COBISS: 1.01

SHIFTING PARADIGMS OF THE EVOLUTION OF CAVE LIFE

SPREMEMBA PARADIGME O EVOLUCIJI JAMSKEGA ŽIVLJENJA

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Abstract UDC 591.522:551.44

David C. Culver & Tanja Pipan: Shifting Paradigms of the Evolution of Cave Life

The unique morphology of cave animals has interested biologists at least since the time of Lamarck. After a number of non-adaptive explanations for the morphology of cave animals, especially with respect to eye and pigment loss, a neo-Darwinian explanation, emphasizing constructive morphological changes rather than losses, was put forward by Barr, Christiansen, and Poulson in the 1960's. Emphasizing convergent evolution (troglomorphy), this paradigm has recently been challenged, with evidence of divergent selection and the widespread occurrence of morphologically similar species in other subterranean habitats, including ones with abundant food and relatively strong environmental fluctuations. An emerging paradigm emphasizes the central role of darkness in convergent evolution, and the role of habitat size and interspecific competition in divergent evolution of subterranean species.

Keywords: adaptation, cave life, natural selection, neo-Darwinism, shallow subterranean habitats, troglomorphy.

Izvleček UDK 591.522:551.44

David C. Culver & Tanja Pipan: Sprememba paradigme o evoluciji jamskega življenja

Edinstvena morfologija jamskih živali, predvsem v povezavi z izgubo oči in pigmenta, je zanimala biologe že od časa Lamarcka. Kasneje so bile predstavljene tudi druge, t.im. neodarvinistične razlage, s poudarkom na konstruktivnih morfoloških spremembah namesto izgubah, povzete s strani Barra, Christiansena in Poulsona v 60. letih 20. stoletja. Z novejšimi raziskavami o divergentni selekciji in razširjeni prisotnosti morfološko podobnih vrst v drugih podzemeljskih habitatih, vključno s tistimi, kjer je veliko hrane in z relativno velikimi okoljskimi nihanji, se je zamajala paradigma, temelječa na konvergentni evoluciji (troglomorfija). Novo nastajajoča paradigma poudarja osrednjo vlogo teme v konvergentni evoluciji, ter vlogo velikosti habitata in medvrstne kompeticije v divergentni evoluciji podzemeljskih vrst.

Ključne besede: adaptacija, jamsko življenje, naravna selekcija, neodarvinizem, plitvi podzemeljski habitati, troglomorfija.

INTRODUCTION

In any field of science, the foci of research, and the research questions being asked, change over time. Subterranean biology (speleobiology) is no exception. In this review, we trace the history of that most basic of research topics in speleobiology – how is the morphology of subterranean-dwelling animals different from the morphology of surface-dwelling animals? Not only is the morphology of subterranean animals unique, even bizarre (Fig. 1), its causes remain elusive.

We review the rise and development of neo-Darwinian explanations for the morphology of subterranean animals, beginning with the first explicitly neo-Darwin-

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Received/Prejeto: 30.01.2015

Fig. 1: Photograph of the European cave salamander Proteus anguinus *(Photo: G. Aljančič).*

ian speleobiologists, writing in the 1960's and later. We trace its origin, the development of its central paradigm of convergent evolution, the "hardening" of this synthesis, critiques of this paradigm, and the beginnings of a new paradigm, still neo-Darwinian, but with a focus away from convergence.

QUESTIONS RESULTING FROM THE MORPHOLOGY OF SUBTERRANEAN ANIMALS

Known from at least the time of Lamarck in the late eighteenth and early nineteenth centuries, the European cave salamander *Proteus anguinus* (Fig. 1) is the iconic cave animal. It was known by Darwin, but he probably did not see a living specimen, even though he was offered one by Falconer in 1861 (Shaw 1999). The most obvious and unusual features of *Proteus* are its lack of eyes and pigment, so-called regressive features. However, a closer examination reveals constructive features, including relatively elongated appendages, a large head, elaborated inner ear receptors, and an overall increase in the lateral line system (Bulog 2004). Nonetheless, the overall aspect of *Proteus* is one of loss.

In the late nineteenth and early twentieth century, the widely accepted explanation for the loss of features was the Lamarckian idea of "use and disuse." According to Lamarck:

> *… it becomes clear that the shrinkage and even disappearance of the organ in question are the result of permanent disuse of that organ* (Lamarck 1809).

