

CLIMATE, ABIOTIC FACTORS, AND THE EVOLUTION OF SUBTERRANEAN LIFE

KLIMA, ABIOTSKI DEJAVNIKI IN EVOLUCIJA PODZEMELJSKEGA ŽIVLJENJA

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Abstract

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David C. Culver & Tanja Pipan: Climate, abiotic factors, and the evolution of subterranean life

Climate, and more generally the physical conditions in caves and other subterranean habitats have a profound influence on the biota. At longer time scale (centuries), climate change can force and/or isolate species in subterranean habitats. Not only Pleistocene climate changes, but earlier ones as well, such as the Messinian salinity crisis were important in this regard. While many speleobiologists assume that caves are nearly constant environmentally and with scarce organic carbon, this is not the case, especially in non-cave subterranean habitats. Many shallow subterranean habitats, such as epikarst, seepage springs, and talus harbor highly modified organisms, ones without eyes and pigment and with elongated appendages. Yet these habitats are highly variable with respect to temperature and other environmental factors, and often have high levels of organic carbon. Overall, the role of these shallow subterranean habitats in the evolution and biogeography of subterranean species may be crucial. On smaller spatial scales, environmental differences, such as differences in chemistry of epikarst water, may be important in allowing large numbers of species to coexist.

Keywords: shallow subterranean habitats, cave environments, climate change, stygobionts, troglobionts.

Izvleček

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David C. Culver & Tanja Pipan: Klima, abiotiski dejavniki in evolucija podzemeljskega življenja

Klima ter fizične značilnosti jam in drugih podzemeljskih habitatov pomembno vplivajo na podzemeljski življenjski svet. Klimatske spremembe so, gledano z vidika daljših časovnih obdobij (stoletja) lahko vzrok, da organizmi naselijo podzemeljske habitate in tam ostanejo izolirani. V tem pogledu so bile pomembne klimatske spremembe v pleistocenu in predhodna mesinska kriza slanosti. Čeprav mnogo speleobiologov meni, da so jame skoraj stabilno okolje, revno po količini organskih snovi, temu ni tako, kar velja zlasti za nejamne habitate. V plitvih podzemeljskih habitatih, kot so epikras, hipotelminorejki (močila) in gručnata pobočja, živijo visoko specializirani organizmi, taki brez oči in pigmenta ter s podaljšanimi okončinami. Temperatura in drugi okoljski parametri so v tovrstnih habitatih zelo spremenljivi, in količina organskih snovi je pogosto visoka. Vloga plitvih podzemeljskih habitatov bi lahko bila ključna pri evoluciji in biogeografiji podzemeljskih vrst. Na manjšem območju so okoljske spremembe, kot so razlike v kemijskih parametrih epikraške vode, morda pomembne pri sobivanju večjega števila vrst.

Ključne besede: plitvi podzemeljski habitati, jamsko okolje, klimatske spremembe, stigmatobionti, troglobionti.

INTRODUCTION

Climate, and more generally the physical environment, has a profound effect on the distribution, evolution, and even the invasion of species into caves and other subterranean spaces. Overall, the subterranean environment

has a simple physical definition – it is below the surface and, at least from a biological point of view (Culver & Pipan 2009), it is aphotic. Speleobiologists often add several other differentiating physical characteristics – the

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absence of temporal variation, especially cyclical variation, both diurnal and annual. The purported absence of cycles implies an absence of cues for physiological processes such as reproduction. Although not strictly a characteristic of the physical subterranean environment, low amounts and fluxes of organic carbon are typically included in the characterization of subterranean habitats, especially caves (Culver & Pipan 2008).

The effect of climate and the physical environment on the subterranean fauna, to a large extent, depends on spatial and temporal scale. It is convenient to consider three such scales. The first is the impact of long-term climate change on the colonization of subterranean habitats by elements of the surface-dwelling fauna. Temperature change resulting from Pleistocene glaciations is often held to be the factor forcing animals into caves and causing the extinction of surface-dwelling populations (e.g., Peck 1980; Holsinger 1988). Other large-scale climate changes, such as the closing and subsequent drying of the Mediterranean Sea around 6.6 million years ago, are also invoked to explain the colonization and distribution of subterranean fauna. The second scale that is important to consider is the physical environment as a selective

agent that molds the morphology, physiology, behavior, and life history of subterranean organisms. Beginning with Christiansen (1961) and Poulson (1963), and continuing with Culver *et al.* (1995) and Jeffery (2005), neo-Darwinian speleobiologists have used factors of the physical environment either directly (absence of light) or indirectly (low levels of organic carbon resulting from the absence of light) as explanatory factors for adaptation to subterranean life. Third, spatial variation in environmental conditions can be a way of dividing up the subterranean habitat so that competition is reduced, and niche separation increases. Among the variation in the physical environment can be important in this regard are sizes of gravels in streams in the case of amphipods (Culver 1976) and chemical differences in water dripping from epikarst in the case of copepods (Pipan 2005, Pipan *et al.* 2006).

In this contribution, we review the temporal and spatial patterns of the physical environment of subterranean habitats at the three scales of importance, and consider whether these patterns have had a major impact on the distribution, ecology, and evolution of the subterranean fauna.

