Diversity patterns of fauna in dripping water of caves from Transylvania

Ioana N. Meleg[1], Oana T. Moldovan[1]*, Sanda Iepure[1], Frank Fiers[2] and Traian Brad[1]

[1] "Emil Racoviţă" Institute of Speleology, Department of Cluj, Romanian Academy, Clinicilor 5, PO Box 58, 400006 Cluj-Napoca, Romania
[2] Royal Belgian Institute of Natural Sciences, Vautierstraat 29, B-1000 Brussels, Belgium

Abstract – Recent studies substantiate the importance of the unsaturated zone in ground-water biodiversity of karst areas. Few investigations, however, have addressed the temporal changes in community composition in relation to water physico-chemical features. We provide information on the distribution pattern of the fauna in dripping water at spatial and temporal scales. This is related to variation in water chemistry and other environmental features in five caves within two hydrographic basins of the Pădurea Craiului Mountains (northwestern Romania). The analysis revealed no major pollution in the dripping water. The physico-chemical parameters varied within and between caves over one year. The dripping water fauna is heterogeneously distributed within and between the caves, containing a mixture of epigean and hypogean species. This emphasizes high microhabitat partition and also underlines the influence of physico-chemical parameters. The species composition among the two hydrographic basins was different. Crustaceans are the best represented in terms of both abundance and species richness. Six out of 15 crustacean species are endemic to the Pădurea Craiului Mountains and four are new to science. All these point to the importance of unsaturated karstic habitats as biodiversity hot spots in ground-water ecosystems.

Key words: Biodiversity / karst unsaturated zone / Pădurea Craiului Mountains / Romania

Introduction

The epikarst is the uppermost unsaturated zone of carbonate rocks. It extends several meters underground and is characterized by high porosity and permeability, potentially containing large water reserves (Mangin, 1994). Water infiltrating from the surface or from the soil percolates through the epikarst before reaching the vadose zone. It then continues downward and reaches the saturated or phreatic zone of caves (Jones *et al.*, 2004).

Despite an apparent scarcity, the fauna inhabiting the fissures and cracks of the unsaturated zone (epikarst and vadose zone) is diverse and often exceeds the diversity of other habitats such as pools, subterranean streams or lakes (Juberthie, 2000; Pipan and Brancelj, 2004a; Galassi *et al.*, 2009). This high diversity, combined with the presence of a mixture of soil, epikarstic and typical hypogean fauna, makes the study of the upper layer of the karst highly interesting for biologists (Pipan and Culver, 2007a).

The fauna inhabiting the water in fissures first came to the attention of Bou (1979), Delay (1968) and Rouch (1968), who sampled the dripping water from stalactites and the temporary water flowing on cave walls. Recently, the unsaturated zone has been extensively investigated at different scales, revealing a high biodiversity associated with a high degree of endemism, mainly among crustacean copepods (Pipan and Brancelj, 2001, 2004a, 2004b; Brancelj, 2002; Bobić, 2003; Sket *et al.*, 2004; Pipan, 2005; Pipan and Culver, 2005, 2007a, 2007b, 2007c; Moldovan *et al.*, 2007; Pipan *et al.*, 2008). All studies emphasized the heterogeneity of the faunal distribution in the unsaturated zone. Some demonstrated that the faunal composition is influenced by certain abiotic features of the ecosystem, mainly temperature, electrical conductivity, precipitation and thickness of the cave ceiling.

This contribution provides an overview of the aquatic communities in dripping water of several caves by investigating the α-diversity (within caves) and the β-diversity (between caves) in the regional context of the Romanian Western Carpathians. This is coupled with an attempt to define spatial and temporal patterns of

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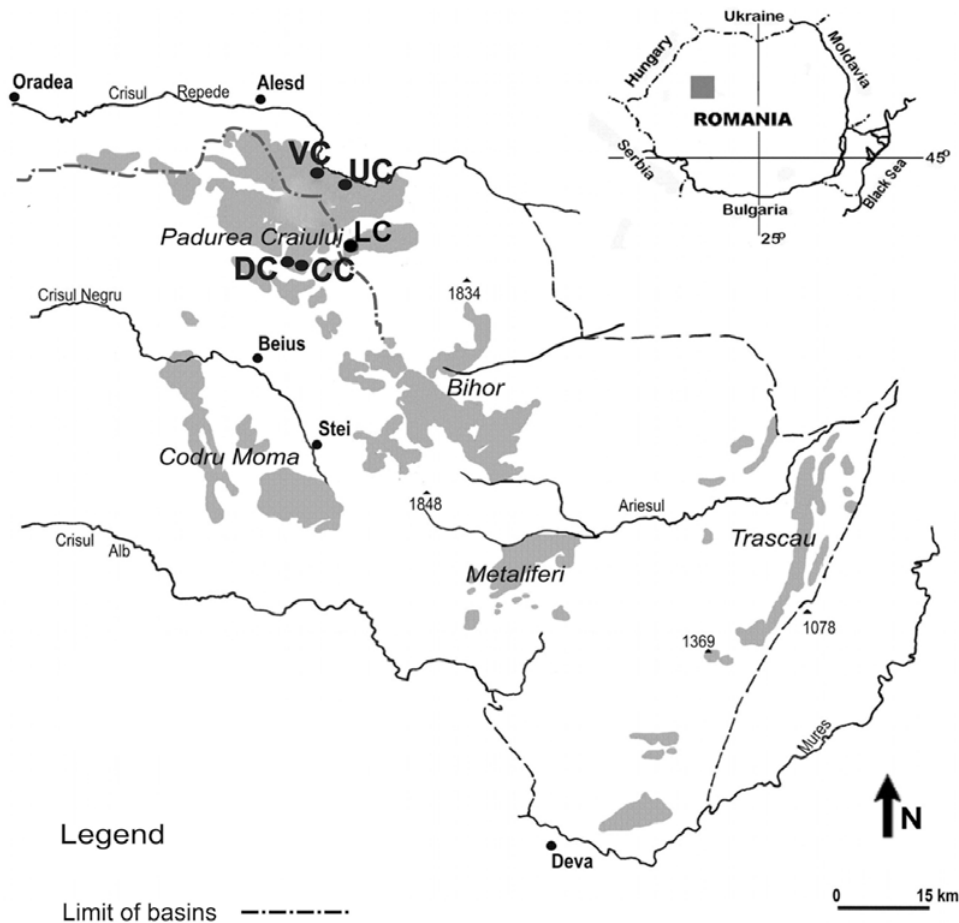*Corresponding author: oanamol@hasdeu.ubbcluj.ro

Fig. 1. Locations of investigated caves in the Pădurea Craiului Mountains (northwestern Romania): grey surfaces are limestone areas (for abbreviations see Table 1).

distribution and abundance of subterranean communities in relation to environmental parameters.