More importantly, Darwin himself took a distinctly Lamarckian view of the morphology of cave animals, rather than an adaptationist view:

> *It is well known that several cave animals that inhabit the caves of Carniola [Slovenia] and Kentucky are blind … As it is difficult to imagine that eyes, though useless, could be in any way injurious to animals living in darkness, their loss may be attributed to disuse* (Darwin 1859).

Small wonder that for decades following Darwin, adaptation was not associated with subterranean organisms.

What then were explanations for the evolution of cave animals? For North American neo-Lamarckians such as Packard (1888), the theory of use and disuse provided an obvious answer (Culver *et al.* 1995). The related theory of orthogenesis, evolution toward a "perfect form", determined by factors internal to the organism, was later taken up by some French speleobiogists, especially Vandel (1964). For Vandel, animals were not eyeless because they were in caves, but were in caves because they were blind! According to Vandel, animals were in senescent phyletic lines, destined to extinction. Other speleobiologists, while not denying the occurrence of natural selection, minimized its importance. Most prominent, Wilkens (1971), building on ideas developed by Kosswig and Kosswig (1940) and the emerging theory of neutral mutation (Kimura 1983), held that losses of eyes and pigment were independent of natural selection and the result of structurally reducing, selectively neutral mutations. Still other speleobiologists, such as Magniez (1985) emphasized the importance of the retardation of somatic development, *e.g.*, neoteny, in determining morphology of subterranean animals. Some early speleobiologists, especially Racoviță (1907), did argue for an adaptationist explanation, but their views took decades to gain support.

TROGLOMORPHY—THE NEO-DARWINIAN PARADIGM

Independently, in the 1960's, three North American subterranean biologists–Barr, Christiansen, and Poulson–developed a neo-Darwinian paradigm of the evolution of cave life. While they each took different approaches and studied different subterranean animals, their work *in toto* offers a thorough demonstration of how neo-Darwinism can explain the morphology, as well as physiology, ecology, and behavior of subterranean animals.

Christiansen (1961, 1965) studied adaptation of cave Collembola (with reduced or absent eyes) to darkness and to walking on wet surfaces, including pools. Christiansen very deliberately set to establish a neo-Darwinian example from the cave fauna, which is clear from the title of his 1961 paper, "Convergence and parallelism in cave Entomobryinae". Using the comparative approach, the preferred method of analysis at that time, he showed consistent morphological differences in claw structure and differences in locomotory behavior between cave-modified and unmodified species (Fig. 2), differences he found to be repeated in different lineages of Collembola. Hence he demonstrated that there was convergent evolution (the independent evolution of similar traits) among cave Collembola. In 1962, he also coined the term "troglomorphy" for characters showing convergence as a result of convergent selective pressures in the cave environment. This concept has been widely used in the speleobiological literature and Christiansen himself recently updated the list of troglomorphic characters (Tab. 1). Other terms in widespread use, namely aquatic stygobionts and terrestrial troglobionts, refer to ecological/distributional rather than morphological attributes, where species are limited to subterranean habitats. Most, but not all stygobionts and troglobionts are troglomorphic. As our colleague Daniel Fong pointed out to us, troglomorphy is a hypothesis about the morphology of stygobionts and troglobionts, rather than just a morphological definition. It is the core of the neo-Darwinean paradigm of adaptation to cave life.

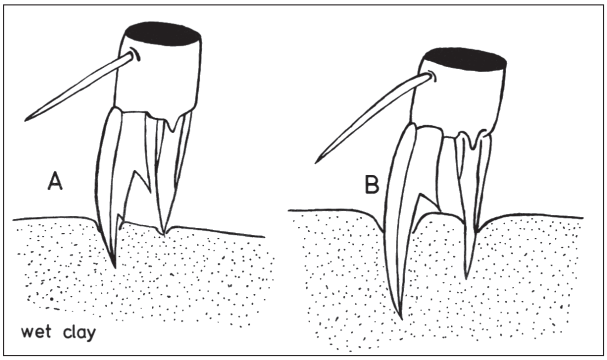

Fig. 2: Convergent changes in structure of collembolan claw as a result of adaptation to walking on wet substrates. (A) Claw of non-troglomorphic species and (B) Claw of troglomorphic species. The unguis is on the right and empodium is on the left in each drawing. Modified from Culver and Pipan (2009).

