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Chapter 10

Historical and Ecological Factors Determining Cave Diversity



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10.1 Introductory Background

In this chapter, we do not aim to review the historical views on the origin and evolution of cave fauna, of which there are several excellent accounts (see, e.g. Bellés 1987; Culver et al. 1995; Romero 2009; Culver and Pipan 2014), but to try to understand the origin of some persistent ideas that have traditionally shaped the study of the subterranean fauna and its diversity and that still have a recognisable influence. We will mostly refer to terrestrial fauna and mostly to the groups with which we are most familiar through our own work (Coleoptera Leiodidae and Carabidae), which are also the ones with the highest diversity in the subterranean environment. For the evolution of the stygobiontic fauna, see, e.g. Marmonier et al. (1993), Culver et al. (1995), Danielopol et al. (2000), Lefébure et al. (2006) or Trontelj et al. (2009).

The origins of most of the current views on the evolution of the subterranean fauna can be traced back to Emil Racovitza and René Jeannel (e.g. Racovitza 1907; Jeannel 1926, 1943), which were the first to document extensively and systematically the diverse fauna of the European caves. They were strongly influenced by the earlier work of North American biospeleologists (e.g. Packard 1888), but they reframed their ideas according to the evolutionary views prevalent in the first decades of the twentieth century. The work of R. Jeannel and other biospeleologists

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had by then firmly established some generalities about the subterranean fauna that demanded an evolutionary explanation, which could be summarised as follows:

1. Species living in the deep subterranean environment tend to share similar morphological characters, such as reduction or lack of eyes, wings and pigment, elongated appendages, and in many cases extremely similar body shapes (Jeannel 1943; Vandel 1964).
2. These characteristics (the troglomorphic phenotype, Christiansen 1962, 2012; see Chap. 4) evolved independently from epigeal ancestors in multiple zoological groups, showing a high degree of convergence (Fig. 10.1).
3. Some zoological groups are more prone than others to colonise the subterranean environment. In the Northern Hemisphere, the terrestrial fauna in caves is dominated by a few groups, mostly among insects and spiders. Two lineages show a clear dominance in terms of number of species with troglomorphic characters, both of them among the Coleoptera: tribe Trechini within the Carabidae (ground beetles) and, in Europe and the Middle East up to the Caspian Sea, tribe Leptodirini within the Leiodidae (Jeannel 1911, 1928).
4. Most species with troglomorphic characters have very restricted distributions. They are often known from a single cave or a group of nearby caves and are likely to be restricted to their surrounding subterranean system (e.g. Jeannel 1911, 1928; Barr 1968; Assmann et al. 2010). This is in sharp contrast to many epigeal species of the same groups that often have wider distributions.

The usually restricted ranges of subterranean species would imply that, once underground, they do not tend to move, at least not outside the area directly accessible through subterranean fissures or cavities. This means that “islands” of subterranean habitat, isolated from other such islands by substratum not suitable for subterranean species, would have an independent subterranean fauna. The difficulty with this scenario is that in some cases, subterranean species in different units (“islands”) of subterranean habitat, and thus of supposed independent origin, can have extremely similar morphologies, forming an apparently well-defined lineage with very close phylogenetic relationships among species and without any easily identifiable epigeal relative. In these cases, an independent origin would require first a parallel evolution of their morphology and second the extinction of all epigeal relatives. It is well established that there were many independent colonisations of the subterranean environment in different groups of organisms, and many of them had also unquestionably converged to a similar troglomorphic phenotype (e.g. Racovitza 1907; Vandel 1964). As an extension of these two generally accepted facts, it was assumed that the independent evolution of troglomorphy occurred not only in distantly related lineages but in virtually all subterranean species. In addition to this, the increasing knowledge on the history of the effect of the glaciations in Europe and North America provided a convenient driving force to explain the extinction of the epigeal relatives: with the onset of a glaciation, all species that took no refuge in caves would have been extirpated from the surface (Peyerimhoff 1906; Jeannel 1943). The joint extinction of all surface ancestors would account for the lack of easily identifiable close relatives of many troglomorphic species, and

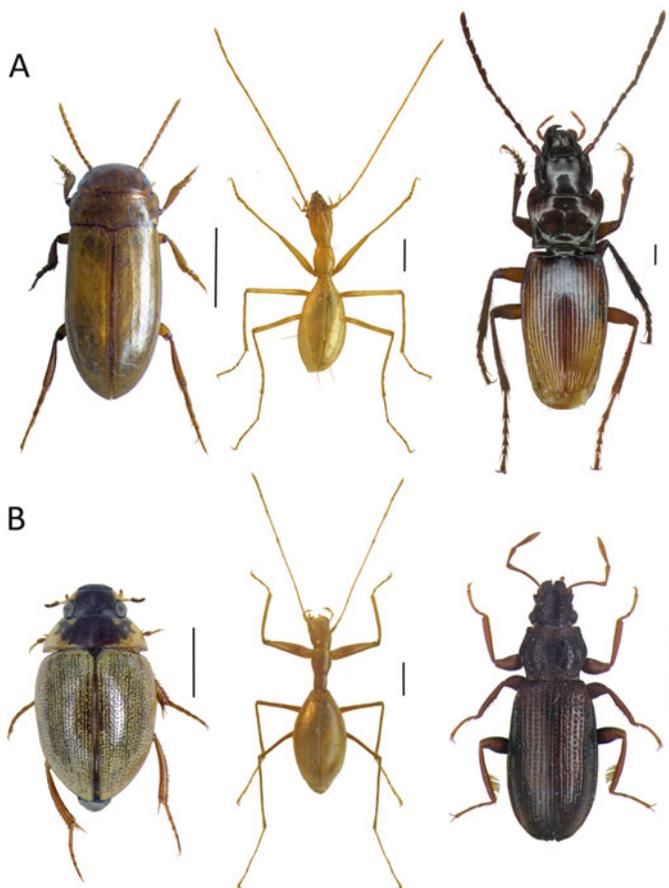


Fig. 10.1 Convergence in body shape in species typical of the deep subterranean environment. **(a)** Species of suborder Adephaga, from left to right *Graptodytes eremitus* (Dytiscidae) from the High Atlas of Morocco, *Aphaenops pluto* (Carabidae, Trechinae) from the French Pyrenees and *Oscadytes rovira* (Carabidae, Pterostichinae) from the Spanish Pyrenees; **(b)** species of suborder Polyphaga, from left to right *Laccobius fresnedai* (Hydrophilidae) from the Western Mediterranean, *Anthroherpon charon* (Leiodidae, Cholevinae, Leptodirini) from Bosnia-Herzegovina and *Hydraena exasperata* (Hydraenidae) from the Iberian Peninsula. Note the extreme similarity between the two middle species despite belonging to different suborders; this similarity cannot be solely due to the fact that they are both subterranean, as the three represented species of Adephaga share the subterranean habits (*G. eremitus* is a stygobiont species, and *O. rovira* is found in MSS, Ribera & Faille 2010; Fresneda et al. 1997). Scale bars, 1 mm; Photos by © Javier Fresneda

given that each colonisation was independent, there was no need to explain how subterranean species diversify or disperse.

