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PROTAPHORURA TETRAGRAMATA (GISIN, 1963) AND *KALAPHORURA CARPENTERI* (STACH, 1920) (COLLEMBOLA: ONYCHIURIDAE) – TWO NEW RECORDS FOR ROMANIAN FAUNA

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During the collection of Collembola between 2006 and 2009, two new species for Romania were found: *Protaphorura tetragramata* (Gisin, 1963) and *Kalaphorura carpenteri* (Stach, 1920). Notes on their taxonomical status, ecology and distribution are given.

Key words: springtails, faunistics, Romania.

INTRODUCTION

The Onychiuridae family is represented in the world fauna by nearly 600 species (Bellinger *et al*., 2013) and is divided into three subfamilies: Onychiurinae Börner, 1901, Tetrodontophorinae Stach, 1954 and Lophognathellinae Stach, 1954.

Studies on the Onychiuridae of Romania dating back to 19th century were first started by Tömösváry (1882) who mentioned few species for Transylvania region.

A total of 13 genera with 56 species have been recorded from Romania, which belongs of the first two subfamilies. Until now, 17 species of the genus *Protaphorura* Absolon, 1901 have been identified from our country (Fiera, 2007; Dányi & Traser, 2008 a). The number is still incomplete, because some species of the genus *Protaphorura* require a modern revision.

Of the 10 species of *Kalaphorura* listed by Bellinger *et al.* (2013) in the world checklist, 3 were reported from Romania till now: *K. burmeisteri* (Lubbock, 1873), *K. paradoxa* (Schäffer, 1900) and *K. tuberculata* (Moniez, 1890).

Due to the diversity of biogeographical regions (Alpine, Continental, Pontic, Steppe and Pannonian) which occur in Romania, many habitats are still unexplored from the faunistic point of view. Opportunistic sampling, carried out on survey trips or in the course of other studies, provided two new records for Romanian springtail fauna: *Protaphorura tetragramata* (Gisin, 1963) and *Kalaphorura carpenteri* (Stach, 1920).

MATERIAL AND METHODS

Our investigations have been conducted in the period 2006-2009 in different localities of Romania. The sampling of soil and litter samples was done using Mac

Fadyen cylinder, 3×10 cm; 10 samples of soil from each site were collected. The extraction of the biological material was done at Berlesse-Tullgren, keeping for about five or six days. After extraction, the biological material was preserved in ethanol 96% and then it was labeled. The specimens were clarified using KOH 10% and after that they were laid on slides in Schwann medium for identification at species level (Rusek, 1975).

RESULTS

PROTAPHORURA TETRAGRAMATA (GISIN, 1963)

Material examined. The species was collected from three sites:

1. Sovata (Mureş County), Lacul Ursu, Natural Reserve, IUCN cat. III, (46°36'14"N; 25°05'07"E), 502 m altitude, mixed forest with *Quercus robur* L., *Q. petraea* (Mattuschka) Liebl., *Fagus sylvatica* L. and *Carpinus betulus* L., soil (♀♀♀), 2.10.2008, leg. C. Fiera;
2. Retezat Massif, Nucşoara (Sălaşu de Sus village, Hunedoara County) (45°30'20"N; 22°54'24"E), dump site, soil (♂), 10.09.2006, leg. D. Constantinescu;
3. Doftana Valley, Glodeasa Reserve, 650-890 m altitude, *Luzulo–Fagetum* forest, aged between 200-300 years, in mosses (♂, ♀, 1 juv.) and lichens (♂), 13.05.2009, leg. C. Fiera.

Taxonomy. *Protaphorura tetragramata* belongs to *octopunctata* group, with four and more pseudocelli at antennal base; the group is included in the tribe Onychiurini. In Romania there are other three species from *octopunctata* group: *P. quadriocellata* (Gisin, 1947), *P. octopunctata* (Tullberg, 1876) and *P. sakatoi* (Yosii, 1966).

P. tetragrammata is characterised by presence of pseudocelli on upper subcoxa of all legs, 2+2 pseudocelli on both II and III thoracic terga and with anterolateral pseudocelli on abdominal tergum IV. Among octopunctata group this species has distinctly differentiated macro- and mesochaetae, which are apically retused and a little knobbed.

Postantennal sense organ consists of 37-40 simple vesicles (42 in the Romanian material). Pseudocellar formula dorsally: 43/022/33343; ventrally: 1/000/00000, all subcoxae 1 of I, II, III pair of legs with one pseudocellus and one parapseudocellus. Dorsal chaetotaxy is very distinctly differentiated into apically retused and slightly knobbed macrochaetae and pointed mesochaetae. Microchaetae very short, apically pointed. Thoracic tergum I with 12–16 chaetae (chaetotaxy type i2–3m). Abdominal terga I–III without chaetae s'. Abdominal tergum V with p0 chaeta present. Abdominal tergum VI with 2 medial chaetae (Fig. 1) (sometimes with only one). Straight lines, passing through bases of short chaetae situated above anal spines, convergent. A detailed re-description was provided by Kaprus'& Pomorski (2008).

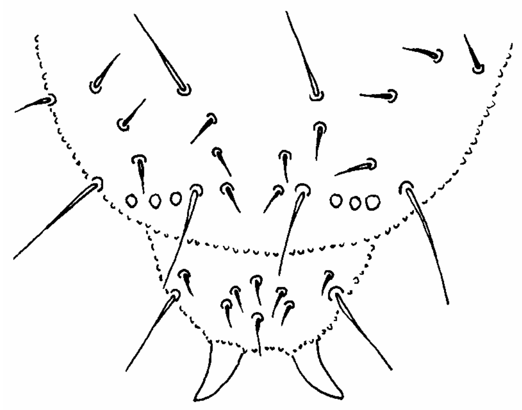

Fig. 1. *Protaphorura tetragramata* (Gisin, 1963)
Chaetotaxy of abdominal terga V and VI (orig.).

P. tetragramata is close to *P. sakatoi* (Yosii, 1966) and *P. octopunctata* (Tullberg, 1876) with its pseudocellar formula dorsally: 43/022/33343, 1/000/00000 ventrally, but differs in the number of pseudocelli from subcoxae 1. *P. sakatoi* has 1,0,0 pseudocelli on subcoxae 1 of I, II, III pair of legs and at *P. octopunctata* they are absent. If we compare *P. tetragramata* with *P. quadriocellata* these two species distinctly differ in pseudocellar formula dorsally which is 43/022/33333 in *P. quadriocellata*. Besides, *P. tetragramata* has ventral tube with 9-10+9-10 chaetae and 2+2 chaetae at the base and at *P. quadriocellata* the ventral tube is with 13-19+13-19 chaetae.

Distribution and ecology: Bosnia and Herzegovina, Serbia and Montenegru, Poland, Ukraine. The species lives in mountain forests, in soil and litter; bisexual (Kaprus' & Pomorki, 2008).

KALAPHORURA CARPENTERI (STACH, 1920)

Material examined. The species was collected from the following sites:

1. Odorheiu Secuiesc (Harghita county), 46°16'55"N; 25°15'41"E, beech forest, soil (♂♂) and litter (♂♂, ♀), 03.10.2008, leg. C. Fiera;
2. Sinaia (Prahova county), 45°21'2"N; 25°33'29"E, mixed forest with *Abies alba* Mill. and *Fagus sylvatica* L., litter (♂), 29.08.2009, leg. C. Fiera;
3. Posada Gorges (Prahova county), 45°17'43.5"N; 25°35'40.9"E, mixed forest with *Fagus sylvatica* L., soil, (1 ex. juv.), 14.08.2009, leg. C. Fiera.

The studied specimens are stored at the Institute of Biology Bucharest of Romanian Academy.

Taxonomy. Habitus typical of the genus *Kalaphorura* Absolon, 1901; abdominal VI elongated with anal spines on distinct papillae. Dorsal side of the body covered with very strong granulation, especially coarse on terga.

PAO is in a deep, long cuticular groove, with 22-25 simple vesicles. Dorsal pseudocellar formula (pso) per half tergum: 20/011/11122, ventral pso absent. Each subcoxal with one parapseudocellus. Dorsal chaetotaxy often asymmetrical not differentiated into macrochaetae and microchaetae, most of setae knobbed. Thorax II-III with lateral microsensilla. Subcoxae usually with 5, 6, 6 setae. Tubus ventralis with 8-9 setae. Male ventral organ absent.

Medial seta a_0 on abdominal tergum VI situated above row of p setae is the only way to separate it to *K. paradoxa* Schäffer, 1900 (Fig. 2 A). These two species have the same number of papillae in sensory organ of antennal segment III and the same dorsal pseudocellar formula (pso): 20/011/11122.

K. carpenteri (Fig. 2 B) is related to *K. tuberculata* (Moniez, 1890), with which it agrees in: the shape of the body, coarse granulation of the skin, the form of vesicles in PAO, the presence of rudimental furca and tenaculum. The tergits of *K. carpenteri* are covered with very variable large granules which are arranged in another way (Stach, 1954). If we compare *K. carpenteri* with the other two Romanian species (*K. burmeisteri* Lubbock, 1873 and *K. tuberculata* Moniez, 1890), the first species differs in the number of papillae in sensory organ of antennal segment III (5 in *K. carpenteri*, 4 in K. *burmeisteri* and *K. tuberculata*) (Massoud, 1968).

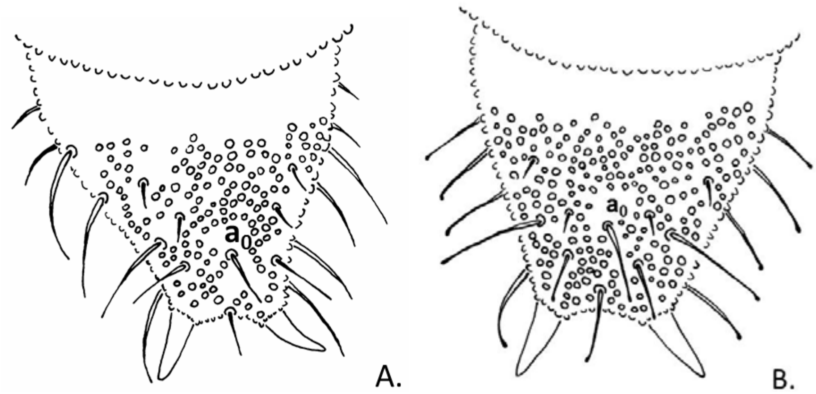

Fig. 2. Abdominal tergum VI.
A) *Kalaphorura paradoxa*; B) *K. carpenteri* (orig.).

Distribution and ecology. Poland, Slovakia, Ukraine (Sterzyńska *et al.*, 2007; Raschmanová *et al.*, 2008; Kaprus *et al.*, 2006). The species was found in different mountain forests (lime, beech, maple-hornbeam and cornel-oak wood (Raschmanová *et al.*, 2008) and it occurs in humid litter, under stones and pieces of wood; bisexual (Pomorski, 1998).

Broader analysis of the biogeographic data and faunistic lists from neighboring countries: Moldova (Buşmachiu, 2010); Ukraine (Kaprus' *et al.*, 2006); Hungary (Dányi & Traser, 2008 b); Poland (Sterzyńska *et al.*, 2007) indicates the possibility of occurrence of several more species in our country.

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COMPARATIVE STUDY CONCERNING SOIL MITES COMMUNITIES (ACARI: MESOSTIGMATA) FROM SOME ANTHROPIZED MARSHLANDS IN THE INSULA MARE A BRĂILEI (ROMANIA)

MINODORA MANU

Insula Mare a Brăilei is a famous agricultural area in Romania. This is the result of 45 years of communist agricultural policy, when the soil drainage transformed natural marshlands in agroecosystems. The main task of this paper is to make for the first time in Romania a comparative analysis of the predatory soil mites communities from different types of anthropical ecosystems: grasslands, arable fields and one planted forest. Study was made in 2005-2006. 280 samples were collected, with 23 species and 350 individuals. From the total number of identified species, 71.42% were found in grasslands, 57.14% in agroecosystems and 26.08% in planted forest. *Hypoaspis aculeifer* and *Rhodacarellus kreuzi* are the most frequent species. Taxonomical classification and applied statistical methods used (Jaccard index), showed the similarities as well as the differences between predators mite's communities, taking account of the specific environment conditions.

Key words: soil mites, marshlands, vegetal associations, community, similarity.

INTRODUCTION

Biodiversity refers to all species of plants, animals, and microorganisms existing and interacting within an ecosystem (McNeely *et al.*, 1990). Global threats to biodiversity should not be foreign to agriculturalists, since agriculture, which covers about 25 to 30 percent of world land area, is perhaps one of the main activities affecting biological diversity. It is estimated that the global extent of cropland increased from around 265 million hectares (ha) in 1700 to around 1.5 billion hectares today, predominantly at the expense of forest habitats. Very limited areas remain totally unaffected by agriculture-induced land-use changes. Clearly, agriculture implies the simplification of the structure of the environment over vast areas, replacing nature's diversity with a small number of cultivated plants and animals (Altieri, 1999; Altieri & Nicholls, 2004; Knop *et al.*, 2006).

In Romania, during the communism period, some main changes over natural ecosystems were made, in order to extend the agricultural fields. So, for 45 years, soil drainage works in the Insula Mare a Brăilei transformed natural marshlands in anthropical ecosystems. This involved modifications on ecosystem's structures and functions, especially on species diversity. In this area, researches concerning soil invertebrates diversity are few (Fiera, 2006; Vasiliu Oromulu *et al.*, 2009). In soil fauna there are included invertebrates with an important role in decomposing the vegetal material (soil mites), participating to the soil formation, influencing its fertility, and indirectly to the production and productivity of cultures. Through activity of different trophical components from soil web, the edaphical fauna provided over 75% of total assimilated energy by plants (Coleman *et al.*, 2002; Adl, 2003; Moore *et al.*, 2005).

The aim of the present paper is to make, for the first time in Romania, a comparative analysis of the soil mites community structures from different types of ecosystems of some anthropized marshlands from Insula Mare a Brăilei: grasslands, arable fields and one planted forest. An actual study concerning the soil predator mites provides new, original and precious informations, which can be used to assess the anthropical ecosystems in comparison with natural ones.

MATERIAL AND METHODS

The research was made in the Insula Mare a Brăilei, in 2005-2006 period. Insula Mare a Brăilei is situated on the lower course of the Danube river, in Brăila district. It is 60 km long and 20 km wide, with a total surface of 710 km. It is separated from the continent by the two river's arms: Măcin and Cremenea. 681.3 km² (94.6% from the total area) are arable fields, from which 70.84 km² are irrigated and protected by a dam of 23.5 km length (Fig. 1).

The soil is alluvial and is characteristic for the Insula Mare a Brăilei. This soil has many nutritive elements, such as: humus 4.68%; pH 8.4; N total 0.372%; PAL (potentially assimilable phosphorus) 46.8 ppm; KAL (potentially assimilable potassium) 108.7 ppm; carbonates 10.9% (Năstase *et al.*, 2001).

The soil samplings were collected randomly, with MacFadyen soil core, by 5 cm diameter. The sampling was made up to 10 cm depth. The extraction of the mites was made in 10–14 days by the Berlese-Tullgren method, modified by Balogh (1972). The samples were kept in a refrigerator till the next extraction. 280 samples, 23 species with 350 individuals were analysed in total. Counting and identification of mites were made under a Zeiss binocular and microscope using the keys of Ghiliarov & Bregetova (1977); Karg (1993); Mašán (2003); Mašán & Fenďa (2004); Mašán (2007); Gwiazdowicz (2007); Mašán & Halliday (2010). Preservation of the gamasid mites was made in an alcohol and glycerin mixture. All identified specimens are deposited in the mite collection of the Institute of Biology Bucharest, Ecological Stationary from Posada.

The populational parameters were analysed: numerical density (ind./sq.m.) and species diversity (number of species). In order to compare the species composition of predatory mites populations (Acari - Mesostigmata), three types of ecosystems were analysed: grassland ecosystems (site I, II, III); agriculture fields (IV, V, VI) and planted forest (VII) (Fig. 2). Data were collected during the vegetation period, for two years (Table 1).

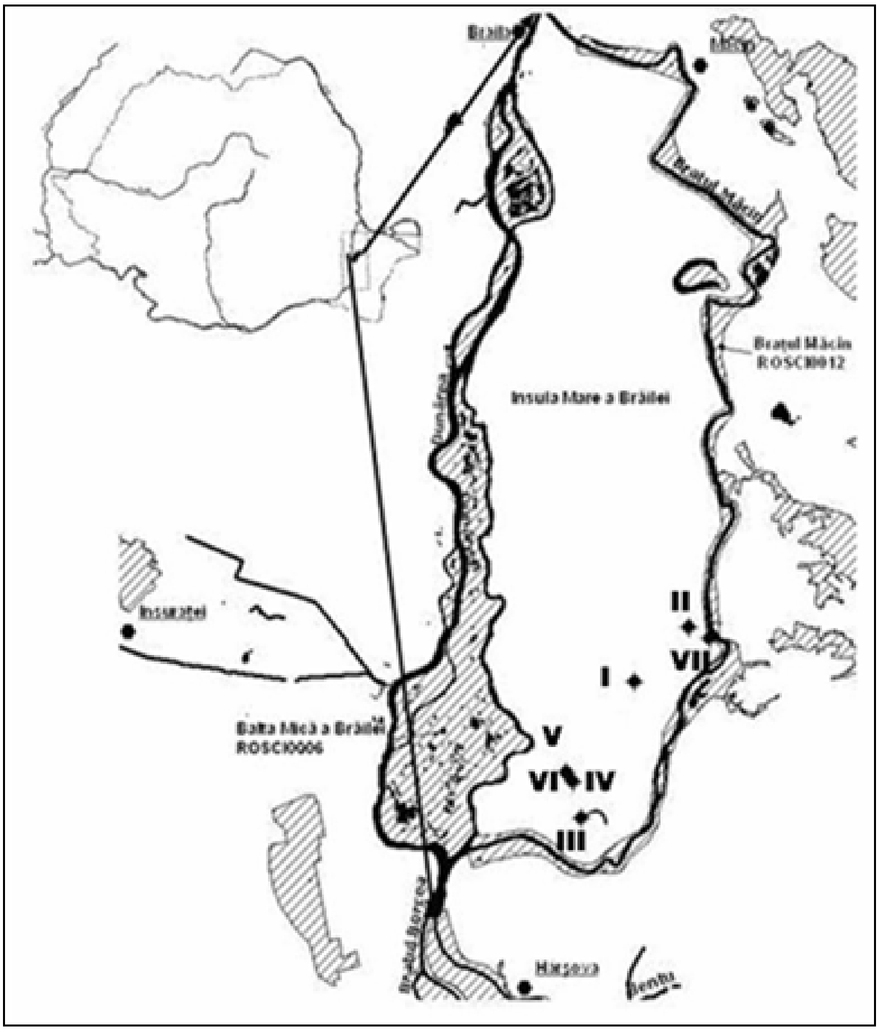

Fig. 1. The geographical position of the Insula Mare a Brăilei with studied sites
(I, II, III = grasslands; IV, V, VI = arable fields; VII = planted forest).

Site I - grassland **Site IV – arable field** **Site VII – planted forest**

Fig. 2. Types of investigated ecosystems from Insula Mare a Brăilei.

Table 1
The investigated sites from the Insula Mare a Brăilei

Site	Vegetal association	GPS Coordinate
I	*Hordeetum murini* Libbertt 1932 em. Pass. 1964 *Agropyretum pectiniformae* (Prodan 1939) Dihoru 1970	N $44^0$87'19.40"; E = $28^0$05'86.10"
II	*Xeranthemo cylindracei –Brometum arvensis* Popescu Gh. 1992 *Typhetum laxmannii* Nedelcu 1969	N $44^0$90'37.10"; E = $28^0$10'88.50"
III	*Conietum maculati* I.Pop 1968 *Arctio –Ballotetum nigrae* (Felfoldy 1942) Morariu 1943	N $44^0$78'99.10"; E = $28^0$00'65.50"
IV	*Triticum aestivum* L.	N $44^0$81'20.70"; E = $27^0$99'98.60"
V	*Glycine max* (L.) Merr.	N $44^0$81'84.60"; E = $27^0$99'43.30"
VI	*Zea mays* L.	N $44^0$81'84.40"; E = $27^0$99'41.90"
VII	*Salicetum albae* Issler 1924 s.l.	N $44^0$89'69.00"; E = $28^0$12'73.80"

In order to show similarities between mite populations from investigated ecosystems, the Jaccard index (q) was calculated using the BioDiversityPro software (McAleece *et al.*, 1997).

$$q = c / a+b–c,$$

where, a = number of species from ecosystem A; b = number of species from ecosystem B; c = number of common species from ecosystems A and B.

RESULTS AND DISCUSSION

In all six investigated sites, 23 species were identified, with a numerical density about 37.000 ind./sq.m. The highest values of these populational parameters were obtained in sites I, II and the lowest in site VI. In sites III, IV, V medium values were recorded. Making a complete analysis in all types of ecosystems, the species number and numerical densities were much higher in grasslands, in comparison with planted forest and with arable fields, where the lowest values were recorded. From all described species from sites I–VI (taking account of the equitability of the number of sites: three grasslands and three arable fields), 71.42% were identified in grasslands, 57.14% in agroecosystems and 26.08% in planted forest (Table 2).

Hypoaspis aculeifer and *Rhodacarellus kreuzi* are the most frequent species, being identified in six sites and respectively in four areas. These species have the capacity to adapt to specific conditions to any type of ecosystems, due to their wide ecological plasticity (*Hypoaspis aculeifer*) or to their ability to migrate in soil up to 20 cm (species from *Rhodacarellus* genera), in unfavorable conditions such as: dryness, decreased quantity of organic matter, chemical pollution (Salmane, 2003; Ruf

& Beck, 2005; Honciuc & Manu, 2010; Manu, 2012). The species characteristic for each site were: site I – *Epicriopsis horridus, Pachyseius humeralis, Trachytes aegrota* (which represent 30% from total number of identified species); site III – *Melichares pomorum, Pachylaelaps imitans* (66.6%); site IV – *Pergamasus laetus, Pergamasus quisquiliarum* (33.3%); site V – *Pergamasus barbarus, Zercon peltatus* (40%), site VI – *Geholaspis longispinosus* (50%), site VII – *Proctolaelaps pygmaeus* (18.8%). These mesostigmatids are common species for a temperate area, being identified in different types of ecosystems such as: forests, shrubs, meadows, urban areas (Skorupski, 2001; Salmane, 2003; Gwiazdowicz & Kmita, 2004; Manu, 2012) (Table 2).

