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THE EPIGEAN FRESHWATER MALACOSTRACANS (CRUSTACEA: MALACOSTRACA) OF THE RIVERS IN THE ANINA MOUNTAINS (SW ROMANIA)

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SUMMARY. This paper represents a biogeographic study on the distribution of freshwater crustacean species of the Astacidae (Decapoda), Gammaridae (Amphipoda) and Asellidae (Isopoda) Malacostracans in the rivers of the Anina Mountains, in Southwestern Romania. Two species of crayfish, three species of Amphipods and one species of aquatic Isopods have been identified. The specimens were captured by hand from the river bad (crayfish) or using Surber collector (for small malacostracans). The data come to complete the existent data as to the distribution and the relative size of the population in the two national parks located in the investigated area - the Semenic-Cheile Caraşului (Semenic - Caras Gorges) National Park and the Cheile Nerei-Beuşniţa (Nera Gorges - Beuşniţa) National Park. The georeferenced data acquired can be used in the policymaking process for the protected areas located within this mountain range.

Keywords: Anina Mountains, *Asellus*, *Astacus*, *Austropotamobius*, distribution, *Gammarus*

Introduction

Freshwater malacostracans residing in the Romanian aquatic ecosystems belong to the Decapoda, Amphipoda and Isopoda orders (Băcescu, 1967), and they also represent water quality indicators (Chapman and Jackson, 1996). Of all the decapods, the stone crayfish *Austropotamobius torrentium* is the most relevant indicator of the habitat's health (Streissl and Hödl, 2002) due to the fact that it is very sensitive to water pollutants (Machino and Füreder, 2005). In the European Council's Directive 92/43, they are rated as "prioritary species" and thus a series of management measures are necessary within the protected areas, amongst which the periodic measurement of population density is of great importance (Pârvulescu, 2007). Moreover, both *A. torrentium* and *Astacus astacus* are considered to be "vulnerable" (IUCN, 2008). Thus, crayfish remain vulnerable to various threats: overexploitation, habitat modification and loss, pollution, the spread of non-indigenous crayfish species, crayfish plague (Holdich and Pöckl, 2005).

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The majority of amphipod species reside in marine or brackish environments, but they also inhabit a wide range of freshwater habitats, in close relation to the environmental conditions (Ciubuc, 1985). Species diversity is higher in flowing waters, either cold, or subterranean, while the number of epigeal species is lower (Väinölä *et al.,* 2008). As to isopods, the majority of species are marine, some species are adapted to terrestrial environments and very few of them prefer freshwater environments exclusively (Müller and Tomescu, in Godeanu, 2002).

The data published in regard to freshwater crayfish species in the Anina Mts. area are few and obsolete, and many of the locations mentioned are no longer in accordance with present reality. First records of the *Austropotamobius torrentium* in the Anina Mts. place this species in the Minis River, in Buhui and around Anina (Entz, 1912). In the Decapoda volume of the "Romanian Fauna", the species is mentioned in the Minis River, in the Righidia stream (affluent of the Minis, in the area around the village of Bozovici), as well as in the Ponicova stream – an affluent of the Caras that crosses the Comarnic Cave (Băcescu, 1967). Bănărescu and Oprescu (1971) mention the species in the affluent of the Nera River in the mountainous area, but without specifying the exact locations. The distribution map presented by Machino and Füreder (2005) for *A. torrentium*, refers only to a broad European context. Machino and Holdich (2006) mention the species in Southwestern Transylvania and in Western Walachia. The *Astacus astacus* species is mentioned in the literature as present in the Anina Mts. in the Buhui Lake (cohabitating with *A. torrentium*), in the Minis river (Băcescu, 1967) and in the Nera river (Bănărescu and Oprescu, 1971). There is no mention of the presence of *A. leptodactylus* or any other decapods in the Anina Mts. In the work of Machino and Holdich (2006), the only reference is that related to the distribution of *A. astacus* and *A. leptodactylus*. The most recent monograph on crayfish species of Europe, "Atlas of crayfish in Europe" (Souty-Grosset *et al.*, 2006) reflects the deficiency in data on the distribution of the three native crayfish species of Romania.