Poulson (1963) studied the species in the fish family Amblyopsidae, which at the time of his study comprised five species, three from caves, one from springs and caves, and one from swamps. In contrast to Christiansen, Poulson's focus was more physiological, behavioral, and demographic, which he showed to have changed in predictable ways with increasing cave adaptation. For example, cave fish lived longer and had fewer, but larger eggs per clutch. He did not neglect morphology and demonstrated changes in brain anatomy for cave species, with extra-optic sensory structures, *e.g.,* the olfactory lobe, increased in size and optic sensory structures, *e.g.,* optic lobe, decreased in size. Interestingly, he largely attributed eye and pigment degeneration to

> … *accumulation of loss mutations with reduction of selection pressure* (Poulson 1963).

Tab. 1: List of troglomorphic features, from Christiansen (2012).

Specialization of sensory organs (*e.g.,* touch, chemoreception)
Elongation of appendages
Pseudophysiogastry
Reduction of eyes, pigment, and wings
Compressed or depressed body form (Hexapoda)
Increased egg volume
Increased size (Collembola, Arachnida)
Unguis elongation (Collembola)
Foot modification (Collembola, planthoppers)
Scale reduction or loss (teleost fishes)
Loss of pigment cells and deposits
Cuticle thinnng
Elongate body form (teleost fishes, Arachnida)
Depressed, shovel-like heads (teleost fishes, salamanders)
Reduction or loss of swim bladder (teleost fishes)
Decreasing hind femur length, crop-empty live weight ratio (crickets)

Neither he nor Christiansen emphasized eye degeneration but rather looked at characters that either increased in complexity or changed in the course of isolation in caves.

Barr (1960, 1968) proposed models of speciation in which speciation (and adaptation) occurred after the extirpation of surface populations, and hence was allopatric, *i.e.,* with geographic separation. Barr, based both on his extensive taxonomic work on the beetle genus *Pseudanophthalmus* (Barr 2004) and his work on the discovery, mapping, and description of caves (Barr 1961), showed that subterranean dispersal was highly restricted, and in many cases, species only occurred in a single cave. One implication of restricted dispersal was that isolation and adaptation happened many times independently.

STRENGTHENING OF THE NEO-DARWINIAN PARADIGM OF TROGLOMORPHY

Most of the evidence for natural selection and adaptation in Barr, Christiansen, and Poulson's studies was indirect, using the comparative approach. Culver *et al.* (1995) used a more direct approach, and measured the intensity of natural selection on a number of populations of the amphipod *Gammarus minus* living in springs and in caves, using newly developed techniques for the estimation of selection coefficients using regression models. They did not measure overall selection but rather the components connected to mating and fecundity. Predictions can be made about the direction of natural selection in both cave and spring populations. Large size should be selected for in caves perhaps because of the increased range of available food and the absence of predator; small size should be selected for in spring populations to reduce predation by trout (Culver *et al.* 1995). Long antennae should be selected for in cave populations because a greater sensory spatial range should be advantageous in darkness; small antennae may be selected for in spring populations because longer antennae maybe a disadvantage in streams. Large eyes should be selected for in spring populations because of the advantage of increased visual acuity; small eyes may be advantageous in cave populations because of pleiotropic effects (see below). The results are a mixed bag with respect to predictions (Tab. 2). Large body size was selected for in both habitats, but the effect of predators on fitness was not directly measured. If it had been, it is likely that selection would favor small individuals. Eye size selection was in the direction predicted. The puzzle is antennal length. The positive selection for antennal length in spring populations is unexpected and makes it difficult to explain why spring populations have longer antennae than cave populations because there was no difference in selection in the two habitats.

A possible explanation of this anomaly was provided by Christman *et al.* (1997). Using a technique that allows the subtraction of phylogenetic effects from morphology, they analyzed the residuals that remained. Any pattern of the residuals is best explained by convergent selective pressures. Phylogenetically subtracted eye size was in the direction predicted for two independent drainage basins (Fig. 3), in agreement with convergent selective pressures. For antennal size, one cave population was larger than the sister spring population, but the

Tab. 2: Standardized natural selection gradients for Gammarus minus *from caves and springs. Modified from Culver and Pipan (2009).*

Character	***Habitat***	***Mean Selection***	***Predicted Selection***
Head Length	Cave	+0.18	+
Head Length	Spring	+0.19	-
Antenna Length/ Number of segments	Cave	+0.06	+
Antenna Length/ Number of segments	Spring	+0.06	-
Eye Size/ Ommatidia number	Cave	-0.08	-
Eye Size/ Ommatidia number	Spring	+0.06	+

