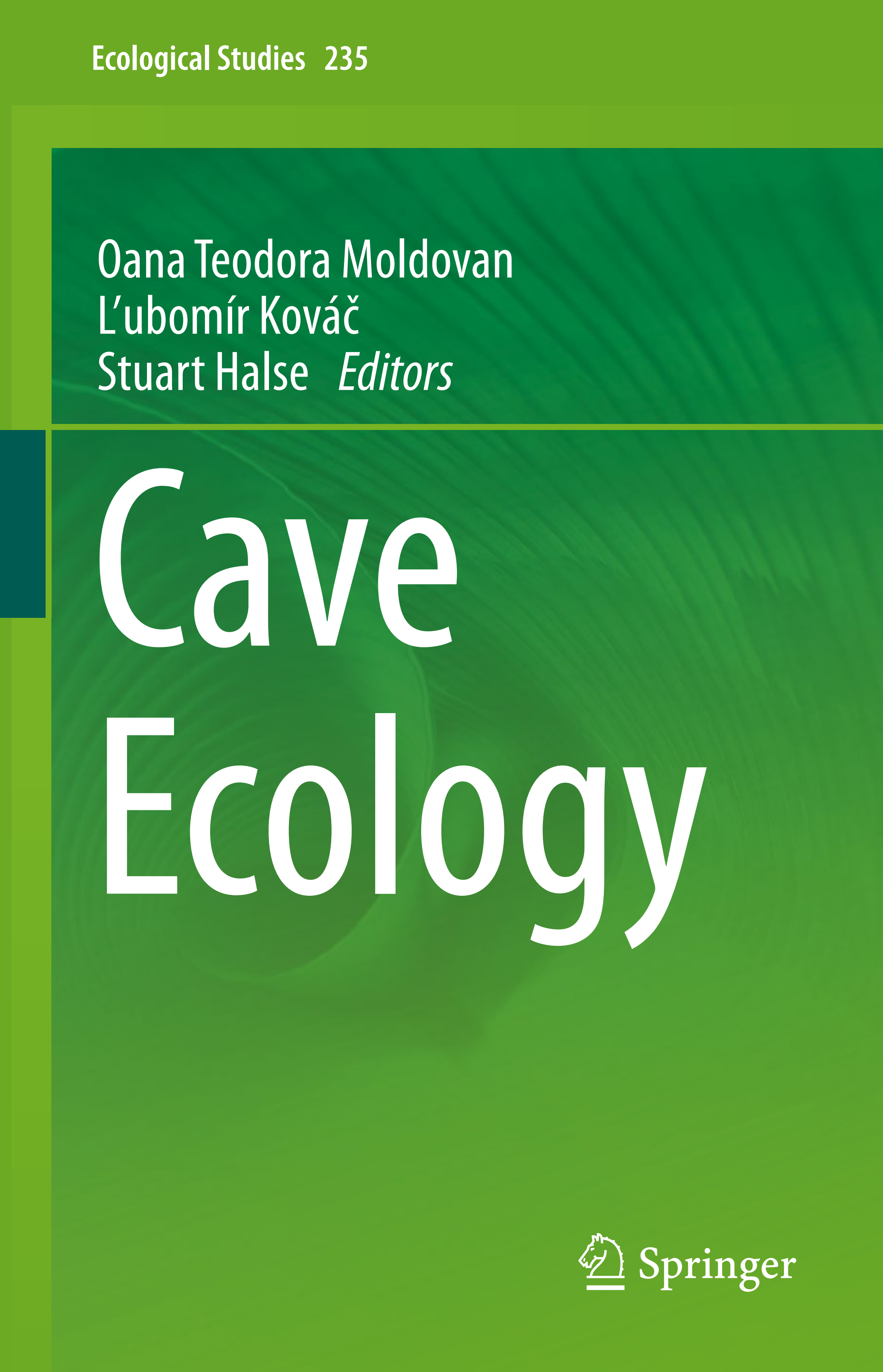

Ecological Studies 235

Oana Teodora Moldovan
L'ubomír Kováč
Stuart Halse *Editors*

Cave Ecology

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Volume 235

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Contents

Part III About Communities, Populations and Food

Part IV Particular Cave Ecosystems

Part V Conservation and Protection of Cave Habitats and Cave Fauna

Part VI Cave Ecology for the Twenty-First Century

Chapter 1
Preamble

Oana Teodora Moldovan, Ľubomír Kováč, and Stuart Halse

> *Wherefore each of you, when his turn comes, must go down to the general underground abode, and get the habit of seeing in the dark. When you have acquired the habit, you will see ten thousand times better than the inhabitants of the cave, and you will know what the several images are, and what they represent, because you have seen the beautiful and just and good in their truth.*
> *Plato, Republica*

1.1 On Caves, Cave Ecology, and Cave Inhabitants

Caves are seen as natural openings large enough to allow entrance of humans. Caves are formed in many types of rock and by many processes, although the largest and most common caves are those formed in limestone, dolomite, and solidified lava. Nevertheless, when considering caves as living spaces, the size of the cave is often less important than the size of the cave inhabitants. Most cave organisms are a few millimeters or even less in size, and they can colonize any void of larger size than this, especially where there is no light and environmental conditions are relatively constant throughout the year. “The subterranean environment would be as insignificant by the small space it occupies on Earth, as by the low number of beings

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inhabiting here; it would only be a kind of nature's oddity. Now, there is no falser idea!" (translated from French) was a statement by Racovitza in 1907, and he added further that subterranean fauna consists of a heterogeneous mixture of very different forms in origin, hereditary aptitudes, degree of organization, and time of immigration into caves.

We use the term 'cave' for any space or void that fulfills the environmental requirements of living organisms for their survival. The anthropocentric view of a cave size has been reconsidered in the last three decades, and the syntagm *cave ecology* was replaced by *subterranean ecology*, not always in a justified way.

Ecology is the science that studies the interactions between organisms and their relationship with the environment, and *cave ecology* studies the connections of cave organisms with their cave environment, at multiple levels. The abiotic environment is represented by the cave habitats, and the organisms are those typical troglobionts or stygobionts, which live permanently in caves. The focus of the book is less on troglophiles/stygophiles and guanobionts although all these categories are mentioned when appropriate.

1.2 Cave Ecology as the Center of Biospeleological Researches

It is difficult to think about the beginning of cave ecology without referring to the "Essai sur les problèmes biospéologiques" (Essay on biospeleological problems), which was published by Racovitza in 1907 and ignored for a long time simply because it was written in French. It is the manifesto of biospeleology, marking the birth of this science, where the author defines the terms to be used, the habitats, and the adaptation of cave inhabitants and outlines the ecological research to be done.

As knowledge of the fauna and habitats increased, details of the life histories of various species were published by different authors (Delamare-Deboutteville 1960; Vandel 1964; Ginet and Decou 1977), and almost one century later, the encyclopedias (Juberthie and Decu 1994, 1998, 2001; Gunn 2004; Culver and White 2005; White and Culver 2012) and books (Culver 1982; Camacho 1992; Wilkens et al. 2000; Romero 2009; Culver and Pipan 2009, 2014) brought together information on the subterranean world, including its ecology.

1.3 What Can Bring a Multi-author Book on Cave Ecology?

We want this volume to regenerate interest in the study of caves and their living organisms by highlighting that caves are spaces of any size as long as they support life. "Traditional" caves are still the places to which we have access, where organisms can be studied in situ and where communities can best be observed and sampled. In caves we find the specimens used for laboratory experiments and many other different studies in astrobiology, human health, paleoclimatology, geomicrobiology, etc. The smaller caves, often referred to as micro- and mesocaverns, may occur as a network of spaces around traditional caves or below surface streams. The vast majority of these spaces, however, occur in groundwater or weathering parts of the vadose zone elsewhere in the landscape, and the study of these systems is in its infancy.

Cave ecology, as with all other areas of biological study, has numerous hypotheses and theories regarding cave inhabitants and the processes that sustain them. A book with multi-authored opinions provides a more comprehensive overview of the subject. Although it is the duty of the authors to provide an objective and embracing view on what they write as scientists, the diversity of authors and their different opinions enrich our understanding of cave ecology and its importance, as well as increasing the range of topics covered and the perspectives on subterranean life.

This is a book about the state of the art and future directions in biospeleological research addressed to students, teachers, and specialists who need simple definitions, clear explanations of existing knowledge and theories, and informed opinions about future directions for research in a single book. We do not pretend to have extensively covered all the possible aspects and topics of cave ecology. Foremost in our aims was to thrust the cave and the cave organisms into the spotlight in the hope that this will lead to greater conservation and protection of this wonderful environment that is under the increasing pressure from human activities.

Acknowledgment We would like to express our special thanks to Francis G. Howarth for the reading of the book and useful suggestions and comments. OTM acknowledges the financial support from the Romanian Academy and the grant of the Romanian Ministry of Research and Innovation, CNCS—UEFISCDI, project number PN-III-P4-ID-PCCF-2016-0016, within PNCDI III.

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Part I
Into Karst, Caves and Cave Habitats

Chapter 2
Physiography of the Caves

Stein-Erik Lauritzen

2.1 Introduction

This chapter deals with the physiography (formation, morphology and internal environment) of underground habitats (see also Chap. 3), generally known as "caves", and smaller interstitial spaces that can be occupied by organisms (e.g. Poulson and White 1969). Caves are, by definition, natural underground voids (White and Culver 2012), and passable caves (that are accessible to humans) are just fragments of the hydrogeological network (Fig. 2.1). Caves exist in a variety of geological materials, but they share many common characteristics with respect to environmental factors: total darkness except in entrance zones, relatively constant temperature and humidity and a compartmental geometry over a wide range of dimensions.

The most commonly known "cave" types are karst caves, which are formed by chemical dissolution of the host rock. These are so-called "true" karst caves (Fig. 2.1). Other "pseudokarst" caves are lava tubes and various fissure and talus caves that are formed in rocks that do not dissolve fast enough in water to make them "karstic". They are thus formed by processes other than chemical dissolution alone. Caves may connect into vast, interconnected systems of complex architecture but can also consist of physically isolated units, to be likened with islands, and can therefore host endemic biota.

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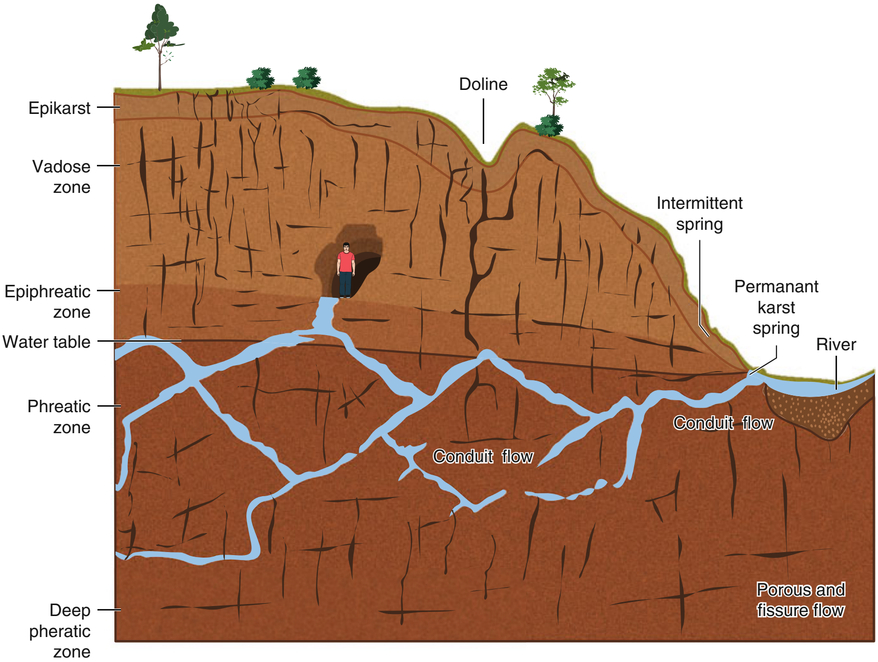


Fig. 2.1 Conceptual cross section of a karst system

2.2 Cave Patterns

Depending on bedrock structure, and on hydrologic regimes, solutional caves develop into several distinct architectures or patterns (Fig. 2.2). A basic difference of pattern exists between caves developed along bedding planes and along fracture networks. This difference is depicted by the top and bottom rows in Fig. 2.2. Dendritic caves are the basic pattern formed on stream passages that converge as tributaries, similar to surface rivers (Fig. 2.2a and e). Mazes or labyrinths consist of a network of interconnected passages that have evolved simultaneously. They are generally interpreted as created by periodic flooding that pumps into and drains all available fractures with water, thus enlarging all fractures at the same time. An alternative mechanism is diffuse recharge through a porous caprock or an underlying porous formation, thus feeding every fracture with aggressive (corrosive) water. This is particularly effective in situations where two waterbodies of different origin (and chemistry) meet. Their different chemical composition may enhance the rate of dissolution as a result of their mixing (mixing corrosion, Bögli 1964). The water flow can be quite slow. Spongework and ramiform patterns are characteristics of so-called hypogenic caves, where ascending water moves through porous rocks and the voids are created by release of chemical aggressiveness due to mixing. In particular, this may happen when saturated fresh and haline waters meet, forming

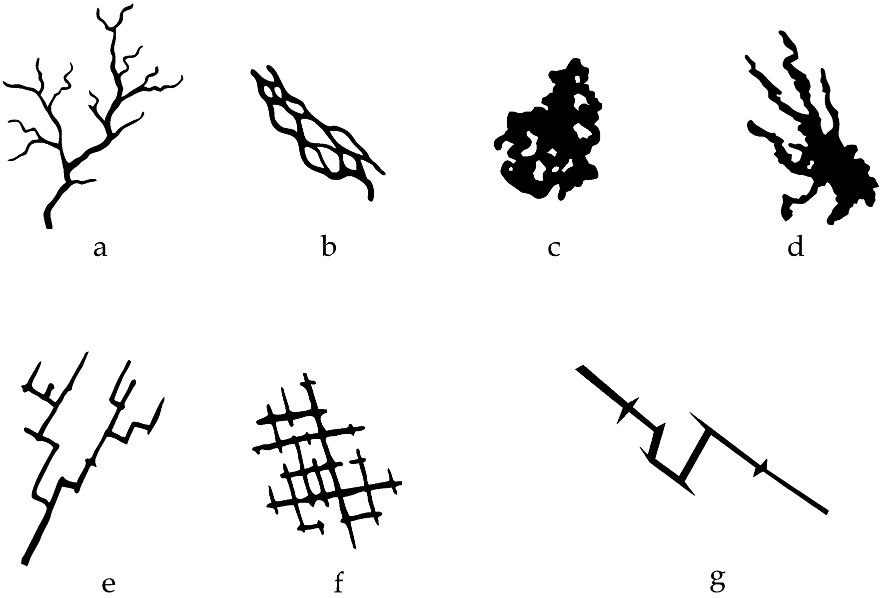


Fig. 2.2 Various plan-view patterns or architectures of solutional caves. Upper row shows caves formed along bedding planes or in porous rock; lower row depicts joint- or fracture-controlled caves. (**a**) and (**e**) dendritic or branchwork caves; (**b**) and (**f**) anastomotic and network mazes; (**c**) spongework cave; (**d**) ramiform cave; (**g**) linear, joint-controlled cave. Adapted from Palmer (2007)

halocline caves, and where hydrogen sulphide (as gas or dissolved in water) encounters oxygenated waters and produces sulphuric acid, which boosts the dissolution rate (ramiform caves).

2.3 Size Distribution of Cave Voids

Apart from the absence of light and, as a consequence, photosynthesis, the cave habitat can be characterized by the size distribution of individual voids. A "cave" may be defined in two ways, either anthropomorphic or hydraulic. In the first case, a cave is defined as a natural underground space that is passable by man, i.e. it is at least 30–60 cm diameter; in the second case, a (karst) cave is a continuous underground space that allows turbulent water flow, i.e. >10 mm diameter. From a biological point of view, caves are voids large enough to host cave-dwelling organisms. The distribution of elements (voids) with diameter δ for proper caves, sensu Curl (1986), follows a power or fractal law:

$$n_\delta = a\delta^{1-D}$$

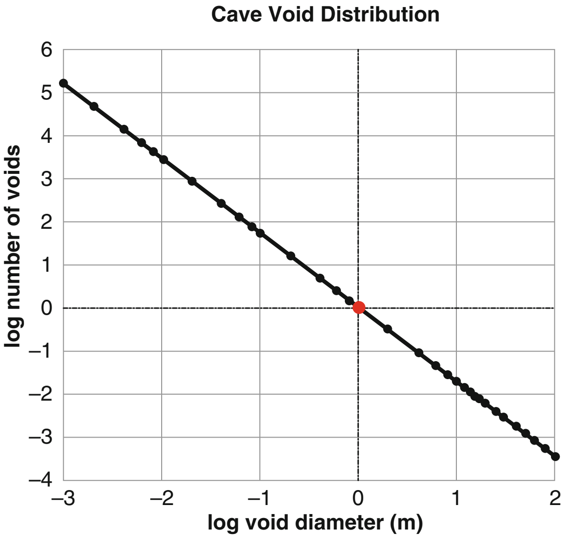


Fig. 2.3 Size distribution of cave elements (voids) relative to a (spherical) unit, 1 m in diameter (the red dot at 0,0). Assuming that this particular cave obeys a fractal dimension, $D \approx 2.73$, i.e. the Menger sponge, the size distribution of smaller voids would follow the graph. Associated with this 1 m diameter void, we may expect that there exists about 3000 and 150,000 voids greater than 1 cm and 1 mm, respectively. This model illustrates the vast habitat available in a cave where only a few passages are in fact passable to man

where n is the number of elements of diameter δ, a is a scaling constant and D is the so-called fractal dimension. In practice, there are a lot smaller elements than there are large ones. Empirically, many dissolutional caves have $D \approx 2.73$, which means that the cave is partially space filling, and with a dimension similar to the so-called Menger sponge (Curl 1986). In consequence, if a person enters a cave with a diameter of 1 m (e.g. Fig. 2.1), there may be another 10^5 voids in the surroundings with a diameter down to about 1 mm (Fig. 2.3). The cave habitat is therefore vastly larger than a human can reach, and it can accommodate space for small organisms.

The *entrance* of a cave is an intersection between the land surface and an underground void. The formation of cave entrances is a stochastic process (Curl 1958, 1960); surface erosion processes may open or close entrances over time. The longer the cave exists as a system, the more likely it is to have one or more entrances. The likelihood is obviously greater for shallow caves than for systems deeply beneath the land surface. In consequence, most caves in a region have no known entrance and are therefore only indirectly accessible, e.g. through springs, boreholes or construction works. This effect is not restricted to karst caves but applies to pseudokarst voids as well.

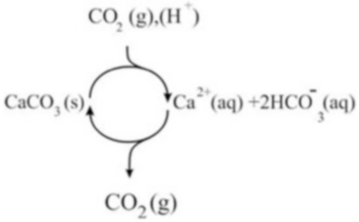


Fig. 2.4 The carbonate karst cycle

Box 2.1

Karst caves result from groundwater dissolving a rock mass consisting of relatively easily dissolvable minerals: calcite, dolomite, gypsum and rock salt. Carbonate karst is by far the most common type globally, followed by evaporate (gypsum) karst (Ford and Williams 2007). For carbonates, the process is an acid–base reaction, involving carbonic acid or other organic or inorganic acids (Fig. 2.4).

The process from left to right creates caves (i.e. speleogenesis) and consumes CO_2, whilst the reverse process, from right to left, where H^+ or CO_2 is removed from the system, actually fills the cave void—with speleothems. From this, we realize that karstification is a CO_2 sequestration process that, in fact, is of global importance.

2.4 Karst Caves

2.4.1 Speleogenesis

Speleogenesis occurs when water penetrates joints, fissures or bedding plane partings. There is a considerable latency (10^3–10^5 years), named “inception” or “breakthrough time”, during which the downstream aperture of the proto-cave voids stays below 10 mm (White 1977; Dreybrodt et al. 2005). This is because a relatively rapid, first-order dissolution process consumes most of the aggressiveness over the first few metres of flow along a tight fracture. After that, dissolution is extremely slow along the remaining distance of the fracture, attenuated by a factor of 10^4–10^5. The key issue is that the process approaches thermodynamic equilibrium very slowly, so that the water emerging at the downstream end still has a minute dissolution potential and consequently widens the downstream aperture (“bottleneck”) at a correspondingly slow rate. When the downstream aperture has attained a diameter of 10–20 mm, highly aggressive water emerges, and the aperture expands rapidly. This change in process regime is named “chemical breakthrough”. At the same time, the conduit has attained a dimension that permits the onset of turbulent flow, which greatly speeds up the dissolution process. For a 200 m long fracture or bedding plane parting, the nominal breakthrough time is of the order of 30,000 years. From this moment, the conduit may grow to metre size in a few thousand years (Fig. 2.5). After this, the

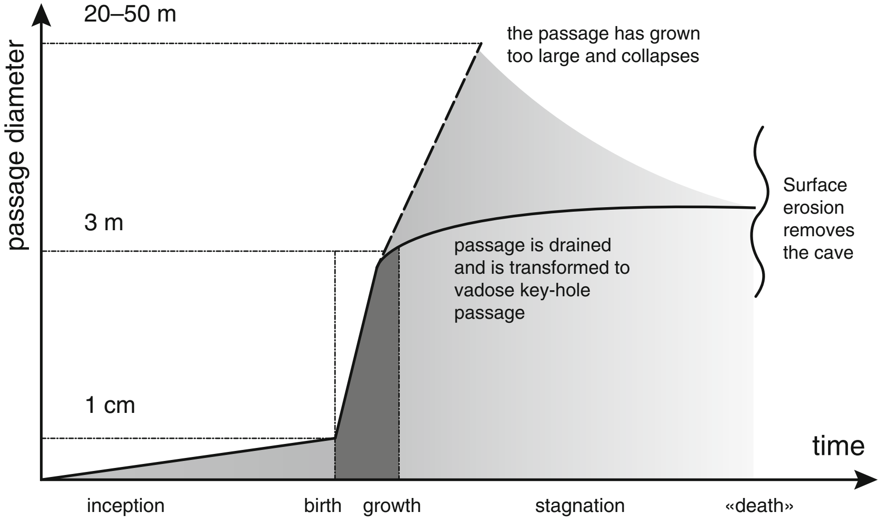


Fig. 2.5 The life cycle of a dissolutional (karst) cave passage; diameter as a function of time. Time of growth from being a tight fracture (0.1 mm) to a 1 cm diameter proto-conduit may take 10^4–10^5 years, depending on flowpath length. After that, growth is very rapid and may attain ≤ 1 mm year^{-1}. A diameter of >ca 3 m is often too large for natural recharge to keep it filled, and it turns into a vadose passage. If situated deeply below the water table, the passage may continue to grow until it becomes mechanically unstable and collapses. The ultimate speleothanatosis is when the surface erosion destroys the cave. Compiled from White (1988) and Dreybrodt et al. (2005)

conduit may become too large for most surface streams to keep it completely full, and it enters the vadose zone.

Alternatively, if the conduit is situated deep beneath the water table, it may continue to grow until its diameter exceeds the mechanical strength of the host rock, resulting in collapse. Such collapses may evolve through boulder chokes and breccia pipes and eventually reach the surface. Collapses are high-porosity zones which are not easily accessed by man. The cave's life cycle ends when the surface erosion destroys it (speleothanatosis, Šušteršič 1999) (Fig. 2.5). Caves are therefore the most resistant elements of a landscape, and they do not disappear before the surrounding rock is gone.

2.4.2 Hydromorphic Zones in Karst

Epikarst defines the interface between soil cover and the *endokarst*. It is generally a ≤10 m thick zone of intensely fractured and dissolved rock. With numerous, interconnected fissures and narrow channels, the epikarst is more porous than the bulk rock beneath it. Where there is scanty or no soil cover, the epikarst is exposed as a barren surface with karren and grikes (lapiés).

The epikarst comprises a separate, perched aquifer that is poorly connected to the vadose and phreatic zones deeper in the endokarst massif. The epikarst aquifer is recharged through every fissure and hole, and water is transported horizontally towards zones of denser and deeper fractures where water is conveyed vertically into the vadose zone of the endokarst. These sites of capture are often situated beneath dolines (closed depressions), which are also formed by this process through a positive feedback loop (Williams 1985). The epikarst contain both air-filled and water-filled voids.

The *vadose zone* occupies the upper endokarst in the vertical space between the epikarst and the *water table*. The vadose zone is partially air-filled, and water movement is mostly vertical as a result of gravity flow. There is a wide range of types of water movement in the endokarst, including waterfalls and low-gradient streams, trickles, seepage and slowly moving water films. Higher flow rates are linked to inlets with correspondingly large catchments: streamsinks and dolines feed streams and trickles, whilst fissures and percolation paths give rise to drips or film movements. Vadose water is more or less directly connected to the surface environment and is an important nutrition vector for the cave habitats (Culver and Pipan 2009). Depending on climatic zone, cave geometry and ventilation, the vadose zone may be quite wet or completely dry, as revealed by effervescence of moisture-sensitive secondary minerals (Hill and Forti 1997; White 1997). The vadose zone is the main locus for speleothem deposition, where carbonate speleothems are precipitated by degassing CO_2 (Fig. 2.4).

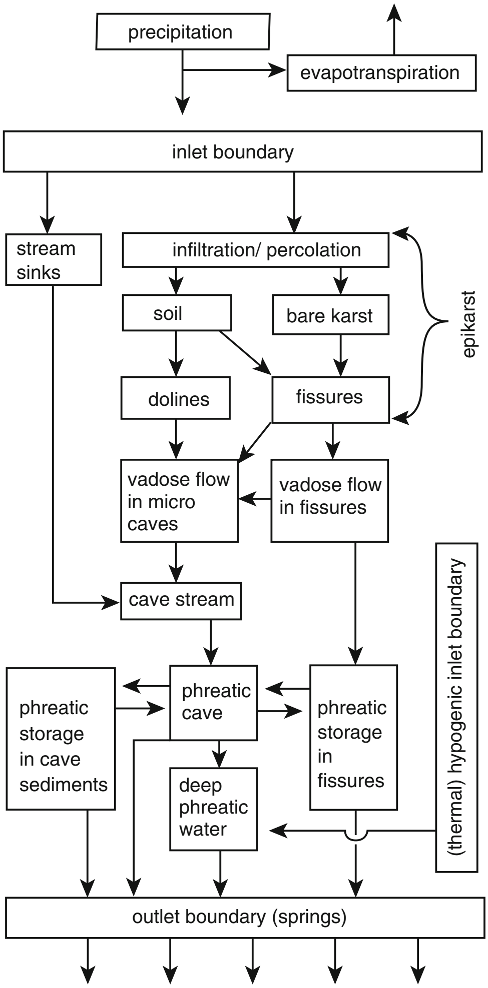
Water levels in caves may be viewed as a simple water table or, more correctly, a piezometric level, below which all available voids are completely water-filled. There is no continuous water table in karst; the water surface is only observed in passages (syphons) which may be compared to a complex system of interconnected U-tubes, with a piezometric level applying across them. Due to dynamic (venturi) effects, the water table in karst does not always slope in a simple manner, and it will also vary with recharge and local passage flow rate. This zone of variable water filling is named the *epiphreatic* or floodwater zone (Fig. 2.1). Due to the pumping effect of the fluctuating water table, aggressive water is replenished periodically, so that dissolution may be more intense here than elsewhere in the phreatic zone.

The *phreatic zone* is the zone of permanent water saturation, below the lowest water level in the epiphreatic, and thus there are no airspaces (Fig. 2.1). The phreatic zone in karst is distinguished from groundwater in, for example, gravel by having much larger spaces (conduits) and that water movement in well-developed tubes may be turbulent. In general, phreatic water circulation is slow, and residence times may be 10^2–10^3 years (Ford and Williams 2007). Deep circulation may go down to about 300 m.

The deep phreatic zone designates the deepest limits of karstification, where water movement is very slow in curved paths—downward at first, then rising again towards the spring outlets at the boundary of the karst body. If the limestone extends deep enough, there may be a relatively stagnant zone known as the *nothephreatic* zone (Grimes 1999).

Even deeper-seated karst may form from ascending, thermal water, forming hypogenic caves (Klimchouk 2007). The source of this water is *connate*

Fig. 2.6 Flow chart depicting water recharge and discharge in a karst system. The meteoric water circulation penetrates through the epikarst, the vadose zone and phreatic zones before discharging at the karst boundary zones. In the hypogenic case, deep-seated, thermal groundwater enters the karst



(i.e. trapped within sedimentary rocks) or *juvenile* (of magmatic origin). Being formed by ascending water, hypogenic caves display a characteristic, bulbous or labyrinthic morphology (Ford and Williams 2007). It appears that most caves may have a little recognized but significant, hypogenic prehistory, so that later karstification tends to inherit older flowpaths and develop them further. Hypogenic cave architectures are shown in Fig. 2.2c and d.

The cycle of meteoric water through a karst system is depicted in Fig. 2.6. Infiltration from surface precipitation follows three main vectors, infiltration through soil and through open fissures in bare karst or through discrete streamsinks. The further movement through the karst is distributed among voids of a wide range of dimensions (see Fig. 2.3), basically classified as epikarst fissures and micro-caves, vadose fissures and shafts, sediment interstices and larger cave passages. The discharge boundary of karst waters often coincidences with the lithological boundary of the karst, and discharge occurs through discrete springs feeding surface drainage and diffuse seepage into neighbouring sediments or adjacent waterbodies. Deep-seated karst has another input boundary of hypogene, thermal waters that may reach the surface through the phreatic zone (Fig. 2.6). Thus, in terms of energy and nutrition transport into the subterranean ecosystem, there are two principally different vectors: the meteoric, surface-derived pathway that conveys organic matter connected to photosynthesis and the hypogenic, thermal pathway that is connected to chemoautotrophic biosynthesis.

2.5 The Physics and Chemistry of Caves

In principle, one should expect the cave to pick up the geothermal gradient of the surrounding rock mass, so that ambient temperature would increase monotonically with depth. This is rarely the case, as ground heat is intercepted by flowing groundwater and air currents (Badino 2005). Long or deep caves may show interior thermal gradients that reflect geothermal influence (e.g. Ford et al. 1976). The distribution of temperature and humidity in the epikarst and vadose zone is driven by air and water movement.

Box 2.2
If the cave air is warmer than the surface air, e.g. a winter scenario, the density of the cave air is less than surface air; there is a positive pressure into the lower entrance, forcing air upwards and out of the higher-level entrance. The lower entrance is the locus of lowest temperatures and potential for perennial ice formation. In summer, the situation is opposite, and the air current is reversed (Wigley and Brown 1976).

2.5.1 Cave Ventilation

Cave ventilation may have several origins. Air movement in multi-entrance caves is commonly caused by seasonal, thermal differences between the cave and the surface, the so-called chimney effect (Box 2.2. Fig. 2.7). The chimney effect is driven by difference in air density between the cave and the surface. (Air density depends on temperature and moisture content, so that the virtual temperature is the crucial parameter.) The density difference results in a pressure difference between the inside and outside of the cave at its entrances.

Air velocities depend on difference in elevation between entrances, temperature contrasts and entrance diameter but can exceed 10 m s^{-1}. In extreme cases, this ventilation effect (also named Balch ventilation) may result in the formation of

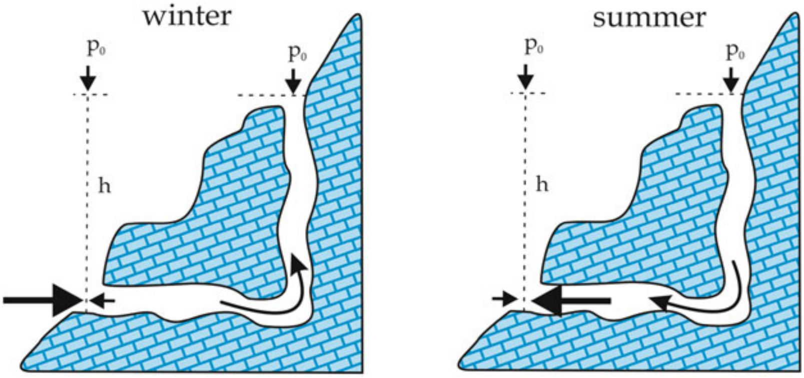


Fig. 2.7 The principle of the chimney effect of cave ventilation in a simple cave model. Figure modified after Wigley and Brown (1976)

perennial ice behind the lower entrance, i.e. a "dynamic ice cave". Such ice caves are in fact pockets of local permafrost and occur in locations with mean annual temperatures far above zero degrees (Luetscher and Jeannin 2004; Perşoiu 2018; see also Chap. 15). Single-entrance shafts may trap cold, dense air during winter, whilst the summer condition will cap the opening with warm, dry air, preserving the low temperature at the bottom. This effect may also sustain perennial snow or ice and is the "static ice cave" type of Luetscher and Jeannin (2004). Also, deep dolines or other karst depressions particularly when connected to caves that have other entrances at higher elevation may function as cold traps and have inverted temperature gradients. Such caves host particular fauna and microbiota (Iepure 2018; Purcarea 2018; see also Chap. 15).

Atmospheric pressure variations may also control ventilation in single-entrance caves. Depending on the total volume of the cave system, the diameter of the entrance and the range and speed of external pressure change, quite strong winds may occur (Deike III 1960). The phenomenon is called "cave breathing", as exhalation and inhalation often alternate with the pressure change (Wigley 1967). Running water may entrain air currents through streamsinks into vadose passages and cause return flow along other parts of the cave or along the ceiling of the same passage.

2.5.2 Attenuation of Surface Temperature

Surface diurnal and seasonal temperature variations are attenuated in the cave environment, but they rarely attain complete constancy (Cigna 2002), although an asymptotic mean can be identified. The deep interior of well-ventilated caves attain the surface annual mean temperature, and the lower opening of multi-entrance caves may develop a lower temperature regime than the annual mean surface (Wigley and Brown 1971, 1976).

Neglecting latent heat effects, it can be shown that the amplitude is attenuated exponentially along the cave passage from the entrance. Exponential damping is characterized with a relaxation length, L_0, over which the amplitude is attenuated by $1/e$. L_0 is a function of the passage diameter, d (in metres), and air velocity, v (m/s) (Wigley and Brown 1976):

$$L_0 = 100\, d^{1.2} v^{0.2},$$

which means that L_0 is much more sensitive to the diameter of the void than to the air velocity through it. Temperature attenuation approaches an asymptotic value (T_a) which, for a well-ventilated cave system, is close to the mean annual surface temperature, although local effects, like ground heat flux, may cause deviation from it. When introducing latent heat and humidity, the attenuation deviates significantly from the Wigley and Brown (1976) equation, although the concept of L_0 is still applicable. The attenuation depends on the relation between the external temperature (T_e), the asymptotic deep-cave temperature (T_a) and the dewpoint temperature (T_d). From this, three principally different situations may be identified (Fig. 2.8).

In the summer situation, T_e and T_d are both greater than T_a, and condensation at shallow depths may in some cases lead to a slight temperature rise (Fig. 2.8a). In winter, T_e and T_d are both smaller than T_a, and the air is simultaneously warmed by direct contact with the walls and cooled by evaporation of wall moisture so that a cold zone forms next to the entrance where the temperature is lower than both T_e and T_a (Wigley and Brown 1976). In a similar way, humidity in caves depends on ventilation and on the humidity of the external air. The model (Fig. 2.8b) suggests that during summer, a well-ventilated cave never reaches saturation, although local effects, like annexes and dead-end passages may override the model expectation. During the transition regime, saturation is reached within one or two relaxation lengths; in winter this distance is much shorter.

2.5.3 The Chemistry of the Cave Environment

Due to the carbon dioxide equilibria, the chemical environment in carbonate karst water is buffered to pH 7–9, depending on the total hardness and hydrological history, e.g. Figs. 2.4 and 2.5. Total hardness ($[Ca^{2+}] + [Mg^{2+}]$) depends on climate and respiration in the environment (Atkinson 1977) and ranges from a few mMol L^{-1} in arctic and alpine environments to several hundred mMol L^{-1} in a humic tropic environment (Ford and Williams 2007). Similarly, the CO_2 content of cave air depends on respiration and ventilation and may vary from atmospheric values

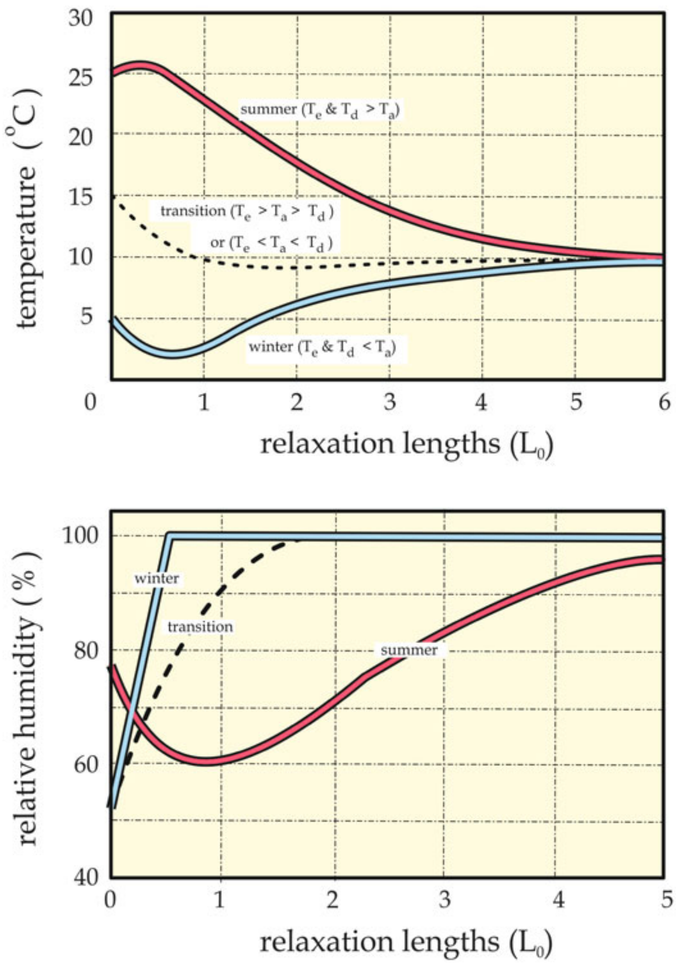


Fig. 2.8 Season-dependent attenuation curves for temperature (**a**) and humidity (**b**) amplitudes through a well-ventilated cave system. Figure modified after Wigley and Brown (1971). See text for further discussion

(~400 ppm) to >10^4 ppm or more in closed chambers. Respiration-derived CO_2 levels are independent of whether the cave is karstic or not.

In the case of noncarbonate host rocks (silicate, gypsum, halite), or where accessory minerals that may oxidize and form acids are present (e.g. sulphides and skarn minerals), the pH of the dry and aquatic cave environment may have extreme values. For instance, the oxidation of pyrite produces sulphuric acid, as revealed by minerals like jarosite (pH 1–2) and copiapite (pH < 1) (White 1997). Oxidation of base-metal sulphides may release metallic ions of high toxicity (e.g. Zn^{2+}, Pb^{2+}, Hg^{2+}), whose concentration gradients may provide conditions where there is selection for metal tolerance. Similarly, oxidation of hydrogen sulphide as expelled as gas or solute from petroleum reservoirs or from sulphate reduction may produce quite low pH in condensation and drip water (Macalady et al. 2007).

Soils in karst caves are often, but not always, basic and provide excellent conservation for bones, which will otherwise dissolve in acidic soils (e.g. Shahack-Gross et al. 2004).

2.6 Non-karstic Caves

Lava tubes are remnants of the internal drainage conduits of lava under a hardened crust (see also Chap. 17). Lava tubes occur most commonly in low viscosity, "pahoehoe lava", but are also found in the coarser, "aa lava". Lava tubes form when the surface crust of a lava flow freeze, but liquid lava still flows underneath. Lava tubes may form in two ways, by crusting over an active lava channel and, more commonly, by liquid lava continuing to flow under a hardened crust. Hydrostatic pressure from liquid lava may inflate the soft roof and enlarge the conduit ("inflation"). When the upstream supply of lava ceases, the conduit may empty and leave the roofed cave. Diameters can extend to >6 m, and many are branched. Lava tubes can attain great lengths and complexities; the longest on Hawaii is some 65 km long and 1000 m deep. However, the tubes are generally quite shallow; some 1–20 m below the land surface, sloping lava flows may however give them "depth". Lava tubes are of the same age as the lava flow, and most are quite young, as the thin roof makes them fragile and new lava flows often erode into them. At a temperature of 700–1200 °C in flowing lava, the tubes are sterile and pristine at the moment of formation.

Due to tensile fracturing during cooling and partings between individual lava flows, basalts host transmissive aquifers. Collapse of cave roofs and occasional stream capture emphasize the pseudokarstic appearance of lava flows. Due to elevation differences between entrances, many lava tubes are well ventilated and may host perennial ice.

Silicate Karst Caves Complex solutional cave systems, containing branchwork, anastomotic and network patterns, exist in quartzites and quartz-sandstone rocks, of which the most famous examples are those of the tepuis in Venezuela and in western Brazil. Various modes of genesis have been attributed to such cave types, which display morphology that is strikingly similar to carbonate karst caves of solutional origin. Quartz is slightly soluble in water (ca 4 mg L^{-1}), amorphous silica cement is more soluble, and the dissolution rate of quartz in water at moderate pH is in the range of 10^{-12} mMol cm^{-2} s^{-1} (Ford and Williams 2007). For comparison, the corresponding rates for calcite and dolomite are in the order of 10^{-7} and 10^{-11} mMol cm^{-2} s^{-1}. Although slower, but comparable to dolomite dissolution rates, given sufficient time, quartzite karst caves can form by simple dissolution. However, it is sufficient to dissolve only the silica cement in quartzite sandstones in order to create a cavity, as the loose grains can then be evacuated through incipient fissures (arenization, e.g. Auler 2012) or simply by erosion through zones of lateritization or incomplete cementation (Aubrecht et al. 2011). The latter views have been challenged by Sauro et al. (2013). Deep weathering of feldspar- and mica-rich rocks, like granite, gneiss and arkosic sandstones, will produce clay mineral residues that are easily removed and so can also produce complex cave systems, as with quartzite.

Fissure and Talus Caves Tectonic stresses, due to faulting or unloading, may result in open fractures and voids in any kind of rock. Along escarpments, tension joints are likely to develop into fissure caves. Likewise, large talus deposits may host complex underground cavities. For instance, the so-called Bodagrottorna in Sweden are 2.6 km long over four levels and cover an area of 4 ha (Sjöberg 1986). In steep fjord walls of Western Norway, deep talus caves contain ground ice and are efficient aquifers. Weathered granite may host complex cavities of biospeleological importance, e.g. Sallstedt et al. (2014) and Bauer et al. (2015). There are also caves formed by piping (resulted from removal of particles by channelled flow of groundwater; e.g. Halliday (2007)).

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Chapter 3
Where Cave Animals Live

Francis G. Howarth and Oana Teodora Moldovan

3.1 Introduction

Subterranean habitats support discrete ecosystems composed of communities that often include species highly specialized to live only underground. The physical environment is rigidly constrained by the geological and environmental setting, and because it is surrounded and buffered by layers of rock, it can often be defined with great precision. These enclosed habitats represent rigorous, high-stress environments for most surface organisms, and they are difficult for humans to access and study. Nevertheless, because the environment is discrete, rigorous, and easily defined, accessible subterranean habitats provide exemplary systems in which to conduct biological studies (Culver 1982; Howarth 1983). Although they interact and share many attributes (Fig. 3.1), the terrestrial and aquatic habitats are described separately in this chapter.

Communities of specialized cave-adapted species are known from unglaciated areas in the temperate zones to the humid tropics. In fact, they have been found wherever suitable voids have been searched; however, many regions have yet to be adequately surveyed. Biodiversity of troglobites is highest at mid-latitudes in the north temperate zone (Culver et al. 2006), but this pattern may be an artifact of incomplete sampling in tropical caves or other regions.

Only a small subset of the local surface taxa have successfully colonized subterranean habitats in each region. The ancestors appear to have been preadapted for cave life; that is, they already preferred to live in damp, dark habitats on the surface. In temperate regions, many cave species were considered relictual since they have survived severe climatic changes during glacial episodes, while their surface

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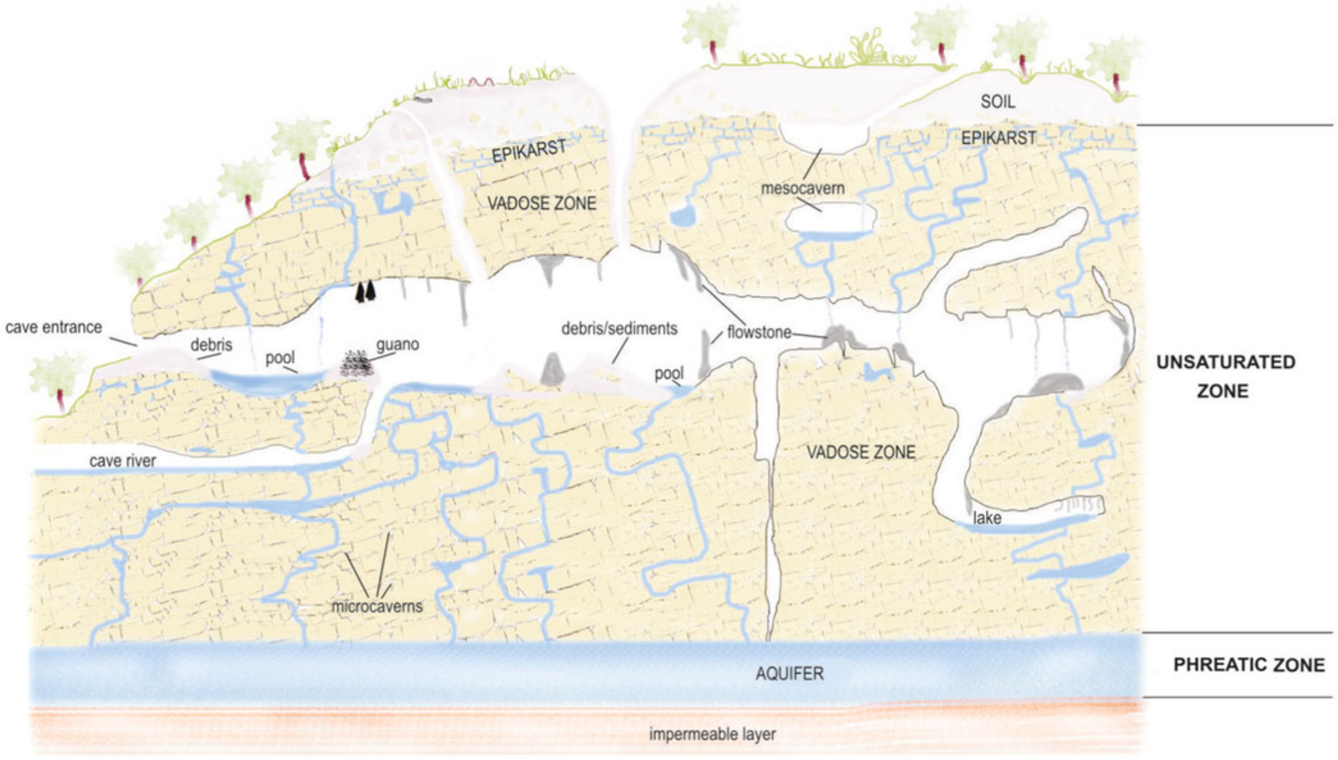

Fig. 3.1 Diversity of cave terrestrial and aquatic habitats and microhabitats. Original drawing by Oana T Moldovan

relatives became locally extinct (Barr 1968; Barr and Holsinger 1985). Recent advances in molecular phylogenies (see Chap. 12) contradict this view, and active colonization of subterranean habitats is advocated.

Box 3.1
In caves, habitats are of different sizes, but the degree of habitat interconnectivity (the so-called permeability of rocks) makes possible the dispersal of cave fauna throughout large and small voids in a karst massif. The common features of cave (and other subterranean) habitats are constant darkness; relative constancy of temperature and high relative humidity; food resources that are primarily allochthonous and scattered; often limited potential for subterranean dispersal, as this ability is contingent on the presence of suitable interconnected voids; and absent or weakened environmental cues used by surface species for dispersing, locating food, and reproduction in the three-dimensional dark maze.

There is a continuum between caves and the other subterranean habitats (Table 3.1) with barriers represented by different types of rocks, water, or sediments that limit animals migration. Despite this continuum, the diversity of cave habitats is reduced compared to the surface, with an increase of the physical inertia with depth according to Gibert et al. (1994). Nevertheless, slight differences between different parts of the cave habitats are discernible, and these define the *microhabitats* (Table 3.1; Fig. 3.2). Spatial diversity of microhabitats within a three-dimensional profile of a karstic massif can be much higher than supposed because environmental factors, intrinsic population processes, and disturbances can shape spatial biodiversity on very small scales. They also provide spatial and temporal protection from disturbance and advantages in biotic interactions (Keppel et al. 2011). The use of different microhabitats is one mechanism that makes possible the coexistence of species with the same environmental requirements and using the same resources (defined as niches), especially in caves where living conditions and food are patchily

Table 3.1 Examples of terrestrial subterranean microhabitats where cave-adapted species are often found. MSS = mesocavernous shallow substratum; see also explanation is the text and Fig. 3.2

Microhabitats	Caves	MSS
Damp mud, clay, and other sediments	√	√
Under and near rocks and debris	√	√
Decomposing organic material	√	√
Guano deposits	√	
Wet flowstone	√	
Damp walls	√	
Hygropetric habitat	√	
Biomineral oozes	√	
Plant roots	√	√
Water surface	√	

Fig. 3.2 Examples of microhabitats in caves: (**a**) biomineral ooze with larval trails of the crane fly, *Dicranomyia jacobus*, on a wall in a Hawaiian lava tube; (**b**) tree roots (probably *Ficus* sp.) in the stagnant air zone of Bayliss Cave, Queensland, Australia; (**c**) *Hadesia* sp. (Coleoptera) feeding on the film of water flowing on calcite and defined as the hygropetric habitat. Photos by © Francis G Howarth (**a**, **b**) and © Jana Bedek (**c**)

dispersed. Cave microhabitats were defined in studies on cave invertebrates and vertebrates based on environmental characteristics for both terrestrial and aquatic representatives, for example, substrate and the available space (Christiansen 1961, 1965; Trontelj et al. 2012); physicochemical characteristics of the water (Moldovan et al. 2012; Brancelj 2015); water depth and flow regime (Culver 1970; Moldovan et al. 2012; Jourdan et al. 2014); concentration of H_2S in water (Sârbu et al. 1996; Jourdan et al. 2014); abundance of food, such as carbon and nutrients (Jourdan et al. 2014); presence of biomineral oozes (Barton and Northrup 2007); and tree roots (Howarth et al. 2007); and distribution of predators (Jourdan et al. 2014). Microhabitats in caves can be temporary due to changing microclimatic features, such as drying of pools or of wet surfaces. Some species can be found in different microhabitats, while others are microhabitat specialists. The best examples are given by microhabitat partitioning in the caves with the highest diversity in cave fauna. One of these caves is Vjetrenica Pećina in Bosnia and Herzegovina with more than 60 cave species (Culver and Sket 2000), where some of the species can be found in a single place of no more than few square meters (OTM, personal observation).

3.2 Terrestrial Cave Habitats

3.2.1 Abiotic Factors Characteristic of Cave Communities

Substrate Subterranean habitats occur in a broad range of sizes from tiny voids to exceptionally large caverns and from shallow to great depth beneath the surface. They also are found in many different rock and substrate types, but solution caves in limestone and volcanic caves in basaltic lava are the best known. Subterranean habitats can be divided into three size classes based on the environment and communities supported. These are *microcaverns* (generally <5 mm in width), *mesocaverns* (~5–500 mm in width), and *macrocaverns* (i.e., caves >50 cm in width) (Howarth 1983).

Microcaverns rarely support terrestrial species because these tiny spaces quickly fill or become blocked by debris. However, flowing water can keep such spaces open and interconnected as well as transport food resources.

Mesocaverns are intermediate-sized spaces, large enough to remain open and serve as dispersal corridors for cavernicolous animals yet small enough to restrict airflow and gas exchange. Food resources can also be limiting especially in smaller passages. Recent studies using new sampling methods have demonstrated that mesocaverns provide a major part of the habitat as well as dispersal routes between caves for many cave-adapted species (Howarth 1993; López and Oromí 2010). Mesocavernous networks are especially important in young basaltic lava flows, unconsolidated river deposits, and talus slopes. In addition, they can occur in almost any rock type in which erosional or depositional processes create and maintain interconnected air-filled voids. Based on faunal characteristics and environment, mesocavernous shallow substratum (MSS) is considered distinct from deeper mesocavernous profound substratum (MPS) voids (Juberthie 1983). If the MSS merges with deeper caves, these shallow habitats can harbor a subset of the cave fauna (Uéno 1987) as well as many unique species (Derkarabetian et al. 2010; Olmi et al. 2014; Jiménez-Valverde et al. 2015).

Finally, macrocaverns include the accessible cave passages familiar to most people. They commonly occur in soluble rock types (such as limestone, dolomite, gypsum) and in basaltic lava flows, but they can also be created in other rock types by a variety of geologic processes (see also Chaps. 17, 19, and 21). The fauna of limestone caves is best known partly because these are the most accessible and long-lasting stable caves. In the past few decades, lava tubes have been recognized as also harboring highly diverse cave ecosystems (Ashmole et al. 1992; Peck and Finston 1993; Howarth 1996). Accessible cave passages also often harbor large colonies of vertebrates (e.g., birds and bats) that introduce large quantities of food resources into caves (Price et al. 2004; Holland et al. 2009; Ladle et al. 2012).

Darkness The single overarching characteristic of subterranean environments is the absence of light. Total darkness affects nearly all aspects of how organisms adapt and respond to environmental signals. Cues used by surface species (such as sight, orientation, air motion, odor plumes, sound) are absent or act inappropriately in caves and mesocaverns. Special behavioral and physiological characteristics must be important for animals to avoid or escape harmful circumstances, locate food, and reproduce in the dark three-dimensional mazes.

Atmosphere The atmospheric variables affecting cave ecosystems include temperature, relative humidity and potential evaporation and condensation rates, diurnal and seasonal periodicity, air movement, and gas concentrations (see also Chap. 2). These factors vary according to the size, shape, and location of the passage within the cave as well as by biogenic activity and climatic events. Fortunately, these factors can be understood by considering caves as zonal habitats, with each zone defined by these variables.

3.2.2 Environmental Zones

Box 3.2

Terrestrial subterranean habitats are strongly zoned, with five environmental zones recognized, each with different communities of organisms and defined on the basis of its physical environment, especially the amount of light, moisture, air flow, gas concentration, and evaporative power of the air. These are the *entrance*, *twilight*, *transition*, *deep*, and *stagnant air* zones (Fig. 3.3 and Table 3.2) (Howarth 1993). Since potential evaporation rate increases nearly exponentially with temperature, the boundaries between cave zones are often more pronounced in tropical caves. Also, the boundaries between zones are often highly dynamic within given caves, and animals living in one zone may make brief forays into neighboring zones for food and dispersal or even by accident, making static descriptions and predictions on animal distributions tenuous. Nevertheless, the zonation provides a useful classification scheme to understand cave ecology.

The entrance zone (euphotic) includes the area with sufficient light for vascular plant life. It is the zone of mixing of surface and underground communities, and since the more mesic environment can produce more food resources, this zone often supports a larger number of species of plants and animals than is found in nearby surface or cave habitats. The twilight zone, as the name implies, is the area with reduced light between the limit of vascular plants and the region of total darkness. It is alternately wet and dry, with a high and variable potential evaporation rate. Species diversity is low and mostly composed of waifs from neighboring zones, surface animals seeking shelter, scavengers, and predators. Beyond the twilight zone, there is often a transition zone, which is characterized by total darkness and a variable abiotic environment, especially changing humidity levels, airflow, and potential evaporation

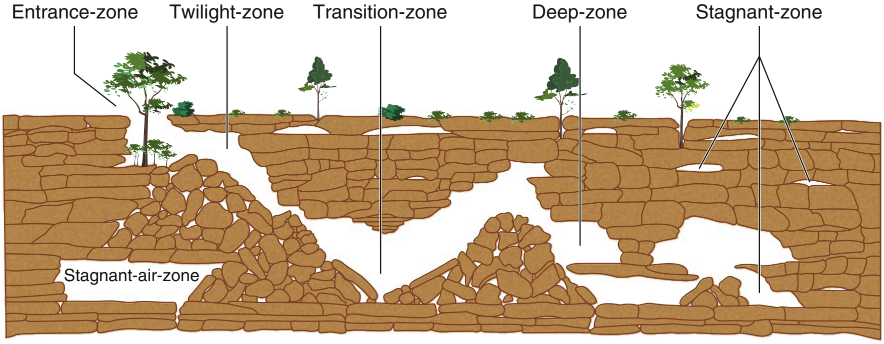

Fig. 3.3 Stylized profile view of a cave showing the five environmental zones. Scale for length greatly condensed relative to height. Figure modified after Howarth (1993), with permission

Table 3.2 Abiotic and biotic parameters of each of the five zones found in caves

			Dark		
Zones	Entrance	Twilight	Transition	Deep	Stagnant air
Light	Sunshine/ vascular plants	Shade to edge of darkness	Total darkness	Total darkness	Total darkness
Temperature	Ambient surface	Reduced variation	Nearly stable	Stable	Stable
Humidity	Variable/ desiccating	Reduced variation/ desiccating	Reduced variation/ desiccating	Saturated	Saturated/ condensing
CO_2 (volume %)	≥0.04%	0.04–0.5%	0.04–1%	0.1–3%	3–>6%
Fauna	Surface	Trogloxenes, troglophiles, waifs	Trogloxenes, troglophiles	Troglobites, troglophiles, trogloxenes	Predominately troglobites

rate. The variability is produced by diurnal and seasonal climatic cycles as well as by local weather events on the surface. The habitat is generally dry, and species diversity is generally low and composed of trogloxenes, scavengers, predators, and waifs. However, locally large colonies of trogloxenes, such as bats or cave crickets (Lavoie et al. 2007), can support a high diversity of guanophiles and guanobionts. Twilight and transition zones are sometimes recognized as disphotic zone.

The deep cave zone (aphotic) lies beyond the transition zone where light is also completely absent; the air remains still and saturated with water vapor, the substrate remains moist, and potential evaporation rate is negligible, at least for relatively long periods of time. There is usually a constriction in the passage that creates a barrier to air exchange and marks the boundary between the transition and deep cave zones. Caves that have multiple entrances or do not have such an obstruction may never develop a deep cave zone. The most stable deep cave zone environments often occur in dead-end upper-level passages since water vapor is roughly 0.625 as dense as air and thus can become trapped in such passages. This is the zone in which cave-adapted animals typically are found. Many troglophiles and trogloxenes, including both invertebrates and vertebrates, also inhabit this zone.

The stagnant air zone occurs where air exchange is restricted even further; the atmosphere periodically stagnates and gas concentrations, particularly carbon dioxide, become stressful. There is increasing evidence that this is the primary habitat of troglobitic species (Howarth and Stone 1990). This zone is believed to be the principal zone present in mesocavernous cracks and voids (Howarth 1993).

Behavioral, ecological, and environmental evidence all indicate that many of the specialized cave animals live primarily within these medium-sized voids and enter cave-sized passages only where the stagnant conditions are approximated. Thus, important habitats may extend significantly beyond accessible cave passages. Caves allow researchers to enter only the accessible fringes of subterranean habitats to conduct studies on the underground community.

3.3 Aquatic Cave Habitats

Part of the precipitation that falls as rain or snow seeps underground and percolates downward through soil and rocks until it reaches the water table or saturated zone (Fig. 3.4). Subterranean water is called groundwater, which can fill voids completely or only partially as it sinks to the water table (see also Chap. 2). The water-filled voids comprise the groundwater habitats, which occur at different scales (Danielopol 1989), from the largest, the *macrohabitat* (known as aquifers), that can cover from 1 to more than 100 square kilometers in area; through *mesohabitats*, intermediate in size and covering few square meters up to less than one square meter in area; to *microhabitats* that are small in sizes that can be measured in square centimeters.

Macrohabitats are more heterogeneous and represent the principle living space of most cave-living aquatic animals. Mesohabitats have relatively uniform physico-chemical features and represent part of the species' living space that is used during different periods of the year or their life cycle. Microhabitats define the habitat of an animal at a certain moment in time. Within microcaverns, liquid water movement is controlled largely by capillary forces; however, flowing water under sufficient pressure can keep such spaces open and interconnected as well as transport food resources, which can provide suitable microhabitats for tiny aquatic species. Consolidated and unconsolidated rocks provide distinct microhabitats for different species assemblages (Gibert et al. 1994; Malard et al. 2009). In consolidated rocks (limestone, dolomite, granite, basalt, and sandstone), the voids can be from big (especially in limestone and dolomites) to small in size, different from unconsolidated rocks (gravel to sand) where voids are generally small but can be increased by bioturbation (Datry et al. 2003).

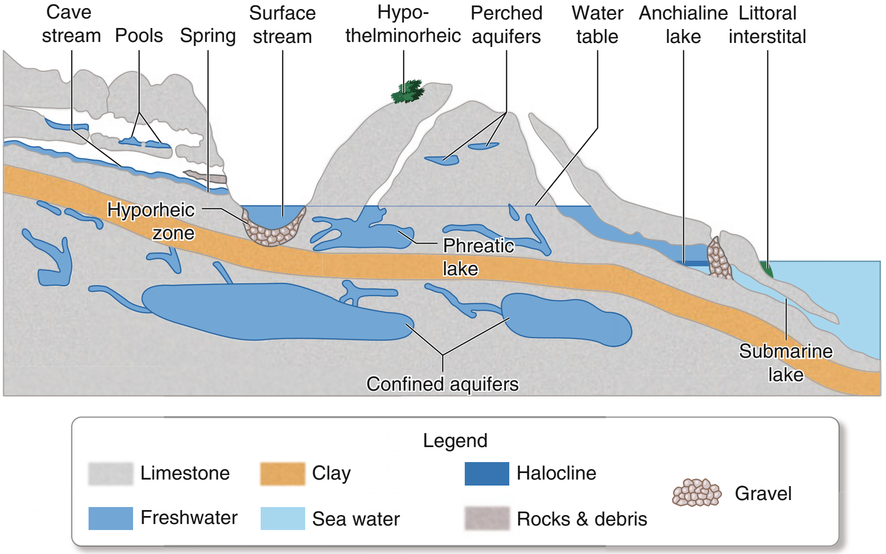

Fig. 3.4 The relationship between aquatic cave habitats and other subterranean aquatic habitats in karstifiable limestone

3.3.1 The Aquatic Living Space

Groundwater habitats in unconsolidated sediments and in various rocks (Table 3.3) represent one of the most extensive ecosystems on our planet (Gibert 2001). The degree of connectivity between cave habitats and other types of groundwater habitats depends of the rock permeability for animal migration and for nutrients, organic carbon, and dissolved oxygen input (Cornu et al. 2013). Therefore, groundwater habitats form a domain that extends from the superficial hypothelminorheic to the interstitial of the marine littoral (Fig. 3.4). Groundwater animals (i.e., stygobionts; see also Chap. 4) were considered to have much larger distribution areas than troglobionts. However, more recent molecular analysis has brought a different view on the distribution of groundwater species to less than 200 km in linear extent (Trontelj et al. 2007; see also Chap. 9).

Box 3.3
Carbonate dissolution in karstic systems results in highly heterogeneous rock permeability. Rock permeability is important because it defines the living space of cave animals, including stygobionts. In karst massifs two major zones are defined according to the hydrologic behavior: the saturated and the unsaturated zones (Fig. 3.1). The *unsaturated zone* has two main parts, the *epikarst* and the *vadose zone*, the last containing also most of the caves accessible to humans.

Table 3.3 Examples of aquatic cave habitats/microhabitats and connected groundwater habitats where stygobionts were found

Cave and microcave aquatic habitats/microhabitats	Other groundwater habitats
Dripping water	Hypotelminorheic
Small pools (gours)/puddles	Springs
Big pools/lakes	Wells
Streams	Hyporheic zone = freshwater interstitial
Riffles	Littoral interstitial
Rocks in water	
Sediments next to water bodies	
Underwater/marine caves	
Calcretes (see Chap. 18)	

The epikarst is a highly weathered zone (see Box 3.3) where water can be stored for long periods with a leaky capillary barrier at its base (Mangin 1975) that can reach a depth of 0.5–2 m on average (Bonacci 1987). The epikarst is characterized by slow percolation of air and water into narrow fissures and water storage. In the vadose zone, water percolates from the surface to the water table by rapid drainage through connected pipes (Ford and Williams 2007). The saturated zone is often sandwiched between the unsaturated zone and an impermeable level. The saturated karst is also known as the "flooded karst" (or *phreatic zone*) and represents the main water storage of the *karst aquifer* (see also Chap. 2). Aquifers are rock formations that can store, transmit, and yield economically significant amounts of water (Ford and Williams 2007). Karst aquifers like those of other rocks are of three types: unconfined, confined, and perched. The *unconfined aquifers*, known as water table aquifers, extend to the surface and are more vulnerable to climatic and human influences. *Confined aquifers* are isolated from the surface by overlying and underlying impervious geologic formations, but they can also be limited by thick layers of un-karstified rocks (Ford and Williams 2007). *Perched aquifers* are suspended above the water table, within the vadose zone, and they are of small volumes, vulnerable to climate conditions, and therefore highly variable in time. Natural and artificial *wells* are the windows to the confined aquifers, and *springs* are the outlets of the karst aquifers. Both wells and springs represent accessible habitats for research and fauna observations.

Aquatic cave habitats in karstic rocks were classified and defined into four categories by Leruth (1939). Here, we redefine these categories and add a fifth one (see also Fig. 3.1):

1. *Highly dynamic flowing waters (sinking streams)*: these can be permanent or temporary cave rivers with high flow that bring gravel from the surface or rework the rocks inside the cave, shaping the walls and the riverbed by forming smooth surfaces and few hiding places for aquatics. These waters provide few habitats suitable for cave animals, although they can bring important amount of organic matter and debris from the surface. The fast-flowing water and the lack of

constancy hamper the establishment of aquatic cave microhabitats, and they are usually dominated by surface aquatic invertebrates and even vertebrates (salamanders, fishes, and frogs; Ginet and Decou 1977).

2. *Slow-flowing waters and lakes* are characterized by the fine sediments deposited on the bottom. Slow or no flow and the presence of sediments of different sizes are the prerogatives for forming microhabitats for aquatic fauna and even terrestrial fauna on the water surface. The diversity of this type of habitat can be very high, from free-swimming large stygobionts, such as crustaceans and fishes, to minute animals, which rest in hidden places, such as under rocks or in sediments. Slow-flowing waters are the most stable aquatic cave habitats because with their high volume, they are less prone to complete drying. This type of habitat can be fed by surface water, groundwater, or films of water flowing through the vadose zone.
3. *Gours or pools formed on flowstone* are of different volumes and are present in most limestone caves. They are formed by the dripping water and, thus, can be permanently or temporarily filled with water; also, they can be filled during flooding periods if a cave river flows nearby. These pools can have clay and/or organic matter brought by water from the karstic voids above. However, such gours can have important communities that belong both to aquatic and terrestrial fauna: amphipods and harpacticoids hiding in the sediments, swimming cyclopoids, and collembolans and acari on the surface of the water. They can form a microcosm where dripping water brings new prey or predators and food from the vadose zone and sometimes even an unexpected terrestrial visitor such as beetles or pseudoscorpions can hide under the calcite and sediments near the water.
4. *Small pools on clay or mud* can be dynamic aquatic habitats because they can form only during periods of very intense percolation or by the flooding rivers. During the periods with water, they can contain crustaceans from the vadose zone (Brancelj 2015).
5. *Dripping or percolation water* brings inside caves both epigean fauna from the surface and hypogean fauna from the unsaturated zone above. Dripping water is an extremely rich domain that can contain hundreds of individuals of different species, especially crustaceans, and its richness was for the first time mentioned by Rouch (1968) and Delay (1969), followed by many other researchers in the last decade. The ways taken by the water from the surface to the collection point inside caves can be very different, even for drips few meters apart. Water crosses different limestone layers, fractures, and voids of different sizes; it can remain longer periods in the unsaturated zone, from minutes to days and even years depending on the voids size and their interconnectivity.

Uniformity and relative stability characterize cave waters that are not in direct contact to the surface waters (Camacho 1992). However, differences between microhabitats exist and are sometimes measurable inside a single cave where one can find different species if it collects different dripping points or pools (Moldovan et al. 2012; Brancelj 2015; Fig. 3.5).

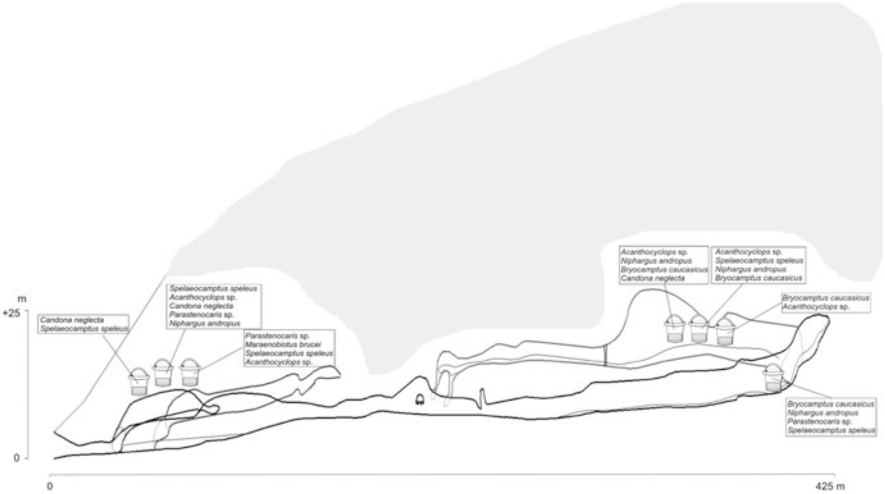

Fig. 3.5 Distribution and abundance of species in seven dripping stations of the Ciur Izbuc Cave (Romania); species are in abundance order in each station. Modified from Moldovan et al. (2012)

3.3.2 Groundwater Habitat Features

Box 3.4
Aquatic subterranean habitats potentially include all the voids with water and conditions of survival and reproduction. What best defines the groundwater habitats, beside the features common with terrestrial subterranean habitats, are usually the relatively constant chemical composition, low concentration of organic matter, complex relationship with surface water (Schmidt and Hahn 2012), flow rate, permeability, and void size (Cornu et al. 2013). The constancy of physicochemical features seems to be more important for the survival and range extension of groundwater fauna than the seasonality and availability of habitats (Zagmajster et al. 2014).

Darkness is absolute in cave waters, as in all subterranean habitats. *Water temperature* is relatively stable in deep habitats and very similar to air temperature inside the cave. The slight difference is no more than 1 °C lower (Camacho 1992). Dripping water can have more variable temperatures that are in the order of few degrees in the deep zones (e.g., ~ 5 °C maximum in Meleg et al. 2012). *Flow rate*, *velocity*, and *water level* in aquatic habitats can be variable if in direct contact to the surface waters. High flow rates bring organic matter underground, but it destabilizes the constancy of cave habitats, which in turn may promote the short-term colonization of epigean species. Slow flow limits the input of nutrients inside caves that become even scarcer with depth. *Water pH* is neutral or slightly acidic in limestone/

dolomite caves due to the presence of carbonic acid buffered by carbonates. *Oxygen content* is generally low mainly because primary producers, the main water oxygenators, are absent in cave waters. Cave waters with highly dynamic flows can have more oxygen produced by physical processes. *Hardness* of the water is given by the calcium ions that also give the karstic water the beautiful turquoise color. *Water mineralization* depends on five independent variables (Bakalowicz 1974): karstification degree, aquifer confinement, sediments cover, surface washaway and its loss, and bioclimatic conditions on the surface.

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Part II
There is Rich and Diverse Life in Caves

Chapter 4
The Ecological Classification of Cave Animals and Their Adaptations

Francis G. Howarth and Oana Teodora Moldovan

4.1 History of Classification Schemes

During the earliest studies of cave biology, researchers soon recognized that animal species living permanently in caves often shared a suite of similar morphological features, even when only distantly related (Racovitza 1907; Vandel 1964). The most notable features are the loss or reduction of structures normally considered necessary for life, such as eyes, bodily color, and robustness. To better understand cave ecology, early workers attempted to classify cave animals by correlating the different degrees of morphological change with the observed levels of their association with caves. However, the high variability in morphology displayed by cavernicoles, lack of ecological data for most species, and the subjective nature inherent in determining their status in caves make ecological classification of some species problematic.

One of the earliest attempts to classify cave animals was presented by the Danish biologist Schiødte (1849). He proposed four levels or categories distinguished by the amount of light and the nature of the walls in the preferred habitat: (1) shadow animals (*Skygge-Dyr*), (2) twilight animals (*Tusmorke-Dyr*), (3) obscure area animals (*Hule-Dyr*), and (4) obscure area with flowstone animals (*Drypsteenshule-Dyr*).

Later, Schiner (1854) visiting several caves in southeastern Europe recognized three categories of cave animals based on their ethology: (1) occasional hosts, animals encountered in caves but also living in surface habitats; (2) troglophiles, animals inhabiting regions where daylight still penetrates but which can be rarely encountered on the surface; and (3) troglobionts, animals living exclusively in caves

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and which are only encountered in epigean habitats following exceptional events such as floods.

Joseph (1882) was a German biologist who proposed three categories corresponding to topography: (1) inhabitants of cave entrances, i.e., well-lit regions with variable temperature; (2) inhabitants of middle areas, i.e., where the noon sun produces a kind of twilight; and (3) inhabitants of deep areas, with complete darkness and constant temperature.

These early attempts were reviewed by Racovitza in 1907 who subsequently provided more precise definitions of each category. Racovitza's system proved useful in understanding the evolutionary ecology of cavernicoles and his scheme, which is called the *Schiner-Racovitza classification*, became the basis for all subsequent classifications (see Box 4.1).

Box 4.1

Racovitza's original definitions are as follows:

1. Trogloxenes are lost or occasional visitors that do not live or reproduce in caves. They rarely display any special adaptive features for life in caves, and moreover, they usually stay near cave entrances.
2. Troglophiles are able to live and reproduce in subterranean environments but are also able to live in surface habitats. In caves, they generally prefer areas near entrances. They may have reduced eyes and other adaptations to life in darkness.
3. Troglobionts exclusively inhabit subterranean environments and usually are found only in the deepest areas of caves. They are highly modified morphologically for underground life. Racovitza believed troglobionts were among the oldest cave animals.

Unfortunately, placing cavernicoles in a classification scheme is often subjective until their true association to caves is better known. Given the variability and differing degrees of adaptation to caves and other subterranean habitats, it is often not possible to determine the status of all species based on morphology or the environment where they are found. Many species found in caves also live in soil or cryptic habitats on the surface and their occurrence in caves may be accidental or facultative. In cases where the status is uncertain, the general terms *epigean* and *hypogean* are often used. Sket (2008) and Trajano and Carvalho (2017), among other authors, discussed the difficulties and pitfalls in the application of the Schiner-Racovitza classification. Trajano and Carvalho (2017) identified difficulties in the "separation of subterranean organisms from accidentals, use of troglomorphisms to infer the troglobitic status, distinction between troglophiles and trogloxenes, detection of obligatory trogloxenes." Sket, retrieving a classification proposed by Ruffo (1957), proposed to split the troglophiles in two categories, the subtroglophiles (species inclined to perpetually or temporarily inhabit a subterranean habitat but intimately associated with epigean habitats for some biological functions like

Fig. 4.1 Habitus of the epigean *Catops fuliginosus* (left, Photo by © Udo Schmidt) compared to cave species at different degrees of adaptation to life in caves: *Ptomaphagus hirtus*, *Speonomus hydrophilus*, *Closania winkleri*, and *Leptodirus hochenwartii* (Photos by © Pawel Jaloszynski)

feeding, seasonally or during the life history) and the eutroglophiles (epigean species able to maintain a permanent subterranean population). Further discussions on classifications are also presented in Chap. 7.

Christiansen (1962, 2012) coined troglomorphy as a general term for adaptive traits commonly associated with cave-adapted species. An animal displaying at least some troglomorphies and also known only from caves can be considered troglobiont/stygobiont (Fig. 4.1). The term troglomorphic is roughly equivalent to “le facies morphologique cavernicole” (= the cavernicole morphological appearance) used by Vandel (1964). How and why troglomorphies have occurred has long intrigued both evolutionary biologists and laymen.

Preadaptation was originally proposed by Cuénot in 1911. As used here, the term refers to a set of characters that evolved within a population, which allowed the population to survive in a specific environment and which subsequently facilitated the colonization of a novel habitat with a similar environment. For example, many cave species have relatives living in cryptic dark and moist habitats on the surface, such as in soil, under rocks, among moss, etc.

4.2 Overview of Common Features of Cave Inhabitants

The relationship of cavernicoles to subterranean environments ranges from being casual visitors to highly specialized animals that are restricted to life underground. Consequently, the traits displayed by cavernicoles are frequently related to their

Table 4.1 Common troglomorphic features for cave representatives. Modified after Langecker (2000) and Christiansen (2012)

Features	Troglobionts	Stygobionts
Morphological features		
Pigmentation loss	All groups	All groups
Loss or reduction of eyes/ocelli and eye structures	All groups	All groups
Increased body size/body elongation	Arachnids, Coleoptera, Collembola, etc	Some crustaceans, fishes
Elongation of appendages	All groups	Some crustaceans
Elongation/increased number/different spatial distribution of sensory receptors	All groups	Cave *Asellus aquaticus* and some other crustaceans, fishes
Cuticle thinning/scale reduction	All groups	All groups
Physiological features		
Low metabolism rate	All groups	All groups
Fasting/lipid storage	All groups	All groups
Dietary changes	All groups	All groups
Loss of water balance mechanisms	All groups	–
Tolerance to high CO_2/low O_2	All groups	All groups
Behavioral features		
Loss of circadian rhythm	All groups	All groups
Changes in mating behavior	All groups	All groups
Special features		
Life cycle reduction	All groups	All groups
Egg number reduction	All groups	All groups
Egg volume increase	All groups	All groups

degree of association with caves (Howarth 1983; Trontelj et al. 2012). These traits range from no discernible adaptation among waifs and casual visitors in caves to the often-bizarre modifications displayed by obligate cave species. The morphological, physiological, and behavioral modifications displayed by obligate subterranean animals are convergent and arose independently in different taxa (see also Table 4.1 and Fig. 10.6). Convergent morphologies of cave (subterranean) animals are highly predictable (Derkarabetian et al. 2010), although explanations of their function and fitness are rarely provided (Hüppop 2000; Jeffery 2009). In caves, there is one major selective environmental feature whose extreme value gives predictability and convergent traits, and this is the lack of light (Culver et al. 2010; Derkarabetian et al. 2010).

4.3 Adaptations to Caves by Terrestrial Animals

4.3.1 Morphological Adaptations

Box 4.2
Loss of sight, bodily color, and fragile appearance are the classic hallmarks displayed by cave-restricted species and initially were considered examples of enigmatic regressive evolution (see also Chap. 10). Additional reductions of morphological features include degeneration of wings and flight among insects and thinning of the cuticle of arthropods. These traits are often convergent, that is, evolved independently in different taxa inhabiting caves (Figs. 4.2 and 4.3).

Although reductive troglomorphies are often the most conspicuous features of obligate cavernicoles, there also are many "positive" changes. Examples include an increase in size and number of sense organs (receptors); elongation of the body, legs, antennae, and other appendages (Figs. 4.1 and 4.3); and specialization of foot structures in Collembola and other terrestrial arthropods. The reduction of the pronotum displayed by the most evolved cave Leptodirini and Trechini beetles is a feature probably linked to the mechanics of locomotion associated with elongated legs. The increase of the antennal surface is not by widening but by lengthening of the antenna (Fig. 4.3a). This lengthening allows for an increase in the total number of receptors, especially those responsible of mechanical reception (Moldovan et al. 2004). In springtails, the tarsal specializations allow the animals to walk on water and climb the meniscus and thus escape from pools of water (Christiansen 1965).

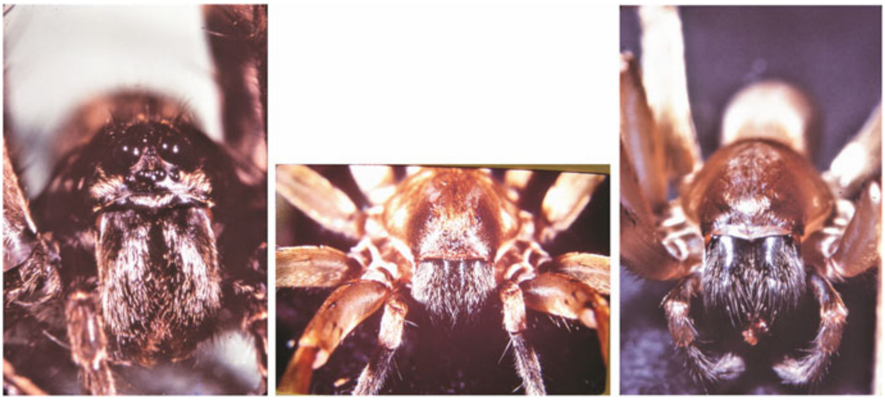

Fig. 4.2 Hawaiian Lycosidae spiders comparing epigean, *Lycosa hawaiiensis* (left), and two cave species: *Lycosa howarthi* with vestigial eyes (center) and *Adelocosa anops* with no eyes (right) (Photos by © William P Mull)

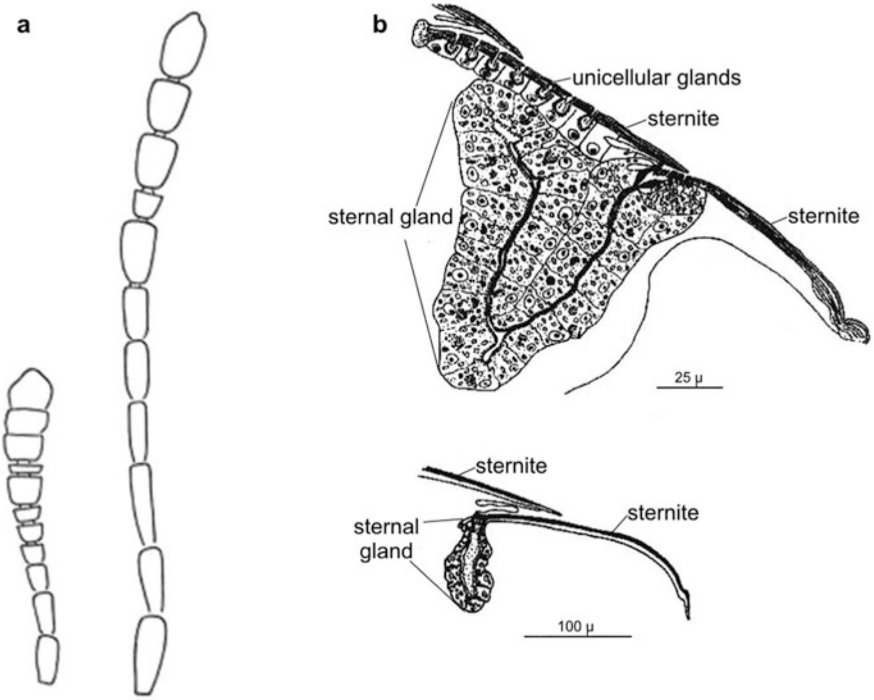

Fig. 4.3 Examples of morphological adaptations in cave beetles (Leiodidae) compared to their troglophilic relatives: (**a**) antennal length in troglophilic *Ptomaphagus elabra* (left) and cave *P. troglomexicanus* (right); (**b**) sternal exocrine gland in endogean *Bathysciola meridionalis* (top) and cave *B. delayi* (bottom) [Figures from Peck (1973; a) and Moldovan (1998; b)]

Changes in pretarsal structure also occur in some cave planthoppers (Howarth 1981; Hoch and Howarth 1989). In this group, the arolium (a flexible pad at the apex of the tarsus) is vestigial, whereas the claws are greatly elongated and adapted for walking on barren wet rock instead of walking on vegetation (Fennah 1973). In beetles, reduction of the sternal exocrine gland, which probably produces pheromones, has been documented in cave representatives of *Bathysciola* (Fig. 4.3b; Moldovan and Juberthie 1994). More about the specific adaptations in some groups can be found in Chaps. 7 and 8.

4.3.2 Physiological Adaptations

Dietary Changes Troglobionts often broaden the types of food eaten compared to their surface relatives; e.g., many species become omnivores, and some predators add carrion to their diets. In addition, many troglobionts are able to consume prodigious amounts of food and then are able to survive long periods without eating.

In caves, food resources are often scattered and difficult to find and exploit; therefore, cavernicoles cannot afford to be selective and adapt to take advantage of a broad range of resources discovered.

The Leptodirini group of cave beetles are mainly detritivorous or saprophagous. Differences in their mouthparts are obvious among genera and even among cave species that display the same level of adaptation to life in caves. The main modifications concern the shape and structure of the different parts, as well as their length and both position and number of hairs, bristles, and setae (Moldovan et al. 2004). Some species show modifications of the mouthparts associated with their dietary niche, being adapted to a semiaquatic way of life such as found in hygropetric habitats on cave walls (Fig. 4.4). The adaptation to an aquatic niche has modified the mandibles, which acquired a spoonlike form to bring water near the mouth, the lacinia, which has taken on the role of stirring, and the galea, which can filter organic particles with its modified fine and very dense hairs. Other terrestrial groups, such as Diplopoda and Collembola, may also be adapted to take food from water with modified mouthparts, especially structures evolving toward a spoonlike form (Deharveng and Christian 1984; Enghoff 1985a, b; Adis et al. 1997; Enghoff et al. 1997).

Low Metabolism Rate Troglobionts often exhibit low levels of activity and low mobility even during active periods. This is usually correlated with a low metabolic rate (Hüppop 1985), but in some studies comparing closely related cave and surface species, this relationship is unclear. In *Caconemobius* rock crickets in Hawaii, the surface species has a high metabolic rate when active at night but rests in a torpid state during daylight, whereas its cave-adapted relative is continuously active at a

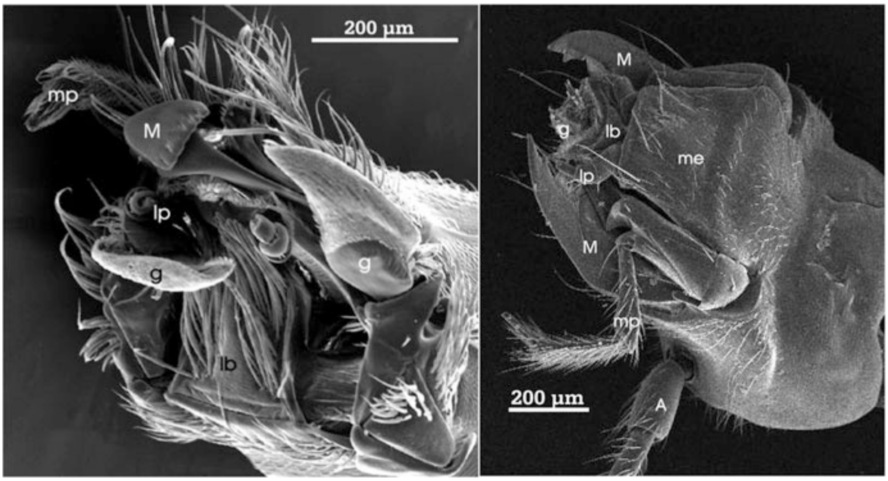

Fig. 4.4 Frontal view of the mouth of *Hadesia vasiceki* (left) and ventral view of the head of *Tismanella chappuisi* (right), both cave beetles (Leiodidae Leptodirini): *A* antenna, *g* galea, *lb* labium, *lp* labial palp, *M* mandible, *me* mentum, *mp* maxillary palp [From Moldovan et al. (2004)]

low metabolic rate, as would be expected. However, averaged over 24-h periods and corrected for weight, the two morphs have a similar overall rate of oxygen consumption (Ahearn and Howarth 1982).

The reduced metabolic rate, as well as conspicuous loss of normally critically important traits such as eyes and pigments, has been considered evidence that troglobionts evolved to exploit food-poor environments (Hüppop 2005). This hypothesis appears corroborated by the apparent barren rocky substrate usually present in deep cave passages. However, energy conservation is a universal trait shared by nearly all organisms in adapting to their environment, and therefore, such adaptations may not indicate a food-poor environment. In fact, other evidence suggests that food-rich environments may also foster reductions in morphological and physiological traits. For example, among polymorphic arthropods living in resource-rich habitats, the greatest reduction in morphological features often is correlated with the presence of the largest amount of stable food resources, and food scarcity triggers a switch to individuals with greater dispersal ability (Steffan 1973; Roff 1986). Also, the degree of cave adaptation found among troglobionts is not closely linked to the amount of energy sources available, nor does it indicate the age or time since the colonization of the cave system. Studies in Hawaiian (Hoch and Howarth 1999) and Australian caves (Hoch and Howarth 1989) demonstrated that the degree of cave adaptation is strongly correlated with the cave environment and extent of geographic area available rather than with age of the cave or amount of available food. The relative abundance of food resources, however, appears to be correlated with the numbers of individuals and species that can share the habitat.

Fasting for long periods is possible due to the capacity of large body for fat storing, a depressed metabolism, during which they mainly subsist on lipid stores, a prolonged state of glycogen- and protein-sparing, and low energetic requirements (Hervant and Renault 2002). This ability to store fat is found in all life stages, i.e., eggs, juveniles, and adults (Fig. 4.5).

Water Balance Deep cave environments, where troglobionts live, remain at or near 100% relative humidity, and occasionally the air becomes supersaturated. Saturated atmosphere is stressful for most epigean organisms, and cavernicoles have altered their water balance mechanisms to cope. Since saturated air is above the equilibrium humidity of bodily fluids, troglobionts must deal with excess water rather than desiccation. In doing so, they have lost many attributes (such as reduction and change in composition of cuticular hydrocarbons and of thinning of the cuticle) that conserve water and have become highly sensitive to desiccation (Howarth 1980; Ahearn and Howarth 1982; Hadley et al. 1981).

CO_2 Tolerance Decomposition of organic material can increase the concentration of carbon dioxide to stressful levels in underground environments. Most invertebrates can tolerate brief exposure to high CO_2 concentrations. Organisms living in soil and shallow mesocaverns may be able to migrate to less stressful conditions, but species living in deeper caves, especially those in deep mesocaverns, would be unlikely to find refuge habitats since the dark, three-dimensional maze provides

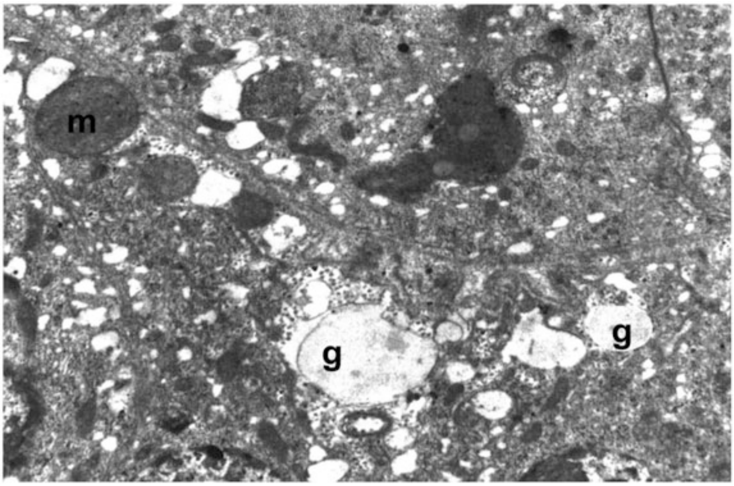

Fig. 4.5 Electron microscope details of fat stored in the form of glycogen vesicles in the posterior abdomen of cave beetle *Drimeotus bokori* (male; ×11,400): g = glycogen, m = mitochondria (Photo by © Oana T Moldovan)

few cues for escape. Humid bad air passages have been found to harbor highly diverse communities of troglobionts (Howarth and Stone 1990).

4.3.3 Behavioral Adaptations

The perpetually dark, complex three-dimensional mazes in caves and mesocaverns (see also Chap. 3) present special problems for organisms attempting to find food, mates, and safe havens. Many important cues for life (e.g., light\dark cycles, air currents, temperature changes, odor dispersion, and acoustics) are absent or provide inappropriate signals in subterranean habitats. Thus, behavior must play a big role in adaptations to live permanently underground. For example, an animal following a scent plume, whether a pheromone or food odor, would spend most of its effort walking away from the source since the animal must walk around or over numerous irregularly shaped obstacles along the path. The main special behaviors exhibited by troglobites are described below.

Loss of Circadian Rhythm Troglobites have lost the ability to entrain their activity to daily cycles but instead exhibit a continuous level of activity, albeit movement is often infrequent and irregular. The loss of daily rhythm results from the relaxed selection pressure to maintain it, whereas the irregular activity allows the animal to better exploit resources in the cave. Some cavernicoles living in shallow mesocaverns or airy caves have reduced eyes and can entrain to light/dark daily cycles.

Random Walks Troglobites move slowly in a random path, following a fractal pattern (Moldovan and Paredes Bartolome 1998/1999). This behavior minimizes energy consumption while maximizing the chance of finding food or mates. It also minimizes the chance of falling victim to dangers, e.g., many troglobites actively avoid entering pitfall traps (Kuštor and Novak 1980). Numerous pits and crevices in cave passages often act as natural pitfalls, and selection to avoid these has been harsh. Similarly, cave crickets and other jumping arthropods have greatly reduced their ability and propensity to jump since this behavior is hazardous in total darkness. Many, if not most, volant cave insects characteristically fly forward slowly with legs extended to contact the substrate first (Howarth 1983).

Mating Behavior Finding mates and successfully reproducing in caves has been little studied but is likely an important factor in the isolation and evolution of troglobites. Visual cues are the primary means used to locate and recognize suitable mates among many epigean animals, but of course this cue would be ineffective in total darkness.

Some troglobites have elaborate pheromone glands (exocrine sternal gland and isolated exocrine glands in cave beetles; Fig. 4.3), which indicate that pheromones are important for sexual communication in some species (Cazals and Juberthie-Jupeau 1983; Juberthie-Jupeau and Cazals 1984; Moldovan and Juberthie 1994). Similarly, cuticular hydrocarbons, which are molecules with reduced or no volatility, would function over short distances for intraspecific communication and mate recognition (Moldovan 2003). Each species has a specific mixture of cuticular hydrocarbons that is influenced by phylogenetical constraints and external factors, and they might promote speciation in a changing climate much faster than genetic isolation alone (Moldovan 2003).

Except for sonar used by cave-roosting bats and birds, airborne sound communication is exceptionally rare among cavernicoles, which is probably a result of the poor acoustical properties within cave passages. However, substrate-borne sound may be widely used at least for short range communication. This has been studied in troglobitic cixiid planthoppers (Hoch and Howarth 1993; Hoch and Wessel 2005; Wessel et al. 2013; see also Chap. 7). Epigean planthoppers duet on the host plant; receptive females remain stationary, while males fly to potential host plants and sing. When a receptive female hears the appropriate song, she responds and then the two alternately sing until the male locates the female. In contrast, receptive female cave cixiids initiate calling. They remain stationary on the host root and periodically call. Their species-specific songs are transmitted along plant roots. Males wander among patches of host plant roots and listen. When they hear a female song, they answer. Upon hearing the appropriate male song, the receptive female responds, and the two sexes then sing alternately until the male locates the female (Hoch and Howarth 1993).

4.3.4 Special Adaptations

Behavior May Precede Morphology Christiansen (1965) reported a stepwise sequence in the evolution of the ability to climb a meniscus and thus escape from pools of water in cave Collembola. Christiansen found that initially a small change in behavior, which improved the animal's ability to walk on water, was followed by morphological change, which further improved the ability and which set the stage for a subsequent behavioral change. The fully formed and functional troglomorphic foot required several sequential behavioral and morphological steps.

Life History Adaptations to life in cave habitats often involve convergent changes in life history traits. Troglobionts exhibit K-adapted traits, such as producing a few large eggs (i.e., low fecundity; Vandel 1964; Poulson and White 1969). In addition, the number of immature stages is reduced, and lifespan of the adult stage is extended. For example, epigean wolf spiders (Lycosidae) produce more than 300 eggs per clutch and the young ride on the female for a month or more until ready to disperse (Fig. 4.6a). The small-eyed cave species (*Lycosa howarthi*) has about 40 spiderlings per clutch, which stay with their mother for about 2 weeks (Fig. 4.6b). The no-eyed wolf spider (*Adelocosa anops*) has about 25 spiderlings, which are able to leave the mother in a few days (Fig. 4.6c) (Howarth and Mull 1992).

Increased lifespan also makes possible a longer investment in reproduction that compensates in some groups the low number of offspring. Cave beetles show the modification of life cycles at different levels of adaptation to life in caves (Deleurance-Glaçon 1963; Deleurance and Deleurance 1964). Embryonic and larval phases are longer in troglobionts compared to endogean relatives (Fig. 4.7). An increase in the quantity of vitellus allows the development of the embryo for a longer period. A larger egg ensures larger juveniles that have higher fitness; e.g., larger young can cope better with the restrictive environment, have a higher resistance to starvation, and exploit a broader range of food resources (Roff 1992; Hüppop 2000; Wilhelm and Schindler 2000). Unless triggered by seasonal or regular pulsed input of food resources, the timing of reproduction by cavernicoles is generally not

Fig. 4.6 Hawaiian Lycosidae comparing fecundity with each female carrying young. Epigean wolf spider, *Lycosa hawaiiensis* (left; photo by © William P Mull), and two cave species, small-eyed wolf spider, *Lycosa howarthi* (center), and no-eyed wolf spider, *Adelocosa anops* (right) (Photos by © Francis G Howarth)

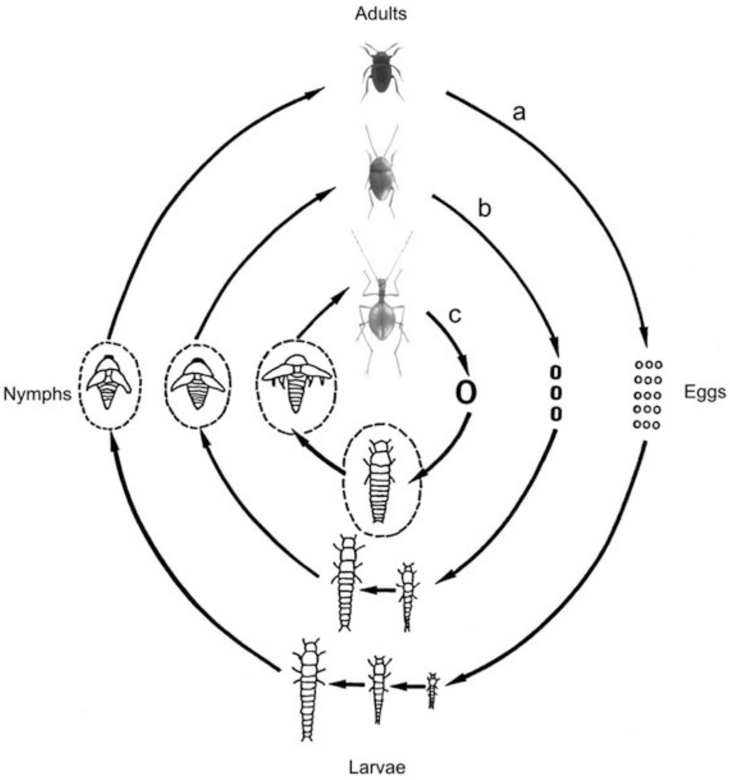

Fig. 4.7 The development cycle of cave Leptodirini (Coleoptera, Leiodidae): normal cycle (**a**) of epigeans and intermediary (**b**) and contracted (**c**) cycles of cave beetles [Modified after Deleurance-Glaçon (1963)]

synchronized, and all life stages are often present simultaneously throughout the year.

Neoteny or Pedomorphosis Many of the troglomorphic changes commonly found in cavernicoles may have evolved by neoteny (the retention of juvenile characters into adulthood) (Culver 1982; Howarth 1993). Matsuda (1982), studying amphibians and terrestrial amphipods, proposed that, in these groups, adaptive shifts to new habitats are facilitated by stress-induced neoteny. Neoteny can arise rapidly as it would require changes in only a few regulatory genes. Natural selection may favor neoteny as an energy-saving mechanism, e.g., reduction of eyes and other traits by changes in allometric development (Matsuda 1982). Neoteny may be positively selected in cavernicolous species whose immature stages already live underground, e.g., cixiid planthoppers. Cixiid nymphs feed on plant roots and characteristically display many troglomorphies. In addition, the nymphs feed on xylem sap, which is very dilute; thus, they have well-developed physiological and morphological adaptations to deal with excess water. Adults of epigean species metamorphose into typical surface planthoppers and emerge from their underground lairs to disperse and reproduce. Adults of the cave species have retained many features found in the nymphal stage and remain underground (Howarth 1993).

Pseudo-physogastry The swelling of the posterior part of the more cave-adapted Leptodirini (Coleoptera) body is due to a false physogastry caused by the enlargement of the elytra volume, while the abdomen remains normal (Fig. 4.1). One possible explanation is that a spherical chamber formed by elytra can regulate the hydric equilibrium of the body since cave animals living in saturated air humidity are very sensitive to desiccation.

4.4 Adaptations to Caves by Aquatic Animals

4.4.1 Morphological Adaptations of Stygobionts

Pigmentation The lack of pigmentation (also called albinism) occurs not only in stygobionts but also in some stygophiles that spend most of their life inside caves. Some of the aquatic cave vertebrates are pigmented when young but then lose bodily color as they age. For example, *Proteus anguinus* salamanders have black chromatophores as larvae but lose these and maintain a pink coloration of the body during their entire life. The pink color is not due to pigments but to blood circulating under a thin and depigmented skin (Durand 1983). Fish belonging to *Typhlichthys* and *Amblyopsis* lose their grayish color during growth. Aquatic crustaceans may be completely transparent (*Troglocaris*) or white and opaque, their integument being impregnated by calcium salts (e.g., *Proasellus*, *Niphargus*, *Cambarus*). Some aquatic invertebrates can also recover color when exposed to light, and some populations are colored by pigments that are ingested together with the food, such as carotenoids (Ginet and Decou 1977).

In cave populations of *Asellus aquaticus,* albinism (Fig. 4.8 and Chap. 8) can be achieved in two different genetic ways involving three different genes within the same population (Protas et al. 2011), unlike the cave fish *Astyanax mexicanus* where the same gene is involved in the evolution of the albinism in three independently evolved cave populations (Protas et al. 2006). Albinism might make stygobionts more vulnerable to ultraviolet light with deleterious effects (Langecker 2000), and direct light exposure is deadly or stressful for cave planarians (Merker and Gilbert 1932), ostracods (Maguire 1960), and for some amphipods, i.e., *Niphargus virei* (Ginet 1960) and *N. stygius* (Simčič and Brancelj 2007).

Vision *(Eyes or Ocelli in General)* In completely dark caves, selection will strongly favor individuals with a reduced visual system to reduce overall energy expenditure (Moran et al. 2015), although some species retain remnants of these organs (Fig. 4.9). Accordingly, the nerve centers are also reduced, and the reduction is believed to reflect the degree of adaptation to caves or to the ancientness of

Fig. 4.8 The isopod *Asellus aquaticus* lives in surface waters (pigmented populations – upper left) and inside caves (depigmented or translucent populations – center and right) (Photo by © Boris Sket)

adaptation to cave life; some crustaceans have eye structures as embryos or young. All blind cave vertebrates exhibit initial eye development followed by secondary degradation during later development stages (Durand 1976; Jeffery 2009; Jeffery and Strickler 2010). *Proteus* has normally pigmented eyes when they hatch, but these become reduced and the structure altered during development. The two black spots at the side of the head eventually disappear completely (Durand 1971), and this is an ontogenetic regression rather than a phylogenetic regression, which occurs in some fishes and crustaceans.

Eye loss and eye reduction have different genetic bases within populations of *Asellus aquaticus* and in different populations of *Astyanax mexicanus* (Wilkens and Strecker 2003; Jeffery 2005; Borowsky 2008; Protas et al. 2011). Nevertheless, many cave species that were considered blind prove to be microphthalmic and respond to light stimuli in a more buffered way than their stygophilic relatives living in springs (Langecker 2000; Borowsky 2013; Soares and Niemiller 2013).

Other Senses It was assumed that other senses must compensate for the reduction or complete lack of sight, e.g., the sensorial compensation proposed by Poulson (1963) for amblyopsid fishes. The lateral line, olfaction, taste, and touch and electro-sense are hypertrophied in cave species (Langecker 2000) together with drastic modification of the brain (Fig. 4.10). *Proteus anguinus*, with its flattened and enlarged head, can carry a larger number of sensory receptors (Schlegel et al. 2006) and use

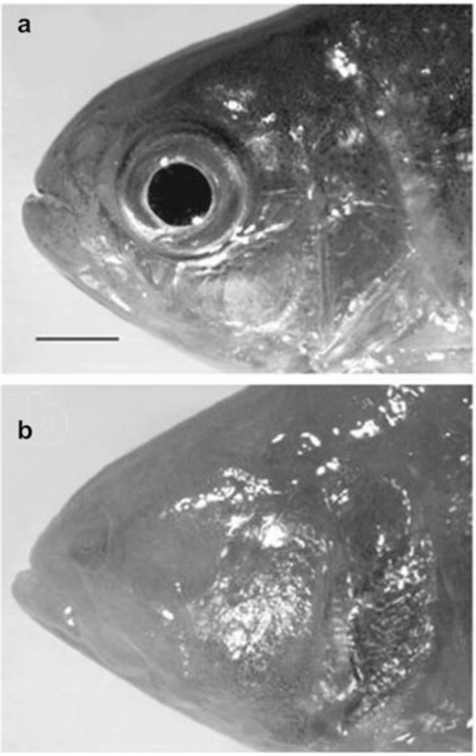

Fig. 4.9 The normal eye in surface *Astyanax mexicanus* (**a**) is reduced in cave populations (**b**), and lack of eyes is compensated by the neuromasts (not shown here) [Photos from McGaugh et al. (2014), with permission]

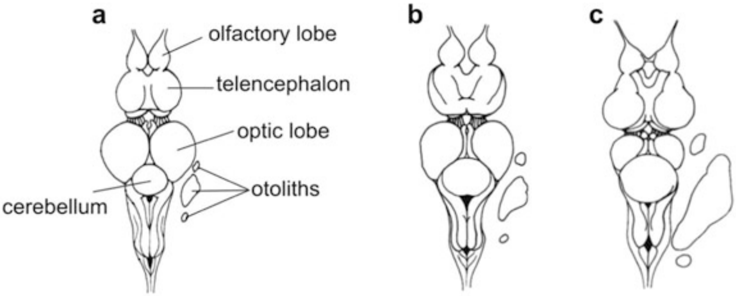

Fig. 4.10 Adaptation of the brain in amblyopsid fishes to life in caves involves the reduction of the optic lobes and the volumetric increase of both the cerebellum and otoliths from the epigean *Chologaster cornuta* (**a**) to the cave adapted *Forbesichthys agassizii* (**b**) and *Amblyopsis rosae* (**c**) [Modified after Culver (1982)]

chemical clues (Uiblein et al. 1992) as well as mechanoreceptors and electroreceptors (Schlegel and Bulog 1997). The cave *Astyanax* have larger jaws and more taste buds that together with a modified brain induce changes in behavior (Yamamoto et al. 2009). The lateral line of fishes consists of a canal and superficial neuromasts; the superficial neuromasts in cave *Astyanax mexicanus* are several times more numerous than in surface fish (Schemmel 1967; Teyke 1990; Jeffery et al. 2000). Cave fishes neuromasts are also larger and contain more sensory hairs than those of surface fish. The superficial neuromasts cupulae (hair cells covered by a gelatinous case) are almost eight times longer in cave fishes compared to surface fishes (Teyke 1990), which enhance detection and reaction to vibrating stimuli in cave fishes (Yoshizawa et al. 2010). In populations of *Asellus aquaticus*, the aesthetascs acting as chemoreceptors are longer, and setal patterns change in cave populations compared to surface relatives (Turk et al. 1996; Prevorčnik et al. 2004). In the cave shrimp *Troglocaris*, lengthening of the rostrum and significant enlargement of the postorbital carapace represent defense mechanisms against predation by *Proteus*. This adaptation has been observed in places where these two taxa co-occur (Jugovic et al. 2010).

Some species show reduction of senses in addition to vision, e.g., the lack of statocysts in stygobiontic Bathynellacea and the scarcity of aesthetascs in stygobiontic isopods (Camacho 1992), although in-depth studies are still scarce and incomplete for most groups. The reduction of the optic lobe is correlated with enlarged deutocerebral olfactory centers in malacostracans (Crustacea), which implies an important role of the olfactory sense in this group (Stegner et al. 2015). On the other hand, within Peracarida (Crustacea), the missing optic sense is not correlated with an emphasized mechanosense.