An important aspect of biodiversity conservation is the biocontamination of benthic macro invertebrates with alien species (Arbačiuskas *et al.*, 2008). Currently, nine species of freshwater crayfish are known to reside in Europe. These species were intentionally introduced here during the XIXth and XXth century. Four of these species belong to the *Orconectes* genus, one to *Procambarus*, three to *Cherax* and also one to *Pacifastacus* (Holdich and Pöckl, 2007; Souty-Grosset *et al.*, 2006; Henttonen and Huner, 1999). In many cases, these species have their own way of spreading from one area to another, through canals and rivers or during floods (Holdich and Pöckl, 2007). Among the countries that are close to Romania and are found upstream of the Danube, Hungary, Croatia and Slovakia have already been infested with 2 species: *Pacifastacus leniusculus* and *Orconectes limosus* (Puky *et al.*, 2005; Janský and Kautman, 2007; Petrusek and Petruscová, 2007; Maguire *et al.*, 2008; Maguire and Klobučar, 2008; Puky, 2009); in Serbia, *O. limous* is present (Pavlović

et al., 2006; Simić *et al.*, 2008). These species are potential competitors of the native species (Holdich, 2003) as well as potential carriers of the deadly crayfish plague *Aphanomyces astaci* (Kozubiková *et al., in press*). On the other hand, the Danube river acts like a passage for the upstream spreading of several amphipod species. During the last few years, these species have largely expanded from the Balkan and the Ponto-Caspian regions towards northwestern Europe (Grabowski *et al.*, 2007a), Thus, some species native to Romania have managed to colonize western Europe through the Rhine-Danube system, connected for the first time by the Ludwigskanal in 1845: *Echinogammarus ischnus, Pontogammarus robustoides, Obesogammarus crassus, Dikerogammarus haemobaphes, D. villosus* (Grabowski *et al.*, 2007c); *D. villosus* has even reached the alpine lake Lac du Bourget in France (Grabowski *et al.*, 2007b).

The investigated area, i.e. the Anina Mountains, is located in southwestern Romania; it has a surface of 770 km^2 (Sencu, 1978) mostly included in two National Parks: Semenic-Cheile Caraşului (Semenic – Caras Gorges) National Park and Cheile Nerei-Beuşniţa (Nera Gorges - Beusnita) National Park. The relief is mainly calcareous (Bleahu and Rusu, 1965). This geographical unit is drained by three main water courses: the Bârzava River, the Caraş River and the Nera River. The Bârzava River collects its waters from the northern and northeastern part of the Anina Mountains and from the western part of the Semenic Mountains, crossing a substratum of crystalline schists. The upstream stretch of the Caraş River drains the central-western Anina Mountains, of calcareous composition. The Nera River crosses the southern sector of these mountains, draining the waters from the southern and central-eastern sector; its main tributary, the Miniş River, crosses a calcareous substratum. The Caraş and the Nera rivers are the direct tributaries of the Danube River, and the Bârzava River flows into the Danube River, after the confluence with the Timiş River (Ujvari, 1972).

Since the existing data related to the distribution of freshwater crustacean species is rather obsolete, it is necessary to update this data in view of a better management of this region.

Materials and methods

Sampling sites and collection of specimens. Qualitative and quantitative biologic samples were collected in August 2007 and in August 2008 respectively, in a total of 52 sampling stations, on all the permanent waters in the upper sector of the Bârzava, Caraş and Nera rivers (Figure 1). Most of these sampling stations are located within two National Parks (Semenic-Cheile Caraşului National Park and Cheile Nerei-Beuşniţa National Park).

Each sampling station was comprised of at least 100 m of river under investigation. On this occasion, observations were carried out with regard to the riverbed morphology and the surrounding habitats. The crayfish were collected using

active methods (Dorn *et al.*, 2005), i.e. direct hand sampling from the water bed, by checking the spaces between rocks, roots and galleries within banks, and by sampling over 100 meters along each of the rivers investigated. If specimen collection was rendered impossible by the morphological features of the substratum, we declared the species as being absent when, after sampling over 200-300 meters, we found no specimens.

For the quantitative collection of small freshwater crustaceans (Amphipods and Isopods), we used a Surber sampler with a catchment area of 300 cm^2 and a mesh size of 350 μm, followed by further research among solid objects and plants on the bottom of the river. The collecting surface was established at five randomly chosen different squares per sampling station, on the 100-metre river sector, for covering more microhabitats.