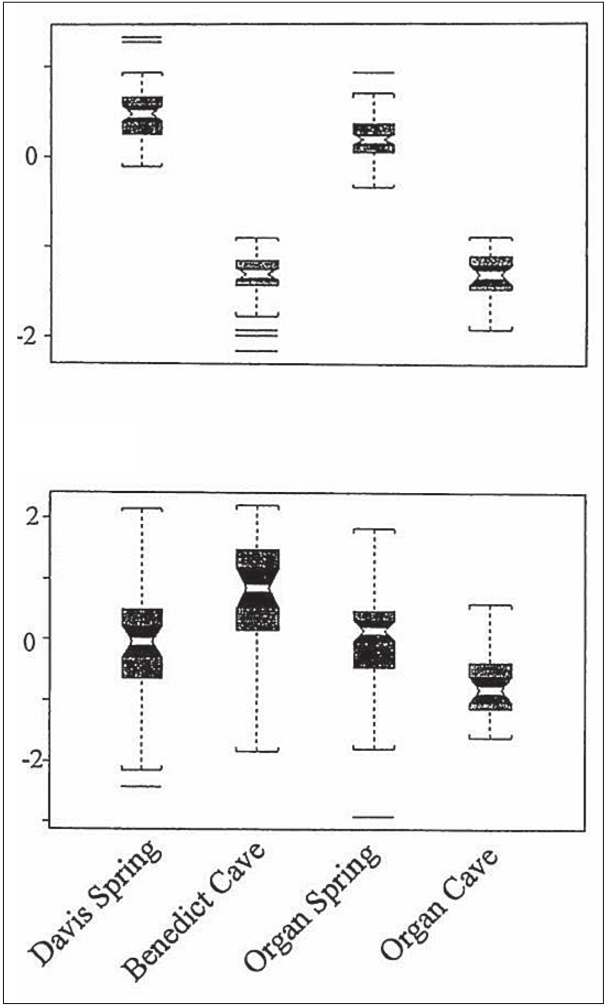

Fig. 3: Box plots of the residuals following phylogenetic subtraction, of eye size (top) and antennal size (bottom) for populations of Gammarus minus *from two drainage basins in West Virginia, U.S.A. The lines in the middle of the polygon identify the median, the rectangle encloses the interquartile range, and whisker include 90 percent of the data. Modified from Christman* et al. *(1997).*

other was not, suggesting divergent selective pressures. This work, although the authors did not recognize it at the time, anticipates the weakening of the neo-Darwinian synthesis.

Over the past 20 years, a group of developmental biologists, centered at the Jeffery laboratory at University of Maryland, have been examining the pathways of eye and pigment development in the cavefish *Astyanax mexicanus*, and thus have returned to the question of whether the reduced features of eye and pigment are themselves subject to natural selection. *Astyanax mexicanus* is a particularly useful model organism for the study of evolution and development because closely related surface populations exist with which cave populations can interbreed, and both cave and surface populations can be reared in the laboratory. A series of studies have repeatedly demonstrated that there are two aspects of eye and pigment development in *A. mexicanus* that cannot be easily explained without invoking natural selection. Implicitly at least, netural mutation (e.g., Wilkens 1971) is less important.

The first aspect is that the eye and pigment development pathways are often blocked at the same point, a “convergent defect” in the apt phrase of Bilandžija *et al.* (2012). In the case of pigment, melanin synthesis is blocked at the same step in a diverse set of subterranean animals, including planthoppers (Bilandžija *et al.* 2012) and *A. mexicanus* (Bilandžija *et al.* 2013), specifically at the early stage of melanin synthesis (L-tyrosine to L-Dopa) and this results in an increase in the catecholamine synthesis pathway via an increase in norepinephrine. Enhancement of norepinephrine and the catecholamine pathway may confer several advantages of cave populations of *A. mexicanus*, including increased foraging efficiency, decreased sleep, and stress response (Bilandžija *et al.* 2013).

The second pattern that is difficult to explain without recourse to natural selection is increased expression of some genes along the eye development pathway in cave populations of *A. mexicanus*. Yamamoto *et al.* (2004) showed that the sonic hedgehog (*shh)* gene increased in expression in cave populations. The increase in expression of *shh* leads to an increase in the density of taste buds in cave populations (Yamamoto *et al.* 2009).