CLIMATE CHANGE AS A FORCING AGENT FOR COLONIZATION OF SUBTERRANEAN HABITATS

For speleobiologists working in north temperate regions, the effects of climate change associated with Pleistocene glaciations on the subterranean fauna have been obvious. An obligate cave fauna is nearly absent from glaciated regions, and the fauna that remains apparently survived in groundwater underneath the ice sheets (e.g., Holsinger 1980). This is in many ways a self-evident effect of climate change – species cannot survive in caves filled with ice. Climate change as a result of glacial advances and retreats extended far beyond the ice sheets themselves, and many speleobiologists (e.g., Jeannel 1943; Barr 1968) supported what is called the “climatic relict hypothesis”, that climate changes associated either with glacial advances or declines forced animals into caves. Those that did not enter caves went extinct, according to this hypothesis. Many scenarios have been presented to explain the current distribution of animals in caves as a result of patterns of glaciations. One example comes from Barr (1960), who suggested that the presence of large numbers of species of troglotrophic beetles in the genus *Pseudanophthalmus* in Indiana was the result of the intense climate change in the area, which is near the boundary of the Wisconsin glacial maximum. Care must be taken with these kinds of

explanations however. For example, the Wisconsin glacial maximum also reached karst areas in central Pennsylvania, which have no troglotrophic species near the glacial boundary. In other cases where troglotrophic beetle distribution was thought to be the result of Pleistocene climate change, estimates of age of the subterranean lineage extend well beyond the Pleistocene. A particularly well studied case is that of the ground beetles in the tribe Trechine in the Pyrenees (Faille *et al.* 2010). Based on mitochondrial DNA sequence differences, they estimate that the lineage originally was isolated underground approximately 10 million years ago, perhaps associated with the Messinian salinity crisis at the Miocene-Pliocene boundary. In this case it seems that climate change did force beetles into caves, but that until the work of Faille *et al.* (2010), the wrong climate change was identified.

The best documented case supporting the climatic relict hypothesis is that of a diverse assemblage of diving beetles in the family Dytiscidae found in calcrete aquifers in south western Australia (Leys *et al.* 2003). Calcrete aquifers are a feature of arid landscapes in Australia, and were formed between 37 and 30 million years ago during a cool, dry period in the Eocene. From 30

million years ago until 10 million years ago, there was a warm temperate climate in this part of Australia. Beginning in the Miocene, there was a period of drying that began in the northwest and moved southeast over the next 5 million years. Leys *et al.* (2003) argue that if the climatic relict hypothesis is correct, species should become isolated in caves (as a result of extinct of surface populations) only during this period of maximum aridification. Using an estimate of a 2.3 percent pairwise divergence rate of mitochondrial DNA per million years and a phylogeny determined using Bayesian methods of tree building, they determined the age of divergence of sympatric pairs of species within a calcrete aquifer. These divergence times range from 3.6 to 8.1 million years ago, and the differences in estimated time of divergence have a strong latitudinal component. Species from northwestern calcretes diverged earliest (Fig. 1) and it was in the northwest that aridification began. Overall, latitude accounted for 83 percent of the variance in estimates of divergence times.

Climate change cannot explain all cases of isolation of species in caves, as both Mitchell (1969) and Howarth (1972) forcefully pointed out, using examples from tropical caves, climate forcing and the climatic relict hypothesis could not explain all stygobionts and troglobionts. Howarth (1987) proposed the adaptive shift hypothesis, in which climate change plays no role. In essence, he

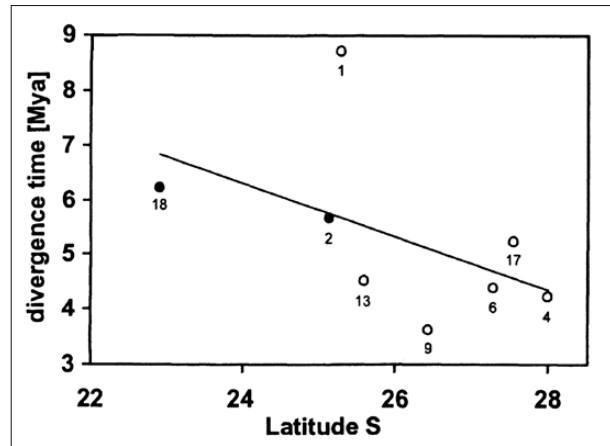


Fig. 1: Latitudinal variation in divergence times of eight sympatric sister pairs of stygobiotic dytiscid beetles in Western Australia. The open circles show species pairs belonging to the Bidessini; the black circles show species pairs belonging to the Hydroporini. Numbers refer to different calcrete aquifers (see Leys *et al.* 2003).

argued that species colonize caves because of more resources and that it was a more active invasion of the subterranean realm. Nevertheless, since the bulk of stygobionts and troglobionts are in the temperate zone rather than the tropics, it is likely that most stygobionts and troglobionts occur in caves as a result of climate change (Culver & Pipan 2009).