The modification of the pineal organ is variable in different cave fishes, ranging from disorganization to complete reduction, as in *Typhlichthys subterraneus* (Langecker 2000). The pineal photoreceptors in fishes have the combined roles of photoreception and as neuroendocrine cells for the synthetization and secretion of the melatonin hormone under the control of light.

Body Size The void sizes of groundwater microhabitats strongly select the size of the inhabitants resulting in giants or dwarfs of the cave world (Ginet and Decou 1977). In some Harpacticoida (Crustacea), that live in small and narrow spaces, the body size, length and number of the swimming legs, and setae number are reduced [e.g., in *Parastenocaris*, *Speocyclops*, and *Morariopsis*; Brancelj (2004)]. In some genera, body size is highly variable even within species inhabiting the same cave (Fig. 4.11).

4.4.2 Physiological Adaptations of Stygobionts

Metabolism The reduced metabolic rate and energy-saving behaviors of stygobionts have been experimentally demonstrated in cave fishes and crustaceans (Hervant et al. 1997, 1998); it is as an adaptation to food and oxygen stress in cave habitats (Hervant and Renault 2002) or a response to energy economy (Hüppop 2005). In other species there is apparently no difference in the metabolic rates of cave species and surface relatives, as in *Astyanax mexicanus*, a species that is not experiencing food limitation (Culver 1982).

Fig. 4.11 Ecomorphs of *Niphargus* showing that the elongation of the appendages and the shape of the body depend on the occupied microhabitat [Modified after Trontelj et al. (2012)]

Oxygen Consumption The first observations of low oxygen consumption in cave inhabitants were made by Eigenmann in 1909 through observing the opercula movement of *Amblyopsis spelaeus*. Deep cave waters, especially in the phreatic zone, are often hypoxic (1 mg O_2/L), but hypoxia can be also found few meters below the water table (Malard and Hervant 2012). The response of the crustaceans *Niphargus* and *Stenasellus* to hypoxia is a rapid reduction of locomotory and respiratory activities, which allows longer survival. Recovery after periods of low or no oxygen is also rapid, and the lactate resulting from the anaerobic metabolism is reused, unlike surface *Gammarus* and *Asellus* crustacean relatives which oxidize the lactate (Malard and Hervant 2012).

Fasting Stygobionts can store more fat than their epigean spring relatives (Hüppop 2000) and makes fasting for long periods a characteristic of stygobionts. *Niphargus* can survive for more than a year (Gibert and Mathieu 1980; Mathieu and Gibert 1980), *Stenasellus virei* for more than 200 days (Hervant et al. 1997), *Troglocaris* up to 2 years (Vogt and Štrus 1999), and *Proteus* up to 96 months of starvation without showing any signs of disease (Vandel 1964; Hervant et al. 2001).

4.4.3 Behavioral Adaptations of Stygobionts

Circadian/Seasonal Rhythm Experiments on the cave *Niphargus virei* and the epigean *Gammarus pulex* demonstrated that the cave species moved one-tenth as much as the epigean species and that *Niphargus* had fewer periods of activity during daylight than *Gammarus* (Ginet 1960; Camacho 1992). Cave fishes and crustaceans have their own internal rhythms not linked with changes at the surface (Poulson and Jegla 1969); *Proteus* is another cave representative that does not display a circadian rhythm (Schatz et al. 1977). Weak activity patterns are connected to low oxygen consumption in *Orconectes pellucidus* (Jegla and Poulson 1968), but these crayfish still kept a rhythm of molting and reproduction in response to changes in both water temperature and volume (Jegla and Poulson 1970).

The existence of periodicities in the life of aquatic cave animals might be inherited from their epigean ancestors (Trajano and Menna-Barreto 1995) and subsequently evolve in the absence of light inside caves as well as to the scarcity of food sources, at least during some periods of the year. Photosensitivity or photokinesis (defined as sensitivity to light) experiments showed that cave fishes and crustaceans react to a certain intensity of light by an avoiding reflex and by hiding, which proved to be a conserved ability to detect light of the pineal eye in cave fishes (Yoshizawa and Jeffery 2008). Surface amphipod species showed a weak photophobic, photoneutral, or in one case, photophilic response, whereas all cave species showed a strong photophobic response (Fišer et al. 2015). On a regional scale, photophobia could explain limited dispersal and a high degree of endemism observed among subterranean species (Fišer et al. 2015) that cannot use surface waters for dispersal.

Agonistic Behaviors Such behaviors include the reduction in aggressive and submissive reactions observed in cave fishes. The highly cave-adapted *Amblyopsis rosae* and *Typhlichthys subterraneus* engage in much shorter, simpler, and less intense agonistic bouts, most frequently being the tail-beating (Bechler 1983). The explanations for reduced agonistic behavior are the reduced selection pressures, decreased metabolic rate, and increased longevity that all confer the advantage of energy conservation (Parzefall 1992). In contrast, aggressive behavior linked to territoriality was observed at *Proteus anguinus* for short periods of time (Parzefall 1976).

Active search for cryptic microhabitats in cave waters (under rocks, in clay, and within small voids in the calcite formations) was observed in *Proteus,* which otherwise rest most of the time by "hiding" under rocks; and they preferred places formerly used by other individuals, which had deposited a substance at the resting place (Parzefall et al. 1980).

Schooling Behavior Fish schooling and aggregation behaviors are social and group activities exhibited by fishes for foraging, reproduction, and defense from predators. Fish in schools can also obtain a hydrodynamic advantage, thus reducing the cost of locomotion. A reduction of this behavior has been observed in cave fishes and is believed to be an adaptation to the lack of visual orientation or to a change in stabilizing selection for schooling (Parzefall 1992). Tests on different generations on *Astyanax fasciatus* led to the conclusion that the reduction of this behavior is genetically based (Kowalko et al. 2013).

Feeding Behavior The studied cave fishes search for food by holding their body at an angle of 55° to the bottom substrate (Schemmel 1980); they also use a much larger area of dense taste buds on the ventral side of the head, compared to epigean species that have such buds only at the tip of the mouth (Wilkens 1992). Some stygobionts occasionally may use resources outside of caves during the night, i.e., *Proteus* has been observed feeding overnight outside caves (Sket 2008), and *Niphargus timavi* stomach content was full of ephemeropteran larvae that live outside caves (Fišer et al. 2010).

Predation The lack of predators may account for the observed reduction of defensive behaviors in some cave fishes, although cannibalism by bigger individuals is present in *Astyanax* (Parzefall 1983). The decapods *Orconectes* and *Procambarus* have a more intense reaction to the presence of predators than their epigean relatives if water movements accompany their presence, but not in the presence of chemical cues. In the presence of chemical cues, epigean species responded quicker than cave species (Ginet and Decou 1977).

4.4.4 Special Adaptations

Life Cycles The adult stage is exceptionally long in cave species, being up to 35 years for *Niphargus* (Turquin and Barthelemy 1985), more than 2 years for harpacticoids (Ginet and Decou 1977), and reaching dozens of years for the cave crayfish *Orconectes australis australis* (Cooper and Cooper 1976). *Proteus* females become mature at an average age of 15.6 years and reproduce every 12.5 years for a lifespan of 68.5 to 102 years (three times higher when compared to its most closely related species; Voituran et al. 2011). The timing and rhythm of reproduction is easy to check for many aquatics as ovigerous females are easily identified and censused. For example, the cave isopod *Stenasellus* reproduces once every two years in good conditions, but with low food supply and the interval between reproductions can be much longer (Fig. 4.12). Nearly every phase of the development cycle is much longer in cave animals than in related surface animals, as was demonstrated for amphipods and harpacticoids (Ginet and Decou 1977; Rouch 1968). In contrast, the life cycle of epigean invertebrates is normally completed within a single year or few months during one year.

Number and Size of Eggs The number of eggs produced by stygobionts is often one-tenth of their epigean relatives. There are also cases in which females carry a single egg, as in Microparasellidae (Coineau 1971). *Niphargus* females lay more voluminous eggs compared to their surface relatives (Fišer et al. 2012) but invest less in a single brood, similarly to cave fishes from different genera (Poulson 1963). Nevertheless, investment in egg quality and high survival rate in *Niphargus* is not correlated with low number of eggs but to an increase in the body size of adults, which can lay bigger eggs (Fišer et al. 2010); the increased size of the coxal plates in *Niphargus* females provides better aeration of the large eggs (Fišer et al. 2012). Coevolution of traits, such as egg size, aeration capacity, and number of eggs, minimizes the overall costs and contributes to lifetime fitness (Fišer et al. 2012). A female of *Amblyopsis spelaea* produces 40–60 large, heavily yolked eggs (Poulson 1963) and the cave *Poecilia mexicana,* with smaller females than their surface relatives, produces larger offspring (Riesch et al. 2011). For *Proteus*, the clutch comprises about 35 eggs of which less than 50% hatch (Voituran et al. 2011).

Reduced sleep is a novel trait aquired by stygobionts, with genes underlying the behavioral regression independent from those responsible for eye and pigment

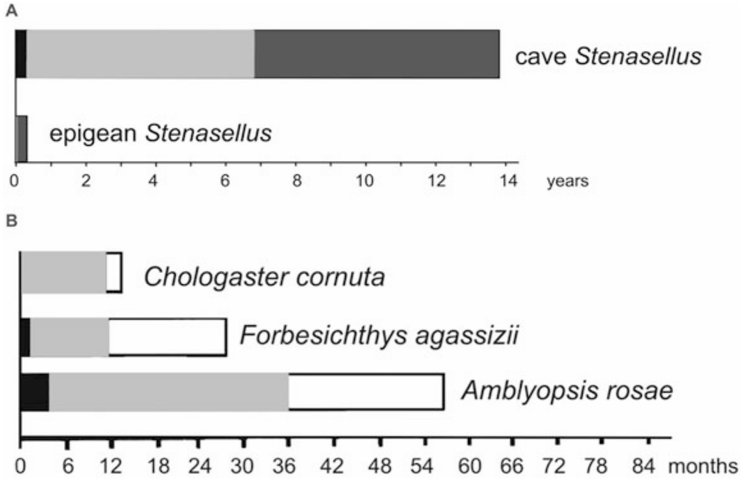

Fig. 4.12 The lifespan of the stygobiont *Stenasellus* (**a**), black = time the female carries the embryos, light gray = juvenile period, dark gray = adult period, and of cave fishes at different levels of adaptation to life in caves (**b**), from the epigean *Chologaster* to the more cave adapted *Forbesichthys* and *Amblyopsis,* black = time to hatching, light gray = time from hatching to first reproduction, white = reproductive lifespan [Modified after Ginet and Decou (1977; **a**) and Poulson (1963; **b**)]

regression. In the same time, sleep regression evolved convergent in different, isolated cave populations (Duboué et al. 2011). Cave *Astyanax mexicanus* have drastically reduced sleep periods, and both daytime and nighttime sleep are reduced, compared to their surface relatives (Soares et al. 2004).

Resistance to high concentration of sulfides in the water by *Niphargus* has been associated with the resistance to low oxygen, or with traits involved in detoxification, such as symbiosis with *Thiothrix* bacteria (Dattagupta et al. 2009). Adaptive behaviors that improve oxygen uptake were proposed for *Niphargus* and a population of *Poecilia mexicana* from Cueva de Villa Luz (Mexico), respectively (Plath et al. 2007; Tobler et al. 2011, 2014).

Box 4.3

A comparison between cave-adapted *Asellus* from freshwater in Slovenia and sulfuric waters in Romania shows that there is a parallel evolution of eye loss, depigmentation, and elongation of some appendages. Water chemistry, nutrient availability, and other factors like ancestral genetic variability and population genetic structure are not hampering the convergent adaptation to caves (Konec et al. 2015).

Thermal Tolerance Experiments on three cave *Proasellus* isopods show different response for species that live in a habitat with less than 1 °C yearly temperature amplitude. Two of the species were sensitive to changes of less than 2 °C, while the third species tolerated a variation of 11 °C, which shows that thermal physiology is not solely shaped by the temperature in the natural environment for all groundwater species (Mermillod-Blondin et al. 2013).

Salinity Tolerance Salinity tolerance is characteristic for the complex anchialine systems where cave fauna is separated by salinity-stratified water layers (Iliffe and Kornicker 2009; see also Chap. 17). Remipeds are restricted to the marine component of the systems, and some atyid shrimps can be found in both marine and less saline layers. The effect of salinity tolerance on habitat connectivity and dispersibility of species living in these complex systems remains to be tested (Moritsch et al. 2014).

Neoteny or pedomorphosis is striking for some of the cave salamanders. *Eurycea spelaea*, *Gyrinophilus subterraneus*, and *Proteus anguinus* are neotenic, keeping the gills outside the body and an aquatic way of life even as adults.

Ethophysiological Adaptations Cave animals display strong stereotropism (a reflex in which the contact with a rigid surface is the orienting factor) and other ethophysiological strategies (Ginet and Decou 1977; Holsinger 1988). For example, *Niphargus* adapt to dry periods by digging a water-filled hole in wet clay that allows survival of individuals even when water dries out (Ginet and Decou 1977).

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Chapter 5
The Microbial Diversity of Caves

Olivia S. Hershey and Hazel A. Barton

5.1 Introduction

In attempting to describe microbial diversity, Baas-Becking famously stated that “Everything is everywhere, but the environment selects” (Baas-Becking 1934). His hypothesis was simple—the small size of microorganisms allows their broad environmental distribution, with the appropriate conditions selecting species growth to dominate niche space (Baas-Becking 1934). Given the many types of caves, and hence a myriad of environmental conditions, it is difficult to describe a broadly relevant microbial diversity; varying cave conditions provide opportunities to select a multitude of adaptations and hence community diversity.

Despite this drawback, caves do represent an important environment for studying microbial ecology, chiefly due to community adaptations to the resource limitation of the subsurface; the lack of photosynthetic activity in caves requires microorganisms to rely on either the heterotrophic breakdown of scant allochthonous organic carbon or autotrophic growth using in situ redox-active compounds (Peck 1986; Northup et al. 2003; Carmichael et al. 2013; Desai et al. 2013; Parker et al. 2013; Jones and Macalady 2016). While some caves, including sulfidic systems, are dominated by chemolithotrophic primary production, such cave systems are relatively rare (and their microbial diversity is reviewed in Chap. 15). In more common epigenic caves, nutrients entering from surface-derived ecosystems are limited, with allochthonous organic carbon delivered by vadose-zone groundwater generally measured below 0.5 mg/L (Barton 2015). This puts cave environments firmly in the oligotrophic (<2.0 mg/L) spectrum of energetic systems; for simplicity, we will to refer to such systems as *oligotrophic* caves (Engel et al. 2010; Harmon et al. 2013; Barton 2015).

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Fig. 5.1 A passage within Poor Farm Cave, West Virginia, USA. Based on the age of fossil skeletons found within cave sediments, it is unlikely that the environmental conditions within this passage have significantly changed in the last 400,000 years (Grady et al. 2000). Energy for microbial growth likely comes from allochthonous organic carbon entering via cracks or fissures in the bedrock, or the presence of redox-active compounds [Mn(II), Fe(II), NO_3^-, or NH_3].

Another unique feature of caves, and one commonly overlooked by microbiologists not used to working in such systems, is the static nature of the environment; once cave forming processes have ended, the environmental conditions of a particular niche might not change for thousands (to potentially millions) of years (Fig. 5.1; Klimchouk et al. 2000; Palmer 2007). This is in stark contrast to surface ecosystems, where plant species, foraging animals, anthropogenic impacts, and even the weather can have a profound influence on microbial community structure in short (daily, seasonal) time frames (Barton 2015; Palmer 2007). The absence of these dynamic processes in caves means that the selective pressures driving diversity are dependent on the variables that are present and strongly influenced by the geologic setting and geochemical (environmental, local and regional) nature of the cave (Ortiz et al. 2013).

This review will not try to provide a synthesis of the ~400 papers published on cave microbiology (Fig. 5.2). Instead, it will focus on the microbiology of oligotrophic caves in limestone (carbonate; $CaCO_3$) settings. We will also not produce a comprehensive list of the microbial species found in caves, as this has recently been reviewed elsewhere (Vanderwolf et al. 2013; Tomczyk-Żak and Zielenkiewicz 2016). Instead, we will attempt to outline how the research history has led to our current understanding of microbial ecology in caves, the potential for a *core microbiome,* and the common ecological themes that might drive microbial diversity in caves.

5.2 Microbial Diversity in Caves Prior to 1996

Our understanding of the microbial ecology of caves is influenced by the technology of the time (Engel 2015). As microbiology was built upon the ability to culture microorganisms within the laboratory, early cave researchers used the same cultivation techniques as soil scientists and (somewhat unsurprisingly) found that caves were a weak reflection of the microbiology of surface soils (Hess 1900; Scott 1909; Høeg 1946; Caumartin 1963). The interpretation of microbial activity in caves was therefore limited, and it seemed to be of little interest to the scientific community, with less than 40 papers published prior to 1997 (Fig. 5.2; Engel 2015). Yet these papers defined our understanding of cave microbiology, suggesting that caves were essentially lifeless due to an absence of photosynthetic input, or simply home to transient microbial species introduced by the activity of animals or humans (Caumartin 1963).

When endemic cave microorganisms were putatively identified, it was through unusual metabolisms that were (incorrectly) thought to distinguish them from soil species, such as iron oxidation (Caumartin 1963).

The primary limitation of cultivation-based approaches is that the vast majority (>99%) of environmental microorganisms cannot be cultured; as in other microbial environments, the ability to accurately describe microbial diversity within caves required cultivation-independent techniques (Amann et al. 1996). Some early non-cultivation approaches did support the idea that microbiology in caves was more complex than originally thought: Fliermans and Schmidt (1977) used antibodies to identify non-culturable *Nitrobacter* in Mammoth Cave sediments; the microscopic techniques of Cunningham et al. (1995) demonstrated a rich structural diversity from samples deep within Lechuguilla Cave; and Gonzalez et al. (1999) demonstrated a rich diversity of actinobacteria in Spanish caves using fatty acid methyl ester (FAME) analyses (Fliermans and Schmidt 1977; Cunningham et al. 1995; Gonzalez et al. 1999). Nonetheless, it wasn't until the use of molecular phylogenetics in the 1990s that the potential diversity of microorganisms in cave environments emerged (Fliermans and Schmidt 1977; Cunningham et al. 1995; Pace 1997; Gonzalez et al. 1999; Barton 2006; Barton and Northup 2007; Engel 2010; Lee et al. 2012).

5.3 Microbial Diversity in Caves: The Molecular Era (1997–2012)

First introduced in the 1980s as a revolutionary way of identifying microorganisms in the environment, Pace et al. used the 16S small ribosomal subunit rRNA gene sequence as a genetic marker (phylotype) to distinguish previously uncultured species (Stahl et al. 1984; Pace et al. 1986). It was also Pace (himself an avid cave explorer) who facilitated the first molecular analysis of a microbial cave community (Fig. 5.2). These investigators used molecular phylogenetic approaches to examine the filamentous biofilms of a sulfidic stream within Sulfur River Cave,

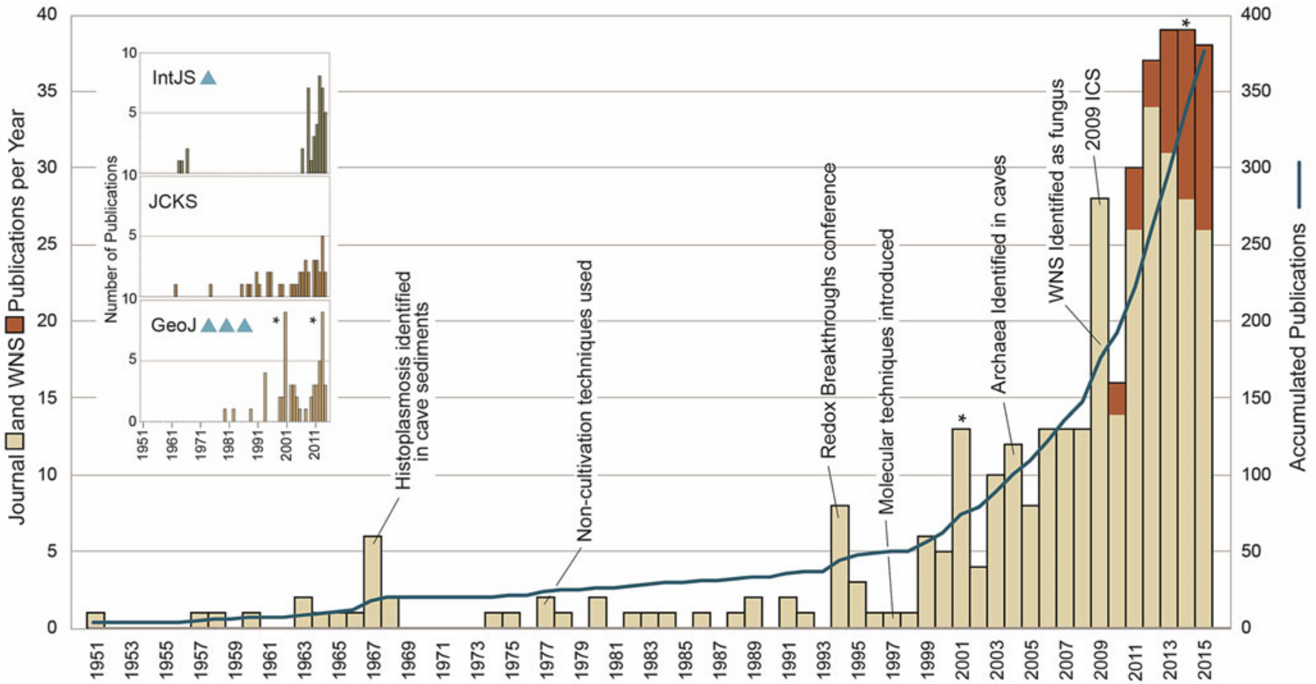

Fig. 5.2 Impact of major events in cave microbiology on research in the field, as measured by publications in peer-reviewed journals (abstracts and conference proceedings are not included in cumulative totals). The number of publications per year is indicated, cumulative total of publications, as well as the impact of the WNS epidemic on cave microbiology publications. Redox Breakthroughs Conference = Breakthroughs in Karst Geomicrobiology and Redox Geochemistry Conference (see Sasowsky and Palmer 1994). WNS = White-Nose Syndrome. 2009 ICS = International Congress of Speleology Special Section: Microbiology and Geomicrobiology of Cave and Karst Environments. The "*" symbol indicates the publication of a special issue on Cave Microbiology by the *Geomicrobiology Journal*. Inset: histogram of publication rates by the primary journals in the field, including *International Journal of Speleology* (IntJS), *Journal of Cave and Karst Studies* (JCKS), and *Geomicrobiology Journal* (GeoJ); blue triangles correspond to the number of cave microbiologists who serve as associate editors

Kentucky (Angert et al. 1998). This study revealed the surprising dominance of the *Epsilonproteobacteria,* which were previously seen only in deep, oceanic hydrothermal systems; it was also the first clue to the important influence that members of this phylum have within sulfidic cave environments (Campbell et al. 2006). Most importantly, the study also demonstrated that microbial cave communities could be remarkably distinct from their surface counterparts (Angert et al. 1998).

Through the 1990s molecular phylogenetics was the most powerful tool to study microorganisms within the environment (Pace 1997). But the tool remained limited to labs with both the molecular expertise and computing resources necessary to translate genetic difference into the robust phylogenies necessary to identify uncultivated microorganisms, with only two labs carrying out such analyses in caves (Vlasceanu et al. 1997; Angert et al. 1998). The ability to analyze cave communities was further complicated by the low biomass of these environments (routinely $<10^6$ cells/g), along with a complex geochemistry, both of which interfered with the ability to obtain sufficient DNA for analysis (Barton et al. 2006). This restricted early analyses to sites with enough biomass to overcome DNA extraction limitations, such as those found in the chemolithotrophic ecosystems of sulfidic caves, as examined by Pace (Sarbu et al. 1996; Angert et al. 1998; Engel et al. 2004a).

A lot changed over the next 15 years: new techniques improved the ability to extract DNA from the environment (Barton et al. 2006; Tan and Yiap 2009); new programs made phylogenetic analysis more accessible (Posada 2003; Wilgenbusch and Swofford 2003; Edgar 2004; Kumar et al. 2006; Pruesse et al. 2007); sequencing technologies reduced costs (Shendure et al. 2004); and high-impact journal articles demonstrated the important contributions that the study of cave microorganisms could provide (Cunningham et al. 1995; Engel et al. 2004b). A number of events further raised the profile of cave microbiology, beginning with the 1994 *Breakthroughs in Karst Geomicrobiology and Redox Geochemistry* Conference (59 conference proceedings), a special issue of the *Geomicrobiology Journal* in 2001 (10 journal articles), and a special session on the *Microbiology and Geomicrobiology of Cave and Karst Environments* at the 2009 International Congress of Speleology (32 conference papers). The cumulative impact of these changes was a fivefold increase in the number of journal articles on cave microbiology between 1997 and 2012 (from 43 to 256; Fig. 5.2).

Traditional molecular phylogenetic approaches involve PCR amplification of 16S rRNA gene sequences from environmental DNA, followed by cloning or denaturing gradient gel electrophoresis (DGGE) to generate libraries of representative 16S rRNA sequences (Pace 1997). These libraries can range in size from a few dozen to a few hundred cloned sequences. Nonetheless, given the tens of thousands to potentially millions of microbial cells in every sample, such “clone” libraries tend to identify the species/phylotypes within an environment that make the greatest contribution to total environmental DNA and/or 16S rRNA copy number (Fig. 5.4; DeSantis et al. 2007; de Araujo and Schneider 2008; Kembel et al. 2012). While this does allow a snapshot of the most successful microorganisms within an environment, it is also a limitation of the technique, potentially missing a large number of organisms that play important roles in ecosystem function (Fig. 5.4).

Box 5.1

This dramatic rise in microbial research also opened our eyes to the potential diversity of microorganisms in cave environments. Rather than supporting the idea that caves were dominated by a few specialized species adapted to nutrient limitation, caves appeared to be home to a diverse assemblage of species from multiple phyla, including the *Alpha-, Beta-, Gamma-*, and *Deltaproteobacteria, Chloroflexi, Planctomycetales, Bacteroidetes*, Acidobacteria, and Actinobacteria, with small but significant contributions from members of the *Nitrospirae, Gemmatimonadetes*, and *Verrucomicrobia* (Fig. 5.3; Northup et al. 2003; Barton et al. 2004; Chelius and Moore 2004; Barton et al. 2007; Zhou et al. 2007; Cuezva et al. 2012; Lee et al. 2012; Porca et al. 2012; Rusznyak et al. 2012; Ivanova et al. 2013; Barton 2015). These data also demonstrated the potentially significant contribution of the archaea to subsurface communities (Northup et al. 2003; Chelius and Moore 2004; Shabarova and Pernthaler 2010).

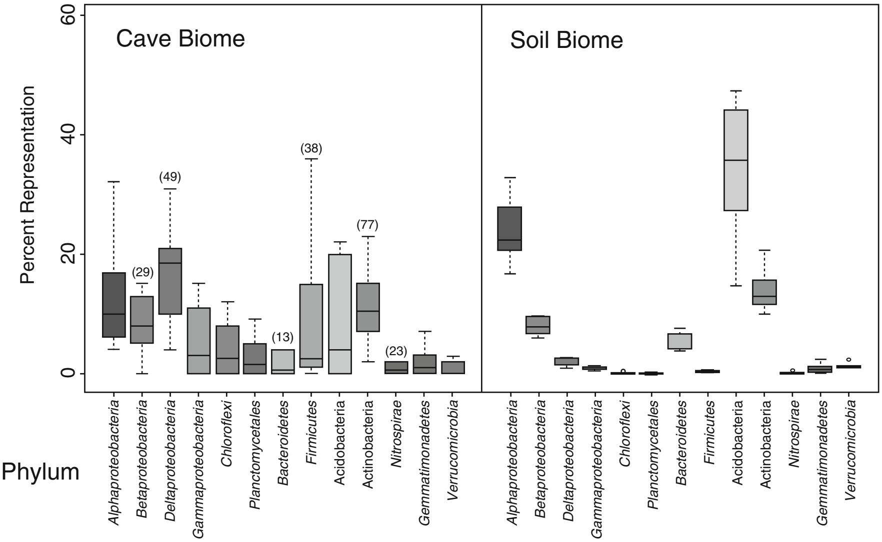

Fig. 5.3 Box plot comparison of soil microbial communities to those found in caves. The representation of major phyla of pooled soil and cave samples is shown. The soil biome was obtained from the collated data of Chu et al. (2010). The cave biome was collated from the data of Northup et al. (2003), Barton et al. (2004, 2007), Chelius and Moore (2004), Zhou et al. (2007), Cuezva et al. (2012), Porca et al. (2012), Rusznyak et al. (2012), and Ivanova et al. (2013). Only datasets including at least 100 cloned 16S rRNA phylotypes are included. The boundaries for the first and third quartile are shown, with the centerline representing the mean and whiskers representing the max/min values (outlier values for the cave biome data are shown in parentheses)

5.4 Microbial Diversity in Caves: The Genomics Era (2013–Present)

Among the many impacts of the Human Genome Project, the most powerful was the development of optically based sequencing methods—collectively referred to as "next-generation sequencing" (NGS) technologies (Ansorge 2009; Lander 2011). The dramatic increase in the number of bases that these technologies could sequence (>15 billion bases in as little as 4 h) combined with their significant cost reductions revolutionized the ability to sequence DNA (Snyder et al. 2009; Forde and O'Toole 2013). Sogin et al. (2006) were the first to use NGS to identify environmental 16S rRNA; rather than restricting the identification of phylotypes within a community to a few hundred cloned 16S rRNA genes, NGS allowed Sogin and colleagues to sequence 120,000 PCR products directly. The results were transformative and demonstrated that microbial ecosystems contained thousands of previously unidentified phylotypes (Sogin et al. 2006). Sogin et al. referred to this extensive collection of previously unidentified microorganisms as the "rare biosphere"—organisms of sufficiently low number that they cannot be identified without deep-sequencing NGS approaches (Fig. 5.4).

Due to limited access to NGS and the advanced statistical methods needed to distinguish unique DNA sequences against a background of inherent PCR error, this technology was also initially limited to a few specialized labs (Sogin et al. 2006). But as

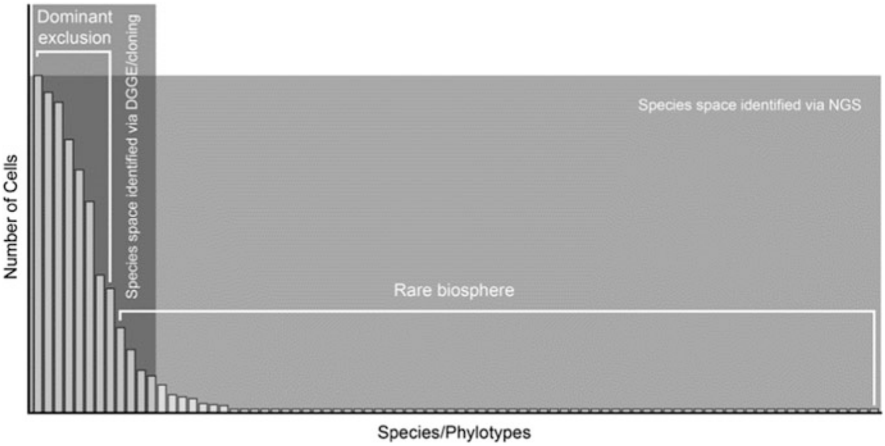

Fig. 5.4 Idealized microbial diversity in cave environments and representative portions screened via different molecular techniques. Some species are able to rapidly utilize the available nutrient and energy sources, providing a competitive advantage that allows them to become dominant in the ecosystem (similar to *r*-type selection). These dominant species are most often identified in shallow-coverage analyses, such as clone libraries and DGGE. The rare biosphere contains a combination of numerically low, slower-growing, poorly adapted, or even viable but non-growing species (similar to *K*-type selection). The rare biosphere can usually only be identified using deep-sequencing approaches, such as Illumina sequencing. NGS, next-generation sequencing

researchers developed more efficient mathematical algorithms to reduce the necessary computational power, and techniques in bioinformatics simplified the analysis of large NGS data sets, this technology rapidly became available to other researchers (Kuczynski et al. 2012; Caporaso et al. 2010; Stamatakis 2014). The first to apply these applications in cave environments were Ortiz et al. (2013) who used 454-pyrosequencing to examine ~400,000 PCR products from Kartchner Caverns, USA (Ortiz et al. 2013). Along with the 13 phyla already identified in caves by cloning approaches, Ortiz et al. demonstrated the presence of an additional 8 described and 12 candidate phyla, suggesting that caves also contained rare biosphere microorganisms. In a significant step forward, these researchers also used NGS to compare microbial communities in the cave with those in surface soils directly above. These data demonstrated that only 16% of the sequences were shared between the surface and the cave, confirming the uniqueness of microbial cave ecosystems (Ortiz et al. 2013).

In the years since this study, there have been no other published 16S rRNA NGS sequence datasets from oligotrophic caves; however, a number of studies have submitted sequence data to public databases, such as the NCBI sequence read archive (SRA) (Fig. 5.5; Leinonen et al. 2010). By processing these datasets, it is possible to expand the work of Ortiz et al. across multiple cave systems with broadly distributed geographical locations, including North America and Asia (Fig. 5.5). The results confirm the robustness of the 13 dominant phyla already identified, along with another 14 phyla consistently represented in these populations (above a 0.1% threshold); these include the *Armatimonadetes* (OP10)*, Chlorobi, Cyanobacteria, Elusimicrobia, Spirochetes*, and the candidate phyla BRC1, GN04, NC10, OP3 (Ca. *Omnitrophica*), TM6 (Ca. *Dependentiae*), WS1, and WS3 (Ca. *Latescibacteria*). Together these data support the existence of a *cave rare biosphere* (Fig. 5.5). The cave NGS datasets also contain a

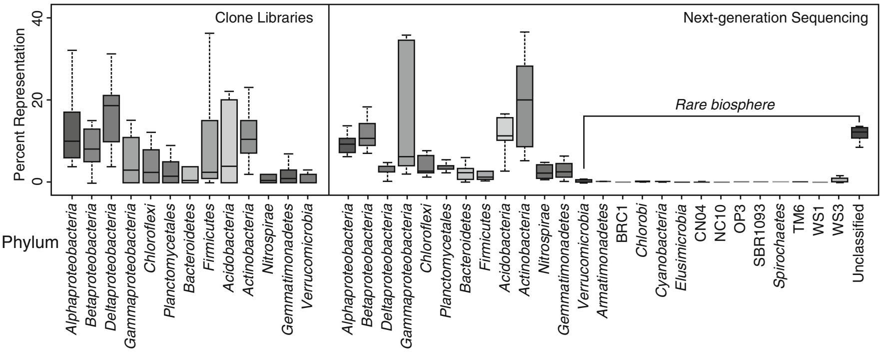

Fig. 5.5 Boxplot comparison of microbial cave community structure analyzed by 16S rRNA gene clone libraries (from Fig. 5.3) or Illumina (next-generation) sequencing. The datasets SRR1686967, SRR1686970, SRR1686976, SRR1693633, SRR1703816, SRR1703817, SRR1703818, SRR1703819, SRR1703820, and SRR1703821, obtained from the NCBI sequence read archive (SRA), were used. The datasets, which contained between 12,901 and 585,434 unfiltered sequence tags, were processed in QIIME (Caporaso et al. 2010) to assign taxonomic identity. The boundaries for the first and third quartile are shown with the centerline representing the mean and whiskers representing the max/min values

significant proportion of sequences that cannot be classified using the taxon reference databases, Greengenes and SILVA (Fig. 5.5; McDonald et al. 2012; Quast et al. 2013). These sequences, which cannot be easily placed within the current taxonomic framework, are known as *microbial dark matter* and represent the currently unexplored diversity of microbial populations (Rinke et al. 2013). Such sequences tend to have a higher representation in caves than other habitats, suggesting that the true diversity of caves requires further description (Sogin et al. 2006; Rinke et al. 2013).

While targeted PCR amplification makes it possible to rapidly screen the 16S rRNA sequences in the environment, it is also susceptible to significant technical issues, including primer and amplification biases that preferentially select certain DNA sequences for amplification (Chandler et al. 1997; Polz and Cavanaugh 1998; DeSantis et al. 2007; Kembel et al. 2012). Overcoming these limitations requires bypassing the PCR amplification step entirely and sequencing the sum of the genetic information in the environment (Miller et al. 2011). This process requires randomly fragmenting DNA into sizes appropriate for NGS sequencing (35–300 bp), either by mechanical means or using transposons (Adey et al. 2010). These fragments are then sequenced, and the overlapping ends are computationally reassembled back into a full-length DNA contig, ranging in size from a few hundred to millions of bases—a technique referred to as "shotgun sequencing" due to the randomness of the initial DNA fragmentation (Sanger et al. 1977; Adey et al. 2010). Prior to the advent of NGS, shotgun methods were not possible using environmental DNA as the complexity of the samples reduced the likelihood of obtaining sufficient coverage for assembly (Venter et al. 2004). But NGS dramatically increased sequence coverage, making it possible to examine all the genes in an environment rather than just one—a technique called *metagenomics*. Such metagenomic approaches allow the interactions that support microbial ecosystem dynamics to be identified through the functional gene composition of the community (Handelsman 2004; Tyson et al. 2004; Venter et al. 2004).

Carrying out metagenomic approaches in oligotrophic caves continues to be problematic, primarily due to the significant amounts of DNA that are needed to create shotgun libraries, from a minimum of a few hundred nanograms to multiple micrograms, depending on the method (Thomas et al. 2012). Despite these limitations, in 2014 Ortiz et al. were able to carry out metagenomic analyses of the microbial communities within Kartchner Caverns. Their data identified over 365,000 gene fragments from the microbial populations found on speleothems and walls within the cave and demonstrated that the enrichment of genes involved carbohydrate metabolism and CO_2 fixation. The enrichment of these genes suggested that both heterotrophic and autotrophic metabolic activity were important in community growth and subsistence, along with potentially novel mechanisms of nutrient cycling, especially in regard to nitrogen.

To date, the technical limitations of low biomass have prevented other researchers from publishing metagenomic studies from oligotrophic caves. Nonetheless, alternate approaches still allow researchers to use NGS to explore evolutionary adaptations, including genomic sequencing of cultured isolates (Lee 2008; Land et al. 2009; Bhullar et al. 2012; Barton et al. 2013; Saw et al. 2013; Gan et al. 2014; Jiao et al. 2015). Land et al. (2009) were the first to sequence the genome of a bacterial species isolated from a cave: *Beutenbergia cavernae*. Their data demonstrated the significant contribution of carbohydrate catabolism and nutrient cycling genes to the genotype of this organism, mirroring the results of Ortiz et al., despite the large geographic distance between the two cave sites (China versus Arizona, USA) (Land et al. 2009). A culture study by Bhullar et al. (2012) on antimicrobial resistance phenotypes in Lechuguilla Cave (USA) sequenced the genomes of a number of isolates, revealing the presence of a novel antibiotic resistance pathway and suggesting the in situ evolution of antibiotic resistance. Finally, a comparative study between cave and soil strains of *Pseudomonas fluorescens* determined that cave isolates had adapted to living within a mineral (rather than soil) environment, demonstrating genomic traits that could be considered evidence of endemism, including horizontal gene transfer events, increased scavenging efficiency using twitching motility, and an increased ability to cycle nutrients, particularly nitrogen (Barton et al. 2013). Thus, while culturable organisms within caves do represent a small minority (<1%), they are still able to provide important clues to microbial adaptation within caves (Land et al. 2009; Bhullar et al. 2012; Barton et al. 2013).

5.5 Ecological Themes in Cave Microbial Communities

Box 5.2

Microbial communities in oligotrophic caves are dominated by the *Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Gammaproteobacteria, Chloroflexi, Planctomycetales, Bacteroidetes, Firmicutes,* Acidobacteria, Actinobacteria, *Nitrospirae, Gemmatimonadetes*, and *Verrucomicrobia* (Fig. 5.3). This distribution appears to be robust across a broad geographic range, indicating that the key drivers of cave community structure are consistent and that this diversity represents a core cave microbiome.

More than 80 years after Baas-Becking developed his theory of "everything is everywhere..." the identification of a rare biosphere appears to provide the necessary empirical support—a cosmopolitan distribution of microorganisms that can proliferate or "bloom" under the appropriate conditions (Lynch and Neufeld 2015). If all environments contain such functionally diverse populations, then the primary drivers of population dynamics and structure are simply those factors that favor one microorganism over another (Lynch and Neufeld 2015).

Yet these same 13 phyla (see Box 5.2) are also dominant in soils, which have remarkably different conditions of light, productivity, disturbance, and pH (Fig. 5.6). The simplest explanation of such similarity is that soil microorganisms seed caves. The seeding hypothesis for cave community structure would certainly provide an explanation of how cave environments are populated: the solvent action of surface (meteoric) water creates the cave, while also carrying microbial species into this newly

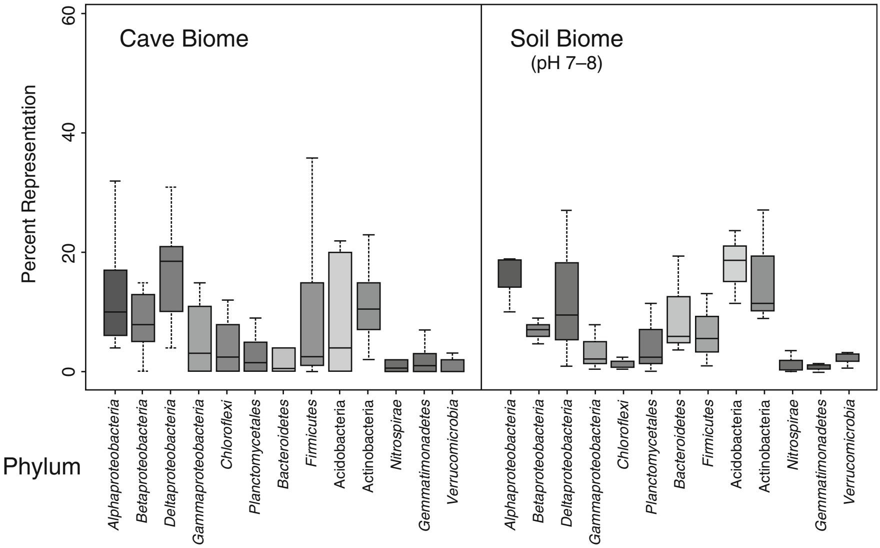

Fig. 5.6 Boxplot comparison of soil microbial communities under alkaline conditions to those found in caves. The cave biome includes the data shown in Fig. 5.3. The soil biome data was obtained from the studies of Lauber et al. (2009), Zhou et al. (2007), Ganzert et al. (2014), and Zhalnina et al. (2015). The boundaries for the first and third quartile are shown, with the centerline representing the mean and whiskers representing the max/min values (outlier values for the cave biome data are shown in parentheses)

forming habitat. Recent studies support this idea, including the identification of surface/epikarst-derived microorganisms in stalactite drip water (based on their covariance with seasonal events) and the scant (<0.1%) population of cyanobacteria observed in cave samples via deep sequencing (Fig. 5.5) (Gerič et al. 2004; Harmon et al. 2013; Yun et al. 2015). Nonetheless, several pieces of evidence suggest that the long-term impact of surface species on cave community structure may be diminished over time, including (1) the finding by Ortiz et al. that less than 16% of the microorganisms found in caves share taxonomic identity with soil species, (2) the significant evolutionary adaptations observed in the genomes of indigenous microorganisms, and (3) a study by Johnston et al., which demonstrated that human commensal species introduced into a cave habitat are quickly lost (Land et al. 2009; Barton and Barton 2012; Johnston et al. 2012; Ortiz et al. 2013). Thus, while soil may seed these environments, unique selective pressures within caves "sort" microbial species into the observed cave populations (Fig. 5.3).

5.6 An Incomplete View

This review has focused on the contributions of bacteria to cave microbial community structure as most studies likewise focus on the members of this domain (Peck 1986; Vlasceanu et al. 1997; Schabereiter-Gurtner et al. 2002; Laiz et al. 2003; Barton et al. 2004; Engel et al. 2004b; Ikner et al. 2007; Zhou et al. 2007; Banks et al. 2010; Iker et al. 2010; Bhullar et al. 2012; Cuezva et al. 2012; Ortiz et al. 2013). Nonetheless, focusing on the bacteria provides an incomplete view of microbial populations, which commonly include contributions from the archaea and microscopic eukarya—in caves, primarily in the form of filamentous fungi (Pace 1997; Barton and Northup 2007; Vanderwolf et al. 2013). While some studies have incorporated the archaea into their analyses and others have focused exclusively on mycology, none have attempted to determine community structure and metabolic relationships across all three domains of life (Woese and Fox 1977, Northup et al. 2003; Tetu et al. 2013; Vanderwolf et al. 2013; Barton et al. 2014; Ortiz et al. 2014). Given the significant contributions that members of these domains can make in other microbial ecosystems, it is reasonable to assume that the archaea and fungi help shape community metabolic interactions and diversity in caves.

5.6.1 Archaea

The first demonstration of archaea in caves was by Northup et al. (2003), who identified members of the *Thaumarchaeota* (at the time still phylogenetically grouped within the *Crenarchaeota*) in the ferromanganese deposits of Lechuguilla Cave, USA. Other studies supported the presence of archaea in caves, including the significance of the *Thaumarchaeota* across multiple cave habitats (Fig. 5.7; Chelius and Moore 2004; Barton et al. 2007; Barton et al. 2014; Ortiz et al. 2014). In all cases, the numerical abundance of the archaea remains small (<2%), although these studies are based on sequence-only approaches, rather than direct cell counts (Fig. 5.7). In our work, which incorporated direct cell counts using archaeal-specific fluorescent in situ hybridization, we have observed a strong correlation between the availability of nitrogen and presence of archaea*;* when nitrogen levels are at their lowest (ng/L), the contribution of the *Thaumarchaeota* to total population size can exceed 15% (Johnston and Barton unpublished data), indicating that the contribution of archaea to population structure covaries with resource limitation. The small size of *Thaumarchaeota* (up to 100-fold smaller than their bacterial counterparts), their slow growth rate, and innate resistance to severe energetic stress could certainly make the archaea more competitive under extreme nutrient limitation and explain why they make a larger contribution to microbial community structure in some cave environments (Könneke et al. 2005; Valentine 2007; Brochier-Armanet et al. 2008; Brochier-Armanet et al. 2012).

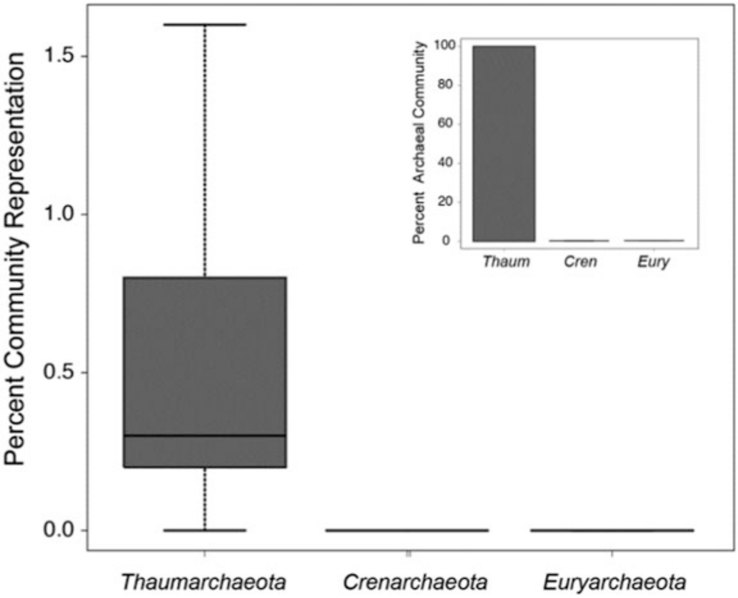

Fig. 5.7 Boxplot comparison of the relative distribution of three major Archaea phyla, *Thaumarchaeota, Crenarchaeota*, and *Euryarchaeota* in cave environments as determined by Illumina sequencing. The datasets used were SRR1686967, SRR1686970, SRR1686976, SRR1693633, SRR1703816, SRR1703817, SRR1703818, SRR1703819, SRR1703820, and SRR1703821, obtained from the NCBI sequence read archive (SRA) and processed in QIIME (Caporaso et al. 2010) as described in Fig. 5.5. The boundaries for the first and third quartile are shown with the centerline representing the mean and whiskers representing the max/min values. Inset: A histogram of the relative distribution of all archaeal 16S rRNA sequences identified from caves

Despite this, the dominance of *Thaumarchaeota* in archaeal populations should be difficult to reconcile with the low levels of nitrogen found in caves (Barton 2015); the *Thaumarchaeota* play an important role in nitrification, where they utilize the oxidation of mineralized nitrogen (NH_3/NH_4^+) to generate energy for autotrophic growth (Brochier-Armanet et al. 2008). In order to compete with bacterial species for available NH_3, the *Thaumarchaeota* express high affinity (nM) transport mechanisms, making them better adapted to the low-nitrogen level found in caves, and providing them an alternate growth strategy in an environment where competition for other resources is likely to be high (Martens-Habbena et al. 2009)**.** Given the increased dominance of nitrogen cycling and recycling metabolic pathways observed in bacterial populations in caves, the almost exclusive identification of *Thaumarchaeota* in archaeal populations may suggest that nitrogen is one of the most overlooked drivers of microbial community structure in caves (Fig. 5.7; Ortiz et al. 2013; Tetu et al. 2013).

5.6.2 Fungi

One aspect of diversity that has generally lagged behind our understanding of cave microbiology is the role of fungi. While the human pathogen *Histoplasma capsulatum* was found in guano from cave hibernacula in 1957, very few papers had examined the mycology of caves beyond this organism (Ajello et al. 1960; Hasenclever et al. 1967; McMurray and Russel 1982; Sterflinger 2000; Burford et al. 2003); however, in 2009 this rapidly changed, when a fungal agent was found to be responsible for the devastating White-Nose Syndrome (WNS) epidemic in bats (Fig. 5.2; Reynolds and Barton 2014b). This disease was first identified in the winter of 2006–2007, when a cave in New York State, USA, contained a number of dead and dying bats. Every one of the sick bats appeared to have an unusual, white-powdery substance on their muzzles and wing membranes (Frick et al. 2010). This powdery substance was subsequently identified as the conidia (asexual spores) of a previously undescribed fungal pathogen, *Pseudogymnoascus* (known as *Geomyces*) *destructans* (*Pd*) (Gargas et al. 2009; Frick et al. 2010; Minnis and Lindner 2013). Since this initial outbreak, WNS has spread to infect bats across 38 US states and 5 Canadian provinces with mortality rates approaching 71%, making it one of the most devastating wildlife diseases of North America in modern history (Boyles et al. 2011; Reynolds and Barton 2014b). The WNS fungus (*Pd*) originated in Europe, where it likely emerged from a *Pseudogymnoascus* sp. endemic to cave environments (Peuchmaille et al. 2011; Warnecke et al. 2012; Reynolds and Barton 2014a; Reynolds et al. 2015, 2016). The identification of such an important mycosis demonstrated a significant lack of our understanding of cave mycology, and there has been a surge in studies attempting to determine how the *Geomyces/Pseudogymnoascus* fit into the ecology of cave systems (Fig. 5.2). By attempting to produce a broader ecosystem prospective, such studies have dramatically increased our understanding of the diversity of fungi in caves (Fig. 5.1; Vanderwolf et al. 2013).

The most commonly identified fungal species in caves are members of the phyla *Ascomycota* (~70%)*, Basidiomycota* (20%), and *Zygomycota* (~7%) (Vanderwolf et al. 2013); however, the relevance of these findings should be viewed with caution, as these studies utilize cultivation-dependent techniques, which are susceptible to the same sample bias that once plagued cave bacteriology (Anderson and Cairney 2004; Tedersoo et al. 2014). The dominance of the phylum *Ascomycota,* which contains filamentous (mold) species such as *Aspergillus* and *Penicillium,* would suggest an environmental advantage for members of these genera; however, these fast-growing species readily utilize the nutrients found in media, often outcompeting other species during cultivation. One cave study based on molecular techniques suggested that the *Ascomycota* and *Basidiomycota* were almost equal in abundance (52 and 48%, respectively), although the investigators could not rule out contamination for dominance of the observed *Basidiomycota* (Connell and Staudigel 2013). Other investigators have suggested that fungal spores found in cave locations have been introduced by human or animal activity, reiterating the hypothesis that microbial populations in caves do not represent endemic species, but environmental

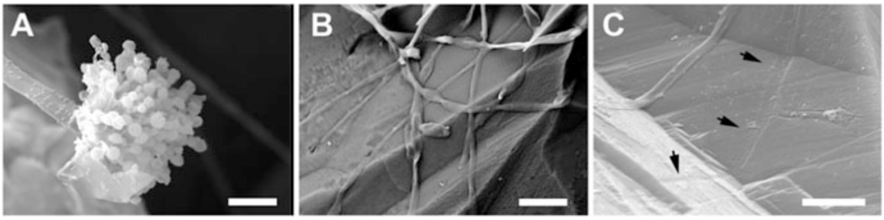

Fig. 5.8 Scanning electron microscopy images of pristine surfaces from Lechuguilla Cave, USA. Clearly visible on calcite mineral surface are fungal conidia (**a**), fungal hyphae (**b**), and etch marks left behind by fungal growth (**c**; arrows), demonstrating how the fungi modify mineral surfaces within the cave. Such etching of calcite by fungal species has been demonstrated before (e.g., Burford et al. 2003). Scalebars = 10 μm

contaminants (Shapiro and Pringle 2009, Vanderwolf et al. 2013). The use of molecular techniques to identify the true fungal ecology of caves therefore remains a high priority (Tedersoo et al. 2014).

Whatever the true diversity of fungi in caves, it is likely that they play a significant role in ecosystem processes. Fungi have been described from pristine cave environments, ruling out the idea that they are introduced contaminants, while there is evidence that they play an important role in altering mineral chemistry (Fig. 5.8; Cunningham et al. 1995). In other geologic settings, fungi are known to be important weathering agents, whether mechanically sugaring or chemically dissolving the surface in an attempt to access nutrients, or concentrating important nutrients and trace metal ions, which can be readily utilized by other microorganisms for growth (Sterflinger 2000; Burford et al. 2003). The growth of these filamentous fungi across mineral surfaces (Fig. 5.8) also generates microfabrics that support the growth of other microbial species (Burford et al. 2003). Together these diagenetic processes change the mineral matrix to form other deposits, such as calcite, goethite, halloysite, and montmorillonite, all of which have been detected in caves (Polyak and Güven 2000, 1996). Within surface soils, fungi play a most dominant role in breaking down macromolecular structures, and it is likely that their dominance in caves is greatest where particulate detritus (such as sticks and leaves) is brought in through flooding or direct anthropogenic impact (Jurado et al. 2010; Schneider et al. 2012). The effective ability of fungi to breakdown these recalcitrant carbon sources could subsequently provide a pool of nutrients for the growth of other microorganisms (Barton 2015).

5.7 Toward a Better Understanding of Microbial Cave Diversity

There has been a rapid increase in the pace of research in cave microbiology (Fig. 5.2). While just a handful of labs in North America, Europe, and Australia published on the topic in the 1990s, a tally of current publications reveals over 47 separate research groups, including numerous labs in Asia and the emergence of the discipline in South America. Such increased contributions have started to impact the broader microbiological sciences, where interest is growing in the ability of cave environments to provide important clues into the emergence of infectious mycoses, novel antibiotics, and the evolution of antibiotic resistance (Bhullar et al. 2012; Fisher et al. 2012; Derewacz et al. 2013, 2014).

Despite this increased interest, there remain several barriers that need to be overcome by new researchers wanting to enter the field. These barriers include the difficulty in accessing and carrying out research in the challenging environment of caves and the technical limitations of working with low biomass samples. Cave access limitations can be overcome by reaching out to local cavers and speleologists, who often have the best information on appropriate caves for access and can even help identify microbial habitats for research; however, working with low biomass samples remains challenging, particularly in regard to preventing contamination, DNA extraction, and low DNA template levels (Barton et al. 2006). Such limitations can be overcome by using targeted cultivation approaches that take into account potential bias or circumvented by access to technologies that make it possible to work with low biomass samples (Summons et al. 2014). Currently, advances in DNA extraction and analysis make it possible to extract and work with nanogram to picogram- levels of DNA from geochemically complex environmental samples, while a new Nextera protocol only requires 1 ng/μL of template DNA to prepare Illumina libraries for metagenomic sequencing (Pel et al. 2009; Grunenwald et al. 2010; Rinke et al. 2013). Low biomass limitations may be overcome in the future by emergent technologies, such as new NGS approaches; PacBio (sequencing individual DNA fragments >40,000 bp in length) or Nanopore (sequencing single DNA molecules up to millions of bases in length) sequencing could be combined with single-cell whole-genome sequencing (SCWGS) (Branton et al. 2008; Rinke et al. 2013; Rhoads and Au 2015). While these methods have not yet been used in caves, such technologies could revolutionize the way low biomass environments are examined.

Over the next decade, it is likely that cave research will adopt many of the technology trends currently advancing the field of environmental microbiology, including the use of metabolomics (studying whole community metabolic products), metatranscriptomics (studying changes in whole community transcription), and metaproteomics (studying whole community protein expression patterns). Such data could help us understand the relative contributions of heterotrophy, autotrophy, mutualism, and competition to community energetics, or the unique role geochemistry plays on microbial community structure, with a goal of integrating the domain-level contributions of bacteria, archaea, and fungi that make it possible for microbial communities to subsist in such nutrient-limited habitats (Barton et al. 2007; Banks et al. 2010; Engel 2010; Lee et al. 2012; Barton 2015). Such studies may identify the fundamental ecological principles and adaptations that drive community dynamics and diversity and provide a more satisfying answer as to what constitutes a cave microbiome.

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Chapter 6
Phototrophs in Caves

Janez Mulec

6.1 Introduction

Cave passages that open to the surface experience some level of direct or indirect illumination by sunlight. Natural light is available not only at the cave entrance but also deeper inwards, and its amount depends upon entrance orientation, type of cave (horizontal cave/shaft) and the surrounding relief around the cave entrance which affects the pattern of light scattering and shading. Under suitable conditions, indirect light reflected from water, ice or snow can penetrate deeper into underground passages. In this illuminated zone, extending to the zone of complete darkness, phototrophic organisms can utilize light as an energy source (Dobat 1998).

Vegetation commonly masks cave openings, but in some cases, its presence is indicative of a cave, for example, where humidity from underground reaches the surface in arid areas. With gradual light decrease, the zonation of phototrophs reduces sequentially from phanerophytes, pteridophytes and bryophytes to algae and cyanobacteria. Microscopic phototrophs including lichens and, especially, algae and cyanobacteria colonize rocky and sedimentary surfaces at entrances. Colourful vertical streaks named also ink streaks or tintenstriche (originally described as Tintenstriche in German) are clearly visible on exposed limestone and dolomite surfaces dominated by cyanobacteria (Luttge 1997). Their colour is intensified when seeps become active. Cyanobacteria in particular live not only on the surface (epilithic) but also beneath the surface (endolithic) and in crevices within stony substrata as euendoliths (Komárek and Anagnostidis 2000). Differently coloured patinas of biofilm on walls, ranging between greenish, bluish and brownish, span

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from the cave mouth deep into the twilight zone. Phototrophs submerged in entrances of water cave colonize rocky surfaces as a part of periphyton. Researchers have to improvise many times during sampling in caves. Once a proper sample is collected, it is generally subjected to well-established laboratory methodologies from plant and microbial ecology and lately more and more from molecular biology.

Light is a prerequisite for growth of phototrophs (Figs. 6.1a and b), but commonly in cave entrances, there is no clear zonation of vegetation (Mulec et al. 2008). In the Frasassi Caves (Italy), the cyanobacterium *Phormidium* sp. exhibited a light compensation point at 13 μmol photons/m^2/s and captured an average photosynthetic photon flux density (PPFD) only between 6 and 10 μmol photons/m^2/s (Giordano et al. 2000). The quantity of energy (PPFD) available for photosynthesis is photosynthetically active radiation (PAR) between 400 and 700 nm. The quantity of photons available for photosynthesis does not correspond to illuminance sensed by the human eye, which is expressed in luminous flux (lux). In extreme habitats, the light compensation point of bryophytes is similar to that of algae (Glime 2007). Some algae and cyanobacteria can readily switch photoautotrophic metabolism to override the lack or insufficient quanta of light by adopting photoheterotrophic or entirely heterotrophic metabolic pathways (Droop 1974; Lloyd 1974; Pentecost and Whitton 2012).

In some caves, phototrophic organisms are actively involved in lithogenesis (Figs. 6.1a and c). These formations commonly include stromatolitic stalagmites, stalactitic tufas (Taboroši 2006) and biocalcification associated with the mosses *Eucladium verticillatum* and *Didymodon tophaceus* termed eucladioliths and didimodontoliths, respectively (Dalby 1966).

In show caves equipped with electric lighting, there are areas around lamps that are colonized by lampenflora (Fig. 6.1d). Lampenflora also referred to as lamp flora is a result of light eutrophication and is composed predominantly of algae, cyanobacteria, commonly also with bryophytes and ferns (Mulec 2012). Because the community of lampenflora is responsible for the deterioration of substrata, this issue is particularly urgent in artificially lit historic underground sites (Asencio and Aboal 2001; Saiz-Jimenez 2010; Zammit et al. 2011; Albertano 2012; Zucconi et al. 2012).

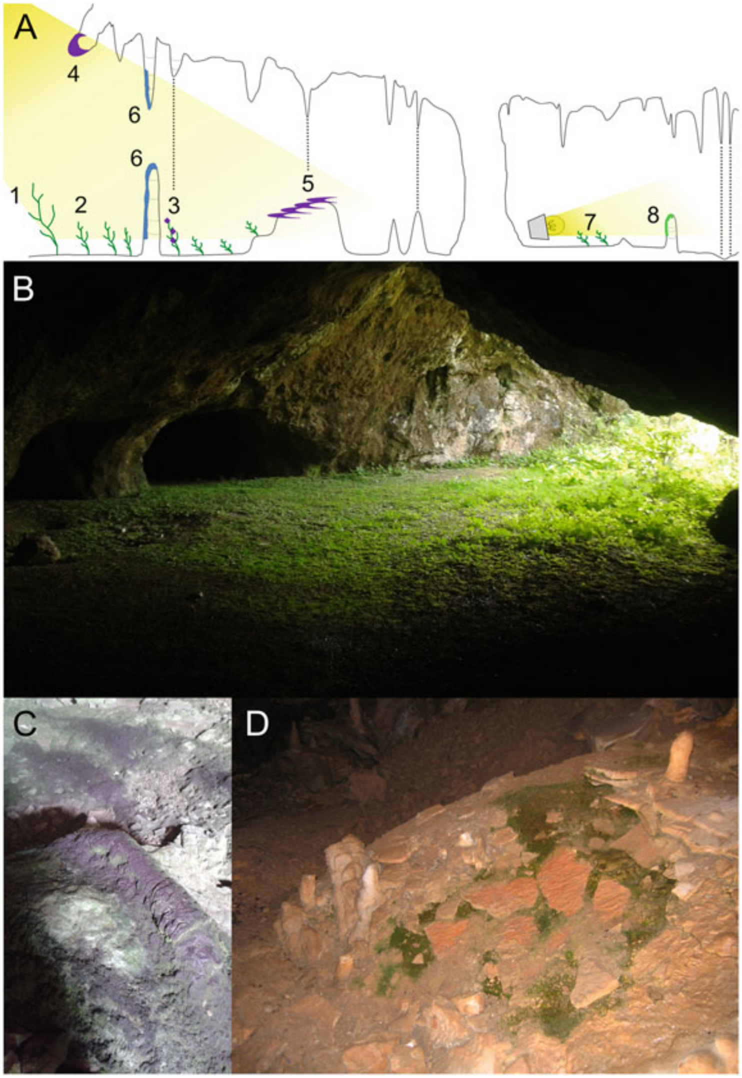

Fig. 6.1 Phototrophs in caves: (**a**) schematic representation of natural flora in a cave entrance (*1* phanerophytes, *2* bryophytes and ferns, *3* biocalcification on a moss under a seepage, *4* a tuffaceous stalactite oriented towards incoming sunlight, *5* a stromatolitic stalagmite under a seepage, *6* surfaces colonized predominantly by cyanobacteria) and lampenflora (*7* bryophytes, *8* a speleothem predominantly colonized by algae); (**b**) cave entrance colonized by phototrophs, Osojca Cave, Slovenia; (**c**) stromatolitic stalagmites, Škocjan Caves, Slovenia; (**d**) lampenflora, preferential colonization of sediment surfaces, Postojna Cave, Slovenia

6.2 Zonation of Phototrophic Communities

Box 6.1

The pattern of decrease in light and increase in humidity towards the cave interior, accompanied by zonation of vegetation (Fig. 6.1b), gives four distinct regions of the phototrophs distribution (Dobat 1970, 1998). The outermost region, the "access zone", can be rather shady but is always well illuminated, and some soil is present; phanerophytes predominate over pteridophytes and bryophytes. The "entrance zone" has less soil, receives indirect sunlight and is distinguished by a more hygrophilous and ombrophilous vegetation, which still includes phanerophytes, ferns and bryophytes. Etiolated growth of plants is also activated and is expressed as bigger or sparser leaves, weak stems, elongated internodes or even chlorosis (Dalby 1966; Burgess 1985; Dobat 1998; Whippo and Hangarter 2006). Cytological changes, such as alteration of chloroplasts, grana and thylakoids, were observed in mosses cultivated in complete darkness inside a cave (Rajczy 1978–1979). In the "transition zone", which receives weak, indirect light, there are phanerophytes, but fairly abundant communities of ferns and bryophytes also occur. Non-colonized patches between these organisms clearly show a greenish patina of algae and cyanobacteria. Finally, the "deep twilight zone" ends with the absence of surface colonization of algae and/or cyanobacteria. As the distance down the light gradient increases, there is a corresponding decrease in cyanobacterial diversity. There are also reports of a lowering of the proportion of coccoid vs. filamentous cyanobacteria in the deep zone in some caves (Vinogradova et al. 1998), whereas in some other caves, there are reports of predominance of *Oscillatoriales* over *Chroococcales* (Lamprinou et al. 2012a).

There are also changes in the phototrophic community related to eukaryotic algae. For example, towards the inner part of Sefunim Cave in Israel, the diversity of diatoms was greater than that of Chlorophyta, and Xanthophyta gradually disappeared from the community (Vinogradova et al. 2009). Additionally, an ideal clear zonation related to species diversity and their abundance in cave entrances is not always expressed for bryophytes (Pentecost and Zhaohui 2001), nor for algae and cyanobacteria (Mulec et al. 2008; Lamprinou et al. 2012a). In show caves, lampenflora can colonize wide surfaces, up to or locally exceeding a few square metres around an individual lamp. Distinct light-related zonation of different groups of phototrophs is not common among lampenflora (Mulec et al. 2008).

Light and climatic conditions differ between horizontal caves and shaft systems. Stronger climatic gradients are generally better expressed in shafts, with a more clearly delimited zonation of vegetation (Rosselló and Ginés 1980; Fiol 1995) giving favourable conditions for some uncommon bryophytes (Pericàs et al. 2009). This is particularly important in hot places such as the Mediterranean region, where the summer is extremely dry and the shaft microclimate offers a refuge to species such as *Asplenium scolopendrium* which is most common in shaded places, moist soil and damp crevices (Ginés and Ginés 1992). Interestingly, in this respect, approximately 25% of the Balearic (Spain) bryoflora is reported from karst caves and shafts (Rosselló and Pericàs 2011). Furthermore, because of microclimatic peculiarities and passage morphology at cave entrances, such as in the Škocjan Caves (Slovenia), glacial floral relicts (e.g. *Primula auricula*, *Saxifraga incrustata*, *Viola biflora*) and thermophilic taxa (e.g. *Adiantum capillus-veneris*, *Asparagus acutifolius*) can be found at the same location (Martinčič 1973). It seems that some cave entrances in SW China act as a refuge for diverse angiosperms (flowering plants) due to deforestation of the surrounding areas in the past, which caused drastic changes in the microclimate and landscape (Monro et al. 2018).

6.3 Ecology and Relevance of Cave–Associated Phototrophs

Phototrophs fix carbon dioxide into biomass (Berg 2011) which becomes available higher in the food web (Briand and Cohen 1987). In situ fixed biomass in cave entrances and the eventual fate of its organic carbon in a cave ecosystem have not yet been evaluated, though probably its significance is not negligible. Prokaryotes, as well as (micro)eukaryotes, enter into interactions with phototrophs in these habitats, where there is the potential to discover new organisms, such as the amoebae *Allovahlkampfia spelaea*, first described from an illuminated stromatolitic stalagmite (Walochnik and Mulec 2009). Independent of photosynthetic reactions, there are also cave ecosystems based upon in situ microbial chemolithoautotrophy (Sarbu et al. 1996; Porter et al. 2009; Por et al. 2013).

6.3.1 Diversity

At least 278 unique cyanobacteria species have been documented from caves in the temperate zone (Hauer et al. 2015). Various authors have pointed out the relative dominance of cyanobacteria in the community of cave entrances (e.g. Golubić 1967; Vinogradova et al. 1995; Asencio and Aboal 2000; Selvi and Altuner 2007; Uzunov et al. 2007; Mulec et al. 2008; Czerwik-Marcinkowska and Mrozińska 2011; Mazina and Maximov 2011; Lamprinou et al. 2012a; Popović et al. 2015). The most commonly documented cyanobacteria were from the genera: *Aphanocapsa*, *Aphanothece*, *Chroococcus*, *Gloeocapsa* (Fig. 6.2), *Lyngbya*, *Phormidium* and *Scytonema*. The evidence suggests that caves may be diversity hotspots for some cyanobacteria, especially *Hapalosiphonaceae* and *Symphyonemataceae*, and some species representatives are believed to be obligatorily cavernicoles. This could be

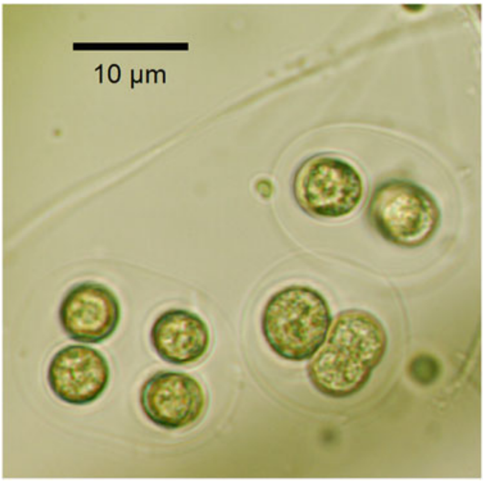

Fig. 6.2 *Gloeocapsa* sp., a common cyanobacterium in cave entrances

attributed to the fact that in comparison to subaerial habitats, where cyanobacteria are not exposed to high UV irradiation, desiccation and major temperature oscillations, the only stressor in cave entrances is low PPFD (Hauer et al. 2015). Additionally, in cave, micro-niches with higher PPFD values, large filaments of algae and cyanobacteria can physically protect any underlying small phototrophs (Golubić 1967).

Mosses, liverworts and ferns in cave entrances are common species that also occur in adjacent areas (Buckallew 2003; Moseley et al. 2013). Flowering plants are restricted to the outermost parts of cave entrances (Dobat 1998). Several bryophytes are commonly found in cave entrances, for example: *Anomodon viviculosus*, *Conocephalum conicum*, *Ctenidium molluscum*, *Encalypta streptocarpa*, *Homalothecium sericeum*, *Plagiochila asplenioides* and *Porella platyphylla*. Taxa such as *Fissidens cristatus*, *F. minutulus*, *Leiocolea muelleri*, *Mnium stellare*, *Pedinophyllum interruptum* and *Thamnobryum alopecurum* have a larger ecological tolerance for the dim light conditions in caves. The most commonly reported representative of pteridophytes in cave entrances is *Asplenium trichomanes* (Dobat 1998). Some caves provide a habitat for endangered and protected species, for example, the fern *Polystichum aculeatum* in Nagi Vizes Cave, Hungary (Buczkó and Rajczy 1989). Fern prothalli and moss protonema are commonly encountered deeper inside cave entrances (Rajczy et al. 1986).

In lampenflora, the percentage of cyanobacteria is generally smaller, commonly with essentially equal proportions of cyanobacteria, Chlorophyta and Chrysophyta. Chrysophyta are represented mostly by diatoms (Mulec 2015). A typical representative of lampenflora is an ubiquitous, fast-reproducing aerophyte terrestrial alga (Chlorophyta or Chrysophyta) or cyanobacterium that is adapted to low PPFD values (Mulec 2015). In lampenflora, mosses *Fissidens taxifolius* and *Amblystegium serpens* are frequently identified (Glime 2007) among other moss protonema and fern prothalli.

Cave habitats are sources of species new to science; some recent examples are the cyanobacteria, *Iphinoe spelaeobios* and *Loriellopsis cavernicola* (Lamprinou et al. 2011), *Toxopsis calypsus* (Lamprinou et al. 2012b) and *Culatella subterranea* (Zammit et al. 2012) and the diatoms: *Cholnokyella aerophila* (Taylor and Lange-Bertalot 2013), *Nupela troglophila* (Falasco et al. 2015), *Mayamaea cavernicola* and *Sellaphora barae* (Van De Vijver and Cox 2013). Algal diversity in submerged marine caves is, as yet, understudied (Gerovasileiou et al. 2015); also there is only limited information about psychrophilic algal diversity in ice caves (Kol 1964; Hillebrand-Voiculescu et al. 2014), fumaroles and lava tubes (Goff et al. 1979; Van De Vijver and Cox 2013). Molecular methods provide opportunities to extend understanding of the total diversity of subaerial and underground communities (Pentecost and Whitton 2012).

6.3.2 Environmental Conditions and Estimates for Colonization

Box 6.2

There are many environmental parameters crucial for the development of aerophytic communities in cave entrances: light, type of substrata (e.g. limestone, lava, ice, soil, sediment) and their characteristics (e.g. porosity, inclination), water and nutrient availability, concentration of carbon dioxide and biological factors such as the availability of inoculum (cells, spores and thalli), ecological interactions and organic debris including excrements (Mulec 2015). For colonization by higher plants, other important considerations are the cave entrance morphology (e.g. horizontal cave or shaft) and its geographic position (altitude and orientation), the characteristics of the surrounding area (such as whether it is open, shrub-covered or forested) and the past and ongoing activities of humans and animals (Fiol 1995).

The inoculum of phototrophs from adjacent areas reaches cave entrances either with air currents, normal water flow, floods of sinking rivers and seeps through crevices in the rock mass or by the intervention of animals and humans (Mulec 2015). Typical planktonic species are sometimes found in cave phototrophic communities, for example, a nonmotile coenobial green alga *Pediastrum boryanum* was identified as a part of lampenflora and in a community from the cave entrance. Their presence indicates not only the efficient transport but also the existence of a suitable niche in water droplets in aerophytic habitats (Mulec et al. 2008). Alongside light levels and the depth of the cave entrance, hydrodynamics is the most important factor in the establishment of phytobenthos within submerged caves (Alongi et al. 2012). In Vlychada Cave, Greece, the distribution of cyanobacteria in a water gallery was influenced predominantly by concentration of carbon dioxide, in a dry part of the cave by relative humidity and PPFD, and at the cave entrance by temperature (Lamprinou et al. 2014). In Arubota'im Cave, Israel, low light, water deficit and peculiarities of the halite bedrock were responsible for extremely low cyanobacterial diversity (Vinogradova et al. 2011). In the Alpine show Cave Bossea, in Italy, the presence of seeping water and the distance from the cave entrance had significant impacts on the growth of lampenflora, but tourist presence did not (Piano et al. 2015).

Concentration of photosynthetic pigments per unit of surface is a measure of colonization and organic biomass of phototrophic biofilms. In the illuminated cave entrance of Božana Cave, Serbia, the concentration of chlorophyll *a* was up to 7.07 μg/cm^2 (Popović et al. 2015), whereas in Škocjan Cave, Slovenia, the maximum concentration was 1.71 μg/cm^2. Using a pulse amplitude modulated fluorimeter on lampenflora in Cave Bossea, the chlorophyll *a* concentration for cyanobacteria was up to 5.90 μg/cm^2, 6.14 μg/cm^2 for diatoms and 4.32 μg/cm^2 for green algae (Piano et al. 2015). These are higher values compared to the maximum concentration of total lampenflora chlorophyll *a* (2.45 μg/cm^2) in Pekel pri Zalogu Cave, Slovenia, where pigment extraction procedures were used (Mulec et al. 2008). Furthermore, in Cave Bossea, illuminance (lux) was the major factor that correlated positively with the presence and productivity of these main groups of phototrophs (Piano et al. 2015).

6.3.3 Adaptations of Phototrophs for Light–Deprived Cave Environments

Cyanobacteria can live in caves at minuscule PPFD values, ranging as low as 0.0008–0.06 μmol photons/m^2/s (Martínez and Asencio 2010). Changes of the ultrastructure of photosynthetic membrane systems (generally they become densely packed) in light-deprived cave environments have been documented for several genera and organisms: *Chlorella* (Cox 1977), *Chroococcus* (Cox 1977), *Gloeocapsa* (Cox et al. 1981), *Geitleria calcarea* (Couté 1982), *Chroococcidiopsis*, *Cyanosarcina*, *Leptolyngbya*, *Phormidium* and *Pseudocapsa* (Asencio and Aboal 2004). The authors suggested that the loss of a raphe system in *Diprora* can also be attributed to the cave environment (Kociolek et al. 2013).

Box 6.3

Physiological changes, such as increased biosynthesis of photosynthetic pigments (Mulec et al. 2008) and chromatic adaptation, for example, in *Phormidium valderianum* (Pentecost and Whitton 2012), were recognized among the isolates from some caves. Algae and cyanobacteria in caves can change their cell morphology, and this makes microscopic identification

(continued)

Box 6.3 (continued)
problematical; thus, the use of cultures is essential. Various morphological modifications have been observed, for example, among the cyanobacteria: sheath pigments, cell walls and envelopes and formation of resting or dormant cells (Komárek and Anagnostidis 2000; Komárek and Anagnostidis 2005; Komárek 2013).

One of the well-known cytological adaptations of bryophytes to low levels of light is the example of the moss *Schistostega pennata*. It commonly grows in cave entrances in the northern temperate zone and, due to its characteristic light reflection, it is known as "dragon's gold". The lens-shaped cells of the moss protonema are curved to focus the light, and chloroplasts orient themselves so that they are always located at the most intensely illuminated point at the inner wall of the cell (Glime 2007). In the southern hemisphere, the moss *Mittenia plumula* exhibits similar protonema characteristics (Dobat 1998).

6.3.4 Lampenflora: A Problem for Show Caves

The term lampenflora is applied to the phenomenon of the proliferation of mainly phototrophic organisms in newly formed ecological niches close to artificial lights in caves (Fig. 6.1d). Vascular plants are rarely present at these locations, but if they do occur, they are normally found as germinating shoots (Mulec 2012). Additionally, mass tourism and artificially induced air circulation can enhance the expansion of lampenflora (Mulec 2015). Surfaces with lampenflora are likely to develop more quickly in show caves with an active underground stream. For example, two show caves in Slovenia with comparable visitor numbers and lighting regimes (annual illumination of >60 h/sector and average PPFD of 28.0 μmol photons/m^2/s in Kostanjeviška Cave and > 80 h/sector and average PPFD of 30.3 μmol photons/m ^{2}/s in Županova Cave) differ in terms of their lampenflora colonization. In Županova Cave, 58.2% of lamps are colonized by algae and 7.7% by bryophytes, whereas in Kostanjeviška Cave, with an active underground river causing occasional floods, 85.5% of lamps are colonized by algae and 43.6% by bryophytes. Bryophytes, as well as algae and cyanobacteria, can proliferate at low PPFD values. For example, *Eucladium verticillatum* was identified at PPFD ranging from 1.4 to 530.0 μmol photons/m^2/s (Mulec and Kubešová 2010).

The effects of lampenflora may differ between cave ecosystems. Surfaces covered by phototrophic lampenflora that are a source of organic carbon can become the physical and nutritional base for niche ecosystems populated by higher trophic levels of heterotrophs. Abundant lampenflora biomass might impact the ecology of fauna and their interactions. The potentially irreversible deterioration effect of lampenflora biofilm on underlying substrata is especially serious in caves with painted rock art

(Albertano and Urzi 1999; Saiz-Jimenez 2010; Albertano 2012; del Rosal et al. 2014).

Different physical methods have been employed in attempts to control lampenflora: cleaning of colonized surfaces with brushes and water jets, shortening the lighting period, reducing the light intensity, partially altering the emission spectra of lamps and the use of UV lamps (Mulec and Kosi 2009). UVC-emitting lamps have a proven deleterious effect on a mixture of Chlorophyta from lampenflora (Borderie et al. 2011). UVC irradiation causes thymine-thymine dimers leading to mutations (Sinha and Häder 2002) and thus also has a deleterious effect upon other cave biota in a range of irradiation. Various chemicals have been applied in attempts to kill lampenflora, for example: Atrazine (6-Chloro-N-ethyl-N′-(1methylethyl)-1,3,5-triazine-2,4-diamine), bromine compounds, calcium hypochlorite, cupric ammoniac compounds, Diuron (N-3, 4-dichlorophenyl-N′-dimethyl urea), formalin, hydrogen peroxide, quaternary ammonium derivatives, Simazine (6-Chloro-N,N′-diethyl-1,3,5-triazine-2,4-diamine) and sodium hypochlorite (Mulec and Kosi 2009; Bastian et al. 2010). Sodium hypochlorite and hydrogen peroxide are nowadays the most widely used chemicals (Hebelka 2014). At the moment, a buffered (pH 7.0–7.5) solution of hydrogen peroxide (15–20%) seems to be the most environmentally friendly method for the remediation of insensitive calcite surfaces affected by lampenflora (Mulec 2014).

Some progress towards the long-term preservation of delicate, historically important, surfaces that are artificially lit might be achieved by installation of a tuneable LED lighting system. This would have the capability to modulate the spectral distribution to minimize damage (such as colour fading) by the effective irradiance, would partially inhibit biodeterioration and would improve the apparent colour reproduction of the illuminated objects (de Luna et al. 2015). The use of lamps with altered emission spectra is only partially successful in inhibiting biodeterioration because some phototrophs can readily and rapidly modify synthesis of (accessory) photosynthetic pigments (Roldán et al. 2006; Mulec 2014). Alongside regular removal of lampenflora, in order to reduce lampenflora growth and expansion, the lighting regime should also be restricted; illumination of speleothems and artefacts should be as brief as possible, with the minimum PPFD (Mulec 2014).

6.4 Phototrophs Impact Cave (Micro)Morphology

Biota participates actively in changes to the cave environment as a biogeomorphological factor that involves litholysis (rock weathering, erosion) and lithogenesis (mineral formation). Light availability undoubtedly enhances biologically mediated erosion in marine caves via the formation of notches (Coombes et al. 2015). Biodeterioration of colonized illuminated surfaces in caves with rock-art paintings is an especially urgent problem (Urzi et al. 2010). Mineral deposits such as gypsum, halite and calcite related to biochemical activities contribute to biodeterioration of artworks in underground archaeological sites (Zammit et al. 2011). In some caves, especially those close to the surface, plant roots can penetrate deep and impact upon cave formations. On the other hand, lithogenesis attributed to phototrophs in caves results in the development of some particularly obvious and peculiar formations (e.g. Fig. 6.1c).

Few biologically influenced speleothems are known in caves (Taboroši 2006), some of them particularly related to phototrophs, calcified plants (e.g. eucladioliths—*Eucladium verticillatum*, didimodontoliths—*Didymodon tophaceus*), generally irregularly shaped stalactitic tufas (in literature also termed tuffaceous stalactites or calcareous tufa stalactites) and stromatolitic stalagmites (Fig. 6.1c), referred also as crayback stalagmites (Dalby 1966; Cox et al. 1989a). Calcite deposited on *E. verticillatum* as eucladioliths appears first on leaves, as isolated crystals, then increases gradually and coalesces until the whole plant is embedded in calcareous sheaths (Dalby 1966). All such biogenic structures are oriented towards the incoming sunlight. The term microbialite was applied to a formation containing the cyanobacterium *Phormidium laysanense* and hanging from a cave ceiling on Tikehau atoll, French Polynesia (Sprachta et al. 2001). These biolithogenic structures are normally soft, fragile and commonly porous. Until they are not completely lithified, they can physically protect organisms against desiccation and excessive light quanta (Mulec et al. 2007). Only a few cyanobacteria that actively precipitate carbonates are documented in caves, for example, *Geitleria calcarea* (Couté 1989), *Scytonema julianum* (Couté and Bury 1988), *Loriella osteophila* (Hernández-Mariné et al. 1999) and *Herpyzonema pulverulentum* (Hernández-Mariné and Canals 1994).

Laminae of stromatolitic stalagmites resemble those of stromatolites formed in shallow lakes and seas, but they differ in their mode of formation. The proposed model based on observations at Wombeyan and Jenolan caves (New South Wales, Australia) includes an active seep within the illuminated zone at the cave entrance. Wind helps to shape the size of the formation, but most importantly, fixation of carbon dioxide by cyanobacterial biofilm results in an increase of pH during photosynthesis and consequently in biocalcification. During drier periods, grains of aeolian sediment are lithified together by microbial exopolysaccharides on stalagmites, creating typically laminar strata. Low molecular weight fatty acids, amino acids and *n*-alkanes derived from cyanobacteria were identified within the structures (Cox et al. 1989b; James et al. 1994). Recent observations revealed a variety of crayback morphologies that reflect different local conditions, e.g. wind velocity, drip level, and activity of phototrophs (Dodge-Wan et al. 2012). Formation of unique phosphatic stromatolites of hydroxyapatite (Deer Cave, Sarawak, Malaysia) requires photosynthetic activity of cyanobacteria and a low pH (2.4) of guano drainage water that dissolves bedrock (Lundberg and McFarlane 2011). It has been suggested that microbiota also has an important role in the formation of silica stromatolites in a volcanic cave in the Azores, Portugal (Brunet and Revuelta 2014). Nevertheless, in some caves, there are also old and inactive stromatolites from past geological periods, which can be used to aid reconstruction of environmental conditions during their development (Lisker et al. 2009).

The importance of phototrophs as a potential food source for the cave fauna is part of Chap. 14.

6.5 Conclusions

Biodiversity and the genetic pool within naturally illuminated parts of caves are still understudied, especially in lava tubes, ice caves, submerged caves and cenotes, i.e. open water pools of groundwater. Particularly, for certain higher plants, cave entrances represent life at an extreme as well as refuge against extinction. Exactly what, and how much, phototrophs do for the cave ecosystem in conjunction with other biota remain open questions. There is considerable potential for biotechnological applications among cave phototrophs, for example, some cyanobacteria from caves have displayed antibacterial activity against some human pathogens (Lamprinou et al. 2015). Phototrophs play an active role in weathering processes that are especially undesirable in show caves and caves with rock-art paintings. Regular removal of lampenflora in such places is recommended for bioremediation of insensitive surfaces. The lighting regime in underground places should be severely restricted. In addition, in some caves, phototrophs can be the main driver in the formation of specialized types of speleothems.

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Chapter 7
Diversity of Terrestrial Invertebrates in Subterranean Habitats

Louis Deharveng and Anne Bedos

7.1 Introduction

The most comprehensive contribution to the knowledge of world subterranean biodiversity is the monumental *Encyclopaedia Biospeologica* in three volumes coordinated by Juberthie and Decu (1994, 1998, 2001). Since then, a very large number of subterranean taxa have been described, while the understanding of diversification processes has considerably progressed (see also Chap. 1). The diversity of terrestrial subterranean fauna has been summarized more recently in dedicated chapters of several books, generally limited to large taxa or large regions (Culver and Pipan 2009, 2014; Romero 2009; White and Culver 2012). In this chapter, we shall give a broader overview at the world scale of the patterns of morphological diversity among terrestrial subterranean invertebrates of the world, in their taxonomic and adaptive aspects.

Subterranean habitats encompass all kinds of voids in mesoporous below-ground substrate, including caves, cracks, MSS, upper hypogean voids, and various kinds of screes (see Chap. 3). Interstitial habitats, where voids are less than a few mm in their larger size, are traditionally dealt with soil habitats and will not be considered here (Deharveng and Bedos 2012; Culver and Pipan 2014).

Cave invertebrates are classically categorized as troglobites, troglophiles, and trogloxenes (but see below for definitions). We shall focus here on troglobites. Troglophiles will be touched on for species of uncertain ecological status, or when they belong to lineages where both lifestyles are represented. Amphibious species and free stages of parasites when only known from caves will be considered as well.

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Table 7.1 World troglobitic Hexapoda by order. Source of the total number of species; Zhang: values extracted from Zhang (2011) (ed.); text: values extracted from various sources, see text; total: total number of species in the world. Tb: number of troglobites in the world, see text; Orthoptera: rough estimate including troglophiles

Group	Source	Total	Tb	%
Grylloblattodea	Text	32	7	21.8750
Diplura	Text	984	131	13.3130
Zygentoma	Text	568	45	7.9225
Collembola	Text	8128	500	6.1516
Orthoptera	Text	27,267	250	0.9169
Coleoptera	Zhang	386,500	2500	0.6468
Blattodea	Zhang	7314	40	0.5469
Psocoptera	Text	10,880	30	0.2757
Dermaptera	Zhang	1978	2	0.1011
Hemiptera	Zhang	103,590	85	0.0821
Diptera	Zhang	155,477	22	0.0142
Lepidoptera	Zhang	157,338	2	0.0013
Hymenoptera	Zhang	116,881	1	0.0009
Zoraptera	Zhang	37	0	0
Rhaphidioptera	Zhang	254	0	0
Embioptera	Zhang	463	0	0
Megaloptera	Zhang	354	0	0
Mecoptera	Zhang	757	0	0
Protura	Zhang	804	0	0
Strepsiptera	Zhang	609	0	0
Ephemeroptera	Zhang	3240	0	0
Mantodea	Zhang	2400	0	0
Microcoryphia	Zhang	513	0	0
Neuroptera	Zhang	5868	0	0
Odonata	Zhang	5899	0	0
Phasmatodea	Zhang	3014	0	0
Phthiraptera	Zhang	5102	0	0
Plecoptera	Zhang	3743	0	0
Siphonaptera	Zhang	2075	0	0
Thysanoptera	Zhang	5864	0	0
Trichoptera	Zhang	14,391	0	0

7.1.1 Statistics, Patterns, Sampling

Globally, terrestrial subterranean diversity represents only a small fraction of surface diversity (Gibert and Deharveng 2002). Except rare root-feeders, animals linked to green plants, which constitute the largest part of biodiversity on earth, are absent underground. Other invertebrate groups have colonized the subterranean environment with unequal success (Table 7.1), for reasons that remain largely unknown.

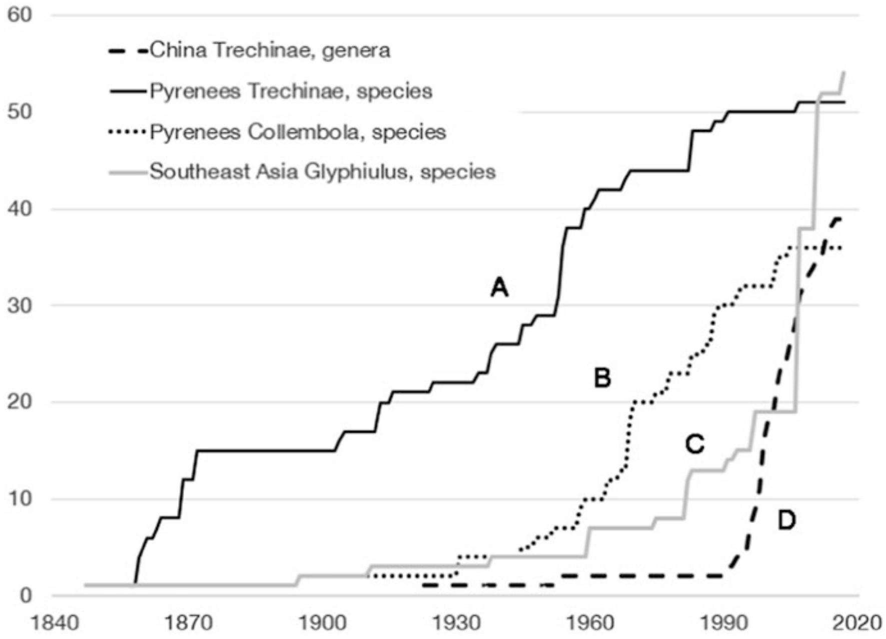

Fig. 7.1 Cumulated number of taxa along years. (**a**) Genera of Chinese troglobitic Trechinae (data from Tian et al. 2016); (**b**) species of Pyrenean cave Trechinae (data from Faille et al. 2010b); (**c**) species of French Pyrenean cave Collembola (data from Thibaud 2017); (**d**) species of Southeast Asian cave Diplopoda, genus *Glyphiulus*. (Data from Golovatch et al. 2007a, b, 2011a, b, 2012; Likhitrakarn et al. 2017)

Sampling efforts continue to be considerably uneven, and the underlying diversity patterns generated by biogeographical history are emerging only slowly (Christiansen 2012). Hard data on cave species number are scattered in the literature, uneven and incomplete, and the statistics given here are indicative.

It is clear that several zoological groups or regions of Japan, the USA, or Europe, thoroughly investigated for more than a century, are progressively running dry of discoveries for many groups. For instance, of the 47 species and subspecies of the large beetle genus *Anophthalmus* that occur in the Dinarides, only three have been described after 2000, against 9 from 1910 to 1920; of the 36 current species and subspecies of the mostly Romanian beetle genus *Drimeotus*, none has been described after 2000, against 11 between 1910 and 1920 (Hlaváč et al. 2017). Some taxa escape however from this trend, even in the best documented hotspots like the beetle genus *Typhloreicheia*: 35 of its 50 Sardinian species, some of them cave restricted, have described after 2000 (Magrini et al. 2013). Nevertheless, globally, the contribution of such regions to global cave biodiversity is decreasing while that of newly surveyed areas is growing rapidly (Fig. 7.1).

The fast pace of hypogean species discoveries that Australia has experienced for several years is for instance not likely to slow down with the total number of subterranean invertebrate species estimated to be 10 times their current number (Guzik et al. 2011). In Russia, the number of described troglobites doubled since 2001 (Turbanov et al. 2016), with an emerging hotspot in Caucasus. A similar progress occurred in parts of the Iberian Peninsula (Reboleira et al. 2011; Sendra et al. 2011). It is nevertheless in the tropics and subtropics that the progress for now two decades has been the fastest, particularly in China, Southeast Asia, and Brazil (Fig. 7.1). Recent discoveries have reinforced the demise of the paradigm of a very low diversity of terrestrial non-guanobitic fauna in these regions, first questioned by Howarth (1972) based on the fauna of Hawaiian lava tubes: troglobites, some clearly troglomorphic, have been discovered in Cuba (Silva Taboada 1974), Jamaica (Peck 1975), New Guinea and Borneo (Chapman 1976, 1980), Central America (Reddell 1981), Sulawesi and Thailand (Deharveng and Leclerc 1986), and many other regions. The lower richness of South America underlined by Deharveng and Bedos (2012) does not hold anymore with the discovery of a rich troglobitic fauna in Brazil (Souza-Silva and Ferreira 2016; Trajano et al. 2016). The knowledge of southern China and Southeast Asia cave diversity has experienced a similar growth (Deharveng et al. 2009b; Golovatch 2015; Tian et al. 2016). Both Brazil and China have now emerged as world-class diversity hotspots for subterranean fauna, at the level of the traditional hotspots of temperate regions (Culver et al. 2006).

In spite of these advances, large blanks subsist on the map. Particularly puzzling is the paucity of Africa in non-guanobitic troglobites, probably a result of insufficient sampling and rarity of favorable habitats. A second gap concerns high altitude tropical caves, where limited available information suggests a high richness in troglomorphic species (Chapman 1976 and Smith 1980 for New Guinea; Reddell 1981 for Guatemala and Mexico).

7.1.2 Original Features of Terrestrial Subterranean Diversity

Aside from the low and uneven species richness evoked above, terrestrial subterranean biodiversity departs considerably from that of other ecosystems by a higher proportion of narrow endemics, a higher contribution of radiations to diversity, a much higher frequency of relictual taxa, and a very original taxonomic composition.

The high representation of short-range endemics in subterranean compared to surface habitats and the much smaller size, on average, of their distribution range are documented in many publications (Gibert and Deharveng 2002; Harvey 2002).

A number of speciose lineages contribute substantially to terrestrial subterranean diversity, as illustrated later on for various groups, like *Aphaenops* beetles in Pyrenean or *Telema* spiders in Southern China (see also Fig. 7.2).

The high contribution of relicts to overall diversity is one of the most exciting features of cave fauna. With major climate changes having generated species extinction much more rapidly in surface than in subterranean habitats, many

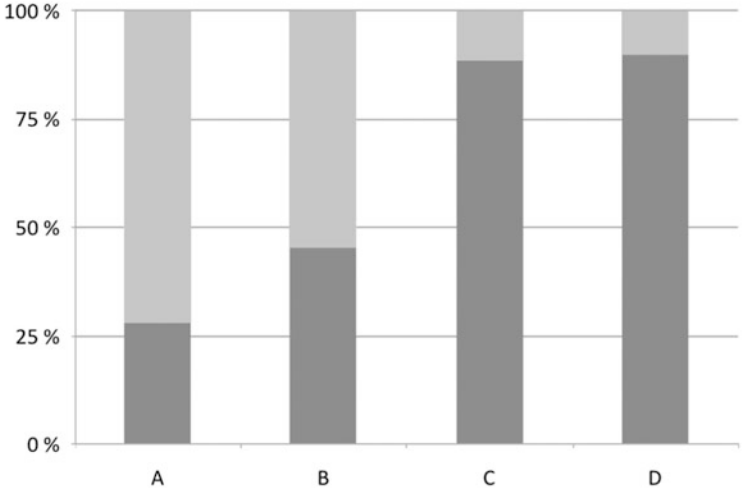

Fig. 7.2 Contribution of the dominant genus to the total richness in troglobites of different suprageneric taxa and regions. (**a**) Coleoptera of the USA (248 species, *Pseudanophthalmus* the richest with 157 species); (**b**) Araneae of the USA (86 species, *Cicurina* the richest with 47 species); (**c**) Trechini of China (104 species, *Dongodytes* the richest with 12 species); (**d**) Leptodirini of the world (935 species, *Bathysciola* the richest with 95 species); richest genus in light grey, pooled richness of other genera in dark grey. **a** and **b** from Culver et al. (2000), **c** from Tian et al. (2016) and **d** from Perreau (2000, and personal communication)

subterranean species are today geographically disconnected from their closest relatives and considered as relicts (Peck 1990; Humphreys 2000).

Four kinds of relicts can be recognized in terrestrial cave fauna: (1) cold climate relicts, generated by the glacial reflux after Pleistocene glaciations, are surprisingly rare; (2) tropical climate relicts, witnesses of warmer or more humid climates, are frequent. The cave fauna of Cape Range in Western Australia, under arid climate today, is for instance clearly related to the fauna of rainforest of Eastern Australia, 3500 km apart (Humphreys 1993); (3) most often, disjunct distributions cannot be explained in climatic terms, and such patterns are much more common in caves than in any other habitat. Many remarkable examples are listed later in the text for each taxon, like the spider *Telema tenella* (Wang et al. 2012) from Catalonia, or the beetle *Dalyat mirabilis* (Ribera et al. 2005) from Spain; (4) phyletic relicts, i.e., taxa without "close" relatives at world level (Humphreys 2000), are also numerous in caves, like the monospecific and micro-endemic millipede genera *Niphatrogleuma* (Switzerland) and *Marboreuma* (Pyrenees).

7.1.3 Taxonomic Composition

The prime characteristic of subterranean diversity is to be severely truncated, in the absence of green plants, which constitute the largest component of surface terrestrial diversity on earth (Gibert and Deharveng 2002). Second, invertebrate groups that colonized underground habitats do not contribute to cave diversity in proportion to their diversity in surface habitats, as clearly illustrated for Hexapoda in Table 7.1: troglobites are in particular absent or rare in all large groups of insects except Coleoptera. Non-insects Arthropods (arachnids, millipedes, terrestrial isopods, and springtails) often linked to soil have a higher proportion of cave-related species. However, several groups such as mites, Symphyla, Pauropoda, Protura, many Collembolan families, a large diversity of Coleoptera, though largely dominating in deep soil communities at the interface with subterranean environments, have almost no troglobitic representatives. The interface between subterranean and soil habitats is perhaps a severe filter rather than a major evolutionary pathway for cave colonization.

7.1.4 Temperate Versus Tropical Diversity

Terrestrial diversity is significantly higher in tropics than that in temperate regions. This is not the case for cave diversity (Deharveng and Bedos 2012), though recent discoveries and the integration of guanobites in statistics may shake this view. Tropical and temperate terrestrial subterranean diversity differs as follows (Deharveng and Bedos 2000):

A—among troglobites and troglophiles not related to guano: (1) higher diversity of arachnids except mites; (2) lower diversity of springtails; (3) lower diversity and lower levels of troglomorphy in beetles; and (4) higher diversity of several other cave groups, like crickets, cockroaches, millipedes, and woodlice.

B—among guanobites and guanophiles: (5) much higher richness at all taxonomic levels.

Trajano and Bichuette (2010) retrieved several of these features in Brazil, but rightly stressed that (points 1 and 4 above) are for a part correlated to the characteristics of local epigean faunas as source of colonizers rather than to that of subterranean ecological factors per se. In subtropical regions, as well as at high altitude in the tropics, diversity patterns exhibit a mix of tropical and temperate subterranean elements, such as local diversification of troglobitic beetles and lower diversity of higher Arachnid taxa and guano species.

7.1.5 Lifestyles and Life Forms

Lifestyles Clearly delimited ecological categories are useful for describing and understanding the diversity, origin, and dynamics of cave communities. Subterranean animals are most often classified according to the strength of their link to subterranean environment, under the classical terms of troglobites, troglophiles, and trogloxenes. We understand here ecological categories as defined below, as a trade-off between practicality, scientific relevance, etymological coherence, and commonness of use. The terminology used by Trajano and Carvalho (2017) is given in brackets when relevant: (1) troglobite: a species only reported from cave and (2) troglophile: a species reported from inside and outside caves. The subdivision of troglophiles into eu- and subtroglophiles proposed by Pavan (1944) and endorsed by Sket (2008) is retained when possible, as meaningful information: eutroglophile: a species with permanent populations inside and outside caves (=troglophile); subtroglophile: a species linked to cave for part of its life cycle (seasonal or circadian) (=trogloxene); (3) trogloxene: a species not linked to caves, whatever its morphology (=accidental).

Some species, of major importance in the tropics, seem to be more dependent on the presence of guano than on cave habitat itself. Following the rationale of "affinity to the environment," Decu (1986) and Gnaspini and Trajano (2000) consider that species should be characterized along two separate gradients, i.e., their dependence on cave habitat and their dependence on guano habitat, in order to meaningfully characterize species ecology. For instance, "guanobites" may be "troglophilic" or "troglobitic." Applied to invertebrate fauna, this might greatly change the current statistics on terrestrial subterranean diversity. For Orthoptera, Desutter-Grandcolas (1998) uses an even more elaborated system (see below).

Assignation of a species to an ecological category faces in many cases with lack of biological information (Giachino et al. 2011; Trajano and Carvalho 2017). The use of morphological traits as indication of troglobitic lifestyle is discussed below.

Life Forms, Facies, Troglomorphy Life forms are combinations of morphological traits that are only or most frequently encountered in species of unrelated groups living in similar environments. They are assumed to result from convergent evolution, acquired under a same set of selective pressures (Trontelj et al. 2012). Their description and interpretation in terms of convergence and divergence, as well as of regressive and progressive evolution, is a major focus of current investigation in subterranean biology (Culver and Pipan 2015, see also Chap. 4).

The concept of facies or "life form" (morphotypes of Golovatch and Kime 2009) is actually widely used in ecology. Gisin (1943) characterized different life forms in Collembola that were characteristic of various habitat types. A similar system has been devised for Diplopoda (Golovatch and Kime 2009). This categorization was greatly improved by Christiansen (1964), who redefined the terminology on a purely morphological basis and introduced the term troglomorphic. Life forms and lifestyles are considered separately in this conception, allowing a double characterization, ecological and morphological, of a species.

Based on the screening of all cave terrestrial groups of invertebrates, we propose here a system of life forms characterized as follows (Table 7.2): (1) hypogeomorphic: regressed eyes, pigment, and wings, without significant modification of body size, appendage length and claw thickness compared to surface relatives; (2) troglomorphic: regressed eyes, pigment, and wings + larger size, more slender body, longer appendages, thinner claws; (3) euedaphomorphic: regressed eyes, pigment, and wings + smaller size, more compact body, shorter appendages, thicker claw (Coiffait 1958); (4) epigeomorphic: no regressive evolution, no particular modification of body size and appendage length.

Troglomorphy as defined above applies more easily to arthropods than to other invertebrates. Troglomorphic and euedaphomorphic life forms are well recognizable in these groups, being at the opposite ends of gradients of appendage and body size (Pipan and Culver 2017). All intermediates exist nevertheless with hypogeomorphic life forms.

Life forms can be used as proxy for assigning species to ecological or functional categories, as far as they have been sufficiently documented in the group. However, such ecological assignation on purely morphological grounds can be misleading as mismatch between life forms and habitat is not exceptional. Culver and Pipan (2009, 2015) point out that the springtail *Acherontides eleonorae* is troglomorphic by its claw elongation but ecologically a guanobite and, based on such mismatch, questioned the use of the term troglomorphy. In fact, guanobites with more or less reduced eyes and pigment compared to their epigean relatives are not exceptional in Diplopoda and Collembola (see later). Nevertheless, the concept of troglomorphy remains statistically robust across a large array of taxa and remains an integrative and descriptive tool to approach evolutionary issues related to convergence and divergence hypotheses.

Troglomorphy illustrates striking convergent morphological evolutions that occurs in many unrelated living groups (see also Chap. 4). However, several of these traits are also observed separately in epigean life forms, from completely different habitats (Christiansen 2012 and Table 7.2). Thus, eye, pigment, and wing regression are shared with interstitial species and are not sufficient to qualify as troglomorphic. They need for that to be combined with appendage or claw elongation in the definition adopted here.

As stated in Chap. 4 and by Christiansen (2012), morphological changes associated with cave life may affect various traits: body size, body slenderness, eyes, pigment, appendage length, foot complex, and wing development. Other more taxon-specific traits are recognized by taxonomists as cave related, like cuticle thinning, pseudophysogastry in beetles, or development of specialized sensory organs in Palpigradi (Condé 1998), Rhagidiidae mites (Zacharda et al. 2011), and Diplura (Bareth and Pagès 1994), increase in number and sensorial organs in Collembola.

Some of these characters may be extremely modified in a few cave species that are qualified as highly troglomorphic. Several examples are given later on. Appendage elongation is particularly spectacular in this respect, in a large variety of groups (Ricinulei, Collembola, Zygentoma, Diplura, Carabidae, and Leiodidae). Such exaggerated traits raise evolutionary and functional questions beyond the scope of this chapter. The last point to be stressed is that, in all diversified terrestrial groups,

Table 7.2 Terminology and definition of terrestrial life forms of Arthropods with most common trends in morphologic traits, and preferential habitats. Derived from Gisin (1943) and Christiansen (1964). 0 very reduced or absent; 1 reduced or short; 2 intermediate or present; 3 large, long, slender, or well developed. Foot complex is too group dependent to be considered here. In brackets, organs which concern only some groups

Life form	Size	Eyes	Pigment	(Antennae), legs, (pedipalps)	(Furca)	Claw	(Wings)	Preferential habitats	Lifestyle best match
Atmobiomorphic	3	3	3	3	3	2	3	Vegetation in open space	Epigean
Hemiedaphomorphic	2	2	2	2	1 or 2	2	1 or 2	Superficial soil	Hemiedaphic, guanobitic
Hygrophilomorphic	2	3	3	2	3	3	3	Wet habitats	Epigean
Troglomorphic	3	0	0	3	3	3	1	Caves, (MSS)	Troglobitic
Hypogeomorphic	2	0 or 1	0 or 1	2	2	2	1 or 2	Caves, MSS, soils	Troglobitic, guanobitic
Euedaphomorphic	1	0	0	1	0 or 1	1	0	Deep soil, interstitial habitats, MSS, caves	Euedaphic, troglobitic

regressive and troglomorphic characters do not evolve at the same pace within a lineage. It is this decoupling that generates the observed diversity in the expression of troglomorphy.