Materials and methods

Study area

The spatial and temporal dynamics of the fauna at micro- (within-cave, ranging from 3 to 500 m) and mesoscales (part of river basins, ranging from 1 to 15 km) were analyzed in the Pădurea Craiului Mountains, located in the western part of the Carpathian ridge in Romania (the Apuseni Mountains). The massif is mainly composed of Mesozoic sediments, whereby the karst landscape is highly represented, up to 37% of the entire surface (Rusu, 1988).

Our study focused on five active caves (Fig. 1) of different lengths (320–4000 m) within a range of approximately 200 km^2 between the catchment areas of two hydrographic basins: Crişul Repede (three caves) and Crişul Negru (two caves). Details on the geographical position of the caves, the sampling periods, the number of sites in each cave and the total number of collected samples are given in Table 1 and Figure 2. Ungurului Cave (UC) is

developed in Ladinian (middle Triassic) limestone and Vadu Crişului Cave (VC) in Barremian (Cretaceous) limestone (Orăşeanu, 1991). The entire area has been intensively exploited for fireclay. Ciur Izbuc Cave (CC), developed on two levels, and Doboş Cave (DC) are located on the karstic plateau of Runcuri. These two caves, together with the Cave cu Apă din Valea Leşului (LC), have developed in Neocomian–lower Aptian (lower Cretaceous) limestone (Orăşeanu, 1991). Two household deposits are located above DC, and the wastewater enters the cave downstream of the selected sampling sites. The area was intensively exploited for bauxite and for fireclay with iron and aluminum oxides.

Sampling protocol

Preliminary investigations were conducted in the selected caves in order to identify the permanent or quasi-permanent character of the drips. Fauna and water samples were taken monthly from January to December 2008, with the exception of one cave (Table 1). Samples omitted due to external weather conditions (*i.e.*, frozen or dry periods, high water levels) are specified in Table 1. The water from trickles was directed through a funnel

Table 1. Investigated caves from the Pădurea Craiului Mountains (northwestern Romania) (see also Figs. 1 and 2).

Cave	Abbrev.	Basin	Altitude (m above sea level)	Geographic coordinates	Length (m)	Sampling period	Number of sites	Total number of samples collected	Samples not collected
Ungurului Cave	UC	Crişul Repede	305	N 46° 55'.989" E 22° 32'.890"	1500	I–XII 2008	3	31	Site 1 in I, II, III, IX, X
Vadu Crişului Cave	VC	Crişul Repede	305	N 46° 57'.714" E 22° 30'.679"	4000	I–XII 2008	9	105	Site 2 in XI Site 9 in III, IV, XI
Cave cu Apă din Valea Leşului	LC	Crişul Repede	650	N 46° 49'.528" E 22° 33'.419"	800	I–XII 2008	6	71	
Ciur Izbuc Cave	CC	Crişul Negru	395	N 46° 51'.100" E 22° 24'.002"	1030	I–XII 2008	9	98	
Doboş Cave	DC	Crişul Negru	467	N 46° 50'.658" E 22° 23'.584"	320	III 2008– II 2009	4	45	Site 4 in IX, X
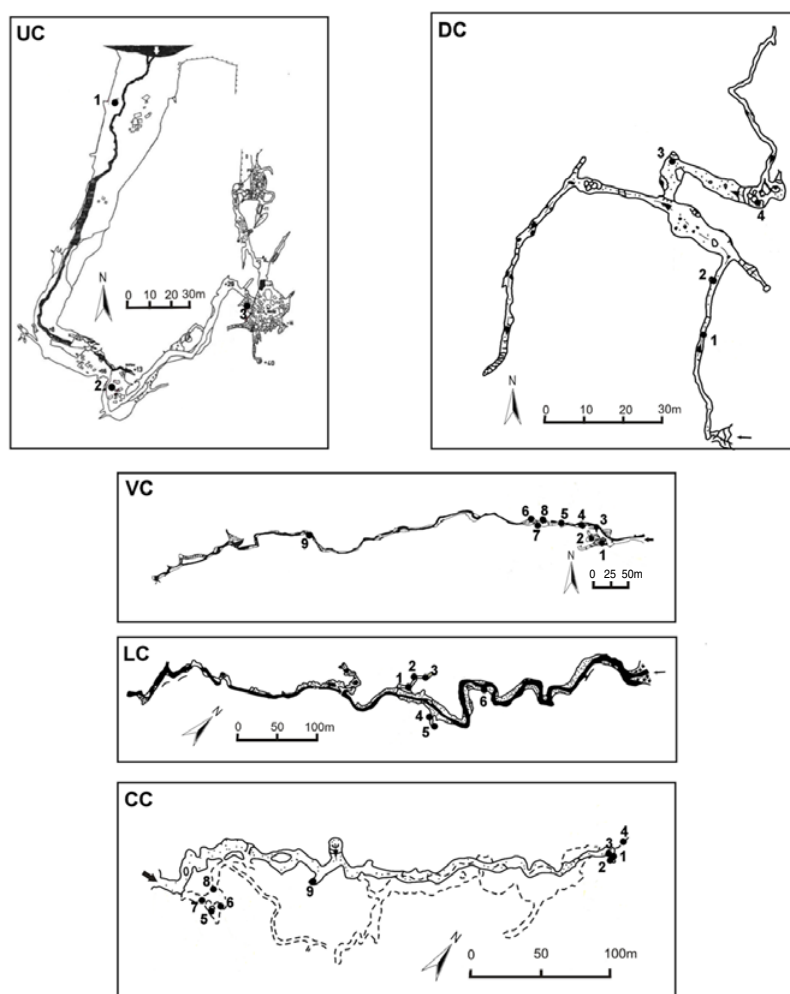
Fig. 2. Cave maps and sampling site position (UC map – modified from Vălenaş and Iurkiewicz, 1981; VC map – modified from Pleşa, 1969; LC, CC, DC – modified from Rusu, 1988).

into containers whose sides were covered by a 100 μm mesh plankton net, following the protocol described and utilized by Pipan and Brancelj (2001, 2003). Animals were able to survive for one month in the 1 cm deep water at the bottom of the container. Animals were fixed in 96% ethanol and sorted to different taxonomic levels using a stereomicroscope (Olympus SZX16): phylum (Nematoda), class or subclass (Gastropoda, Oligochaeta), infraclass (Acarina), order (Collembola) and family (Chironomidae). Crustaceans were identified to species level under an Olympus BX51 microscope. Collembolans and isopods were removed from the

statistical analyses because the identified species proved to be terrestrial or semiterrestrial, not aquatic.