THE PHYSICAL ENVIRONMENT AS A SELECTIVE AGENT

Caves are extensively replicated habitats, with the deep portions of caves in a region having nearly identical climates, especially with respect to temperature, which approximates the mean annual surface temperature (Palmer 2007). Many investigators have taken advantage of these characteristics to use caves as ecological and evolutionary laboratories, a phrase first used by Poulson and White (1969). Perhaps the most important contribution of the study of caves as evolutionary laboratories has been in the area of adaptation. Poulson (1963, 1985) investigated parallel and convergent changes in amblyopsid cave fish, including increases in the lateral line system, and reduced metabolic rate. In Poulson's view, the key features of the selective environment were darkness, scarce organic matter, and reduced environmental cyclicality. Christiansen (1962, 2005) also developed the idea of troglomorphy – the suite of morphological changes, e.g., appendage lengthening and eye reduction, that are hallmarks of adaptation to caves.

If subterranean habitats are defined as aphotic habitats with eyeless, depigmented species with elongated appendages, then caves are only one of several kinds of subterranean habitats, and in many situations may be a minor part of the subterranean ecosystems. Moreover, even cave environments are by no means all homogeneous and food-poor. The selective environment that results in typical troglomorphic features needs to be re-examined, and the morphological consequences (e.g., appendage elongation) need to be reconsidered.

What are non-cave subterranean habitats? Botosaneanu (1986) and Juberthie (2000) recognise two primary categories of subterranean habitats: large cavities (caves) and small interstitial cavities (gravel and sand aquifers and the underflow of streams and rivers). These habitats share two important characteristics – the absence of light and the presence of species both limited to and modified for subterranean life (troglobionts for terrestrial species and stygobionts for aquatic species). While species in both habitat types are typically without

eyes and pigment, large cavity species have elongated appendages while animals limited to interstitial habitats are often miniaturized with shortened appendages (Coineau 2000). However, there is at least one more category, the shallow subterranean habitats (SSHs), defined as aphotic habitats such as seeps and the spaces between rocks in talus slopes, less than 10 m from the surface, and with cavities considerably larger than their inhabitants (Culver & Pipan 2008). Animals limited to caves and SSHs have similar morphologies, such as appendage elongation in amphipods (Culver *et al.* 2010) and a modified claw complex in Collembola (Christiansen 1998).

We hypothesize that the barrier to colonization of and adaptation to subterranean environments is not as formidable as it sometimes appears. For example, in the neo-Darwinian views of Christiansen and Poulson, absence of light, scarcity of food, and the absence of seasonal cues for reproduction make the environment extreme so that successful adaptation occurs rarely. We argue that the main barrier to successful establishment of populations in caves is the total absence of light (which is not the case in the deep sea because of bioluminescence and some light production at deep sea vents), rather than the absence of food or environmental cues, and is less extreme than that suggested by Christiansen and Poulson. Much of the reason for our hypotheses is the nature of SSHs and the species that inhabit them.

SSHs share two important characteristics with both large cavities and small cavities—the absence of light and the presence of species both limited to (troglobionts for terrestrial species and stygobionts for aquatic species) and modified for subterranean life (troglomorphs) as well as epigeal fauna that may be present and abundant in some instances. They share with many small cavities a proximity to the surface (< 10 m) and the presence of seasonal cues. They share with large cavities a habitable space large enough that organisms are not in contact with solid surfaces in all three dimensions. SSHs have a number of features, including: (1) the areal extent of an individual habitat is small, usually < 0.1 km², but many replicates exist that are more or less widely distributed geographically; (2) the habitable space is intermediate in size between interstitial habitats (typically with spaces < 1 cm in diameter) and caves (typically with spaces > 50 cm in diameter); (3) they are rich in organic matter relative to other subterranean habitats, in part because of their close proximity to the surface; and (4) they have intimate connections to the surface resulting in greater environment variation than other subterranean habitats. Of course, caves can also be close to the surface, but for the purposes of this review, we want to emphasize the other subterranean habitats. As we discuss below, hyporheic habitats share all of the features of SSHs except the

size of the habitable space. For this reason we include them in our discussion.

Among important SSHs are (1) spaces between rocks and cracks in rocks, given the general term *milieu souterrain* superficiel by Juberthie 2000 (Fig. 2), (2) epikarst the uppermost layer of karst with poorly integrated solution cavities (Fig. 3) (Pipan 2005), (3) the underflow of streams and rivers, the hyporheos and associated groundwater (Malard *et al.* 2000) (Fig. 4), and (4) seepage springs (Fig. 5), also called the hypotelminorheos (Culver *et al.* 2006). We have presented data, especially temperature records, that indicates that these habitats are both variable and not particularly impoverished with respect to organic carbon (Culver and Pipan 2008; Pipan *et al.* 2010). In this review we highlight seepage springs because they are the most superficial of all SSHs and they harbor a highly modified troglomorphic fauna.



Fig. 2. *Milieu souterrain superficiel* (MSS) site at Mašun, Slovenia (Photo: T. Pipan).

Culver *et al.* (2006) defined the hypotelminorheic as (1) a persistent wet spot, a kind of perched aquifer fed by subsurface water in a slight depression in an area of low to moderate slope; (2) rich in organic matter; (3) underlain by a clay layer typically 5 to 50 cm beneath the surface; (4) with a drainage area typically < 10,000 m² and (5) with a characteristic dark colour derived from decaying leaves which are usually not skeletonized. The habitat can occur in a wide variety of geologic settings anywhere outside of arid regions where there is a layer of impermeable sediment but it is probably less common in karst landscapes because of the extensive occurrence of an impermeable clay layer would prevent the downward movement of water and the development of karst landscapes. Most of the available habitat for the animals comprises spaces between decomposing leaves and sedi-



Fig. 3: An active ceiling drip in Organ Cave, West Virginia, USA, which shows water exiting epikarst (Photo: H. H. Hobbs III, used with permission).

ment, and the animals literally live in their food. The studied examples of hypotelminorheic habitats are all from forested landscapes in temperate regions, but we suspect they can also occur in grasslands and tropical forests, as long as there is a layer of dead leaves or grass on the ground.

