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PODONEVADNE TRIGONA OVUM (ZERNOV, 1901) (CLADOCERA), AN IMMIGRANT SUBSPECIES WITH CASPIAN ORIGIN, NEWLY PENETRATED IN ROMANIAN WATERS

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The analysis of zooplankton in the Danube-Black Sea Canal revealed the presence of a new species for the Romanian fauna: Caspian cladoceran *Podonevadne trigona ovum.* Its appearance occurred at the end of the first year of the ecosystem (1985). In this phase of the canal colonization, its presence was limited to the mixing zone of marine and freshwaters. Further, its adaptation at the ecological conditions of the new ecosystem had major difficulties, which led to its disappearance from the zooplankton community structure in the next year (1986). The renewal of the studies after two decades (2005) reveals that the immigrant overcomes these difficulties, populating the entire biotope, except the contact area with the river waters. The analysis of the frequency and abundance of the immigrant shows that it became one of the main components of the predator zooplankton. The invasive behavior of some species belonging to Onychopoda Infraorder, resulted in significant changes in the structure of some ecosystems, suggests the usefulness of monitoring the evolution of *Podonevadne trigona ovum* in the Danube-Black Sea Canal.

Key words: *Podonevadne trigona ovum*, immigrant species, zooplankton, predator, parthenogenetic, gamogenetic, resting eggs.

INTRODUCTION

The researches focused on the forming and development processes of the zooplankton community in the Danube-Black Sea Canal have revealed the presence of a new species for the Romanian waters: the cladoceran *Podonevadne trigona ovum* (Zernov, 1901).

The origin of the cladocerans fauna belonging to the Onychopoda Infraorder, which includes the mentioned species, is located in the Caspian basin. The particular ecological characteristics of this group reflect, to a significant extent, the complexity of the genesis of this basin.

This process starts in Miocene, 5 million years ago. The formation of a deep depression area with tectonic origin in the south-eastern extremity of the Sarmatian Lake, led to the primordial Caspian basin. Its location coincides with the southern end of the current pool.

The new tectonic events created conditions for further expansion of the primary basin in an area corresponding to the middle sector of the current basin.

The strong orogenetic movements registered at the end of the Miocene resulted in the fragmentation of the Sarmatian Lake in two separate pools: the Caspian and Pontic region.

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The important hydrological contribution of some tributary waters determined, finally, the emergence of the northern sector. Unlike the middle and especially the southern ones, the northern sector is characterized by low depth and salinity (Dumont, 1998; Rivier, 1998).

Repeated transgressions and regressions recorded in the Pliocene, during 2 million years, have generated major changes (within 25–120 m) of the water levels.

The registered increases in the transgression phases have produced the considerable expansion of the Caspian basin. Therewith, there were created the conditions to establish some temporary communication channels with the Azov, Pontic and Aral basins.

In interglacial periods, the melting of glaciers also caused a considerable increase of water flow from the northern tributaries of the basin. A consequence of this fact was the establishment of a temporary communication route between the Caspian and Baltic basin.

The long geographical isolation of the Caspian basin created a preponderant endemic fauna and regional and temporal variations in salinity have been an important factor of its speciation.

The ability of species to live in waters with different concentrations of salinity could be improved during the temporarily emergence periods of the connection lines with other basins. It is remarkable that some of these species currently maintain the tendency to populate new ecosystems.

The Infraorder Onychopoda includes 33 species and subspecies. Currently, two prefer freshwaters, 7 marine waters and 24 are brackish species. The first two ecological categories no longer reflect the structure of the fauna of the Caspian basin. In the same situation, there are two brackish species. One of these is *Podonevadne trigona ovum.* As a result, currently only 46% of species remain endemic any longer, 21% inhabit both native basin and other basins, and 33% have disappeared from the structure of the cladoceran fauna of the mentioned basin.

In the first stage, the saltwater species left the Caspian basin and entered the Azovo-Pontic, Aralic or Baltic basin. In the next stage, some of them colonized more distant seas, such as the Mediterranean Sea or the North Sea, and eventually spread in the Atlantic, Pacific and Indian Oceans. Integrated in the biocenotic structure of the new ecosystems, this species became dominant, due its high prolificity and short generation time.

These are the species *Evadne nordmanni* from the Baltic Sea, or *Pseudoevadum tergestina*, *Evadne spinifera*, *Podom intermedius* and *Pleopis polyphemoides* that inhabit different areas of the planetary ocean (Zenkevich, 1963; Gieskes, 1970; Della Croce & Venugopal, 1972; Longhurst & Seibert, 1972; Brosch & Taylor, 1973; Onbe, 1974; Thiriot, 1974; Kos, 1977; Frontier, 1979).

The colonization of the remote areas has been achieved mainly through resting eggs transported by birds. A wide and circumpolar spread, favored by the existence of a short life cycle and a high prolificacy, exhibits also the freshwater species *Polyphemus pediculus* (Ekman, 1904; Keilchack, 1906; Butorina, 1963, 1971, 1993).

In a recent period, the anthropogenic factor also exerted an important role in the spread (active or passive) of some species, as a result of the construction of canals, reservoirs, intensive practice of shipping or the cultivation of alien species. A surprisingly, fast and wide spread was recorded for freshwater species *Bytotrephes longimanus*. It recently settled (during 1970-1980) the great lakes of North America, where it got the ballast water of ships. In Lake Michigan, even if it is not very abundant, this species has already produced changes in the structure of planktonic community and fisheries (Marlene, 1990). It also maintains the role of dominant species in the zooplankton of some large lakes in the manner recorded in Europe half a century ago (Gerd, 1947; Drako & Stetsenko, 1956; Fenyuk, 1960; Mordukhai-Boltovskaya & Mukhordova, 1970; Glamadza, 1971; Buschman, 1978).

Among the cladocerans of the Onychopoda Infraorder that currently continue to expand, there are listed some low brackish water species, also tolerant in freshwater, such as *Cercopagis pengo, Corniger maeoticus* or *Podonevadne trigona ovum.* In their case, the construction of canals and reservoirs represented an opportunity to expand their habitat in tributary waters of the Black, Azov and Caspian Seas (Rivier, 1998).

Researches carried out in the last half century evidenced the entering of some immigrant species belonging to Onichopoda in the Romanian Black Sea sector (*Podon leuckarti*, *Pleopis polyphemoides*, *Pseudoevadne tergestina*, *Evadne nordmanni*, *E. spinifera*, *Cercopagis pengoi* and *Cornigerius maeoticus*). The last two species were also found in some lakes with marine or Danube floodplain origin (Mordukhai-Boltovskoi & Negrea, 1965; Negrea, 1983). The extending process of some species in the Romanian spaces continues in the current period. *Podonevadne trigona ovum* is the cladoceran species that recently entered in the Danube-Black Sea Canal.

In the opinion of the specialists, the genesis of species occurred 77.000 years ago, during a major transgression (Zernov, 1901). It was adapted to environments with low salinity or freshwaters, and colonized the outskirts of huge flooded areas in the northern part of the Caspian basin. The flooding of the Manych depression area, which temporarily stopped the isolation phase of the Caspian basin, has allowed its migration in the Azov basin. As a result of the flooded area stint and the break of the contact with Azov basin during the following regression period, *Podonevadue trigona ovum* disappeared from the Caspian basin. Instead, it has maintained its presence in the Azov Sea and Chalkar Lake, located within the former floaded areas (Behning, 1928).

In the next phase, it came in the Black Sea, where successively populated the lagoons of Bug, Dnieper and Dniester rivers. The construction of the Volga-Don canal and some reservoirs of the Volga course, Don, Dnieper and the Dniester in the period 1930-1975 provided the species a new opportunity to populate other ecosystems (Tseeb, 1962; Mordukhai-Boltovskoi & Galinskii, 1974; Gusynskaya, 1989).

MATERIAL AND METHODS

The researches were conducted during 1985–2005 in the Danube-Black Sea Canal. In the hierarchy of Earth's major channels it ranks third (after Panama and Suez) and is located at 44°11'46.78" N latitude, 28°39'46.62" E longitude. Its main branch (Danube km 299 – Agigea Seaport) has a length of 64 km, and the secondary one (Poarta Albă-Midia Năvodari Seaport) has 31 km (Fig. 1).

Fig. 1. The map of Danube – Black Sea Canal – the sampling sites of zooplankton (www.earthgoogle.com).

The minimum depth of two branches is 7 and 5.5 m, respectively, their width varies between 140 and 100 m, and the flow of water transit between 0.3 to 0.9, respectively from 0.13 to 0.23 m^3/s.

The sampling of zooplankton in the canal was carried out, initially, in 4 stations in 1985 and 11 sampling points, in 2005 (Table 1) with seasonal periodicity. To this goal we used a Schindler Patalas device with 5-liters capacity. For each sample, there were taken 50 liters of entire water column. The samples were concentrated by filtering through a plankton net with a mesh of 65 mm. The preservation of samples was done with 4% formaldehyde solution.

Table 1

Sampling sites of zooplankton

No st.	Canal km	Period	Geographic landmark	Canal section
1	64+000	2005	Cernavodă City	Danube – Black Sea
2	61+000	2005	Upstream Cernavodă lock	Danube – Black Sea
3	47+600	2005	Mircea Vodă	Danube – Black Sea

Table 1

(continued)

4	40+000	2005	Medgidia City	Danube – Black Sea
5	37+900	1985-1986, 2005	Medgidia City	Danube – Black Sea
6	28+700	2005	Poarta Albă City	Danube – Black Sea
7	23+000	2005	Basarabi City	Danube – Black Sea
8	20+700	2005	-	Poarta Albă – Năvodari
9	15+100	2005	-	Poarta Albă – Năvodari
10	10+000	2005	Downstream Ovidiu lock	Poarta Albă –Navodari
11	2+000	2005	Navodari City	Poarta Albă – Năvodari
12	0+000	1985 -1986	Upstream Agigea lock	Danube – Black Sea
13	-	1985 -1986	Black Sea	Agigea Zone

The material analysis revealed the presence of a new cladoceran species for the Romanian waters: *Podonevadne trigona ovum* (Fig. 2). Based on data obtained from the analysis of zooplankton samples (Tables 2-4), we could determine where and when entering the immigrant in canal, its abundance frequency (Table 5) and the prevalence in the numerical structure of the total (Table 6) and predator zooplankton (Table 7).

Fig. 2. *Podonevadne trigona ovum* (orig.).

RESULTS AND DISCUSSION

The low velocity of water flow, the heterogeneous nature of the environment, caused by the presence of submerged and emerged macrophytes in coastal area, the river water intake and the influence of the marine terminal area of the canal, have created favorable conditions for the existence of zooplankton.

The low values of species richness (Fig. 3) and abundance (Tables 2-4), recorded in the first year of existence of the ecosystem (1985), showed significant increases over the next two decades.

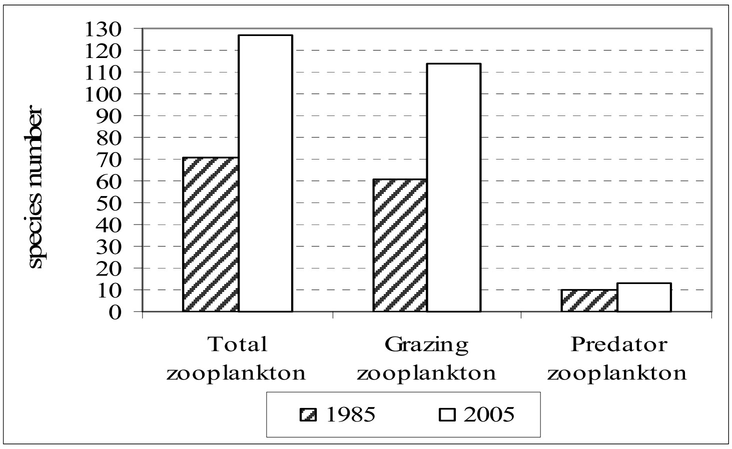

Fig. 3. The dynamics of zooplankton species richness on trophic levels (species number).

Table 2

The dynamics of total zooplankton abundance (ind./l)

Station	V	VI	X	XI	Xa V-XI
1985					
2	42.2	34.6	10.0	4.8	22.9
5	1067.0	102.1	203.2	37.3	352.40
12	260.2	34.4	60.6	3.0	89.55
13	29.2	52.8	34.7	2.1	29.70
Xa	349.65	55.98	77.13	11.80	123.64
2005					
Station	III	VIII	IX	XII	Xa III -XII
1	7.3	156.8	4.9	13.7	45.67
2	13.2	139.7	5.34	3.7	40.48
3	13.3	196.7	262.5	2.0	118.62
4	7.1	456.7	266.96	12.3	185.76
5	10.4	412.1	299.7	22.0	186.05
6	4.3	1131.6	372.2	0.7	377.20
7	1.9	1044.0	258.6	1.8	326.75
8	109.3	1825.4	462.7	3.1	600.12

Table 2

(continued)

9	5.3	1538.5	227.9	5.43	444.28
10	213.5	1387.6	1108.8	655.8	841.43
11	533.7	710.8	369.0	248.6	465.52
Xa	83.57	818.17	330.78	88.10	330.16

Table 3

The dynamics of herbivore zooplankton abundance (ind./l)

Station	V	VI	X	XI	Xa V-XI
1985					
2	42.2	33.4	10.0	4.8	22.6
5	1067.0	91.32	203.2	34.0	348.88
12	258.1	31.9	54.8	2.2	86.75
13	26.1	50.5	23.0	1.0	25.15
X a	348.35	51.78	72.75	10.5	120.85
2005					
Station	III	VIII	IX	XII	Xa III-XII
1	7.3	154.7	3.8	13.7	44.87
2	13.0	135.7	5.0	3.5	39.30
3	13.3	180.9	256.6	2.0	113.2
4	7.1	446.2	264.7	12.3	182.57
5	10.4	386.5	287.3	22.0	176.55
6	4.3	1049.3	370.6	0.7	356.22
7	1.9	1001.8	245.5	1.8	312.75
8	109.3	1766.7	451.7	3.1	582.7
9	5.3	1482.0	225.7	4.93	429.48
10	213.5	1283.1	1099.2	654.4	812.55
11	533.7	671.7	357.0	248.6	452.75
Xa	83.55	778.05	324.28	87.91	318.45

Table 4

The dynamics of predator zooplankton abundance (ind./l)

Station	V	VI	X	XI	Xa V-XI
1985					
2	-	1.2	-	-	0.3
5	-	10.78	-	3.3	3.52
12	2.1	2.50	5.8	0.8	2.8
13	3.1	2.30	11.7	1.1	4.55
Xa	1.3	4.24	4.2	1.3	11.17
2005					
Station	III	VIII	IX	XII	Xa III -XII
1	-	2.1	1.1	-	0.80
2	0.2	4.0	0.34	0.2	1.18
3	-	15.8	5.9	0.2	5.47
4	-	10.5	2.26	-	3.19

Table 4

(continued)

5	-	25.6	12.4	-	9.50
6	-	82.3	1.6	-	20.97
7	-	42.2	13.1	-	13.83
8	-	58.7	11.0	-	17.43
9	-	56.5	2.2	0.5	14.80
10	-	104.5	9.6	1.4	28.77
11	-	39.1	12.0	-	12.78
Xa	0.02	40.12	6.5	0.21	11.70

Table 5

The dynamics of the *Podonevadne trigona ovum* frequency (%)

Year	Months	%	Ecological significance
1985	V	-	-
	VI	-	-
	X	25.00	accidental species
	XI	-	-
	Xa V-XI	6.25	accidental species
2005	III	9.09	accidental species
	VIII	81.82	persistent species
	IX	54.54	persistent species
	XII	-	-
	Xa III-XII	36.36	accessories species

Table 6

The dynamics of the percentage of *Podonevadne trigona ovum* in the total zooplankton abundance (%)

Year	Station	Months			
		V	VI	X	XI
1985	2	-	-	-	-
	5	-	-	-	-
	12	-	-	1.81	-
	13	-	-	0.30	-
2005	Station	III	VIII	IX	XII
	1	-	-	-	-
	2	2.26	-	-	-
	3	-	0.71	-	-
	4	-	1.36	-	-
	5	-	2.69	3.20	-
	6	-	2.89	0.05	-
	7	-	2.02	2.74	-
	8	-	1.05	1.21	-
	9	-	3.01	0.96	-
	10	-	0.49	-	-
	11	-	0.12	0.54	-
	Xa 1-11	0.21	1.30	0.79	-

Table 7

The dynamics of the percentage of *Podonevadne trigona ovum* in the predator zooplankton abundance (%)

Year	Station	Months			
		V	VI	X	XI
1985	2	-	-	-	-
	5	-	-	-	-
	12	-	-	23.40	-
	13	-	-	0.86	-
2005	Station	III	VIII	IX	XII
	1	-	-	-	-
	2	100	-	-	-
	3	-	8.86	-	-
	4	-	59.04	-	-
	5	-	64.45	77.42	-
	6	-	30.62	12.50	-
	7	-	50.00	54.20	-
	8	-	32.71	50.91	-
	9	-	82.12	100	-
	10	-	6.51	-	-
	11	-	2.05	16.67	-
	Xa 1-11	9.09	30.58	26.82	-

The taxonomic analysis of the zooplankton recorded the emergence of *Podonevadne trigona ovum* in the Danube-Black Sea Canal at the end of the first year of ecosystem existence (1985), in the terminal sector of the canal (upstream, Agigea sluice). This is a subspecies of *Podonevadne trigona* species, belonging to the *Podonevadne genus*, *Podonidae* family, *Onychopoda* infraorder, *Cladocera* suborder (Boxshall, 2001; Martin & Davis, 2001). The concomitant presence of species in the adjacent marine coastal zone (Table 1) offered an explanation for the source of the immigrant in the canal. In the structure of zooplankton constituted in the first year of ecosystem existence the immigrant cladoceran presented the characteristics of accessories species (Table 5).

In this phase, the adaptation to the ecological conditions of the new ecosystem encountered major difficulties, which could not be overcome. As a result, the species could not be identified in the structure of zooplankton in the following year (1986).

The renewal of the studies in 2005 noted the recurrence of *Podonevadne trigona ovum*. Its presence in 10 of the 11 analyzed sampling sites in this year reflected the upturn in the process of adaptation of the species to the ecological conditions of the ecosystem. Its absence could be recorded only in the confluence area between the fluvial and canal waters. The avoiding of the fluvial environment is a feature also noted in other onychopod species regarded as typical planktonic organisms. With high capacity of buoyancy and diminished adaptations for active moving, they show positive selectivity only for the current free waters (Egloff *et al.*, 1996).

The dynamics of the frequency index of the species showed significant differences during the annual cycle of life. In the early spring the presence of the cladoceran in our samples was relatively low (9.09%), corresponding to the accidental species type. Instead, in early autumn and, especially in late summer, there were recorded high values (54.54% and 81.82%) (Table 6), reflecting the constant status of species and holding a significant role in the structure of the zooplankton in canal.

The populational structure of the immigrant cladoceran was formed, exclusively, by the parthenogenetic females (Fig. 2). The dominance of the parthenogenetic females is a common situation for the cladocerans living in permanent aquatic ecosystems characterized by favourable environmental conditions (Berg, 1936). The gamogenetic females and the male individuals are characterized by a much smaller presence, usually located in the terminal life cycle (from October to December) or during unfavourable ecological periods. Their proportion is significantly higher in the temporary aquatic ecosystems (Negrea, & Negrea, 1975; Negrea, 1983).

The comparative analysis of the parthenogenetic females from the Azov Sea (Mordukhai-Boltovskoi & Negrea, 1965) and the Danube-Black Sea Canal revealed the existence of similar dimensions of body length limits. In contrast, the height and volume of the brood poach were clearly higher for the canal specimens. They express the existence of some particular ecological conditions.

For the species belonging to *Podonevadne* genus, characterized by reduced possibilities of movement, the brood poach has an important hydrostatic role. In the dense environment of salt water the volume and height of brood poach is relatively low and clearly higher in freshwater, where water density is lower. For the species *P. camptonyx* and *P. angusta* (whose optimum salinity of water varies within 11 to 13.5 ‰) the brood poach is narrow and less bulky. For *P. trigona trigona* (optimal salinity 3–8 ‰) the brood poach is wider and slightly higher (Rivier, 1998). The population of *P. trigona ovum* (optimal salinity 3–4 ‰) from the Azov Sea have a globular shaped brood poach. It is much higher and the protective valves are provided with deformations that increase the floating ability (Mordukhai-Boltovskoi & Negrea, 1965). The population of the Danube-Black Sea Canal, which lives exclusively in freshwater, has a very tall and bulky brood poach (Fig. 2).

The fecundity of the parthenogenetic females is much higher than that of gamogenetic females. However, the gamogenesis plays an extremely important role in the survival of the species by resting eggs, able to remain viable in the most difficult environmental conditions. Transported by birds, they contribute to the spread of the species into new ecosystems (Rivier, 1998).

The absence of effective organs of movement, the small size of the individuals and the large volume of the brood poach, drastically restrict the speed of movement for the species belonging to *Podonevadne* genus. The speed of *P. trigona trigona* is 1m h^{-1} (Egloff *et al.*, 1996) and, probably *P. trigona ovum* has a similar value. However, the researches performed in 2005 showed that it is present in almost the entire basin of the Danube-Black Sea Canal.

Reported at that speed, the colonization of 64 km of the canal by *P. trigona ovum* would have lasted 87 months. Due to the transportation of the resting eggs by birds, the colonization process of the Canal lasted, probably, a much shorter period. Also, presumably, the crossing distance between the origin basin of immigrant and the mouth of the Danube-Black Sea Canal was made in a similar manner.

The *Podonevadne* genus is a nocturnal predator filtered type. They feed mainly on ciliates, rotifers, nauplii, small cladocerans and, in addition, nanoplanktonic algae (Egloff *et al.*, 1996).

The percentage of the abundance of the mentioned cladoceran in the structure of total zooplankton stands at low level (Table 6). However, the emphasized values are comparable with those of other predatory species found in the zooplankton structure of different aquatic ecosystems (Zinevici & Parpală, 2007).

Instead, the analyzed species showed a much higher percentage as part of the predator zooplankton. It represented 30.58% of the abundance of the predator zooplankton, in August and 26.82%, in September (Table 7).

Very high values have emerged in the middle of the canal, located between 4-9 stations. The analysis of these data reveals that *P. trigona ovum* became a numerically dominant species in the structure of the predator zooplankton.

These data highlight the strength of *P. trigona ovum* reflected in zooplankton community trophic relationships in two decades of existence in the Danube-Black Sea Canal ecosystem.

CONCLUSIONS

The cladoceran *Podonevadne trigona ovum* is a Caspian immigrant subspecies and entered the terminal area of the Danube-Black Sea Canal in the first year of the ecosystem (1985).

Adapting to the new ecological conditions, over the next 20 years it has spread to almost all parts of the ecosystem, except for the river confluence area.

The annual dynamics of the abundance presented the highest values during summer and slightly lower in early autumn.

The analysis of the frequency and percentage of the immigrant in the structure of the predator zooplankton emphasized an integrating process in the structure and trophic relationship of the new ecosystem.

In the next stage, there can be predicted that *Podonevadne trigona ovum* will populate a series of lakes, with lagoon origin, from the Romanian Black Sea sector.

Invasive behavior of some species belonging to the Onychopoda infraorder resulted in significant changes in the structure of some ecosystems, suggests the usefulness of monitoring the evolution of *Podonevadne trigona ovum* in the Danube-Black Sea Canal.

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MAIN CHARACTERISTICS OF WEED-BED INVERTEBRATE COMMUNITIES IN AQUATIC SYSTEMS OF THE SMALL ISLAND OF BRĂILA DURING 2009 SUMMER SEASON

EMILIA RADU*, GETA RÎŞNOVEANU**

As a result of structural changes recorded in the last half of century in the Lower Danube River System, the aquatic ecosystems remained under a natural flooding regime showed a pregnant tendency of transition toward hypertrophy. As a consequence, the energy input into the shallow lakes of the Small Island of Braila (SIB) increased through excessive development of macrophytes-epiphytic complex. Changes in the composition and structure of the weed-bed invertebrate fauna are poorly documented. Therefore, the weed-bed aquatic invertebrates were investigated during the 2009 summer season in eight representative sites of the SIB. The main purpose of the study was to collect information on the composition and distribution of the main weed-bed invertebrate taxonomical groups. Structural indices like taxonomic richness, organism densities, percentage numerical abundances and frequencies of occurrence of identified taxa were calculated. The structural features that may describe the studied weed-bed fauna communities are: a total of 27 taxonomical groups identified along the sampled sites in the whole study period, the pregnant dominance of one to four taxa, (among which chironomids and oligochaetes) and a better level of representation of invertebrate communities in lakes, as compared to channels.

Key words: shallow lakes, macrophyte stands, weed-bed fauna communities, taxonomic richness, abundance, Small Island of Brăila.

INTRODUCTION

The Lower Danube River System (LDRS) represents a complex ecological structure consisting of a set of aquatic and terrestrial ecosystems closely and multidirectional interconnected, and functioning as a key buffer component, between the whole Danube river basin and the Black Sea (Vădineanu, 1991; Adamescu, 2004). Over the last half of the twentieth century, following the implementation of various policies and management plans for wetlands in the LDRS (drainage and damming activities, intensive industrialization, urbanization and agricultural use practices), the structure and functions of the LDRS were significantly affected (Vădineanu *et al.*, 1992, 1998). The major consequences of these changes were the disappearance of large natural areas (lakes, ponds, wet meadows and marshes, channels and flood plains), accompanied by the transition of the remnant aquatic ecosystems to eutrophic states. These changes have resulted in the simplification of the trophic networks and a significant reduction of the subsequent energy flows through ecosystems (Vădineanu *et al.*, 1992, 1998; Rîşnoveanu & Vădineanu, 2000; Adamescu, 2004).

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In the case of primary producers, the density of the energy input into ecosystems increased through excessive development of either phytoplankton, a process correlated with the reduction or extinction of the aquatic macrophytes and weed-bed fauna (*e.g.* deeper lakes in costal Danube Delta), or macrophytes – epiphytic complex (a typical case in the shallow lakes in the Small Island of Brăila) (Vădineanu *et al.*, 1992; Dinu & Cristofor, 1993; Adamescu, 2004). After 1990, the tendency in the dynamics of LDRS was to rebalance the various biotic compartments of the affected ecosystems (Vădineanu *et al.*, 1998) and an approach to establish their ratio in the integrative systems and the specific factors that are controlling their dynamics at present became challenging.

If the structure and dynamics of some biotic compartments of the ecosystem in the area are well documented, the weed-bed fauna communities have received only a marginal attention, usually in connection to the dynamics of the adjacent aquatic macrophytes (Cristofor, 1989; Dinu & Cristofor, 1993; REDI, 1999). The data and knowledge base concerning the weed-bed invertebrates are particularly poor for the SIB area, whereas for the coastal delta there are several studies over the time (Popescu-Marinescu & Zinevici, 1968, 1969; Tatole, 1983; Ciubuc & Ciolpan, 2006).

It is generally known that aquatic plants provide a physically complex habitat in aquatic ecosystems, and architectural features of this habitat as well as water main characteristics can affect invertebrate communities taxonomical composition, density and distribution (Cyr & Downing, 1988; Cheruvelil *et al.*, 2002; Tessier *et al.*, 2004; Papas, 2007). Depending on the type of interaction with the weed-bed support, the attached aquatic invertebrates are able to form different types of associations with distinct levels of diversity and complexity (Cyr & Downing, 1988; Lalonde & Downing, 1992; Bogut *et al.*, 2010).

According to this conceptual framework and considering that the impact of described changes on the composition and structure of the weed-bed invertebrates in the area is poorly documented, revealing its principal present characteristics – in terms of distribution, composition and structure – became the aim of this paper. This purpose also attempts to complete the scientific databases in a long term socio-ecological research site, represented by the Small Island of Brăila.

MATERIAL AND METHODS

The Small Island of Brăila (SIB) (Fig. 1) is the largest area remained under the natural hydrological regime of flooding from the former Inner Delta of the Danube (an important component of LDRS). Because of its attributes, this wetland with various ecosystems types received a statute of protected area (170 km^2) and is appreciated to be a buffer zone (between river and sea), well known as an important reference system and acknowledged as an international socio-ecological long-term research area (ILTER). Our researches were focused on two complexes of aquatic ecosystems representative for the SIB, namely: Fundu Mare and Popa (Fig. 1).

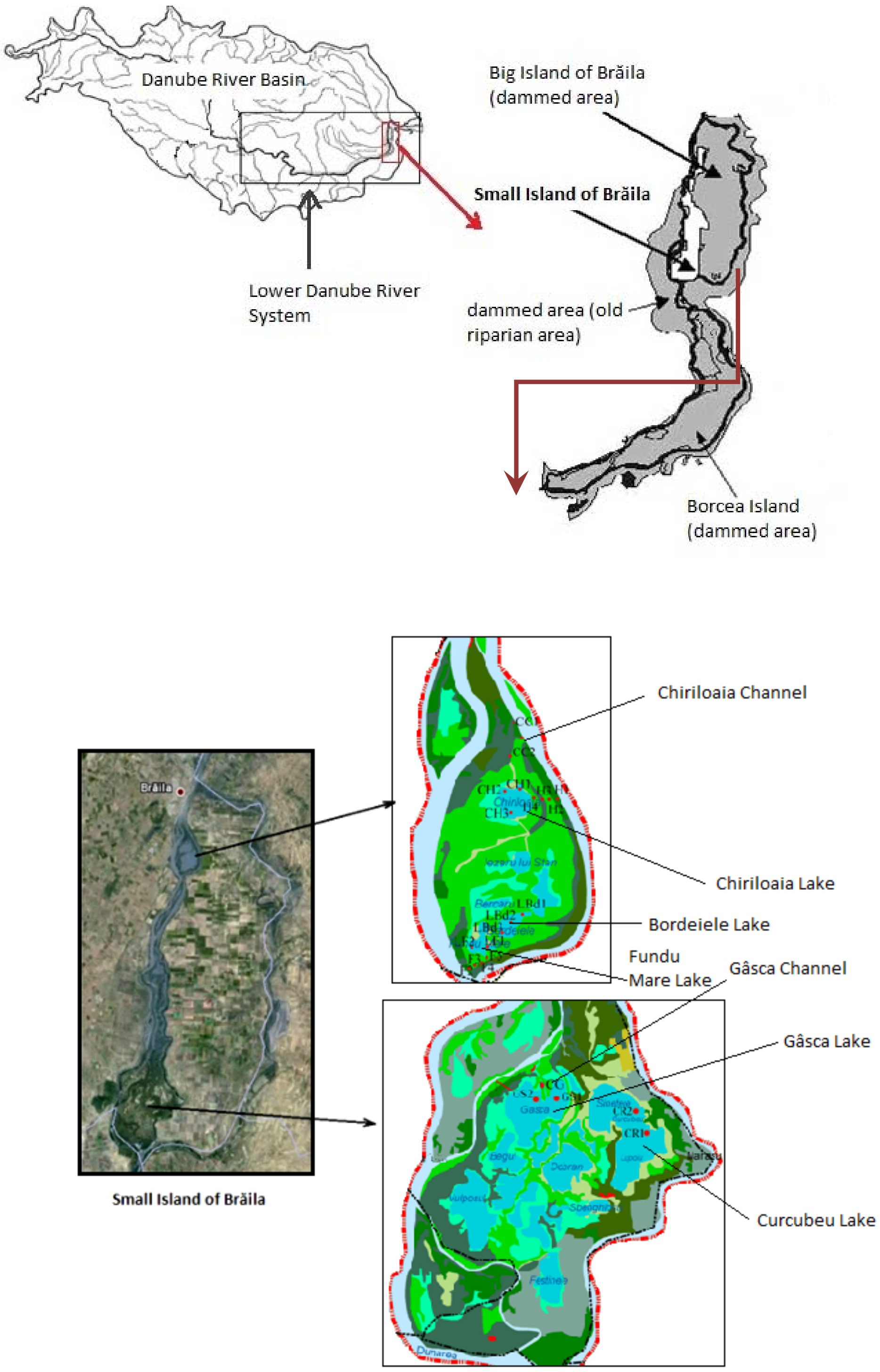

Fig. 1. Location of the study sites and placement of the stations.