Under this view, troglomorphic species would have originated from ancestors living in the soil or forest litter, a dark, cold and humid environment in some aspects similar to that of a cave (see also Chap. 3). These ancestors may already have been

adapted for the subterranean life (in fact, under some criteria the deep layers of litter or the upper layers of soil may be considered a shallow subterranean environment, see below), as they are frequently wingless, partially unpigmented and sometimes even blind. Once underground, species would go through the fixed pathways of morphological modification due to the effect of the environment and phyletic evolution. The different morphologies we observe would thus be due to the different stages in which the species are in this path from epigeal to extreme troglomorphy, which in turn depends on the time elapsed since the colonisation (a *morphological clock*; e.g. Poulson 1963; Wilkens 1987). Extremely modified species would be the most ancient colonisers, including the only survivors of lineages of which all epigeal relatives are long extinct, the “antequaternaires” (i.e. before the Quaternary) of Racovitza (1907) or the “living fossils” of Darwin (1859, p. 107) or Jeannel (1943). On the contrary, those with still incomplete modifications (e.g. partly pigmented or microphthalmic) would be the most recent newcomers.

On a more theoretical ground, to fundament this view of the origin of the subterranean species, R. Jeannel argued that regressive troglomorphic characters are not adaptive and appear as a result of the lack of use of the affected organs in the caves. This view can be traced at least to Lamarck (1809, p. 242), mentioning the *Proteus anguinus*, together with the mole and other vertebrates of subterranean habits, as examples of the reduction or loss of eyes through disuse. In Darwin (1859, p.138), there is also mention to the loss by disuse, “By the time that an animal had reached, after numberless generations, the deepest recesses, disuse will on this view have more or less perfectly obliterated its eyes”. However, Darwin and others also argued that some characters commonly present in troglomorphic species were the result of natural selection (e.g. elongation of appendages, increased sensitivity of some sensorial organs). Thus, the second part of the sentence quoted above reads: “and natural selection will often have effected other changes, such as an increase in the length of the antenna or palpi, as a compensation for blindness”.

Contrary to Darwin’s view, the role of natural selection was minimised, or plainly rejected, by early biospeleologists (e.g. Packard 1888), but most prominently by R. Jeannel and most of the biospeleologists of the early twentieth century. Instead, they argued that there was a genetic tendency of lineages to follow fixed evolutionary paths, going through different phases (origin, expansion, diversification and senescence, Jeannel 1950; see also Vandel 1964) until the last stage, which is characterised by a lack of variation causing their ultimate extinction. The number of these orthogenetic paths is also limited, leading to further convergence onto certain morphological “syndromes”.

Jeannel developed multiple hypotheses on the geographical and temporal origin of different subterranean lineages, based on their morphology, their current distribution and the geological knowledge of the time (he was one of the first biogeographers to consider the then highly unorthodox theories of Wegener on continental drift; e.g. Jeannel 1942, 1959). There were no fossils or other evidence to support many of his biogeographic hypotheses, but still many of them have proved to be surprisingly accurate and have been corroborated by recent studies using molecular data and an improved knowledge of the geological history of the areas involved (e.g. Faille et al. 2013; Andújar et al. 2016).

To understand the evolutionary hypotheses developed by R. Jeannel, E. Racovitza and other early biospeleologists, it is necessary to interpret them in the context in which they were first formulated. In the early decades of the twentieth century, there was a general acceptance of the evolution of the species, but the mechanisms driving this evolution were still highly controversial. Darwinian natural selection was out of favour, even among some of the founders of modern genetics and evolutionary theory, such as T.H. Morgan, which were—at least in their earlier works—strong advocates of the mutationist theories of De Vries and followers, dismissing the role of adaptation and natural selection in evolution (Bowler 1983). The observation that eyeless and wingless flies could suddenly appear in a “glass milk bottle” (the type used for the laboratory cultures of *Drosophila*) in one generation led Morgan to deny the role of natural selection in the origination of new phenotypes (and specially of characters considered to be regressive), which needed not to have any apparent adaptive value to become established in a population (Morgan 1919). Even more relevant for shaping the views of E. Racovitza and R. Jeannel was the lack of experimental evidence against the inheritance of acquired characters, which was assumed by default to be one of the prevalent mechanisms of inheritance even by Darwin himself (Bowler 1983).

It was also known that in some species, subterranean populations showing typical troglomorphic characters could at least partially reverse to an epigeal phenotype, and in particular to recover pigmentation, when exposed to light for long periods (e.g. in *Proteus*, Dubois 1892). Individuals of epigeal species in a subterranean environment could also develop troglomorphic characters, as was known for some cave fishes (e.g. Packard 1888; see Mazza et al. 2014 for a recent example). The assumption was then that these modifications could become fixed and be transmitted to the descendants. Although not in this context, it is noteworthy that the evolutionary role of phenotypic plasticity as originator of morphological diversity has been recently vindicated (e.g. Gómez-Mestre and Jovani 2013).

The definitive ostracism of the neo-Lamarckism did not arrive until the discredit of Paul Kammerer’s experiments with midwife toads (which are still subject of controversy, e.g. Vargas 2009). But even then—and until the end of his life (see, e.g. Jeannel 1950)—R. Jeannel argued that troglomorphic characters were the direct result of a neo-Lamarckian inheritance. He acknowledged the lack of experimental evidence but considered that laboratory experiments were maintained for a time too short to observe any appreciable effect, which should only be visible at much larger temporal scales (Jeannel 1950) (something that, incidentally, sounds as a rather reasonable objection). The problem was that the lack of experimental evidence was accompanied by a lack of any plausible molecular mechanism, which led to the complete rejection of neo-Lamarckism in the standard view established after the evolutionary synthesis of the neo-Darwinism.