Chemical and physical conditions vary considerably between sites (Culver *et al.* 2006), but conductivity tends to be high, indicating that the water had been underground for some time. Although oxygen concentrations varied considerably, the fauna did not seem to be especially sensitive to this parameter. Organic carbon was not measured but is presumably high because of the high concentration of decaying leaves. Based on a ten

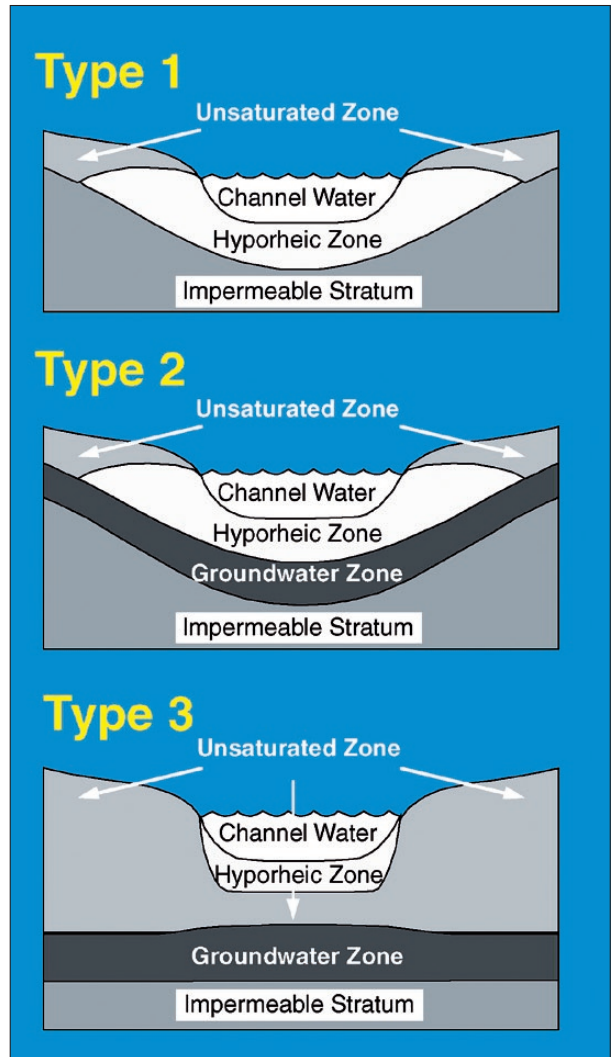


Fig. 4: Conceptual cross-sectional models of surface channels and beds showing the relationship of channel water to hypoheic, groundwater, and impermeable zones. From Malard *et al.* (2000).



Fig. 5: A seepage spring in Scotts Run Park, Virginia, USA (Photo: W. K. Jones, used with permission).

month monitoring period (March 2007 to January 2008) of a hypotelminorheic habitat in Prince William Forest Park in Virginia, USA, the habitat was temporally variable (Fig. 6). From May to September, hypotelminorheic

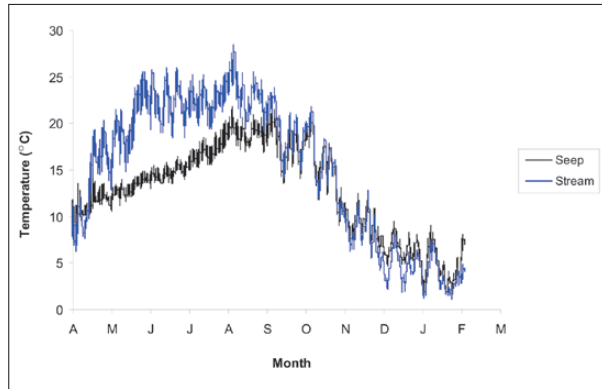


Fig. 6: Hourly temperatures from April 7, 2007 to February 4, 2008 in a seepage spring and adjoining stream in Prince William Forest Park, Virginia, USA. Because of the scale, the thickness of the line indicates the extent of daily fluctuations.

temperatures were depressed compared to the nearby surface stream, and approximated surface water temperatures for the rest of the year. In spite of the variability, the amplitude of variation in hypotelminorheic temperatures is less than that of surface waters. The maximum recorded temperature in the hypotelminorheic was 22 °C compared to 28 °C in a nearby (< 10 m) stream (Tab. 1). The coefficient of variation of stream temperature for the data in Fig. 6 was 49.8% and the coefficient of variation

Tab. 1: Statistical properties of temperature (in °C) time series for a seepage spring in Prince William Forest Park, Virginia, USA.