Physico-chemical parameters (pH, electrical conductivity (EC) (μS.cm^{-1}) and temperature (T) ($^\circ$C)) were measured on-site using a portable HANNA Combo, HI 98129 instrument. Chemical analyses of the drip water in caves UC, VC and CC were performed at the Research Institute for Analytical Instrumentation (Cluj-Napoca): dissolved metals (Al, Cr, Fe) with an inductively coupled mass spectrometer Perkin Elmer, ELAN DRC II; NO_2^- with an ion chromatograph 761 Compact Metrohm; and chemical oxygen consumption (COD) by potassium permanganate titration in acid medium.

Dripping water contamination was established by comparing the results with the standard limits established by the Romanian and European Standards for drinking water quality.

Statistical analyses

Multivariate analyses were performed using the software XLSTAT Version 2010 (Addinsoft SARL). Samples with less than three individuals were excluded from the data set. To visualize the relationship between the identified taxa and physico-chemical parameters, Canonical Correspondence Analyses (CCA) were computed.

The sampling effort was tested by constructing a species accumulation curve (SAC) based on the Mao–Tau procedure (Colwell *et al.*, 2004) and computed with EstimateS version 7.5 (Colwell, 2005).

α-Diversity was calculated as the diversity of the crustacean community associated with each cave using the Shannon–Wiener index (H). β-Diversity was analyzed by comparing species richness between caves and was calculated using Whittaker's β diversity measure, based on the presence/absence of crustacean species. These tests were performed with PAST (PAlaeontological STatistics), ver. 0.93 (Hammer *et al.*, 2002).

A matrix was created for the biological data set based on the presence/absence of crustacean species. Then, the same matrix was transformed using the Bray–Curtis similarity index in order to evaluate the degree of similarity among samples. The latter analysis was performed with PRIMER 6 (Primer-E Ltd, Plymouth Marine Laboratory, UK) (Clarke and Warwick, 2001).

Results

Water chemistry

All physico-chemical parameters differed between and within caves throughout the year (Table 2). The dripping water from UC, VC and CC had no significant contamination from the surface. The highest standard deviations of Al, Cr, Fe, NO_2^- and COD values were found in CC drips.

The pH was slightly alkaline in all samples; the highest pH and highest temperature variations were measured in UC, at sites 3 and 1. Seasonal variation in the temperature of drips located close to the cave entrances appeared to be strongly affected by the external climatic conditions (*e.g.*, site 1 in UC). With regard to electrical conductivity, the highest amplitude was registered in CC, where spatial and temporal variations were observed; in most cases, the samples from sites 1, 2 and 3 showed low electrical conductivity, measured in winter and early spring, whereas the same sites revealed high electrical conductivity during summer and autumn; at the other sites (4–9), high electrical conductivity values were measured during the entire year. The greatest constancy of pH, temperature and electrical conductivity was measured in LC.

Fauna composition

The 350 biological samples collected during the sampling period contained 1726 individuals. The following major taxonomic groups were identified: Oligochaeta, Nematoda, Cyclopoida, Harpacticoida, Ostracoda, Collembola, Acarina, Amphipoda, Isopoda, Gastropoda and Insecta larvae (mainly Diptera Chironomidae). The total number of taxonomic groups varied among the five investigated caves from 8 to 11 (Table 3). Taxa composition (at higher taxonomic rank) and abundance varied within and between caves (Table 3).

The distribution of taxa with the highest number of individuals ($N > 3$) and high frequency was analyzed for UC, LC and CC. In UC (Fig. 3a), the poor fauna at site 1 was represented by insect larvae (chironomids) in June and July. Site 3 was the richest, with a constant high number of harpacticoids in almost all months (except April and September). In LC (Fig. 3b), insect larvae were more abundant at sites 1, 2 and 6. Harpacticoids were encountered in high number at almost all sites. The harpacticoids were the best-represented group at site 5, except for October when amphipods were dominant. Site 6 was represented only by insect larvae in October. In CC (Fig. 3c), harpacticoids and cyclopoids were represented in almost all sites, with high abundance at sites 3, 6 and 7. Ostracods were mainly distributed at site 7, except in March and April, when they were also present at sites 3 and 4.

The spatial and temporal variation of taxa related to the physico-chemical parameters of the dripping water was examined for UC, VC and CC. In the resulting plot (Fig. 4), major taxonomic groups and sites were ordered in the taxa–environment space. The cumulative percentage variance of the taxa–environment relationship was 80.90% for the first two canonical axes. The CCA axis 1 was strongly correlated with increasing electrical conductivity and decreasing pH, and accounted for 54.56% of the taxa–environment relationship. The best-represented taxa (harpacticoids, insect larvae and nematodes) are distributed along this axis. Axis 2 (26.38%) is a gradient of decreasing temperature and increasing Al, being related

Table 2. Physico-chemical features of dripping water in the selected caves during the one-year sampling period ("–" = not measured) (for cave abbreviations see Table 1).

Parameter/cave	UC	VC	LC	CC	DC
pH					
Mean	8.70	8.36	8.58	8.49	8.35
SD	0.35	0.23	0.26	0.22	0.19
Min	7.90	7.80	8.00	7.80	8.00
Max	9.60	8.90	9.00	8.90	8.80
EC (μS.cm^{-1})					
Mean	256.13	380.84	180.61	265.31	281.70
SD	73.97	60.49	28.16	85.53	67.77
Min	118.00	273.00	132.00	71.00	178.00
Max	437.00	506.00	279.00	482.00	382.00
T ($^{\circ}$C)					
Mean	9.98	11.80	8.77	10.62	10.05
SD	3.17	2.09	0.97	1.92	1.48
Min	4.70	9.00	6.50	7.80	4.90
Max	18.20	23.60	11.40	15.7	12.6
Al (mg.L^{-1})					
Mean	0.42	0.11	–	0.54	–
SD	0.71	0.07	–	0.73	–
Min	0.02	0.02	–	0.00	–
Max	2.63	0.32	–	2.68	–
Cr (mg.L^{-1})					
Mean	0.01	0.01	–	0.01	–
SD	0.02	0.01	–	0.02	–
Min	0.00	0.00	–	0.00	–
Max	0.06	0.06	–	0.09	–
Fe (mg.L^{-1})					
Mean	0.63	0.31	–	2.94	–
SD	0.73	0.63	–	6.21	–
Min	0.19	0.03	–	0.01	–
Max	2.81	4.00	–	21.70	–
NO$_2^-$ (mg.L^{-1})					
Mean	0.25	0.46	–	0.44	–
SD	0.24	0.35	–	1.15	–
Min	0.05	0.05	–	0.05	–
Max	0.74	1.14	–	4.00	–
COD (mg.L^{-1})					
Mean	9.21	6.95	–	8.17	–
SD	10.57	10.61	–	12.08	–
Min	1.60	0.10	–	1.00	–
Max	38.30	45.80	–	51.40	–

to oligochaetes and ostracods. The samples where high electrical conductivity was measured are grouped in the right part of the plot. Two samples are isolated in the left part of the plot: UC in January, due to the lowest temperature measured there (5.8 $^{\circ}$C), and CC in July when a high value of Fe was measured (14.6 mg.L^{-1}) and many insect larvae were found.