Table 2

Numerical density (ind./sq.m.) of mesostigmatid mites from investigated sites

Species	**Site I**	**Site II**	**Site III**	**Site IV**	**Site V**	**Site VI**	**Site VII**
Arctoseius semiscissus (Berlese, 1892)	2000						400
Epicriopsis horridus (Kramer, 1876)	800						
Geholaspis longispinosus (Kramer, 1876)						400	
Hypoaspis aculeifer (Canestrini, 1883)	2800	1200	2600	600	400		200
Macrocheles decoloratus (C.L. Koch, 1839)	800	400					
Melichares sp.			800				
Olopachys vysotskajae Koroleva, 1976		600				400	200
Pachylaelaps imitans Berlese, 1921			800				
Pachyseius humeralis Berlese, 1910	1400						
Pachylaelaps furcifer Oudemans, 1904					200		
Parasitus kraepelini (Berlese, 1905)	800						200
Parasitus sp.	400	1200					
Pergamasus barbarus Berlese, 1904	800				200		
Pergamasus quisquiliarum (G. and R. Canestrini, 1882)	800			400			
Pergamasus sp.	400			200			
Proctolaelaps pygmaeus (Muller, 1859)							400
Rhodacarellus kreuzi Karg, 1965	2400	3200			600		400
Trachytes aegrota (C.L. Koch, 1841)	1200						
Uropoda sp.	1000			400			
Veigaia nemorensis (C.L. Koch, 1939)		2800					200
Vulgarogamasus oudemansi (Berlese, 1903)		600		400			
Zercon peltatus C.L. Koch, 1836					200		
Zercon romagniolus Sellnick, 1944	400			400			
Total no. of species	14	7	3	6	5	2	6
Total no. of individuals	80	50	21	12	8	4	10
Total no. of ind./sq.m.	16.000	10.000	4.200	2.400	1600	800	2000

Other specialists have recorded different values of these two populational parameters, in relation with the same type of studied ecosystems (agriculture field, grassland and planted forest). The highest values of numerical densities and of species diversity was recorded in grasslands, in comparison with agroecosystems. The values of the two populational parameters from the seven sites are comparable with those obtained in Europe, even in Argentina, Canada and the USA, although in these last three countries the climate and the type of soil are different (Koehler 1997, 1999; Gulvik, 2007; Bedano & Ruf, 2007, 2010; Salmane & Brumelis, 2010). In Poland, studies on soil mite's communities from the converted mixed forests (beech, fir and spruce) showed a smaller number of species, in comparison with natural ones from Romania, similar to that obtained in investigated ecosystems from the Insula Mare a Brăilei (Skorupski *et al*., 2009; Manu, 2012) (Table 3).

Table 3
Populational parameters on mesostigmatid mites
from various type of ecosystems in different countries

Country	**Mesostigmata index**	**Ecosystems**			**References**
		Arable field	Grassland	Planted Forest	
Germany	no. sp. ind./sq.m.	7-57 5000	15-50 10000	50	Koehler, 1999 Ruf & Beck, 2005
Latvia	no. sp. ind./sq.m.	83-104 5016	73-141 654		Salmane, 2001 Salmane & Brumelis, 2010
France	ind./sq.m.	2000-3660			Cortet *et al.*, 2002
Poland	no. sp. ind./sq.m.			29-39 3950-6363	Skorupski *et al.*, 2009
Argentina	no. sp. ind./sq.m.	13 897	15 2737		Bedano *et al.*, 2005 Bedano *et al.*, 2006 Bedano & Ruf, 2007
Canada	no. sp.	7	14		Behan-Pelletier, 2003 Osler *et al.*, 2008 Behan-Pelletier & Kanashiro, 2010
USA	no. sp. ind./sq.m.			40 1997	Minor & Norton, 2004

Abbreviations: no. sp. = number of species; ind./sq.m. = numerical density.

Species number and diversity measures were the highest in natural ecosystems and very low in the arable site. So, in terms of species number, the arable field did not have an impoverished community in comparison with the natural undisturbed grassland. In general, total mesostigmatid densities in organic horizons are negatively affected by cultivation (Bedano & Ruf, 2007).

Taking account of the similarities of species composition from the investigated sites in Insula Mare a Brăilei, we observed that between sites I–II and V–VII the highest values of Jaccard index (q= 45.794 and respectively q = 38.888) were recorded (Fig. 3). Mesostigmata is very much influenced by microclimate, which depends on the structure of the herbaceous plants, shrubs or trees and on the litter layer. The similarities of the environmental conditions (the same type of soil, the vegetal associations, characteristic for grasslands) create specific habitats for these mesostigmatid mites in sites I, II and III. An interesting relation was observed on mites from sites V and VII. It is possible that the relative small distance between these two areas offers possibility of migration, being known that mesostigmatids are very mobile mites, permanently looking for the food (Krantz & Walter, 2009).

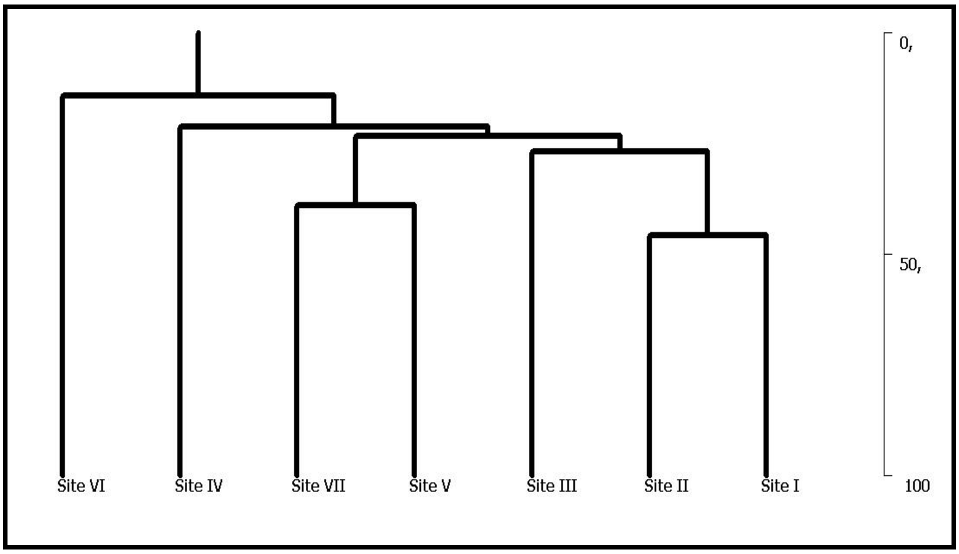

Fig. 3. Bray-Curtis Cluster Analysis of the mesostigmatid species from Insula Mare a Brăilei.

On the opposite, there are the species of mesostigmatids from these groups of sites: VI–II and III–VII, where the lowest values of similarities index (q = 5.556 and q = 9.677) indicated the high differences of the species diversity between all three types of ecosystems: grassland, arable field and planted forest. The distances between these four sites are quite big (about 20 km) and the environmental conditions could be different. The different vegetal associations, the poor composition in organic matter (due to the monocultures from arable fields, in comparison with grassland, where vegetation is spontaneous) and the presence of a litter–fermentation layer in the planted forest, could be factors that influence the species diversity in the studied sites.

CONCLUSION

This study highlighted taxonomical and structural differences on mesostigmatid communities. The taxonomical structure of mesostigmatid populations revealed the presence of 23 species, with 36.000 ind./sq.m. Taking account of the species diversity and the numerical density, in grasslands these parameters were much higher, in comparison with planted forest and with arable fields, where the lowest values were recorded.

From all identified species, *Hypoaspis aculeifer* and *Rhodacarellus kreuzi* are the most common species, being described in majority of sites studied. These are predator species, with wide ecological plasticity, which easily migrate from one site to another, searching for food. Each studied area is characterized by characteristic species, which are common for temperate ecosystems.

The similarities of the environmental conditions (the same type of soil and of vegetal associations, characteristic for grasslands) create specific habitats for mesostigmatid mites in sites I, II and III. The Jaccard index revealed two groups of sites, with the mesostigmatid recorded similarities of species diversity: I–II and V–VII. On the other hand, high differences were obtained between species diversity from three types of ecosystems: grassland, arable field and planted forest. These differences were highlighted by the lowest values of similarities index from these groups of sites: VI–II and III–VII.

Specific environmental factors, such as: type of soil, the type of vegetal associations, which influence indirectly the quantity and quality of organic matter (especially on monocultures where input of organic matter is lower in comparison with grassland or forest) could influence the species composition from each studied sites, this fact being demonstrated using Jaccard similarity index.

Natural restoration of the arable field from Insula Mare a Brăilei is a benefic process for soil mites, natural grassland providing favorable habitats for these soil invertebrates.

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HOST ASSOCIATIONS OF THYSANOPTERA WITH *MEDICAGO SATIVA*

DANIELA BĂRBUCEANU*, LILIANA VASILIU-OROMULU**

Studies conducted during June-September 2012 on an alfalfa crop of an agroecosystem from Vişina (Dâmboviţa District) highlighted the taxonomic and ecological structure of thrips fauna. To the thrips coenosis contributed 18 species, which showed great specific richness. The specific core was represented by polyphagous *Frankliniella intonsa* and *Thrips tabaci*. *F. intonsa* proved the highest abilities in operating the trophic substrate, accounting for 67.84%, followed by *T. tabaci* with 19.62 %. High numerical abundances of the two species on the alfalfa plants showed their attachment to the host plant, which provides optimal shelter, food and breeding conditions. The Shannon-Wiener diversity index presented low values in the studied site. *Kakothrips robustus, Thrips validus, Haplothrips setiger* are mentioned for the first time on alfalfa in Romania, complementing the list of taxa inhabiting the alfalfa inflorescences. The current study represents the first ecological research of Thysanoptera on *Medicago sativa* in our country.

Key words: thrips, alfalfa, biodiversity, ecological indices.

INTRODUCTION

Knowledge of the biodiversity of living organisms and their niches is a fundamental objective of taxonomic and ecological research worldwide. Compared to other groups of insects, there are few entomologists specialized in thrips study. Abundance of floricolous thrips enables their use mainly to study their population dynamics. Research on thrips inhabiting the inflorescences of *Medicago sativa*, a Fabaceae species with forage importance, was addressed in terms of taxonomy by Jenser (1990) in Hungary. The latter mentioned five species on *Medicago sativa* in a compendium of thrips species inhabiting Fabaceae.

In Croatia, Raspudić *et al*. (2009) identified 8 species on this host. Priesner (1964) and Lacasa & Lloréns (1996) mentioned the *Odontothrips confusus* species on alfalfa, and Bournier & Kochbav (1965) described its effect in the sterilization of alfalfa flowers.

In Romania, Knechtel (1951) quoted 15 species of Thysanoptera on inflorescences of *M. sativa* collected from different geographical areas, and Vasiliu & Burlacu (1970) and Vasiliu-Oromulu (2002) completed the list with *Aeolothrips intermedius* and *Odontothrips confusus* species.

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MATERIAL AND METHODS

The observations were carried out during June-September 2012 on inflorescences of *Medicago sativa* on a crop of about 400 sqm from a private garden from Vişina (Dâmboviţa District), located in the Găvanu-Burdea plain, 44° 20' 53" north latitude and 25° 08' 41" east longitude. In its neighbourhood there were vegetable crops, fruit trees and vines. Collections were made during the flowering of alfalfa. Ten samples each were collected every month, and a sample consisted of 10 inflorescences. Adult individuals and larvae were collected from inflorescences and preserved in vials with AGA, a mixture of 60% ethyl alcohol (10 parts), glycerine (one part) and glacial acetic acid (one part).

The thrips species was identified using the following keys of determination: Knechtel (1951); Schliephacke & Klimt (1979); zur Strassen (2003); Kucharczyk (2010).

In order to assess the diversity of the ecosystem, the Shannon-Weaver diversity index was calculated, using the formula improved by Lloyd and Ghelardi (1964):

$$H(S)=\frac{K}{N}\left(N\log10N - \sum_{p=1}^{S} Nr\log10Nr\right)$$

where: H = index; S = total number of species; K = 3, 321928; N = total number of individuals; *Nr* = total number of individuals in species *r*. (Simionescu, 1984).

RESULTS AND DISCUSSION

Data from this study reveal aspects related to the biodiversity and ecological indices of Thysanoptera fauna on *Medicago sativa* plants.

Biodiversity

Ecological research of thrips on *M. sativa* consisted in revealing their structural peculiarities, which required in a first phase knowledge of the taxonomic composition of these insects.

Thus, the study highlighted the presence of thrips from two suborders, three families, eight genera and eighteen species, with a total numerical abundance of 489 adults and 94 larvae (Table 1).

Four of them, *Frankliniella intonsa, F. tenuicornis, Kakothrips robustus*, and *Thrips tabaci* were mentioned by Raspudić *et al.* (2009) on *Medicago sativa*, in Croatia.

Ecologically, the taxonomic spectrum of Thysanoptera on alfalfa is diversified: floricolous, folicolous, gramineous, mesophyllous and xero-thermophyllous species.

Table 1
Specific diversity of Thysanoptera fauna on *Medicago sativa*

Suborder	Family	Species	No. ind.	A (%)	Geographical distribution
Terebrantia	Aeolo-thripidae	*Aeolothrips intermedius* Bagnall, 1934	10♀♀; 10♂♂	4.17	PAL
	Thripidae	*Chirothrips manicatus* Haliday, 1836	2♀♀	0.41	HOL
		Frankliniella intonsa (Trybom, 1895)	295♀♀; 30♂♂	67.84	EUS
		Frankliniella pallida Uzel, 1895	9♀♀	1.87	EUR
		Frankliniella tenuicornis Uzel,1895	1♀	0.2	COS
		Kakothrips robustus Uzel,1895	4 ♀♀	0.83	EUR
		Odontothrips confusus Priesner , 1928	1♀	0.2	EUS
		Tenothrips frici Uzel , 1895	2 ♀♀	0.41	WPAL
		Thrips atratus Haliday, 1836	1♀	0.2	PAL
		Thrips flavus Schrank, 1776	1♀	0.2	COS
		Thrips physapus Linne, 1761	1♀; 2♂♂	0.62	EUS
		Thrips tabaci (Lindeman, 1888)	94♀♀	19.62	COS
		Thrips validus Uzel, 1895	6♀♀	1.25	EUS
		Terebrantia larvae	93		–
Tubulifera	Phlaeo-thripidae	*Haplothrips acanthoscelis* Karny, 1909	3♀♀; 2♂♂	1.04	EUS
		Haplothrips aculeatus (Fabricius, 1803)	1♂	0.2	PAL
		Haplothrips leucanthemi (Schrank 1781)	7♀♀; 2♂♂	1.88	EUS
		Haplothrips reuteri Karny, 1907	2♀♀; 2♂♂	0.84	PON-MED
		Haplothrips setiger (Priesner, 1921)	1♀	0.2	WPAL
		Tubulifera larvae	1	–	–

COS=Cosmopolite; EUR= European; EUS=Euro-Siberian; HOL= Holarctic; PAL= Palaearctic; PON-MED = Ponto-Mediterranean; WPAL= West-Palaearctic.

All species are polyphagous, an efficient adaptive strategy, that ensures survival in terms of reducing the trophic spectrum. Most of them are primary consumers and only two species belong to the secondary consumers, the polyphagous *Aeolothrips intermedius* and *Thrips flavus*.

As expected, typical flower species dominate, three species being a gramineous species – *Chirothrips manicatus, Frankliniella tenuicornis*, and *Haplothrips aculeatus* known on Poaceae. *Thrips tabaci* stands out through its ability to inhabit both the flowers and leaves of plants.

The same diversity is also found in terms of geographical distribution. The Euro-Siberian elements dominate, a situation encountered in Thysanoptera coenosis from other ecosystem types studied in Romania (Vasiliu-Oromulu & Bărbuceanu, 2010).

Sex ratio falls within the specific range, knowing female dominance in this order of insects (Table 1). The most abundant species of this study, *Frankliniella intonsa*, presents a percentage of 90.7% females and sex ratio of 0.11, while for the zoophagous *A. intermedius* we notice a balanced ratio of 1:1.

According to Lewis (1973), in many species in which individuals of the two sexes are produced in equal numbers, females are apparently prevalent because they live longer than males.

One of the collected species represented only by females is the cosmopolitan species *Thrips tabaci*. It seems that in the center of origin of the species (Central Asia) the same as of the favorite host – *Allium cepa*, the sex ratio is about 1:1 (Lewis, 1973). In Romania, *T. tabaci* is known only by the parthenogenetic females. This situation may be due to the spread of thrips in certain areas only under this form.

Ecological indices

The “basic nucleus” of Thysanoptera association consists of two species: *Frankliniella intonsa* and *Thrips tabaci*, having the highest values of structural indices (Fig. 1,Table 2). Jenser (1990) mentioned the two species in a study on thrips species on various Fabaceae, expressing their preference for alfalfa in a ratio of 16% for *F. intonsa* and only 2% in the case of *T. tabaci*.

Collection from June to September showed high specific diversity on this host plant, ranging from 7 to 12 species. In collection from June 2012, populations of thrips coenosis belong to 8 species, showing great specific richness. The polyphagous *Frankliniella intonsa* proves the greatest abilities in the exploitation of the trophic substrate, accounting for 85.15%, actually being the only dominant species. We notice the presence of typical floricultural species, *F. intonsa, Kakothrips robustus, Thrips physapus, Haplothrips leucanthemi, H. reuteri.* Additionally, we can mention the gramineous thrips *Chirothrips manicatus*, probably brought on the alfalfa inflorescences by anemochory.

Reduced values of constancy, in relation to this host, i.e. alfalfa, reflect a dynamic coenosis, these insects being extremely mobile, to such behaviour contributing their polyphagia. As expected, the value of the Shannon-Wiener diversity index is influenced by low equitability: 17.13, the lowest of all collections.

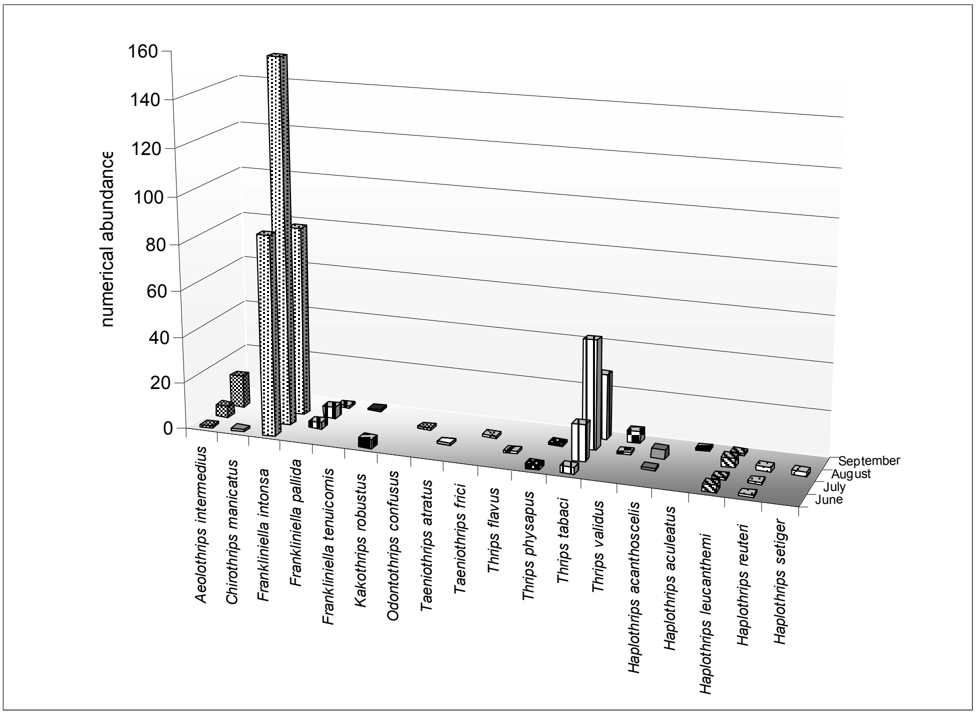

Fig. 1. Numerical abundance of thrips populations on *Medicago sativa*.

In July, the high degree of alfalfa flowering attracts the greatest numerical abundance during the study period: 186 individuals/inflorescence. The Thysanoptera association is composed of 9 species, the species with an important role in creating the coenosis are the polyphagous *F. intonsa* and *T. tabaci*, dominant and euconstant. Such conditions are also favourable for secondary consumers, represented by *Aeolothrips intermedius* and *Thrips flavus* species.

However, although the number of species involved in the coenosis structure is large, their numerical abundance is low, the two dominant species totalling 93.01% of the collected individuals. This fact is reflected in the low value of equitability: 30%. Phytophagous species *Thrips atratus, Haplothrips acanthoscelis, H. leucanthemi, H. reuteri* have minimal numerical abundances, falling into the category of accidental species.

The highest specific diversity, 12 species, is found in the August collection, the numerical abundance keeping high rates: 164 individuals/inflorescence. The trophic offer attracts many species of Terebrantia and Tubulifera, thus favouring the zoophagous *Aeolothrips intermedius*, which along with *F. intonsa* and *T. tabaci* falls into the category of dominant species, with high values of frequency in samples: 80-100%.

Table 2

The structural indices of the thrips populations on *Medicago sativa*

June 2012	**no. ind/flower**	**x**	**Stdev**	**A%**	**C%**	**$p_i \log p_i$**
Aeolothrips intermedius	1	0.1	0.3	0.99	10	-0.020
Chirothrips manicatus	1	0.1	0.3	0.99	10	-0.020
Frankliniella intonsa	86	8.6	4.3	85.15	100	-0.059
Kakothrips robustus	4	0.4	0.5	3.96	40	-0.056
Thrips physapus	2	0.2	0.6	1.98	10	-0.034
Thrips tabaci	3	0.3	0.7	2.97	20	-0.045
Haplothrips leucanthemi	3	0.3	0.7	2.97	20	-0.045
Haplothrips reuteri	1	0.1	0.3	0.99	10	-0.020
Σ	101	10.1	4.5	100.00		-0.155
	H(S)	**=0.514**	**H_{max}**	**=3.0**	**E%**	**=17.13**
F. intonsa larvae	1					
July 2012						
Aeolothrips intermedius	5	0.50	0.7	2.69	40	-0.042
Frankliniella intonsa	157	15.7	7.6	84.41	100	-0.062
Frankliniella pallida	3	0.3	0.5	1.61	30	-0.029
Thrips atratus	1	0.1	0.3	0.54	10	-0.012
Thrips flavus	1	0.1	0.3	0.54	10	-0.012
Thrips tabaci	16	1.6	1.4	8.60	70	-0.092
Haplothrips acanthoscelis	1	0.1	0.3	0.54	10	-0.012
Haplothrips leucanthemi	1	0.1	0.3	0.54	10	-0.012
Haplothrips reuteri	1	0.1	0.3	0.54	10	-0.012
Σ	186	**18.6**	6.8	100.00		-0.286
	H(S)	**= 0.95**	**H_{max}**	**=3.0**	**E%**	**=30.0**
F. intonsa larvae	17					
August 2012						
Aeolothrips intermedius	14	1.40	1.0	8.54	80	-0.091
Frankliniella intonsa	82	8.2	9.4	50.00	100	-0.151
Frankliniella pallida	5	0.5	0.7	3.05	40	-0.046
Odontothrips confusus	1	0.1	0.3	0.61	10	-0.014
Tenothrips frici	1	0.1	0.3	0.61	10	-0.014
Thrips physapus	1	0.1	0.3	0.61	10	-0.014
Thrips tabaci	47	4.7	3.6	28.66	90	-0.156
Thrips validus	1	0.1	0.3	0.61	10	-0.014
Haplothrips acanthoscelis	4	0.4	0.7	2.44	30	-0.039
Haplothrips leucanthemi	4	0.4	0.7	2.44	30	-0.039
Haplothrips reuteri	2	0.2	0.4	1.22	20	-0.023
Haplothrips setiger	2	0.2	0.4	1.22	20	-0.023
Σ	164	**16.4**	9.2	100.00		-0.623
	H(S)	**=2.06**	**H_{max}**	**=4**	**E%**	**=58**
	no. ind/flower	**x**	**Stdev**	**A%**	**C%**	**$p_i \log p_i$**
F. intonsa larvae	14					
September 2012						
Chirothrips manicatus	1	0.1	0.3	2.63	10	-0.042
Frankliniella pallida	1	0.1	0.3	2.63	10	-0.042
Frankliniella tenuicornis	1	0.1	0.3	2.63	10	-0.042
Thrips tabaci	28	2.8	1.6	73.68	90	-0.098
Thrips validus	5	0.5	0.7	13.16	40	-0.116
Haplothrips aculeatus	1	0.1	0.3	2.63	10	-0.042
Haplothrips leucanthemi	1	0.1	0.3	2.63	10	-0.042
Σ	38	3.8	2.2	100.00	10	-0.421
	H(S)	**=1.4**	**H_{max}**	**=3**	**E%**	**=50**
F. intonsa larvae	61					
Haplothrips larvae	1					

Odontothrips confusus known as an alfalfa pest (Bournier and Kochbav, 1965) has an accidental presence, although Vasiliu & Burlacu (1970) noticed it in a ratio of 33%. Jenser (1990) also highlighted its presence on the alfalfa in a ratio of 69% compared to other Fabaceae.