	Seepage Spring	Surface Stream
Mean	12.80	15.10
Standard Error	0.06	0.09
Median	13.46	17.15
Standard Deviation	4.88	7.51
Coefficient of Variation	38.15	49.75
Range	20.03	27.47
Minimum	1.79	1.02
Maximum	21.82	28.49
Count	7274	7274

of hypotelminorheic temperature for the same period was 38.2%. This is a remarkable difference given the superficial nature of seepage springs. In other areas, such as Nanos Mountain in Slovenia where winters are more severe, hypotelminorheic sites have higher winter temperatures as well as lower summer temperatures compared to surface waters (Pipan & Culver unpublished). The differences may become more important given predictions of climatic variability and change.

Based on a study of 50 seepage springs in the lower Potomac River basin that drain hypotelminorheic habitats within a radius of 45 km, a total of 15 macroinvertebrates have been recorded, including twelve amphipod, two isopod, and one gastropod species (Tab. 2, Culver & Pipan 2008). Four of the amphipods were probably accidentals – they were uncommon and showed no evidence of reproduction. Of the remaining eleven macroinvertebrate species, seven were stygobionts – species living

Tab. 2: Species of amphipods, isopods, and gastropods found in seepage springs (hypotelminorheos) in the lower Potomac River drainage in the environs of Washington, DC. Data from Culver and Pipan (2008). Stygobionts are obligate aquatic subterranean species; stygophiles are facultative aquatic subterranean species.

Group	Species	Ecological Category	Hypotel–minorheic specialist	Troglophobic
Amphipoda:	<i>Stygobromus tenuis potomacus</i>	stygobiont	no	yes
	<i>Stygobromus pizzinii</i>	stygobiont	no	yes
	<i>Stygobromus hayi</i>	stygobiont	yes	yes
	<i>Stygobromus kenki</i>	stygobiont	yes	yes
	<i>Stygobromus sextarius</i>	stygobiont	yes	yes
	<i>Crangonyx floridanus</i>	stygophile	no	no
	<i>Crangonyx palustris</i>	accidental	no	no
	<i>Crangonyx serratus</i>	accidental	no	no
	<i>Crangonyx shoemakeri</i>	stygophile	no	no
	<i>Crangonyx stagnicolous</i>	accidental	no	no
	<i>Gammarus fasciatus</i>	accidental	no	no
	<i>Gammarus minus</i>	stygophile	no	no
	Isopoda:	<i>Caecidotea kenki</i>	stygobiont	yes
<i>Caecidotea nodulus</i>		stygophile	no	no
Gastropoda:	<i>Fontigens bottimeri</i>	stygobiont	yes	weakly

exclusively in subterranean habitats. Of these seven, five are exclusively found in seeps: they are hypotelminorheic specialists (Fig. 7). Five of the seven stygobionts were troglomorphic. Hypotelminorheic sites in Croatia and Slovenia have a similar mixture of specialized and non-specialized amphipods, although with lower numbers of species (Culver *et al.* 2006).

The nature of the physical environment and the biota of seepage springs as well as other SSHs strongly suggests that the key feature of the selective environment leading to the evolution of troglomorphy is the absence of light, not lack of environmental cyclicality and not scarce organic carbon. Recent work by Jeffery (2009) on the evolution and development of the Mexican cavefish *Astyanax mexicanus*, which lives in relatively food-rich, environmentally variable habitats, has shown that it has not only evolved increased extra-optic sensory structures but also lost its eyes and pigment as a direct result of selection in an aphotic environment.



Fig. 7: The stygobiotic blind, depigmented amphipod *Stygobromus tenuis* from a seepage spring in Scotts Run Park, Virginia, USA (Photo: W. K. Jones, used with permission).

SPATIAL VARIATION IN ENVIRONMENTAL CONDITIONS

Long ago, Hawes (1939) pointed out that flooding was an important component of the cave environment, and that floods provided both organic carbon and cues for reproduction. The increased discharge and velocity of water during flooding also represents significant risks to many cave-stream dwelling organisms. In a series of experiments in laboratory streams, Culver (1971) demonstrated that amphipods and isopods in cave streams incurred significant mortality by living in moving water, and this was also supported by field observations that showed that abundance was positively correlated with velocity and discharge (Culver 1971).

Culver and Ehlinger (1980), for two species in the isopod genus *Caecidotea*, showed that this washout and mortality was dependent on the size of the gravels in a stream. Small *Caecidotea* had lower mortality

rates in laboratory streams with small rocks, and large *Caecidotea* had lower mortality rates in laboratory streams with large rocks. The two species they investigated, *C. cannula* and *C. holsingeri*, were highly variable in body size, and this suggested that there might be a “match” between body size and gravel size. In a study of four caves in West Virginia, USA, they did find a correspondence between the shape of the distribution of isopod sizes and gravel sizes (Tab. 3). Caves with two species had gravel sizes with a bimodal distribution and caves with one species had gravel sizes with a uni-modal distribution. It was these differences in the physical environment that allowed the presence of two species, and there was no evidence that the two isopod species competed or that any character displacement took place. The absence of competition was later confirmed in laborato

Tab. 3: Characteristics of gravel size and body sizes of two isopod species (*Caecidotea cannula* and *C. holsingeri*) in four caves in West Virginia, USA. Modified from Culver and Ehlinger (1980).