There may well be aspects of eye and pigment degeneration that are largely the result of the accumulation of structurally reducing neutral mutations, but these cases are becoming fewer and fewer (Jeffery 2010).

What may appropriately be termed the troglomorphy paradigm, resulting from the work summarized above as well as many other studies has three main components:

- Cave animals are highly convergent because of a convergent selective environment;
- Convergent morphology includes both reduced characters (eyes and pigment) and elaborated characters (antennae and other sensors); and
- The convergent selective environment includes little food, little environmental variation, and no light.

THE WEAKENING OF THE TROGLOMORPHY PARADIGM

In their discussion of adaptation to subterranean life, Barr, Christiansen, and Poulson focused almost exclusively on caves. European researchers, especially Racoviță (1907) emphasized the cracks and crevices in caves that were inaccessible to humans but which he thought harbored the majority of cave-dwelling individuals. Juberthie *et al.* (1980) expanded the subterranean realm beyond caves and associated cracks to non-karst areas with the description of shallow terrestrial habitats such as covered talus slopes, the *milieu souterrain superficiel (MSS).* Culver and Pipan (2014) formally introduced the concept of *shallow subterranean habitats (SSHs),* pointing out the unifying characteristics of aphotic habitats that are close to the surface. All of these habitats are more closely connected to the surface than caves, and all are more environmentally variable than caves, although less than surface habitats. In addition, most shallow subterranean habitats have high levels of nutrients and organic matter. SSHs include:

- Seepage springs and the hypotelminorheic habitat
- Epikarst—the soil-rock interface in karst
- Intermediate-sized terrestrial shallow subterranean habitats (including the MSS)
- Calcrete aquifers, arid land aquifers typically formed in soils subject to a moisture deficit
- Interstitial habitats along rivers and streams
- Soil
- Lava tubes

With respect to environmental conditions, they share with caves only darkness.

Shallow subterranean habitats also share with caves the occurrence of a troglomorphic fauna, a fauna with eyeless, pigmentless species, and depending on the dimensions of the habitat (pore size), elongated

Tab. 3: List of shallow subterranean habitats and caves where all resident species have been recorded, along with the frequency of troglomorphic stygobionts and troglobionts. The total number of species for each habitat is sometimes less than the total number of species recorded because some species were found in more than one site. Modified from Pipan & Culver 2012.

Habitat	***Site***	***No. of species***	***Group***	***Percent troglomorphs***
Epikarst	Organ Cave, W.Va., U.S.A.	10	Copepoda	40
	Črna jama, Slovenia	8	Copepoda	100
	Dimnice, Slovenia	8	Copepoda	100
	Huda luknja, Slovenia	2	Copepoda	100
	Pivka jama, Slovenia	11	Copepoda	73
	Postojnska jama, Slovenia	5	Copepoda	80
	Škocjanske jame, Slovenia	9	Copepoda	89
	Snežna jama na planini Arto, Slovenia	2	Copepoda	100
	Županova jama, Slovenia	14	Copepoda	93
	TOTAL	37		70
	Overall Community Mean - Epikarst			86
Hypotelminorheic	Lower Potomac, U.S.A.	15	macro-Crustacea	47
	Medvenica Mountain, Croatia	4	macro-Crustacea	75
	Nanos, Slovenia	4	macro-Crustacea	50
	TOTAL	23		52
	Overall Community Mean - Hypotelminorheic	9		57
MSS	La Ballongue, France	98	Arthropoda	16
	La Guancha, Canary Islands	26	Arthropoda	15
	Průčelská Gorge, Czech Rep.	39	Chelicerata	5
	Teno, Canary Islands	73	Arthropoda	14
	TOTAL	229		14
	Overall Community Mean - MSS	4		12
Caves	12 small caves in Greenbrier Co., W.Va., U.S.A.	91	terrestrial invertebrates	11
	Cueva del Mulo, Canary Islands	29	terrestrial Arthropoda	41
	Peak-Speedwell Cave System, England	28	aquatic invertebrates	0
	Mammoth Cave, Kentucky, U.S.A.	51	terrestrial invertebrates	51
	Mammoth Cave, Kentucky, U.S.A.	16	aquatic vertebrates and macroinvertebrates	69
	Organ Cave, W.Va., U.S.A.	15	aquatic vertebrates and macroinvertebrates	33
	Vjetrenica, Bosnia & Hercegovina	51	terrestrial invertebrates	69
	Vjetrenica, Bosnia & Hercegovina	34	aquatic vertebrates and macroinvertebrates	97
	TOTAL	315		48
	Overall Community Mean - Caves	8		46