Crustacea

The crustaceans contributed the most to the total biodiversity of the study area, being highly diversified at all taxonomic levels: seven families, 12 genera and 15 species (Table 4). The sampling completeness, based on 11 crustacean species and 46 samples collected every month from all five caves, was tested (Fig. 5). The SAC did not reach an asymptote. Twenty-seven samples were sufficient to find eight species, while about 10 species were found after collecting 44 samples out of 46. Even if the sampling continues since 2008, no new species were found.

The proportion of species richness varied within and between caves. α-Diversity was the highest in CC and DC. β-Diversity was higher between the caves from Crişul Negru Basin (1.54) compared with the values in caves from the Crişul Repede Basin (1.21) (Table 5).

Among crustaceans, the harpacticoid copepods dominated in terms of both species richness (seven species) and total number of crustacean individuals (74%). Species known to be adapted for life in subterranean habitats (*i.e.*, stygobionts) were found: among the Harpacticoida, *Parastenocaris* sp. (in CC), the widely distributed *Spelaeocamptus spelaeus* (Chappuis, 1925) and *Maraenobiotus brucei carpathicus* Chappuis 1928 (in UC, VC and CC); among the Cyclopoida, *Speocyclops troglodytes* (Chappuis, 1923) (in VC), *Acanthocyclops* sp. (in UC, LC, CC and DC); and among the Amphipoda, *Niphargus andropus* Schellenberg, 1940 (in LC and CC).

The epigean crustacean fauna was represented by species widely distributed in all the caves: *Bryocamptus* (*Rheocamptus*) *caucasicus* Borutzky, 1930, or species found in only one cave: *Maraenobiotus vejdovskyi vejdovskyi* Mrazek, 1893, *Diacyclops bisetosus* (Rehberg, 1880) (in VC) and *Paracyclops fimbriatus* (Fischer, 1853) (in UC). The epigean *Candona neglecta* Sars, 1887 was the only ostracod identified in CC and DC.

The spatial and temporal dynamics of epigean and hypogean crustaceans along the physico-chemical gradients are defined in Figure 6. The two axis of the CCA accounted for 80.89% of the total variance. The first axis is a gradient of increasing temperature, while the second axis is a gradient of decreasing electrical conductivity and pH. The first axis (52.35%) is related to *B. caucasicus*, *C. neglecta* and *P. fimbriatus*, suggesting that their distribution is influenced by temperature and pH; the second axis (28.54%) is related mainly to the hypogean species *S. spelaeus* and *M. b. carpathicus*, whose distribution might be influenced by electrical conductivity.

The spatial distribution pattern within and between caves based on Bray–Curtis (BC) similarity analysis (Fig. 7) showed three main clusters. Clustered at the lowest level of similarity (40%) are stations from the same cave (CC); these stations are about 8 m apart and share one endemic hypogean harpacticoid (*S. spelaeus*). The next cluster (68%) grouped sites from VC and LC caves in the same basin but about 15 km apart, sharing another endemic harpacticoid, *Bryocamptus* sp. 1. The third cluster grouped, at 46% similarity, sites from all caves based on the widely distributed harpacticoids *B. caucasicus* and *S. spelaeus*.

Table 3. Relative abundance (A%) and relative frequency (F%) of major taxonomic groups recorded in the five caves during the one-year sampling period.

	UC		VC		LC		CC		DC	
	A%	F%	A%	F%	A%	F%	A%	F%	A%	F%
Oligochaeta	4.52	19.35	1.67	4.90	2.62	15.49	6.47	18.37	4.29	6.67
Acarina	0.85	3.23	0.32	0.98	2.82	16.90	3.11	11.22	0.00	0.00
Amphipoda	0.85	3.23	0.00	0.00	3.66	19.72	3.13	7.14	0.00	0.00
Isopoda	0.00	0.00	0.00	0.00	0.00	0.00	0.47	2.04	2.86	2.22
Cyclopoida	4.80	12.90	7.00	15.69	2.68	14.08	24.67	27.55	8.57	6.67
Harpacticoida	52.54	54.84	64.47	34.31	31.35	53.52	29.14	36.73	25.71	20.00
Ostracoda	0.00	0.00	0.00	0.00	0.00	0.00	10.09	12.24	1.43	2.22
Collembola	4.24	19.35	23.67	35.29	11.96	36.62	9.52	16.33	24.29	22.22
Insect larvae	30.23	29.03	1.27	2.94	42.96	15.49	6.10	7.14	2.86	2.22
Gastropoda	0.00	0.00	0.64	0.98	0.00	0.00	0.61	3.06	0.00	0.00
Nematoda	1.98	6.45	0.95	1.96	1.95	14.08	6.68	16.33	30.00	11.11

Discussion

The present study focused on the spatial and temporal structure of invertebrate communities in the unsaturated zone of the karst and revealed a patchy distribution within and between caves at different scales.

The heterogeneous distribution of the fauna inhabiting the ground-water habitats reflects biotic and abiotic interactions that took place from the microscale to megascale during historical times (Gibert *et al.*, 1994, 2009). Ground-water habitats are comparatively more conservative than aquatic surface habitats due to their environmental stability. Despite the old conception of ground-water as a species-poor environment, it has been demonstrated that its species richness is greater than formerly assumed and that the fauna (mainly invertebrates) is unexpectedly diverse and unique (Botoşăneanu, 1986; Juberthie and Decu, 1994, 1998, 2001; Gibert *et al.*, 1994, 2009; Stoch, 1995; Juberthie, 2000; Galassi, 2001; Gibert and Deharveng, 2002; Galassi *et al.*, 2009).