In the second half of the twentieth century, the evolutionary views of some European biospeleologists became increasingly marginalised, but perhaps due to the enormous importance of the taxonomic and biogeographic publications of R. Jeannel, his unorthodox evolutionary views did not affect the recognition or the relevance of his work. In any case, and despite the influence of the early

biospeleologists, most studies on the diversity and ecology of cave faunas done in the last decades are in perfect agreement with contemporary evolutionary ideas, especially in North America (among others the works of T. C. Barr, K. Christiansen, D. C. Culver or T. L. Poulson; see References below and Culver and Pipan 2015 for a review). The discovery of troglomorphic species in tropical caves (Howarth 1972) introduced a full new field of research and helped to shape the different views on how cave colonisation could have proceeded. As seen above, the prevalent view at the time involved strong climatic fluctuations as a driver of colonisation, something that clearly did not apply to tropical faunas. However, the two proposed modes of colonisation (Howarth 1972; Peck and Finston 1993; Hampe and Jump 2011) can be seen as extremes of a continuum in which the different factors (gene flow between epigeal ancestors and subterranean species, ecological differences between the ancestral and subterranean habitat) merge and combine in each particular circumstance. But these differences refer only to the process of colonisation, not to the subsequent evolution of subterranean lineages, for which there was hardly any solid evidence available.

In the last years, there have been huge advances in the understanding of the origin of troglomorphic characters, thanks mostly to the research conducted on some species with epigeal and subterranean populations (e.g. Culver et al. 1995), and in particular the cave fish *Astyanax mexicanus*. This is a species with multiple populations, some of them living in caves and with typical troglomorphic modifications: fishes living in caves are unpigmented and blind, with different sensorial capabilities and with some other ecological and physiological modifications (see, e.g. Jeffery 2008, 2009 or Protas and Jeffery 2012 for reviews). There are different cave populations with an independent origin showing these modifications, which has allowed to investigate convergence in some characters (Protas et al. 2006; Bilandzija et al. 2012; Kowalko et al. 2013).

The focus on species with epigeal and cave populations allows the direct comparison of modified and unmodified phenotypes and the identification of the precise genetic and physiological origin of troglomorphic characters. It is, however, necessarily limited to fast-evolving characters (those first evolving after the colonisation) and is silent on the long-term evolution and diversification of the subterranean species and lineages.

10.2 The Crucial Dilemma: No Dispersal but Multiple Origins, or Single Origin but Geographic Dispersion and Diversification

Despite the growing understanding of the origin of the morphological characters typical of the subterranean fauna (Culver and Pipan 2015), until recently there was still little evidence on the long-term evolution of subterranean species. The dilemma framed by the early biospeleologists was still unsolved: either subterranean species

cannot disperse, they have independent origins, and their similarity is due to generalised convergent evolution or there is a single origin of the subterranean adaptations at least in some cases, with the implication that they can disperse over non-subterranean habitats and diversify to generate species-rich and widely distributed lineages with only subterranean species. To distinguish between the two possibilities, it would be necessary to know the detailed phylogeny of the lineages in which subterranean species occur and to have a time estimate of their evolution. In most cases this was not possible. There is little—if any—fossil data that could be of any use, and the extensive occurrence of phenotypic convergence makes difficult the estimation of phylogenetic relationships based on morphological data only (Wiens et al. 2003; Fresneda et al. 2007).

The increasingly widespread use of molecular data in the last years allowed addressing both issues for a number of subterranean lineages. Molecular data provide a set of characters to reconstruct robust evolutionary relationships between species (phylogenies), which are in principle neutral and thus not affected by the strong convergence that preclude the use of morphological characters to reconstruct relationships between subterranean species. The use of molecular clock techniques allows establishing a broad temporal framework for the reconstructed phylogenies, even with the absence of fossil data and despite unavoidable uncertainties (Kumar 2005; Ho and Duchêne 2014).

Molecular data have been used to find the phylogenetic position of isolated and highly modified cave species, without apparent relatives in the geographic area in which they are found. These species are sometimes referred to as “living fossils” (see Sect. 10.1), although this term may be more appropriate to species (or genera) that have been first described as fossils to be subsequently discovered alive and well, such as the coelacanth (genus *Latimeria*) or the Mallorca midwife toad (*Alytes muletensis*; Alcover and Mayol 1980) (also referred as “Lazarus taxa”). In the case of subterranean species, there is usually no fossil record, and the term “living fossils” refers to the fact that they are presumably relicts, the only survivors of an ancient fauna that became extinct on the surface and of which only some isolated examples remain, thanks to the protection offered by the caves and the surrounding subterranean environment (Assmann et al. 2010). Some highly modified subterranean species do seem to be highly isolated phylogenetically, with the only known distant relatives living in remote geographical areas. This is the case, for example, of *Dalyat mirabilis*, the only Palaearctic Promecognathinae (Coleoptera Carabidae), which otherwise have some species living in the forests of western North America and South Africa (although with a Cretaceous fossil in Central Africa; Ribera et al. 2005), or *Ildobates neboti*, a Zuphiini (also a Carabidae), which closest relatives are unknown (Ribera et al. 2006). Other cases of likely isolated highly troglomorphic species (or a small group of closely related taxa) within Trechini are the genera *Paraphaenops* in the Iberian System, *Sardaphaenops* in Sardinia or *Jeannelius* in the Caucasus (Faille et al. 2013; see Assmann et al. 2010 for examples in other groups). However, it should be stressed that the actual age and origin of the colonisation of the subterranean environment of these species are unknown, so it may well be that it happened only recently and that the role of the subterranean

environment for the survival (or extinction) of their respective lineages was absolutely irrelevant.

Of more interest for the understanding of the evolution of the subterranean fauna are the studies of the phylogenetic relationships and the diversification of large clades with abundant subterranean species. There have been a number of works on stygobiontic groups, most of them Crustaceans (e.g. Zakšek et al. 2007; Fišer et al. 2008; Bauzà-Ribot et al. 2012; Botello et al. 2012; Meleg et al. 2013; Esmacilli-Rineh et al. 2015) but also diving beetles (Dytiscidae, e.g. Leijs et al. 2012). Terrestrial groups include mostly spiders (e.g. Arnedo et al. 2007; Mammola et al. 2015) and beetles, which, as already noted, will be treated in more detail and are the main subject of this chapter.