7.2 Non-arthropod Invertebrates

7.2.1 Annelida: Hirudinea (Leeches, Fig. 7.3a)

Hirudinea (more than 680 species distributed worldwide, Sket and Trontelj 2008) mostly live in freshwaters, but are also very common in terrestrial habitats in South Asia and Australasia. Troglobitic species of the family Haemadipsidae are known from Southeast Asian caves (Chapman 1976; Deharveng et al. 2007), but only two have been described: *Leiobdella jawarerensis* from Papua New Guinea and *Haemadipsa cavatuses* from China. Both have reduced skin pigmentation, unlike surface leeches, but retain pigmented eye patches. They may be found in rather large populations on cave walls and have been observed sucking the blood of bats.

7.2.2 Gastropoda (Snails, Fig. 7.3b)

Gastropoda include 80,000 species (Bouchet et al. 2005). Among them, only 80 were considered troglobitic or troglophilic by Bernasconi (2004). They belong to Pulmonata, characterized by the absence of operculum and a portion of the mantle cavity modified for respiration, as adaptations to terrestrial life.

Cave species are distributed in 12 families (Culver 2012), but cave-restricted ones are probably few. They include carnivorous species like the troglobitic slug *Troglolestes sokolovi* from Caucasus and several troglophilic *Oxychilus*. Most detritivorous troglobites were described from the Dinarides, but some species are also known from other Mediterranean regions like the Pyreneo-cantabric range. The genus *Zospeum* (Ellobiidae) is the richest in species, all of minute size, with a disjunct distribution Eastern and Dinaric Alps—Pyreneo-cantabric range. Other microsnails were recently described from Eastern Asia, some less than one mm long, hence among the smallest snails on earth (Páll-Gergely et al. 2015). Several of them (e.g., *Koreozospeum nodongense* from Korea) are only known from caves (Weigand et al. 2013; Jochum et al. 2015). The Subulinidae so frequent on the guano of tropical caves are considered eutroglophiles.

Eye and pigment reduction are retrieved in all troglobitic species, and partly in cave Subulinidae. Elongation of tentacles is also obvious in some species, and the shell is often thinner than that of surface species (Vandel 1964).

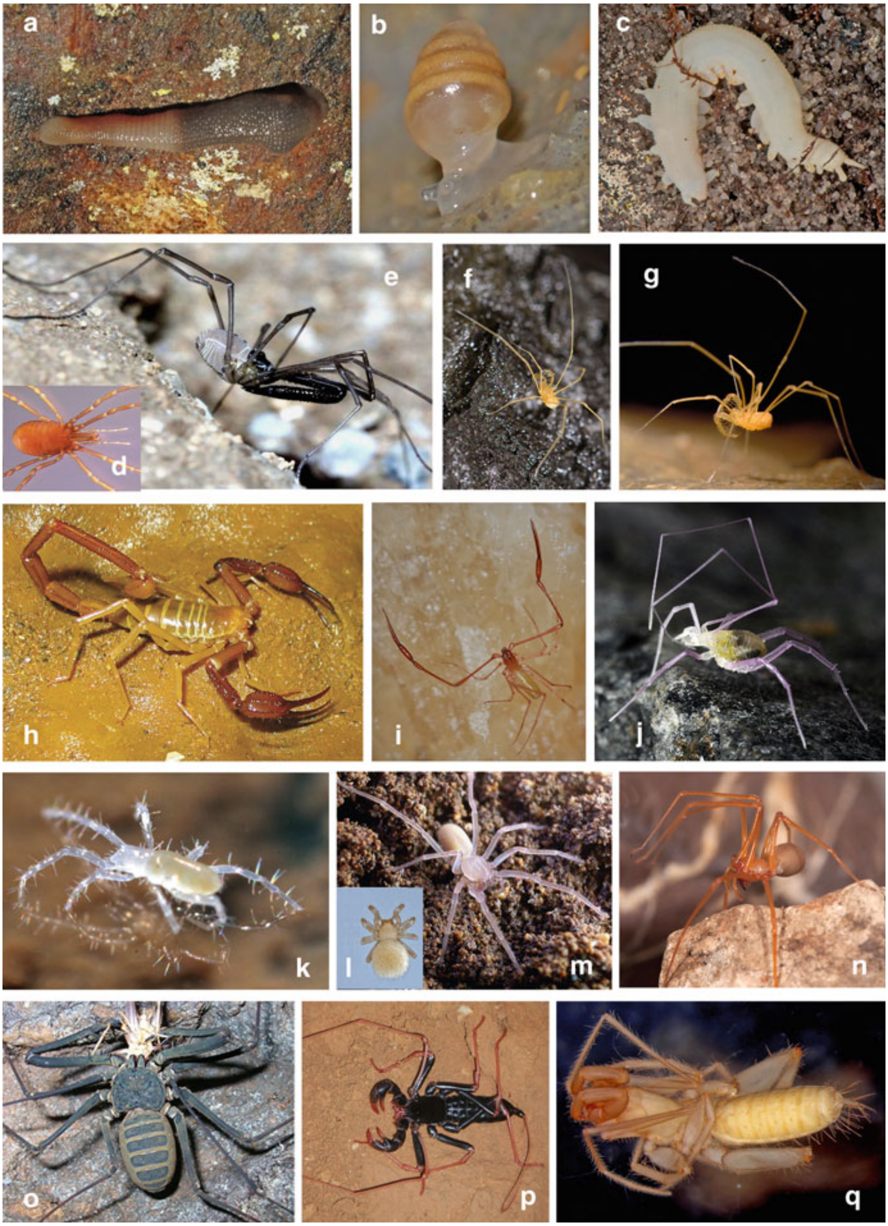

Fig. 7.3 Cave non-arthropods and Arachnida; not at the same scale. (**a**) *Haemadipsa* sp. (Hirudinea; Sarawak); (**b**) *Zospeum bellesi* (Gastropoda Ellobiidae; Pyrenees); (**c**) *Peripatopsis alba* (Onychophora; South Africa); Opiliones (**d** to **g**): (**d**) *Shearogovea mexasca* (Cyphophthalmi; Mexico), (**e**) *Taracus silvestrii* (Ischyropsalidae; USA), (**f**) *Speleonychia sengeri* (Travuniidae; USA), (**g**) *Trojanella* serbica (Travuniidae; Serbia); (**h**) *Alacran tartarus* (Scorpions; Mexico); (**i**) *Troglobisium racovitzai* (Pseudoscorpiones; Spain); (**j**) *Siamacarus* sp. (Opilioacari; Vietnam); (**k**) *Rhagidia (Deharvengiella) paralleloseta* (Rhagidiidae; France); Araneae (**l** to **n**): (**l**) *Anapistula ataecina* (Symphytognathidae; Portugal), (**m**) *Amauropelma matakecil* (Ctenidae; Indonesia), (**n**) *Trogloraptor marchingtoni* (Trogloraptoridae; USA); Amblypygi: (**o**) Whip spider eating a cricket

7.2.3 Nematoda

The phylum Nematoda includes 24,773 extant species (Hodda 2011) of which 295 have been reported underground (Du Preez et al. 2017). The two species considered as troglobites (*Mylonchulus cavensis* and *Stenonchulus troglodytes* both from Europe) were recently retrieved in soil.

7.2.4 Onychophora (velvet worms, Fig. 7.3c)

The phylum Onychophora has 180 extant species and 49 genera (Mayer and Oliveira 2011). All are terrestrial ambush predators and show a number of highly peculiar morphological and biological features. They are distributed in two families, each with one troglobite: Peripatopsidae in the southern hemisphere with *Peripatopsis alba* from South Africa and Peripatidae in the tropics with *Speleoperipatus spelaeus* from Jamaica. Both are devoid of eyes and pigment contrary to all surface species except one, *Tasmanipatus anophthalmus* from Tasmania, completely white and blind.

7.3 Arachnida

Arachnida encompasses 112,201 extant species (Zhang 2011) in 16 orders, all represented in subterranean habitats except Solifugae. They comprise parasites, guanobites, troglophiles, and troglobites, but in very uneven proportions. In spite of their huge diversity, mites (Parasitiformes + Acariformes, formerly Acari) are very poorly represented in oligotrophic cave habitats, being only diversified in guano or as parasites. On the contrary, spiders and Opiliones, rare in guano, have successfully colonized subterranean oligotrophic habitats.

Fig. 7.3 (continued) (Amblypigi; Indonesia), (**p**) *Typopeltis cf. magnificus* (Thelyphonida; Laos); (**q**) Hubbardiidae (Schizomida; Vietnam). Photos by © A Cressler (**a**), © O Gargominy (**b**), © G Giribet (**c**), © G Giribet (2011) (**d**), Shear and Warfel (2016) (**e**), © M Hedin (**f**), I Karaman in Kury et al. (2014) (**g**), P Sprouse in NSF (2017) website (**h**), © A Meseguer (**i**), © M Lukić (**j**, **k**), Cardoso and Scharff (2009) (**l**), Miller and Rahmadi (2012) (**m**), Griswold et al. (2012) (**n**), © C Rahmadi (**o**), © L Deharveng and A Bedos (**p**, **q**)

7.3.1 Opiliones (Fig. 7.3d–g)

Statistics and taxonomic hierarchy for this order follow Kury (2017) unless specified. Opiliones contain more than 6650 extant species in ca. 1670 genera and four monophyletic suborders: Cyphophthalmi, Eupnoi, Dyspnoi, and Laniatores (Fernández et al. 2017). Most are predators of small arthropods. Troglobites and troglophiles are numerous, but very few are guano dwellers. Eupnoi has subtroglophiles not dealt with here, but no troglobites. The other suborders include troglophiles and troglobites, some of them strongly troglomorphic. In 1994, Rambla and Juberthie estimated that Opiliones included 82 troglobites and 33 troglophiles, of which 50 belonged to Gonyleptoidea and 17 to Triaenonychidae. These numbers have since enormously increased, though detailed statistics are not available.

Cyphophthalmi suborder (147 species, six families) is patchily distributed worldwide. Its species live in soil and leaf litter and a few are recorded from caves. They are homogeneous in habitus, small, uniformly and often darkly pigmented, oval, compact, and relatively short-legged. Many species have been described during the last two decades, with considerable progress in the knowledge of their phylogeny and biogeography (Giribet et al. 2016). Stylocellidae family of Southeast Asia includes seven troglobites, three of which are eyeless species of *Fangensis* (Clouse 2012). Among the 61 species of Petallidae, of Gondwanan distribution, only *Purcellia argasiformis* from South Africa is troglobitic, but slightly modified for cave life. *Troglosiro aelleni*, endemic to New Caledonia, is the only cave representative of this genus of 13 species. It is eyeless, but darkly pigmented and without appendage elongation. The Afro-American Neogoveidae include the Brazilian troglobite *Canga renatae*, eyeless but short-legged. The relictual troglobites *Marwe coarctata* from Kenya and *Shearogovea mexasca* from Mexico combine anophthalmy, pale coloration, and elongated appendages. Sironidae family (52 species, eight genera), distributed in the northern Mediterranean region with isolated spots in Japan and the USA, is the richest in troglobites, all members of the large Northeastern Mediterranean genus *Cyphophthalmus*, and representing more than half of its 32 species (Karaman 2009). Anophthalmy and depigmentation are shared by all species of the genus; appendage elongation, including chelicerae, is significant in some species like *C. paradoxus.*

Eye reduction and depigmentation in troglobitic Cyphophthalmi are associated with subterranean life in some lineages (Stylocellidae), but phyletic characters in others (Sironidae). Appendage elongation is at most moderate in troglobitic species.

The suborder Dyspnoi (ca. 390 species) is mostly Holarctic in distribution. Tens of species have been reported from caves as troglobites or troglophiles (Schönhofer 2013). In southern Europe, half of the ca. 23 species of the genus *Ischyropsalis* are cave related, and several like *Ischyropsalis pyrenaea* from Pyrenees are weakly modified troglobites (Schönhofer et al. 2015). Eye reduction, depigmentation, and appendage elongation are common in other genera, with more than 10 eyeless species, including seven of the Caucasian genus *Nemaspela*.

Laniatores suborder (ca. 4248 species, 29 families) is extremely diverse morphologically and includes many eutroglophiles or troglobites (but no guano species). Since the 1990s, its taxonomy has progressed at a fast pace, through taxonomic rearrangements (Kury 2015), phylogenetic studies (Sharma and Giribet 2011), and new taxa descriptions. The richest continent in cave Laniatores is America. They are considerably less diversified in Africa, the Palaearctic, and tropical Asia. The eight superfamilies recognized by Kury (2017) include cave-obligate species.

About 13 troglobites, mostly African, are known in Assamioidea superfamily (535 species). Three of them are eyeless, whereas anophthalmy is reported in at least ten non-cave species of the group (Starega 1992). Two species of the superfamily are highly troglomorphic: *Maiorerus randoi* from Canary Islands and *Jarmilana pecki* from Belize, the only American species of the African family Pyramidopidae.

Gonyleptoidea superfamily (2039 species), mostly American, contributes largely to the overall diversity of troglobitic Laniatores. Several families are concerned, with Stygnopsidae the richest. Of its more than 30 troglobites, about one-third are blind. A few species have significantly elongated appendages associated with anophthalmy and depigmentation, like *Giupponia chagasi* from Brazil or *Mictlana inops* from Mexico.

Phalangodoidea superfamily (132 species) is as rich as Gonyleptoidea in troglobites (>30), but they represent a much higher proportion of the total richness. Most European Phalangodoidea are subterranean, i.e., about ten species including two rare, eyeless, highly troglomorphic ones in relictual monospecific genera of Balkans: *Paralola buresi* and *Lola insularis*. North America is much richer than Europe, with a remarkable hotspot in California where about 50 species and four endemic genera are known (Derkarabetian et al. 2010), including non-cave and cave species at various levels of eye reduction and appendage elongation. Among them, *Banksula* comprises ten species, nine of which are troglobites and blind or quasi-blind. The most troglomorphic species of northern America, like *Texella mulaiki,* have very long appendages.

Only 4–5 troglobites are known among Epedanoidea superfamily, mostly diversified in the Indo-Malayan and Australasian regions (ca. 410 species). None is troglomorphic, though *Sungsotia uenoi* from Vietnam exhibits some eye and pigment reduction. Samooidea superfamily (215 species) is a small group of Laniatores with a few troglobites, mostly diversified in tropical America. A single species, *Stygnomma fiskei*, is eyeless and has elongated appendages. More than half of the 78 species of Travunioidea superfamily are cave dwellers, linked to oligotrophic habitats. They are concentrated in two regions: the USA and Mediterranean Europe. The USA has a dozen of weakly cave-adapted troglobites, all oculated, in two genera *(Erebomaster* and *Sclerobunus)*, and three troglomorphic eyeless species in two genera (*Speleonychia*, monospecific, and *Speleomaster*, bispecific). In Europe, more than 20 cave species are known, all in Travuniidae, including about 15 troglobites in mono- or oligospecific relictual genera scattered across northern Mediterranean regions. They are highly troglomorphic in facies, like *Trojanella serbica* from Serbia. Adaptive traits include reduction or absence of eyes (about ten species are eyeless), very pale coloration, and elongated appendages.

Triaenonychoidea superfamily (ca. 490 species), temperate Gondwanan in distribution, is poorly diversified in caves with only four troglobites. Two of them are eyeless and troglomorphic: *Picunchenops spelaeus* from Patagonia and *Lomanella troglodytes* from Tasmania. Zalmoxoidea superfamily (272 species), widely distributed in tropical regions, have troglobites in America, and one in Australia. Reduced eyes or anophthalmy are reported in several of them, but also in some soil species. The cave-restricted *Relictopiolus galadriel* from Brazil has moderately elongated appendages and minute size (Pérez-González et al. 2017).

Troglomorphy among Opiliones is mostly expressed through the classical trends of eye reduction, depigmentation, and appendage elongation, while body size may increase or decrease. Eye reduction up to disappearance is also reported in non-cave species of Assamioidea in Africa (Kauri 1985) or Zalmoxoidea in Brazil (Pinto-da-Rocha and Kury 2003), associated with soil or termite nests.

7.3.2 Scorpiones (Fig. 7.3h)

Scorpiones (1947 species, 18 families, Prendini 2011a) are all predators. Lourenço (1994) mentioned 13 troglobitic species in four families, of which 11 are from Mexico. Sixteen years later, Prendini et al. (2010) considered as unequivocally troglobitic 23 species, in 16 genera and 10 families. Today, we know about 40 cave-restricted species in 25 genera and 12 families and a few eutroglophilic ones.

More than half of the troglobitic scorpions have eyes and pigment reduced or absent, often associated with appendage elongation. Anophthalmy is however retrieved in non-cave species, like *Chaerilus telnovi* from Halmahera. Cave scorpions are patchily distributed. They are absent in temperate regions, except the relictual genera *Belisarius* in Catalonia, almost blind but troglophilic, and *Akrav* in Israel, troglobitic and blind. Most cave scorpions are distributed in two spots: Mexico and central Indochina. In Mexico, troglobites (ca. eight species in four genera, all eyeless) belong to Typhlochactidae. *Alacran* species are rather large (6–7 cm) and highly troglomorphic, and two of them are the only known amphibious scorpions (Santibáñez-López et al. 2014). Troglobites of the three other genera, like *Typhlochactas reddelli*, are often minute (less than 2.5 cm). In Cuba, the guanobitic-troglobitic *Alayotityius delacruzi* is not morphologically modified. Central Indochina between 15°N and 20°N is the second spot of diversity, recently discovered, with five cave species in the small relictual family Pseudochactidae (Lourenço and Pham 2010), including three blind *Vietbocap*. In the tropics south of 15°N, scorpions are highly diverse in surface habitats, but rare underground. The only blind cave species in this area is *Chaerilus sabinae* from Sulawesi. No troglobite is known from Africa, only a few, weakly modified, from Madagascar (Lourenço and Goodman 2008), South America (Gallão and Bichuette 2016), and tropical Asia. At least, Australia has a single troglobite, *Aops oncodactylus*, which is small, blind, and depigmented.

7.3.3 Pseudoscorpiones (Fig. 7.3i)

Pseudoscorpiones (3533 extant species, 26 families in 2012, Harvey 2013) are small-size predators of arthropods, frequent in soil, mosses, under barks, and in caves, where most of their 26 families are represented. Cave-dwelling species can be allocated to two ecological categories: guano species and oligotrophic habitat species. Guano species, mostly Chernetidae and Cheliferidae, are moderately diversified, not troglomorphic and often widely distributed through phoretic behavior.

Troglobites of oligotrophic habitats are narrow endemics and much more diverse, though often extremely rare. Most have reduced or no eyes, and elongated appendages, to an extreme degree in some Ideoroncidae and Bochicidae. On the other hand, several eyeless troglobites are euedaphomorphic in facies.

Troglobitic species are especially numerous among Chthoniidae and Neobisiidae. Edward and Harvey (2008) list 49 species of blind cave-dwelling Chthoniidae in the world, most of them troglobitic members of *Lagynnochthonius* and *Tyrannochthonius*, diversified in various regions like the USA and Spain (Zaragoza 2017), Australia, or the Dinarides (Ozimec 2004). The family Neobisiidae exhibits a similar richness in the latter region, with many cave endemics.

Relictual Pseudoscorpiones are frequent in caves. The Iberian Peninsula is of particular interest, with two highly troglomorphic relicts, *Titanobochica magna* and *Troglobisium racovitzai*, only representatives in Europe of the American family Bochicidae (Reboleira et al. 2010), and *Arcanobisium comasi*, the only species of the subfamily Arcanobisiinae within Syarinidae. In Southeast Asia, the subfamily Cybellinae was recently defined for two non-troglomorphic cave species of Feaellidae, a family mostly diversified in Africa (Judson 2017).

7.3.4 Palpigradi

Palpigradi (82 species, two families, Prendini 2011b) are small (less than 2.2 mm), delicate, white and eyeless, and live in soils and caves of tropical, Mediterranean, and more rarely temperate regions (Condé 1996). In the northern part of their distribution range, they are only found in caves. In the tropics, they are more frequent in soils. They seem to prey on small microarthropods like Collembola (Condé 1996). Most cave species are members of the cosmopolitan genus *Eukoenenia*. Europe has more than 25 described troglobitic species (Condé 1998), while the two richest tropical countries, i.e., Brazil and Thailand, have seven and five species of *Eukoenenia* (Souza and Ferreira 2016).

Troglomorphic characters observed in cave species include larger size and longer appendages, and an increase in the number of blades in the lateral organ of the prosoma (Condé 1998), of unknown function. These characters change at different pace during evolution. Thus, *E. thais* from Thailand which has long appendages and the highest number of blades in the lateral organ is a small-size species (about 1 mm).

7.3.5 Ricinulei

Ricinulei (58 species, a single family, Prendini 2011c) are predators of nematodes and small arthropods. They are well diversified in tropical America, less in Africa, and absent in Asia. Cave species, restricted to tropical America, are usually guanobitic, blind but pigmented like surface species, and of moderate size (10 mm at most). A wide gradient of appendage elongation is known in *Pseudocellus*, which contains the most troglomorphic species, *Pseudocellus krejcae*.

7.3.6 Acari (Mites) (Fig. 7.3j, k)

Though no more recognized as monophyletic, mites are very abundant and highly diversified in energy-rich habitats of caves, while very few have adapted to energy-poor habitats. Troglomorphic species are exceptional, limited to a few Opilioacari and Rhagidiidae. This low representation of mites in cave oligotrophic habitats illustrates that soil has not been a highway for the colonization of these habitats by this invertebrate group. Two superorders and four orders contain troglobitic species: Parasitiformes with the orders Opilioacarida and Gamasida, and Acariformes with the orders Sarcoptiformes and Trombidiformes.

Opilioacarida order includes ca. 35 species in a single family (Beaulieu et al. 2011), that live in soil or under tree barks mainly in the tropics. Four species in two genera are known as cave dwellers. Two are *Opilioacarus* from Cuban caves; they have eyes, pigment, and non-elongated appendages. Two are *Siamacarus* from southern Thailand caves where they live in large populations on guano; they have delicate and long legs and are almost devoid of pigment, though eyes are still present. This genus is present in Vietnam with undescribed troglobitic species (Deharveng et al. 2009a). Gamasida, the largest order of Parasitiformes (11,424 species, 109 families; Beaulieu et al. 2011), are distributed worldwide. A large number of cave species are permanent or temporary ectoparasites of bats, but most are free living. Parasitidae and several related families are active predators in high-energy habitats. Many Gamasida are only known from caves. Thus, 60% of the 50 subterranean species of Uropodidae are cave restricted, with 12 of them in the genus *Uroobovella* alone, but we ignore in most cases to which extent these non-modified species are dependent on subterranean environment. Many species of Gamasida disperse by phoresy, which may explain their frequently wide distribution range.

Among Sarcoptiformes the suborder Oribatida contains 16197 extant species in 249 families (Schatz et al. 2011). Its species, all decomposers, are the most diversified and abundant mites in soil. They are represented in caves as troglophiles or guano dwellers, but cave-restricted species are surprisingly rare: some species of *Schwiebea* (Acaridida) from humid hypogean habitats, devoid of adaptations to subterranean life, and a few European Belbidae, such as *Metabelbella* *phalangioides*—pale and long-legged. Trombidiformes (25821 extant species, 151 families; Zhang et al. 2011) is one of the most diverse order of mites. Its larger subdivision, Prostigmata (more than 20,000 species), includes a few poorly known cave species (Palacios-Vargas et al. 1998) in the families Leeuwenhoekiidae, Trombiculidae, and Trombidiidae, frequent on or close to guano accumulations in tropical caves, and bat or invertebrate parasites at larval stage. They are large, unpigmented, eyeless, and long-legged, i.e., of typical troglomorphic facies. The family Proterorhagidiidae has a single relictual troglobite, *Proterorhagia oztotloica* from Mexico. Rhagidiidae is the only mite family that has colonized cave oligotrophic habitats. Its species are hunting predators frequent in soils worldwide, all blind and unpigmented (Zacharda 1980). Troglobitic Rhagidiidae are often linked to cold caves in the Holarctic. They may exhibit substantial appendage elongation. Peculiar to the family, the rhagidial organ, a group of solenidia of unknown function, shows significant elongation or multiplication in most cave-obligate species.

7.3.7 Araneae (spiders, Fig. 7.3l–n)

In 2017, extant Araneae encompassed almost 47,000 species in ca. 4060 genera and 112 families (World Spider Catalog 2017). Spiders have colonized most terrestrial habitats and are a major faunistic element in caves and subterranean voids. While they are second to mites in soil layers and guano, spiders are the main predators in parietal and often in oligotrophic habitats. Two syntheses on cave spiders diversity and biology were recently published (Mammola et al. 2017; Mammola and Isaia 2017). At the world level, these authors provide an estimate of 1000 cave-related species, in at least 48 families.

Northern temperate regions are the richest in cave spiders, but increasing sampling in the tropics may change the story. No less than 40 eyeless cave spiders are already described from tropical regions (Mammola and Isaia 2017), and we know that a same cave may harbor several strongly modified troglobitic species (Bloom et al. 2014; Deharveng and Bedos 2000). Sparassidae (*Heteropoda* and related genera), prominent predators of the giant arthropod communities of Southeast Asia, have diversified into a huge number of mostly epigean species worldwide (Jäger 2014), with several cave species, some of them with eye reduction up to anophthalmy (*Sinopoda scurion* from Laos).

In Europe, 486 cave-related species are known, of which 194 are considered troglobites (Mammola et al. 2017). This modest number of troglobites compared to the huge number of troglophiles is related to the large contingent of darkness-loving species that exist all over the world. Among troglobites, the families Linyphiidae and Dysderidae are dominant, like in other temperate regions, in contrast to many tropical and southern hemisphere regions, where other families contribute more to biodiversity.

Like the cited *Sinopoda*, most genera of troglobitic spiders have epigean and troglophilic counterparts (Ribera 2004). Regional radiations involving surface and cave species contribute for a large part to spider diversity, like that of the genus *Dysdera* in Canary Islands. In southern China, the recently disclosed radiation of the genus *Telema* encompasses dozens of species, probably all troglobitic, some eyed and some eyeless (Lin and Li 2010; Wang and Li 2010). The radiation of *Cicurina (Cicurella)* in Texas includes 80 surface and troglobitic species with extensive gradients of eye regression, from full-eyed to eyeless species (Paquin and Dupérré 2009). Conversely, relictual species that have no close relatives in surface fauna are surprisingly rare compared to other cave groups. The case of *Telema tenella* is the best documented: the species limited to a few caves of Catalonia (Spain) is thousands km away from other species of other Telemidae in Africa, America, or Asia. A second remarkable species, *Anapistula ataecina* from Portugal, is the only member of the family Symphytognathidae in Europe. A last remarkable relict is *Trogloraptor marchingtoni* recently discovered in caves of Oregon (USA), for which a special family (Trogloraptoridae) has been erected.

Morphological diversity within cave spiders is illustrated among various features by their size range (Mammola and Isaia 2017): from 0.4 mm (*Anapistula ataecina* from Portugal, one of the smallest spiders in the world) to 30 cm of legspan (*Heteropoda maxima* from Laos). The troglomorphic "syndrome" is a classical combination of eye reduction, depigmentation, size increase, and appendage elongation, but is less obvious in spiders than in other arthropods for the last two characters. Tiny species, particularly in the families Oonopidae and Tetrablemmidae, frequently encountered in tropical caves, have a euedaphomorphic facies, and may prove to be actually soil dwellers, except *Bacillemma leclerci*, a troglobite from Thailand, which combines very small size and very long legs.

7.3.8 Amblypygi (Whip Spiders, Fig. 7.3o, p)

Amblypygi comprise ca. 190 extant species grouped into five families (Seiter and Wolf 2014) and two unequal suborders: Paleoamblypygi and Euamblypygi. Statistics on the different families are drawn from Prendini (2011d). The group is widespread in the tropics. All species are predators using a "sit-and-wait" hunting strategy. Till recently, only two or three cave species of Amblypygi were considered troglobitic (Weygoldt 2000). A number of new species have been described since, mostly in the genus *Charinus* in Brazil, several troglobitic with reduced eyes and pigment (Giupponi and Miranda 2016).

Paleoamblypygi suborder contains a single extant species of small size (*Paracharon caecus*), which lives in termite nests in Guinea-Bissau and is completely blind (Weygoldt 2000). Almost half of Euamblypygi species are troglophiles and few are troglobites. Most troglobites belong to Phrynidae and Charinidae. Phrynidae (70 species, four genera) are often of large size and are all American except *Phrynus exsul* from an Indonesian cave. Phrynidae include troglobites from Mexico and Cuba with eyes variously reduced, like *Paraphrynus reddelli*, an eyeless species of Mexico. Charinidae (ca. 90 species, three genera) are small-size circumtropical Amblypygi, including at least 30 short-range troglobites or troglophiles, mostly from Southeast Asia and mostly in the genera *Charinus* and *Sarax*. Cave species tend to be depigmented and their eyes variously reduced, up to anophthalmy for instance in *Charinus caribensis* from Jamaica or *C. stygochthobius* from Socotra Island. Charontidae (14 species, two genera) are limited to Southeast Asia, Australasia, and the Pacific. Some large species are components of the giant arthropod cave fauna of Southeast Asia. None exhibits clear morphological adaptations to cave life.

The only certain cave-adaptive characters of cave Amblypygi are the reduction of eyes and pigment. Both traits are however also observed in some non-cave species. The long appendages are similar in cave and non-cave species. Adaptation of Amblypygi to cave life is therefore weakly expressed in their morphology.

7.3.9 Thelyphonida (Giant Whip Scorpions, Fig. 7.3p)

Thelyphonida (110 species, one family; Prendini 2011e) are large-size tropical Arachnida. No cave dwellers were reported by Juberthie and Decu (1994, 1998). The recently described *Typopeltis magnificus* is however, in spite of its dark coloration, a regular inhabitant of caves in southern Laos, so far unnoticed outside caves.

7.3.10 Schizomida (Microwhip Scorpions, Fig. 7.3q)

Schizomida (ca. 260 species, two families; Prendini 2011f) are fast-moving predators of moderate size (5–10 mm), living in soils and in caves, preferentially in high-energy habitats. Protoschizomidae, restricted to Mexico and Texas, has 16 species in two genera, all troglobitic. Hubbardiidae, widely distributed in tropical and subtropical regions up to Japan and the USA, include the remaining species, with troglobites or troglophiles. Several show weak troglomorphic traits such as loss of eyes or relatively elongated appendages (Cokendolpher and Reddell 1992).

7.4 Myriapoda

Of the four classes of Myriapoda, one has no cave species (Pauropoda) and another a few uncertain troglobites (Symphyla; Juberthie-Jupeau 1994).

7.4.1 Chilopoda (Centipedes, Fig. 7.4a)

Chilopoda (ca. 3110 extant species, 24 families; Minelli 2011) is divided into 5 orders. Scutigeromorpha and Scolopendromorpha are mostly diversified under warm climates, Lithobiomorpha and Craterostigmomorpha in temperate regions, while Geophilomorpha are present worldwide. All Chilopoda are carnivorous and play a major role in soil and cave communities. Cave species are known in six families: Scutigeridae (Scutigeromorpha), Lithobiidae (Lithobiomorpha), Cryptopidae, Scolopendridae and Scolopocryptopidae (Scolopendromorpha), and Geophilidae (Geophilomorpha). Scutigeridae and Scolopendridae include troglophiles, but no troglobites. An estimate of 55 troglobitic Chilopoda was given by Negrea and Minelli (1994). This number has increased to at least 70 species today.

Lithobiidae (1000 species, 43 genera, Minelli 2011) are mostly, though not only, diversified in the western Palaearctic. Bonato et al. (2016) list 519 species for the genus *Lithobius* alone, which is the richest in troglobites and troglophiles. In France, 16 of its 62 species are troglobites (Geoffroy and Iorio 2009). Eye reduction and appendage elongation are frequent among cave species, where elongation of antennae and legs may be extreme, like in *L. matulicii* (Dinarides) or *L. lorioli* (Cantabric range). Other troglomorphic species are known in the eastern Mediterranean genus *Eupolybothrus*, like *E. cavernicolus* from the Dinarides.

Cryptopidae and Scolopocryptopidae have a few troglobites in Europe, Canary Islands, and Australia, and more in tropical and subtropical America with ca. five taxa of *Cryptops* (Azara and Ferreira 2014) and ca. eight of Scolopocryptopidae (Chagas and Bichuette 2015). Strongly elongated antennae and legs are known in *Scolopocryptops troglocaudatus* and several *Newportia*. Only two troglobitic Geophilidae with significant antennal elongation exist in Europe: *Geophilus persephones* from Pyrenees and *Geophilus hadesi* from Croatia (Stoev et al. 2015).

7.4.2 Diplopoda (Millipedes, Fig. 7.4b–i)

Diplopoda is a mega-diverse group of mostly saprophagous soil species (7753 species, 147 families, Shear 2011). They are present in all regions of the world, with a large number of cave-related species in 11 of the 16 orders of the class, taxonomically arranged as follows (Shear 2011): subclass Penicillata (Polyxenida), subclass Chilognatha (infraclass Pentazonia (Glomeridesmida, Glomerida), infraclass Helminthomorpha (Siphonophorida, Julida, Spirostreptida, Spirobolida, Callipodida, Chordeumatida, Stemmiulida, Polydesmida)).

Polyxenida order (86 extant species, four families, Shear 2011) is distributed worldwide. Its species are abundant in dry soils and under barks, with ca. three troglobites: *Lophoproctus pagesi* from Balearic Islands and two recently described species from Christmas Island (*Lophoturus speophilus* and *L. humphreysi*). A weak elongation of appendages is noted in *L. pagesi* and *L. speophilus*.

Fig. 7.4 Cave Myriapoda, Isopoda, Collembola, Diplura, Zygentoma; not at same scale. (**a**) *Eupolybothrus cavernicolus* (Chilopoda; Croatia); Diplopoda (**b** to **i**): (**b**) *Illacme tobini* (Siphonorhinidae; USA), (**c**) *Glyphiulus* sp. (Cambalopsidae; China) feeding on a dead bat, (**d**) *Pseudonannolene lundi* (Pseudonannolenidae; Brazil), (**e**) *Biokovella mauriesi* (Biokovellidae;

Glomeridesmida order (31 species, two families, Shear 2011) is locally frequent in tropical forest of Asia and America. Unidentified species are cited from various caves of Southeast Asia (Shelley and Golovatch 2011), and two troglobitic *Glomeridesmus* species have been described from Mexico and Brazil.

Glomerida order (pill millipedes; 212 species, two families, Shear 2011) is widely distributed in East Asia and the Euro-Mediterranean region, with a few representatives in central and northern America (Shelley and Golovatch 2011). In America, only the genus *Glomeroides* is present underground, with six microphthalmic troglobites from Mexico (Palacios-Vargas et al. 2015). The Euro-Mediterranean region is much richer, especially its western part, with several oligospecific genera including cave species of euedaphomorphic facies, such as *Doderia*, *Geoglomeris,* and *Spelaeoglomeris* (Mauriès 1994). Four troglobites are reported from southern Spain, northern Africa, and Canary Islands in the large, ordinarily epigean genus *Glomeris*. *Hyleoglomeris* known till recently by a few cave species from southeastern Europe and Southeast Asia, unpigmented and blind or microphthalmic, is in fact diversified, with more than 20 troglobitic species described since 2006 in these regions (Golovatch et al. 2012). Cave Glomerida are depigmented, their eyes are reduced or absent, but their appendages not elongated.

Siphonophorida order (ca. 70 species, 14 genera, Shear 2011) includes three subterranean species: *Yucatanium sabachana* from Mexico (Palacios-Vargas et al. 2015) and two species of the relictual Californian genus *Illacme*: *I. plenipes*, an MSS inhabitant and the leggiest animal of the world with up to 750 legs, and *I. tobini* known by a single specimen from cave. They are phyletically eyeless, but not troglomorphic.

Julida order (ca. 740 extant species, 163 genera, Shear 2011) is mostly distributed in the Holarctic and Oriental regions. Since the review of Mauriès (1994), a number of cave species and genera have been described in the families Julidae, more diversified in eastern Mediterranean regions, and Blaniulidae, more diversified in western Mediterranean regions. In Julidae, the genus *Typhloiulus* has ca. 30 cave species (WoRMS 2017), scattered in the Balkans and surroundings, eutroglophilic or troglobitic (Mauriès 1994). Cave species are known in several other genera, like

Fig. 7.4 (continued) Croatia), (**f**) *Dobrodesmus mirabilis* (Dobrodesmidae; Brazil), (**g** and **h**) *Pacidesmus* spp. (China), non-troglomorphic (above) and troglomorphic (below), (**i**) *Eutrichodesmus astrisimilis* (Vietnam); Crustacea (**j** to **m**): (**j**) *Erebusa calobates* (Brachyura; Laos), (**k**) *Trogloniscus trilobatus* (Oniscidea; China), (**l**) *Iuiuniscus iuiuensis* (Oniscidea; Brazil), (**m**) *Xangoniscus odara* (Oniscidea; Brazil); Collembola (**n** to **t**): (**n**) *Yuukianura judithae* on bat guano (Vanuatu), (**o**) *Verhoeffiella longicornis* (Bosnia-Herzegovina), (**p**) *Sinella longiantenna* (China), (**q**) *Lepidonella lecongkieti* (Vietnam), (**r**) *Oncopodura* sp. (Spain), (**s**) *Tritomurus veles* (Croatia), (**t**) *Galeriella liciniana* (Bosnia-Herzegovina); (**u**) *Gollumjapyx smeagol* (Diplura; Spain); (**v**) *Texoreddellia* sp. (Zygentoma; USA). Photos by Stoev et al. (2013) (**a**), Marek et al. (2016) (**b**), Latella and Hu (2008) (**c**), Iniesta and Ferreira (2015) (**d**), Antić et al. (2016) (**e**), Shear et al. (2016) (**f**), © L. Deharveng and A. Bedos (**g, h, j, k, p, q, r**), D VandenSpiegel in Golovatch et al. (2016) (**i**), Souza et al. (2015) (**l**), Campos-Filho et al. (2016) (**m**), © B Lips (**n**), © M. Lukić (**o**, **s**, **t**), JM Azkarraga in Sendra et al. (2006) (**u**), Espinasa et al. (2016) (**v**) eyeless *Dolichoiulus* in Canary Islands (Enghoff 2012). The six species of the Pyreneo-Cantabric genus *Baskoiulus* are troglobites, as well as the two species of the Catalan genus *Paratyphloiulus*. Several monospecific genera have also troglobitic species, like *Mammamia* from Italy. Blaniulidae have more than 50 cave-dwelling species, particularly diversified in southwestern Europe (Antić et al. 2015). Of the 11 species of *Blaniulus*, six are troglobitic (Kime and Enghoff 2017), and others are known in a number of oligo- or monospecific genera of western (e.g., *Euzkadiulus*, *Iberoiulus*, *Tarracoblaniulus)* and eastern Mediterranean regions (*Cibiniulus*, *Thassoblaniulus*). Nemasomatidae includes some remarkable troglobites, like *Antrokoreana gracilipes* from Korea, and two species of the genus *Thalassisobates* (continental Spain and Canary Islands) closely related to a littoral species. Some Mongoliulidae from the Palaearctic Far East seem to be troglobitic as well (Mikhaljova 1997).

All Julida are cylindrical and devoid of well-marked integument ornamentation with a homogeneous habitus, accentuated in cave species by convergent regressive evolution of eyes and pigment and absence of appendage elongation or shortening. A few species like *Vascoblaniulus cabidochei* (Pyrenees) and *Typhloiulus* spp. (Balkans) have modified mouthparts suggesting life in hygropetric habitats.

The tropical order *Spirostreptida* is subdivided into Cambalidea (242 species, five families) and Spirostreptidea (772 species, five families, Shear 2011). Cambalidea includes a high proportion of cave-related species, while Spirostreptidea has only two troglobites from Yucatan Peninsula caves. Aside from a few eutroglophiles known in and outside caves, many Spirostreptida, listed as troglophiles due to lack of troglomorphic traits, are probably cave restricted, as most were so far not found outside caves. Cambalopsidae (noodle millipedes), widespread in continental Southeast Asia and southern China, are the main contributor to cave species richness of Cambalidea. Two of its genera are highly diversified underground: *Glyphiulus* and *Plusioglyphiulus*. Of their 81 species, 57 have been described since 2007 (see for instance Golovatch et al. 2011a, b). Most live in eutrophic cave habitats, reaching impressive density on guano. They exhibit various degrees of pigment and eye reduction up to anophthalmy in several *Glyphiulus* species, without clear appendage elongation. Endemism, usually low among guano species, is very high in cave Cambalopsidae. Cambalidae includes several eyeless troglobitic species, distributed in Central America and southern USA (*Cambala*, *Mexicambala*, *Jarmilka*). They are replaced in South America by Pseudonannolenidae which includes several recently described troglobites, all members of the large genus *Pseudonannolene*. They prefer high-energy habitats (debris or guano), are depigmented, have reduced eyes, but their size is smaller than that of their epigean relatives (Iniesta and Ferreira 2015).

The pantropical order Spirobolida (506 species, 13 families, Shear 2011) has at least two troglobites: the troglomorphic guanobite *Reddellobus troglobius* in Mexico and *Speleostrophus nesiotes*, depigmented and eyeless, in Australia.

Callipodida order (ca. 140 species, seven families, Shear 2011) has a patchy distribution in northern hemisphere. Its species, very large—up to 10 cm long, live preferentially in rocky habitats including caves. About 30% are troglobites or troglophiles (Stoev et al. 2008). However, they are not or are only weakly troglomorphic (depigmentation and eyes sometimes reduced). Their appendages are elongated, like those of their epigean relatives. The main genera which contain troglobites are *Tetracion* (USA), *Sinocallipus* and *Paracortina* (continental Southeast Asia), *Acanthopetalum*, *Apfelbeckia*, *Balkanopetalum* with six troglobites, and *Callipus* (Europe, especially Balkans).

Chordeumatida order (ca. 900 species, 50 families and four suborders, Shear 2011) are absent in Africa and South America, and patchily distributed elsewhere, with a peak of richness in Europe (Shelley and Golovatch 2011). About 40% of its 50 families include troglobitic or troglophilic species, usually linked to oligotrophic habitats. This high taxonomic diversity is paralleled by a wide morphological range of adaptive traits, with gradients of pigment and eye reduction till complete loss within several genera and families. Cave Chordeumatida are particularly diversified in the Pyreneo-cantabric range, Dinarides, and Eastern United States (Culver and Shear 2012), with many oligospecific genera. Caucasus has recently emerged as a new spot of diversity, with 11 troglobitic Anthroleucosomatidae species described at once, several clearly troglomorphic for appendage length (Antić and Makarov 2016). Surprisingly, cave Chordeumatida are only moderately diversified in southern China, a hotspot for cave Diplopoda (Golovatch 2015) and are absent in Macaronesia.

Several species of Chordeumatida are restricted to cold habitats (Golovatch and Kime 2009). *Brolemanneuma gayi* and *Biokovella* spp. live for instance in cold caves of Alps and Croatia. Two other species are remarkable relicts of uncertain phyletic position: *Niphatrogleuma wildbergeri* from northwestern Alps and *Marboreuma brouquissei* from high altitude shafts in Pyrenees. All these cryophilic troglobites are markedly troglomorphic.

Stemmiulida (ca. 150 species, a single family; Mauriès et al. 2010) is an order distributed in tropical regions of Africa and America, with a few occurrences in tropical Asia and New Guinea (Shelley and Golovatch 2011). Three species have been recently described as cave restricted. Two, one from Brazil, and one from Malukku associated with bat guano belong to the widespread genus *Stemmiulus* (Mauriès and Golovatch 2006). They are eyed and pigmented. A third one from Vietnam is the only species of the relictual genus *Eostemmiulus* and the only blind species of the order.

With 3374 species in 28 families (Shear 2011), Polydesmida is the richest order of Diplopoda. Distributed worldwide, it has subterranean representatives in all temperate and tropical regions except Sub-Saharan Africa. No species seems to be guano dependent, but troglobites and troglophiles are plenty. All four suborders and at least 16 of the 28 families of Polydesmida have cave species. Troglophilic Polydesmida, including tramps, are numerous in cave ecosystems. Many are congeneric with troglobitic species within large genera, like *Polydesmus* (ca. 250 species, including several weakly modified troglobites in Europe). The genus *Eutrichodesmus* of East and Southeast Asia is constituted of ca. 50 microendemics of which 44 are troglobitic. Such a mix of troglobitic and non-troglobitic species is also found in smaller genera. On the other hand, relictual oligospecific genera, like *Caucasodesmus* from Crimea or *Cantabrodesmus lorioli* (Chelodesmidae) from

Cantabric range, are rare. The most remarkable is *Dobrodesmus mirabilis* recently discovered in a Brazilian cave, and sole species of the family Dobrodesmidae.

Polydesmida are phyletically blind but show large gradients in other cave-related traits, from tiny, short-appendaged *Nevadesmus* from the USA to large, long-appendaged troglomorphic *Pacidesmus* from China. Most troglobites range between these extremes. Depigmentation is very common, and appendages are elongated in troglomorphic species of various families, like *Balkanodesmus biokovensis* from Croatia (Trichopolydesmidae), *Selminosoma chapmani* from New Guinea (Paradoxosomatidae), or *Nepalella grandoides* from China (Megalotylidae).

Troglomorphy and regressive morphological evolutions in Diplopoda were recently the object of a detailed analysis based on several Chinese examples (Liu et al. 2017). Like most arthropods, cave Diplopoda frequently show eye and pigment reduction, elongation of legs and antennae, and body slenderness (Golovatch and Kime 2009). Body size increase is frequent, but the reverse is also reported in some lineages (Enghoff 1992).

Interesting features of cave Diplopoda diversity can be summarized as follows: (1) a very important contribution to global subterranean diversity; (2) a diversification in all subterranean environment, from guano to the most oligotrophic habitats; (3) an exceptionally large number of relicts, including phyletic relicts, especially in Blaniulidae and Chordeumatida; (4) a hotspot of diversity and troglomorphy in southern China caves (Deharveng et al. 2009b); (5) an overwhelming importance of Cambalidea, especially Cambalopsidae and Pseudonannolenidae, in many caves with guano of Asia and to a lesser extent of the Neotropics; and (6) a frequent occurrence of regressive features among guanobitic Cambalopsidae, undermining the paradigm of the morphological stasis of guano-dependent invertebrates (Culver and Pipan 2009).

7.5 Crustacea Malacostracea

7.5.1 Decapoda: Brachyura (Crabs, Fig. 7.4j)

Crabs are usually omnivorous and aquatic Crustacea, mostly diversified in seawater, with more than 7000 species (Ng and Davie 2017). A strong minority is constituted of nonmarine species (1476 species, 238 genera, Yeo et al. 2008), rich in cave dwellers, mostly freshwater species. Cave terrestrial species, mainly known from Southeast Asia, belong to the families Chasmocarcinidae, Gecarcinidae, Gecarcinucidae, Potamidae, and Sesarmidae, with probably less than 20 species. The status of troglo- or stygobite is inappropriate for some of them, which are amphibious, like the Sesarmidae *Karstarma balicum* from Bali. The genus *Karstarma* is the richest in troglobites, all narrow endemics. Surprisingly, the highly troglomorphic *Trogloplax joliveti* (Chasmocarcinidae) has a large distribution, being present in caves 200 km apart in New Guinea (Guinot 1994).

Morphological modifications of troglobitic crabs are eye and pigment reduction and leg elongation. These trends are moderately expressed in most species; eye loss in particular is exceptional. The species *Erebusa calobates* from Laos, one of the most modified with its elongated legs and pale bluish coloration, still has small eyes. Highly troglomorphic facies combining anophthalmy, pigment loss, and very long legs are known in several freshwater crabs (Guinot 1994), but limited among terrestrial species to *Trogloplax joliveti* from New Guinea.

7.5.2 Amphipoda: Talitridae

The only troglobitic Amphipods are three Talitridae, a small family of ca. 320 species (WoRMS 2017): the microphthalmic species *Palmorchestia hypogaea* from Canary Islands, and two eyeless species, *Spelaeorchestia koloana* from Hawaii and *Minamitalitrus zoltani* from Japan (White et al. 2013). These species have elongated appendages and can be qualified to be troglomorphic.

7.5.3 Isopoda: Oniscidea (Woodlice; Fig. 7.4k–m)

Oniscidea, the most successful group of terrestrial Crustacea, included 3710 species in 37 families distributed worldwide in 2014 (Sfenthourakis and Taiti 2015). Many are troglophilic, and about 10% are troglobitic. Half of the families include troglobites, with Trichoniscidae the richest (about 70% of all known troglobitic species, Taiti 2004). The number of subterranean Oniscidea was estimated at 270 by Argano (1994) and at 300 by Taiti (2004). They are probably more than 350 today, significant discoveries having been recently made in caves of Brazil (Campos-Filho et al. 2017), Socotra (Taiti and Checcucci 2009), Greece (Alexiou and Sfenthourakis 2013), and Portugal (Reboleira et al. 2015).

Eutroglophiles and guanophiles are well represented among cave Oniscidea. Guano species, sometimes largely distributed, lack troglomorphic traits, but some, like several tropical *Trichorhina*, have reduced eyes and pigment. Many troglobitic Trichoniscidae, not attracted by guano, have a euedaphomorphic facies, i.e., short appendages, eyes reduced or absent, no pigment, and small size. They are well represented in Western Europe in the genus *Trichoniscoides*.

Troglomorphic or hypogeomorphic facies are common as well in meso- and oligotrophic habitats. Among them, Trichoniscidae and to a lesser degree Armadillidiidae dominate cave assemblages in temperate regions, usually replaced by Styloniscidae, Philosciidae, and Armadillidae in the tropics. In Crimea, however, troglobitic Ligiidae are more diverse than Trichoniscidae (Gongalsky and Taiti 2014). Most of these species (e.g., *Scotoniscus* in Pyrenees) are eyeless and depigmented, but appendage elongation is always moderate.

Several troglobitic Oniscidea are secondarily adapted to life in freshwater. From 1994, their number has more than doubled. They belong to the families Trichoniscidae, Styloniscidae, Philosciidae, and Scyphacidae. Recently described are *Macedonethes stankoi* from the Balkans, *Haloniscus* spp. from Australia (Taiti and Humphreys 2001), two species of *Trogloniscus* from China (Taiti and Xue 2012), and several species of *Xangoniscus*, and *Iuiuniscus iuiuensis* from Brazil (Souza et al. 2015). Adaptation to freshwater is apparently not associated with particular morphology, but these amphibious species are often clearly troglomorphic.

Several genera of cave Oniscidea have given local radiations. *Oritoniscus* of southwestern Europe radiated in the Pyrenees, where about 20 troglobitic, soil, and epigean species are known, from minute and blind euedaphomorphic cave-restricted species to eyed and well-pigmented epigeomorphic ones. Within *Alpioniscus* (ca. 30 species), a probably nonadaptive radiation involves more than 25 species in the Balkans, all eyeless troglobites.

Oligospecific or monospecific genera are numerous in Oniscidea, and a few of them are considered phyletics relicts, like the amphibious *Cantabroniscus primitivus* from northwestern Spain, or *Thaumatoniscellus* spp. from southeastern Europe. At a finer scale, disjunct distributions and local relicts are common. Within *Alpioniscus* for instance, one species (*Alpioniscus (Illyrionethes) escolai*) is known from Catalonia, while its congeners are distributed from Alps and Sardinia to Greece.

7.6 Collembola (Springtails; Fig. 7.4n–t)

Collembola (8128 species in 30 families; Janssens and Christiansen 2011) are divided into four orders: Poduromorpha (3154 species), Entomobryomorpha (3753 species), Symphypleona (1188 species), and Neelipleona (33 species). Further taxonomic statistics in the text are drawn from Bellinger et al. (2017). Collembola are terrestrial arthropods, rarely neustonic, abundant, and diversified worldwide from tree canopy to soil and caves. Most are decomposers, feeding on microorganisms or vegetal debris. More than 400 troglobitic species are known from subterranean habitats, where they are preys for various predators. Eutroglophilic species are also well represented. Many species are linked to guano, where they are second to mites numerically (Deharveng and Bedos 2012). In terrestrial oligotrophic cave habitats, Collembola usually dominate invertebrate communities in abundance: Entomobryidae and Paronellidae in tropical caves, Hypogastruridae, Onychiuridae, and Entomobryidae in temperate caves.

Cave species exist in four of the nine families of Poduromorpha order: Gulgastruridae, Hypogastruridae, Onychiuridae, and Neanuridae. Gulgastruridae, phyletically isolated, has a single species, *Gulgastrura reticulosa*, only known from cave entrances in Korea (Lee and Thibaud 1998). Hypogastruridae and Onychiuridae (ca. 690 and 660 species, respectively) have successfully colonized the subterranean environment, mostly in temperate climate of the northern hemisphere. Troglobitic Hypogastruridae of oligotrophic habitats are often characterized by slender (*Taurogastrura* in Crimea, *Typhlogastrura* and *Bonetogastrura* in the Holarctic) or extremely slender claw (the relictual *Ongulogastrura longisensilla* from Pyrenees), but elongation of appendages is moderate. Guanobitic Hypogastruridae have usually reduced eyes and pigment and sometimes elongated claw like several *Pseudacherontides*.

Guano-related Onychiuridae are few, but species of meso- or oligotrophic habitats are plenty. They mostly belong to the speciose genera *Deuteraphorura* and *Onychiurus*. At least four relictual monospecific genera are also cave restricted, including *Ongulonychiurus colpus* from Spain which has the most slender claw among Poduromorpha and *Absolonia gigantea* from Slovenia of unusually large size.

Neanuridae (ca. 1480 species) differ from other Collembola by strongly modified, often sucking mouthparts. Cave species are known in three subfamilies. Frieseinae contain a single relictual, non-cave-adapted troglobite, *Gisinea delhezi*, from Belgium. Pseudachorutinae include ca. 10 troglobitic *Anurida* in the Coreo-Japanese region and one in Caucasus, blind, white, and of large size like some of their epigean congeners, but without morphological modifications. Troglobitic Neanurinae are few: *Yuukianura judithae*—a guanobite from Vanuatu, several *Coecolob*a—Japan, *C. plumleyi*—New Guinea, the only Neanuridae with elongated claws, and two eyeless and unpigmented *Deutonura* in France (*D. anophthalma*) and in Austria (*D. mirabilis*).

Entomobryomorpha order is constituted by nine extant families (Deharveng 2004). Six of them include cave species. Isotomidae (ca. 1380 species) have many guanophiles in the tropics, one cosmopolitan troglophile (*Folsomia candida*) and three troglobites restricted to oligotrophic habitats: *Gnathofolsomia palpata* from Austria, an isolated relict, and two *Isotomiella* species from France. All have moderately elongated claws. Entomobryidae (ca. 1850 species) have a few troglophiles, tropical guanophiles, and many troglobites. Cave-restricted species are members of the large widespread genera *Pseudosinella*, *Coecobrya*, and *Sinella*, which all have epigean, edaphic, and cave species. Less speciose genera, more restricted geographically, are entirely constituted of troglobites, like *Verhoeffiella* in the Balkans. Subterranean radiations are known for all the cited genera, in various regions of the world (Pyrenees for *Pseudosinella*, southern China for *Coecobrya* and *Sinella*, Dinarides for *Verhoeffiella*). Within each of these genera, several species exhibit spectacular troglomorphic characters, i.e., extremely long antennae (like *Verhoeffiella longicornis* from the Dinarides, *Pseudosinella christianseni* from the USA, *Sinella longiantenna* from China, *Coecobrya polychaeta* from Thailand), others very elongated claw (like *Pseudosinella cabidochei* from Pyrenees, *Sinella longiungula* from China). The highly troglomorphic *Bessoniella procera,* sole species of the subfamily Bessoniellinae, is a phyletic relict restricted to cold Pyrenean caves.

Paronellidae (ca. 400 species), mostly diversified in tropical regions, include large colorful species living on the vegetation, and a strong minority of soil and cave species. This last component, widespread in tropical and Mediterranean regions, is constituted of guanobites and troglobites of the genera *Cyphoderopsis*, *Lepidonella*, *Troglopedetes*, and *Trogolaphysa*. They are the most diversified Collembola in many tropical caves but are absent in large areas like southern Sunda Islands. In Mediterranean regions, *Troglopedetes* species have been described from caves and are considered as climatic relicts. Troglomorphic species with eyes and pigmentation reduced or absent, elongated appendages, and thin claws are frequent, like *Troglopedetes multispinosus* in Thailand or *Trogolaphysa jacobyi* in Belize. In many cases however, especially among guano dwellers, appendages are not elongated (Jantarit et al. 2013).

Cyphoderidae (ca. 140 species) are all phyletically blind and unpigmented. Most are myrmecophilous or termitophilous, but *Cyphoderus* and *Troglobius* have troglobitic-guanobitic species in tropical caves. None exhibits appendage or claw elongation.

Tomoceridae (ca. 170 species), diversified in northern and southern temperate regions with an extension into Southeast Asia, include cave species in Holarctic regions not affected by Quaternary glaciations. They are especially numerous in North America and eastern Asia (Yosii 1967). They include troglophiles, many troglobites, but no guanophiles. Some troglobitic species do not exhibit differences with their outside relatives in eyes, pigment, or appendage length, like *Plutomurus unidentatus*, widespread in Western Europe. Many show various regressions of eyes and pigment (Yu and Deharveng 2015). Several eyeless species are known, like *Tritomurus scutellatus* (Slovenia, Croatia), *Lethemurus missus* (USA), *Plutomurus ehimensis* (Japan), or *Tomocerus caecus* (China). None of them exhibit significant appendage elongation, and claws are moderately slender. Two relictual troglobites only, *Tritomurus falcifer* (Pyrenees) and *T. veles* (Croatia), combine anophthalmy and very elongated claw (Lukić et al. 2010).

Oncopoduridae has 49 species in three genera, of which 35 are cave restricted, mostly in oligotrophic habitats. Described troglobites are Holarctic, but undescribed species are reported from tropical and Australian caves. All are phyletically blind, and some like *O. lebretoni* (Pyrenees) are troglomorphic with long claw and elongated antennae.

Cave Symphypleona belong to Arrhopalitidae and Sminthuridae. More than 80 species are known today, living mostly in oligotrophic or mesotrophic habitats, a number that increased to about two-thirds since Thibaud and Deharveng (1994). Arrhopalitidae, with the genera *Arrhopalites* and *Pygmarrhopalites*, are the richest in troglobites. Especially diversified in temperate regions of northern hemisphere (Christiansen and Bellinger 1996; Vargovitsh 2009), they are present in South America (Zeppelini 2006) and Southeast Asia (Nayrolles 1990), but absent in Africa. In tropical and southern hemisphere caves, Arrhopalitidae are usually replaced by Sminthuridae: *Adelphoderia* in Australia, *Troglospinotheca* in Argentina, *and Pararrhopalites* in the tropics, all poorly known taxonomically. All cave Symphypleona are microphthalmic or blind, weakly or not pigmented, but their appendages and claws are moderately or not elongated. Long antennae and very slender claws are however known in recently described troglobites from Caucasus like *Arrhopalites macronyx*, but they reach their highest expression in *Galeriella liciniana* from Dinaric caves, which has extremely elongated antennae and legs.

Neelipleona order, with its single family Neelidae, includes six genera and 47 species, distributed worldwide, of which 15 are troglobites. The genus *Spinaethorax* has a highly disjunct distribution with two species in Mexico and one in Vietnam (Schneider and Deharveng 2017). Several troglobites belong to *Megalothorax*, the smallest cave Collembola (often less than 0.5 mm long). All Neelidae are phyletically blind and devoid of cuticular pigment, but cave species show larger size, more slender claw, and hypertrophy of some antennal sensillar organs (Kováč and Papáč 2010). Appendage elongation is significant in *Neelus lackovici* (Croatia) and *N. klisurensis* (Kosovo).

The high taxonomic diversity of troglobitic Collembola is associated with a high diversity in troglomorphic and regressive traits. Troglomorphy is expressed by larger body size, reduced eyes and pigment, elongated appendages and claws, and modifications more specific to Collembola: shorter, thinner, and pointed tenent hairs on tibiotarsi, reduction and basal shift of inner teeth on claw, hypertrophy or multiplication of antennal sensilla (Christiansen 2012). Each trait evolves at its own pace within lineages. Anophthalmy without appendage elongation is for instance frequent among cave and soil species, while some cave species are oculated, weakly pigmented, but have elongated appendages (e.g., *Pseudosinella theodoridesi* from Pyrenees). Extreme elongation of appendages is not paralleled by extreme elongation of claws. These differences in evolutionary changes result in complex patterns of troglomorphy among Collembola.

7.7 Diplura (Fig. 7.4u)

Sendra (2015) reports 984 species of Diplura in ten families and two orders, Dicellurata (484 species, short sclerotized cerci) and Rhabdura (500 species, segmented filiform cerci). Most Dicellurata are predators in deep soil layers. Rhabdura are decomposers or predators from upper soil layers. Six Dicellurata are troglobitic against more than 125 Rhabdura. Since the last review of subterranean Diplura (Bareth and Pagès 1994), a single new cave Dicellurata has been described, against about 35 cave Rhabdura.

Troglobitic Dicellurata are members of the family Japygidae. *Gollumjapyx smeagol* from Spanish Catalonia, the most recently described, is remarkable by its slender forebody and elongated legs (Sendra et al. 2006). Other troglobitic species do not exhibit troglomorphic features.

Subterranean Rhabdura belong to Campodeidae, represented underground by many eutroglophilic and troglobitic species. MSS habitats may host various species, but only *Campodea alluvialis*, a weakly adapted species from Spain, is considered restricted to this habitat (Sendra et al. 2017b). Troglobitic campodeids are mostly known from mesotrophic or oligotrophic cave habitats. They are much less diverse under tropical (ca. 10 species, Lepidocampinae and Campodeinae) than temperate climate (more than 115 species, Campodeinae and Plusiocampinae). Diversity is concentrated in the northern Mediterranean region with about 70% of the troglobites, and in the southern Nearctic region with about 13%. Elsewhere, troglobitic Rhabdura are rare.

Blindness and lack of cuticular pigment are phyletic traits shared by all Diplura. Troglomorphy is expressed in other traits: increase in body size, slenderness, elongation of appendages, increase in number and/or size of antennal and cercal articles, and modifications of claw structure. An original troglomorphic "syndrome" is the increase of sensorial receptors, especially in the cupuliform organ of antennae (Sendra et al. 2017a). These different trends are variously combined. In *Oncinocampa* for instance, minute euedaphomorphic troglobites with short appendage like *O. trajanoae* from Brazil (less than 2 mm, with cerci less than 1/3 of body length) contrast sharply with highly troglomorphic species like *O. asonensis* from Spain (5–7.4 mm long, with cerci three times longer than body and very slender claws). Extreme appendage elongation is encountered in other European species, such as *Paratachycampa hispanica* (Spanish Catalonia) and *Plusiocampa christiani* (Serbia). Similarly modified species are also known in tropical Lepidocampinae (e.g., *Lepidocampa beltrani* from La Réunion).

7.8 Zygentoma (Fig. 7.4v)

Zygentoma included 568 extant species, all decomposers, and two families in 2014 (Gaju-Ricart et al. 2015). Sixteen troglobites were recognized by Mendès (1994), and 45 are known at present. Most belong to Nicoletiidae, except two Protrinemuridae from Greece and Thailand (Mendes 2002). Nicoletiidae is subdivided into four subfamilies: Atelurinae (a single species from Philippines), Coletiinae, Subcoletiinae, and Cubacubaninae. Coletiinae have about 12 troglobites in the Mediterranean basin, and three outliers in South Africa (Mendès 1994), Brazil (Mendès and Ferreira 2002), and Thailand (Mendes 2002). Subcoletiinae have three troglobites in Southeast Asia, one in New Guinea and one in Australia, where several euedaphomorphic species are known from deep bores. Cubacubaninae, more diverse underground than the other subfamilies, have 25 troglobites distributed from Central America to southern USA.

Ecologically, cave Zygentoma live preferentially in oligotrophic habitats, except two guano-troglobitic species of Cuba, of which one, *Cubacubana decui,* has elongated appendages. Anophthalmy and depigmentation are phyletic traits shared by all Zygentoma, but body size and appendage length increase in troglobites, like *Coletinia capolongoi* (Spain) and *Squamatinia algharbica* (Portugal), which are among the largest species of the group in the Palearctic. Appendage elongation is remarkable in several unrelated lineages, like *Speleonycta anachoretes* (USA), *Lepidospora (Brinckina) makapaan* (South Africa), or *Coletinia longissima* (Turkey). Antennae and caudal appendages are three times longer than the body in *Cubacubana negreai* (Cuba) and two times longer in *Anelpistina levidensis* (Guatemala), while they do not usually exceed body length in non-cave species of *Anelpistina*.

7.9 Insecta Except Coleoptera

7.9.1 Orthoptera (Fig. 7.5a–c)

Unless specified, statistics on Orthoptera are drawn from Cigliano et al. (2017). The taxonomic arrangement of the order is provisional (Chintauan-Marquier et al. 2016). Orthoptera encompass 27,267 extant species in 43 families distributed in two suborders of similar specific richness, Ensifera and Caelifera. All cave species are members of Ensifera, which are omnivorous or scavengers, and none of Caelifera, which are phytophagous. They are known in half of Ensifera families, representing hundreds of troglobitic and troglophilic species. Many are nonpermanent cave dwellers, i.e., obscuricole and "cavicolous" sensu Desutter-Grandcolas (1993, 1998), which forage at night for feeding outside cave. Some are cave restricted. Crickets are the most important primary consumers in many subterranean food webs, as stressed for caves of North America (Lavoie et al. 2007) or Southeast Asia (Deharveng and Bedos 2000). Most belong to the large families Gryllidae, Phalangopsidae, Trigonidiidae, and Rhaphidophoridae. A noticeable exception is the two cave Anostostomatidae from Venezuela (Derka et al. 2013), which are the only aquatic or semi-aquatic cave Orthoptera.

Gryllidae is rich in species, but few have colonized caves. The troglobite *Anurogryllus typhlos* from Galapagos has reduced eyes, elongated legs, and no epigean congener in the island. Conversely, *Thaumatogryllus cavicola,* a reduced-eye species from Hawaii, has relatives in epigean habitats. Several weakly modified Petaloptilini (moved to Phalangopsidae; Chintauan-Marquier et al. 2016), such as *Petaloptila pyrenaea*, have been described from northern Mediterranean caves.

Phalangopsidae are mainly tropical, with extensions in Mediterranean regions and Asia (if Gryllomorphini and Petaloptilini are included). They comprise numerous cave crickets, which are more often cavicolous than strictly troglobitic (Desutter-Grandcolas 1998). The African genus *Phaeophilacris* is probably the most diversified, with at least 30 of its 74 species troglobitic or troglophilic-cavicolous and narrowly distributed. The monospecific genus *Socotracris* from Socotra (Yemen) is troglobitic, but fully eyed. In tropical Asia, Phalangopsidae number five or six genera that include both cave and epigean species. They are usually absent when Rhaphidophoridae are dominant. None of them exhibits well-marked troglomorphic traits, apart from reduced eyes in some cases, like *Arachnomimus microphthalmus* from Malaysia. Australasia has very few troglobitic Phalangopsidae and all are epigeomorphic in facies, like *Endacusta irrorata*.

Neotropics are the richest region in epigean and cave Phalangopsidae. Desutter-Grandcolas (1998) lists 40 cave species, a number that has significantly increased with recently described Brazilian species (Souza-Dias et al. 2014). Many are troglobitic or subtroglophilic species, with wings, pigmentation, and eyes variously reduced (*Longuripes* in Mexico, *Eidmanacris* and *Endecous* in Brazil). *Paracophus caecus* from Mexico is probably the most troglomorphic species, eyeless, wingless, depigmented, and with elongated appendages.

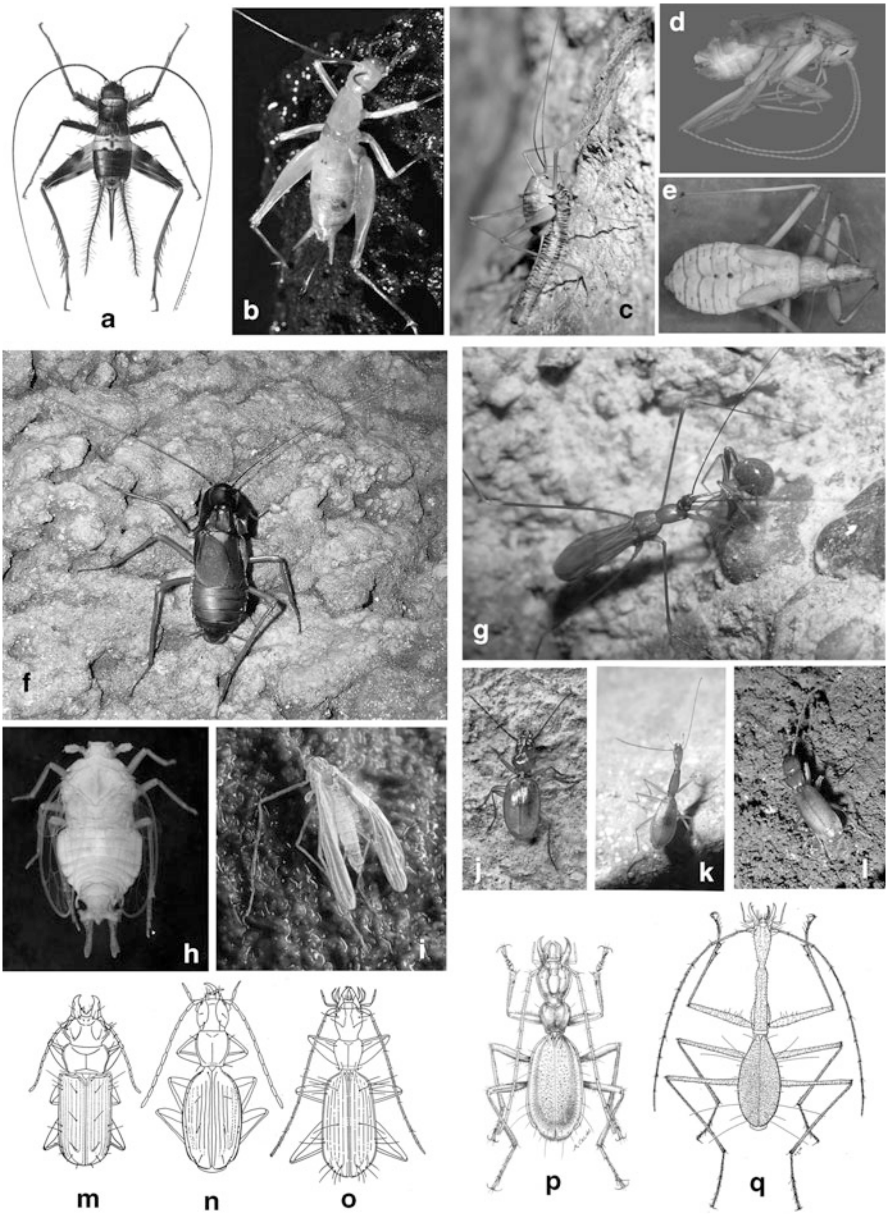

Fig. 7.5 Cave Insecta including part of Coleoptera; not at same scale. Orthoptera (**a** to **c**): (**a**) *Cophonemobius faustini* (Trigonidiidae; Vanuatu), (**b**) *Thaumatogryllus* sp. (Gryllidae; Hawaii), (**c**) *Diestrammena* sp. (Rhaphidophoridae; China) eating a dead Diplopoda; Blattodea (**d** and **f**): (**d**) *Helmablatta louisrothi* (Nocticolidae; Vietnam), (**f**) *Miroblatta baai* (Blaberidae; Indonesia); Hemiptera (**e**, **g**, **h**): (**e**) Reduviidae larva (Vietnam), (**g**) *Phasmatocoris labyrinthicus* (Emesinae; USA), (**h**) *Kinnapotiguara troglobia* (Kinnaridae; Brazil); (**i**) *Troglocladius hajdi* (Diptera Chironomidae; Croatia); Coleoptera Carabidae (**j** to **q**): (**j**) *Duvalius lucidus* (Trechinae; Croatia), (**k**) *Rhadine exilis* (Platyninae; USA), (**l**) *Coarazuphium tapiaguassu* (Dryptinae; Brazil), (**m**) *Subilsia senenti* (Trechinae, Morocco), (**n**) *Goedetrechus damperi* (Trechinae; Tasmania), (**o**) *Duvalius iblis*

Distributed worldwide, Trigonidiidae include two subfamilies, Nemobiinae and Trigonidiinae. Troglobites are only known in the former, scattered in Spain, India, Australia, and Pacific islands. Most have an epigeomorphic facies, without marked eye reduction, like *Nemobius interstitialis* (Spanish MSS), *Speonemobius decoloratus* (Assam), several *Caconemobius* (Hawaii), or *Cophonemobius faustini* (associated with guano in Vanuatu). In contrast, *Ngamarlanguia luisae*, the only truly troglobitic cricket of Australia, is eyeless, pigmentless, and wingless (Humphreys 2012).

Rhaphidophoridae include the largest number of cave Ensifera. It is widespread in the Nearctic, Central America, Europe, southern and eastern Asia, and Gondwanan lands. Half of its ca. 300 species are cave dwellers (Sbordoni and Cobolli 2004). Of its nine extant subfamilies, six include cave species: Aemodogryllinae, Ceuthophilinae, Dolichopodinae, Macropathinae, Rhaphidophorinae, and Troglophilinae, each restricted to a well-delimited region.

Aemodogryllinae, from tropical Asia, eastern Asia, and Western Europe with a small patch in North America, uncover a large array of life forms from epigeomorphic to troglomorphic. They include the only Rhaphidophoridae strongly modified for cave life, i.e., eyeless, unpigmented, and with elongated appendages: *Eutachycines cassani* (Laos), *Eutachycines caecus* (Assam), and *Diestrammena (Gymnaeta) omninocaeca* (Southern China).

Ceuthophilinae are limited to America north of Mexico. Many species in the genera *Ceuthophilus, Hadenoecus*, and *Euhadenoecus* are found in caves, often in large populations. All are troglophiles except possibly *Ceuthophilus longipes* which has reduced pigmentation. Interestingly, a blind species exists in the subfamily, *Typhloceuthophilus floridanus*, but it lives in rodent burrows, not in caves.

Dolichopodainae comprise 53 species in a single genus, *Dolichopoda*. One-third of them have been described after 2001, and half of them from Greece. They are the dominant crickets in caves of the northern Mediterranean region but are absent in Iberia south and east of Catalonia and represented by a single species in the Dinarides. When ecologically studied, these troglobites usually turn out to be subtroglophiles, being active outside caves at night.

Macropathinae have a typical Gondwanan distribution with a large number of cave species (some troglobitic) in Gondwanan lands (Di Russo and Sbordoni 1998). These species do not exhibit strong morphological modifications related to cave life.

Rhaphidophorinae are distributed across tropical Asia, Australasia, and eastern Asia. Its cave species are large in size (4 cm for *Rhaphidophora oophaga* from Sarawak) and the trophic basis of the giant arthropods community of Southeast Asian caves (Deharveng and Bedos 2000). They may live in very dense populations

Fig. 7.5 (continued) (Trechinae; Algeria), (**p**) *Velebitaphaenops giganteus* (Trechinae; Croatia), (**q**) *Giraffaphaenops clarkei* (Trechinae, China). Photos/drawings by Desutter-Grandcolas (2009) (**a**), Howarth et al. (2007) (**b**), © MY Tian (**c**), © L Deharveng and A Bedos (**d**, **e**, **f**), Pape (2013) (**g**), Hoch and Ferreira (2013) (**h**), J Bedek in Andersen et al. (2016) (**i**), Jalžić et al. (2013) (**j**), Krejca and Weckerly (2008) (**k**), Pellegrini and Ferreira (2011) (**l**), Español (1967) (**m**), PM Giachino in Eberhard and Giachino (2011) (**n**), Peyerimhoff (1910) (**o**), Casale et al. (2012) (**p**), Deuve (2002) (**q**) on walls and ground, from the entrance to the deepest recesses of caves as soon as guano or food resources are plenty. Some are suspected to forage outside at night for feeding (Chopard 1969), but this is unlikely to occur for deep-cave populations. None of them exhibits modifications related to cave life (Di Russo and Rampini 2017).

Troglophilinae, with its single genus *Troglophilus* (21 species, all troglophiles or at least obscuricoles), are the dominant crickets in Eastern Mediterranean regions.

The classical regressive traits, i.e., reduction of wings, eyes, and pigment in cave compared to non-cave species, can be found in all Orthoptera lineages (Chopard 1969; Di Russo and Sbordoni 1998; Desutter-Grandcolas 1999), but less strongly marked than in other cave insects. Anophthalmy is rare. Size increase, change in leg tip structure, and appendage elongation are not observed or tenuous. The strikingly elongated appendages are largely a phyletic character for most cave species. Leg elongation has been reported to occur within groups of closely related species, but it needs to be confirmed (Leroy 1967; Di Russo and Sbordoni 1998; Desutter-Grandcolas 1999). In this context, evolutionary reversion from troglobitic to epigean life, as suggested by Desutter-Grandcolas (1993) from phylogenetic analyses of Neotropical Phalangopsidae, seems plausible. The importance of initial exaptations for subsequent cave adaptation has been stressed by the same author who showed that straminicolous-cavicolous Phalangopsidae are more prone to colonize subterranean habitats than other species.

Some interesting characteristics of cave Orthoptera diversity may be summarized as follows: (1) a large number of subtroglophiles that use caves as daily refuge (Desutter-Grandcolas 1993); (2) a large contribution of radiations to overall biodiversity, like in several other Arthropod groups. These radiations involve in various proportions cave and non-cave species, as illustrated by European *Dolichopoda* or Southeast Asian *Diestrammena*; (3) a low proportion of relicts compared to several Arthropod groups; and (4) moderate levels of troglomorphy compared to most Arthropod groups.

7.9.2 Grylloblattodea

Grylloblattodea are large predatory insects, restricted to the northern Pacific region, and have 32 extant species (Wipfler et al. 2014) living in mountain forest litter, some near the snow, except seven that are cave dwellers. These troglobites have reduced eyes and larger size than their epigean relatives, like the two blind species of *Namkungia* from Korea.

7.9.3 Blattodea (Cockroaches, Fig. 7.5d, f)

In 2011, extant Blattodea (not including Isoptera) contained 4779 species, 9 families (Beccaloni 2014). Cockroaches are almost absent in caves of temperate regions, but well represented in tropical caves, with many troglophiles and guanophiles (often tramp species of large size), and about 40 troglobitic species, all saprophagous. The troglobitic Blaberidae *Miroblatta baai,* endemic to a Kalimantan cave, is one of the largest cockroaches (up to 6 cm long). It is dark pigmented, eyed, and long-legged but moves slowly, with the body elevated up over the substrate (Bell et al. 2007). Another rather large troglobite is *Neostylopyga jambusanensis* (Blattidae) from Borneo, which has reduced eyes and pigmentation, but no appendage elongation. All other troglobites are smaller in size. They belong to Ectobiidae (ca. 18 species, on a total of ca. 2840; Beccaloni 2014), particularly diversified in Canary Islands and Australia, and Nocticolidae (ca. 22 species, on a total of 34; Vidlička et al. 2017) particularly diversified in Southeast Asia and Africa. They may be encountered near guano (the Nocticolidae *Helmablatta louisrothi* from southern Vietnam), but live preferentially in oligotrophic habitats with stagnant air (Stone 1988). Troglobites of both families have more or less reduced eyes, many being highly troglomorphic, eyeless, unpigmented, wingless, and with very long appendages, like *Nocticola flabella* (Australia). Several have lost the specialized adhesive structures of the tip of the legs usually present in epigean species, a possible adaptation to cave life (Bell et al. 2007).

7.9.4 Dermaptera (Earwigs)

Extant Dermaptera contain 1978 species (Zhang 2011), present worldwide, though more diversified in the tropics. Two families (ca. 16 species) are parasitic on or linked to mammals, including bats. All others are free-living omnivores, more rarely predators. Very few are strictly associated with caves, and none has been described since the synthesis of Brindle and Oromi (1994). There are only two troglobites with well-marked troglomorphic traits, *Anataelia troglobia* (Canary Islands) and *Anisolabis howarthi* (Hawaii). Their eyes are much reduced, their appendages elongated, and they are depigmented and wingless. Three other Dermaptera species from deep soil layers are blind and wingless, but of much smaller size and with shorter appendages (Brindle and Oromi 1994).

7.9.5 Psocoptera

Johnson and Smith (2017) report 10,880 extant species of Psocoptera in 64 families. In Badonnel and Lienhard (1994), 12 species were listed as associated with caves at world level. About 40 species are known today, distributed in two suborders: Trogiomorpha and Troctomorpha. Cave Psocoptera live preferentially in dry habitats, often on guano. The most common are a few widespread troglophilic species, like *Prionoglaris stygia*, a quasi-troglobite of Europe. Northern America also has a single troglobite, *Speleketor flocki*. Tropical regions are richer in troglobitic species, most of them recently described: three in Malaysia (Moseley et al. 2012), six or seven in Africa (Lienhard and Ashmole 2011), and more than 20 in the Neotropics, mainly in the genus *Psyllipsocus* in Brazil (Lienhard and Ferreira 2015). All belong to the suborder Trogiomorpha and are weakly or not modified in relation to cave life, except for three Afro-Atlantic Troctomorpha, which include the only eyeless cave Psocoptera known so far (*Sphaeropsocopsis myrtleae* from St. Helena Island).

7.9.6 Hemiptera: Heteroptera (Fig. 7.5e, g)

Schuh and Slater (1995) estimated that Heteroptera comprised 38,000 species worldwide. They are highly diversified in all terrestrial and freshwater habitats on earth and feed in sucking a large range of living plants or animals. A small proportion of them are found in subterranean habitats, as guanophiles (Leptopodidae, Lygaeidae, and Reduviidae), bat ectoparasites (Cimicidae), or troglophiles and troglobites of parietal associations (Reduviidae Emesinae) (Maldonado Capriles 1994). Emesinae have 44 cave species (24 cave-restricted) on a total of 900 (Pape 2013). All are immediately recognized by their very long appendages, but this character is lineage dependent and not an adaptation to cave life. All species have well-developed eyes and most are pigmented, with the exception of three troglobites: *Collartida anophthalma* and *C. tanausu* from Canary Islands, and *Nesidiolestes ana* from Hawaii. Other Reduviidae, poorly represented underground, are mostly troglophilic-guanophilic species, with eyes, pigment, and normal appendages. Southeast Asian caves also host large, unpigmented, reduced-eyes Reduviidae larvae (possibly *Oncocephalus* sp., Cai pers. comm.), frequently found wandering on or around guano.

7.9.7 Hemiptera: Fulgoromorpha (Fig. 7.5h)

About 7000 species of Fulgoromorpha are known on earth. Four families include cave species: Cixiidae, Meenoplidae, Kinnaridae, and Delphacidae. All are rhizophagous and most are troglobites. Since the last review of Hoch (1994) who listed ca. 40 cave species, at least 20 additional ones have been described. Fulgoromorpha have given local radiations with nice series of eye and wing regression, without significant appendage elongation, in Australia (e.g., *Solonaima*), Canary Islands (e.g., *Cixius*), and Hawaii (e.g., *Oliarius*). Several species are eyeless with well-developed wings. Recently, species with regressed eyes, pigment, or wings have also been described from temperate regions (Italy, Croatia), tropical Asia (Sulawesi, Vietnam), and South America (Brazil).

7.9.8 Hymenoptera (Ants)

Hymenoptera is the fourth largest order of insects (116,881 species; Zhang 2011). Various troglophilic ants are regularly encountered in guano of tropical cave (Decu et al. 1998a). *Leptogenys khammouanensis*, collected in oligotrophic habitats of a Laotian cave, is possibly the only troglobitic ant.

7.9.9 Lepidoptera (Butterflies)

The 157,338 species of Lepidoptera (Zhang 2011) include very few cave species. Only two of them are troglobitic: the reduced-eyes Tineidae, *Tinea microphthalma* (Philippines; Robinson 1980), and the Erebidae *Schrankia howarthi* (Hawaii; Howarth and Hoch 2012), which presents several regressive traits: flightlessness, reduced pigmentation, and reduced eye size.

7.9.10 Diptera (Flies, Fig. 7.5i)

Diptera (155,477 species, Zhang 2011) are poorly diversified underground with about 100 species of which 22 are troglobites after Matile (1994). Aside from parasites of bats, Diptera include many guanobites or guanophiles associated with guano in tropical caves, as well as a number of non-guano-dependent troglophiles.

Among non-hematophagous Diptera, the emblematic *Mormotomyia hirsuta* from Kenya, a remarkable relict of aberrant morphology, is actually not troglobitic contrary to literature claims (Copeland et al. 2011). *Clisa australe* from Australia and *Spelobia tenebrarum,* the only troglobitic Diptera of northern America, are cave restricted. This last species has reduced eyes, like its congeners living in mammal burrows. *Gymnomus troglodytes* from the Dinarides is the only troglobitic Heleomyzidae. Among Sciaridae, several troglobitic or troglophilic species, like *Allopnyxia patrizii* from Italy, have a minute size, regressed eyes and pigment, and shortened appendages, i.e., euedaphomorphic attributes. Six troglobitic species of the large genus *Crumomyia* (Sphaeroceridae) have reduced eyes, elongated antennal arista and legs, weak sclerotization, pale pigmentation of body, and sometimes reduced wings (Roháček and Papp 2000). The last discovered troglobite is a Chironomidae collected from a deep pit of Croatia, *Troglocladius hajdi*. It combines pale color, strongly reduced eyes, and very long legs, but its antennae are short and its wings well developed.

Hematophagous Diptera include many troglophilic and some troglobitic species of Psychodidae and Culicidae. Psychodidae, abundant in tropical caves, are well studied as vectors of Leishmaniasis. Several species of *Phlebotomus* and *Chinius* from Africa, Asia, and Australia are considered troglobites (Matile 1994). However, most have been found outside caves when searched for, and should be considered as troglophilic (Carvalho et al. 2013). The only species firmly established as a troglobite, *Deanemyia maruaga* from Brazil, is parthenogenetic, nonvector, and can complete its full life cycle in cave. A few tropical Culicidae seem be troglobitic in a way similar to Psychodidae (hematophagous-guanophagous).

All troglobitic Diptera are not or weakly modified for cave life.

7.10 Insecta: Coleoptera (Beetles)

With 386,500 species in 29,500 genera and 176 families (Slipinski et al. 2011), Coleoptera is the most species-rich order of living organisms. Beetles are present in all terrestrial and freshwater habitats and in all climatic zones. They are common in terrestrial subterranean habitats worldwide, as troglophiles, troglobites, or associated with guano. Most species are terrestrial, estimated at 1927 by Decu and Juberthie (1998), and probably outnumbering today 2500 species. Understanding of cave Coleoptera diversity and evolution is hampered the lack of data on the larval instars of troglobitic species, which seem to have developed adaptive traits different from those of adults.

Three families, Carabidae, Staphylinidae (including Pselaphinae and Scydmaeninae), and Leiodidae count for 98% of the diversity of troglobitic and troglophilic beetles, with 1180, 110, and 599 species (Decu and Juberthie 1998), for total species richness of 40,000, 56,000, and 3700 species, respectively (Slipinski et al. 2011). This taxonomic unbalance is retrieved at lower taxonomic levels. It is also striking at the geographical scale, with lineages developing large subterranean radiations locally and being almost absent from caves in other regions.

Guanobites and guanophiles, largely neglected taxonomically, represent a significant component of subterranean Coleoptera diversity. Thus, 28 cave beetles associated with guano from 10 families are listed by Peck et al. (1998) for Cuba, and 82 species from 19 families by Moulds (2004) for Australia. The diversity of guano beetles is much higher than that of non-guanobite troglobitic beetles at single-cave scale in the tropics, but not in temperate regions.

7.10.1 Adephaga: Carabidae (Groundbeetles; Figs. 7.5j–q and 7.6a–d)

All terrestrial cave species of Adephaga belong to Carabidae. We follow here Anichtchenko (2017) for the taxonomic hierarchy of the family. Casale et al. (1998) published a major synthesis of cave Carabidae diversity and mentioned 14 subfamilies (of a total of ca. 33) having representatives underground: Anthiinae, Brachininae, Dryptinae, Harpalinae, Lebiinae, Nebriinae, Paussinae, Platyninae, Promecognathinae, Psydrinae, Pterostichinae, Scaritinae, Siagoninae, and Trechinae.

Trechinae comprise 90% of all described species of cave Carabidae (Decu and Juberthie 1998). Of their six tribes, three contain troglobites (Trechini, Bembidiini, and Zolini). Distributed worldwide, they are however mostly diversified in temperate climates. Half of their more than 2000 species are cave dwellers (Casale et al. 1998).

The basic outlines of the taxonomy of the subfamily were set up by Jeannel (1926–1928). Since the last review of Casale et al. (1998), numerous new taxa have been described, and molecular phylogenies of several major lineages of the subfamily have been published (Faille et al. 2010a, 2011, 2013), bringing new insight on their origin and evolution.

Of the four subtribes of Trechini, only Trechodina and Trechina include cave species. Trechodina have a few troglobites of euedaphomorphic facies, limited to Macaronesia and eastern Spain. Three anophthalmic ones are Canarian endemics in the genera *Canarobius* and *Spelaeovulcania*. The others are microphthalmic and belong to the European genus *Thalassophilus*.

Trechina includes all other cave Trechini. More than any other cave group, this subtribe illustrates the major features of the diversity patterns known among cave fauna: unbalanced distribution, large evolutionary radiations, high number of relicts, and a broad range of morphologies from euedaphomorphy to extreme troglomorphy.

Cave-restricted Trechini inhabit almost exclusively caves of temperate and subtropical climates. They are absent north of 47°N and south of 45°S, probably impacted by Quaternary glaciations. They are also extremely rare in tropical caves. Though present in the southern hemisphere, they are more diversified in the Holarctic. At fine grain, pattern of taxonomic richness is complex and uneven, characterized by intense but scattered local radiations (Casale et al. 1998). Recent phylogenetic analyses (e.g., Faille et al. 2010b) combined with a fast increase in taxa discoveries and species distribution have allowed robust hypotheses on divergence and biogeographical history of various lineages.

Ecologically, most cave Trechini are troglobites of oligotrophic habitats. Their absence in guano parallels their rarity in tropical regions. Surprisingly, troglophilic species also are few.

Till recently, Southern Europe was known as the major diversification area for cave Trechinae on earth (Casale et al. 1998). Its species richness is mostly a result of local evolutionary radiations, reflected in the occurrence of 10 genera having more than 15 troglobites each. On the other hand, the large number of phyletically isolated and oligospecific genera recognized by taxonomists (of which more than

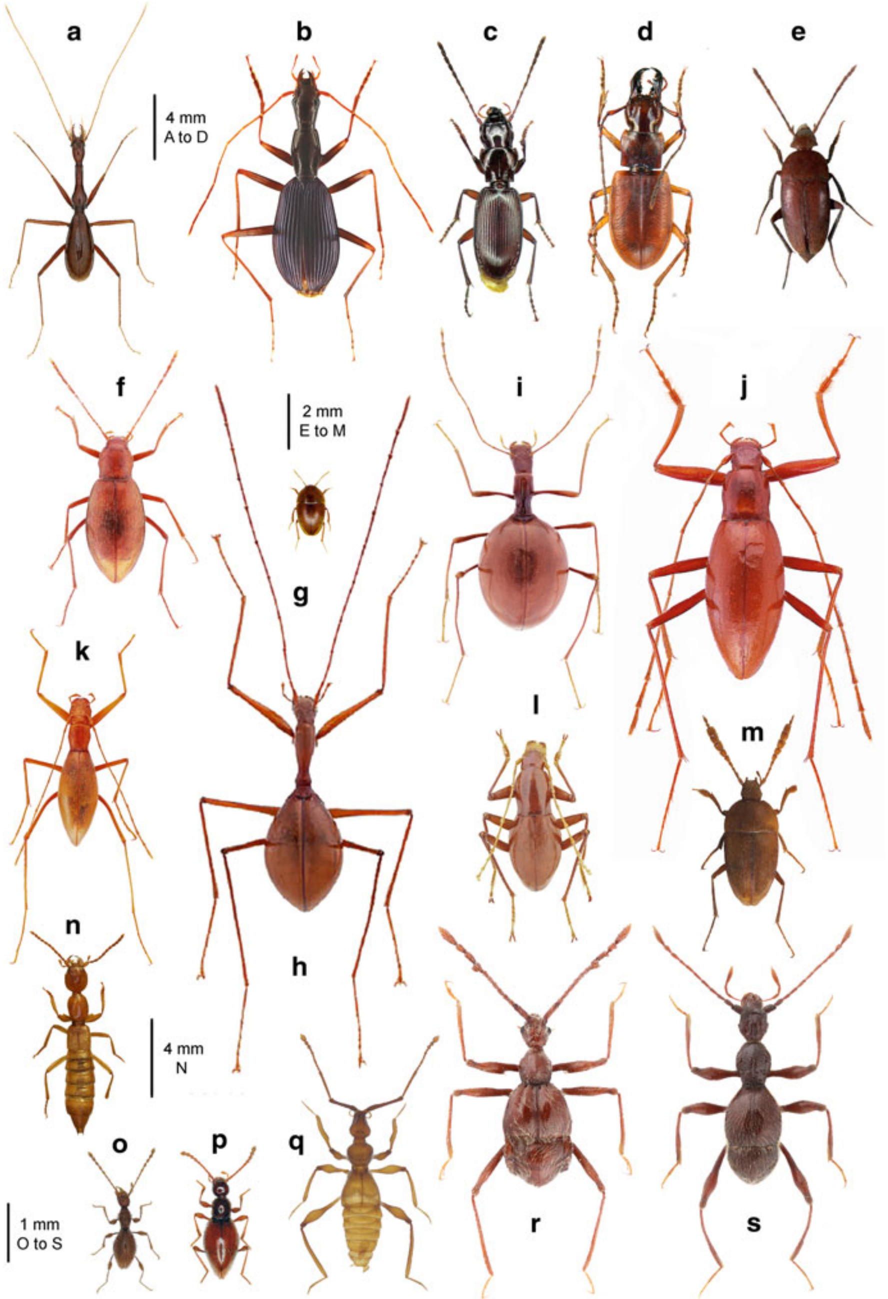

Fig. 7.6 Cave Coleoptera, Carabidae and Staphylinidae; scales as specified. Carabidae (**a** to **d**): (**a**) *Xuedytes bellus* (Trechinae; China), (**b**) *Morimotoidius zhushandong* (Platyninae; China), (**c**) *Zariquieya boumortensis* (Pterostichinae; Spain), (**d**) *Dalyat mirabilis* (Promecognathinae; Spain); Leiodidae (**e** to **m**): (**e**) *Ptomaphagus trezzii* (Guatemala), (**f**) *Pholeuon (P.) knirschi* (Romania), (**g**) *Nafarroa sorogainensis* (Spain), (**h**) *Graciliella ozimeci* (Croatia), (**i**) *Leptodirus hochenwartii croaticus* (Croatia), (**j**) *Speoplanes giganteus biokovensis* (Croatia), (**k**) *Remyella*

20 monospecific genera) illustrates a high phyletic richness. Cave Trechini diversity is maximal on the biodiversity ridge between 42 and 46° of latitude (Culver et al. 2006), more particularly in Pyrenees and the Dinarides. Pyrenees is home of the *Aphaenops* lineage, with 87 mostly troglobitic species in two genera, *Aphaenops* and *Geotrechus* (Queinnec 2014). This lineage has no clearly relatives with *Trechus* surface lineage or European Trechinae genera (Faille et al. 2010a, b). All species are blind and depigmented, and most are troglomorphic regarding body slenderness and appendage elongation.

The *Duvalius* lineage spreads from northeastern Spain to China (Moravec et al. 2003), but is absent in Pyrenees. It is richer in species than the *Aphaenops* lineage but spread across a much larger area. It ranges from epigean winged and eyed species (life forms that do not exist in the *Aphaenops* lineage) to highly troglomorphic ones, like *Duvalius (Trechopsis) iblis* from Algeria or Alpine *Trichaphaenops*. The genus *Anophthalmus*, closely allied to *Duvalius*, represents a third important radiation diversified across southeastern Europe.

Casale et al. (1998) recorded nine cave genera of Trechini in southern China. This number has since increased impressively. The region is today the richest on earth for troglobitic Trechini, with more than 130 species in ca. 45 endemic genera (Tian et al. 2016, 2017). Like for the Pyrenean radiation, species of the Chinese radiation(s) are all troglobites, blind, wingless, and unpigmented. They exhibit an extremely large range of morphologies, reflected in the small number of species by genus, many being monospecific and the richest, *Dongodytes*, having only 12 species. Their maximal diversification area stretches from 23°N to 30°N, more southerly than the Japanese, European, or American hotspots. Outside this hotspot, the single troglobitic Trechini known in 1998 for the region was the reduced-eyes monospecific genus *Trechiamiotes* from Thailand. Recent discovery of blind and highly troglomorphic species in lowlands caves, south of the southern China hotspot, has been a surprise: three species of *Lanxangaphaenops* in Vietnam and Laos, and the monospecific genera *Tonkinaphaenops* in Vietnam, *Laosaphaenops* in Laos, and *Birmaphaenops* in Burma. The origin of this large radiation is unclear, no species having been related so far to eyed Trechinae. Most species are troglomorphic. Several, in the genera *Giraffaphaenops, Dongodytes*, and *Xuedytes* are the most modified troglobitic ground beetles known so far in the world due to extreme elongation of appendages.

Fig. 7.6 (continued) scaphoides scaphoides (Serbia), (**l**) *Radziella styx* (Croatia), (**m**) *Baronniesia delioti* (France); Staphylinidae (**n** to **s**): (**n**) *Domene (D.) lencinai* (Paederinae; Spain), (**o**) *Euconnus (Tetramelus) longipedes* (Scydmaeninae; Croatia), (**p**) *E. (T.) tronqueti* (France), (**q**) *Metopiellus painensis* (Pselaphinae; Brazil), (**r**) *Pseudophanias spinitarsis* (Pselaphinae; Nepal), (**s**) *Zopherobatrus tianmingyii* (Pselaphinae; China). Photos by Tian et al. (2017) (**a**), Pang and Tian (2014) (**b**), Faille et al. (2011) (**c**), © A Anitchenko (**d**), Perreau (2009) (**e**), Hlaváč et al. (2017) (**f, i, j**, **k, l**), Fresneda and Dupré (2010) (**g**), Njunjić et al. (2016) (**h**), Fresneda et al. (2009) (**m**), Vives (2010) (**n**), Hlaváč and Jalžić (2009) (**o**), Orousset (2014b) (**p**), Asenjo et al. (2017) (**q**), Yin et al. (2015a) (**r**), Yin and Li (2015) (**s**)

Though close to Southern China, the Japanese hotspot of cave Trechinae biodiversity is located more northerly, centered on the island of Shikoku at about 33°N (Uéno 2001). It hosts speciose lineages, especially in the *Trechoblemus* and *Trechiama* groups. Contrary to the radiations of southern China, the richest lineages of cave Trechinae in Japan comprise edaphic and troglobitic species: *Rakantrechus* and *Yamautidius* with c.a. 20 species each, *Kurasawatrechus* with more than 30 species, and *Trechiama* with more than 130 species (Moravec et al. 2003). Though many species lack eyes and pigment, a few only are highly troglomorphic.

In North America, the highest diversity of cave Trechini occurs at 35–40°N (Culver et al. 2006). There, 145 species of the genus *Pseudanophthalmus* (Barr 2004) contribute for almost 85% to the total richness of cave Carabidae of the USA (Culver et al. 2000). Most species are obligate cave dwellers; a few are edaphic. Their eyes are absent or very reduced and their appendages only moderately elongated, though some species like *Nelsonites jonesi* are more strongly troglomorphic. Further south subterranean Trechini are confined to high elevation caves and have more elongated appendages: several *Mexaphaenops*, and four species of oligospecific genera: *Mayaphaenops*, *Chiapadytes*, *Guatemalatrechus*, and *Mamesdytes*.

Cave Trechini are much less diversified in the southern than in the northern hemisphere. They include troglomorphic species of uncertain affinities in New Zealand, which combine vestigial eyes and rather long appendages (Townsend 2010), like *Erebotrechus infernus* or *Scototrechus orcinus*. In Tasmania, they are also frequent, with local radiations of the genera *Goedetrechus* and *Tasmanotrechus*, almost entirely constituted of troglobites which exhibit mild degrees of eye reduction and moderate appendage elongation (Eberhard and Giachino 2011).

Zolini include six troglobites of the genera *Idacarabus* and *Pterocyrtu*s in Tasmania, all oculated and weakly or not modified for cave life (Eberhard and Giachino 2011). Bembidiini have cave species in five of their six subtribes, most of them troglophilic of euedaphomorphic habitus, i.e., small, blind, and with short appendages. Anillina, particularly in the northern Mediterranean region (Hlaváč et al. 2017), include a large number of such species, living in soil or MSS, and a few troglobites that exhibit weak troglomorphic facies (slenderness, appendages slightly elongated), like *Microtyphlus alegrei* from Spain or *Hygranillus kuscheli* from New Zealand. Six genera and 17 species of Anillina have been recently discovered from deep bores in Western Australia.

The subtribe Bembidiina has troglophilic species, but probably no troglobites. Some have regressed eyes, but none has significantly elongated appendages. Horologionina is a monospecific subtribe, established for a subterranean species of atypical morphology from the USA. The four troglobitic species of the enigmatic Balkanic subtribe Lovriciina also show, aside from anophthalmy, moderate troglomorphic traits, like *Paralovricia beroi* (Hlaváč et al. 2017). Among troglophilic Tachyina, especially in the tropics, a few linked to soil or caves are blind and euedaphomorphic in facies (like some *Lymnastis* of Canary Islands; Machado 1992).

Of the three tribes of Dryptinae, only Zuphiini are present in caves with ca. 15 species in seven genera, besides a number of epigean and edaphic forms.

Ecologically, most cave Zuphiini are troglobites and none is guanophile. Three of them are relicts of outstanding interest. The monospecific genus *Ildobates* from Catalonia, blind and highly troglomorphic, is the only cave representative of Zuphiini in Europe (Ortuño et al. 2004; Ribera et al. 2006). The two Australian species from the Nullarbor karst *Speothalpius grayi* and *Speozuphium poulteri*) belong to monospecific genera of uncertain affinities and are by far the most troglomorphic Carabidae of Australia. Other cave species of the tribe have shorter appendages, but remain slender, unpigmented, and blind or microphthalmic: eight troglobitic *Coarazuphium* in Brazil and one *Parazuphium* in Canary Islands. Several species with a similar morphology have also been collected in deep soil in Morocco and in deep bores in Australia.

Though one of the richest subfamily of Carabidae, Harpalinae has only a few cave species, restricted to Australia and New Zealand. Australian *Notospeophonus*, probably guanophilic, are fully oculated and devoid of cave-adapted traits (Moore 1964). New Zealand species of *Syllectus* and *Pholeodytes* live in oligotrophic cave habitats, have reduced eyes, and have a typical aphaenopsian habitus (Larochelle and Larivière 2005).

Paussinae include highly modified species living as commensals of ants, and free-living species, mostly Ozaenini, with several tropical troglobites. *Itamus cavicola* from New Ireland is a guanobitic troglobite. The genus *Eustra,* sporadically distributed in soil and caves of Southeast Asia, includes several troglobites living of oligotrophic habitats, unpigmented and with eyes reduced or absent. They are morphologically euedaphomorphic, except two species from northern Thailand with relatively elongated appendages (Deuve 2001).

Platyninae are subdivided into three tribes, of which two include cave species: Platynini and Sphodrini. Platynini have colonized subterranean habitats, to the exclusion of guano, in various regions on earth. Troglobites are numerous. Most belong to radiations including epigean species: *Rhadine* in North America (ca. 50 species; Gómez et al. 2016), *Jujiroa* in eastern Asia, *Gastragonum* in Australasia, *Mexisphodrus* in Mexico, or *Blackburnia* in Hawaii. They are moderately modified morphologically, though eyes and pigmentation are often reduced. Some species however, like *Rhadine exilis,* have a typical troglomorphic facies, with vestigial eyes, depigmentation, very slender forebody, and long appendages. Aside from these speciose genera, a few mono- or oligospecific genera contain highly troglomorphic species like *Speocolpodes franiai* from Guatemala or *Speagonum mirabile* from New Guinea, both at high altitude. In eastern Asia, some eyed and pigmented species exhibit surprisingly long appendages, like *Morimotoidius* from China. The only cave Platyninae of Europe is *Galiciotyphlotes weberi* from Spain, considered as a relict (Ortuño and Gilgado 2010). Many of the ca. 700 species of Sphodrini are linked to subterranean habitats. Since the revision of Casale (1988) a lot of new species have been described, but the main lineages, the global patterns of distribution, and the troglomorphic gradients have only changed a little. The group seems to be absent from caves in the tropics and in the southern hemisphere. Within Sphodrini, the *Laemostenus* lineage is the richest and most widespread, distributed in West Palearctic and Central Asia. Its remarkable radiation of ca. 200 species of relatively uniform habitus includes epigean, guanobites, troglophiles-guanophiles, and troglobites, often of large size compared to other cave Carabids. Troglomorphic traits are not or weakly marked in species of the subgenera *Actenipus* and *Pristonychus*, usually troglophilic or guano dwellers, but troglobites of the subgenus *Antisphodrus*, like *L. (A.) navaricus* from Pyrenees, are clearly modified (eye and pigment reduced, appendages elongated). No species is eyeless. Aside from *Laemostenus*, a few oligospecific genera of the Palaearctic (Canary Islands, Morocco, Turkey, Japan) comprise also weakly modified troglobites. In America, Sphodrini are represented by a single relictual troglobite of Mexico, *Miquihuana rhadiniformis*, which is also the most modified of the tribe with an unusual combination of depigmentation, vestigial eyes, long and slender forebody, but moderate elongation of antennae.

Several lineages of Pterostichinae have colonized caves (Casale et al. 1998). Of the eight tribes of Pterostichinae, two contain troglobites: Abacetini and Pterostichini. Abacetini include two weakly modified troglobitic species of Indonesia: *Mateuellus troglobioticus,* from Sulawesi and *Metabacetus willi*, from Java. Pterostichini, distributed in Canary Islands, the Pyreneo-Iberian region, and Eastern Europe, have subterranean species in three subtribes (Molopina, Euchroina, and Pterostichina). Molopina includes rare relictual troglobites, not attracted by guano, in four mono- or bispecific genera (Faille et al. 2011), all blind, but without appendages elongation: *Speomolops sardous* in Sardinia, *Henrotius jordai* in Balearic Islands, *Oscadytes rovirai*, *Zariquieya boumortensis*, and *Z. troglodytes* in Catalonia and eastern Pyrenees. The subtribe Euchroina is present underground only in Canary Islands, with three species of the genus *Orthomus* from caves or MSS (Machado 1992). Their eyes and pigmentation are reduced, but their appendages are not significantly elongated. The subtribe Pterostichina, rich in epigean species, has several representatives underground. In France, *Stomis benoiti* from Massif Central is oculated, pigmented, and with normal appendages, but cave restricted. Several species of *Pterostichus* (subgenera *Rambousekiella*, *Troglorites*, *Lianoe*, and *Tinautius*) are scattered in Europe. They are blind or microphthalmic and have relatively elongated appendages, but a rather stout body. The genus *Speluncarius* is the richest, with 27 species in the eastern Mediterranean region, of which about one-third are troglobites of hypogeomorphic facies.

Scaritinae are represented underground by several species of the tribe Clivinini, subtribes Reicheina and Clivinina. Within Reicheina, the radiation of *Typhloreicheia* in Sardinia includes a majority of soil species and several cave species of euedaphomorphic facies (Magrini et al. 2013). Eight anophthalmous Clivinini have been described from southern Europe, Mexico, Papua New Guinea, and China (Tian 2013). Other reduced-eye species and a few troglophiles mentioned in Casale et al. (1998) are known in the tribe. All these species have the same strong burrowing forelegs as their epigean relatives and an overall euedaphomorphic facies. A single species, *Italodytes stammeri*, is clearly troglomorphic, with much less robust forelegs and slender forebody.

Other cave species, interesting in various respects, are scattered in several subfamilies of Carabidae. Of outstanding interest, *Dalyat mirabilis*, unique species of the subfamily Promecognathinae in Europe, is a large-size relictual beetle of southern Spain, related to South African and North American taxa (Mateu and Bellès 2003). Brachininae include a few endogean species, and *Brachynillus varendorffi* from Tanzania, the only troglobitic Carabidae of Sub-Saharan Africa, long considered as a relict. Nebriinae at least include hygrophilic-cryophilic species in Holarctic region, of which the troglobitic *Nebria nudicollis* from Algeria, with reduced eyes and pigment, and slightly elongate appendages.