α- and β-diversity

The water percolating through the unsaturated karst harbors a wide range of organisms, from surface or soil species to highly specialized subterranean fauna (Pipan, 2005). Our study supports this observation by demonstrating the presence of five higher taxa with epigean and hypogean species common in all five caves: oligochaetes, nematodes, cyclopoids, harpacticoids and insects. Their presence indicates biological activity in the network of fissures of the unsaturated karst, where animals move by both active and passive dispersal.

At a larger scale, the contribution of both α- and β-diversity to total diversity of the area increased with spatial scale, from north to south: from the Crişul Repede Basin (the lowest β-diversity) (UC, VC and LC) toward the Crişul Negru Basin (the highest β-diversity) (CC and DC).

Gibert and Deharveng (2002) and Malard *et al.* (2009) sustain that ground-water diversity is a consequence of differences recorded between sites rather than within-site diversity. Our results show considerable differences both within and between caves. All recorded major taxonomic groups and species have different distribution inside caves and some are found at only one sampling site (*i.e.*, *Bryocamptus* sp. 1, *S. troglodytes*, *P. fimbriatus*). Also crustacean community composition varied within caves and over a larger scale, as confirmed by Bray–Curtis similarity analysis.

The community assemblages from the dripping water in the investigated caves are characterized by high diversity but few individuals. Low numbers of individuals in dripping water were also mentioned by Pipan and Brancelj (2001) in a study that compared different subterranean habitats (*i.e.*, drips, basins on calcareous sinter, and puddles on clay), and by Moldovan *et al.* (2007) in drips from caves.

The crustaceans contribute considerably to species diversity of dripping water communities, being diversified at both taxonomic and ecological levels. Our findings confirm the patterns previously observed in various ground-water habitats from the temperate regions (Ferreira *et al.*, 2007). Crustaceans are the dominant group in ground-water and account for 71.9% and 80.3% of the species and records, respectively, in the European database (Malard *et al.*, 2009). In the present study, crustaceans represent 73% from the total fauna. Each cave holds between five and eight species in drips, and the highest diversity was recorded in CC, with eight crustacean species. The trend of the species accumulation curve for crustaceans assumes that a major part of the total diversity was covered by spatial and temporal samplings.

Among the variety of ground-water habitats, the unsaturated karstic zone is known to be the richest in stygobiotic copepods (Pipan and Brancelj, 2004a; Pipan and Culver, 2007a; Galassi *et al.*, 2009). The copepods are the most abundant and frequent in the unsaturated karstic zone of our study area, except in LC. This result is supported by their high specialization, compared with other groups, for narrow spaces (Pipan and Brancelj, 2001, 2004a, 2004b; Pipan, 2005; Camacho *et al.*, 2006;
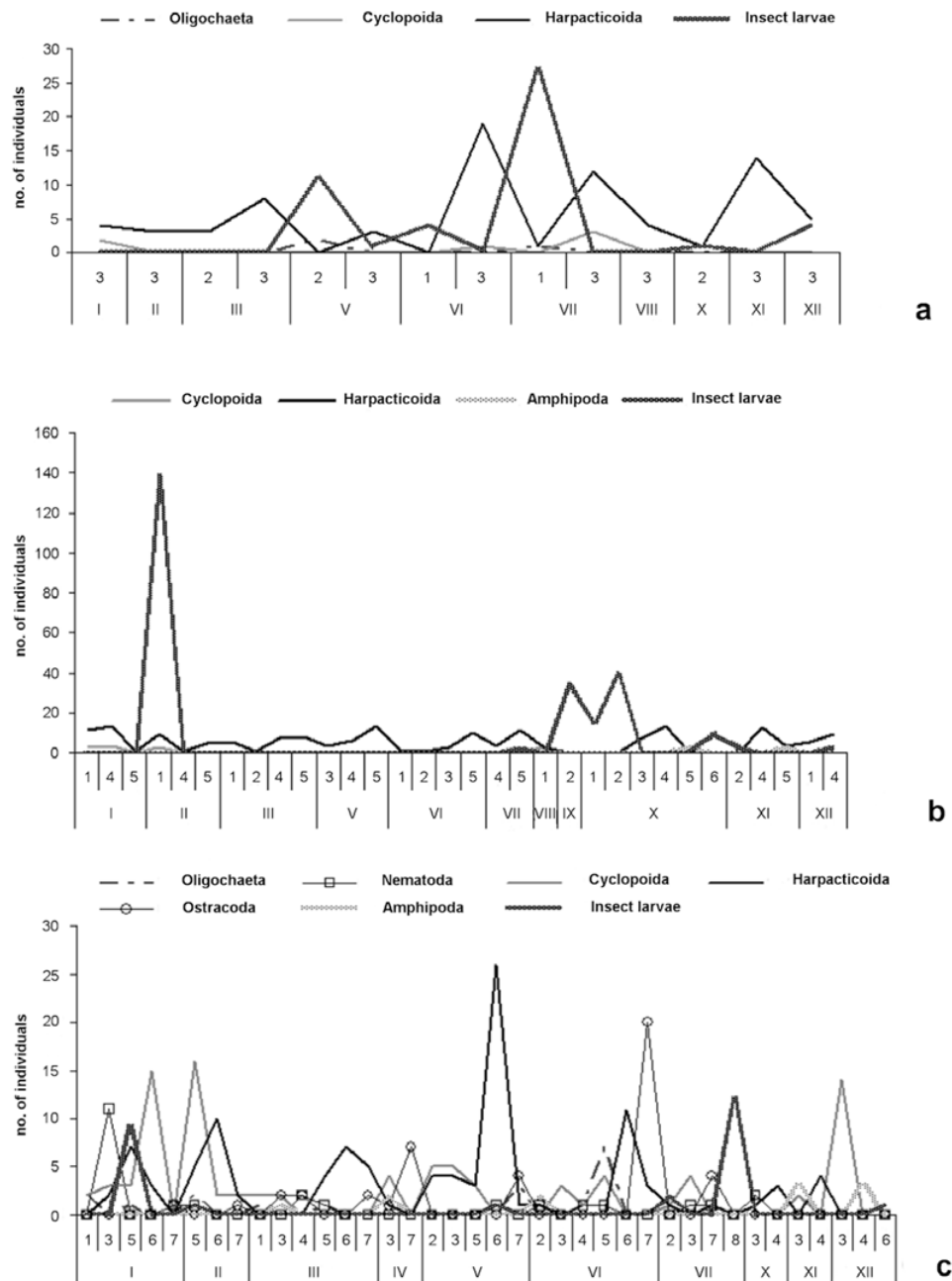
Fig. 3. Number of individuals ($N > 3$) of major taxa recorded in UC (a), LC (b) and CC (c) over the one-year sampling period; sampling month: I–XII; site number: 1–8.