10.3 Once or Many? Single Versus Multiple Origin of the Subterranean Colonisation

When trying to answer this question, there is a consideration to be made which, despite its formal appearance, is of crucial importance. Imagine we have a group of species which are presumably each other closest relatives and of which some are subterranean and some are epigeal, and we want to test if there are single or multiple origins of the subterranean way of life. When our initial hypothesis—i.e. the null hypothesis that has to be falsified—is that there is a single origin of the subterranean species, to falsify, it we only need to demonstrate that one of the epigeal species is the closest relative of any of the subterranean ones, as this would require at least two origins and possibly more. On the contrary, when the null hypothesis is that all subterranean species have colonised the subterranean environment independently but that their epigeal closest relatives are all extinct, there is no simple observation that allows us to reject it and accept the alternative hypothesis of a single origin. Even when all subterranean species are found to be monophyletic and phylogenetically nested within the epigeal ones, there is always the possibility that the epigeal relatives that should have appeared interspersed among them had become extinct. Only additional evidence could make this hypothesis increasingly unlikely, but not falsify it unquestionably.

In this sense, it may be considered that, when hold to the extreme, the hypothesis of multiple origins with the corresponding extinction of epigeal relatives is unfalsifiable. Fortunately, in practice this rarely happens, as in some cases, the additional evidence is strong enough to make the multiple origins extremely unlikely.

Box 10.1

We assume that the morphological modifications leading to troglomorphy are in general irreversible, in particular the loss of eyes, wings or pigmentation. Note that we refer to lineages with subterranean species with fixed troglomorphic characters, without polymorphisms or known phenotypic plasticity. It is known that in some species it is possible to recover pigmentation (e.g. the already noted example of *Proteus*) and, at least under laboratory conditions, it is even possible to partially recover some functionality of the eyes (Porter and Crandall 2003; Niven 2008). But, to our knowledge, there is no evidence of the reversal of troglomorphic characters implying the recovery of completely lost complex structures. There have been some claims of recovery of wings in otherwise wingless lineages (Whiting et al. 2003), but these are controversial (e.g. Stone and French 2003; Collin and Miglietta 2008; Goldberg and Igic 2008) and in any case do not refer to subterranean species, in which there is always an association of multiple modified characters. We also refer to morphological modifications, not to changes in the habitat or the way of life: it is known that within subterranean lineages, some species may become secondarily epigeal, but to our knowledge, this never implies the reversal of their troglomorphic characters.

Starting with the simplest case and assuming the irreversibility of the troglomorphic characters (see Box 10.1), there is now strong evidence of the multiple independent origin of the subterranean way of life within different lineages of Coleoptera and especially in Trechini. This was the case of the genus *Duvalius* (Faille et al. 2013), the species of the *Trechus fulvus* group (Faille et al. 2014) or the Cantabrian species of *Trechus* (Faille et al. 2012; Fresneda et al. 2015). In all these examples, subterranean species with different degrees of morphological modification (or in some cases small clades of closely related species) are sisters to epigeal species, demonstrating their independent colonisation of the subterranean environment. *Trechus fulvus* is a good model to study the process of colonisation of the subterranean environment, as it is widely distributed in Western Europe and North Africa, with many isolated populations under different ecological conditions, although always requiring high humidity. The existence of multiple replicates of isolated populations leads to morphological variability, with some populations having only apterous individuals with more or less reduced eyes, whereas other populations include only macropterous individuals (Jeannel 1920). The evolution of the Cantabrian lineage of *Trechus* within the Iberian Peninsula seems to follow a similar pattern, with *T. escalerae*, *T. valenzuelai* and a clade including some species of *Apoduvalius* having independently developed some troglomorphic characters (Faille et al. 2013; Fresneda et al. 2015).

Contrary to the examples above, there is evidence for the single origin of the colonisation of the subterranean environment—and of the origin of the associated modifications—in a number of diverse lineages both within Trechini and

Leptodirini. Within Trechini, the largest and most unexpected (so far) clade of subterranean species with troglomorphic characters is the Pyrenean lineage (Faille et al. 2010), with all 87 species of the 2 genera *Aphaenops* (including subgenus *Hydraphaenops*) and *Geotrechus*, the latter previously considered to belong to a distinct lineage (Jeannel 1919). In addition to a strong support of the monophyly of the Pyrenean subterranean lineage including most of the species of those genera in all phylogenetic analyses (Faille et al. 2010, 2011, 2013), there is also considerable additional evidence confirming the single origin of the colonisation of the subterranean environment and the relatively simple evolution of the troglomorphic characters. Thus, the topologies observed might suggest a gradation in the specialisation of the species inside the karstic massif, associated with morphological and ecological changes and modification of the life cycle (Faille and Pluot-Sigwalt 2015, Faille et al. manuscript in preparation; Fig. 10.2). There are other instances in various geographic areas in which an unexpected clade of subterranean species of Trechini turned out to be monophyletic, such as the Dinaro-Alpine hypogean clade gathering all the highly troglomorphic cave Trechine of the Eastern Alps and Dinarids (Faille et al. 2013).

Within Leptodirini there is also strong evidence to support the single origin of the subterranean colonisation, and the troglomorphic characters, of many of the geographically localised radiations (Ribera et al. 2010; Fresneda et al. 2011; Cieslak et al. 2014). In addition to the strong support of the monophyly of the exclusively subterranean lineages in the molecular phylogenies, there is additional support from morphological and physiological characters. Thus, in the Pyrenean lineage (the *Speonomus* clade, Ribera et al. 2010), including 141 described species, the evolution of the life cycle from the ancestral 3 instars to a reduced larval development with only 2 instars and finally a larvae that does not moult, is not active and does not feed follows a simple path with a single transition from 3 to 2 instars at the end of the Oligocene, and from 2 to 1 in the early Miocene (Fig. 10.2). To explain the monophyly of all known species with one instar cycle from the Pyrenees, we could always argue that from an ancestor with three instars, there were multiple independent origins of the modifications of the life cycle to produce species with one instar, and what we observe is just the result of the extinction of all the species living in the surface that would be interspersed within the clade. However, in addition to the extinction of all epigeal species, this would require that, depending on their phylogenetic placement, subterranean species independently (but in coordination) either did not modify their cycle, evolved to a two-instar cycle or to a one-instar cycle. This would require the widespread occurrence of orthogenesis in its most extreme version. The evolution of the life cycle followed similar simple patterns in other geographic areas, although data are scarcer than for the Pyrenees and the patterns were less firmly established (Cieslak et al. 2014).