Great specific richness and equitability lead to a higher diversity index, H(S) = 2.06. Towards the end of alfalfa flowering season, in September, Thysanoptera coenosis was composed of 7 species, with the lowest values of numerical abundance throughout the entire collection period. *T.tabaci* dominates with 73.68% and a frequency in samples of 90%, replacing *F. intonsa*. Even though *T. validus* participates in the creation of the coenosis from the position of dominant species (13.16%) it shows a low attachment towards the host plant, being present only in 40% of samples.

The gramineous species, *Chirothrips manicatus, Frankliniella tenuicornis*, and *Haplothrips aculeatus* have sporadic presence, recording frequency values of 10%. Although reduced, the somewhat balanced participation of species in building the coenosis is reflected in the average values of equitability (50) and diversity index: 1.4.

Temporal dynamics of thrips populations on *Medicago sativa*

This study highlights large populations of the xerophilous species *F. intonsa*, known due to its attachment to inflorescences of a great number of herbaceous plants.

Following adult population dynamics we notice that it presents increased values of abundance in July-August, with a peak in July. If during the first months of collection *F. intonsa* dominates, in September its population regresses in favor of cosmopolitan *T. tabaci*.

In terms of demographic structure, the dynamics showed an increase of numerical abundance of the immature from 1 specimen in June to 61 specimens in September. The ratio adults/larvae for the *F. intonsa* species was favourable to adults in June-August at a rate of 10:1, indicating the presence of a mature population. Instead, in September, the massive presence of larvae, expresses a dynamic coenosis, a renewal of *F. intonsa* populations (Fig. 2).

Thus, during September collection, the *F. intonsa* adults were no longer present in samples, instead we noticed a very large number of larvae, the largest in the whole study period (Table 2). Such situation may be due to unusually high temperatures in autumn 2012 that influenced the thrips life cycle. According to Lewis (1973), in temperate regions, in natural environment, most species have 1 or 2 generations per year. It is possible that due to long and warm autumn, *F.intonsa* continues its life cycle by the emergence of a new generation.

Regarding the *T. tabaci* species, its population had increased abundance values in August and September, recording a peak in August. Complementary values of numerical abundance highlighted by the temporal dynamics of these two dominant species, *F. intonsa* and *T. tabaci*, may be due to inter-specific competition occurring between them.

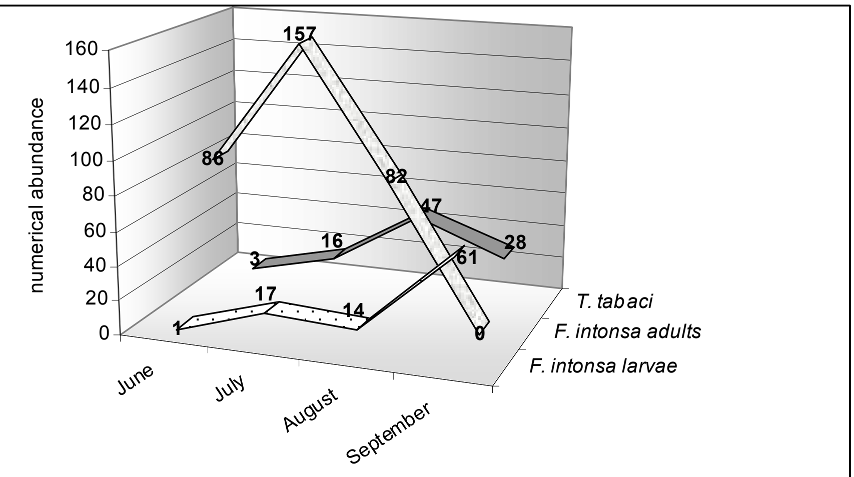

Fig. 2. Dynamic of thrips populations on *Medicago sativa.*

The temporal dynamics of taxonomic structure and numerical abundance of thrips populations was influenced by variation in abiotic factors (T, U) and biotic (numerous alfalfa plants got dried causing an aggregation of thrips), just as in the case of *F. intonsa* species. The temporal dynamics curve reached a peak in July and a minimum in September. The phenomenon of aggregation of thrips populations on *M. sativa* is obvious due to the concentration of insects on an ever decreasing number of flowering plants in warm and dry autumn, highlighting the negative binomial distribution of thrips (Vasiliu-Oromulu, 1999).

CONCLUSIONS

Populations of the Thysanoptera association on *Medicago sativa* belong to 18 species, pointing out high specific diversity. The presence of the two tropho-dynamic modules, primary and secondary consumers, ensures the balance of this community of Thysanoptera.

The specific nucleus of the thrips coenosis is represented by *Frankliniella intonsa* and *Thrips tabaci*, with the highest values of ecological indicators; they dominate the coenosis with 67.84 % and 19.62 %, respectively.

High numerical abundances of *Frankliniella intonsa* and *Thrips tabaci* species on the alfalfa plants show the attachment of these species to the host plant, providing optimal shelter, food and breeding. *Kakothrips robustus, Thrips validus, Haplothrips setiger* are mentioned for the first time on alfalfa in our country and complete the list of Thysanoptera species inhabiting the alfalfa inflorescences.

The current study is a pioneering ecological research of Thysanoptera on *Medicago sativa* in our country, by temporal dynamics analysis of the structure of those populations. The study of the population structure dynamics of *F. intonsa*, adults and the immature, represents another contribution to ecological research of this species.

Such studies highlight that, under certain conditions, anthropogenic ecosystems can contribute, too, to the conservation of the biodiversity of these insects.

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THE ROTIFER PRODUCTION IN RELATION TO EXTRINSIC FACTORS IN THE SFÂNTU GHEORGHE BRANCH (THE DANUBE DELTA)

LARISA FLORESCU, ALINA DUMITRACHE, MIRELA MOLDOVEANU

The rotifers are known as cosmopolitan species, with a high capacity to adapt to different environmental conditions, however, few species can adapt to major fluctuations. Inter-annual variations (2008-2010) in the dynamics of primary and secondary production are affected by a large scale of biotic and abiotic variability. During the first two years of the study, the primary production ranged between 0.037 and 3.759 mg O_2/L/24h. The rotifer production presented high values in spring (7.00 µg/L/24h) and summer (4.24 mg/L/24h, annual average) which suggests that the life cycles of these organisms are more intense in these seasons. The correlation matrix showed that O_2, dissolved inorganic nitrogen (DIN), total phosphorus (TP), organic phosphorus (P_{org}), total organic carbon (TOC) and nitrites (NO_2) fluctuations had effects on the rotifer development. Also, there is a direct correlation between primary production and secondary production of the rotifers ($R^2 = 0.079$, $R = 0.28$, $p < 0.05$). It can be noticed an inverse tendency of variation, the rotifers production increasing with a lower DIN/TRP ratio. The aim of this paper is to identify the seasonal and spatial dynamics of the rotifers production in different areas (natural and altered due to hydraulic works) on Sfântu Gheorghe branch, the Danube Delta, in relation to the key physical-chemical and biological drivers.

Key words: rotifers, secondary production, Sfântu Gheorghe branch, the Danube Delta.

INTRODUCTION

The zooplankton communities are characterized by a certain complexity, being in a constant changing of their ecological parameters (abundance, biomass and generation time), due to seasonal variations, abiotic factors, predator pressures or competition mechanisms. Although the rotifers are known as cosmopolitan species, with a high capacity to adapt to different environmental conditions, however, few species can adapt to major fluctuations. Thus, the production of species/populations may be particularly important in assessing the ability to provide resources and services to socio-economic systems (Cristofor *et al.*, 2010).

The secondary production has been described in detail in the literature since the early period of the ecological research. Most studies refer to "biomass produced in a given time period and area" (Edmondson, 1974; Benke & Wallace, 1980). However, the relationship between control factors and secondary production is less discussed.

In freshwater ecosystems the rotifers play an important role, both the abundance and specific diversity and their function in the food web. The physiology of rotifers is strongly influenced by their ability to interact with the environment, thus, depending on the physical and chemical variations, the populations can develop or not. Inter-annual variations in the dynamics of primary and secondary production is affected by large scale abiotic variability such as climate swings (Gerten & Adrian, 2000; Wallace *et al.,* 2006).

For example, the water movement causes variations of the dissolved oxygen concentration or temperature that will affect the development of the rotifers communities and then, further into the food web. The human impact and natural factors are the main responsible of the long-term changes of the specific diversity and short-term of the abundance, biomass and productivity (Wrona *et al.*, 2006). In the recent decades, the influence of human and social components on various natural and semi-natural ecosystems increased significantly, which caused imbalances. This forced change either direct or indirect of the ecological structures of the systems led implicitly to change their functions. Such ecosystems cannot provide the same services, in addition to loss of utility, even causing damage, by increasing the risk of certain pollutants, potentially toxic or damaging and reducing of the resources (plants, animals, landscape) provided by the natural capital (Vădineanu, 2004).

The aim of this paper is to identify the seasonal and spatial dynamics of the rotifers production in different areas (natural and altered due to hydraulic works) on Sfântu Gheorghe branch, in relation to the key physical-chemical and biological drivers. Also, it is important to know the answer of the biocenoses to the anthropogenic impact in the three studied zones.

MATERIAL AND METHODS

The study site

Sfântu Gheorghe branch is the oldest branch of the Danube river. Between the years 1985-1990, some meanders of the Sfântu Gheorghe branch were cut off, to improve the water run-off, the navigation, the discharge of sediments. After these hydrotechnical changes, the branch acquired another geomorphologic configuration formed by new canals, cut-off meanders and natural sectors.

For this study, 7 sampling stations with coastal and medial points were established: 1, 4, 7 – in natural sectors; 2, 5 – in meanders; 3, 6 – in canals (Fig. 1).

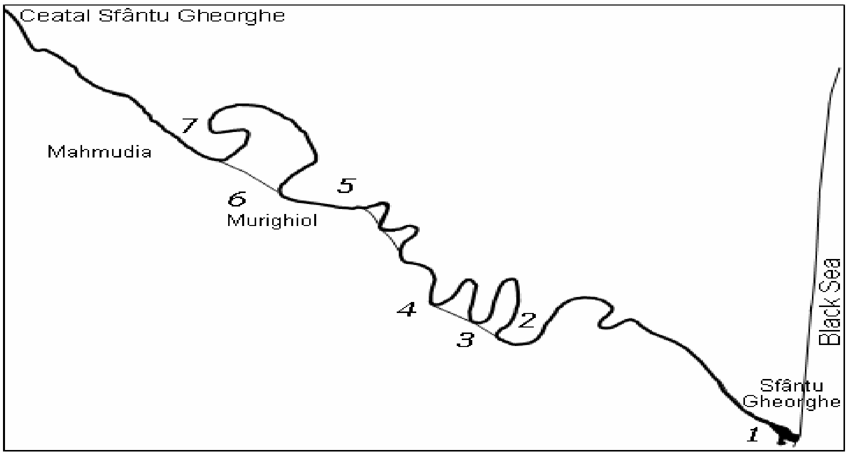

Fig. 1. Sfântu Gheorghe branch with the sampling sites.

Methods

Water samples have been taken for the physical-chemical variables determinations. The transparency was established with Secchi disk and the depth with Humimbird 260 sonar. The temperature, pH, dissolved oxygen content were measured in the field with a multiparameter WTW 340i (Germany). Samples for chemical analyses were frozen for further analyses in the lab. Nutrients were determined spectrophotometrically (CECIL 1100, UK): NH_4^+ – as yellow compound with Nessler reagent, NO_2^- – as red compound with sulphanilic acid and α-naphthylamine, NO_3^- – as yellow compound with sodium salicylate (Tartari & Mosello, 1997), total reactive phosphorus (TRP) – as blue phosphomolybdate, reduced by ascorbic acid, total phosphorus (TP) – by oxidation with potassium peroxodisulphate (Tartari & Mosello, 1997); the organic matter content was estimated from the chemical oxygen demand determined by oxidation with $K_2Cr_2O_7$ (COD-Cr).

The primary production was estimated by *"in situ"* experiments using the "black and white bottles" method. This method is based on the principle of quantitative determination of the released oxygen in the process of photosynthesis and consumed by respiration within 24 hours (Vollenweider, 1969; Philips, 1973; Marton *et al.*, 1981). For measuring the dissolved oxygen in water the Winkler method was used. The principle is the oxidation of manganese hydroxide by the dissolved oxygen to manganic hydroxide, which remove, in the acidic environment, the iodine potassium iodide in an equivalent amount to the dissolved oxygen and then, titrated with sodium thiosulfate.

The rotifer samples were taken seasonally (spring, summer and autumn), from 2008 to 2010, in water column with a Patalas Schindler plankton trap. The rotifers samples were collected by filtering 50 liters of water through standard plankton net (65 µm Ø mesh) and the samples were fixed in 4% of formalin. For the species identification a Zeiss inverted microscope was used according to the following references: Voight, 1956; Rudescu, 1960. The density was calculated as individuals L^{-1} and for the biomass calculations, the wet weight of the organism (µg wet weight L^{-1}) was used according to Dumont *et al.* (1975). Production per day was assessed based on method described by Edmondson & Winberg (1971) and expressed in (µg wet weight L^{-1} /24h).

For statistical analysis, Microsoft Office 2007 and XLSTAT (trial version) were used.

RESULTS

Physical-chemical parameters

In the aquatic ecosystems there are important interactions between abiotic and biotic components. It turned out in several studies that physical-chemical factors have a strong influence on zooplankton and rotifers too (Fantin-Cruz *et al.*, 2011).

To evaluate the importance of different control parameters which act at the level of rotifers communities in the Sfântu Gheorghe branch, and the way they influence, the following physical and chemical parameters were measured (Table 1).

Table 1

Descriptive statistics of the physical and chemical parameters

Parameters	N	Minimum	Maximum	Mean	Std. Deviation
Depth (m)	63	1.000	14.600	7.085	3.655
Transp. (m)	63	0.100	0.950	0.480	0.191
T/D	63	0.013	0.476	0.136	0.094
Temp. (C)	63	9.500	29.100	18.136	6.178
pH	63	7.645	10.635	8.390	0.685
O_2 (mg O/L)	63	0.000	11.710	7.716	2.373
TOC (mgC/L)	63	0.024	22.875	6.537	5.198
NH_4 (mgN/L)	63	0.145	1.450	0.310	0.173
NO_2 (mgN/L)	63	0.005	0.462	0.074	0.105
NO_3 (mgN/L)	63	0.380	3.455	1.390	0.675
DIN (mgN/L)	63	0.662	17.400	4.640	4.667
TRP (µg/L)	63	24.000	88.500	52.786	15.261
TP (µg/L)	63	55.500	141.000	87.310	20.050
Porg (µg/L)	63	3.000	94.000	32.008	24.595

The most important pressure on ecological systems is the increasing concentration of nutrients associated with variations in the hydrological and temperature regime, that can lead to increased trophic level to mesotrophy, eutrophy, even to the hypertrophy (Parpală *et al.*, 2008).

The variation of DIN/TRP ratio (dissolved inorganic nitrogen/total reactive phosphorus) shows the changes in the ratio of the two nutrients, N and P, and may represent a measure of eutrophication (Vădineanu *et al.*, 1987).

In the Sfântu Gheorghe branch, the available nutrients (N and P forms) had a wide range of fluctuation (Table 1).

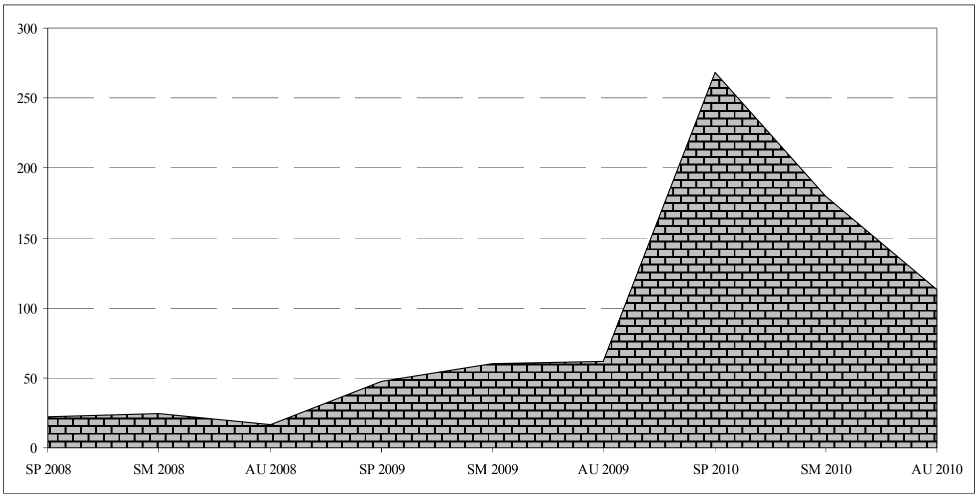

Fig. 2. The seasonal dynamics of DIN/TRP ratio in the studied ecosystems
(SP – spring; SM – summer; AU – autumn).

The seasonal average of DIN/TRP ratio in the studied period ranged from 16.967 to 268.41 (Fig. 2), which leads to the classification of Sfântu Gheorghe branch as mesotrophic stage (DIN/TRP > 10) (Postolache, 2006).

Phytoplankton community

The primary producers (mainly phytoplankton) are the food source for other trophic levels and one of the important factors that depend on the structural and functional characteristics of the higher trophic levels (Moldoveanu & Ionică, 2011).

In general, in aquatic ecosystems, the primary productivity depends on hydrological factors, climate, food supply, the cycling capacity of biogenic elements and the structure and functionality of biocenosis at system level (Botnariuc, 1999). In lotic systems, the primary productivity is dependent on the solar radiation, temperature, chlorophyll concentration, euphotic zone depth, current velocity, water and solids flow and the structure of bottom (Takahashi *et al*., 1973).

The primary production of the phytoplankton has a periodicity related to seasonal succession. During the first two years of the study, the primary production ranged between 0.037 and 3.759 mg O_2/L/24h. From the histogram of production is noted that the most frequent are the low values (≤ 0.8) (Fig. 3). Due to unfavorable conditions, the lotic ecosystems are less productive, only nutrient enrichment derived from human activities lead to increased productivity (Odum, 1971; Lair, 2005).

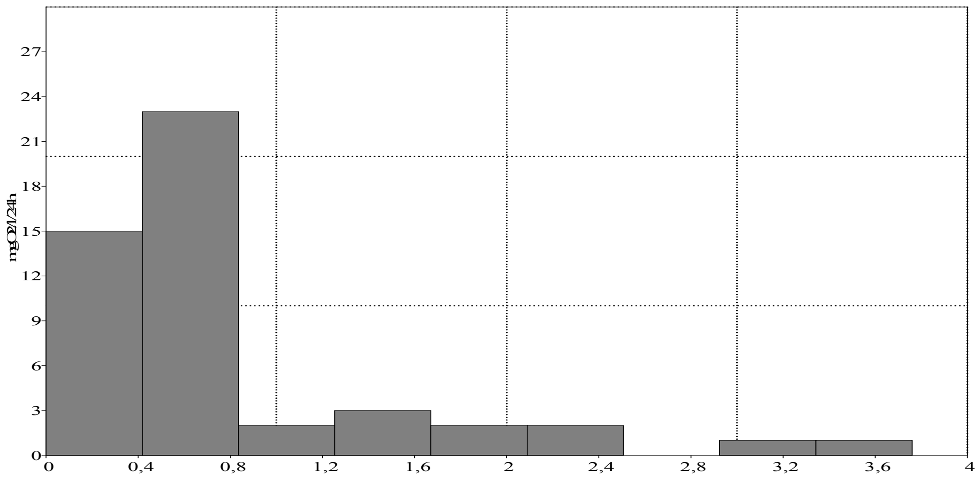

Fig. 3. The histogram of the seasonal phytoplankton primary production.

The highest value of primary production was recorded in summer 2008 (3.759 mg O_2/L/24h) in the two years of study in the sectioned meanders, where the conditions are more stable (less water velocity, low turbidity, lower shipping). The other two areas do not offer optimal conditions for primary production, being zones with high flow velocity of water, strong currents, large solids load of water (Fig. 4).

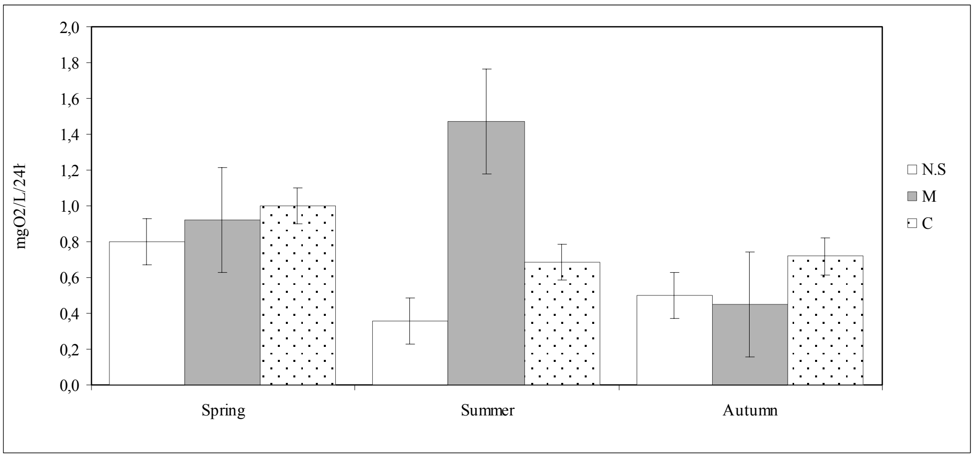

Fig. 4. The seasonal variation of primary production in the three investigated zones with standard errors (NS – natural sectors; M – meanders; C – canals).

Rotifers community

The rotifers presented high values in spring (7.00 µg/L/24h) and summer (4.24 mg/L/24h, annual average) which suggests that the life cycles of these organisms are more intense in these seasons. Towards the end of the year, in general, the production is characterized by a decline (1.00 µg/L/24h). The process decline may be delayed if the thermal regime is maintained at a high level. This may be an explanation for higher values of production in autumn 2008 (2.824 µg/L/24h) compared with the other two similar periods in the following years (in 2009 – 0.14 and 2010 – 0.04 µg/L/24h) (Fig. 5). In the three years of sampling, the production showed a gradual decrease from spring to autumn. The exception is 2008, in the natural sectors, where the production significantly increased from spring to summer and, as has been mentioned, remained high in autumn.

Instead, 2009 is a modest year regarding the production (1.03 µg/L/24h, annual average). The spring 2010 is a particular season, with much higher production values than other studied periods (9.78-12.83 µg/L/24h) (Fig. 5).