Moldovan *et al.*, 2007). The copepods in dripping water are mostly represented by members of the families Canthocamptidae and Parastenocarididae (Harpacticoida) and of the Cyclopidae (Cyclopoida). These are the most successful groups, together with the malacostracans, in colonizing inland ground-water (Galassi, 2001; Boxshall and Defaye, 2008; Galassi *et al.*, 2009). This reflects their wide range of morphological (small size, eye loss), physiological (increased lifespan, low fecundity) and ecological (various trophic niches) features. They are "preadapted" (Galassi *et al.*, 2009) to subterranean environments and hence were able to invade and colonize repeatedly the patchy habitats of the ground-water.

Our results support the view that habitat physico-chemical heterogeneity and isolation of microhabitats are determinants for copepod diversity in terms of species richness (Galassi *et al.*, 2009). The most abundant and frequent fraction of the copepods is composed of species with a local distribution. Nonetheless, the significant components of the unsaturated zone are species that display an evident morphology (such as *Acanthocyclops* sp., *S. spelaeus*, *Parastenocaris* sp.) of true hypogean inhabitants: appendage reductions, small-sized body. *S. spelaeus* is endemic to the Apuseni Mountains and is widespread in caves of one massif of these mountains (Fiers and Moldovan, 2008). *S. troglodytes* is known
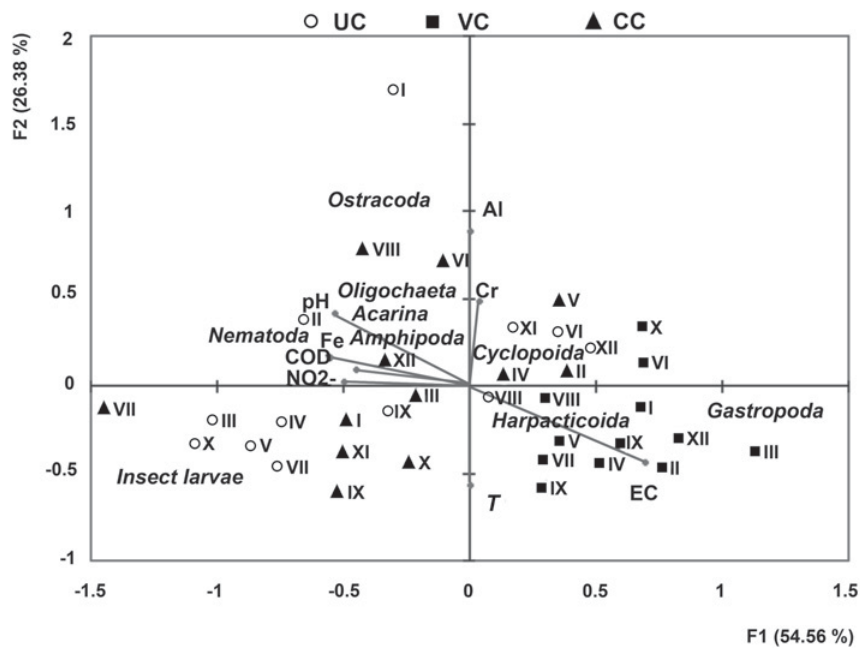
Fig. 4. CCA based on the total number of major taxa/month and the mean values of the physico-chemical parameters measured in UC, VC and CC over the one-year sampling period; sampling month: I–XII; *T* – temperature, EC – electrical conductivity, COD – chemical oxygen consumption.

from Romania only from the Pădurea Craiului Mountains (Damian-Georgescu, 1963; Pleşa, 1985; Iepure, 2007; Moldovan *et al.*, 2007). Two of the new species belong to two highly specious ground-water genera, *Parastenocaris* (in CC) and *Acanthocyclops* (in UC, LC, CC and DC).

At a small scale, species are presumed to interact and compete for similar limiting resources (Gray, 2000); to reduce competition between species, they will use different parts of the habitat (Whittaker, 1972). This view was supported particularly in CC, where species with wide ecological valence (*B. caucasicus*, *S. spelaeus* and *Acanthocyclops* sp.) did not interfere with the distribution of more specialized species (*M. b. carpathicus*, *Parastenocaris* sp.). Ostracods – attributable to the species *C. neglecta* – are represented in high numbers in CC and by only a single individual in DC; this is a common species with a Holarctic distribution. The occurrence of this epigean ostracod in dripping water samples is probably due to seepage from the surface.

Among other fauna groups, oligochaetes and nematodes were frequently recorded and present in relatively high numbers in all caves. Their presence was already assessed for UC and VC (Moldovan *et al.*, 2007) but also for other cave systems (Pipan and Brancelj, 2001; Pipan and Culver, 2005; Camacho *et al.*, 2006; Pipan *et al.*, 2008). The insect larvae are represented mainly by chironomids, and their higher abundance in LC can be related to the more direct inflow of water from the surface inside the cave, and therefore a direct input of surface species (especially chironomids, but also the amphipod *Gammarus balcanicus* Schäferna, 1922). When high

numbers of chironomid larvae and gammarids typical for moist soil and vegetation are present, few or no hypogean species occur in LC. This can be related more to direct discharge of meteoric water underground.

The presence of four new species (*Parastenocaris* sp., *Bryocamptus* sp. 1, *Bryocamptus* sp. 2 and *Acanthocyclops* sp.) (taxonomic descriptions in progress) and of six endemics supports the observation that cracks and fissures from the unsaturated zone harbor numerous undiscovered species (Pipan *et al.*, 2006; Culver and Pipan, 2007). These species are high contributors to both α- and β-diversity and consequently increase the conservation value of the caves and of the karstic area of the Pădurea Craiului Mountains. The presence of many copepods and numerous endemics in a country at 45° northern latitude can be explained by the patchy distribution of limestone in the Carpathian Mountains. This leads to small continental islands among non-karstic areas, which function as natural barriers to hypogean migration and allow the radiation of lineages (Moldovan *et al.*, 2005).

Spatial and temporal distribution patterns and relation to environmental parameters

The meroclimate, and the regional and geological conditions can significantly influence the variation of physico-chemical parameters inside caves (Musgrove and Banner, 2004). In the caves studied here, the measured physical and chemical parameters of the water varied spatially (between and within caves) and temporally,

Table 4. List and number of identified taxa in the investigated caves (* – endemic species; bold – hypogean species).