Statistical analysis. Once the number of individuals captured on the river sector under investigation was established, the approximate dimension of the freshwater crayfish population was determined for each sampling station and employed in statistical analyses. To perform the statistical analysis and create the diagrams, we used the PAST software (version 1.88). Similarity between the preferences of the 6 malacostracans species, from the 52 sampling stations, were represented by the cluster correlation analyzes (Ceapoiu, 1968).

Identification of specimens. The crayfish were identified *in situ* according to their morphological features, sexed and photographed. Tissue for subsequent molecular analysis was sampled, by detaching the last pereiopod on the right and conserving it in alcohol (80%). Moreover, an inspection was carried out, to determine the specimens' health status and potential parasites (Holdich, 2003). Subsequently, the specimens were set free in the same location where they had been captured. To identify the specimens, we used the keys in Băcescu (1967) and Ingle (1997).

The amphipods and the isopods were brought to the laboratory, processed and analyzed using the optic stereomicroscope; for details, micro dissections and digital photography were carried out. The identification of amphipods was carried out on the species level, using the Karaman and Pinkster (1977, 1987) and the Cărăuşu *et al.* (1955) determination keys, and the Racovitza (1919) and Negoescu (1987, 1989) publications, respectively. Subsequently, we updated the data systematically, in accordance with the data in the Fauna Europaea project (Geoffrey, 2007; Geoffrey and Ronald, 2007). The collected specimens have been conserved in alcohol (70%), in our Faculty collection.