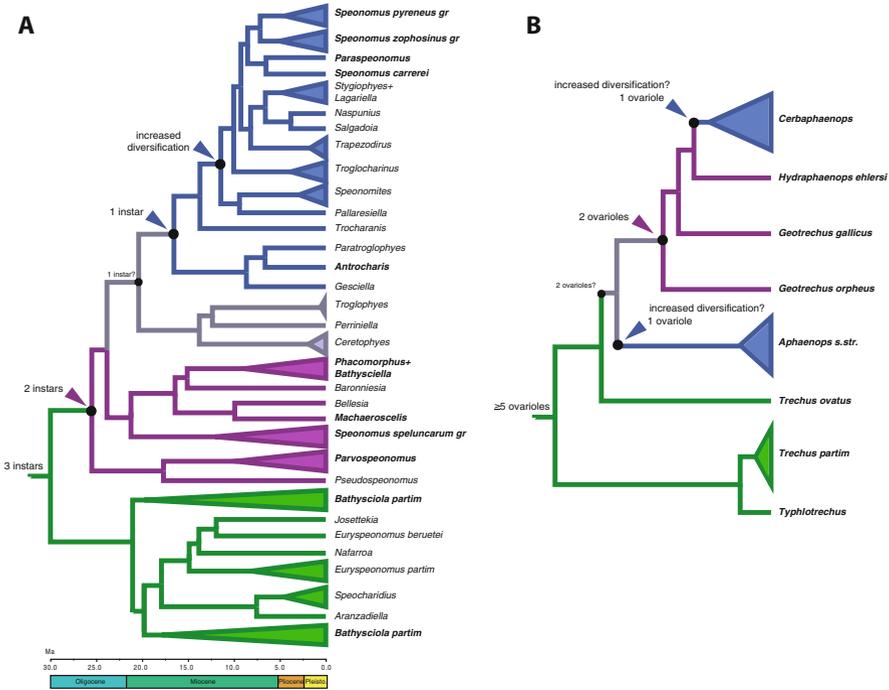


Fig. 10.2 Schematic phylogeny of the Pyrenean clades of (a) Leptodirini and (b) Trechini, showing the reconstructed transitions from the ancestral life cycle (3 instar larvae in Leptodirini, ≥ 5 ovarioles in Trechini) to an intermediate cycle (2 instars in Leptodirini, 2 ovarioles in Trechini) and a highly modified cycle (1 instar Leptodirini, 1 ovariole in Trechini), in which the larvae are not active and do not feed. In the Leptodirini this transition was estimated to have occurred only once, associated with an increased diversification rate, but in Trechini it likely occurred twice, probably also associated with shifts in diversification rates. In bold, clades for which actual data of the life cycle is known. Modified from Cieslak et al. (2014) and Faille and Pluot-Sigwalt (2015)

10.4 Relation Between Age and Troglomorphy

The single origin of the colonisation of the subterranean environment in large, diverse lineages questions the relationship between the age of a species and its degree of troglomorphy, i.e. the extent of the morphological and physiological modifications from the ancestral phenotype. When the assumption is that virtually all subterranean species had an independent origin from epigeal ancestors, it follows naturally that the more modified the species, the more ancient the colonisation of the subterranean environment (e.g. Jeannel 1950; Poulson 1963). However, in a monophyletic lineage in which all species share a single ancestor with subterranean habits (i.e. a single colonisation of the subterranean environment), the relationship between the degree of troglomorphy and time underground is meaningless, as for all species of the lineage this time would be the same. The age of the extant species

may differ, but this is irrelevant, as the important factor is how much a species differs from its direct ancestor, that is, the rates of phenotypic evolution. These rates may depend on the environment or way of life of a species and their ancestors, and thus the relationship of interest is not between phenotype and age but between phenotype and habitat or ecology. Old species may be less troglomorphic than younger ones if they have slower rates of phenotypic evolution but also if the ancestor of the younger one was already a troglomorphic species or if they have a different ecology.

There may still be continuous trends in the evolution of troglomorphy, but not in individual species (or closely related species) but in whole lineages. Thus, as seen above (Fig. 10.2), among Pyrenean Leptodirini, there was a single evolutionary path from forest litter species with three-instar larvae to subterranean species with a two-instar cycle and finally species with a highly modified, one-instar cycle (Cieslak et al. 2014). Among Pyrenean subterranean Trechini, there were two independent developments of the same type of contracted life cycle, with two evolutionary paths starting with endogean species of the genus *Geotrechus* (Faille and Pluot-Sigwalt 2015; Fig. 10.2). But extant species of the unmodified lineages can be as old, or older, than any of the highly troglomorphic ones. In fact, in these cases it can be argued that the older phenotypes are the less troglomorphic ones, which have remained closer to the ancestral type, while the more derived—and thus more recent—phenotypes are those of the highly troglomorphic species. The importance of considering the evolution of a lineage and not only of the individual species is also clear in the case of recently diverged species which still are very similar to each other (and likely to their most recent common ancestor). Thus, the highly modified and isolated genus *Sardaphaenops* has two extant species (and one subspecies) of very recent origin, as are many of the species of the highly diverse and not very modified genus *Duvalius* (Casale 2004; Faille et al. 2013). In both cases, what is of interest is not the age and morphology of the extant species, which in both cases are of recent divergence and likely very similar to their direct ancestors, but those of the whole lineage.

10.5 Dispersal and Diversification

It is generally accepted that subterranean species can disperse to some degree through the network of fissures and cavities within a karst massif (e.g. Barr and Holsinger 1985), and there have been attempts to quantify the movement of subterranean species through mark-recapture methods (Cabidoche 1966; Delay 1975; Fejér and Moldovan 2013) or to estimate gene flow (e.g. Crouau-Roy 1989). The generally accepted single origin of groups of closely related species in close geographical proximity also implied the possibility of some dispersal with subsequent isolation. What is usually questioned is the possibility of range expansions at large geographical scales, implying displacements over areas without subterranean environment.

However, if we accept that lineages that occupy large geographic areas—such as the Pyrenees—are monophyletic and have colonised the subterranean environment only once, it necessarily follows that species with subterranean habits and some degree of troglomorphy were able to expand their ranges and colonised new isolated subterranean units by epigeal dispersal.