The location of the stations was established along a longitudinal gradient, according with the water loading into the lakes from the Danube (Adamescu, 2004). This included the Lakes Chiriloaia, Bordeiele and Fundu Mare that belongs to the Fundu Mare complex of ecosystems and Lakes Gâsca and Curcubeu placed in the Popa complex of ecosystems. Six to nine sample units taken randomly, at approximately equal distances (Trivinho-Strixino *et al.*, 2000) by hand collecting of submersed and floating plants (Popescu-Marinescu & Zinevici, 1968, 1969), in two or three fixed stations were sampled in each lake. The hydrologic connectivity issues in the area determined us to sample also one to two sampling stations in the Danube Strand, and Chiriloaia and Gâsca channels (Fig. 1). The samples were fixed in the field in polyethylene plastic bags using formalin 4% (Albertoni, 2005). The sampling period was associated with the aquatic macrophyte vegetation season (Trivinho-Strixino *et al.*, 2000; Colon-Gaud *et al.*, 2004; Albertoni, 2005) and was conducted monthly (June, July and August) during 2009 summer season. In some aquatic ecosystems, the access in the area was not possible due to the low water level or to the extreme coverage with aquatic vegetation (*e.g.* Lakes Bordeiele and Fundu Mare in June, and Lakes Chiriloaia, Bordeiele, Fundu Mare in August). In other sites the macrophytes stands were not present at all (*e.g.* Danube strand in July and August, and Channel Chiriloaia in July).

In the laboratory, the macrophytes samples were washed through a griddle with pores of 250 µm and taxonomically identified. The dry weight was determined as the weight of the weed-bed plants, after 7 days of air exposure at the room temperature (Colon-Gaud *et al.*, 2004). The weed-bed invertebrates collected on sieves were further preserved in ethyl alcohol 70% and the analyses were done using a binocular (16X, 40X) (Tessier *et al.*, 2004; Colon-Gaud *et al.*, 2004). The main invertebrate groups were identified at different taxonomic levels (classes, orders, families, genera) according to different authors (Cry & Downing, 1988; Tessier *et al.*, 2004; Colon-Gaud *et al.*, 2004) by using the identification guides of Chiriac & Udrescu (1965) and Barnes *et al.* (2001).

Visual estimates were used for assessment of the aquatic vegetation stands coverage in each station and as an overall view at the ecosystem level, as well as for the assessment of the dominant species of macrophytes (Sutherland, 2006). A particular set of physical-chemical parameters of the water column was measured by YSI 600R Multi-parameter Water-Quality device (water temperature, pH, turbidity, total dissolved solids and dissolved oxygen concentrations) along with particular field observations (*e.g.* atmospheric and hydrological conditions in the area, the morphological features of the lakes and adjacent channels). The depth and transparency of water were measured by using the Secchi disc and nitrogen and phosphorous concentration were measured in laboratory.

In order to evaluate the structure and composition of weed-bed fauna communities, invertebrate numerical density (N.d.) was determined as the number of individuals per weed-bed grams dry mass in samples, and taxonomic richness as the total number of taxa in each sample. Numerical percentage abundance (A%)

was calculated as the percentage of one invertebrate group numerical density out of the total invertebrate density.

To establish the dominant weed-bed fauna groups percentage abundances were used and for revealing the constant ones, the frequencies of occurrence (F%) index (as the percent ratio of the number of samples where a taxonomic invertebrates group was present to the total number of samples taken per month or per studied system) was calculated.

The spatio-temporal fluctuations of the weed-bed community structure were generally expressed using average values of the structural parameters (counted per station, ecosystem and month of sampling or for the entire study period).

RESULTS AND DISCUSSION

SPATIAL AND TEMPORAL CHARACTERISTICS OF THE AQUATIC MACROPHYTE STANDS

A total of 13 macrophyte species were identified in the studied systems during the period of study. Their spatio-temporal distribution is presented in Table 1. *Nymphaea alba*, *Trapa natans* and *Nymphoides peltata* were the most frequent species in lakes and *Sagittaria*, *Ranunculus*, *Myosotis*, *Salvinia* and *Lemna* species mostly in channels and in the Danube strand. *Nymphaea* and *Trapa* species represented constant components of the macrophyte communities over the entire study period (June to August).

Table 1

Spatio-temporal distribution of the aquatic macrophyte stands in the eight aquatic systems studied in SIB during 2009 summer season (◊ – June, ○ – July, □ – August)

Aquatic system	**Danube strand**	**Chiriloaia Cannel**	**Lake Chiriloaia**			**Lake Bordeiele**			**Lake Fundu Mare**		**Gâsca Channel**	**Lake Gâsca**		**Lake Curcubeu**	
Taxonomic composition	**I0**	**CC2**	**CH1**	**CH2**	**CH3**	**LBD1**	**LBD2**	**LBD3**	**LF1**	**LF2**	**CG**	**GS1**	**GS2**	**CR1**	**CR2**
Sagittaria sagittifolia L.	◊		◊												
Ranunculus lingua L.	◊														
Nymphaea alba L.	◊					○		○	○	○		◊ ○ □	◊ ○		□
Veronica anagallis-aquatica L.	◊	◊												◊ □	
Myosotis sp.		◊												◊	

Salvinia natans (L.) All.		◊ □									□				□
Lemna minor L.		◊ □									○ □				□
Equisetum sp.		◊													
Trapa natans L.			◊ ○	◊ ○	◊ ○		○	○				○ □	◊ ○ □	◊ ○ □	◊ ○
Potamogeton pusillus L.			◊	◊	◊							◊	◊	◊	◊
Schoenoplectus lacustris (L.) Lye			◊												
Nymphoides peltata (Gmel.) Kuntze					◊			○		○	○ □			□	
Oenanthe aquatica (L.) Poir.					◊	○			○				◊		

THE STRUCTURE AND DYNAMICS OF THE WEED-BED FAUNA COMMUNITIES

The qualitative taxonomical composition of weed-bed fauna communities in SIB in the summer of 2009 consists in a total number of 27 taxonomic groups, with a higher diversity in the Popa ecological complex where 22 groups were present in both Lake Gâsca and Lake Curcubeu as compared to Lake Chiriloaia where only 19 groups were identified during the entire study period (Fig. 2A).

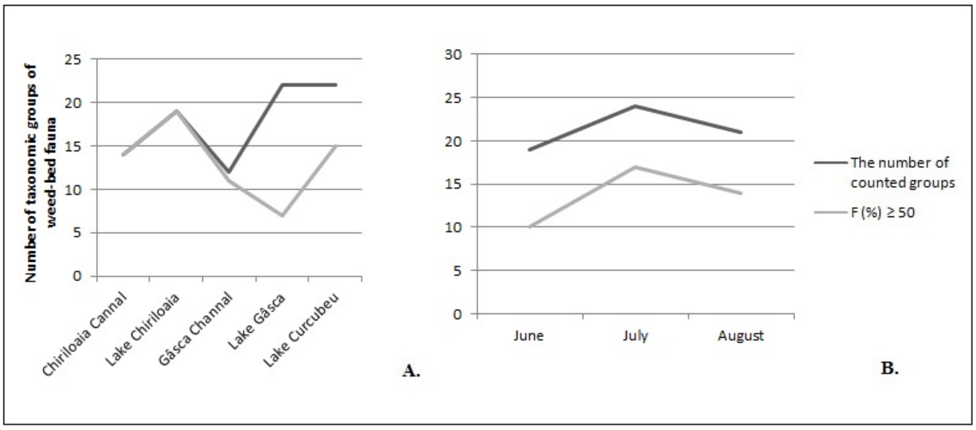

Fig. 2. The spatial (A) and temporal (B) frequency of occurrence of the total number of weed-bed invertebrate groups and the number of constant ones in studied systems during June-August 2009.

The weed-bed fauna communities were better represented in July (24 groups) and August (21 groups) as compared to June, when only 19 different invertebrate groups were counted (Fig. 2B).

Analyzing the spatial frequency of occurrence of weed-bed groups in sites where sampling process was repeated in two or three months of the season it is interesting to remark a higher taxonomic diversity in the Popa complex of ecosystems along with a lower number of constant groups (Fig. 2A) that may suggest a higher variability of the community's composition in this region. In this case, the taxonomical composition of macrophyte stands changed across sampling moments (nine different plant species identified during the study) and according with Hanson (1990), this could greatly alter the structure of the macroinvertebrate communities. At the same time, dense homogeneous beds (like *Trapa* sp. stands, prevalent in lakes in Popa complex, especially in July) are known to harbour a high number of macroinvertebrate taxa (Dvorak & Best, 1982).

Excepting Lakes Gâsca and Curcubeu, the number of invertebrate groups that met the criteria of constancy (F% ≥ 50) showed a similar tendency of variation with the total number of wed-bed taxonomical groups identified in the structure of communities (Fig. 2A, B).

The number of taxa identified in the structure of weed-bed fauna of each aquatic system at each sampling moment varied between 8 (Channel Chiriloaia in June) and 17 (in Lakes Chiriloaia and Bordeiele in July, and Lake Curcubeu in August). The taxonomic diversity was higher in lakes as compared to channels, the higher values being recorded in July (Fig. 3).

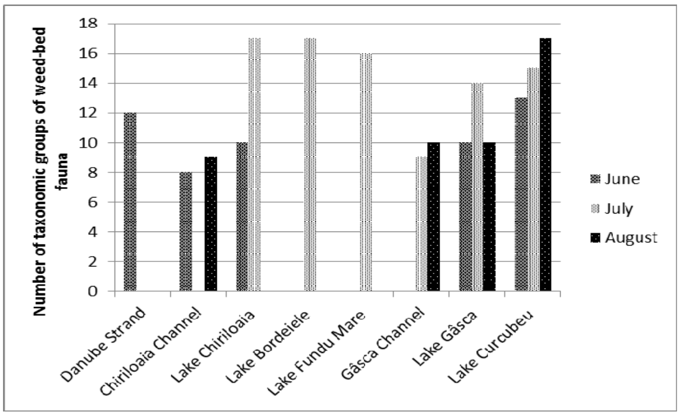

Fig. 3. The dynamics of taxonomical richness of the weed-bed fauna in studied ecosystems across 2009 summer season.

Taking into account the total frequency of occurrence registered by groups across all the studied sites and sampling moments, 16 taxons gained values over 50% (Fig. 4), representing thus constant components of the communities studied.

Among these, eight macroinvertebrate groups were constant at each of the three sampling moments: Chironomidae, Coleoptera, Ephemeroptera, Gammaridea, Gastropoda, Isopoda, Oligochaeta and Trichoptera (Fig. 5). At the same time, Chironomidae, Coleoptera and Gastropoda were the groups that meet the constancy criteria in all sites, during the summer. Oligochaeta and Isopoda were constant across the studied systems also, excepting the Danube Strand for Oligochaeta and Lake Gâsca for Isopoda.

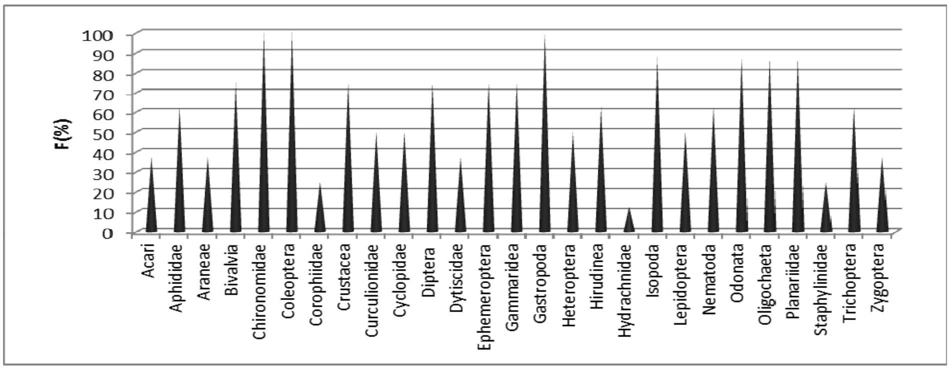

Fig. 4. The total frequency of occurrence registered by weed-bed fauna groups across all the sites and sampling moments in the 2009 summer season.

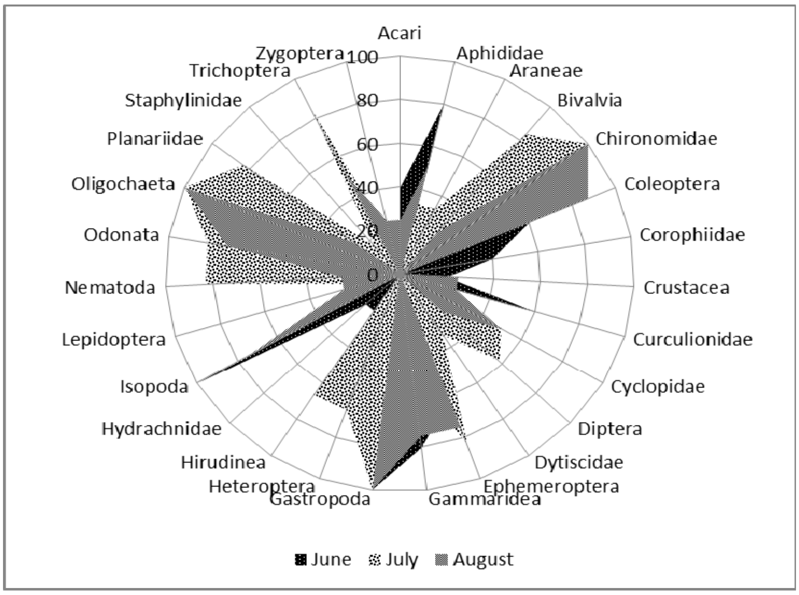

Fig. 5. Frequency of occurrence (F%) of the weed-bed fauna taxonomic groups across the studied area in different months in 2009 summer season.

The highest frequencies of occurrence were recorded by Chironomidae, Gastropoda (100 % in all three months) and Oligochaeta (80 % in June and 100 % in July and August), while the lowest (F < 20 %) by Hydrachnidae, Lepidoptera, Staphylinidae and Zygoptera (Fig. 5).

Percentages of numerical abundance (A%) of different taxonomic groups varied across space and time (Fig. 6). In every aquatic system 1 to 5 weed-bed taxonomic groups were dominant or co-dominant (the most characteristic groups in the biotic community) out of which, in each lake, 2–3 groups represent together over 80 % in the community's structure. Generally, Chironomidae and Oligochaeta were the most dominant, but in some ecosystems, Isopoda, Aphididae and Gastropoda met the dominance criteria. In channels the equitability of different groups is higher.

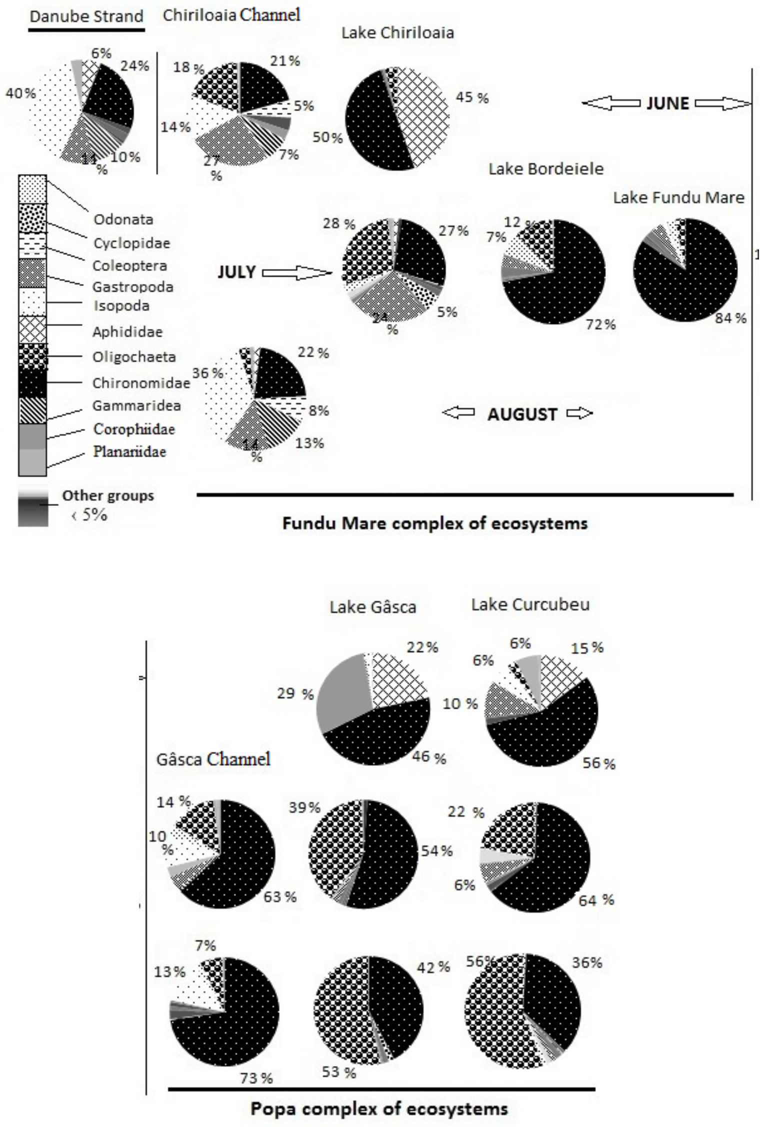

Fig. 6. Numerical abundance (A%) of weed-bed fauna taxonomic groups identified in studied ecosystems for the 2009 summer season.

Chironomidae were dominant in all eight sampled systems, with average values of abundance per system ranging between 21 % (Channel Chirloaia, June) and 84 % (Lake Fundu Mare, July) (Fig. 6). This invertebrates group is almost always found to be numerically predominant, both in lotic and lentic environments, due to its tolerance under widely different environmental conditions (Albertoni *et al.*, 2007; Morais *et al.*, 2010).

Oligochaeta reached the highest percentage numerical abundances in lakes Gâsca (53 %) and Curcubeu (56 %) in August, but ranged over 10 % in the other four aquatic systems (Fig. 6). Oligochaeta is a typical detritivorous group (Albertoni *et al.*, 2007) and according to Trivinho-Strixino *et al.* (1997), the roots and other submersed parts of macrophytes accumulate detritus and large quantities of organic matter, favouring the establishment of populations of detritivorous and collector feeding organisms.

Aphididae had important abundance values in June, in the Lakes Chiriloaia (45 %), Gâsca (22 %) and Curcubeu (15 %). Isopoda registered high distinct values in Danube Strand (40 %) and Channel Chiriloaia (14 %) in June, Channel Gâsca (10 %) in July and Channels Chiriloaia (36 %) and Gâsca (13 %) in August. In terms of numerical abundance, Gastropoda, peeked in Channel Chiriloaia in June (27 %), but ranged between 24 and 10 % in other three aquatic systems at different sampling moments (Fig. 6).

Gammaridea had smaller abundance levels in the analyzed communities, being represented better in channels and Danube strand, with values ranging up to 13 % (Channel Chiriloaia, August), but had a good frequency of occurrence in all studied sites during the summer season, excepting Lakes Chiriloaia and Fundu Mare.

The weed-bed fauna groups that counted both small abundance levels (less than 5 %) and a sporadic frequency of occurrence (equal or less than 25 %) were: Hydrachnidae, Lepidoptera, Staphylinidae and Zygoptera (Figs. 5, 6).

The most dominant groups, Chironomidae and Oligochaeta recorded high numerical densities (Fig. 7).

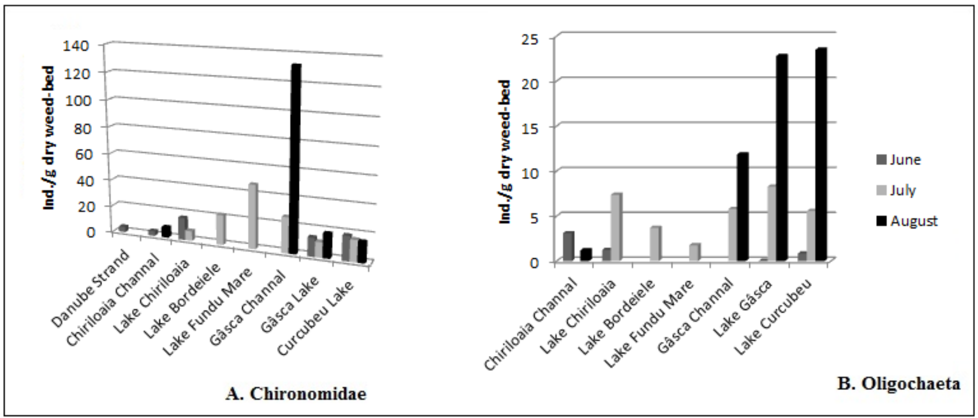

Fig. 7. Chironomidae (A) and Oligochaeta (B) numerical densities (N.d.) dynamics.

The highest density values were recorded for Chironomidae. Their numerical densities ranged between 3.48 (Channel Chiriloaia) and 18.4 ind./g dry weed-bed (Lake Curcubeu) in June, 7.27 (Lake Chiriloaia) and 47.37 ind./g dry weed-bed (Lake Fundu Mare) in July, and 7.73 (Channel Chiriloaia) and 132.62 ind./g dry weed-bed (an extreame peak in Channel Gâsca) in August 2009.

Oligochaeta numerical densities ranged between 0.03 (Lake Gâsca) and 3.1 ind./g dry weed-bed (Channel Chiriloaia) in June, 1.76 (Lake Fundu Mare) and 8.29 ind./g dry weed-bed (Lake Gâsca) in July, and 1.2 (Channel Chiriloaia) and 23.49 (Lake Curcubeu) in August. These values are considerably lower than those recorded for Chironomidae and are comparable with those of other numerically representative groups, like Aphididae, Isopoda, Gastropoda and Gammaridea, which appear abundant in some weed-bed communities structure (Fig. 6).

CONCLUSIONS

The present study was carried out in several shallow lakes and adjacent channels characteristic and representative for the Small Island of Brăila regional system of wetlands.

Aquatic vegetation in studied sites was relieved as an important habitat for weed-bed fauna communities.

27 different macroinvertebrate groups of various taxonomic levels were recorded in the sampled aquatic vegetation stands. Chironomidae larva and Oligochaeta were both dominant and constant groups in the weed-bed fauna communities. The subdominant status was gained by the Isopoda, Aphididae and Gastropoda taxa.

Permanent inhabitants of the weed-bed communities were considered the highly frequent groups: Chironomidae, Gastropoda and Oligochaeta, while Hydrachnidae, Lepidoptera, Staphylinidae and Zygoptera groups were very scarce within the communities.

In 2009, the weed-bed macroinvertebrate communities were better represented in lakes and, in July, in close relation to the appropriate habitats.

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FEATURES OF *IXODES RICINUS* (ACARI: IXODIDAE) PHENOLOGY IN DIFFERENT ECOREGIONS OF ROMANIA

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Ixodes ricinus abundance and seasonal activity patterns were investigated in three sites belonging to different ecoregions of Romania in a two-years longitudinal study (2007–2008). The sites were investigated monthly and 1466 ticks (296 adults, 912 nymphs and 258 larvae) were collected by dragging technique. Ticks abundance differed between sites and years. According to drag captures nymphs and adults displayed a bimodal pattern (May-June and September) with strong dominance of spring activity. In the attempt to assess the influence of climatic factors on the questing activity of ticks we analyzed the cumulative and peak tick densities for each of the sites in relation to the mean temperature and saturation deficit of the air. We found that there is a negative correlation between the cumulative tick density and the saturation deficit that could account for the differences in the observed tick densities in the selected sites.

Key words: *Ixodes ricinus*, phenology, temperature, saturation deficit, Romania.

INTRODUCTION

Ticks transmit the greatest variety of pathogens of any bloodsucking arthropod (Nuttall & Labuda, 2003), and they are second only to mosquitoes in their medical importance as disease vectors (Dennis & Piesman, 2005). The patterns of vector distribution, abundance and seasonality are major determinants of the spatially and temporally variable risk of infection by the transmitted parasites (Randolph, 1998). As *Ixodes ricinus* is the most common tick in Europe (Aeschlimann, 1981) and in Romania (Feider, 1965), and vector for more pathogens than any other tick in the Old World (Jongejan & Uilenberg, 2004), the seasonal dynamics of questing ticks is of the greatest importance for determining the risk of tick-borne diseases in particular areas.

I. ricinus phenology varies considerably throughout its wide distribution range (Gray, 1991), from unimodal (Yousfi-Monod & Aeschlimann, 1986; Randolph *et al.*, 2002; Dsouli *et al.*, 2006; Burri *et al.*, 2007) to bimodal patterns (Gray, 1984; Korenberg, 2000; Randolph *et al.*, 2002; Burri *et al.*, 2007) and also among years (Tälleklint & Jaenson, 1996; Perret *et al.*, 2000).

The ideal habitat of this tick is in a deciduous forest with damp soil covered by a dense and thick layer of undergrowth (Lane *et al.*, 1991) but, the population density and activity pattern of *I. ricinus* vary even between different deciduous forests, being influenced by several interacting abiotic and biotic factors (Lindgren *et al.*, 2000). Thus, studies have shown that the beginning of tick activity in spring is linked to temperature (Perret *et al.*, 2000; Randolph, 2004). Relative humidity as well as saturation deficit (a measure of the drying power of the atmosphere) are important factors influencing tick behavior and mortality rates (Randolph & Storey, 1999; Perret *et al.*, 2003, 2004).

The purpose of this work was to compare questing tick dynamics in different areas of Romania in two consecutive years, within the same habitats type (deciduous forest with *Quercus* sp. as dominating species in the tree layer), in relationship with climate variables.

MATERIAL AND METHODS

Tick sampling. Three study sites (Comana, Brateiu, Ciucurova) were selected based on the historical data for ticks and/or tick-borne pathogens in those regions and their positioning in different ecoregions (Fig. 1). Questing *I. ricinus* ticks were sampled by pulling a 1-m^2 flag over 100 m of vegetation every month from March to October in 2007 and 2008 respectively. Questing tick density was expressed for each stage as the number of individuals collected per 100 m^2.

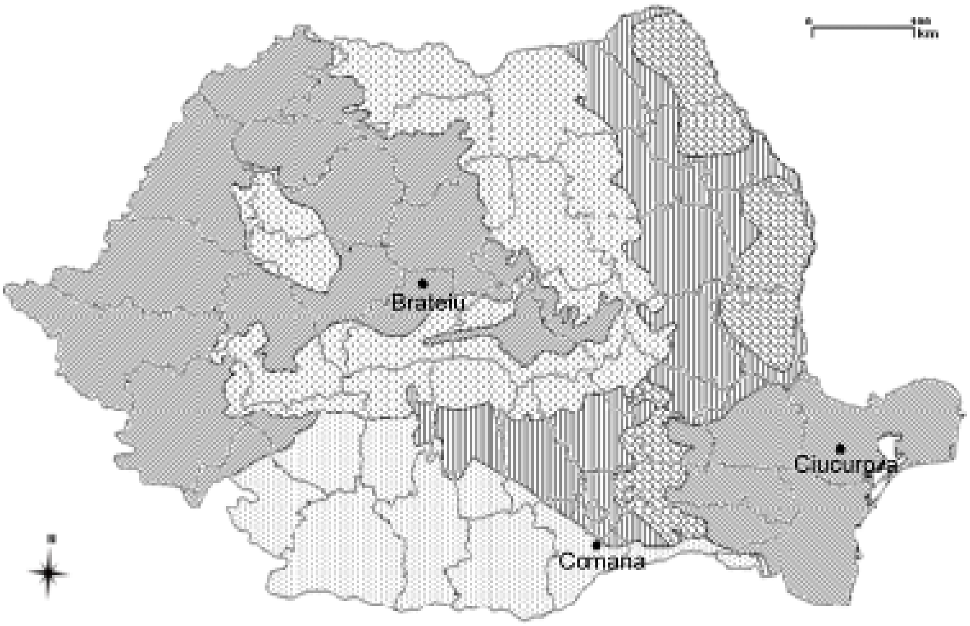

Fig. 1. Sampling sites within the ecoregional context.
Legend: ● sampling site, ⊠ East European forest steppe, ▨ Pannonian mixed forests, ▧ Pontic steppe, ⁙ Balkan mixed forests, ⁘ Carpathian montane forests, ▥ Central European mixed forests.

Climate data. Climate data were obtained as daily means for the three counties in which the sites are located: Giurgiu (Comana), Sibiu (Brateiu), and Tulcea (Ciucurova) from the National Meteorological Administration. Collected data included mean temperature (°C), mean relative humidity (%), and precipitations amount (mm). From temperature and relative humidity the saturation deficit (mmHg) was calculated from the formula given by Randolph & Storey (1999). From the daily values monthly means and totals were computed for each of the four variables.

Statistical analysis. To characterize the seasonal dynamics of *I. ricinus* tick we used several variables (Eisen *et al.*, 2003; Jouda *et al.*, 2004 a, b): (a) cumulative tick density (CTD) obtained by integrating the linearly interpolated curve of the measured questing tick densities over one year, and (b) peak tick density (PTD) defined as the maximal tick density, with a spring peak (SPTD) and an autumn one (APTD). Nymphs and adults were treated separately. Differences in tick activity dynamics within sites and between the two years were evaluated with t test while differences between sites within the same year were evaluated using Kruskall-Wallis test (both tests considered significant at $p < 5\%$). Saturation deficit was compared to the cumulative tick density for every location and Pearson correlation was calculated for the yearly means. Statistical tests were performed using PASW® Statistics 17.0 (IBM SPSS®).

RESULTS

A total of 1466 ticks were collected during the two years, 589 in 2007 and 877 in 2008, of which 296 were adults, 912 nymphs and 258 larvae. The number of collected ticks did not differ significantly between the two consecutive years except at Ciucurova, in the spring and early summer months (April–June) for adults (t stat = – 2.71746, p = 0.036) as well as for nymphs (t stat = – 3.92695, p = 0.0147). Most adults and nymphs were collected in the spring and early summer. Thus, the PTD was reached in most of the cases in May, for nymphs and adults, with one exception each year: in 2007 at Brateiu and in 2008 at Comana, the nymphal PTD appeared in June. In July and August nymphal and adult tick density dropped considerably (Fig. 2a–c).

The larvae became active later than the other stages. Larval PTD was recorded generally in August, except for 2008 at Ciucurova, when it appeared a month earlier (July). At Brateiu, in 2007, larval activity seemed to have extended from July to September.