7.10.2 Polyphaga

Polyphaga are present in caves with ca. 30 families and a very large number of species (Decu et al. 1998b). Most troglobites and guanobites are distributed in 10 families according to the following taxonomic hierarchy: superfamily Hydrophiloidea (Histeridae), Staphylinoidea (Ptiliidae, Leiodidae, Staphylinidae), Scarabaeoidea (Trogidae, Scarabaeidae), Derodontoidea (Jacobsoniidae), Tenebrionoidea (Aderidae, Tenebrionidae) and Curculionoidea (Curculionidae); several other families include troglophiles or guano species of uncertain ecological status (Elateridae and Cantharidae among Elateroidea, Salpingidae and Zopheridae among Tenebrionidae).

Histeridae (4300 species, 350 genera, Slipinski et al. 2011) have various feeding habits from carnivory to saprophagy. They include, aside from soil and inquiline species, several guanophiles and troglophiles, and about 15 troglobites. These last species, distributed in the Mediterranean region, Africa, and Central America (Vomero 1998; Lackner 2013), small in size and wingless have reduced or absent eyes, with sometimes weak appendage elongation.

Ptiliidae (650 species, 80 genera, Slipinski et al. 2011) are saprophagous soil dwellers, present worldwide. They may be numerically important in the guano of tropical caves. Two species are listed as troglobitic by Decu et al. (1998b). One of them (*Malkinella cavatica* from South Africa) is clearly guanobitic, eyeless, pigmentless, and wingless, but its appendages are not elongated, traits that are retrieved in several epigean Ptiliidae.

Leiodidae (Fig. 7.6e-m). Statistics for Leiodidae taxa are drawn from Perreau (2000, updated). Leiodidae is a moderately diverse family among beetles, with ca. 4000 species worldwide in 450 genera. Giachino et al. (1998) published a reference synthesis at world level on its underground diversity. The rough lines of its phyletic structure are well documented (Fresneda et al. 2011). Leiodidae encompasses a higher proportion of cave-restricted species than any other large beetle family and is only second to Carabidae in number of troglobites. Since 1998, our taxonomic knowledge of the family has considerably progressed. The last available estimate gave 599 species of cave Leiodidae (Decu and Juberthie 1998). In the Balkans alone, 369 species are currently recorded, about the same number as Carabidae (368 species, Hlaváč et al. 2017). All cave Leiodidae are scavengers, and most are linked to oligotrophic habitats.

Leiodidae comprises six subfamilies of unequal species richness. Camiarinae and Coloninae have no cave representatives. Platypsyllinae group a few pholeophilous species including troglophilic *Leptinus* spp. which are blind and wingless.

Catopocerinae group five genera and ca. 50 species of which four are cave dwellers. Three of them belong to the genera *Catopocerus* and *Pinodytes*, which are blind and wingless, but without significant appendage elongation (Fresneda et al. 2011). The fourth troglobite is *Glacicavicola bathyscioides* from western USA, a blind, troglomorphic, relictual endemic of leptodiroid facies (see below for explanation). Leiodinae have more than 1700 species, but less than 10 cave species, of which none is markedly modified for cave life. Regressive evolution of wings and eyes is not exceptional among non-cave species of the subfamily. Thus, 32 of the ca. 825 species of *Agathidium* have reduced eyes or are eyeless (Švec 2014), but none inhabits cave.

Cholevinae (ca. 2500 species in seven tribes) have a large number of cave species, mostly in Leptodirini and Ptomaphagini. Sciaphyini from Eastern Asia (one genus, three species) are blind, but not cave dwellers. Anemadini, with ca. 100 species distributed worldwide except in Africa and South America, are poorly diversified in subterranean habitats. Its cave species considered as troglobites (*Speonemadus escalerae* from Spain for instance) have wings, eyes, and pigment, but two anophthalmic species of the tribe (*Anemadus kabaki* and *A. imurai*) have been recently described from soil or MSS-like habitat in China. Ptomaphagini (ca. 360 species) contain a large proportion of troglobitic species of bathyscioid facies in two main areas: Southeast Asia and northern and central America. Southeast Asia caves host a few *Ptomaphaginus* and several *Ptomaphaminus*. They are attracted by decaying organic matter, but rarely guanobites. They are not or hardly troglomorphic, having eyes and short appendages, but wings are sometimes reduced. In America, the genus *Ptomaphagus* (ca. 140 species) includes soil and cave species, with more than 35 troglobites. Morphological gradients from fully eyed, fully winged, small size, and short appendages to eyeless, wingless, large size, and moderately long appendages species have been documented in the genus *Ptomaphagus* (Peck 1986). Marked troglomorphic cave species, like *Ptomaphagus (Adelops) trezzii* from Guatemala, are rare. Surprisingly, the only indubitably blind species of the genus, *P. troglodytes*, is a troglobite from Spain (Fresneda et al. 2011).

Leptodirini (ca. 950 species, 235 genera) is the richest tribe of Cholevinae. It is mostly distributed in Europe and western Asia, with only three genera in eastern Asia and one genus in the Nearctic. Its species are eyeless and depigmented, with very rare exceptions (Jeannel 1924; Fresneda et al. 2011). About three-fourths of them are troglobites, living in oligotrophic habitats of cave and in the MSS, the remaining ones living in soil. Eutroglophiles are not rare, but guanobites are infrequent.

Balkans is the richest spot of Leptodirini, followed by the Pyreneo-Cantabric range and Alps (Perreau 2000). The whole tribe constitutes a remarkable radiation of the northern Mediterranean region, including local radiations of several clades like *Antroherpon* in the Dinarides (Njunjić et al. 2016), *Quaestus* and *Speonomus* in the Pyreneo-Cantabric range (Cieslak et al. 2014), and *Drimeotus* in Romania (Moldovan

2000). On the other hand, half of the Leptodirini genera are monospecific, reflecting a relictual status (like *Speophyes* in France) or rapid rates of morphological evolution. Most species of the tribe are narrow endemics, with more than one-third known from a single site (Moldovan 2008). Life forms of Leptodirini, extremely diverse, have been allocated to four facies defined by Jeannel (1943) and illustrated by Laneyrie (1967): bathyscioid, pholeuonoid, leptodiroid, and scaphoid, characterized by different combination of slenderness, appendage elongation, and body outlines. The bathyscioid facies, considered as primitive, characterizes most non-Leptodirini Leiodidae, and among Leptodirini, many soil and litter species, as well as troglobites like *Pholeuonella* from the Dinarides, *Quaestus* from Spain, *Sophrochaeta* from Romania, or *Speonomus* from Pyrenees. The pholeuonoid facies, characterized by longer appendages, more slender body, wider head, and thorax more elongated and sinuous, is retrieved in many "normal" troglobites of various phyletic lines, like *Pholeuon* from Romania, *Antrocharis* from Pyrenees, or *Speoplanes* from Croatia. The spectacular leptodiroid and scaphoid facies are limited to Dinaric species of the subtribes Leptodirina and Antroherponina. Antennae are much longer than the body, legs very long, and head and thorax narrow and sometimes elongated like in the most troglomorphic Trechinae. Abdomen is distinctly swollen in the leptodiroid facies, versus narrow in the scaphoid facies. These life forms are known among Leptodirina (like *Leptodirus hochenwartii*, *Albanodirus trezzii*, *Nonveilleriella ognjenovici*) and Antroherponina (like various species of *Antroherpon*, *Leptomeson*, and *Graciliella).*

Species of hygropetric environment often show intermediate facies between pholeuonoid and leptodiroid, associated with strongly modified mouthparts (Sket 2004; Polak et al. 2016). They are known in Italy (*Cansiliella*) and the Balkans (*Croatodirus*, *Hadesia, Kircheria, Nauticiella, Radziella, Tartariella*, and *Velebitodromus*).

Staphylinidae (Fig. 7.6n-s). With more than 55,000 species, Staphylinidae is the largest family of the whole animal kingdom. It comprises a low number of subterranean species, including guanobites, very few troglophiles, and more than 220 troglobites (Hlaváč et al. 2006; Yin et al. 2015a).

Guanophilic Staphylinidae are common in northern temperate regions (Bordoni and Oromi 1998) but rarer in the tropics and do not exhibit modifications related to cave life, with the exception of the large-size Scydmaeninae *Clidicus gracilipes* from Sumatra.

Non-guano troglobitic Staphylinidae, much more diverse, are known in seven subfamilies: Omaliinae, Tachyporinae, Paederinae, Aleocharinae, Pselaphinae, Scydmaeninae and Scaphidiinae. Among them, several troglobites are clearly troglomorphic, i.e., blind, depigmented, long-legged, and large-sized. Cave Omaliinae include two troglobites (*Uenohadesina styx* from South Korea and *Lesteva (Lestevina) sbordonii* from southern Italy), while a single one is known among Tachyporinae (*Ischnosoma spelaeum* from Spain) after Hlaváč et al. (2006). None of these species show troglomorphic features.

Paederinae and Aleocharinae contain 28 and 15 troglobitic species (Hlaváč et al. 2006; Perreau and Faille 2012), as well as several eutroglophiles. They include cave species nested in large genera, with local spots of underground diversification in

Canary Islands (*Domene*, *Medon, Micranops* for Paederinae, *Alevonata* for Aleocharinae), Northwestern Spain (*Domene* for Paederinae), and perhaps Japan (*Lathrobium* for Paederinae*)*. Remaining species belong to less speciose genera, some relictual, in Galapagos (*Pinostygus galapagoensis)*, India (*Typhlomalota glenniei*), and Spain (*Cantabrodytes vivesi*). All show eye and pigment reduction, body slenderness, and appendage elongation, with several highly troglomorphic species like *Domene vulcanica* from Canary Islands or *Anopsapterus bordati* from Sahara.

The ca. 8600 species of Pselaphinae (Poggi et al. 1998) are predators of smaller arthropods and live mostly in soils (Fig. 7.6q–s). About 170 cave species have been described (Yin et al. 2015a) mostly from northern temperate regions. Africa has a single genus (*Speobaxyris*) with a few eyed and weakly modified species (Poggi et al. 1998), and a single species, slightly troglomorphic, was recently described from Brazil (*Metopiellus painensis*). The world hotspot of cave Pselaphinae diversity is the Balkans. The number of cave taxa in this region increased at a fast pace during recent years, from 25 species in 11 genera in 1998 to ca. 64 species in ca. 18 genera in 2017 (Hlaváč et al. 2017). Among recently described troglobites, some show euedaphomorphic or atypical facies, but two species of the monospecific genera *Pauperobythus* and *Velebythus* are highly troglomorphic.

In other Euro-Mediterranean regions, cave Pselaphinae number more than 25 species in ca. 10 genera, almost all in the tribe Bythinini. Most species have reduced eyes and weakly elongated appendages Besuchet 1974) A few are eyeless and strongly troglomorphic, such as *Bathybythus bleyi* (Pyrenees). Since 1998, many species have been described from East Asia (China and northern Indochina), which emerges today as a novel spot of diversity for cave Pselaphinae. Batrisini dominate this fauna, with for instance 13 species of *Tribasodites* in Southern China, microphthalmic and more or less troglomorphic (Yin et al. 2015b), or the Chinese genus *Zopherobatrus*, which is blind and has very elongated appendages. North America is rich in troglobitic Pselaphinae. Barr and Holsinger (1985) recognized three components in its fauna, a pattern still valid today: (a) troglobites lacking pronounced troglomorphic features, e.g., about 20 troglobitic *Batrisodes* on the ca. 85 North-American species of this genus*;* (b) a few troglomorphic troglobites, eyeless, and with often slightly elongated appendages, e.g., the two monospecific genera *Speleobama* and *Texamaurops,* and cave species of the genus *Arianops*, a radiation of 42 species from southeastern USA, all blind and wingless, either edaphic or troglobitic (Carlton 2008); and (c) minute eyeless euedaphomorphic species of the genus *Speleochus* (Carlton 2012).

Life forms of cave Pselaphinae vary from euedaphomorphic to highly troglomorphic. Regressive characters are, as usual in beetles, eye reduction, pigment reduction, and loss of wings. However, eyes are often retained in troglobites, and appendages elongation is limited: antennae as long as body are only known in the phyletically isolated species *Decumarellus sarbui* from Romania.

Two supertribes, Scymaenitae and Mastigitae, include 98 and 2% of the species of Scydmaeninae (Orousset 2014a, b). Mastigitae have one dubiously guanobitic species from a Sumatran cave, *Clidicus gracilipes*, with large eyes and wings but elongated appendages. Five troglobitic species, eyeless and wingless, are known. *Scydmaenus (Heteroeustemmus) aelleni* from New Caledonia was already listed in Decu et al. (1998b). Four species have been described since, three from Croatia [*Euconnus* (*Tetramelus*) *bazgoviensis*, *E. (T.) longipedes*, and *Scydmoraphes speluncarius*], and one from French Pyrenees [*E. (T.) tronqueti*]. *S. speluncarius* has a facies of soil Scydmaeninae, but the three others, especially *E. (T.) longipedes*, have more slender body and longer appendage than their epigean relatives. Two species of Scaphidiinae, only known from caves in Madagascar (*Toxidium cavicola*) and Thailand (*Bironium troglophilum*), do not exhibit any character linked to cave life (Löbl and Faille 2017).

Trogidae and Scarabaeidae species linked to cave guano are known from many tropical regions (Decu et al. 1998b). They do not show characters associated with cave life, except for the Scarabaeidae *Mozartius uenoi* from a Japanese cave, which is wingless and has regressed eyes.

Jacobsoniidae (22 species, three genera; Hava 2016) includes the minute guanobites *Derolathrus cavernicolus* and *D. troglophilus*, both fully oculated, respectively in the USA and Fidji.

Aderidae (ca. 900 species worldwide, Slipinski et al. 2011) are sometimes in large numbers on guano piles or organic debris in caves of Africa (*Zarcosia*) and tropical Asia (*Aderus* and *Euglenes*), with up to five species in a single cave (Moseley et al. 2012). None of them is blind or troglomorphic. Tenebrionidae (ca. 20,000 species worldwide, Slipinski et al. 2011) usually live in dry and warm habitats, but some colonized caves where they often dominate guano assemblages in biomass. Most are eutroglophiles, winged, fully eyed, dark pigmented, and with short appendages, like their epigean congeners. A few are dark pigmented but with reduced eyes and moderately elongated appendages, like some cave-restricted North American *Eleodes (Caverneleodes)*.

Curculionidae (ca. 51,000 species, 4600 genera worldwide, Slipinski et al. 2011) are mostly phytophagous, those found in caves being apparently root feeders. Osella and Zuppa (1998) list 34 troglobitic or troglophilic species. Since this review, most generic assignations have changed, and at least 35 additional species have been described (Morrone and Hlaváč 2017). They are so far limited to the Euro-Mediterranean region, Canary Islands, and North America, though undescribed species are reported from the tropics or China. Within Europe, 50% of the species are described from Italy (Morrone and Hlaváč 2017) and few from the Balkanic or Pyreneo-Cantabric hotspots. The vast majority of cave Curculionids belong to the subfamily Entiminae (54 species), with the large genera *Baldorhynchus* and *Otiorhynchus*, almost entirely European. Molytinae have eight species from Canary Islands and North America, and the subfamilies Brachycerinae and Cossininae eight European species (Morrone and Hlaváč 2017). Morphologically, these cave root feeders tend to have a hypogeomorphic or euedaphomorphic facies, i.e., eyes reduced or absent, no pigmentation, relatively small size, and appendages not elongated.

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Chapter 8
An Overview on the Aquatic Cave Fauna

Oana Teodora Moldovan

8.1 Introduction

The community of organisms that live in groundwater, which is the obligate subterranean water community, was defined as stygon by Husmann (1966, 1967) who gave the name stygobiology to the science that studies groundwater life. Earlier, in 1925, Thienemann proposed a terminology for groundwater animals that paralleled the ecological classification of terrestrial cave fauna (see also Chaps. 1 and 4): (1) stygoxenes—occasionally found in groundwater, (2) stygophiles—live both in groundwater and in epigean habitats; (3) stygobionts—live only in groundwater.

The first discovered and described cave inhabitants were aquatic animals. In 1436, in Yunnan (China) a local doctor mentioned a cave fish, the stygophile *Sinocyclocheilus grahami* described centuries later (1904) by C. Tate Regan (Ma and Zhao 2012). In 1540, also in China, a stygobiont fish was mentioned by the local governor of Guangxi province and described as *Sinocyclocheilus hyalinus* in 1944 by Chen and Yang (Ma and Zhao 2012). Two centuries later, in 1768, the baby dragon—*Proteus anguinus*—was described by Laurenti from a small karstic source in Carniola (parts of the present-day Slovenia). This dragon was for a long time thought to be the first described cave species in the world.

In 1986, Botosaneanu stated that more than 7000 groundwater species occur worldwide, a number that was soon recognized to be an underestimate (Gibert and Culver 2005), and there has been a steady upward trend each year in the number of species known. Even without the description of new taxa the discovery of cryptic species, due to improvements in molecular techniques, is steadily increasing the number of known and described species (Trontelj et al. 2009; Fišer et al. 2018). Groundwater species richness is higher in Europe (~2000 species) compared with the other continents, ~561 species in Asia, ~500 species in North America, ~335

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species in Africa (Gibert and Culver 2005), and ~500 in Australia (Stuart Halse, personal communication), although this can be the result of a more concentrated effort of identification of new groundwater species in the Old Continent until relatively recently. Europe was the center of biospeleological researches for almost a century and the first researches on groundwater fauna were concentrated mostly on cave aquatic habitats, especially cave rivers and pools, and on wells and springs outside the caves (see also Chap. 3). It was only later that the hyporheic zone was described (Orghidan 1959; Motaş 1963) and studied more intensively than caves (reviews in Danielopol and Rouch 1991; Rouch 1992; Boulton et al. 2003; Di Lorenzo et al. 2013). The deeper aquatic zones, such as the phreatic zone (see Chap. 3) known to harbor specific fauna (Marmonier et al. 1993; Stoch et al. 2009), have been less studied due to limitations such as difficulties of access, boundaries assignment, and spatial heterogeneity (Larned 2012).

8.2 Groundwater Unicellular Organisms (see Table 8.1)

8.2.1 Protista: Protists

Protists are a group of unicellular eukaryotic organisms ranked as a separate kingdom. More than 180 species have been described from caves, of which ~20% occur in guano, decomposing organic matter, and humid clay (Golemansky and Bonnet 1994). They are also abundant in show caves, in pools and also around lampenflora (Varga 1959, 1963). Most of the studied groundwater protists are from interstitial habitats and display some adaptations such as small, fine, and transparent shells of Foraminifera that inhabit deep groundwater of the Kara-Kum desert (Brodsky 1928, 1929; Nikoljuk 1948, 1968 cited by Golemansky and Bonnet 1994). Protists are mostly introduced in caves by waters that come from the surface. The species found in deserts groundwater are sometimes remnants of ancient seas that covered the respective areas (Delamare-Debouteville 1960). In an unpublished study of Sheila Seale (cited by Lavoie 2015), protists were found in all aquatic pools and streams sampled in Mammoth Cave (USA) but not in drip water. González-López et al. (2013) also found protists on stalagmites in different caves around the world but all were edaphic species. Protists have also been found in the microbial mats of Movile Cave (Romania) together with bacteria and fungi (see also Chap. 16). Epibiontic and endoparasitic species of protists were found on and in cave animals and some are specific for their stygobiont hosts, such as *Lagenophrys monolistrae*and *Spelaeophrya troglocaridis* (Golemansky and Bonnet 1994).

Table 8.1 The main groundwater organismal groups of this chapter (in italics) and their simplified systematic position

Kingdom	Phylum	Subphylum	Class	Subclass	Superorder	Order	Suborder
Protista		*Foraminifera*					
Animalia	*Porifera*						
	Cnidaria		*Hydrozoa*				
	Platyhelminthes					*Tricladida* *Temnocephalida*	
	Rotifera						
	Kinorhyncha						
	Nematoda						
	Nemertea						
	Gastrotricha						
	Tardigrada						
	Annelida		*Aphanoneura*				
			Clitellata	*Oligochaeta* *Hirudinea*			
			Polychaeta				
	Mollusca		*Gastropoda* *Bivalvia*				
	Arthropoda	*Crustacea*	Branchiopoda			*Cladocera*	
			Remipedia				
			Maxillopoda	Copepoda		*Calanoida*	
						Cyclopoida	
						Harpacticoida	
						Gelyelloida	
			Ostracoda				
			Malacostraca		Eucarida	*Decapoda*	
					Peracarida	*Isopoda*	

(continued)

Table 8.1 (continued)

Kingdom	Phylum	Subphylum	Class	Subclass	Superorder	Order	Suborder
						Amphipoda	
						Spelaeogriphacea	
						Theormosbaenacea	
						Mysidacea	
						Bochusacea (Mictacea)	
					Syncarida	*Anaspidacea* *Bathynellacea*	
		Chelicerata	Arachnida	Acari		Trombidiformes	Prostigmata-*Hydrachnidia*
		Hexapoda	Insecta			*Coleoptera*	
	Chordata	Vertebrata	*Pisces*				
			Amphibia			*Urodela*	

8.3 Groundwater Multicellular Animal Groups (see Table 8.1)

8.3.1 Porifera: Sponges

Sponges, multicellular organisms with a body full of pores and channels, are poorly represented in caves or other subterranean environments, and the few that inhabit groundwaters are considered to be stygoxenes. The only true stygobiont sponge found to date occurs in Croatia, *Eunapius subterraneus*, with the following adaptive characteristics: gemmule reduction and reduced thickness of both skeleton and body consistence (Sket and Velikonja 1984, 1986; Fig. 8.1a).

There are sponges that inhabit marine caves, because of their constant conditions, without particular adaptations to life in caves (Vacelet 1990; Manconi et al. 2013).

8.3.2 Hydrozoa: Hydrozoans

The only known cave hydrozoan is *Velkovrhia enigmatica* described by Matjašič and Sket in 1971 from caves of the Dinaric karst. Other hydrozoans found in caves of Mexico, the USA, the Czech Republic, Austria, Australia, and Dinarides belong to the genus *Hydra* and are not adapted to subterranean life (Zagmajster et al. 2011).

8.3.3 Platyhelminthes: Flat Worms

The order Tricladida order contains stygobiont planarians that are depigmented, lack eyes or have reduced eyes and have slower biological processes (ontogenesis, respiration, and metabolism) than their epigean relatives (Fig. 8.1b). One of the peculiar characteristics of cave planarians is that they have a higher number of chromosomes (Gourbault 1968, 1970). Their origin can be either freshwater or marine waters, and half of the described freshwater species are hypogean. Among stygobionts the genus *Dendrocoelum* contains ~57 species inhabiting groundwaters in the Palearctic, including the special ecosystem of the Movile Cave (Stocchino et al. 2017; see also Chap. 16). Stygobiont planarians have also been described from the Americas and Australia. For example, *Hausera hauseri* is a typical stygobiont, unpigmented and eyeless, presumably an oceanic relict found in Brazil (Leal-Zanchet et al. 2014), while the genus *Girardia* has diversified in caves of Brazil and Mexico (Souza et al. 2016).

Most planarians, however, are considered stygophiles because the lack of eyes and depigmentation are the group characteristics and no other adaptations to groundwater were observed. There are studies indicating that the presence of planarians in groundwater could be a good indicator of organic pollution (Holsinger 1966; Eberhard 1990).

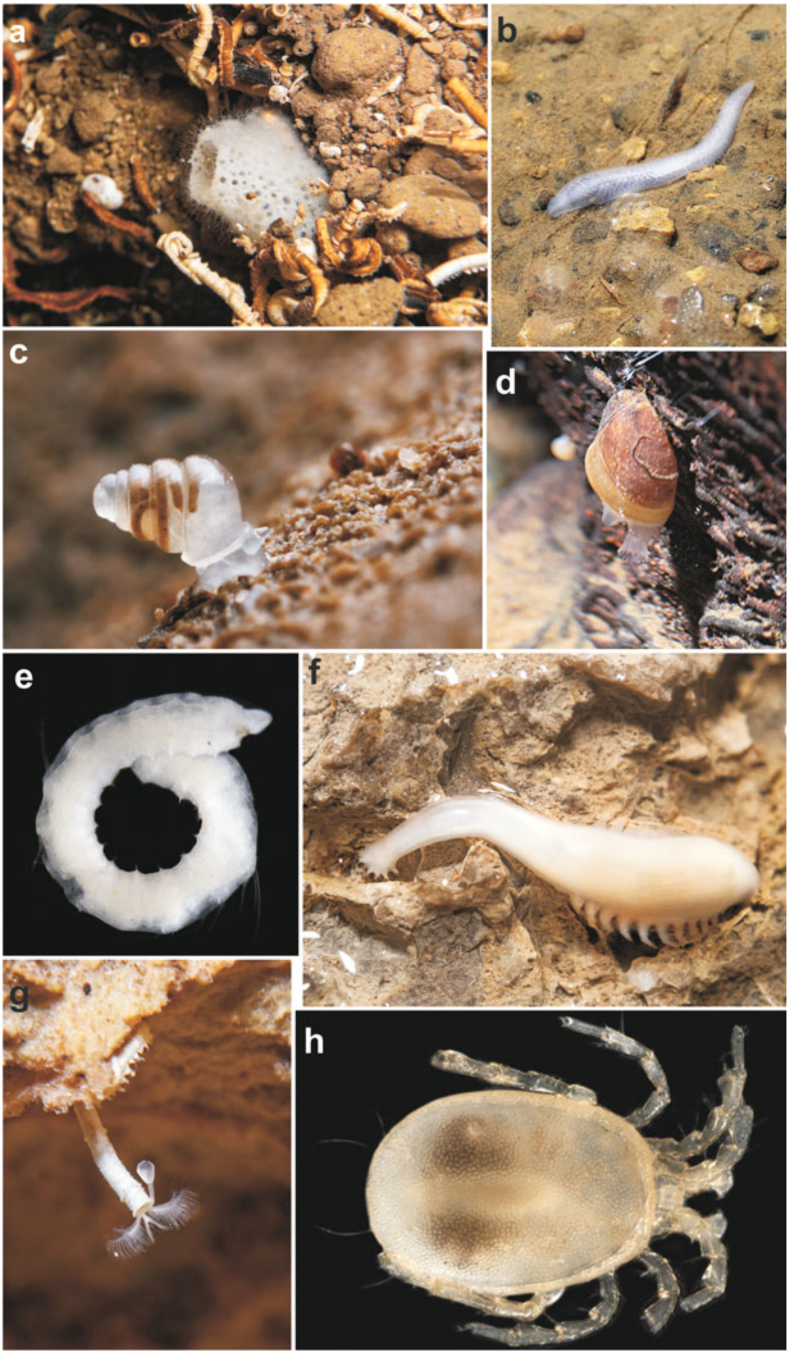

Fig. 8.1 Stygobiont invertebrates from caves and calcrete aquifers; (**a**) *Eunapius subterraneus* (Porifera), (**b**) *Dendrocoelum* sp. (Tricladida), (**c**) *Zospeum tholussum* (Gastropoda), (**d**) *Congeria* sp. (Bivalvia), (**e**) *Pristina pastoral* (Oligochaeta), (**f**) *Croatobranchus mestrovi* (Hirudinea), (**g**) *Marifugia cavatica* (Polychaeta), (**h**) *Arenurus* sp. (Hydrachnidia). Note that the relative size of the

The order Temnocephalida contains smaller parasitic species that live on cave crustaceans, with the exception of *Bubalocerus pretneri*, which is a predator. All subterranean described Temnocephalida lack eyes and most of the genera have been found in caves of the Dinaric range—*Stygodyticola*, *Scutariella*, *Subtelsonia*, and *Troglocaridicola*—, other than a single unidentified species from Papua New Guinea (Matjašič 1994).

8.3.4 Rotifera: Rotifers

Many rotifers have been observed in cave waters but none seem to be groundwater adapted, with the exception of some interstitial species, found outside caves (Pourriot 1994). Nevertheless, the presence and diversity of Rotifera in caves and other groundwater might be underestimated because most of the sampling has been done with larger mesh of planktonic nets unable to catch these extremely tiny organisms. A study on fauna in dripping water in caves in Brazil found rotifers to be more diverse than Copepoda as usually found in such habitats in Europe (Simões et al. 2013).

8.3.5 Kinorhyncha: Mud Dragons

Species of kinorhynch found in marine caves in the Mediterranean regions of Europe and Australia belong to *Echinoderes* (Sørensen et al. 2000), and those in Japan belong to *Ryuguderes* (Yamasaki 2016).

8.3.6 Nematoda: Roundworms

Nematodes colonized different environments and do not have pigments or eyes, which makes it difficult to distinguish stygobionts from epigean or stygophile species. There are a few species that were described from continental and marine caves that are considered to be cave adapted. They belong to genera such as *Desmocolex*, *Cylindrolaimus*, *Plectus*, *Halalaimus*, and *Thalassoalaimus* (Delamare-Deboutteville 1960). In Movile Cave, five species live in the microbial mats: *Chronogaster troglodytes*—endemic to this cave—, *Panagrolaimus* sp., *Protorhabditis* sp., *Udonchus tenuicaudatus*, and probably a species of *Monhystrella* (Riess et al. 1999). Their

◀

Fig. 8.1 (continued) animals is not respected. Photos by © Marko Lukić (**a**), © Jaroslav Stankovič (**b**), © Jana Bedek (**c**, **d**, **f**, **g**), © Jane McRae (**e**, **h**) population is controlled by the abundant copepod *Eucyclops subterraneus scythicus*, revealing a trophic linkage between nematodes and copepods (Muschiol 2009).

8.3.7 Nemertea: Ribbon Worms

There is no evidence of subterranean adaptations in marine species, while some freshwater cave species are depigmented and lack eyes. Eyeless species of *Prostoma* have been described from cave waters in Europe (Botosaneanu 1998), as have species of *Potamonemertes* from gravel riverbeds in New Zealand (Moore and Gibson 1972).

8.3.8 Gastrotricha: Hairybacks

A single species can be considered as stygobiontic, *Marinellina flagelata*, a gastrotrich that lives in the hyporheic zone. This interstitial species has reduced size, reduced number of eggs, is depigmented, and lack eyes (Kisielewski 1998). Few gastrotrichs have also been recorded in continental cave waters, such as *Chaetonotus antrumus* from a cave in Montenegro (Kolicka et al. 2017). The only comprehensive study of the gastrotrich fauna in a cave habitat was carried out in an Italian sea cave by Todaro et al. (2006).

8.3.9 Tardigrada: Water Bears

Only interstitial stygobiont species are known from this group; there are no occurrences in continental caves. They are depigmented and lack eyes. Tardigrades in marine caves are more frequent although there is no information on their adaptation to the environment. However, Jørgensen et al. (2014) recently found dozens of species in marine caves, with nine from Australia; Fujimoto and Yamasaki (2017) described a new genus and species collected from sandy beaches of Ryukyu Archipelago, Japan.

8.3.10 Annelida: Segmented Worms

Representatives of the class Aphanoneura and subclass Oligochaeta (Clitellata class) occur frequently in continental and marine caves. Some terrestrial taxa also live in cave waters, such as *Fridericia* and *Allolobophora* (Dumnicka and Juberthie 1994). Cave Annelida do not show any adaptations to subterranean life, because they already lack eyes and appendices (Fig. 8.1e), except for the smaller size and shorter hairs of some Naididae, or longer hairs of some Enchytraeidae (Dumnicka 1986; Dumnicka and Wojtan 1989). However, in a recent study Gonzalez et al. (2018) have shown that Aphroditiformia suborder show a significant elongation of sensory parapodial cirri (bundles of cilia), while lacking eyes and pigmentation (eyes could be also lost during colonization of deep-sea habitats).

In caves, the oligochetes prefer the sediments at the bottom of the water bodies, leaving only their posterior parts free, above the sediments, for respiration (Dumnicka and Juberthie 1994).

Some families, such as Potamodrilidae (Aphanoneura) and Dorydrilidae (Oligochaeta), live exclusively in groundwater habitats. Other representatives of Aelosomatidae (Aphanoneura) have been described from caves in Europe (*Rheomorpha neiswestnovae*, *Aeolosoma gineti*) and Cuba (*Aeolosoma cubana*), although their typical habitats are interstitial waters. Among oligochetes, Dorydrilidae has one groundwater genus (*Dorydrilus*), Haplotaxidae has three genera known only from caves, Lumbriculidae has seven genera with cave taxa, and Tubificidae has 14 genera of which five are known only from groundwater. Enchytraeidae, although known as soil inhabitants, has subterranean species that live exclusively in water, while stygobiont species of Naididae have been described only from the Antilles (Dumnicka and Juberthie 1994); groundwater appears to be an important habitat for Phreodrilidae in northern Australia (Pinder 2008).

Hirudinea subclass has few cave-adapted leeches around the world, and their adaptations to life in caves include depigmentation and lack or reduction of eyes. The slight widening of the oral sucker in *Dina absoloni* was proposed as another cave-specific adaptation by Sket (1986). In the family Haemopidae, *Haemopis caeca* is the only known stygobiont and was found in Movile Cave (see also Chap. 16). Most of the aquatic cave leeches belong to Erpobdellidae in the Northern Hemisphere; within this family the stygobionts belong to *Dina*, *Trocheta*, and *Croatobranchus* which occur in the Dinaric karst, the last with an unique mouth morphology (Sket et al. 2001) (Fig. 8.1f). *Erpobdella borisi* is a possible new stygobiont found in Iran (Cichocka et al. 2015).

Polychaeta class are marine species with few representatives living in anchialine caves (see also Chap. 18) or in freshwater cave habitats. There are two well-known polychaetes from freshwater cave habitats: *Troglochaetus beranecki* and *Marifugia cavatica*. The minute and transparent *T. beranecki* is widely distributed in Europe, and a related species was found in Japan and attributed to *Speochaetes* (although never described according to Uéno 1957; from Juberthie and Decu 1998). *M. cavatica* is depigmented and eyeless, lives in a calcified tube, and probably colonized cave waters during Pliocene or Pleistocene from freshwater lakes in the Dinaric karst region (Sket 1997; Mihevc et al. 2001; Fig. 8.1g). Twelve of the 17 described *Namanereis* species inhabiting subterranean habitats were mentioned from Australia, Yemen, Canary Islands, and Mexico (Williams 2004; Glasby et al. 2014; Conde-Vela 2017).

8.3.11 Mollusca: Mollusks

Among several classes of mollusks, some are marine and others only fossils; only two have subterranean representatives: Gastropoda and Bivalvia. Cave adaptations are reflected in features such as a thin and fragile shell, white-depigmented body, fine and semi-transparent tegument, and more or less reduced eyes. The small size of the groundwater species can also be an adaptation (Ginet and Decou 1977).

Gastropoda (snails). Unlike epigean snails, the groundwater species belong to a group that can breathe under water with the aid of gills positioned in front of their internal cavity (Ginet and Decou 1977). Altogether, 350 stygobiont gastropods have been described around the world, with 97% of the taxa belonging to Hydrobiidae (Culver 2012), of which 168 species inhabit caves in the Balkan Peninsula (Sket et al. 2004; Fig. 8.1c). Most of the described species were found in the western Palearctic.

Bivalvia (clams). The cave clams belong to the genera *Congeria* and *Pisidium*. Only one species was assigned to *Congeria* until 2013, when C. *kusceri* was split and two other species added: *C. jalzici* and *C. mulaomerovici* (Bilandžija et al. 2013). They all inhabit groundwater of the Dinaric region (Fig. 8.1d). Four species of *Pisidium* are known to inhabit caves of Caucasus, Turkey, and Japan (Bole and Velkovrh 1986). However, the number of described species underestimates true species richness since various cave populations of a species have different shell shape and organization of the hinge teeth, which are usually specific characters, so that each cave is likely to have at least one endemic species (Chertoprud et al. 2016).

8.4 Arthropoda (see Table 8.1)

8.4.1 Crustacea

Crustaceans are the most diverse stygobiont group. Crustaceans live in marine and freshwater environments and their adaptations to groundwater include reduction of eggs number (less than 10 compared to dozens at epigean species), reduction of the reproductive rhythm, longer development, longer life, depigmentation, eye reduction, and reduction of the swimming legs and hairs (see also Chap. 4).

There are only a few stygobiont Cladocera (water fleas) worldwide, and their adaptations to subterranean life include valve depigmentation and eye regression. To date, five stygobiontic chydorid species belonging to *Alona* and *Brancelia* are known (Negrea 1994; Van Damme and Sinev 2011), while other cladoceran species belonging to *Macrothrix*, *Neothrix*, *Moina*, and *Leptodora* require further research.

Stygobiont Copepoda belong to the orders Platycopioida, Calanoida, Misophrioida, Cyclopoida, Harpacticoida, and Gelyelloida, although the number of stygobiont species is not evenly distributed among and within these orders (Galassi 2001; Fig. 8.2). Stygobiont species are particularly common within

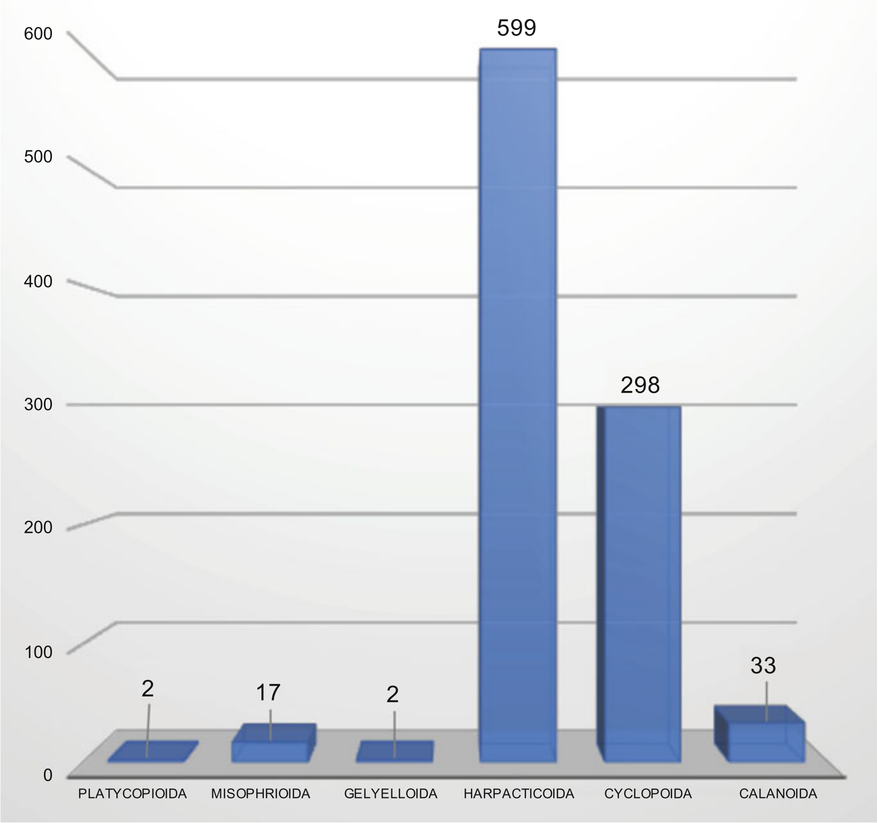

Fig. 8.2 Number of species and subspecies of different orders of cave Copepoda (modified after Galassi 2001)

Harpacticoida, Cyclopoida, and Calanoida. The Cyclopoida and the Harpacticoida have almost 900 species and subspecies (Galassi 2001) distributed worldwide. Most of the stygobiont Harpacticoida (Fig. 8.3e) belong to the families Ameiridae, Canthocamptidae, and Parastenocaridae, with *Praeleptomesochra*, *Pseudoleptomesochrella*, *Parapseudoleptomesochra*, *Nitocrellopsis*, *Stygonitocrella*, *Nitocrella*, *Antrocamptus*, *Spelaeocamptus*, *Gulcamptus*, *Stygepactophanes*, *Ceuthonectes*, *Parastenocaris*, *Forficatocaris*, *Paraforficatocaris*, and *Potamocaris* being exclusively subterranean genera. Chappuisidae has only two exclusively subterranean species (Rouch 1994). The exclusively subterranean Cyclopoida (Fig. 8.3g) belong to genera *Speocyclops*, *Graeteriella*, *Allocyclops*, and *Kieferiella*, with *Speocyclops* as the most specious genus. The 11 known freshwater stygobiont Calanoida belong to Diaptomidae (Bowman 1986; Shu et al. 2017). Freshwater calanoids are distributed from Mexico to Europe and to the Far East, including Australia (Shu et al. 2017), although marine and anchialine cave Calanoida are much more diversified (see also Chap. 18).

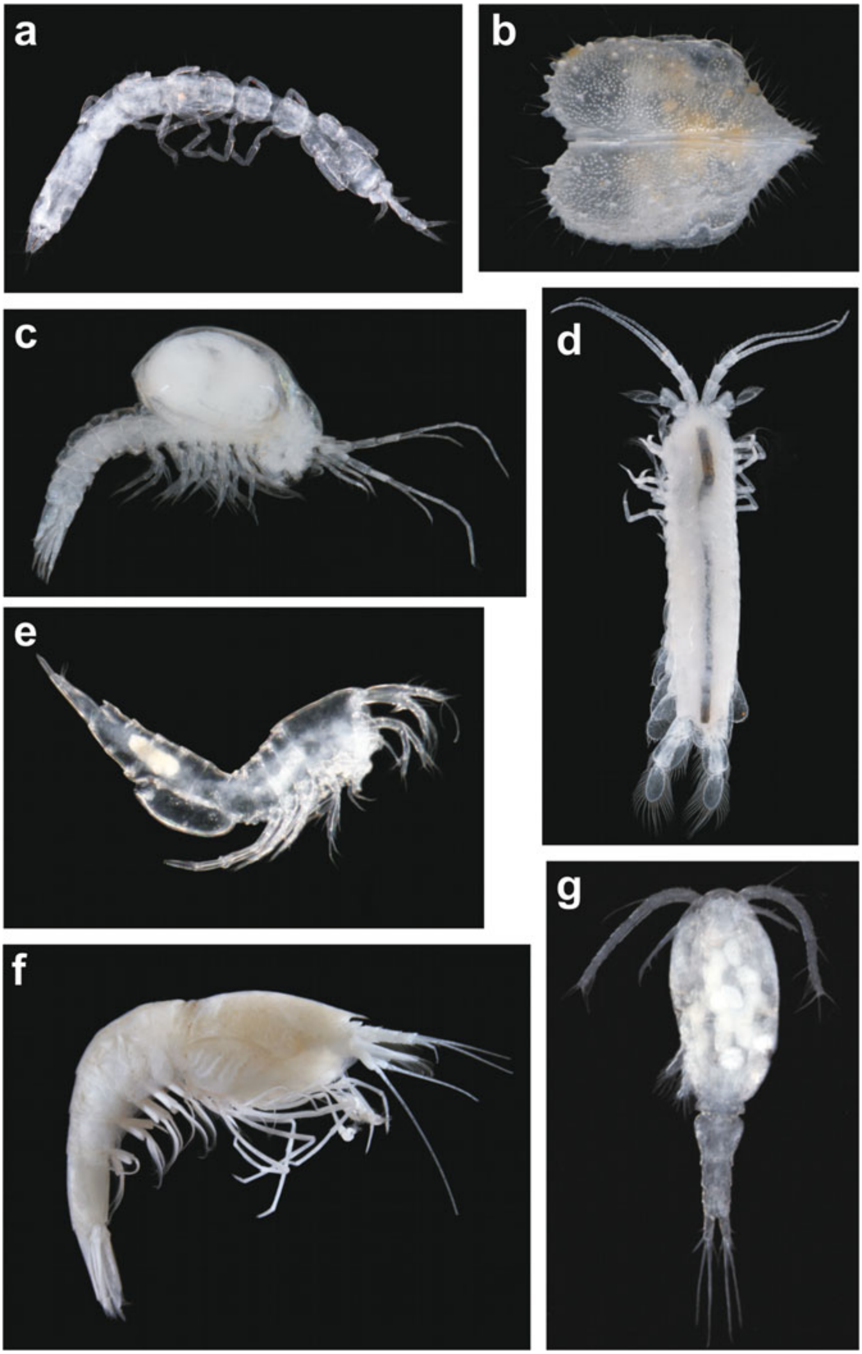

Fig. 8.3 Stygobiont crustaceans from caves and calcrete aquifers; (**a**) Microcerberidae (Isopoda), (**b**) *Gomphodella yandi* (Ostracoda), (**c**) *Mangkurtu* sp. (Spelaegriphacea), (**d**) *Halosbaena tulki* (Thermosbaenacea), (**e**) *Phyllopodopsyllus wellsi* (Harpacticoida), (**f**) *Stygiocaris stylifera*

Stygobiont orders Platycopioida and Misophrioida are found only in marine and anchialine caves (see also Chap. 18). The order Gelyelloida is represented by two species that live in groundwater in France and Switzerland and by another species living in the interstitial sediments of a stream in the USA (Galassi 2001).

The class Remipedia was first described from an anchialine cave (Yager 1981). They lack eyes and are depigmented and have been found only in marine cave systems in the Caribbean Sea, Australia, Canary Islands, Mexico, and Dominican Republic (Koenemann et al. 2018; see also Chaps. 18 and 19). There are 29 described species (Koenemann et al. 2018) and most speciose genera are *Speleonectes*, *Cryptocorynetes,* and *Morlockia*.

According to Danielopol and Hartmann (1985), 310 species and subspecies of the class Ostracoda (seed shrimps) were known from hypogean habitats in 1986, of which only 50 were reported from karst and caves habitats (Fig. 8.3b). Most of subterranean ostracods belong to the order Podocopida and some of the genera have only stygobiont species (i.e., *Mixtacandona*, *Caribecandona*, *Danielocandona*, *Phreatocandona*). Since 1986, dozens of species have been described from freshwater and marine cave habitats. The most notable is the discovery of more than 80 stygobiont ostracods in the Pilbara region of Australia (see also Chap. 20), most of them belonging to endemic genera (e.g., *Deminutiocandona*, *Pilbaracandona*, *Areacandona*; Karanovic 2007). Other interesting discoveries have enlarged not only the number of cave ostracods and their biogeographic distribution but also the knowledge on the origin and history of the group; for example, the discovery of a *Frambocythere* species in a South Korean cave, a genus considered extinct in Eocene (Smith et al. 2017). Subterranean ostracods are mostly blind and depigmented, their size is reduced, and chaetotaxy simplified, while some of the chemosensory aesthetascs are enlarged (Martens 2004).

Groundwater Mysidacea (opossum shrimps) are known from coastal caves, phreatic waters, and anchialine habitats (see also Chap. 18) mostly in the Caribbean region, Mexico, and Mediterranean basin, with only a few species described from south-east Africa and southern India. Stygobiont species belong to *Spelaeomysis*, *Stygiomysis*, *Antromysis*, *Troglomysis*, *Heteromysoides*, *Burrimysis*, and *Palaumysis*. They are blind and depigmented and have a reduced carapace; their sensory setae also show some differences from their epigean relatives in terms of their fine morphology (Crouau 1978, 1980).

Three stygobiont species of Bochusacea (formerly Mictacea) have been described from marine caves of the Caribbean Sea: *Mictocaris halope* and two species of *Thetispelecaris* (Bowman and Iliffe 1985; Ohtsuka et al. 2002).

Eyeless and unpigmented spelaeogriphaceans and thermosbaenaceans are represented by few species from subterranean waters. The Spelaeogriphacea are found only in continental waters, in limestone or sandstone caves, or in calcrete

Fig. 8.3 (continued) (Decapoda), (**g**) *Thermocyclops decipiens* (Cyclopoida). Note that the relative size of the animals is not respected. Photos by © Jane McRae aquifers where they are represented by three genera, *Mangkurtu* (Fig. 8.3c), *Potiicoara*, and *Spelaeogriphus* (Jaume 2008). Thermosbaenacea are marine crustaceans and most taxa occur in the anchialine environment. Only 18 species of Thermosbaenacea have been recorded in limnic or brackish waters, in caves, or in interstitial habitats (Jaume 2008), and they belong to *Thermosbaena*, *Tethysbaena*, *Limnosbaena*, *Halosbaena* (Fig. 8.3d), *Theosbaena*, and *Tulumella* (Boutin 1998). Subterranean thermosbaenaceans have a larger distribution, matching precisely the area covered by the ancient Tethys Sea (Jaume 2008).

One of the most diverse orders of crustaceans, Isopoda, is found in all subterranean marine and freshwater habitats (see also Chap. 7 for a review of terrestrial isopods). The order contains small individuals that live in interstitial habitats and bigger individuals that occur in larger groundwater bodies. They lack eyes, are depigmented, have relatively longer antennae than their epigean relatives and sometimes very elongated uropods, have slower development and produce fewer eggs, —even one single bigger egg that is richer in vitellus as in Microparasellidae (Coineau 1998). With one exception all suborders have also colonized groundwater habitats. Few species belong to the most primitive suborder of isopods, the Phreatoicidea (Coineau 1998; Knott and Halse 1999; Wilson 2008). The rest of groundwater representatives belong to Asellota [i.e., *Caecidotea*, *Proasellus*, *Synasellus*, *Stenasellus*, *Microcerberus* (Fig. 8.3a), *Microcharon*, etc.], Cymothoida (i.e., *Cyathura*, *Stygocyathura*, *Thyphlocirolana*, *Faucheria*), Sphaeromatidea (i.e., *Monolistra*), and Calabozoida (from Coineau 1998). In the world, the most diverse regions in aquatic isopods are Caribbean and Mediterranean. *Asellus aquaticus* is a special example of a largely distributed isopod inhabiting various aquatic habitats in Europe, with only two subspecies restricted to caves, one in Movile Cave and another in the Dinaric karst caves. The importance of this species is that it shows how surface populations diverge after subterranean colonization and that only about one-third of all changed traits can be considered as troglomorphisms (Konec et al. 2015).

Amphipoda is another diverse order of crustaceans characterized by the lack of both eyes and body pigmentation and a diversity of body and appendage sizes depending on the groundwater habitat it occupies. They also have longer life and lay larger and fewer eggs. More than half of the known species (~750 species) occur in caves (Holsinger 1994; Hobbs 2004). Groundwater amphipods belong to 32 families; among the most speciose genera are *Niphargus*, *Stygobromus*, *Metaniphargus*, *Bogidiella*, *Pseudoniphargus*, *Ingolfiella*, *Gammarus*, *Salentinella*, *Metacrangonyx*, *Paramelita*, etc. Two broad regions are especially rich in amphipods, namely, a region comprising eastern and southern North America and West Indies and the Mediterranean region of Europe. *Niphargus* species are used in biospeleology as models for phylogeographical and evolutionary studies (see a recent paper by Delić et al. 2017).

The most evolved crustacean order, the Decapoda, have numerous cave representatives in the infraorders of Brachyura (crabs), Caridea (shrimps), Astacidea (crayfishes), and Anomura. The cave decapods have thin tegument, fine and long pereiopods and antennae with increased number of aesthetascs and are depigmented, except for some anchialine shrimps that are orange, red, or pink in color. The visual organs are reduced or animals are completely blind with a slow metabolism and increased longevity (Cooper and Cooper 1978; Hobbs 1998).

More than 30 species of crabs with marine or freshwater origins have been described from caves. *Sesarmoides*, *Trogloplax*, and *Cancrocaeca* are the main cave genera with a marine origin, while the freshwater crabs belong to *Cerberusa*, *Isolapotamon*, *Phaibulamon*, *Stygothelphusa*, *Adeleana*, *Thelphusula*, *Phricothelphusa*, *Sendleria*, *Holthuisana*, *Rouxana*, *Typhlopseudothelphusa*, *Neostrengeria*, *Chaceus*, and *Rodriguezia*. The cave crabs are distributed in Indonesia, Papua-New Guinea-New Britain, Thailand (the species of marine origin), Jamaica, Mexico, Guatemala, Belize, and South America (the species of freshwater origin) (from Guinot 1994).

Cave shrimps belong to different families and some genera are especially speciose: *Procaris*, *Caridina*, *Parisia*, *Troglocaris*, *Typhlatya*, *Macrobrachium*, *Troglomexicanus*, and *Typhlocaris* (Fig. 8.3f). They are distributed in North America, West Indies, Australia, and the Mediterranean regions (Hobbs 1998; Page et al. 2008).

Freshwater crayfishes are considered successful in colonizing caves, with the Cambaridae as the best represented family. *Cambarus*, *Orconectes*, and *Procambarus* are the most diversified genera distributed in North America and north of Mexico (Hobbs 1998).

Syncarida superorder have subterranean representatives in both its orders, Anaspidacea and Bathynellacea. Anaspidacea has families restricted to caves or interstitial habitats, like Psammaspididae and Stygocarididae, that lack eyes and have reduced appendages (Coineau 1998). They were identified in Tasmania, Australia, and South America. Bathynellacea have exclusively groundwater representatives distributed on all continents. They lack eyes and statocysts and have reduced appendages. The order has more than 95 species, most of them belonging to *Bathynella* with more than 50 species and subspecies (Camacho and Valdecasas 2008). *Hexabathynella* has a cosmopolitan distribution being as rich in species as *Iberobathynella* with a limited distribution to the Iberian Peninsula (Coineau and Camacho 2004; Camacho 2003). Most genera have small geographic distribution (Camacho and Valdecasas 2008).

8.4.2 Hydrachnidia: Water Mites

Water mites are known only from phreatic and hyporheic zone waters (Fig. 8.1h). Specializations to these habitats include eye reduction or lack of eyes, depigmentation and thinner body cuticle, smaller and elongated body, shorter legs than their epigean relatives, and reduction of the number of eggs (Teschner 1963). Only one of the 10 suprafamilies of this subclass has no subterranean representatives. The most frequent genera in phreatic waters are *Stygotrombidium*, *Cerberotrombidium*, *Wandesia*, *Tartarothyas*, *Bandakia*, *Torrenticola*, *Neomamersa*, *Kawamuracarus*, *Atractides*, *Frontipodopsis*, *Aturus*, *Stygomomonia*, etc. (Schwarz et al. 1998).

Fig. 8.4 Stygobiont beetles from a single calcrete aquifer in the Australian desert (Dytiscidae: Hydroporini), from left to right: *Paroster macrosturtensis*, *P. mesosturtensis*, *P. microsturtensis*. Photo by © Chris Watts

8.4.3 *Insecta, Coleoptera aquatica: Aquatic Beetles*

The first stygobiont beetle (*Siettitia balsetensis*) was discovered in 1904, in France, by Abeille de Perrin and until the end of the twentieth century only 16 more species and one subspecies were reported from Japan, China, Thailand, Indonesia, and Ecuador (Spangler and Decu 1998). They belong to Dytiscidae, Elmidae, Hydrophilidae, and Noteridae families. The number of stygobiont species increased dramatically with the study of the calcrete aquifers in Western Australia (see also Chap. 20) where approximately 100 dytiscid species with regressed or absent eyes have been found within 45 separate calcrete bodies (Leys et al. 2003; Watts and Humphreys 2009; Watts et al. 2016).

Along with the typical adaptations of subterranean inhabitants, stygobiont beetles have unique adaptations that are not found in their epigean water beetle relatives, such as the change of pupation habitat from terrestrial to the bottom of the subterranean waters (Uéno 1957). Smaller size was also considered as one of the general adaptations of stygobiont beetles until the discovery of calcrete Dytiscidae of very different sizes (Fig. 8.4).

8.5 Chordata

8.5.1 *Pisces: Fishes*

Fish are the most studied group of cave inhabitants, especially in relation to processes of adaptations. All stygobiont fishes belong to the Teleostei and have a small and slender body. More than 80 taxa were described but new species are added each year. Cave fishes belong to the orders Characiformes, Cypriniformes, Siluriformes, Gymnotiformes, Percopsiformes, Ophidiiformes, Cyprinodontiformes, Synbranchiformes, and Perciformes. The richest in species are Cypriniformes and Siluriformes. The most diversified genera are *Sinocyclocheilus*, *Triplophysa*, *Rhamdia*, *Trichomycterus*, *Ancistrus*, and *Lucifuga*. Cave fishes are most common in tropical and subtropical caves, but a few have also been described from temperate caves (Weber et al. 1998; Romero 2001; Trajano et al. 2010). The most recent discovery was the first cave fish in Europe, found in the cave labyrinth called the Danube-Aach System, in southern Germany (Behrmann-Godel et al. 2017).

Adaptations of cave fish include the ones common for all stygobiont animals—depigmentation and reduction or lack of eyes, as well as some typical adaptations for the group: reduction of the pineal organ in some species, enhanced olfaction, taste and touch senses, and the development of the lateral line (see also Chap. 4).

8.5.2 Amphibia

Only the order of Urodela (salamanders) has cave representatives. The most advanced adaptations include the development of the lateral line, slender legs, a flat and wide head, and neoteny. *Eurycea*, *Typhlotriton*, *Haideotriton*, and *Gyrinophilus* are known from North America; the species of these genera are depigmented and have reduced eyes. The only European cave salamander is *Proteus anguinus* which reaches sexual maturity at 11–14 years for the males and 15–18 years for the females, while hatching takes place more than 100 days after oviposition (Juberthie et al. 1996; Durand 1998). A first black *Proteus* was found almost three centuries after the description of this species by Von Valvasor in 1689, and described as a subspecies endemic to a small area in Slovenia (Sket and Arntzen 1994).

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Chapter 9
Subterranean Biodiversity Patterns from Global to Regional Scales

Maja Zagmajster, Florian Malard, David Eme, and David C. Culver

9.1 Introduction

Interest in the geographic pattern of species richness became a central theme in macroecology in the late 1980s (Wilson 1988). The quantitative study of biodiversity was made possible by (1) the development of spreadsheets and databases that could store and manipulate large amounts of data, (2) GPS devices and detailed digital maps that allowed accurate and rapid georeferencing, and (3) mapping software that made display and analyses of georeferenced occurrence data straightforward. In the light of rapid environmental changes due to human impacts, which may bring species close or even to extinction, the need to map biodiversity patterns and improve the understanding of the mechanisms underlying the patterns became obvious.

Caves and other subterranean habitats offer many challenges and opportunities in tackling general biodiversity questions, but large-scale biodiversity studies started

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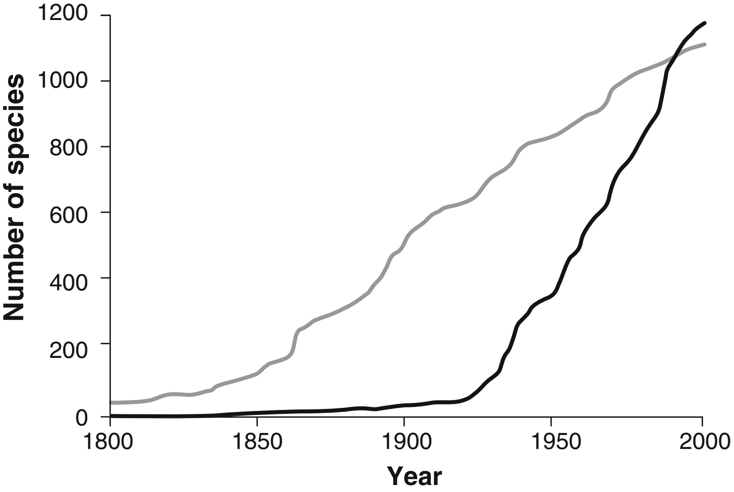

Fig. 9.1 Cumulative number of aquatic crustacean species described, by year, for surface species (thin line) and subterranean species (thick line) in Europe (Modified from Stoch and Galassi (2010))

much later below ground than in other habitats for several reasons. First, discovery and description of subterranean species lagged behind the description of surface species, at least by several decades (Fig. 9.1; Stoch and Galassi 2010). Second, until relatively recently, the number of stygobiotic and troglobiotic species seemed so low that quantification was unnecessary, as can be seen in Fig. 9.1. In past decades this changed significantly. There are some taxonomic groups that are even richest in the subterranean realm; for example, in Europe, the number of stygobiotic crustacean species exceeds the number of surface-dwelling crustacean species (Stoch and Galassi 2010: 1174 vs. 1111 species, respectively). There are more troglobiotic palpigrades (Arachnida) than surface ones in temperate regions (Giribet et al. 2014). The most speciose genus of Amphipoda, *Niphargus*, has more than 400 species, all but a handful of which are strictly subterranean (Fišer et al. 2008; Horton et al. 2017). Third, because many species have very small ranges, local species richness is low compared to regional species richness (Gibert and Deharveng 2002), which in turn means large amounts of data are required for large-scale analysis.

In past few decades, there has been a substantial progress in mapping biodiversity patterns of subterranean taxa, as presented in this chapter. But there is one aspect that becomes obvious in such an overview—there is a large bias toward analyses of groundwater taxa, compared to terrestrial subterranean taxa, especially on global and continental scales.

9.2 Challenges in Describing and Explaining Subterranean Biodiversity Patterns

Biodiversity studies foremost depend on the quality of species distribution data, which are inevitably incomplete. At any point in time, data can be improved, more records collected, and additional species discovered, so that "perfectly complete datasets" can never be obtained and waiting to get them may lead to biodiversity loss prior to discovery. Even incomplete datasets can give valuable information for scientific studies, as well as the development of conservation strategies, if interpreted and evaluated properly. This is especially true of subterranean biodiversity studies, which deal with habitats that are difficult to access, species that are difficult to sample and, even when collected, mostly difficult to identify (Fišer and Zagmajster 2009; Delić et al. 2017). Consequently, studies of subterranean biodiversity may be more challenging. But if the characteristics of such environments are taken into account, and advantage is taken of the data resources and analytical capacity currently available, robust conclusions are possible. An overview of potential pitfalls in comparative studies of subterranean biodiversity was prepared by Culver et al. (2012), and we refer to this work at several places. In the following paragraphs, we describe challenges related to documenting biodiversity patterns, which are followed by challenges in explaining these patterns.

9.2.1 Spatial Scale and Availability of Data

One of the main challenges is to improve accuracy and availability of data. It was only relatively recently that databases on subterranean species distributions developed from species lists per country/region to sets of species occurrences with accurate spatial coordinates defined at the level of sampling sites (Culver et al. 2001; Zagmajster et al. 2008, 2014; Deharveng et al. 2009; Christman et al. 2016). While species lists per country/region can be used for inferences at global or continental scales, spatially accurate species occurrences enable studies at regional and local scales as well as application of dedicated analytical tools for the study of spatial patterns (Christman and Zagmajster 2012). Databases with georeferenced records on subterranean species distributions have been developed, but are not freely available. Metadata on some databases can be found online, with instructions on how to retrieve the data (e.g., CKmap2000 2003; BioFresh Portal 2014). In rare cases, publicly available databases can also be downloaded (e.g., for Bathynellacea in Iberian Peninsula, Camacho et al. 2014). Finally, occurrences of species at the country level are directly available, for example, in Fauna Europaea database (de Jong et al. 2014).

9.2.2 Not All Species Are Troglobionts

An issue that is somewhat specific to subterranean habitats is that not all species are exclusively subterranean, as some live underground only during some parts of their life cycle or can occur there accidentally (see Chap. 3 for discussion on classification of subterranean fauna). Comparative studies of distributional patterns of subterranean biodiversity should be done with species or combination of species that have the same level of dependency on subterranean habitats. This is because troglobiotic and non-troglobiotic species differ in their responses to different environmental factors. This does not imply that only troglobionts should be considered in analyzing subterranean biodiversity patterns. Indeed, it could be useful to include data on troglophiles, but such data are very rarely gathered in databases. The same is the case with guanobionts, species that are specialized to live in bat guano but do not exhibit troglomorphies—they can present a substantial amount of species in guano-rich tropical caves (Deharveng and Bedos 2012).

9.2.3 The Omnipresence of Undescribed Species

A recurrent problem in subterranean research is that a lot of specimens remain determined to the level of "undescribed species" due to the lack of taxonomists. Yet, not all undescribed species become new valid species when checked by taxonomists—some of them turn to be already described species. This issue was referred to by Culver et al. (2012) as the fallacy of "equality of the described and undescribed species," with recommendation that only formally described species should be used for regional comparisons. This may represent a problem for some regions, for example Brazil, where many new recent discoveries yielded many specimens attributed to undescribed species based on visual morphological differences (Trajano and Bichuette 2010; Souza and Ferreira 2016). But, compared to morphological estimation, molecular methods may be better in distinguishing different "units" that could be considered as separate species (Guzik et al. 2011). Indeed, molecular approaches can facilitate the discovery of species diversity, and molecular data offer valuable distinctive characters which can be used for species diagnosis (Delić et al. 2017).

9.2.4 Dealing with Sampling Difficulties

Even though the problem of sampling incompleteness is common to biodiversity studies in general (Beck and Schwanghart 2010), it is more apparent in caves and other subterranean habitats. Indeed, accessing subterranean habitats is challenging and may require special physical skills from collectors (vertical caving, cave diving) or specialized equipment to reach the animals where humans cannot physically enter. As abundance of species in caves is generally low (Culver and Pipan 2009), repeated visits or trapping techniques are needed to collect most of the present animals. Repeated visits with comparable sampling effort can be used to estimate species detectability—this is the probability that the species will be sampled when present (Tyre et al. 2003). However, taking this into account in multispecies subterranean studies is fairly uncommon due to the difficulty of evaluating the detectability of each species.

Even working with incomplete datasets can lead to sound and firm conclusions, if differences in sampling are considered properly. On large scales, robustness of the pattern can be evaluated by comparing the patterns at different snapshots in time (Culver et al. 2004a; Bregović and Zagmajster 2016). Species accumulation curves enable comparisons of regions considering their potential for additional species—the steeper the curve, the more species are still expected to be found (Gotelli and Colwell 2001). Species richness estimators, which predict missing species on the basis of rare species (Colwell and Coddington 1994), have been widely used in subterranean comparative studies (e.g., Culver et al. 2006; Zagmajster et al. 2008; Deharveng et al. 2009). Using the ratio of observed species richness to estimated species richness has been proposed as a direct measure of sampling/completeness (Soberón et al. 2007). This approach was applied to large-scale subterranean biodiversity studies, to get the subsample of “well-sampled” areas (Zagmajster et al. 2014). A different approach to identify such areas was proposed by Zagmajster et al. (2010), who used the number of single-cave species per quadrat as indicators of sampling completeness when quadrats of similar species richness were compared.

Some approaches used in subterranean studies can lead to biased conclusions. One is the use of best expert opinion rather than sampling results to estimate the expected number of species, which was referred to by Culver et al. (2012) as “misuse of expert opinion.” Guzik et al. (2011) treated the expected numbers of subterranean species richness in Western Australia, given by taxonomic experts, as a number for comparisons with other regions of the world, where no such expert estimates had been made. Another false approach is presuming one should not expect new species in areas where high numbers of described species already occur. Treating various regions differently in this respect was termed by Culver et al. (2012) as fallacy of “provincialism.” The point is that new species are being discovered not only in species-poor but also in species-rich areas. This has been statistically confirmed in the Dinaric karst in the Balkans, where additional species are not to be expected only in species-poor but also within species-rich areas (Zagmajster et al. 2010). On the other hand, this does not imply that eventually all regions of the same size will yield the same number of subterranean species (termed fallacy of “isotropy” by Culver et al. 2012). This means that number of species per certain area cannot be simply extrapolated to other areas of the same size.

9.2.5 Area Matters

In comparative studies, surface areas that are used to report number of species should be taken into account—this is termed as fallacy of “scale invariance” by Culver et al. (2012). Clearly, species richness tends to increase with increasing area, also known as the species-area relationship (Rosenzweig 1995). Thus, only species richness of similar or comparable areas should be compared. If this is not possible, corrections due to differences in area should be included. For example, Stoch and Galassi (2010) used adjusted formula controlling for area to investigate groundwater crustacean biodiversity patterns in Europe.

9.2.6 Spatial Autocorrelation

There are also challenges in studies that attempt to explain the patterns of species richness. First is the consideration of spatial phenomena termed spatial autocorrelation (Cressie 1993), referring to the observations that spatially closer areas are more related than the ones further apart. Spatial autocorrelation, which is the result of dispersal (or the lack of it), should be considered when inferences in explaining the mechanisms behind the observed patterns are sought. Different approaches to considering spatial autocorrelation exist and have been employed in subterranean biodiversity studies, including Moran’s I (Christman et al. 2005; Zagmajster et al. 2008), conditional autoregressive models (Christman and Culver 2001), simultaneous autoregressive models (Zagmajster et al. 2014; Eme et al. 2015), generalized least squares models (Eme et al. 2017), and spatial eigenvectors (Bregović and Zagmajster 2016).

9.2.7 Selection of Relevant Variables

Selection of variables used to explain subterranean biodiversity patterns should be done with consideration of their potential effect on subterranean communities. For example, in cave/subterranean habitats, daily and even yearly fluctuations of temperature are attenuated (e.g., Freeze and Cherry 1979; Šebela and Turk 2011), so such variables are not expected to have direct impact on subterranean communities. In species richness studies, number of caves and cave length were initially used as a measure of habitat availability (Christman and Culver 2001; Culver et al. 2001, 2004a, b), but recent publications of the distribution map of groundwater habitats in Europe (Cornu et al. 2013) and the karst map database in the United States (Weary and Doctor 2014) enabled the development of additional variables (Zagmajster et al. 2014; Eme et al. 2015, 2017; Christman et al. 2016). Bearing in mind the recent increase in availability of different environmental variables for statistical modeling, it is important to select those variables that are related to the central question and scale of analyses (Zagmajster et al. 2014; Eme et al. 2015, 2017; Bregović and Zagmajster 2016; Mammola and Leroy 2017).

9.2.8 The Importance of Spatial Scale and Spatial Non-stationarity

The importance of different explanatory variables may differ according to the scale of analysis (Field et al. 2009), as well as to the region under study (Eme et al. 2015). The latter, termed spatial non-stationarity, acknowledges that the relationship between response variable (biodiversity metrics) and environmental factors may vary across space. Geographic weighted regression models that take into account spatial non-stationarity (Brunsdon et al. 1996) were used by Eme et al. (2015) to reveal geographic variation in the relative contribution of environmental factors to species richness of groundwater crustaceans in Europe.

9.3 Global Patterns of Species Richness: The Tropics Are Not Hotspots

Owing to the lack of comparable data on cave and other subterranean species from most countries, little is known on global patterns of subterranean biodiversity. Attempts to collect all known data on subterranean species of the world started already in the first half of twentieth century. Species lists of Wolf (1934–1938) and numerous heterogeneous country lists of taxa were included in publications within *Encyclopaedia Biospeologica* (Juberthie and Decu 1994, 1998, 2001). The per country distributions of subterranean fishes indicate that their diversity is highest between 16 and 25 N (Culver and Pipan 2009). This case is specific to rather low number of cave fishes on a world scale (Proudlove 2010) and may reflect also some other specificities of the group. A few global overviews of species richness of other subterranean taxa [Amphipoda, by Holsinger (1993) and Nematoda, by Du Preez et al. (2017)] are also interesting but of little use to evaluate global subterranean biodiversity patterns.

9.3.1 Global Distribution of Biodiversity Hotspots

In 2000, Culver and Sket published a list of caves and wells of the world where at least 20 troglobionts, both terrestrial and aquatic, had been recorded. Their list and the map they produced was the first global overview of the distribution of hotspots of species richness per site. Since then, the number of such sites quickly doubled (Culver and Pipan 2009; Souza and Ferreira 2016). Culver and Pipan (2013) set a more stringent criterion to define the hotspots in cave biodiversity and listed the sites with at least 25 species, where aquatic and terrestrial troglobionts were treated separately. The list became much shorter, including only nine aquatic and seven terrestrial sites on the globe (Fig. 9.2). These two lists show that the distribution of hotspots is not uniform around the globe and that areas with largest aggregations of hotspots do not occur in the tropical belt. Only one site in Australia is situated at the edge of the tropical belt in the Southern Hemisphere, while all the others are located in temperate latitudes. Deharveng and Bedos (2012) brought forward the potential for increased species richness in the tropics, if guano-specialized species (guanobionts) were included in the lists.

9.3.2 Global Patterns of Groundwater Crustacean Species Richness

Mapping sites containing at least 25 troglobionts is only a rough approximation of the global patterns in subterranean biodiversity. Additionally, there are resources available that enable mapping of biodiversity of at least some subterranean taxa at global scale.

We tentatively mapped the global distribution of groundwater crustaceans (Fig. 9.2), using first, data from Botosaneanu (1986) and second, data from various sources published over the last two decades (Africa: Kayo et al. 2012; Asia: Brancelj et al. 2013; Australia: Guzik et al. 2011; Halse et al. 2014; Europe: Zagmajster et al. 2014; North America: Peck 1994; Mejia-Ortiz et al. 2013; Niemiller and Culver, unpublished; Southern America: Pérez-González and Yager 2001; Trajano and Bichuette 2010; Rios-Escalante et al. 2016). Even though species richness data were not available for some countries, the maps provide valuable insights into the global pattern of groundwater crustacean species richness. The number of species per country is rising in time, supporting the need for further worldwide species inventories. The updated map of groundwater crustacean species does not indicate any rise in species richness near equator or in tropical regions. Highest numbers of species can be found at temperate latitudes, especially in the Palearctic and Nearctic regions.

9.4 Striking Features of Subterranean Biodiversity Patterns at Continental Scales

The first initiatives in mapping continental subterranean biodiversity patterns started in North America, covering contiguous states of the United States (Culver et al. 1999, 2000). An important impetus in promoting the mapping of subterranean biodiversity on larger scales was presented by a thematic workshop on *Mapping Subterranean Biodiversity* (Culver et al. 2001). The first large-scale quantitative study was the PASCALIS

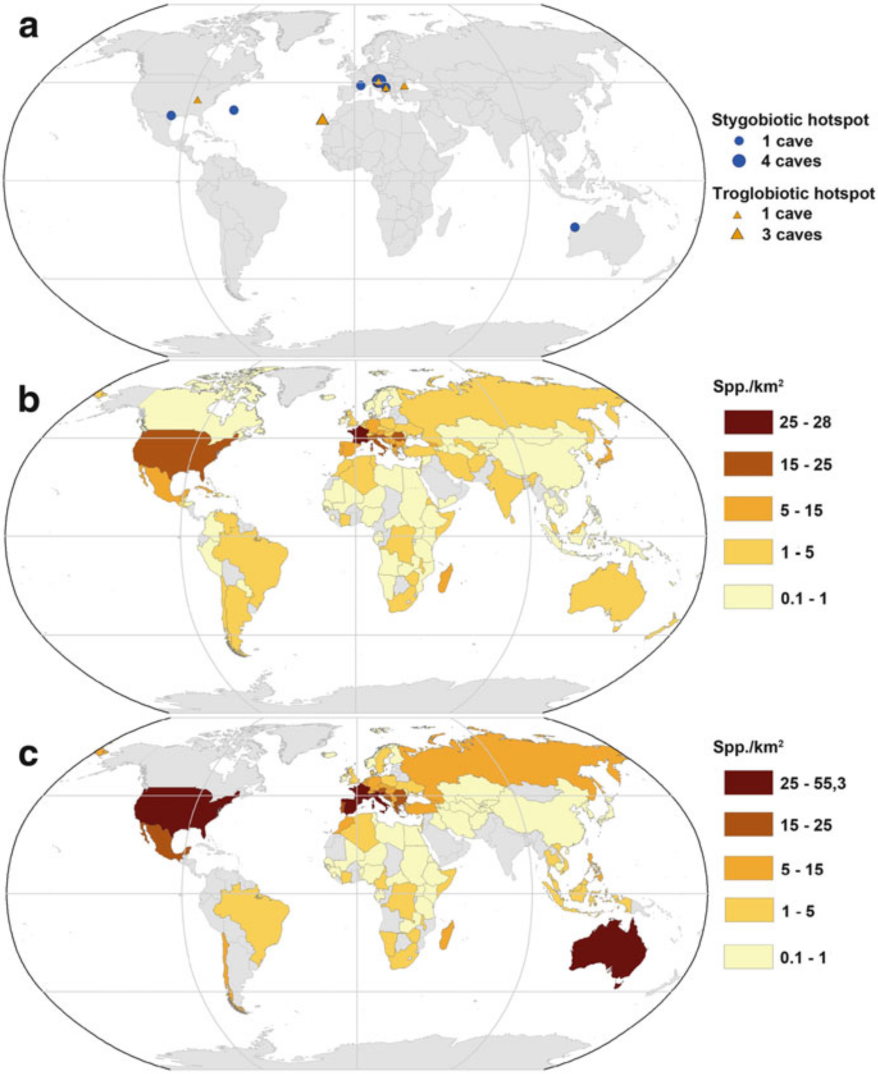

Fig. 9.2 Different presentations of global patterns of subterranean biodiversity: (**a**) distribution of caves with at least 25 troglobionts and/or stygobionts (data from Culver and Pipan 2013); (**b**) distribution of groundwater crustacean species per km^2, based on data from Botosaneanu (1986); (**c**) distribution of groundwater crustacean species per km^2, based on data from publications in the last two decades (see text for the list). Species richness per country (SR_c) in (**b**) and (**c**) is corrected for the area effect using the following formula $SR_c = SR_{obs}/A^{0.15}$ where SR_{obs} is the raw species count for each country and A is the country area (Stoch and Galassi 2010). Class delimitation in (**b**) is done according to the highest value, this is >85%, >60%, >40%, >20% and less than 20% of the richest area. In (**c**) class delimitation follows limits from (**b**). Grey colour in (**b**) and (**c**) refers to the countries with no data

project (*P*rotocols for the *AS*sessment and *C*onservation of *A*quatic *LI*fe in the *S*ubsurface), a western-central European-wide study (Gibert et al. 2004). Recently, studies reporting on high subterranean biodiversity areas have been reported from Brazil (Trajano et al. 2016) and Western Australia (Guzik et al. 2011; Halse et al. 2014), but they are of limited use to evaluate continental patterns. Most of what we know about spatial patterns of subterranean species richness at continental scale is from studies conducted in Europe, which are almost exclusively limited to groundwater habitats.

9.4.1 Scale Dependence of Species Richness Patterns

Not surprisingly, patterns depend on the spatial grain used to map species richness. Coarse-grain studies revealed a northward monotonic decline in the number of species in Europe (Fig. 9.3). This latitudinal gradient of aquatic species richness was first documented by Hof et al. (2008) for the groundwater fauna, based on 934 groundwater species taken from *Limnofauna Europaea* (Illies 1978). Stoch and Galassi (2010) documented the pattern of groundwater crustacean species richness in Europe using data from Fauna Europaea (de Jong et al. 2014). Their analysis included 1174 groundwater crustaceans. They calculated species richness for each country, corrected for area, and clustered the countries into three latitudinal bands. For each band, they calculated the number of species per area. The pattern of decreasing species richness with increasing latitude became visible (Fig. 9.3a). In Fig. 9.3b, we used a more recent dataset of 1570 groundwater crustacean species in Europe (Zagmajster et al. 2014), calculated number of species per each bioregion (sensu Illies 1978), and corrected for area of mainland within each region (species

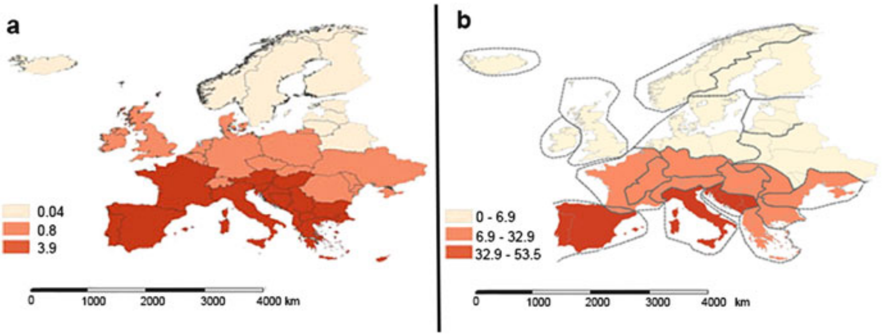

Fig. 9.3 Coarse-grain patterns of species richness for the groundwater crustaceans in Europe. (**a**) Latitudinal bands of species richness, joining countries with similar groundwater crustacean species richness. The legend presents species richness per area [spp/km^2] within each band (Stoch and Galassi 2010). (**b**) Groundwater crustacean species richness from the European groundwater crustacean dataset (Zagmajster et al. 2014), calculated per each biogeographical region (sensu Illies 1978). Species richness was corrected for the area of mainland within each region [spp/km^2]. Dashed lines denoted region borders, and pale gray denoted country borders for reference

richness [SR_c] is corrected for the area effect using the formula $SR_c = SR_{obs}/A^{0.15}$ where SR_{obs} is the species count and A is the area of the region; as in Stoch and Galassi 2010). Presented in three classes according to species richness, the latitudinal decrease in species richness is again visible (Fig. 9.3b).

In contrast to coarse-grain studies of continental biodiversity patterns, once a finer spatial grain was used, a ridge of high terrestrial and aquatic species richness at latitudes ranging from *c.* 42 to 46° N became visible (Culver et al. 2006; Zagmajster et al. 2014). Culver et al. (2006) assembled georeferenced records of terrestrial cave species for three European regions and four north-American regions ranging in size from 2000 to 6300 km^2. By mapping the number of species for a total of 23 regions, Culver et al. (2006) hypothesized that the pattern for Europe was one of a ridge of high species richness (Fig. 9.4). In North America, the ridge of high species richness was reduced to a single region (northeast Alabama), later expanded to south central Tennessee (Niemiller and Zigler 2013).

Zagmajster et al. (2014) assembled in the European groundwater crustacean dataset (EGCD) a total of 21,700 database records collectively representing 1570 species and subspecies of Crustacea. To analyze the spatial patterns of species richness at a European scale, the authors used bands of 0.9° latitude, with cells in the bands having an area of 10,000 km^2 (Fig. 9.5). The average species richness per cells within 0.9° latitudinal band exhibited a hump-shaped pattern: species richness for groundwater crustaceans peaked at roughly the same latitudes (*c.* 42–46° N; Fig. 9.5) as richness of terrestrial cave species (Fig. 9.4). Zagmajster et al. (2014) showed that the hump-shaped pattern of groundwater crustacean species richness was robust to sampling bias.

9.4.2 Range Size Follows a Rapoport Rule in the Palearctic

The species richness patterns are inherently related to the patterns of range size that were explored in groundwater crustaceans (Zagmajster et al. 2014). The "Rapoport rule" was first coined by Stevens (1989) to describe the pattern of increasing species range size at higher latitudes observed among a number of plants and animals. Zagmajster et al. (2014) measured the range size of groundwater crustaceans by calculating the maximum linear extent (MLE) of each species—defined as the straight-line distance between the two most distant known localities of a species. Then, they computed and mapped the median MLE of species contained in 10,000 km^2 cells across Europe (Fig. 9.6). The groundwater crustacean ranges were found to exhibit a marked Rapoport rule, as the cell average of median MLE per 0.9° latitudinal band increased markedly with latitude above a latitudinal threshold of 43° N (Fig. 9.6).

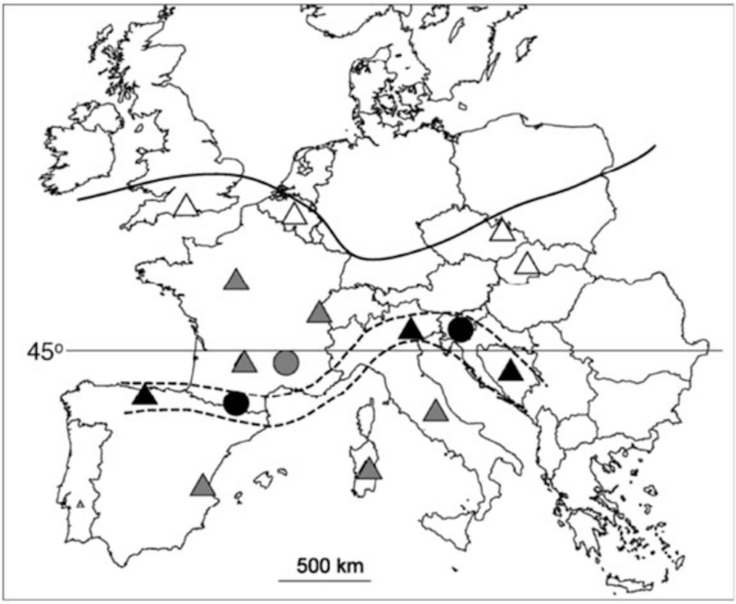

Fig. 9.4 Map of species richness patterns of European terrestrial cave species. The open triangles are areas with few if any terrestrial cave species, the gray triangles are areas with <50 species, and the gray circle is Ardèche with <50 species in 5000 km^2 of area. The black circles are the diversity hotspots in Slovenia and Ariège (France). Black triangles are other possible diversity hotspots. The boundary of the Pleistocene ice sheet is shown as solid line. A pair of dashed lines indicates the hypothesized position of the high diversity ridge (From Culver et al. (2006))

9.4.3 A High Spatial Turnover in Species Composition Over Short Distances

Subterranean fauna typically consist of narrow range species, which affect patterns of change in species composition (i.e., beta diversity). Culver et al. (2000) provided data on species composition of stygobionts, troglobionts, and single-county endemics for the contiguous counties of the United States, based on 927 species. Endemism was high and 54% of the species were known from a single county. High endemism of troglobionts was reported for calcrete bodies of Western Australia, with very little common species also among closely positioned calcrete bodies (e.g., only 8 and 20% shared species among three calcrete bodies; Humphreys et al. 2009). Of a total of 313 groundwater species collected in six regions of Central Europe as part of the PASCALIS project, only 1, 3, 4, and 27 species were shared by 6, 4, 3, and 2 regions, respectively (Dole-Olivier et al. 2009).

Zagmajster et al. (2014) quantified the latitudinal pattern in the intraregional turnover component of beta diversity of groundwater crustaceans across Europe using large squares of equal area (Fig. 9.7a). The turnover component of beta diversity represents differences in species composition caused by species replacements as

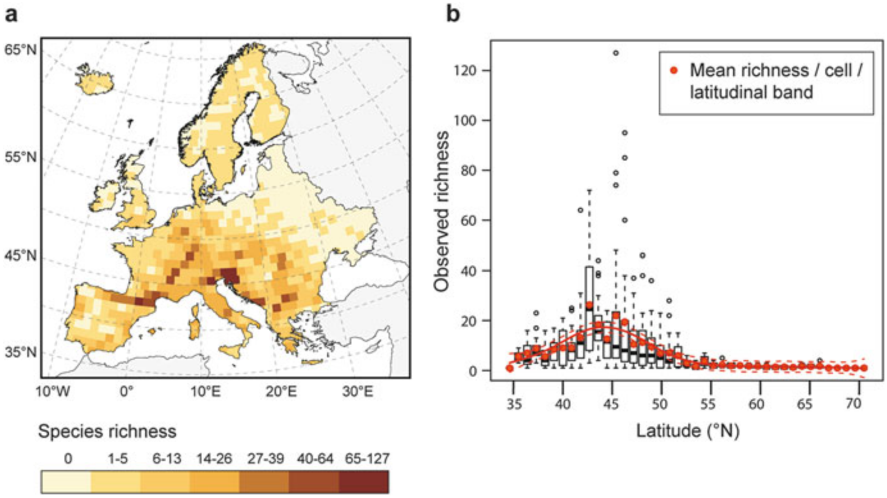

Fig. 9.5 Map of species richness patterns of European groundwater crustacean species. (**a**) Species richness of 10,000 km^2 cells. (**b**) Relationship between the cell average of species richness per 0.9° latitudinal band and latitude. Black horizontal bars and boxes show the median and interquartile range, respectively, for latitudinal bands. The maximum length of each whisker is up to 1.5 times the interquartile range, and open circles represent outliers. The thick red line represents the fit of generalized additive model to the averages of latitudinal bands (From Zagmajster et al. (2014))

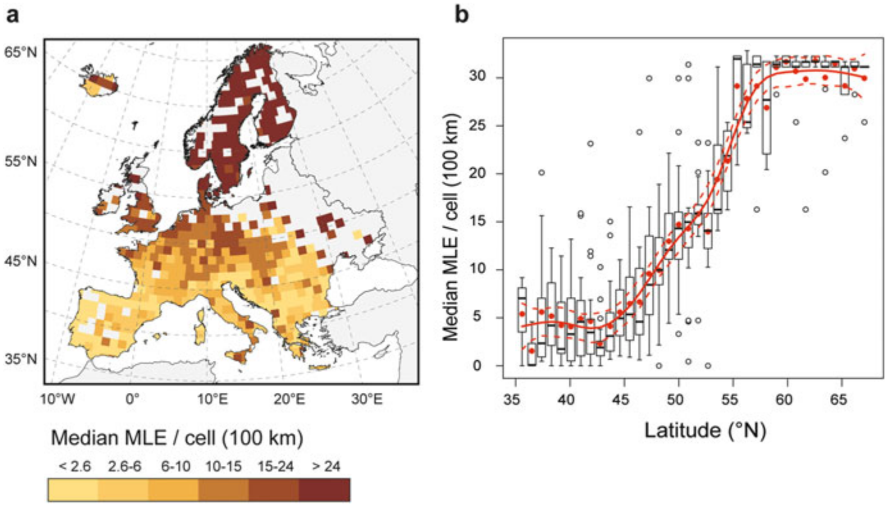

Fig. 9.6 Map of range size patterns of European groundwater crustacean species. (**a**) Median maximum linear extent (MLE) of species contained in 10,000 km^2 cells. (**b**) Relationship between the cell average of median maximum linear extent per 0.9° latitudinal band and latitude. Black horizontal bars and boxes show the median and interquartile range, respectively, for latitudinal bands. The maximum length of each whisker is up to 1.5 times the interquartile range, and open circles represent outliers. The thick red line represents the fit of generalized additive model to the averages of latitudinal bands (From Zagmajster et al. (2014))

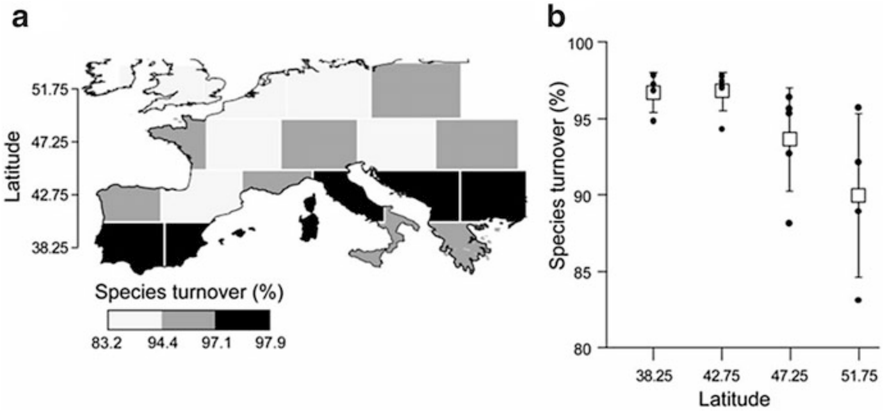

Fig. 9.7 Spatial variation of intraregional species turnover of groundwater crustaceans in Europe. (**a**) Map of species turnover expressed as a proportion of beta diversity (Jaccard index of dissimilarity). Species turnover was calculated by resampling 11 cells of 10,000 km^2 in each 250,000 km^2 square 1000 times. (**b**) Plot of intraregional species turnover versus latitude. White squares and whiskers show means and standard deviations, respectively (From Zagmajster et al. (2014))

opposed to the nestedness-resultant component of beta diversity, which represents differences in species composition caused by species losses or gains (Baselga 2012). Zagmajster et al. (2014) showed that the mean intraregional beta diversity was as high as 0.95 ± 0.019 ($n = 19$ squares), and it was almost entirely due to species replacement (mean species turnover component = 94.6% ± 4) (Fig. 9.7a). Moreover, the proportion of beta diversity explained by species replacement was found to decrease with increasing latitude (Fig. 9.7b).

9.5 Subterranean Biodiversity Patterns at Regional Scales

There are some general objectives that can be pursued based on existing studies of regional patterns in subterranean biodiversity. The first is to determine the overall number of species in a region. It is undisputable that there are not enough human and financial resources to sample in a reasonable time frame any large region to properly delineate the distribution range of all species. Therefore, different approaches have been employed to estimate the size of regional species pools from known local communities, and they have been extensively used in subterranean studies. The next interesting objective is to explore the differences and similarities between different regions—and the contribution of regional diversity to overall species richness. Within regions, spatial patterns of species richness are not uniform—aggregations of species in certain parts can be recognized.

9.5.1 Regional Species Accumulation Curves Rarely Reach Saturation

Over the last decade, considerable effort has been made to identify the most efficient strategy for assessing the size of the regional species pool (Dole-Olivier et al. 2009). An efficient strategy must provide an unbiased estimate of the number of species present in a region while minimizing the sampling effort. Subterranean communities are characterized by an overrepresentation of rare species, which makes it difficult to obtain an unbiased estimate of the regional species pool. Extensive sampling carried out as part of the project PASCALIS in six European regions of less than 10,000 km^2 in size showed that the proportion of rare groundwater species (i.e., species occurring in less than 3% of the 192 sites sampled in each region) varied from 40 to 77% depending on the region (Dole-Olivier et al. 2009). Terrestrial subterranean communities also support large proportions of rare species. For example, single-site species represented 45% of all terrestrial troglobionts in the Eastern United States (Christman et al. 2005); almost 70% of all terrestrial troglobionts in Tennessee are known from five or fewer caves (Niemiller and Zigler 2013). It is therefore not surprising that despite extensive sampling of subterranean fauna carried out in a number of regions in Europe and North America, species accumulation curves began to level off but never reached an asymptote (Fig. 9.8) (Culver et al. 2006; Castellarini et al. 2007; Zagmajster et al. 2008; Dole-Olivier et al. 2009, 2015; Stoch and Galassi 2010).

There are various reasons why species accumulation curves are unlikely to saturate at the regional level. When the same data on distribution of subterranean beetles in the Dinaric region in the Balkans were associated with grids of different sizes, species accumulation curves started to level off only for the grids of the smallest size, 5 × 5 km (Zagmajster et al. 2008). The size of the unit at which species accumulation curves can reach an asymptote depends on the dispersal capacity of organism studied. Generally, stygobiotic species have higher dispersal capacity than troglobionts (Lamoreux 2004). In Tennessee, species accumulation curves for terrestrial troglobionts were rising, while the curves for stygobionts reached an asymptote (Niemiller and Zigler 2013). Dole-Olivier et al. (2009) showed that the efficiency of sampling design for estimating the number of species in any region could also be improved by accounting for different sources of environmental heterogeneity such as catchment boundaries, aquifer types (i.e., karst versus porous aquifers), and habitat types within aquifers. However, it is not possible to provide a universal sampling design that is efficient in all regions because the most relevant sources of heterogeneity to be considered are region-specific.

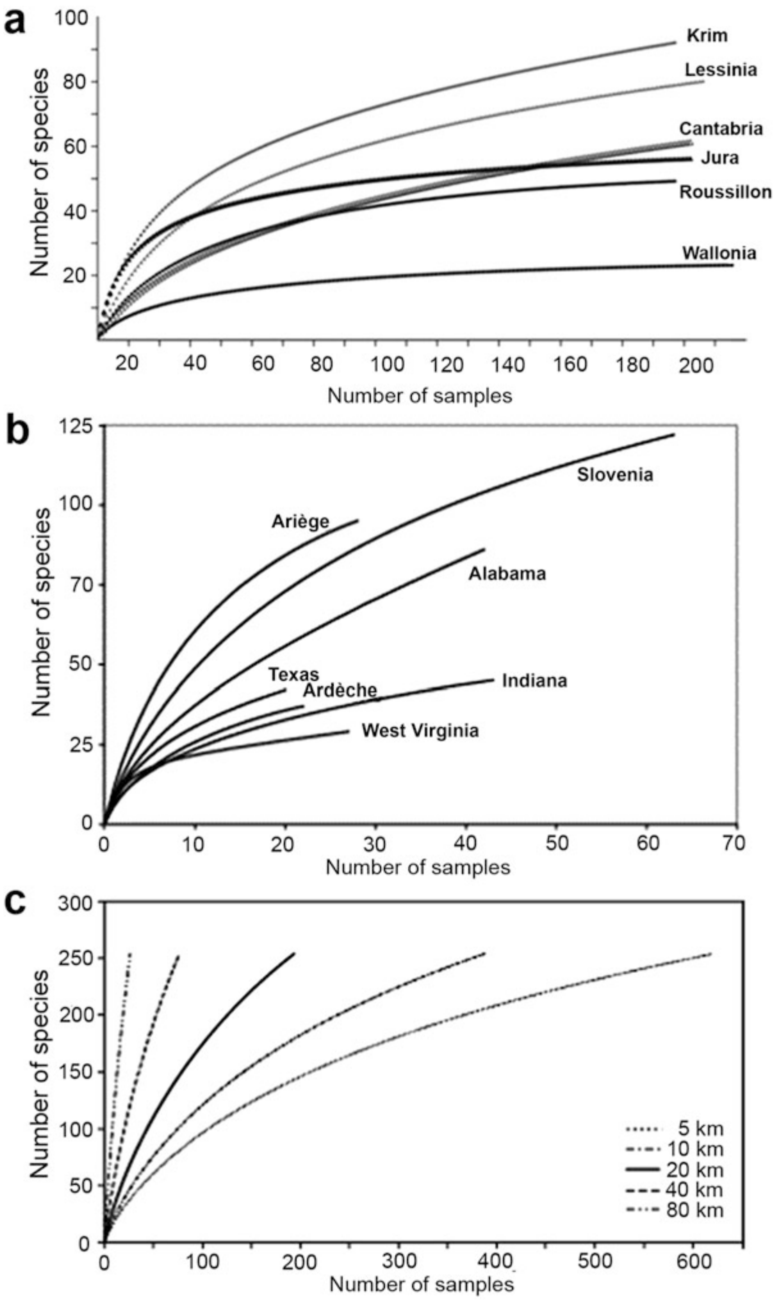

Fig. 9.8 Regional species accumulation curves for (**a**) groundwater species in six European regions (from Dole-Olivier et al. 2009), using sites as units; (**b**) terrestrial cave species in seven regions in Europe and the United States (from Culver et al. 2006), using 100 km^2 hexagons as units; and (**c**) subterranean beetles in Dinarides in the Balkans, using grid cells of different sizes (from Zagmajster et al. 2008)

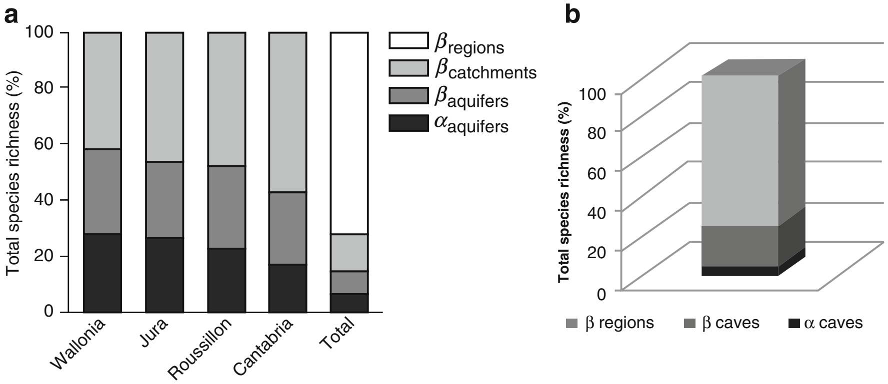

Fig. 9.9 Additive partitioning of groundwater crustacean diversity in (**a**) four European regions (from Malard et al. 2009) and (**b**) the eastern Alpine area, Italy (from Stoch and Galassi 2010); α = alpha diversity, β = beta diversity

9.5.2 *The Disproportionally High Contribution of Regional Diversity to Overall Richness*

Total diversity at any spatial scale can be additively partitioned into a within-sample component (alpha diversity, α) and a between-sample component (beta diversity, β) (Lande 1996). Additive partitioning can be extended across multiple hierarchical spatial levels to determine the contribution of each level to total richness (Crist et al. 2003). Additive partitioning was applied by Malard et al. (2009) to analyze hierarchical patterns of groundwater crustacean diversity in four European regions and by Stoch and Galassi (2010) to analyze the hierarchical patterns of groundwater crustaceans in the eastern Alpine area, Italy. In both datasets, the contribution of each spatial level to total species richness increased as its size increased (Fig. 9.9): $\beta_{regions} > \beta_{catchments} > \beta_{aquifers} > \alpha_{aquifers}$, for the four European regions and $\beta_{regions} > \beta_{caves} > \alpha_{caves}$ for the eastern Alpine area. However, beta diversity between regions made by far the highest contribution to total richness in both datasets (i.e., 71.8% for the four European regions and 75.2% for the eastern Alpine area). The disproportionally high contribution of beta diversity between regions to total richness implies that differences in community composition between similar habitats in different regions are typically more pronounced than between different habitats (i.e., karst and porous habitats) of the same region. From a conservation perspective, it implies that a high proportion of groundwater species in Europe can be protected by focusing conservation efforts on a few species-rich aquifers distributed in distinct regions (Michel et al. 2009).

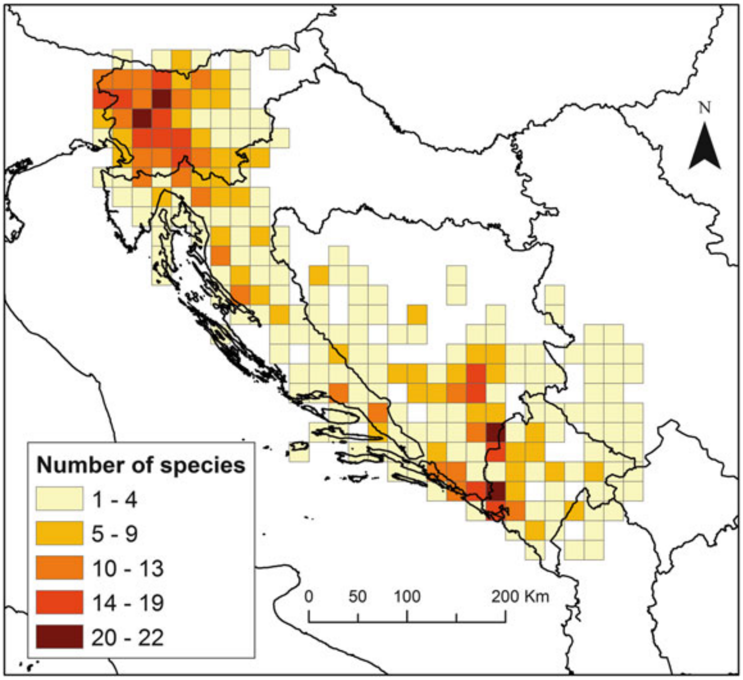

Fig. 9.10 Species richness of subterranean beetles in the Western Balkans, using grid cells of 20 × 20 km (From Bregović and Zagmajster (2016))

9.5.3 Hotspots Within Hotspots

When species are mapped onto a grid within regions, the pattern is not uniform—there are hotspots of species richness, as well as areas with low species richness. Even within generally high diverse region, areas of low species diversity can be recognized—as well as peaks in subterranean biodiversity (Fig. 9.10).

However, the number of species accounted for by the hotspots within any region represents a small proportion of the regional species pool. In the updated dataset on subterranean beetles of the Western Balkans, the highest number of species per 20 × 20 km cell reached only 5% of all species known in the study region (Bregović and Zagmajster 2016). The contribution of the species richness within hotspots in Tennessee was somewhat higher, 15–20% to the overall species richness of troglobionts (Niemiller and Zigler 2013).

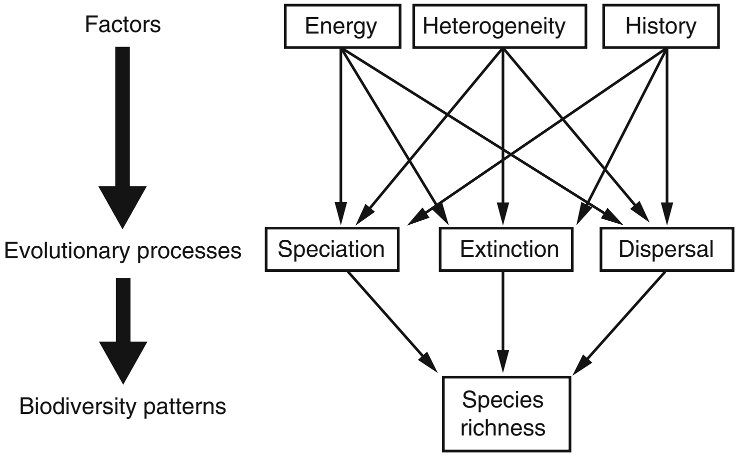

Fig. 9.11 Relationships between environmental factors, evolutionary processes, and resulting patterns of species richness

9.6 Explaining Patterns of Subterranean Biodiversity

A major goal in subterranean ecology is to understand the determinants of species richness at a range of spatial scales (herein, from global to regional scales). The recent availability of different environmental variables has increased immensely in recent decade, enabling complex modeling using statistical tools.

9.6.1 Environmental Factors: Energy, Spatial Heterogeneity, and History

The number of species in a region ultimately depends on three evolutionary processes—speciation, extinction, and dispersal (Wiens 2011; Fig. 9.11). Yet, a multitude of environmental factors act upon each of these three evolutionary processes. For the sake of clarity, environmental factors have been grouped into three broad classes of environmental factors: energy, spatial heterogeneity, and history (Field et al. 2009; Tisseuil et al. 2013; Fig. 9.11). This broad classification echoes the main hypotheses that were proposed in the macroecological literature to explain spatial variation of species richness at global to regional scales.

The energy hypothesis proposes that climate controls species richness by acting directly on the physiology of organisms (e.g., via temperature) or by acting indirectly on the amount of food resources available to them (e.g., via temperature and water availability) (Brown et al. 2004; Evans et al. 2005). Under the heading "energy" (Fig. 9.11), actual evapotranspiration (AET), temperature, and precipitation are often used in studies of surface ecosystems as surrogates of productive energy, ambient energy, and water availability, respectively (Evans et al. 2005).

Heterogeneity across space or time is associated with a number of hypotheses (Brown 1995; Bonn et al. 2004; Ohlemüller et al. 2008) which propose that environmental heterogeneity can either increase speciation through increased specialization, decrease dispersal by restricting the movement of individuals across space, or decrease extinction by promoting local survival. For subterranean taxa, number of caves, surface area of aquifers, surface area of karstic aquifers, and the karst connectivity index were used as surrogates for area (Christman and Culver 2001; Zagmajster et al. 2014; Bregović and Zagmajster 2016). Subterranean habitat diversity was calculated by Eme et al. (2015) at the scale of Europe using the areal proportion of 12 groundwater habitat types as defined in the groundwater habitat map of Europe (Cornu et al. 2013).

History refers to the long-lasting effect of past climatic and paleogeographic events on present-day pattern of species richness. A popular hypothesis states that species extinction has been more severe in regions that experienced large Quaternary climatic oscillations (Dynesius and Jansson 2000; Leprieur et al. 2011). However, the effect of history is intrinsically linked to dispersal constraints. In species showing low dispersal ability, the effect of extinction on present-day species richness is less likely to be compensated by subsequent dispersal phases (Foulquier et al. 2008; Hof et al. 2008). Under the heading "history" (Fig. 9.11), a commonly used surrogate of historical climatic variability is temperature anomaly, defined as the difference in mean annual temperature between the present and last glacial maximum. In regional studies of groundwater species richness, the distance from historical sea margins (e.g., late Cretaceous) is also used as a factor because present-day species richness may also reflect past colonization events of groundwater by marine species ancestors (Boutin and Coineau 2000; Culver et al. 2003). Speleogenesis, the process in which a cave was formed, was used as an explanatory variable in study of species richness of caves in the Iberian Peninsula (Jiménez-Valverde et al. 2017).

9.6.2 *Multi-causality and Spatial Non-stationarity*

The concept of multi-causality acknowledges that geographic variation in species richness is more likely shaped by multiple factors (Fig. 9.12). Eme et al. (2015) showed that productive energy, spatial heterogeneity, and historical climate variability were equally important in explaining geographic variation of groundwater crustacean species richness in Europe when the importance of each factor was tested separately (i.e., single-hypothesis testing). However, when the three factors were analyzed simultaneously (i.e., multiple-hypothesis testing), historical climate variability was only marginally important because its influence became hidden in the variation shared with productive energy and spatial heterogeneity (Eme et al. 2015).