Some examples are species of *Anophthalmus* (Faille et al. 2013), different populations of *Aphaenops cerberus* (Faille et al. 2015) or, at a larger geographic scale, the genus *Troglocharinus*. This genus belongs to the Pyrenean lineage of Leptodirini with a highly modified 1-instar life cycle (Cieslak et al. 2014). It was estimated to have an origin in the late Miocene, derived from a lineage in which all species have troglomorphic characters and estimated to have colonised the subterranean environment in the late Oligocene (Cieslak et al. 2014). *Troglocharinus* has some 20 species, mostly distributed in the pre-Pyrenees, but there is a monophyletic group of species living in the coastal mountain ranges of Barcelona and Tarragona (Rizzo et al. 2013). The linear distance between the closest localities of the Pyrenean and coastal clades is not exceedingly large (some 60 km), but they are separated by Miocene sediments of the upper Ebro basin without any trace of subterranean environment (Fig. 10.3). The only possibility is thus that the ancestor of the coastal lineage expanded its range from the Pyrenees, necessarily crossing the upper reach of several rivers and some non-karstified areas. During the estimated time for this range expansion, the end of the Miocene-early Pliocene (Rizzo et al. 2013), temperatures were decreasing, but precipitation remained high—higher than today—and seasonality was low, as the Mediterranean climate, with its high seasonality, would not become established until ca. 2.3 Ma (Suc 1984). It seems likely that most of Catalonia was at that time covered by a dense forest, with a high humidity and no temperature extremes. Physiological experiments have demonstrated that coastal species of *Troglocharinus* can tolerate temperatures up to 20 °C for long periods of time, provided that humidity is maintained close to saturation (Rizzo et al. 2015). There are some records of the presence of specimens of *Troglocharinus*—and some other highly troglomorphic species—closer to the entrance of caves or directly outside (Rizzo et al. 2013), and the possibility of finding troglomorphic species typical of the deep subterranean environment close to the surface, under stones or in the MSS has been always recognised. For example, Packard (1888), quoting Bedel, states that “It appears that not all the species [of Leptodirini] live in caves, but occur in the open air under large stones, moss, vegetable detritus, or at the entrance to caves”. A particularly interesting case are species that at lower altitudes occur exclusively in caves but at high elevation can be found under stones or in shallow environments (among others, species of *Duvalius*, *Trechus* or some Leptodirini; Jeannel 1943; Hernando et al. 1999). More recently, Pipan and Culver (2012) and Culver and Pipan (2014, 2015) have comprehensively documented the regular presence of troglomorphic species in the shallow subterranean habitats (SSHs), questioning the relationship between troglomorphy and the deep subterranean environment (see Fig. 10.4 for some examples with terrestrial fauna).

Although still insufficient, the available data on the physiological limits of troglomorphic species point to humidity as a more stringent limiting factor than

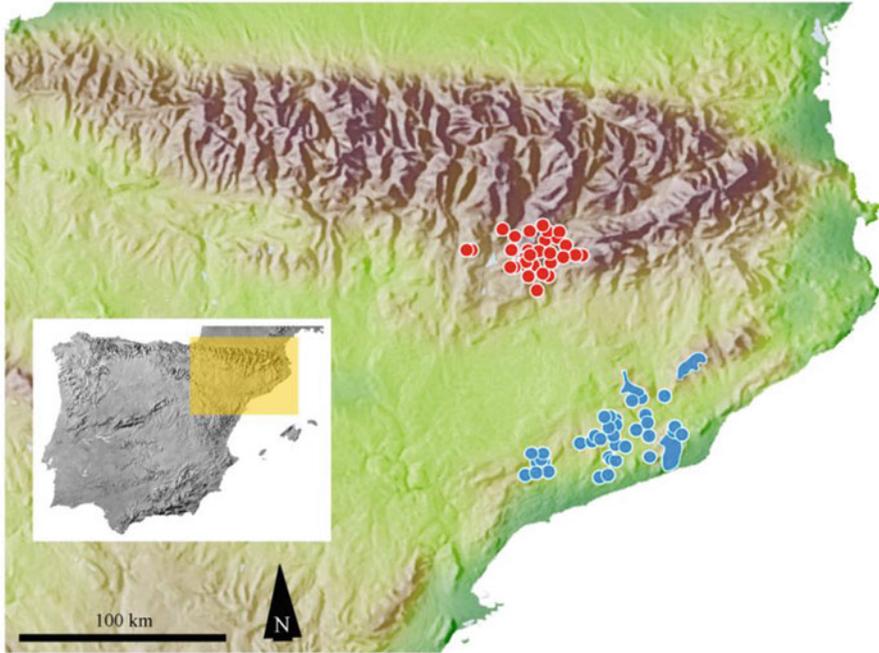


Fig. 10.3 Distribution of the species of the genus *Troglucharinus*. In red, species occupying the ancestral range of the lineage, in the Spanish Pyrenees. In blue, species in the Catalanian coastal ranges (see Rizzo et al. 2013 for details). The shortest distance between them, ca. 60 km, crosses Miocene sedimentary deposits of the upper Ebro valley

temperature (Rizzo et al. 2015; Sánchez-Fernández et al. 2016). The importance of humidity as a limiting factor for subterranean species has been stressed by many authors (e.g. Peyerimhoff 1906; Racovitza 1907; Jeannel 1943, see also Chap. 3). It is likely that during climatic periods with low seasonality, without extreme temperatures, species could move through microenvironments maintaining a high humidity, such as the deep layers of forest litter. In the northern temperate regions, this may have been the case during some periods of the late Miocene or the early-middle Pliocene, with temperatures similar or slightly above the present but higher precipitations (Jiménez-Moreno et al. 2010). The Pleistocene glaciations, and, in the Mediterranean area, the onset of the strong seasonal variation typical of the Mediterranean climate, ended these favourable climatic window, reducing the possibility of range movements on the surface and confining subterranean species to their current areas of distribution (Rizzo et al. 2013). Even if occasionally subterranean species can disperse across non-subterranean medium, in general their geographic ranges are extremely reduced, suggesting that most species have a very restricted mobility most of the time. One of the most robust macroevolutionary patterns is the strong negative correlation between range size and extinction probability (Ribera 2008), so for old lineages of subterranean species to persist, either they have somewhat escaped extinction (becoming highly