The CTD values were generally higher in 2008 than in 2007 except for the nymphs in Brateiu and the larvae in Comana (Table 1).

Table 1

Cumulative tick densities calculated as area under curve

Site	Year	Cumulative tick density (CTD)		
		Adults	Nymphs	Larvae
Comana	2007	633	1960.5	2255.5
	2008	1621.5	3776.5	1119.5
Brateiu	2007	1343	6545	242
	2008	2033.5	6071.5	1020.5
Ciucurova	2007	912	2024.5	1161
	2008	1647	6430	1986.5

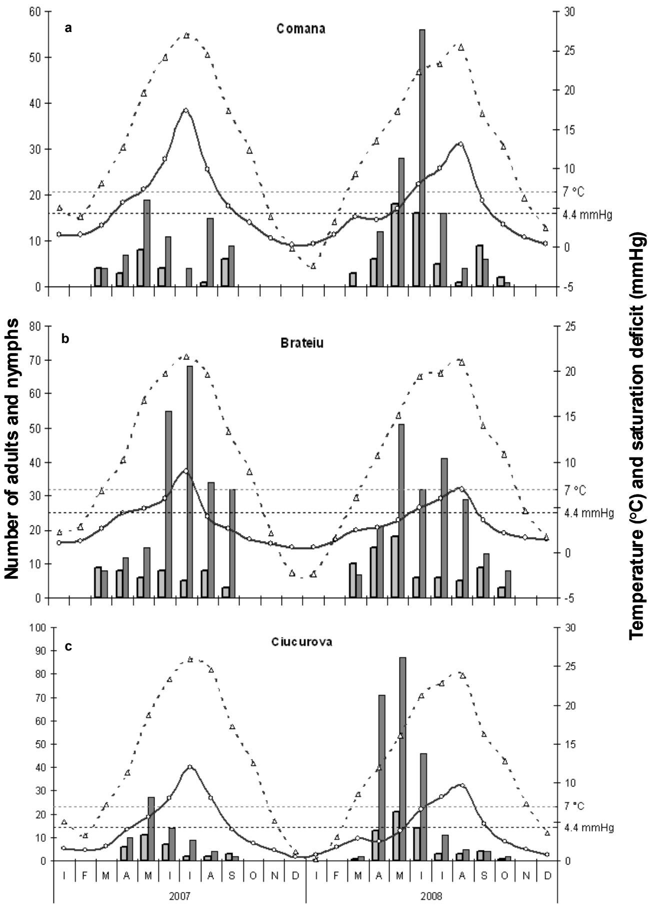

Fig. 2. Tick density for nymphs (■) and adults (▣) overlapped by monthly mean temperature (- - △) and SD (—○). Legend: The dotted lines (- - - -) represent the border thresholds for SD (4.4 and 7 mmHg) and temperature (7 °C).

No significant differences were observed in the average monthly temperatures between years at the 3 meteorological stations corresponding to the three counties in which our investigations were performed (Chi-square test: $p > 0.05$). The effect of climate on nymph and adult tick dynamics is illustrated for the 3 locations (Fig. 2).

DISCUSSION

In most of the locations the activity pattern appeared as bimodal, with a second, smaller peak in September, except for the adults in Brateiu which displayed a rather constant activity throughout 2007 (Fig. 2b). This observation is in agreement with studies performed in other European countries regarding tick phenology (Randolph, 2002), and the explanation for the second peak would be that newly developed stages emerge in the autumn in the same year (if the previous stage feeds before July) or in the next year autumn (if they feed after July). The explanation for the lower values of the second peak is that most hatched ticks do not become active in the autumn, but enter a behavioral diapause and do not feed until next spring.

The critical period for the onset of ticks' questing activity in Romania appears to span over the months of March and April, when mean temperatures rise above the minimum thershold of 7 °C (Fig. 3). An earlier onset of tick activity was registered in 2007 at Brateiu – in March (Fig. 2b). Brateiu also displayed a second activity peak earlier than other sites in 2007 – in August.

The summer break in the questing activity of ticks may be caused by the fact that many ticks that start questing in spring find their hosts by summertime; on the other hand, during the summer months the temperatures increase highly and the humidity decreases, resulting in maximum values of saturation deficit which determine the ticks to descend to the leaf litter for water sorption (Perret *et al.*, 2000; Randolph, 2002, 2004).

The end of questing appears to be close to the date at which mean air temperatures drop below 7 °C and even earlier. This advance in the offset of ticks activity may be due to the differences in the temperatures recorded at the weather station comparing to those at the study sites.

When compared between the three sites the CTD's revealed that in both years Brateiu had the highest values for adults, followed by Ciucurova and Comana (Table 1). In the case of nymphal CTD's the situation was similar in 2007 to that of adults, but in 2008 there was a switch of ranks between Brateiu (6071.5) and Ciucurova (6430), with Comana still on the last place. The CTD increased in 2008 comparing to 2007 in all three sites both for adults and nymphs (Table 1), except for the nymphs in Brateiu which remained at a constant level. The highest increments were noticed for adults in Comana (2.56) and for nymphs in Ciucurova (3.18).

With larvae the situation was completely different in that Brateiu had the lowest CTD in both years and in most cases larval CTD was smaller than that of adults and nymphs. This is not in concordance with the theory on tick ecology that gives a ratio of 100 larvae: 10 nymphs: 2 adults for the tick stages (Randolph & Craine, 1995). Due to the fact that larvae are found particularly in the superficial soil layer and in the leaf litter, most of the times the dragging technique fails to capture them, especially when they are not at very high densities (Ginsberg & Ewing, 1989). Moreover, their distribution is extremely fragmented, being determined by the sites where the females laid the eggs (Ruiz-Fons & Gilbert, 2010). These two observations could explain the very low number of larvae captured in comparison to the other stages and the consequent failure to have a good estimation of larvae density.

We tried to find explanation for the observed differences in nymphal and adult tick densities between sites in the variation of climate parameters such as mean air temperature, relative humidity and saturation deficit. Temperature and saturation deficit data for the 3 sampling sites are compared (Figs. 2, 3). We noticed that at Brateiu the mean SD for March-October was lower than that of the other sites (Fig. 2), with a bi-annual average of 4 mmHg comparing to 7.2 at Comana and 5,3 at Ciucurova. Monthly means of SD pass the threshold of 4.4 mmHg (the optimum for tick questing; Perret *et al.*, 2000) just 3 months a year at Brateiu while at Comana 6 months and at Ciucurova 3 months (Fig. 2). Also, at Brateiu SD values rarely pass the threshold of 7 mmHg (in some years they do not even reach it), which is considered to diminish greatly the questing activity of ticks (Perret *et al.*, 2000). Thus, the decreased value of SD may be responsible for the increased tick densities observed at Brateiu. The fact that in 2007, at Brateiu the peak of SD overlapped the peak of questing nymphs' density (Fig. 2b) could be explained by the fact the climate data we used are drawn from weather stations that are more or less distant of the investigation sites and thus, may not always reflect the microhabitat climatic conditions.

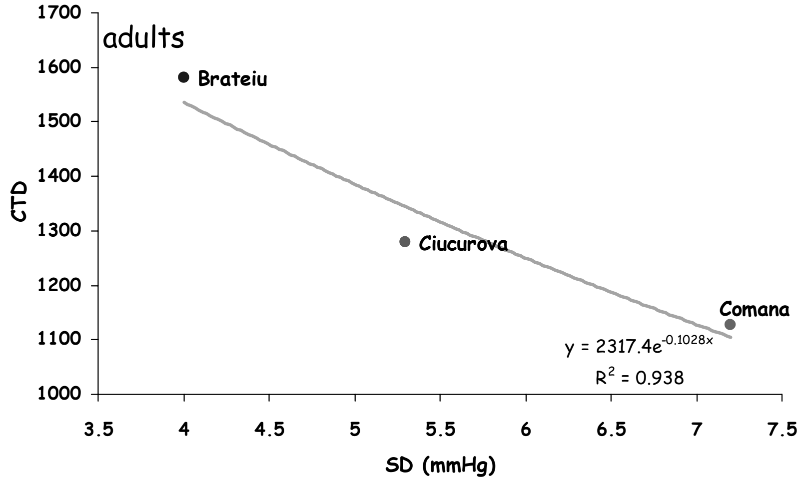

Fig. 3. Negative correlations of saturation deficit and Cumulative Tick Densities for adults and nymphs.

Nevertheless, from our data there seems to be a negative correlation between average saturation deficit during the tick activity months and average cumulative tick density considered for the two years of study; this seems to be the case for both adults and nymphs (Fig. 3).

Ixodes ricinus phenology is known to vary from one year to another (Tälleklint & Jaenson, 1996; Perret *et al.*, 2000; Randolph *et al.*, 2002) which was also the case in our study. Annual variations in ticks activity can be associated with the fluctuations of biotic (vertebrate hosts' density and behaviour, vegetation structure) and abiotic (climate) factors. In all three sites the winter of 2006-2007 was warmer than that of 2007-2008 which should have resulted in a higher survival rate of ticks and implicitly higher densities of ticks in 2007, but, on the other hand, 2008 had higher moisture levels during the months of ticks' questing activity. Consequently, ticks questing activity seems to be better explained by humidity and saturation deficit than by temperature alone.

CONCLUSIONS

This study confirms the presence of *Ixodes ricinus* ticks in various ecoregions of Romania. The activity pattern appears to be a bimodal one for nymphs and adults in all the investigated sites with some exceptions for Brateiu, where even in the summer the ticks' effectives remain at rather high rates.

Climate variables like saturation deficit and mean monthly temperatures are able to explain the observed differences in cumulative tick densities between the three sites and the length of the questing period, but fail to explain the unusual activity patterns observed at some sites (Brateiu).

Thus, we may assume that there could be some climatic conditions or vegetation and host assemblages that differ between the three ecoregions and that interfere with seasonal dynamics of ticks activity, remaining to be the subject of further studies.

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ACAROFAUNA (ACARI-MESOSTIGMATA: GAMASINA) FROM AN ADJACENT AREA TO THE CLIFF ECOSYSTEM FROM BREBU GORGES (PRAHOVA DISTRICT, ROMANIA)

MINODORA MANU

This study was made in 2010, near to a cliff area from Brebu gorges, from Prahova district, Romania. In studied area adjacent to the cliff ecosystem, the taxonomical structure was represented by 10 families (Parasitidae, Veigaidae, Ascidae, Rhodacaridae, Macrochelidae, Pachylaelaptidae, Laelaptidae, Eviphididae and Zerconidae), with 19 genera and 34 species. The identified species were common for a terrestrial temperate ecosystem. *Pergamasus crassipes* was recorded as species with the most wide biogeographical distribution and on the opposite, *Zercon foveolatus,* which was signaled only in Slovakia, Ukraine and Romania. *Zercon foveolatus* and *Zercon hungaricus* were identified as xerotolerant and thermophilous. The maximum number of species was recorded on the north side of cliff ecosystem (26), in comparison with south side (19). This study is the first step of a careful and comprehensive sampling of individual cliffs, in order to determine if cliffs select for particular groupings of soil mites, on the basis of their functional ecological characteristics.

Key words: gamasid, habitat, distribution, cliff, Brebu Gorges, Romania.

INTRODUCTION

Studies regarding the gamasid fauna from different types of ecosystems (forests, meadows, shrubs, agroecosystems, urban parks, dunes, seashores, waste dumps) are many (Dunger *et al.*, 2001; Madej & Skubala, 2002; Wanner & Dunger, 2002; Masan, 2003; Masan & Fenda, 2004; Masan, 2007; Gwiazdowicz, 2007; Madej & Skodolka, 2008; Skorupski *et al.*, 2009; Manu & Honciuc, 2010 a, b; Manu, 2010; Masan & Halliday, 2010; Ruf & Bedano, 2010; Salmane & Brumelis, 2010). Studies of the invertebrate communities in cliff environments are not numerous. Cliffs provide a unique habitat, for plants as well as for invertebrates, rarely investigated from an ecological viewpoint. This “uniqueness” is due to the specifical microclimate (increased temperatures, presence of the wind, absence of direct precipitations, constance battles with the force of gravity), vegetation (lack of space for roots) or type of soil (lack of soil cover, presence of carbonate-based rocks). Soil invertebrates have an important ecological role in an autochthonous nutrient cycle. The large mass of rich organic debris (which sustains the productivity of plants) from cracks and crevices are the results of biological activity of some soil invertebrates such as earthworms, isopods and mites that feed on dead and decaying plant material. The gamasids, which are predators, have an important role to regulate these groups populations (Maser *et al.*, 1979; Larson *et al.*, 2000). The task of the research is to investigate, for the first time in Romania, the acarofauna from adjacent area to the cliff ecosystem.

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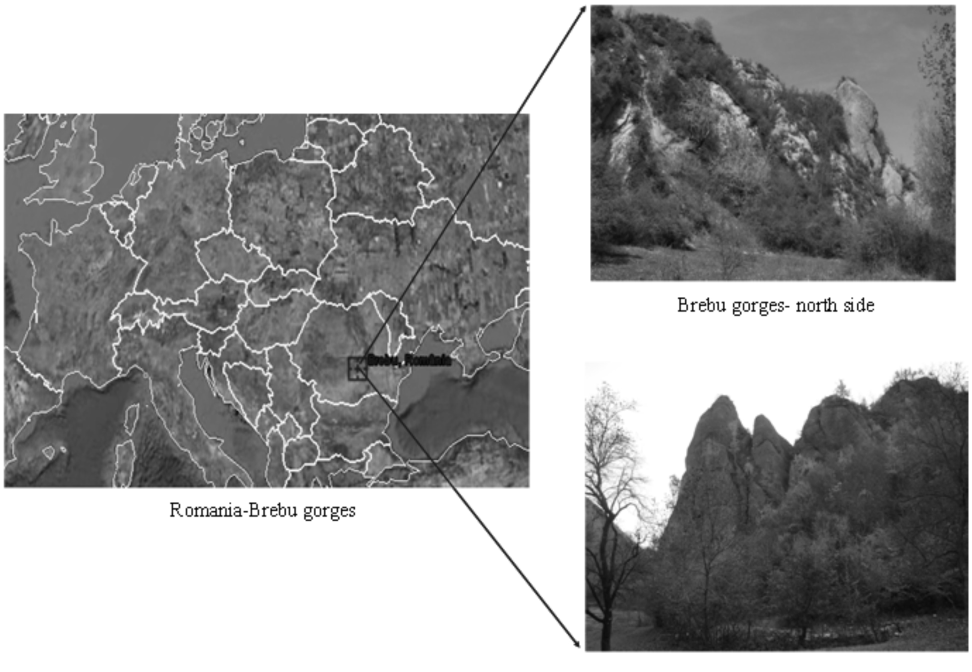
MATERIAL AND METHODS

This ecological study was made in 2010, near to a cliff area from Brebu gorges, from Prahova district, Romania (N: 45° 12' 31,1"; E: 25° 44' 23,5") (Fig. 1). Altitude was by 537 m. Vegetation was represented by different types of elements: euroasiatic: *Rubus saxatilis*, *Rubus caesius*, *Hippophaë rhamnoides*, *Populus tremula*, *Salix caprea*, *Salvia glutinosa*, *Campanula sibirica* (44.18%); european: *Valeriana montana, Crataegus monogyna*, *Berberis vulgaris, Taxus baccata* (18.6 %); central-european: *Centaurea stoebe*, *Cornus sanguinea, Cytisus nigricans* (9.3 %); carpathian: *Sesleria heuflerana*, *Thymus pullcherimus*, *Silene nutans* subsp. *dubia* (11.62 %); mediterraneous: *Cnidium silaifolium*, *Hedera helix*. The highest representation had mesophytes species (42 %), followed by xeromesophytes (37 %) and mesohygrophytes (9 %), and finally xerophytes (7 %). More that 20 % are pioneer species, such as: *Cytisus nigricans*, *Rosa canina* and *Hippophaë rhamnoides.*



Romania-Brebu gorges

Brebu gorges- north side

Brebu gorges- south side

Fig. 1. The geographical position of the investigated area (Brebu gorges, Prahova district, Romania).

Soils are classified in three classes: clayey till argillaceous on the moderate and strongly inclined peaks, which are seriously affected by erosion; brown eumesobasic till pseudogleic, which have a mineral component formed at soil surface, connected to a thin humified organic matter layer and typically alluvial soil.

20 samples/month were collected with MacFadyen corer (5 cm diameter), on 10 cm deep. The soil samples were taken in April, June, and October, 2010, around of the cliff area, taking account of its two geographical orientations: north and south. The extraction was performed with a modified Berlese-Tullgren extractor, in ethyl alcohol and the mites samples were clarified in lactic acid. The identification of the mites from the Mesostigmata order was made up to the species level. In total, there were analysed 120 soil samples, with 34 species and 158 individuals (Ghiliarov & Bregetova, 1977; Karg, 1993; Masan, 2003; Masan & Fenda, 2004; Gwiazdowicz, 2007; Masan, 2007; Masan & Halliday, 2010).

RESULTS AND DISCUSSION

Analysing the taxonomical structure of the mite populations, 34 species were identified, belonging to the 19 genera and 10 families: Parasitidae, Veigaidae, Ascidae, Rhodacaridae, Macrochelidae, Pachylaelaptidae, Laelaptidae, Eviphididae and Zerconidae. The families Parasitidae, Macrochelidae and Zerconidae are represented by the most increased number of species, in comparison with Rhodacaridae, Ascidae and Eviphididae (Table 1).

Table 1

Identified soil mite families (Acari: Mesostigmata-Gamasina) and the number of species from the Brebu gorges (Prahova district, Romania)

Families	North	South	Total
PARASITIDAE	9	2	9
VEIGAIDAE	3	2	4
RHODACARIDAE	0	1	1
ASCIDAE	0	1	1
MACROCHELIDAE	4	5	5
LAELAPIDAE	2	3	4
PACHYLAELAPIDAE	2	2	4
EVIPHIDIDAE	1	0	1
ZERCONIDAE	5	3	5
Total	26	19	34

The number of recorded species is comparable with those obtained in other studied ecosystems (as urban parks, shrubs, spoilt areas, meadows, arable fields). Rarely more than 60 species are recorded at a single site. Generally, the species number of gamasids is much lower in other types of ecosystems than in forests, but the number of species varies between habitats (Koehler, 1997; Madej & Skubala, 2002; Ruf & Beck, 2005; Gulvik, 2007; Manu, 2010). It is possible that some similarities of environmental conditions to be the cause of this phenomenon. Mites of the family Ascidae, together with those from Rhodacaridae, are the first species of mesostigmatid mites found in degraded habitats. Due to the small size and morphological adaptation they move easily in soil with tiny interstitial gaps. Species *Asca bicornis* is considered pioneer species (Gwiazdowicz, 2007). Species from the family Eviphididae are predatory, especially on nematodes. Species from the genus Eviphis are known as nematophagous, and it is possible that this tiny trophical spectrum to be a limitative factor for population development. Species *Eviphis ostrinus* is known as edaphically detricolous and could be considered as secondary inhabitant of soil substrates (Masan & Halliday, 2010).

Parasitids and macrochelids are a cosmopolitan group of free-living mites with a wide ecological and behavioral diversity (Masan, 2003). They are specialized predators of nematodes, enchytraeids, eggs and larvae of insects. They are capable to adapt at unfavorable conditions as those from cliff ecosystems (the poor vegetation, the sandy soil, the lack of organic matter, the increased air and soil temperature, the decreased soil humidity). The wide trophical spectrum assured them the possibility of survival in such environment.

Zerconids are predators, too, and constitute an important zooedaphon component in all soil microhabitats of the temperate zone. Species *Zercon foveolatus* and *Zercon hungaricus* are xerotolerant and thermophilous (Masan & Fenda, 2003).

Taking account of the spatial dynamics (the two geographical orientations of the cliff area), on the north side there was recorded the most increased number of species (76.5% from the total number), in comparison with the south area (23.5%). On the north side the humidity of soil is most increased and the temperature is not so high, in comparison with the south part, being a proper habitat for gamasid developing.

The identified gamasids are common species for terrestrial temperate ecosystems. 35.3 % of species have distribution in Europe; 17.6 % in Central Europe; 14.7 % in Europe and Asia; 8.82 % in Europe, Asia, USA, Australia and New Zealand; 2.94 % in East Europe; 2.94 % in Central and East Europe.

Species *Pergamasus crassipes* had a wide biogeographical distribution, being identified in holarctic region. On the opposite, species *Zercon foveolatus* was signaled only in Slovakia, Ukraine and Romania (Masan & Fenda, 2004) (Table 2).

Being very mobile and predators, investigated soil mites occur in various habitats, even in the adjacent areas to a cliff ecosystem. Through their adaptations to the rough environmental conditions (structural – body size and functional – mobility, being capable to run about 20 cm/min) they easily migrate, searching proper habitats and food source (Walter & Kaplan, 1991; Koehler, 1997; Gwiazdowicz, 2007).

Table 2

Identified soil mite species (Acari: Mesostigmata-Gamasina) from the Brebu gorges (Prahova district, Romania)

Taxa	**North**	**South**	**Habitat preferences**	**Biogeographical distribution**
PARASITIDAE				
Leptogamasus obesus Holzmann, 1969	+	+	Coniferous and deciduous forests	Central Europe
Lysigamaus lapponicus Tragardh, 1910	+		Coniferous and deciduous forests	Europe
Lysigamasus neoruncatellus Schweizer, 1961	+		Litter of forests, in orchards	Central Europe
Lysigamasus sp.	+			
Pergamasus crassipes Linne, 1758	+		Coniferous and deciduous forests	Holarctic region
Pergamasus barbarus Berlese, 1904	+	+	Coniferous and deciduous forests	Central Europe
Vulgarogamasus kraepelini Berlese, 1905	+		Humus, moss, organic matter, compost, in orchard	Europe
Poecilochirus carabi G. &. R. Canestrini, 1882	+		Humus, moss, litter, agroecosystems	Europe, Asia
Parasitellus sp.	+			
VEIGAIDAE				
Veigaia exigua Berlese, 1917	+		Agroecosystems, meadows, silvicolous, compost	Europe
Veigaia planicola Berlese, 1892	+	+	Compost, agroecosystems, silvicolous, organic matter	Europe, Asia
Veigaia nemorensis C.L. Koch, 1939		+	Orchards,fields, meadows, forests, compost, litter	Europe, Asia
Veigaia propinqua Willmann, 1936	+		Coniferous and deciduous forest	Europe, Asia
RHODACARIDAE				
Asca bicornis Canestrini and Fanzago, 1887		+	Moss, litter, meadows, dunes, sand, seashore, dumps	Europe
ASCIDAE				
Arctoseius resinae Karg, 1969		+	Litter, rotting wood	Central Europe
MACROCHELIDAE				
Macrocheles sp.	+	+		
Macrocheles matrius Hull, 1925	+	+	Litter, nests, decaying organic matter	Europe, Asia, USA, New Zealand
Macrocheles montanus Willmann, 1951	+	+	Litter, humus, soil detritus, lichens, nests, mouldering wood substrates	Europe, Asia

Table 2

(continued)

Geholaspis longisetosus Balogh, 1958		+	Litter and heterogeneous soil substrates	Central Europe
Geholaspis mandibularis Berlese, 1904	+	+	Litter, humus, soil detritus, lichens, nests, ant-hills, mouldering wood substrates	Europe, Asia, Australia
LAELAPIDAE				
Hypoaspis aculeifer Canestrini, 1883		+	Litter, humus, orchards, fields, forests	Europe, Asia, South and North America
Pachyseius humeralis Berlese, 1910	+		Litter, soil, raw humus, moss, ant-hills, nests, rotting wood, subcorticolous detritus, wood-destroying fungi, rotting straw, decaying organic remnants and cadavers	Europe
Olopachys vysotskajae Koroleva, 1976	+	+	Litter, soil and wood substrates,of various broad leaved deciduous forests	East Europe
Olopachys suecicus Sellnick, 1950		+	Litter, soil, raw humus, moss, ant-hills, nests, rotting wood, subcorticolous detritus, wood-destroying fungi, rotting straw, decaying organic remnants and cadavers	Europe
PACHYLAELAPIDAE				
Pachylaelaps furcifer Oudemans, 1903	+		Litter, soil detritus, rotting wood substrates, moss, nests	Europe
Pachylaelaps dubius, Hirschmann & Krauss, 1965	+		Litter, humus, moss, nests	Europe
Pachylaelaps pectinifer G. and R. Canestrini, 1882		+	Litter, soil and wood detritus, decaying organic substrates, nests	Europe
Pseudolaelaps doderoi Berlese, 1910		+	Fields, orchards, meadows	Europe
EVIPHIDIDAE				
Eviphis ostrinus C.L.Koch, 1836	+		Litter, heterogeneous soil detritus, moss, rhizosphere of herbs, ant-hills, nests, bark, rotting wood.	Europe
ZERCONIDAE				
Zercon peltadoides Halaskova, 1970	+	+		
Zercon foveolatus Halaskova, 1969	+		Litter, soil detritus, various woodland habitats	Slovakia, Ukraine and Romania
Zercon hungaricus Sellnick, 1958	+		Litter, soil detritus, various woodland habitats	Central and Eastern Europe
Prozercon traegardhi Halbert, 1923	+	+	Litter, humus, soil detritus, moss, nests, ant-hills	Europe
Prozercon carsticus Halaskova, 1963	+	+	Various woodland habitats	Central Europe
Total	**106**	**52**		

CONCLUSIONS

The taxonomical structure was represented by 34 species, belonging to 19 genera and 10 families. The identified species were common for a terrestrial temperate ecosystem. The highest representation had species with a wide distribution in Europe; followed by those from Central Europe; Europe and Asia; Europe, Asia, USA, Australia and New Zealand; East Central Europe and East Europe. Species *Pergamasus crassipes* had a wide biogeographical distribution, being identified in the Holarctic region. On the opposite, species *Zercon foveolatus* was signaled only in Slovakia, Ukraine and Romania.

In spatial dynamics, on the north side of the cliff ecosystem there was recorded the most increased number of species, in comparison with the south side.

An important task for the future research is to identify the specifical microenvironment conditions of the cliff area and their influence on the structure and dynamics of soil predator mites.

In the future it would be very useful if careful and comprehensive sampling of individual cliffs could be carried out in order to determine if cliffs select for these particular groupings on the basis of their functional ecological characteristics.

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ANOPHELES MACULIPENNIS COMPLEX (DIPTERA: CULICIDAE) IN COMANA AREA (GIURGIU COUNTY, ROMANIA)

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Romania is a malaria-free country since 1967; nevertheless, in Europe this disease remains a problem due to the presence of the former vectors, belonging to the *Anopheles maculipennis* complex (Diptera: Culicidae). The current study was carried out between 2005 and 2008 in Budeni village and Comana Forest, Giurgiu County, a former malaria endemic area, and was designed in order to provide further evidence on the status of species composition of the *Anopheles maculipennis* complex. Morphological and molecular identification, blood meal analyses, and ovary dissection for determining the females' physiological age were performed in order to evaluate the risk of malaria re-emergence in this area. A total number of 8.910 adult mosquitoes was collected, 8.733 out of them belonging to the *Anopheles maculipennis* complex. Three *Anopheles maculipennis* s.l. species were recorded: *Anopheles maculipennis* s.str., *Anopheles messeae* and *Anopheles atroparvus*. Our study revealed that *Anopheles messeae* is the most abundant species, the mosquito female's physiological age decreased from 4–6 to 2–3 egg batches and, after blood meal analyses, that the anopheline species are highly zoophilic. In conclusion, on the basis of all these facts, we may assume that the re-emergence of endemic malaria in Romania is an unlikely but not an impossible event.

Key words: Romania, malaria re-emergence, *Anopheles maculipennis* complex, physiological age, zoophily.

INTRODUCTION

WHO officially declared Europe malaria free in 1975 (Bruce-Chwatt & De Zulueta, 1980). Nevertheless, events of the last years, including the general increase of the imported malaria cases and the re-appearance of malaria in countries of the former USSR (Sabatinelli *et al.*, 2001; Romi *et al.*, 2002), autochthonous cases in some Southern European countries (Baldari *et al.*, 1998; Kurdova *et al.*, 2001; Sabatinelli *et al.*, 2001; Kampen *et al.*, 2003) or permanent malaria presence in Turkey (Alten *et al.*, 2000), led to the concern regarding the increasing risk of malaria re-emergence in Europe. In addition, the new aspects of the presence and distribution of European *Anopheles* vectors in relation to climate change (Kuhn *et al.*, 2002) represent the main element of this risk.

In the 1920s–30s, *Anopheles maculipennis* was recognized as the first example of a sibling species complex among mosquitoes and the permanent interest in its systematic appraisal leads gradually to a more natural classification based on morphological similarity and phylogenetic relationships confirmed by

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molecular studies (Hackett & Missiroli, 1935; White, 1978; Linton, 2004; Harbach, 1994, 2004).

Three species of the *maculipennis* complex: *Anopheles atroparvus* Van Thiel, 1927, *Anopheles labranchiae* Falleroni, 1926 and *Anopheles sacharovi* Favre, 1903, are known to be the main efficient historical vectors of malaria in the West Palaearctic Region and remain as principal potential vectors in their present and presumably extended distribution areas in Europe (Bruce-Chwatt & de Zulueta, 1980; Jaenson *et al.* 1986; Ribeiro *et al.*, 1988; Kasap, 1990; Jetten & Takken, 1994; Romi, 1999; Ramsdale & Snow, 2000; Romi *et al.*, 2001; Capinha *et al.*, 2009). *Anopheles sacharovi* is still the primary vector in malaria endemic areas in Turkey (Kasap, 1990; Alten *et al.*, 2000).

In Romania, malaria transmission used to be attributed to four vectors in the past, *Anopheles atroparvus, Anopheles messeae* Falleroni, 1926*, Anopheles maculipennis* s.str.. Meigen, 1818 and *Anopheles sacharovi* Favre, 1903 (Martini & Zotta, 1934; Zotta, 1938, 1943; Zotta *et al.*, 1940). *Anopheles atroparvus* was the main malaria vector with a distribution over all the former endemic areas. Both *An. messeae* and *A. maculipennis* s.str. were weak vectors with a medium index of spontaneous infection not bigger than 0.07 % (Barber & Rice, cited by Zotta in 1943). *A. sacharovi* was the strongest malaria vector with a medium index of spontaneous infection of 1.3 % on the Black Sea shore in 1935, seven times bigger than *A. atroparvus* (Zotta, 1943). *A. sacharovi* was spread only along the Black Sea coast (Lupaşcu *et al.*, 1958), where it disappeared from after malaria eradication (Bîlbîe *et al.*, 1978).

Comana area was a part of the flooded zones of “malaria stratification” in Romania, with *A. messeae* as the numerically dominant species and *A. atroparvus* as the main vector (Zotta, 1938) of malaria in the valley of the Neajlov River. At the beginning of 1950, the Argeş River basin had a proportion of 6.11% malaria cases out of 317,530 inhabitants and Budeni settlement had a proportion of 14.7 % of malaria cases among 1277 inhabitants (Radacovici *et al.*, 1959). The sprayings against *Anopheles* species in Comana area, carried out between 1950 and 1952, led to a decrease of malaria cases to 0.32% (5 cases) in 1952. Beginning with 1953 local transmitted malaria cases were not registered in this area anymore (Radacovici *et al.*, 1959).