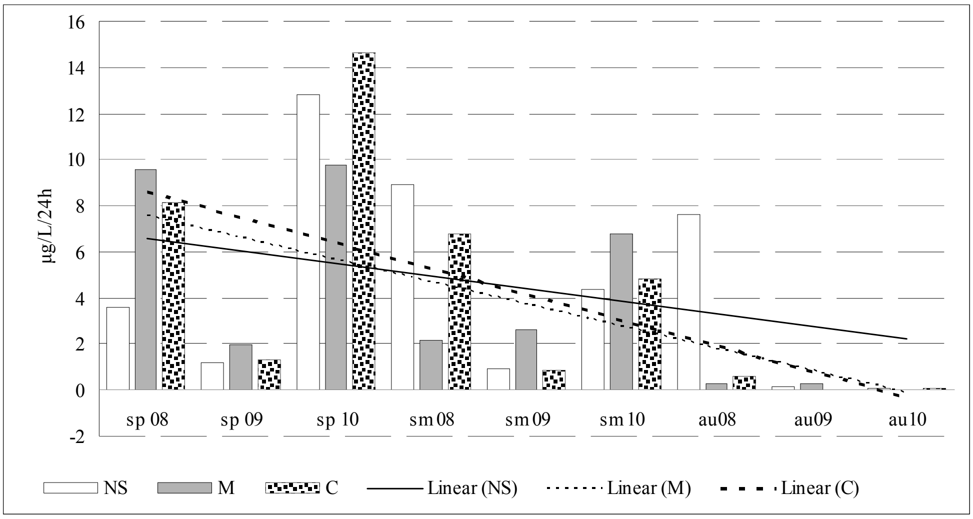

Fig. 5. The seasonal distribution of rotifers community production in Sfântu Gheorghe branch (NS – natural sectors; M – meanders; C – canals; SP – spring; SM – summer; AU – autumn).

DISCUSSION

The study of interactions between the rotifer production and physical-chemical parameters is based on statistical analysis to establish the degree to which abiotic factors have influenced the community development. The distribution of rotifers, the abundance and their production is closely related to specific physiological requirements and their ability to adapt to existing conditions.

Based on the correlation matrix the statistically significant physical and chemical factors that influenced rotifer production were established. The results showed that O_2, dissolved inorganic nitrogen (DIN), total phosphorus (TP), organic phosphorus (P_{org}), total organic carbon (TOC) and nitrites (NO_2) fluctuations had effects on the rotifer development (Table 2).

An increase of phosphorus concentration produces an increase of phytoplankton and implicitly the zooplankton community increased (Dzialowski & Smith, 2008; Muylaert *et al.*, 2010). The phytoplankton assimilates the phosphorus and result in high primary production, resulting, through the food web, an increase of the secondary production (Sterner *et al.*, 1993). Phosphorus is a limiting factor in the development of phytoplankton, some species require specific amounts of phosphates for development, others being less influenced, the algal diversity is closely linked to fluctuations of this parameter (Sterner *et al.*, 1992). The dominance of some phytoplankton species will consequently influence the zooplankton structure due to the feeding selectivity of these organisms (Townsend *et al.*, 2003).

The algae are characterized by increased carbon content as a result of fixation processes through photosynthesis, in addition to phosphorus and nitrogen (Le Borgne, 1982; Sterner, 1990). The transfer of matter and energy from the primary producers to the consumers depends largely on the grazing efficiency (Elser & Goldman, 1991).

Therefore, it indirectly explains the statistically significant correlations in the correlation matrix obtained. Also, there is a direct correlation between primary production and secondary production of the rotifers ($R^2 = 0.079$, $R = 0.28$, $P < 0.05$) (Fig. 6).

Through their feeding and metabolism, rotifers, as part of the zooplankton, play an important role in the cycling of carbon and nutrients. Also by grazing and their involving in the nutrients sequestration and regeneration, the zooplankton components influence the algal development.

In addition to phytoplankton, the bacterioplankton is another important trophic base for rotifers. The populations of this group of organisms feed on bacterial aggregates or attached to some particles (Branco & Senna, 1996). Hence, the trophic structure of a biocoenosis is the result of nutrition relationships of the component species of trophic level: primary producers, consumers, decomposers.

To assess the statistical significance of the relationships between the above-mentioned control factors and rotifer production, a simple linear regression for each parameter was applied. TOC, DIN showed a positive correlation with production changes that means they have stimulated the rotifer development, instead P_{org}, NO_2, TP, O_2 had the reverse effect (Table 2).

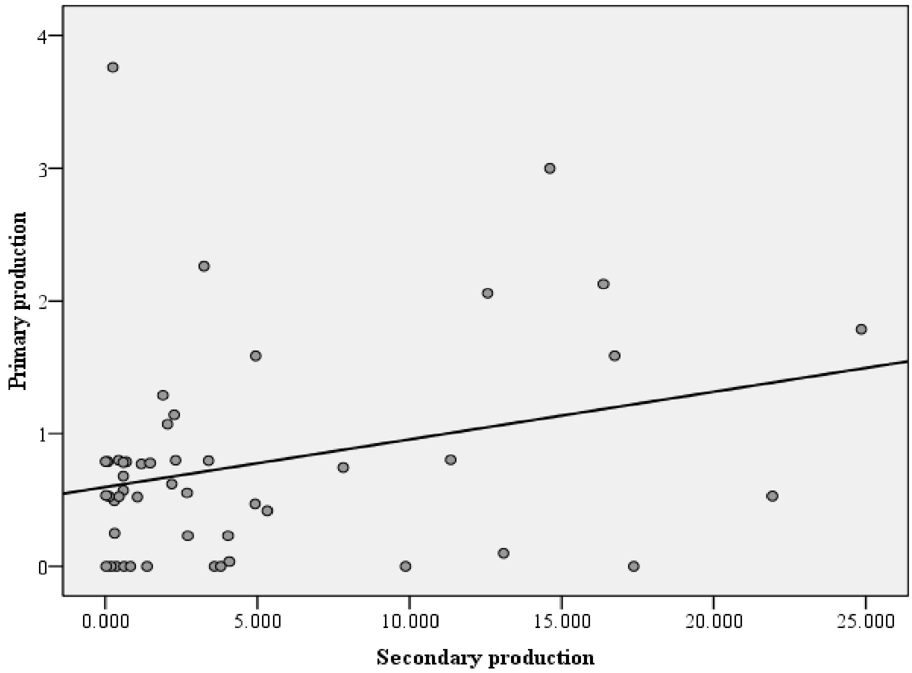

Fig. 6. Relationship between primary production and secondary production of rotifers.

Table 2

The linear regression results of the key chemical and physical parameters in the secondary production of rotifers

Label	N	Slope	R^2	Intercept	P	Significance
NO_2	63	-0.0057	0.0983	0.0987	0.0120	*
O_2	63	-0.1895	0.2103	8.5157	0.0000	***
TOC	63	0.3163	0.1220	5.2032	0.0050	**
DIN	63	0.2566	0.0996	3.5578	0.0110	*
P org	63	-1.7666	0.1700	39.4595	0.0000	***
TP	63	-1.7666	0.1343	92.7078	0.0030	**

Due to eco-physiological characteristics, the planktonic algae are “the first users” of any type of nutrients in the water (Klein & Chorus, 1991; Lau & Lane, 2001). Consecutively, the phytoplankton level increases and the species composition may provide significant information on the trophic status of the water or changes over time. There are attempts to define the trophic state considering phytoplankton composition and quantitative indices. Vollenweider (1968), taking into account the correlation between the amount of nutrients and quantitative development of phytoplankton, considers the biomass value of 10 mg/L as the lower limit of eutrophication. Oltean (1985) established at 5 mg/L the threshold of "algal bloom". The nutrient elimination in lakes may even escape to detection because the phytoplankton is able to sequester with high affinity the occasional pulses of nutrients (Kisand *et al.*, 2001).

The DIN/TRP ratio is used both to assess the trophic status of ecosystems and to identify the limiting factors of the phytoplankton development. Thus, the values of DIN/TRP>10 show the role of phosphorus as limiting factor, and the values of DIN/TRP<10, the role of nitrogen as limiting factor. Also, the last condition is characteristic of strongly eutrophic systems, and the first of the oligo-mesotrophic stage (Postolache, 2006). In the Sfântu Gheorghe branch, the values of DIN/TRP ratio are generally maintained above the threshold of 10. The phyto-plankton production is not significantly influenced by this ratio, remaining almost constant over the studied period, with some exceptions (the highest value, 3.76 mg O_2/L/24h in summer 2008 followed by 2.13 and 2.26 mg O_2/L/24h in spring 2008) (Fig. 7).

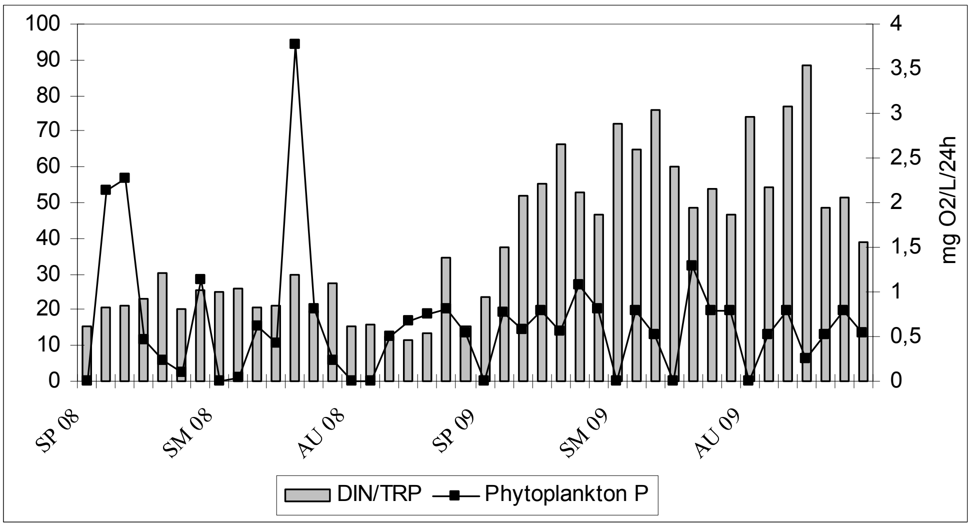

Fig. 7. The spatial and temporal variation of the phytoplankton primary production and the DIN/TRP ratio in the Sfântu Gheorghe branch (SP – spring; SM – summer; AU – autumn).

The simple correlation between rotifer production and DIN/TRP ratio showed an inverse relationship between these two parameters (N = 42, R = -0.42, P = 0.006). It can be noticed an inverse tendency of variation, the rotifers production increasing with lower DIN/TRP ratio (Fig. 8). It is known that the low values of this ratio indicate a tendency of nitrogen to become the limiting factor of phyto-plankton development, which causes changes in the taxonomic composition. Because they are able to fix atmospheric nitrogen, Cyanobacteria develop despite Chlorophyceae group, the favorite food source of rotifers. They are forced to switch to other trophic resources such as detrito-bacterial aggregates. In the Sfântu Gheorghe branch, the phytoplankton biomass values are reduced and the detritus amount is high. Thus, it appears that rotifer production is carried out mainly due to detrito-bacterial aggregates development.

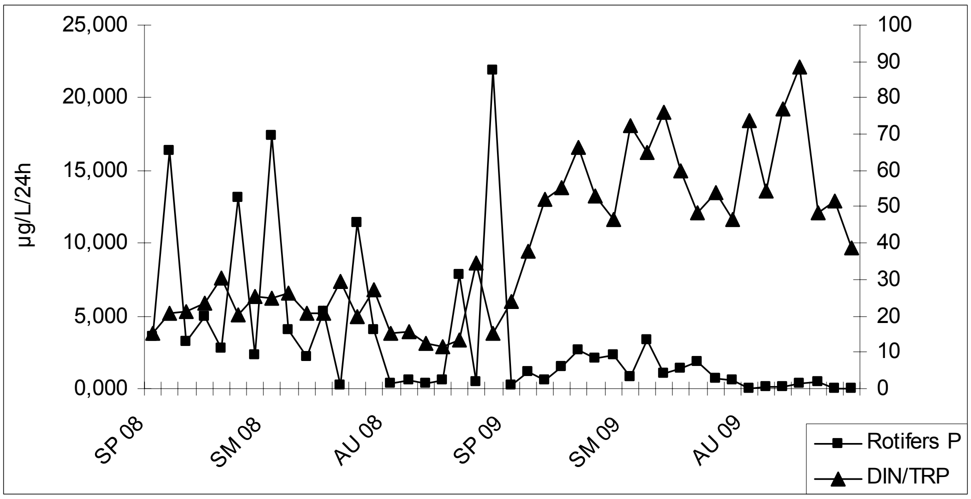

Fig. 8. The spatial and temporal variation of rotifer production and DIN/TRP ratio in the Sfântu Gheorghe branch (SP – spring; SM – summer; AU – autumn).

The productivity or production rate is defined by the speed that production is achieved in the ecosystem. Starting from biomass (B) and productivity (P) and reporting to time and space we get two indices: P/B and B/P (Battes *et al.*, 2003). In order to see the influence of physical-chemical factors on rotifer productivity expressed by these indices, a correlation matrix was performed.

Based on this matrix it has been established the temperature and oxygen concentration influenced the seasonal dynamics of productivity. Statistically significant results were obtained for the index B/P (Table 3). The water temperature and O_2 content are recognized as important factors in the dynamics of rotifer communities (Bogaert & Dumont, 1989).

Table 3

The statistical significance of the influence of temperature and O_2 on rotifers productivity

Slope	Slope	Intercept	R^2	Mean	F	DFn	DFd	P	Significance level
Temp.	-0.922	21.4075	0.119	3.547	8.252	1	61	0.005	**
O_2	0.257	6.80252	0.063	3.547	4.102	1	61	0.047	*

However, in addition to direct effects of temperature and oxygen, the effects of these variables on rotifer fluctuations could be mediated via other biotic and abiotic factors.

CONCLUSIONS

In the three years of sampling, the production showed a gradual decrease from spring to autumn, the highest values were in spring (7.00 µg/L/24h) and summer (4.24 mg/L/24h, annual average) and in autumn (1.00 µg/L/24h).

The interactions among the rotifer production and physical-chemical parameters showed that the distribution of rotifers production is closely related to O_2, dissolved inorganic nitrogen (DIN), total phosphorus (TP), organic phosphorus (P_{org}), total organic carbon (TOC) and nitrites (NO_2).

In the Sfântu Gheorghe branch, the values of DIN/TRP ratio are generally maintained above the threshold of 10, the phytoplankton production was not significantly influenced by this ratio, remaining almost constant over the studied period.

Also, it can be noticed an inverse tendency of variation, the rotifers production increasing with lower DIN/TRP ratio, showing an inverse relationship between these two parameters ($N = 42$, $R = -0.42$, $P = 0.006$).

Through their feeding and metabolism, rotifers by their grazing influenced the algal development. There were found significant correlations between primary production and secondary production of the rotifers ($R^2 = 0.079$, $R = 0.28$, $P < 0.05$).

Acknowledgements. Our study was part of the project named "The impact of hydraulic works on the ecological systems of Sfântu Gheorghe branch, in the context of sustainable development". The study was funded by project no. RO1567-IBB02/2010 from the Institute of Biology Bucharest of Romanian Academy. We thank Victor Zinevici, PhD, for the taxonomical revision of rotifers and Stela Sofa for technical support.

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FISH SPECIES OF COMMUNITY INTEREST IN THE CIUC BASIN (TRANSYLVANIA, ROMANIA) AND THEIR DISTRIBUTION AREA BASED ON THE LATEST SURVEYS

IMECS ISTVÁN*, NAGY ANDRÁS ATTILA**, DEMETER LÁSZLÓ***, UJVÁRI KRISZTIÁN-ROBERT****

In the Ciuc Basin (Transylvania, Romania) only two Natura 2000 sites were designated based on fish species (among other species): *Cottus gobio* for ROSCI0007 and *Cottus gobio* and *Eudontomyzon danfordi* for ROSCI0323. Our study aimed to present the distribution within and next to the existing Natura 2000 sites (in the Ciuc Basin) of community interest fish species based on the most recently available data and to identify the best umbrella species out of these for the local ichthyofauna. We found that in the last four years seven communities of interest fish species were identified in the Ciuc Basin: *Eudontomyzon danfordi, Aspius aspius, Barbus petenyi, Misgurnus fossilis, Cobitis elongatoides, Sabanejewia aurata* and *Cottus gobio*. Their distribution showed that *Cottus gobio* is the most widespread in the Basin and it can act as the best umbrella species during conservation measures for other fish species. As a result we can say that due to the lack of proper information few fish species were included in the Standard Data Forms of the existing SCIs which does not cover properly the distribution area of community interest fish species.

Key words: Natura 2000, community interest fish species, umbrella species, distribution area, Ciuc Basin.

INTRODUCTION

The aim of the Natura 2000 Network is to protect vulnerable habitats and species across their natural range in Europe and to ensure that they are maintained in a favorable conservation status. Two types of areas are included in the Natura 2000 network: Special Protection Areas (SPA – areas with significant numbers of wild birds and their habitats) designated by the Member States under the Birds Directive (79/409/EEC), and Special Areas of Conservation (SAC – rare, endangered or vulnerable natural habitats and species of plants or animals, others than birds), designating first Sites of Community Interest (SCI) under the Habitats Directive (92/43/EEC) and then turned them into SACs (Caluşeru *et al*., 2011; Cioacă, 2012).

The Directives on Habitats and Birds were introduced into Romanian legislation under Emergency Government Ordinance no. 57 of 2007 on the regime of protected natural areas, conservation of wild fauna and flora, natural habitats, and later, with amendments, under Law No. 49/2011. In Romania, with its highly valuable biodiversity, 557 Natura 2000 sites were registered, classified into 149 SPAs and 408 SCIs, covering all the 5 bioregions in the country: continental, alpine, pannonic, pontic and steppic. Romania is the most biogeographically diverse member state of the EU (Bănăduc, 2007; Cioacă, 2012).

In the Ciuc Basin (Transylvania, Romania) two Natura 2000 sites were designated based on fish species (among other species): the Lower Ciuc Basin Natura 2000 site (Bazinul Ciucului de Jos ROSCI0007), with an area of 2693 ha, proposed and designated in 2007 on the basis of the existing specific literature, and the Ciuc Mountains (Munții Ciucului ROSCI0323 – only covers a part of the Basin), with an area of 59641 ha, proposed and declared in 2011 mainly on the basis of the latest surveys in the region (Csergő *et al*., 2011). Only two fish species were included in the Standard Data Forms of these SCIs: *Cottus gobio* for ROSCI0007 and *Cottus gobio* and *Eudontomyzon danfordi* for ROSCI0323 (Annex II, Habitats Directive 92/43/EEC).

It is important to know that Natura 2000 sites do not exclude human presence or investments but certain activities with a significant negative impact on the species or types of habitats for which the site has been designated must be restricted or stopped (Cioacă, 2012). Every investment needs environmental authorization, and in every case the Environmental Protection Agency must know first if the investment is planned in one of the Natura 2000 sites or not. If it is, they examine the impact of the investment on the species and habitats, and if it turns to be significant then the appropriate assessment procedures will start for the investors. These procedures can result the rejection of the authorization, but it can result also the implementation of alternative solutions for the investment which has no significant impact on the species or habitats (Groza *et al.*, 2011).

On this basis we can say that if there is an investment that affects the natural water system in the Ciuc Basin within these two designated Natura 2000 sites, there are two fish species in ROSCI0323 and one in ROSCI0007 through which it is possible to regulate these investments. But this attempt is successful only if these species are widespread in this region and if so, they can act as umbrella species for the local ichthyofauna (Bănăduc, 2007; Moreno-Opo, 2011). Umbrella species are species whose habitat needs large areas or multiple habitat types and it can encompass the habitat requirements of many other species (Noon & Dale, 2002). It is important in landscape ecology that umbrella species are used to undertake broad conservation based around the habitat needs of a single species, thus allowing whole ecosystems to be conserved under the umbrella of one species (Hunter & Gibbs, 2007). Umbrella species habitats affected by implementing projects will have potential effects on the integrity of the whole Natura 2000 system (Machar, 2010).

The low number of fish species that were included in the Standard Data Forms of these two sites is due to the lack of data about their distribution when the proposals were submitted 2-6 years ago. The goals of this paper are: 1) – to present the distribution within and next to the existing Natura 2000 sites (in the Ciuc Basin) of priority fish species based on the most recent available data; 2) – to identify the best umbrella species out of these.

MATERIAL AND METHODS

The study area

The Ciuc Basin is in the middle part of the eastern Carpathian Mountains with approximately 60 km length and 30 km width. The region is a distinct inter-Carpathian Basin with separate tectonic and geomorphic units (Kristó, 2002) and it is included entirely in the Alpine Bioregion (Tatole *et al*., 2008; Căluşeru *et al*., 2011). The Basin is divided into 3 sub-basins: the Upper Ciuc Basin, the Middle Ciuc Basin and the Lower Ciuc Basin (Jánosi, 2002). The streams, which are flowing down from the East Mountains (Ciuc Mountains) and the West Mountains (Harghita Mountains), arrive in the Olt River (river that takes its source in the Basin) and that makes a closed hydrography for the Olt River in the Ciuc Basin (Kristó, 2002).

Data collection

We have little information about the fish fauna of the Basin before 21st century. The first data is from Vitos (1894) from the end of the19th century when 12 fish species were described, data collected mostly from fishermen. 70 years later Bănărescu (1964) elaborated the fish fauna volume of the country where 15 species were mentioned from the Basin. At the end of the 70-80’s the floodplain of the Basin was entirely drained and the course of the Olt River regulated totally, as well as all the streams that entered the floodplain, which negatively influenced the flora and the fauna (Demeter, 2002). Bănăduc (1999) made an ichthyological survey on the Olt River which included 3 sampling stations in the Ciuc Basin resulting only 6 species.

These data did not prove relevant for the Natura 2000 system, because they were old and meanwhile the floodplain was reconstructed too. So we had to rely on the analysis of the latest ichthyological surveys (Imecs & Újvári, 2009; Imecs *et al.*, 2011; Imecs & Újvári, 2012; Imecs *et al*., *in manuscript*) and evaluate them. These surveys were conducted exclusively in the Ciuc Basin. Maps were created using the existing data of the community interest fish species within the Ciuc Basin to evaluate the role of their presence in the SCIs and further conservation importance of their distribution.

RESULTS AND DISCUSSION

During the last four years (2008-2012) data were collected from 122 sampling stations using a standard fish collecting method (Imecs & Újvári, 2009; Imecs *et al*., 2011; Imecs & Újvári, 2012; Imecs *et al*., in manuscript).

The majority of these stations were in the Lower Ciuc Basin, because the floodplain on this section is expanded, with a lot of drainage channels, some backwaters and small ponds, unlike the northern part (Upper Ciuc Basin), which is much narrower and is dominated by small streams (Fig. 1).

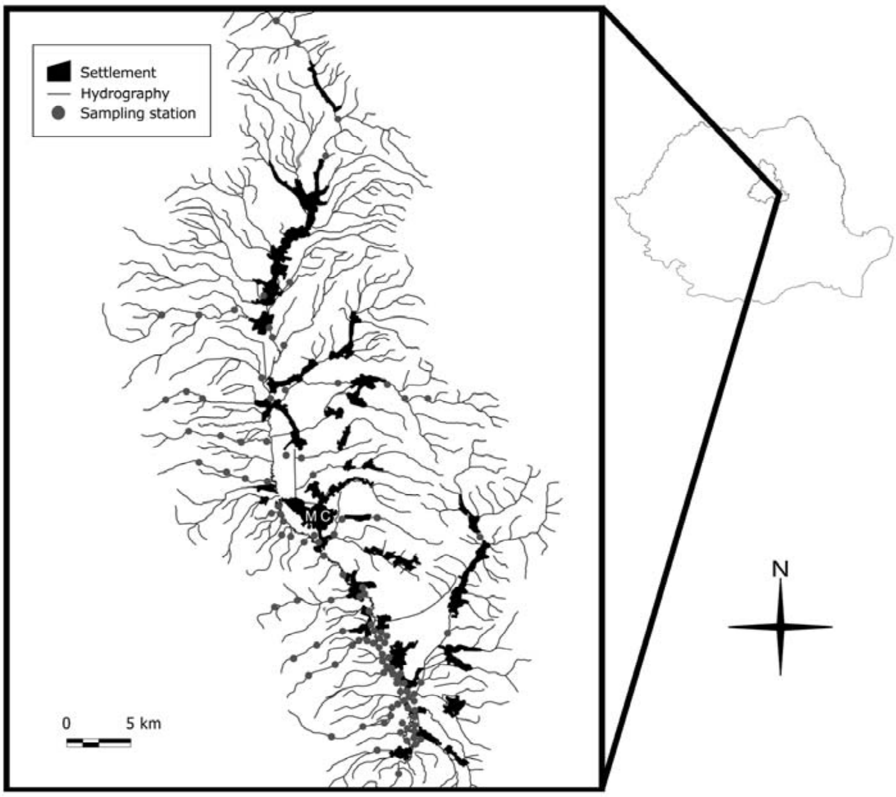

Fig. 1. Sampling stations from the last four years in the Ciuc Basin (Imecs *et al*., in manuscript) (MC – Miercurea Ciuc city).