appendages. In Table 3, we summarize the relative frequency of troglomorphic stygobionts and troglobionts in some well-studied caves and SSHs. The highest frequency of stygobionts and troglobionts within a community was found in epikarst (86 percent and 70 percent of all species) and the lowest in the MSS (12 percent and 14 percent of all species). Caves were intermediate at 46 percent troglomorphy (with 48 percent of all species). Not too much credence should be put in the numerical values because there is high variability within groups. What is abundantly clear is that stygobionts and troglobionts, that is, obligate subterranean inhabitants, are widespread in shallow subterranean habitats.

The second aspect of the occurrence of many stygobionts and troglobionts in shallow subterranean habitats is the degree of morphological specialization, that is, the degree of troglomorphy. For SSHs with very small habitat dimensions, *i.e.*, interstitial aquifers and soil, it is well known that both size and shape of stygobionts and

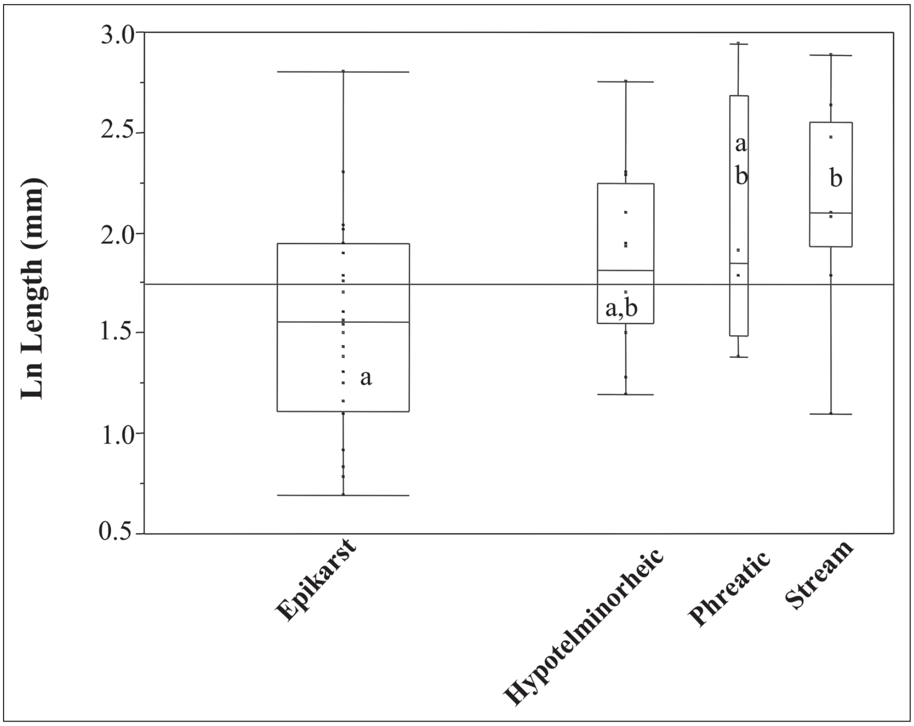

Fig. 4: Box and whiskers plots of ln female body length of species of the amphipod genus Stygobromus for epikarst, hypotelminorheic, cave streams, and phreatic habitats in North America. Overall mean represented by the line across the entire plot. Boxes: 50 percent of the data; line across each box: group median; whiskers: minimum and maximum values. The widths of the rectangles are proportional to sample size. Dots are individual data points. Plots with the same letter (a or b) do not differ according to the Tukey-Cramer HSD test. From Culver et al. (2010).