The aim of the present study is the evaluation of the *A. maculipennis* complex structure in Comana area under the aspects of the main elements of their present vector capacity. The *Anopheles* potential vectors are present in relevant densities in this area and the investigation performed between 2005 and 2008 puts in evidence the present level of the risk of malaria re-emergence in one of the former malarial areas of the Romanian Plain.

MATERIAL AND METHODS

Investigated area. The investigations on *A. maculipennis* complex populations have been performed in Budeni village and its surroundings, in

Comana Lake and Comana Forest located in the agricultural and touristic Comana area (26°00'-26°10' E and 44°08'-44°14' N), in the South of the Romanian Plain (Fig. 1). Comana area including Budeni and other 4 villages is located in the South-Eastern corner of the middle basin of Argeş River at the confluence of Neajlov and Argeş rivers. Comana Lake, generated and crossed by Neajlov River, has a variable surface (between 700 and 1900 ha) depending on the climate factors, especially rainfall and temperature values. In different parts of this area the presence of the levigated black soils (chernozems) with high concentration of salts favors the *A. atroparvus* larvae development. Comana area has a continental temperate climate with the highest annual mean temperature (10–11 ºC as in Romanian Plain) but with a quite low level of rainfall (450–600 mm/year) irregularly distributed in space and time.

The fauna of Comana area is characterized by a high diversity including 31 mammalian species and 108 bird species among them being numerous migrating birds that breed in summer in different wet habitats.

Mosquito collections. Resting adult mosquitoes were collected from a cattle shelter in Budeni village, monthly, from March to October, in 2005 and 2006, from May to October in 2007 and from March to August in 2008, using battery powered aspirators. During the study period, the mosquito fauna from Comana Forest was also investigated occasionally in order to establish the proportion of the anophelines within outdoor fauna, using different methods: battery powered aspirators, CDC-light traps and Bird-Baited-Traps.

Mosquitoes' analysis. Taking into account that the species of *Anopheles maculipennis* complex are a group of closely related species, difficult to distinguish on the basis of their adults' morphology, the females were put in individual tubes in order to lay eggs and then they were identified up to species level, based on the eggs exochorion ornamentation under a Nikon stereoscopic zoom microscope.

Previous studies of the *A. maculipennis* complex indicate that differences in vector capacity are due to host feeding preferences (Fantini, 1994). Thus in 2005, the abdomens of 150 freshly fed females were separate from the rest of the body with a scalpel, squashed individually on confetti filter papers and kept for blood meal analysis. Feeding preferences were identified by ELISA (Beier *et al.*, 1988) using seven antibodies: anti-bovine, anti-chicken, anti-dog, anti-horse, anti-human, anti-pig, and anti-sheep. The head-thorax parts of the same females were kept for molecular identification (Proft *et al.*, 1999).

The physiological age of the females was established by dissections and control of the state of their ovaries: after the females laid eggs, a fraction of them were killed, immobilized for removing the legs and the wings and then placed on a slide in a drop of physiological saline, arranged in a more suitable position for dissecting the ovaries (Beklemishev *et al.*, 1959; Detinova, 1962) and counting the number of permanent dilatations of the ovarian duct. Observations about the female's parity rate were also made.

RESULTS AND DISCUSSION

In the past, in Comana area, the *A. maculipennis* complex was represented by mixed populations of *A. messeae, A. atroparvus* and *A. maculipennis* s.str. species (Zotta 1938; Zotta *et al.* 1940; Radacovici *et al.* 1959, Nicolescu 2000). A new species of *maculipennis* complex, *Anopheles daciae* Linton, Nicolescu & Harbach 2004, most similar to and sympatric with *A. messeae*, was identified in Budeni (and in other former malaria endemic areas in Romania) and a single specimen of *A. melanoon* was once recorded (Nicolescu *et al.*, 2004).

A great difference, in species' number and composition, and especially of the numerical abundance was registered between outside mosquito (Comana Forest) and inside mosquito fauna (Budeni village).

Outside mosquito fauna. In Comana Forest 16 mosquito species were identified, belonging to *Anopheles, Aedimorphus, Culex, Ochlerotatus, Dahliana* and *Coquillettidia* genera. *Culex pipiens* Linnaeus, 1758 was the most dominant mosquito, but moderate dominance registered also *Ochlerotatus annulipes* Meigen, 1830 and *Ochlerotatus cantans* Meigen, 1818. The abundance of the *Anopeles maculipennis* s.l. (3.28 %) within outside fauna was very low (Fig. 2).

Fig. 1. The investigated area including the collecting sites.

Inside mosquito fauna. Between 2005 and 2008, one animal shelter was investigated for the presence of the *maculipennis* complex species. A total number of 8.910 mosquitoes was collected, 8.864 (99.48 %) out of them were anophelines, of which 8.733 (98.52 %) belonging to the *Anopheles maculipennis* complex. Eight species belonging to five genera were identified resting inside the animal shelter: *Anopheles atroparvus* Van Thiel, 1927, *Anopheles maculipennis* s.str., *Anopheles messeae* Falleroni, 1926, *Anopheles claviger* Meigen, 1804, *Aedimorphus vexans*

Meigen, 1830, *Culex pipiens* Linnaeus, 1758, *Culiseta annulata* Schrank, 1776 and *Ochlerotatus caspius* Pallas, 1771.

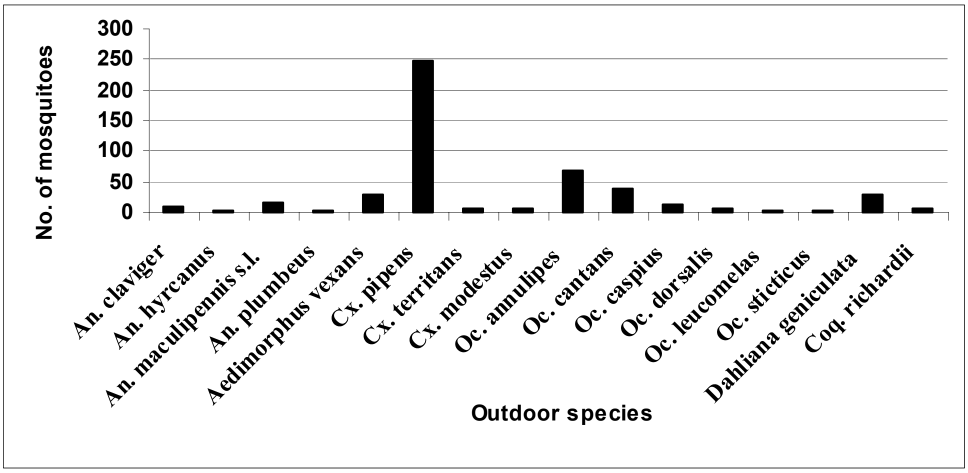

Fig. 2. Mosquito species collected in Comana Forest.

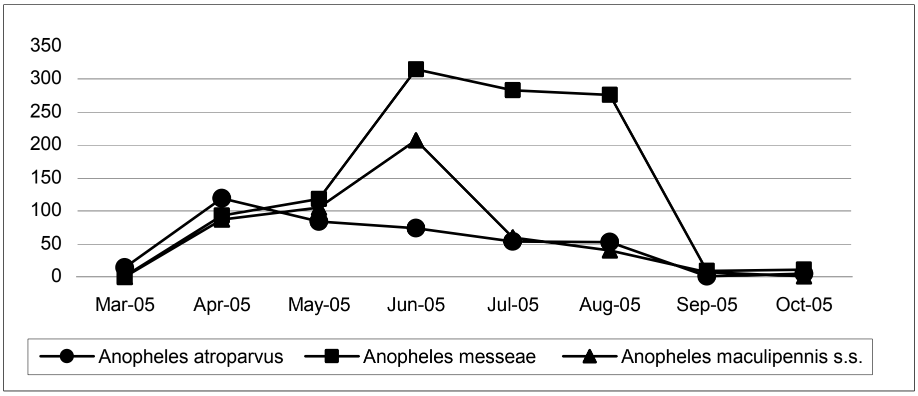

Fig. 3. Seasonal dynamics of *An. maculipennis s.l.* adults in 2005.

Our study was focused on *A. maculipennis* complex, which dominated the inside mosquito fauna, by establishing its presence, abundance and dynamics, host preferences, and the females physiological age.

During the 4-year study 4.329 anophelines (49.57 %) out of the total number was identified by the egg morphology and only 150 (1.72 %) by PCR technique. The rest of the 48.71 % (4.254) *Anopheles maculipennis* species died after arriving in the laboratory and because they could not be identified to species were only registered as mortality rate.

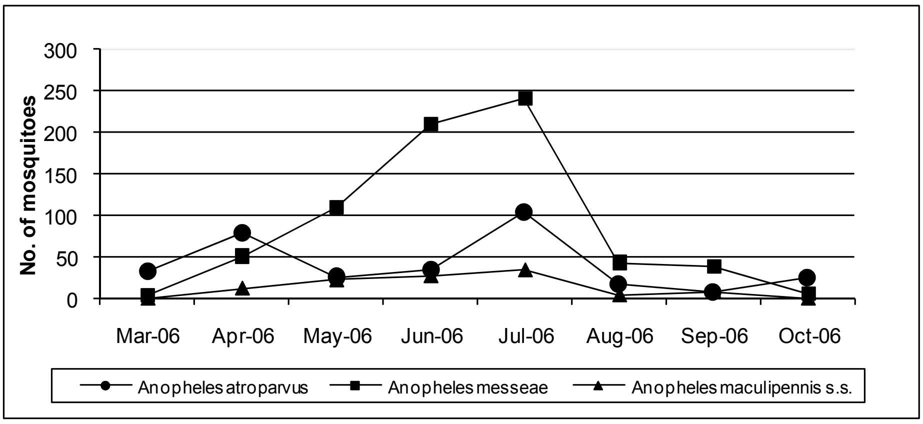

Fig. 4. Seasonal dynamics of *An. maculipennis s.l.* adults in 2006.

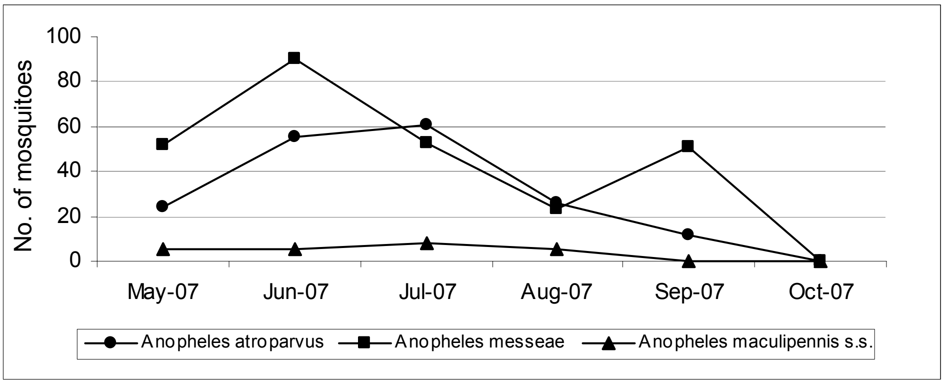

Fig. 5. Seasonal dynamics of *An. maculipennis s.l.* adults in 2007.

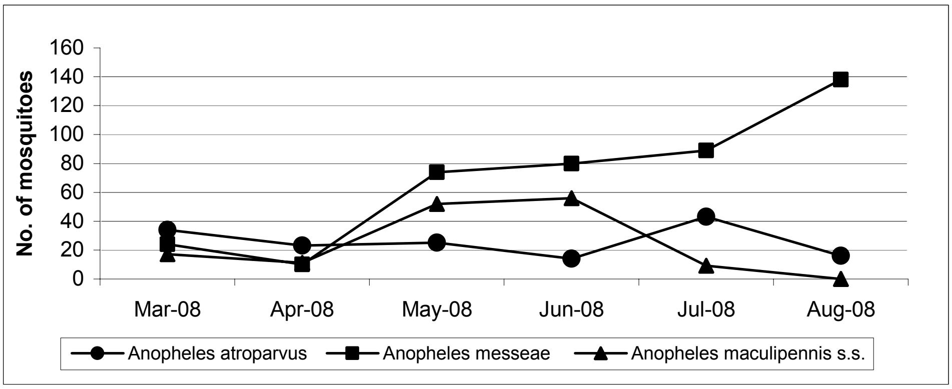

Fig. 6. Seasonal dynamics of *An. maculipennis s.l.* adults in 2008.

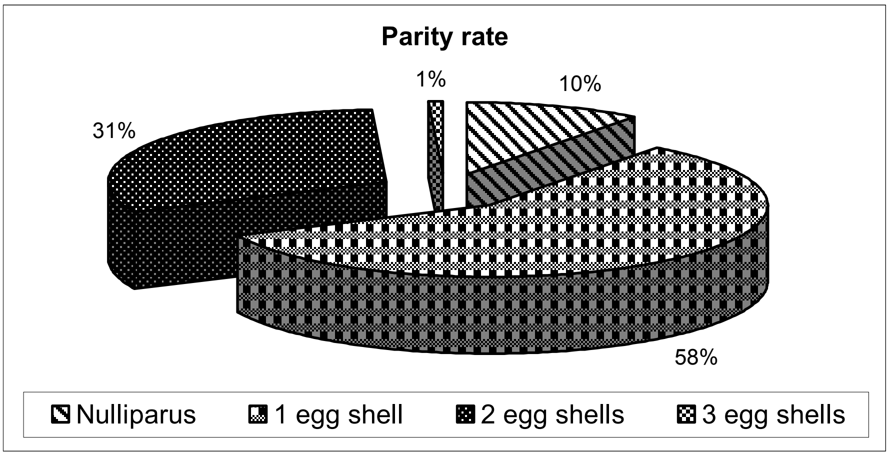

Fig. 7. *Anopheles* parity rate.

Only three species from the *Anopheles maculipennis* complex (*A. atroparvus*, *A. messeae*, *A. maculipennis* s.str.) were found by morphological and molecular identification.

As dominance, *A. messeae* was the most dominant species (57.58%), *A. atroparvus* (24.42%) and *A. maculipennis* s.str. (18%) were less abundant being more accessory species.

Seasonal and annual dynamics of the adults' abundance. We observed that the annual dynamics of the local *A. maculipennis* s.l. species followed a similar trend during all the 4 years. Comparatively with 2005, between 2006 and 2008 was recorded a numerical decrease for all three species, three times in 2007 and 2008 for *A. atroparvus*, five times in 2007 for *A. messeae* and in 2006 for *A. maculipennis* s.str., and even over twenty times for *A. maculipennis* s.str. in 2007 (Table 1).

The comparison of the historical data (Table 2), accumulated between 1933 and 2003, revealed that for more than 50 years (1933–1984), *A. messeae* was the dominant species, with a higher level of abundance between 1933-1940 and 1974–1984, and a lower level of abundance between 1985 and 2003. Eighteen years (1985–2003), *A. messeae* was surpassed in abundances by *A. atroparvus*. A lower level of abundance registered *A. maculipennis* s.s in 1933–1940 and *A. atroparvus* in 1933–1940 and 1974–1984.

Adults' seasonal dynamics, assessed between March–October 2005–2006, May–October 2007 and March–August 2008, is different for all the three species (Figs. 3, 4, 5, 6). The mosquitoes appeared first in March (excepting 2007) and their activity was extended until September in 2005 and October in 2006 and 2007. In August 2008 the dynamics survey was called off. *A. messeae* adults' abundance was high from June till August 2005, from May till July 2006, in June and September 2007 (bimodal dynamics) and from May till August 2008. During the survey period *A. atroparvus* adults' abundance was low, excepting April 2005, April and July 2006, June and July 2007 and July 2008. Regarding *A. maculipennis* s.str., its adults' abundance was high in June 2005, and May–June 2008, and low during the entire 2006–2007 period.

Table 1

Dymamics of *Anopheles maculipennis* species abundance between 2005 and 2008

Year	*A. atroparvus*		*A. messeae*		*A. maculipennis* s.str.	
2005	454	9.81 %	1201	25.96 %	533	11.52 %
2006	446	9.64 %	696	15.04 %	111	2.4 %
2007	179	3.87 %	269	5.81 %	23	0.5 %
2008	155	3.35 %	415	8.97 %	145	3.13 %

Table 2

Dymamics of *Anopheles maculipennis* species abundance identified in Comana area between 1933 and 2003

Period	*Anopheles* sp. (%)		
	A. maculipennis s.str.	*A. messeae*	*A. atroparvus*
1933–1940	3.6	89.0	7.4
1950–1952	12.5	65.3	22.2
1974–1984	14.8	84.1	1.1
1985–2003	13.1	37.8	49.1

Breeding sites. During the whole period, the potential anopheline breeding sites situated close to the selected resting site were regularly visited. Only once in 2005 and twice in 2006 these sites were positive for anophelines showing a very low larvae density of 1–2/5 dips. The adults emerged from these larvae were not identified to species level.

Host preference. The estimation of the preferential feeding habits has been carried out on blood from the gut of freshly-fed *Anopheles* sp. by ELISA technique, using seven antibodies (anti-bovine, anti-chicken, anti-dog, anti-horse, anti-pig, anti-sheep and anti-human), we established that the species belonging to the *An. maculipennis* complex fed predominantly on cattle (85.5 %), but had also some preferences for pigs, horses and chickens. None of the investigated mosquitoes fed on humans (Fălcuță *et al*., 2010).

Physiological age. Parity rate showed an analogous trend during the entire period. It was studied by correlating the observations obtained by dissections on the Christophers' ovarian developmental stages, the appearance of the Malpighi tubes, and the skeins of tracheoles on the surface of ovary and especially by the number of dilatations which remain on the ovarian tube after each oviposition.

We observed that the number of nulliparous over-wintering females gradually decreased until the end of April, when we registered an increase due to the appearance of the first generation of mosquitoes. The maximum number of dilatations found by us was two, with the exception of a small number of *Anopheles* females (n = 26) which were found with three dilatations at the end of August 2006 (Fig. 7). The number of ovipositions by an *Anopheles* female shows the physiological age of the respective female and if the female could infect or not the humans. The *Anopheles* species female could be infectious for humans at about 18 days old, namely when it has three or more dilatations of the ovarioles.

Historical data show that in the Romanian climate conditions, the duration of *Plasmodium* species' sporogony is about 14–18 days, and the *Anopheles* female completes its gonotrophic cycle in 5–6 days. The same historical data show that, during the endemic period, in Romania (including the Romanian Plain) the *Anopheles* females used to lay about 4–6 and even more egg batches (Cristesco, 1966). During our study we detected that at present this number decreased to 2–3 egg batches.

CONCLUSIONS

Our study reveals that in Comana, a former malaria-endemic area, the *Anopheles* mosquito fauna comprises the same species of the *A. maculipennis* complex detected during malaria-endemic period (*Anopheles atroparvus* van Thiel 1927, *Anopheles maculipennis* s.str. Meigen 1818, *Anopheles messeae* Falleroni, 1926). The species level was identified based on the eggs chorion ornamentations and by PCR technique.

We found changes in the species proportion of the *A. maculipennis* complex reported to the past: while *A. messeae* decreased 1.54 times, *A. atroparvus* and *A. maculipennis* s.str. increased over three, respectively five times.

Comparing our results with the previous ones we observed that *A. messeae* is still numerically dominant within the *A. maculipennis* complex but also that *A. atroparvus* alarmingly increased. Taking into account that *A. atroparvus* was a strong malaria vector in Romania this finding could be an important one in case of some future changes regarding malaria spreading in Europe.

An important thing found by us is that the *Anopheles* mosquito female's physiological age decreased; we did not find more than three egg batches, comparatively with 4–6 in the past.

Correlating all data with the results of the blood meals analysis, which show that the *Anopheles* species are highly zoophile and that none of the mosquitoes of the *maculipennis* complex had fed on human blood even if humans were available, we may assume affirmation that in Romania malaria reintroduction based on autochthonous transmission is an unlikely but not an impossible event.

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XYLOPHAGOUS INSECT SPECIES, PESTS IN WOOD COLLECTION FROM THE ROMANIAN PEASANT MUSEUM

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The results integrated in this paper present analysis of 1300 wood pieces from Romanian Peasant Museum wood collection, to detect wood boring insects attack. The wood pieces, from eight categories (furnishings, house wares, distaffs, hank reels, musical instruments, cart man tallies, twisting spindles, handicrafts) belong to 20 forest species. From the investigated pieces, wood type was identified for 797. In the absence of the alive or dead insects (probably due to an old attack), to establish species responsible for the attack, the size and shape of adults exit holes were used. A number of 368 wood pieces were damaged by xylophagous species, and insect species were determined on 222 of these. Thus, attack of seven insect species all belonging to Coleoptera order were identified: *Anobium punctatum*, *Hadrobregmus pertinax*, *Ptilinus pectinicornis,* and *Xestobium rufovillosum* (Anobiidae family), *Lyctus linearis* and *Trogoxylon impressum* (Lyctidae family), and *Hylotrupes bajulus* (Cerambycidae family). Single insect species attack (particularly of the Anobiidae family) was involved in 81 % of the damaged pieces. Of the remaining 19 %, representing associated attacks by two or three species, dominants were attacks involving *Anobium punctatum* species. The most noxious pests identified by our analysis were *Anobium punctatum*, *Hadrobregmus pertinax*, *Xestobium rufovillosum* (detected on 62 % of all damaged pieces), and *Hylotrupes bajulus*.

Key words: wood destroying insects, wood pests, wood-degrading organisms, biodeterioration, xylophagous attack.

INTRODUCTION

The Romanian Peasant Museum, part of the European family of Museums of Popular Art and Traditions, is a national museum, under the Ministry of Culture's patronage. In 1996, the Museum receives the European Museum of the Year Award (EMYA). It is in possession of an especially rich collection (about 900,000 objects): pottery, hardware, popular clothes, interior tissues, religious exhibits, furnishings and other wood objects. Of these, wood pieces, furnishings and hardware count over 8,000 pieces.

In museums special conditions, the main wood degrading organisms are fungi, insects, and bacteria, and their action is categorized as biodeterioration of wood (Highley, 1999). For wood museum collections, the insect pests (coleopterans) are the main wood-degrading organisms and can cause the most of the damages. One of the first evidence for insect attack of wood pieces (artifacts, specimens) is the presence of particular signs on the wood surface, named "exit holes" (emergence holes, flight holes or shot holes). It is not the "entrance" holes, because in the oviposition period, the insect females remain outside of the wood, depositing their eggs into the surface wood pores, in cracks and crevices in the wood, or placing them by boring into the wood. The eggs hatch and the larvae then begin to eat and pupate. Once the metamorphosis is complete, the pupae become adults, which make these exit holes through the surface of the wood, with their mandibles, emerge from the wood, fly off, mate, and start new infestations.

The species that destroy the wood objects in museums, houses or buildings belong to Coleoptera orders, Anobiidae, Cerambycidae, Lyctidae and Bostrichidae families. The adults do not damage the wood and only have reproductive role, but their larvae excavate burrows in hardwoods and softwoods, producing powder-like sawdust (named frass), that is pushed out of the emergence holes.

In Anobiidae family (deathwatch beetles) the larvae of some species are able to directly digest wood cellulose without the aid of a protozoan (and the growth of stain fungi within the galleries can serve as a source of protein), but other species can digest the cellulose, with a special enzyme. As a result, Anobiids infest all types of seasoned wood, although they prefer the sapwood of softwoods.

The species of family Lyctidae (true powder post beetles) infest hard woods. Their larvae are unable to digest cellulose and hemicellulose which form the wood cell walls and the sources of their food are different cell contents (starch, sugars, and proteins). The undigested cellulose and hemicellulose pass through the larvae digestive tract and account for the large amount of powdery frass. Because heartwood is practically free of starch, and softwoods usually have low starch content, they are not infested by Lyctidae.

In Cerambycidae family (long-horned beetles), the larvae hollow out extensive galleries in seasoned softwood (*e.g.*, pine, spruce and fir) but can re-infest seasoned lumber in houses or museums. Even though their larvae can directly digest cellulose (without the aid of yeasts or protozoans) they still need some other cell contents (sugars, starches and protein).

In Romania, the wood-degrading insects were detected in Museums, Wooden Churches, historic monuments (Mustață, 1998; Bucşa *et al.*, 2005; Moşneagu & Gămălie, 2006; Antonie & Teodorescu, 2009; Bucşa & Bucşa, 2005, 2009; Moşneagu, 2010).

MATERIAL AND METHODS

We performed investigations (2009-2010) in Wood Collection pieces of the Romanian Peasant Museum, to detect wood boring insects. The insect wood pest genus or species identification usually requires the examination of the adult insect itself. Because the wood attack is old, manifested probably in the original place (rural households) or in the museum, the adult beetles were usually missing from the analyzed pieces. The adult exit holes form and size are characteristic, and help to place the insects that cause the attack, in their families, genera and even species. The exit holes of Anobiidae and Lyctidae are perfectly rounded while those of *Hylotrupes bajulus* are oval (Leary, 2002).

RESULTS AND DISCUSSION

Of 1300 wood objects controlled, from Romanian Peasant Museum wood collection, 368 (28 %) were damaged by wood-infesting, xylophagous insects species. Many categories of wood pieces were controlled: furnishings, house wares, distaffs, hank reels, musical instruments, cart man tallies, twisting spindles, and handicrafts. The evidence of insect activities is not the presence of alive or dead adults, but that of the special signs on wood surface – the adult exit holes. Indeed, the surface of infested wood pieces appears to be perforated by few or many holes. The size and shape of adult exit holes were circular, 1.6 to 3 mm in diameter in Anobiids (1.5-2.0 mm in *Anobium punctatum*, 2-3 in *Hadrobregmus pertinax,* about 1-1.5 mm in *Ptilinus pectinicornis*, 3-4 (2-4) mm in *Xestobium rufovillosum*), circular, feeble oval, in Lyctidae (1 mm (0.8-2) in *Lyctus linearis*, 2-3 mm in *Trogoxylon impressum*) and broadly oval, 4-5 (2-4, 3-6) mm in diameter in *Hylotrupes bajulus*.

Wood preferance are hard-wood and soft-wood to *Anobium punctatum* and *Trogoxylon impressum*, coniferous to *Hadrobregmus pertinax* and *Hylotrupes bajulus*, broadleaved (especially *Quercus* and *Fagus*), to *Ptilinus pectinicornis*, broadleaved to *Lyctus linearis*, broadleaved and hard-wood with fungal decay already present to *Xestobium rufovillosum*.

From 222 of the 368 damaged wood pieces (60.32 %) the wood-boring insect species responsible for the attack were identified: 184 were detected with adult exit holes produced by Anobiidae species, 63 by Lyctidae and 49 by Cerambycidae (in single or associated attack).

Seven insect species were detected: *Anobium punctatum* (De Geer, 1774), *Hadrobregmus pertinax* (Linnaeus, 1758) (= *Anobium pertinax*), *Ptilinus pectinicornis* (Linnaeus, 1758), *Xestobium rufovillosum* (De Geer, 1774) (Anobiidae family), *Trogoxylon impressum* (Comolli, 1837) (= *Lyctus impressum*), *Lyctus linearis* (Goeze, 1877) (Lyctidae family) and *Hylotrupes bajulus* (Linnaeus, 1758) (Cerambycidae family).

The most noxious pests were Anobiidae species that produced serious damage of wood pieces (62% of all damaged pieces). The most common and economically damaging Anobiids are *Anobium punctatum*, *Hadrobregmus pertinax, Xestobium rufovillosum*. We also found *Hylotrupes bajulus*, one of the most destructive insects from Cerambycidae family.

In 81 % of the 222 damaged pieces we registered attacks of single species, especially Anobiidae while in the remaining 19 % we detected associated attacks of two or three species (Figs. 1, 2, 3). In associated attack with two beetle species *Anobium punctatum* was dominant, being present in 91 % of these associations, from this 56% with other anobiid species, and especially with *Xestobium rufovillosum*. *Hylotrupes bajulus* was present in associated attack with one species of *Anobiidae* on 28 % of wood pieces (Fig. 4). In three beetle species associated attacks, *Anobium punctatum* and *Hylotrupes bajulus* were 100 % present (Fig. 5). Lyctidae species were present in 16 % of two beetle species associated attacks and in 25% of three beetle species associated attacks.

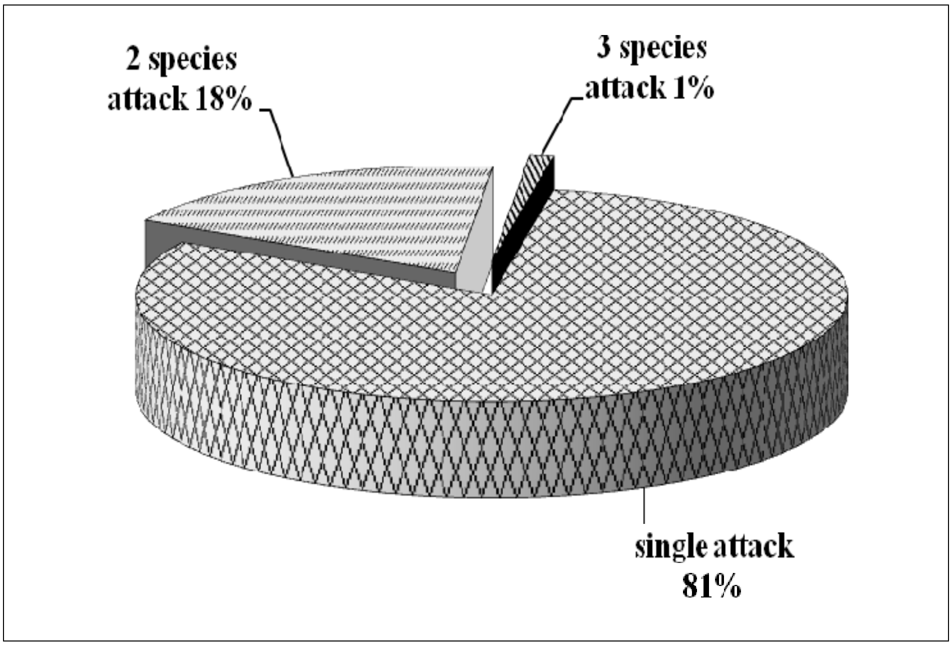

Fig. 1. Ratio between the number of pieces with single and multiple attacks.

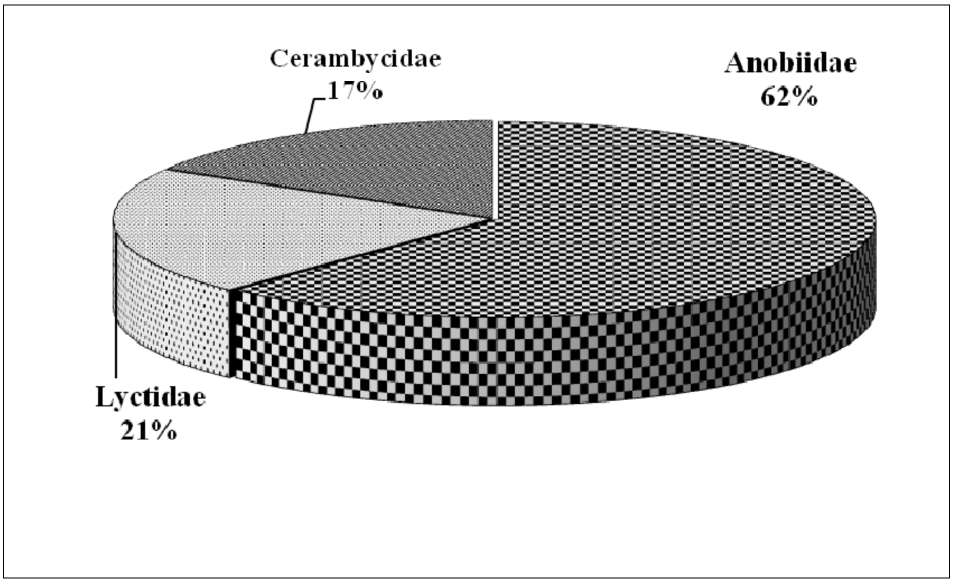

Fig. 2. Ratio between the three families involved in single attacks.