Species	UC	VC	LC	CC	DC
		No. of individuals			
HARPACTICOIDA					
Fam. Canthocamptidae					
Bryocamptus (Rheocamptus) caucasicus Borutzky 1930	51	65	96	16	3
Bryocamptus (Rheocamptus) sp. 1*	–	–	1	–	–
Bryocamptus (Rheocamptus) sp. 2*	1	–	1	–	–
Maraenobiotus vejdovskyi vejdovskyi Mrazek, 1893	–	1	–	–	–
Maraenobiotus brucei carpathicus Chappuis 1928	2	2	–	30	–
Spelaeocamptus spelaeus (Chappuis 1925)*	6	–	16	34	14
Fam. Parastenocarididae					
Parastenocaris sp.*	–	–	–	24	–
Harpacticoid copepodids (indet.)	14	118	48	6	1
Total number of individuals	74	186	162	110	18
Total number of species	4	3	4	4	2
CYCLOPOIDA					
Fam. Cyclopidae					
Acanthocyclops sp.*	1	–	5	45	5
Speocyclops troglodytes (Chappuis 1923)	–	8	–	–	–
Diacyclops bisetosus (Rehberg 1880)	–	1	–	–	–
Paracyclops fimbriatus (Fischer 1853)	4	–	–	–	–
Cyclopoid copepodids (indet.)	1	1	3	36	–
Total number of individuals	6	10	8	81	5
Total number of species	2	2	1	1	1
AMPHIPODA					
Fam. Gammaridae					
Gammarus balcanicus Schäferna 1922	1	–	12	–	–
Fam. Niphargidae					
Niphargus andropus Schellenberg 1940*	–	–	9	11	–
Juveniles (indet.)	–	–	14	4	–
Total number of individuals	1	–	35	15	–
Total number of species	1	–	2	1	–
OSTRACODA					
Fam. Candonidae					
Candona neglecta Sars 1887	–	–	–	32	1
Juveniles (indet.)	–	–	–	1	–
Total number of individuals	–	–	–	33	1
ISOPODA					
Fam. Mesoniscidae					
Mesoniscus graniger (Frivaldsky, 1865)	–	–	–	2	2

as already observed by Moldovan *et al.* (2007) and Pipan *et al.* (2008) in similar studies on dripping water fauna. The temperature variations reflect the cumulated influence of both position along a cave and season. The variation of electrical conductivity can reflect the different residence times of the water in the subsurface, as emphasized by Culver and Pipan (2007). The residence times indicate the degree of interconnectivity of the voids in the unsaturated zone, which can enhance or decrease survival and reproduction of biological communities in the unsaturated karstic zone.

The dripping water here showed no contamination from the surface, although the content of Al, Cr and Fe is higher than the standards for drinking water. The presence of these elements in the dripping water is associated with the regional composition of the rocks and deposits, formed by impure limestone with overlying bauxite and pyrites (Onac, 2002).

High numbers of individuals and taxa were observed at sites with constant dripping over the year. This supports the observations made by Pipan (2005) and Camacho *et al.* (2006). Numerous hypogean species occurred at sites supplied with a constant slow input of water (UC, CC and DC), whereas numerous epigean species were collected from sites with a fast infiltration of water (LC). The different spatial distribution of the fauna in UC probably reflects the constancy of dripping at sites situated more than 50 m from the entrance: more taxa and more
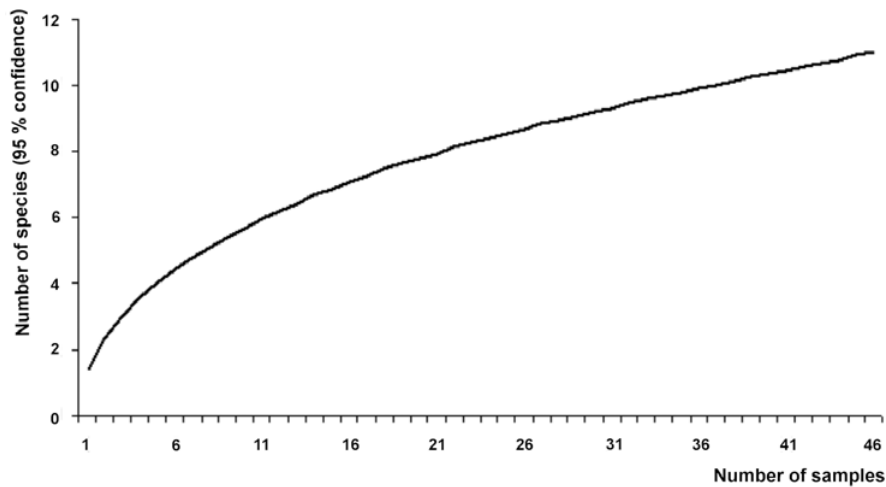
Fig. 5. Crustacean species accumulation curve based on the Mao–Tau procedure.

Table 5. Shannon–Wiener index (H) and Whittaker's β-diversity measure of crustaceans in the investigated caves, CR – Crişul Repede Basin, CN – Crişul Negru Basin.

Basin	CR			CN		CR and CN
β	1.21			1.54		1.33
Cave	UC	VC	LC	CC	DC	
H	0.88	0.60	1.01	1.85	1.03	

individuals were recorded there. In LC, the spatial distribution of harpacticoids was constant, with high population densities at almost all sites. This might be explained by the low variations in electrical conductivity and temperature. Amphipods, mainly *G. balcanicus*, were sampled from only two sites, which were characterized by fast infiltration pathways of the water. In CC, the distribution of almost all the major taxonomic groups was very heterogeneous, with no observable spatial trends. One exception was the harpacticoids, which preferred sites with low yearly variations in electrical conductivity, as in the previous cave.

Paran *et al.* (2005) and Dole-Olivier *et al.* (2009) reported that water chemistry only minimally influences faunal distributions. According to Pipan (2005), Pipan *et al.* (2006) and Moldovan *et al.* (2007), certain copepod species preferred high conductivity, high temperature and a high concentration of different ions. In our study, electrical conductivity was also the significant factor that influenced taxa composition and distribution: (1) more hypogean harpacticoids were present at sites with high electrical conductivity; (2) more epigean species (cyclopoids and harpacticoids) were present at sites with higher variations of electrical conductivity and temperature. At the species level, two hypogean harpacticoids (*M. b. carpathicus* and *S. spelaeus*) were related to high electrical conductivity (200–350 μS.cm^{-1}). Such high electrical conductivity indicates lengthier water residence times, underlining that the distribution of hypogean species is influenced by the rock porosity and the available habitats.

pH, dissolved metals, nitrite and chemical oxygen consumption were related mainly with oligochaetes, nematodes, cyclopoids, amphipods and mites, but the effects were not statistically significant. The distributions of the epigean *B. caucasicus*, *P. fimbriatus* and *C. neglecta* are influenced by temperature, the first one being found in samples with wide temperature values (3.7–12.1 °C), the others being related to high temperatures (10.0–14.5 °C).