Fig. 10.4 Some circumstances in which troglomorphic species can be found close to the surface. (a) Doline in Sardín, Asturias, NW Spain. Some dolines are likely to act as “gates” to the deep subterranean environment, providing a direct connection to the surface; (b) below stones like the one in the image in the lowest part of the doline, there were troglomorphic species which have also been associated with caves: *Apoduvalius franzi* (Carabidae), *Cantabrodytes vivesi* and *Domene* sp. (Staphylinidae); (c) MSS in the Spanish Pyrenees (Barruera), only partially covered by soil. In a pitfall trap placed below the stone marked with the arrow, *Strygiophyes aldomai allomorphus* was found; (d) doline in Collau de la Plana del Turbón (Spanish Pyrenees), type locality of *Trapezodirus orobios orobios*. The last two are highly derived troglomorphic species (Cieslak et al. 2014), the first also found in caves (Fresneda and Salgado 2016); photos by © Ignacio Ribera (a and b) and © Javier Fresneda (c and d)

isolated due to the extinction of most of their close relatives, Assmann et al. 2010) or have to compensate a high extinction probability with an also high speciation rate. Without fossil data the only evidence to estimate evolutionary turnover are phylogenies of extant species, from which it is notoriously difficult to estimate speciation and extinction rates (Rabosky 2015). But what can be estimated is the net diversification rate, i.e. the result of speciation minus extinction, and it is also possible to identify significant changes in this diversification rate and associate them to particular nodes in the phylogeny (Rabosky and Huang 2016). There is still few data on diversification rates for subterranean lineages, but for some groups, there is good evidence of an increased diversification in highly troglomorphic species associated with changes in the life cycle. This is evidenced in Leptodirini (Cieslak et al. 2014) and suspected in Trechini (Faille and Pluot-Sigwalt 2015).

10.6 General Overview and Concluding Remarks

In the last years, different lines of research have converged in a renewed view of the biology and evolution of species living in subterranean environments and on the subterranean environment itself. The discovery of highly troglomorphic species in lava tubes was the first challenge to the established paradigm on the origin of subterranean species but, in the last years' comprehensive studies of what were considered marginal habitats, has further faded the limits of the subterranean environment. Thus, the SSHs of Culver and Pipan (2014) include a wide range of habitats for which the limits between them, and with more "traditional" subterranean environments, are impossible to draw precisely. Similarly, among more terrestrial environments, the limits of the MSS as originally understood (Juberthie et al. 1980) have been challenged by the recent discovery of highly troglomorphic species in areas and habitats in where their presence was not suspected (Giachino and Vailati 2010; Fig. 10.4). Although without highly troglomorphic species, other shallow environments have been recognised as subterranean due to their physical conditions and some peculiarities of the fauna (e.g. Ortuño et al. 2013). There is a basic dichotomy when defining and limiting the subterranean environment, which is whether using the characters of the species or the physical conditions of the habitat. There does not seem to be a one-to-one correspondence, and as seen above, there are many examples of subterranean environments without troglomorphic species and troglomorphic species in habitats that do not have the typical characteristics of the subterranean environment. These discordances are of particular interest to understand the evolution of the subterranean fauna (Culver and Pipan 2014).

Despite the progress made in recent years in the study of the subterranean species and their adaptations, as seen here there are still a number of questions that remain largely unanswered. Without claiming to be comprehensive, in what follows we list some of them, delineating some potential areas of research.

Why Troglomorphic Species Are More Frequent in Some Lineages? As already noted, the number of species with troglomorphic features is highly unevenly distributed through the terrestrial invertebrates, suggesting that some of them have a stronger propensity to colonise the subterranean environment. In some cases, there are obvious reasons for what species cannot successfully colonise the underground, such as for species strongly depending on green plants (e.g. leaf beetles) or with a predominantly aerial adult way of life (e.g. dragonflies, butterflies). There are some known cases of troglomorphic species in otherwise almost entirely epigeal groups, such as some Diptera (Andersen et al. 2016), Hymenoptera (Juberthie and Decu 1998) or even Plecoptera (López-Rodríguez and Tierno de Figueroa 2012), but these are anecdotic and of little quantitative importance.

It is commonly assumed that groups with multiple independent invasions of the subterranean environment (e.g. spiders, pseudoscorpions, springtails, some families of beetles) are somehow "preadapted" to it, mostly because species living in forest litter or the upper soil layers show some typical troglomorphic characters (e.g. reduced eyes, lack of pigment and wings), suggesting that these environments

may be stepping stones for the colonisation of the subterranean environment. The abundance in the subterranean environment of trophic resources adequate for some groups of forest litter species, such as fungi or decayed organic matter for Collembola or Leptodirini, or the appropriate prey for Trechini may also be a key factor facilitating their colonisation. However, there is little phylogenetic data to support this hypothesis, and there are many other possible routes for the origin of subterranean lineages. They may directly derive from a fully epigeic ancestor, from which forest litter and subterranean lineages may have derived independently, or from endogean species (see Fig. 10.5 for some possibilities). It could also be that the key characters facilitating a subterranean life are less apparent and may have originally evolved for an unrelated reason, such as changes in sensory organs, type of development or other physiological characters.

What Are the Factors Driving the Colonisation of the Subterranean Environment?

Or in other words, what pushed species to the underground? There are many hypotheses broadly related to abiotic (e.g. climatic) or biotic factors (mostly ecological, such as resource availability or to avoid competition), as emphasised by the climatic relict or adaptive shift hypotheses, respectively (Howarth 1972; Peck and Finston 1993). It can, however, be questioned that the colonisation of the subterranean environment needs a particular explanation more than, say, the colonisation of the aquatic environment, the forest canopy or a particular species of plant. It may seem rather unattractive and inhospitable, but, like any other habitat, it has advantages and disadvantages that have to be overcome by the species living in it. Why did species colonise subterranean habitats in the first place is probably a question with little biological sense, unless it is reframed to ask why some colonisations are more successful than others, which is the same as to ask why some groups have more troglomorphic species than others.

What Are the Selective Advantages, if Any, of the Troglomorphic Phenotype? There is still little hard evidence as to what could be the advantage, if there is any, of some common troglomorphic characters that have repeatedly evolved in different groups. Prominently among them are some body shapes found among Coleoptera (the morphotypes of R. Jeannel; see above and Fig. 10.1), in which potential adaptive value is unknown. The same applies to some modification of the life cycle, such as the reduction of larval instars and the loss of activity that has evolved multiple times in different groups (Deleurance 1963; Cieslak et al. 2014; Faille and Pluot-Sigwalt 2015). It has been hypothesised that by concentrating all the foraging and the resource acquisition in the more mobile stage (the adult), species can survive in environments with a lower density of resources, as a less mobile larva would not be able to cover all the surface needed to gather the resources needed for the development into an adult. This would allow these species to colonise the deeper part of the caves, or caves in which resources are scarcer, for example, at higher altitudes (Cieslak et al. 2014).