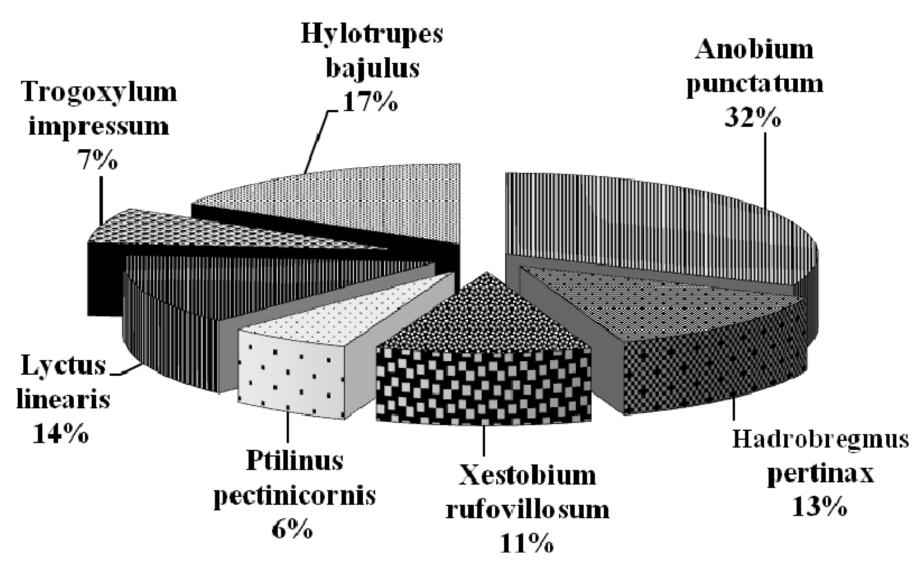

Fig. 3. Insect species involved in single attack.

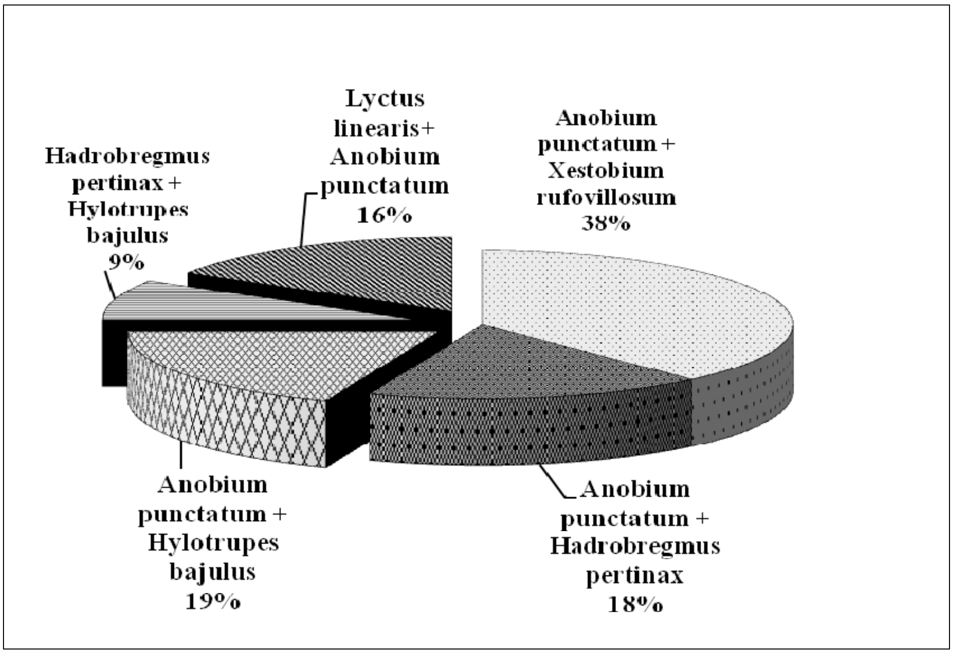

Fig. 4. Insect species involved in double attack.

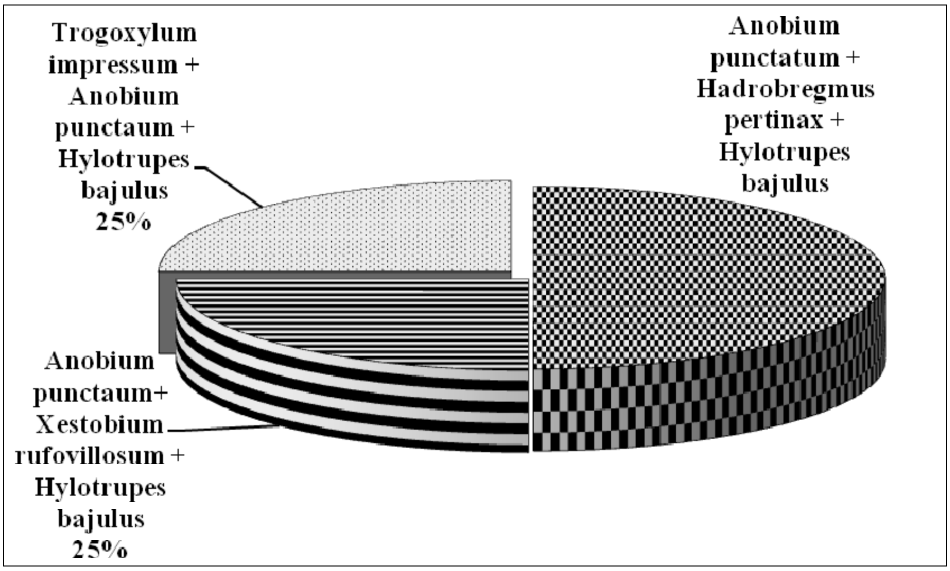

Fig. 5. Insect species involved in triple attack.

Because the etiquettes were missing, the wood forest species could be determined in only 61% of the wood pieces. Thus, wood pieces belonging to 20 tree species were established and controlled in the museum: *Abies* sp., *Acer pseudoplatanus*, *Acer campestre*, *Betula pendula*, *Carpinus betulus*, *Cerasus avium*, *Cerasus vulgaris*, *Cornus mas*, *Corylus avelana*, *Fagus sylvatica*, *Fraxinus* sp., *Juglans regia*, *Populus* sp., *Prunus cerasifera*, *Prunus domestica*, *Pyrus communis*, *Robinia pseudacacia*, *Salix* sp., *Tilia* sp., *Quercus* sp. Of the 222 damaged wood pieces, a number of 201 (90.54%) were from *Fagus sylvatica*, *Abies*, *Corylus avellana*, *Acer pseudoplatanus* and *Fraxinus* wood (Figs. 6, 7, 8, 9).

The attack of *Hylotrupes bajulus*, single or in association with one or two anobiid species, was registered only on *Abies* wood pieces. Even if *Hylotrupes* attack was registered on 38% of *Abies* pieces, Anobiidae attack remained dominant (52%) (Fig. 10). On *Fagus sylvatica* wood pieces, dominant was Anobiidae species attack (79%), single species or in association; Lyctidae attack was also significant (Fig. 11). On *Acer pseudoplatanus*, *Tilia* and *Quercus* wood pieces, Anobiidae were dominant on 90%, 87%, respectively 86% of pieces, as single and double species or in association with Lyctidae (Figs. 12, 13, 14). On *Corylus avellana* and *Cornus mas* wood pieces, Anobiidae dominance (single species, double or in association) decreased (66%, respectively 61%) and Lyctidae dominance increased to 45%, respectively 35% (Figs. 15, 16).

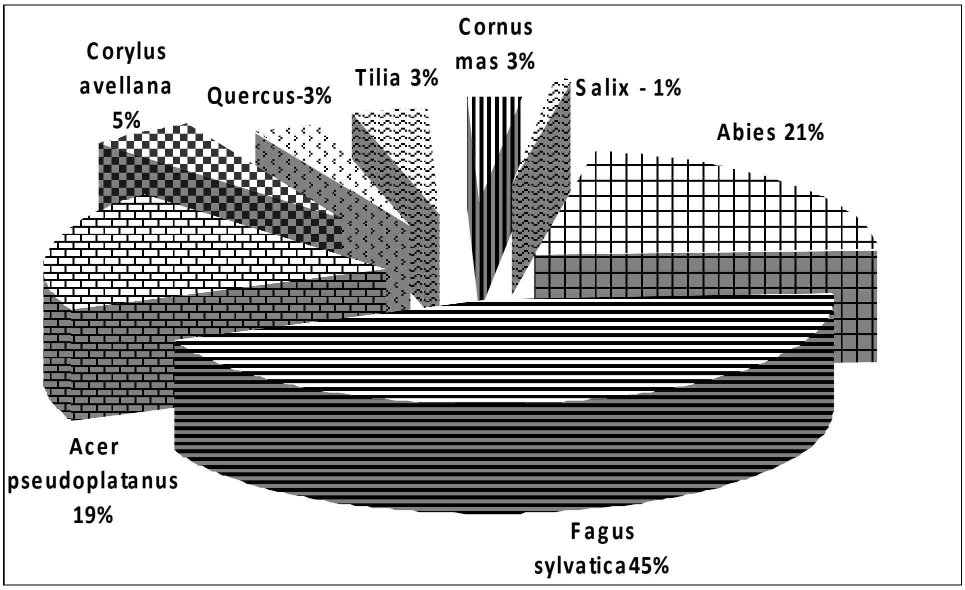

Fig. 6. Wood type with Anobiidae single attack.

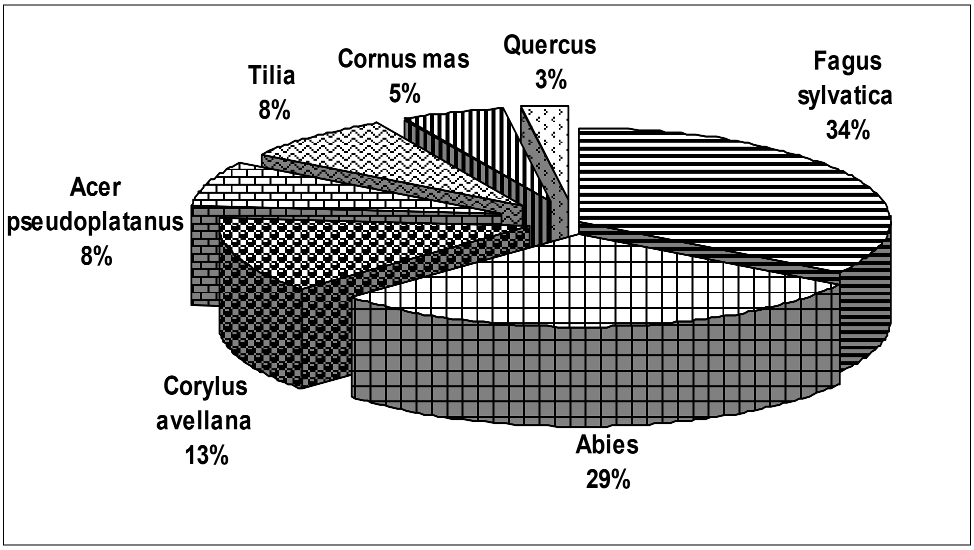

Fig. 7. Wood type with Anobiidae associate attack.

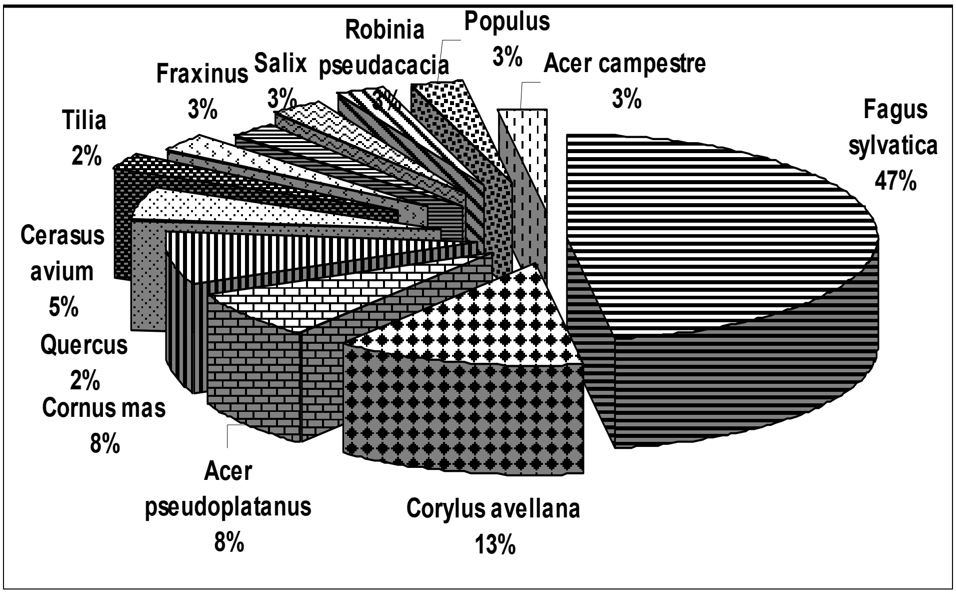

Fig. 8. Wood type with Lyctidae single attack.

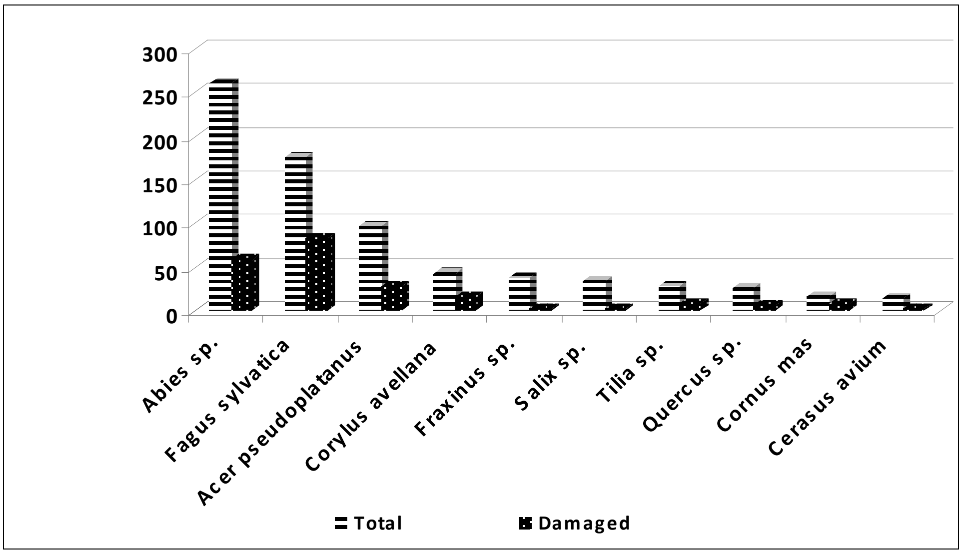

Fig. 9. Ratio of the damaged wood pieces types.

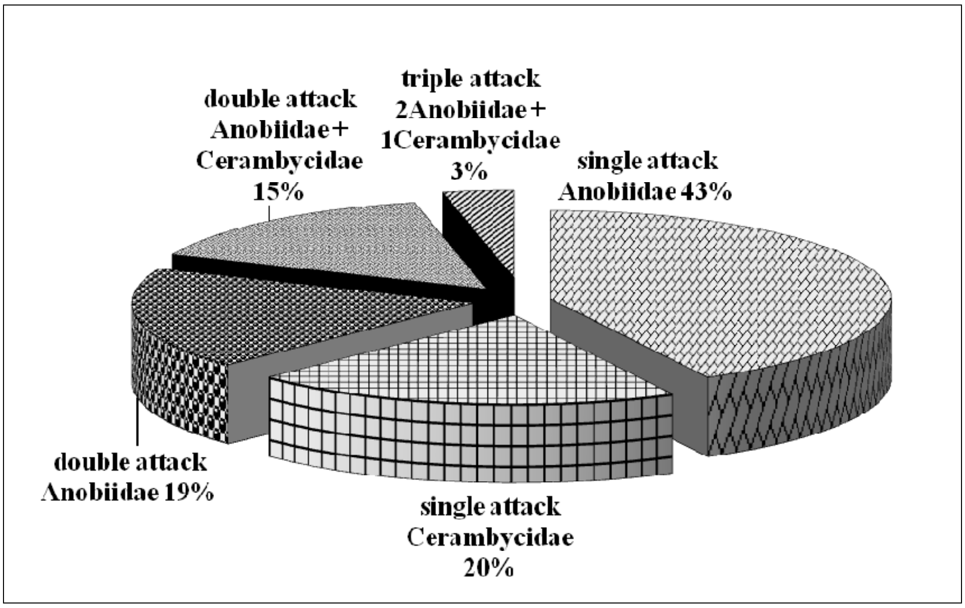

Fig. 10. Single and associate attack on *Abies* wood pieces.

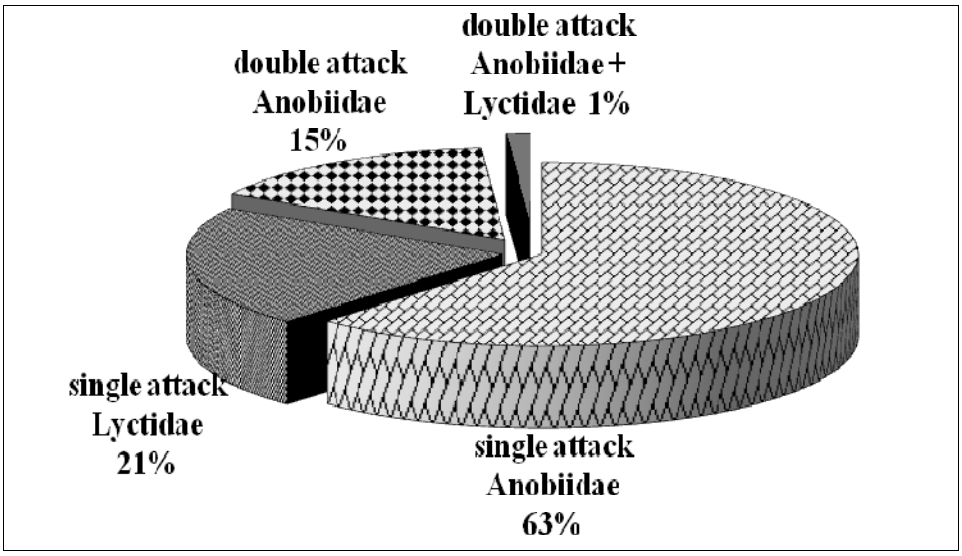

Fig. 11. Single and associate attack on *Fagus sylvatica* wood pieces.

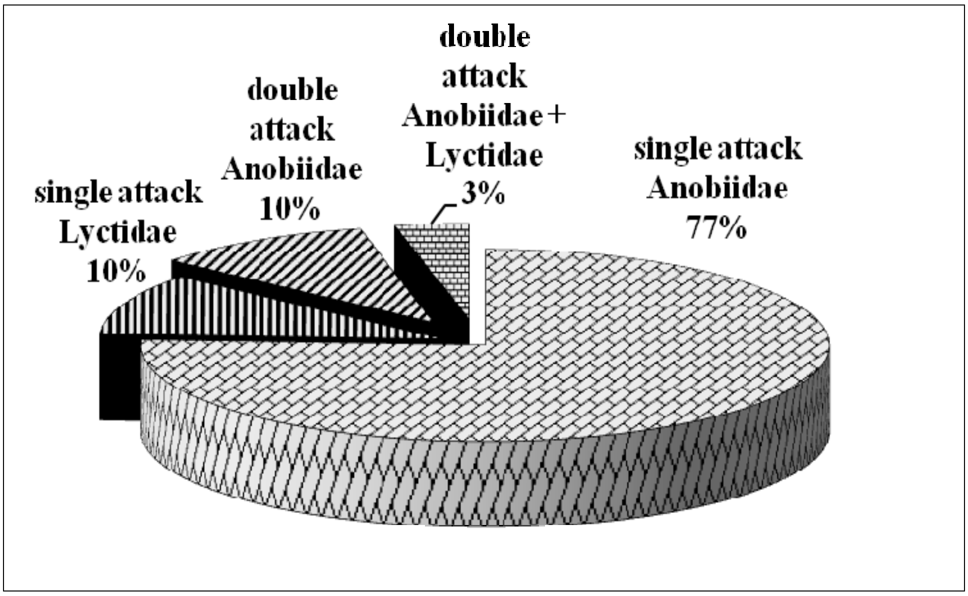

Fig. 12. Single and associate attack on *Acer pseudoplatanus* wood pieces.

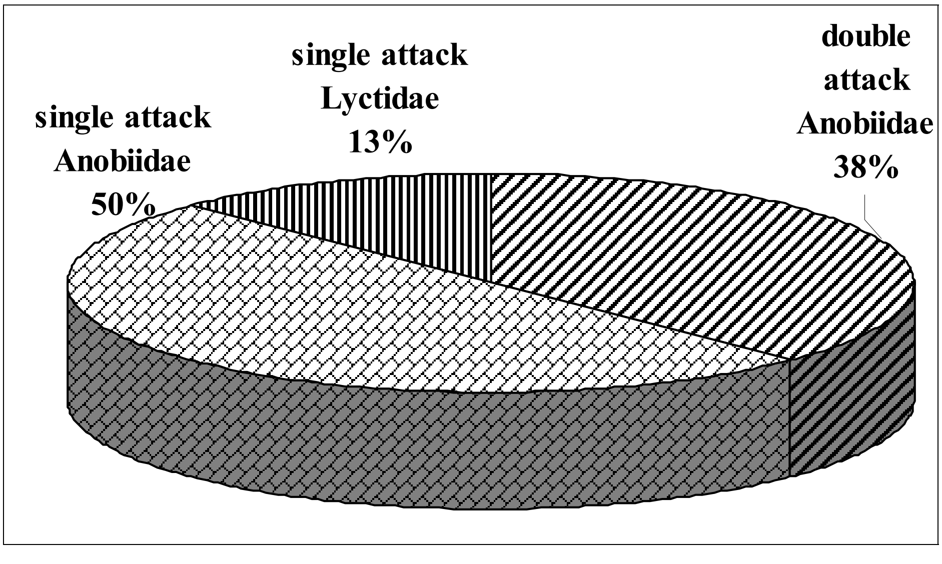

Fig. 13. Single and associate attack on *Tilia* wood pieces.

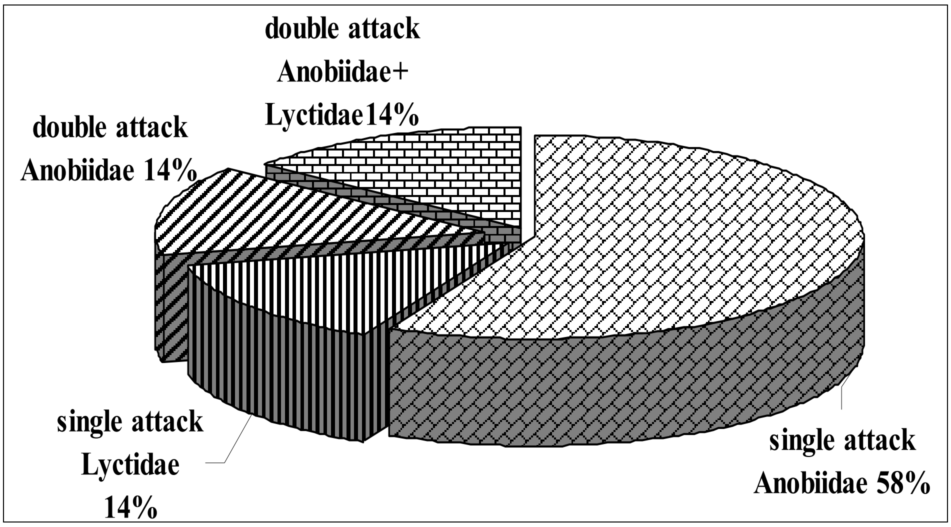

Fig. 14. Single and associate attack on *Quercus* wood pieces.

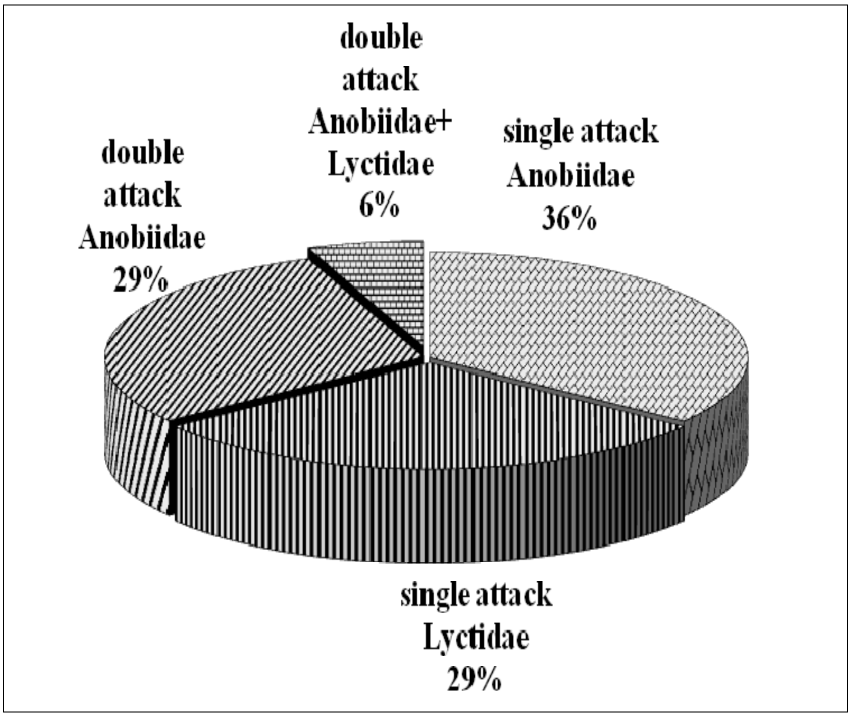

Fig. 15. Single and associate attack on *Corylus avellana* wood pieces.

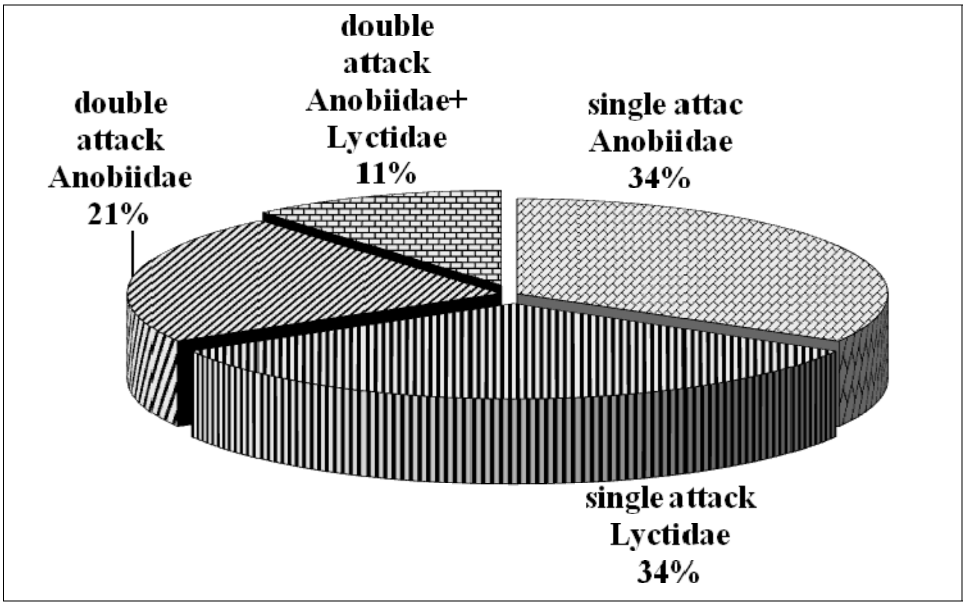

Fig. 16. Single and associate attack on *Cornus mas* wood pieces.

Our investigations underline danger of insect pest attack upon Romanian Peasant Museum wood pieces. The biological degradation of the wooden monuments and objects is both a serious and a sensitive arising issue, which sometimes can only be solved by interdisciplinary collaboration with participants from different fields, such as conservator-restorers, biologists specialized in entomology, engineers (Chacula, 2009). To resolve pests problem in museums it is necessary to prevent the introduction of wood pieces bearing alive insects (by inspection, isolation and treatment of all the pieces), and to eliminate danger of the newly installed wood insect pests from outside sources. In storage areas, coolness, dryness and warmth can prevent insects' development. According to Odegaard (1991), Pinniger & Winsor (2004), the Integrated Pest Management approach in museums includes assessment of insect problems, prevention of infested materials introduction into museums, careful control of entry points, and eradication of pests detected in the museum.

CONCLUSIONS

In Romanian Peasant Museum wood collection, 1300 wood pieces (furnishings, house wares, distaffs, hank reels, musical instruments, cart man tallies, twisting spindles, handicrafts) were controlled to detect wood-boring insect attacks.

There were established wood pieces belonging to 20 tree species: *Abies* sp., *Acer pseudoplatanus*, *Acer campestre*, *Betula pendula*, *Carpinus betulus*, *Cerasus avium*, *Cerasus vulgaris*, *Cornus mas*, *Corylus avelana*, *Fagus sylvatica*, *Fraxinus* sp., *Juglans regia*, *Populus* sp., *Prunus cerasifera*, *Prunus domestica*, *Pyrus communis*, *Robinia pseudacacia*, *Salix* sp., *Tilia* sp., *Quercus* sp.

Of wood pieces, 28 % were damaged by seven insect xylophagous species belonging to Coleoptera order, from Anobiidae family (*Anobium punctatum*, *Hadrobregmus pertinax*, *Ptilinus pectinicornis*, *Xestobium rufovillosum*), Lyctidae family (*Lyctus linearis*, *Trogoxylon impressum*) and Cerambycidae family (*Hylotrupes bajulus*).

Single species attack (81% of attacked pieces) was dominant, especially by Anobiidae family (*Anobium* and *Xestobium* species). The most noxious insect pests were Anobiidae (especially *Anobium punctatum*, *Hadrobregmus pertinax* and *Xestobium rufovillosum*) and Cerambycidae (*Hylotrupes bajulus*) species.

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* * *, http://www.kendal.bioresearch.ca.uk/domest.htm
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* * *, http://species.wikimedia.org/wiki/Hadrobregmus_pertinax
* * *, http://www.kendalluk.com/woodworm.htm Wood-Boring Beetles
* * *, http://termite-office.web.officelive.com/insectesxylophages.aspx
* * *, http://zipcodezoo.com/Animals/H/Hylotrupes_bajulus/use Longhorn Beetle.