The concept of spatial non-stationarity acknowledges that the relative contribution of factors may vary among regions (Fig. 9.12). Geographical variation in the

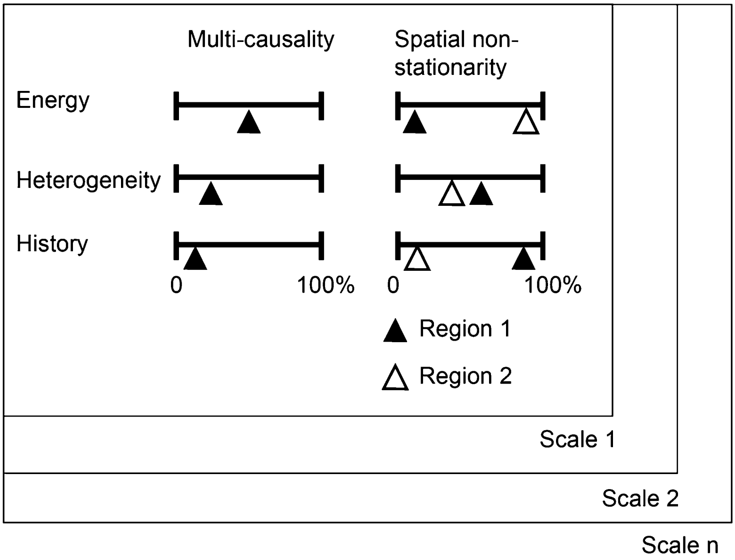

Fig. 9.12 Biodiversity patterns are shaped by multiple environmental factors (i.e., multi-causality), merged here into three broad classes of factors, but the relative influences of factors on biodiversity patterns vary among regions (i.e., spatial non-stationarity). The three boxes in the panel indicate that concepts of multi-causality and spatial non-stationarity can be applied at different scales

relative contribution of factors can be analyzed using local regressions models (Gouveia et al. 2013; Eme et al. 2015). And last, multi-causality and spatial non-stationarity in the determinants of species richness can be analyzed across a series of nested spatial scales.

9.6.3 The Importance of Historical Climate Variability

Historical climate variability has long been viewed by subterranean biogeographers as a primary factor driving both the pattern of species richness and the pattern of species range in the Palearctic (Hof et al. 2008; Stoch and Galassi 2010). Indeed, until the 2000s, the disproportionate extinction of narrow range species at higher latitudes during cold Pleistocene climate was put forward to explain both the pattern of decreasing species richness and the pattern of increasing range size with increasing latitude. Recent data and analyses by Culver et al. (2006), Zagmajster et al. (2014), and Eme et al. (2015) provided a broader perspective of subterranean biodiversity patterns. Although the latitudinal increase in range size north of the ridge of high species richness undoubtedly reflects the long-lasting effect of cold Pleistocene climate (Zagmajster et al. 2014), historical climate variability alone is a poor predictor of geographic variation in species richness (Eme et al. 2015, 2017). In Northern Europe, the long-lasting effect of cold Pleistocene climates adds up to the

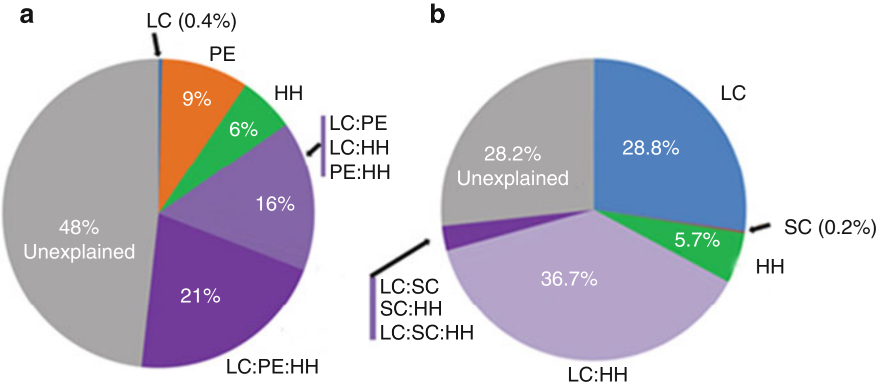

Fig. 9.13 Multi-causality in the determinants of species richness (**a**, data from Eme et al. 2015) and range size (**b**, data from Zagmajster et al. 2014) of groundwater crustaceans in Europe. Pie charts show the proportion of variance attributed to different environmental factors in the explanation of geographic variation of species richness and range size of groundwater crustaceans in Europe; *LC* long-term climate variability, *PE* productive energy, *HH* habitat heterogeneity, *SC* short-term climate variability. Colons denote shared variance between factors

effects of reduced spatial heterogeneity and lower productive energy to limit the number of species. In Southwestern Europe, the increasing aridity since the last glacial maximum potentially adds up to the lower productive energy to limit the number of species. Species richness peaks in regions of mid-latitude where the combined beneficial effects of a high productive energy and high habitat heterogeneity have not been counteracted by cold or arid historical events.

A study by Zagmajster et al. (2014) testing the contribution of the three broad classes of environmental factors to the range size pattern of European groundwater crustaceans found that historical climate variability, more especially the long-term variability of temperature rather than precipitation, had the highest independent contribution (i.e., 28.8%) to geographic variation in range size (Fig. 9.13). Historical climate variability also shared a substantial amount of variance with habitat heterogeneity (i.e., 36.7%), which by itself explained a relatively small proportion of variation in range size (i.e., 5.7%; Fig. 9.13). It has proven very difficult to tease apart the relative importance of temperature seasonality and the amplitude of long-term climate oscillations in shaping patterns of range size in other taxa, because the two factors covary in most environments (Veter et al. 2013). The study by Zagmajster et al. (2014) has shown that groundwater organisms offer very useful systems to resolve this issue because they lack strong thermal seasonality. Groundwater and its fauna is one of the systems where historical climate can be decoupled from present seasonality and its importance in forming large-scale range size patterns can be evaluated.

9.6.4 Productive Energy as a Driver of Species Richness at Large Scales

Low food supply has long been regarded as a major factor constraining subterranean life (Gibert and Deharveng 2002). The role of productive energy in controlling subterranean species richness has long been restricted to studies conducted at local spatial scales (Strayer et al. 1997; Datry et al. 2005; Foulquier et al. 2011). Culver et al. (2006) were the first to suggest that continental patterns of subterranean species richness might be driven by geographic variation in productive energy. They hypothesized that the mid-latitude ridge of high species richness for the terrestrial cave fauna in Europe and the United States (see Fig. 9.4) might correspond to regions where density of caves (habitat availability) was high and where productivity remained high over recent geological time. The productivity hypothesis was corroborated by Eme et al. (2015) for the groundwater crustacean fauna of Europe (see Fig. 9.5). They found that energy, herein AET, explained spatial variation in species richness better than spatial heterogeneity and history (Fig. 9.13).

Considering the inferred importance of productive energy at continental scale, the reason why tropical regions of high productive energy contain fewer groundwater crustacean species remains unclear. One hypothesis may be that a high food supply to groundwater may restrict the acquisition of troglobiotic traits and hence the diversification of obligate groundwater lineages arising from the colonization of groundwater by surface ancestors. On the other hand, cave fish tend to show the opposite pattern to groundwater invertebrate fauna, as they seem to be most diverse in tropical regions (Culver and Pipan 2009). As there are many other past and present factors that can influence diversification processes, this question remains open.

9.6.5 Explanations Vary Among Regions

Spatial non-stationarity in the importance of environmental factors was shown in the aforementioned continental study of Eme et al. (2015). These authors studied the contribution of several environmental factors to spatial variation in species richness at regional scale (regional meaning 10,000 km^2 squares), and regional differences in the relative contributions of factors became apparent. Notable differences in factors explaining species distributions among regions in Europe also differed (Gibert et al. 2009). In a study of factors that drive terrestrial cave biodiversity pattern in Dinarides in the Western Balkans, productive energy had very little effect on the observed species richness pattern of cave beetles within the region (Bregović and Zagmajster 2016) (Fig. 9.14). The highest proportion of variance was explained by habitat heterogeneity. Even though Dinaric region compared to other parts of the continent is the area with high surface productivity (Zagmajster et al. 2014; Eme et al. 2015), low variation in productive energy within the region contributed little to explaining the biodiversity pattern within the region (Bregović and Zagmajster 2016). In a study

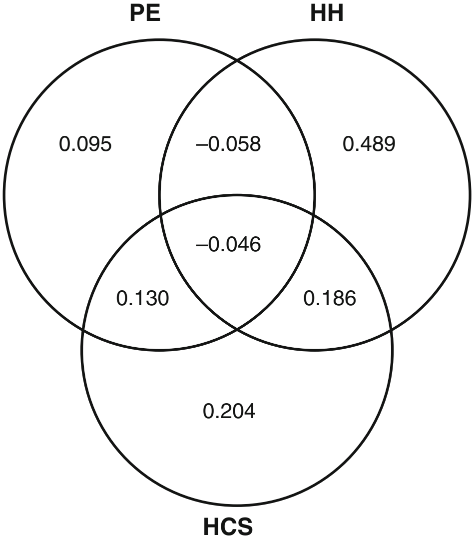

Fig. 9.14 Variance partitioning in nonspatial model of subterranean beetles in the Balkans; *PE* productive energy, *HH* habitat heterogeneity, *HCS* historical climate stability (From Bregović and Zagmajster (2016))

in Portugal, comparing individual caves, the presence of organic material in the cave and the origin of the cave were the most important variables in explaining terrestrial species richness (troglobionts and troglophiles combined) among caves (Jiménez-Valverde et al. 2017).

9.7 Subterranean Macroecology in the Molecular Era

Molecular methods have become a widely used tool also in subterranean biology, in studies of evolution and distribution of various taxa (see also Chap. 13). Due to high levels of convergence in many morphological characters, molecular approaches have turned out to be crucial in revealing hidden (cryptic) biodiversity. Detection of species-specific molecular sequences represents the base for studies of species distribution via environmental DNA approaches that are starting to be used in subterranean studies. Incorporating phylogenetic information represents a promising but not yet used approach in studies of evolutionary processes shaping subterranean biodiversity patterns.

9.7.1 Cryptic Species Do Not Challenge Biodiversity Determinants

Recent developments in molecular species delineation methods have enabled highly distinct genetic lineages to be identified within morphologically similar taxa. Those lineages represent putative species commonly described as “cryptic species” (Bickford et al. 2007). A review by Trontelj et al. (2009), based on 14 groundwater species, revealed that small distribution ranges among 51 cryptic species were the rule, while large range sizes above 200 km were extremely rare. However, a few phylogeographic studies also revealed that cryptic species have similar distribution range sizes as their corresponding morphologically described species (Lefébure et al. 2006; Eme et al. 2013, 2014).

Eme et al. (2017) conducted one of the first studies to test the effect of different species delimitation methods on the richness and range size patterns using data for groundwater crustaceans in Europe (Niphargidae and Aselloidea). Despite doubling the number of putative species when shifting from morphological to molecular methods, the spatial patterns of species richness were qualitatively similar among delimitation methods. All delimitation methods supported a ridge of high species richness between 42° and 46° N with a smaller peak between 49° and 51° N (Fig. 9.15). The number of species delimited by the different molecular methods increased linearly with the number of morphologically described species along the latitudinal gradient. These results supported the controversial view that cryptic species might be evenly distributed across large geographic regions (Pfenninger and Schwenk 2007; Trontelj and Fišer 2009; Vodă et al. 2015). For range size pattern, the shift from morphologically to molecularly delimited species led to a tenfold decrease in the average range size of species. However, the increase in median range size per cell with increasing latitude (i.e., the Rapoport rule) was typically more pronounced for molecularly delimited species (Fig. 9.15). The presence of widely distributed species at northern latitudes confirmed the differential fragmentation of species ranges between Southern and Northern Europe (Fig. 9.15). Furthermore, they supported the view that large-scale dispersal is an important driver of species range size in Northern Europe (Lefébure et al. 2006; Eme et al. 2013).

Interestingly, Eme et al. (2017) found that the relative proportions of variation in species richness explained by productive energy, historical climate, and spatial heterogeneity were very stable regardless of the species delimitation methods used, which is in line with the conclusions of Eme et al. (2015). For range size pattern, historical climate variability, more especially temperature anomaly, remained by far the most important predictor (as in Zagmajster et al. 2014). Although these results suggest that the presence of cryptic species may be evenly distributed along large geographical regions and environmental gradients, they do not provide any evidence that the mechanisms driving cryptic diversity are invariant across space and environmental gradients. The lack of morphological distinguishability between species may be due to recent divergence, niche conservatism, or morphological convergence (Trontelj et al. 2012; Meleg et al. 2013). These three mechanisms may be structured along space or environmental gradients in such a way that they compensate each other to produce the homogeneous distribution of cryptic species as described by Eme et al. (2017).

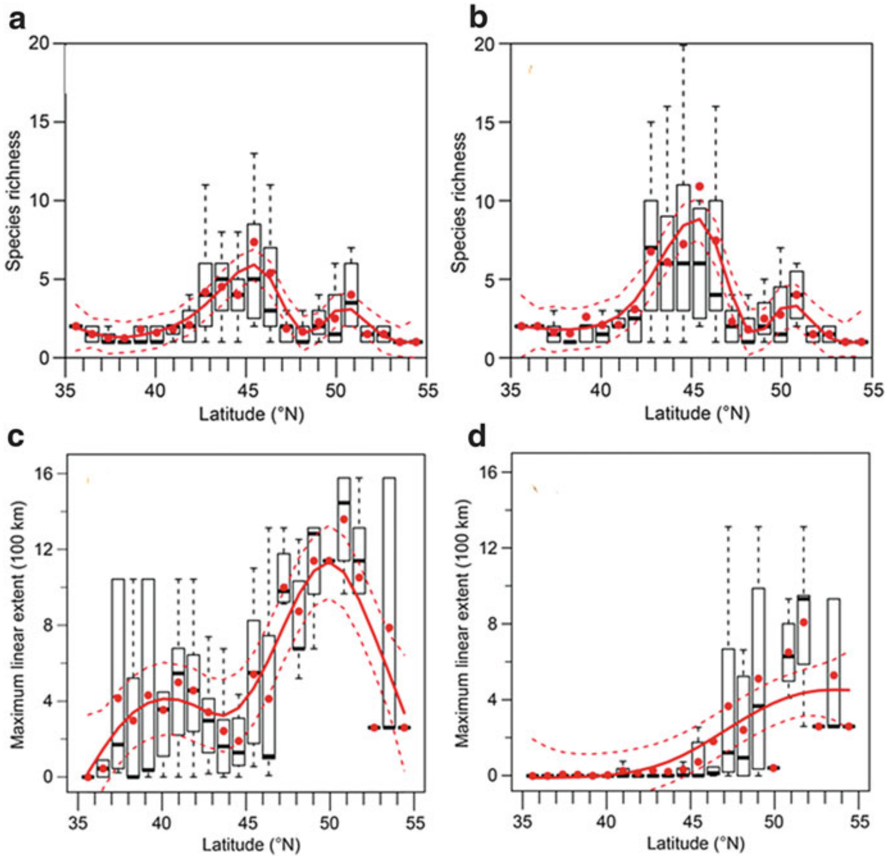

Fig. 9.15 Relationships between species richness per latitudinal band and latitude for the morphologically (**a**) and molecularly delimited (**b**) species and relationships between median range size (maximum linear extent) per latitudinal band and latitude for both sets of morphologically (**c**) and molecularly delimited (**d**) species. In all panels, black horizontal bars, red dots, and boxes show the median, average, and interquartile range, respectively, for 0.9° latitudinal bands. The maximum length of each whisker is up to 1.5 times the interquartile range. Continuous red lines represent the fit of a generalized additive model to the averages of latitudinal bands, and dashed red lines show 95% confidence intervals (From Eme et al. (2017))

9.7.2 Environmental DNA Can Speed Up Species Inventory

Detecting environmental DNA (eDNA) is a noninvasive sampling procedure that relies on the presence of DNA in the environment to detect species' presence (Ficetola et al. 2008; Bohmann et al. 2014). It has started to be successfully applied also to detecting subterranean species. The pioneering use of eDNA for subterranean species detection was the work of Gorički et al. (2017) on the olm *Proteus anguinus*, distributed in caves of Dinaric karst in Southeastern Europe. The eDNA approach proved to be successful in detection of the presence of two stygobiotic amphipod species (*Stygobromus tenuis potomacus*, *S. hayi*) in shallow subsurface habitats in the vicinity of Washington, DC (USA; Niemiller et al. 2017). The potential of eDNA analyses in biodiversity sampling in subterranean habitats is enormous because of the rarity of many subterranean species and the difficulty in accessing subterranean habitats.

9.7.3 Making Use of Phylogeny to Foster Our Understanding of Biodiversity Patterns

In continental-scale studies of subterranean biodiversity patterns, the inferences about the relative role of evolutionary processes remain extremely limited, which is mostly due to considering all species as equivalent evolutionary and functional entities. Phylogenetic diversity metrics which integrate the evolutionary relationships among species hold much promise to disentangle the relative influence of different evolutionary processes (Tucker et al. 2017). Geographic variation of alpha phylogenetic diversity can provide insight into the relative contribution of speciation and extinction (see Davies et al. 2007 for further details). Species-rich areas associated with a low phylogenetic diversity (closely related species) reflect a process of recent and rapid speciation in situ. These areas are commonly referred to as "cradles of diversity" (Jablonski et al. 2006). For a given species richness, an increase in phylogenetic diversity (species with long independent evolutionary histories) suggests a decrease in the extinction rate occurring in regions often qualified as "museums" because they preserve diversity through time (Jablonski et al. 2006). It is not yet clear which of the two hypotheses better support the ridge of high species richness in groundwater crustaceans (Zagmajster et al. 2014; Eme et al. 2015, 2017).

Phylogenetic diversity metrics can also be used to tease apart the role of dispersal in shaping biodiversity patterns. Some regions—which we refer to as "Noah's Arks"—can be characterized by high species richness and high phylogenetic diversity, both because they preserve lineages through time and because they receive phylogenetically distant migrants from neighboring regions. Recent decomposition of phylobetadiversity indices offered the possibility to tease apart the effects of spatial turnover and gradient of phylogenetic diversity caused by differences in species richness among communities (Baselga 2010, 2012; Leprieur et al. 2012). For a given species richness, the spatial turnover of phylogenetic diversity is expected to be higher in a museum than in a Noah's Ark. Phylobetadiversity indices may thus be used to test whether the ridge of high subterranean species richness might also have served as refugia during glacial periods and/or as a source of colonizers during interglacials.

Developing a phylogenetic framework of subterranean communities, which combines the use of both alpha and beta phylodiversity metrics, can provide new insights into the links between environmental factors, evolutionary processes, and biodiversity patterns (see Fig. 9.12). The development of georeferenced molecular datasets over large spatial scale as proposed by Eme et al. (2017) and the ever-increasing availability of robust phylogenetic trees for a number of subterranean taxa offer an exciting perspective to apply this macro-phylogenetic framework.

9.8 Conclusions

Sampling difficulties, numerical rarity, and high levels of endemism make the analysis of geographic patterns of subterranean species especially difficult. Nevertheless, there are now georeferenced datasets with thousands of individual records from hundreds of sites that make it possible to describe and begin to explain global, continental, and regional patterns. For all countries and all regions, there remain many undescribed and undiscovered species, and this makes comparisons difficult. But, even incomplete datasets can be used for analyses, if critically evaluated and properly compared. Different statistical procedures can be applied to take into consideration differences in sampling effort as well as spatial properties of the different datasets being compared.

There are some general conclusions that can be briefly summarized at each spatial scale. At the global scale, there is a consistent pattern of highest species richness in north temperate regions of Europe and North America, as well as Australia. At the continental scale, the pattern profoundly depends on the size of the grain used for analysis. Using a coarse grain, like countries or large biogeographical regions, groundwater diversity in Europe declines with increasing latitude, but a ridge of high species richness becomes apparent between 42 and 46° N when using a finer grain. The contribution of beta diversity to total diversity increases with spatial scale, but beta diversity between regions disproportionally contributes to total diversity. Patterns within regions are not uniform, but hotspots represent only a small proportion of the regional species pool.

A number of environmental factors, lumped into energy, spatial heterogeneity, and history, affect species richness. While productivity, spatial heterogeneity, and severity of the Pleistocene climate are all important, productivity is the most important at least in explaining the European subterranean ridge of species richness. However, the spatial non-stationarity of explanations really predominates because the contribution of different factors appears to vary substantially among regions. Although severity of the Pleistocene climate alone is a poor predictor of subterranean species richness pattern at the whole of Europe scale, it exerts a major control on the pattern on increasing range size with increasing latitude in Europe (i.e., the Rapoport rule). Once again, scale of analysis is important. For example, the importance of productivity in shaping patterns of species richness disappears in smaller areas such as the Balkans. The inclusion of cryptic species appears not to change overall geographic patterns or their explanations. It does of course greatly increase the number of species. A more thoroughly phylogenetic approach is likely to produce greater clarity with regard to the geographic patterns of subterranean biodiversity as well as for their explanations.

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Chapter 10
Historical and Ecological Factors Determining Cave Diversity

Ignacio Ribera, Alexandra Cieslak, Arnaud Faille, and Javier Fresneda

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 epigean 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 epigean 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 epigean relative. In these cases, an independent origin would require first a parallel evolution of their morphology and second the extinction of all epigean 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 epigean 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

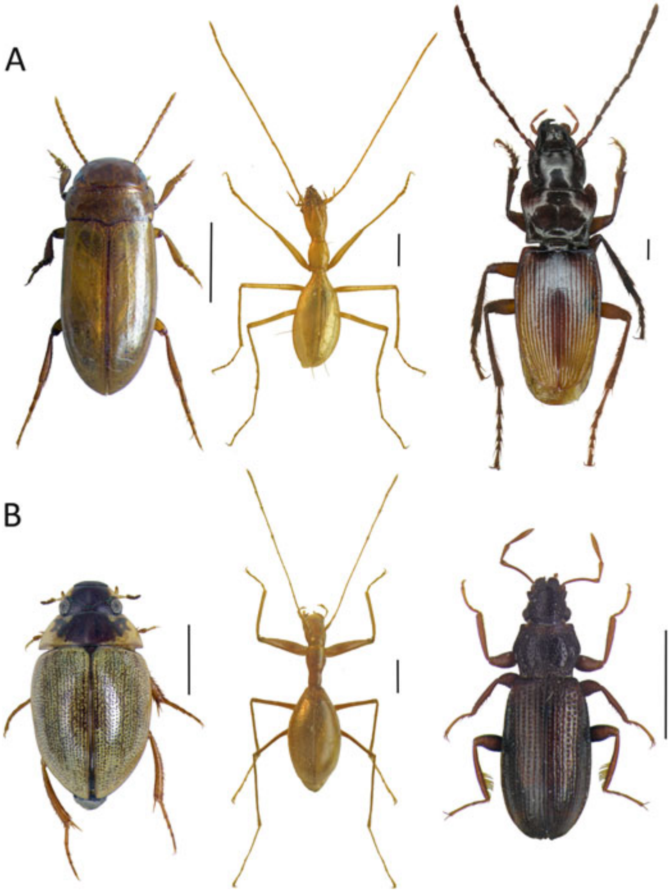

Fig. 10.1 Convergence in body shape in species typical of the deep subterranean environment. (**a**) Species of suborder Adephaga, from left to right *Graptodytes eremitus* (Dytiscidae) from the High Atlas of Morocco, *Aphaenops pluto* (Carabidae, Trechinae) from the French Pyrenees and *Oscadytes rovirai* (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. rovirai* 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 epigean 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 epigean 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 epigean phenotype, and in particular to recover pigmentation, when exposed to light for long periods (e.g. in *Proteus*, Dubois 1892). Individuals of epigean 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 epigean 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 epigean 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 epigean 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 coelacanths (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; Esmaeili-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 epigean, 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 epigean 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 epigean 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 epigean ones, there is always the possibility that the epigean 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 epigean 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 troglomorphism 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 epigean, 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 epigean 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 mould, 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 epigean 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).

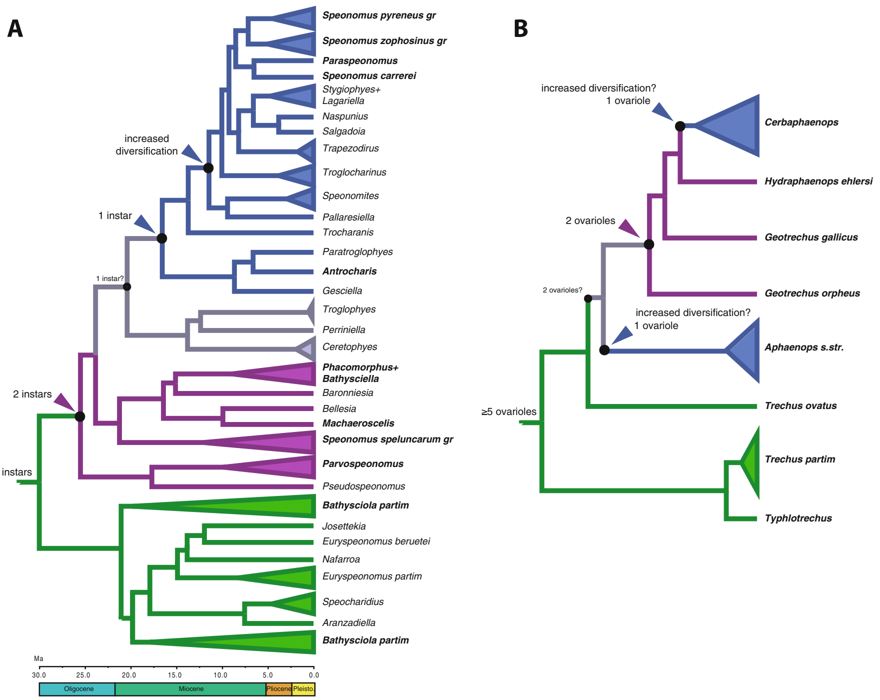

Fig. 10.2 Schematic phylogeny of the Pyrenean clades of (**a**) Leptodirini and (**b**) Trechini, showing the reconstructed transitions from the ancestral life cycle (3 instar larvae in Leptodirini, ≥5 ovarioles in Trechini) to an intermediate cycle (2 instars in Leptodirini, 2 ovarioles in Trechini) 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 Troglomorphism

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 troglomorphism, 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 epigean 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 troglomorphism 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 troglomorphism, 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 troglomorphism were able to expand their ranges and colonised new isolated subterranean units by epigean 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

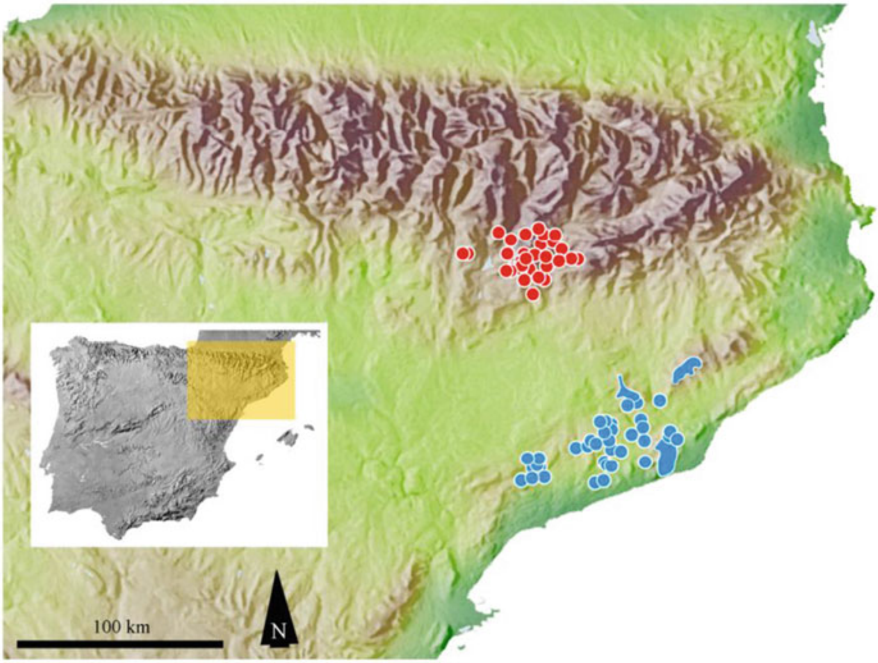

Fig. 10.3 Distribution of the species of the genus *Troglocharinus*. In red, species occupying the ancestral range of the lineage, in the Spanish Pyrenees. In blue, species in the Catalonian coastal ranges (see Rizzo et al. 2013 for details). The shortest distance between them, ca. 60 km, crosses Miocene 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, *Stygiophyes 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 epigean groups, such as some Diptera (Andersen et al. 2016), Hymenoptera (Juberthie and Decu 1998) or even Plecoptera (López-Ródriguez 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 epigean 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 ecologic, 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

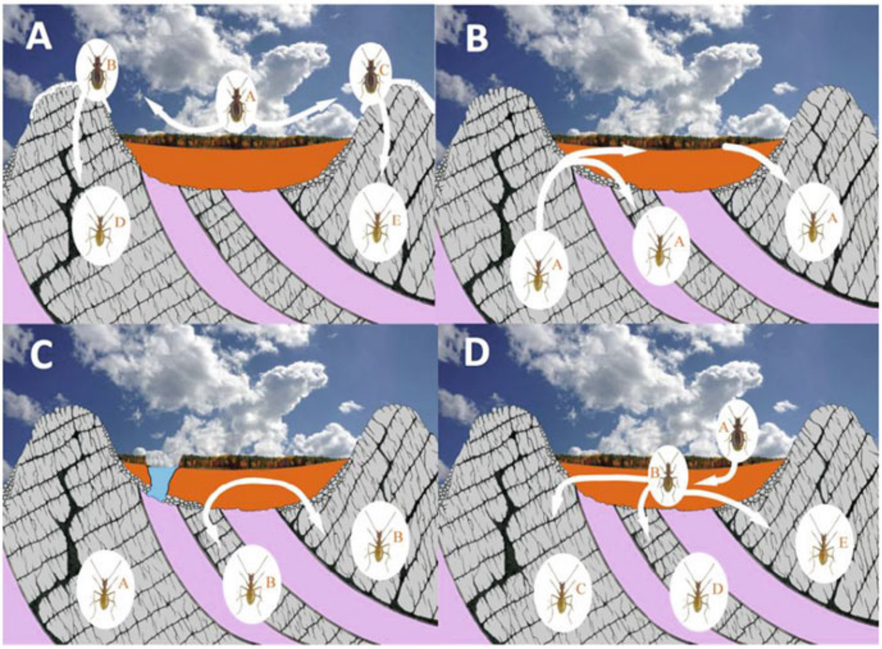

Fig. 10.5 Schematic representation of some among many possible modes of colonisation, range expansion and diversification within the deep subterranean environment (DSE). (**a**) In alpine conditions, epigean species may be forced to seek refuge in superficial subterranean environments (MSS), colonising 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 epigean 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 epigean 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

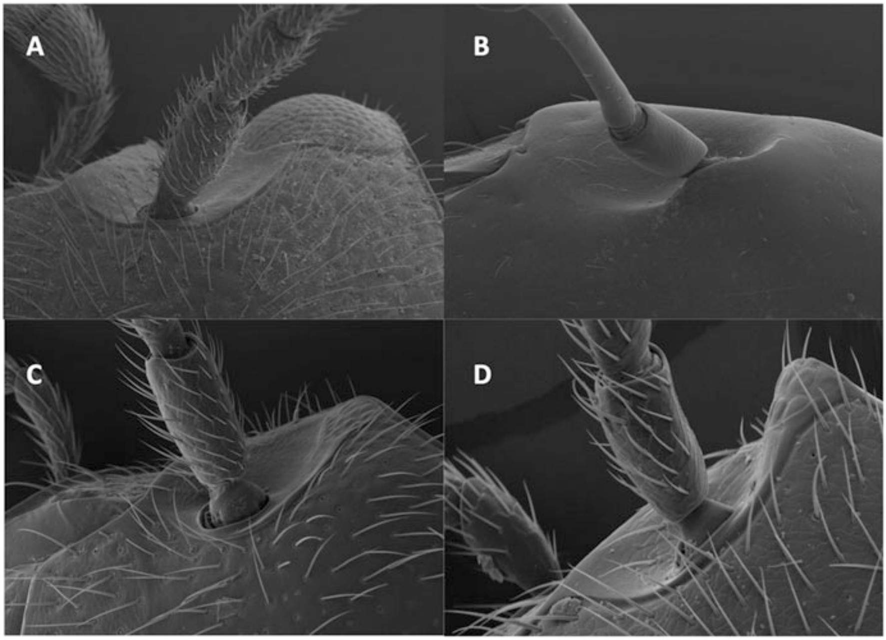

Fig. 10.6 Convergence in the eye and chaetotaxy reduction in some Cholevinae. (**a**) *Speonemadus bolivari* (Anemadini); (**b**) *Leptodirus hochenwartii* (Leptodirini); (**c**) *Anemadus smetanai* (Anemadini); (**d**) *Notidocharis calabrezi* (Leptodirini). C and D have independently developed reduced 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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Part III
About Communities, Populations and Food

Chapter 11
Cave Communities and Species Interactions

Stefano Mammola and Marco Isaia

11.1 Cave Communities

In ecology, a community—or also a biocoenosis—is defined as an assemblage of different species living in a particular space and time. Entering a cave and moving toward the inner sections, the abundance and the diversity of organisms progressively decrease, going from the highly populated and diverse fauna crawling in the twilight zone to the energy-deprived and poorly populated habitats of the deeper parts (see also Chap. 3). The explanation for the high abundance and diversity of organisms dwelling at the cave entrance has to be generally referred to the fact that the entrance is an input site of potential food materials (Poulson and White 1969; see also Chap. 12). Moreover, an array of organisms exploits this sheltered habitat for a variety of functions—trophic purposes, reproduction, etc.—thus increasing local diversity. While some subterranean specialized elements from the superficial subterranean habitat (SSH) fauna may be present (Novak et al. 2012), the communities of the twilight zone are usually dominated by species exhibiting a poor degree of adaptation to subterranean life. In contrast, true troglobionts dominate in the deep subterranean domain, where strong selective pressures promote a highly specialized community and, at the same time, limit diversity and abundance (Fig. 11.1).

It is, however, an open question whether species within a cave are interdependent on each other for keeping balance and stability of the system—recalling Clement's (1916) holistic view—or whether the community is primarily defined by the environmental conditions following the individualistic concept of Gleason (1926). While both concepts may apply to cave communities in different circumstances, neither of them has been demonstrated to apply entirely. In a third, controversial view proposed by Hubbell (2001), differences between members of an ecological community

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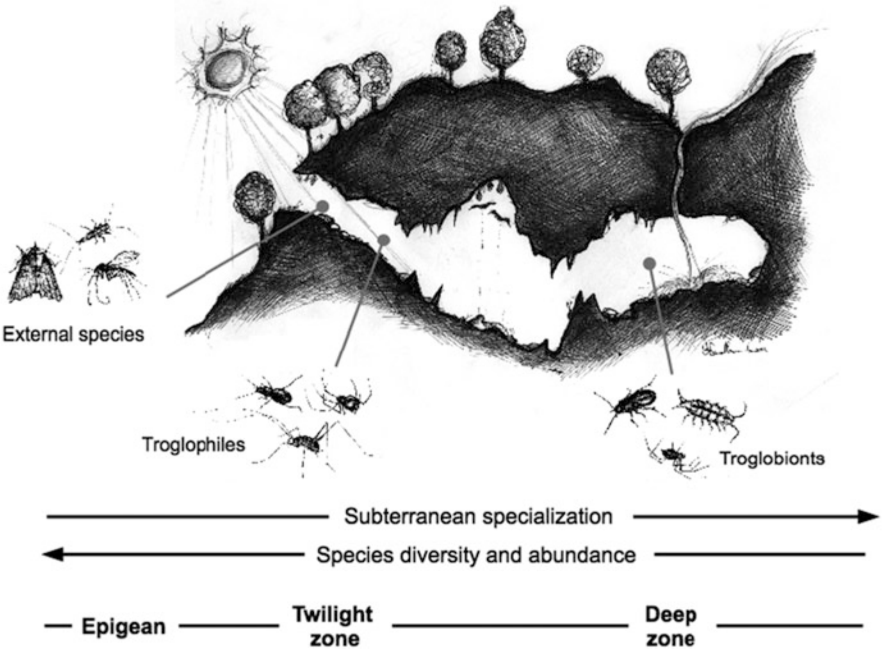

Fig. 11.1 The classic representation of cave communities. In the twilight zone, species exhibiting a poor degree of subterranean adaptation dominate. In contrast, true troglobionts are preferentially found in the deep zone, where high selective pressures promote highly specialized communities and, at the same time, limit diversity and abundance (original illustration by Stefano Mammola)

of trophically similar species are "neutral" or irrelevant to their success. The neutral view has been criticized because it requires all species to be equivalent and in constant environmental equilibrium, which is a rare condition in nature, where climatic and geographical conditions change too frequently for this to be attained (Ricklefs 2006). The existence of semi-stable conditions would make cave ecosystems ideal candidates for testing this theory, although no direct support for this concept has ever been demonstrated in cave communities to date.

Support for the organismic view of the cave community is offered by authors who postulate the existence of so-called species associations in caves, which form discrete units. One of the first authors stressing this concept was Jeannel (1926), who focused on spatial variations in the community from the cave floor, walls, and ceilings. In particular, he noticed how the animal community thriving on wall surfaces—that he termed *parietal association*—differed greatly from the one dwelling on the cave floors. Pushing this concept further, Bourne (1976) described local variations of parietal associations according to different wall morphologies, vertical height, and distances from the cave floor. Recently, Moseley (2009) retrieved this idea, identifying and defining different associations of species within the cave microhabitats, such as the parietal association, the pool surface association, the guano pile association, etc.

On the other hand, the occurrence of a transition between the communities of the twilight and the dark zones, characterized by species commuting to the surface—Poulson and White's (1969) "middle zone"—supports an individualistic rather than a holistic view of cave communities. Under this perspective, transitions in cave communities are gradual and not easily defined, and the relations between species are mostly the results of similar requirements and tolerances.

11.2 Diversity of Cave Communities

The number of species within a cave is likely to depend on the carrying capacity of the system and the biogeographic context of the area under consideration. Furthermore, being strongly related to external inputs—as well as to the extent of the system of rock fissures in which the cave develops (Culver and Pipan 2014; Mammola et al. 2016a)—estimations of species richness can be ambiguous. However, as a rule of thumb, species richness in caves is orders of magnitude lower than in neighboring epigean ecosystems. Caves have indeed low energy inputs and are subjected to strong environmental filters, which pose a challenge to the adaptation of organisms (see also Chaps. 3, 4, and 10).

Culver and Sket (2000) published a very influential contribution, in which they documented species richness in 20 hot spots of subterranean biodiversity across the world, hosting 20 or more stygobionts and troglobionts. Later, more sites were added to this list (Culver and Pipan 2009), including a few subterranean hot spots in the tropics (Souza-Silva and Ferreira 2016; Trajano et al. 2016). The high richness in these caves—up to 84 troglobiont species in the case of the Postojna-Planina Cave in Slovenia—possibly represents the maximum numbers of obligate species comprising a hypogean community. Moreover, the number of species almost doubles when considering troglophile and occasional species interacting with the deep hypogean assemblage of obligate cave species. In many circumstances, occasional species have indeed been reported far inside the cave entrance, testifying to the potential exchanges between epigean and hypogean communities. An emblematic case is the occurrence of troglophile flies *Trichocera maculipennis* (Diptera, Trichoceridae) in the deepest sections (–2140 m) of the deepest abyss in the world (Krubera-Voronja Cave, Western Caucasus) (Sendra and Reboleira 2012).

However, compared to epigean ecosystems, the average number of species in a cave community is generally low, especially when considering intra-guild diversity. For example, the coexistence of multiple troglobiont spiders in the same cave is rare and often mediated by niche partitioning (Mammola and Isaia 2017 and unpublished data). Typically, deep cave communities host a single troglobiont spider or rarely two or three species. As far as we are aware, the maximum number of troglobiont spiders documented from a single cave in the temperate zone is four (Balićeva špilja, Croatia; Martina Pavlek, personal communication) and two in the case of the tropical regions (Bloom et al. 2014).

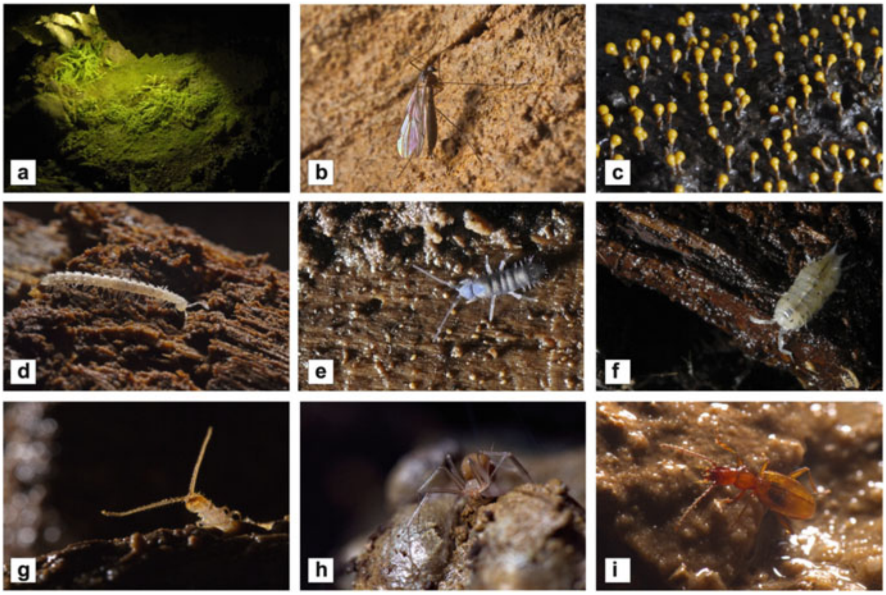

Fig. 11.2 Members of cave communities belong to different trophic and functional groups. (**a**) Phototrophic organisms may be present in the twilight zone or in areas artificially illuminated; (**b**) occasional external elements such as winged insects can colonize the cave entrance and neighboring areas; (**c**) fungi represent the base of the typical detritus-based food chain of the cave environment; (**d**–**f**) detritivorous and microbivores, such as diplopods, collembolans, and isopods, generally dominate the arthropod communities in caves; (**g**–**i**) predators such as chilopods, spiders, and carabid beetles occupy the apical positions in the food chain. All photos by courtesy of © Francesco Tomasinelli

11.3 Functional Composition of the Community

Because of the considerable hindrance of the low flux of energy in cave ecosystems (see also Chaps. 13 and 14), caves generally host simplified communities, less diverse and less functionally redundant than their epigean counterparts (Fig. 11.2). Deprived of primary photosynthesizing producers, herbivores are therefore absent in subterranean habitats, cave root-feeders being the exception (Howarth 1983). Accordingly, subterranean communities are considered to be “disharmonic” (Gibert and Deharveng 2002). A complication to this general pattern is provided by microbivores organisms, small arthropods which are able to ingest bacteria or fungi—microfloras—but exclude detritus from their guts. However, the larger the animal, the less able it is to distinguish between microflora as food and the plant or animal detritus on which these are growing. In fact, the majority of the detritivorous animals involved in the decomposition of dead organic matter are generalist consumers of both the detritus itself and the associated microfloral populations.

Furthermore, there is evidence that in energy-deprived ecosystems such as most of the cave/subterranean ones, obligate predators may decrease their disappearance being due to lack of prey, as well as secondary evolutionary shifts toward polyphagy (Gibert and Deharveng 2002). For example, in the troglobiont beetle *Aphaenops*, the mouthparts are highly adapted for predation, to feed on dead plant matter and to ingest earth to extract nutrients (Gers 1995).

It can be argued that the richness and the abundance of detritivores in a cave act as regulating factor for the entire cave community through a bottom-up control mechanism—strongly dependent on external energy inputs—which, in turn, limit the abundance and the diversity of predators. As in other detritus-based food chains, the most important interspecific interactions are competition—among decomposers and among predators—and predation. However, variations to these general patterns are expected in relation to the type and nature of the trophic inputs. It is self-evident, for instance, that guanobionts—organisms feeding on bat guano—will be present only if there are bat colonies within the cave and that specific parasites and parasitoids will be part of the community only in presence of their hosts.

Primary producers and true herbivores may be also locally present in cave entrances. Phototrophic organisms generally occur at the entrance zone, where they may support small groups of true herbivores. Primary producers exploiting different sources of energy such as chemolithotrophic bacteria (e.g., Northup and Lavoie 2001)—especially ferrobacteria, sulphobacteria, and nitrificant bacteria—may be also present in caves.

Another exception to the general pattern can be found in caves that are show caves, where primary production dramatically increases because of the installation of artificial lighting systems (Albertano 2012; see also Chap. 6).

11.4 Turnover in Space

Beta-diversity or species turnover is the extent of change in community composition along a gradient (Whittaker 1960). At the regional scale, the species turnover is the species loss/replacement from site to site—also referred to as nestedness. Most of the studies focusing on species turnover in caves have focused on single taxonomic groups (e.g., Malard et al. 2009; Stoch and Galassi 2010; Cardoso 2012; Zagmajster et al. 2014) and not on subterranean communities as a whole. Considering that most subterranean species exhibit restricted ranges, we may expect that even at a small geographical scale, caves should display higher levels of beta-diversity in comparison to most epigean ecosystems. In other words, it is theoretically expected that communities in caves relatively close to each other should exhibit faunal differences. The high level of endemism of most subterranean species seems to confirm this hypothesis in most cases (Sharratt et al. 2000; Christman et al. 2005).

More throughout considerations can be drawn when considering the spatial community turnover at the local scale—e.g., along a certain gradient within a single cave. It is generally observed that in a typical cave, there is a gradient of subterranean specialization of the biological community from the entrance zone toward the deepest sectors (see, e.g., Tobin et al. 2013; Fig. 11.1)—or, more generally, from shallow toward deep subterranean spaces (see Mammola et al. 2016a). Nevertheless, this perspective does not take into account the connections that exist between the habitats accessible to humans—the caves themselves—and the habitats that are not directly accessible (e.g., the SSHs; Culver and Pipan 2014).

Accordingly, the existence of a linear turnover in the community with the distance from the entrance has been recently questioned by Novak et al. (2012). By studying cave communities in Slovenia, they observed and characterized two distinct communities, one dwelling close to the surface (0–10 m depth) that is characterized by non-troglobionts and a major group of troglobionts belonging to the SSH fauna and another consisting of a minor group of troglobionts, dwelling in the deeper parts of the caves. Such spatial separation in the community directly implies sharp variations in the beta-diversity from the surface toward the subsurface (i.e., a bimodal distribution), rather than a constant variation along the cave gradient (i.e., a linear distribution). Although the generality of this pattern remains to be proven, other authors have reached similar conclusions. For instance, in their studies on ecotones, Prous and colleagues noticed how the animal community in the twilight zone presents higher diversity in respect to the neighboring epigean and deep hypogean habitats, with sharp decreases in both directions (Prous et al. 2004, 2015; see also Chap. 3). In particular, they interpreted the entrance zone as an ecotonal area harboring an enriched community, capable of exploiting its unique and intermediate characteristics (Fig. 11.3).

11.5 Turnover in Time

There is a very old paradigm in cave-based science that subterranean communities are temporally stable. However, it is nowadays well acknowledged that subterranean habitats are characterized by a constant flux of “invaders” (Culver and Pipan 2009) and that there is a temporal variability in the distribution of invertebrates found in caves (Delay 1969; Tobin et al. 2013). For instance, subterranean invertebrates move actively in search of food in the larger chambers and move back to the more stable fissures in response to physiological stress—e.g., variations in relative humidity. Moreover, external species enter the subterranean habitat for a number of specific purposes, e.g., day/night refuge and seasonal refuge. As a consequence, in a typical cave community, a significant temporal turnover in the species composition is observed, particularly near the surface (Bento et al. 2016).

Twilight zones are also frequently colonized by external elements—e.g., winged epigean arthropods—which cause greater fluctuations in species composition over the year. This is particularly true in the case of epigean species that are linked to cave habitat in a certain period of their biological cycle. For instance, lepidopterans such as *Scoliopteryx libatrix* (Erebidae) and *Triphosa* spp. (Geometridae) hibernate in European caves in winter, whereas *Vanessa* spp. (Nymphalidae) enter caves in summer, possibly to avoid extreme temperature (Mammola S., personal observations). Similarly, epigean harvestmen and crickets commonly overwinter in

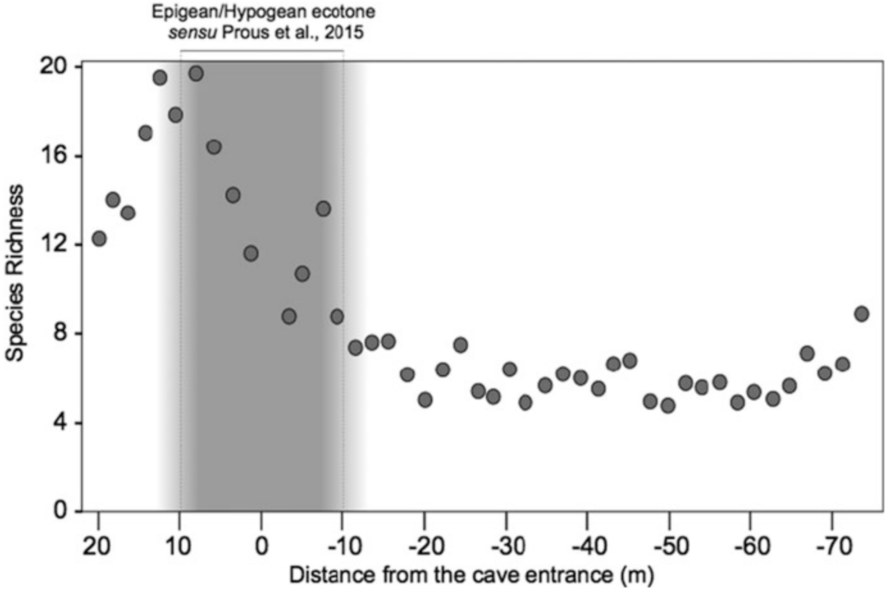

Fig. 11.3 The epigean/hypogean ecotone: in a recent study on the community of a Brazilian limestone cave (Lapa do Mosquito), Prous et al. (2015) sampled the animal biocoenosis from the exterior of the cave toward the dark zone—up to 70 m inside. By this they identified a transition area of approximately 20 m (10 m inside the cave and 10 m outside) acting as an epigean/hypogean ecotone and hosting 55 species. Out of these species, 49 were shared with the epigean community, 37 with the hypogean community, and 14 species shared by the 3 communities. Modified from Prous et al. (2015)

gregarious formations on cave walls and ceiling in the twilight zone, both in temperate (Kozel et al. 2015; Lipovšek et al. 2016) and subtropical areas (Chelini et al. 2011). Variations in the community may also be detected within day-night cycles, especially with respect to nocturnal species taking shelter in caves during the day. Classic examples in this sense are bats, but other organisms such as nocturnal lepidopterans can display a similar behavior.

Although less documented, temporal turnover may also occur in communities occupying the deeper sections of caves. Crouau-Roy et al. (1992) observed how the abundance of the subterranean beetle *Speonomus hydrophilus* (Leiodidae, Leptodirini) underwent a significant decline both in winter and summer, in relation to temperature variations. They argued that this species was able to take shelter in the network of fissures when the climatic conditions were not favorable. Chapman (1985) observed a similar pattern in the Hawaiian Islands in certain highly vagile specialized species, which were able to appear and disappear rapidly in the cave, visiting the larger chamber from the adjacent labyrinth of voids and vice versa. In a similar way, Mammola et al. (2015) documented the occurrence in troglophile beetles of local seasonal migrations from the cave entrance toward the inner parts of the cave in response to mean daily temperature and humidity drops. Such evidence directly implies that, even in the most stable sections of caves, the composition of the animal community cannot be considered stable, and the study of cave communities should preferably incorporate a temporal perspective.

11.6 Ecological Niche and Interspecific Competition

The biological structure of a community is defined by its composition of species, namely, their number and relative abundance. However, to be part of a community, individuals of a certain species have to maximize their survival in the given environmental conditions characterizing the habitat in which the community develops. The probability of surviving in certain conditions is the consequence of an array of physiological, ethological, and morphological adaptations, which from one side may allow the survival of a certain species but from the other may preclude survival of another.

We define the ecological niche of a species as the array of adaptations determining its success in terms of survival, growth, and reproduction in a certain habitat. Therefore, along environmental gradients, species may exhibit distinctive bell-shaped abundance patterns, which reflect their differential survival probability as the environmental conditions change. In caves, for example, troglobionts exhibit an array of characters, which make them more competitive—and more abundant—in total absence of light and in energy-deprived habitats. Conversely, troglophiles tend to be more abundant in the twilight zone, either because their adaptations are efficiently exploited or because they are poorly suited to the darkest parts of the cave.

In one of the most influential contributions on subterranean biology, Poulson and White (1969) argued that microclimate, habitat structure, and food availability are the primary and secondary factors which determine subterranean biodiversity, rather than competition- and niche-based processes. Remarkably, however, a few years later, Poulson himself (1977) demonstrated the existence of spatial niche partitioning in two linyphiid spider species—*Phanetta subterranea* and *Anthrobia monmouthia*—in North American caves. He documented how the two species were able to exploit distinct spatial niches, in order to avoid competition for food resources. Parallel observations by Culver (1973, 1975) led to similar results, underlining that competition in caves was indeed more common and important than previously thought.

In line with these early observations, recent studies unraveled that niche-based processes operating in cave/subterranean ecosystems are more the rule than the exception (e.g., Fišer et al. 2012; Mammola et al. 2016b; Resende and Bichuette 2016). Accordingly, Culver and Pipan (2015) included competition among the three most important factors of the selection pressures existing in the subterranean environment. They argued that (p. 442): "[. . .] Interspecific competition, a divergent selective force, is important in shaping morphology when competing species are present."

Commonly, ecological niches and species interactions are represented as normal functions of the abundance a species—or its probability density—along a certain

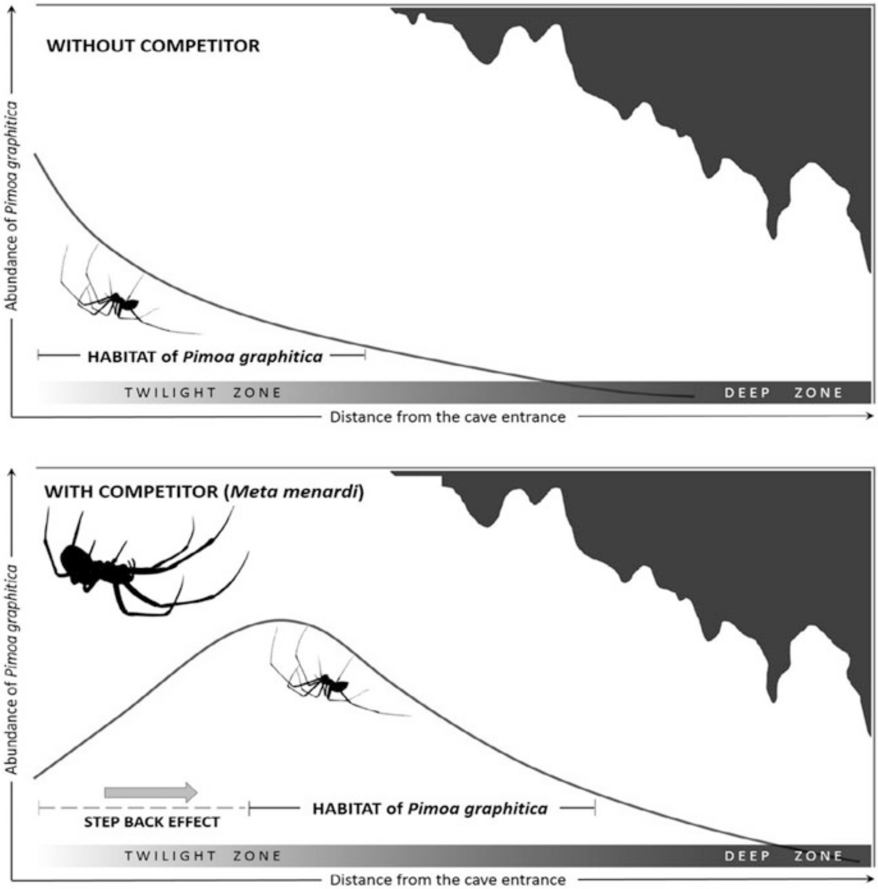

Fig. 11.4 An example of species interaction in a cave: the abundance of a competitor affects the distribution pattern of other species of the cave community. In this example, the abundance of the spider *Pimoa graphitica* (Pimoidae) is highest in the vicinity of the entrance, where trophic resources are more abundant (top panel). As the abundance of the competitor *Meta menardi* (Tetragnathidae) increases (bottom panel), *P. graphitica* is forced to "step back," rearranging its niche deeper inside the cave, a suboptimal habitat in terms of trophic availability. Modified from Mammola et al. (2016b)

gradient. For instance, in a study in caves in the Alps focusing on top predators in the twilight zone, it has been shown that niche overlaps lead to strong species interactions, which determine the rearrangement of the niche of the less competitive species toward suboptimal habitats (Mammola et al. 2016b; Fig. 11.4).

More recently, thanks to the advances in the mathematical characterization of the niche, Blonder et al. (2014) developed a simple method to calculate the *n*-dimensional hypervolume (sensu Hutchinson 1957) of a certain species and to quantify its reciprocal intersection (i.e., overlap) with other species sharing the same habitat. By this means, it is possible to use field collected data to map the species distribution in

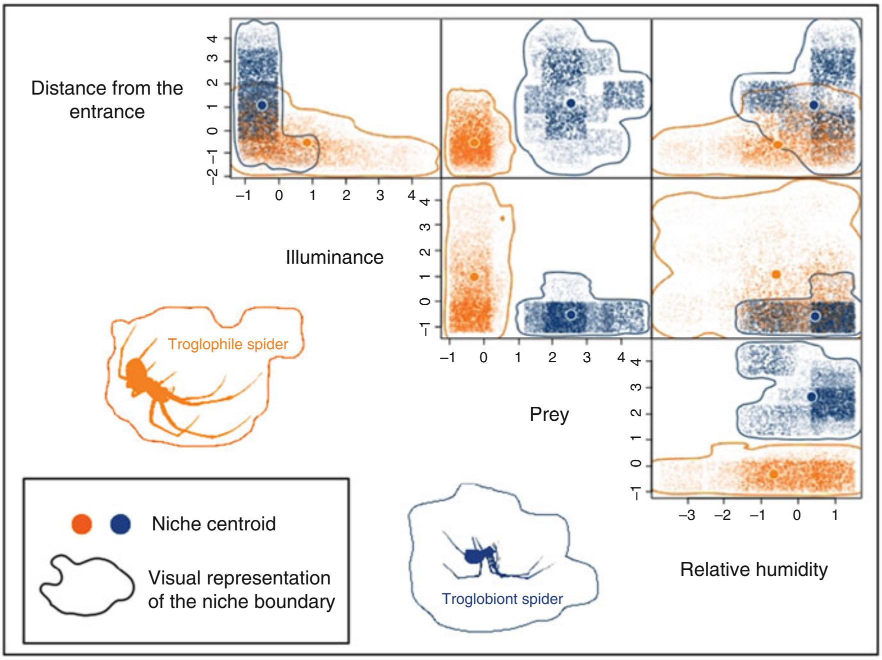

Fig. 11.5 The niche hypervolume of two cave predators. Graphical representation of the four-dimensional niche hypervolume (*sensu* Blonder et al. 2014) of two spiders showing different degrees of subterranean adaptation and coexisting within the same cave habitat. The combination of multiple environmental factors determines the niche of the two species—all variables are rescaled. For instance, in the central row, the first panel illustrates high partitioning in the niche of the two species concerning the optimal sites for hunting. The troglophile species finds its prey in most illuminated area of the cave (twilight zone), whereas the troglobiont hunts in the darker area. Similarly, the right panel in the same row shows that the troglobiont species has a preference for a narrow range of relative humidity, while the troglophiles is more tolerant. Data derived from Mammola and Isaia (2016)

relation to environmental features of the cave habitat and to use these data to represent the species niche in a mathematical space (e.g., see Fig. 11.5).

From a more morphologically oriented perspective, competitive exclusion can be studied by analyzing variations in functional morphological traits in multidimensional morphospace. By these means, it is possible to detect signatures of character displacement and divergent evolution due to interspecific competition. In recent times, using species pairs of subterranean amphipods of the genus *Niphargus* coexisting within the same habitat, Fišer et al. (2012, 2015) demonstrated that functional morphological diversity within subterranean communities was higher than expectation, suggesting that extreme habitats maintain a high potential for diversification via niche partitioning.

These examples testify the ecological potential of simplified ecosystems, such as caves, as model systems for the advance of niche theory. However, despite their potential, the use of subterranean organisms as models to study competition has lagged behind the use of their epigean counterparts. This is perhaps surprising, given that in complex epigean ecosystems, confounding effects may increase the complexity in defining and quantifying the niche. These include higher species diversity, multi-species interactions, and the intrinsic variability of the environmental conditions. On the other hand, due to their intrinsic simplicity and the absence of most confounding effects, caves offer unique natural laboratories. We are indeed convinced that the idea of basing niche-based studies in cave/subterranean habitats has strong potential in improve ecological theory.

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Chapter 12
Structure and Genetics of Cave Populations

Peter Trontelj

12.1 Introduction

12.1.1 The Genetics of Cave Populations Reflects Evolutionary History

Genetics of cave species can be and occasionally has been studied in its own right, just for the sake of the genetic and genomic mechanisms behind their captivating biology. But far more often, genetics in all its forms has served to support or reject concepts and hypotheses within the current paradigm or some particular research program of biospeleology. Over the past four decades, the predominant view has been that rare and localized colonization events give rise to small, isolated subterranean populations. These eventually become species of their own with hardly any opportunity to disperse beyond the bounds of an aquifer or a fissured, permeable geotectonic unit. The resulting biodiversity pattern is a patchwork of tiny ranges with large gaps in-between, as well as high allopatry of closely related and morphologically similar taxa (Sbordoni et al. 2000; Culver and Pipan 2014). Over the years, evidence from numerous studies from around the globe has only consolidated this model, despite occasional reports about larger ranges and subsurface dispersal (reviewed below).

There is a major, albeit largely overlooked, conceptual problem with the current model. While each single troglobiotic (used here for both troglobionts and stygobionts, unless specified) species has a small range, groups of closely related and morphologically very similar species can be distributed over much larger areas. This could have happened either by independent cave invasion and convergent or parallel evolution, or by a single troglomorphic ancestral species spreading over the

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entire, vast range that subsequently became fragmented. The principle of Ockham's razor, or parsimony, advises us to be cautious when postulating copious repetitions of a complex evolutionary transition. On the other hand, it is hard to imagine how a specialized cave species might traverse belts of non-permeable substratum, be it terrestrial or aquatic. In a few cases, empirical genetic evidence points toward long-range subsurface dispersal (e.g., Lefébure et al. 2006; Zakšek et al. 2007; Eme et al. 2013), but the biological means remain speculative. The most solid explanation, still far from being universally applicable, seems to be dispersal via the hyporheic interstitial habitat along gravel-filled riverbeds (Ward and Palmer 1994), which might occasionally work also for terrestrial species (Ortuño et al. 2013). In Lefébure et al. (2006), we have asked ourselves "whether wide distributions should always be bent to accommodate a nondispersal paradigm", or perhaps some other hypotheses should be given priority. More than 10 years later and in the light of overwhelming genetic evidence, it has become clear that the non-dispersal is the winning paradigm, as exemplified by the studies discussed in this chapter.

12.1.2 The Paradox of Large Subterranean Ranges

But how can the paradox of large ranges be resolved for all those species that do not disperse via the interstitial? On their evolutionary transition from surface to subterranean life, populations are believed to undergo four stages: (1) colonization, (2) a "trial phase" when their success or failure is determined, (3) speciation, and (4) dispersal of the new subterranean population (Culver and Pipan 2014). Unfortunately, this hypothesized linear chain of events will not help us find a solution. On the contrary, it strengthens the paradox, because it permits only subterranean dispersal.

A sobering example comes from recent molecular phylogeographic work on the widespread Cuban cave fish *Lucifuga dentata*. Hernández et al. (2016) have shown that the species occurs in five disjunct clusters of populations in isolated karst areas spanning nearly 400 km of western Cuba. The population clusters are genetically completely isolated from each other, as inferred from mitochondrial DNA (mtDNA) sequences. Genetic differences between them are not much larger than the divergence within, implying that they still belong to the same species, and that whatever events caused that structure must have taken place quite recently. Using approximate Bayesian computation, Hernández and his co-workers estimated that the present distribution was reached through gradual westward dispersal following a stepping-stone model and starting from the eastern-most population cluster. Paradoxically, the population clusters are separated by tens, sometimes hundreds of kilometers of unsuitable substratum. So, how could *L. dentata*, as an obligate subterranean fish, disperse so far westwards? The authors adhere to some unknown means of subterranean dispersal, mentioning that, under certain hydrological conditions, barriers to underground dispersal can be transiently overcome. Such an explanation is not far from the "just-so" stories of dispersal from the pre-vicariance era of historical

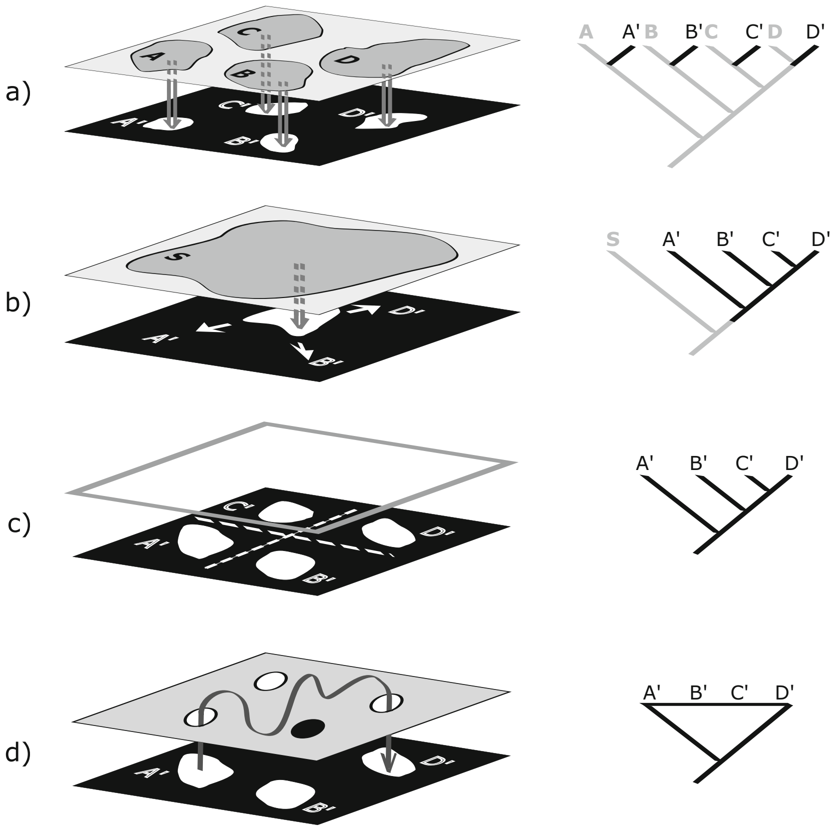

Fig. 12.1 Hypotheses explaining wide and discontinuous ranges of subterranean populations. (**a**) Structuring took place already among ancestral population at the surface, followed by multiple independent and geographically separate (polytopic) invasions; (**b**) a single surface population gave rise to a single invasion, and the new subterranean population subsequently dispersed or expanded endogenously; (**c**) whether a group of allopatric subterranean populations evolved by polytopic invasions, by subterranean dispersal, or by vicariant fragmentation of a once larger contiguous subterranean range cannot be inferred by phylogeny alone, once the surface ancestor has gone extinct; (**d**) occasional chance dispersal of troglobionts via the surface could partly homogenize gene pools of disjunct cave populations

biogeography (Morrison 2014). It shows that subterranean biology has yet to search for answers to some of its basic questions.

As a side note, the usual biogeographic alternative of vicariant fragmentation of a once large and contiguous subterranean range faces exactly the same limitation as any “just-so” story about dispersal. For subterranean vicariance to occur, a troglobiont would have first to spread over a large area of subterranean habitat.

To illustrate another aspect of the problem, let us take the example of a widely distributed but strongly structured subterranean species (Fig. 12.1). The linear model of cave species evolution can be applied in two ways to explain this pattern. The first is through a single colonization, followed by subterranean dispersal over a wider area of subterranean habitat and, finally, vicariant fragmentation of the subterranean range creating the well-known pattern of patchy, isolated subterranean populations. The second one is to postulate a strongly structured surface species, each of its population giving rise to a separate subterranean population in the same way as postulated above but not followed by any substantial subterranean dispersal and range fragmentation. The problem starts when the surface ancestor goes extinct, which is the rule for cave species (Culver et al. 2009). A phylogeographic analysis of the cave populations will not be able to discern between the two scenarios. The limitations of molecular phylogenies in tests of classical biospeleological hypotheses have been noticed and described before by Desutter-Grandcolas (1997) and Culver et al. (2009). Researchers therefore focused on questions (see examples discussed below) that can be addressed more easily and that do not require explicitly differentiating between processes taking place at the surface or during colonization and those taking place after the subterranean realm has been colonized. This essentially means that despite the use of sophisticated phylogeographic tools, we are often still not able to tell whether the most basic ecological and evolutionary events that shape the characteristics of cave species are taking place at the surface or underneath it.

The mentioned paradox is mostly a feature of continental subterranean habitats, both aquatic and terrestrial. The marine and coastal subterranean fauna from habitats such as anchialine caves and the coastal interstitial is governed by different spatial and temporal scales of dispersal and differentiation. It is dealt with in another chapter of this book (see Chaps. 18 and 19).

12.1.3 Exogenous and Endogenous Processes Determine the Structure of Subterranean Populations

Culver et al. (2009) pointed out that, in order to reconstruct these historical processes, one needs to obtain additional information, like times estimated by molecular clocks or data on paleoclimatic changes. The list can be expanded by data on genetic signatures of demography and genetic diversity of populations, pointing to hypothetical bottlenecks or founder events during colonization and population growth during phases of range expansion but also to events that increase diversity such as multiple invasions and secondary contact of subterranean populations. Further hints might be obtained by studying biological traits related to dispersal and subterranean lifestyle as well as comparative phylogenetic reconstruction of their evolution. The third category of data in this non-exhaustive list contains past and current abiotic and biotic factors enabling or obstructing dispersal above or below the surface, including changes in the availability of subterranean habitat, and vicariant events causing fragmentation of a wider, contiguous historical range.

Using these data, innumerous scenarios can be coined to formulate testable hypotheses. Some of them have been tested (described below) but hardly ever in a formal conceptual framework that would address the central problem in an explicit and comparable way: “Which of the processes that shape subterranean biodiversity take place at the surface and which within the subterranean realm?” And, as an extension to this question: “How did large subterranean ranges form?”

In its simplest form, such a framework might imply that the studied processes and events belong to either of two classes with respect to the ecological space in which they take place. In an operational sense, it requires the formulation of hypotheses that discriminate between these two classes:

1. *Exogenous* processes: they take place at the surface or during colonization and are generally common (e.g., fragmentation of ancestral populations, cave invasions, and the accompanying evolutionary changes).
2. *Endogenous* processes: they take place in the subterranean realm, after a new troglobiotic population has been established. They are comparatively rare (e.g., subsurface dispersal, vicariant fragmentation of subterranean ranges, secondary sympatry by two closely related troglobionts, evolving into new subterranean niches).

The idea that evolutionary processes shaping subterranean populations act at both levels, surface and subterranean, has been put forward already in the 1960s by Thomas Barr and has been later explicitly elaborated by John Holsinger (reviewed in Holsinger 2000). Although often cited and illustrated in biospeleological texts, the “two-phase model”, as Holsinger called it, has not received much consideration in empirical studies using genetic and phylogenetic approaches. Nevertheless, as will be shown in this chapter, our ability or failure to identify the level at which certain processes take place may have important consequences for our understanding of various biospeleological problems as well as practical implications, for example for the assessment of conservation priorities.

12.2 Genetic Diversification of Subterranean Populations

12.2.1 Brief Historical Account

In the early years of molecular population genetics, allozyme electrophoresis and the allele frequency data derived from it were the preferred tools to study population structure, diversity and differentiation. Studies of the 1960s to 1990s have shaped a picture of strongly structured populations of troglobionts with low to very low amount of gene flow between them (reviewed by Kane 1982; Sbordoni et al. 2000). Comparative analyses revealed much lower rates of gene flow taking place over considerably shorter distances in cave species than in closely related surface taxa. Interestingly, in populations that were examined over a range of geographical distances, e.g., cave crickets, beetles, spiders and amphipods, only negligible gene flow was detected whenever average distances between populations exceeded 20–40 km. That was one of the first empirical indications of a phenomenon that would two decades later become popular under the expression “cryptic species”. Population genetic research in caves has soon proven a valuable new tool in biospeleology, and development of the discipline has gone hand in hand with the advancement of genetic methodology ever since.

While allozyme data and the analytical methods applied to them enabled profound insight into population genetic structure and diversity, they fell short when it came to the detection of the temporal dynamics of population and lineage-level processes. The measure used (Wright’s *F*-statistics) presumes an equilibrium situation where gene flow by migration counteracts drift and other factors that tend to drive allele frequencies of different populations apart. But what if we knew that migration between physically isolated subterranean populations is impossible or nearly impossible? Then, the amount of gene flow, determined based on measured allele frequencies, “was supposed to reflect the historical gene flow that occurred between cave and epigean populations” (Sbordoni et al. 2000). This, of course, is a conceptual oversimplification that obfuscates the temporal and spatial dimension of evolution. We have accurately described the pattern but remain largely ignorant about which of many possible scenarios of surface and subterranean processes (Holsinger 2000) might have produced it.

With the start of routine use of DNA sequence methods in the 1990s, a temporal treatment of genes and their genealogies became possible. DNA sequences from individual specimens could be arranged to phylogenetic trees and networks. By mapping the distribution of genealogical lineages, one could draw conclusions about colonization and fragmentation history in an approach known as phylogeography (Avise 2000). First attempts at resolving questions of colonization history and isolation showed that simple cladistic approaches are too naïve to accommodate the complexity of possible scenarios (Rivera et al. 2002). Rivera and his co-workers, studying troglobiotic Hawaiian oniscidean isopods, concluded that “the known cave species of *Hawaiioscia* evolved from a widespread ancestral surface species or a group of closely related species through multiple, independent adaptive shifts on each of the islands separately”. Their paper can be seen as the beginning of modern, DNA-based molecular biospeleology. It tentatively announced that cave invasions by the same surface species are frequent in both time and space, leaving a complicated imprint on the molecular architecture of cave populations. The study showed that the question of whether isolated cave populations represent independent species is important not just from a taxonomic point but also for our understanding of the evolution of subterranean biodiversity. Furthermore, it underscored the need for an explicit temporal framework for the evolutionary dynamics of cave population. The temporal component was soon addressed by other authors (Caccone and Sbordoni 2001; Leys et al. 2003; Ketmaier et al. 2003; Verovnik et al. 2004), and it is presented in more detail in another chapter of this book (see Chap. 10).

12.2.2 Genetic Structure Results from Historical Processes and Concurrent Brakes in Connectivity Between Cave Populations

The term genetic structure is applied loosely, to denote any spatial or ecological departure of complete genetic homogeneity. In most cases, genetic structure is detected by comparing the genetic setup of several individuals from different caves. When in a population distributed across several caves individuals from different caves differ genetically more than individuals from the same cave this population is said to be structured into smaller populations (also subpopulations or demes). This is primarily done by measuring (counting) allele frequencies, be it in the form of allozymes, microsatellites, or single-nucleotide polymorphisms (SNPs), and by comparing DNA sequences and calculating average within- and between-population genetic distances. Alternatively, geographic population structure can be revealed by a phylogeographic approach, which is essentially a phylogenetic study of DNA sequences sampled from many individuals from different caves. Phylogeographically, the clearest case of population structure is obtained when members of different populations constitute mutually exclusive monophyletic groups and thus constitute separate species, at least under the phylogenetic species concept. It is the most commonly reported form of genetic structure, often in association with taxonomic proposals regarding cryptic or unrecognized species (Fig. 12.2a). This, however, is not the type of genetic structure that is particularly interesting for the ecology of cave populations.

Essentially, the ecologically challenging patterns of differentiation between cave populations are produced in two ways or a combination thereof:

1. As an historical imprint of exogenous processes, determined by the genetics of the surface ancestor and the way and number of times it invaded the caves (Fig. 12.2c)
2. As a consequence of concurrent endogenous factors acting upon the connectivity between caves, such as physical or other ecological barriers, limited biological dispersal abilities of troglobionts, and their potential to disperse over the surface (Fig. 12.2b)

Thus, we are looking at cases where cave populations are actually or at least theoretically in contact with each other via ongoing or recent gene flow. Such cases are remarkably rare. One reason for this is that, when researches look at subterranean populations at ever smaller geographical scales, they keep discovering new cryptic species instead of moderately structured populations (Zakšek et al. 2009). Other reasons are discussed below.

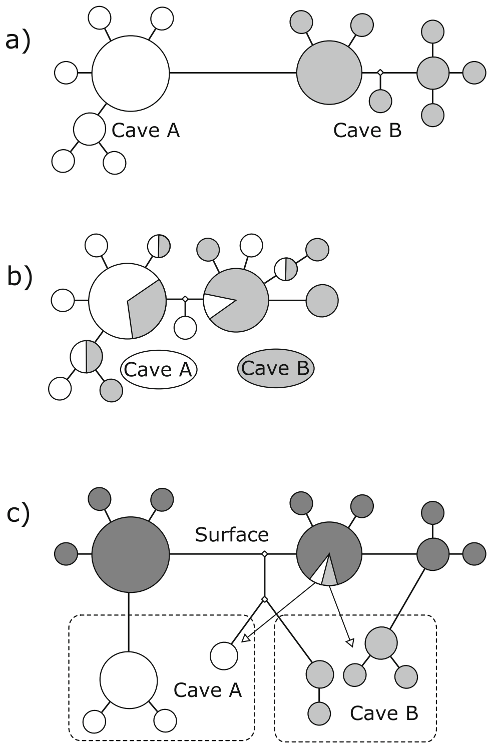

Fig. 12.2 Different types of genetic structure of cave populations as revealed by DNA sequence networks. (**a**) Mutually exclusive monophyletic clusters of sequences, indicating two separate species; (**b**) several sequences shared between populations, differences mostly in terms of haplotype frequencies; this network suggests ongoing gene flow between subterranean populations; (**c**) similar or same sequences in two separate cave populations can stem from a shared surface ancestor or from ongoing polytopic immigration from a widespread surface population. Most analytical methods would indicate gene flow, although no migrants are being exchanged between caves. Note how multiple invasion events, although low in numbers, can lead to high diversity by introducing highly divergent sequences. Each circle represents a DNA sequence haplotype, the diameter reflecting its gene pool frequency and the color the population of origin. The length of the path between circles is proportional to the number of substitutional differences between haplotypes. The networks are hypothetical but based on examples from studies discussed in this chapter

12.3 Population Structures in Different Ecological and Evolutionary Settings

12.3.1 The Sluggish Interstitial Highway Produces Strong Genetic Structuring and Isolation by Distance

An illustrative example is the small-scale genetic structuring of aquatic fauna in calcrete aquifers in the arid zone of central Western Australia. Technically, calcretes are not caves but shallow subterranean habitats formed in carbonate deposits in the groundwater of paleodrainages (Humphreys 2008; see also Chap. 18). They are completely isolated from each other for more than 5 million years (Leys et al. 2003), each harboring its own assemblage of endemic species (e.g., diving beetles, amphipods and parabathynellids) and therefore called a *subterranean archipelago* of groundwater islands in the desert (Cooper et al. 2007). The distribution of genetic diversity between calcretes is clearly a matter of exogenous processes taking place millions of years ago and in no relation to genetic connectivity between them. Yet, calcretes extend over tens and even hundreds of kilometers, which is huge for a contiguous area of subterranean habitat (Trontelj et al. 2009). Therefore, single calcretes are an ideal place to look for "internal" subterranean population structure. Inaccessible to humans, they are sampled via boreholes. In two recent studies, dense fields of orthogonally arranged boreholes were used to gain insight into the small-scale genetic structure of selected aquatic troglobionts. First, at the 3.5 km^2 large borefield of the Sturt Meadows calcrete, three sympatric species of dytiscid diving beetle were examined for genetic structure using mtDNA sequences (Guzik et al. 2009). In all three species, a remarkable diversity of mtDNA haplotypes was discovered that was highest within single bores and showed an isolation-by-distance-like behavior at a distance of maximally 2.5 km in the larger two species. A lack of population structure in the smallest species was interpreted of consequences of its better dispersal capability across matrix with small pore size. A similar study in the same area on chiltoniid amphipods using allozymes in addition to mtDNA (Bradford et al. 2013) revealed the possibility of an east-west bipartition and a weak (F_{ST} up to 0.14) structuring by some of the individual bores for the most common species. With the second most common species showing isolation by distance, the amphipods, just as diving beetles appeared to be slightly limited in their dispersal through the subterranean matrix. Guzik et al. (2011) chose a larger window to look at potential population structure of dytiscid diving beetles, an amphipod, and an isopod in the Laverton Downs calcrete. They sampled the fauna via a series of boreholes at three different points over a distance of 15 km and looked at mtDNA sequences. Just as in Sturt Meadows calcrete, the majority of genetic diversity was within bores, and some structuring was observed around groups of boreholes, in part possibly because of the influence of a nearby salt lake that restricted dispersal. Isolation by distance was detected for most of the species but only at distances larger than 6.5 km. This second study confirmed that limited dispersal and habitat heterogeneity shape the genetic structure within continuous subterranean habitats at the scale of 5–10 km in a similar way as they do at a smaller scale.

Strong genetic structuring at much shorter geographical distances (<50 m) was reported for small crustaceans, the parabathynellid Bathynellaceans, in an alluvial interstitial habitat in New South Wales, Australia (Asmyhr et al. 2014; Fig. 12.3). The entire studied borefield was no longer than 300 m. Inferred migration rates between more distant bores were below one percent, which, upon taking into account an average sample size of about 20, can be translated to an F_{ST} value of about 0.9 (very high). It is noteworthy that the mtDNA sequences of the sampled

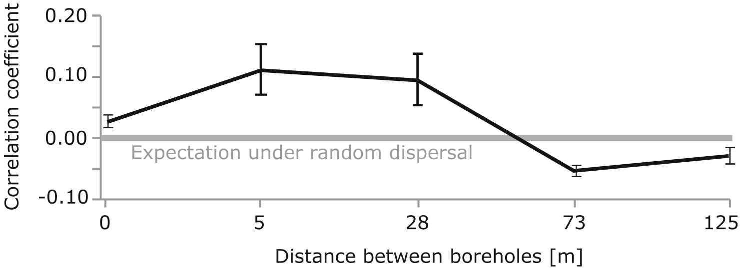

Fig. 12.3 Fine-scale genetic structure of small-bodied crustaceans in interstitial groundwater. Spatial autocorrelation serves as measure of the genetic similarity between pairs of individuals within a specified distance range. Spatial autocorrelation coefficient = 0 (gray line) corresponds to the null model of random dispersal within a given distance. Only for boreholes within the range of about 30 m are individuals more similar than expected by chance. At larger distances, migration in the interstitial is slower than expected by chance (Based on microsatellite data of an unspecified species of *Parabathynellidae* from the alluvial interstitial in New South Wales (Australia; Asmyhr et al. 2014))

parabathynellids averaged at about 2% and did not indicate a deep taxonomic structure, demonstrating the superiority of highly variable nuclear markers (microsatellites) for population-level studies.

The work by Asmyhr et al. (2014) is not directly comparable with the previous ones as the type of molecular markers and analytical methods (Bayesian assignment tests) applied were different, but the basic message is the same. Both the alluvial interstitial and the calcrete matrix are small-pore aquatic subterranean habitats, and both show very low migration rates and slow dispersal despite a continuous distribution in a continuous habitat. From the point of view of the interstitial highway hypothesis (Ward and Palmer 1994), the "highway" may still validly describe the riparian or hyporheic interstitial as continuous linear habitat, but certainly not the speed at which its inhabitants disperse. With respect to the new model, the important message is that the endogenous process of subterranean dispersal, while possible along continuous habitats, is likely to be extremely slow compared to surface dispersal and thus in a purely numerical sense is a less probable scenario. Furthermore, recently developed mathematical models suggest that when dispersal proceeds at a very slow pace and the reach of single individuals is short compared to the entire range, new species may evolve parapatrically without the need for ecological discontinuities (Hoelzer et al. 2008), especially in linear habitats (De Aguiar et al. 2009). This possibility, so far not yet considered by biospeleologists, offers an additional and elegant explanation to the all-pervading phenomenon of subterranean cryptic species.

12.3.2 Cave Populations Tend to Have an "All-or-Nothing" Structure

In caves and karstic (i.e., large pore) aquifers, a number of studies revealed two possible patterns of genetic structuring of populations. One is a nearly panmictic or admixed pattern; the other one is strong structuring according to local and regional hydrological boundaries.

A typical situation is that found by Carlini et al. (2009), who sampled three caves and several surface populations of the gammarid amphipod *Gammarus minus* along a 50 km stretch of the Greenbrier River in West Virginia, USA. They sequenced mitochondrial DNA and nuclear internal transcribed spacers (ITS1) from up to 20 individuals per sampling site. The three caves contained completely different sequences, belonging to separate clades, and were obviously colonized at three separate occasions from different ancestral populations. They are completely isolated from each other. Conversely, within Organ Cave, at a distance of about 0.5 km, no genetic structuring was detectable, meaning that the amphipods disperse and mate without restriction inside the large cave.

On the other side of the Atlantic, another widespread and common aquatic crustacean, the asellid isopod *Asellus aquaticus*, is known for its rich and complex history of cave invasions. In the northwest Dinaric Karst, studies using mitochondrial and nuclear DNA sequences (Verovnik et al. 2004), randomly amplified polymorphic DNA (Verovnik et al. 2003), and microsatellites (Konec et al. 2015) revealed deep genetic differentiation between cave population from different drainage basins. A phylogeographic analysis based on mtDNA suggested that caves in each basin have been invaded separately, which, besides the lack of hydrological connections, explains the deep structure. In the central part of the Slovenian Dinaric Karst, inside the Postojna-Planina cave system, a seemingly different pattern popped up. Inside a single cave system, three separate cave populations coexist in a parapatric manner (Verovnik et al. 2003, 2004). At a first glance, this looked like a rare or possibly the first known case of endogenous, subterranean fragmentation within a single cave. However, later microsatellite studies (Konec et al. 2015 and unpublished data) suggested that the three populations are simply three independently founded and evolved subterranean populations in secondary contact and partially reproductively isolated. A tentative example of a vicariant range fragmentation within a subterranean species was nevertheless identified in the nearby subterranean Reka river system, inhabited by the recently described close relative *Asellus kosswigi* (Konec et al. 2016). In the uppermost part of the system, about 400 m higher than other known sites, a small population with different mitochondrial haplotypes has been discovered. It is believed that such an altitudinal difference at a short horizontal distance presents an insurmountable barrier for the aquatic isopods. The area is tectonically very active, so today's disjunct distribution could have resulted from times when the piezometric levels where more even.

A similar system of a widespread surface population and numerous but localized cave populations as in both aforementioned crustaceans is presented by the famous

Mexican characid cave fish, *Astyanax mexicanus*. All cave populations live within a relatively small area (about 150 km by 40 km), the Sierra de El Abra region of east-central Mexico. No other cave animal has been studied as thoroughly and by as many researches as the Mexican cave fish. Three recent comprehensive studies, building upon previous mtDNA phylogeographies, used microsatellite (Bradic et al. 2012; Strecker et al. 2012) and genome-wide SNP data (Coghill et al. 2014) to unravel the complex story of multiple invasions from multiple surface lineages to multiple cave populations and occasionally the reverse. The three studies partly disagree about the number of lineages that independently invaded the caves (from three in Strecker et al. 2012 to five or more in Bradic et al. 2012) and on some details in timing of gene flow and dispersal. Nonetheless, they all uncovered a common pattern. Some cave populations exchange genes with surface population in both directions. Hence, these cave populations are directly or indirectly connected by migration via the surface, where there is hardly any genetic structuring (Bradic et al. 2012). Cave populations or groups of cave populations are genetically either completely distinct from each other (the El Abra, Guatemala, and Micos cave populations), or they show almost no structure at all. In the central group of six El Abra caves, spanning about 30 km, microsatellite-based F_{ST} values are mostly insignificant and rarely exceed 0.1 (Bradic et al. 2012). Strong structure ($F_{ST} \approx 0.3$) arises only compared to the Pachón Cave population that is 60 km away, but this might well stem from a separate invasion or recent genetic exchange with different surface populations.

12.3.3 Dispersal of Evolutionarily Old and Large-Bodied Cave Species Probably Proceeds via the Surface

Just like the crustaceans *G. minus* and *Asellus*, the cave fish *A. mexicanus* is not the typical, evolutionary mature cave animal that has ceased exchanging genes with its surface ancestor. The genetic structure of their cave populations is still determined exogenously, by the genetics of their surface ancestors and the dynamics of colonization events rather than by intrinsic conditions of the cave environment. Large ranges, beyond the boundaries of physical connectivity of the cave habitat, are readily imaginable as consequence of multiple, spatially separate colonizations followed by parallel or convergent troglomorphic evolution. Conversely, there is no easy answer to the question how evolutionarily old cave species that have lost contact to their surface relatives long ago reach and maintain their large distributions. For example, the last surface ancestor of *Niphargus*, a diverse genus of European cave and groundwater amphipods, was marine and lived in the Mesozoic. There probably never was a surface proto-*Niphargus* to disperse over the continent and colonize the karst areas one by one. It all had to be achieved by fully troglobiotic animals.

The next interesting question therefore is whether such evolutionarily old cave species undergo some active concurrent processes that maintain their populations structured and yet not entirely separated from each other. In theory, these processes are endogenous, because they must have originated from an already evolved troglobiotic population and should be controlled by the conditions of the subterranean environment only. Potential aquatic examples that have been extensively studied for their mtDNA and nuclear gene variation include cave amphipods of the genus *Niphargus* (Niphargidae) and atyid cave shrimps *Troglocaris* sp. Most of these species as adult measure several centimeters in length and are thus bound to large-pore habitats, usually karstic aquifers and caves. Nominal species like *Niphargus virei* and *N. rhenorhodanensis* from western France, *N. laticaudatus* from the Western Carpathians and *T. anophthalmus* from the Dinaric Karst all have relatively large ranges, reaching up to several hundred kilometers (Lefébure et al. 2006, 2007; Zakšek et al. 2009; Meleg et al. 2013). All these nominal species harbor considerable cryptic diversity. At a closer look, even within some of the cryptic species a further, hierarchically lower level of mutually exclusive clades exists that contains further cryptic species. Such is the pattern within two of the geographically largest clades of *N. virei* (Lefébure et al. 2006) and some of the *N. rhenorhodanensis* clade, while in at least one of the *N. rhenorhodanensis* clades, a geographically admixed DNA haplotype distribution suggested recent dispersal (Lefébure et al. 2007). Similarly, one of the phylogeographic clades of *T. anophthalmus* sensu lato extending across 300 km of hydrographically fragmented karst terrain displayed a genetic pattern of recent dispersal, while other large-range clades were genetically structured either in an isolation-by-distance-like way or by allopatric fragmentation (Zakšek et al. 2009). Where sampling was dense enough, e.g., in the W-Slo clade, good connectivity and dispersal within existing hydrological connections were found, as a small number of common haplotypes were shared among interconnected caves.

Thus, we are observing two types of genetic and phylogeographic patterns in these evolutionarily old subterranean species. One is a hierarchically arranged subclustering from large to smaller geographical areas. The other is the lack of structure within small but occasionally also quite large areas. While the lack of structure in small areas of contiguous habitat is expected, all distributions crossing hydrological boundaries require some unknown means of dispersal. Vicariant fragmentation of a wide ancestral range, like the one of *Niphargus virei* sensu lato, spanning over 800 km, is no solution to the puzzle, because it still requires wide-range dispersal of a subterranean ancestor to start with. Therefore, most authors have considered the possibility of some form of passive or active dispersal via surface habitats. Some are very cautious about it, e.g., “it seems that its ancestor was more ubiquitous than today, and that it was able to disperse through surface water” (Lefébure et al. 2006). Others are more explicit: “Stygobitic crayfishes are severely limited in dispersal abilities by both subterranean and surface barriers, except during high water levels when they can migrate (or wash) out of caves” (Buhay and Crandall 2005). Whoever is more correct, occasional occurrence of troglobionts outside caves is a well-known phenomenon (Bressi et al. 1999; Graening et al. 2006), and it is only a matter of time when one of them will reach the next section of subterranean habitat. As for young troglobionts independently invading multiple caves, the key to understanding large subterranean ranges here, too, lies on the surface. When we are interested in exclusively endogenous processes, we should focus on smaller areas of presently or historically contiguous subterranean space.

Freshwater stygobionts with phylogenetic, ecological or geographic affinities to marine environments often have large, disjunct ranges. A number of scenarios have been proposed about how they might have dispersed across such large areas using coastal or marine waters as medium (Notenboom 1991; Boutin and Coineau 2000). The proposed scenarios consist of two major evolutionary transitions, one from marine to freshwater and the other from pelagic or epibenthic lifestyle to a subterranean one. While the order by which they occur may vary, they are usually supposed to be irreversible, i.e., a population, once it has colonized the subterranean environment and become stygobiotic, does not return to the sea. However, for young, non-relictual but disjunct populations, this may be a valid alternative hypothesis to consider. The five disjunct population clusters of the Cuban cave fish *Lucifuga dentata* mentioned at the beginning of this chapter have evolved by gradually "jumping" from one isolated karst area to the other (Hernández et al. 2016). All five areas are close to the current shoreline; thus past sea-level fluctuations might have created occasional connections to the sea, enabling the fish to disperse via a non-subterranean habitat. In that way, exogenous processes might shape the population structure of widely distributed stygobionts with marine affinities. Here, too, the real barriers to dispersal seem to lie between caves, not between the surface and the cave.

12.3.4 Non-dispersal Has Different Meanings in Karstic and in Interstitial Groundwater

A general problem with most of these studies is that they were not designed for detecting fine-scale genetic structure within caves or cave systems. Other molecular markers, preferably microsatellites or SNPs, and a much denser sampling would be required for this purpose. But from what has been found so far, we can infer the following. Evolutionary old, large-bodied cave species and troglobionts from deep cave/subterranean habitats, both terrestrial and aquatic, are highly mobile within the limits of their interconnected subterranean habitat. In single caves and cave systems, even very large ones, genetic structure is weak or lacking. They have evolved locomotory, sensory, and energetic mechanisms optimally adapted to moving through the large-pore subterranean space. The proof of existence of such mechanism was one of the great achievements of the American neo-Darwinian school of subterranean biology (Poulson 1963; Christiansen 1965; Culver 1982; Culver et al. 1995).

On the other hand, hydrological divides, geomorphological brakes, non-permeable bedrock, and other discontinuities of their habitat represent formidable barriers that sharply shape the structure of populations and species. Therefore, in aquatic organisms, the non-dispersal paradigm does not mean the same for karstic and interstitial groundwaters. In the latter, low dispersal results from an intrinsic combination of small body size and physical or ecological limitations of the small-pore labyrinth structure of habitat, which in itself is not necessarily fragmented. In the former, locomotion is probably just as fast as in similarly sized and built surface organisms, but their dispersal is restricted extrinsically, by insurmountable ecological barriers.

12.3.5 *Terrestrial Troglobionts Are Less Studied but Seem to Share Basic Patterns of Genetic Structuring with Aquatic Ones*

There is much less recent data available for the genetic population structure of terrestrial troglobionts than for aquatic ones. A lot of work has been done in the allozyme era on rhaphidophorid cave crickets of the genus *Dolichopoda* (Sbordoni et al. 2000) and leiodid (*Speonomus*, e.g., Crouau-Roy 1989) and carabid (*Pseudanophthalmus*, *Darlingtonea*, e.g., Kane et al. 1992) cave beetles.

Box 12.1

The results of these studies agree remarkably well with what has recently been found for aquatic subterranean fauna using DNA sequences and other modern molecular markers. For instance, the small *Speonomus* beetles living in caves as well as in deep soil and MSS showed a pattern of genetic differentiation among populations resulting from slow dispersal, just as in the interstitial. The population structure of larger-bodied cave beetles responded to geological features and rivers, indicating brakes in the connectivity of the subterranean habitat and possibly barriers to surface dispersal. In the large *Dolichopoda* cave crickets, surface dispersal played an essential role for the genetic structuring of populations. Populations in areas with unfavorable surface habitat were strongly structured, while those in areas with favorable surface habitat were only weakly structured.

A small number of population-level phylogeographic studies on terrestrial troglobionts using mitochondrial and sometimes also nuclear DNA sequences are in agreement with the forgoing suggestions: cave populations form interconnected clusters that are completely isolated from each other (all-or-nothing structure). The current subterranean distribution is mostly believed to reflect the distribution and structure of ancestral surface populations (Villacorta et al. 2008). A detailed phylogeography of Caribbean Amblypygi of the genus *Phrynus*, which are not exclusively cave bound, suggested that barriers to surface dispersal such as geological substratum, unsuitable habitat, and climate are responsible for the island-like structure of cave populations (Esposito et al. 2015).

Alternatively, disjunct distributions separated by extensive areas of non-karstic habitat can be explained by surface movements of animals that are otherwise deemed strict troglobionts during short periods of favorable climatic conditions (Rizzo et al. 2013). Both scenarios require hypothesizing exogenous processes to explain an endogenous pattern. A noteworthy possible exception has been reported for cave plant hoppers of the *Oliarus polyphemus* species complex (Wessel et al. 2013). The authors suggest that the island-like population structure resulted from a combination of very low random dispersal through the subterranean lava tube network of Hawai'i island and very fast, non-ecological speciation. It is questionable, however, whether this model is applicable to other cave species.

Two recent phylogeographic and molecular systematic studies on cave beetles illustrate how difficult it can be to distinguish between dispersal and fragmentation, past and present habitat connectivity, as well endogenous and exogenous processes. Both studies are primarily systematic and not intended to disentangle population structure. However, since they cover small areas (>100 km), densely packed with even smaller allopatric ranges of closely related species, these can be used as proxy for strongly structured populations. The first case study, conducted in the eastern Pyrenees on the carabid genus *Geotrechus*, suggested that the strictly allopatric distribution of seven closely related species resulted from a series of vicariant events that, over a period of about 10 million years, had fragmented a once contiguous, larger ancestral range (Faille et al. 2015). Since these beetles are quite capable of dispersal, as they are found also in artificial caverns, occasional dispersal events across geomorphological boundaries cannot be ruled out. Ancestral dispersal might have proceeded during wetter and therefore more favorable conditions for surface dispersal. The processes behind the current allopatric and highly fragmented structure were therefore probably exogenous or mixed rather than exclusively endogenous. In a superficially very similar situation in the southern Dinaric Karst, the cholevid genus *Hadesia* has evolved into five allopatric species during the past 3 million years (Polak et al. 2016). However, circumstantial evidence clearly points toward a purely endogenous process ever since the ancestral *Hadesia* evolved. All species of the genus share a highly specialized way of life, filter-feeding on cave walls in films of flowing water. Their complexly elaborated mouthparts and feet are identical in all five species and thus very unlikely to have evolved independently. One cannot imagine these animals surviving and migrating in any other habitat but wet caves. The most likely scenario thus foresees an endogenous evolutionary origin of the ancestral *Hadesia*, followed by subterranean dispersal over the entire range of the genus during the warm and wet mid-Pliocene, when cave development in the Dinaric Karst was at its height. The possibility that there existed a huge, at least, temporarily interconnected cave and crevice system is supported by the fact that today this is one of the most highly karstified areas in the world (Milanović 2015). During later period of cooler and drier climate, the range gradually became restricted to isolated habitat fragments, where there was enough percolating water to sustain a permanent cave hygropetric environment. The five known *Hadesia* species, although descendant from a single endogenous ancestor, thus probably evolved allopatrically in what may be one of very few documented cases of genuine subterranean vicariance.

The above examples demonstrate that the inferential procedure to discern between exogenous and endogenous evolutionary processes is not a straightforward one. Adequate background knowledge on the biology of organisms is crucial for the study of genetic connectivity (Pante et al. 2015). Explaining large ranges, structured populations and high diversity of subterranean populations requires data on the geological, hydrological and climatic history (Rizzo et al. 2013). Only then can we incorporate our population genetic and phylogenetic data into hypotheses in a way that makes biological sense. An essential part of these hypotheses is the origin and evolutionary dynamics of genetic variability of cave populations, which is the subject of the next section.

12.4 Genetic Diversity of Cave Populations

12.4.1 Conflicting Theoretical Predictions and a Paradox

Genetic diversity determines a population's fitness and adaptive potential (Reed and Frankham 2003; Allendorf and Luikart 2007). The question of how subterranean populations obtain and maintain their genetic diversity is therefore central to the understanding of the evolutionary success of subterranean life. Over the past four decades, it has been the matter of some debate and paradigm shifts. Even nowadays, researchers keep reporting genetically surprisingly diverse as well as unexpectedly uniform populations, reflecting the uncertainties of theoretical predictions.

Before the first empirical data on genetic diversity of cave populations became available in the form allozyme data, two contrasting views had formed (Fig. 12.4). Barr (1968) suggested a model in which a small founder population becomes isolated underground and, if successful, gradually recovers from the reduced diversity by expanding into the unoccupied subterranean space. Conversely, Poulson and White (1969) proposed that genetic variability of a cave population gradually decreases because of the constant selective environment, isolation, small population size and longer generation times as compared to the surface ancestor.

Soon thereafter, the results of several allozyme studies announced that a simple and unequivocal solution is not to be expected. Some researchers found evidence in support of the reduced genetic variability hypothesis (e.g., Avise and Selander 1972; Laing et al. 1976; Swofford et al. 1980). Others detected high genetic diversity that equaled or exceeded that of related surface populations (Turanchik and Kane 1979; Sbordoni et al. 1980; Culver et al. 1995). These studies were not without drawbacks. Only two of them—on *Astyanax* cave fish (Avise and Selander 1972) and the amphipod *Gammarus minus* (Culver et al. 1995)—were able to compare surface populations with their derived cave populations. All others had to use surface populations of different species, with no guarantee that they truthfully represent

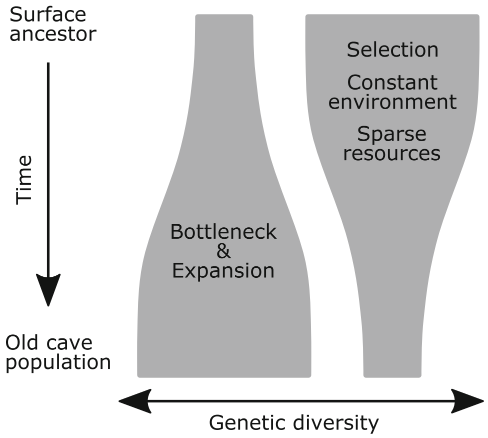

Fig. 12.4 Two contrasting models of the dynamics of genetic diversity of cave populations. Left: low diversity after colonization results from a genetic bottleneck; if successful, the population gradually adapts and expands into the new habitat, eventually restoring its genetic variability (Barr 1968). Right: the initial genetic variability of the surface ancestor decreases after colonization as consequence of selection in an isolated, unvarying environment that is spatially limited and poor in food (Poulson and White 1969)

the genetic diversity of the direct surface ancestor. Reliable age estimates for cave populations are crucial for testing the two hypotheses but were mostly lacking. Culver (1982) reformulated the hypotheses explaining genetic diversity of cave populations to incorporate the neutralist-selectionist debate that was very popular at that time. This is important because the DNA-based genetic markers that became widely used in the late 1990s are mostly selectively neutral. The prediction for neutral genetic variation is to be low in small populations and to reflect temporary reduction in population size (low effective population size, N_e = an idealized measure of the average number of individuals of a population that contribute their genes in equal shares to the gene pools of succeeding generations) that is believed to have occurred during cave colonization. Culver (1982) commented that genetic variability in cave populations shows no consistent pattern and that it is unlikely that this pattern will become any simpler when new data become available. It turned out he was right.

The one thing most authors seem to agree with is the existence of some form of genetic bottleneck or founder event reducing the initial genetic diversity of a new cave population. Paradoxically, such a restriction in population size and available genetic variation for selection to act on is incompatible with the observed multitude of highly successful, replicated, and ongoing cave invasions as well as the fast and consistent evolutionary change taking place. This is a dilemma well known from invasion biology: how do newly founded populations overcome the low genetic diversity and the expected low evolutionary potential, typically associated with extinction risk, to become established outside their native habitat (Roman and Darling 2007)? The paradox has been largely resolved by discovering that successful biological invasions tend to be driven by high propagule pressure (Simberloff 2009). Propagules are the invading individuals, and high propagule pressure means that these are invading in higher numbers and diversity than expected by a single chance dispersal event. This can happen simply because the numbers of invading individuals are high, through ongoing migration between the original and the new habitator by augmentation through repeated invasions or by any combination thereof. Because colonization of caves is nothing but a specific form of biological invasion, it is reasonable to expect that the success of cave colonization is determined by propagule pressure, too.

12.4.2 Exogenous and Endogenous Factors Affect Genetic Variability in Different Ways

There are three ways by which genetic diversity becomes available to a cave/subterranean population:

1. Directly from an ancestral surface population. Since no new mutations are involved, this kind of genetic variability is called standing genetic variation. It is more important in young populations than in old ones, and it probably plays a major role in early stages of adaptation. It determines the potential of a population to adapt. Standing genetic variation of a new cave population depends entirely on exogenous factors: the genetic diversity of the ancestral population, random sampling of alleles (drift) and propagule pressure. During the later course of its subterranean existence, a cave population will inevitably loose some its initial genetic variability due to drift. This is controlled by endogenous factors such as effective population size, mating system, and demography of the cave population. Several molecular studies (see next section) provide insight into the genetic diversity of young cave populations and the demography of cave lineages.
2. By endogenous exchange with other cave populations. Separate cave populations could, in theory, become interconnected when barriers to subterranean dispersal between them break down. The resulting genetic admixture or gene flow would increase the genetic diversity of the populations receiving migrants. Although this is a pattern commonly encountered in surface species, no such case has been documented for troglobionts so far. The handful of troglobiont populations that are known to exchange genes by underground migration, e.g., *Astyanax mexicanus* in Mexico (Bradic et al. 2012; Strecker et al. 2012) and *Asellus aquaticus* in Romania and Slovenia (Verovnik et al. 2004; Konec et al. 2015), are geographically close and relatively young populations with their surface ancestor still present. For these populations it is difficult to disentangle exogenous or mixed from purely endogenous processes.
3. By new mutations. This is a purely endogenous process. The accumulation of new (neutral) mutations is expected to proceed at slightly lower rate when the generation cycle becomes longer. There are suggestions that this might be

compensated by a higher mutation rate, for example, because of higher radon concentrations in caves (Allegrucci et al. 2015). This subject remains speculative. It is much clearer that the available subterranean habitat is usually much smaller than surface habitats, as is the available energy. This, in combination with low connectivity, is expected to lead to lower N_e on the long term than in surface populations of taxonomically and functionally similar species. However, using various methods for reconstructing demographic history, researchers have found that populations of old troglobionts can be more dynamic and experience population growth long after they have invaded the subterranean realm. This could happen when ecological conditions are not as constant as usually assumed, for example after a long-term rise of the groundwater table in calcrete aquifers of Western Australia (Guzik et al. 2009; Bradford et al. 2013).

Of these three groups of processes that are generating genetic diversity of cave populations, only the first—the exogenous one—has been sufficiently explored. However, new molecular data seem to be more in support of the high propagule pressure hypothesis of invasion biology than of the traditional genetic bottleneck view of subterranean biology. This dilemma has rarely been addressed by biospeleologists.

12.4.3 *Exogenous Propagule Pressure Appears to Shape the Genetics of Young Cave Populations More than Bottlenecks*

The most direct test of the genetic bottleneck hypothesis is to compare the gene pool of recently established cave populations to that of the surface population from which it has been derived. This kind of analysis is hampered by the scarcity of known cases where troglobiotic populations exist alongside their direct surface relative.