Thirty fish species were recorded from the natural water system of the Ciuc Basin, among which there are six species captured only with fishing rod by fishermen, and not during the collections (Imecs *et al*., in manuscript). The following community interest Natura 2000 fish species were recorded from the Ciuc Basin during the last four years:

1. *Eudontomyzon danfordi* (code: 4123, formerly code: 9903) (cyclostome).

The species lives only in the upper stretch of small rivers and in mountainous streams, requiring cold and clean water. The main threatening factor is the habitat destruction and pollution (Pintér, 2002). It is on the Standard Form of the ROSCI0323, but it was not described from any water body covered by the

ROSCI0323 in the Ciuc Basin. The species appeared only in 3 streams, mainly from the territory of the ROSCI0007 (Imecs & Újvári, 2012; Imecs *et al*., in manuscript) (Fig. 2). This does not mean that the species is not present in the ROSCI0323, especially outside the Ciuc Basin, but it means that its distribution area is small in the Ciuc Basin. The conservation of this species will depend on the management of the Lower Ciuc Basin Natura 2000 site streams that hosts the *Eudontomyzon danfordi*.

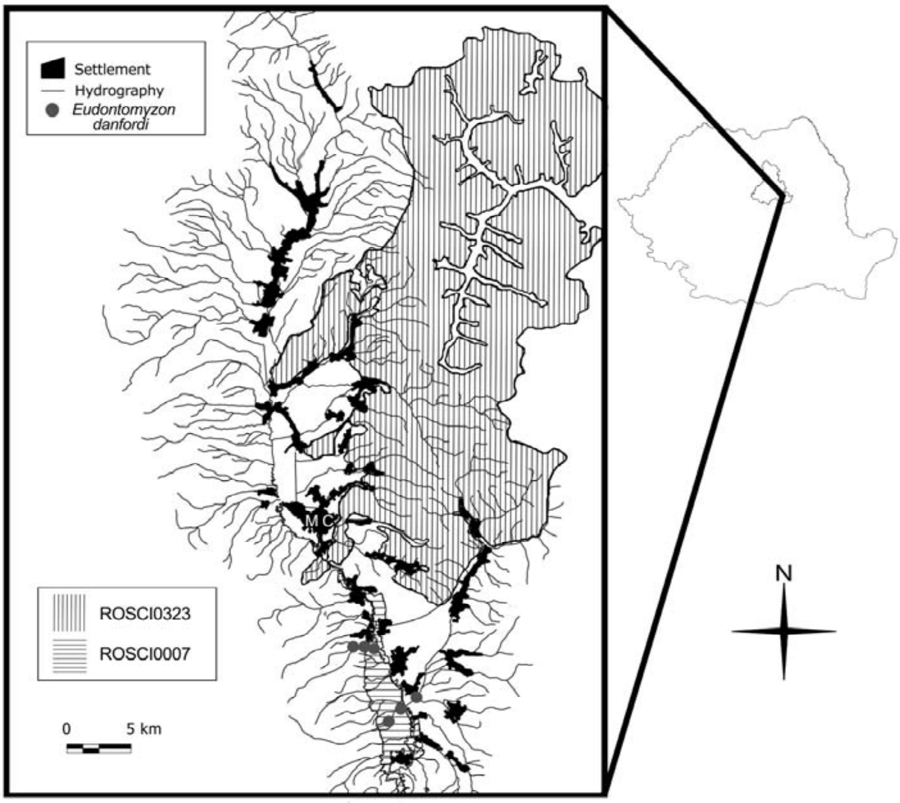

Fig. 2. The distribution area of *Eudontomyzon danfordi* in the Ciuc Basin.

2. *Aspius aspius* (code: 1130)

The species lives in the lowland up to hilly big rivers, also in big freshwater or brackish ponds and lakes. It is a day light active predator fish, which is threatened by poaching, damming and pollution (Bănărescu, 1964; Bănărescu & Bănăduc, 2007). *Aspius aspius* is not listed for Romania in the Reference List for the Alpine Region and as a result it will be protected in the Alpine region (including the Ciuc Basin), only if it falls under the geographic “umbrella” of other species of community interest. In the Ciuc Basin its distribution area is limited to a single location, where it was caught by anglers but it was not found during our surveys (Imecs *et al*., in manuscript) (Fig. 3). The total distribution area of this species in the Ciuc Basin and its conservation is uncertain.

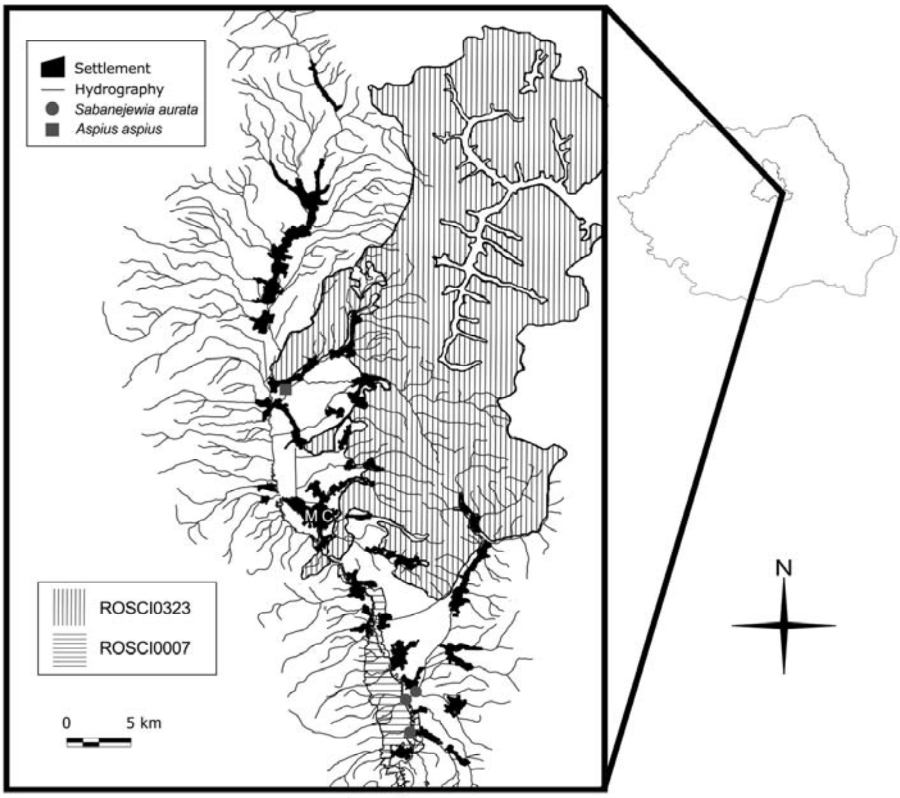

Fig. 3. The distribution area of *Aspius aspius* in the Ciuc Basin.

3. *Barbus (meridionalis) petenyi* (code: 1138)

It is a benthopelagic freshwater fish, which is found in mountainous and hilly rivers, with springs in this area. It prefers clear and fast flowing water sectors with hard substrate. The main threatening factors are pollution, habitat destruction and water takeout (Bănărescu, 1964; Bănărescu & Bănăduc, 2007). The species was introduced under the name of *Barbus meridionalis* in the Habitats Directive, but after that its scientific name was clarified, as being *Barbus petenyi* (Tsigenopoulos *et al*., 1999; Tsigenopoulos & Berrebi, 2000; Machordom & Doadrio, 2001; Kotlík *et al*., 2002). In the Ciuc Basin its distribution covers the Olt River and the lower sections of bigger streams (Fig. 4). ROSCI0007 is well represented by this species, but the ROSCI0323 is less, because the site covers mainly smaller streams in higher zones. The species is relatively widely distributed in the Ciuc Basin covering the low-lying flowing waters. It is on the Reference List for the Alpine Region of Romania, but it was not included in the Standard Form of the SCIs in the Ciuc Basin. The management of the ROSCI0007 can influence positively the conservation of this species in the Lower Ciuc Basin, maintaining its habitat requirements.

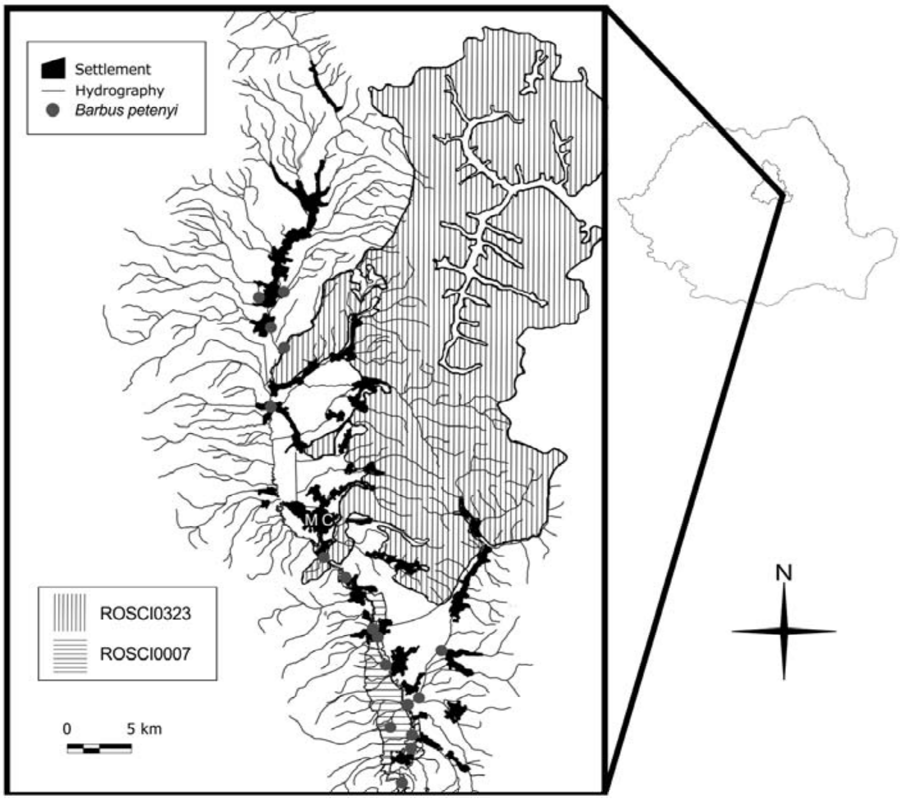

Fig. 4. The distribution area of *Barbus petenyi* in the Ciuc Basin.

4. *Misgurnus fossilis* (code: 1145)

Misgurnus fossilis is an inconspicuous limnophilic European species, whose distribution area spans from Spain to the Volga River (Meyer & Hindrichs, 2000; Pintér, 2002) but at the same time it is one of the most threatened fish species in the world (Hartvich *et al*., 2010). The IUCN Red List of Threatened Species classified this species in the Least Concern category, with a decreasing population trend (IUCN 2010). This species is included in the Red List in the Czech Republic (Lusk *et al*., 2004), Vulnerable status in Croatia (Mrakovčić *et al*., 2008) and Critically Endangered status in the Red List of Austria (Wolfram & Mikschi, 2007). In Romania the species is not included in the Red Book of Vertebrates (Bănărescu, 2005), but it is considered a vulnerable species with decreasing area and population size (Wilhelm, 2000). Its occurrence is linked to specific biotopes in floodplains of larger rivers (Meyer & Hinrichs, 2000), but the river regulations lead to a significant decrease in these original natural biotopes (Mendel *et al*., 2008). In the Ciuc Basin first it was described by Orbán (1869), an ethnographer, who describes it as the most abundant fish species of the marshy floodplain of the Olt River, later Vitos (1894) mentions it as well. From the 20th century we have no information of the presence of this species (Bănărescu, 1964; Bănăduc, 1999). During the last four years the species was detected mainly from the floodplain of the Olt River in the Lower Ciuc Basin. The species was found in drainage channels, regulated stream sections, but also in the Olt River (Imecs & Újvári, 2009; Imecs *et al*., 2011; Imecs & Újvári, 2012; Imecs *et al*., in manuscript) (Fig. 5). Its distribution area is concentrated to the Lower Ciuc Basin which covers the ROSCI0007 territory therefore the management of this site will determine the conservation of this species. In spite of the fact that it is not on the Reference List for the Alpine Region of Romania, specific conservation measures are indicated for this species, as habitat rehabilitation (Demény *et al*., 2009).

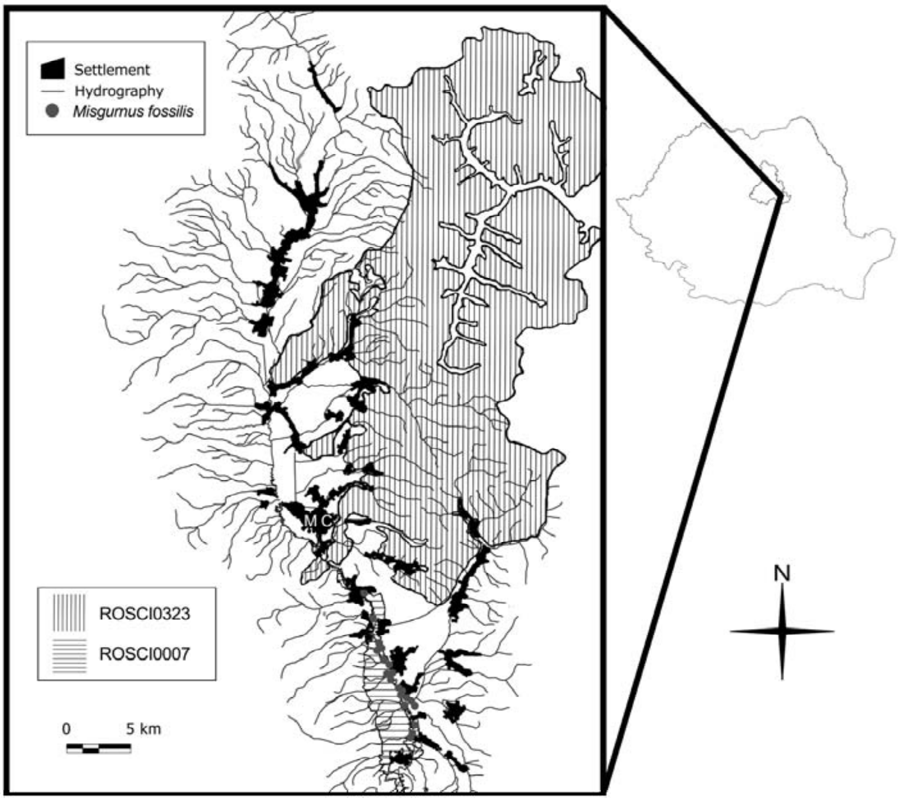

Fig. 5. The distribution area of *Misgurnus fossilis* in the Ciuc Basin.

5. *Cobitis (taenia) elongatoides* (code: 1149)

This species was introduced under the name of *Cobitis taenia* in the Habitats Directive, but after genetic studies it has been clarified that only *Cobitis elongatoides* lives in the Danube water system (Culling *et al*., 2006). *Cobitis elongatoides* lives in slow-flowing and standing waters, with sand or clay substrate, rarely on stony substrate. The main threatening factors are pollution and habitat destruction (Bănărescu, 1964; Bănărescu & Bănăduc, 2007). The species is not on the Reference List for the Alpine Region of Romania, but it is present on an acceptable level in the Olt River and its floodplain in the Lower Ciuc Basin (including totally the ROSCI0007) and less in the Upper Ciuc Basin. There were almost no data from the ROSCI0323 territory (Imecs & Újvári, 2009; Imecs *et al*., 2011; Imecs & Újvári, 2012; Imecs *et al*., in manuscript) (Fig. 6). The species needs management measures to preserve its habitat, eliminating the pollution sources and habitat destruction.

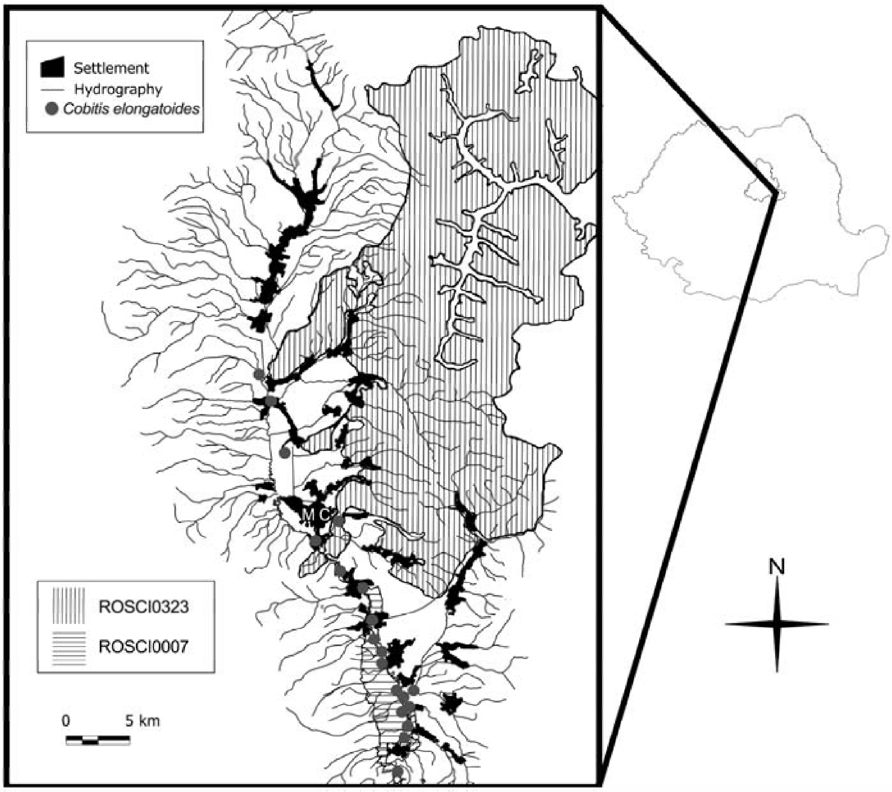

Fig. 6. The distribution area of *Cobitis elongatoides* in the Ciuc Basin.

6. *Sabanejewia aurata* (code: 1146)

It is a freshwater and bottom-living fish, appearing usually in the upper and middle parts of the rivers. The presence of sand in the river bed is an important habitat condition, the individuals stand a long time in the sand. This species is threatened by habitat destruction and pollution (Bănărescu, 1964; Bănărescu & Bănăduc, 2007). *Sabanejewia aurata* is listed in the Reference List for the Alpine Region of Romania, but it was not included in the Standard Data Forms of

ROSCI0007 and ROSCI0323. In the Ciuc Basin the species has a very small distribution area (Fig. 3), near the ROSCI0007. Increased attention is recommended for this species conservation in the Lower Ciuc Basin, maintaining its habitat.

7. *Cottus gobio* (code: 1163)

It lives exclusively in cold, mountain freshwater streams, very rarely in lakes. Usually it stays hidden under the rocks taking advantage of its camouflage colors. *Cottus gobio* is threatened by pollution and habitat destruction (Bănărescu, 1964; Bănărescu & Bănăduc, 2007). The species was included in the Standard Data Forms of ROSCI0007 and ROSCI0323. The *Cottus gobio* has the widest distribution area of all the community interest species from the Ciuc Basin, being present in almost all the examined streams and in the Olt River. Its distribution area covers ROSCI0007 and an important area from ROSCI0323 (from the Ciuc Basin part of the site) (Fig. 7). Its wide distribution area and the presence of numerous unexamined streams which satisfies the habitat requirements of the species, suggests that the *Cottus gobio* could be an umbrella species for the local ichthyofauna in the Ciuc Basin, including the other fish species of community interest. Its wide distribution also suggests that the current area of the two SCIs in the Ciuc Basin does not cover the distribution area of this species in an appropriate level.

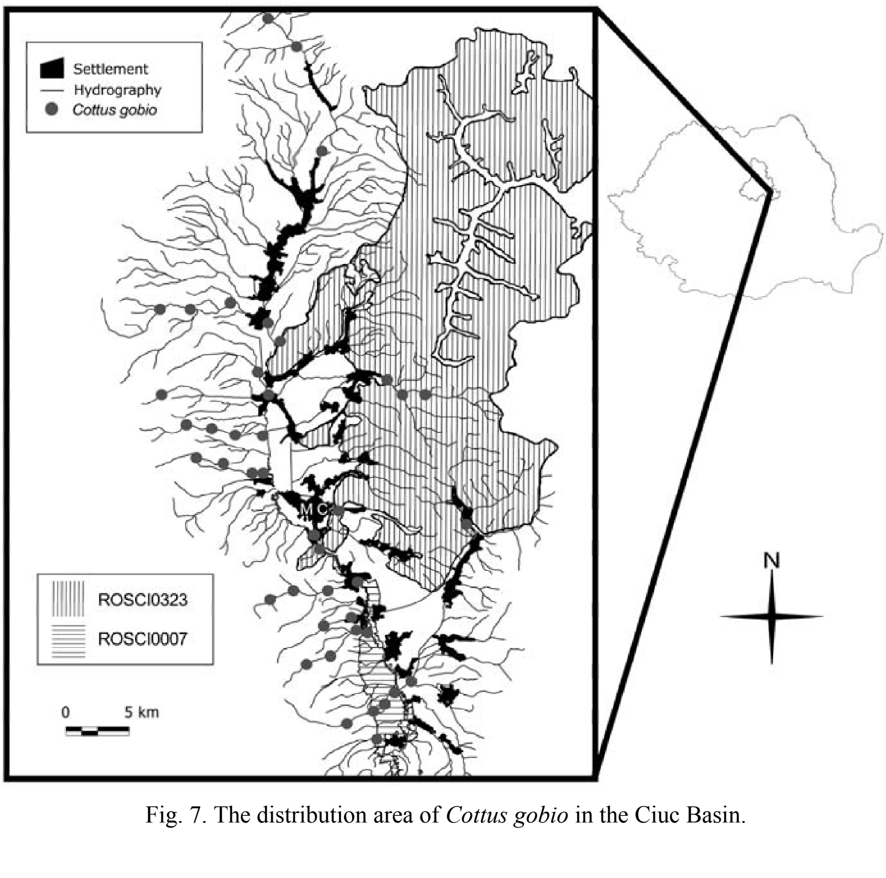

Fig. 7. The distribution area of *Cottus gobio* in the Ciuc Basin.

CONCLUSIONS

Seven species of community interest were described during the latest surveys from the Ciuc Basin (Imecs & Újvári, 2009; Imecs *et al*., 2011; Imecs & Újvári, 2012; Imecs *et al*., in manuscript). From these, four species were included in the Reference List for the Alpine Region (Ref List 2010) of Romania and only for two of them there were declared Natura 2000 sites in the Ciuc Basin (*Eudontomyzon danfordi* and *Cottus gobio* for ROSCI0323 – part of the Basin and *Cottus gobio* for ROSCI0007).

Due to the lack of proper information about the presence of the other two listed species (*Barbus petenyi* and *Sabanejewia aurata*), they were not included in the Standard Forms of any SCI in the Basin. For further management measures it is needed for community interest species to be umbrella species too because broad conservation measures are based around the habitat needs of a single species, thus allowing whole ecosystems to be conserved under the umbrella of one species (Hunter & Gibbs, 2007).