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METRIOPTERA (*ZEUNERIANA*) *AMPLIPENNIS* (BRUNNER VON WATTENWYL, 1882) – NEW ORTHOPTERA SPECIES FOR THE DANUBE DELTA BIOSPHERE RESERVE

GABRIEL LUPU

For the first time observed in Romania in August 2006, near the Cernavodă city, and indicated like new genus and species for Romanian territory, *Metrioptera* (*Zeuneriana*) *amplipennis* was found again in 2009 in the Danube Delta Biosphere Reserve, near the Maliuc village, this area being the newest eastern known limit of distribution for this species belonging to Orthoptera order.

Key words: *Metrioptera* (*Zeuneriana*) *amplipennis*, Orthoptera, Danube Delta Biosphere Reserve.

INTRODUCTION

The *Metrioptera* genus, *Zeuneriana* subgenus (*Tettigoniidae* family, *Platycleidini* tribe) includes 4 species outspread in south Europe: *Zeuneriana abbreviata* (Pirinei Mountains), *Zeuneriana amplipennis* (Serbia and Romania), *Zeuneriana burriana* (Spain) and *Zeuneriana marmorata* (Italy and Slovenia) (Iorgu, 2009).

Metrioptera (*Zeuneriana*) *amplipennis* Brunner von Wattenwyl, 1882 was considered endemic to northern Serbia, and has been recorded within the narrow zone of mesophyllic and hygrophyllic communities surrounding the former Yugoslavian part of the Danube and Sava rivers. It is highly unusual that this species has never been recorded outside this region, although the appropriate habitats are relatively widespread (http://www.biopolitics.gr/HTML/PUBS/VOL6/HTML/radovic.htm).

The species prefer area with abundant vegetation-plants with no more than 30-50 cm tallness, near the river shore. It is a very shy bush-cricket, which quickly jumps toward the ground and hides in the thick bushes. For the first time it was observed in Romania in August 2006, near the Cernavodă city, and is indicated like a new genus and species for the Romanian territory (Iorgu, 2009).

On the other hand, the Danube Delta Biosphere Reserve is characterized by having a rich and diverse fauna of Orthoptera with about 80 known species (Iorgu *et al*., 2008), one species (*Isophya dobrogensis*) being endemic on the northern part of Lake Razim (Kis, 1994).

MATERIAL AND METHODS

The Danube Delta is a region that was intensely studied in the past century by some researchers, Bela Kis being the one who made the most complex

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investigations. He described two new Orthoptera taxa in this area: *Saga campbelli gracilis* (new subspecies at Histria) (Kis, 1962) and *Isophya dobrogensis* (new species on Popina Island) (Kis, 1994).

It is an area of great biogeographical interest, because it is the meeting point between the European, Mediterranean and central Asiatic-European elements of orthopterofauna. The Danube Delta is characterized by the permanent presence of a large water mass, the influence of the Black Sea neighborhood being at the same time important, for climate and for the influence of the presence and the distribution of the species and their characteristic habitats.

The investigations were made inside of some research projects concerning the realization of the atlas of Orthoptera species from the Danube Delta and the reconsideration of the limits for the functional area from the Danube Delta Biosphere Reserve, the entomofaunistic investigations giving some interesting elements.

In August 2009, some investigations that were made near the Maliuc village (26 mile from the Sulina Channel) (Fig. 1), revealed the presence of one new Orthoptera species for this geographical area – *Metrioptera* (*Zeuneriana*) *amplipennis* (Brunner von Wattenwyl, 1882), the species being known as spread along the Sava river basin in Vojvodina and in the surroundings of Belgrad area (Gomboc & Šegula, 2005).

The investigations were made by itinerary method, from east to west on the right shore of Sulina channel, on the internal part of the Danube Delta Biosphere Reserve territory.

The species was identified by stridulating sound, this being different from the sound of the species normally present and found in this area, the investigation revealing the presence of two grasshopper males belonging to the *Metrioptera amplipennis* species.

The images were collected with Canon S5IS photo camera.

RESULTS AND DISCUSSION

Metrioptera (*Zeuneriana*) *amplipennis* is synonym with *Platycleis amplipennis* Brunner von Wattenwyl (Prodr. Eur. Orth., 1882); *Metrioptera amplipennis* Ramme (Mitt. Zool. Mus. Berlin, 24, 1939); *Sphagniana amplipennis* Zeuner (Roy. Ent. Soc. Lond. Trans., 91, 1941).

Our investigation reveal the presence of *Metrioptera* (*Zeuneriana*) *amplipennis* species in hygrophilous habitat dependent on seasonal fluctuation of Danube waters, in the hottest month of the year, in one area where, from plants, predominant was *Xantium strumarium*. The temperature was circa 30°C and the cover with clouds was minimal. The human intervention is minimal, the area being not interesting from economical point of view.

The area where species was found has “Rivers with muddy banks with *Chenopodion rubri* p.p. and *Bidention* vegetation” type of habitat, this being characterized by *Xanthium strumarium* as dominant species (Sanda *et al.*, 2008).

The cover with vegetation is about 70-75%. The most important vegetal species from this type of habitat are: *Xanthium strumarium*, *Urtica dioica*, *Chenopodium album, C. urbicum, Atriplex hastata, Amaranthus retroflexus, Althaea officinalis, Plantago major, Bidens tripartita*, *Polygonum hydropiper, P. lapathifolium, Rorippa austriaca, Symphytum officinale, Chlorocyperus glomeratus, Rumex palustris.* These have 40-50 cm height being the tall herbaceous layer, the lower layer being characterized by the presence of: *Alopecurus aequalis, Mentha arvensis, Chenopodium botrys* or *Ranunculus sceleratus* (Sanda *et al*., 2008).

The presence and distribution of this type of habitat was observed, regular on the shore area of channels and Danube branches with organic material. The relief is regular plane or very low inclined. The conservative value of this type of habitat is low.

Fig. 1. The Danube Delta Biosphere Reserve – Maliuc area (modified and added satellite image) (http://maps.google.com/).

The species was observed in dense vegetation formed especially by herbaceous species *Xanthium strumarium*, *Arctium minus, Cirsium vulgare, Urtica* *dioica* and from ligneous species *Amorpha fruticosa*, near the water limit of the right shore of Sulina Branch, upstream the Maliuc village.

The observed individuals sitting on not more than 50 centimeters tall plants are hiding, at the smallest signal of danger, on the basis of the plant where they were found.

Fig. 2. *Metrioptera* (*Zeuneriana*) *amplipennis* (orig.).

The type of habitat where *Metrioptera* (*Zeuneriana*) *amplipennis* (Fig. 2) was observed is in a great measure similar to that where Iorgu (2009) has found it for the first time in the Romanian fauna.

CONCLUSIONS

Considered endemic from Belgrad area (Serbian part of the Danube and Sava rivers), *Metrioptera* (*Zeuneriana*) *amplipennis* extends its distribution area from east, along the Danube river valley, being found in the summer of 2009 (in July) in the Danube Delta Biosphere Reserve, near the Maliuc village, on the right shore of Sulina branch.

The point of observation from the Danube Delta Biosphere Reserve represents the newest known eastern limit of distribution for the species.

The biodiversity from the Danube Delta Biosphere Reserve is growing touching the 80 taxa belonging to Orthoptera order.

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CORRELATION BETWEEN THE INCIDENCE OF HUMAN PARASITIC DISEASES AND THE VALUES OF ANNUAL MEAN TEMPERATURE (ROMANIA, 1989–2009)

IRINA TEODORESCU[*], MARIAN GHERVAN GOGOAȘE[**], CLAUDIA COIPAN[***]

The correlation (simple correlation coefficient Bravais-Pearson) between the incidence of parasitic diseases and the annual mean values of the temperature from 1989 to 2009 was calculated using the data issued by the Ministry of Health Statistics Commission and the National Agency for Meteorology with the help of STATISTICA 9.1 software. Considering a significance level of p = 0.2, correlations could be established for 11 specified parasitic diseases and some other unidentified protozoiases and helminthiases taken into account. The correlation was statistically significant positive in the case of amoebiasis, giardiasis, visceral leishmaniasis, toxoplasmosis, some undiagnosed intestinal protozoiases, ascariasis, and enterobiasis. The correlation was statistically highly significant in the case of amoebiasis produced by *Entamoeba hystolitica*, statistically significant for giardiasis and enterobiasis, weaker but nonetheless statistically significant, for ascariasis, toxoplasmosis, visceral leishmaniasis, and other intestinal protozoiases. For malaria, trichomoniasis, trichinellosis, hydatidosis, taeniasis, and other not clearly established helminthiasis, there was no positive correlation with the temperature values. Determining some statistically significant positive correlations between the incidence of some parasitic diseases and the annual mean temperature represents a powerful argument in supporting the assessment that the global warming process favors the survival, development, multiplication, extends natural range of many disease agents, of their vectors, intermediate hosts or of the infection reservoirs, resulting in the raise of human infection cases.

Key words: climate changes, parasitic diseases, statistical correlation, global warming.

INTRODUCTION

Large-scale and global environmental hazards to human health include climate change, stratospheric ozone depletion, loss of biodiversity, changes in hydrological systems and the supplies of freshwater, land degradation and stresses on food-producing systems (WHO, 2011). Global warming and climate change refer to an increase in average global temperatures. Climate can have wide-ranging and potentially adverse effects on human health *via* direct pathways (*e.g.*, thermal stress and extreme weather/climate events) and indirect pathways (diseases incidence and vectors population increase, vectors, parasites and infectious agents natural range extended, food production decrease).

The United Nations Climate Change Conference (Cancún, Mexico, 29 November – 10 December 2010) affirms that climate change is one of the greatest challenges of our time. It recognizes that climate change represents an urgent and potentially irreversible threat to human societies and the planet. It recognizes that warming of the climate system is unequivocal and that most of the

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observed increase in global average temperatures since the mid twentieth century is very likely due to the observed increase in anthropogenic greenhouse gas concentrations.

The world's climate system is an integral part of the complex of life-supporting processes (McMichael *et al.*, 2006). Today, the human influence has attained a global scale. This reflects the recent rapid increase in population size, energy consumption, intensity of land use, international trade and travel, and other human activities (WHO, 2003).

The health sector component of the first United States National Assessment, published in 2000, synthesized the anticipated health impacts of climate variability and change for five categories of health outcomes: impacts attributable to temperature, extreme weather events (*e.g.*, storms and floods), air pollution, water- and food-borne diseases, and vector- and rodent-borne diseases (Ebi *et al.*, 2006).

NASA's Goddard Institute of Space Studies (GISS) tracks atmospheric global temperature climate trends. GISS established annual mean temperature between 1951 and 1980 as baseline mean temperature and named "anomalies" the difference between this baseline mean and the temperature annual mean. Using this baseline there was determined yearly global temperature anomaly. In the 1880-1935 periods, the temperature anomaly was consistently negative, and in the 1980-2010 periods, the anomaly has been consistently positive.

Climate and weather have always had a powerful impact on human health (McMichael *et al.*, 2006). Human life is dependent on the dynamics of the Earth's climate system (Githeko *et al.*, 2000). Many scientific community members consider that global warming disturbs the natural balance and contributes to many diseases emergence and spread in areas (Brower, 2001). Consensus exists among scientists all over the globe that the world's climate is changing and that these changes can affect human health. Efforts at the Centers for Disease Control and Prevention (CDC) are focused on addressing how environmental changes can affect people's health.

Global warming and wider fluctuations in weather help to spread the diseases: temperature constrains the range of microbes and vectors, and weather affects the timing and intensity of disease outbreaks (Epstein, 2005). Climatic conditions affect diseases transmitted via vectors (vector-borne disease) or through rodents (rodent-borne disease). Net climate change-related increases in the geographic distribution of the vector organisms of infectious diseases and changes in the life-cycle dynamics of vectors and infective parasites would, in aggregate, increase the potential transmission of many vector-borne diseases (Watson *et al.*, 1997). By 2100 it is estimated that average global temperatures will have risen by 1.0–3.5 ^{o}C, increasing the likelihood of many vector-borne diseases in new areas (Githeko *et al.*, 2000). Increases in non-vector-borne infectious diseases could also occur because of climatic impacts on water distribution, temperature increased and micro-organism proliferation (Watson *et al.*, 1997). Some infectious diseases are well known to show apparent seasonal changes and would seem to be very sensitive to global warming (Izrael *et al.*, 1990). Parasitic and viral diseases have the potential for the increase and reintroduction in many countries (Dobson & Carper, 1989).

It is not easy to establish temperature impact upon human health. However, quantifying the projected health impacts is difficult because the extent of climate-induced health disorders depends on other factors, such as migration, provision of clean urban environments, improved nutrition, increased availability of potable water, improvements in sanitation, the extent of disease vector-control measures, changes in resistance of vector organisms to insecticides, and more widespread availability of health care (Watson *et al.*, 1997).

MATERIAL AND METHODS

Through processing the information from the Ministry of Health Statistics Commission and correlating them with the variations of the annual mean temperature values obtained from the National Agency for Meteorology, we assessed the incidence of some infectious diseases in Romania under the influence of climate changes over the period of 1989-2009 (incidence defined as the number of new cases of disease in a given time period).

The calculation of the correlation between the incidence of some parasitic diseases and the annual mean temperature was made using parametric correlations, namely Bravais-Pearson correlation coefficient (*r*); due to the fact that the variables were quantitative, interval scaled ones, and approximately normal distributed), at a significance level of $p = 0.2$ (with values ranging in a confidence level of 80%), in STATISTICA 9.1. software, based on the formula:

$$r_{xy} = \frac{\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2 \sum_{i=1}^{n}(y_i - \bar{y})^2}}$$

RESULTS AND DISCUSSION

Values of over 10 °C were registered for 15 of the 21 years for which the existence of a correlation was tested between the incidence of several human parasitic diseases and the mean annual temperatures in Romania, while in 11 years the temperatures exceeded 10.5 °C. The lowest temperature was recorded in 1997 (9.2 °C) and the highest one in 2003 (11.3 °C). For five years (1995, 2000, 2003, 2007, 2009) the mean annual temperatures exceeded 11°C (Table 1, Fig. 1).

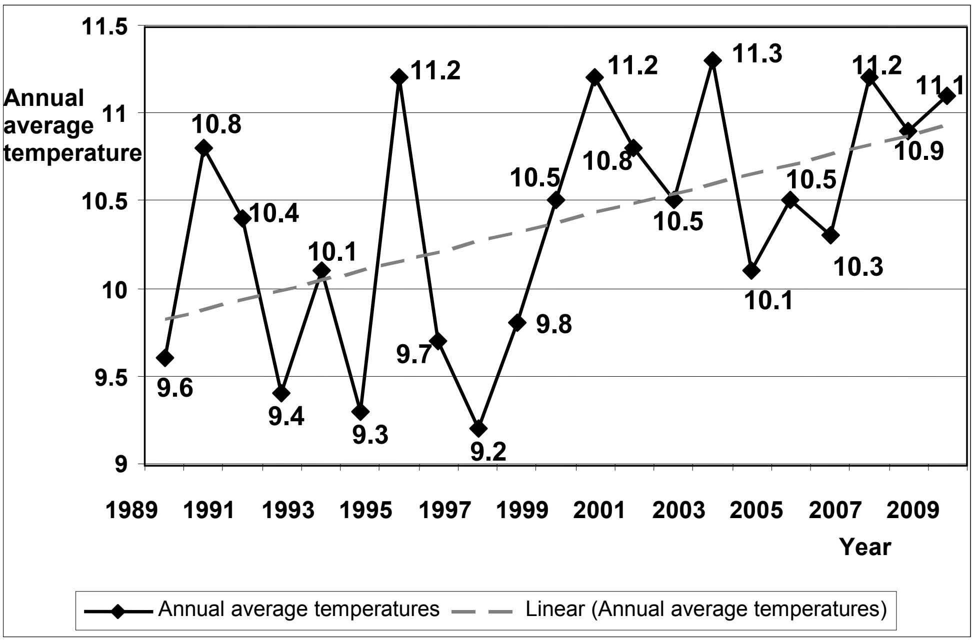

Fig. 1. Dynamics of the mean annual temperature (1989-2009).

Table 1

Dynamics of parasitary diseases incidence between 1989 and 2009

Year	Annual average temperatures	AMOEBIASIS	GIARDIASIS (LAMBLIASIS)	LEISHMANIASIS	MALARIA	TRICHINELLOSIS	OTHER INTESTINAL PROTOZOIASIS	TRICHOMONIASIS	TOXOPLASMOSIS	HYDATIDOSIS	TENIASIS	ASCARIDIASIS	OXIURIASIS	OTHER HELMINTHIASIS
1989	9.6	41	33200	4	5	947	877	5682	1390	82	386	46224	65724	23286
1990	10.8	78	36828	4	11	1031	357	6045	1165	108	486	43461	70694	27462
1991	10.4	19	36394	3	11	1527	118	4897	2123	132	620	46653	74421	20048
1992	9.4	12	41803	2	19	2147	425	5593	4346	53	667	50744	78769	29311
1993	10.1	41	49142	3	21	3649	255	29755	16993	787	3642	61131	34091	161
1994	9.3	52	70507	0	21	3014	3023	6812	10015	56	587	61221	82243	40966
1995	11.2	92	85610	1	25	1965	1174	11512	9204	36	275	53990	91005	53088
1996	9.7	25	69923	1	50	980	1232	8932	7135	43	431	50746	68393	48148
1997	9.2	55	66467	0	39	2037	1092	7853	3043	84	604	41883	56268	37480
1998	9.8	167	79111	0	53	1653	3445	11774	4297	123	652	54182	71174	52886
1999	10.5	106	79774	1	32	1517	1427	8036	6368	548	1101	70748	78693	39179
2000	11.2	271	85380	0	40	1175	2008	8399	6259	81	637	52627	82021	43202
2001	10.8	744	81786	0	19	1387	14629	10769	8394	150	714	60544	75488	49527

Table 1

(continued)

2002	10.5	280	75919	0	10	665	6665	7819	13357	168	667	43105	80901	44229
2003	11.3	1244	86889	0	16	431	3850	9634	14128	150	724	77473	96495	41620
2004	10.1	829	70382	0	9	780	6291	8434	13311	147	681	58597	85029	55639
2005	10.5	595	81299	2	18	574	6066	6442	12698	108	764	57336	86173	41071
2006	10.3	366	76527	1	16	350	5839	6857	12275	90	56	48756	79718	34695
2007	11.2	709	95551	12	38	698	7214	7359	14005	378	824	71386	89917	36762
2008	10.9	741	90738	4	23	515	5987	11062	14134	365	791	60014	83201	36010
2009	11.1	783	93201	7	16	394	6462	9118	15792	416	843	68925	87162	39521

We focused on diseases that have protozoans (*Entamoeba histolytica, Giardia intestinalis, Leishmania* sp., *Plasmodium* sp., *Trichomonas urogenitalis, Toxoplasma gondii*) and helminths (*Trichinella spiralis, Echinococcus granulosus, Taenis* sp., *Ascaris lumbricoides, Enterobius vermicularis*) as etiologic agents.

To test the correlation, we selected diseases that are directly influenced by the increased temperatures (that over the biological cycle entail the contact of some parasite life stages with the soil or the water), diseases that are indirectly affected by the temperature (vector borne diseases, with direct temperature action on vectors biology), and diseases with influence less meaningful (diseases that are transmitted directly from one host to another).

Analyzing the correlation of the disease incidence with the mean annual temperature values for 11 specified parasitic diseases and some unidentified etiologic agent or other protozoiases and helminthiasis we noticed a statistically significant positive correlation in the case of amoebiasis, giardiasis, visceral leishmaniasis, toxoplasmosis, ascariasis, and enterobiasis, other intestinal diseases caused by protozoans (Table 2).

Table 2

Correlation between the incidence of parasitic diseases
and the values of the mean annual temperature (1989-2009)

PARASITOSIS	r	r^2	p	Constancy	Slope
AMOEBIASIS	0.591322	0.349661	0.004755	–2961.3	318.67
GIARDIASIS	0.538175	0.289632	0.011847	–91563.8	15646.03
VISCERAL LEISHMANIASIS	0.334783	0.112080	0.137962	–13.0	1.46
MALARIA	–0.115154	0.013260	0.619156	47.4	–2.31
TRICHINELLOSIS	–0.491082	0.241161	0.023783	7913.0	–636.70
OTHER INTESTINAL PROTOZOIASIS	0.360767	0.130153	0.108130	–15950.9	1897.23
TRICHOMONIASIS	0.049629	0.002463	0.830833	5284.3	375.46
TOXOPLASMOSIS	0.408137	0.166576	0.066251	–22718.1	3063.38
HYDATIDOSIS	0.233788	0.054657	0.307740	–501.6	67.18
TENIASIS	–0.025899	0.000671	0.911271	1062.0	–25.90
ASCARIASIS	0.464840	0.216076	0.033740	–15404.3	6898.75
OXIURIOSIS	0.533821	0.284965	0.012687	–33969.3	10697.27
OTHER HELMINTHIASIS	0.162558	0.026425	0.481418	5914.6	3075.19

For *Entamoeba histolytica* s.l. (*Entamoeba histolytica* s.str. + *Entamoeba dispar*) the number of new cases was low (between 12 and 55) in the interval 1989-1997 when the temperatures varied between 9.2 °C and 10.8 °C, except for the years 1990 and 1995 when the mean temperature was 10.8 and 11.2 °C respectively, when 78, respectively 92 cases were registered. In the following years the number of cases increased, reaching a maximum in 2003 (1244 cases), which is an over 100 fold increase comparing to 1992 when the lowest number of cases was registered. In six of the years (2001, 2003, 2004, 2007, 2008, and 2009) there were over 700 cases recorded annually (Table 1, Fig. 2).

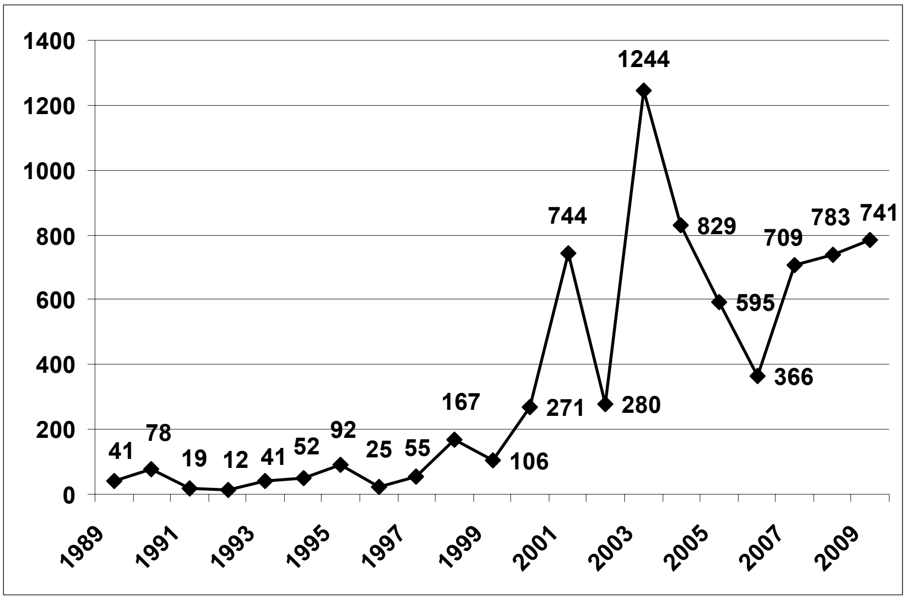

Fig. 2. Dynamics of the amoebiasis incidence values (1989-2009).

In the case of amoebiasis, the correlation between incidence and annual mean temperature was *statistically highly significant*, **r** = 0.591 and p = 0.005 (Fig. 3).

For *Giardia intestinalis*, the number of recorded cases was lower than 40,000 only in the first three years. In 1994, the incidence of giardiasis grew at over 70,000 and in the next 15 years it varied between 66,000 and 95,000 approximately. Incidences of over 80,000 were registered for eight years (1995, 2000, 2001, 2003, 2005, 2007, 2008, and 2009). In six years (1995, 2000, 2003, 2007-2009), with high mean annual temperature between 10.9 °C and 11.3 °C, the number of cases was higher (between 85,380 and 93,201) (Table 1, Fig. 4).

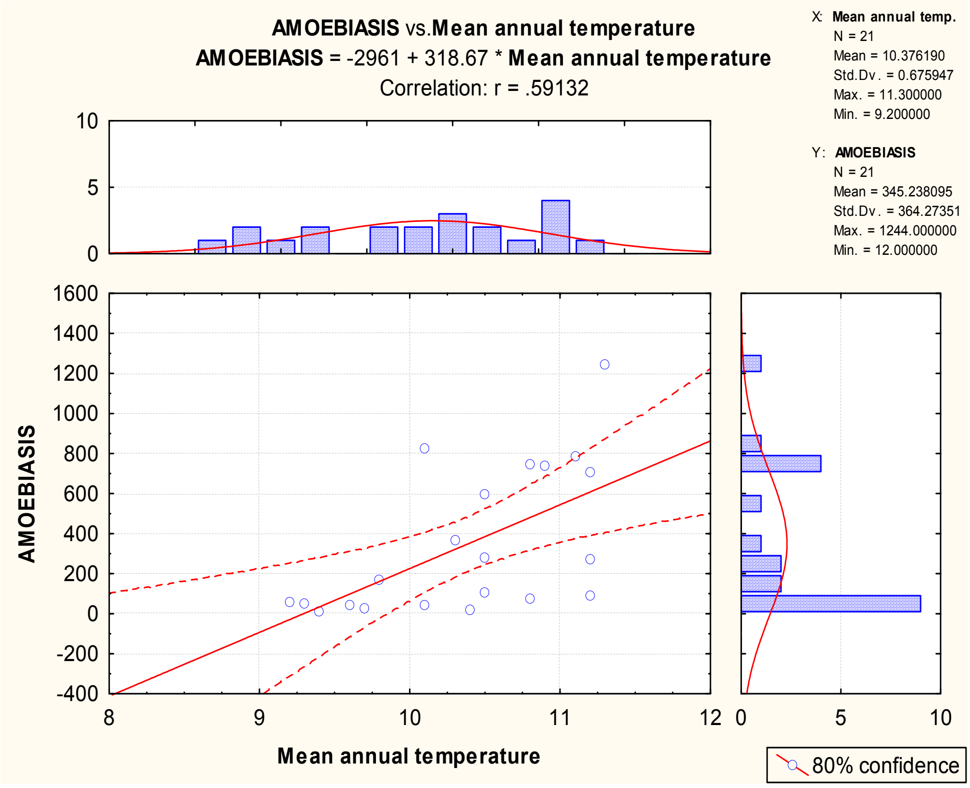

Fig. 3. Correlation of the amoebiasis incidence with the mean annual temperature over the period 1989-2009.

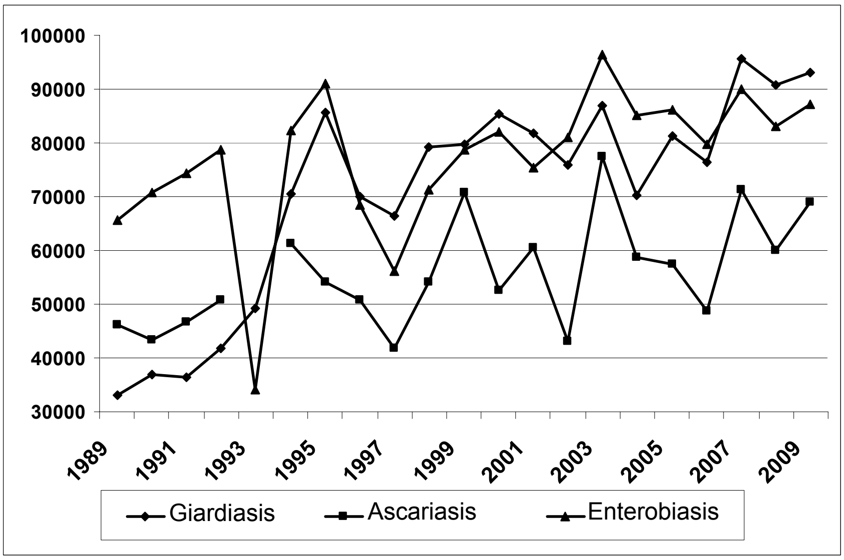

Fig. 4. Dynamics of giardiasis, ascariasis and enterobiasis incidence values (1989-2009).

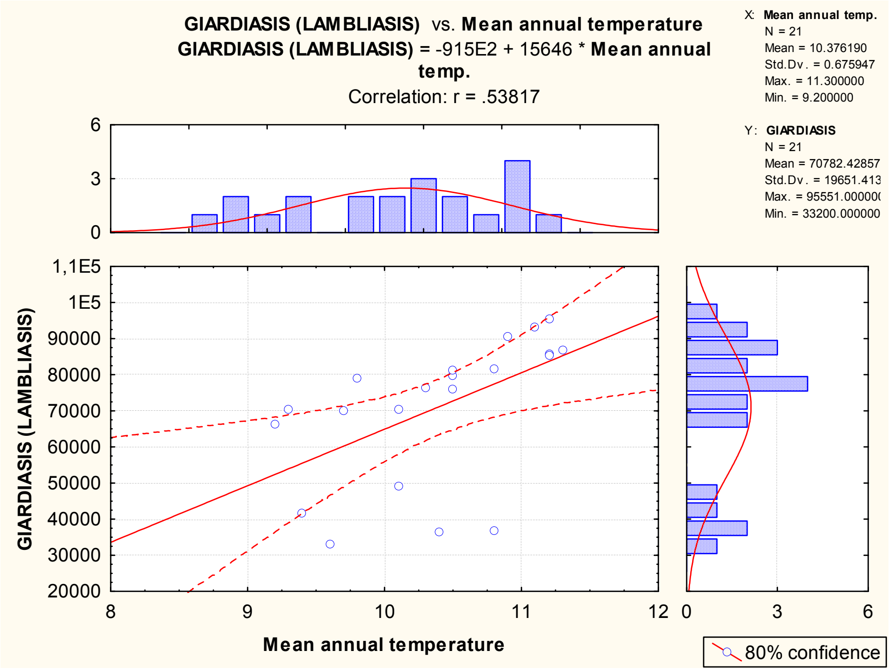

Fig. 5. Correlation of the giardiasis incidence with the mean annual temperature over the period 1989-2009.

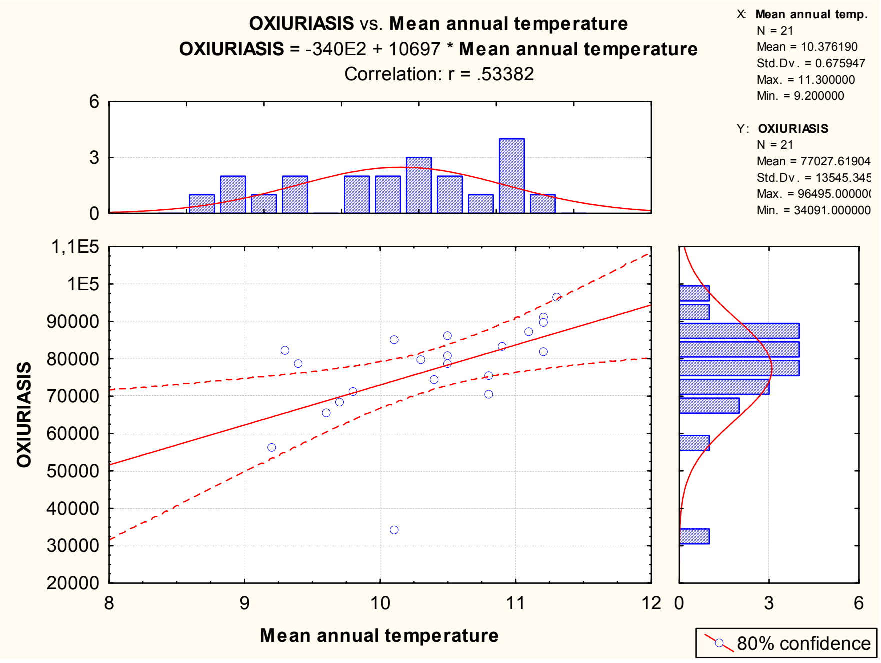

Fig. 6. Correlation of the oxiuriasis (enterobiasis) incidence with the mean annual temperature over the period 1989-2009.