troglobionts differ from those in other subterranean habitats. In particular, animals are often miniaturized, with shortened appendages (Coiffait 1958; Coineau 2000; Culver & Pipan 2014). Additionally, soil and interstitial organisms can become very thin and elongate, relative to other species (Coineau 2000; Thibaud & Coineau 1998). There are other size differences among subterranean habitats. An example is shown in Fig. 4, for the amphipod genus *Stygobromus.* Body sizes in two SSHs (epikarst and hypotelminorheic) and two cave habitats (streams and deep pools) differed roughly according to the size of the habitats. The smallest species were in epikarst and the largest species were in cave streams. However, body size is not necessarily a troglomorphic character and it is not included in Christiansen's (2012) recent list. Relative antennal length and number of antennal segments are troglomorphic characters and should increase with increased adaptation to subterranean life. Culver *et al.*'s (2010) analysis of these characters for *Stygobromus* indicated that cave species were no more troglomorphic than SSH species. If this result holds for other groups, then the part of the troglomorphic paradigm concerning a shared selective environment (see above) is restricted the shared environment of no light, because SSHs are more variable, cyclical and food rich than nearly all caves (Culver & Pipan 2014).

The third important weakness in the troglomorphic paradigm is evidence for divergence, not convergence, in some subterranean communities. Implicit in the troglomorphy paradigm is that the primary selective agents are abiotic factors, and relative fitness of individuals in a population is determined by darkness, reduced food, etc. A major selective agent in many if not most epigean communities, are interactions with other species-competition and predation. Studies of competition and predation have received surprisingly little attention in caves and other subterranean communities. The predator-prey pairof the beetle *Neaphaenops tellkampfi* feeding on eggs of the cricket *Hadenoecus* has been well studied (*e.g.* Kane & Poulson 1976) and predation has apparently resulted in longer ovipositors in the crickets. There are many predators in caves but their role as agents of natural selection for morphological change in their prey remains unstudied except for the above example. Interspecific competition has, until very recently, also been little studied. Culver (1976) and Culver *et al.* (1991) demonstrated that interspecific competition among cave stream amphipods had a large impact on microdistribution and even species composition. However, they provided no examples of morphological change, such as character displacement. Van Zant *et al.* (1978) did find a case of character displacement in cave beetles. The pair of species *Pseudanophthalmus menetriesii* and *P. pubscens* have nearly identical lengths when occurring alone; when together *P. menetriesii* is smaller. Vergnon *et al.* (2013) found that the size ratios of Dytiscidae beetles in Austrailian calcrete aquifers were approximately 1.6, the general result predicted by the theory of limiting similarity, a result of interspecific competition. Presumably, the dyiscids diverged in body size as a result of the selective pressure of interspecific competition.

Vergnon *et al.*'s work implies that there can be divergent selection in a subterranean habitat, and Fišer *et al.* (2012) addressed the question directly. They investigated the impact, if any, interspecific competition had on communities of *Niphargus* living in interstitial aquifers in rivers and shallow wells (subterranean habitats) in the Balkans. Up to six species of *Niphargus* co-occur in the same habitat. They used the multivariate statistical procedure of convex hulls, to represent the morphometrics of each species, which included size and relative length and width of appendages. Using all *Niphargus* species in the region as a source of randomly drawn samples of species, they showed that the morphology of co-occurring *Niphargus* was consistently more disparate than species drawn at random (Fig. 5). Thus divergence, not convergence, was occurring.

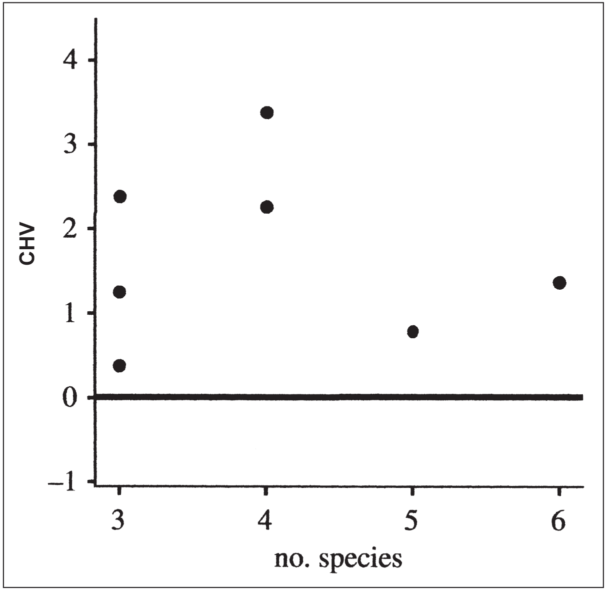

Fig. 5: Convex hull volumes (CHV, y-axis) as measures of functional diversity of Niphargus communities in interstitial sites in the Balkans. Positive values indicate higher morphological diversity than expected if an equal sized community were assembled at random. From Fišer et al. (2012).