The heterozygosity of *Astyanax* populations estimated on 26 microsatellite loci was significantly lower in caves of the El Abra region (0.55 ± 0.07) than in adjacent surface populations (0.82 ± 0.04); so was the allelic number corrected for sample size, 2.54 ± 0.26 in El Abra caves and 3.63 ± 0.14 in surface populations. By comparison of gene pools, Bradic et al. (2012) found out that the allelic contents of cave populations are subsets of alleles of the surface stock. In agreement with the genetic diversity, data were estimates of effective population size, which averaged about 1000 in caves, and were about two to three times larger in surface populations. However, error rates of these estimates were high and largely overlapping. It needs to be mentioned that N_e is a tricky parameter to estimate, and estimates depend heavily on the method and its underlying assumptions. Comparisons between estimates based on different methods and markers can be misleading (Skrbinšek et al. 2012). While differences in genetic diversity were mostly significant, their magnitude is not what one would expect to be after a severe bottleneck. An analysis of migration rates between surface and cave populations explains why this is so: all

Table 12.1 Genetic diversity indices of cave populations and co-occurring surface populations with the relative extent of diversity in caves expressed in %

Taxon	Nucleotide diversity (π)			Observed heterozygosity (H_O)			Source
	Surface	Cave	%	Surface	Cave	%	
Astyanax mexicanus	0.0011[a]	0.0002	18	0.76	0.53	70	Panaram and Borowsky (2005), Bradic et al. (2012)
Poecilia mexicana	–	–		0.50	0.28	56	Plath et al. (2007)
Asellus aquaticus Romania	0.0170[b]	0.0180	106	0.34	0.19	56	Konec et al. (2015)
Asellus aquaticus Postojna-Planina cave system, Slovenia	0.0035[b]	0.0025	71	0.15	0.05	33	idem
Gammarus minus	0.0035[b]	0.0071	203	–	–		Carlini et al. (2009)
Georissa filiasaulae/ G. saulae	0.0013[c]	0.0025	192	–	–		Schilthuizen et al. (2012)

Presented are simplified average values; for original figures, standard errors, and population sizes, the cited references should be consulted

[a] π-values are derived from a genome-wide analysis of randomly amplified polymorphic DNA (RAPD)

[b] π-values are derived from mitochondrial COI gene sequences and their frequencies

[c] π-values are derived from mitochondrial 16S rRNA gene sequences and their frequencies

cave populations exchange genes with surface populations, either directly or indirectly via other cave populations. Altogether, recent studies agree that the current genetic setup of *Astyanax* cave populations resulted not only from a number of temporally distinct invasions but also from ongoing genetic exchange with surface populations. One can safely say that subterranean *Astyanax* populations are under strong exogenous influence. Nevertheless, the gene flow from the surface has to be counterbalanced by strong natural selection to maintain the troglomorphic traits.

The cave molly (cave form of *Poecilia mexicana*) is less differentiated and structured than the cave *Astyanax*, but the pattern of genetic diversity of the only known troglobiotic population in southern Mexico is quite similar. The Cueva del Azufre is a sulfidic cave, which means that colonizers are facing a double ecological gradient—the permanent darkness of the cave combined with toxic hydrogen sulfide (H_2S). Measured by allele frequencies of ten microsatellite loci, the cave population was significantly less diverse than the surface reference population form non-sulfidic water (Table 12.1). No migration from the surface into the cave could be detected by analysis of gene flow. Even though genetically isolated, the population showed no convincing signs of a recent genetic bottleneck (Plath et al. 2007).

On the invertebrate side, *Asellus aquaticus* with several independent pairs of ancestral surface and derived cave populations offers a powerful study system. Konec et al. (2015) compared population pairs in Slovenia and Romania. Mitochondrial DNA diversity was not (Romania) or only slightly (Slovenia) lower in caves than at the surface. Microsatellite diversity, measured at eight loci, was up to three times lower in caves (Table 12.1). High mtDNA diversity of the Romanian cave population is the consequence of at least two independent invasion events, delivering two groups of highly divergent mitochondrial DNA haplotypes into the cave gene pool (Konec et al. 2015). For the Slovenian cave population, the number of colonization events cannot be inferred from the haplotype network, but shared high-frequency haplotypes speak against a founder bottleneck. Natural selection seems to be uncoupled from mitochondrial and microsatellite genetic variability. Neither did the added selection pressure of toxic H_2S in the sulfidic Romanian cave restrict genetic variation, nor did the smaller genetic variability of the Slovenian cave population affect the evolutionary potential, as shown by the overall higher number of changing morphological traits. The *Asellus* example highlights the importance of choosing the correct populations for comparison. By matching, for example, the Romanian surface and the Slovenian cave population one would get the impression of a strongly reduced genetic diversity in caves. In reality, the Romanian cave population is just as diverse as the surface population.

In *Gammarus minus*, two of the cave populations showed very low mtDNA diversity, which is consistent with a genetic bottleneck. The others got colonized twice from different sources, which is more consistent with the propagule pressure hypothesis of cave invasion. Carlini et al. (2009) looked at codon usage bias as a pointer to population size (small populations are expected to have low bias due to drift) and found it was lower in cave than in surface populations. While this is consistent with a low long-term population size, it is not necessarily a consequence of a genetic bottleneck.

The only terrestrial example comes from a nonstandard cave species. The tropical cave snail *Georissa filiasaulae* from Borneo lives in contact with its surface ancestor *G. saulae*. It is thought to have evolved in a process of ecological speciation and has experienced recent gene flow from the surface species (Schilthuizen et al. 2012). The important point is that, although genetically distinct, it shows a similarly shaped mtDNA haplotype network and more genetic variability than the surface species. Apparently, one-way gene flow from the surface is genetically enriching the cave population. The concepts of speciation with gene flow and ecological speciation are compatible with the propagule pressure hypothesis but much less so with founder effects and genetic bottlenecks.

12.5 Conclusions and Perspectives

The predominant, traditional view of the genetics of cave populations is that they are strongly structured because of the patchiness of subterranean habitats and low dispersal of cave species. When a group of populations or closely related species is distributed over a larger area, they are usually explained as the result of multiple separate invasions with subsequent extinction of the surface ancestor. Genetic bottlenecks caused by small numbers of invading individuals additionally contribute to genetic differences between populations.

There is a discrepancy between large and disjunct ranges of troglobionts and low migration capability inferred by genetic analyses, somewhat similar to the mysterious occurrence of nonswimmers and non-fliers on remote oceanic islands. Also, the notion of countless replicated successful invasions is incompatible with the genetic bottleneck hypothesis because low genetic diversity means low evolutionary potential and high extinction risk. The past two decades of molecular population genetics and phylogeography have provided solid solutions to these controversies but left some points unanswered.

A dual approach that differentiates between exogenous and endogenous processes can provide the necessary new perspective. Exogenous processes take place at the surface or during the invasion phase. Although having a strong impact on cave life, they can be studied and understood using a conventional ecological and evolutionary framework. Endogenous processes, on the other hand, are governed by the specific conditions of cave/subterranean environments. Cave life is shaped by both, but it is essential to separate the two classes of processes, if we wish to find out what is really special about caves. With this distinction in mind, the main conclusions of this overview paper are the following:

1. Genetic similarity between allopatric cave populations is rarely the consequence of contemporary gene flow but rather reflects shared ancestry. Such populations can be considered as cryptic species under the phylogenetic species concept. Whether these species evolve by exogenous or endogenous processes is one of the key questions of subterranean biology.
2. The central question, whether a wide range is reached by dispersal via the surface or subterranean migration, cannot be answered directly by molecular phylogenetic and phylogeographic analyses. Geological, biological and ecological information needs to be combined with data points from molecular clocks and demographic analyses in order to formulate testable hypotheses about genetic connectivity of subterranean populations.
3. Evidence for endogenous vicariance—the breakup of a large, contiguous subterranean range through the action of extrinsic forces—is exceedingly rare. This might be in part because of epistemological reasons, in part because it requires a large and interconnected subterranean population to begin with.
4. Evidence for contemporary gene flow between allopatric cave populations is sparse. Most known cases of recent migration between non-neighboring populations entail the possibility of dispersal via the surface, even if the species is an obligate cave dweller. Superficial and small-pore subterranean habitats do not seem to contribute much to the genetic connectivity between cave populations. Genetic data suggest gene flow and dispersal within these habitats are very low. The real barriers to dispersal lie between caves, not between the surface and the cave.
5. Time since colonization is an essential parameter though difficult to measure for older cave species. Young populations are those with their direct ancestors or

sister species still present at the surface. The gene pools of young cave populations often show signs of multiple colonization events and/or recurring gene flow from the surface. This is compatible with the high propagule pressure hypothesis of successful biological invasions. Conversely, convincing molecular evidence for the genetic bottleneck hypothesis of cave colonization is lacking.

6. The dynamics of genetic diversity of old cave populations is poorly understood. Published studies suggest that it is shaped exogenously, by ecological influences from the surface (e.g., long-term groundwater fluctuations), and not by endogenous selective forces. These fluctuations might induce genetic bottlenecks in already established troglobiotic/stygobiotic species and leave similar genetic imprints as founder events. That the same genetic pattern can result from two fundamentally different processes remains a challenge for future investigations.

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Chapter 13
Caves as Oligotrophic Ecosystems

Ľubomír Kováč

13.1 Introduction

Box 13.1
Hypogean systems are usually relatively energy-limited (Poulson and Lavoie 2000). Most caves are *oligotrophic* with low amounts of available organic matter and low energy density. The trophic structure of these caves is less complex than that of the less common energy- and nutrient-rich *eutrophic* caves, which are characterized by larger amounts of animal faeces (such as bat guano) and plant debris transported into caves by floods and/or gravity. Longer caves, where sections differing in amounts of organic material may be recognized, are called *poecilotrophic* (Humphreys 2000a). Generally, food scarcity along with darkness seems to be the main factors shaping the selective regime in caves (Trontelj 2012).

Oligotrophic systems are environments with low amounts of trophic resources in the form of organic substances. The limited trophic base of such systems leads to low diversity and biomass and less developed biotic communities. Such systems occur in the Arctic, Antarctic and high-mountain tundra where harsh conditions result in very low primary production in both aquatic and terrestrial habitats. Similarly, oligotrophic systems are characteristic of subterranean environments that are relatively isolated from the surface, thus having very limited organic input. Chemolithoautotrophy by bacterial communities in oligotrophic systems is usually negligible. Despite the low organic supply resulting in less developed and less

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diverse biotic communities, oligotrophic cave systems have been in the focus of attention from the beginning of cave explorations. Recently, this attention has even increased as scientists study the composition of the biotic communities of oligotrophic caves, the peculiar adaptations of organisms to this extreme environment and the functional roles of different species in these systems. The focus of this chapter is primarily on cave passages that appear oligotrophic, while examples from food-rich or exceptional caves are included for comparison.

Organization of the trophic structure of oligotrophic caves largely depends on their connectivity and interactions with the epigean environment. Oligotrophic cave systems represent relatively isolated subterranean spaces without extensive aboveground connectivity. In most cases, there is a complicated structure with numerous links between the hypogean environment and its surrounding epigean one (Dunne et al. 2002; Romero 2009). The connectivity between epigean and hypogean environments is relatively high in limestone massifs, where the vadose zone plays an important role as an ecotone. Mostly vertical downwards movement of percolating water is characteristic of this zone.

There are two main routes of organic carbon input to caves: (1) localized flow through sinks and shafts, and (2) diffuse flow through soils, epikarst and the vadose zone. Oligotrophic caves, and their characteristic low-energy biota, are mostly associated with diffuse flow (Simon et al. 2007a). The food base for cave biota in nutrient-poor caves is mostly represented by organic matter transported by percolating water. This includes dissolved organic carbon (DOC), represented by complex molecules of fulvic and humic acids that are metabolized very slowly by actinobacteria. It also includes leached particulate organic matter (POM) that is of low quality and only slowly used (Poulson 2012). Transport of organic matter from the surface is limited, with the quantity progressively reduced with depth below the ground surface. The low amounts of organic matter in the remote and isolated parts of caves create a kind of biotope suitable for troglobionts, which are adapted to cope with low food resources (Deharveng and Bedos 2000, see also further text). In Organ Cave (USA) and Postojnska jama (Slovenia), Simon et al. (2007a) found that most of the organic carbon entering through the epikarst was DOC at concentrations averaging around 1 mg/L. Overall, the amount of DOC in percolation water depends on surface ecosystems and residence times of the water in soil, epikarst and the vadose zone. The amount of DOC in percolation water may be strongly reduced in arid areas such as Cape Range, Australia (Humphreys 2000b; Simon et al. 2007b). DOC and POM are further processed within caves, and probably in all voids of the vadose zone, with an important element for their transformation being microbial films on rocks—“epilithion” (Culver et al. 2012). In addition, percolating water mediates transport of living organisms into subterranean habitats, such as microorganisms, aquatic meiofauna and terrestrial microarthropods (Culver and Pipan 2009; see Chap. 14 for more information on this topic).

13.2 Food Variability and Communities of Biota

Food variability and predictability in caves are different for aquatic and terrestrial habitats. Abiotic input of food by water is represented by plant detritus degraded through the activity of surface decomposers and leaching by water, so bacterial and fungal decomposers are also much reduced. On the other hand, irregular and heavy floods can bring in unleached detritus that may be a food base for the cave communities for 10–100 years. Biotic input of organic material from trogloxenes moving into caves is more predictable, and, although it does not bring as much food, the quality is high (Poulson 2012).

Aquatic species are limited by food that is diluted and dispersed widely, whereas terrestrial species rely on food that remains concentrated in a local area. Intervals at which detritus is renewed at the base of the aquatic food web can be of the order of decades, so there is the selection for long life (Poulson 2012). According to Weber (2000), food scarcity is stronger in the terrestrial than in the aquatic cave habitats. In groundwater, low oxygen supply and food availability are overlapping aspects determining the development of adaptive strategies and distribution patterns of animals (Hervant and Malard 2012).

Generally, in deep subterranean systems food webs depend on diffuse and continual input of very small amounts of dissolved organic matter in percolating water. These cave sections are more oligotrophic and support a small number of species that tend to be long-lived troglobionts which are adapted to cope with low food resources (Deharveng and Bedos 2000; Poulson and Lavoie 2000). Specialized troglobionts can persist in very low-energy environments, being documented even from isolated spaces of the deepest vertical caves such as Lukina jama–Trojama (−1421 m) in Croatia (Bedek et al. 2012; Andersen et al. 2016) and Krubera–Voronja (−2140 m) in Abkhazia, Georgia (Sendra and Reboleira 2012).

Box 13.2

In subterranean environments, food input is not uniformly distributed in space or time. Cave animals share numerous adaptations to the relative food scarcity in their habitat. Hüppop (2012) distinguishes three aspects of food scarcity in caves. *General food scarcity* holds for nearly all caves and occurs especially in caves with limited food input. *Periodic food supply* characterizes caves that are regularly flooded or caves that regularly receive food input from visiting animals. During the wet season, food supply can be very high and even abundant for some weeks or months. After exhaustion of these food reserves, animals in such caves suffer food scarcity like animals in generally food-poor caves. Some cave animals have to cope with *patchy food scarcity*. This means that food is not necessarily limited but is difficult to find and exploit. Under such conditions, cave organisms can be observed aggregated at patchy food resources.

Oligotrophic caves occur in bedrock of all types, i.e. limestones, volcanic (lava tubes) and ferruginous rocks (see Chaps. 17 and 21), and also sandstones. Recent exploration in Venezuelan tepuis, table-top mountains in the Guiana Highlands of South America, provided surprising discoveries of caves and cave systems in quartzite rocks that were formerly assumed to be insoluble substrate. These caves, mainly created by the water currents submerging from the upper plateau and flowing through the tectonic faults, are very scarce in food. Orthoquartzite is characterized by poor buffering capacity, when compared to carbonates, and limited nutrient availability. Barton et al. (2014) observed a significant microbial activity on exposed surfaces within a quartzite cave on Roraima Tepui that appeared to be linked to a stream flowing through the cave. Biospeleothems were described in several Venezuelan tepui caves (Aubrecht et al. 2012), along with a few cave animals such as the troglophilic orthopteran *Hydrolutos breweri*, numerous individuals of which were observed in Cueva de Charles Brewer, one of the greatest quartzite cave systems in Venezuela (Derka and Fedor 2010).

Oligotrophic conditions may be also associated with hypogenic caves (see also Chap. 2). These caves are usually created by the ascending flow of reactive fluids, characterized by deep-seated solutional aggressiveness in soluble lithologies. Hypogenic caves may be also the result of the circulation of meteoric groundwater flow in the uppermost zones of the earth crust. Hypogenic karst thus originates from void-conduit systems occurring at depth and evolves without direct geological linkage with the surface (Palmer 2011; Klimchouk et al. 2017). Hypogenic caves developed by fluids with high concentrations of carbon dioxide, but without sulfidic emanations, are very poor in trophic sources. Such caves are isolated deeper underground and may represent larger networks of fissures and voids or great chambers. Based on the first zoological observations carried out in the eastern Iberian Peninsula by Sendra et al. (2014), it appears that this type of hypogenic cave has a depauperate fauna, consisting of non-cave adapted species. This composition is the result of limited opportunity for faunal colonization during and after genesis of the hypogenic habitats because of their isolation from the surface, i.e. the lack of connection between voids and the surface during the entire history of this type of cave.

13.3 Microorganisms in Oligotrophic Caves

Many caves appear to represent an essentially oxidized and nutrient-limited environment. Consequently, any subaerial microorganisms in these systems likely subsist by scavenging sparse nutrients (Barton et al. 2004, 2007; see also Chap. 5). Cave microorganisms use varying metabolic pathways to obtain nutrients and gain energy. In *autotrophs*, carbon for cellular growth originates by converting inorganic carbon (CO_2, HCO_3^-) to organic carbon. In contrast, some microorganisms, including most fungi, have *heterotrophic metabolism* and use organic compounds for most or all of their carbon requirements. The physiological mechanisms for capturing chemical energy are diverse, and the distinction between a chemosynthetic and a photosynthetic organism is based on whether the initial source of energy is from inorganic chemicals (litho) or light (photo). Microbes that gain energy through chemosynthesis and fix inorganic carbon are *chemolithoautotrophs*. Some microbes are *mixotrophs*, in which both chemolithoautotrophy and heterotrophy are expressed simultaneously. Microorganisms that gain cellular energy from chemical transformations but use organic carbon compounds for their carbon source are *chemoorganotrophs* (Engel 2012).

Cave microbiota include Bacteria, Actinobacteria, Archaea and Fungi and more rarely some algae and Cyanobacteria. Bacteria often establish their colonies in caves without evident sources of organic material (Northup and Lavoie 2004). Moist surfaces of cave walls and speleothems are colonized by microorganisms in the form of biofilm. In this way, microorganisms contribute significantly to both the creation of speleothems and the erosion of limestone (Cuezva et al. 2009).

Subterranean microbial communities were originally considered to be a subset of soil communities flushed underground by meteoric drip waters, surface streams and air currents or carried by animals into caves. Because of oligotrophic conditions, microbial biomass in caves was considered to be low and insufficient to impact most geological or geochemical processes (Engel 2012). The studies from recent decades, using modern methods for detecting microbial forms and revealing their metabolism pathways, have brought much deeper insight into cave microbial communities in oligotrophic caves and revealed more complex systems than previously thought. Many of the cave microbes are genetically divergent from surface microbes and adapted to the aphotic and oligotrophic cave environment. It was shown that in nutrient-poor caves heterotrophic bacteria dominate the microbial community, accompanied by a number of chemoautotrophs. Bacteria present under the oligotrophic environment of caves survive using complex metabolic pathways (Ortiz et al. 2014; Oliveira et al. 2017; De Mandal et al. 2017). Thus, despite being starved of nutrients, caves contain surprisingly diverse microbial communities. Chapters 5 and 6 provide more details about the diversity of microorganisms in caves.

In addition to having values in their own right, microorganisms play a very important role in subterranean ecosystems as a major food source for other, more complex organisms. Microorganisms stand at the base of the subterranean food web (see further text).

13.4 Faunal Communities in Oligotrophic Caves

In contrast to assemblages of guano and other energy-rich microhabitats (flood debris, root mats) with rich communities of non-troglobiotic/stygobiotic species, oligotrophic microhabitats are characterized by high proportions of the community being troglobiotic or stygobiotic. In Southeast Asia, troglobionts are mainly found foraging around scattered micropatches of food resources, mostly dispersed faeces of swiftlets, bats or rhaphidophorid crickets, and never colonize guano accumulations. Stygobionts are found in subterranean water flows and pools, in phreatic and percolating waters and more rarely in surface streams which have been filtered through fine boulders. They are usually absent in large tropical sink-resurgence systems, where epigean fauna is often abundant (Deharveng 2004a). This is, however, not the case of some large temperate hydrological systems, such as Postojna-Planina Cave System, which hosts 62 stygobionts (Sket 2012). The pattern of high proportions of troglobionts and stygobionts being associated with oligotrophic habitats is not universal, since a high proportion of troglobionts may sometimes be associated with high amounts of organic debris. For example, root mats often support diverse community of troglobionts, as is the case of Bayliss Cave, a lava tube in North Queensland, Australia (Howarth and Stone 1990; see also Chap. 3).

The food supply in tropical and subtropical caves is richer than in temperate ones because of the higher production of biomass in the tropical biomes (Poulson and Lavoie 2000). As a consequence, selection pressure can be expected to be weaker, the rate of evolution slower and the occurrence of troglobionts limited when compared to caves with low energy input such as temperate ones (Hüppop 2012; see also Chap. 4). However, as emphasized above, patterns are not universal and the rarity of troglobionts in tropical caves may be only the result of sampling error; very few tropical caves have been appropriately sampled.

In spite of the different nature and origin of the various sources of nutrients, the composition of terrestrial troglobiotic communities is rather similar in all oligotrophic habitats for a given underground system, provided that humidity is high (see also Chap. 3). The amount, rather than the nature, of the food supply seems to control the presence of troglobionts and the composition of their assemblages. In most cases, troglobiotic faunas have fewer species than guano communities. In tropical oligotrophic caves, larger predators, such as amblypygids and scorpions, are mostly absent, probably because of the low density of potential prey (Deharveng and Bedos 2000), although some exceptions are found among scorpions (see Chap. 7). Most primary consumers are insects, while predators are arachnids, mainly small spiders. Most of these species exhibit some degree of troglomorphy, particularly the Collembola (Deharveng and Bedos 2000).

13.5 Morphological and Biological Adaptations of the Cave Fauna

In caves, food scarcity acts as a selective force and requires evolutionary adaptations (Deharveng 2004b; Trontelj 2012). Food scarcity in caves is associated with development of morphological adaptations, so-called troglomorphisms (Christiansen 1965, 2012; see also Chap. 4) or more appropriately troglobiomorphisms (Juberthie 1984). The most obvious morphological modifications are longer legs and antennae in arthropods and fins and barbels in fishes. Elongated appendages combined with enlarged or multiplied sensory areas may improve or change food-finding ability and foraging behaviour of animals in hypogean environments through better sensory perception. Thus, cave animals can detect food faster and at a greater distance from their bodies than their epigean relatives and spend less energy searching for food. As a consequence, when foraging, they have abandoned shoaling and aggregation behaviour and adopted a continuous moving mode to cover a greater area. The benefits of shoaling behaviour in terms of reduced predation risk are lacking in most cave fishes (Timmermann et al. 2004; Hüppop 2012). In contrast to the situation in caves, movement through an interstitial medium is presumably facilitated by shortened appendages and elongated bodies that some troglobionts and stygobionts possess (Hüppop 2012). Reduced scales in stygobiont fish and reduced cuticle in troglobionts are other characteristic traits of cave animals. Weber (2000) argued that the body size of stygobiotic fishes and salamanders, which are usually small in comparison to epigean relatives, is an adaptation to the low food supply. However, the considerable reduction in morphological features is often correlated with the presence of the large amounts of stable food resources. And finally, food scarcity may trigger a switch to individuals with greater dispersal ability (see Chap. 4 for more details).

Special morphological traits have evolved in the inhabitants of moist hygropetric habitats (see also Chap. 3). Conspicuously, troglobiotic leptodirine beetles of the genera *Tartariella*, *Hadesia*, *Nauticiella*, *Radziella* and *Cansiliella* occupy such places in caves of the Eastern Alps and Balkan Peninsula. The hygropetric leptodirines have enlarged and curved claws, and modified mouthparts, characterized by a marked setation and by knee-shaped mandibles. The modified mouthparts are an adaptation for filtering organic particles from the percolating water. A similar set of morphological traits occurs in semiaquatic Diplopoda and in some aquatic crustacean genera of the families Asellidae and Niphargidae (Moldovan et al. 2004; Sket 2004). Another set of morphological modifications for hygropetric habitats is found in the troglobiotic collembolan *Pygmarrhopalites aggtelekiensis*, which is endemic to the Western-Carpathian caves. It has significantly extended antennae and legs with elongated claws that are adapted to move over the slippery surfaces of sediments and speleothems covered with a percolating water film (Kováč et al. 2014). The same habitat is occupied by the strikingly troglomorphic collembolan *Disparrhopalites tergestinus*, found in a single cave in the Eastern Alps (Italy) and *Disparrhopalites* sp., which occurs in several caves in Croatia and Bosnia and Herzegovina and has extremely long antennae.

An extreme case of adaptation to oligotrophic habitats is the chironomid dipteran *Troglocladius hajdi* discovered in Lukina jama–Trojama Cave System in Croatia at 980 m depth. The combination of strongly reduced eyes and large, broad wings is unique and might indicate that the species is able to fly or hover in the total darkness of the cave (Andersen et al. 2016). In vertebrates, there are also extreme cases of adaptations to low food in underground streams. These include the waterfall climbing loach *Cryptotora thamicola*, a troglobiotic fish distributed in a few caves of northern Thailand (Trajano et al. 2002; Fenolio 2016), and the climbing troglophilic catfish *Chaetostoma microps* from Ecuador (Hoese et al. 2015). The loach lives out of water for extended periods, using its well-developed pectoral and pelvic fins to crawl out of the water to feed on bacteria growing on damp rocks; the caves where this species lives often have elevated concentrations of carbon dioxide gas.

Besides a general food scarcity, many cave animals are faced with temporal periodicity of food. The inputs of food in oligotrophic caves may be seasonal, based on periodic flooding, or result from animals, such as bats, visiting the cave sporadically. Hence, cave animals need the ability to survive long periods of starvation, which may be the capacity to accumulate and store large energy reserves as a way of coping with food scarcity (see also Chap. 4). Cave animals can enable their young to survive periods of food shortage by producing eggs with more yolk (Thibaud and Deharveng 1994; Hüppop 2012). This phenomenon has been studied in many invertebrates, such as amphipods, decapods, collembolans and beetles, as well as in salamanders and fishes.

A reduced energy demand is also highly adaptive in caves with limited food sources, and many cave species exhibit striking energy economy (Hüppop 2012). Poulson (1963) demonstrated a decreasing trend in the metabolic rate from the epigean species of the Amblyopsidae family through troglophiles to gradually more cave-adapted troglobionts. The body temperature of troglobionts, which are exclusively ectotherms, is determined primarily by passive heat exchange with its environment. Thus, troglobionts do not spend energy for thermoregulation. Owing to mostly low metabolic rates, the troglobionts are successful in zones with limited resource supplies (food, oxygen, water). Reduction of metabolic rate is associated with prolonged lifetime and iteroparity (multiplied reproduction) (Hüppop 2012).

Another adaptation is that most cave animals exhibit feeding generalism, or dietary shift, compared with surface relatives (Hüppop 2000, 2012; Gibert and Deharveng 2002; Moldovan 2004). To cope with the general food scarcity in caves, the life history of terrestrial and aquatic animals changes towards K-selection and limited energy loss in the relatively predictable and stable habitats. K-selected species have low or no population growth, a reproductive strategy based on fewer but larger eggs, increased hatching time, prolonged larval stage, decreased growth rate, delayed reproduction, iteroparity and increased longevity (Thibaud and Deharveng 1994; Hüppop 2000, 2012). Other features enhancing energy economy include late hatching, protection of eggs and embryos, viviparity and dormancy during dry or cold periods (tardigrades, nematodes). In addition, some aquatic organisms are able to regulate their relatively low oxygen consumption (Albuquerque and Coineau 2004). In amblyopsid fishes, Poulson (1963) observed that population growth rate and density of amblyopsid fishes decrease with phylogenetic age of the cave species, and the population structure shifts towards adults.

Food scarcity and stability of the environment also help explain the tendency for paedomorphosis and neoteny (retention of larval characters) in some subterranean vertebrates (Culver 1982). However, neoteny was also observed in a food-abundant cave microhabitat, namely in tree root mats in Hawaii Island lava caves occupied by cixiid planthopper *Oliarus polyphemus* that have evolved from an ancestor by retaining the beneficial nymphal characters to adulthood (Howarth 1993). Besides the morphological features that characterize this retardation of somatic development, some anatomical changes have been observed in cave fish and amphibians, including reduced muscles and ossification (Moldovan 2004).

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Chapter 14
Food Webs in Caves

Michael P. Venarsky and Brock M. Huntsman

14.1 Introduction

A fundamental goal of ecology is to understand the factors influencing spatial and temporal patterns of biodiversity. A central tool in these efforts is the quantitative and/or qualitative description of food webs (see Hall and Raffaelli 1991; Ings et al. 2009; Baiser et al. 2013). Food webs can be used to visualize how energy and materials flow through ecosystems and are complex adaptive systems, meaning that they represent the emergent properties governing community dynamics (Power and Dietrich 2002; Shurin et al. 2006). Food web studies, for example, have identified multiple mechanisms that influence population demographics and/or community structure, including bottom-up versus top-down forces (Power 1992), donor-controlled resource dynamics (Polis and Strong 1996), and the importance of both grazer- and detritus-based energy pathways in supporting community productivity (see Moore et al. 2004; Shurin et al. 2006). In this chapter, we explore cave food webs to elucidate the potential mechanisms that structure these communities. Given the fundamental differences in energy sources (see Box 14.1), we discuss detritus-based and chemolithoautotrophically based cave ecosystems separately.

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Box 14.1
Two types of food webs can be found in cave ecosystems, those based on detritus (dead animal or plant matter) and those based on chemolithoautotrophic bacteria. While each of these food webs is ultimately supported by autotrophic primary production, the pathway by which inorganic carbon (carbon dioxide or bicarbonate) is transformed into organic compounds (prokaryotic and eukaryotic biomass) differs. Detritus-based cave ecosystems are supported via classic photosynthetic pathways, whereby plants and algae utilize solar light energy to transform carbon dioxide into the primary producer biomass that supports cave consumer productivity via detrital inputs. In contrast, chemolithoautotrophically based cave ecosystems are supported by bacteria that acquire energy via the oxidation of inorganic compounds, including sulfide (most common), methane, ammonium, iron, and manganese. This energy is then used to transform carbon dioxide or bicarbonate into the microbial biomass that supports cave consumer productivity (for details see Sarbu 2001; Engel 2007; Madigan et al. 2010).

14.2 Detritus-Based Cave Ecosystems

Detritus-based cave ecosystems rely on detritus (dead animal or plant matter and associated microbial decomposer assemblages) imported from surface environments to support in situ biological productivity (Poulson and Lavoie 2001; see also Box 14.2). These types of cave ecosystems are donor-controlled, meaning that consumers (bacteria to top predators) do not influence the rate of detritus input (Polis and Strong 1996). This contrasts with food webs in surface environments that are supported by primary producers, where consumers at all trophic levels can either directly (top-down control) or indirectly (trophic cascade) influence the quantity, quality, and productivity of the primary producers (algae, macrophytes, and terrestrial plants; Hairston et al. 1960; Fretwell 1977; Oksanen et al. 1981; Power 1992). While detritus-based food webs are common in surface environments (forested headwater streams and leaf-litter communities; Chen and Wise 1999; Wallace et al. 1999), caves are unique by having relatively weak connections to their surface donor habitats, which ultimately limit the detrital inputs that fuel cave productivity (see Moore et al. 2004 for a review of detritus in ecosystems processes).

Box 14.2
When detrital inputs/availability is compared among detritus-based ecosystems, caves appear to anchor the extreme low end of the detrital spectrum, while surface ecosystems occupy the opposing high end (Venarsky et al. 2018). Thus, the structure and productivity of detritally based cave communities are strongly influenced by the factors that control the type, quantity, and

(continued)

Box 14.2 (continued)
quality of detritus inputs (see Moore et al. 2004 for a review of detritus in ecosystem processes).

In this section we begin by discussing the input dynamics of dissolved and particulate organic matter and then examine the role of microbial communities in transferring these detrital resources to higher trophic levels. We end this section with an examination of how detritus inputs influence various aspects of cave community structure, including biodiversity, taxa abundance, and community productivity.

14.2.1 Dissolved Organic Matter

Dissolved organic matter (DOM) dominates detritus budgets in both cave and surface ecosystems (Graening and Brown 2003; Simon et al. 2007; Tank et al. 2010). DOM originates in the soil horizons from the breakdown and leaching of plant organic matter, exudates from plant roots, and the by-products of microbial metabolism (Schiff et al. 1997; Birdwell and Engel 2010; Simon et al. 2010). The concentration of DOM decreases as water percolates through the soil horizon and the complex matrix of fissures within the vadose zone (Baker and Genty 1999; van Beynen et al. 2000; Pabich et al. 2001; Tatár et al. 2004; Simon et al. 2010). Upon reaching the phreatic zone, DOM concentrations decrease further with increasing depth below the groundwater table (Pabich et al. 2001; Datry et al. 2005; Foulquier et al. 2010). The composition of DOM also changes along transport pathways, with concentrations of humic, fulvic, and large molecular weight compounds generally decreasing from the soil horizons to the groundwater table (Baker and Genty 1999; van Beynen et al. 2000; Simon et al. 2010). These changes to the DOM detritus pool are due to both biotic and abiotic processes, including microbial consumption, sorption to clay, and flocculation (see Schiff et al. 1997).

The complex hydrologic setting in karst systems (see Bakalowicz 2005) can strongly influence DOM inputs to caves. Water flows relatively slowly through the soil horizons and vadose zone, causing the DOM to be in contact with biologically active surfaces (biofilms on soil particles and rock surfaces) for extended periods of time. In contrast, the rapid transport of water through large conduits (large fissures and sinking streams) limits the time that water is in contact with microbial biofilms, which potentially limits DOM consumption (Simon et al. 2010). These differences in transport pathways were suggested to influence DOM concentration and composition between water from drips (i.e., from soil horizon and vadose zone) and a cave stream (i.e., from large conduits). Furthermore, DOM concentration and composition can differ among epikarst drips within the same cave, indicating that large amounts of variability in DOM inputs are also found at much finer spatial scales

(Ban et al. 2008; Simon et al. 2010). Thus, the heterogeneity of water flow paths at both small and large spatial scales influences DOM inputs.

DOM inputs are also influenced by local precipitation patterns. Several studies have reported that high concentrations of DOM are flushed from soil horizons and the vadose zone during storms (Baker and Genty 1999; van Beynen et al. 2002; Tatár et al. 2004; Datry et al. 2005; Tissier et al. 2013; Foulquier et al. 2010). These changes in DOM concentration caused by fluctuating hydrologic conditions are also accompanied by shifts in DOM composition (Simon et al. 2010; Tissier et al. 2013). DOM inputs to cave ecosystems are thus likely influenced by complex antecedent hydrological conditions, including the length of time between and the intensity of each precipitation event (see Baker and Genty 1999; van Beynen et al. 2002; Tissier et al. 2013; Shabarova et al. 2014).

14.2.2 Particulate Organic Matter

Particulate organic matter (POM) comes in various forms, including plant material (leaves, wood, fruit, seeds), guano, and animal carrion. Measures of quality [commonly reported as carbon/nitrogen/phosphorus (C/N/P)] ratios can differ greatly among types of POM but follow the general pattern: plant-POM < guano < animal carrion (see Sterner and Elser 2002; Cross et al. 2005 for consumer-resource C/N/P comparisons). Importantly, quality can vary within each POM type. For example, leaf litter is higher in quality than wood debris, and leaf quality can differ significantly among and within plant species (Webster and Benfield 1986). Litter quality also changes throughout the stages of decomposition, generally increasing in the early stages of decomposition and then decreasing in quality during the later stages (Webster and Benfield 1986). Similar to leaf litter, differences in diet can cause guano quality to vary considerably among animal species, with bat diet (frugivorous, hematophagous, and insectivorous) strongly influencing guano C/N/P ratios (Shahack-Gross et al. 2004; Emerson and Roark 2007; Wurster et al. 2015). While we are unaware of studies that have reported C/N/P ratios for cave cricket guano, their herbivorous feeding habits suggest that their guano would be of lower quality than bat guano (Lavoie et al. 2007).

In most cave ecosystems, POM is a small portion of the detrital budgets (Graening and Brown 2003; Simon et al. 2007). However, the type and quantity of POM inputs can vary substantially among caves within the same geographic region (Simon and Benfield 2001; Huntsman et al. 2011a; Venarsky et al. 2012a). The primary factor that appears to control POM inputs is the number and size of large conduits (cave entrances and large fissures) in the vadose zone that connects caves to surface environments. For example, large quantities of plant-POM can accumulate in cave passages near entrances, pits, large fissures, and inflowing and sinking streams (Simon and Benfield 2001; Souza-Silva et al. 2011, 2012; Venarsky et al. 2012a). Cave pits can trap animals (rodents, snakes, frogs, deer, and turtles), while large cave entrances provide access to bat roosting habitats where significant amounts of guano can accumulate. Additionally, large root mats can form in caves as plant roots snake through the maze of fissures in the vadose zone in search of water (Jasinska et al. 1996; Eberhard 2004; Howarth et al. 2007).

The strength of connection to surface environments also appears to dictate temporal patterns in POM inputs. Souza-Silva et al. (2011, 2012) examined inputs of animal- and plant-POM in neotropical caves that have strong connections to surface environments (e.g., inflowing stream and multiple cave entrances), finding that POM inputs were six times higher during the rainy season compared to the dry season. Input rates of bat guano can also substantially fluctuate throughout the year due to bat migrations. Iskali and Zhang (2015) reported that monthly guano deposition rates ranged from 66 to 11,000 kg, with the highest deposition rates occurring when the maternity bat colony was present (20 million individuals). In contrast, several cave stream studies in the eastern United States found that plant-POM standing stocks varied little throughout the year (Simon and Benfield 2001; Venarsky et al. 2012a, 2018). While not a direct measure of input rate, these studies suggest that plant-POM inputs were constant on an annual basis.

14.2.3 The Gatekeeper: The Role of Microbes in Detritus-Based Cave Food Webs

Box 14.3

While caves receive a diverse array of detritus inputs, the DOM and plant-POM that dominate most cave energy budgets do not directly support invertebrate or vertebrate consumer growth and reproduction. Instead, these low-quality detrital resources are first converted into higher-quality microbial biomass by bacteria and fungi (Cummins and Klug 1979; Simon et al. 2003).

Detritally based microbial communities support the entire food webs (small crustaceans to vertebrate predators) in both cave and surface ecosystems (Hall and Meyer 1998; Simon et al. 2003, 2007; Graening and Brown 2003; Venarsky et al. 2018; see also Box 14.3). Cave microbial communities are composed of two groups, bacteria and fungi. Both bacteria and fungi contribute to the decomposition of plant-POM, with fungi typically dominating microbial biomass in the early stages of leaf litter decomposition, followed by bacterial dominance in the latter stages (Webster and Benfield 1986; Simon and Benfield 2001). In contrast, bacteria appear to dominate the uptake of DOM, transforming these resources into both bacterial biomass and a porous polysaccharide matrix that is the structural backbone for biofilm development (Hall and Meyer 1998; Simon et al. 2003; Madigan et al. 2010). The relative importance of bacteria and fungi among cave habitats, ecosystems, or consumers remains poorly understood because bacteria and fungi are generally lumped into a single “microbial” trophic level. However, two lines of evidence suggest that cave macroconsumers (i.e., large-bodied consumers such as insects or amphipods) likely rely on bacteria more than fungi. First, the bacterial uptake of DOM can support the entire cave food webs (Simon et al. 2003). Second, plant-POM is an ideal substrate for the growth of fungi (Webster and Benfield 1986), but the low quantity of plant-POM in most cave ecosystems likely minimizes the role of fungi in cave food webs. However, the role of fungi could be disproportionately important compared to bacteria as some aquatic surface species will selectively feed on fungi over bacteria (Kostalos and Seymour 1976; Arsuffi and Suberkropp 1989). Thus, studies that explore the relative roles of bacteria and fungi will provide a more nuanced understanding of the role of "microbes" in cave food web dynamics.

Some detritus inputs can be directly assimilated by macroconsumers and thus do not require a microbial intermediate. Animal carrion is a high-quality resource that may quickly attract high numbers of macroconsumers and is often used in both aquatic and terrestrial bait traps for cave survey studies (Hunt and Millar 2001; Huntsman et al. 2011b; Schneider et al. 2011). Several studies have reported that animal guano can be directly consumed and assimilated by macroconsumers (Salgado et al. 2014). The most interesting of these studies is Fenolio et al. (2006) who reported that a population of cave salamanders was partially supported by the direct consumption and assimilation of bat guano. However, microbial colonization of guano can likely increase the quality of some guano types, such as herbivorous cave crickets that likely produce low-quality guano (Lavoie et al. 2007).

14.2.4 Detritus Quantity

Detritus-based cave food webs are broadly similar to their surface counterparts (e.g., soil habitats and forested headwater streams) in that caves support three broad trophic levels: detritus and associated microbes, primary consumers, and predators (Gers 1998; Simon et al. 2003, 2008; Graening and Brown 2003; Souza-Silva et al. 2013). Classic bottom-up forces (i.e., limited detrital quantity) are thought to control community structure and productivity within each trophic level (Poulson and Lavoie 2001; Hüppop 2001). This hypothesis is broadly supported by observational and experimental studies. Bacterial productivity, for example, can be stimulated by increased DOM availability (Cooney and Simon 2009; Chelius et al. 2009; Foulquier et al. 2010, 2011a, b), and invertebrate and vertebrate consumer abundance is positively correlated with organic matter availability (Humphreys 1991; Datry et al. 2005; Fenolio et al. 2006, 2014; Huntsman et al. 2011a; Venarsky et al. 2014, 2018). Rapid increases in organic matter availability have also been linked to broad shifts in community composition (Sinton 1984; Smith et al. 1986; Madsen et al. 1991; Notenboom et al. 1994; Simon and Buikema 1997; Sket 1999, 2005; Wood et al. 2002, 2008; Culver and Pipan 2009; Venarsky et al. 2018). However, this appears to be a relatively simplistic view of the mechanisms that structure detritus-based cave food webs. Rather, we suggest that cave communities are structured through a complex array of bottom-up forces where detrital quantity interacts with other factors, including the strength of surface connectivity, size of detrital surpluses, spatial distribution of detrital resources, and consumer-resource stoichiometry.

14.2.5 Surface Connectivity, Detritus Surpluses, and Spatial Distribution of Detritus

The strength of surface connectivity appears to be one of the most important factors that can interact with detritus quantity to structure cave communities. While surface connectivity affects detritus input rates (see above), it also controls the migration rate of surface species (sensu troglophile and trogloxene; Humphreys 2001) into cave ecosystems. Surface and obligate cave species (sensu troglobite; Humphreys 2001) have distinct biological traits that have been shaped by their respective evolutionary histories. Obligate cave species have low metabolic rates, increased starvation resistance, and K-selected life-history traits (long life span, slow growth rate, reduced fecundity; see also Chap. 4), which are presumed adaptations to the energy-limited cave environment (Hüppop 2001; Venarsky et al. 2012b; Craig 2013). In contrast, surface species are adapted to survive in energy-rich surface environments and thus have more r-selected life-history traits, including fast growth rates and high fecundities (Hüppop 2001). Surface species often dominate community biomass and biodiversity in caves with little or no anthropogenic influences (e.g., low pollution; Ferreira et al. 2000; Souza-Silva et al. 2011, 2012; Venarsky et al. 2012a, 2018; Pellegrini and Ferreira 2013) and are thus important in several ecosystem processes, including organic matter decomposition (Brussock et al. 1988; Galas et al. 1996; Simon and Benfield 2001; Venarsky et al. 2012a), predator productivity (Huntsman et al. 2011a; see also the Glossary for more explanations on productivity), and food web energetics (Huntsman et al. 2011a; Venarsky et al. 2014, 2018). Given the proper environmental conditions (temperature and food resources), many vertebrate (salamanders) and invertebrate (crayfish, amphipods, isopods, insects, arachnids) surface species are capable of completing their life cycle in caves. Furthermore, large portions of cave microbial communities have surface origins (e.g., soil and streams; Chelius and Moore 2004; Griebler and Lueders 2009; Engel 2010).

Two factors appear to allow obligate cave and surface species to coexist: the size of the detritus pool and its spatial distribution. In detritus-based surface ecosystems (e.g., soils, forested headwater streams, and large rivers), detritus inputs generally exceed consumer demand (Smock and Roeding 1986; Wallace et al. 1999; Hall et al. 2000, 2001; Stagliano and Whiles 2002; Cross et al. 2007), leaving a large pool of surplus detritus that is either exported from (e.g., streams) or stored in (e.g., soils and floodplains) the ecosystem. This is in contrast to some cave ecosystems, where nearly all resources (detritus and detritivorous prey) are consumed (Venarsky et al. 2014). The near-complete consumption of all detrital resources creates an energy-limited environment, where little surplus of detritus is available to support additional cave macroconsumer productivity. Thus, obligate cave species, with their adaptations to energy limitation, have the competitive advantage in cave ecosystems where detrital surpluses are limited. However, rapid increases in detrital availability cause a corresponding increase in the detrital surplus pool which transfers the competitive advantage to the surface species that are better adapted (higher growth rates and fecundities) to exploit the newly available energy (Venarsky et al. 2014). This is the likely mechanism that is responsible for the extirpation of obligate cave species following organic pollution episodes (Sinton 1984; Smith et al. 1986; Madsen et al. 1991; Notenboom et al. 1994; Simon and Buikema 1997; Sket 1999, 2005; Wood et al. 2002; Culver and Pipan 2009). Venarsky et al. (2018) recently provided experimental support for this mechanism using a stream-reach scale litter amendment experiment, which showed that surface species responded strongly to litter amendment, while no response was seen in obligate cave species.

While the total amount of energy within caves influences the outcomes of competitive interactions among obligate cave and surface species, the spatial distribution of energy throughout a cave ecosystem likely dictates if obligate caves species can persistently coexist with surface species. Cave ecosystems with large bat colonies can contain large deposits of guano that support high abundances of both guano specialist taxa (i.e., exclusively inhabit guano) and guano generalist taxa (i.e., inhabit plant and animal detritus; Gnaspini and Trajano 2000) (Ferreira et al. 2000; Gnaspini and Trajano 2000; Iskali and Zhang 2015). However, measures of species abundance and biodiversity decrease with the increasing distance from guano piles (Pellegrini and Ferreira 2013), indicating the influence of guano on cave community structure is spatially restricted. Similarly, the litter amendment and control (i.e., no litter amendment) reaches in Venarsky et al. (2018) were separated by <25 m, but the control reach contained ambient species densities, while the litter amendment reach had significantly higher densities of surface species.

14.2.6 Nutrients and Detrital Quality

Our discussion has thus far approached the topic of detritus inputs and cave community structure from a strictly energy- or organic carbon-based perspective. However, the growth and reproduction of consumers in detritus-based ecosystems are controlled by several elements (carbon, nitrogen, phosphorous, calcium, potassium) and compounds (fatty acids, vitamins), of which the inadequate supply of one or more can significantly limit consumer productivity (Sterner and Elser 2002; Torres-Ruiz et al. 2007). Two elements, nitrogen and phosphorus, have received considerable attention in the ecological literature, and their availability (forms include nitrate, nitrite, ammonium, orthophosphate, animal and plant detritus) has been directly linked to the productivity of bacteria, fungi, and invertebrate and vertebrate consumers (Sterner and Elser 2002; Cross et al. 2003, 2006, 2007; Johnson et al. 2006). To date, however, little attention has been given to the roles of inorganic nutrients and/or detrital quality (C/N/P ratio) in cave community dynamics.

In cave streams, dissolved inorganic nutrient concentrations can be relatively high and thus likely do not limit microbial productivity (Simon and Benfield 2001). Instead, DOM quality and/or lability appear to strongly influence microbial productivity in cave streams. Using microcosms, Cooney and Simon (2009) provided cave bacteria communities either high (glucose and soil and leaf extracts) or low (tannic acid) lability DOM, finding that bacterial productivity was only stimulated with the highly labile forms of DOM. In contrast to aquatic cave environments, microbial communities in terrestrial cave environments can potentially be co-limited by both detrital quality and nutrients. A study by Chelius et al. (2009) reported that microbial abundance increased with the separate additions of low (lint)- and high (nutrient-rich rat guano)-quality sources of POM. The strongest response, however, was found in combined lint-rat guano treatments which supplied large quantities of both carbon and nutrients. Interestingly, the productivity in terrestrial cave environments can be limited by factors other than carbon or nutrients (e.g., nitrogen or phosphorus). Humphreys (1991) conducted litter addition experiments in an arid Australian cave and found that macroconsumers only responded following the addition of water to the litter, suggesting that water availability was the primary factor limiting both microbial and total community productivity. Collectively, these studies suggest that cave stream microbial communities are limited by detrital quantity and quality rather than inorganic nutrient availability, while cave terrestrial microbial communities could potentially be limited by a multitude of interacting factors, including carbon, nutrient (e.g., nitrogen or phosphorus), and water availability.

To date, only one study has explicitly examined the role of detrital quality in structuring macroconsumer cave communities. Schneider et al. (2011) experimentally manipulated detrital quality in cave pits by supplying food webs with either high (rat carrion)- or low (leaf litter)-quality detritus. While species richness (i.e., troglophiles) did not differ among treatments, higher abundances of different species were present on the high-quality rat carrion. The response in this study bears some resemblance to the patterns in community structure observed in bat guano cave communities (Ferreira et al. 2000; Gnaspini and Trajano 2000; Pellegrini and Ferreira 2013; Iskali and Zhang 2015), suggesting that detrital quality plays an important role in structuring cave communities in bat guano caves. However, the results of these studies should be viewed within the context of surface connectivity, which is strong in both cave pits and bat caves.

Another approach to viewing the role of detritus quality in cave community dynamics is to examine consumer-resource stoichiometry (Sterner and Elser 2002). In detritus-based cave and surface ecosystems, macroconsumers (shredders and collector-gatherers) usually have much lower C/P and C/N ratios than either the plant-POM or epilithic biofilms that support their productivity (Cross et al. 2003; Schneider et al. 2011). These large stoichiometric imbalances indicate that P and N availability probably limits macroconsumer growth and reproduction. On short timescales, consumers can cope with these large imbalances by increasing consumption rates, selectively feeding on high-quality microbes, and dispersing to more suitable foraging habitats or through trophic omnivory (see Schneider et al. 2011). Prolonged exposure to these nutrient imbalances, however, can be a strong evolutionary force that selects for life histories (more K-selected) and physiologies (modified body C/N/P ratios; Elser et al. 2000; Fagan et al. 2002) that reduce the organism's demand for limiting nutrients. Thus, detrital quality is likely an important evolutionary driver in both cave and surface ecosystems (Elser et al. 2000; Fagan et al. 2002; Schneider et al. 2011; Craig 2013).

Adaptation to large stoichiometric imbalances appears to have occurred for some detritivorous insects, which have C/P and N/P ratios that are 2–3 times higher (which indicates lower nutrient demand) than other terrestrial or aquatic insects (Cross et al. 2003). The potentially lower quality of detritus resources in caves suggests that obligate cave species would likely have even higher C/N/P ratios than their surface counterparts (Schneider et al. 2011). Support for this hypothesis remains tentative. Detritus quality is rarely quantified in cave ecosystems, with recent studies suggesting that resource quality is similar among cave and surface ecosystems (Schneider et al. 2011; Craig 2013). Using different approaches, both Schneider et al. (2011) and Craig (2013) suggest that obligate cave species nutrient demand is lower than surface species but that large stoichiometric imbalance between obligate cave species and their food resources is a factor that likely limits the growth of obligate cave species.

14.2.7 Beyond Detritus

The discussion above outlined how bottom-up forces structure cave communities. However, several lines of evidence suggest that cave community structure is also under the influence of top-down forces. Similar to surface ecosystems (Pace and Cole 1994), microbial populations in both cave and groundwater environments can be strongly affected by the feeding activities of heterotrophic nanoflagellates and larger macroconsumers (Edler and Dodds 1996; Kinner et al. 1998; Sintes et al. 2004; Kinsey et al. 2007; Cooney and Simon 2009). Interestingly, Cooney and Simon (2009) found that amphipods influence bacterial production on rock surfaces but not in fine sediments, suggesting that substrate characteristics (e.g., fine sediments vs rock surfaces) dictate whether epilithic microbial biofilms are top-down controlled (e.g., Foulquier et al. 2010). Venarsky et al. (2014) found that nearly all detrital and prey resources needed to be consumed to support community productivity, which implies cave communities could simultaneously be under both bottom-up and top-down control.

14.3 Chemolithoautotrophically Based Cave Ecosystems

Our understanding of population and community dynamics, in both cave and surface ecosystems, has been built around the assumption that complex food webs can only be supported via photosynthetically derived organic compounds. This notion, however, was overturned in the 1970s and 1980s with the discovery of diverse consumer communities (grazers, filter feeders, and predators) surrounding geothermal deep-sea vents and in sulfidic caves (Engel 2007). These complex food webs are supported by chemolithoautotrophic primary production rather than classic photosynthetic primary production. Since their initial discovery, chemolithoautotrophically based ecosystems have been documented in numerous habitats, including phreatic aquifers and marine sediments (Engel 2007). In this section we begin with a general description of the factors influencing chemolithoautotrophic primary production in caves and then explore what factors structure communities in these especially harsh environments. We end with a discussion of cave food webs that are supported by both detrital- and chemolithoautotrophic energy pathways.

14.3.1 Chemolithoautotrophic Primary Production

Rates of chemolithoautotrophic primary production reported within caves (1.8–281 g C m^{-2} $year^{-1}$; Porter et al. 2009) fall within the lower range of global photosynthetic primary production estimates (<1 to ~10,000 g C m^{-2} $year^{-1}$; Cebrian and Lartigue 2004). Similar to how light availability limits photosynthetic primary production, the availability of an energy source also limits chemolithoautotrophic primary production. Porter et al. (2009) found that rates of chemolithoautotrophic primary production in four sulfidic caves were positively correlated with the concentration of hydrogen sulfide (energy source). Local redox conditions also influence chemolithoautotrophic primary production by dictating the amount of energy generated from the energy source (e.g., oxidation of inorganic compounds; Pohlman 2011). The highest energetic gains are attained when oxygen is present, with gains decreasing as other compounds are used as terminal electron acceptors (e.g., nitrate or sulfate; Madigan et al. 2010).

Other factors that limit photosynthetic primary production, such as inorganic carbon and nutrient (N and P) availability (Elser et al. 2000), do not appear to limit chemolithoautotrophic primary production in caves. Inorganic carbon in chemolithoautotrophic caves is abundant, being supplied via the dissolution of carbonate bedrock, oxidation of methane, or respiration (Sarbu 2001; Engel 2010). Inorganic nutrient availability in chemolithoautotrophic caves is not well documented, but high nitrogen content of chemolithoautotrophic bacteria suggests that nitrogen is not limiting productivity (Kinkle and Kane 2001; Sarbu 2001).

14.3.2 A Complex Story: The Role of Chemolithoautotrophic Primary Production in Structuring Cave Communities

Similar to our previous discussion of detritus-based caves, energy availability is not the only factor influencing community structure in chemolithoautotrophically based cave ecosystems. Redox conditions for chemolithoautotrophic primary production commonly include low oxygen and high hydrogen sulfide concentrations, which produces a toxic environment for most eukaryotes (Tobler et al. 2006; Engel 2007; Tobler 2008). Coping with these extreme conditions requires specific physiological and behavioral adaptations. Some taxa are capable of excreting hydrogen sulfide and have enlarged gills to allow more efficient extraction of oxygen from the low-oxygen environment (Tobler 2008). The Mexican cave molly (*Poecilia mexicana*), for example, uses a behavior called active surface respiration to occupy the air-water interface of some sulfidic caves where concentrations of oxygen and hydrogen sulfide are relatively low and high, respectively (Plath et al. 2007; see also Chap. 4). Both terrestrial and aquatic taxa have also been observed exploiting areas of chemolithoautotrophic primary production for only short time periods to minimize exposure to toxic conditions (Sarbu et al. 1996; Dattagupta et al. 2009). Interestingly, Dattagupta et al. (2009) described a symbiotic relationship between an amphipod and an epibiotic chemolithoautotrophic bacterium (*Thiothrix*) where the bacterium assists with detoxification. Undoubtedly, the harsh conditions in these caves strongly dictate community structure by acting as a filtering mechanism on regional species pools.

While most chemolithoautotrophically based cave ecosystems are considered energy-rich and can support a highly diverse and abundant community, the communities in these caves could still be energy-limited. Behavioral strategies to cope with the extremely harsh conditions in sulfidic caves are thought to be energetically costly. Plath et al. (2007) reported that Mexican cave mollies in sulfidic caves spend 73% of their time engaged in aquatic surface respiration, which leaves a small amount of time for foraging activities. Further exacerbating this issue are the increased energetic costs likely associated with the need to detoxify after hydrogen sulfide exposure (Plath et al. 2007; Tobler 2008). The increased energetic demands of living in this harsh environment are reflected in the physical condition of Mexican cave mollies, with populations in sulfidic caves having lower rates of fecundity and reduced body conditions compared with populations in non-sulfidic environments (Tobler 2008; Riesch et al. 2010). Whether these communities are living at a carrying capacity set by limited energy availability or the physiological demands of living in a harsh environment is an exciting avenue of future research.

14.3.3 The Linked Conceptual Model: Chemolithoautotrophically Based and Detritus-Based Ecosystems

In previous sections we discussed chemolithoautotrophically based and detritally based cave ecosystems separately. However, some cave and groundwater ecosystems are supported by both chemolithoautotrophic and detrital energy pathways (Pohlman et al. 1997; Sarbu 2001; Opsahl and Chanton 2006; Roach et al. 2011; Pohlman 2011; Neisch et al. 2012; Hutchins 2013; Tobler et al. 2013). These types of cave ecosystems are similar to many surface ecosystems in that they are simultaneously supported by both living autotrophic (photo- vs. chemoautotrophs) and detrital energy pathways. However, the role of detritus appears to differ between ecosystems. In surface ecosystems, photoautotrophic primary production can be spatially and temporally variable, while the large detrital pool is comparably constant and thus stabilizes food web dynamics (Moore et al. 2004). In contrast, detrital inputs to cave ecosystems are generally low and can fluctuate substantially on both spatial and temporal scales (see Sect. 14.2). Instead, chemolithoautotrophic production can provide a large, continuous input of high-quality resources to the base of cave food webs.

The presence of both detritus and chemolithoautotrophic energy pathways can strongly influence cave community structure. The heterogeneous redox conditions allow multiple microbial metabolic pathways to exist in sympatry, which increases microbial biodiversity (Pohlman 2011; Porter et al. 2009). The Edwards Aquifer in Texas (USA) offers an excellent example of how the presence of two energy pathways can influence macroconsumer community structure. Macroconsumer biodiversity is highest in areas where both detritus inputs and chemolithoautotrophic primary production support in situ productivity and lowest in areas where detrital inputs are the sole energy source (Hutchins 2013). The higher biodiversity near areas with both energy sources appears to be the result of trophic niche differentiation (Hutchins et al. 2014), where the increased productivity at the base of the food web creates more trophic niches for macroconsumers to exploit (Pianka 1966; Abrams 1995). High amounts of biodiversity have also been reported in several Mexican sulfidic caves whose communities are supported by both detrital and chemolithoautotrophic energy pathways (Engel 2007; Roach et al. 2011; Tobler et al. 2013). The cave communities appear to be dominated by surface species, indicating that the strength of surface connectivity plays a similar role in structuring communities in these types of caves as it does in detritus-based cave ecosystems (see Sect. 14.3.2).

14.4 The Donor Habitat: The Role of Surface Ecosystems in Structuring Cave Food Webs

Rates of photosynthetic primary production in surface environments are controlled by multiple factors, including floral community structure (see also Chap. 6), temperature, precipitation, light, and nutrient availability (Begon et al. 2006). Given that most cave ecosystems are partially supported by detritus inputs from surface ecosystems, the factors that govern primary productivity in surface donor habitats can influence cave community structure by modifying detritus inputs. Both local- and regional-scale patterns in obligate cave species biodiversity have been qualitatively linked to patterns in surface ecosystem productivity (see Culver and Sket 2000; Culver et al. 2003; Christman et al. 2005). Culver et al. (2004) found that measures of cave biodiversity were higher in caves at lower elevations and suggested that this was the result of increased surface productivity, which was assumed to be negatively correlated with elevation. Culver et al. (2006) observed that cave biodiversity in Europe and North America peaked in areas that corresponded with warm and wet regions which were assumed to have the highest rates of surface productivity (see also Chap. 9). To date, however, we are not aware of studies that have quantitatively linked cave productivity or biodiversity to rigorous estimates of primary or secondary (animal) production (g C m^{-2} $year^{-1}$) in surface ecosystems. While this is a difficult task, quantitatively linking cave ecosystem processes (food web and community structure and productivity) to both surface donor ecosystem productivity (land use, vegetation type, local climate; Goldscheider 2012) and the physical aspects of karst systems (soil type and depth, conduit size, depth, and distribution; Williams 2008; Tuttle and Stevenson 1977) would allow the identification of "hot spots" or "hot times" of cave productivity (sensu Simon 2008; Simon et al. 2008). Furthermore, these studies would provide a potential link between cave ecosystem processes and the most pressing problems of our time, global climate change and biodiversity loss.

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Part IV
Particular Cave Ecosystems

Chapter 15
Ice Caves

Ľubomír Kováč

15.1 Introduction

Ice caves and glacier caves contain perennial ice, representing unique types of oligotrophic subterranean habitats characterised by low temperature as well as low amounts of organic material. The broad topic of the ice caves is central in a recent comprehensive monograph by Perşoiu and Lauritzen (2018) that is recommended for further reading.

Glacier caves are formed in subpolar zones or high mountains by melting of the ice of glaciers and may be categorised as glacier pseudokarst. The caves form along the crevices in the ice and along its contact with bedrock, through the action of invading water that is usually derived from ice melting on the surface of the glacier. As glaciers melt, this water drains down into the glacier through fissures. This water then moves along the base of the glacier and, because it is slightly warmer than the freezing point of water, it gradually carves out long tunnels that open at the front of the glacier forming the rivers that drain the glaciers. Glacier caves are ice tunnels with floors of rock and walls and ceilings of ice. When the surface of the glacier is below freezing, the tunnels drain and become open to exploration. When the glacier is melting, the tunnels are often filled with water (White and Culver 2012). Glacier caves tend to be ephemeral, but some form quite large integrated systems, e.g. Paradise Ice Caves System in Washington State, USA, which extended for over 19 km. However, by the 1990s, its glacier had melted completely (Halliday 2004).

Contrary to glacier caves, ice caves can occur in various climatic zones, with a few occurrences in the hot arid climates, such as the Southwestern USA and central Turkey (Perşoiu and Onac 2012). However, the global distribution of ice caves,

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Fig. 15.1 Location of important ice caves in Europe. (1) Casteret Cave, Pyrenees (France); (2) Grotte de la Glacière, Jura Mts (France); (3, 4) Eisriesenwelt and Dachstein-Rieseneishöhle, Alps (Austria); (5, 6) Dobšinská and Silická ľadnica ice caves, Western Carpathians (Slovakia); (7, 8) Ghețarul Focul Viu and Ghețarul de la Scărişoara, Apuseni Mts (Romania); (9) Kungurskaya Ice Cave, Ural (Russia). Map source: http://www.gifex.com

containing perennial ice accumulations, is limited to the Northern Hemisphere, and their occurrence follows a general trend of decreasing altitude with increasing latitude. In Europe, ice caves are found generally at mid-altitudes, where temperatures are below freezing for a long-enough period that (1) dripping water will freeze to ice and (2) summer warming will be insufficient to completely melt the accumulated ice (Perşoiu 2018). Two major types of ice are recognised in ice caves: firn and congelation ice. Firn ice results from the regelation of snow accumulated at the base of the cave entrance during the winter season. Congelation ice develops preferentially during spring, when exterior snowmelt and precipitation result in the infiltration of water which refreezes in the cave (Luetscher and Jeannin 2004).

Many caves in European mountains at altitudes over 1500 m harbour snow and ice deposits whole year in their entrance sections (Pyrenees, Alps, Carpathians, Dinarides and others). Well-known sites, with high volumes of ice, are Dobšinská Ice Cave in Slovakia, Ghețarul de la Scărişoara (see details for these two caves at Sect. 15.5) and Ghețarul Focul Viu in Romania, and Eisriesenwelt and Dachstein-Rieseneishöhle in Austria (Fig. 15.1). Eisriesenwelt is a multiple-entrance dynamic cave system with a classical bidirectional airflow pattern developed in the Upper Triassic Dachstein Limestone in the Tennengebirge massif in Austria. The cave consists of over 42 km of passages; the cave entrance is situated at 1641 m asl in a steep rock wall. In the first 700 m from the entrance, the cave houses a perennial ice body with an overall surface area of 10,000 m^2 and estimated volume of about 33,000 m^3. Inside the cave a clear daily cycle in air temperature was observed from late spring until autumn with a maximum temperature of 0.7 °C in summer that is independent of the weather outside the cave (Schöner et al. 2011). Analysis of an ice core revealed the age of the glacier to be several thousand years (May et al. 2011). The neighbouring massif of Dachstein hosts another show cave with ice, Dachstein-Rieseneishöhle. It is a rare static type of ice cave that contains underground glaciers. The ice is not more than 800 years old, as shown by ^{14}C dates of organic debris from the ice base (Silvestru 1999; Audra and Pavuza 2004). The Casteret Ice Cave in the Pyrenees, France, occurs in 2640 m asl, being the highest ice cave in the world (Fig. 15.1). It is a multiple-entrance dynamic cave, holding 220 m^3 of ice along its entire length. Another well-known ice cave in France, Grotte de la Glacière in the French Jura Mountains, is located at only 525 m asl. Silická ľadnica Ice Cave in Slovakia (Fig. 15.2a–c) is a static cave containing a glacier (213–340 m^3) in its entrance section at 470 m asl and is the lowest-lying perennial ice cave in the temperate climatic zone (less than 50° north). Formation of ice in this abyss (light hole) started between 400 and 0 years BC (Bella and Zelinka 2018). In Asia, the best-known ice cave is the 4700 m long Kungurskaya Ice Cave in the Ural Mountains. This multiple-entrance dynamic cave is carved in sulfidic sediments (gypsum rock) of the Lower Permian at 700 m of elevation, with an estimated ice volume of 500 m^3 (Mavlyudov and Kadebskaja 2018). Fuji Fuketsu in Japan is a lava tube with the entrance opening at 1120 m of elevation, formed during a volcanic eruption in 864 BC. The ice-covered section has a static climate with an ice volume of 3000 m^3 (Silvestru 1999).

Ice caves may be found in different geological conditions, most often in karst caves and lava tubes. Caves at high latitude or altitude are often under permafrost conditions. As a consequence, the air temperature is below 0 °C, which provides favourable conditions for ice preservation in the cave. But permafrost conditions restrict water inflow, and therefore only a limited amount of ice forms in these conditions (Perşoiu and Onac 2012). Perennial ice accumulations in caves may occur at medium altitudes where the presence of ice is restricted to vertical or downward sloping spaces with only one entrance in which the dense, cold air sinks and remains trapped (Racoviță and Onac 2000). A peculiar combination of climatic, hydrologic and cave morphology factors is required for the formation of ice in caves in conditions that are otherwise not favourable for its formation. Cave cooling is a prerequisite for ice genesis, which can be achieved by (1) trapping of cold air, the most common mechanism, (2) unidirectional ventilation and (3) evaporative cooling or (4) as a consequence of the cave's geographic setting in the subpolar climatic zone. Trapping of cold air is most common in caves with a single entrance and descending passages, in which cold air sinks in winter, displacing the warm air, which is pushed out through the same entrance or through other entrances. The direct cooling under the influence of sinking cold air is accompanied by evaporative cooling, induced by evaporation of moisture from the walls. In summer, the air inside the cave is colder and thus heavier than the external air and is trapped inside the cave. Cooling begins in early autumn and lasts until late spring. In summer, the cave air temperature begins to rise, due to the heat conduction through the walls and air column in the entrance shaft(s), and the warmer percolating water. Thus, the heat

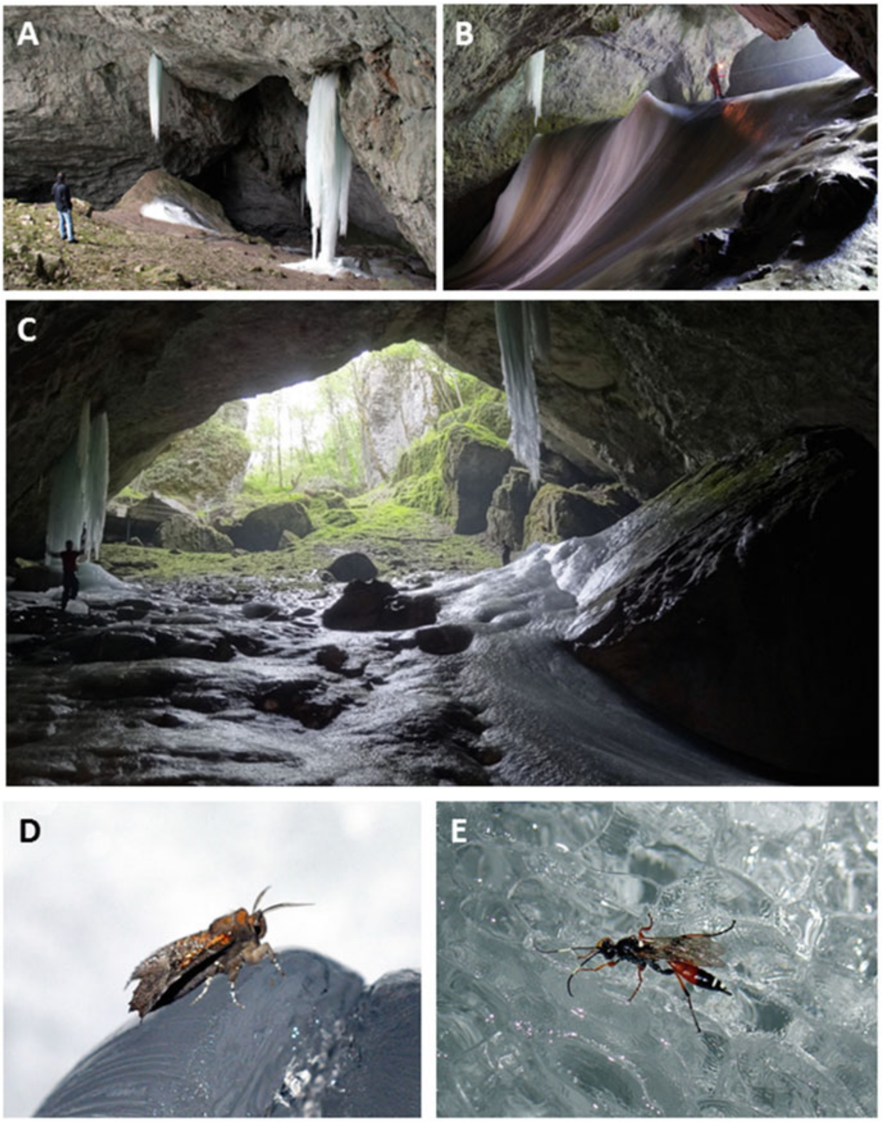

Fig. 15.2 Silická ľadnica Ice Cave, Slovak Karst, Slovakia (**a**–**c**), a lowest-lying perennial ice cave in the temperate climatic zone: (**a**) entrance ice decorations, (**b**) a glacier behind the cave entrance, (**c**) entrance section of the cave with a collapse doline. Inhabitants of subalpine ice cave Ľadová pivnica, Belianske Tatry Mts, Slovakia (**d**, **e**): (**d**) herald moth *Scoliopteryx libatrix*, (**e**) ichneumonid parasitoid wasp *Exephanes ischioxanthus*. Photos by © Jaroslav Stankovič (**a**–**c**), © Ľubomir Kováč (**d**), © Zuzana Višňovská (**e**)

delivered to the cave leads to ice melting and hence cooling of air, so that cave atmosphere is maintained at 0 °C and the cold air remains trapped inside the cave (Perşoiu and Onac 2012). The ice is formed mainly from recrystallisation of snow, from refreezing of percolation water or, less significantly, from deposition of cave-air vapour (Luetscher and Jeannin 2004). If ice formation exceeds loss due to ice melting and ice evaporation (sublimation), a layered ice body will be formed, similar to mountain glaciers. Therefore, the term glacier is extended to perennial ice accumulations in caves that represent large, stratified ice blocks with marked seasonal mass fluctuations and active flow (Perşoiu and Onac 2012).

15.2 Ice Caves as Ecosystems

Studies on cave fauna have a long history, but biospeleological surveys on caves with perennial ice are still unusual, and very little is known about the diversity and activity of their biota (Iepure 2018; Purcarea 2018). According to Poulson (2005), the food supply in glaciated caves is variable, and there are few or no cave-adapted species. If they were present, most became extinct when glaciers covered the caves. Current ice caves tend to be poor in terms of biodiversity (even considering species not adapted to caves), essentially as a consequence of the high latitude and/or altitude (Romero 2009). The small number of studies reflects the perception that environmental conditions of ice caves are too harsh to sustain terrestrial and aquatic populations, yet biological communities in ice caves consist of a variety of organisms (Iepure 2018).

Among microorganisms commonly found in frozen environments, phototrophic prokaryotes and eukaryotes from light-exposed environments play an important role in carbon and nitrogen enrichment of the cave environment by photosynthetic assimilation of atmospheric nitrogen and CO_2, respectively (Hillebrand-Voiculescu et al. 2014). The specific microclimate of ice caves, with their low air temperatures, leads to low concentration levels of fungal spores in the air (Ogórek et al. 2017). In general, perennial cave ice deposits host a lower microbial biomass than other cave sediments (Purcarea 2018). A thorough review of microbiome diversity (Bacteria, Archaea, Fungi and Algae) in ice caves is provided by Purcarea (2018).

Box 15.1

Ice caves at lower elevations have microclimate conditions that are distinctly different from the surrounding surface and may serve as a significant refuge for cryophilic fauna. Data from several ice caves in the Western Carpathians, Slovakia, indicate that habitats in contact with ice deposits are inhabited by sparse invertebrate communities compared with other, non-glaciated parts of the caves. This is the case of Demänovská Cave System where, however, some invertebrates occasionally prefer sites close to the ice. Several species are even

(continued)

Box 15.1 (continued)
active on the ice surface, including the troglophilic beetle *Choleva glauca* and the ichneumon wasp *Exephanes ischioxanthus* (Fig. 15.1e, Kováč et al. 2014).

Animals most often enter ice caves to avoid unsuitable external conditions and usually become inactive in a dormant stage during summer (aestivation) or winter (hibernation), for example, hymenopterans (ichneumons; Fig. 15.2e). Moreover, entering the ice caves probably helps animals to avoid predators. From the early spring, various insects may be seen directly on ice in caves, such as the troglophilic moth *Scoliopteryx libatrix* (Fig. 15.2d). It is not clear if they are attracted by the surface of the ice itself, or by specific internal microclimatic conditions. Sometimes insects are observed to copulate on ice, such as *Choleva glauca* in Demänovská Ice Cave. This behaviour is known in some arthropods active on snow, such as stoneflies (Plecoptera), scorpionflies (Mecoptera) and chironomid midges (Diptera) (Hågvar 2010).

Castleguard Cave, a cave system in the Rocky Mountains, Alberta, Canada, extends underneath the Columbia Icefield glacier for total length of about 20 km as a meteoric water dissolutional cave in limestone. The internal air temperature of the most insulated parts is approx. 3 °C. Two species of troglobiotic or fully adapted crustaceans live in pools, where the water apparently never freezes. Whilst the isopod *Salmasellus steganothrix* is also known elsewhere in the Canadian Rockies, the amphipod *Stygobromus canadensis* has been found only in Castleguard. It is possible that the cave served as a subglacial refuge for these stygobionts during the last glaciation or even longer (Holsinger et al. 1983).

Terrestrial and aquatic invertebrates in ice caves are mainly represented by arthropods (Iepure 2018), including beetles of the tribe Leptodirini (Leiodidae, Cholevinae) and subfamily Trechinae (Carabidae). Examples of troglobionts that populate ice caves at temperatures of 0–2 °C are *Isereus xambeui* which is recorded from the French Alps (Trou du Glaz cave near Grenoble), *Arctaphaenops angulipennis* from the Austrian Alps (Dachstein Mts), *Pholeuon knirschi* from Romania (Apuseni Mts), and *Bathyscimorphus globosus* and congeners of the genus *Astagobius* from Dinaric Mountains (Vandel 1965; Decu and Juberthie 2004; Sket 2014). Figure 15.3 shows Ledena Pećina Ice Cave in Durmitor, Montenegro, as an example of a high-altitude ice cave inhabited by coleopterans.

Another example of beetles adapted to ice caves is the genus *Pretneria*, which includes seven species spread over a karst area extending from north-eastern Italy to western Slovenia. All species have been found in caves and superficial subterranean habitats characterised by low air temperature (0–5 °C), high relative humidity and the presence of temporary or permanent deposits of snow and ice. *P. ternovensis* occupies two caves, Ledenica pri Dolu, a 180 m long and 80 m deep subhorizontal cave, as well as the 2.2 km long and 650 m deep Velika ledena jama v Paradani. Entrances of both caves are situated on the karst plateau of Trnovski gozd, at

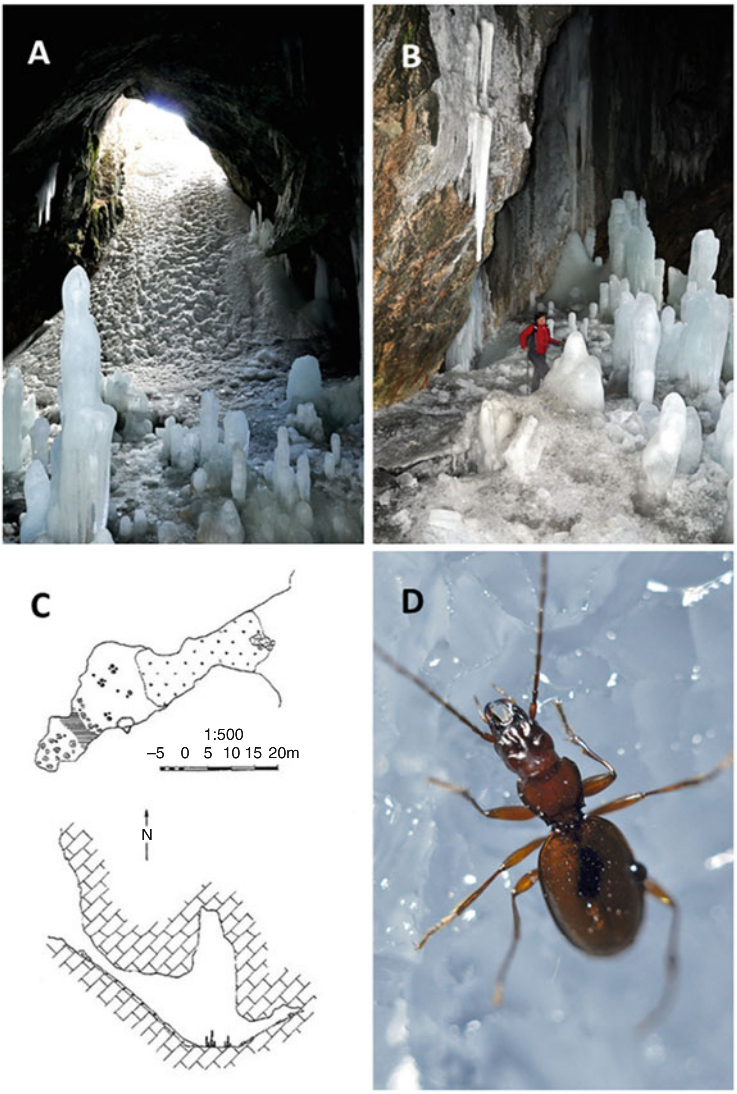

Fig. 15.3 Ledena pećina Ice Cave in Durmitor, Montenegro, an example of a high-altitude ice cave (*ca*. 2100 m asl): (**a**) firn ice slope at the cave entrance and ice fillings at the bottom, (**b**) detail of ice fillings, (**c**) horizontal and vertical cross section of Ledena pećina (Barović et al. 2018), with permission of Elsevier, (**d**) *Neotrechus suturalis*, a subterranean carabid beetle active on cave ice in early summer. Photos (**a**, **b**, **d**) by © Ľubomir Kováč

altitudes of 1000–1100 m on the bottom of deep dolines, characterised by microclimate inversion and deposits of snow that persist until late spring. The beetles are concentrated in the twilight zones within a range of about 50 m from cave entrance. The seasonal activity is intense from May to July, in association with a slight increase of temperature (from 1 °C to 4–5 °C), when the deposits of snow and ice melt and the relative humidity is close to saturation (78–84%; Bognolo 2016).

Westcott (1968) discovered a remarkable blind cholevid beetle *Glacicavicola bathyscioides* in three separate lava tube ice caves in southern Idaho, USA. Based on the degree of troglomorphy, this beetle is a troglobiont and a likely descendant of an old lineage. All the individuals have been found either on ice, where they crawled slowly, or floating in meltwater above the ice floor.

Diptera is another group of invertebrates in which some species are found to be associated with ice in caves. Two new taxa of Sphaeroceridae were recently described from the high-alpine Obstanser Eishöhle (2320 m asl), an ice-bearing cave in East Tyrol, namely, the troglophilic subspecies *Crumomyia cavernicola oculea* and the possibly troglobiont *Crumomyia microps* (Christian and Spötl 2010). The Buso del Valon cave is one of the few karstic caves of the Venetian Prealps in Italy that has a permanent ice body, fed by seasonal snowfall entering from the wide entrance. The cave opens with a shaft of about 30 m in diameter and about 50 m deep, having a vertical trend with a depth of about 70 m. The period of major activity of *Chionea* dipteran coincided with the period of major extent of the snow cover. The cave serves as a seasonal refuge for nivicolous species of dipterans during summer when the outside snow cover is absent. The constant temperature and the presence of snow and ice in the cave, together with the presence of organic material coming from the outside, make this cave an optimal environment for the survival of *Chionea* (Avesani and Latella 2016).

In western North America, 11 species of ice crawlers of the genus *Grylloblatta* (Grylloblattodea, Notoptera, Insecta) are known for their adaptation to cold conditions. These cryophilic insects are found on north-facing talus slopes and snow patches at high elevations (1500–3000 m asl), and in caves with permanent ice at low elevations (300–1000 m asl) (Jarvis and Whiting 2006).

Collembola is another group frequently found near ice in caves. A cryptic collembolan species has been discovered in large numbers in Silická ľadnica Ice Cave, Slovakia (Fig. 15.2a–c). The collembolan aggregations occur regularly in the vicinity of the floor ice with air temperature of approximately zero throughout the year. The species is morphologically identical to *Folsomia manolachei*, which is associated with thermophilous deciduous forests. In a laboratory experiment, the cold-adapted cryptic *Folsomia* species survived significantly lower temperatures than forest congeners from the same area (Raschmanová et al. 2017).

The examples of fauna associated with ice caves may be found also among aquatic crustaceans. Kungurskaya Ice Cave in the Ural Mountains is occupied by the stygobiotic amphipod *Crangonyx chlebinkovi*, endemic to groundwater habitats of the Priuralye karst area, living under constant low water temperatures (0–5.5 °C) (Sidorov et al. 2012). The stygobiotic syncarid *Bathynella glacialis* is associated with an interstitial habitat in a subterranean lake in Ledyanaya Ice Cave near Khabarovsk in the Russian Far East (Birstein and Ljovuschkin 1967). *Stygobromus allegheniensis* is a stygobiotic amphipod commonly found in caves of the Northeastern USA. Its several populations occupy the unique tectonic ice caves in the Shawangunk Ridge in New York, USA; these small caves represent simple faults and cracks in the bedrock, covered with ice until spring. During the warmer months, some of the ice caves have small streams and several 2 m deep pools where the amphipods can be found in great numbers. In the winter months, the ice caves freeze, and the floor and walls become covered in solid ice. The amphipods may seek warmer waters within the cave but can also survive being frozen in solid ice (Espinasa et al. 2015). This has been experimentally documented in another aquatic subterranean amphipod, *Niphargus rhenorhodanensis*. After inoculation at high sub-zero temperatures, cold-acclimated specimens survived. The accumulation of cryoprotective molecules such as glycerol and free amino acids may be the mechanism allowing the survival of this species when it is cold-acclimated (Issartel et al. 2006; see Box 15.2).

Novak et al. (2014) found that the resistances to freezing had a decreasing trend from trogloxenes over troglophiles to troglobionts since trogloxenes are not adapted and troglophiles not completely adapted to thermally stable subterranean environments. A weak resistance was preserved in nearly all troglobionts. The authors found that troglobionts inhabiting the superficial subterranean habitats withstand freezing to a limited degree but have increasing freezing tolerance in winter. Troglobionts inhabiting deep subterranean or other thermally buffered subterranean habitats had equal (limited) tendency to withstand freezing in both summer and winter.

Box 15.2

Life conditions in ice caves are extreme for most living creatures, and colonisation of ice caves by biota requires specific adaptations. A few cavernicoles can develop and exist at temperatures near to freezing point. The adaptations to low temperatures are well known in invertebrates inhabiting cold climatic zones or high mountain regions.

The freeze tolerance strategy in cave invertebrates appears to have evolved according to the habitats species occupy. For example, in two caves in the Venetian Prealps (NE Italy), the leptodirine beetle *Neobathyscia pasai*, abundant close to the entrances where the temperature is variable, was strongly freeze tolerant. On the contrary the congener *N. mancinii*, confined to the deeper part of the caves where the temperature is constant throughout the year, was only moderately freeze tolerant (Lencioni et al. 2010).

Some insects are physiologically able to tolerate the formation of ice in their extracellular body fluid. This freeze tolerance is widespread in some families of Coleoptera, Diptera, Hymenoptera and Lepidoptera, especially in larvae and pupae. However, most species are freeze susceptible and depend on supercooling to survive lower temperatures. These adaptations are usually associated with the evacuation of body water and gut content to reduce freezing of body liquids. In overwintering insects, the degree of supercooling is increased by the accumulation of cryoprotective substances in the haemolymph, which are low molecular weight compounds, such as polyhydric alcohols (e.g. glycerol) and sugars. Antifreeze proteins (thermal hysteresis proteins) stabilise the supercooled state of insects and prevent the inoculation of ice from outside through the cuticle and protect the gut fluids from spontaneous nucleation (Sømme 1999; Willmer et al. 2005). We may expect that these adaptations are less pronounced in arthropods dwelling ice caves because this environment is protected against very low external temperatures. Generally, knowledge of the phenological and behavioural adaptations of invertebrates inhabiting ice caves is very limited. Studies of population dynamics of *Pholeuon knirschi* in Scarişoara Ice Cave in Romania, conducted by G. Racoviţa, provide one of the few examples of this kind of observations (see the further text).

15.3 Ice Caves and Palaeoclimate Reconstruction

The rather stable environments within caves have long been recognised to have the potential to preserve past environmental information in various types of sediments (speleothems, fluvial sediments, guano, bones, etc.). Perennial ice accumulations in caves may also host a wide range of palaeoclimatic and palaeoenvironmental proxies, of which the most important one is the stable isotopic composition of water (ice). Many past climate studies have examined the oxygen and hydrogen stable isotope variation in ice on the assumption that the isotopic composition of ice mirrors that of meteoric water before freezing inside the cave. Thus, there is a strong correlation between outside temperature and the oxygen isotopic composition in the ice. By examining the ages of the ice at different depths with high-resolution stable isotope analyses along the ice core gained by drilling the ice block, a detailed record of climate changes in the cave's region (mean annual temperature, source of moisture, etc.) can be derived for the period since ice accumulation began (May et al. 2011; Perşoiu and Onac 2012; Perşoiu 2018). However, obtaining a climatic signal from cave drip water and associated ice is not a straightforward process because of the complex climatic regime of a given cave, specifically due to a strong influence of various ice mass-balance change-related processes on cave air temperature (Perşoiu et al. 2011a).

Among the available techniques, short-lived radionuclides, such as tritium, radiocaesium, americium and radiolead, are useful dating tools that provide an objective control in age-accumulation models based on cave ice core analyses (Kern 2018). Fossil remains (e.g. pollen grains, plant fossils, charcoal) can also be excavated from cave ice deposits and provide very useful numerical radiocarbon (^{14}C) data (Holmlund et al. 2005; Feurdean et al. 2011). Radiocarbon analysis of surface-derived ephemeral plant and animal remains is currently the most potentially accurate dating approach for the older cave ice deposits (Kern 2018). However, melting phenomena may cause great limitations in the potential analysis of ice deposits, making it impossible to calibrate the palaeoclimatic signals recorded (Kern and Perşoiu 2013).

The radiocarbon ages of cave ice blocks (glaciers) from Europe and North America indicate that they are younger than 1000 years. Eisriesenwelt and Scărişoara are notable exceptions with the age of their ice blocks estimated as ~4000 and 10,000 years, respectively (Perşoiu and Onac 2012).

15.4 Ice Caves as Show Caves

Fascination with ice caves was the main reason for their opening to the public more than 100 years ago. There are about 20 ice caves worldwide that function as show caves (Oedl 2018) and require proper and sensitive conservation management.

Human activities associated with the construction of tourist pathways and cave maintenance may directly threaten the whole ice cave ecosystem. Artificially created ice corridors, tunnels and new cave openings may induce new air currents and subsequently undesired decline in the amount of ice. Moreover, the ice surface is polluted by dirty deposits that are transported inside the cave directly by the visitors or as the natural dust, which stems from water entering the cave or being deposited by drafts. Electric lighting can be a significant problem when the light bulbs emit heat in close proximity to the ice formations creating small melting depressions. Recently, LED lamps as a new technology have become the standard system for lighting in show caves that considerably reduce heat emissions (Oedl 2018).

The high number of visitors may potentially increase the cave air temperature adjacent to human traffic by up to half a degree centigrade for short periods of time. However, in dynamic caves, there is a pronounced natural exchange of air which means that even large numbers of visitors influence the temperature minimally. Even in static ice caves with a much lower level of air exchange, such as Scărişoara Ice Cave, the influence of visitors on the temperature is minimal. The temperature levels inside the cave are influenced to a significantly greater extent by natural causes such as warm rainwater. The internal cave climate is continuously monitored, and visitors have access only during a limited part of the year, for example, for 6 summer months in Eisriesenwelt and 4.5 months in Dobšinská Ice Cave, currently with 180,000 and 80,000 annual visitors, respectively (Oedl 2018; Bella and Zelinka 2018).

Detailed studies are lacking on the potential effect of the management of ice show caves on local biological communities which are considered to be less sensitive to disturbance because of extreme temperature conditions around freezing point. Thus, it is thought that installation of biological materials, such as wooden pathways, does not alter significantly the composition of ice cave biota (Oedl 2018).

15.5 Examples of Ice Caves Ecosystems

In the next section, three examples of complex biotic communities of ice caves are provided: Gheţarul de la Scărişoara in Romania, Dobšinská Ice Cave in Slovakia and fumarolic ice caves on Mt Erebus, Antarctica.

The cave *Gheţarul de la Scărişoara* (700 m long, 105 m deep) belongs to the most intensively studied ice cave systems. It is situated at 1165 m of elevation in the Apuseni Mountains, NW Romania, in Upper Jurassic limestone. At the bottom of the circular shaft, 60 m in diameter and 48 m deep, the cave hosts 1–3 m thick layer of perennial snow. Beyond the entrance, an ice block with a volume of more than

100,000 m^3 forms the floor of the Great Reservation hall, making it one of the world's largest subterranean glaciers. The surface area of the top of the ice block covers approximately 3000 m^2, and its thickness exceeds 22 m. The ice block is preserved due to unusual microclimate and permafrost conditions within the cave. There is seasonal bidirectional ventilation in which the active phase is limited to the winter season when cold air is trapped inside the cave. During summer the vertical temperature gradient becomes steep in the shaft: the upper part has temperatures close to the surrounding outside area, whilst the lower part is close to freezing. Below this level the cold air masses fill the cave as a result of negligible air mixing between the systems. Thus, the internal air temperature is governed by the winter cold and the cooling effect of the ice block, and only to a minor extent influenced by summer temperatures. The average annual mean temperature at the ice surface in the Great Reservation is −0.9 °C, which is almost 8 °C lower than in the surroundings of the cave. At the ice surface, temperatures may drop as low as −14.5 °C during the winter season; during summer the temperature is around 0 °C, rarely exceeding +0.5 °C. The radiocarbon age of the block in the Little Reservation of Scărişoara is ~1200 cal year BP; the total age of ice mass in the cave was estimated around 10,500 years, based on radiocarbon dating of the containing debris (Perşoiu et al. 2017). The accumulation of the perennial ice block must have started after the collapse of the passage in the Great Reservation that was linking to the nearby cave, Pojarul Poliței Cave, a passage that allowed free circulation of air through the cave and thus preventing its warming. A period of rapid and almost continuous melting of the ice block was observed in the middle and late twentieth century (Silvestru 1999; Holmlund et al. 2005; Perşoiu and Pazdur 2011; Perşoiu et al. 2011a, Perşoiu and Onac 2012). Investigations of the behaviour of oxygen and hydrogen stable isotopes ($\delta^{18}O$–$\delta^{2}H$) during the formation of ice by the freezing of water were carried out in Scărişoara Ice Cave. A model for the reconstruction of the initial isotopic composition opens a new direction in palaeoclimatic research in ice caves (Perşoiu et al. 2011b). Derived from a precisely dated isotopic record (^{14}C, $\delta^{18}O$, $\delta^{2}H$ values) of cave ice deposit in Scărişoara, Perşoiu et al. (2017) presented a reconstruction of late autumn through early winter air temperature and moisture source changes in East-Central Europe (ECE) for the Holocene. It was found that during this time period winter temperature in ECE was mainly controlled by insolation changes.

Studies of pollen, charcoal and macrofossil recovered from Scărişoara ice provided valuable palaeoecological data (Fig. 15.4e–f). The collected data provide a picture of past vegetation dynamics and composition at both the local and regional scale, along with clear signals of human impact over the past 1000 years (Feurdean et al. 2011).

Microorganisms belonging to Bacteria and Eukarya domains were found in ice layers of different age (recent, 400, and 900 years). A small section of the ice block surface (~10 m^2) exposed to sunlight promotes the development of an abundant population of phototrophic prokaryotes and eukaryotes living in the supraglacial pond at the water-ice interface. The chemical composition and organic content of both deeply buried (>10 m inside the ice block) and surface (supraglacial pond water) parts of the ice block were analysed in relation to their age and organic composition. The composition of cold-adapted ice embedded microbiota varied with

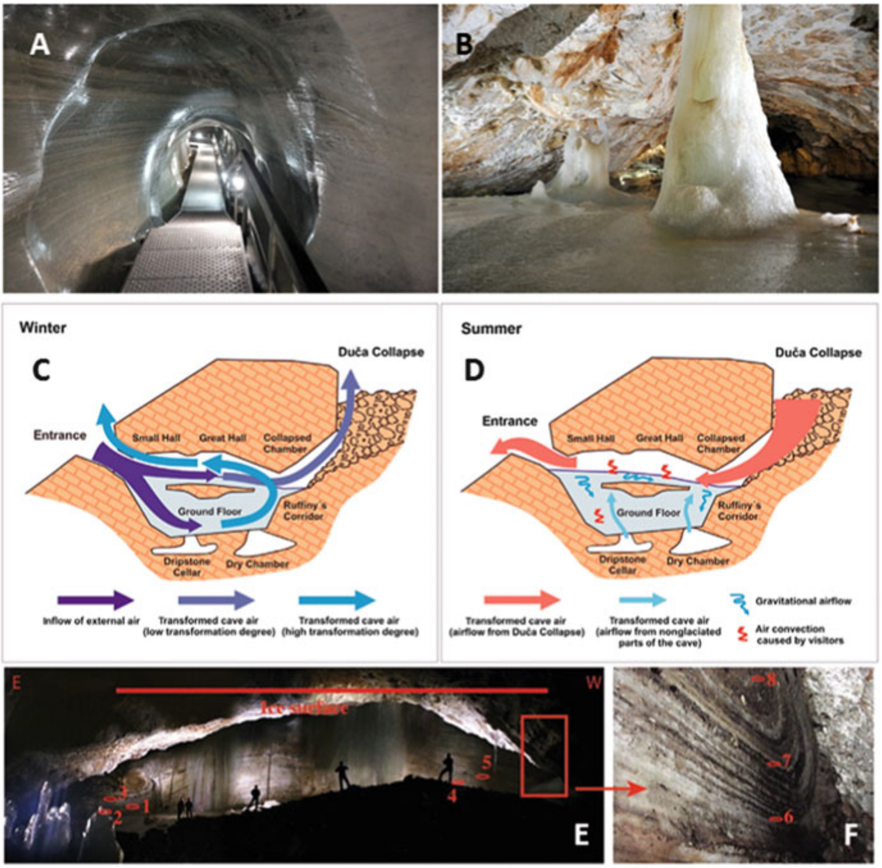

Fig. 15.4 Dobšinská Ice Cave, Slovak Paradise, Slovakia (**a**–**d**): (**a**) tourist passage carved in a compact ice block of the cave glacier, (**b**) upper part of the glacier with ice decorations. Photos (**a**, **b**) by Ľubomir Kováč. (**c**, **d**) Seasonal patterns of air exchange in Dobšinská Ice Cave, schematic cross section (Korzystka et al. 2011). With permission of the Slovak Caves Administration. Scărişoara Ice Cave, Apuseni Mountains, Romania: (**e**, **f**) ice layers of an exposed ice wall in the Little Reservation. The red dot indicates the position of the pollen profile, whereas the numbers in red represent the position and number of the samples used for the radiocarbon dating (from Feurdean et al. 2011). With permission of Elsevier

age and organic content of the section (Hillebrand-Voiculescu et al. 2014; Iţcuş et al. 2016).

The cavernicolous leptodirine beetle *Pholeuon knirschi glaciale* is a subspecies endemic to four caves in Bihor massif of the Apuseni Mountains, which have an internal air temperature of 6–7 °C. The species also frequently occupies warmer zone of Scărişoara where temperature is usually 2–5 °C throughout the year. A population occupying the periglacial zone of the Little Reservation has unique dynamics since it is periodically exposed to low winter temperatures (ca. –1 °C) which lead to emigration of the beetle during this season. In summer the population is re-established when the temperature rises to ca. 0.6 °C. This cave is thus a borderline habitat in terms of the beetle's cold tolerance. Mark-recapture techniques showed that only ca. 1% of beetles were recaptured in a 5-month period, indicating that a large population was present in the cave and interconnected fissures of the surrounding karst. The winter population decrease is associated with the migration of individuals from cave to fissures and crevices in bedrock to avoid less suitable microclimate. *P. k. glaciale* is considered to be a strictly troglobiotic animal. More recent studies, carried out in mesovoid shallow substratum (MSS) in a neighbouring area, found that this beetle may migrate closer to superficial layers of karst and also non-karst bedrock. The summer population maximum in the cave is thus most probably the result of seasonal immigration of individuals from different sectors of the massif, including MSS (Racoviță 1976, 1980, 1987, 2000).

Dobšinská Ice Cave (Dobšinská ľadová jaskyňa, Fig. 15.4a–d) is a famous tourist cave located in the Slovak Paradise karst area, Slovakia, in an area of temperate climate. The entrance of this static-dynamic ice cave is a collapsed doline at an elevation of 969 m asl in a north-facing slope covered by a coniferous forest. It is the isolated end of the Stratenská jaskyňa Cave System, a 23.6 km long multilevel cave formed by sinking palaeo river in the Middle Triassic Steinalm and Wetterstein limestones. The collapse separated the cave with 1483 m of passages and vertical span of 112 m from the remainder of the system, probably in the Middle Quaternary. The beginning of the ice filling development is estimated to ca. 250–140,000 years ago. The main part of the cave consists of a large collapsed chamber containing perennial ice masses, descending to a depth of 70 m. Most of its volume is filled with a glacier with an ice volume of more than 110,132 m^3, glaciated surface of 9772 m^2 and a maximum depth of 26.5 m, making it the largest known compact ice monolith in a cave in the world (Fig. 15.4a–b). The average air temperature in the glaciated Great Hall is −2.7 to −3.9 °C in February and around +0.2 °C in August; in lower parts of the cave, the air temperature is below freezing year-round (Bella and Zelinka 2018). The Dobšinská Ice Cave features a different winter and summer regime of air circulation and thus is classified as a statodynamic cave. The colder air circulates from the surface into the cave during the winter season and reverses during the summer season (Korzystka et al. 2011; Fig. 15.4c–d). The ice monolith grows in the upper parts and melts on the contact with the bedrock, a process that is in equilibrium. The ice is slowly moving in the direction of the floor at a rate of 2–4 cm/year. Continuous replacement of the glacier is estimated to take around 1280 years. The typical stratification of ice with distinct layers is formed in relation to the cyclic freezing of cave seepage water over the years during winter season (Bella and Zelinka 2018; Fig. 15.4a, f).

A comprehensive study of ice cores has been undertaken in Dobšinská Ice Cave by Vrana et al. (2007) and Clausen et al. (2007). Six stages were specified in the development and degradation of ice crystal sediment (Pflitsch et al. 2007). Diverse colonies of microfungi were isolated from sediments and cave air (Nováková 2006). It was observed that the arthropod diversity at glaciated sections is markedly lower than in warmer deeper parts of the cave (air temperature between +0.8 °C and +3.5 °C) as a consequence of the oligotrophic conditions and low air temperature limiting decomposition of organic matter and the development of microbial colonies, which are direct food sources for microbivorous arthropods. The cave is occupied by four troglobiontic collembolans, *Protaphorura janosik*, *Deuteraphorura kratochvili*, *Pygmarrhopalites aggtelekiensis* and *Megalothorax carpaticus*, and by two crustacean stygobionts, *Bathynella natans* and *Elaphoidella* sp. (Kováč et al. 2006). An aggregation of the cryophilic collembolan *Protaphorura janosik* was observed in the vicinity of the ice floor. The species is endemic to the Western Carpathians where its isolated populations preferentially inhabit colder caves. According to the recent molecular study (Parimuchová et al. 2017), populations of *P. janosik* diverged during the Early Pleistocene between 1.3 million and 800,000 years ago.

The fumarolic ice caves on Mt Erebus (Antarctica) are a unique example of an oligotrophic cave system, characterised by volcanic bedrock as a substrate. The Mt Erebus ice caves are moist, cold habitats (on average ~0 °C) at high altitude in one of the most remote and oligotrophic environments on Earth. Subglacial fumaroles issue air-dominated gasses with 80–100% humidity and up to 3% CO_2; some contain also CO and H_2 but are essentially devoid of CH_4 and H_2S. Many of the caves are completely dark and therefore unable to support photosynthesis. In these dark oligotrophic volcanic ecosystems, the only possible sources of organic carbon are from atmospheric deposition or ice algae that may grow on the surface of the ice during summer and subsequently being introduced into the caves through burial from above and melting from below. The microbial communities in these caves are composed primarily of bacteria and fungi. Low phylum-level bacterial diversity confirms a commonly found adaptation to extreme environmental conditions; the energy driving CO_2 fixation and supporting the ecosystem is likely derived from reducing volcanic rocks and gas emissions. Thus, the fundamental support for biological systems in fumarolic Antarctic ice caves is provided by chemolithoautotrophy (Tebo et al. 2015). For more details on this phenomenon, see Chap. 5.

15.6 Ice Caves and Global Change

Perennial ice masses can accumulate in caves far outside of the boundaries of the permafrost climatic zone which makes them especially vulnerable to any warming trend. It has been highlighted that radiocarbon dating showed relatively young age of ice blocks of European and North American ice caves, being mostly younger than 1000 years. Caves hosting ice bodies have been reported to suffer significant loss of ice mass worldwide over the past decades (Kern and Perşoiu 2013; Colucci et al. 2016). This widely observed process threatens cave ice deposits with tremendous volume loss or ultimately total disappearance. It is suggested there is an urgent need to investigate the probably unique palaeoenvironmental information stored in cave ice deposits (Kern and Perşoiu 2013). Cave glaciers have a complex response to external climate variations; therefore their future behaviour under changing climatic conditions is more difficult to predict than for surface glaciers (Perşoiu et al. 2011a).

Warmer and more intense precipitation may be crucial in the future mass balance evolution of permanent cave ice deposits. The close relationship between global and local climate change and the evolution of the ice deposits has been highlighted in the dynamic ice caves, especially in regard to the extreme weather events (Colucci et al. 2016). Mammola et al. (2018) pointed out the importance of subterranean organisms as model species for ecological studies dealing with climatic changes. We may expect that populations of the cryophilic fauna of ice caves, often representing glacial relicts, will decline in ice cave systems, and species may even be threatened with progressive melting of the cave ice due to climate warming.

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Chapter 16
Researches in Sulphide-Based Ecosystems

Alexandra Hillebrand-Voiculescu

16.1 Introduction to Sulphide-Based Ecosystems

The concept of chemolithotrophy, as the ability of bacteria to obtain energy by the oxidation of reduced inorganic compounds such as H_2S, NH_3 and Fe_2^+, which led eventually to the entire concept of sulphur and nitrogen cycles in nature (Dworkin 2012) was discovered by Sergei Winogradsky (1887), at the end of the nineteenth century, when microbiology was taking off as an independent science. The process was initially considered insufficient to support ecosystem-level processes (Summers Engel 2007), and life in the absence of light was thought to be impossible until the 1970s when the deep-sea hydrothermal vents and their associated biocenoses were discovered. These vents showed that in extreme conditions, such as complete darkness, low temperatures and extraordinary high pressure, where no one imagined life to be possible, life not only exists but presents an astonishing abundance and diversity. The dogma according to which life exists only in the presence of light was abolished, but the sustaining processes were still not very well understood until biologists, geologists and chemists put their efforts together to explain the richness and great biodiversity in the vents. As a result, new C/N and energy sources as well as pathways alternative to photosynthesis were found to be enough to sustain ecosystems. One-carbon compounds, other than CO_2, were shown to function as source of C, while oxidation of reduced compounds such as NH_4^+ and H_2S provides almost all the energy requested for biomass formation. Still, the hydrothermal vent ecosystems do receive some organic matter from the water column, plankton, various types of detritus, dead animals, etc.

Chemolithotrophy was confirmed to exist also in habitats associated with sulphurous water, but again, these systems are also open, receiving organic matter from

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the surroundings. In contrast, at the end of the 1980s, a secluded, peculiar chemosynthesis-based ecosystem, isolated from any source of organic matter, was discovered in Romania, inside a cave. This is Movile Cave, the first terrestrial chemolithotrophy-based ecosystem discovered (Sarbu et al. 1996).

Caves are habitats characterised by partial or complete darkness, constant climate (constant air/water temperature, relative humidity near saturation) as well as a restricted input of nutrients (see also Chap. 3). Caves are classified based on various criteria, one being the mechanism of their formation, the speleogenesis. Based on this, caves formed by bedrock dissolution can be epigenic or hypogenic. The majority of caves, known also as solution caves, are epigenic, being formed by the movement of water from overlying or immediately adjacent recharge surfaces to springs in nearby valleys (Palmer 1991; see also Chap. 2). Atmospheric CO_2, as well as CO_2 from soil and dissolved in water, forms carbonic acid ($CO_2 + H_2O \rightarrow H_2CO_3$) that corrodes the carbonate ($CaCO_3$) bedrock ($H_2CO_3 + CaCO_3 \rightarrow 2HCO_3^- + Ca^{2+}$).

16.2 Hypogenic Caves

In contrast to the solution caves formed by meteoritic water dissolving the limestone in karstic regions, caves of hypogenic origins are formed by fluids ascending through various geological and tectonic settings at different depths (ranging from a few tens of metres to several kilometres) by different dissolution mechanisms operating in various lithologies (Klimchouk 2009). Klimchouk found that hypogene speleogenesis is a complex hydrogeological process, with deeper groundwaters in regional or intermediate flow systems interacting with shallower and more local systems to support multiple dissolution mechanisms that differ in their physical and chemical properties. Despite the variability of the speleogenesis mechanisms and of the geological conditions, hypogenic caves can be recognised by specific meso-morphological features indicating sluggish flow conditions (Klimchouk 2009).

Hypogenic caves differ depending on the origin and thus on the chemical composition and temperature of the rising waters as well as on the nature of the host rock. In the case of caves with inputs of gases such as hydrogen sulphide (H_2S) and/or methane (CH_4), which could provide energy sources for microbial communities, H_2SO_4 corrosion and condensation corrosion promoted by HCO_3^- coexist and reinforce each other (Kumaresan et al. 2014).

The deep-seated aquifers rich in H_2S and/or CH_4 are usually thermal as compared to the average temperature of the regional groundwater and have a higher temperature than the underground environment. This results in water condensation on the substrate and sulphuric acid formation due to H_2S exposure to atmospheric O_2 (Eq. 16.1). The limestone ($CaCO_3$) of the walls is then rapidly corroded by H_2SO_4, while gypsum ($CaSO_4 \cdot 2H_2O$) deposits form to cover the walls, and CO_2 is released (Eq. 16.2). The CO_2 further reacts with H_2O vapours and the condensed water with H_2CO_3production (Eq. 16.3), which further forms HCO_3^- that will again corrode the walls (Eq. 16.4) (Kumaresan et al. 2014).

$$H_2S + 2O_2 \rightarrow H_2SO_4 \text{(sulphide oxidation)} \tag{16.1}$$

$$H_2SO_4 + CaCO_3 + H_2O \rightarrow CaSO_4 \cdot 2H_2O + CO_2 \uparrow \text{(sulphuric acid driven corrosion)} \tag{16.2}$$

$$CO_2 + H_2O \rightarrow H_2CO_3 \text{(carbonic acid formation)} \tag{16.3}$$

$$H_2CO_3 + CaCO_3 \rightarrow 2HCO_3^{-} + Ca^{2+} \text{(condensation corrosion)} \tag{16.4}$$

16.2.1 Trophic Webs in Hypogenic Caves

Epigenic cave biocenoses are strongly dependent on photosynthesis-derived organic matter. Subterranean trophic chains are typically short and based on detritivores. In contrast, hypogenic caves usually have a high degree of isolation from the surface, being confined between layers with low porosity. The energy in these systems is mainly provided by the rising fluids and gases (Forti et al. 2002), with the input of exogenous organic matter being insignificant. The energy sources for chemolithotrophic bacteria are provided by dissolved reduced compounds, such as H_2S and CH_4, in the ascending water.