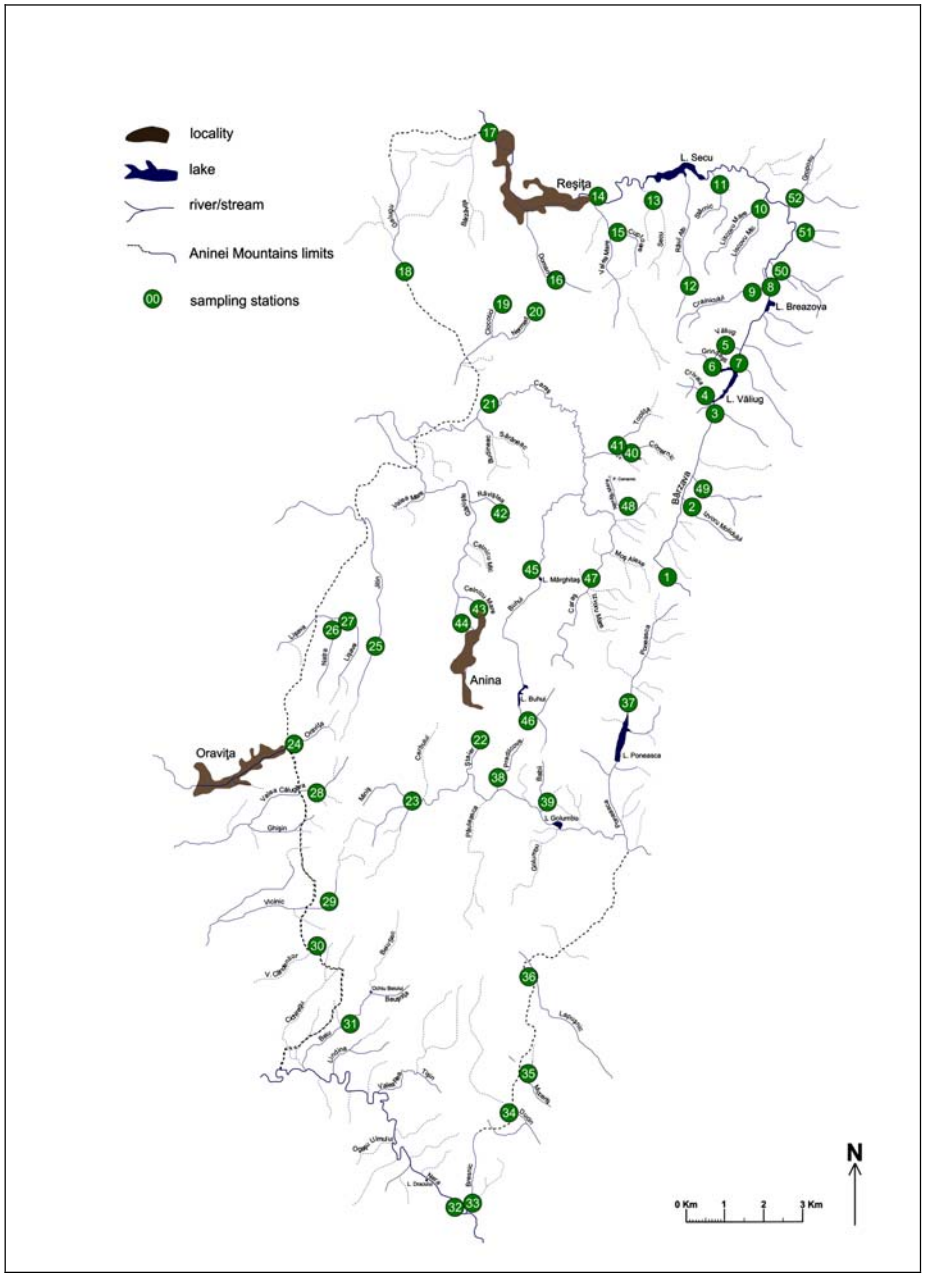

Fig. 1. Distribution of the 52 sampling stations in the lotic aquatic habitats of the Anina Mountains (see Table 1 for geographical coordinates and toponyms)

Results

Based on the findings of the 52 sampling stations dispersed over all the permanent rivers in the Anina Mountains, we were able to create a high-resolution image of the epigean species distribution and the relative dimension of the populations belonging to the Astacidae, Gammaridae and Asellidae groups. For a more detailed image, we will go over each hydrographical basin and its tributaries. To be noted that the rivers are presented according to their geographical position in Table 1.

The Bârzava hydrographical basin was investigated starting from the upper part (near the spring) up to the exit from the Anina Mountains (downstream from the town of Reşiţa) representing a total of 21 sampling stations, 6 on the main course of the river and 15 on its tributaries. As a result to the investigation of the main course, the *Astacus astacus* (Linnaeus 1758) decapod was identified in one location, upstream from the Văliug Lake. The amphipods were present in all the sampling stations (except downstream from the town of Reşiţa and downstream from the Văliug Lake) by means of *Gammarus balcanicus* Schäferna 1922 and *Gammarus fossarum* Koch, in Panzer 1835 species, while the aquatic isopods, by means of *Asellus aquaticus* (Linnaeus 1758), were present only downstream from the town of Reşiţa, in a oozy habitat. The 15 investigated tributaries of the Bârzava River revealed the presence of *A. astacus* and *Austropotamobius torrentium* (Schrank 1803) decapods. *A. astacus* is also present in the river's tributaries which flow directly into the Văliug Lake (Crivaia and Grindeşti) and also in the Doman River, while *A. torrentium* is present in the majority of the tributaries (Dignacea, Crainicului, Radomir, Bogatu, Liscov, Stârnic, Râul Alb, Cuptoare creeks). The *G. balcanicus* amphipod was found in Molidului, Dignacea, Crivaia, Grindeşti, Văliug, Crainicului, Radomir, Bogatu, Râul Alb, Groposu, Liscov, Cuptoare, Stârnic, Secu, while *G. fossarum* was found only in the Doman river.

The Caraş hydrographical basin was investigated in the upper part up to the exit from the mountain range, downstream from the Caraş Gorges, as well as its tributaries with a permanent water course, representing a total of 20 sampling stations. The *A. astacus* decapods were present in 13 sampling stations (Clocotici, Oraviţa, Jitin, Natra, Lişava, Călugăra, Ponicova, Comarnic, Toplita, Răviştea, Celnicu Mare, Buhui downstream from Mărghitaş Lake and also upstream from Buhui Lake), while *A. torrentium* was present in only 2 locations at Cândeni and Buhui creek, upstream from Buhui Lake; in the last sampling station both species were captured, the specimens of *A. torrentium* being numerically predominant. The amphipods were identified in most of the locations: *G. balcanicus* (Buhui, Ponicova, Caraş creek, Răviştea, Comarnic, Toplita, Celnicu Mare, Jitin, Oraviţa, Lişava, Călugăra, Cândeni, Vicinic and Caraş downstream from the Gorges) and *G. fossarum* (Oraviţa, Lişava, Natra, Nermed and Clocotici), while *Gammarus roeseli* Gervais 1835 was identified in two stations (Caraş downstream from the Caraş Gorges and Gelugu creek). The captured aquatic isopods (*A. aquaticus* species) were present in one of the sampling station, Gelugu River, where the habitat was represented by a dim water course with a muddy substratum.

