which have outer hemidesmosomal plaque, only mature hemidesmosomes exhibited the inner hemidesmosomal plaque which connects keratin intermedium filaments cytoskeleton (Mirancea et al., 2001). A subbasal dense plaque underlines both immature and mature hemidesmosomes. Moreover, anchoring filaments connect hemidesmosomes to the basal lamina and anchoring fibrils connect the basal lamina to the dermal counterpart. Epithelial cells have relatively large nuclei. Euchromatine is prevalent and only small patches of heterochromatine usually attached to the inner membrane of the nuclear envelope can be detected. The nuclei have prominent nucleoli.

At this stage of 10 mm length golden hamster, an unusual aspect can be seen: some epidermal basal cells exhibit large abnormal cell extensions invadopodia-like which penetrate into the dermal stroma. At that level, basal lamina is missing and no hemidesmosome can be detected at the edge of the cell protrusion (Figs. 3 and 4). The content of the cytoplasmic side of such a cell protrusion is very rich in cytoskeleton filaments.

The earliest sign of hair development appears at the stage of 10 mm length of golden hamster foetus as a crowding of cells in the basal layer of the epidermis (pre-germ stage) (Breatchnach, 1971). From place to place, the epidermis exhibited some epithelial conglomerates (hair germ) (Fig. 5) which protrude inside of the subjacent dermal tissue and become associated with an aggregation of mesenchymal cells. The lowermost portion of the epithelial protrusion invaginates

and a cup of epithelial cells is formed. The virtual space of the epithelial cup encloses the finger-like mesenchymal cell aggregate to form dermal papilla (Fig. 6) which does not lose its continuity with the surrounding dermal tissue from which it originates. Throughout subsequent development these two fundamental components of the hair follicle remain closely associated.

The epithelial cells which form the hair follicle wall are not very narrowed to each other (wide intercellular spaces and even microfibrils can be seen in some areas). Close contacts between epithelial cells are limited. Epithelial cells showed a well developed rough endoplasmic reticulum but keratin intermedium filaments are scarce (Figs. 7 and 8).

A clearly distinct continuous basal lamina (which normally in case of mouse or human hair follicle separates epithelial cells from dermal papilla) is missing even at the perinatal stage(not shown), but very seldom, some patches of basal lamina can be detectable (compare Fig. 9 – hamster to Figs. 17 and 18 – mouse). Moreover, epithelial plasma membrane facing the dermal papilla does not show mature hemidesmosomes, but some hemidesmosomal equivalents electrondensities (spots) (termed primitive or type II hemidesmosomes) are detectable (Fig. 10).

Mesenchymal cells from dermal papilla have large nuclei in which euchromatin is prevalent and 1–3 big nucleoli can be seen. Their cytoplasm is rich in free ribosomes and a rough endoplasmic reticulum. Mesenchymal cells exhibited cell microextensions which ended with an electrondense zone (probably junctional sites to anchor cells to the extracellular matrix). Sometimes, electrondense short profiles can be seen at the limited contact zones between two adjacent dermal papilla cells (Figs. 11 and 12).

Inside of the central area of dermal papillae, some mesenchymal cells are vacuolised (probably oedemateous) (Figs. 13 and 14) and other mesenchymal cells follow an apoptotic process. This aspect is persistent in case of perinatal golden hamster stage (not shown). Such morphological aspects are not detectable inside of the mouse dermal papillae prenatal or immediately postnatal (post-partum). While the mesenchymal cells papilla of the golden hamster hair follicle are randomly distributed, the mouse dermal papilla shows an elongated shape and the columns of dermal mesenchymal cells have an ordered arrangement (compare Fig. 7 – hamster to Fig. 15 – mouse). Like in case of epithelial sheath of the mouse hair follicle (Fig. 16), some epithelial cells of the golden hamster hair follicle undergo a mitotic process. Different from mouse dermal papillae, extracellular matrix of golden hamster dermal papilla is very rich in numerous microfibrils randomly oriented (compare Figs. 12–14 – hamster to Fig. 17 and Fig. 18 – mouse).

Similar to murine dermal papilla, but different from the human hair papilla, which has abundant small blood vessels, inside of the golden hamster hair follicle no microvasculature can be detected. Nevertheless, mention must be made that in the close vicinity of the hamster hair bulb some capillaries can be seen (not shown).

Golden hamster embryo development has a very short period of time: in two weeks and two days from the egg fertilisation a normal new born animal is at term (Whitney, 1963). During this very short period time, all organs, including the skin, should be formed.

After gastrulation the surface of the embryo is covered by a single ectodermal pluripotent cell layer which subsequently, under subjacent mesenchym influence, will differentiate to give rise to epidermal cells and epidermal derivatives (for example the pilosebaceous system).

In most mammals, hair follicles begin to form in the skin during prenatal life (Hardy, 1992). Various epithelial layers which are partly in morphogenic continuity with the epidermis and develop therefrom during early fetal life, build the hair follicle (Brandner *et al.*, 2003).