There are hypogenic caves, such as Movile (Romania) and Ayyalon (Israel), which host an astonishingly rich and diverse fauna despite their complete isolation from the surface (see review in Summers Engel 2007). In these caves, the organic matter required to support the entire trophic web is synthesised in situ, by chemolithotrophic bacteria performing chemosynthesis (Sarbu et al. 1996; Levy 2007). The microbial communities form biofilms on the surface of the sulphurous water and on the walls/rocks in the proximity of the water. Since the nutrients are derived from the water, and because organic matter also forms here, owing to the chemosynthetic bacteria, the cave species are clustered mainly near the water, with the rest of the galleries being almost depleted of fauna. The trophic net is a surprisingly complex one, with primary producers (chemolithotrophic sulphoxidant and methylotrophic bacteria using the dissolved compounds in the water), primary consumers (heterotrophic bacteria, fungi), secondary consumers (protozoa as well as grazers feeding on the microbial pellicle and plankton), tertiary consumers and quaternary consumers (or predators, feeding on grazers and on lower-level consumers). In other less isolated, open caves, such as Cueva de Villa Luz (Mexico) and Frasassi (Italy), in which sulphurous waters flow, fauna is more uniformly distributed among the galleries, but specific groups of animals tend to cluster depending on the particular features of the cave habitats (Hose and Pisarowicz 1999). Such caves present several diverse habitats, localised in specific galleries and/or chambers that differ by the amount of light coming through the skylights and the entrance, the presence/absence of sulphurous water, the air currents and the anthropogenic influences (tourism, visits by local people for various rituals, etc.).

Owing to their isolation as well as to the peculiar habitats they host, hypogenic caves can be used as natural laboratories where biological researches of sulphide-based ecosystems mainly cover topics such as biodiversity of both micro- (prokaryotic and eukaryotic microorganisms) and macrobiota, energy and C/N sources, food web/trophic net (more precisely trophic chains), the toxicity of the environment and the detoxification mechanisms developed by the organisms to overcome this threat as well as morphological adaptations of those troglo-/stygobitic organisms. Of course, there has also always been a quest for new metabolic pathways, for new potentially valuable microorganisms, able, for instance, to degrade various materials or/and potentially valuable sources for enzymes useful in different biotechnologies. Currently, more than 20 years after the ALH84001 meteorite brought what appeared to be first evidence of primitive life on early Mars, when exo- and astrobiology gravitates mainly around the concept of habitability, the sulphide-based ecosystems—especially in caves—are providing valuable information on evolution of life on Earth and in the Universe through insights about conditions apparently incompatible with life but in which life actually thrives (Schirmack et al. 2015).

16.3 Examples of Sulphide-Based Caves

16.3.1 Movile Cave: The First Terrestrial Chemosynthesis-Based Ecosystem Ever Discovered

Movile Cave is located in south-eastern Dobrogea, a region of Romania limited to the east by the Black Sea and well known for its thermomineral sulphurous waters, which have been a spa facility since antiquity. The artificial shaft drilled during geological prospection works in 1986 became the entrance to the system of natural passages partially flooded with hydrogen sulphide-rich thermal water that is Movile Cave. The cave represents the access to a deep captive sulphurous aquifer located in Barremian-Jurassic limestone, extending 15 km north and 50 km to the south of Mangalia. Through a system of geological faults, the deep water ascends towards the surface and mixes with the Sarmatian oxygenated waters. Movile Cave is part of a broad underground maze of fissures and passages associated with the sulphurous aquifer in Mangalia as is shown by the wide distribution of Movile Cave fauna in hand-dug wells in nearby localities, sometimes at distances of tens of kilometres from the cave (Sarbu et al. 1994b).

Movile Cave is developed at a depth of 21 m (Lascu et al. 1995), in oolitic- and fossil-rich limestone of Sarmatian age (i.e. late Miocene, about 12.5 Ma) that contains numerous mollusc fossils (Lascu 1989). Almost from the very entrance in the vertical shaft that leads to the main gallery, the visitor is struck by a smell of rotten eggs, which becomes ever stronger on the way to the end of the cave. The underground passages form a network, with only about 200 m length accessible to humans. In the deep part of the cave, the galleries become wider and higher, their

Fig. 16.1 Movile Cave main gallery; arrows indicate the cupolas of the ceiling. Photo by © Petr Zajíček

heights ranging from 40 cm to 2.5 m; the walls are soft, easily penetrable by finger, and the ceiling becomes cupola shaped (Fig. 16.1). This feature reflects severe corrosion of the bedrock, as confirmed also by petrographic analysis. The difference of about 0.5°C between the wall temperature (19–21°C) and the atmosphere, with the latter being warmer (Sarbu and Lascu 1997), results in water condensation on the cave walls/ceiling and formation of carbonic acid that accelerate the dissolution of the surface of the limestone walls.

The superficial corroded layer of bedrock reaches a thickness of up to 8 cm and consists of uncemented oolites (Horoi 1994). To this condensation corrosion process is added the sulphuric acid corrosion, as previously described (see Eqs. 16.1 and 16.2), and as a consequence of sulphide dissolved in the water condensed on the walls, gypsum crystals form and cover the limestone surfaces (Fig. 16.2).

The passages are distributed on two levels, merging in one chamber—the Lake Room (Fig. 16.3). The upper level is very dry and cooler than the lower one that is warmed by thermal sulphide-rich water. Due to this temperature regime, warm vapours produced at the surface of the sulphidic lake rise along the passages into the upper level. Here, the atmosphere is enriched in CO_2 (up to 1.5%), resulted mainly from methane oxidation and metabolic activity of the cave biota as demonstrated by C isotopic analysis (Sarbu et al. 1996).

The lower, flooded level (approx. 40 m in length) is accessible from the Lake Room. This water accumulation is actually not a true lake, since the water flows, with a velocity of 5 L/s at the bottom (Sarbu 2000) and makes the flow barely noticeable. The water has a milky aspect, due to white sulphur suspended particles

Fig. 16.2 Gypsum crystals on the walls of Movile Cave. Photo by © Alexandra Hillebrand-Voiculescu

Fig. 16.3 The Lake Room in Movile Cave. Photo by © Cristian Lascu

(Fig. 16.4), has a temperature of 21–22°C, pH near neutral (7.2–7.4) and is rich in H_2S (0.2–0.3 mM), CH_4 (0.02 mM) and NH_4^+ (0.2–0.3 mM). Dissolved oxygen is measurable (9–16 μM) only in the first 5 cm from the surface; deeper than that, the conditions are anoxic. The ceiling of the flooded galleries rises in several places forming air pockets between the surface of the water, the walls and ceiling of the

Fig. 16.4 Microbial biofilm on the surface of the sulphurous water in AirBell II, Movile Cave. Photo by © Alexandra Hillebrand-Voiculescu

gallery—the so-called air bells—with air unusually rich in CO_2 (up to 2.5%) and CH_4 (1–2%) and poor in O_2 (7–10%; Sarbu 2000). In this lower level of the cave, almost no condensation occurs since the walls and ceiling are warmed by the thermal water (Sarbu and Lascu 1997).

No seasonal variations in the chemical composition of the cave atmosphere were registered, and no infiltrations from the surface occur since the fissures and cracks in the limestone are sealed by clay and loess. The isolation from the surface was proven by the lack of radioactive artificial nuclides ^{90}Sr and ^{137}Cs that are otherwise abundant in the region since the nuclear accident at Chernobyl in 1986. Faecal streptococci that should normally infiltrate underground as a result of the intense grazing occurring on the surface are also absent from the cave environment (Sarbu et al. 1994a). This surprising isolation raised questions about: (1) how a void in limestone can be so secluded and (2) what is the energy base of the very rich and diverse biocenosis, characterised by an abundance of predators in the trophic structure, unusual for a cave ecosystem (Sarbu 2000).

At the time of Movile Cave discovery, its biocenosis was represented by 48 species of invertebrates, of which, at that time, 31 were not described (Sarbu and Kane 1995). Later, even more new species endemic to the Movile Cave and/or the sulphidic aquifer associated with Movile Cave were identified. The last identified species was *Dendrocoelum obstinatum* by Stocchino et al. (2017). The species is unpigmented and lacks eyes and is one of the top predators of the aquatic community of the sulphidic groundwaters at Mangalia, along with the leech *Haemopis caeca* and the heteropteran *Nepa anophthalmia*. In contrast to any other species that are restricted either to sulphidic or non-sulphidic environments, *D. obstinatum* occurs both in sulphidic and non-sulphidic wells, a fact that allows two scenarios of subterranean colonisation. According to the first scenario, *D. obstinatum* used to live exclusively in a sulphidic aquifer that in time became fragmented, thus being presently found in areas that once were sulphidic but are not any more. The second hypothesis proposes that cave colonisation occurred more recently, from non-sulphidic areas. *D. obstinatum* is the first triclad species living in both sulphidic and non-sulphidic conditions, as it is the first subterranean planarian species recorded to be infested by a nematode (Stocchino et al. 2017).

Currently, there are 51 species of invertebrates from which 35 are endemic to Movile Cave or the sulphidic aquifer associated (Sarbu et al. 2018). The fauna of the cave is concentrated in close proximity to and within the sulphidic water (i.e. in the Lake Room and the Air bells). The aquatic species living in the cave belong to the phyla Platyhelminthes (flatworms), Nematoda (round worms), Rotifera, Annelida (segmented worms), Mollusca (snails) and Arthropoda (ostracod, copepod, isopod, amphipod crustaceans and insects) (Sarbu and Kane 1995). A peculiarity of the aquatic organisms in Movile Cave is the ability to survive high concentrations of H_2S and very low concentration of O_2/anoxic conditions (see also Chaps. 4 and 11). Organisms exposed to H_2S (Sarbu and Kane 1995) have developed several detoxification/resistance mechanisms such as an association with sulphur bacteria that may be involved in the transformation of the toxic gas (*Nepa anophthalmia*), lowered ventilation rate (*Asellus aquaticus*) and increased tolerance to H_2S poisoning (Tobler et al. 2011; Greenway et al. 2014).

Referring to the terrestrial fauna, 30 new species of terrestrial invertebrates belonging to the classes Arachnida, Crustacea, Myriapoda and Insecta appear to be endemic for the Movile Cave- associated ecosystem (Sarbu et al. 2018; Sarbu and Kane 1995). They have morphological adaptations to the dark environment that are typical for obligate cave-dwelling organisms, such as lack of eyes, depigmentation and elongated antennae and legs (e.g. *Agraecina cristiani*, *Hahnia caeca*, a new species of *Nesticus*, *Trachelipus troglobius*).

While aquatic fauna has probably colonised the cave continuously through the points of discharge of underground springs located along the Black Sea coast and on the bottom of sulphide- rich lakes, two major events could have isolated cave populations from their surface ancestors. These are the Messinian Salinity Crisis (MSC) about 5.96–5.33 Mya (Sarbu and Kane 1995), the onset of the Pleistocene and later glacial periods (Sarbu and Kane 1995; Falniowski et al. 2008). During the MSC, the connection of the Mediterranean Sea with the Atlantic Ocean was closed, and due to intense evaporation, the Mediterranean basin was reduced to hypersaline lakes. The Black Sea level dropped about 3000 m compared with the present level, and the climate in Dobrogea became extremely dry. Passages at depths of about 200 m were formed by underground waters and were later flooded by deep sulphide-rich waters (Sarbu and Kane 1995). Data obtained using the molecular clock technique indicate that the aquatic snail *Heleobia dobrogica* invaded the underground voids associated with the thermal sulphurous water about 2.172 Mya ago, when both temperature and precipitations decreased, initiating the Pleistocene in Europe (Falniowski et al. 2008). Much later, the Würm glaciations in quaternary (115,000–11,700 years ago), when the level of the Black Sea dropped with about 100–110 m (Panin and Strechie 2006), provided ideal conditions for new colonisation. Species such as *Heteromurus nitidus* and *Cryptops anomalans* are abundant in the cave and are also found at the surface.

In the Air bells, but not in the Lake Room where the O_2 concentration is close to normal, the surface of the water is covered by a biofilm as thick as up to 2 cm, kept afloat by CH_4 bubbles, which sinks as soon as it is disturbed (Fig. 16.4). The biofilm consists of bacteria, archaea and fungi and represents both habitat and food for various protozoans and metazoans. The protozoan microbiota consists of flagellates and ciliates. About 95% of total metazoans living in the microbial mat are nematodes (*Poikilolaimus* sp., *Monhystrella* sp., *Panagrolaimus* c.f. *thienemanni*, *Udonchustenui caudatus* and the endemic *Chronogaster troglodytes*), followed by cyclopoid copepods (*Eucyclops subterraneus scythicus*, 3.5%) and ostracods (1.1%). Harpacticoid copepods (*Parapseudoleptomesochra italica*), gammarids, isopods, acarids, rotifers and gastropods occur in very low densities (Muschiol et al. 2015).

When incubated with ^{14}C-labelled bicarbonate, the microbial biofilm was shown to incorporate radioactive carbon in its lipids (Sarbu et al. 1996). Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO, the key enzyme of the Calvin cycle, responsible with CO_2 fixation) was determined to be present and active both in the microbial pellicle floating on the surface of the water and covering the walls of the galleries and in lysates obtained from bacterial cultures isolated from water/ sediments samples (Sarbu et al. 1994a). These data demonstrated that Movile Cave is a self-sustained ecosystem dependent on chemoautotrophically fixed carbon (Kumaresan et al. 2014; Sarbu et al. 1996). At the time of this work, Movile Cave ecosystem was the first terrestrial ecosystem known to be almost entirely based on in situ production of organic matter through chemosynthesis. In 2006, another similar terrestrial ecosystem (Ayyalon Cave) was discovered in Israel. A detailed overview on the animal life in this hypogenic groundwater cave is given by Por et al. (2013).

A preliminary microbiological characterisation of the Movile ecosystem was provided by Sarbu et al. (1994b) who, based on specific morphological and physiological features, provided evidence of the presence of *Thiobacillus* and *Thiosphaera* genera as well as of filamentous bacteria from the genus *Beggiatoa*. Later, while the sulphur cycle in Movile Cave was investigated, it became apparent that the main group of primary producers are the sulphur oxidisers, both aerobic and facultatively anaerobic, and that these bacteria might account for the high concentration of sulphide at the surface of the water (Rohwerder et al. 2003). Sulphate-reducing bacteria were also shown to occur, the sulphur cycle being complete and seemingly connected with the nitrogen cycle through denitrification linked to sulphur oxidation. Detection of sulphur-oxidising bacteria in cultures enriched with thiosulphate and nitrate and incubated in anoxic condition, together with the presence of 16S rRNA gene sequences related to *Denitratisoma* denitrifiers in microbial biofilm samples from the cave, supports the hypothesis that sulphate and nitrogen cycles are linked. The use of NO_3^- by facultatively anaerobic sulphur oxidisers such as *Thiobacillus denitrificans*, as an alternative electron acceptor for respiration, might account for the depletion of nitrate from this environment, which is otherwise very rich in ammonium (Rohwerder et al. 2003; Kumaresan et al. 2014). Microbial N_2 fixation might also occur in the cave, many of the bacteria like *Beggiatoa* and *Methylocystis* being known N_2 fixers; nevertheless, further work in the area is needed because reduction of N_2 to NH_4^+ is highly energy-consuming and generally carried out during nitrogen-limited conditions (Kumaresan et al. 2014).

Along with sulphide oxidisers and sulphate-reducing bacteria, the second most important primary producers are methano- and methylotrophic bacteria, using as sole source of carbon and energy, CH_4 and C1-compounds, respectively, such as methanol and methylated amines resulted from the degradation of the microbial biofilm, as sole source of carbon and energy (Rohwerder et al. 2003; Chen et al. 2009; Wischer et al. 2015). The first methanotrophs identified in Movile Cave were retrieved from the water and the microbial pellicle and belong to *Alpha-* (*Methylocystis*/*Methylosinus*) and *Gammaproteobacteria* (*Methylomonas*, *Methylococcus*) (Hutchens et al. 2004). The methanol released by methanotrophs, as a result of methane oxidation, is probably used by methylotrophs as source of C. Among the obligate methylated amine users present in the microbial biofilm *Methylotenera mobilis* is dominant (Kumaresan et al. 2014). Other abundant methylotrophs are *Methylobacterium extorquens* and *Methylovorus* but also bacteria that were not known to grow methylotrophically, such as *Catellibacterium* (98% identity to *Catellibacterium caeni*), *Cupriavidus* (99% identity to *Cupriavidus necator*), *Porphyrobacter* (99% identity to *Porphyrobacter neustonensis*) and *Altererythrobacter* (99% identity to *Altererythrobacter epoxidivorans*) (Wischer et al. 2015). For extensive description of bacterial metabolism of methylated amines as well as for an overview on novel methylotrophs in Movile Cave refer to Wischer et al. (2015).

Small-scale metagenomic sequencing of DNA from mat samples provided approximately 960,000 sequences with a mean length of 360 bp, from which 36.8% corresponded to annotated proteins, 33.7% to unknown proteins and 1.9% to ribosomal sequences. Of the annotated sequences, 96.5% were of bacterial origin, 1.8% eukaryotic, 1.3% archaeal and 0.2% were viral sequences. Phylum-level phylogenetic classification revealed that 60% of the total annotated sequences belong to *Proteobacteria*, alongside Bacteroidetes (12.1%) and Firmicutes (7.6%). Nearly 3% of the total sequences retrieved were representative of cyanobacterial sequences, which is not expected in a deep cave ecosystem (Kumaresan et al. 2014).

Data about microbiota from sediments, i.e. the microoxic and anoxic sites of Movile, are still scarce. For the presence of methanogens in the sediment accounts, amplicons of the *mcrA* gene from DNA extracted from sediment samples (Kumaresan et al. 2014); to our knowledge, *Methanobacterium movilense* (Schirmack et al. 2014) and *Methanosarcina spelaei* (Gazert et al. 2014) are the only methanogens isolated so far from the anoxic sites of the cave. *Methanobacterium movilense* is a hydrogenotrophic, secondary alcohol-utilising methanogen isolated from sediments at the bottom of the cave water (Schirmack et al. 2014), while *Methanosarcina spelaei* is a strictly anaerobic archaeon, growing autotrophically on H_2/CO_2 and additionally using acetate, methanol, monomethylamine, dimethylamine and trimethylamine (Gazert et al. 2014).

An inventory of the cultivable microscopic fungi has been recently published by Nováková et al. (2018). Samples of dry material, including sediment and clay scrapped from the walls/ceiling of the upper level galleries, isopod faeces and invertebrate carcases (dead isopodes and spiders) were collected, while the wet samples, taken from the lower level (Lake Room and AirBell II), included water, microbial biofilm floating on the surface of the water and underwater sediment. Air from both dry and flooded parts of the cave was assessed for airborne fungi by the gravity settling method. To evaluate the degree of cave contamination with exogenous fungi, the underground microfungal community was compared with the surface, by collecting air and soil samples from the proximity of the cave entrance and similarly processed. For *Aspergillus* and *Trichoderma*, the morphological, physiological and chemical analyses were combined with molecular techniques. 90 and 28 fungi species were recovered from the dry part of the cave and from AirBell II, respectively.

Sixty-seven species were found only in the dry part of the cave, while 20 were found both in dry galleries and AirBell II. The most diverse genera were *Penicillium*, *Aspergillus* and *Cladospora*. Undescribed species of *Aspergillus* sect. *Candidi*, *Talaromyces kendrickii* and *Talaromyces ruber* are confined to AirBell II. The presence of *Aspergillus* sp. sect. *Candidi* in the anoxic sediment might indicate its involvement in denitrification processes. *Aspergillus thesauricus*, *Cylindrocarpon didymum*, *Penicillium griseofulvum* and *P. janthinellum* were isolated only from invertebrate cadavers while *Spiniger meineckellus*, *Aspergillus sydowii*, *Cadophora malorum* and *Paecilomyces* sp. from isopod faeces only. *Aphanocladium album*, *Aspergillus creber*, *Cladosporium variabile*, *Lecanicillium psalliotae*, and *Stachybotrys chartarum* were only isolated from the dry part of the cave and not from AirBell II nor from outside the cave. The new species *Aspergillus movilensis* and *A. europeus* were isolated from outside the cave, isopod faeces and cave sediment and from outside the cave, respectively (Hubka et al. 2015, 2016). Of great abundance and variability in the cave is also the fungi *Trichoderma*. The association with the chemoautotrophic bacteria and archaea present in the microbial pellicles floating on the surface of the water might have ecological importance, in the consumption of organic material produced chemoautotrophically and in dead cell degradation (Nováková et al. 2018).

Classical microbiological and molecular biology methods, together with stable isotope probes (SIP), have enabled a broad understanding of the ecosystem associated with the sulphurous aquifer present in South Dobrogea, to which Movile Cave is only an access gate. Strategies combining classical methods, SIP and metagenomic sequencing can provide genome information for various microbes, especially that these are difficult to cultivate in the laboratory (Kumaresan et al. 2014). Active microbial communities in the cave ecosystem could be further documented using metatranscriptomics and metaproteomics (Kumaresan et al. 2014). Ongoing as well as future research, focussing on the anoxic niches of the cave, namely, the sediment and the anoxic water, may answer questions such as whether there is any biogenic methane production or anaerobic methane oxidation. Still to be investigated are the trophic interactions between various microbial functional groups. Next-generation sequencing, combined with a variety of molecular ecology techniques and a concerted effort to isolate novel organisms, will improve our understanding of the functional diversity of the microbial communities and allow us to study the contributions of different functional guilds in maintaining this self-sustaining chemoautotrophic ecosystem (Kumaresan et al. 2014).

16.3.2 Cueva de Villa Luz (Mexico): A Cave Where Speciation Occurs in Its Multiple Habitats

Another sulphidic cave in which complex research has been undertaken is Cueva de Villa Luz (also called Cave of the Lighted House/Cueva de las Sardinas/Cueva de la Sardina/Cueva del Azufre) located 2 km south of the village Tapijulapa, Municipio de Tacotalpa, Tabasco, Mexico on the northern edge of the Chiapas Highlands (Palacios-Vargas et al. 2015; Borgonie et al. 2010). La Cueva de Villa Luz (Fig. 16.5) began to be scientifically studied only in the 1960s although its occurrence has been known for a very long time and it was used for various ceremonies and rituals by the native Zoque (Soque) population (Tobler et al. 2008, 2011). Decades before scientific studies began, unidentified specimens of a diverse fauna in the cave were collected both alive and preserved (Gordon and Rosen 1962; Palacios-Vargas et al. 2011). However, the first description of the cave as well as detailed studies on the cave molly fish (*Poecilia mexicana*) alongside with a partial inventory of the bats species living here was completed by the biologists Gordon and Rosen (1962). Unfortunately, their work was put aside for over 20 years, until 1987, when speleologists Jim Pisarowicz and Warren Netherton started to explore the region of Teapa (Tabasco). This is when the systematic and scientifically research at Cueva de Villa Luz really took off. A sketch of the entrance segment as well as the geological profile of the area where the cave is located was presented by Gamboa and Ku (1998), and the first accurate map and in-depth description were provided by Hose and Pisarowicz (1999).

The cave is located in a region with very well-represented endokarst, developed in lower cretaceous limestone and bounded to the south by a normal fault, a structure that probably controls the cave inlet's location (Hose et al. 2000). Cueva de Villa Luz is situated at an altitude of 100 m asl in a valley (Arroyo del Solpho) in between Amatán and Oxolatan streams, before they join to form the Tacotalpa river, about 100 km away from the Campeche Bay. The cave is surrounded by semi-tropical jungle as a consequence of both high temperatures and heavy rains resulted from the cooling of the moist winds coming from over the bay, uplifted by the Chiapas Highlands (Sierra Madre de Chiapas; Gordon and Rosen 1962).

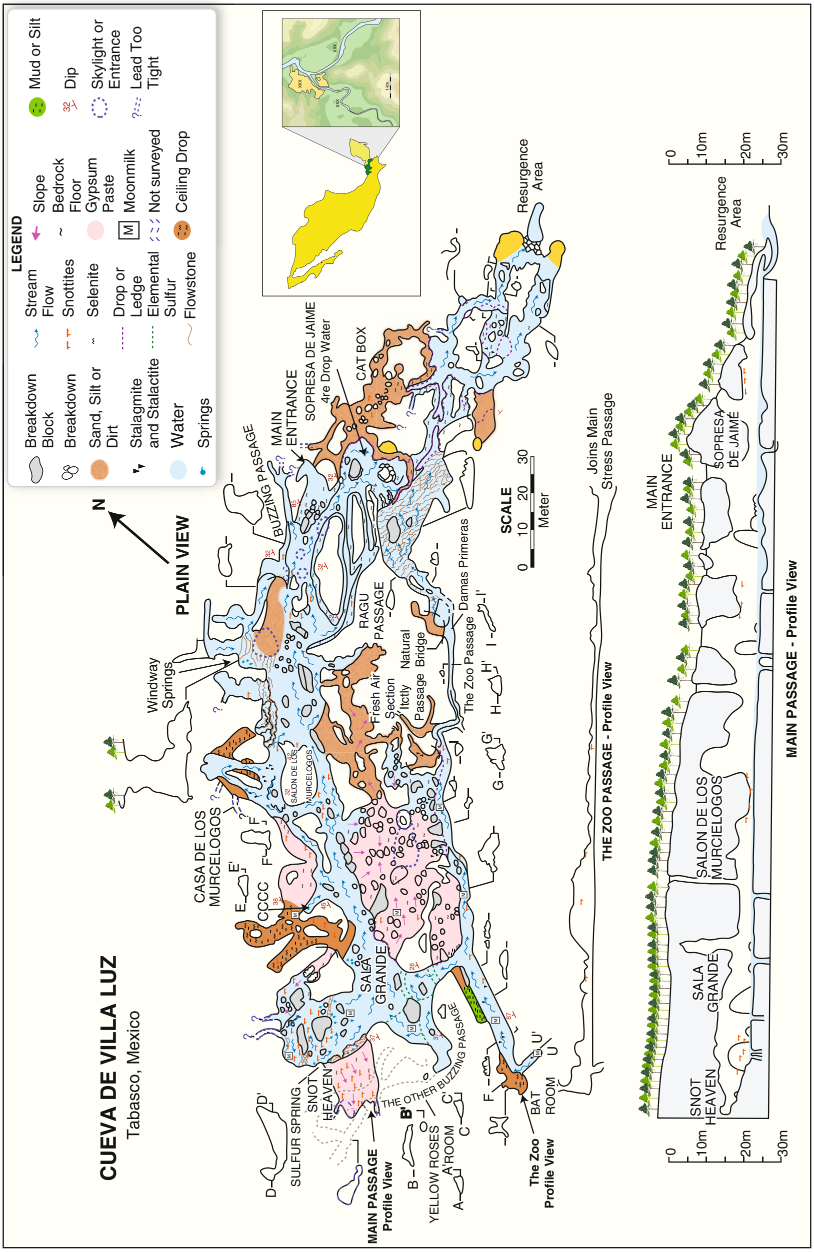

Fig. 16.5 Map of Cueva de Villa Luz; with permission from Hose and Pisarowicz (1999)

Regarding its speleogenesis, Cueva de Villa Luz was formed mainly by the slightly rise of thermal and sulphur-rich water from multiple springs through discrete conduits or gravel deposits on the floor of the cave (Hose et al. 2000). These inlets merge into a low-gradient, milky coloured stream of about 10–30 cm deep that flows through the cave (Hose and Pisarowicz 1999) at a rate of 200–300 L/s (Hose et al. 2000). Along with the H_2S-rich inlets (containing up to 300–500 mg/L H_2S and no measurable O_2), there are also discharge inlets of oxygenated water (4.3 mg/L O_2) and no detectable H_2S. The origin of the cave water is still under debate. The source of the H_2S-rich water might be the rich petroleum fields near Villahermosa, within 50 km of the cave, as indicated by the nature of dissolved solids in the cave water, as well as El Chichón volcano (also about 50 km away from the cave) which has sulphur-rich waters in its caldera (Hose et al. 2000).

The stream discharge occurs year round at a constant level, with flow discharges measured between 290 L/s (January 1998, early in the dry season) and 210 L/s (August 1999, early in the wet season); even after heavy rains, when the discharge in the Río Xoxocotlan, east of Tapijulapa, rose from 186 to 538 m^3/s (Hose et al. 2000) changes in the stream discharge at the resurgence and within the cave were not significant. The pH of the water is nearly neutral, with values of 7.2 and temperature of 27.5–28.38°C—slightly thermal as compared with the regional groundwater temperature of about 22–24°C (Hose et al. 2000).

The length of the explored galleries is of about 1.9 km (Borgonie et al. 2010), most of them being flooded by the sulphurous stream. The heights of the passages range from 25 cm to 15 m with several vertical tubes extending tens of metres upward from the stream level, intersecting the surface to form skylight entrances (Hose et al. 2000). The presence of these holes in the ceiling gave the name of the cave Cueva de Villa Luz (Cave of the Lighted House) and together with the sulphidic inlets determine various habitats, which differ drastically in terms of the presence/absence of the light and the presence/absence of H_2S. These factors can represent sources for natural selection and shape the structure of fauna populations as well as the trophic web in the cave.

The biocenosis of the cave is very diverse, represented by invertebrates and vertebrates. The trophic chains benefit of three sources of energy, namely, the litter that enters the cave through the skylights, the entrance or/and brought by animals, the bats guano and the chemosynthetic bacteria forming the snottites (biofilms in the shape of filamentous, gelatinous, elastic stalactites; Palacios-Vargas 2009). Among the invertebrates, ubiquitously present in huge densities is the dipteran *Goeldichironomus fulvipilus*, whose larvae are in their highest densities within the bacterial mats, where they graze on the bacteria (Palacios-Vargas et al. 2015). Within the snottites, live the nematode *Mesorhabditis acidophila* whose life cycle is tightly linked to the acidity of this microbial structures (pH 0–3), probably to escape predation by mites (Borgonie et al. 2010). The giant water bug *Belostoma* (Heteroptera, Belostomatidae) occurs also in high numbers in the cave. Planarians of the genera *Dugesia*, crabs *Trichodactylus bidens* (Trichodactylidae) and *Avotrichodactylus bidens*, whip spiders *Phrynus* (Amblypygi) and different spiders of the genera *Tetragnatha* (Tetragnathidae)*,* *Maymena* (Mysmenidae), *Eidmannell*a

(Nesticidae), *Tinus* sp. (Pisauridae), *Hemirrhagus pernix* (Theraphosidae) and a Ctenidae representative (Palacios-Vargas et al. 2011; Horstkotte et al. 2010; Palacios-Vargas 2009) are also present in the cave. The majority of acari live in guano (Mesostigmata and Astigmata, 79% and 6% of the microarthropods, respectively) or in litter. Associated with bacteria are only two species of Histiostomidae (Astigmata) and Oppidae (Cryptostigmata), in low number of individuals (Palacios-Vargas 2009).

From vertebrates, one species of fish was recorded in the cave, *Poecilia mexicana*, family Poeciliidae, notorious for being intensively studied for their pronounced ecomorphological and geographic variation (Tobler et al. 2006) and as models for speciation process triggered by peculiar environmental conditions such as H_2S and/or rotenone (a well-known inhibitor of the mitochondrial complex I, NADH coenzyme Q reductase; Palacios et al. 2013). As an adaptation to the high concentration in the water of the extremely toxic H_2S, cave mollies perform aquatic surface respiration (ASR) to exploit the more oxygenated topmost layer of the water column (Horskotte et al. 2010). The synbranchid eel *Ophisternona enigmaticum* was occasionally reported from the cave (Tobler et al. 2007). The other vertebrates are the bats from the families Mormoopidae (*Mormoops megalophylla*, *Pteronotus davyi*, *Pteronotus parnellii*, *Pteronotus personatus* and *Pteronotus gymnonotus*), Emballonuridae (possible *Baliantopterix*), Phyllostomidae *(Carollia)* and the vampire *Desmodus rotundus* (subfamily Desmodontinae; Palacios-Vargas et al. 2011).

In contrast to Movile Cave, Cueva de Villa Luz has three sources of energy, namely, the bat guano, the organic matter (leaves, soil, wood, detritus) that enters the cave through the skylights and the microbial communities formed of chemoautotrophic bacteria (Palacios-Vargas 2009). A pellicle of condensed or infiltrated fresh water covers the walls resulting in droplets in which hydrogen sulphide outgassed from the sulphidic water dissolves. In the droplets, the H_2S reacts with dissolved O_2 forming sulphuric acid. The pH value of the droplets is around 1.4. Sulphur-oxidising bacteria such as *Acidithiobacillus thiooxidans* facilitate this process (Hose et al. 2000) and form white deposits, called snottites that drip sulphuric acid at their extremities and grow quickly in length, up to a centimetre per day (Palacios-Vargas et al. 2015). The biofilm covering the walls of the galleries, as well as the snottites are dominated by the genus *Acidithiobacillus* (Jones et al. 2016) serving as one of the food bases in the cave, directly eaten by very abundant larvae of midges (Hose et al. 2000). While both prokaryotic microbiota and macrofauna of Cueva de Villa Luz are well characterised, fungal and protists communities have been neglected. To our knowledge, no study of these two components of the cave ecosystem has been undertaken yet, so that Cueva de Villa Luz remains still a very fertile subject for future investigation.

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Chapter 17
Researches in Lava Tubes

Pedro Oromí

17.1 Introduction

Volcanic terrain usually lacks limestone and other karstifiable rocks, but the formation of caves is frequent, though in a very different way from karstic processes. The most common caves in volcanic terrain are lava tubes, which form only in smooth, ropy basaltic flows ("pahoehoe") very soon after eruption, while molten lava is still flowing. Once a lava tube has cooled, it becomes habitable by a specialized fauna only through a process of ecological succession, on a variable time span depending on the local climate. Due to collapses, erosion and silting, lava tubes persist as suitable habitats for much shorter periods than limestone caves, usually not exceeding a few hundred thousand years (Howarth 1973).

The Canary archipelago in the eastern Atlantic is exclusively of volcanic origin and includes seven main islands with a variety of ages (from 1 to 21 Ma), volcanic rocks, type of eruptions and local climates. Tenerife is the largest, highest and most diverse island, with abundant lava tubes and other subterranean environments which harbour a rich troglobiont fauna (Oromí 2004). The highest density of lava tubes on the island is found in the lavas emitted by the initial basaltic eruptions of Pico Viejo, a stratovolcano in the central-western part of the island. Three caves located in these lavas, Cueva del Viento-Sobrado, Cueva de Felipe Reventón and Cueva de los Roques, have been the most attractive lava tubes for scientific studies in the Canary Islands (Montoriol and de Mier 1974; Wood and Mills 1977; Martín and Oromí 1986; Oromí 1995; Arechavaleta et al. 1996, 1998, 1999) together with the anchialine part of Corona lava tube on the island of Lanzarote (Wilkens et al. 2009; Martínez et al. 2016 and references therein).

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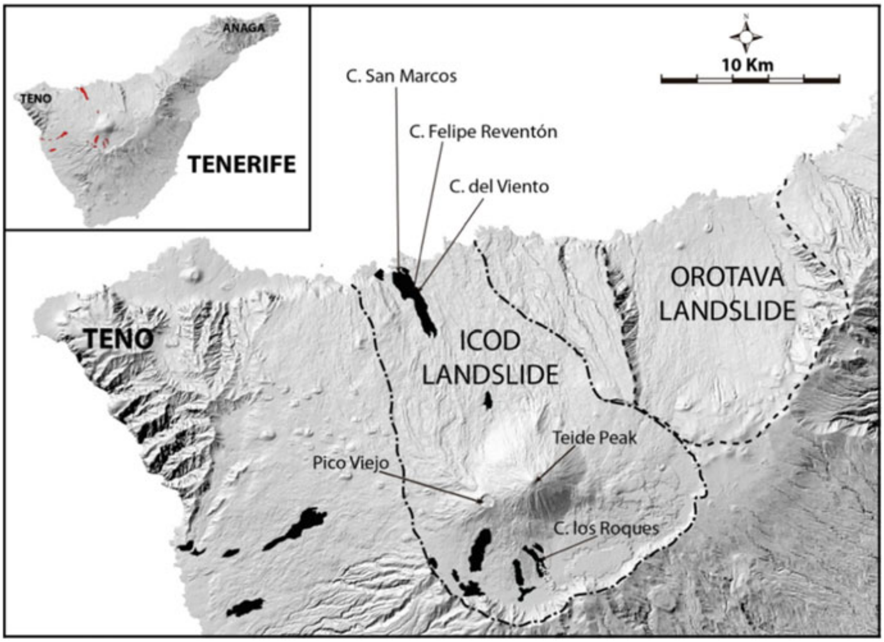

Fig. 17.1 Map of the northwest part of Tenerife. Dark spots: present outcrops of Pico Viejo pahoehoe lavas where the studied lava tubes are located. Dashed lines: boundaries of Icod and Orotava landslides. Modified after Carracedo et al. (2008)

17.2 Geological Aspects

Between 2.5 and 0.2 Ma, there were several cycles of volcanic activity in the centre of Tenerife, with large increases in height and three or more major explosive events, with the consequent steep sloping and instability. At least three huge landslides occurred during the last million years, at 0.8 Ma, 0.6 Ma and 0.15 Ma, respectively (Masson et al. 2002). The two older ones created the Güímar and La Orotava valleys, but the third one, known as the Icod landslide, released the island of such weight that volcanism was strongly reactivated, refilling the landslide scar and building up the existing Teide stratovolcano (3714 m) and its twin Pico Viejo (3135 m). These two volcanoes filled the upper part of the landslide scar and formed the northern limit of the modern 16 × 9 km Las Cañadas caldera (Fig. 17.1).

The initial eruptions of Pico Viejo were exclusively basaltic, producing fluid pahoehoe flows which readily form lava tube caves. Lava flows dated at 27,030 ± 430 years ago extended towards the west and the north, reaching the coast at both sides of the island, but a few short flows went southwards inside Las Cañadas caldera at over 2000 m.a.s.l. (Carracedo et al. 2008). The northern branch spreads over a large area and gave rise to many lava tubes, constituting the highest cave concentration in the archipelago. However, later eruptions of Teide and Pico

Fig. 17.2 Cueva de Felipe Reventón has many interconnected labyrinthine passages, typical of lava tubes originated on steep slopes. Photo by © Juan S. Socorro

Viejo produced extensive intermediate lavas and phonolites which covered most of the northwest of the island (except the ancient Teno massif), leaving exposed on the surface only some big kipukas (outcrops of the older pahoehoe lava surrounded by later flows). The entrances to the most important lava tubes in Tenerife—Cueva del Viento-Sobrado, Cueva de Felipe Reventón and Cueva de San Marcos—are in outcrops of this kind in the vicinity of Icod de los Vinos. The more limited Pico Viejo pahoehoe flows inside the caldera were hardly covered by newer lavas and contain a highly interesting lava tube, Cueva de los Roques (see locations in Fig. 17.1).

As Wood and Mills (1977) pointed out, Cueva del Viento is characterized by unusually complex planimetric form (see Fig. 17.2), sinuosity of the main passages, steep, multilevel long profile and great variety of passage forms. These features are also found at Felipe Reventón and to a lesser extent at Cueva de los Roques. The multilevel profiles were formed by lavas flowing over one another, and their passages were sometimes connected by the lower tube capturing the lava flowing through an upper, newly forming tube. Such captures, indicated by solidified lava falls up to 12 m high, connect as many as three distinct levels in Cueva del Viento-Sobrado (Fig. 17.3). Environmental parameters can differ between shallow and deep passages, with significant ecological consequences.

The known extent of Cueva del Viento-Sobrado (see Table 17.1), the world's fifth longest lava tube (Gulden 2015), must be just a small part of its real dimensions, given that its highest known passage is more than 10 km away from the eruptive vent on Pico Viejo. Felipe Reventón is actually an independent cave from a strict

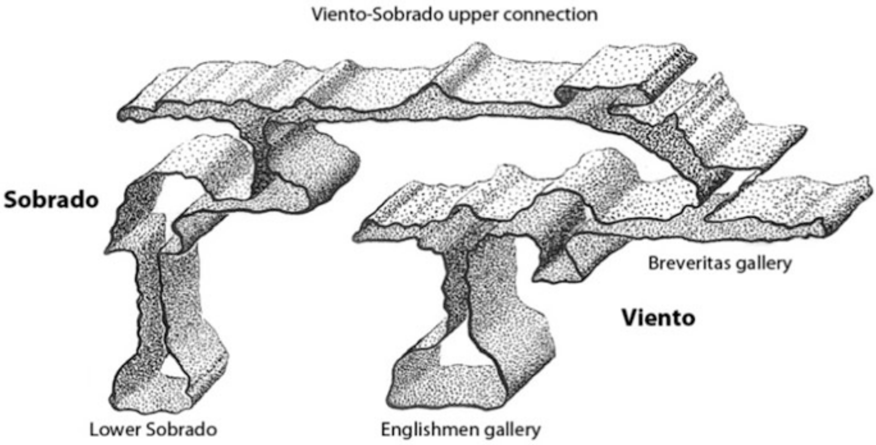

Fig. 17.3 Cross section of interconnected galleries in Viento-Sobrado cave complex, showing the three different levels formed by consecutive overlapping flows of the same eruption. Drawing by © Ángel Vera

Table 17.1 Data on the most important lava tubes from Pico Viejo pahoehoe lavas. Temperatures measured in galleries away from entrances

Cave	Linear extent (m)	Altitude range (m)	Temperature (°C)	No. of entrances
San Marcos	2130	30–99	15.0–16.0	2
Viento-Sobrado	18,500	480–1040	13.8–15.2	6
Felipe Reventón	1845	595–734	14.5–15.5	1
Los Roques	900	2160–2200	13.0–13.5	4

speleological point of view, but its projection on the map reveals its continuity at some distance with one of Cueva del Viento-Sobrado main branches, now interrupted by a lava siphon.

17.3 Ecological Features

Lava tube caves that have not been covered by later flows are usually shallow, forming a few metres below the surface of a solidifying flow and running parallel to the slope. While in limestone caves ecological succession advances downwards creating new habitats ever deeper, in lava tubes the situation is reversed, and succession progresses upwards as soil develops on the surface and vegetation grows on it, and the cave environment becomes humid and stable (Howarth 1996). Conditions in the deepest passages in the lowest levels of Cueva del Viento-Sobrado differ from those in shallower ones, the former with scarcer food resources, more

Fig. 17.4 Roots hanging from the ceiling in one of the main passages of Cueva del Viento (Photo, Juan S. Socorro). Upper left: an individual of the planthopper *Tachycixius lavatubus* on the roots. Photo by © Pedro Oromí

constant temperature and humidity and higher CO_2 concentrations. Conditions in deep tubes are unsuitable for non-adapted animal species and lead to dominance of troglobionts (Arechavaleta et al. 1994).

Young lava tubes are usually dry and are occupied by particular epigean species shared with recent lavas and known as lavicoles; as ecological succession progresses, the caves mature, and this fauna is replaced by troglobionts (Ashmole et al. 1992). Most caves from Pico Viejo lavas are in a mature stage due to the time elapsed since their origin (appr. 27,000 years) combined with the mesic climate prevailing on the island. Pahoehoe lavas from the Icod region are mostly covered by soil, with a mixed pine forest on those at intermediate altitudes (Cueva del Viento-Sobrado and Cueva de Felipe Reventón). Stable temperatures (see Table 17.1) and high humidity prevail throughout the year in these caves, suiting them for cave-adapted fauna. The pahoehoe flows containing Cueva de los Roques are in an earlier stage of succession due to the drier climate at this altitude; here there is generally little soil on the surface and only scattered shrub vegetation. Nevertheless, environmental stability in Cueva de los Roques is adequate in galleries where drying air currents are absent (Martín and Oromí 1986).

The shallow situation of lava tubes and the abundant cracks in the lava allow plant roots to reach these caverns, dangling from the ceiling and walls (Fig. 17.4). This is an essential resource for sapsucking and rhizophagous insects, which are usually absent or very rare in limestone caves from geographically comparable areas. These species are significant contributors to the cave community and its trophic network, and are probably the most abundant cave-dwelling animals. The profuse network of cracks and small collapses also facilitate entry by many trogloxene animals, which form an important resource for predatory species in many parts of the cave, far from any large entrance. The abundant epigean flies captured in traps demonstrate the easy connection with the outside and may account for the striking abundance and variety of troglobiotic web spiders, which could not survive feeding only on other troglobiont arthropods.

Relative humidity is usually high and ceiling drip very frequent, but water is rarely present on the cave floor due to the abundance of cracks connected with a network of voids in the host rock. Thus, no significant water ponds are present, and no stygobiont species have ever been found because the water table is much deeper than the caves. The absence of water streams within the caves is a major cause of the poverty of passages at deeper levels, where scarce food supplies arrive with percolating water and only a few troglobionts can survive.

Environmental features in Viento-Sobrado and Felipe Reventón caves are very similar since they are in the same lava flow, mainly at the same altitude and at an identical stage of maturity, sharing most of the troglobiont species. From the ecological and the speleogenic points of view, they should be considered as a single cave, even though they lack connections for humans. Accumulations of soil in some passages provide the only habitat better represented in Felipe Reventón and may explain the occurrence of some species never found in Cueva del Viento-Sobrado.

Cueva de los Roques is within Teide National Park; its entrances are fenced and visits highly restricted, so it is in a very good state of conservation. However, Viento-Sobrado and Felipe Reventón caves are in an inhabited area, with houses on the surface, so they are threatened to some extent. The lower part of Viento (Piquetes) is absolutely polluted by raw sewage, the central part (Breveritas) is reasonably well preserved but endangered, while the upper part (Sobrado) is in a natural area under pine forest and is owned by the local government, gated and in very good condition (Oromí 1995). A 150 m long stretch of Sobrado is dedicated to public visits.

17.4 Animal Communities

The diversity of terrestrial troglobionts in the three lava tubes is really remarkable, competing with the richest caves in the world (see Table 17.2) and being the highest among volcanic caves (Culver and Pipan 2013). The communities in these cavities are by far the most varied in cave-adapted animals within the Canary archipelago (see Table 17.3), the only one on a comparable level being the community in the anchialine Cueva de la Corona on Lanzarote, which only harbours stygobiont species (Martínez et al. 2016).

Due to the shallowness of these Tenerife caves, which facilitates the input of accidental trogloxenes, the total number of arthropod species found in them is roughly double the number of troglobionts (see Table 17.4, bottom), with the exception of Cueva de los Roques which is in a dry subalpine zone with a poorer epigean fauna. The deepest levels of Cueva del Viento-Sobrado are usually free of these trogloxenes.

Table 17.2 Caves with more than 25 troglobionts (modified from Culver and Pipan 2013). Cueva del Viento and Cueva del Sobrado, actually the same cave system, harbour a total of 36 species between them

Cave	Country	No. of species	Cave type
Postojna-Planina Cave	Slovenia	38	Karst cave
Cueva de Felipe Reventón	Canary Islands	37	Lava tube
Vjetrenica	Bosnia and Herzegovina	30	Karst cave
Movile Cave	Romania	29	Chemoautotrophic
Cueva del Viento	Canary Islands	28	Lava tube
Cueva del Sobrado	Canary Islands	28	Lava tube
Mammoth Cave	USA	26	Karst cave

Table 17.3 Number of cave-adapted species (troglobionts and stygobionts) so far known in the top ten richest lava tubes and one artificial mine ([a]) on the Canary Islands

Cave	Island	Habitat	No. of species
Felipe Reventón	Tenerife	Terrestrial	37
Viento-Sobrado	Tenerife	Terrestrial	36
La Corona	Lanzarote	Anchialine	33
Los Roques	Tenerife	Terrestrial	24
Labrada	Tenerife	Terrestrial	19
Palmeros	La Palma	Terrestrial	19
Grande de Chío	Tenerife	Terrestrial	16
Bucio	Tenerife	Terrestrial	15
Salto de Tigalate	La Palma	Terrestrial	15
Los Llanetes[a]	Gran Canaria	Terrestrial/freshwater	11

Viento-Sobrado and Felipe Reventón have a total of 42 species if considered as a single cave.

Local troglophiles are usually widespread species, some clearly introduced like the millipedes *Blaniulus guttulatus* and *Choneiulus subterraneus* and some probably native like the spider *Meta bourneti*, the psocid *Psyllipsocus ramburii* and also the moth *Schrankia costaestrigalis*, whose caterpillars are active root eaters. None of the mentioned troglophiles except the psocid are present in Cueva de los Roques, probably due to the colder climate at this altitude.

The cave-dwelling communities of Cueva del Viento-Sobrado and Cueva de Felipe Reventón are very alike and probably constitute a single unit for the animals because of their proximity and underground connection through mesocaverns (see Chap. 3). No less than 42 troglobiont species have been found in them together (see Table 17.4), which represents 60% of the total species known from the central, young part of Tenerife at low and intermediate altitudes. Most of these troglobionts have spread out along the island through different subterranean environments (mesocaverns, MSS, etc.), occupying most of the island except the old, caveless Anaga Peninsula in the NE, which has remained isolated in terms of subterranean dispersal (Oromí and Martín 1992). The troglobiont community from Cueva de los Roques includes six species only found at over 1800 m.a.s.l. on the island; some of

Table 17.4 Troglobiont arthropods occurring in the studied caves of Pico Viejo lavas

Fauna group	Family	Species	Trophic spectrum	Lava tube		
				Viento Sobrado	Felipe Reventón	Los Roques
Pseudoscorpiones	Chthoniidae	*Paraliochthonius setiger*	Predator	x		
		Paraliochthonius superstes	Predator	x	x	
		Paraliochthonius tenebrarum	Predator		x	x
		Lagynochthonius curvidigitatus	Predator		E	
	Syarinidae	*Microcreagrina subterranea*	Predator	x	x	x
Araneae	Dysderidae	*Dysdera ambulotenta*	Predator	x	x	x
		Dysdera esquiveli	Predator	x	x	
		Dysdera labradaensis	Predator	x	x	x
		Dysdera sibyllina	Predator	x	x	
		Dysdera unguimmanis	Predator	x	x	
		Dysdera gollumi	Predator			E
		Dysdera chioensis	Predator			x
	Pholcidae	*Spermophorides reventoni*	Predator	x	x	
		Pholcus roquensis	Predator			E
	Liocranidae	***Agraecina canariensis***	Predator	x	x	
	Linyphiidae	***Metopobactrus cavernicola***	Predator	x	x	
		Troglohyphantes oromii	Predator	x	x	x
		Walckenaeria cavernicola	Predator	x		x
	Nesticidae	***Canarionesticus quadridentatus***	Predator	x	x	
Diplopoda	Glomeridae	*Glomeris speobia*	Detritivore	x	x	x
	Iulidae	*Dolichoiulus labradae*	Detritivore	x		
		Dolichoiulus ypsilon	Detritivore	x	x	x
Chilopoda	Cryptopidae	*Cryptops vulcanicus*	Predator		x	
	Lithobiidae	*Lithobius speleovulcanus*	Predator	x	x	x
Isopoda	Porcellionidae	*Porcellio martini*	Detritivore	x	x	
	Armadillidae	*Venezillo tenerifensis*	Detritivore	x	x	x
	Trichoniscidae	*Trichoniscus bassoti*	Detritivore	x	x	

Collembola	Paronellidae	*Troglopedetes cavernicola*	Detritivore	x	x	
		Troglopedetes vandeli	Detritivore	x	x	
Blattaria	Blattellidae	***Loboptera subterranea***	Omnivore	x	x	
		Loboptera troglobia	Omnivore	x	x	
		***Loboptera* sp.**	Omnivore			E
Hemiptera	Cixiidae	*Tachycixius lavatubus*	Rhizophagous	x	x	x
Coleoptera	Carabidae	***Lymnastis subovatus***	Predator	x	x	x
		Lymnastis thoracicus	Predator	x	x	
		Spelaeovulcania canariensis	Predator	x	x	
		Canarobius oromii	Predator			x
		Gietopus martini	Predator	x	x	x
		Wolltinerfia tenerifae	Predator	x	x	x
	Histeridae	*Aeletes oromii*	Predator		E	
	Staphylinidae	*Ocypus* n. sp.	Predator			E
		Domene alticola	Predator	x	x	x
		Domene vulcanica	Predator	x	x	x
		Micranops bifossicapitatus	Predator		x	
		Micranops spelaeus	Predator		E	
		Micranops mlejneki	Predator			x
		Alevonota oromii	Predator	x		
		Alevonota outereloi	Predator	x		
		Alevonota canariensis	Predator			x
	Curculionidae	***Oromia hephaestos***	Rhizophagous	x	x	
Troglobionts		**50**		**36**	**37**	**24**
Total no. of species				**86**	**77**	**38**

In bold: taxa with no congeneric epigean species on the archipelago. (x) presence; (E) exclusive to one cave. Total species: non-adapted + troglobionts.

them are exclusive to this cave (see Table 17.4 and Arechavaleta et al. 1998). Some of these high-altitude species like *Dysdera gollumi*, *Alevonota canariensis*, *Micranops mlejneki* and *Ocypus* sp. are vicariants to other congeneric troglobionts occurring at lower levels.

It is remarkable the high diversity of predators (37) occurring in these caves in relation to the total number of troglobiont species (50). Particularly interesting is the presence of 4–5 species per cave of *Dysdera* spiders (Fig. 17.5), a genus with nine troglobiont species in Tenerife, all but two with different epigean sister species (Arnedo et al. 2007). This implies that at least eight independent colonizations of the underground have taken place, with eight different evolutionary events towards troglomorphism. Only the ninth species derived from an already troglobiont sister taxon. Multispecies lava tubes are generally inhabited by large-, medium- and small-sized *Dysdera* species, as happens with *D. ambulotenta*, *D. chioensis* and *D. gollumi* in Cueva de los Roques (Arnedo et al. 2007). *Agraecina canariensis* is also a wandering spider, while the remaining six spiders found in these caves are web-building species.

Beetles are the most varied order, the majority being predatory species. The ground beetles are all eyeless but not highly evolved with respect to other troglomorphic characters, as is usual with other cave-adapted Canary species of this family. However, most of the nine rove beetles listed in Table 17.4 are highly troglomorphic (see Fig. 17.6), a common feature of the 24 troglobiont rove beetles from the archipelago, which together with those from Morocco form the top world's hot spot for this group (Hlaváč et al. 2006).

Omnivore species are represented by three *Loboptera* cockroaches, completely troglomorphic like three other species of the genus occurring in central Tenerife,

Fig. 17.5 *Dysdera unguimmanis*, the most troglomorphic among nine cave-adapted species of the genus occurring in Tenerife. Photo by © Pedro Oromí

Fig. 17.6 The rove beetle *Domene vulcanica* is one of the most troglomorphic arthropods occurring in Tenerife, with a marked "aphaenopsid" appearance. Photo by © Pedro Oromí

while in the MSS of the older Anaga peninsula, there are two-eyed ambimorph species (Oromí and Martín 1992). Three permanent cave-dwelling insects feed on living roots inside these lava tubes: the caterpillar of the troglophilic moth *Schrankia costaestrigalis*, the scarce eyeless weevil *Oromia hephaestos* and the very abundant planthopper *Tachycixius lavatubus.* The latter is frequent in most lava tubes on the island, showing subtle morphologic but marked bioacoustic differences across its range, which suggests the existence of at least two allopatric species on the island (Hoch and Asche 1993). Detritivore species are represented by *Venezillo tenerifensis* woodlice and *Glomeris speobia* pill millipedes, both occurring in most Tenerife caves, and two true millipedes of the diverse genus *Dolichoiulus*, which has given rise to a swarm of 53 endemic species on the archipelago, including 7 troglobionts (Enghoff 2012 and references therein). Two troglomorphic springtails of the genus *Troglopedetes* have been recorded for these caves (da Gama and Ferreira 2000), but their identity should be checked since it is difficult to accept that continental troglobionts have colonized oceanic islands.

Concerning their possible evolutionary origin, 18 out of 50 troglobionts occurring in these caves (Table 17.4, in bold) have no epigean close relatives and can be considered as relicts. The Canaries have never been glaciated, but other climatic changes like droughts could have promoted isolation underground (Rando et al. 1993). The classic Climatic Relict Hypothesis could explain the origin of these troglobionts although not necessarily promoted by severe cooling. The remaining 32 taxa belong to genera also including epigean species living in the archipelago, often on Tenerife and even on the surface near the caves. The Adaptive Shift Hypothesis fits many Canarian troglobionts which live in sympatry even with their epigean sister species (Contreras-Díaz et al. 2007).

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Chapter 18
Research in Anchialine Caves

Thomas M. Iliffe and Fernando Alvarez

18.1 What Are Anchialine Caves?

Prompted by his discovery of a variety of unusual shrimps from coastal pools in diverse tropical locations, Dutch carcinologist Lipke Holthuis (1973) coined the term anchialine for habitats “with no surface connection with the sea, containing salt or brackish water, which fluctuate(s) with the tides.” Finding similar pools in the interior of otherwise dry caves and associated networks of extensive submerged passageways accessible only to specialized cave divers prompted Stock et al. (1986) to refine the definition of anchialine as “bodies of haline waters, usually with a restricted exposure to open air, always with more or less extensive subterranean connections to the sea, and showing noticeable marine as well as terrestrial influences.” Most recently, Bishop et al. (2015) proposed a further revision of the definition as “a tidally-influenced subterranean estuary located within crevicular and cavernous karst and volcanic terrains that extends inland to the limit of seawater penetration.” Future diving exploration and scientific investigations of anchialine caves are likely to further broaden our understanding of this concept such that new and expanded definitions will result.

Anchialine caves occur primarily on oceanic islands and peninsulas in tropical to subtropical regions around the globe (Fig. 18.1). However, the greatest numbers of anchialine caves are in the Caribbean and Western Atlantic, especially the Bahamas Archipelago and Mexico’s Yucatan Peninsula. Although most anchialine caves form in limestone by solution, they can also occur in volcanic rock as submerged lava

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Fig. 18.1 World map showing the general locations (light gray circles) of regions where anchialine ecosystems occur

tubes (e.g., the Canary Islands and Hawaii) or as tectonic cracks (e.g., Galapagos Islands and Iceland). The world's longest underwater caves are found along the Caribbean coast of the Yucatan Peninsula where entrances to such caves are locally referred to as "cenotes." In this area, more than 1300 km of submerged cave passages has been explored and surveyed. Two of the longest are Sistema Sac Actun at 347 km and Sistema Ox Bel Ha at 270 km (QRSS 2018). Similar underwater caves in the Bahamas and Belize, known as "blue holes," occur both on land and beneath the seafloor. While many blue holes are formed by solution, some occur in straight line and are thought to originate through slump faulting (linear fractures originating from mass wasting by glacio-eustatic changes and gravitational stresses, then enlarged by karstic dissolution along the margin of shallow-water limestone platforms with the deep ocean (Palmer 1986).

One of the defining characteristics of anchialine caves is their subterranean tidal exchange with the ocean, typically with lower salinity water outflowing on a falling tide, while higher salinity water floods inland on the rising tide. Characteristically, tides in anchialine cave pools have diminished amplitude and occur later than the peaks for the corresponding tide in the adjacent ocean.

Another distinguishing feature is the presence of moderate to well-developed haloclines and resulting vertical stratification with meteoric fresh to brackish surface waters overlying denser seawater. One or more salinity interfaces or haloclines separate the cave water column into multiple layers, each with their own distinctive water chemistry and biology. In coastal aquifers, the thickness of the freshwater lens and resulting depth of the halocline increase with distance inland.

In most Yucatan cenotes, water temperature increases below the halocline in the saltwater layer, while in many Bahamian blue holes, the opposite occurs, with temperatures dropping beneath the halocline. In some caves, a sharp spike in temperature is found at the halocline, possibly as a result of increased microbial metabolism of particulate organic matter settling out on the density interface. Open sinkhole-type caves often receive large inputs of organic matter (e.g., leaves, etc.) such that the water becomes anoxic for several meters or more below the halocline, clouds of hydrogen sulfide form, and a strong “rotten egg” odor is apparent to divers. In those caves with horizontal circulation of seawater from the ocean, dissolved oxygen levels recover below the hydrogen sulfide, but in most cases, the underlying seawater clears but remains anoxic with only obligate anaerobic microbes able to exist. For those caves in which the influx of organic matter is lower, the saltwater layer tends to be hypoxic, but fauna with specialized metabolic adaptations are able to survive and sometimes flourish (Bishop et al. 2004).

Low pH is commonly associated with haloclines in anchialine caves, likely as a result of CO_2 produced there by microbial oxidation of particulate organic matter. Passages in anchialine caves commonly occur at haloclines due to dissolution of limestone by low-pH waters, in conjunction with mixing corrosion, whereby the combining of two water masses (e.g., fresh- and saltwater), both saturated with dissolved $CaCO_3$, results in an undersaturated mixture, capable of dissolving more limestone.

Marine caves containing conduits connecting the open ocean with neighboring, more restricted water bodies such as nearly enclosed bays (e.g., Harrington Sound in Bermuda), shallow-water interior of carbonate platforms (e.g., the Bahamas), or saltwater lakes in the interior of islands (e.g., Palau) can exhibit strong tidal currents. In such cave systems, it may only be possible for divers to enter during a brief period at slack high tide when currents in the cave slow, stop, and then change direction. The exact time when slack cave tides occur depends on a complex combination of factors including nature and size of the cave passages, hydrography and distance between the ocean and adjacent water bodies, wind direction and speed, phase of the moon, etc.

18.2 Exploration and Investigation of Anchialine Caves

Submerged caves are one of the few remaining global environments that can only be explored and studied by direct human participation, while cave diving, especially for the untrained or ill equipped, is considered one of the most dangerous activities (Potts et al. 2016). Cave diving involves all of the hazards of open-water diving but adds the risks of becoming lost, running out of breathing gas, getting stuck, or not being able to make a direct ascent to the surface. Conducting scientific research during cave dives further increases the complexity and potential danger (Iliffe 2018).

Cave divers use a guideline, marked with directional arrows, to navigate through complex underwater labyrinths. They reserve at least two-thirds of their initial gas supply to exit and thus need additional air tanks. Due to depth and long duration of cave dives, many of them involve decompression stops to allow accumulated inert gases, e.g., nitrogen or helium, to slowly and safely escape from divers’ tissues. Many cave divers move scuba tanks from the back to positions under each arm so that they can negotiate their way through low areas in the cave passage. An increasing number of cave divers are using closed-circuit rebreathers which recycle exhaled gas through a carbon dioxide scrubber and then add additional oxygen and/or diluent gas to maintain the PO_2 in an appropriate and safe range. Rebreathers release no exhaust bubbles so are less disruptive to the cave environment and cause fewer disturbances to cave fauna.

18.3 Diversity of Anchialine Fauna

A wide variety of invertebrates and vertebrates have invaded and colonized anchialine habitats (Fig. 18.2), becoming specialized to live either in the freshwater or in the marine layer. Only a few species occur both above and below the halocline, although this varies according to the salinity gradient between the layers. The majority of species found in anchialine environments are adapted to cave life, showing the common responses of pigment loss, reduction of eyes, elongation of appendages, increased number of sensory structures such as setae or aesthetascs, reduced fecundity, and absence of dispersing larvae. Other adaptations, to hypoxic conditions or to withstand food deprivation, may include varying oxygen consumption, enzyme activity, and lipid concentration (Bishop and Iliffe 2012).

Stygobitic groups that have been recorded from anchialine habitats include protists, sponges, chaetognaths, annelids, molluscs, crustaceans, mites, echinoderms, and fishes. Cnidarians, ctenophores, nematodes, priapulids, echiurids, and insects have also been collected in anchialine environments but are considered as accidental occurrences. The most diversified group in anchialine habitats is the crustaceans with more than 500 species representing 4 classes, 16 orders, and 57 families. Crustaceans are remarkable in that radiation into this habitat has resulted in the differentiation of major lineages, originating from both freshwater and marine ancestors (Iliffe and Bishop 2007). Characteristic styogobitic groups (see also Fig. 18.2) inhabiting anchialine caves include:

Remipedia The Remipedia, a class of crustaceans exclusively found in anchialine caves, are eyeless, unpigmented, predatory crustaceans with a head and elongate trunk composed of up to 42 similar body segments. Swimming appendages are located laterally on each segment, with animals slowly swimming on their backs. Paired, hollow-tip fangs, connected to secretory glands, can inject venom composed of hydrolytic enzymes and a neurotoxin into their prey (von Reumont et al. 2014). The preys, typically small shrimp or other crustaceans, are held by the mouthparts while being digested externally. Although they have a generally primitive body plan, their brain is highly organized and well differentiated with a large olfactory region.

Remipedes are a prime example of an anchialine stygobite with a Tethyan distribution, inhabiting caves on opposite side of the Atlantic Ocean and from the eastern side of the Indian Ocean (Koenemann and Iliffe 2013). Of the 29 described species, 20 are from the Bahamas Archipelago, 3 from the Yucatan Peninsula, 2 from the Canary Islands, and 1 each from Belize, Cuba, the Dominican Republic, and Western Australia. A recent molecular phylogenetic revision has subdivided extant remipedes into 8 families containing 12 genera (Hoenemann et al. 2013;

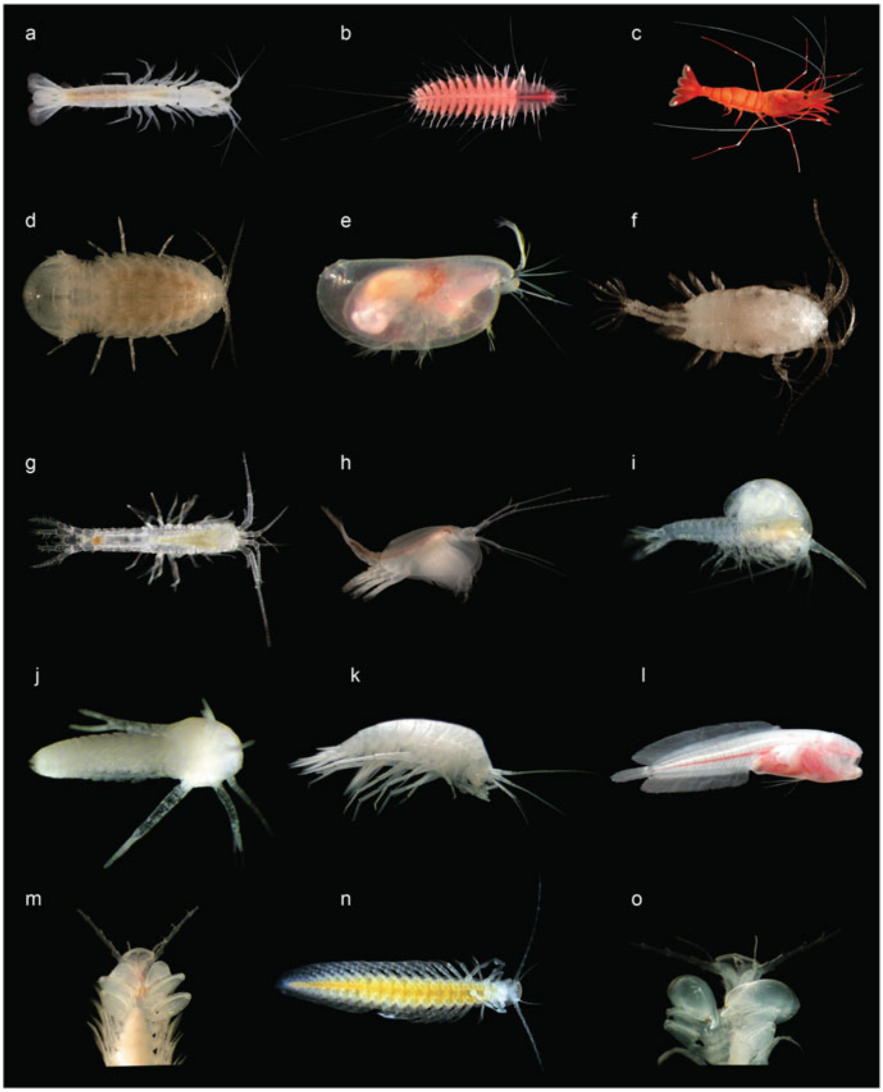

Fig. 18.2 Characteristic anchialine species: (**a**) *Stygiomysis cokei* (Stygiomysida, Stygiomysidae), (**b**) *Pelagomacellicephala iliffei* (Polychaeta, Polynoidae), (**c**) *Parhippolyte sterreri* (Decapoda, Barbouriidae), (**d**) *Exumalana reptans* (Isopoda, Cirolanidae), (**e**) *Spelaeoecia capax* (Ostracoda, Halocyprididae), (**f**) *Bomburiella gigas* (Calanoida, Epactriscidae), (**g**) *Mictocaris halope* (Mictacea, Mictocarididae), (**h**) *Speonebalia* sp. (Leptostraca, Nebaliidae), (**i**) *Tulumella grandis* (Thermosbaenacea, Tulumellidae), (**j**) Remipedia larva—*Pleomothra apletocheles*, (**k**) *Tuluweckelia cernua* (Amphipoda, Hadziidae), (**l**) *Typhliasina pearsei* (Ophidiiformes, Bythitidae), (**m**) *Godzillius fuchsi* (Remipedia, Godzilliidae), (**n**) *Cryptocorynetes elmorei* (Remipedia, Cryptocorynetidae), (**o**) *Pleomothra apletocheles* (Remipedia, Pleomothridae), (**m**) and (**o**)—anterior ventral view

Olesen et al. 2017). Furthermore, multiple lines of morphological and molecular evidence indicate that remipedes are the closest living crustacean sister group of the hexapods or insects (Moura and Christoffersen 1996; Ertas et al. 2009; von Reumont et al. 2011; Misof et al. 2014; Oakley et al. 2012; Stemme et al. 2013, 2015; Christie 2014).

While many remipede species occur only in a single cave, for yet unknown reasons, seven or more species inhabit Dan's Cave on Abaco, Bahamas. Typically, remipedes occur in low density, although there a few exceptions such as Cenote Crustacea in Yucatan, Mexico, where many hundreds of remipedes inhabit a restricted section of this anchialine cave. Larvae of only one species of remipede, *Pleomothra apletocheles*, are known (Olesen et al. 2014a), having been collected exclusively from Dan's Cave. At least nine and likely more developmental stages have been observed, all free-living, nonfeeding, and carrying out only small changes between stages.

Mictacea The peracarid crustacean order Mictacea is represented by a single species from anchialine caves in Bermuda. Although originally grouped with several similar crustaceans from the deep sea, it has now been separated (Poore 2015). Ovigerous females of *Mictocaris halope* from Bermuda have a typical ventral marsupium, but little else is known about the larvae or their development (Olesen et al. 2014b).

Copepoda Calanoida with Anchialine Representatives The basal calanoid family Epacteriscidae contains 18 anchialine genera inhabiting caves in widely distributed locations (Fosshagen et al. 2001; Bradford-Grieve et al. 2014). They include *Azygonectes* from the Caicos Islands and the Bahamas; *Balinella* from the Bahamas and Yucatan; *Bofuriella*, *Bomburiella*, *Caiconectes*, and *Erebonectoides* from the Caicos Islands; *Bunderia* from Western Australia; *Cryptonectes*, *Enantronoides*, *Minnonectes*, and *Oinella* from the Bahamas; *Edaxiella* from Jamaica; *Enantronia* from Lanzarote, Canary Islands; *Enantiosis* from the Bahamas, Bermuda, Belize, Galapagos, Fiji, and Palau; *Epacteriscus* from Belize and Bermuda; *Erebonectes* from Bermuda; and *Gloinella* and *Iboyella* from Cuba. The calanoid family Pseudocyclopidae contains 11 anchialine genera including stygobitic species of *Badijella* from Croatia; *Boholina* from Korea, the Philippines, and Sulawesi; *Brattstromia* from Belize; *Exumella* from the Bahamas, Yucatan, and Balearic Islands; *Exumellina*, *Normancavia*, *Robpalmeria*, and *Stargatia* from the Bahamas; *Hondurella* from the Bay Islands of Honduras; *Ridgewayia* from Bermuda, the Galapagos Islands, and Palau; and *Stygoridgewayia* from Western Australia (Bradford-Grieve et al. 2014).

Copepoda Cyclopoida with Anchialine Representatives The cyclopoid family Speleoithonidae contains three species of *Speleoithona* from anchialine caves in the Bahamas and Bermuda (da Rocha and Iliffe 1991, 1993); Smirnovipinidae with the genus *Costanzoia* inhabits anchialine caves from Italy and the Balearic Islands (Zagami and Brugnano 2012); Cyclopinidae with the monotypic genus *Oromiina* occurs in an anchialine lava tube cave in the Canary Islands; and Cyclopidae with two monotypic genera, *Troglocyclops*, occurs in a single cave in the Bahamas and, *Prehendocyclops*, in multiple caves in the Yucatan Peninsula.

Copepoda Harpacticoida with Anchialine Representatives The harpacticoid family Superornatiremidae includes three genera and nine species from anchialine caves in Bermuda, the Canary Islands, and the Balearic Islands (Huys 1996; Jaume 1997), while Novocriniidae contains the monotypic genus *Novocrinia* inhabiting anchialine and marine caves on the Belize Barrier Reef.

Copepoda Misophrioida Nine genera of misophrioid copepods are found exclusively in anchialine habitats: *Boxshallia*, *Dimisophria*, and *Palpophria* from Lanzarote; *Expansophria* from the Galapagos, Lanzarote, Palau, and Sardinia; *Huysia* and *Protospeleophria* from the Bahamas; *Mexicophria* from Yucatan; *Speleophria* from the Balearic Islands, Bermuda, Croatia, northern Western Australia, the Nullarbor in southern Western Australia, Palau and Yucatan; and *Speleophriopsis* from the Balearic Islands, Bermuda, the Canary Islands, Croatia, and Palau (Boxshall et al. 2014).

Copepoda Platycopioda Two anchialine genera of platycopid copepods, *Antrisocopia* and *Nanocopia*, are known only from a single Bermuda cave (Fosshagen and Iliffe 1985, 1988).

Ostracoda Halocyprida with Anchialine Representatives Most cave-adapted species from the ostracod order Halocyprida belong to four exclusively anchialine genera. The genus *Danielopolina* from the family Thaumatocyprididae has recently been revised by Iglikowska and Boxshall (2013) such that anchialine cave species were separated into *Humphreysella* with 11 species from the Bahamas, Canary Islands, Christmas Island, Cuba, Galapagos, Jamaica, and Yucatan and the monotypic *Welesina* from Western Australia. The family Deeveyinae contains *Deeveya* with seven anchialine species all from the Bahamas Archipelago and *Spelaeoecia* with 11 species from the Bahamas, Bermuda, Cuba, Jamaica, and Yucatan (Kornicker et al. 2007).

Mysida Noteworthy anchialine mysids include the genera *Aberomysis* from Palau; *Antromysis* from Cuba, Jamaica, and Yucatan; *Bermudamysis* and *Platyops* from Bermuda; *Burrimysis* from the Balearic Islands; *Heteromysoides* from the Bahamas, Cayman Islands, Canary Islands, and Okinawa; *Palaumysis* from the Bahamas, Okinawa, Palau, and the Philippines; *Spelaeomysis* from Cuba, Italy, and Zanzibar; and *Stygiomysis* from Italy, Jamaica, and Mexico (Pesce and Iliffe 2002).

Isopoda with Anchialine Representatives Atlantasellidae contains a single genus, *Atlantasellus*, with two anchialine species from Bermuda and the Dominican Republic. Anchialine species from the family Cirolanidae are clustered in Mexico and the Caribbean region (Iliffe and Botosaneanu 2006) and include *Cirolana (Anopsilana)* from Cuba, Grand Cayman Island, Haiti, Indonesia, Jamaica, Madagascar, Palau, the Philippines, and Yucatan; *Arubolana* from Aruba, Bermuda, and Jamaica; *Bahalana* from the Bahamas and Cuba; *Creaseriella* from Yucatan; *Exumalana* from the Bahamas; *Haptolana* from Belize, Cuba, Somalia, Yucatan, and Western

Australia; *Lucayalana* from the Bahamas; and *Yucatalana* from Yucatan. The Leptanthuridae includes the monotypic *Bunderanthura* from Western Australia (Poore and Humphreys 2013). The Paranthuridae contains *Curassanthura* from Bermuda, Canary Islands, and Curaçao. The Stenetriidae includes *Stenobermuda* from the Bahamas and Bermuda, while the Gnathostenetroididae has *Neostenetroides* from the Bahamas and Cuba.

Amphipoda with Anchialine Representatives Anchialine members of the amphipod suborder Gammaridea include the families Hadziidae with *Bahadzia* from the Bahamas, Cuba, Dominican Republic, Haiti, and Yucatan and *Liagoceradocus* from Fiji, Lanzarote, Solomon Islands, Western Australia, and Western Samoa; Pardaliscidae with *Spelaeonicippe* from the Bahamas and Lanzarote; and Pseudoniphargidae with *Pseudoniphargus* from groundwater around the Mediterranean Basin, in Atlantic drainage systems of Portugal and Spain, on Madeira, Azores, Canary and Balearic Islands, as well as Morocco, France, and Bermuda. The suborder Ingolfiellidea includes Ingolfiellidae with anchialine species of *Ingolfiella* from Bermuda, Lanzarote, the Maldives, and Socotra.

Stygiomysida This order has recently been removed from mysids and placed in its own order, the Stygiomysida (Meland et al. 2015). The genus *Stygiomysis* contains seven species—one from the Mediterranean and the others from anchialine caves and coastal groundwater in the Caribbean region.

Thermosbaenacea Two genera of anchialine thermosbaenaceans are noteworthy. The genus *Halosbaena* is represented by nine species (only four of them described)—five inhabiting anchialine and inland caves in Western Australia, plus one species each from Ryukyu, Christmas and Canary Islands, as well as interstitial beach environments around the Caribbean Basin (Page et al. 2016). *Tulumella* on the other hand is more geographically restricted with three anchialine species, two from the Bahamas and one from Yucatan (Wagner 1994).

A unique feature of thermosbaenaceans is their dorsal brood pouch, significant since all of their presumed close relatives have a ventral brood pouch (Olesen et al. 2014b). The collection from a Yucatan cave of a *Tulumella* female with a brood pouch containing 12 embryos in an advanced developmental stage provided an opportunity to observe previously unknown structures (Olesen et al. 2015).

Phyllocarida A blind, unpigmented leptostracan, *Speonebalia cannoni*, occurs in anchialine caves in the Caicos Islands.

Decapoda Anchialine shrimps include at least 18 genera from 10 families (De Grave and Sakihara 2011):

Agostocarididae with *Agostocaris* from the Bahamas and Yucatan;

Alpheidae with *Bermudacaris* from Bermuda and the Balearic Islands; *Metabetaeus* from Christmas Island, Funafuti, Hawaii, Jaluit and Arno Atolls, Kiribati, Loyalty Islands, Rapa Nui, Ryukyu Islands, Sulawesi, Tokelau, and Tuvalu (Anker 2010); and the monotypic *Yagerocaris* from Yucatan;

Anchialocarididae with the monotypic *Anchialocaris* from Cozumel Island (Mexico);

Atyidae with *Antecaridina* from Daito and Ryukyu Islands (Japan), Entedebir Island (Red Sea), Europa Island, Fiji, Guam, Hawaii, Mozambique, the Philippines, Saudi Arabia, and the Solomon Islands; *Halocaridina* including two species from Hawaii; *Stygiocaris* from Western Australia; *Typhlatya* from Ascension Island, the Bahamas, Barbuda, Bermuda, Cuba, Curaçao, the Dominican Republic, Galapagos, Herzegovina, Honduras, Puerto Rico, Spain, and Yucatan;

Barbouriidae with *Barbouria* from the Bahamas, Bermuda, Cayman Islands, Cuba, Jamaica, and Yucatan; *Calliasmata* from Funafuti Atoll, Hawaii, Ryukyu Islands, Sinai Peninsula, and Yucatan; *Janicea* from Antigua, the Bahamas, Bermuda, Brazil, Canary Islands, Cape Verde Islands, São Tomé, and Yucatan; and *Parhippolyte* from Aldabra, the Bahamas, Bermuda, Fiji, Funafuti Atoll, Hawaii, Indonesia, Loyalty Islands, Mexico (Gulf of California and Yucatan), Molucca Islands, the Philippines, and Sulawesi;

Laomediidae with *Naushonia* from the Bahamas, Cuba, and Palau;

Palaemonidae with the monotypic *Creaseria* from Yucatan (Anker 2014);

Macromaxillocarididae with the monotypic *Macromaxillocaris* from the Bahamas;

Pontoniinae with *Periclimenes* from Fiji, Hawaii, Loyalty Islands, the Seychelles, and Sulawesi,

Procarididae with *Procaris* from Ascension Island, Bermuda, Cozumel, and Hawaii and the monotypic *Vetericaris* from Hawaii.

Within the Anomura, the normally deep-sea family Munidopsidae contains only a single shallow-water species from *Munidopsis* that inhabits an anchialine lava tube in the Canary Islands (Wilkens et al. 2009).

Other Invertebrates After the very diverse crustaceans, the annelids and molluscs follow as groups with moderate levels of diversity in anchialine systems. Annelids are represented in anchialine habitats by polychaetes of the families Fauveliopsidae with *Fauveliopsis* from the Canary Islands; Nerillidae with *Leptonerilla* from Bermuda and the Canary Islands, *Speleonerilla* from the Bahamas, Bermuda, the Canary Islands, Cuba and Yucatan, and *Meganerilla* and *Mesonerilla* from the Canary Islands; Polynoidae with *Gesiella* from the Canary Islands and *Pelagomacellicephala* from the Bahamas; Protodrilidae with *Megadrilis* from the Canary Islands; Scalibregmatidae with *Speleobregma* and *Axiokebuita* from the Canary Islands; and Spionidae with *Prionospio* from Western Australia and the oligochaete family Tubificidae with *Phallodriloides* from Bermuda. The molluscan diversity in anchialine habitats is composed of both truly anchialine species and marine species that exploit anchialine environments close to the sea. Stygobitic anchialine molluscs belong to the gastropod families Tornidae with *Teinostoma* from Yucatan, Caecidae with *Caecum* from Bermuda, and Neritiliidae with *Laddia* from Christmas Island, Hawaii, Nauru, Saipan, and Tinian; *Micronerita* from Palau, the Philippines, Saipan, and Tinian; *Pisulina* from Christmas and Cocos Islands; *Siaesella* from Palau; and *Teinostomops* from Indonesia, Palau, and Tahiti.

Cave Fish The only fish families that have become established in anchialine habitats are the Bythitidae with the genera *Ogilbia* from the Galapagos, *Lucifuga* from the Bahamas and Cuba, and *Typhliasina* from Yucatan; the Synbranchidae with *Ophisternon* from Western Australia and Yucatan; and the Eleotridae with *Milyeringa* from Western Australia.

Most anchialine species have a very narrow range that can be as small as a single cave or even a specific section of one cave. In many areas, flooded passages or caves are not always interconnected, and they may often be hydrologically isolated, creating patterns of high endemicity. This isolation and limited distribution of anchialine fauna, coupled with their proximity to the coastline where active touristic development is commonly occurring, means that anchialine caves and their fauna are frequently under serious threat from construction activities, water pollution, and limestone quarrying. As such, a number of anchialine species are currently on the IUCN Red List of endangered species.

Although anchialine systems are found throughout the world, diversity is highest in areas such as the Bahamas Archipelago, Caribbean Basin, Bermuda, and the Canary and Balearic Islands, where in general, the levels of biodiversity correspond to the size of the cave systems. At a regional scale, these locations represent biodiversity hot spots for anchialine fauna. The extremely fragmented distributions of anchialine fauna pose intriguing questions about their origin and distribution patterns while presenting challenges for adequate protection and conservation. In geographical regions where anchialine systems are better developed (e.g., Bermuda, the Bahamas, Yucatan, Australia), beta diversity can be high since species replacement over short distances may be high. Even though the same groups are usually present in distant and geographically isolated localities, the species representing each group are usually different.

18.4 Anchialine Cave Food Webs and Functioning

One of the most important questions regarding the functioning of anchialine systems is where the energy comes from since there are no obvious primary producers. Organic matter enters cave systems through natural entrances either from the sea or from the land above the cave, as well as through smaller crevices and cracks in the bedrock. Some anchialine caves with air-filled chambers above the water level may host large colonies of bats, with their guano providing a rich food source. Cave-adapted crustaceans living deep within anchialine caves have conspicuous stable carbon isotopic values suggesting that carbon and energy from chemoautotrophic pathways linked to a microbial loop shuttling methane and dissolved organic carbon are fueling the cave food web within the extensive coastal cave systems in the Yucatan Peninsula (Pohlman et al. 1997; Brankovits et al. 2017) and in an anchialine sinkhole in Australia (Humphreys 1999). The concept of channeling carbon and energy from dissolved organic compounds into heterotrophic bacterial biomass to higher-order metazoans is a departure from the classical view that subterranean ecosystems are a linear chain from plant detritus to consumers (Dickson 1975) or are based on chemolithoautotrophic processes derived from thermomineral sources (Sarbu et al. 1996; see also Chaps. 11 and 15).

18.5 Future Studies

A series of unique questions have arisen from the initial studies of anchialine ecosystems. Here we outline some that are of considerable scientific significance. Based on the discovery of chemosynthesis-based trophic webs in anchialine habitats (Pohlman 2011), future research in the biogeochemistry of anchialine systems should aim at describing the steps involved in the cycling of organic compounds.

A great diversity of species and of higher taxonomic categories has been described from anchialine systems. Although some species were known from the first half of the twentieth century, most discoveries have occurred in the last 20–30 years. It is interesting to note that while exploration of these flooded caves has advanced greatly, increasing the number and extent of systems known throughout the world, biological reconnaissance and, more so, detailed specific studies of single species have only just began. For example, it has been empirically estimated that less than 5% of the topographically recognized anchialine systems of the Yucatan Peninsula have been explored. Improved diving techniques and equipment and a growing number of scientifically trained cave divers will advance biological exploration in the near future.

From the biogeographic and phylogenetic point of view, anchialine fauna represent an element of enormous importance for several reasons, some of which have been outlined already. Anchialine systems may have operated as refuges through geologic time conserving relict groups that are absent from other aquatic ecosystems. The Remipedia is one example, with species in the Greater Caribbean, the Canary Islands, and Australia that serve as a model to define types of anchialine fauna in different parts of the world and to link distant geographic areas that were connected in the past geologic eras (Hoenemann et al. 2013). Atyid shrimps of the genus *Typhlatya* also show a highly disjunct global distribution suggesting a very old origin with the separation of the main lineages occurring by both vicariance (the Atlantic opening) and dispersal (Jurado-Rivera et al. 2017). The affinities of some anchialine species, like the misophrioid copepods of the genus *Expansophria*, have linked species present in shallow coastal anchialine caves with deep-sea species. These unique relationships have prompted novel hypothesis about the vertical separation of ancestral stocks to produce the observed pattern (Boxshall and Iliffe 1986).

Since anchialine fauna is generally restricted to caves in a band no more than 10 km inland from the coastline, such species would have been forced to migrate in pace with tectonic uplift and glacio-eustatic sea-level change, thus implying the persistence of a vertical continuum of suitable habitats in both time and space. Sea-level position has been a controlling factor in the formation and subsequent development of coastal anchialine caves. The average water depth of anchialine cave systems along the Yucatan's Caribbean coast parallels increasing halocline depth with distance inland. This indicates such caves formed when sea level was at or near its current position, i.e., during the last interglacial (Marine Isotope Stage 5e). Subsequently, the caves drained as sea level receded and were completely dry during the last glacial minimum, as evidenced by the prevalence and size of now submerged speleothems. Alternate habitats, at least 100+ m below current sea level, must have provided a refuge for anchialine fauna at that time. At sites in Yucatan and the Bahamas, massive collapse has broken though to remnant cave passages nearing that depth, but further explorations are required to determine if these caves served as an ice age retreat for anchialine fauna.

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Chapter 19

Volcanic Anchialine Habitats of Lanzarote

Alejandro Martínez and Brett C. Gonzalez

19.1 Introduction

Anchialine systems predominantly occur in karstic limestone and volcanic rock throughout tropical locales (Iliffe et al. 2000; Iliffe and Kornicker 2009; see also Chap. 18). However, these systems can be found as far north as Iceland and as far south as Australia. Most anchialine habitats occur in caves, but they may also be present in a variety of other geological formations such as small depressions, submerged faults, and even in areas of uplifted geology (Iliffe and Bishop 2007).

The most extensive anchialine systems are known from the Caribbean. The largest of these systems are present in the Yucatán Peninsula of México, but large systems are also known from elsewhere, including the Bahamas, Bermuda, and the Mediterranean. Caves in these areas are all formed in limestone by solutional processes, being further shaped by mixing corrosion, and typically containing both fresh meteoric and intruding marine waters. Karstic regions are known for their scarcity of surface water features, making nearly all drainage subterranean (Iliffe et al. 2000). Since most of these caves lie perpendicular to the coast, they are thought to be major drainage conduits into the surrounding marine waters (Iliffe et al. 2000). Sistema Sac Actun (Yucatán Peninsula) is the largest submerged anchialine system, which has more than 347 km of surveyed passages interconnecting with more than 100 entrance pools. However, the majority of anchialine caves are much smaller, ranging in lengths from several meters to few kilometers. Small caves are typically associated with locations outside the Caribbean and Mediterranean, mostly found

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throughout the Indo-Pacific (Iliffe and Bishop 2007). In general, karstic caves are hundreds of thousands if not many millions of years old, persisting far longer than those caves formed in volcanic rock.

Anchialine volcanic caves are known from several island locations including the Canary, Galapagos, and the Hawaiian Islands. Most of these caves are in the form of lava tubes formed during eruptions of fluid basaltic pahoehoe lava, in which the slow-moving surface of the lava is cooled, forming a conduit below that becomes the tube after the lava flow ceases (Bravo 1964; Carracedo et al. 2003; Martínez et al. 2016a). Typically, lava tubes are formed on land and flow toward lower elevations. In island settings, when lava reaches the coastline it is suddenly cooled by the surrounding marine waters, preventing the tube from forming further (Iliffe and Bishop 2007). Under special conditions lava tubes may also be formed along the sea floor or in submarine environments (Fornari et al. 1985). However, these two conditions are not present in Lanzarote. Lava tubes are often relatively short-lived (tens of thousands of years) due to their proximity to the surface where they are exposed to damaging elements (i.e., erosion) (Iliffe and Bishop 2007).

19.2 Anchialine System in Lanzarote

Lanzarote is one of seven major volcanic islands situated off the west coast of Western Sahara (~160 km), being the second oldest among the Canary Islands (about 15 million years) and harboring the most diverse anchialine ecosystems in the Eastern Atlantic. There are three types of surface features providing access to the anchialine systems throughout Lanzarote, namely, exposed lava tubes, pools, or hand dug wells scattered throughout the island.

The main anchialine environment in Lanzarote lies within La Corona lava tube (Fig. 19.1). This cave was formed from a lateral vent off La Corona Volcano at the

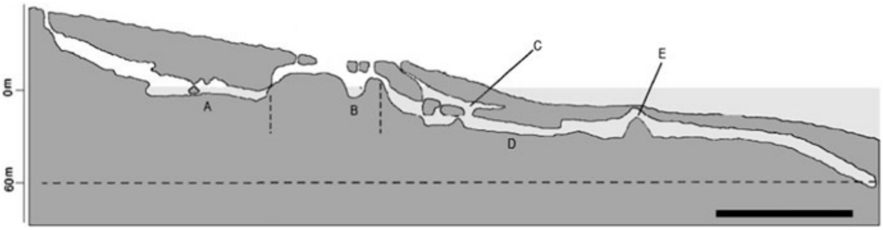

Fig. 19.1 Schematic cross section of the anchialine sections of La Corona lava tube. (**A**) Cueva de Los Lagos; (**B**) Los Jameos del Agua. Vertical dashed lines represent the approximate area occupied by the tourist complex; (**C**) Lago Escondido, upper section of the Túnel de la Atlántida; (**D**) Túnel de la Atlántida; (**E**) Montaña de Arena. Areas shaded in light gray represent the current sea level; horizontal dashed line indicates possible position of the sea level during the formation of the lava tube. Horizontal black scale bar 500 m; vertical scale on left axis exaggerated. Modified from Wilkens et al. (2009)

northern tip of the island. La Corona lava tube traverses over 6.2 km in a southeasterly direction, whereby it continues an additional 1.6 km into the Atlantic Ocean beyond the present coastline of the island. The occurrence of upper and lower sections throughout the lava tube suggests that several volcanic episodes were involved during speleogenesis (Jantschke et al. 1994). Secondary collapses or "jameos" currently divide the cave into several sections, however, only three of them are flooded by anchialine waters (Martínez et al. 2016a). The most inland of them, known as Cueva de Los Lagos, opens 600 m from the coastline into a partially flooded passageway ending in a short sump. Downstream, this sump leads through non-navigable galleries to the second known anchialine section, represented by the tidal lake of Los Jameos del Agua, which has been developed into a major tourist attraction (Fig. 19.2a). The third anchialine section, known as Túnel de la Atlántida, opens nearby Los Jameos del Agua and is completely submerged, extending over 1.6 km under the sea floor without any conspicuous connection to the overlying ocean. Túnel de la Atlántida ends abruptly at its maximum depth of 64 m (Martínez et al. 2016a). Maximum extension and depth of Túnel de la Atlántida fits with palaeocoastlines of Lanzarote, and age estimates based on Ar/K radiometric methods date formation of La Corona lava tube to the last glacial maximum (~21,000 ± 6500 years) (Carracedo et al. 2003). This estimate suggests that the formation of the lava tube was subaerial (formed on the surface) and stopped when the lava flow was abruptly interrupted due to the sudden cooling by contact with the Atlantic Ocean. Flooding of La Corona lava tube was more recent and after the last glacial maxima when sea level rose to its present level.

Anchialine environments of Lanzarote are not limited to those of the flooded portions of La Corona lava tube but can also be found at the surface around the island in the form of landlocked bodies of water known as anchialine pools or ponds (Fig. 19.2b). These occur mostly in two localities, Montaña Bermeja on the southwest coast, and Órzola on the northeastern tip of the island (Martínez et al. 2016a). Typically, the anchialine pools of Lanzarote consist of small depressions that penetrate the subterranean marine waters of the island. Similarly, in the anchialine habitats in La Corona, these anchialine ponds fluctuate with respect to the surrounding tides. Given the proximity to abandoned cinder mines, it is not currently known if all anchialine pools in Lanzarote are natural, or if some are man-made (Wilkens et al. 1993).

In addition, several other man-made access points to anchialine waters exist in Lanzarote (Fig. 19.2c). Hand dug wells were built during the early eighteenth and nineteenth centuries along the coastline, pumping the subterranean anchialine marine waters into saltpans for salt production (Martínez et al. 2016a). Most of these wells are relatively small, ranging between 2 and 3 m in diameter and averaging 10 m in depth. Since construction, the majority of these wells have been destroyed and those that remain are mostly abandoned. Active wells can only be found in the town of Costa Teguise and in the saltworks of Guatiza and Los Agujeros.

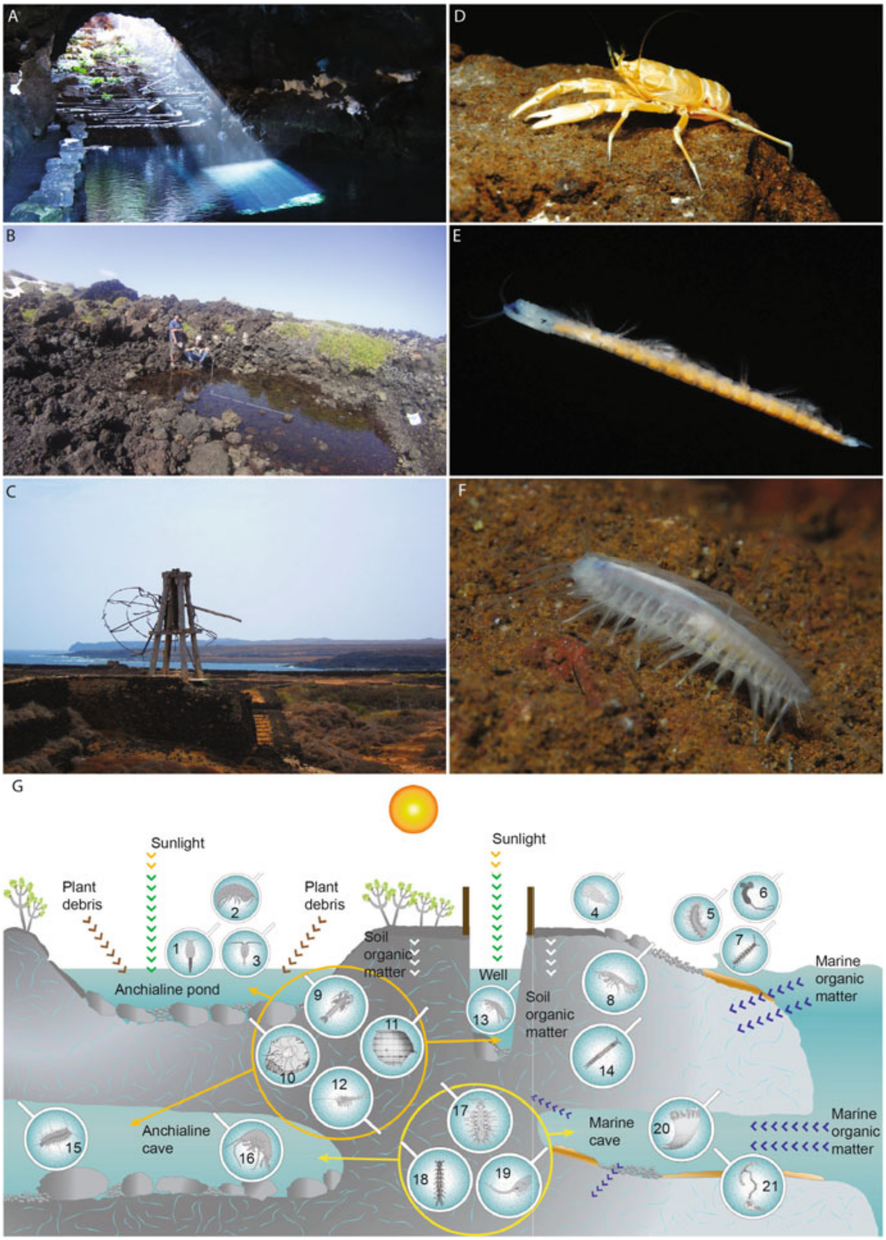

Fig. 19.2 Types of anchialine environments seen throughout Lanzarote including several well-known anchialine endemic species. (**a**) Los Jameos del Agua anchialine lake during early morning; (**b**) Charcos de Luis anchialine ponds, near Órzola, at the northern tip of Lanzarote; (**c**) an anchialine well in Los Agujeros near Mala on the northeast coast of Lanzarote; (**d**) Galatheid squat lobster *Munidopsis polymorpha* at the bottom of Los Jameos del Agua lake; (**e**) Remipede *Morlockia* sp. swimming in the water column of Túnel de la Atlántida; (**f**) Polynoid annelid *Gesiella jameensis* on the bottom of Los Jameos del Agua lake; (**g**) Summary of the interactions between the different types of anchialine habitats and species in Lanzarote (adapted from Martínez et al. 2016b). Numbers

19.3 Biodiversity and Ecological Studies in La Corona Lava Tube: A Hotspot of Diversity for Anchialine Subterranean Fauna

La Corona lava tube has been, and continues to be, the focus for most of the faunistic studies in anchialine ecosystems throughout Lanzarote. Studies in La Corona started with the description of the squat lobster *Munidopsis polymorpha* from Los Jameos del Agua by Koelbel in 1892 (Fig. 19.2d) and continued with several additional species descriptions by various European scientists during the first half of the twentieth century (Calman 1904; Harms 1921; Fage and Monod 1936). Parzefall and Wilkens (1975) conducted the first systematic investigation focusing on the biology and behavior of *Munidopsis polymorpha* in Los Jameos del Agua (Wilkens et al. 1990). However, it was the scientific cave diving explorations of Túnel de la Atlántida that yielded the most impressive discoveries of several endemic stygobites including annelids, copepods, thermosbaenaceans, and remipedes (Iliffe et al. 1984).

Most of these endemic stygobites occur in the water column of the aphotic anchialine portions of La Corona, restricted to Túnel de la Atlántida and Cueva de Los Lagos. The water column represents the most specialized habitat in anchialine cave systems. In La Corona, the water column is characterized by low and stable temperatures (~18 °C) and dissolved oxygen (~3.7–5.7 mg/L), with minimal currents due to tidal exchange (Wilkens et al. 2009). In contrast to other anchialine systems, the water column exhibits no stratification in salinity, temperature, or oxygen, yet deployed water profilers (automated water quality samplers that collect data through the water column from quality sensors) do show fluctuation in all parameters that coincide with tidal exchange (Martínez et al. 2016a). La Corona lava tube, like numerous other anchialine cave systems, is regarded as a detritus-based system (see also Chap. 14), where particulate organic matter is introduced through tidal exchange or infiltration across the overlying lava rock. The presence of suspended organic matter within the water column favors a rich assemblage of endemic suspension feeders dominated by stygobitic crustaceans such as thermosbaenaceans, mysids, and amphipods, as well as a few highly specialized annelid species (Martínez et al. 2016b; Worsaae, Martínez, and Gonzalez, pers. obs.). The water column of La Corona is also home to several species of endemic predators (see also Table 19.1), such as the remipedes *Morlockia ondinae* (Fig. 19.2e) and *M. atlantida*, the annelid *Gesiella jameensis* (Fig. 19.2f), and the

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Fig. 19.2 (continued) correspond to the following species (see also Table 19.1): **1** *Boxshallia bulbantennulata*, **2** *Parhyale multispinosa*, **3** Calanoida, **4** *Halosphiloscia*, **5** Syllidae, **6** *Tubiluchus lemburgi*, **7** *Meganerilla cesari*, **8** *Bogidiella uniramosa*, **9** *Heteromysoides cotti*, **10** *Munidopsis polymorpha*, **11** *Humphreysella* sp., **12** *Halosbaena fortunata*, **13** *Hadzia acutus*, **14** Harpacticoida, **15** *Gesiella jameensis*, **16** *Spelaeonicippe buchi*, **17** *Mesonerilla* n. sp. 1, **18** *Leptonerilla diatomeophaga*, **19** Cumacea, **20** *Caryophyllia smithii*, **21** *Bonellia viridis*; see Table 19.1 for more information on the species systematics. Photos by © Brett C. Gonzalez (A, C) and © Juan Valenciano (D, E, F)

Table 19.1 Summary of the stygobiont and endemic species recorded from the anchialine ecosystems from Lanzarote

Taxonomy		Area						Ecology			Origin		
Family	Species	C	J	T	MA	W	P	St	In	En	Tet	De	Sha
Platyhelminthes													
Cheliplanidae	*Cheliplana cavavulcanica*	–	–	T	MA	–	–	–	In	En	–	–	Sha
Schizorhynchidae	*Schizochilus lanzarotensis*	–	–	T	MA	–	–	–	In	En	–	–	Sha
Annelida													
Acrocirridae	*Macrochaeta* n. sp.[a]	–	J	T	–	–	–	St	In	En	–	?	?
Fauveliopsidae	*Fauveliopsis jameoaquensis*	–	J	T	–	–	–	St	In	En	?	?	?
Nerillidae	*Mesonerilla* n. sp. 1[b]	–	–	T		–	–	–	In	En	–	–	Sha
	Mesonerilla n. sp. 2[b]	C	J	T	–	–	–	St	In	En	–	–	Sha
	Speleonerilla isa	C	–	T	–	–	–	St	–	En	–	–	Sha
	Leptonerilla diatomeophaga	–	J	T	–	–	–	St	In	–	Tet	?	?
Polynoidae	*Gesiella jameensis*	C	J	T	–	–	–	St	–	En	–	De	–
Protodrilidae	*Megadrilus pelagicus*	C	–	T	–	–	–	St	–	En	–	–	Sha
Scalibregmatidae	*Speleobregma lanzaroteum*	–	–	T	–	–	–	St	–	En	–	De	–
Spionidae	*Prionospio* n. sp.[c]	C	–	T	–	–	–	St	–	En	Tet	–	–
Syllidae	*Sphaerosyllis iliffei*	–	–	T	MA	–	–	St	In	En	–	–	Sha
Crustacea, *Calanoida*													
Arietellidae	*Paramisophria reducta*	–	–	T	–	–	–	St	–	En	–	?	–
Epacteriscidae	*Enantronia canariensis*	–	–	T	–	–	–	St	–	En	–	?	–
Pseudocyclopiidae	*Stygocyclopia balearica*	C	–	T	–	–	–	St	–	–	–	?	–
Stephidae	*Stephos canariensis*	C	–	T	–	–	P	St	–	En	–	–	Sha
Crustacea, *Cyclopoida*													
Cyclopinidae	*Oromiina fortunata*	–	J	–	–	–	–	St	–	En	–	?	–
Cyclopinidae	*Muceddina multispinosa*	C	–	–	–	–	–	St	–	–	–	De	–
Crustacea, *Harpacticoida*													
Superornatiremidae	*Neoechinophora karaytugi*	–	–	T	–	–	–	St	–	En	Tet	–	–

Crustacea, Misophrioida													
Misophriidae	*Dimisophria cavernicola*	–	–	T	–	–	–	St	–	En	–	De	–
	Expansophria dimorpha	C	–	T	–	–	–	St	–	En	Tet	De	–
Palpophriidae	*Palpophria aestheta*	–	–	T	–	–	–	St	–	En	–	De	–
Spelophriidae	*Speleophriopsis canariensis*	C	–	–	–	–	–	st	–	En	Tet	De	–
	Boxshallia bulbantennulata	–	–	–	–	–	P	St	–	En	–	?	?
Crustacea, Tantulocarida													
Basipodellidae	*Stygotantulus stocki*	–	–	–	–	–	P	St	–	En	–	–	?
Crustacea, Ostracoda													
Polycopidae	*Eupolycope pnyx*	–	–	T	–	–	–	St	–	En	–	–	–
Thaumatocyprididae	*Humphreysella phalanx*	–	–	T	–	W	–	St	–	En	Tet	De	–
	Humphreysella wilkensi	C	–	T	–	W	–	st	–	En	Tet	De	–
Sarsiellidae	*Eusarsiella bedoyai*	–	–	T	MA	–	–	–	–	En	–	–	–
Crustacea, Amphipoda													
Bogidiellidae	*Bogidiella uniramosa*	–	J	–	–	W	–	St	In	En	Tet	–	–
Hyalidae	*Parhyale multispinosa*	C	J	T	–	–	P	–	–	En	–	–	–
Pardaliscidae	*Spelaeonicippe buchi*	C	J	T	–	–	–	St	–	En	–	De	–
Talitridae	*Hadzia acutus*	–	J	T	MA	W	–	St	–	En	Tet	–	–
Crustacea, Isopoda													
Paranthuridae	*Curassanthura canariensis*	–	J	–	–	–	–	St	–	En	Tet	–	–
Crustacea, Mysida													
Mysidae	*Heteromysoides cotti*	–	J	T	–	W	P	St	–	En	Tet	–	–
Crustacea, Thermosbaenacea													
Halosbaenidae	*Halosbaena fortunata*	–	–	T	–	W	–	St	–	En	Tet	–	–
Crustacea, Decapoda													
Galatheidae	*Munidopsis polymorpha*	C	J	T	–	W	P	St	–	En	–	De	–

(continued)

Table 19.1 (continued)

Taxonomy		Area						Ecology			Origin		
Family	Species	C	J	T	MA	W	P	St	In	En	Tet	De	Sha
Crustacea, Remipedia													
Speleonectidae	*Morlockia atlantida*	C	–	T	–	–	–	St	–	En	Tet	–	–
	Morlockia ondinae	C	–	T	–	–	–	St	–	En	Tet	–	–

C Cueva de los Lagos, *J* Jameos del Agua, *T* Túnel de la Atlántida, *MA* Montaña de Arena, *W* Wells, *P* ponds, *St* stygobite, *In* interstitial, *En* endemic, *Tet* Tethyan origin suggested, *De* suggested marine deep-sea affinity, *Sha* attributed marine shallow water affinity, ? currently unknown

[a] In Núñez et al. (1997)

[b] In Worsaae et al. (2009)

[c] In Martínez et al. (2016)

copepod *Enantronia canariensis* (Martínez et al. 2016a). In contrast, anchialine systems throughout the Caribbean are known to support chemoautotrophic production from low concentrations of dissolved inorganic compounds (Pohlman et al. 1997; Brankovits et al. 2017). However, it remains unknown if such processes are, or capable of, occurring within the dark remote sections of La Corona lava tube.