There are, however, many characters showing some degree of convergence for which virtually nothing is known, not even if they are what are usually considered

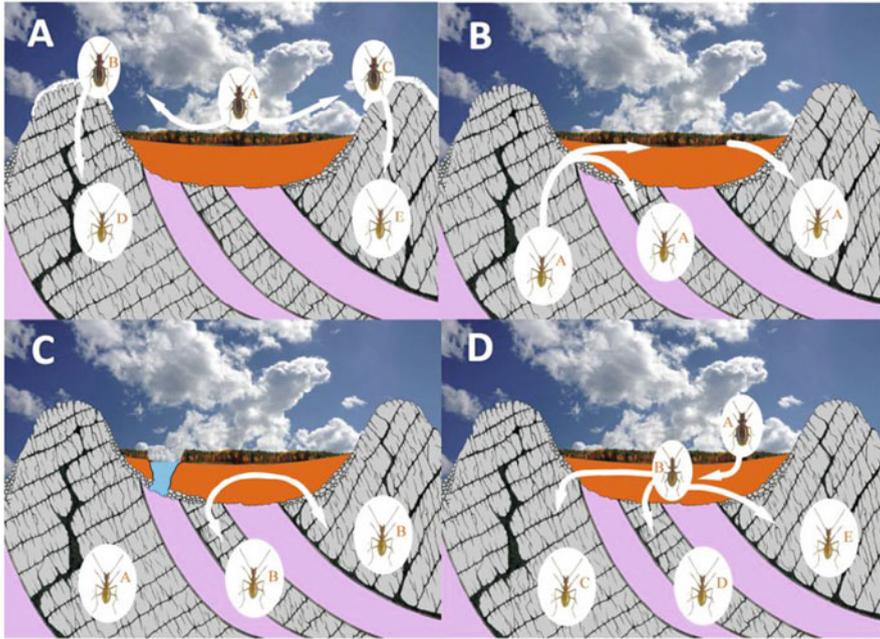


Fig. 10.5 Schematic representation of some among many possible modes of colonisation, range expansion and diversification within the deep subterranean environment (DSE). (a) In alpine conditions, epigeal species may be forced to seek refuge in superficial subterranean environments (MSS), colonising from there the DSE; (b) a subterranean species living in the DSE may reach other geologically isolated DSE units through MSS or forest litter, leading to range expansion and diversification; (c) subterranean species in different geological units may maintain gene flow through soil or forest litter but be isolated from other subterranean species through other geological features (e.g. rivers); (d) an endogean species, derived from an epigeal one, may independently colonise different geological units leading to subterranean species in each of them

“regressive” or “progressive” characters, as, for example, modification in the sensory organs, including the setae (Fig. 10.6).

How Linked Are Troglomorphic Species to the Subterranean Environment? The erosion of the limits between the epigeal and subterranean environments questions the factors that determine the distribution of the troglomorphic species. Physiological experiments can be of great help, although it is difficult to discriminate between different factors or recreate them in controlled conditions. As already noted, available data and recurrent observations suggest that humidity is the main limiting factor. Temperature, at least during periods up to several days, seems to be of lesser importance as far as it is maintained below ca. 20 °C, at least for some Leptodirini (Rizzo et al. 2015). There is, however, no data on the long-term resistance, and the possibility of acclimation of troglomorphic species to environmental conditions. Some of the work done in the Laboratoire Souterrain de Moulis (France) suggests that there may be long-term (at the scale of generations) acclimation to high

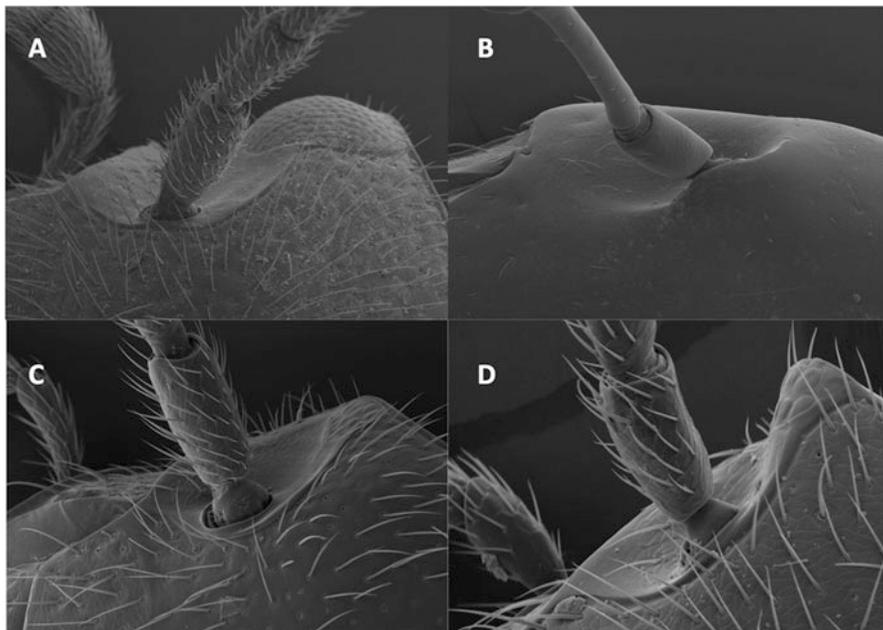


Fig. 10.6 Convergence in the eye and chaetotaxy reduction in some Cholevinae. (a) *Speonemadus bolivari* (Anemadini); (b) *Leptodirus hochenwartii* (Leptodirini); (c) *Anemadus smetanai* (Anemadini); (d) *Notidocharis calabrezi* (Leptodirini). C and D have independently developed reduced eyes and similar modifications of the setae of the head and antennae. Photos by © Ignacio Ribera

temperatures, at least in some developmental parameters (Delay 1978). A better knowledge of the physiological and ecological limits of troglomorphic species will contribute to understand the range expansions that necessarily took place in some of the lineages.

Why Some Colonisations of the Subterranean Environment Gave Origin to Diverse Radiations and Others to Single Species? Some lineages of subterranean species are very diverse, while others remain species poor. To inquiry the reasons for these inequalities would require first to assess the general macroevolutionary pattern of lineage diversification over a large group. Due to random factors in the processes of speciation and extinction, some lineages are expected to be more diverse than others, so that no evolutionary explanation may be required for differences in species numbers unless there are significant deviations with respect to the assumed null model of diversification. There is also the difficulty of precisely determining the moment in which a possible change of diversification rates occurred (i.e. in which node in the phylogeny the diversification rates changed, Vogler and Ribera 2003). Some of the few data available suggest that shifts may be associated not with the original colonisation of the subterranean environment but with changes occurred

when species had already evolved all typical troglomorphic characters (Cieslak et al. 2014; Faille and Pluot-Sigwalt 2015).

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