The hair follicle is a cutaneous organ, mainly an ectodermal derivative. Morphogenesis as well as hair shaft formation and hair growth cycles of the mature follicle are under the control of the dermal papilla which is of the mesenchymal origin (Schlake, 2005; Botchkareva et al., 2006). Indeed, during embryo development as well as during adult life, skin appendages such as pylosebaceous system morphogenesis like the epidermis is governed by reciprocal signalling between the mesenchym and the overlaying epithelial cells (Stark et al., 1990; Nanba et al., 2000). Epithelial appendages share a common developmental program with the interfollicular epidermis. While the importance of the transcription factor p 63 which plays a crucial role both in epidermal and appendage development is evident, the molecular mechanisms by which p 63 functions are largely unknown (Koster & Roop, 2004).

Epithelial cells regulate their contacts with neighbouring cells and associated stroma during both normal development and the acquisition of invasive behaviour during tumor progression (Mirancea et al., 2002). During embryo-foetal golden hamster skin development, some basal epidermal cells extent cell protrusion (invadopodia-like) inside of subjacent dermis and basement membrane is destroyed. The meaning of that cells behaviour which mimic an invasive behaviour (Mirancea et al., 2002; Vosseler et al., 2005) is not clear. In terms of molecular characterisation, invadopodial protrusions are enriched in integrins, tyrosine kinase signalling machinery, soluble and membrane proteases including matrix metalloproteinases, suggesting that these are involved in the surrounding extracellular matrix degradation (Ayala et al., 2006). We do not know yet if this ultrastructural aspect can be related with any event of the initiation of hair follicle morphogenesis. Mention must be made that under normal conditions, during embryo development some epithelial areas (including the corresponding basement membrane) are penetrated by another cell type. This is the case for example with the sensory axons which penetrate into the epithelium of the crista ampullaris of the inner ear (Helling & Merker, 2005).

In response to the first mesenchymal message, the basal cells in some limited areas of the epidermis proliferate faster and agglomerate to form hair placode (s) and this morphologic aspect was termed stage 1 of the hair follicle development. Subsequently, such a conglomerate of epithelial cells grows down to form a hairy plug (termed stage 2). The epidermal messages from the epithelial protrusion induce a dermal mesenchymal cells conglomerate formation which will be surrounded by the bulbous base of the epithelial protrusion. The deepest epithelial cells reorganized their location in such a way to form a virtual inverted cup and the large part of the mesenchymal cells conglomerate will protrude inside of the epithelial cup to form a so-called dermal papilla (stage 3). The second mesenchymal message from the dermal papilla stimulates proliferation of the adjacent epithelial cells of the hair follicle, known as the hair matrix and their differentiation into several types of epithelial cells of the hair follicle (Nanba *et al.*, 2000).

Like other mammalian animals, there are evident regional asynchronic differences of golden hamster skin development probably reflecting the regional differences of adjacent mesenchyme (Byrne, 1997). In a previous study, we reported that during golden hamster development cervico-dorsal skin is most advanced in its differentiation when compared to the caudal skin (Mirancea & Mirancea, 1997).

During hair follicle morphogenesis, a remodelling of desmosomal and hemidesmosomal junctions takes place. There is a body of evidence that the remodelling of the desmosomes and hemidesmosomes (cell-cell and cellextracellular matrix junctions) takes place when epithelium interacts with neighboring mesenchyme during early development or regenerative events (i.e. submandibular or mammary glands). It seems that the adjacent mesenchyme has the ability to disorganize epithelial cell adhesion systems (Nanba et al., 2000). Indeed, in our investigated golden hamster skin specimens, we may note that at the specific sites where the epithelial placodes invade (migrate) inside of the dermal mesenchyme, some desmosomal as well hemidesmosomal junctions become impaired. Moreover, different from early mouse skin and its hair follicle appendages we investigated (unpublished data), the golden hamster hair follicle showed that the basement membrane has some discontinuities and primitive (type II) hemidesmosomal junctions are less numerous even at the perinatal stage. Hamster hair follicle is much bigger than mouse hair follicle because the hamster dermal papilla has more mesenchymal cells and intercellular spaces are larger.

In conclusion, while generally golden hamster hair follicle morphogenesis follows the pattern characteristics to the main mammalian animals, yet some ultrastructural particularities are significant.