A temporal trend was observed only for insect larvae, with abundance peak during summer months, except in LC, where the peak was recorded in late winter. No seasonal pattern was discernible for other taxa; rather than being linked to seasons, the assemblages were related more to physico-chemical features. These, in turn, are potentially influenced by surface parameters.

Copepod composition and distribution are mentioned to be influenced by the amount of precipitation (Pipan and Brancelj, 2001; Pipan, 2005), and the distribution of narrow distributed copepod species to be related to the water chemistry (*i.e.*, sodium, nitrate and potassium) (Pipan, 2005; Pipan *et al.*, 2006). We found no significant correlation between the number of crustacean species from dripping water and precipitation. Also, there was no significant correlation between taxa distribution and water chemistry (*i.e.*, dissolved metals, nitrite and chemical oxygen consumption).

Differences in α-diversity (within caves) and β-diversity (between hydrographic basins) in caves from the Pădurea Craiului Mountains integrate the results on the spatial heterogeneous distribution of taxa in the vadose zone correlated with life strategy, hydrological dynamics and variations in the water physico-chemical parameters. The heterogeneous distribution of aquatic fauna in this ecosystem was also mentioned in previous studies (Pipan and Brancelj, 2001; Sket *et al.*, 2004; Pipan, 2005; Pipan and Culver, 2005; Moldovan *et al.*, 2007). A temporal trend was observed only for epigean species (chironomids), while the hypogean representatives were confined to specific sites or galleries within the caves. The narrow spatial distribution of hypogean species was also observed
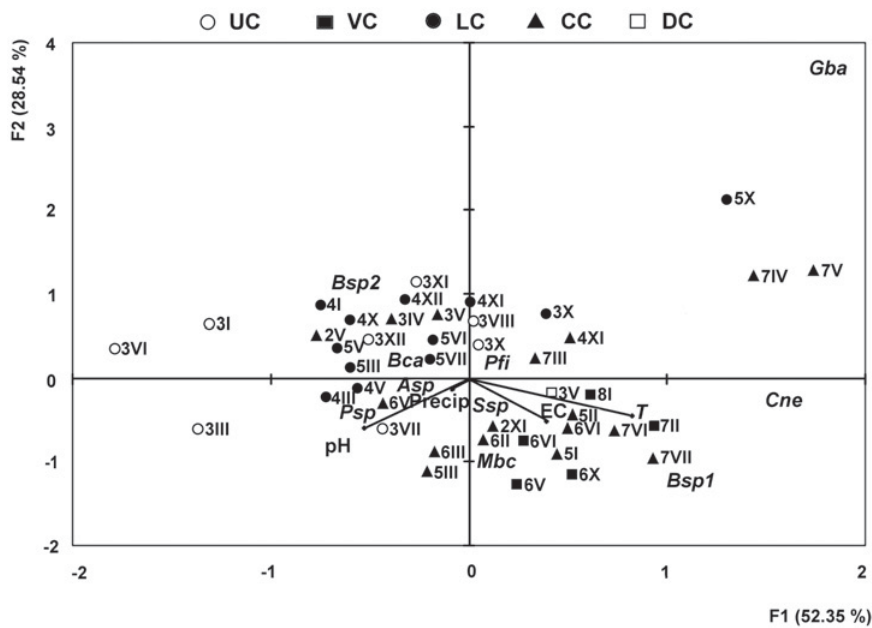
Fig. 6. CCA based on crustacean species, physico-chemical parameters (pH, *T* and EC) and precipitation (Precip.) registered in the five caves over the one-year sampling period: *Bca* – *B. caucasicus*; *Bsp1* – *Bryocamptus* sp. 1; *Bsp2* – *Bryocamptus* sp. 2; *Mbc* – *M. b. carpathicus*; *Ssp* – *S. spelaeus*; *Psp* – *Parastenocaris* sp.; *Asp* – *Acanthocyclops* sp.; *Pfi* – *P. fimbriatus*; *Cne* – *C. neglecta*; *Gba* – *G. balcanicus*; sampling month: I–XII; *T* – temperature, EC – electrical conductivity.
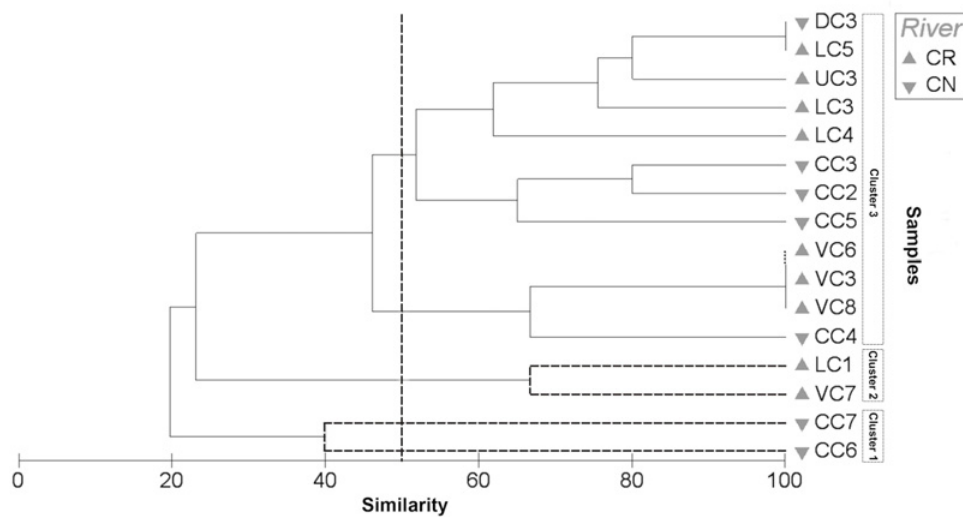
Fig. 7. Bray–Curtis dendrogram based on the presence of crustacean species in the investigated caves over the one-year sampling period. Site number: 1–8; CR – Crişul Repede River; CN – Crişul Negru River.

by Brancelj (2002), Pipan (2005) and Moldovan *et al.* (2007).

The present study emphasizes the importance of electrical conductivity and drip constancy in shaping the distribution and composition of hypogean crustacean species, as an indirect measure of rock porosity, habitat availability and water residence time in karst. In contrast, epigean species are related to high variation in physico-chemical parameters (temperature and electrical conductivity), underlining their transitory status within the vadose zone.

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