Cave	Species present	Median gravel size (mm)	Qualitative Features	Median isopod size (mm)	Qualitative features
Linwood Cave	<i>C. holsingeri</i>	11.9	Unimodal	5.4	Unimodal
Harman Cave	<i>C. holsingeri</i>	5.6	Unimodal	2.1	Unimodal
Bowden Cave	<i>C. cannula</i>	11.9	Bimodal	8.2	Bimodal
	<i>C. holsingeri</i>			2.5	
Gladly Cave	<i>C. cannula</i>	8.7	Bimodal	7.0	Broadly unimodal
	<i>C. holsingeri</i>			3.6	

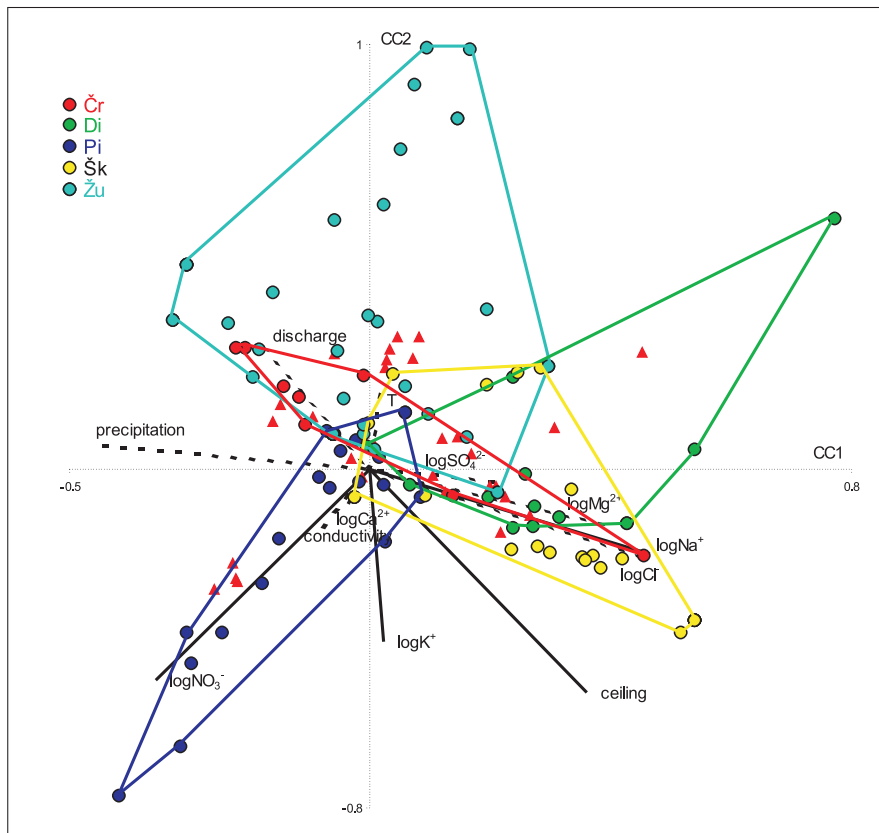
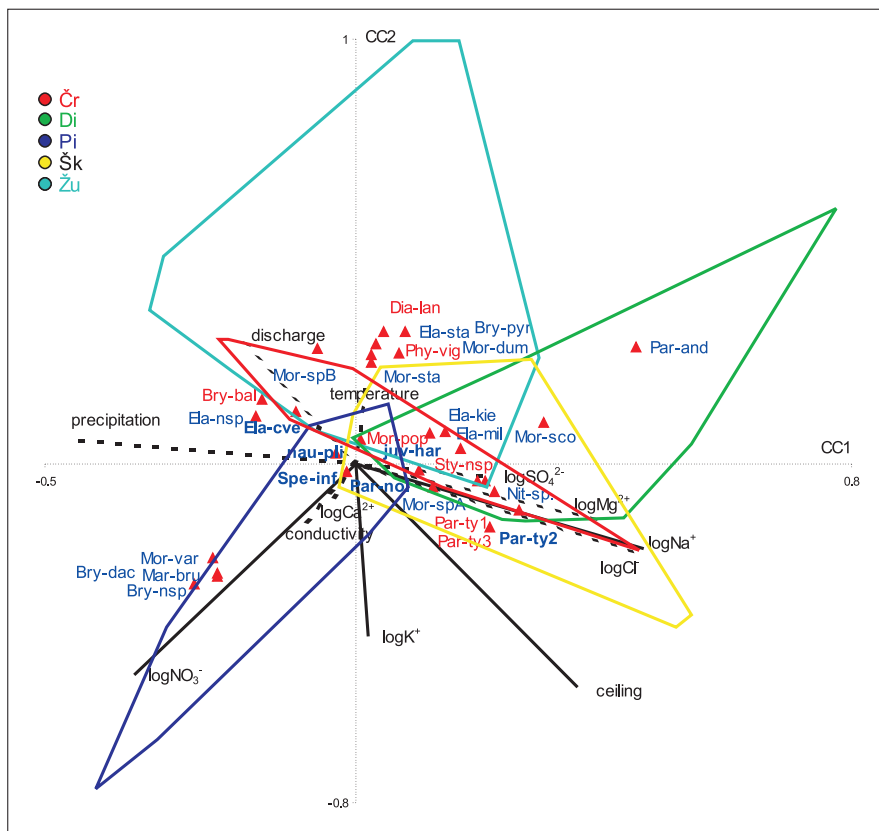


Fig. 8: Ordination diagram based on species composition and abundance data of copepods in epikarst drips showing caves (convex hulls containing sampling sites ○) and species (▲) in relation to the twelve environmental variables (represented as lines) of the five caves.



ry stream and cave stream perturbation experiments (Culver 2005).