In the Ciuc Basin, *Cottus gobio* is the most widespread fish species of community interest (Fig. 7), and it can be found in the Standard Data Forms of both SCIs that are designated in the Basin. The distribution area of *Cottus gobio* just partially overlaps with the distribution area of *Misgurnus fossilis* in the ROSCI0007 (Fig. 5), so this latter species needs alternative management measures, under the "umbrella" of other species or habitats.

There are two main ways through which the Natura 2000 mechanisms can enhance the conservation of these species: the enlargement of the SCIs surface, and the elaboration and implementation of scientifically based management plans for these SCIs. The enlargement of these SCIs areas is less likely, so the accent will fall on the procedures and methods through which management plans will be elaborated. The management measures for these species should allow the human socio-economic system to use resources and services provided by ecological systems, but the maintaining of the favorable conservation status of the species, which is the primary management objective, cannot be compromised under any circumstances (Bănăduc, 2011).

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COMPARATIVE ANALYSIS OF NATURA 2000 SITES FOR PROTECTED BIRDS SPECIES FROM ROMANIA AND BULGARIA

DOINA CIOACĂ

As an argument to include Cernica Lake and Forest (Ilfov County) in the European Ecological Network Natura 2000, between 2006-2009, I made a comparative analysis with protected wild bird species from six Natura 2000 Sites from Romania (Suhaia, Iezer Călărași and Bistreț) and Bulgaria (Srebarna, Orsoya and Ribarnitsi Mechka). I made the comparative analysis as biological and socio-economic importance and the vulnerability of human pressure. All assessed sites have a great biological importance and a good socio-economic importance. The Bray-Curtis Similarity Dendrograme of the assessed Natura 2000 sites revealed about 80% similarity values Cernica – Srebarna and Iezer Călărași – Suhaia and Bistreț. Regarding the vulnerability degree of the human activities (fisheries and aquaculture, forestry, hunting, poaching, small industry, trade and services, transport and household specific activities) high value to human pressure registered for Cernica and Suhaia and less pressure for Srebarna, Orsoya and Iezer Călărași.

Key words: Natura 2000, Birds Directive, ROSPA0122 Cernica Lake and Forest, Romania, Bulgaria.

INTRODUCTION

Like an obligation to adherence to the European Union, Romania and Bulgaria had to create new protected areas on their territories for Natura 2000 Network extension. Romania has a total number of 530 Natura 2000 Sites and Bulgaria covers 349 Natura 2000 Sites.

To obtain Natura 2000 status for Cernica area I accomplished an extensive scientific study during 2001 to 2010, and to search many valuable criteria to sustain this necessity, in 2006-2009, I made a comparative analysis between Cernica and other six Nature 2000 sites from Romania and Bulgaria.

The legal status for Cernica area was obtained in 2011 by Government Decision no. 971/2011, as part of the ecological network Natura 2000 in Romania. So, "The Natura 2000 ROSPA0122 Cernica Lake and Forest" site was proposed by myself in 2006, revised and accepted in 2010 and it was published in The Official Journal of Romania no.715 from 11.10.2011.

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MATERIAL AND METHODS

This comparative analysis is based on an own innovation (Estimating the Efficiency of Management Planning in Natura 2000 Sites), created in 2007, as part of the WWF Germany project (Cioacă, 2007). This method is adapted from the Rapid Assessment and Prioritization for Protected Areas Management (RAPPAM Method of WWF International) and it can be used for any Natura 2000 site, even if it has or not a legal administrator, but at the same time, it needs many information for the assessed sites.

Investigations methods were: visual observations with the naked eye or with binoculars, bird counts in flight, on the ground or on the water, counts and identification of certain bird and sheltering, breeding, feeding or wintering ranges.

To obtain the necessary data for the comparative analysis between the seven Natura 2000 Sites I have used a questionnaire adapted to these types of protected areas and I also made a series of field studies in both countries. The questionnaire contains 9 points (themes), three of them referring to the biological importance, the socio-economic importance and the vulnerability of human pressure. For each of the questions assign points as follows: 5 = yes, 3 = mostly yes, 1 = mostly no, 0 = no, blank = no answer. The sum of the questions can be a number between 0 and 45 (Ervin, 2002). Minimum value can be zero, but maximum value can be 40 for the biological importance, and 45 for the socio-economic point of view and for vulnerability of human activities pressure.

RESULTS AND DISCUSSION

In this paper there are presented the results of a comparative analysis with six Natura 2000 Sites from Romania and Bulgaria as an argument to designate “The Natura 2000 ROSCI0308 Cernica Lake and Forest” site. The investigated Natura 2000 sites were: Cernica Lake and Forest, Bistreț, Iezer-Călăraşi and Suhaia Lake from Romania and Orsoya, Srebarna and Ribarnitsi Mechka from Bulgaria (Table 1).

Table 1

The investigated Natura 2000 Sites

No.	Site name	Site code	Surface (ha)	County / District	Country
1	Orsoya Fishpond	BG0000182	2949.4	Vidin	Bulgaria
2	Bistreț	ROSPA0010	1916	Dolj	Romania
3	Suhaia Lake	ROSPA0102	4473	Teleorman	Romania
4	Ribarnitsi Mechka	BG0002024	2582.4	Ruse	Bulgaria
5	Srebarna	BG0000241	1448.2	Silistra	Bulgaria
6	Iezerul Călărași	ROSPA0051	5001	Călărași	Romania
7	Cernica Lake and Forest	ROSPA0122	3744	Ilfov	Romania

I chose these sites according to several criteria of selection:

1. The legal protection is an important criterion for identification. All sites have been proposed as Special Protection Area (SPA) under the Birds Directive and those have been accepted by the European Commission (in 2011 for Cernica Lake and Forest and in 2007 for the other Natura 2000 sites). Except Cernica, all Sites are Important Birds Areas (IBAs) from BirdLife International criteria. Also, Srebarna from Bulgaria, Iezer-Călăraşi, Suhaia and Bistreț from Romania are Ramsar sites (International Importance Wetlands, especially for aquatic wild birds). In those sites there could be found many wild bird species protected by the international (Bern Convention, Bonn Convention, Agreement on the Conservation of African-Eurasian Migratory Waterbirds – AEWA) and by national legislation from both countries, especially wild birds which are dependent on the deciduous forests or wetlands areas.

2. All sites have a high wild bird biodiversity. Many are migratory, with seasonal migration (some in summer, some in winter, others stop during passage), and some are non-migratory (resident or sedentary) (Cioacă, 2012).

3. The location of these natural protected areas was another criterion. Excepting Cernica Lake and Forest which is located near Bucharest (Ilfov county), all sites are located on the Danube river shore.

4. The types of habitats from these Natura 2000 sites are *important criteria for comparing these sites.* In all selected Natura 2000 sites are natural and seminatural habitats represented by: lakes, temporary and permanent wetlands, riparian and meadow woods, rivers, marshes, peats and agrosystems and floodplain forests. Except for Cernica, the rest of Natura 2000 sites have floodplains areas from the Danube river shore.

5. All sites have large areas (over 1000 ha).

6. Five of the six Natura 2000 sites include some other protected areas, having in the past at least one experience in the management of these natural areas.

7. All sites are used for fishing.

8. All sites are near the residential areas.

The protected wild birds species that can be found in these seven Natura 2000 Sites are: *Ardeola ralloides, Ardea purpurea, Nycticorax nycticorax, Botaurus stellaris, Ixobrychus minutus, Egretta alba, E. garzetta, Podiceps cristatus, Ciconia alba, Aythya nyroca, Cygnus cygnus, Phalacrocorax pygmaeus, P. carbo, Himantopus himantopus, Philomachus pugnax, Vanellus vanellus, Rallus aquaticus, Larus minutus, L. ridibundus, Apus apus, Riparia riparia, Oriolus oriolus, Alcedo atthis, Merops apiaster, Sterna hirundo, S. albifrons, Chlidonias hybridus, C. niger*, *Acrocephalus scirpaceus, Circus aeruginosus, Accipiter brevipes, Upupa epops, Cuculus canorus, Dryocopus martius, Dendrocopos syriacus, D. major, D. minor, Picus canus, P. viridis, Caprimulgus europaeus, Athene noctua, Ficedula albicollis*.

The great and pygmy cormorants (*Phalacrocorax carbo* and *P. pygmaeus*) and some species of herons are found in small groups, nearby fishponds, and also in large colonies from Suhaia and Cernica in Romania, and Srebarna in Bulgaria.

For the Natura 2000 Sites mentioned in Table 2 stood a greater or lesser number of bird species protected by Annexe I of the Birds Directive (Fig. 1). The highest number of bird species listed in the Annexe I of Birds Directive is in Srebarna and Mechka. Between Bistreț, Suhaia and Iezer-Călăraşi there are not significant differences (30-37). The number of species recorded in Orsoya is less than half to Srebarna. Cernica appears with the lowest number of species, but this fact is not real, because in the field there are many more species of birds with different other types of legal protection. Thus, in Cernica I have inventoried a total number of 122 bird species, of which 27 are listed in the Annexe I of the Birds Directive. From these birds, only 10 species were required for the establishment in 2011 of "The Natura 2000 site ROSPA0122 Cernica Lake and Forest", and following the standard data form will be complete later.

The analysis aimed an assessment from the point of view of biological and socio-economic potential of these Natura 2000 sites and also to establish the level of vulnerability of those sites to human pressures.

Analysis of answers to questions referring to biological importance revealed that all seven assessed sites have a high biological importance. Higher value registered in Cernica, Orsoya, Suhaia and Iezer-Călăraşi (32 from maximum value 40), over those of Bistreț, Mechka and Srebarna (Fig. 2).

Table 2

The presence/absence of bird species listed to the Standard Data Forms of the assessed Natura 2000 Sites from Romania and Bulgaria

Species	**Cernica (Ro)**	**Orsoya (Bg)**	**Bistreț (Ro)**	**Suhaia (Ro)**	**Mechka (Bg)**	**Srebarna (Bg)**	**Iezer Călăraşi (Ro)**
Gavia arctica	1	0	0	0	0	1	0
Sterna hirundo	1	1	1	0	1	1	1
Phalacrocorax pygmaeus	1	1	1	1	1	1	1
Aythya nyroca	1	1	1	1	1	1	1
Nycticorax nycticorax	1	1	1	1	1	1	1
Coracias garrulous	1	1	1	1	0	1	0
Lanius collurio	1	1	1	1	1	1	0
Lanius minor	1	1	0	0	1	1	0
Ficedula albicollis	1	0	0	0	0	0	0
Dendrocopos syriacus	1	0	1	0	1	1	0

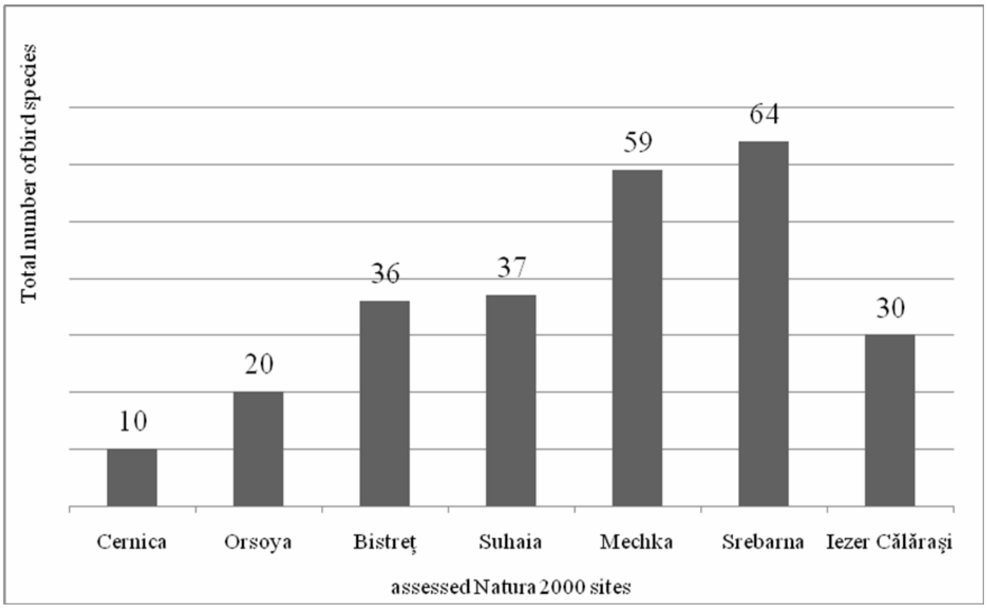

Fig. 1. The total number of protected species by Annex I of Birds Directive.

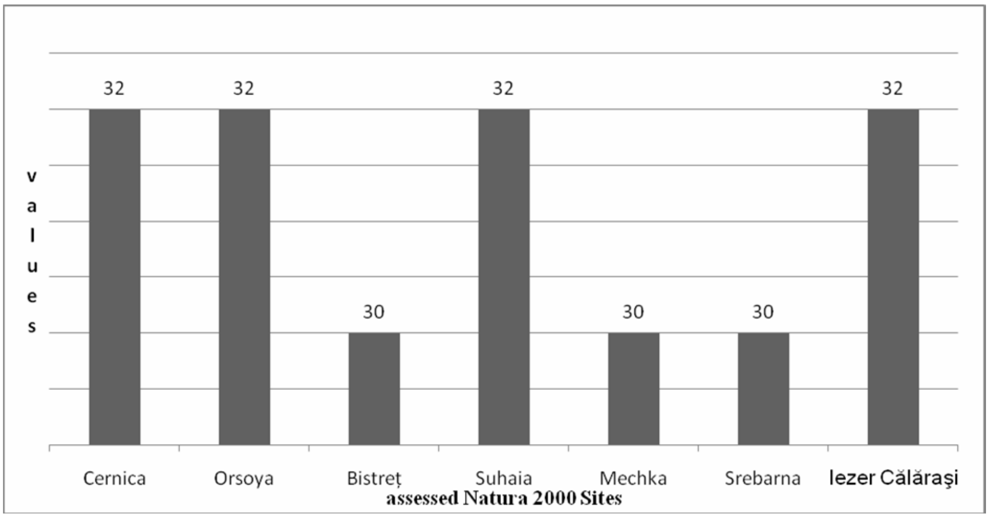

Fig. 2. The estimation of biological importance to Natura 2000 Sites from Romania and Bulgaria.

As an argument for the biological importance of Cernica area, I analyzed the relative presence/absence of 10 species of birds that were the subject of protection of "The Natura 2000 site ROSPA0122 Cernica Lake and Forest", species listed in the Annexe I of the Birds Directive (Table 2). *Gavia arctica* is found only in Srebarna (when one specimen is recorded) and in Cernica (when more than 4 individuals are recorded in the wintering passage) (Cioacă, 2005). *Ficedula albicollis* was found only in Cernica Site. In Orsoya, Bistreț and Mechka the same number of species (seven) was registered. In Iezer-Călăraşi and Bistreț, the number of bird species is the smallest, representing almost 50% of the species in Cernica.

Six sites have presented *Sterna hirundo* and *Lanius collurio,* five sites registered *Coracias garrulous* and four sites *Lanius minor* and *Dendrocopos syriacus*. A higher number of species was registered in Srebarna (nine), Orsoya, Bistreț and Mechka (seven), and a lower number in Suhaia (five) and Iezer-Călăraşi (four).

Phalacrocorax pygmaeus, *Aythya nyroca* and *Nycticorax nycticorax* are present in all seven Natura 2000 Sites. *Coracias garrulus* were recorded in all Natura 2000 Sites, except Mechka (Bulgaria) and Iezer-Călăraşi (Romania). *Lanius collurio* longer meets in all sites, except for Iezer-Călăraşi, and *Lanius minor* were recorded only in Bulgaria. *Dendrocopos syriacus* is present only in three sites (Bistreț, Mechka and Srebarna) and *Sterna hirundo* is present in all sites (excepting Suhaia).

The Bray-Curtis Similarity Dendrograme of the assessed Natura 2000 sites (Fig. 3) shows about 80% similarity values from Cernica with Srebarna (Bulgaria) and Iezer-Călăraşi with Suhaia and Bistreț (Romania).

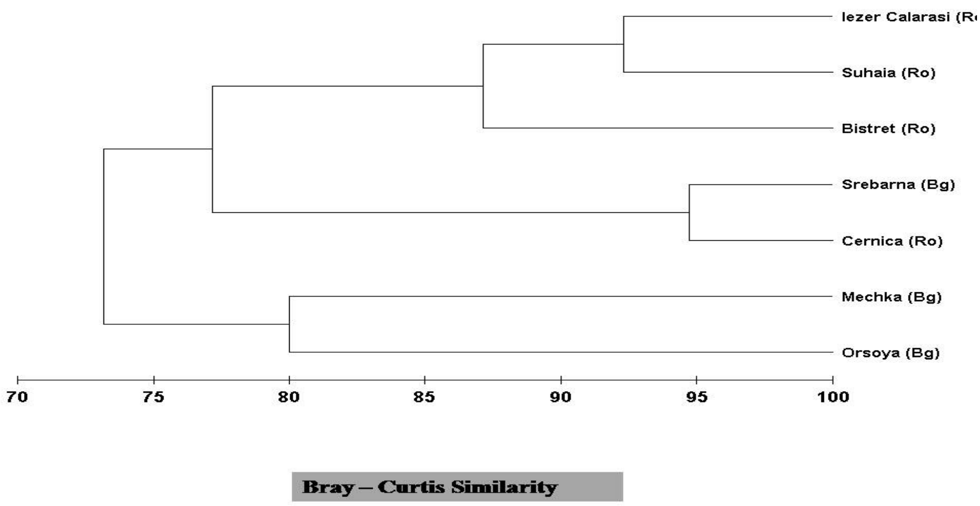

Fig. 3. The Bray-Curtis Similarity Dendrogram of the assessed Natura 2000 sites.

All the seven Natura 2000 Sites are valuable from the socio-economic point of view. Higher values were registered especially in Mechka (37 from maximum value 45), Orsoya, Suhaia Cernica and Bistreț (33-35 from 45), and lower values in Srebarna and Iezer-Călăraşi. Mechka has the highest value because it is located in an economically underdeveloped area and most of the time is used for subsistence (Fig. 4). As a result, all assessed sites have a good socio-economic importance.

Because all these seven Natura 2000 Sites are located nearby residential areas, I considered necessary to make a comparison regarding the degree of human activities vulnerability (Fig. 5). The most human activities in these protected areas are fisheries and aquaculture, forestry, hunting, poaching, small industry, trade and services, transport and household specific activities.

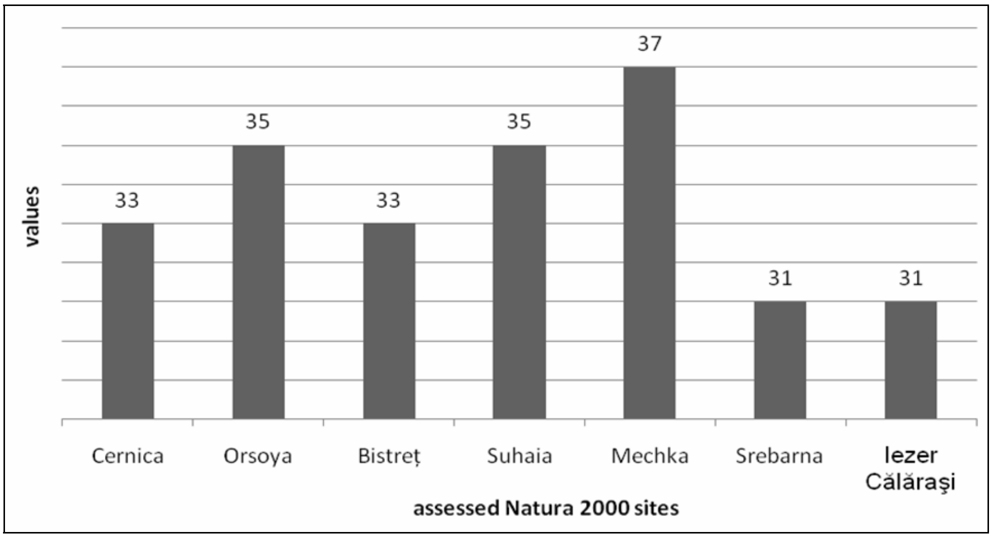

Fig. 4. The estimation of socio-economic importance of Natura 2000 Sites from Romania and Bulgaria.

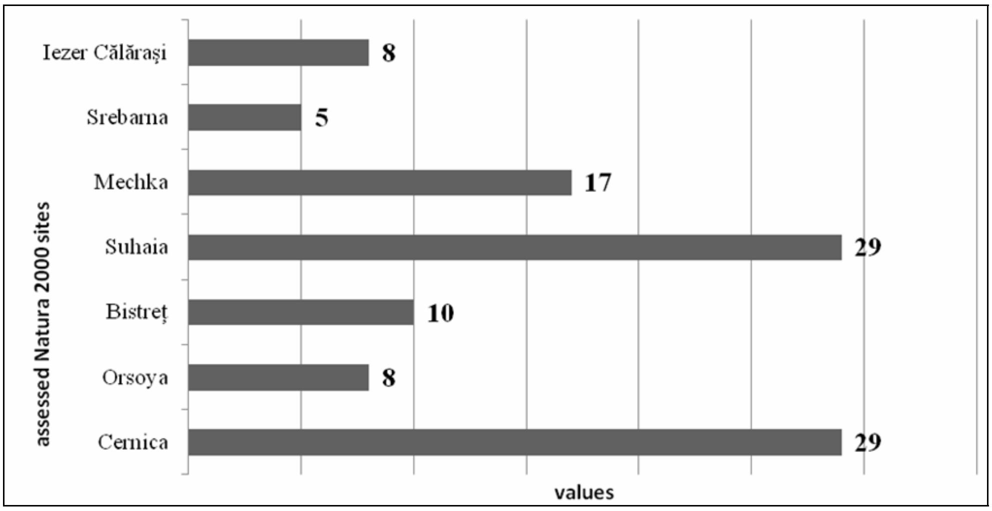

Fig. 5. The estimation of Natura 2000 Sites vulnerability.

Vulnerability level to human pressure appears low especially in Srebarna, Iezer-Călăraşi and Orsoya and higher in Cernica and Suhaia. High vulnerability in

Cernica is explained by the fact that Cernica 2000 site has been newly created in 2011 and in Suhaia because in this Natura 2000 site still remains a conflict between those who manage the natural resources of this area (Cioacă, 2007; 2008).

CONCLUSIONS

To bring additional arguments to designate Cernica area (Ilfov District) in the Natura 2000 Sites, I made a comparison with other six Natura 2000 sites from Romania and Bulgaria, and I demonstrated the similarity between Cernica and the top rated Natura 2000 site SPA's in the two countries (Iezer-Călăraşi and Srebarna).

The investigated Natura 2000 sites were: Cernica Lake and Forest, Bistreț, Iezer-Călăraşi and Suhaia Lake from Romania and Orsoya, Srebarna and Ribarnitsi Mechka from Bulgaria.

To select these Natura 2000 Sites, there were established eight criteria: the existence in zone of the Special Protection Area (SPA) under the Birds Directive and accepted by the European Commission; a high local biodiversity; the location (the majority along the Danube River); the same types of habitats; over 1000 ha surface in all cases; existence in areas of some other protected zones (in five of the six Natura 2000 sites); used for fishing; proximity with the residential zone for humans.

I made an assessment of biological and socio-economic importance to Natura 2000 Sites from Romania and Bulgaria and for the vulnerability of human pressures level.

The estimation of the biological importance showed that all sites have a high importance. Like a result of the comparative analysis between Natura 2000 Sites from the both states, I found a similitude in Cernica, Orsoya, Suhaia and Iezer-Călăraşi, with the same value.