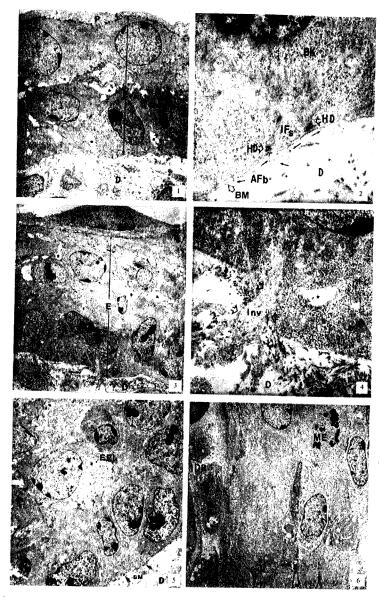


Fig. 1. At the stage of 2-3 cell layers epidermis (E), the desmosomal junctions are scarce, large intercellular spaces being visible (asterisks). Peridermal cells (P) are still attached to the epithelium. D = dermis. (× 1,500). Fig. 2. The dermo-epidermal junction is performed by hemidesmosomal junctions (HD) connecting intermedium filaments (IFs) and anchoring fibrils (AFb). BK = basal keratinocyte. Basal membrane (BM). D = dermis. (× 11,500). Fig. 3 and Fig. 4. A small cell protrusion invadopodia-like (inv) disrupt the basement membrane (detailed in Fig. 4; see the open arrows). P = periderm; E = epidermis; BK = basal keratinocyte; D = dermis. (Fig. 4 × 1,200; Fig. 5 × 5,700). Fig. 5. A conglomerate of epithelial cells (EPL) delimited by a basement membrane (BM) protrude inside of the adjacent dermis (D). (× 1,500). Fig. 6. A sheath of epithelial cells is facing the dermal papilla (DP). M marks a mitotic epithelial cell. (× 1,200).

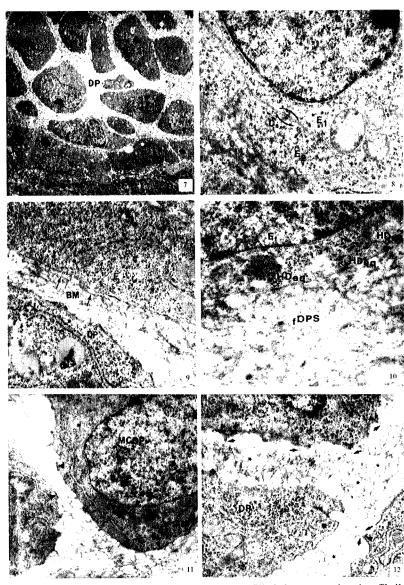


Fig. 7. Inside of dermal papilla (DP), large spaces filled by stromal microfibrils separate mesenchymal cells each other. Below, a sector of epithelial sheath (E) is visible. (× 1,000). Fig. 8. Two epithelial cells (E1 and E2) of the hair follicle epithelial sheath connected by a desmosomal junction (D). (× 10,000). Fig. 9. A discontinuous basement membrane (BM) separates epithelial sheath by the adjacent dermal papilla (D) (×10,000). Fig. 10. A sector of an epithelial cell (E) facing a fibrotic dermal papillae stroma (fDPS) showed type II hemidesmosome equivalents (HDeq) (× 11,500). Fig. 11. Mesenchymal cells of dermal papilla (MCDP) show large euchromatic nucleus. Some endoplasmic reticulum profiles and mitochondria as well as numerous ribosomes filled the cytoplasm. Cell microextensions (triangle). (× 5,700). Fig. 12. Mesenchymal cells from dermal papilla exhibited cell microextensions ended by electronodensities (triangles) which seem to connect cells to the microfibrils (arrows) randomly oriented inside of the large intercellular stroma (asterisks). (× 7,100).

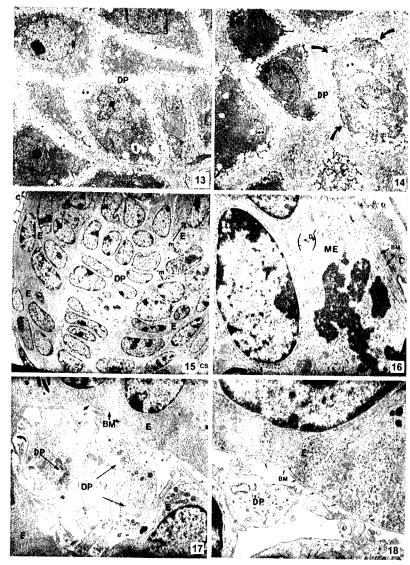


Fig. 13 and Fig. 14. Some areas of dermal papilla cells (DP) with irregular contours and vacuolised cytoplasm (\*). Extensive vacuolised areas (curved arrows) are performed by small vacuoles fusion, detailed in Fig. 13 (Fig  $13 \times 1,200$ ; Fig.  $14 \times 1,300$ ). Fig. 15. A general view of the mouse hair follicle showing an enlarged dermal papilla (DP) blanked by 2–3 cell layers of epithelial cells (E). Note the arrangements of the dermal papilla cells. M = melanosomes; c = collagen as fibrillar material lining the external surface of epithelial sheaths of hair follicle. CS = capillary in close vicinity of the hair follicle (CS). (Mouse). (× 700). Fig. 16. A sector of the epithelial matrix of mouse hair follicle showing an active mitotic cell (ME), still keeping the desmosomal junctions (D) with its neighboring cell. A basement membrane (BM) separates epithelial sheath from the dermis (× 7,100). Fig. 17 and Fig. 18. A continuous basement membrane (BM) separates epithelial cells by the dermal papilla (DP) of the mouse hair follicle. (Fig.  $17 \times 5,200$ ; Fig.  $18 \times 11,500$ ).

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Received November 22, 2006

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