Table 1.

Distribution of freshwater crustaceans, relative dimension of the populations and frequency in Anina Mountains Rivers (2007-2008)

Col. pts.	Location	Geographical coordinates N/E	Relative altitude	*Ast.ast*	*Aus.tor*	*Gam.bal*	*Gam.fos*	*Gam.roe*	*Ase.aqu*
Bârzava basin									
A1	Bârzava creek	45°07’15’’/ 21°59’23’’	900 m			○			
A2	Molidului	45°10’06’’/ 21°59’59’’	700 m			●			
A49	Dignacea	45°10’55’’/ 22°00’19’’	700 m		○	●			
A3	Bârzava (ups. Văliug Lake)	45°12’03’’/ 22°00’42’’	655 m	○		○			
A4	Crivaia	45°12’10’’/ 22°00’38’’	658 m	○		●			
A6	Grindeşti	45°13’14’’/ 22°00’33’’	650 m	●		■			
A5	Văliug	45°13’50’’/ 22°00’49’’	600 m			■			
A7	Bârzava (dws. Văliug Lake)	45°13’11’’/ 22°01’28’’	560 m						
A8	Bârzava (dws. Breazova Lake)	45°15’10’’/ 22°02’58’’	480 m			●			
A9	Crainicului	45°15’23’’/ 22°02’37’’	460 m		○	●			
A50	Radomir	45°15’52’’/ 22°02’54’’	475 m		○	●			
A51	Bogatu	45°16’45’’/ 22°03’45’’	450 m		●	●			
A12	Râul Alb	45°17’32’’/ 21°59’41’’	430 m		○	●			
A52	Groposu	45°17’28’’/ 22°03’43’’	415 m			●			
A10	Liscov	45°17’32’’/ 22°02’33’’	380 m		○	●			
A15	Cuptoare	45°16’36’’/ 21°57’35’’	360 m		○	●			
A11	Stârnic	45°18’13’’/ 22°02’43’’	330 m		○	■			
A16	Doman	45°15’38’’/ 21°54’18’’	320 m	●			●		
A13	Secu	45°17’48’’/ 21°58’14’’	300 m			■			

Table 1. (continued)

Col. pts.	Location	Geographical coordinates N/E	Relative altitude	*Ast.ast*	*Aus.tor*	*Gam.bal*	*Gam.fos*	*Gam.roe*	*Ase.aqu*
A14	Bârzava m.c. (ups. Reşiţa)	45°17'39''/ 21°56'15''	275 m				●		
A17	Bârzava m.c. (dws. Reşiţa)	45°20'36''/ 21°50'08''	190 m						■
	Species frequency in the Bârzava basin (%)			19	38.1	80.9	9.5	0.0	4.7
			Caraş basin						
A46	Buhui creek	45°03'51''/ 21°53'20''	660 m	○	●				
A45	Buhui (dws. Mărghitaş Lake)	45°07'31''/ 21°54'00''	530 m	○		●			
A48	Ponicova	45°09'26''/ 21°57'03''	515 m	●		●			
			Caraş basin						
A47	Caraş creek	45°07'23''/ 21°56'00''	500 m			○			
A42	Răviştea	45°03'10''/ 21°52'35''	475 m	■		●			
A40	Comarnic	45°10'46''/ 21°57'10''	470 m	●		●			
A41	Toplița	45°10'56''/ 21°56'56''	465 m	●		●			
A43	Celnicu Mare	45°06'48''/ 21°51'46''	435 m	■		●			
A44	Gârlişte	45°06'23''/ 21°50'53''	420 m						
A25	Jitin	45°07'13''/ 21°48'02''	395 m	●		●			
A24	Oravița	45°03'07''/ 21°45'02''	390 m	●		●	■		
A27	Lişava	45°06'22''/ 21°46'39''	300 m	●		●	●		
A28	Călugăra	45°01'45''/ 21°45'15''	300 m	○		●			
A30	Cândeni	44°56'49''/ 21°44'08''	295 m		■	■			
A26	Natra	45°06'21''/ 21°46'09''	295 m	■			■		
A29	Vicinic	44°58'28''/ 21°44'37''	292 m			■			
A20	Nermed	45°13'59''/ 21°52'26''	275 m				■		
A19	Clocotici	45°14'43''/ 21°50'28''	270 m	■			●		