A more complex example of how spatial differences in environment allow for the coexistence of species is that of Pipan and colleagues (Pipan 2005; Pipan *et al.* 2006). A series of drips in five caves in Slovenian caves were intensively sampled for copepods using a specially designed continuous sampling device; water in the drips was also periodically measured for Na^+ , K^+ , Ca^{+2} , Mg^{+2} , NH_4^+ , SO_4^{-2} , Cl^- , PO_4^{-3} , NO_3^- , pH, temperature, discharge, conductivity, as well as ceiling thickness and surface precipitation. Canonical correspondence analysis (CCA) was used to associate species data with environmental variables. The overall pattern is shown in Figs. 8 and 9. The first canonical axis (eigenvalue = 0.49) is positively associated with Na^+ and thickness of the cave ceiling, but negatively correlated with NO_3^- . The second canonical axis (eigenvalue = 0.31) was negatively

Fig. 9: Ordination diagram based on species composition and abundance data of copepods in epikarst drips in relation to the twelve environmental variables of the five caves. Species are abbreviated by the first three letters of the genus and species names. For complete names, see Pipan (2005) and Pipan *et al.* (2006).

correlated with K^+ , Na^+ , NO_3^- , and thickness of the cave ceiling.

Several interesting patterns emerge. First, the five caves themselves are partially separated (Fig. 8), especially Županova jama and Pivka jama. Second, several clusters of species were apparent. One, in the lower left in Fig. 9 was positively correlated with NO_3^- , which may indicate a tolerance for elevated levels of nitrate as well as a means of niche separation. Third, some species, especially from Škocjanske jame, were positively cor-

related with Na^+ concentration. Fourth, *Parastenocaris* cf. *andreji* occupies a distinct region in the upper right of CCA plot of the first two axes (Fig. 9). Fifth, most of the species were negatively or not correlated with ceiling height. The exception was four undescribed species in the genus *Parastenocaris*. These species may reduce competition with other copepods by living in the narrow cracks and crevices that are present in the lower part of thick ceilings rather than in epikarst per se.

CONCLUSIONS

On a broad temporal scale, there is strong evidence of the importance of climate change as an agent forcing or isolating animals in subterranean habitats. Climatic changes that have been implicated in the climatic relict hypothesis include temperature increases and decreases during the Pleistocene, the Messinian salinity crisis in the Mediterranean region at the Miocene/Pliocene boundary, and aridification of Australia during the Miocene. While climate change probably explains the majority of cases of isolation in subterranean habitats and the subsequent acquisition of troglomorphic characters, it does not explain all cases, especially in the tropics.

It has been less generally recognized by speleobiologists that subterranean habitats are both temporally variable and include a wide variety of non-cave, non-karst habitats. These habitats, especially shallow subterranean habitats, are less environmentally extreme, with abundant organic carbon and intimate connections with the

surface, including daily and seasonal cues for circadian and annual cycles. These habitats may also hold the key to understanding how organisms successfully invade and adapt to subterranean habitats in general.

Finally, there is considerable spatial variation in both the physical and chemical environment. In the case of isopods living in cave streams, gravel appeared to be the major selective force determining body size. In the case of copepods living in epikarst, small scale physical separation based on different chemical conditions seems to allow the persistence of a remarkably diverse epikarst copepod community.

Speleobiologists should show renewed interest in both the climate, microclimate and the overall physical environment of subterranean habitats, as they are likely to provide many answers concerning the biology of subterranean animals.

REFERENCES

- Barr, T.C., 1960: Synopsis of the cave beetles of the Genus *Pseudanophthalmus* of the Mitchell Plain in Southern Indiana (Coleoptera, Carabidae).- *The American Midland Naturalist*, 63, 307-320.
- Barr, T.C., 1968: Cave ecology and the evolution of troglobites.- *Evolutionary Biology*, 2, 35-102.
- Botosaneanu, L. (ed.), 1986- *Stygofauna Mundi*.- E.J Brill, pp. 740, Leiden, The Netherlands.
- Christiansen, K.A., 1961: Convergence and parallelism in cave Entomobryinae.- *Evolution*, 15, 288-301.
- Christiansen, K.A., 1962: Proposition pour la classification des animaux cavernicoles.- *Spelunca*, 2, 75-78.
- Christiansen, K. A., 1998: New species of *Pseudosinella* from Ascension Island.- *Journal of Natural History*, 32, 149-156.
- Christiansen, K.A., 2005: Morphological adaptations.- In: Culver, D.C. & W.B. White (eds.) *Encyclopedia of Caves*. Elsevier Press, pp. 386-397, Amsterdam, The Netherlands.
- Coineau, N., 2000: Adaptations to interstitial groundwater life.- In: Wilkens, H. *et al.* (eds.) *Subterranean ecosystems*. Elsevier Press, pp.189-210, Amsterdam, The Netherlands.