For *Enterobius* (*Oxyurus*) *vermicularis*, the incidence of the disease caused was generally high, with the lowest number of cases (34,091) registered in 1993. In the five years with the most elevated mean temperatures (11.1 ^{o}C–11.3 ^{o}C) the number of cases was high, getting beyond 82,000 and marking the highest value in 2003 when the mean annual temperature was also at its peak (Table 1, Fig. 4).

For giardiasis (Fig. 5) and enterobiasis (Fig. 6) the correlation coefficients were above 0.5 (0.538 and 0.534 respectively) and the p-values were under 0.05 (0.012 and 0.013 respectively) indicating a strong, statistically significant correlation of their incidence with the mean annual temperatures.

For *Ascaris lumbricoides* the number of reported infestation cases was high through the entire period. The smallest case number (41883) was registered in 1997 when the lowest mean annual temperature (9.2 ^{o}C) of the interval was also reached. A high number of cases (over 70,000) were recorded in 1999, 2003, and 2007, in two of those years the mean temperature reaching values of 11.2 ^{o}C–11.3 ^{o}C (Table 1, Fig. 4).

For *Toxoplasma gondii*, reduced incidence values were registered in 1989-1992 and 1997. In 1993 and the last eight years of the interval, there were over 12,000 cases recorded annually and the mean temperatures varied between 10.1 ^{o}C and 11.3 ^{o}C. For leishmaniasis, in the first 16 years (1989–2004) only19 cases were registered and in the last 5 years (2005–2009) the number increased to 26 (Table 1). We must underline that perhaps all these cases are temporary resident in endemic areas of leishmaniases (especially from southern Europe).

For ascariasis (Fig. 7), toxoplasmosis (Fig. 8), other intestinal protozoiases (Fig. 9), and visceral leishmaniasis (Fig. 10) the correlation coefficients (0.465, 0.408137, 0.360767, and 0.334783, respectively) indicate weaker but nonetheless statistically significant (p = 0.034, 0.066251, 0.108130, and 0.137962, respectively) correlations of the incidence with the mean annual temperature.

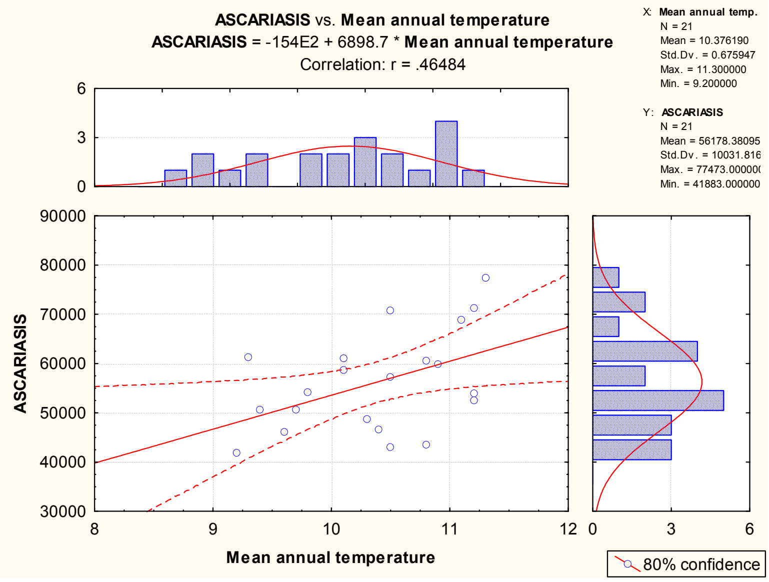

Fig. 7. Correlation of the ascariasis incidence with the mean annual temperature over the period 1989-2009.

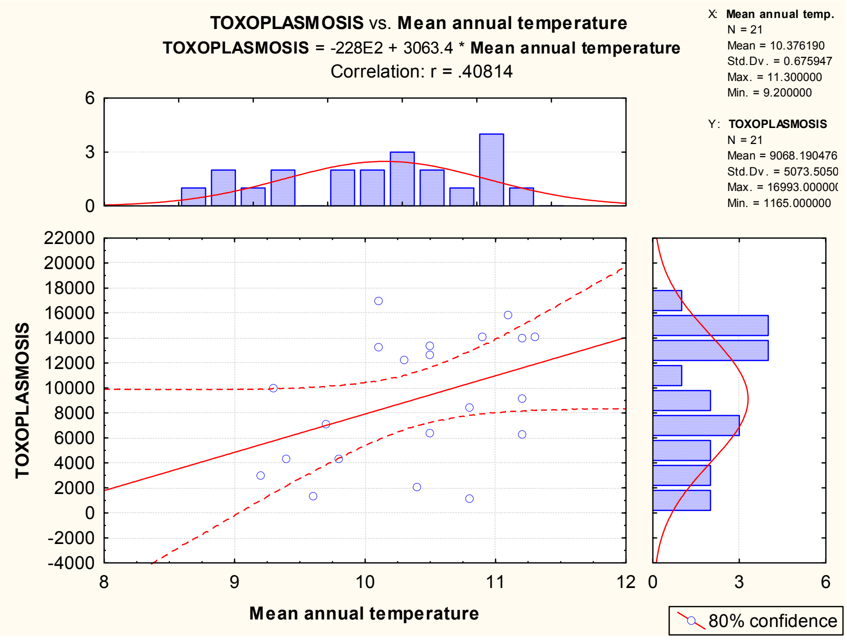

Fig. 8. Correlation of the toxoplasmosis incidence with the mean annual temperature over the period 1989-2009.

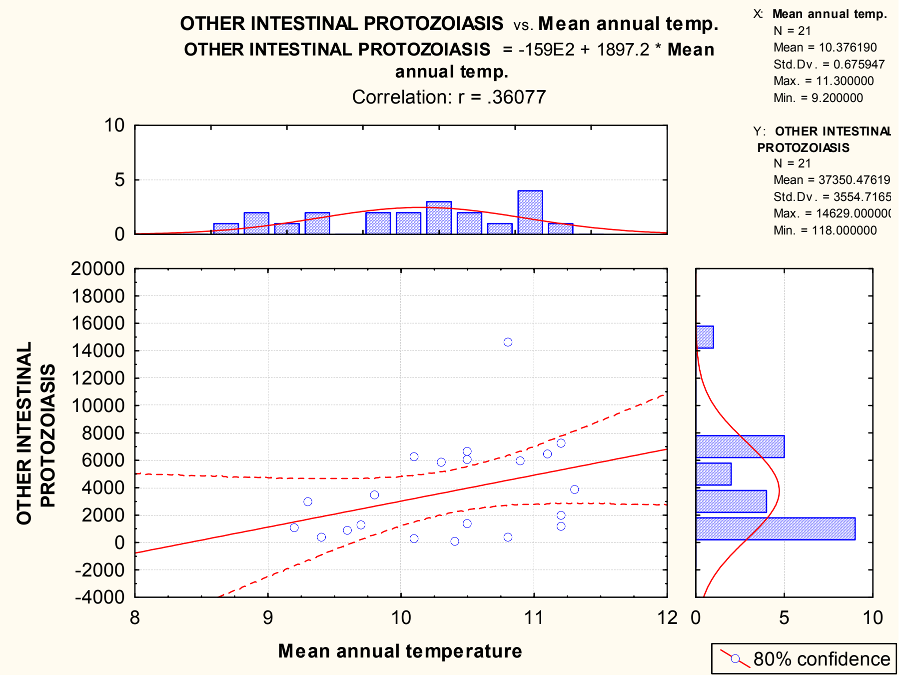

Fig. 9. Correlation of the incidence of other intestinal protozoiasis with the mean annual temperature over the period 1989-2009.

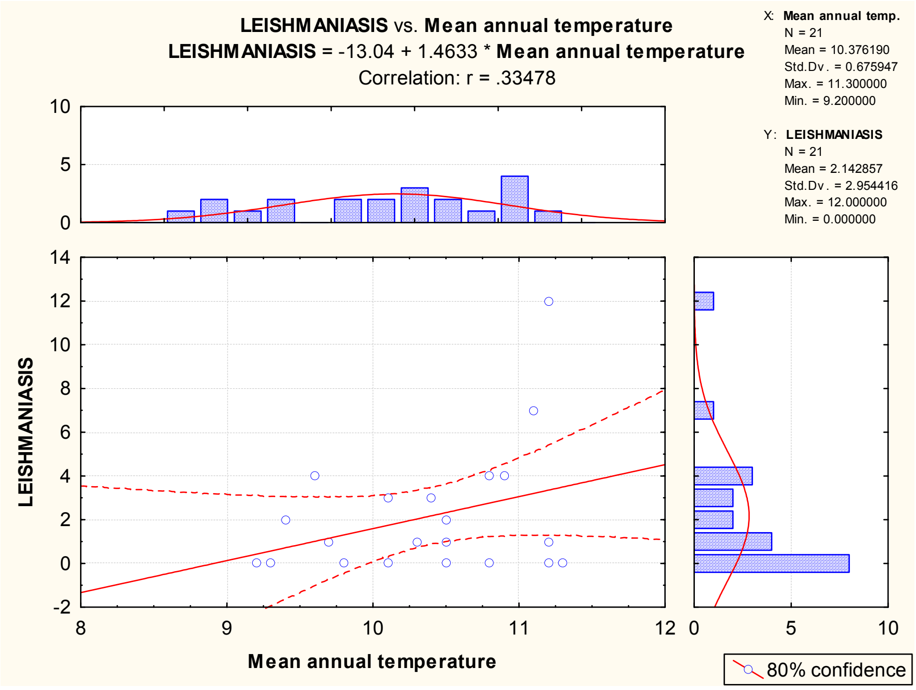

Fig. 10. Correlation of the visceral leishmaniasis incidence with the mean annual temperature over the period 1989-2009.

The incidence of some protozoiases and particularly helminthiases (with undetermined etiologic agent) was generally high, but mainly in the last eight years for the protozoiases and in the last 16 years for helminthiasis (with the highest mean temperature values).

For malaria, trichomoniasis, trichinellosis, hydatidosis, taeniasis, and other not clearly established helminthiasis, based on these data we could not establish a positive correlation of the incidence with the mean annual air temperatures. Although not statistically evinced for the other diseases over a certain period of time the graphs did render some correlation also of the disease incidence with the temperature values.

CONCLUSIONS

To assess the correlation between the incidence of some parasitic diseases and the annual mean values of the temperature from 1989 to 2009 used parametric correlations, namely Bravais-Pearson correlation coefficient (*r*), at a significance level of p = 0.2 (with values ranging in a confidence level of 80%), in STATISTICA 9.1. software, based on the data issued by the the Ministry of Health Statistics Commission and the National Agency for Meteorology.

We assessed correlations for 11 specified parasitic diseases and some other protozoiases and helmithiasis with unidentified etiologic agent and the correlation was a statistically significant positive in the case of amoebiasis, giardiasis, visceral leishmaniasis, toxoplasmosis, ascariasis, enterobiasis, and of some of unspecified intestinal protozoiases.

There were registered different degrees of correlations: a statistically highly significant in the case of amoebiasis, statistically significant for giardiasis and enterobiasis, weaker but nonetheless statistically significant for ascariasis, toxoplasmosis, visceral leishmaniasis, and other intestinal protozoiases.

In the case of malaria, trichomoniasis, trichinellosis, hydatidosis, taeniasis, and other not clearly established helminthiasis, there was no positive correlation with the temperature values.

Determining some statistically significant positive correlations between the incidence of some parasitic diseases and the annual mean temperature values in 21 years (1989-2009) can be an argument to sustain the idea that the global warming process can affect human health favoring the survival, development, multiplication, and areal enlargement of the etiologic agents of the diseases, of their vectors, intermediate hosts or of the infection reservoirs.

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RELEVANT INFRASTRUCTURAL ALTERATIONS AS PREMALIGNANT LESIONS DURING BENIGN TUMORS DEVELOPMENT

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The aim of this study is to know some peculiar infrastructural abnormalities which characterize some benign and malignant cutaneous tumors which can be considered for better understanding of the malignant invasive behaviour acquisition by the epithelial tumor cells. For that, specimens surgically excised as cutaneous tumors (seborrheic keratosis, hemangioma, basal cell carcinoma) were electron microscopically investigated. We focus our interest on the dermal epidermal junction zone. Most important infrastructural abnormalities were recorded: basement membrane and hemidesmosomal junctions as well as cytoskeleton perturbations take place both in benign and malignant tumors. Here, we envisage our observations for better understanding of the malignant invasive behaviour acquisition by the epithelial tumor cells.

Key words: seborrheic keratosis, cavernous hemangioma, basal cell carcinoma, squamous cell carcinoma, basement membrane alteration.

INTRODUCTION

One of the most important aspects of cancer research is deciphering molecular abnormalities which involve normal cells to transform into malignant tumors. Nowadays, it is generally accepted that malignant transformation of a cell is a consequence of multiple accumulation of genomic alterations, mostly as oncogenes activation and tumor suppressor genes inactivation (Woodman & Mills, 2010). In order to discriminate between benign and malignant cutaneous lesions, dermatologist clinical experience is essential. Sometimes, a differential diagnostic is necessary. For this purpose, special modalities of investigation must be applied. Electron microscopic examination is a very useful way to detect the cell phenotypes involved in tumor formation and growth. When electron microscopy is associated with the immune detection for some molecules of interest, the accuracy of diagnostic and prognostic is increased (Mirancea *et al*., 2001, 2002). Here, we investigate at the ultrastructural level some cutaneous tumors, clinically diagnosed as malignant (basal cell carcinoma), or benign (seborrheic keratosis and hemangioma). Our purpose of this comparative study was to find, if possible, some peculiar infrastructural abnormalities as relevant infrastructural alterations for

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premalignant lesions, during benign tumors development, which can be considered for better understanding of the malignant invasive behaviour acquisition by the epithelial tumor cells.

MATERIAL AND METHODS

BIOLOGICAL MATERIAL

Small fragments of tumor skin resulted by surgical therapy from patients suffering from seborrheic keratoma (♂, 54 years), hemangioma (♀, 52 years), and basal cell carcinoma (♀, 57 years), (the surgeon got patients consent) were processed for histological and electron microscopic investigations.

TRANSMISSION ELECTRON MICROSCOPY

Biological material as small pieces for electron microscopy examination were pre-fixed in 3-4.5% glutaraldehyde in 0.05 M sodium cacodylate buffer pH 7.4 at 4 °C for minimum 2 h, post-fixed in 2.5% osmium tetroxide in 0.1 M sodium cacodylate buffer for 2 h at room temperature and washed with 0.05 M sodium cacodylate buffer. After dehydration in graded series of ethanol and infiltration with propylene oxide, specimens were embedded in Glycid ether (Epon 812-equivalent) and finally polymerized at 60 °C for 48 hours. Semithin sections were stained with 1% toluidine blue for light microscopy. Ultrathin sections (80-100 nm) were cut using a diamond knife and, collected on 200 mesh grids, and counterstained with uranyl acetate and subsequently, lead citrate. The grids were examined in a Philips electron microscope operated at an acceleration voltage of 80 kV.

RESULTS AND DISCUSSION

SEBORRHEIC KERATOSIS

Seborrheic keratose (SK) is one of the most common benign human tumors. SK increases with age and old individuals can have many lesions; little is known about their etiology. SK is classified in three histological subtypes: (1) acanthotic, (2) hyperkeratotic, and (3) adenoid. At the microscopic level, they are similar to epidermal nevi, a benign skin lesion that generally occurs at birth or in young children and that also has no malignant potential (Hafner *et al.*, 2010). Basal cell carcinoma (BCC) and seborrheic keratosis (SK) are representative pigmented skin tumors, and they are differentiated as non-melanocytic lesions.

Electron microscopic analysis of the specimens surgically harvested from a patient suffering from seborrheic keratose showed that, usually at the dermal epidermal junction zone (DEJZ) a continuous basement membrane separates epidermis from a fibrotic dermis (Fig. 1). Investigated at the higher magnification, the basement membrane appears with distinct and continuous lamina lucida and lamina densa. Intermedium filaments bundles connect normal hemidesmosomal junctions. Nevertheless, at some places, DEJZ showed infrastructural abnormalities. Redundant patches of basement membrane can be seen (Fig. 2). Hemidesmosomal junctions are missing along the basal profile or they are destructured (Figs. 3-5). However, plasma membrane derived trafficking vesicles (endocytotic caveolae, clathrin coated vesicles) can be seen (Fig. 3, Fig. 6, Fig. 7).

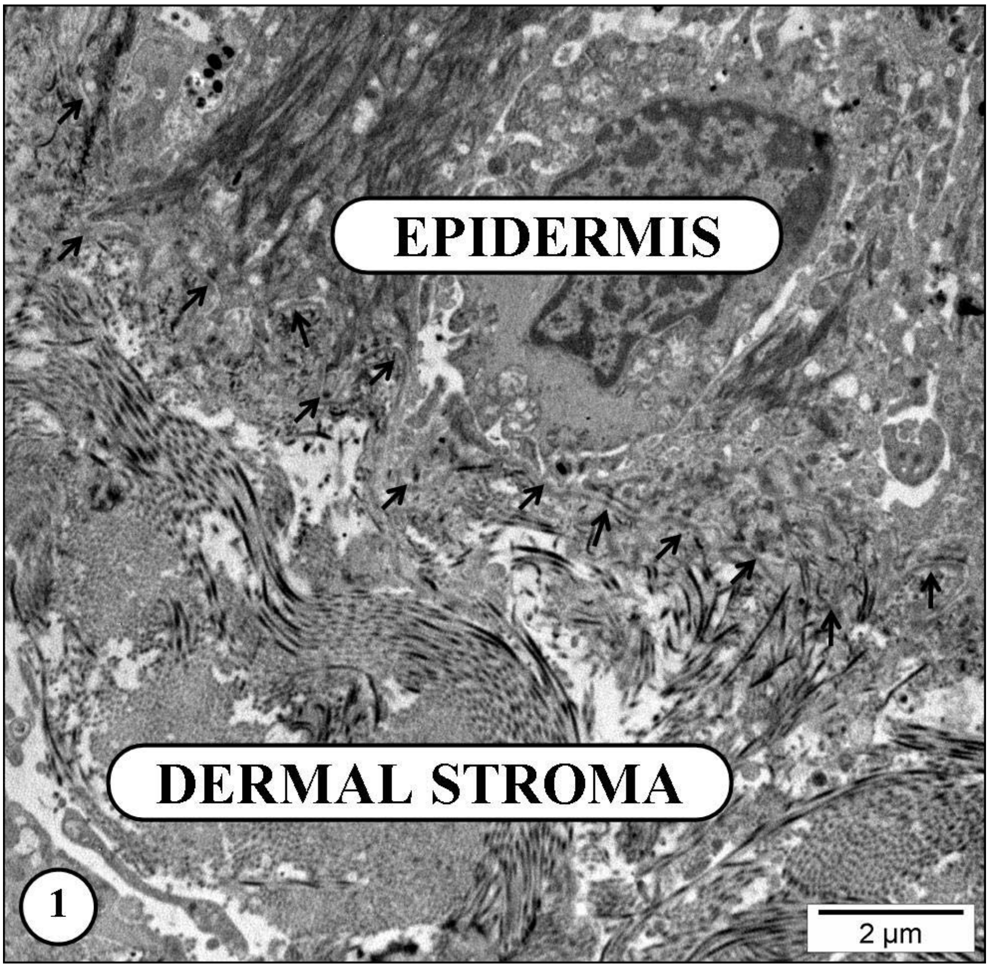


Fig. 1. A continuous basement membrane (arrows) separates the epidermis from the fibrotic dermis (seborrheic keratose).

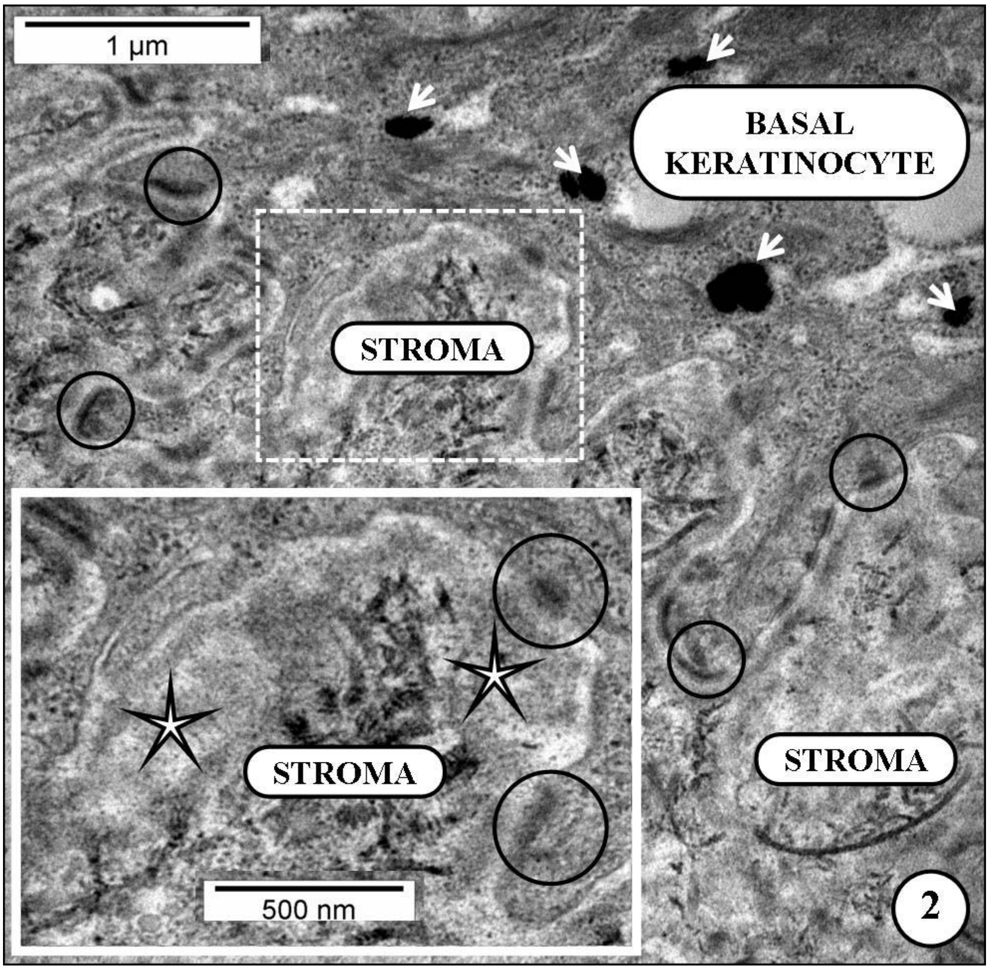


Fig. 2. Dermal-epidermal junction zone: a basement membrane with distinct and continuous lamina lucida and lamina densa as well as normal hemidesmosomal junctions (encircled areas) are clearly visible. Nevertheless, some redundant of basement membrane (framed area by interrupted lines) can be seen. In inset: enlarged framed area. Stars mark redundant basement membrane. Encircled areas mark hemidesmosomes (seborrheic keratose).

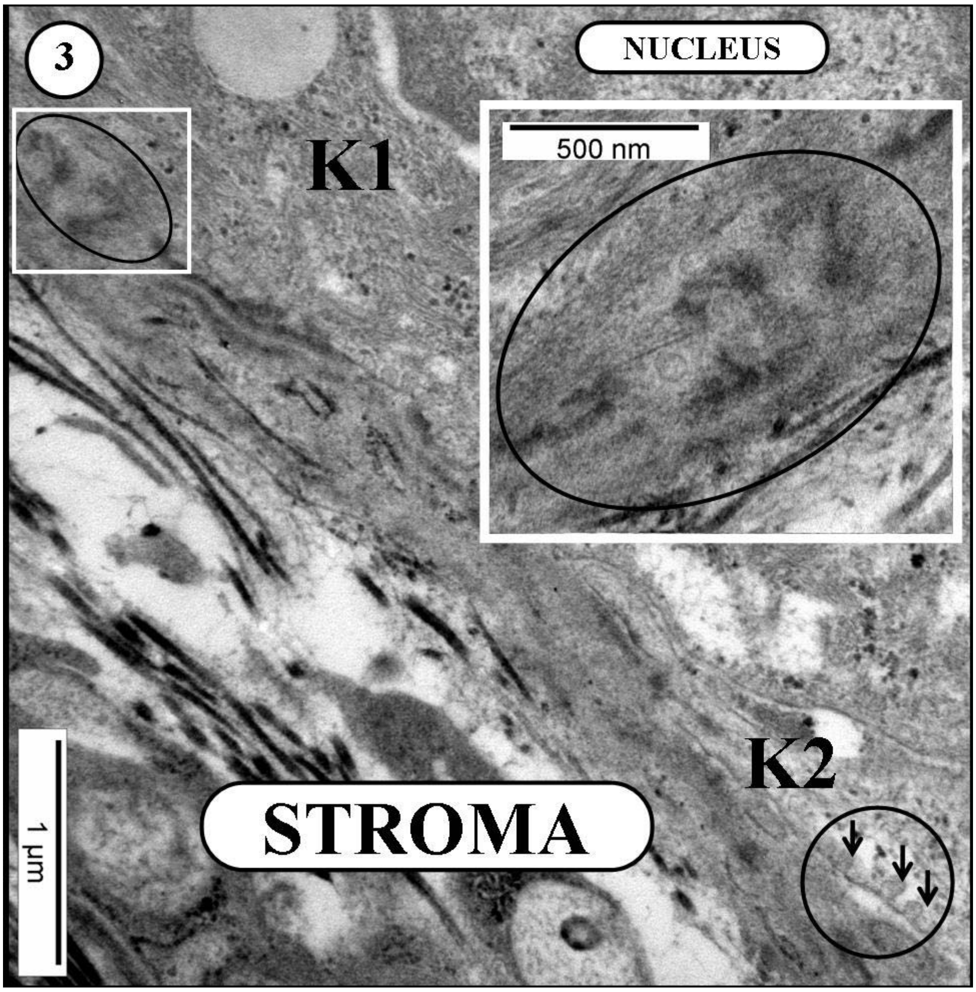


Fig. 3. Two basal keratinocytes (K1 and K2) are separated from the dermal stroma by a basement membrane showing some abnormalities. Hemidesmosomal junctions are missing along the basal profile. Framed area (enlarged as inset) depicted destructured hemidesmosomes. Arrows in encircled area mark endocytotic caveols (seborrheic keratose).

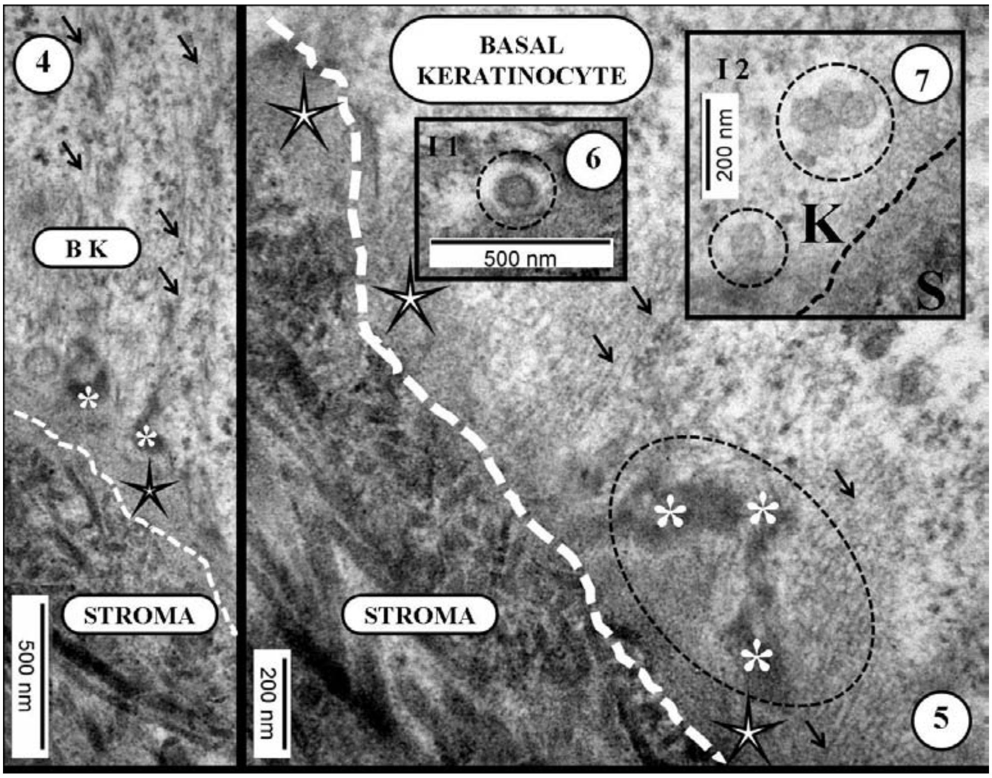


Fig. 4. At the basal profile of a basal keratinocyte (BK) remnants of hemidesmosomal junctions, here visible as amorphous dense material (asterisks) to which still some keratin filaments (arrows) are directed, can be seen. At the dermal-epidermal junction (discontinuous interrupted line) an amorphous deposited material, probably a basement membrane deposition (black star) is visible (seborrheic keratose).

Fig. 5. All the above described ultrastructural abnormalities in Fig. 4 are better visible at another dermal-epidermal junction zone profile. (Seborrheic keratose).

Fig. 6. A clathrin coated vesicle inside of the basal keratinocyte.

Fig. 7. Endocytotic vesicles near the plasma membrane of a basal keratinocyte is facing the stroma.

BBC is a malignant cutaneous tumor while SK is one of the most common benign human tumors. As benign tumor, SK is considered without malignant potential (Hafner *et al*., 2010). However, investigators reported some medical cases when basal cell carcinoma (Akasaka & Kon, 1997) or malignant melanoma (Zabel *et al*., 2000) arise in seborrheic keratosis. So far, it is very difficult to know whether reported cases of basal cell carcinoma or malignant melanoma associated with SK are coincidental or whether malignant transformation occurs. In this context, the present study at the electron microscopic level showed that, to some extent, ultrastructural alterations occurred at the dermal-epidermal junction zone

(DEJZ) in SK mimic those described in BCC and squamous cell carcinoma (the present study on BCC, Mirancea *et al.*, 2009, 2010). Focal destruction of DEJZ in SK, meaning indistinct lamina lucida and lamina densa of the basal lamina (but an amorphous material interposed at the dermal-epidermal limit), and especially, absence of hemidesmosomes and consecutively missing keratin filaments connection to the basal pole (Figs. 3-5) are similar to those described in BCC (Figs. 13-14). In both cases, such alterations of the epithelial cells affronted to the dermal stroma lead to cell depolarization, a prerequisite for invasive cell behavior.