Table 1. (continued)

Col. pts.	Location	Geographical coordinates N/E	Relative altitude	*Ast.ast*	*Aus.tor*	*Gam.bal*	*Gam.fos*	*Gam.roe*	*Ase.aqu*
A21	Caraş (dws. gorges)	45°12'06''/ 21°52'27''	240 m			●		●	
A18	Gelugu	45°16'01''/ 21°48'31''	230 m			○		■	●
Species frequency in the Caraş basin (%)				65	10	75	25	10	5
Nera basin									
A23	Miniş creek	45°01'29''/ 21°49'24''	595 m		●	●			
A22	Şteier	45°02'38''/ 21°51'34''	540 m			■			■
A38	Predilcova	45°01'57''/ 21°52'39''	505 m		●	■			
A37	Poneasca	45°03'29''/ 21°57'36''	465 m		○				
A39	Babii	45°01'18''/ 21°54'24''	350 m		○	●			
A36	Lăpuşnic	44°55'03''/ 21°55'37''	298 m		■	●			
A35	Moceriş	44°53'36''/ 21°53'53''	298 m		●	●			
A34	Ducin	44°52'31''/ 21°53'47''	280 m		●	●			
A31	Beiu	44°55'22''/ 21°46'28''	240 m		●	■			
A33	Bresnic	44°50'27''/ 21°51'21''	205 m			●			
A32	Nera m.c. (dws gorges)	44°50'19''/ 21°51'18''	200 m						
Species frequency in the Nera basin (%)				0.0	72.7	81.8	0.0	0.0	5
Species frequency in the rivers of the Anina Mountains (%)				**32.7**	**34.6**	**78.8**	**13.5**	**3.8**	**5.8**
Total specimens				**69**	**113**	**1567**	**1779**	**139**	**228**
Uncertain juveniles				**3**			**155**		**0**

Observations: the symbols in the cells have the following meanings for crayfish: ○ = rare (<0.04 ind/m^2); ● = present (0.05-1 ind/m^2); ■ = common (>1 ind/m^2); for amphipods and isopods: ○ = rare (<10 ind/m^2); ● = present (11-100 ind/m^2); ■ = common (>100 ind/m^2), empty cell = absent

Abbreviations: *Ast.ast = Astacus astacus*; *Aus.tor = Austropotamobius torrentium; Gam.bal = Gammarus balcanicus; Gam.fos = G. fossarum; Gam.roe = G. roeseli; Ase.aqu = Asellus aquaticus*; m.c. = main course; ups = upstream; dws = downstream

As far as the similarity in the preferences for a specific habitat (Figure 2) is concerned, no significant links were noticed among the six aquatic Malacostraca species in the Anina Mountains. However, affinities are noticed between the *Gammarus roeseli* amphipod and the *Asellus aquaticus* isopod, and between the *Gammarus fosarum* amphipod and the *Astacus astacus* decapod. A weak but positive-evidence association is determined for the *Gammarus balcanicus* amphipod and the *Austropotamobius torrentium* decapod, which were observed at the same sampling stations.

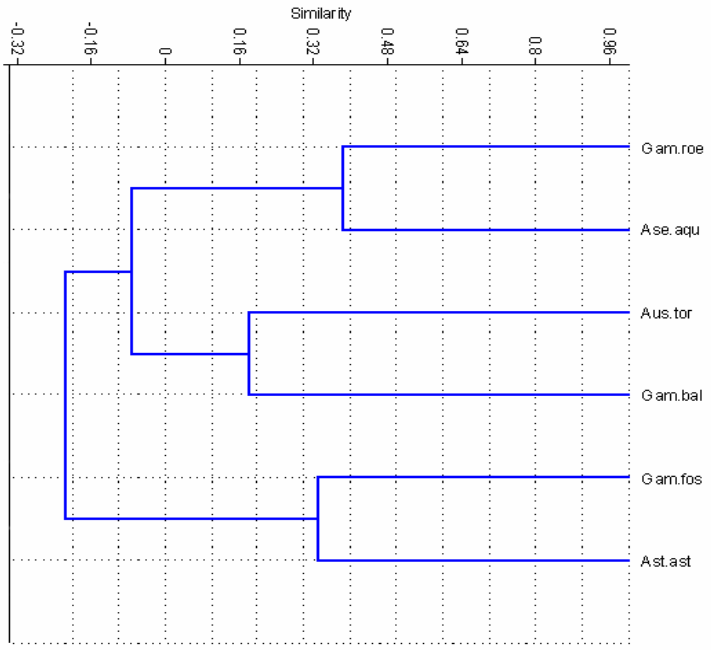

Fig. 2. The association of Malacostraca species in the rivers of Anina Mountains (coph. corr. 0.8434)