- Culver, D.C., 1971: Analysis of simple cave communities. III. Control of abundance.- *American Midland Naturalist*, 85, 173-187.
- Culver, D.C., 1976: The evolution of aquatic cave communities.- *The American Naturalist*, 110, 945-957.
- Culver, D.C., 2005: Species interactions.- In: Culver, D.C. & W.B. White (eds.) *Encyclopedia of Caves*. Elsevier Press, pp. 539-543, Amsterdam, The Netherlands.
- Culver, D.C. & T.J. Ehlinger, 1980: The effects of microhabitat size and competitor size on two cave isopods.- *Brimleyana*, 4, 103-113.
- Culver, D.C., Kane, T.C. & D.W. Fong, 1995: *Adaptation and Natural Selection in Caves*.- Harvard University Press, pp. 223, Cambridge, Massachusetts.
- Culver, D.C., Pipan, T. & S. Gottstein, 2006: Hypotelminorheic—a unique freshwater habitat.- *Subterranean Biology*, 4, 1-8.
- Culver, D.C. & T. Pipan, 2008: Superficial subterranean habitats – gateway to the subterranean realm?- *Cave and Karst Science*, 35, 5-12.
- Culver, D.C. & T. Pipan, 2009: *The Biology of Caves and Other Subterranean Habitats*.- Oxford University Press, pp. 254, Oxford.
- Culver D.C., Holsinger, J.R., Christman, M.C. & T. Pipan, 2010: Morphological differences among eyeless amphipods in the genus *Stygobromus* dwelling in different subterranean habitats.- *Journal of Crustacean Biology*, 30, 68-74.
- Faille, A., Ribera, I., Deharveng, L., Bourdeau, C., Garnery, L., Quéinnec, E. & T. Deuve, 2010: A molecular phylogeny shows the single origin of the Pyrenean subterranean Trechini ground beetles (Coleoptera: Carabidae).- *Molecular Phylogenetics and Evolution*, 54, 97-106.
- Hawes, R.S., 1939: The flood factor in the ecology of caves.- *Journal of Animal Ecology*, 8, 1-5.
- Holsinger, J.R., 1980: *Stygobromus canadensis*, a new subterranean amphipod crustacean (Crangoncytidae) from Canada, with remarks on *Wisoconsin refugia*.- *Canadian Journal of Zoology*, 58, 290-297.
- Holsinger, J. R., 1988: Trogllobites: the evolution of cave-dwelling organisms.- *American Scientist*, 76, 146-153.
- Howarth, F.G., 1972: Cavernicoles in lava tubes on the island of Hawaii.- *Science*, 175, 325-326.
- Howarth, F.G., 1987: The evolution of non-relictual tropical trogllobites.- *International Journal of Speleology*, 16, 1-16.
- Jeannel, R., 1943: *Les Fossiles Vivants des Cavernes*.- Gallimard, pp. 321, Paris.
- Jeffery, W.R., 2005: Adaptive evolution of eye degeneration in the Mexican blind cavefish.- *Journal of Heredity*, 96, 185-96.
- Jeffery, W.R., 2009: Regressive evolution in *Astyanax* cavefish.- *Annual Review of Genetics*, 43, 25-47.
- Juberthie, C., 2000: The diversity of the karstic and pseudokarstic hypogean habitats in the world.- In: Wilkens, H. *et al.* (eds.) *Subterranean ecosystems*.- Elsevier Press, pp. 17-39, Amsterdam, The Netherlands.
- Leys, R., Watts, C.H.S., Cooper, S.J.B. & W. Humphreys, 2003: Evolution of subterranean diving beetles (Coleoptera: Dytiscidae: Hydroporini, Bidessini) in the arid zone of Australia.- *Evolution*, 57, 2819-2834.
- Malard, F., Ward, J.V. & C.T. Robinson, 2000: An expanded perspective of the hyporheic zone.- *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie*, 27, 431-437.
- Mitchell, R., 1969: A comparison of temperature and tropical cave communities.- *The Southwestern Naturalist*, 14, 73-88.
- Palmer, A.N., 2007: *Cave geology*.- Cave Books, pp. 454, Dayton, Ohio.
- Peck, S.B., 1980: Climatic change and the evolution of cave invertebrates in the Grand Canyon, Arizona.- *National Speleological Society, Bulletin*, 45, 53-60.
- Pipan, T., 2005: *Epikarst – a promising habitat*.- Založba ZRC, pp. 101, Ljubljana, Slovenia.
- Pipan, T., Blejec, A. & A. Brancelj, 2006: Multivariate analysis of copepod assemblages in epikarstic waters of some Slovenian caves.- *Hydrobiologia*, 559, 213-223.
- Pipan, T., López, H., Oromí, P., Polak, S. & D.C. Culver, 2010: Temperature variation and the presence of trogllobionts in terrestrial shallow subterranean habitats.- *Journal of Natural History*.
- Poulson, T.L., 1963: Cave adaptation in amblyopsid fishes.- *American Midland Naturalist*, 70, 257-90.
- Poulson, T. L., 1985: Evolutionary reductions by neutral mutations: plausibility arguments and data from amblyopsid fishes and linyphid spiders.- *National Speleological Society, Bulletin*, 47, 109-117.
- Poulson, T.L. & W.B. White, 1969: The cave environment.- *Science*, 165, 971-81.