The analysis of protected bird species presence/absence showed that three species, *Phalacrocorax pygmaeus*, *Aythya nyroca* and *Nycticorax nycticorax*, are present in all Natura 2000 Sites and *Ficedula albicollis* are present in Cernica only. Six sites have presented *Sterna hirundo* and *Lanius collurio,* five sites registered *Coracias garrulous* and four sites, *Lanius minor* and *Dendrocopos syriacus*. A higher number of species was registered in Srebarna, Orsoya, Bistreț and Mechka, and a lower number in Suhaia and Iezer-Călăraşi. The Bray-Curtis Dendrogram of similarity showed that Cernica has almost 80% similarity with Srebarna, which is rated among the best Natura 2000 sites along the Danube River.

The comparative analysis regarding to the socio-economic importance reflects a similitude between Natura 2000 assessed Sites, and the highest value registered in Mechka from Bulgaria.

The highest value of vulnerability to human pressures in the cases of Cernica and Suhaia can be explained by the fact that Cernica was newly created in 2011, and in Suhaia still remains a conflict between those who manage the natural resources of this area.

Based on an extensive scientific study during 2001-2010 and on these comparative analyses between 2006-2009, “The Natura 2000 ROSPA0122 Cernica Lake and Forest”, proposed by myself in 2006, was revised and accepted in 2010 and published in The Official Journal of Romania no. 715 from 11.10.2011 with Government Decision no. 971/2011, as part of the ecological network Natura 2000 in Romania (Cioacă, 2012).

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*** Legea nr. 49/2011 pentru aprobarea Ordonanţei de urgenţă a Guvernului nr. 57/2007 privind regimul ariilor naturale protejate, conservarea habitatelor naturale, a florei şi faunei sălbatice.

*** Directive 2009/147/EC of the European Parliament and of the Council on the conservation of wild birds (2009).

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*** http://ec.europa.eu/environment/nature/natura2000
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CELL-CELL AND CELL-EXTRACELLULAR MATRIX JUNCTIONS ALTERATION ARE PREREQUISITE FOR TUMOR PROGRESSION IN THE HUMAN BASAL CELL CARCINOMA

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FLORIN DANIEL JURAVLE[***], NICOLAE MIRANCEA[*]

The present study deals with the infrastructural changes concerning cell-cell (desmosomes) and cell-extracellular matrix (hemidesmosomes) junctions during basal cell carcinoma development. Transmission electron microscopic investigation of basal cell carcinoma tumors reveals severe alterations in both desmosomal and hemidesmosomal junctions. Desmosomes are missing for long profile cell-cell interface; mostly are impaired, and some are internalized so that large intercellular spaces are formed. Keratin filaments appear as large conglomerate inside of epithelial tumor cells. Hemidesmosomes are almost missing or defective. At the tumor-stroma interface, tumor cells shed a plethora of tumor microvesicles (oncosomes). Inside of peritumoral stroma, fibroblasts are mostly activated showing an epithelioid phenotype. Telocytes are reduced as number. Heterotypic junctions of telocytes with other stromal components as endothelial cells, nerves, mast cells are very limited.

Key words: basal cell carcinoma, desmosome, hemidesmosome, oncosome, telocyte.

INTRODUCTION

The majority of human cancers are carcinomas arising from epithelial cells that line glands, ducts, and surfaces of different organs. In order to become a malignant transformed cell, epithelial cell undergoes multiple and multistage specific alterations (Mueller & Fusenig, 2004).

The human skin is considered the largest organ of the body. Its outermost part, the epidermis – a multi-stratified epithelium, located at the body interface with the aggressive physical, chemical and biological environmental factors, functions as first defence line as well as barrier for water and other plasma components lost (Breitkreutz *et al*., 2009).

Like nervous cells, epidermis has an ectodermal origin and is considered a vital tissue. If large areas of the epidermis are damaged, and consequently, impaired in their function, then a life-threatening problem may occur. To function as a vital barrier, epidermis as a stratifying and keratinizing epithelium is constantly renewed by stem cells, mostly located in basal cell layer in contact with basement membrane (Watt & Jensen, 2009). Sun exposed areas of skin for long time to the aggressive effect of the UV radiation may induce DNA damage as a prerequisite to initiate a carcinogenic process. In humans, basal cell carcinoma is the most common form of skin cancer. This is related, in principal, to the fact that skin, the largest organ of the body, is directly exposed to the sun and carcinogenic chemicals.

Except for some cell types habilitated to move inside of the body (for example, blood cells), the rest of the cells develop stable cell-cell and cell-ECM relationships to behave in a cooperative fashion. In a healthy body, every cell is able to send and to receive various signals. Cancer is a disease in which some body's communication systems function abnormally.

Cell-cell and cell-ECM adhesions play a crucial role in epithelial morphogenesis regulation and homeostasia maintenance by coordinating cell positioning, cell polarization and consequently cell function *via* cell signalling (Mirancea & Mirancea, 2010). In this context, cell-to-cell and cell-ECM adhesions are very important in the maintenance of tissue architecture. The most important and widespread cell-cell junctions are represented by desmosomes, while hemidesmosomes attach epithelial cells to the high specialised component of ECM known as basement membrane. Desmosomes are very early detected during embryo development. They are formed during morula stage of the mouse and during very early stage of golden hamster (*Mesocricetus auratus*) embryo development (Mirancea & Mirancea, 2000). During postlesional regeneration, as well as during *in vitro* (organotypic coculture) new epithelia reconstruction requires new formed desmosomal junctions. Any impairment of desmosomes detected in some inborn or acquired diseases or in case of native or experimental null mutation in animals leads to tissue disruption, some of them being life-threatening or even incompatible with life. Moreover, some specific peptides may block cell-cell adhesions mediated by desmosomal glycolproteins. Hemidesmosomes are specialised infrastructures, coupling the epithelial cells to the basement membrane. Both hemidesmosomes and desmosomes serve as places where some cell signals are transmitted. The disruption of desmosomal adhesion and/or hemidesmosomal junctions might lead to tissue disorganization in diseases such as cancer (Runswick *et al*., 2001; Mirancea *et al*., 2010).

It is well documented that basement membrane (BM) integrity is a prerequisite for epithelia morphogenesis during embryo development. BM regulates epithelial-mesenchymal interactions. Epithelial cells facing basement membrane maintain their morpho-functional phenotype of polarised cell as long as the adjacent basement membrane remains unaltered in their molecular composition. Any mutation in their components and infrastructural alterations of basement membrane lead to a wide variety of clinical phenotypes (specific diseases), including invasive cell growth in case of cancer (Breitkreutz *et al*., 2009; Mirancea *et al*., 2010; Van Agmatel & Bruckner-Tuderman, 2010).

Taking into consideration that, beyond the classically known morpho-structural characteristics of basal cell carcinoma, from time to time, new examinations contribute with relevant data, we investigated by transmission electron microscopy the infrastructural alterations of tumor cell relationships, correlated with the peri-tumoral stroma status, especially ultrastructural aspects of the tumor-stroma interface and telocytes.

MATERIAL AND METHODS

Biological material

Small fragments of tumor skin resulted by surgical therapy from two patients suffering from skin cancer (the surgeon got patient consent) were processed for electron microscopic investigations.

Transmission Electron Microscopy

Specimens for electron microscopy were pre-fixed in 2.5-4% glutaraldehyde in 0.05 M sodium cacodylate buffer pH 7.4 at 4° C for minimum 2h and post fixed in 2.5% osmium tetroxide in 0.1 M cacodylate buffer for 2h at room temperature. The specimens were then over night stained in block with aqueous 0.5% uranyl-acetate and dehydrated in a graded series ethanol, then infiltrated with propyleneoxide followed by embedding in Glycidether 100 (Epon 812 equivalent). 1 μm semithin sections were stained with toluidine blue for light microscopic examination. Ultrathin sections of 80-90 nm were obtained with an ultramicrotome equipped with a diamond knife, double counterstained with uranyl acetate and lead citrate, and investigated in an electron microscope operated at 80 kV.

RESULTS AND DISCUSSION

In both cases investigated, a large tumor of basal cell carcinoma type was developed. Tumor epithelium is represented by non-layered cells showing large nuclei. Tumor stroma limits have a convoluted contour. A very fibrotic stroma comes in direct contact with the tumor (Fig. 1).

Almost all tumor cells have large nuclei occupied by large and small blocks of heterochromatin and euchromatin. Inside of cytoplasm, keratin filaments appear as large conglomerates (Fig. 2). Very seldom, in case of the tumor cells affronted to the stroma, from the keratin filaments conglomerate few keratin rods are connected to the impaired hemidesmosomes.

For very long distances, below the plasma membrane of the tumor cell, large amounts of amorphous material participate to a redundant basal lamina formation. Moreover, numerous trafficking membrane vesicles (some of them attached to the tumor cell plasma membrane) can be seen (Figs. 3-4).

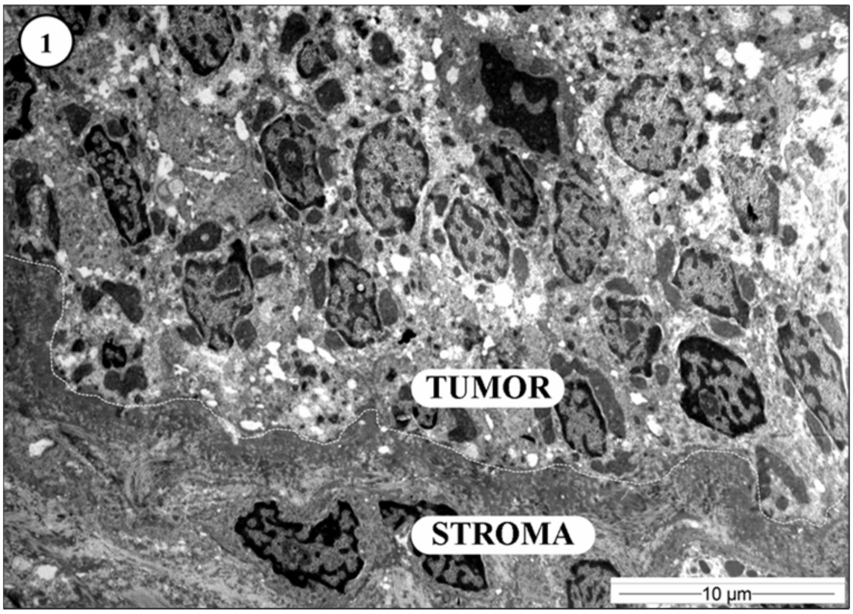

Fig. 1. Overview showing tumor epithelium with nonlayered cells. Dotted line marks the limit between the tumor and peritumoral stroma represented by fibrotic tissue and connective cells with epithelioid aspect.

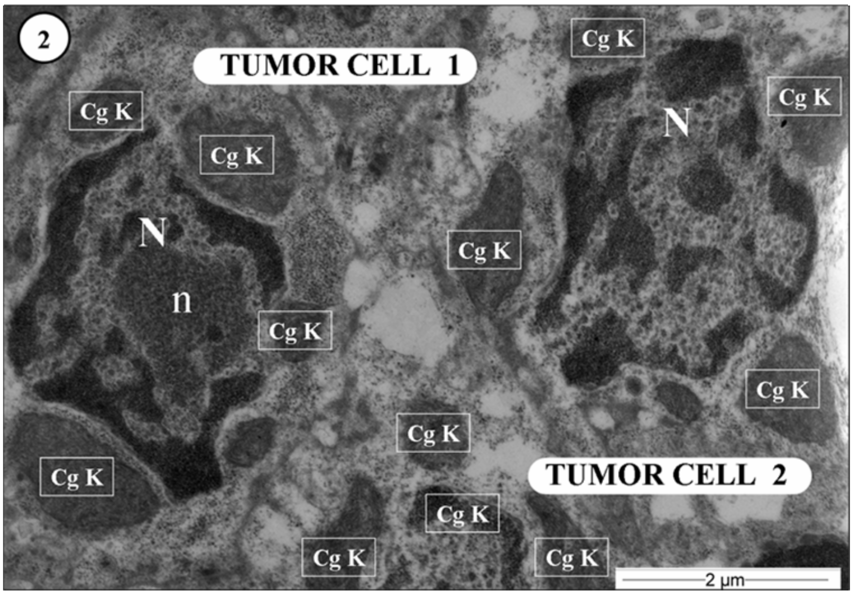

Fig. 2. Two tumor cells with large nuclei (N) occupied by large and small blocks of heterochromatin and euchromatin. Inside of cytoplasm, keratin filaments appear as large conglomerates (Cg K). n = nucleolus

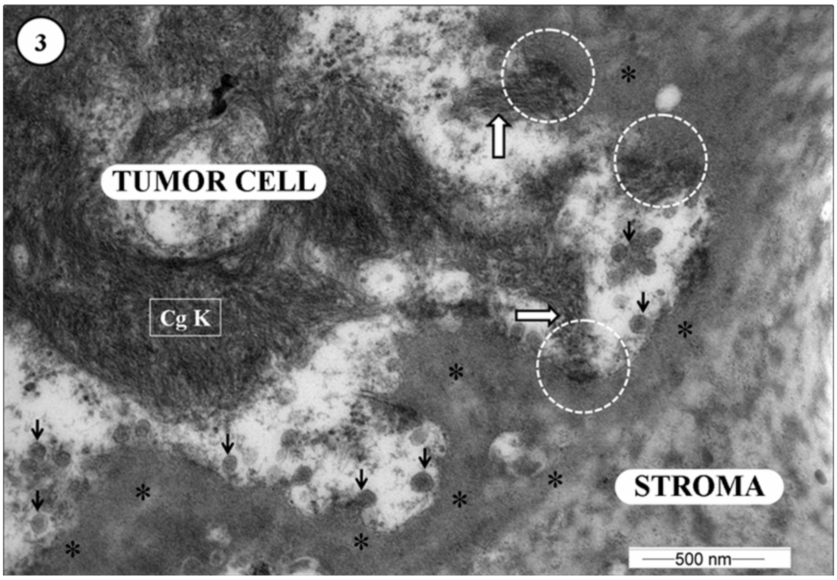

Fig. 3. Sector of a tumor cell affronted by a fibrotic tumor stroma. From a keratin filaments conglomerate (Cg K) few keratin rods (large white arrows) are connected to the impaired hemidesmosomes (encircled areas). Below the plasma membrane of the tumor cell, large amounts of amorphous material participate to a redundant basal lamina formation (asterisks). Small black arrows mark numerous trafficking membrane vesicles (some of them are attached to the tumor cell plasma membrane).

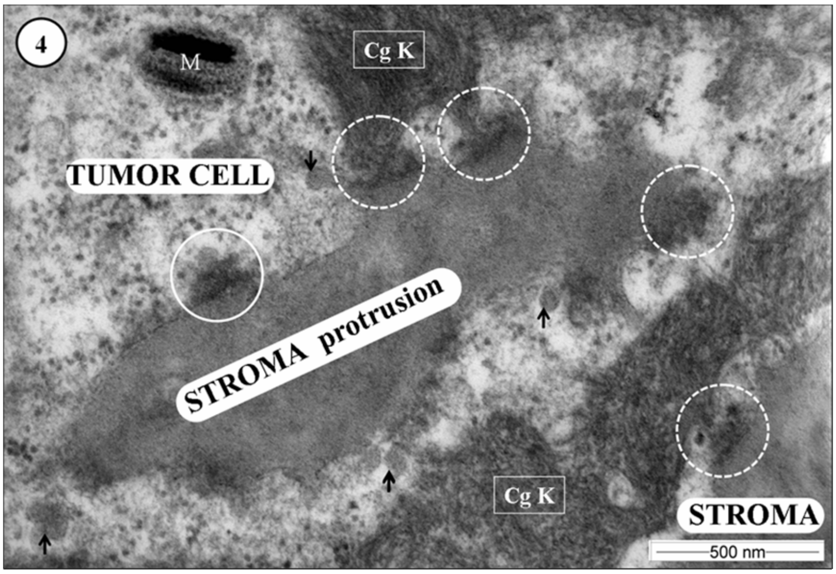

Fig. 4. A stromal protrusion penetrates inside of a tumor cell. At this level, as well as in the rest of the tumor cell contact with the adjacent stroma, from the conglomerate keratin (Cg K) strands of keratin filaments connect some precarious hemidesmosomes (encircled areas). Small black arrows mark the isolated or clustered membrane vesicles. M = melanosome.

Sometimes, also for long distances, the limit between tumor cells and adjacent stroma appears linear, but no basement membrane can be detected. Moreover, hemidesmosomal junctions are totally missing and consequently tonofilaments do not reach the basal pole of plasma membrane (Fig. 5).

Because the basement membrane is missing, from place to place, tumor cell extensions known as invadopodia penetrate deeply inside of peritumoral stroma. Along the peripheral zone of invadopodium with microenvironment, including the tip of this, no hemidesmosomes and no basement membrane can be seen (Fig. 6).

When we analyzed the cell-cell junctions inside of tumor epithelia, in both cases we investigated, large intercellular spaces are visible. TEM investigations revealed that desmosomal junctions are severely damaged, so that cell-cell delimitation becomes illusive (Figs. 7-8).

It seems that huge conglomerates of tonofilaments trapped and internalized desmosomal junctions inside of the cytoplasm. Because of intercellular junctions alteration, including internalization of desmosomes, intercellular spaces become abnormally enlarged (Figs. 9-11).

Another remarkable infrastructural abnormality is related to the shedding membrane vesicles phenomenon which involved the plasma membrane of tumor cells facing the adjacent stroma (Fig. 12).

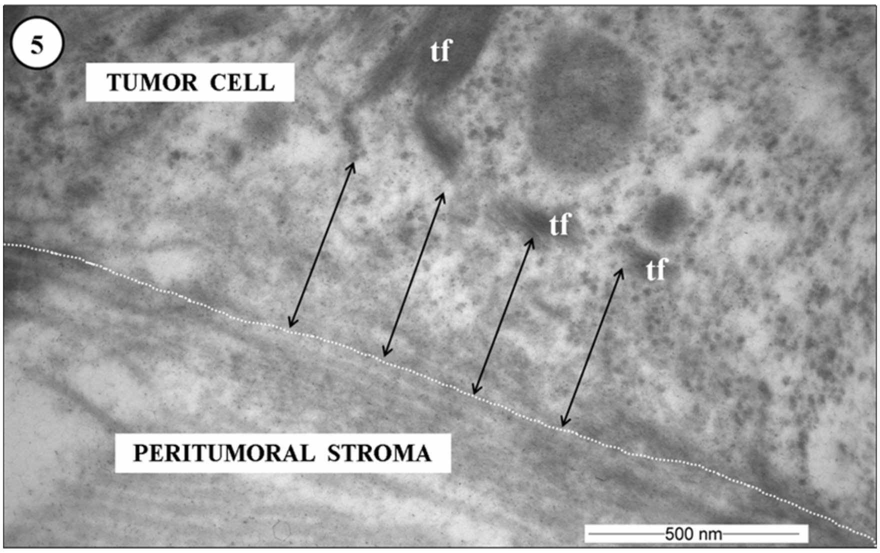

Fig. 5. Small sector of a malignant cell affronted to the peritumoral stroma. Dotted line marks the tumor cell-peritumoral stroma interface where no basement membrane can be detected. Hemidesmosomal junctions are totally missing. Tonofilaments (tf) do not reach the basal pole of plasma membrane so that a remarkable space (double headed arrows) separates the cytoskeleton from the basal profile.

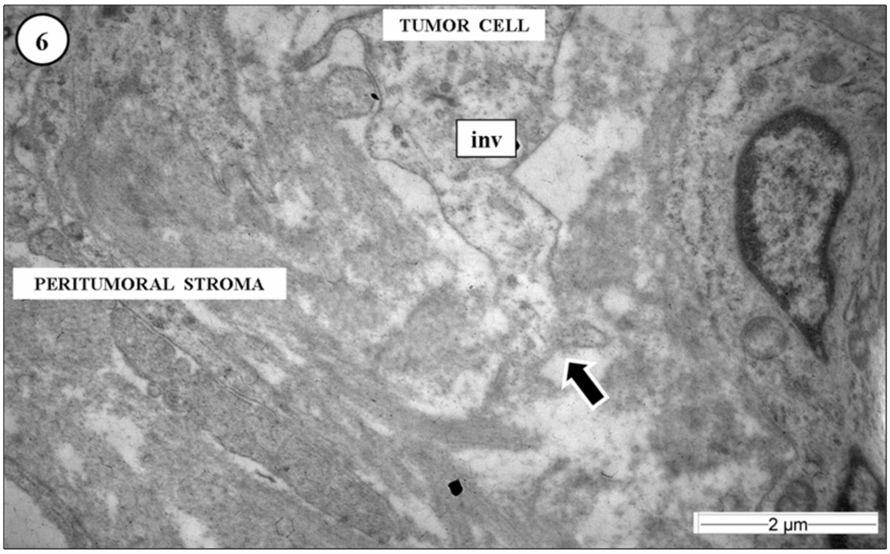

Fig. 6. A tumor invadopodium (inv) penetrates deeply inside of peritumoral stroma. Along the peripheral zone of invadopodium with microenvironment, including the tip (arrow) of this, no hemidesmosomes and no basement membrane can be seen.

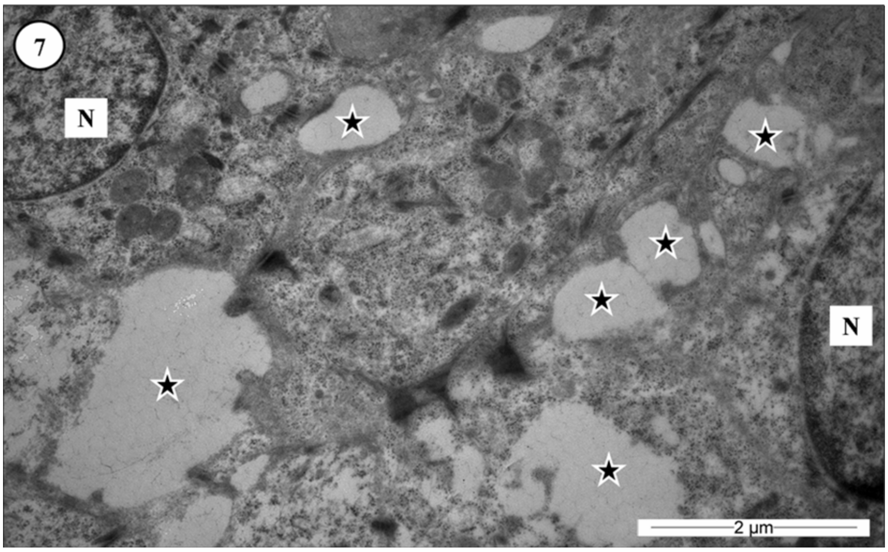

Fig. 7. Large intercellular spaces (stars) between epithelial cells of the tumor mass can be seen. N = nuclei.