CAVERNOUS HEMANGIOMA

Hemangioma is a disease consisting of new formed capillary blood vessels located elsewhere in the body: on the scalp, face, arms and legs skin, but sometimes, the hemangioma may have an inner body location: liver, brain, etc.

Hemangioma is a benign tumor usually developed by new-born children during the first months of life (infantile hemangiomas). The most hemangiomas undergo a rapid proliferation, especially during 8-10 months of their life, and then they follow an involution along the age of 5-7 years. Most hemangiomas occur at birth while others develop after birth.

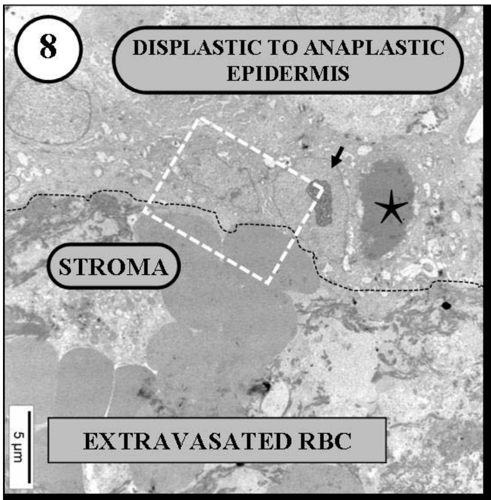
Formation of new blood vessels is essential for several physiological and pathological events, *e.g.* embryogenesis (Mirancea & Mirancea, 2000), tumor growth and metastasis (Vosseler *et al.*, 2005; Miller *et al.*, 2005), lipoid proteinosis (Mirancea *et al.*, 2006).

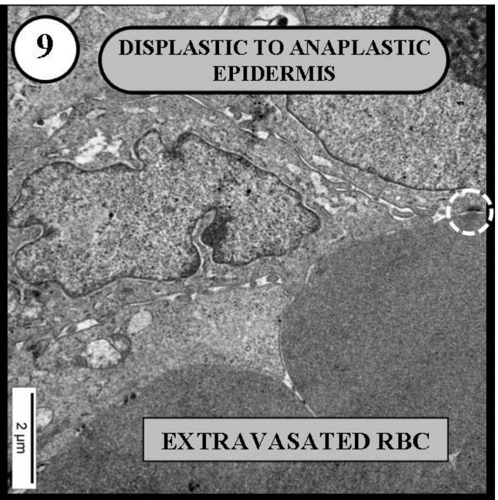
Histopathologically, new formed capillary blood vessels such as hemangiomas exhibit very polymorphic patterns. One of these is so called cavernous hemangioma (also known as cavernoma) which is a benign tumor of blood vessels that rapidly grows over a period of time and does not usually reduce in size.

Causes for the development of cavernous hemangioma are still unknown, but genetic predisposition may play a role. The disorder commonly appears in individuals between 20 and 30 years old, although a small number of cases are seen during birth. Cavernous malformations can occur anywhere in the body, but they usually produce serious signs and symptoms only when they occur in the central nervous system (the brain and spinal cord).

Analyzed by optic microscope, semithin sections from the patient with skin cavernous hemangioma showed a lot of large and irregular in structure capillaries, with abnormally thin walls that are prone to leak. Ultrathin sections examined by transmission electron microscopy showed a lackage by extravasated red blood cells inside of dermal stroma beneath the displastic to anaplastic epidermis. Almost all keratinocytes lost their epithelial phenotype: desmosomal junctions are missing or, when present they are precarious. Intermedium filaments are absent. Moreover, the basement membrane is missing at the dermo-epidermal interface and no hemidesmosomal junction can be detected. Euchromatin is prevalently represented inside of the majority of nuclei of epithelial cells and, huge nucleoli can be seen (Fig. 8). At higher magnification, extravasated red blood cells are in direct contact with the dysplastic to anaplastic epidermal cells. No basement membrane is interposed. Remnants of a hemidesmosomal junction represented by the outer plaque can be detected. No intermedium filament reached basal pole (Fig. 9). Fig. 10 depicted a short profile at the dermal-epidermal junction where fibrotic stroma is in direct contact with the basal keratinocyte. Ultrastructural aspect of the epidermal cells revealed that desmosomal junctions are impaired: not at all or scanty cytokeratin filaments connect such kind of intercellular junctions. Moreover, extensive sectors of the keratinocyte plasma membrane lost completely junctions with neighbour cells; there they express microvilli, oriented to large intercellular spaces. Some epithelial cells were engaged in an apoptotic process (Fig. 11). Along intercellular contacts, no desmosomal junctions can be detected. Sometimes, intracellular channel can be detected (Fig. 12). All the above mentioned infrastructural abnormalities offer a real support for the diagnosis: the epidermis appears as a dysplastic to anaplastic epithelium.

The strange and important finding in this report for a case of cavernous hemangioma is how much the epidermal epithelium is affected by the blood microvasculature abnormalities. It seems that severely infrastructural alterations of microvasculature allowed formation of extensive hemorrhagic areas affronted to the epidermal epithelium. Without normal barrier represented by the endothelial wall and associated basement membrane as well as pericytes, the paracrine factors will induce very intense and unlimited effect to the epidermis. In such circumstances, the basement membrane and hemidesmosomal junctions are the first targets for the lytic enzymes. Aggressive action of such kind of paracrine factors easily delivered by extravasated sanguine cells (Mueller & Fusenig, 2004) to the epidermal cells leads to the epithelial phenotype change: cell-cell and cell-extracellular junctions are destructured, cytoskeleton represented by cytokeratine filaments disappeared, and, consequently cell depolarization is remarkable. Consequently, some epithelial cells enter and follow the apoptotic process (Fig. 11). Using normal and malignant mammary epithelium, in an experimental three-dimensional model, Weaver *et al.* (2002) concluded that formation of polarized, three-dimensional structures driven by basement membrane confers protection to apoptosis in both normal and malignant mammary epithelial cells. By contrast, irrespective of their malignant status, non-polarized structures are sensitive to induction of apoptosis.





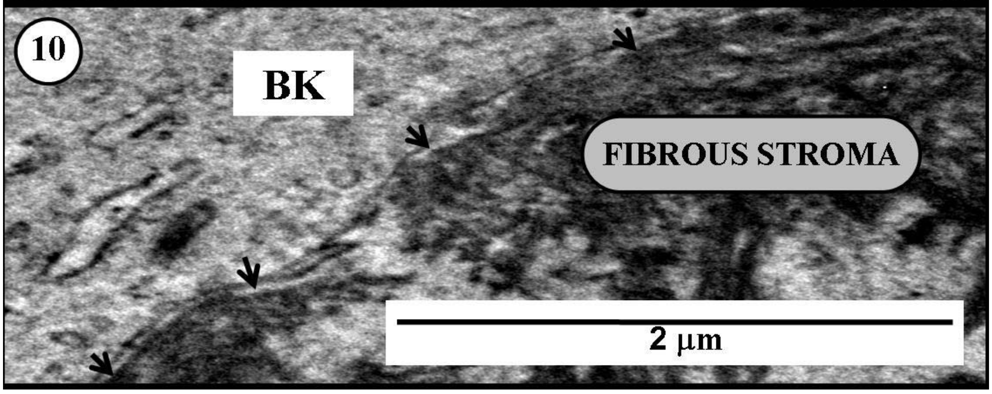
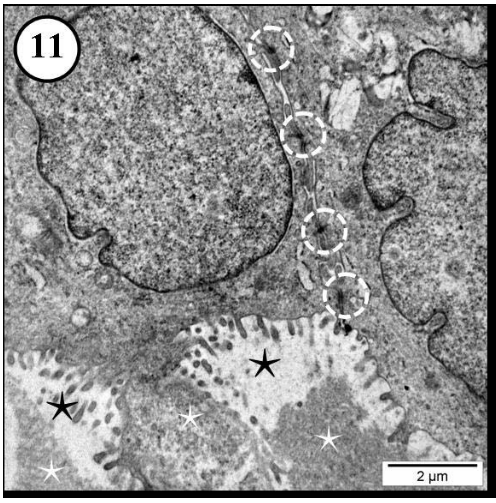


Fig. 8. Extravasated red blood cells (RBC) inside of dermal stroma beneath the displastic to anaplastic epidermis. Keratinocytes almost lost their epithelial phenotype: desmosomal junctions are missing or, when present they are precarious. Intermedium filaments are absent. Arrow marks an anaplastic cell with a huge nucleolus and the star marks an apoptotic cell. Moreover, the basement membrane is missing at the dermo-epidermal interface (black interrupted line) and no hemidesmosomal junction can be detected. (Cavernous hemangioma).

Fig. 9. Detail from the framed area in Fig. 8. Extravasated red blood cells (RBC) are in direct contact with the dysplastic to anaplastic epidermal cells. No basement membrane is interposed. Encircled area delineates a remnant of a hemidesmosomal junction represented by the outer plaque. No intermedium filament reaches basal pole. (Cavernous hemangioma).

Fig. 10. A short profile at the dermal-epidermal junction. A fibrotic stroma becomes in direct contact (arrows) with a keratinocyte (BK). (Cavernous hemangioma).



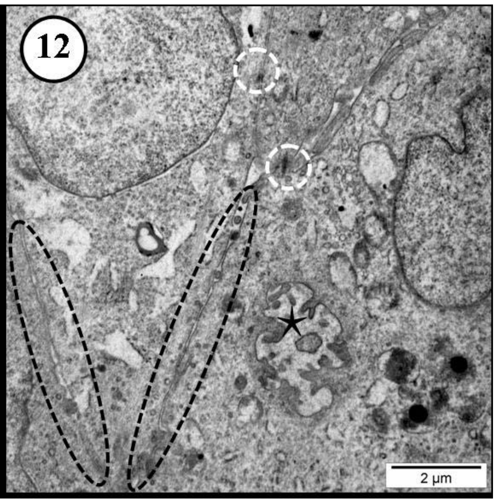


Fig. 11. Ultrastructural aspect of the epidermal cells. Desmosomal junctions are impaired: not at all or scanty cytokeratin filaments connect such kind of intercellular junctions (encircled areas). Moreover, extensive sectors of the keratinocyte plasma membrane lost completely junctions with neighbour cells; there they express microvilli, oriented to large intercellular spaces (black stars). Some epithelial cells are engaged in the apoptotic process (white stars). (Cavernous hemangioma).

Fig. 12. Along intercellular contacts of the epidermal cells, no desmosomal junctions can be detected (elliptic areas). Black star marks an intracellular canalicle. (Cavernous hemangioma).

Histologically and ultrastructurally, the infantile hemangiomas in the skin are represented by endothelial hypertrophic tumor cells. They are similar and analogous to the endothelial cells they originated from (Lebkova & Kodrian, 1977).

Capillary hemangioma in children and cavernous hemangiomas in adults are often grouped together as if they were modulations of the same entity. In order to clarify this nosologic question, Iwamoto and Jakobiec (1979) investigated by transmission electron microscopy capillary hemangiomas in children and cavernous hemangiomas in adults and they concluded that capillary hemangioma is completely different from cavernous hemangioma. In agreement with Iwamoto and Jakobiec (1979) opinion, we observed that cavernous hemangiomas had much larger lumens (which resembles a venous tumor), and inside of the stroma there are more heavily collagenized trabeculae (Fig. 8, Fig. 10).

Congenital cutaneous angiomas in children are going to regression: tumor cells underwent necrosis resulting in regression of angiomas and a fibrotic tissue is formed (Lebkova & Kodrian, 1977). Interestingly, in our case of cavernous hemangiomas in adult, an apoptotic to necrotic process takes place and a fibrotic tissue is also formed, but different, the epidermal epithelium is severely affected: a dysplastic to anaplastic epithelium transition is visible (Fig. 8, Fig. 9, Fig. 11, Fig. 12).

BASAL CELL CARCINOMA

Basal cell carcinoma is a neoplastic lesion occurring at the different tissue types. Electron microscopic investigation of the skin specimens from the diagnosed patient to suffer by basal cell carcinoma showed that the specific epidermal cell phenotype is largely lost: keratin intermedium filaments, as well as desmosomal junctions are almost missing. At the tumor epithelial cell-fibrotic stroma interface, the basement membrane and hemidesmosomal junctions are completely missing. Only occasionally remnants of hemidesmosomal junction can be detected. When present, intermedium filaments can be seen as perinuclear bundles or sparse cytokeratine filaments (Fig. 13, Fig. 14). Mention must be made that some areas of the neoplastic epidermal epithelium showed extensive cell prolongations deeply penetrating into peritumoral stroma. The basement membrane and hemidesmosomes accompany cell prolongations. Nevertheless, some areas of such kind of cell extensions are devoid of basement membrane and hemidesmosomes. Moreover, because of the plasma membrane fragility, cell content tends to herniate into peritumoral stroma (Fig. 15, Fig. 16). Similar aspects were described in another case of basal cell carcinoma (Mirancea *et al*., 2010). Tomakidi *et al*. (1999) reported that defects of basement membrane and hemidesmosome structure correlate with malignant phenotype and stromal interactions in HaCaT- ras xenografts, which behave as a squamous cell carcinoma. An overview from the inside neoplastic epidermal epithelium showed that intercellular junctions are almost missing, nuclei are predominantly euchromatic and a lot of polymorphic vacuoles can be seen inside of the cytoplasm. Fig. 17 depicted a neoplastic epidermal epithelium: intercellular junctions are absent, nuclei are predominantly euchromatic and keratine cytoskeleton is almost missing.

Electron microscopic investigations of both pathological cases diagnosed as seborrheic keratosis and cavernous hemangioma showed, at least for long profiles of dermal-epidermal interface, severely basement membrane alterations. The basement membrane integrity is essential for the epithelia organization and homeostasis (Mirancea & Mirancea, 2007-2008, Breitkreutz *et al*., 2009). The basement membrane is a dense meshwork of laminins, type IV collagen, glycoproteins, proteoglycans (Mirancea *et al*., 2001; McMillan *et al*., 2003; Mirancea *et al*., 2007 ; Mirancea & Mirancea, 2010). Usually, a non-interrupted basement membrane separates the benign epithelia tumor from the surrounding stroma. Different from that, between the neoplastic epithelia and peritumoral stroma, the basement membrane is degraded and, only very seldom, patches of such infrastructure can be detected. Indeed, in all our three cases (two benign and one malignant tumors) the above aspects can be recorded. It is not surprising that in our investigated case of seborrheic keratosis the basement membrane is either missing or even expressed in a redundant manner (compare Figs. 3–5 with Fig. 2).

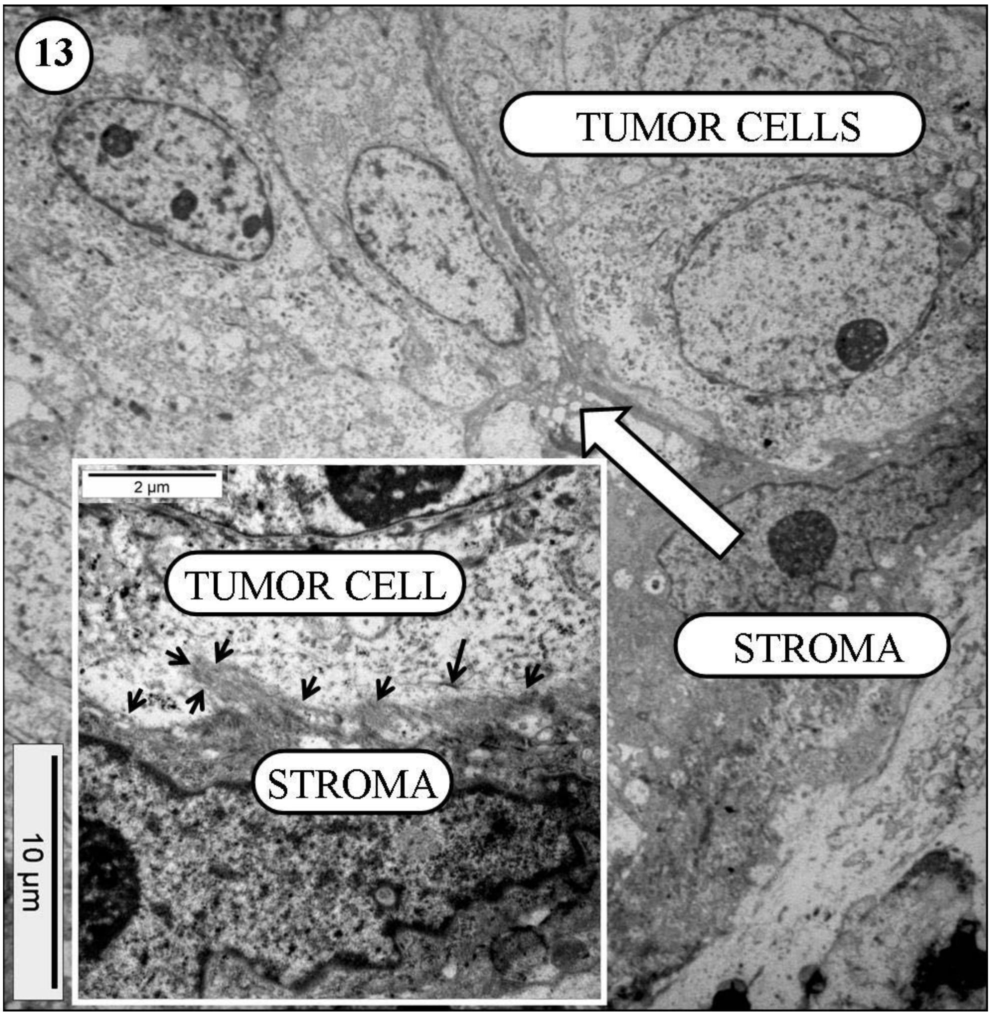


Fig. 13. Neoplastic epithelium (overview). The specific epidermal cell phenotype is largely lost: keratin intermedium filaments as well as desmosomal junctions are almost missing. The basement membrane is absent and a stromal cell tends to penetrate (large arrow) into the neoplastic epithelium. In inset: detail from the neoplastic epithelial cell in direct contact with a stromal cell (head arrows). A remnant of a hemidesmosomal junction can be detected (arrow). (Basal epithelioma).

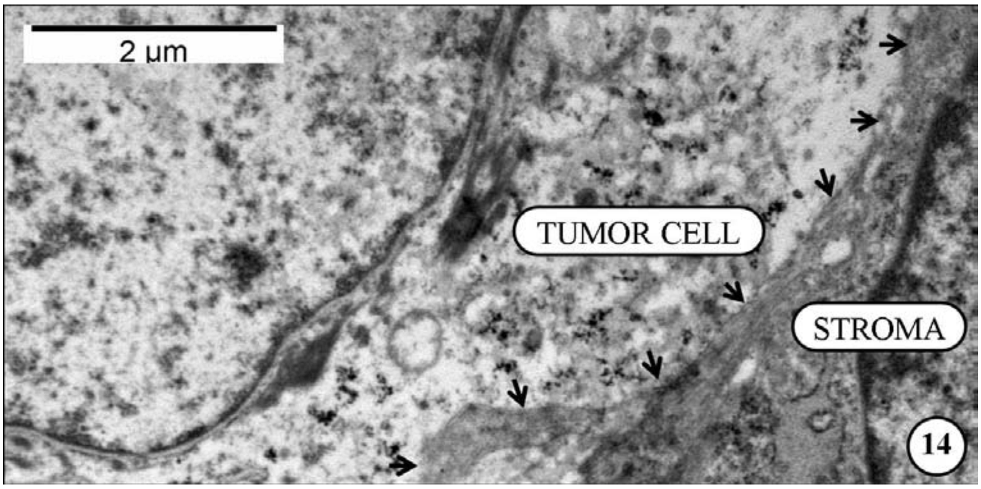


Fig. 14. At the tumor epithelial cell-fibrotic stroma interface (arrows) the basement membrane and hemidesmosomal junctions are completely missing. Perinuclear, some intermedium filaments as bundles of cytokeratines can be seen. (Basal epithelioma).

Weaver *et al*. (2002) showed that, despite of the fact that tumors overexpress extracellular degrading proteases, aggressive tumors often make excess basement membrane. Interestingly, in the cavernous hemangioma in the adult, the severe abnormalities of microvasculature (endothelial cell alterations, missing endothelial wall associated basement membrane and pericytes) and consecutive haemorrhage, meaning prolonged direct contacts between epithelia and blood cells, lead to massive degradation of the dermal-epidermal junction zone. In such circumstances, the whole epidermal epithelium is visibly affected. The remarkable ultrastructural aspect is that suggesting a dysplastic to anaplastic epidermal epithelium. Here, we emphasise the power of the microenvironment: as much as the stroma is altered, the adjacent epithelial tissue is affected in its organization and function (Skobe & Fusenig, 1998). Presence for a long time in massive amount of extravasated blood cells, including inflammatory cells in close vicinity of the epidermis induce by specific molecules (cytokines, enzymes) destabilization of tissue integrity by cell-cell and cell-intercellular junctions. Loss of adhesiveness (downregulation of hemidesmosomes and desmosomes) might result in changes in cell behaviour involving the promotion of tissue invasion (Garrod, 1995). Invasive behaviour results in the development of a diffuse interface between contiguous tissues (Armstrong & Armstrong, 2009). Conversely, when the peritumoral stroma is adequately manipulated in such a way to restore the basement membrane integrity and cell junctions, in spite of the fact that malignant cells still bear the genetic alterations, the normal histo-architecture and functionality of the capillary and epithelia are restored (the tumor phenotype is abolished) (Weaver *et al*., 1979; Vosseler *et al*., 2005).

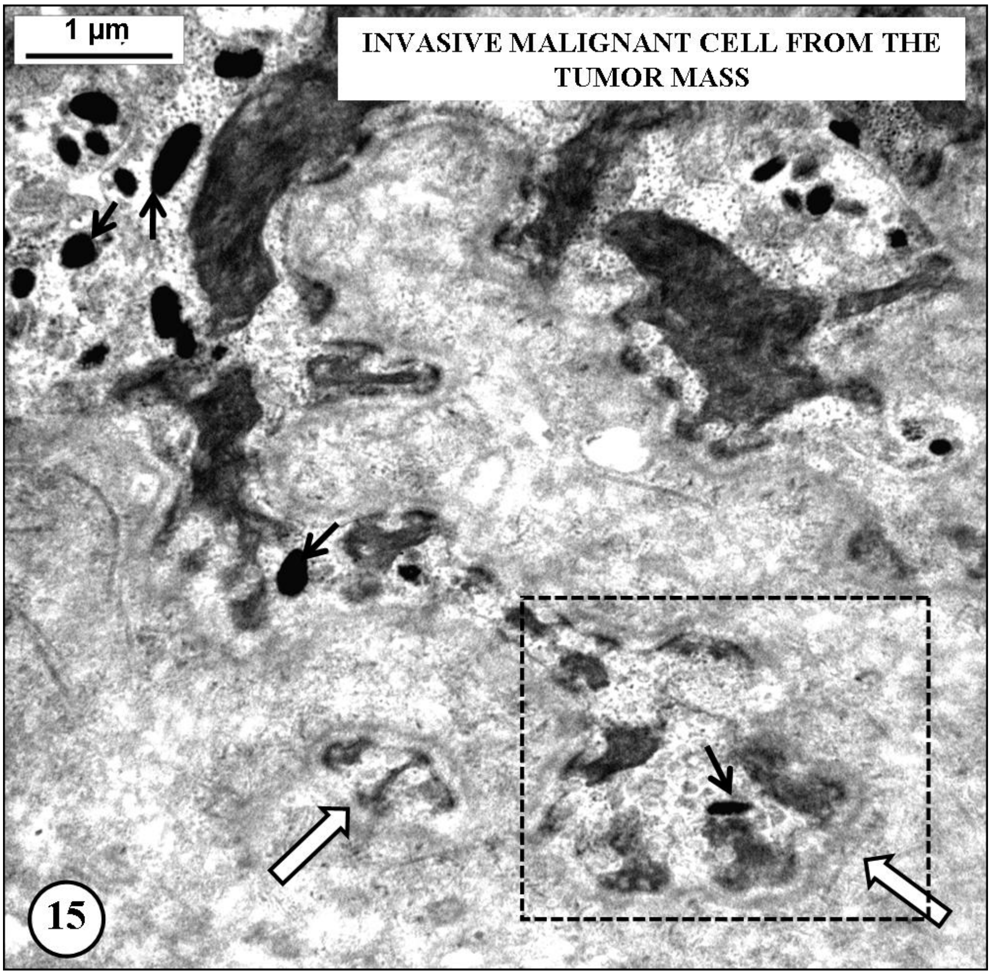


Fig. 15. Extensive cell prolongations of a malignant cell deeply penetrate into the peritumoral stroma (large head arrows). Small arrows depicted melanic granules. Framed area is detailed in Fig. 16. (Basal epithelioma).

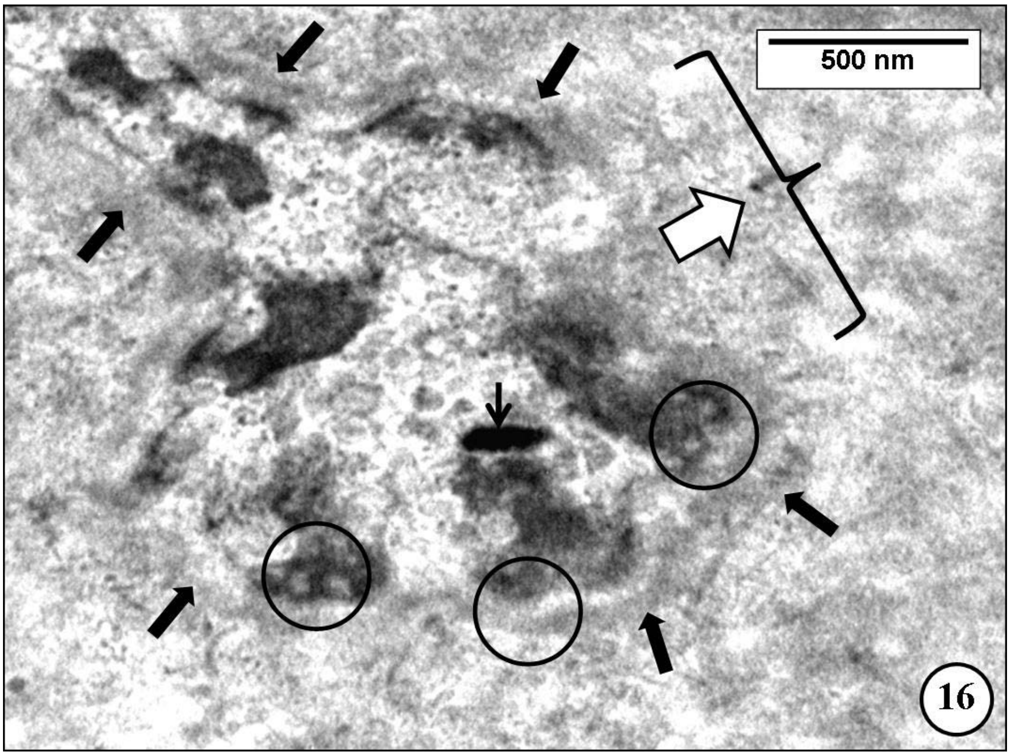


Fig. 16. In spite of the fact that extensive cell prolongations of the neoplastic epithelium (large arrows) deeply penetrate into peritumoral stroma, the basement membrane and hemidesmosomes accompany cell prolongations. Nevertheless, some areas of such kind of cell extensions are devoid of basement membrane and hemidesmosomes. Moreover, because of the plasma membrane fragility, cell content tends to herniate into peritumoral stroma (large head arrow). (Basal epithelioma).

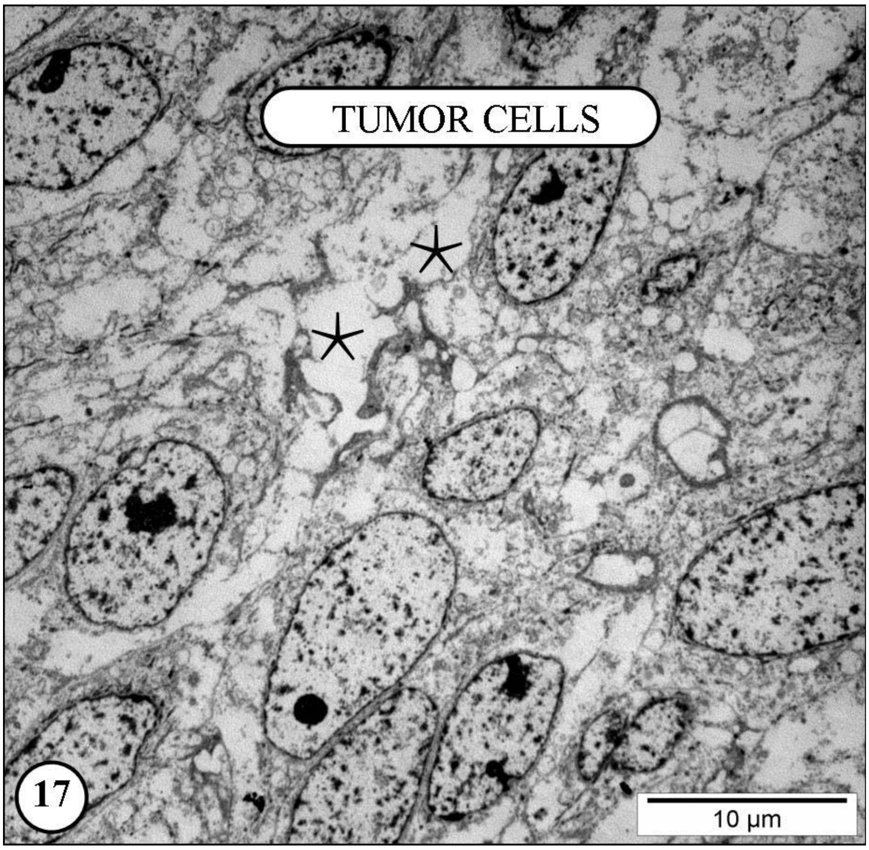


Fig. 17. Neoplastic epidermal epithelium: nuclei are predominantly euchromatic, intercellular junctions are absent, and large intercellular spaces are created (stars). Keratin cytoskeleton is almost missing. A lot of polymorphic vacuoles can be seen inside of the cytoplasm. (Basal epithelioma).

CONCLUSIONS

The reported ultrastructural abnormalities in a case of cutaneous basal cell carcinoma in this paper together with two other cases of benign lesions (seborrheic keratosis and cavernous hemangioma), both, to some extent, in transition to premalignant phenotype, offer an appropriately comparative study deciphering the subtle infrastructural alterations which accompany malignant transformation. Our findings, concerning dysplastic to anaplastic epidermal epithelium as an unusual phenotype in a case of cavernous hemangioma, as well as, unexpected severe alterations of the DEJZ in a case of seborrheic keratose which, to some extent, mimic those described in basal cell carcinoma, underline that alterations of microenvironment exert direct influence to the adjacent epithelia and contribute to a better understanding of the malignant transformation.

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