As a final example of the weakening of the troglomorphic paradigm, consider the Collembola species *Acherontides eleonorae,* an obligate inhabitant of vampire bat guano in caves in Brazil. It is widely held that troglomorphy does not occur in guano communities in caves because of the super abundant food supply (Poulson 1972). Eyeless and without pigment, *A. eleonorae* can reach densities of hundreds per cm^2 (Palacios-Vargas & Gnaspini-Netto 1992). *Acherontides eleonorae* must navigate on the viscous surface of vampire bat guano, and the unquis (part of the claw, see Fig. 2) is modified to be a narrow, smooth structure that provides good purchase on the substrate. The ungual modifications share features with parallel and convergent changes Christiansen (1961,1965) described for Collembola in non-guano cave that enabled them to walk on the surface of pools (see Fig. 2). Thus, it seems reasonable to call *A. eleonorae* troglomorphic. Konec *et al.* (2015) report a case of the evolution of troglomorphy in the isopod *Asellus aquaticus* in a resource rich subterranean environment (the chemoautotrophic Movile Cave in Romania) as well as in resource poor Slovenian caves. Konec *et al.* (2015) also point out that the Romanian and Slovenian caves really only share the characteristic of constant darkness.

A NEW EMERGING PARADIGM

For caves and subterranean habitats in general, there are three factors of the selective environment that have been demonstrated to affect morphology:

- Absence of light is a convergent selective force in the reduction of eyes and pigment in subterranean animals, and it is likely to be important, but not conclusively demonstrated, in the elaboration of extra-optic sensory structures, including appendage elongation. This is recasting of the troglomorphy paradigm.
- Habitat (pore) size is a divergent selective force in the evolution of body size, and this is especially important among different subterranean habitats, but occurs within habitats as well.
- Interspecific competition, a divergent selective force, is important in shaping morphology when competing species are present.

Conspicuously absent from this list is reduced food and reduced cyclicity of the environment. Poulson's (1963) now classic work on Amblyopsidae fish points to many features of their ecology, physiology, and behavior that are best explained as adaptation to low food. However, the generality of his results are not at all clear. Amblyopsid fish are the top predators in the caves they inhabit, and thus are most likely species in the community to have low food supplies, a feature shared with predators in surface environments as well. It may be as important to the understanding of the Amblyopsidae that they are top predators as it is that they are cave animals. Many examples of resistance to starvation among subterranean species, not all of them predators, have been reported (e.g., Hervant & Renault 2002). How common such adaptations are is not clear, and many of the reported cases involve comparisons between phylogenetically distant species, such as the amphipods *Gammarus* and *Niphargus,* which are in different families. In these cases, phylogenetic effects may be confounding apparent convergence (see Christman *et al.* 1997). The presence of species with troglomorphic morphology in SSHs with relatively abundant food argues that food scarcity is not a necessary condition for the evolution of troglomorphic features.

Both habitat size (pore size) and interspecific competition have been demonstrated to result in divergence but at different scales. For habitat size, this divergence is largely between habitats, *e.g.* interstitial compared to cave streams. For competition, divergent selection occurs within a community, as Fišer *et al.* (2012) demonstrated for interstitial communities in the Balkans.

Convergence is especially common with reduced characters, although there are a large number of cave species with elongated appendages. The ongoing work on eye and pigment loss in the Mexican cavefish *Astyanax mexicanus* (*e.g.,* Bilandžija *et al.* 2014) places a stronger role for natural selection in eye loss than most previous work. While the concept of troglomorphy has helped guide research in the subterranean realm for nearly sixty years, it may now be more of a hindrance than a help. It is time to return to the more general terms of convergence and divergence.

This new emerging paradigm should help make subterranean species model systems for the study of more general evolutionary phenomena, such as convergence, divergence, and speciation. As it develops, this paradigm should address the duality of subterranean morphology-convergent yet divergent, and it should look at the totality of species living and reproducing in caves and other subterranean habitats-troglomorphic and non-troglomorphic species alike. In its more than fifty year history, troglomorphy has proved to be a useful way to organize thinking about adaptation to the subterranean environment. But, we think it is incomplete for the reasons we discuss above. Perhaps the concept of troglomorphy has become too hardened or ossified. Christiansen himself has always advocated a flexible view (especially Christiansen 1992), and at least there needs to be recognition that it is not universal among cave organisms.

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