Discussion and conclusions

The researches made between summer 2007 and 2008 in the freshwaters of the Anina Mountains offer a good view regarding the distribution, frequency and relative dimension of the populations of the freshwater crustacean species in this region and the possibility of data interpretation.

By comparing our data to the data provided by previous research, one will notice that *A. astacus* can no longer be found in the Miniş River, any of its tributaries or the main course of the Nera River. *A. torrentium* was completely replaced by

A. astacus in the Ponicova stream. However, the co-existence of the two species upstream from the lake formed on the Buhui River is still confirmed. Taking into account that *Orconectes limosus* is a species which can live in small streams (Pöckl *et al.*, 2006), that it has already been attested in the area where the Nera river flows into the Danube, (Pârvulescu *et al.,* 2009), and that it is a potential carrier of the crayfish plague, which is deadly for the native crayfish species (Kozubiková *et al.*, *in press*), we consider that this species is a potential danger for the aquatic fauna of the Anina Mountains.

Since the diversity of the amphipods is low in the surface waters of Romanian mountains (Cărăuşu *et al.,* 1955), the presence of the three species from the Gammaridae family shows a good diversity of the aquatic habitats in the Anina Mountains. The most frequent species are *Gammarus balcanicus* (with a frequency of 78.8 %) and *Gammarus fossarum* (with a frequency of 13.5 %); the two species are common throughout the Balkan Peninsula. (Živić and Marcović, 2007) In Western Europe it is considered that *Gammarus roeseli* has extended its habitat in southeastern Europe. (Grabowski *et al.,* 2007c) Cărăuşu *et al*. (1955) mentions that *Rivulogammarus triacanthus* (sin. *Gammarus roeseli*) lives in the warmer waters of the Episcopia Bihor area (North-Western Romania).

A. aquaticus is a species living in slow lowland waters which display a higher degree of saprobity. Even if high numbers of specimens were collected at the Steier sampling station situated at an altitude higher than 500 meters, this was caused by the eutrophication of the stream as a result of pollutants released in the village of Steierdorf (Anina); the absence of the common but more pollution-sensitive crayfish (Machino and Füreder, 2005) in the Nera basin confirms this atypical situation. In the other two sampling stations the species was actually collected only in low-altitude streams polluted by various human activities specific to the villages and towns which the streams cross.

As far as the sensitivity of the three groups of freshwater crustaceans is concerned, according to the Biological Monitoring Working Party Score System (Chapman *et al.,* 1996), one notices that the Astacidae (score 8) and the aquatic Asellidae (score 3) do not cohabit. The Gammaridae's score 6 is not relevant for this comparison. We also notice that in the main course of the rivers, downstream from towns or reservoirs the diversity of freshwater crustaceans is very low. In the Bârzava River, downstream from the Văliug Reservoir (in the area right downstream of the dam), the freshwater crustacean are missing; the sample actually contains no insect larvae characteristic of that type of region. The absence of aquatic invertebrates at this sampling station is highly likely to be caused by the water from the deeper layers of the reservoir, which is rich in suspensions and low in oxygen (Pârvulescu and Hamchevici, *submitted manuscript*). *G. balcanicus* was reported at approximately 200 meters downstream from Breazova Lake. In the main course of Bârzava River, downstream from the town of Reşiţa, the aquatic crustacean fauna is represented by only one species and a high number of individuals: *A. aquaticus,* a species which

usually lives in high-nutrient waters. The results of the investigations carried out in the main course of Caraş River showed a normal diversity for that region, probably because there is no village or town upstream from the sampling station (Pârvulescu and Hamchevici, *submitted manuscript*). The absence of freshwater crustaceans in Nera River is most probably caused by the released pollutants upstream in the river, from the towns and villages (Pârvulescu, 2009; Pârvulescu and Hamchevici, *submitted manuscript*).

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