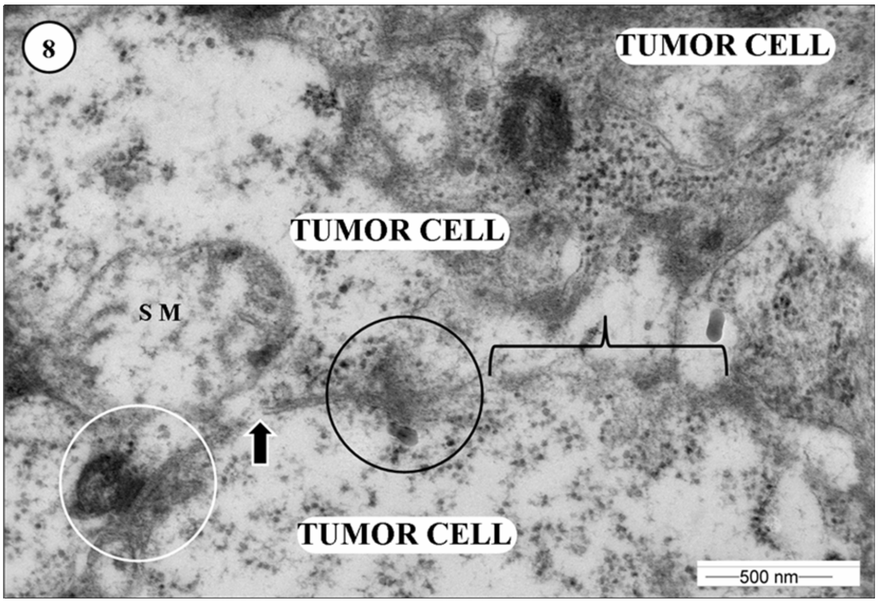

Fig. 8. The limits between tumor cells appear impaired: an altered desmosome (white circle area) is followed by interrupted plasma membranes (large arrow), an impaired desmosome (black circle area) and illusive cell-cell delimitation (accolade). SM = swollen mitochondria.

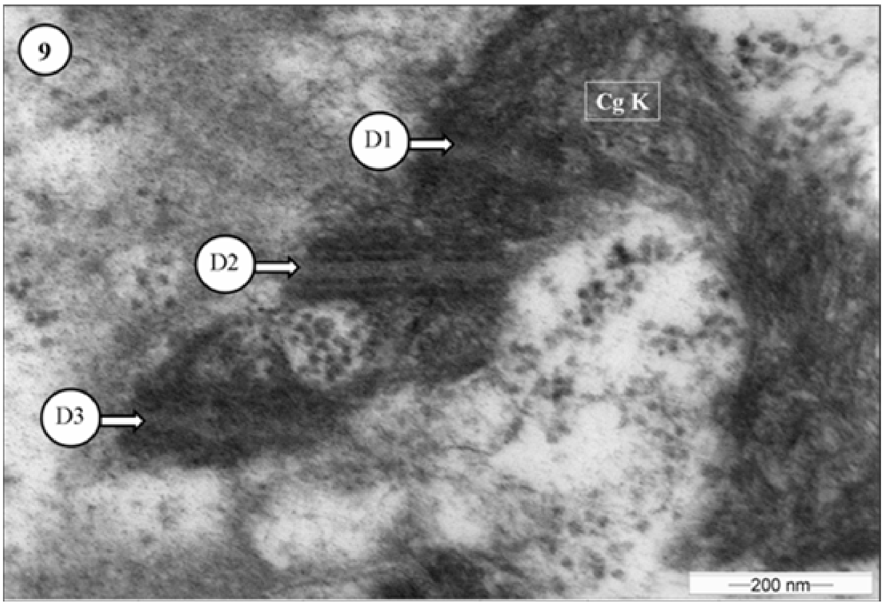

Fig. 9. A huge conglomerate of keratin filaments (Cg K) trapped the internalized desmosomes (D1-D3).

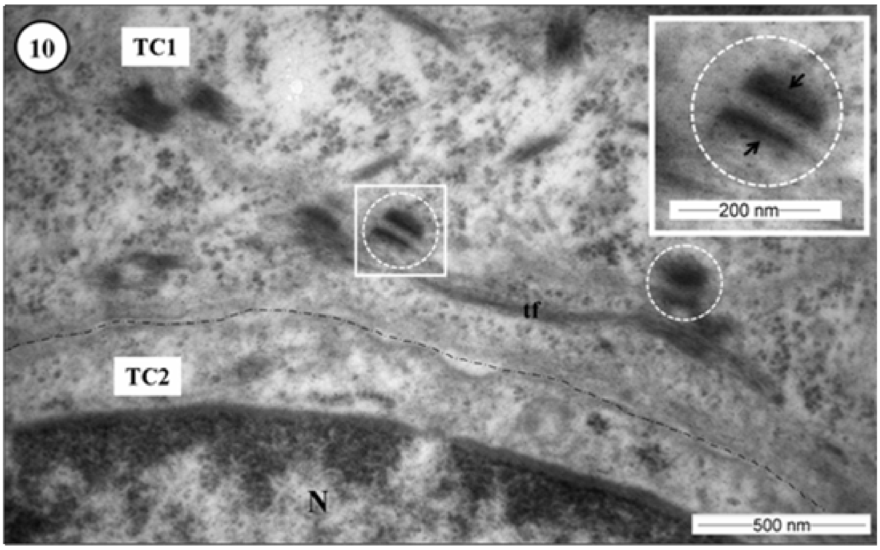

Fig. 10. No desmosomal junction can be detected between two tumoral cells (TC1 and TC2) for a long profile (dotted line). Instead, internalized desmosomes are visible (encircled areas). N = nucleus. tf = tonofilaments. Inset: larger view for a desmosome (delimited area by a white square in Fig. 10). Middle line is not visible but desmosomal plaques are well represented (arrows).

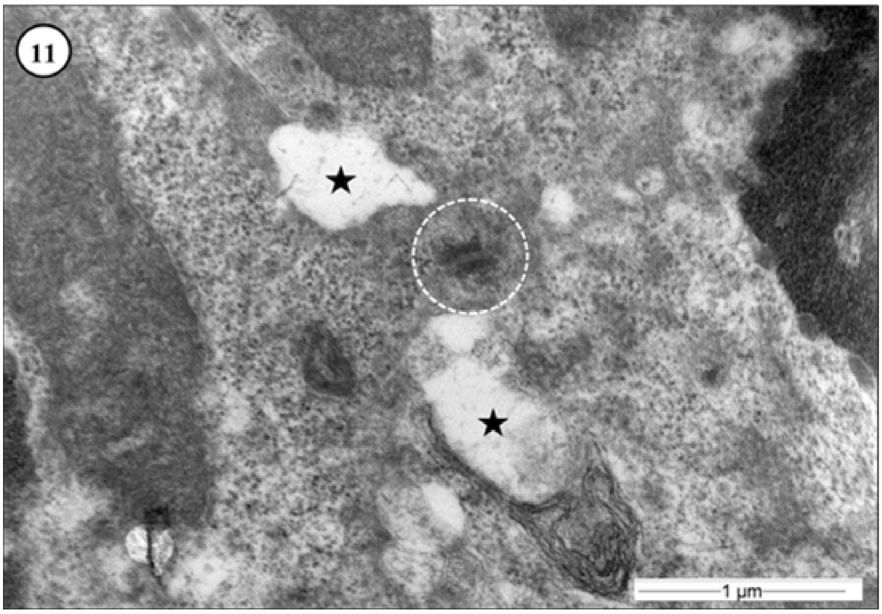

Fig. 11. Intercellular junction alteration: desmosomes are internalized (encircled area) and the intercellular spaces become abnormally enlarged (stars).

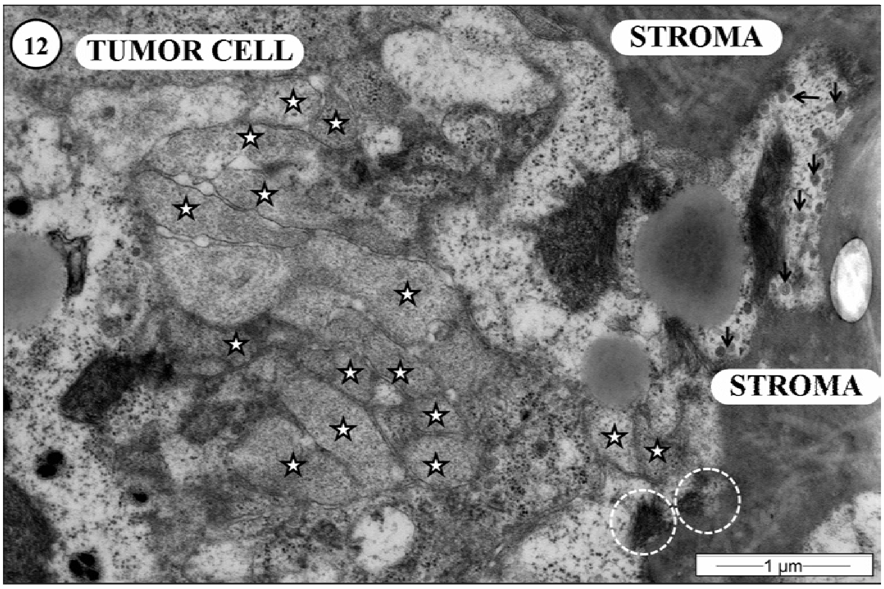

Fig. 12. Numerous shedded membrane vesicles (stars) are visible in close vicinity with peritumoral stroma. Small trafficking membrane vesicles (small arrows) are detectable.

Interesting here is that a basement membrane is still interposed between tumor cells and tumor stroma. Moreover, small trafficking membrane vesicles are detectable. The basement membrane is binding a variety of cytokines and growth factors, serving as a reservoir (Bix & Iozzo, 2005; Breitkreutz *et al.*, 2009). Under normal conditions, BM keeps in a balanced state the control of some cytokines and growth factors for tissue remodelling or repair processes after injury. During initiation and progression of a tumor, malignant cells make their accomplice the surrounding stromal cells and inflammatory cells release bioactive molecules, their amount being enhanced due to a vast BM destruction, as a part of the stroma activation (Mueller & Fusenig, 2004; Bix & Iozzo, 2005).

Concerning the ultrastructural aspect of the tumor stroma, mention must be made this appears as a reactive activated connective tissue. First we underline the presence of epithelioid phenotype of tumor stroma connective cells. Then, we emphasize the presence of a very fibrotic tissue (Fig. 1, Fig. 3 and Fig. 4). Moreover, (mostly occluded) small blood vessels are visible inside of tumor stroma, some of them becoming in very close contact with tumor cells (not shown). Different inflammatory extravasated cells are present inside of tumor stroma. Mastocytes and macrophages are well represented. Another stromal (interstitial) cell type we investigated refers to the telocyte. Telocytes are limited as number.

Inside of fibrotic tumor stroma they are mostly located following the contour of small blood vessels (Figs. 13-15).

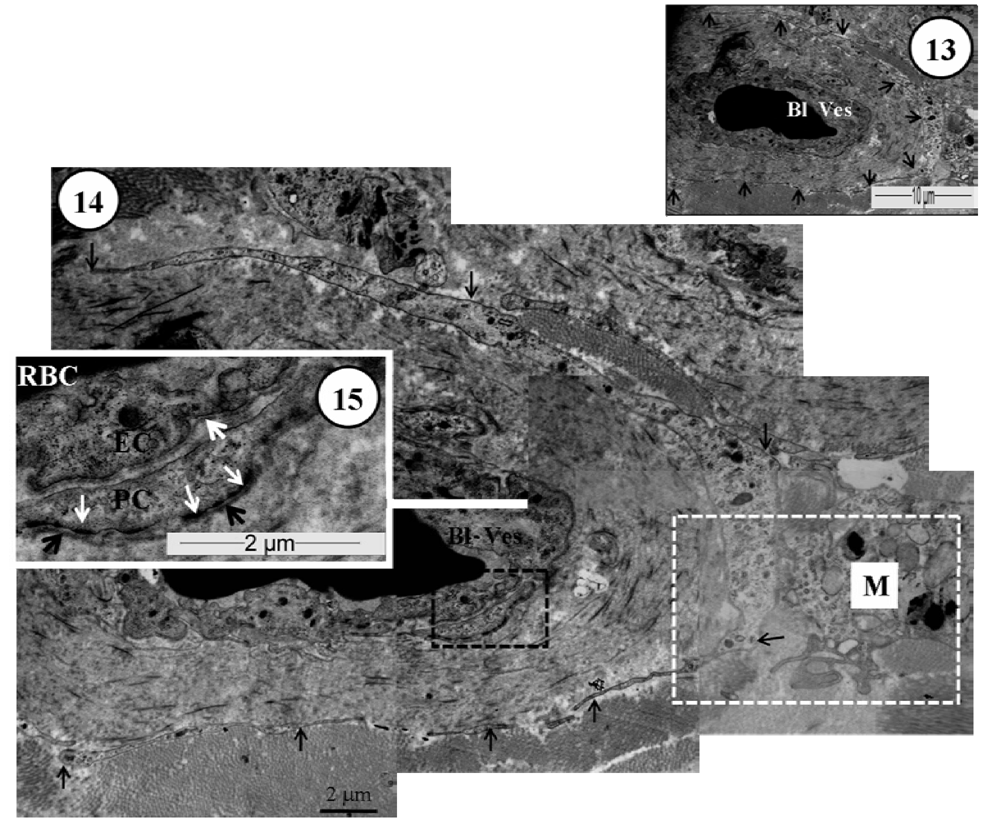

Fig. 13. Overview of a telocyte (small arrows) partially following the contour of a small blood vessel (Bl. Ves.), detailed in Fig. 14 and Fig. 15.

Fig. 14. A very long profile of a telocyte (arrows) represented by podomes and podomeres surrounding a rich fibrotic tissue which separates telocytes from a small blood vessel (Bl. Ves.). The framed area by a black rectangle is detailed in Fig. 15 and the framed area by white rectangle shows a macrophage (M) in close vicinity of telocyte, detailed in Fig. 16.

Fig. 15. A small sector from a capillary (see Fig. 13). Endothelial wall (EC) is surrounded by a basement membrane (white head arrow). The associated pericyte (PC) shows inner dense plaques (small white arrows) and a proper basement membrane (small black arrows).

Different from the normal skin, here a rich fibrotic tissue separates telocytes from the small blood vessel. Instead, telocytes can be visible in association with macrophages (Fig. 14, framed area by white rectangle and Fig. 16). Heterocellular contacts between telocyte and macrophage are depicted in Fig. 16.

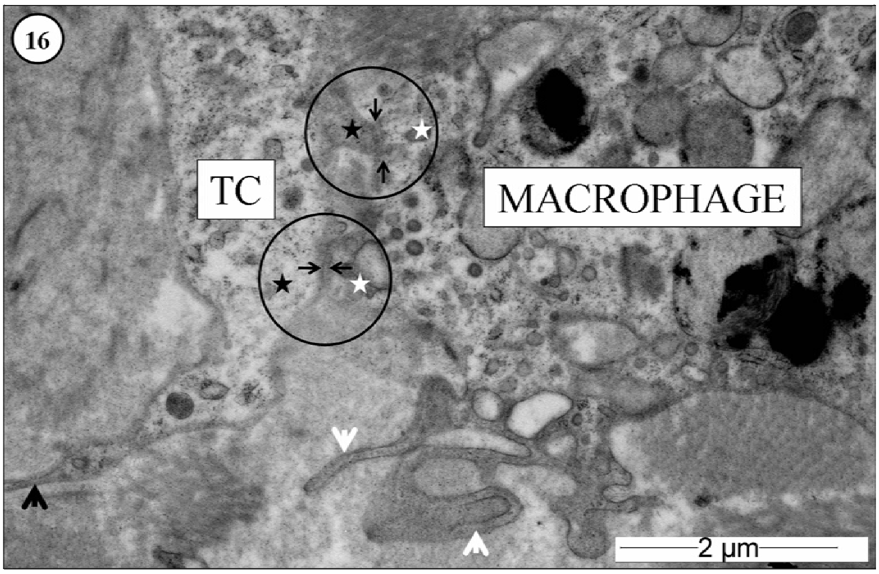

Fig. 16. Two intercellular contacts (encircled areas) established between the plasma membranes of a telocyte (TC) and a macrophage where some cytoplasmic areas of the podome (black stars) are affronted (small black arrows) to the cytoplasmic areas of the macrophage (white stars). Black head arrow indicates a podomer while the white head arrows indicate thin cell extensions of the macrophage involved in endocytosis. Black bodies inside of the macrophage are heterophagosomes.

In the normal human skin, intracellular keratin filaments, *via* desmosomal junctions, form a continuous intra-epidermal network and contribute to mechanical strength of the whole epithelia. In our investigated case, keratin filaments are severely affected: first, because the hemidesmosomes are defective, almost missing, the keratin filaments do not connect basal plasma membrane of tumor cells affronted to the tumor stroma (Fig. 5 and Fig. 6). Because desmosomal junctions are severely impaired or missing for a long profile between the adjacent epithelial tumor cells, tonofilaments formed conglomerates of keratin filaments (Figs. 3-11). While in normal epidermis both desmosomes and hemidesmosomes are important gates in cell signalling (Mueller & Fusenig, 2004; Helfand *et al.*, 2005; Breitkreutz *et al.*, 2009), in case of tumor cells exhibiting junctional abnormalities, cross-talk between adjacent cells and cells-ECM, respectively, is compromised.

In the current paper, we observed a lot of membrane vesicles produced by tumor cells and delivered at the interface with adjacent stroma (Fig. 12), but mention must be made that different from our above mentioned paper, here we have, even abnormal, still a basement membrane represented by a plenty of amorphous material. Moreover, a very fibrotic tissue made by reactive stroma separates the tumor from the stroma (Fig. 1 and Fig. 5). Despite the fact that tumors overexpress extracellular degrading proteases, some aggressive tumors make excessive basement membrane (Fig. 3) (Weaver *et al.*, 2002). Both normal and tumor cells shed membrane vesicles into their microenvironment (Dolo *et al*., 1995; Shedden *et al*., 2003; Mirancea & Mirancea, 2010; Mirancea *et al*., 2010; D'Souza-Schorey & Clancy, 2012). Malignant cells are known to shed tumor membrane vesicles (TMVs), also termed oncosomes, into their microenvironment both *in vitro* and *in vivo*. TMVs can range from 200 nm to a few micrometers in diameter (Mirancea & Mirancea, 2010; Mirancea *et al*., 2010; D'Souza-Schorey & Clancy, 2012). Membrane vesicles contain some enzymes which could be involved in tumor cell invasion inside of peritumoral stroma (Dolo *et al*., 1995; Mirancea *et al*., 2010).

Tumor-derived microvesicles are shed from the surface of tumor cells into the ECM. They represent a deposit of paracrine information, creating paths of least resistance by matrix degradation, facilitating tumor cell proliferation and survival. Tumor shedded microvesicles can be taken up by other cell types such as fibroblasts, endothelial cells (Shedden *et al*., 2003; D'Souza-Schorey & Clancy, 2012). TMVs represent a cargo for many bioactive molecules (proteins, DNA, RNA, micro-RNA) which may promote signaling responses in the target cells by (1) fusion of the TMVs with the target cell or (2) endocytosis of the TMVs (D'Souza-Schorey & Clancy, 2012). In a previous study, we reported about the tumor cell-stroma cell fusion by their plasma membranes (Mirancea *et al*., 2010). It seems that the tumor cell plasma membrane is fragile, prone to perform membrane recombination. Shedden *et al*. (2003) reported an accumulation of drug in membrane domains in which vesicles originate (a drug efflux mechanism potentially involved in drug resistance). Overexpression of genes associated with shed vesicles would correlate with anticancer drug resistance (Shedden *et al*., 2003; Muralidharan-Chari *et al*., 2010).

Dolo *et al*. (1995) reported that shed plasma membrane vesicles of human breast carcinoma cell lines CMF-7 and 870-BC express/carry tumor-associated antigens and HLA class I molecules. These structures could in principle present antigens to the immune system and consequently, shedded membrane vesicles may play an important role in the immunological control escape of the tumor cells (Muralidharan-Chari *et al*., 2010).

In order to proliferate uncontrolled and to perform individual or in group migration, tumor cells are producing a plethora of mediators with paracrine effect onto adjacent stroma. Among them, so called EMPRIN (also termed basigin), a glycoprotein associated to the tumor cell plasma membrane, is able to stimulate the matrix metallo-proteinases (MMPs) production by the tumor-stroma fibroblasts. In agreement with Sidhu *et al*. (2004), in a recently published paper (Mirancea *et al*., 2010) we showed that multitude shedding membrane vesicles may be involved in EMPRIN deliverance and, consequently in the basement membrane destruction.

When we are talking about solid tumors, we cannot dissociate epithelial cells involved in tumorigenesis process from their stromal microenvironment. A solid tumor is assimilated to an organ-like entity comprised of diverse populations of cells: (1) mainly tumor (cancer) cells bearing genetic abnormalities (mutations) heterogeneous multicellular entities containing cells of multiple lineages and (2) other cell types from the so-called tumor stroma represented by activated and/or recruited to the local microenvironment such as fibroblasts, inflammatory cells (innate immune cells, including granulocytes, dendritic cells, macrophages, natural killer cells, mast cells, telocytes) (Matrisian *et al*., 2001; Mueller & Fusenig, 2004; Tlsty & Coussens, 2006; Mirancea *et al*., 2010; Zheng *et al*., 2012 a, b). The fibroblast is the major cell type of stromal compartment. Fibroblasts play a pivotal role in orchestrating epithelial cells-adjacent stroma cross talk (Olumi *et al*., 1999; Mueller & Fusenig, 2004; Bhowmick *et al*., 2004; Martinez-Outschoom *et al*., 2010). Interesting, here, most fibroblasts have an epithelioid phenotype being embedded in a very fibrotic stroma. This morphologic change shows an activated/ reactive state of the fibroblasts.

Numerous *in vivo* and experimental studies emphasize that, concomitant with epithelial cell genomic alterations, changes also occur in stroma cells surrounding the epithelial tumor cells. For example, carcinoma-associated fibroblasts promote tumorigenesis of initiated prostate epithelial cells, but this effect was not observed when normal prostatic fibroblasts were grown with initiated epithelial cells under the same experimental conditions (Olumi *et al*., 1999). Conversely, by manipulating tumor-stroma interactions, stroma can exert a dominant force over the malignant behaviour of the tumor cells still bearing the genetic alterations, so that malignant cells can be reverted to a normal phenotype as morphology and behaviour (Bissell *et al*., 1999; Vosseler *et al*., 2005; Miller *et al*., 2005; Willhauck *et al*., 2007). Like other stromal cells, a recently described stromal (interstitial) cell type named telocyte (Popescu & Faussone-Pellegrini, 2010; Popescu, 2011; Gherghiceanu & Popescu, 2012) seems to play a role in tumor formation and evolution (Zheng *et al*., 2012 a, b). In normal tissue, telocytes perform homo- and heterotypic cell junctions (Popescu & Faussone-Pellegrini, 2010; Rusu *et al*., 2011; Gherghiceanu & Popescu, 2012; Rusu *et al*., 2012 a, b; Popescu & Nicolescu, 2013). Different from the normal skin (Rusu *et al*., 2012 a; Ceafalan *et al*., 2012), in this study we observed that, except for some direct contacts between telocytes and macrophages (Fig. 14 and Fig. 16), homo- and heterotypic cell junctions of telocytes are very limited. Most reduced seem to be the heterotypic junctions of telocytes with tumor cells and, especially with blood vessel wall, probably because of the high amount of amorphous material and the fibrotic tissue interposed between telocytes and tumor epithelium, and blood vessel, respectively (Fig. 13 and Fig. 14). Here we emphasize the paucity of telocytes *per se*, but especially paucity of heterocellular junctions which can be assimilated to the loss of function at the tissue homeostasia (at the stromal level). Our opinion is in agreement with Manetti *et al*. (2013) considerations. They reported that in the skin systemic sclerosis, there is a progressive loss of telocytes which may contribute to the alteration of 3-D organization of the extracellular matrix. In our investigated cases of basal cell carcinoma, there is a reduction of heterotypic junctions of telocytes with fibroblats, mast cells, endothelial cells, nerves, and consequently, abnormalities in skin regeneration and/or repair impairment.

CONCLUSIONS

There are polymorphic ultrastructural aspects of cell-cell and cell-extracellular matrix junction alterations in basal cell carcinoma. Severe infrastructural alterations of cell junctions, both at the tumor epithelia level and stromal cells (mainly telocytes), prepare tumor cells for their invasive growth.

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