The benthic environments in La Corona lava tube are spatially more complex than those of the water column and include rock surfaces as well as patches of different types of sediments that host crevicular or interstitial habitats depending on their origin and grain size. Patches of lava debris are distributed throughout the lava tube, although they are more common in areas of breakdown such as the entrance of Túnel de la Atlántida or in several parts within Cueva de Los Lagos. While these patches might superficially resemble interstitial environments, they mainly comprise lava pebbles, resulting in similar permeability to that of the surrounding subterranean crevicular environment. As a consequence, they do not host typical interstitial species but rather a few stygobites in low abundances, including several species of copepods, the isopod *Curassanthura canariensis*, and several nerillid annelids (Martínez et al. 2009; Worsaae et al. 2009).

True interstitial environments are characterized by the presence of smaller sized sediment particles. In La Corona, they are restricted to Montaña de Arena; a 30 m high sand dune formed approximately 750 m from the entrance of Túnel de la Atlántida. This accumulation of marine coarse sand has been entering the cave ceiling through a non-visible crack for an unknown length of time and contains both marine and cave endemic species (Martínez et al. 2009). In contrast to the patches of lava pebbles, this environment is colonized by a rich fauna and includes typical interstitial meiofaunal groups such as annelids, platyhelminthes, gastrotrichs, gnathostomulids, priapulids, and crustaceans (García-Valdecasas 1985; Núñez et al. 2009; Worsaae et al. 2009; García-Herrero et al. 2017; Gobert et al. 2017). As a result of tidal exchange through this connection to the overlying marine environment, several marine species referred to as “accidentals” or stygoxenes are also present. This connection also contributes nutrients into the system as areas surrounding Montaña de Arena are characterized by an increase in particulate organic matter in both the water column and the dune itself, favoring the rich interstitial diversity.

Within the La Corona lava tube, indirect solar insolation is restricted to the anchialine lake of Los Jameos del Agua. The intensity of light in Los Jameos del Agua varies across the lake and favors the presence of primary production in the form of microscopic algae as well as dense beds of benthic diatoms. This primary production sustains large populations of mysids, copepods, ostracods, and other suspension feeders within the water column and in the benthos where several interstitial cave endemic annelids are present (Núñez et al. 1997; Worsaae et al. 2009). However, the opportunistic squat lobster *Munidopsis polymorpha* is by far the most abundant stygobiont in both Los Jameos and the entrance pool of Túnel de la Atlántida (Wilkens et al. 1990). Stygophilic and accidental stygoxenic species are also common in these localities, taking advantage of the increased concentration of organic matter. In particular, the stygophilic annelid *Bonellia viridis* (Bonellidae, Echiura) has increased its population size in recent decades, enriching the deposits of cinders and lava debris in the lake with organic matter derived from fecal deposits (Brito et al. 2009).

19.4 Biodiversity Studies in Anchialine Pools and Wells in Lanzarote

Anchialine pools host a very particular combination of marine and stygobitic species (see also Table 19.1), some of them exclusive to these pools such as the copepod *Boxshallia bulbantennulata* and the parasitic crustacean *Stygotantulus stocki*. Historically, research on these pools has mostly consisted of species descriptions. Faunal surveys of these pools have often recorded species otherwise known only from La Corona lava tube, providing evidence of connectivity among the island's anchialine habitats, thereby strengthening the foundation for theories regarding dispersal between distant anchialine caves by means of crevicular habitats or "spelean corridors" (Hart et al. 1985; Wilkens et al. 1986; Gonzalez et al. 2017).

Some species present in these anchialine pools, such as the amphipod *Parhyale multispinosa*, can be considered "pond specialists", capable of coping with extreme changes in both temperature and salinity while forming permanent populations. In contrast, true stygobites, such as *Munidopsis polymorpha* and *Heteromysoides cotti*, migrate in and out of the ponds, taking advantage of trophic resources during the most favorable nighttime conditions when temperatures and risk of predation are typically lower. The bottom of these anchialine pools are quite different from the bottom of La Corona, having several layers of fine sediment covered by varying degrees of decomposing organic matter (including terrestrial leaf litter), green algae, and cyanobacteria. Often, several benthic species, many of which also occur in marine intertidal environments, occur in these bottom pool sediments. One of the most extraordinary records from these anchialine pools is that of the endangered European eel *Anguilla anguilla* (Actinopterygii, Anguillidae), which has been frequently observed in these pools in northern Lanzarote (Wilkens et al. 1986).

Hand dug wells are also connected to the subterranean waters of Lanzarote through crevicular spaces and are often directly affected by the surrounding tides. However, the limited water present in these wells is less exposed than that of anchialine pools, being protected by the steep walls from not only the direct sunlight, but also the extreme temperatures occurring daily. The bottoms of these wells mostly consist of gravel and mud, but natural or anthropogenic debris is often present. Few stygobites are known from hand dug wells, but the stygobitic amphipod *Hadzia acutus* can always be found and baited traps have been shown to attract additional stygobiont species from the surrounding crevicular spaces. Again, this illustrates the connectivity (see Fig. 19.2g) across the various subterranean areas scattered among the island of Lanzarote (Wilkens et al. 1986).

19.5 Evolutionary Studies in Lanzarote: Origin of the Anchialine Fauna

The origin of many anchialine lineages remains an open and fascinating evolutionary and biogeographical question. While most anchialine lineages have an unequivocally marine origin, they have been placed often in new genera, families, or even higher taxonomic ranks that are restricted to caves distributed across broad geographic ranges (Wilkens et al. 2009; see also Chap. 18) (Fig. 19.2). Such disjunct global distributions were first described in stygobitic crustaceans, interpreted as the result of cave colonization by their marine ancestors thought to be present along the coasts of the Tethys Sea during the Mesozoic. These ancestral cave populations would have subsequently been divided by plate tectonic vicariance (Stock 1993). This so-called Tethyan origin has been credited to several stygobitic groups, including remipedes, thermosbaenaceans, atyid shrimp, and thaumatocyprid ostracods. However, while this hypothesis may explain the presence and distribution of stygobionts in caves throughout the Caribbean, Australia, and other locations along margins of continental plates, it cannot explain the presence of stygobionts in geologically young oceanic islands of volcanic origin. This evolutionary origin conundrum includes fauna present in Lanzarote, as well as several other oceanic islands such as the Galapagos, Bermuda, and Christmas Island. The presence of several species previously attributed to a Tethyan origin in anchialine habitats in these geologically young islands cannot be explained without addressing alternative forms of dispersal (Jurado-Rivera et al. 2017). Molecular phylogenetic studies attempting to address colonization alternatives are still missing or incomplete for many of these groups in La Corona, but those already published have resulted in topologies incongruent with the known vicariant scenarios (see also Chap. 12), such as *Halosbaena fortunata* (Page et al. 2016), or have yielded very old divergence times compared with the geological ages estimated for the island that the species inhabits (e.g., *Gesiella jameensis*; Gonzalez et al. 2017).

Alternatively, a deep-sea origin hypothesis has also been proposed for numerous anchialine stygobites. This hypothesis offers an alternative to the Tethyan origin as it includes the possibility of dispersal among caves through cave-like habitats in the deep sea, including crevices (i.e., spaces among rocks). Several stygobites endemic to La Corona lava tube have a clear deep-sea affinity, as they belong to groups never preset in shallow water (Martínez et al. 2009; Gonzalez et al. 2017). This affinity, however, does not necessarily indicate that cave colonization occurred from the deep, as independent phylogenetic analyses have actually favored a shallow water origin for some of these species (i.e., several misophrioids and *Paramisophria* copepods; Boxshall and Jaume 2000; Jaume et al. 2000). In other instances (i.e., *Speleobregma lanzaroteum*), a deep-sea origin cannot be unequivocally attributed until more sampling is performed (Martínez et al. 2013). The only case in which phylogenetic analyses cannot reject colonization from the deep are those involving the polynoid *Gesiella jameensis,* and the squat lobster *Munidopsis polymorpha* (Ahyong et al. 2011; Gonzalez et al. 2017).

19.6 Evolutionary Studies in Lanzarote: Adaptations to Lava Cave Anchialine Environments

Adaptations to cave-specific environments, including subterranean anchialine habitats, are known as troglomorphism (see also Chap. 4). However, traits associated with troglomorphism may also be present in non-cave environments that have similar ecological conditions to those of caves. For example, loss of eyes or pigmentation is two of the most iconic regressive troglomorphic adaptations, yet they are conditions commonly seen among deep-sea or interstitial lineages (Danielopol et al. 1996; Giere 2009). Therefore, unraveling true troglomorphic traits often demands specific comparative analyses.

Comparative analyses from two endemic cave annelids from La Corona lava tube have shown that some traits were indeed present prior to cave colonization, thus reducing the number of features that can be regarded as troglomorphic. For instance, *Speleobregma lanzaroteum* is an endemic scalibregmatid annelid that bears palps and is found suspension feeding within the water column of La Corona lava tube. This species belongs to a family dominated by burrowers lacking head appendages (Martínez et al. 2013), and it was thought that the presence of palps was a troglomorphic adaptation to cave suspension feeding. However, comparative analyses showed that these palps were not correlated with cave colonization but were already present in the ancestor shared by *Speleobregma* and the species of the genus *Axiokebuita*, dwelling both in deep-sea and cave crevicular habitats (Martínez et al. 2014). Similarly, recent comparative studies on the endemic polynoid *Gesiella jameensis,* also including the Bahamian anchialine polynoid species complex of *Pelagomacellicephala iliffei,* showed that the loss of eyes was already present in their deep-sea ancestors but the elongation of dorsal cirri, a sensory projection located on the parapodium, in both of these cave polynoids evolved in association with cave colonization (Gonzalez et al. 2018). Additionally, two lineages of primarily interstitial annelids in La Corona, belonging to the families Nerillidae and Protodrilidae, are often interpreted as providing other examples of troglomorphic adaptation (Worsaae 2014; Martínez et al. 2015). Endemic species from both these groups inhabit the water column of La Corona lava tube and exhibit elongated ciliated palps and ciliary bands along their body in conjunction with their adaptation to suspension feed in the water column (Martínez et al. 2016b).

19.7 Conservation of Anchialine Habitats

Anchialine habitats, like many other natural splendors, are highly susceptible to anthropogenic encroachment. One of the largest impacts on anchialine habitats is land use, whether it is for commercialization and construction, mineral exploitation, or water resources (Iliffe and Kornicker 2009). Due to the vastness and interconnectivity of the subterranean realm, even activities several kilometers away from anchialine caves or pools may have detrimental effects, especially with regard to those contaminants capable of being flushed through the system by tidal pumping. Any anthropogenic access to subterranean or anchialine waters via bore holes, wells, quarries, or deep well injection sites have the potential to introduce contamination, ultimately impacting the environmental health of the system accessed and those neighboring it. This is likely to have conservation implications because the majority of species living within these habitats have limited distributions and highly specific habitat requirements. As a result, the majority of anchialine species automatically qualify for inclusion on endangered or protected lists because of these characteristics (Iliffe and Bishop 2007).

The push for development of tourism throughout regions containing anchialine habitats (i.e., tropical oceanic islands and coastal regions) puts subterranean ecosystems at a heightened risk of contamination. Recent booms in tourism, combined with the lack of effective environmental laws or implementation of laws in many of these locations, have complicated conservation efforts, even in areas with developed educational outreach and conservation programs. Fortunately, the entire island of Lanzarote has been protected as a Biosphere Reserve since 1993, and more recently (2015), part of Lanzarote, including the Chinijo Islands, became a UNESCO Geopark. For the most part, urbanization and land use in Lanzarote have been strongly controlled, largely because of the efforts of local artist César Manrique.

Los Jameos del Agua and Túnel de la Atlántida have been a major touristic attraction in Lanzarote since 1966, receiving thousands of visitors each year. While the tourist center attempts to protect these anchialine ecosystems from uncontrolled visitation, dumping of trash, acts of vandalism, and the existence of the center itself expose these fragile ecosystems to other types of anthropogenic threats. These threats mostly revolve around the intentional tossing of coins into the lake by visitors who regard this water body as a natural “wishing well”, despite the posted placards forbidding such activities. Additionally, the occasional dropping of random objects, evening musical events, and periodical festivals may also potentially have detrimental effects on these fragile ecosystems. While to date the populations of many of the endemic species in the lake show no signs of stress, ongoing research supported by the Tourist Center as well as the Governments of Lanzarote and the Canary Islands is attempting to understand the cumulative effects of all these disturbances, with aims of finding ways to further minimize anthropogenic impacts.

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Chapter 20
Research in Calcretes and Other Deep Subterranean Habitats Outside Caves

Stuart Halse

20.1 Introduction

Box 20.1
The outstanding difference between traditional subterranean fauna studies and those carried out recently in Australia is the emphasis in Australia on the fauna outside caves within the network of small cavities that occur deep underground across large parts of the landscape. Much of the arid zone, especially in the western half of Australia, is rich in subterranean fauna. Despite the ancient age of the land mass in which many species occur, it appears that most of the species (or at least the lineages from which they have evolved) moved underground during the past 15 million years seeking moisture as the Australian continent moved north and became increasingly arid (Byrne et al. 2008).

This chapter deals with the subterranean fauna occurring outside caves in the Australian arid zone (Fig. 20.1). It focuses on the characteristics of the habitats in which stygofauna and troglofauna occur, as well as the taxonomic structure of these subterranean fauna communities and some of the more general characteristics of subterranean fauna outside caves. Two other important texts on Australian subterranean fauna are Humphreys (2016), which focuses on biogeography and the origin of the fauna, and Hose et al. (2015), which provides more information about eastern Australia.

Despite the large areas of karstic habitats in Australia, the continent has very few large caves. This is especially so in the arid zone, where there are arguably only two

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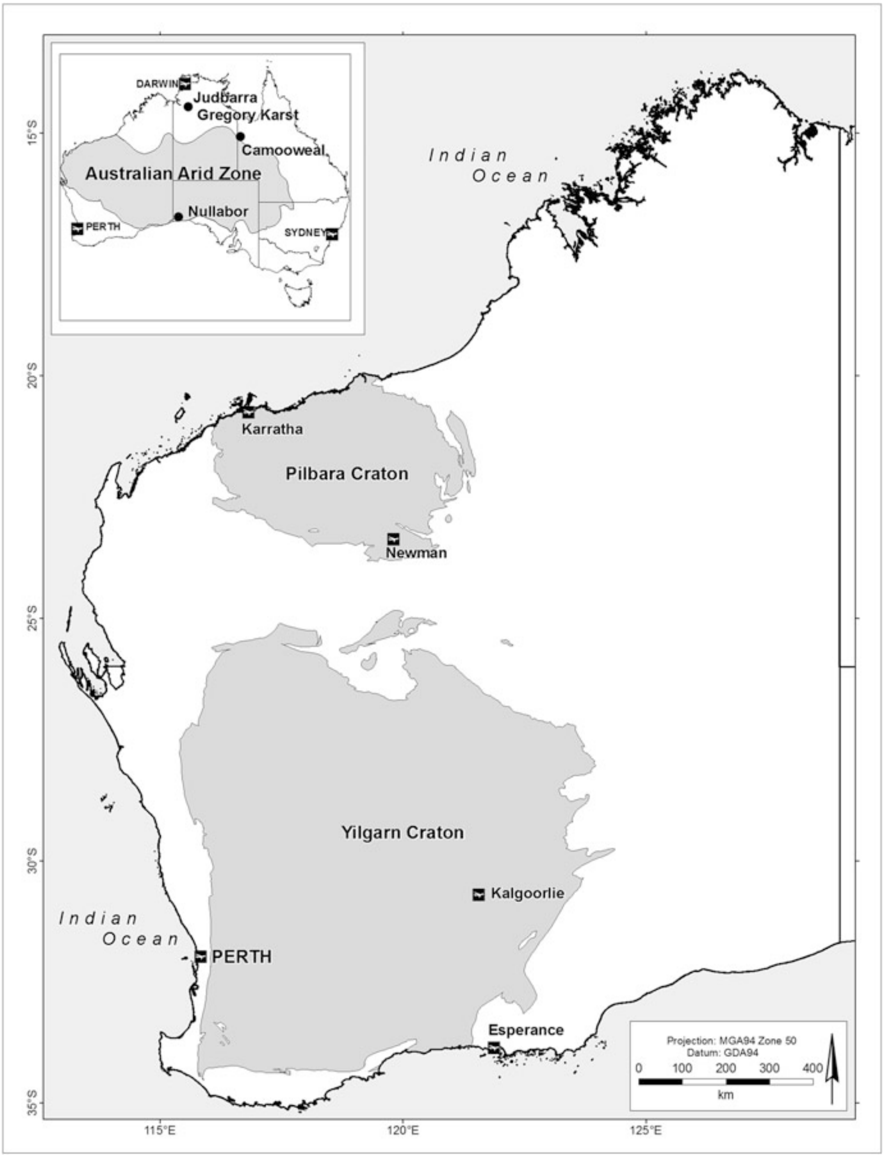

Fig. 20.1 Locations of the Pilbara and Yilgarn regions (or cratons) and other places mentioned in text

large cave systems. One is on the Nullarbor Plain (Webb and James 2006) and the other is around Camooweal, north-west of Mount Isa (Grimes 1988; Eberhard 2003) (Fig. 20.1). Despite their large size, both systems have relatively depauperate subterranean faunas (Richards 1971; Eberhard 2003). The same is true of the

Judbarra/Gregory karst area on the edge of the arid zone in the Northern Territory (Moulds and Bannink 2012) (Fig. 20.1).

In very general terms, stygofauna in the Australian arid zone occurs mostly in unconfined, surficial regional aquifers that extend across the landscape. These aquifers mostly lie within alluvium (sometimes containing calcrete bodies), other detrital geologies, or in fractured or weathered rock. Troglofauna species mostly occur in the vadose (or unsaturated) zone, but there is no commonly used terminology to define their habitat, which extends downward from a meter or more below-ground surface to the water table. Troglofauna are found mainly in mineralized or weathered rock, calcrete, and detritals (consisting of scree, alluvium, or colluvium). The formations in the vadose zone containing troglofauna in Australia share some features with the milieu souterrain superficial (MSS) or shallow superficial habitats (SSH) of the northern hemisphere (Mammola et al. 2016), although the overall scale and geological setting of the Australian formations is quite different.

20.2 Subterranean Habitat Other than Caves

Box 20.2

While the fauna of caves can be collected relatively easily by skilled cavers and these cavers can see where the animals are and what they are doing at the time of collection, sampling areas outside caves is a blind process. Either hauls nets or traps are used to collect stygofauna and troglofauna. The operator is usually remote from the sampling device, and it is rarely possible to collect information about the exact habitat occupied by the animals. Information on their behavior is never available. As a result, the ecological preferences of stygofauna and troglofauna occupying the broader landscape are very poorly understood even at the level of the types of spaces used by the animals and the degree of interconnectedness of these spaces.

The main feature of aquifer and vadose zone habitats used by both stygofauna and troglofauna is that the available subterranean spaces are relatively small and mostly comprise what Howarth (1983) termed microcaverns (<5 mm in width) and mesocaverns (5–500 mm) (Fig. 20.2; see also Chap. 3). Mesocaverns occur mostly in calcrete and in the upper layers of extensively weathered rock formations. Most of the calcrete bodies that have been investigated are of groundwater origin, with the production of calcrete being principally the result of a shallow depth to the water table combined with a climatic regime that comprises low annual rainfall with occasional very heavy rain events and high rate of evaporation (Mann and Horwitz 1979). As groundwater levels rise following recharge from the heavy rain events, calcium and carbonate are transported in groundwater into areas of high evaporation where calcium carbonates are precipitated. As a consequence of changing baseline

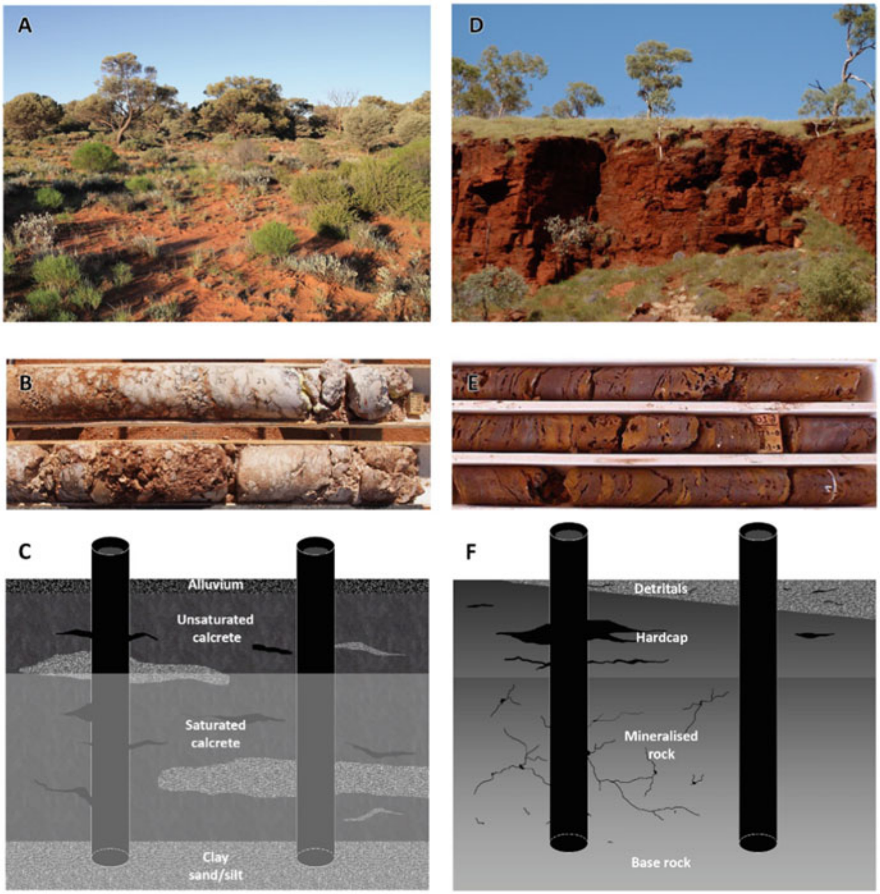

Fig. 20.2 Habitats in which subterranean fauna species occur. (**a**) Palaeochannel containing extensive subterranean calcrete in the Yilgarn; (**b**) drill core through saturated calcrete; (**c**) schematic illustration of subterranean habitat where calcrete is present; (**d**) iron ore range in the Pilbara, with deep gullies and an exposed face of hardcap containing mesocaverns; (**e**) drill core through mineralized iron ore formation; (**f**) schematic illustration of subterranean habitat in iron ore range with a deep water table (not illustrated)

groundwater levels over time because of long-term climatic variation, there is re-working and re-forming of calcrete during wet and dry periods, which creates mesocaverns and smaller spaces both above and below the water table in the calcrete. Spaces tend to be largest around the water table; deeper sections of calcrete are often quite compact and may lack any spaces. It is also common for areas of calcrete to contain substantial pods of clay and silt that lack spaces, so that overall the habitat structure within a calcrete body can be quite heterogeneous. Figure 20.2 shows a 30 cm length of calcrete core from just below the water table; it is composed of porcelain-like clasts of calcrete bound together by cementing carbonate and partially infilled with cream-colored clay and fine sand. The resulting unit has a discontinuous, vuggy texture.

The amount of weathering in rock formations in the Pilbara and Yilgarn is relatively high because of the very old age of these two cratons (Johnson 2009; see also Fig. 20.1). Weathering breaks down rocks and may lead to formation of spaces within the rock (vugginess). The surface of many rock formations in the Pilbara consists of a ferricrete duricrust (commonly called hardcap; Fig. 20.2d) that has resulted from weathering of the exposed host rock and may extend as deep as 60 m. Especially in its upper layers, the hardcap is frequently vuggy and may also contain mesocaverns and even small caves. Occasionally, the hardcap has been folded as a result of tectonic activity and so may occur at depth below unmodified rock formations. The other process that leads to vugginess in rock formations is mineralization, whereby various substances are leached from fresh rock with the consequent enrichment of iron or other mineral elements (Morris 1983). Mineralization can be substantially deeper than weathering because many of the processes leading to mineralization occur at depth during rock formation (Evans et al. 2013).

As already mentioned, the vadose zone habitats in Australia (or at least the upper strata) have some similarities to the MSS zone described by Juberthie (1983) and Ortuño et al. (2013). This is particularly the case in and around low ranges containing iron ore formations, especially where detritals form an important component of the landscape profile (Morris and Ramanaidou 2007), and in palaeovalleys filled with alluvium/colluvium and calcretes (Morgan 1993). There are also analogies between the hardcap in Australia and the canga of Brazilian iron formations (see also Chap. 21). However, a large proportion of the subterranean habitat in most iron ore formations occurs in what is most appropriately regarded as vuggy bedrock.

The relationship between high numbers of stygofauna and the occurrence of calcrete in palaeochannels in the Yilgarn region of Western Australia is well documented (Humphreys 2001, 2008; Guzik et al. 2010), with up to 75 species recorded from an individual calcrete body or cluster of calcrete deposits (EPA 2016). Another relatively well-studied relationship is the occurrence of high numbers of troglofauna species in mineralized banded iron formations and channel iron deposits farther north in the Pilbara region (EPA 2007, 2011, 2012), with more than 100 species having been recorded from sections of the banded iron formation of the Hamersley Range and about 25 species per mesa (flat-topped hill) from individual small mesas in the Robe Valley (unpublished data).

It should also be noted that it is not uncommon for a site to yield high numbers of stygofauna and low numbers of troglofauna, or vice versa. This is sometimes the result of different geologies occurring in the vadose zone and in the underlying groundwater aquifer; in other situations, the flow of water may have kept spaces open in the aquifer, whereas they have been filled by fine sediment in the vadose zone.

Box 20.3

The sampling methods used to collect stygofauna and troglofauna from subterranean habitats across the landscape have been described by Eberhard et al. (2009) and Halse and Pearson (2014). Stygofauna are sampled using groundwater monitoring bores to access the water table and underlying aquifer (s). The bores usually have a slotted PVC casing. This casing prevents the bores collapsing below the water table, while the vertical slots (usually 1–3 mm wide and extending the full depth of the bore below water table) allow stygofauna to migrate into the bore void from the surrounding aquifer. Stygofauna are collected by dropping a weighted haul net made of very fine mesh to the bottom of the bore, agitating the sediments at the bottom, and then slowly retrieving the net back through the water column. Troglofauna are usually sampled in holes drilled for mineral exploration. These holes are uncased and may be open at the ground surface, although sometimes they have a short PVC collar to reduce the likelihood of the hole collapsing. Troglofauna are collected either by trapping or scraping. Traps consist of short PVC cylinders that have slightly smaller diameter than the hole and moderate-sized perforations along their length to allow entry of troglofauna. The traps are baited with leaf material, lowered to the desired depth on a piece of cord and left in place for 6–8 weeks before being retrieved. Scraping consists of lowering a weighted, reinforced haul net to the bottom of the hole (or just into the water table) and then pulling it back to the surface along the wall of the hole, thus scraping troglofauna from the wall.

20.3 Subterranean Fauna Outside Caves

Arid and semiarid regions of Western Australia contain very high richness of stygofauna and troglofauna. Eberhard et al. (2009) estimated, based on a regional sampling program, that 500–550 stygofauna species occur in the Pilbara region alone, while Guzik et al. (2010) used expert opinion to estimate that 4140 subterranean fauna species, comprising 2680 stygofauna and 1460 troglofauna species, occur in the western half of Australia, mostly in the arid zone. More recently, Halse (2016) proposed, based on a combination of sampling results and extrapolation of the pattern of increasing richness from further sampling (mostly for environmental impact assessment), that nearly 3000 species of subterranean fauna occur in the Pilbara (Table 20.1).

Other environmental impact assessment sampling suggests that the Yilgarn has stygofauna richness similar to the Pilbara but fewer troglofauna species. On this basis, it is considered likely that more than 4500 stygofauna and troglofauna species occur in the Pilbara and Yilgarn. This figure more-or-less matches that proposed independently by Guzik et al. (2010) for the larger western half of Australia, but undoubtedly most of the richness is on the Western Shield where the Pilbara and

Table 20.1 Number of subterranean fauna species in the Pilbara, as collected by Bennelongia Environmental Consultants (BEC) or estimated to be present based on extrapolation of the collecting results to date by BEC and other environmental consultants

Faunal group	No. of species	
	Collected by BEC	Estimated
Stygofauna		
Crustacea		
Amphipoda	106	200
Isopoda	31	75
Syncarida	70	300
Copepoda	130	250
Ostracoda	194	300
Other	4	4
Hydracarina	23	40
Annelida	74	150
Mollusca	5	10
Total	637	1329
Troglofauna		
Isopoda	81	200
Pseudoscorpiones	66	150
Schizomida	59	120
Araneae	53	130
Palpigradi	18	40
Diplopoda	24	50
Chilopoda	51	120
Symphyla	38	80
Pauropoda	27	60
Diplura	90	200
Thysanura	47	100
Blattodea	27	40
Hemiptera	23	50
Coleoptera	69	150
Other	7+diptera	21
Total	680	1511

Yilgarn occur (Fig. 20.1). As is a regular feature of subterranean fauna species with their localized distributions, nearly all stygofauna and troglofauna species in the Pilbara and Yilgarn are endemic to the region in which they occur (Humphreys et al. 2008; Halse and Pearson 2014; Halse et al. 2014).

20.3.1 Stygofauna

Box 20.4

Most stygofauna species are crustaceans. The aquifers they use in the Australian arid zone occupy a variety of geologies that provide suitable spaces for animals. Alluvial and colluvial aquifers are important habitat because of their widespread occurrence and frequent high species richness, while areas of saturated calcrete (usually within alluvial or colluvial aquifers) are important habitat because of high species richness and very fine-scale endemism. Some aquifers in iron formations, especially channel iron deposit, may also support moderate numbers of stygofauna species. However, depth to the water table is a constraint on stygofauna occurrence, with assemblages usually being sparse where depth to the water table is much more than 30 m.

A detailed account of the stygofauna of the Pilbara is given by Halse et al. (2014). Although information on the Yilgarn is extensive, it is less consolidated and the first moderately comprehensive overview is provided here. General information about the communities in Yilgarn calcretes is provided by Humphreys (2001) and Humphreys et al. (2008).

A significant feature of both the Pilbara and Yilgarn is the relatively small overlap in species composition of the hyporheic fauna of streams and the stygofauna communities of deeper groundwater aquifers (Halse et al. 2002). While some species typical of the hyporheos are found in regional aquifers, such as darwinulid ostracods, the candonid ostracod *Candonocypris tenuis*, many cyclopoid copepods, and possibly phreatoicid isopods (Knott and Halse 1999; Pinder et al. 2010; Schön et al. 2010), the reverse rarely occurs. The low overlap is probably partly a result of the water associated with the alluvium of the ephemeral rivers and creeks being poorly connected to regional groundwater (Dogramaci et al. 2012), but the absence of deeper groundwater species in the hyporheos also suggests that the ecological and life history characteristics of these stygobitic groundwater species make them unsuited to hyporheic conditions.

Overall, the higher level taxonomic composition of stygofauna assemblages in the Pilbara and Yilgarn is similar, despite some differences in the proportions of major taxonomic groups (Fig. 20.3). Six groups are considered here in more detail. Copepods dominate the fauna of both areas, comprising approximately 60% of the animals in the Yilgarn and 40% in the Pilbara. However, individual species are often represented by large numbers of animals and copepods comprise only 44% and 20% of species in the Yilgarn and Pilbara, respectively (Halse et al. 2014; unpublished data), which is similar to the representation of 20–40% of species in European communities (Galassi et al. 2009). Perhaps of most interest, there appears to have been explosive speciation of harpacticoid copepods in some Yilgarn calcretes where

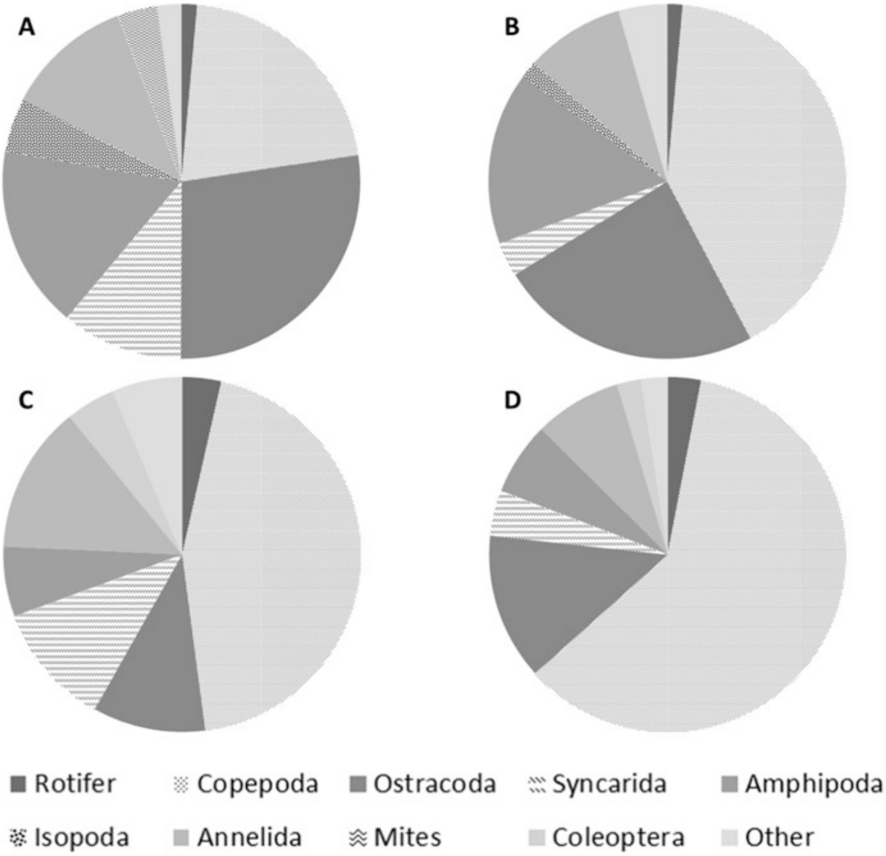

Fig. 20.3 Proportions of stygofauna in the Pilbara and Yilgarn belonging to different taxonomic groups. (**a**) Pilbara species, (**b**) Pilbara abundance, (**c**) Yilgarn species, and (**d**) Yilgarn abundance. Based on collecting results of Bennelongia Environmental Consultants

copepod species may represent almost half the fauna (Karanovic and Cooper 2011, 2012).

Ostracods represent 24% and 13%, respectively, of the animals in the Pilbara and Yilgarn (Fig. 20.3) and 30% and 10% of the species. The greater contribution of ostracods to the fauna of the Pilbara reflects the enormous radiation of candonid ostracods in this region, consisting of 11 described endemic genera and more than 108 collected species (Karanovic 2007; Reeves et al. 2007, unpublished data). By global standards, where ostracods typically constitute about 3% of all species (Eberhard et al. 2005), both the Pilbara and Yilgarn are rich in ostracods, but the Pilbara has exceptional diversity.

Another group showing high species richness is dytiscid beetles in Yilgarn calcretes and some other parts of the arid zone (Watts and Humphreys 2009; Eberhard et al. 2016). Strangely, only one dytiscid species has been recorded from the Pilbara (Watts and McRae 2013). The large number of stygofauna dytiscid beetles collected to date from the western half of Australia (approx. 100), despite single calcretes almost never containing more than three species, is a consequence of the high species turnover between calcretes. Beetles are estimated to represent 2.2% of the animals in the Yilgarn and 4.5% of the species.

Amphipod species have much the same pattern of occurrence in the Yilgarn as dytiscids, but they are more speciose and occur in higher abundance. They are also abundant in the Pilbara and represent 16% and 7%, respectively, of the animals in the Pilbara and Yilgarn (Fig. 20.3) and 17% and 20% of the species (Halse et al. 2014; unpublished data). This is similar to the overall representation of amphipod species in stygofaunal assemblages globally (19%, Eberhard et al. 2005). Much of the stygofaunal research in the Yilgarn and Pilbara has been on amphipods, with species in the Yilgarn considered to be confined to single calcretes (although these may sometimes be more accurately described as a cluster of adjacent calcrete bodies), while species in the Pilbara mostly have ranges confined to the catchments of individual tributaries of major rivers (Finston et al. 2004, 2007; Cooper et al. 2007; Bradford et al. 2010, 2013; King et al. 2012).

Based on limited taxonomic and genetic work to define species units (e.g., Guzik et al. 2008), syncarids comprise 3.1% and 4.1%, respectively, of the animals in the Pilbara and Yilgarn and 11% of the species in both regions. This is a substantially higher proportion of species than recorded globally (Eberhard et al. 2005). Limited surveys have shown that syncarids are also ubiquitous in alluvial aquifers of better watered coastal areas of Australia (Cho et al. 2005; Camacho and Hancock 2012; Cook et al. 2012), and it is likely that more survey will show the Australian fauna is at least as rich as that of Europe (see Camacho and Valdecasas 2008) and with perhaps less of an arid zone focus than most groups of Australian stygofauna.

Oligochaetes represent 9% and 8%, respectively, of the animals in the Pilbara and Yilgarn (Fig. 20.3) and 11% and 8% of the species compared with a global average of 2% of species (Eberhard et al. 2005). As in other parts of the world (Creuzé des Châtelliers et al. 2009), many oligochaetes in the Pilbara and Yilgarn are quite widespread and also have surface occurrences, so that they should be treated as stygophiles (and sometimes possibly stygoxenes). The greater number of stygal species in the Pilbara and Yilgarn is principally attributable to the collection of relatively large numbers of enchytraeid species during sampling (31% of Pilbara and 50% of Yilgarn species compared with 11% of European species). It is also of interest that phreodrilids are quite common as stygofauna in the arid Pilbara and Yilgarn (Pinder 2008; Brown et al. 2015).

20.3.2 Troglofauna

Information on the occurrence of troglofauna outside caves comes almost entirely from environmental impact assessment surveys associated with mining proposals and so data are strongly biased toward the sampling of hard rock geologies. Areas of calcrete have usually been sampled at low intensity, if at all, because of the difficulty maintaining open holes for sampling in soft substrata. Halse and Pearson (2014) have provided a description of the taxonomic composition of troglofauna in the Pilbara, but the first account of the overall composition of Yilgarn troglofaunal assemblages is presented here.

Box 20.5
Troglofauna in the Australian arid zone are taxonomically much more diverse than stygofauna. Sampling has been strongly biased toward iron formations where exploration drill holes (for mining) provide access to subterranean habitat. Nevertheless, it is likely that iron formations provide some of the most important troglofaunal habitat. Areas of unsaturated calcrete also seem to provide important habitat, provided the water table is not too shallow and soil salinity is not too high. While species ranges are still being documented, one of the outstanding characteristics of Australian arid zone troglofauna species is that they appear to have very small ranges, which may sometimes be <1 km^2.

One of the peculiarities of the information on troglofauna in Pilbara and Yilgarn is that there has been no attempt to assess the occurrence of troglofaunal mites and collembolans. Both groups comprise significant components of the fauna in other parts of the world (Ducarme et al. 2004; Kováč et al. 2016), and troglofauna species belonging to these groups have been observed frequently in Pilbara and Yilgarn samples (Greenslade 2002).

Several of the groups that are prominent in troglofaunal assemblages of the Pilbara (cockroaches, schizomids, dipterans) are absent, or nearly so, from the Yilgarn (Fig. 20.4). Isopods are the dominant group in assemblages of the Yilgarn. Based on the number of animals collected, they represent 6% and 43% of the fauna in the Pilbara and Yilgarn, respectively, and 12% and 30% of species. Isopod occurrence is globally variable with 12% of species in the Balkan Peninsula (Sket et al. 2004) and 26% of species in Portugal (Reboleira et al. 2013), and the variation between regions in Western Australia reflects this.

Hemipterans (mostly Meenoplidae) appear to have variable ranges (Fig. 20.5). The group is relatively abundant, representing 23% and 10% of animals in the Pilbara and Yilgarn, respectively, but little more than 3% of species in each region (Fig. 20.4). Some troglophilic species appear to have ranges extending over hundreds of kilometers, while other potentially troglobitic species appear to have small ranges (JM McRae, unpublished data). Culver and Pipan (2008) considered troglobitic hemipterans to be more common in shallow subterranean habitats than caves. Records from the Pilbara, in particular, suggest that hemipterans may occur at considerable depths (Halse and Pearson 2014; unpublished data).

Cockroaches, mostly belonging to the family Nocticolidae, are also abundant in the Pilbara, where they represent 19% of animals but only 4% of species, although cockroaches are one of the many groups in which the use of genetic species concepts

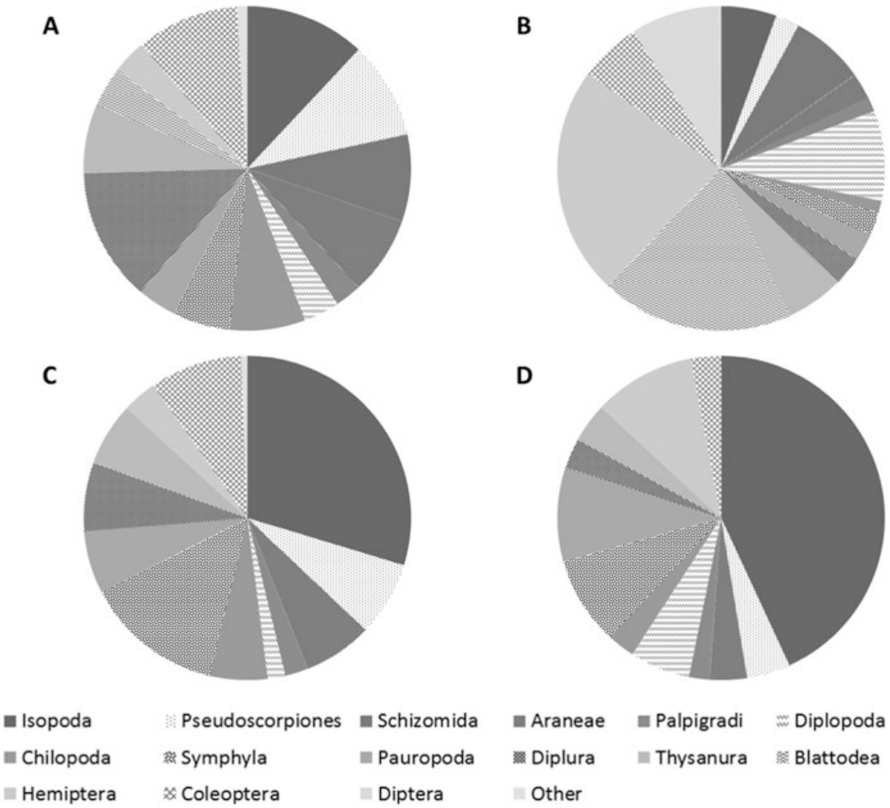

Fig. 20.4 Proportions of troglofauna in the Pilbara and Yilgarn belonging to different taxonomic groups. (**a**) Pilbara species, (**b**) Pilbara abundance, (**c**) Yilgarn species, and (**d**) Yilbara abundance. Based on collecting results of Bennelongia Environmental Consultants

is likely to substantially increase the number of species recognized (Trotter et al. 2017; Fig. 20.5). A single cockroach has been collected from the Yilgarn. While comparative data are difficult to obtain, the diversity of troglofaunal cockroaches in the Pilbara appears to be unusually high (Roth 1991; Moulds and Bannink 2012).

In contrast to the abundance of hemipterans and cockroaches, the proportions of beetles in the troglofauna assemblages of the Pilbara and Yilgarn are surprisingly low, especially when the Yilgarn is comparatively rich in stygofaunal beetles. Only 6% and 3% of troglofaunal animals and 10% and 9% of troglofaunal species are beetles in the Pilbara and Yilgarn, respectively. Typically, beetles comprise more than a third of the species in troglofaunal communities elsewhere in the world (Culver and Sket 2000; Sket et al. 2004; Niemiller and Zigler 2013). While more taxonomic investigation is likely to substantially increase the number of beetles known from the Pilbara and Yilgarn (e.g., Baehr and Main 2016; Table 20.1), their proportion of the known fauna is not expected to change greatly.

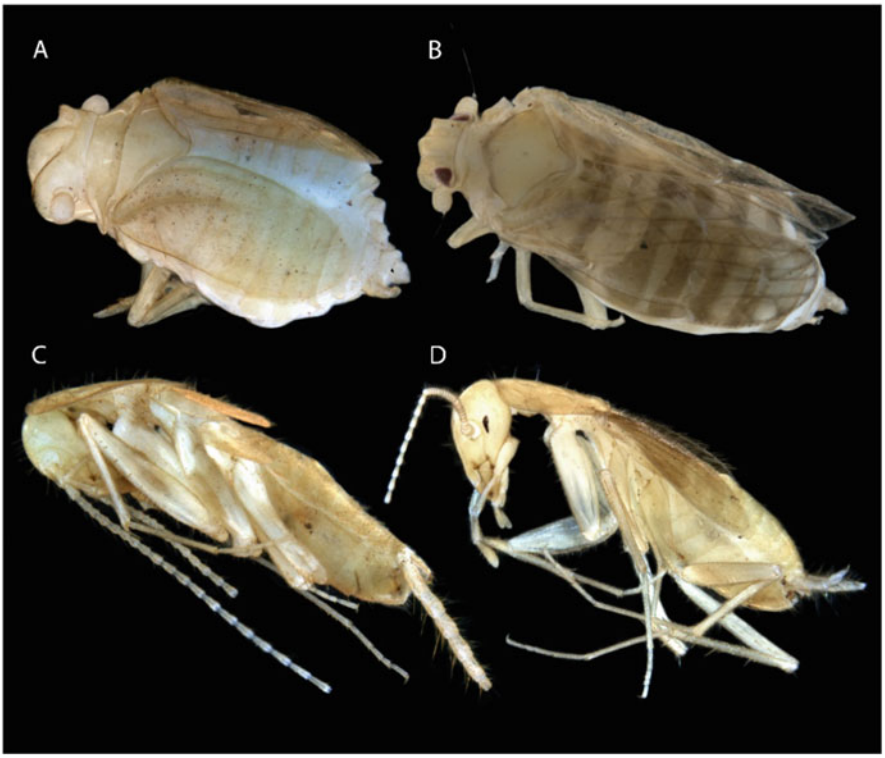

Fig. 20.5 Degrees of troglomorphy in meenoplid hemipterans and nocticolid cockroaches. (**a**) Troglobitic meenoplid, (**b**) troglophilic meenoplid, (**c**) troglobitic nocticolid, and (**d**) troglophilic nocticolid with eyespot

Millipedes represent 9% and 6% of animals in the Pilbara and Yilgarn, respectively (Fig. 20.4), as a result of the widespread occurrence of the circumtropical troglophilic *Lophoturus madecassus* (see Car et al. 2013). The group comprises only 3.5%and 1.7% of species known from these regions compared with 10% of the fauna in the Balkan Peninsula (Sket et al. 2004).

Perhaps the most iconic troglofaunal group in the Australian arid zone is the minor arachnid order Schizomida. Its occurrence is indicative of a taxonomically rich troglofauna community, and collection of schizomid species in mesas of the Robe Valley in the Pilbara led to the first troglofauna-based recommendations against mine approval by the Environmental Protection Authority in Western Australia (EPA 2007). Schizomids occur moderately often in the vadose zone and in caves across northern Australia (e.g., Harvey 2001), as well as in humid surface habitats of the tropics more generally (Monjaraz-Ruedas 2013). They have been collected in high abundance in the iron formation ranges of the central Pilbara and comprise 7% of animals and 9% of species in troglofauna assemblages of the Pilbara as a whole (Fig. 20.4). Some of the diversity of schizomids in the Robe Valley of the

Pilbara has been documented in detail by Harvey et al. (2008) and Harms et al. (2018).

Diplurans usually comprise a small to moderate proportion of troglofauna assemblages (1.1% in the Balkan Peninsula, Sket et al. 2004; 1.4–6% in superficial subterranean habitats, Culver and Pipan 2008; 7% in Portugal, Reboleira et al. 2013). In contrast, they comprise 13% and 7% of species in the Pilbara and Yilgarn, respectively, despite accounting for only 3% of the animals in each region. Some of these species are certainly troglophiles, and determining the proportion of troglobites is likely to require detailed taxonomic investigations and, ideally, life history studies to understand species ranges. However, the estimated median range of 16 km^2 for Pilbara species (Halse and Pearson 2014) suggests the proportion of troglobites may be quite high.

For pauropods, symphylans, and, to a lesser extent, palpigrads, it is difficult to distinguish troglofaunal from epigean species, because all animals of these three groups lack eyes and pigment. Furthermore, collection from subterranean habitats does not necessarily mean a species is troglofauna because most animals collected in drill holes are clearly identifiable as epigean species that have “fallen” into the drill hole. Many holes lack collars and are open at the surface with nothing to prevent surface species falling in. Even when holes are collared with PVC pipe, there is often subsidence around the collar and space for surface species to enter the hole. Bearing in mind the uncertainties associated with interpreting captures of the three groups in the Western Australian context, palpigrads and symphylans that are clearly troglobitic are regularly recorded in other parts of the world (e.g., Sket et al. 2004) and the described palpigradid *Eukoenenia guzikae* from the Yilgarn is considered to be troglobitic (Barranco and Harvey 2008). Halse and Pearson (2014) suggested that at least some of the pauropod species collected from the Pilbara are also likely to be troglobites because of their small ranges and, more particularly, the hostile surface soil conditions in the arid Pilbara. Currently, pauropods, symphylans, and palpigrads are considered to comprise 4.9%, 5.6%, and 2.6% of animals and 6.3%, 13.6%, and 2.3% of species in the Pilbara and Yilgarn, respectively (Fig. 20.4).

20.4 Species Distributions

As a group, subterranean fauna species are characterized by small ranges. This is especially so for troglofauna species (Halse and Pearson 2014), which in the Pilbara appear to have ranges that are mostly at least an order of magnitude smaller than those of stygofauna species (Eberhard et al. 2009; Halse et al. 2014). Linear ranges of <1 km sometimes occur and ranges of 1–2 km are probably quite common among arid zone troglofauna in the Pilbara (Table 20.2). In contrast only about 5% of Pilbara stygofauna species are likely to have linear ranges of <30 km (Halse et al. 2014). There is probably less difference between ranges of stygofauna and troglofauna in calcretes in the Yilgarn where a habitat feature (i.e., the calcrete

Table 20.2 Median linear ranges (recalculated from Halse and Pearson 2014) of different groups of troglofauna species in the Pilbara and the main geologies from which the groups are known in the Pilbara and Yilgarn

Troglofauna group	Median linear range (km)	Major habitats
Pseudoscorpiones	5.3	Mineralized rock, detritals (incl. calcrete)
Palpigradida	21	Mineralized rock, detritals (incl. calcrete)
Schizomida	2.6	Mineralized rock
Araneae	2.2	Mineralized rock (incl. calcrete)
Chilopoda	6.2	Mineralized rock, detritals (incl. calcrete)
Diplopoda	4.5	Mineralized rock, detritals (incl. calcrete)
Pauropoda	6.6	Detritals, mineralized rock (incl. calcrete)
Symphyla	3.2	Detritals, mineralized rock (incl. calcrete)
Isopoda	1.8	Mineralized rock, detritals (incl. calcrete)
Diplura	4.5	Mineralized rock, detritals (incl. calcrete)
Thysanura	3.7	Mineralized rock, detritals (incl. calcrete)
Blattodea	6.1	Mineralized rock
Hemiptera	68	Mineralized rock, detritals (incl. calcrete)
Coleoptera	8.7	Mineralized rock, detritals
Diptera	159	Mineralized rock

body) is often the factor limiting ranges rather than characteristics of the species themselves or the distribution of subtle habitat differences within the calcrete.

The pattern of subterranean fauna species being restricted to single calcretes or calcrete clusters in the Yilgarn led to Steven Cooper and others proposing the *calcrete island hypothesis* in relation to stygofauna (Cooper et al. 2002, 2007). It also seems to apply to troglofauna species (Javidkar et al. 2016). Under this hypothesis, most species in calcretes of the Yilgarn region are expected to be restricted to individual calcrete bodies that may have linear ranges of only tens of kilometers at most. The areas between calcrete bodies, which include intervening sections of the palaeochannel valleys hosting the calcretes, are unsuitable for stygofauna and troglofauna because of high salinity (Humphreys et al. 2008), lack of suitable voids and spaces, or otherwise inhospitable habitat. A series of papers by Tomas Karanovic on the copepods of the Yeelirrie calcrete illustrate the extreme levels of geographic replacement and local endemism that may occur within calcretes, with some stygofauna species appearing to have linear ranges of <5 km (Karanovic and Cooper 2011, 2012; Karanovic et al. 2015).

Another generalization is that weathered and mineralized iron ore deposits provide rich troglofauna habitat. The occurrence of rich troglofauna communities in iron ore ranges in Australia is analogous to the occurrence of troglofauna in iron ore mining areas of Brazil (Silva et al. 2011; see Chap. 21), although in Brazil the animals have mostly been collected from caves rather than from microcaverns within areas of vuggy iron ore (Fig. 20.2). That said, the factors affecting the importance of different types of iron ore deposits for troglofauna in Western Australia are still being studied. For reasons still to be explained, banded iron formations and channel iron deposits in the Pilbara support greater numbers of troglofauna species than banded iron formations in the Yilgarn, with Pilbara communities being more complex and, as already mentioned, supporting groups such as schizomids and cockroaches that are absent (or very nearly so) from the Yilgarn.

The vuggy habitats found in banded iron and other rock formations, especially if the available spaces are mostly microcaverns, probably provide few pathways for significant lateral underground dispersal (Fig. 20.2). Therefore, the troglobitic species in rock habitats would be expected to have smaller ranges than species inhabiting various types of detritals (scree and alluvium/colluvium) where the potential for dispersal through the matrix is likely to be greater. Despite this, and based on the current very limited understanding of the habitats that species occupy, it appears that the ranges of most troglofauna species in the Pilbara (Table 20.2) are small and determined by factors other than the broad type of geology in which the species occurs. For example, species occurring in mineralized rock do not consistently have smaller ranges than species in detritals. Probably the most important factor affecting range is whether species are troglophilic and have a surface dispersal phase, rather than relying on below-ground dispersal as troblobites do, but other intrinsic biological differences between groups may also affect species' ranges. It should be emphasized that groups for which median ranges are relatively large because they include some widespread troglophiles, such as palpigrads and hemipterans, also contain some presumed troglobitic species with very small ranges.

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Chapter 21
Subterranean Biodiversity in Ferruginous Landscapes

Rodrigo Lopes Ferreira, Marcus Paulo Alves de Oliveira, and Marconi Souza Silva

21.1 Introduction

When both their epigean and subterranean components are considered, ferriferous formations are certainly among the most unknown ecosystems of the world. Such formations are widely distributed, even in small portions, covering approximately 5% of the world's land surface (Ollier and Galloway 1990). Important deposits of these iron ore formations, which are of pre-Cambrian origin, are located in Brazil, Australia, India, and South Africa and various other countries (Costa 1993). Although many of these areas have been targeted by mineral exploration for decades, recent studies conducted in Brazil and Australia have revealed a high richness and singularity of the fauna and flora of these ferruginous formations (Jacobi et al. 2007; Souza-Silva et al. 2011; Gibson et al. 2015).

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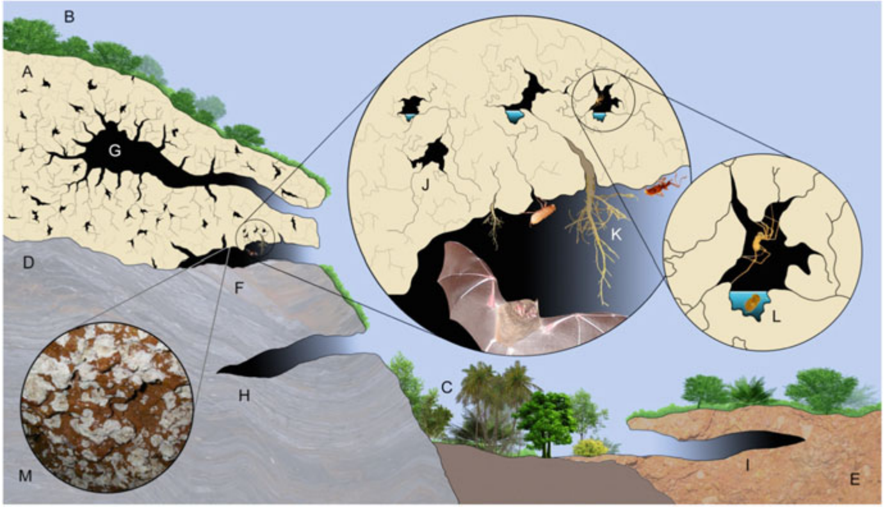

Fig. 21.1 Schematic profile of the ferruginous habitats: (**a**) superficial ferruginous breccia (canga formation), (**b**) metallophilic savannah, (**c**) tropical rainforest, (**d**) banded iron formation (BIF), (**e**) ferricretes, (**f**) cave at the contact between the BIF and the canga, (**g**) cave entirely in the canga, (**h**) cave in banded iron formation (BIF), (**i**) cave in ferricretes, (**j**) voids, (**k**) roots from the external vegetation, (**l**) water stored in voids, (**m**) actinomycetes on the cave walls

21.2 Subterranean Environments in Iron Ore Landscapes

In Brazil, the two largest ferriferous formations differ in their structure. In Carajás (Pará state), the formations are represented by jaspilite interspersed by ore bodies rich in iron. In this area, extensive iron ore plateaus comprising superficial ferruginous breccia (denominated as canga formations; Fig. 21.1a) cover the banded ferriferous formations (Piló et al. 2015). The vegetation covering those formations (800 m asl) is metallophilic savannah, and the slopes are covered by rainforest with medium and large-size trees typical of the Amazon region (Fig. 21.1b, c). In the Iron Quadrangle (Minas Gerais state), the banded formations are represented by itabirites and the landforms are more pronounced, with cangas occurring until 2000 m asl (Salgado and Carmo 2015). The vegetation of the range tops and slopes comprises grass with scrub and small-size trees, sparse or grouped in small forests, while the valleys are dominated by rainforest typical of Atlantic forest (Salgado and Carmo 2015).

The original banded iron formation (BIF), due to its long history of diastrophism and weathering, is rarely observed unaltered near the surface (Fig. 21.1d). The canga formations are usually the main surface layer on top of plateaus and mountains (Fig. 21.1a), unlike the ferricretes (Fig. 21.1e) which are located on the middle and basal portions of the cliffs (Piló et al. 2015). Chemical alterations occur at different depths, thus creating subterranean spaces with a volume and distribution that is controlled by the low rates of dissolution and chemical change found in iron ore, even in tropical regions of high rainfall. The high porosity of the iron ore formations results from the removal of silica and carbonates, which are more soluble than iron (Schuster et al. 2012).

Caves formed in iron ore found in Brazil (more than 2500) are usually small (average size of 30 m), although there are records of caves with 1500 m of extension (Piló et al. 2015). A great number of caves are inserted at the contact between BIF and canga (Fig. 21.1f), but they may occur entirely in the canga (Fig. 21.1g), in the BIF (Fig. 21.1h), and in ferricretes (Fig. 21.1i) (Auler et al. 2014). According to Auler et al. (2014), the higher frequency of caves in the contact zone of canga/BIF may reflect that the documentation of caves is biased toward shallower caves in erosional areas. There are a significant number of voids (or caves) in deep portions of the rock that lack natural entrances for humans. Such facts are indicative of hypogenic speleogenesis linked to slow biospeleogenetic processes (e.g., microbial reducibility of Fe) that require a long time to occur, thus attesting the old age of these subterranean spaces (Parker et al. 2013).

The most relevant characteristics of ferruginous subterranean ecosystems are their old age, occurring relatively close to surface, and also the presence of several interconnected small spaces in the canga formations—the voids in Fig. 21.1j (Ferreira 2005), which differ from other shallow subterranean habitats described in most parts of the world (epikarst, MSS, calcrete, and hypothelminorheic) (Culver and Pipan 2014). They are also found in BIF in Australia (see also Chap. 20). The voids in canga formations represent a distinct type of shallow subterranean habitat because of their similarity to the structural and microclimate conditions found in macro-caves (permanent, old aphotic spaces within the matrix rock), other than their small dimensions (Ferreira 2005). Such voids also differ from other shallow subterranean habitats in the availability of trophic resources. Once the roots of overlying vegetation penetrate the voids (Fig. 21.1k), they are not necessarily oligotrophic environments. Furthermore, the voids networks in canga formations allow the storage and circulation of water (Fig. 21.1l), thus favoring the coexistence of terrestrial and aquatic fauna which move from the surface to the innermost subterranean habitats, frequently accessing the macro-caves. In epikarst, water circulation is limited to small and semi-isolated wet areas, while MSS is usually formed by a mosaic of isolated and unconsolidated rock fragments (Culver and Pipan 2014). Ferruginous voids are connected to the edaphic epigean environment and its components, such as soil, lapidicolous compartments, and leaf litter.

The ecological and evolutionary importance of ferruginous voids is evident in the diversity of the species they harbor, including relict and endemic species, many of them troglobite. The voids play an important role as refugees and corridor for the fauna, thus acting as a fundamental habitat for the conservation of the biodiversity in ferruginous ecosystems.

Fig. 21.2 Some troglobitic species found in Brazilian iron ore caves: (**a**) *Glomeridesmus spelaeus* (Glomeridesmidae), (**b**) *Coarazuphium tapiaguassu* (Carabidae), (**c**) *Pseudonannolene spelaea* (Pseudonannolenidae), (**d**) *Ferricixius davidi* (Cixiidae), (**e**) *Pseudochthonius* sp. (Chthoniidae), (**f**) *Brasilomma enigmatica* (Prodidomidae), (**g**) *Pirassunungoleptes* sp. (Zalmoxidae), (**h**) *Carajas paraua* (Caponiidae), (**i**) *Charinus ferreus* (Charinidae), (**j**) *Circoniscus carajasensis* (Scleropactidae), (**k**) *Trichorhina* sp. (Plathyarthridae), (**l**) *Hyalella* sp. (Hyalellidae)

21.3 Troglobitic Species

Ferruginous caves certainly stand out in terms of the high richness of troglobitic species they support, especially when compared with caves in other lithologies (Souza-Silva et al. 2011; Ferreira et al. 2015). The troglobitic species found in iron ore caves belong to a variety of invertebrate groups, as shown in Fig. 21.2. Among the factors that apparently contributed to the evolution of troglobites in these environments can be mentioned the superficiality of the systems, the external environmental severity, and the time (Ferreira et al. 2015). The superficiality of many ferruginous subterranean spaces allows the access of roots from the external vegetation, what provides trophic resource for several species. Furthermore, the relative severity of epigean environments above these caves (scarce vegetation covering the canga and high exposure of the soil and rock to solar radiation) contributes to the active colonization of subterranean environments. Finally, other important component for the evolution of troglobites in ferruginous caves is the age of the formations and their internal changes. Schuster et al. (2012) conducted a study on the stability of cangas in the Carajás region of Brazil and showed that the canga of this region had a very slow rate of erosion throughout the last million years. The canga surface is extremely resistant to erosional processes, in spite of a humid tropical environment. However, details of the deep profile of the canga suggest that it is internally dynamic with translocation of materials as well as processes of dissolution and precipitation (Schuster et al. 2012).

Similarly, Auler et al. (2014) suggested that the speleogenetic processes through which the Brazilian ferruginous caves developed have many indications of hypogenesis associated with long-term biospeleogenetic processes performed by bacteria (Fig. 21.1m) and other organisms over hundreds of millions of years, demonstrating that dynamic sub-superficial spaces are extremely old in these environments. Many of the spaces may have sheltered ancestral species of several current troglobites. The ancestral species may have colonized these spaces earlier than speculated for the evolution of troglobites in environments such as limestone caves.

The level of endemism of troglobitic species associated with ferruginous landscapes is quite variable. There are species of wide distributions, which may extend beyond the limits of the ferruginous lithology (e.g., the Prodidomidae spider *Brasilomma enigmatica*—Fig. 21.2a—found in ferruginous, limestone, and quartzite caves with a linear range of approximately 300 km) as well as species with restricted distributions, such as the planthopper *Ferricixius davidi* (Fig. 21.2b—Cixiidae) that is endemic to one single cave.

Such differences in the level of endemism result from several factors. The first (and maybe one of the most important) is the local geomorphology, which may favor (or not) subterranean contact among macro-caves, thus increasing (or reducing) the range of a given species. For example, there is a clear difference in patterns of similarity of the troglobitic fauna associated with caves in two important Brazilian ferruginous regions. Figure 21.3 presents two graphs of multidimensional scaling (MDS) ordination based on the composition of the troglobitic fauna of some caves of Carajás (Pará state) and Iron Quadrangle (Minas Gerais state). Such graphs do not consider unique species since they would lead to some stress in the analyses because they only occur in one cave. It is noticeable that caves of Iron Quadrangle do not form distinct groups despite the apparent geomorphological compartmentalizing of this formation. Thus, it is assumed that there are subterranean connections among these macro-caves, allowing faunal migrations along almost the entire Iron Quadrangle, as demonstrated by the distribution of the troglobitic spider *Tisentnops mineiro* (Brescovit and Sánchez-Ruiz 2016). On the other hand, caves located in Carajás present a distinct pattern. Cavities located in a given unit (plateau) are highly similar regarding the troglobitic fauna, indicating the existence of species movement among the macro-caves of the same plateau, but the dissimilarity observed in caves of different units is remarkable and demonstrates that there is no movement of troglobites among plateaus. Therefore, troglobitic species that exist in one plateau are completely different from those that exist in other plateaus of Carajás region.

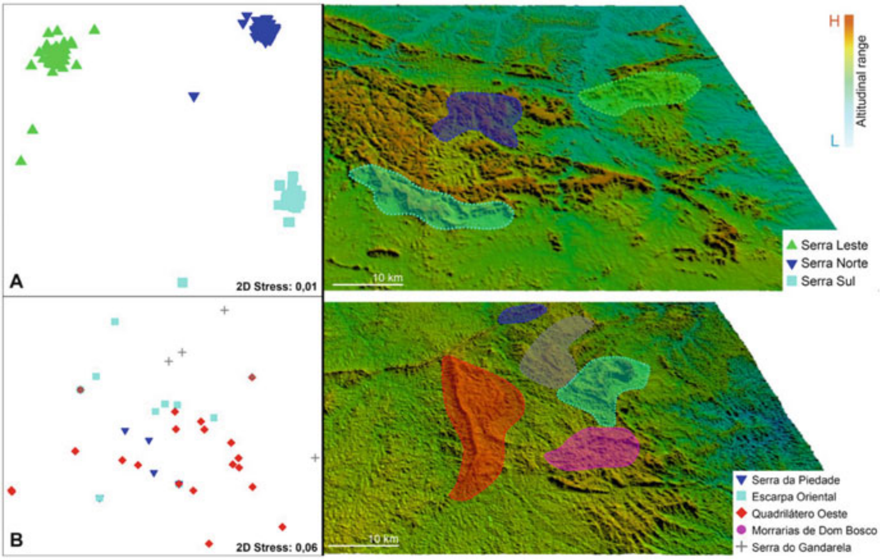

Fig. 21.3 MDS analysis performed for some ferruginous caves of Carajás (**a**) and the Iron Quadrangle (**b**), considering only troglobitic species. The symbols on the graph represent caves, and the colors indicate from which unit they belong to. The units (areas) present in each formation are highlighted in colors (corresponding to the graph) in hypsometric maps of each area: (**a**) Carajás region (Pará state, Brazil): green triangles: Serra Leste unit; blue triangles: Serra Norte Unit; light blue squares: Serra Sul unit; (**b**) Iron quadrangle (Minas Gerais state, Brazil): blue triangles: Serra da Piedade unit; light blue squares: Escarpa Oriental Unit; red diamond: Quadrilátero Oeste unit; pink circle: Morrarias de Dom Bosco unit; gray cross: Serra do Gandarela unit. See further explanations in the text

The similarity of troglobitic species composition observed within each plateau in the Carajás region is probably the result of species migration among caves through voids in the canga. Such spaces may even represent, for many species, their main habitat. Figure 21.4b shows the distribution of some troglobitic species present in a plateau in Carajás. The caves in which a given species occurs (represented by small circles) were grouped by lines indicating a minimum distribution for each species, and this distribution is much wider than the macro-caves per se. Thus, each plateau in that area may, in a sense, represent a great and unique cave formed by macro-galleries interconnected by huge systems of voids.

Another important factor that may determine general patterns of distribution of troglobitic species is biological or environmental preferences. In the Carajás region, analyses performed using troglobitic species present in the same ferruginous plateau revealed patterns of cave use that may reflect preferences for distinct micro-habitats. Figure 21.4a represents a multidimensional scaling analysis conducted considering troglobitic species present in a plateau of Carajás. Each cave is represented by a dot in the graph. Three groups were formed, each one with highly similar caves regarding troglobitic species. Sub-superficial connections that allow the migration

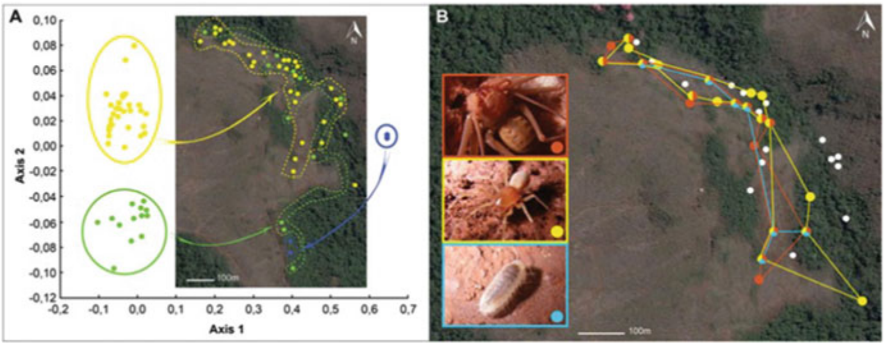

Fig. 21.4 (**a**) MDS analysis performed for the ferruginous caves present in a plateau in the Carajás region, considering only troglobitic species. The caves are represented in the graph by colored dots, and an aerial image of the area was incorporated in the graph, so that each cluster generated by the analysis is linked to their correspondent caves in the aerial image. See explanations in the text; (**b**) Distribution of some troglobitic species in a ferruginous plateau in the Carajás region. Circles in the aerial phonograph indicate the caves in the area. Each color refers to a species (orange—*Charinus* sp.; yellow—*Carajas paraua*; blue—*Circoniscus buckupi*) and the lines connecting the caves indicate the minimum distribution of each species shown in the figure. See further explanations in the text

among macro-caves exist connecting all the system, although not all species are widely or uniformly distributed. An aerial photograph of the area (including the location of caves) was overlapped to the graph, and each group defined by the MDS was associated with the corresponding cave in the aerial image. It is noted that one of these groups comprises more internally located caves in the plateau (yellow group), while other group comprises caves preferentially associated with the breaks in the canga border (green group). Finally, a small group is formed by two isolated caves (blue group). Such pattern suggests that specific traits of each micro-region (maybe related to micro-climate or availability and type of trophic resources) are important in determining which species will occur on each of these "units." Thus, it is noted that even considering each plateau as a large cave, different regions exhibit distinct traits that determine the differentiation in the occurrence and distribution of the cave fauna. In short, these "mega-caves" are not homogeneous in biological terms.

21.4 Iron Ore Formations in Brazil and Australia

Australia and Brazil have the largest ferruginous geosystems in the world, but the characteristics of the systems in each country lead to different habitats being present and, consequently, differences in their subterranean faunas. In Brazil, the occurrence of iron ore macro-caves is extremely common, and they are usually dry, with rare temporary drainages resulting from drip or percolation of pluvial waters through voids or geological discontinuities (Piló et al. 2015). Caves are the main target of subterranean studies in Brazil, and most of the known fauna for Brazilian ferriferous areas came from these habitats. However, in Australia, the lower intensity of erosive and dissolution processes hampered the formation of macro-caves, and the subterranean fauna has been sampled using geological exploration drill holes and bores to access much smaller subterranean habitats (Eberhard et al. 2009; Guzik et al. 2011; Halse and Pearson 2014; see also Chap. 20).

Consequently, the subterranean fauna of Brazilian ferruginous geosystems is almost totally represented by terrestrial invertebrates, while in Australia there are a significant number of stygobiotic species as well in ferruginous formations, although most stygofauna occur in alluvium and a range of other geologies (Halse et al. 2014). Currently, there are approximately 150 troglomorphic species associated with Brazilian ferruginous caves, of which 21 are formally described and only one is aquatic (Ferreira et al. 2015; Brescovit and Sánchez-Ruiz 2016; Zeppelini and Oliveira 2016; Giupponi and de Miranda 2016; Asenjo et al. 2018; Souza and Ferreira 2018). While there are more than 403 formally described species in the western part of Australia (Guzik et al. 2011), most of these species are stygobitic copepods, ostracods, and beetles found in alluvium or calcrete. A few of these species occur in aquifers in ferruginous formations (Halse et al. 2014). There are about 50 described troglobitic species from ferriferous formations, although almost 700 ferriferous troglobites and troglophiles have been collected from these formations and about 1500 species are estimated to occur (see also Chap. 20).

The distance from coastal regions may be an important factor determining the differences in the number of stygobiotic species between Brazil and Australia. The Hamersley Range and sections of the Robe Valley, which together probably embrace the highest concentrations of stygobites in Australian ferruginous areas (Halse et al. 2014), are located within 50 km of the coast at their western extent. Thus, it is plausible to consider a previous connection between the aquifers present in these areas and the ocean that may have favored the migration and evolution of ancestral species, which currently are represented by many endemic stygobites found therein. By contrast, in Brazil, the Iron Quadrangle and Carajás regions are at least 270 km and 1000 km, respectively, from the coast, which may preclude the occurrence of species derived from oceanic ancestors. However, it is important to highlight that the lack of studies regarding the fauna of Brazilian ferruginous aquifers unfortunately prevents any consistent comparison regarding evolutionary processes that have led to differences between these two different ferruginous systems.

21.5 Ecology

The relation between the size of a cave and its invertebrate richness is well known (Souza-Silva et al. 2011; Ferreira et al. 2015; Simões et al. 2015; Jaffé et al. 2016). Large caves tend to be more heterogeneous, which probably results in an increase of microhabitats, and consequently more species may establish themselves in these conditions. Souza-Silva et al. (2011) demonstrated that the relationship between species richness and cave size tends to be more pronounced in ferruginous caves than in other lithologies. Probably the larger ferruginous caves accumulate more organic resources, besides the fact that they potentially can connect to more voids (due to the increase of the subterranean volume). Such conditions make the macro-caves “attractors” of epigean and interstitial fauna, and this attraction may occur exponentially, with a small increase in linear development leading to a large increase in connections and substantially increasing species richness above what is found in other lithologies where such voids do not occur (Souza-Silva et al. 2011; Ferreira et al. 2015).

This cave size/species richness relationship was also demonstrated by Ferreira et al. (2015) in a set of 240 caves sampled in the region of Carajás. Thus, the size of ferruginous caves is important not only for the maintenance of high species richness, but also because it allows the evolution and coexistence of more troglobitic species. Ferreira et al. (2015) also demonstrated that total species richness and richness of troglobitic species are related. This suggests that some of the conditions favoring the occurrence and evolution of troglobites in the subterranean environment also favor the settlement and current occurrence of other non-troglobitic species. When a cave rich in troglobitic species is protected, a significant number of non-troglobitic species associated with these systems are also preserved.

Ferreira et al. (2015) determined the turnover of species through time for the same previously mentioned set of caves in relation to the dry and rainy seasons in the region. The temporal turnover was calculated from data of presence/absence of species through the index of Harrison et al. (1992), modified from Whittaker (1960). In general, caves sampled in the same plateau in Carajás had high turnover values, which suggest there tends to a considerable replacement of species between the dry and rainy seasons. For the analyzed set, 89.8% of caves had turnover values higher than 60%, 56.1% had values higher to 70%, and 19.4% had values higher than 80% (Ferreira et al. 2015). Ferreira et al. (2015) also conducted a multiple regression between the species turnover and the size and number of entrances of the caves. The turnover was negatively related to size of the cave and positively related to the number of entrances. Thus, this species replacement is higher in caves with many entrances, but lower for larger caves. This model corroborates what was expected, since a large amount of entrances increases the contact with the external environment, thus increasing the chances of colonization of the cave by accidental or transient species.

Thus, caves present in the Carajás region tend to have highly variable species composition through time, especially small caves which are continuously accessed by several species from the epigean environment. Finally, it is important to highlight the negative relationship observed between the number of troglobitic species and the turnover (Ferreira et al. 2015). Caves with more troglobitic species are those with lower turnover values, which tend to be considered more ecologically stable.

21.6 Threats

Ferruginous regions present in Brazil and Australia comprise some of the main areas of mineral exploration in the world. Mining extraction removes the ferruginous crust (canga formation) that covers the mineral reserves, thus modifying the landscape and having a potentially huge impact on the local and regional biodiversity (Jacobi and Carmo 2008). According to Gibson et al. (2015), the Australian ferruginous landscapes are characterized by the complexity of fauna and flora, with endemic taxa including cryptic and subterranean species with restricted geographical distributions. The loss of habitat in these areas as a result of mining may lead to irreversible changes in biological communities (see also Chap. 22). In Brazilian ferruginous geosystems, the level of endemism and complexity are similar; however, the scenario is still more alarming, since most of the ferruginous macro-caves occur in the shallowest part of the deposits (Auler and Piló 2005). Due to the depth of mine excavations, sometimes below groundwater, the contamination of groundwater and watercourses by mineral wastes, heavy metals, and toxic elements also must be considered a potentially important impact (Veado et al. 2006).

In November 2015, a huge waste dam associated with an iron ore mine collapsed in central Brazil, causing what has being considered one of the biggest environmental disasters ever registered in the country (Yoemans and Bowater 2016; Porto 2016). The impacts include social problems (an entire village was buried by the waste) as well as environmental problems (a basin of an important Brazilian river—Doce River—suffered silting, heavy metals contamination and fish mortality, as well as other impacts). Although the media and authorities highlighted different impacts, all of them were visible impacts occurring in epigean habitats. To date there have been no studies examining the potentially serious impacts of the accident on ferruginous subterranean environments, which were certainly affected.

In addition to mining, the urban expansion and other anthropogenic activities also pose important threats to the subterranean biodiversity in ferruginous landscapes. In Brazil, the main ferruginous areas are located close to large metropolitan centers or areas of that receive a large number of migrant workers and their families (Ferreira et al. 2015). In the Carajás region, many of the impacts documented for the Amazon region are present, like livestock, agriculture, cutting, and fire associated with the timber harvesting (Veríssimo et al. 1996; Arima et al. 2005; Ferreira et al. 2005). For Australia there are alterations caused by the introduction of feral animals and exotic plants (McKenzie et al. 2006). Besides direct anthropic impacts, the fire may be considered a natural disturbance in these areas, enhanced by the human occupation that increases the frequency of focus of forest fires (Ferreira et al. 2015; Gibson et al. 2015).

The geoferruginous system in Brazil is characterized by the expressive hydric potential, which is essential for the preservation of the fauna with many endemic stygobite species, and also for the public supply, since these areas may be located in arid regions and/or next to large urban centers. The uncontrolled use of water from these systems may alter the balance between the recharge of the aquifer and the residence time of subterranean water, thus precluding the hydric recovery of the system (Gama and Matias 2015). Such condition leads to alterations in the hydric balance with the reduction of water levels and decrease of free flow, thus impacting the whole associated ecosystem. According to Mourão (2007), the time needed for the renewal of an aquifer of Iron Quadrangle (Brazil) may be superior to 500 years, demonstrating that the time of water permanence in these ferruginous systems is high. Such situation demonstrates the stability of these environments, what may be essential for the development of the stygobites, which have been increasingly sampled in ferriferous regions.

Finally, despite the growing number of publications on the biology of caves in iron ore, the knowledge about them is still in its very early stages. It is still important to study the fauna present in ferruginous caves in order to conserve them. Future research will provide more information about the environment factors controlling species occurrence, the processes structuring the communities, and the distribution pattern of troglobitic species found in these caves.

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Part V
Conservation and Protection of Cave Habitats and Cave Fauna

Chapter 22
Conservation of Cave Fauna, with an Emphasis on Europe and the Americas

Matthew L. Niemiller, Steven J. Taylor, and Maria Elina Bichuette

22.1 Introduction

There has been an increasing awareness and concern for subterranean biodiversity over the past two decades (Culver et al. 2000; Danielopol et al. 2000; Elliott 2000; Gibert and Deharveng 2002; Culver and Pipan 2009, 2014; Gibert and Culver 2009). Although the subterranean environment traditionally has been considered species poor, an exceptionally species-rich and phylogenetically diverse community of organisms exists in caves, groundwater, and other subterranean habitats of Europe, North America, Central America, and South America (Culver et al. 2000; Ferreira et al. 2007; Trajano and Bichuette 2009; Cordeiro et al. 2014; Gallão and Bichuette 2015). Subterranean biodiversity is particularly diverse in Europe where some 5000 troglobionts have been described (Gibert and Culver 2009; Deharveng et al. 2012). Most local hotspots of subterranean biodiversity globally, defined by Culver and Sket (2000) as caves or wells with 20 or more troglobionts, are known from southern Europe (Culver and Sket 2000; Culver and Pipan 2009). Five of the six caves with 40 or more species occur in Europe, including Postojna-Planina Cave System in Slovenia and Vjetrenica in Bosnia-Herzegovina, which are the two most biodiverse caves in the world with almost 100 species (Culver and Pipan 2009). The sixth cave

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is the Mammoth Cave System in Kentucky, USA. For South America, specifically Brazil, there are at least three cave systems with high diversity of troglobionts (Deharveng and Bedos 2012). More recently, four cave systems have been identified as areas of high diversity of troglobionts in Brazil: Areias Cave System (26+ troglobionts), Alambari Cave System (16 species), Parede Vermelha Cave (12+ species), and Olhos d'Água Cave (11+ species) (Trajano et al. 2016).

Several life history traits common to many troglobionts, and to some extent, cave-roosting bats, are associated with increased risk of extinction, including low reproductive rates and limited dispersal ability (Culver and Pipan 2009, 2014). Thus, population rescue is often much slower and risk of extinction much greater relative to populations of related surface species. Moreover, many troglobionts may be particularly sensitive to small fluctuations in abiotic variables such as temperature, humidity, dissolved oxygen, and concentrations of heavy metals, among others.

Obligate cave/subterranean fauna, as well as many facultative cave/subterranean species (such as bats), rely heavily on subterranean habitats and thus are highly vulnerable to threats that result in environmental change and habitat disturbance and degradation. These threats vary with respect to scope, source, severity, and timing among species, karst regions, and continents. Some threats, such as climate change and groundwater pollution, are global in scope (Culver and Pipan 2009). However, effects of climate change and sources of groundwater pollution vary at regional and local scales. Other threats affect cave/subterranean populations and species at a local or regional scale, such as mining and quarrying, impoundments, groundwater extraction, commercialization of caves, and amateur and scientific collection. Important threats to subterranean communities in caves, groundwater, and other subterranean habitats in Europe, North America, Central America, and South America have been reviewed and discussed in Juberthie (1995), Elliott (2000), Tercafs (2001), Culver and Pipan (2009, 2014), Reboleira et al. (2011), Trajano (2010), Simoes et al. (2014), and Gallão and Bichuette (2015, 2018), among others, and we direct those interested in specific threats to subterranean biodiversity in general to these publications (Fig. 22.1).

Box 22.1

Several biological factors common to many troglobionts are associated with increased vulnerability to threats and risk of extinction (reviewed in Culver and Pipan 2009, 2014). First, most subterranean species are geographically rare, often having small, highly restricted geographic ranges (Culver et al. 2006; Zagmajster et al. 2008; Deharveng et al. 2009; Gallão and Bichuette 2018). These animals often are endemic to a single or few cave systems (Christman et al. 2005; Deharveng et al. 2009; Niemiller and Zigler 2013). Many species also may be numerically rare. Several troglobionts are known from only one or a few specimens (e.g., Niemiller et al. 2017). However, it is often unclear whether such species are actually comprised of very small

(continued)

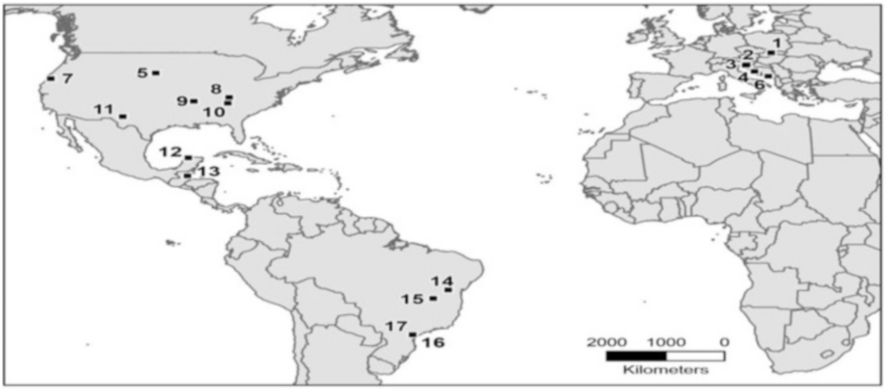

Fig. 22.1 Locations of caves, systems, features, and areas mentioned specifically in this chapter. *1* Caves of the Demänová valley; *2* Postojna-Planina Cave System; *3* Skocjanske Jame; *4* Dinaric Karst; *5* Wind Cave; *6* Vjetrenica; *7* Cave Creek; *8* Mammoth Cave; *9* Buffalo National River; *10* Hubbard's Cave; *11* Carlsbad Caverns; *12* Anillo de Cenotes; *13* Chiquibul Cave; *14* Parede Vermelha Cave; *15* Olhos d'Água Cave; *16* Alambari Cave System; *17* Areias Cave System

Box 22.1 (continued)
populations (i.e., observed rarity reflects actual rarity) or whether rarity reflects undersampling or sampling of suboptimal habitat. For example, some species may be common in epikarst but are very infrequently observed in caves.

Herein, we identify and review several important international and national legislation, policies, and conservation initiatives that have been implemented or proposed related to the protection and conservation of cave/subterranean biodiversity.

Box 22.2
Despite these biological factors and many documented and suspected threats, only a small fraction of subterranean biodiversity receives any direct protection under international, national, or regional legislation. Compared to vertebrates, subterranean invertebrates and other organisms have largely been neglected in conservation studies, assessments, and policy decisions, despite their significantly greater diversity, roles in groundwater and subterranean ecosystem services, sometimes high levels of endemicity, and benefits to mankind.

22.2 International Legislation

Four major international conventions that focus on biodiversity issues are relevant to the protection and conservation of subterranean biodiversity and ecosystems: Convention on Biological Diversity (CBD), Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), Ramsar Convention on Wetlands of International Importance, and World Heritage Convention (WHC). Each of these biodiversity-related conventions aims to implement conservation actions at the international, national, and regional levels. However, few subterranean species are currently protected under the auspices of these conventions.

The *Convention on Biological Diversity* (CBD) is an international treaty among 196 countries today that entered into force in 1993 to develop strategies for the conservation and sustainable use of biodiversity. All countries in Europe, North America, Central America, and South America are member parties, except for the United States. CBD has three main goals: conservation of biodiversity, sustainable use of its components, and fair and equitable sharing of benefits arising from genetic resources. These objectives are of particular importance for developing countries. The convention requires countries to prepare a national biodiversity strategy, called National Biodiversity Strategies and Action Plans (NBSAPs), and to ensure that NBSAPs are implemented into all relevant planning and activities that may have a positive or negative impact on biodiversity. Of the 196 member parties, 94% have developed NBSAPs. Cave and groundwater biodiversity and ecosystems are specifically addressed in several countries' NBSAPs. For example, the Slovenian NBSAPs has a specific objective on cave habitat types "to maintain subterranean habitat types in ecologically important areas, and the entire subterranean fauna, at favorable conservation status." However, the focus on subterranean fauna and ecosystems in NBSAPs appears to be highly variable among countries.

The *Convention on International Trade in Endangered Species of Wild Fauna and Flora* (CITES) is an international agreement signed in 1973 and entered into force in 1975 between governments to ensure that international trade of wild animals and plants of conservation concern does not further threaten their continued survival. Today, 181 countries, including most countries in Europe, North America, Central America, and South America (Table 22.1), are member parties. Some 5600 species of animals are protected by CITES. However, only about 40% of the species listed are invertebrates. These species are listed in one of the three CITES Appendices according to how threatened they are by international trade. No troglobionts from Europe, North America, Central America, or South America are listed; however, some non-troglobiotic taxa that rely on caves are included (e.g., cave-roosting birds, *Steatornis caripensis*).

The *Convention on Wetlands of International Importance (Ramsar Convention)* is the oldest global international environmental agreement adopted in 1971 and entered into force in 1975. Its mission is the conservation and wise use of all wetlands through local and national actions and international cooperation. Some 169 countries are contracting parties to the convention, including most countries in

Table 22.1 Important international and national legislation relevant to the conservation and protection of subterranean biodiversity and ecosystems in countries of Europe, North America, Central America, and South America

Country	Bern	Bonn	CBD	CITES	RC	WHC	Legislation
Europe							
Albania	1999	2001*	1994	2003	1996	1989	Law on Biodiversity Protection (2006) Law on Protected Areas (2002, 2008) Law on Wild Fauna Protection (2008)
Andorra	2001		2015		2012	1997	Law of Protected Species (2001)
Austria	1983	2005	1994	1982	1983	1992	Flurverfassungsgrundsatzgesetz (1951)
Belarus		2003	1993	1995	1991	1988	Law on Protection of the Environment (2002) Law on the Animal World (2007)
Belgium	1990	2003*	1997	1983	1986	1996	Regional policy
Bosnia and Herzegovina	2009		2002	2009	1992	1993	Law on Environmental Protection (2013)
Bulgaria	1991	1999*	1996	1991	1976	1974	Nature Protection Act (1967, 1991) Biological Diversity Act (2002, 2011)
Croatia	2000	2000*	1997	2000	1991	1992	Cave Protection Act (1900) Nature Protection Act (2005) Regulation on Protection of Wild Species (2006)
Cyprus	1988	2001*	1996	1974	2001	1975	Law on the Protection and Management of Nature and Wildlife (2003)
Czech Republic	1998	1994*	1994	1993	1993	1993	Act on the Protection of Nature and Landscape (1992, 2004)
Denmark	1983	1983*	1994	1977	1978	1979	Nature Conservation Act (2009, 2013)
Estonia	1992	2008*	1994	1992	1994	1995	Animal Protection Act (2000) Nature Conservation Act (2004)
European Union	1982	1983	1994	2015			
Finland	1986	1989*	1994	1976	1975	1987	Nature Conservation Act (1923, 1996) Nature Conservation (Amendment) Act (1991)
France	1990	1990*	1994	1978	1986	1975	Environment Code (2010)
Germany	1985	1984*	1994	1976	1976	1976	Federal Nature Conservation Act (2010)
Greece	1983	1999	1994	1992	1975	1981	Biodiversity Law (2011)

(continued)

Table 22.1 (continued)

Country	Bern	Bonn	CBD	CITES	RC	WHC	Legislation
Hungary	1990	1983*	1994	1985	1979	1985	Act on Nature Conservation (1996)
Iceland	1993		1994	2000	1978	1995	Nature Conservation Act (1999)
Ireland	1982	1983*	1996	2002	1985	1991	Wildlife Act (1976) Wildlife (Amendment) Act (2000)
Italy	1982	1983*	1994	1979	1977	1978	Law on Protected Areas (1991)
Latvia	1997	1999*	1996	1997	1995	1995	Law on the Conservation of Species and Biotypes (2000, 2005) Law on Specially Protected Nature Territories (1993, 2007) Law on Subterranean Depths (1996)
Liechtenstein	1982	1997	1998	1979	1991		Law on the Protection of Nature and Landscape (2004)
Lithuania	1997	2002*	1996	2001	1993	1992	Law on the Protected Fauna, Flora and Fungi Species and Communities (1997) Law on Protected Areas (2001) Underground Law (1995)
Luxembourg	1982	1983*	1994	1983	1998	1983	Law on Nature Protection and Natural Resources (2004)
Malta	1994	2001*	2001	1989	1989	1978	Flora, Fauna, and Natural Habitats Protection Regulations (2006, [amended] 2013)
Moldova	1994	2001*	1996	2001	2000	2002	Law on the Animal Kingdom (1995)
Montenegro	2010	2009*	2006	2007	2006	2006	Law on Nature Protection (1977, 1989)
Netherlands	1982	1983*	1994	1984	1980	1992	Nature Conservation Act (1998) Flora and Fauna Act (2002)
Norway	1986	1985	1993	1976	1975	1977	Nature Diversity Act (2009)
Poland	1996	1996*	1996	1989	1978	1976	Nature Conservation Act (2004)
Portugal	1982	1983*	1994	1980	1981	1980	Nature Conservation Act (2008)
Romania	1993	1998*	1994	1994	1991	1990	Law no. 49 (2011) for the approval of Government Emergency Ordinance no. 57 on the regime of natural protected areas, conservation of natural habitats, wild flora and fauna (2007)
Serbia	2008	2008	2002	2006	1992	2001	Law on Nature Conservation (2010)

Slovakia	1997	1995*	1994	1993	1993	1993	Act on Nature and Landscape Protection (2002)
Slovenia	2000	1999*	1996	2000	1991	1992	Nature Conservation Act (1999) Cave Protection Act (2004)
Spain	1986	1985	1994	1986	1982	1982	Law on Natural Heritage and Biodiversity (2007)
Sweden	1983	1983	1994	1974	1975	1985	Environmental Code (1998)
Switzerland	1982	1995*	1995	1974	1976	1975	Federal Act on the Protection of Nature and Cultural Heritage (1966)
The Former Yugoslav Republic of Macedonia	1999	1999*	1998	2000	1991	1997	Law on Nature Protection (2004)
Ukraine	1999	1999*	1995	1999	1991	1988	Law on Animals (2001)
United Kingdom	1982	1985*	1994	1976	1976	1984	Wildlife and Countryside Act (1981) Countryside Rights of Access Act (2000) Nature Conservation (Scotland) Act (2004) Protected Species Act (2003)—Bermuda
North and Central America							
Antigua and Barbuda		2007	1993	1997	2005	1983	Environmental Protection and Management Act (2014)
Bahamas			1993	1979	1997	2014	Forestry Act (2010)
Barbados			1994	1992	2006	2002	
Belize			1994	1986	1998	1990	Wildlife Protection Act (1981)
Canada			1993	1975	1981	1976	Species at Risk Act (2002)
Costa Rica		2007	1994	1975	1992	1977	Biodiversity Law (1997) Law on Wildlife Conservation (2012)
Cuba		2008	1994	1990	2001	1981	Wild Animals Protection Act (1968)
Dominica			1994	1995		1995	Forestry and Wildlife Act (1976)
Dominican Republic			1997	1986	2002	1985	General Law on Environmental and Natural Resources (2000)
El Salvador			1994	1987	1999	1991	Wildlife Conservation Law (1994)
Grenada			1994	1999	2012	1998	Birds and Other Wildlife Protection Act (1957)
Guatemala			1995	1979	1990	1979	Forestry Law (1996)
Haiti			1996			1980	

(continued)

Table 22.1 (continued)

Country	Bern	Bonn	CBD	CITES	RC	WHC	Legislation
Honduras		2007	1995	1985	1993	1979	Forest, Protected Areas, and Wildlife Law (2007)
Jamaica			1995	1997	1998	1983	Endangered Species Act (2000) Wildlife Protection Act (1945)
Mexico			1993	1991	1986	1984	General Wildlife Act (2000)
Nicaragua			1996	1977	1997	1979	Law on Use and Conservation of Biodiversity (2012)
Panama		1989	1995	1978	1990	1978	General Environmental Law (1998) Wildlife Law (1995)
Saint Kitts and Nevis			1993	1994		1986	National Conservation and Environment Protection Act (1987)
Saint Lucia			1993	1982	2002	1991	Wildlife Protection Act (1980)
Saint Vincent and the Grenadines			1996	1988		2003	Wildlife Protection Act (1987)
United States				1974	1986	1973	Endangered Species Act (1973)
South America							
Argentina		1992	1995	1981	1992	1978	Law on Wildlife Conservation (1997)
Bolivia		2003	1995	1979	1990	1976	Law on the Rights of Mother Earth (2010) Supreme Decree 22641 (1990)
Brazil		2015	1994	1975	1993	1977	Environmental Crimes Law (1999)
Chile		1983	1994	1975	1981	1980	Environmental Law (1994) Hunting Law (1996)
Columbia			1995	1981	1998	1983	General Environmental Law (1993)
Ecuador		2004	1993	1975	1991	1975	Forestry and Conservation of Natural Areas and Wildlife Law (1981) Biodiversity Law (2004)
Guyana			1994	1977		1977	Environmental Protection Act (1996)
Paraguay		1999	1994	1976	1995	1988	Law of Wild Life (1992)
Peru		1997	1993	1975	1992	1982	Forest and Wildlife Act (1975) Biological Diversity Conservation and Sustainable Use Act (1997)
Suriname			1996	1980	1985	1997	Nature Conservation Law (1954)

Trinidad and Tobago			1996	1984	1993	2005	Conservation of Wildlife Act (1958, 1980)
Uruguay		1990	1994	1975	1984	1989	
Venezuela			1994	1977	1988	1990	Protection of Wildlife Act (1970) Biological Diversity Act (2000)

International legislation includes several important conventions: the Bern Convention (Bern), Bonn Convention (Bonn), Convention on Biological Diversity (CBD), Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), Ramsar Convention (RC), and World Heritage Convention (WHC). EUROBATS member parties are denoted by an asterisk (*) under the Bonn Convention column. Entry dates for each country are presented

Europe, North America, Central America, and South America. At the time of joining the convention, each contracting party must designate at least one wetland site for inclusion in the List of Wetlands of International Importance. Ramsar Sites are designated based on nine criteria, eight of which are related to biodiversity. When a wetland is officially included in the list, it is recognized as being of significant value for humanity as a whole. Each contracting party must take the necessary actions to ensure that the ecological character of the Ramsar Site is preserved. Many Ramsar Sites are also protected under other international and national protection conventions and schemes, such as the World Heritage List under the UNESCO World Heritage Convention. Karst and other subterranean hydrological systems are broadly defined as wetlands, including marine, inland freshwater, and man-made types. Thirty-seven Ramsar Sites are inland karst wetlands that cover over 850,000 ha and occur in Europe, including notable cave systems such as Skocjanske Jame in Slovenia and Caves of the Demänová valley in Slovakia. In North and Central America, 44 karst and subterranean wetland Ramsar Sites occur that cover over 3.4 million ha, predominantly in Mexico, including the Anillo de Cenotes in the Yucatan. Only two Ramsar Sites in Chile are karst or subterranean wetlands in South America.

The *Convention Concerning the Protection of the World Cultural and Natural Heritage or the World Heritage Convention* (WHC) was adopted by the United Nations Educational, Scientific and Cultural Organization (UNESCO) General Conference in 1972 and came into force in 1975. WHC aims to promote cooperation among nations to protect cultural and natural heritage globally that is of outstanding universal value to humanity. Today, 191 parties have agreed to identify, protect, and conserve World Heritage Sites. A site has outstanding universal value, as defined by the Operational Guidelines for the Implementation of the World Heritage Convention if it has cultural and/or natural significance that is so exceptional that it transcends national boundaries and is of importance to current and future generations of all humanity. Nomination and inclusion in the World Heritage List represents a formal pledge by the host country to take steps necessary to protect a site, which includes the appropriate legal, scientific, administrative, and financial measures for the identification, protection, conservation, preservation, and rehabilitation of sites with outstanding universal value. Listing as a World Heritage Site is often accompanied by increased tourism at some sites and prioritization for funding and technical assistance, but also controversial debates regarding how to protect some threatened sites.

Several sites included in the UNESCO World Heritage List contain significant cave and karst systems (Table 22.2). Of these sites, seven are specifically recognized for their outstanding biodiversity value under the biodiversity criteria (ix) and/or (x): criterion (ix) “to be outstanding examples representing significant on-going ecological and biological processes in the evolution and development of terrestrial, freshwater, coastal, and marine ecosystems and communities of plants and animals”, and criterion (x) “to contain the most important and significant natural habitats for in-situ conservation of biological diversity, including those containing threatened species of outstanding universal value from the point of view of science and

Table 22.2 List of UNESCO World Heritage Sites and Tentative List Sites (T) within cave and karst terrains in Europe, North America, Central America, and South America

Continent	Country	World heritage site	Year inscribed	Natural criteria
Europe	Bulgaria	Pirin National Park	1983	vii, viii
	Bulgaria	Vratsa Karst Nature Reserve	T	vii, viii, ix, x
	Croatia	Kornati National Park and Telascica Nature Park	T	vii, viii, x
	Croatia	Plitvice Lakes National Park*	1979, 2000	vii, viii, ix
	Croatia	Velebit Mountain	T	vii, viii, ix, x
	France	Ensemble de grottes à concretions du Sud de la France	T	vii, viii, ix
	France/ Spain	Pyrenees-Mont Perdu*	1997, 1999	vii, viii
	Greece	Meteora	1988	vii
	Hungary	Hydrothermal Caves and Thermal Karst Systems of the Rozsadomb Area	T	viii
	Hungary/ Slovakia	Caves of Aggtelek and Slovak Karst*	1995, 2000	viii
	Italy	The Murge of Altamura	T	vii, viii
	Montenegro	Durmitor National Park	1980, 2005	vii, viii, x
	Norway	Svalbard Archipelago	T	vii, viii, ix, x
	Serbia	The Tara National Park with the Drina River Canyon	T	x
	Slovenia	Skocjan Caves*	1986	vii, viii
North America	Canada	Canadian Rocky Mountain Parks*	1984	vii, viii
	Canada	Nahanni National Park*	1978	vii, viii
	Cuba	Alejandro de Humboldt National Park	2001	ix, x
	Cuba	Desembarco del Granma National Park*	1999	vii, viii
	Mexico	Reserve de la Biosphere Selva El Ocote	T	n
	Mexico	Sian Ka’an	1987	vii, x
	United States	Carlsbad Caverns National Park*	1995	vii, viii
	United States	Grand Canyon National Park	1979	vii, viii, ix, x
	United States	Mammoth Cave National Park*	1981	vii, viii, x
South America	Brazil	Canyon du Rio Peruaçu, Minas Gerais	T	vii, viii, ix, x
	Venezuela	Canaima National Park*	1994	vii, viii, ix, x

Natural criteria for each site are listed and sites with outstanding universal value are denoted with an asterisk (*)

conservation." There are several additional areas not listed in Table 22.2 that have cave and karst of national rather international significance or have cultural rather than natural value (Williams 2008). Although caves and karst are well represented in World Heritage sites in humid tropical and temperate regions, particularly in the Northern Hemisphere, representation of caves and karst in World Heritage sites is deficient in several regions (Williams 2008), particularly the arid to semiarid zone of the tropics to subtropics, the periglacial zone, and in the Southern Hemisphere, including South America.

22.3 Conservation Organizations

Several organizations are important partners and leaders for conservation worldwide, working to protect and conserve biodiversity, including subterranean fauna. These organizations, highlighted by the International Union for the Conservation of Nature and Natural Resources and NatureServe, work at global, national, and regional scales to assist government agencies and other conservation organizations on many biodiversity conservation issues, setting priorities for funding, research, and conservation efforts and developing sound conservation policies, legislation, and practices.

The *International Union for the Conservation of Nature and Natural Resources* (IUCN) was founded in 1948 and is the world's oldest international environmental and conservation organization, which today includes 200+ government and 900+ nongovernment organizations as members. IUCN assists governments and other conservation organizations with national and international biodiversity policies and initiatives. The IUCN Global Species Programme, in conjunction with the IUCN Species Survival Commission (SSC), produces, maintains, and manages the IUCN Red List of Threatened Species. The IUCN SSC is a group of more than 10,000 volunteer experts that provide valuable information and advice on biodiversity to IUCN. Most experts belong to the more than 140 Specialist Groups, Red List Authorities, Task Forces, and Subcommittees. Although most Specialist Groups focus on particular taxa, some groups are more ecosystem focused, including the Cave Invertebrate Specialist Group. Information on the life history, ecology, status, trends, and threats of species provided by these experts is used in the IUCN Red List, which is a list that highlights species that are at greatest risk of extinction and greatest need of conservation. The IUCN Red List is widely recognized as the most comprehensive, objective global approach for evaluating the conservation status of flora and fauna (Lamoreaux et al. 2003). The IUCN Red List is used to help raise awareness about threatened species, set priorities for and guide conservation efforts and funding, and influence environmental policies and legislation (Rodrigues et al. 2006; Baillie et al. 2008; Cardoso et al. 2011a, b). A species may be classified on the IUCN Red List as critically endangered (CR), endangered (EN), or vulnerable (VU) on a global scale if it meets specific conditions under any one of these five criteria (IUCN 2001): (A) past, present, or projected reduction in population size

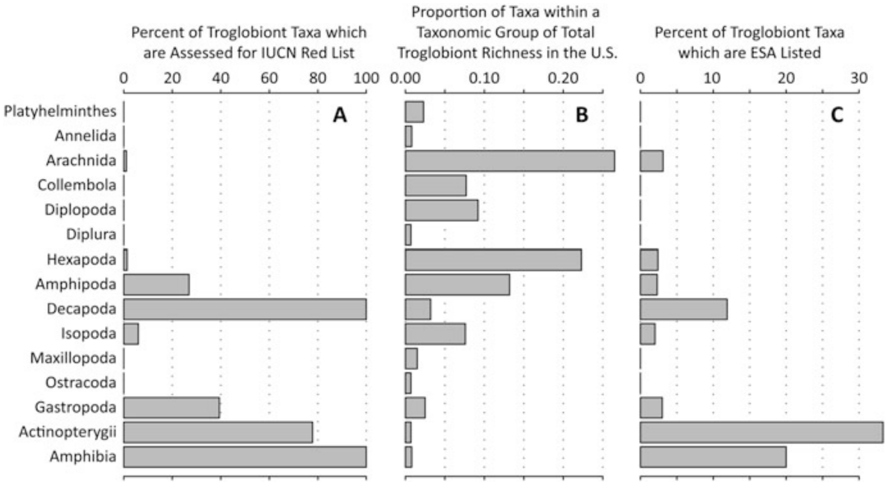

Fig. 22.2 Percentage of species assessed under IUCN Red List criteria (**a**), troglobiont diversity for various groups of invertebrate taxa (**b**), and percentages of troglobionts listed under the United States Endangered Species Act as endangered or threatened (**c**)

over three generations; (B) small geographic range in combination with fragmentation, population decline, or fluctuations; (C) small population size in combination with decline or fluctuations; (D) very small population or very restricted distribution; and (E) a quantitative analysis of extinction risk. Species should be assessed against all criteria, when possible, to confirm that the most accurate threat classification is obtained (IUCN 2001).

Although the IUCN Red List has been widely accepted and has many strengths (Rodrigues et al. 2006), several biases and limitations have been noted (Cardoso et al. 2011a). Taxa that have been evaluated are biased toward those occurring in terrestrial ecosystems and those which are vertebrates (IUCN 2010). Most mammals, birds, and amphibians have been evaluated, yet only 0.5% of described arthropods have been assessed (Cardoso et al. 2011a, b). Subterranean fauna of Europe (outside of Croatia and Slovenia), North America, Central America, and South America are poorly represented in the IUCN Red List, with only 10% of subterranean species having been evaluated. Of the subterranean species evaluated, taxonomic composition is biased toward fishes, salamanders, and decapods, where >75% of taxa within these groups have been evaluated (Fig. 22.2a). In contrast, just 14% of arachnids and hexapods, which collectively account for 48% of subterranean biodiversity overall in the United States, have been evaluated.

Cardoso et al. (2011a) cited two primary reasons for the taxonomic bias against invertebrates on the IUCN Red List. First, even if most necessary biological data could be gathered for Red List criteria, thresholds suggest that they were defined based on the requirements of large vertebrate species and, thus, may not be appropriate for most invertebrates, Second, current Red List criteria are difficult to apply to invertebrates because of four main shortfalls (Cardoso et al. 2011b): (1) most species are undescribed (the Linnean shortfall); (2) the full distributions of most species are unknown (the Wallacean shortfall); (3) the abundances of most species and how they change spatially and temporally are unknown (the Prestonian shortfall); and (4) the ecology of species and sensitivity to habitat changes are largely unknown (the Hutchinsonian shortfall). These shortfalls are the consequence of limited research on—and funding for—invertebrates, especially related to taxonomy and life history (Cardoso et al. 2011b). Consequently, it is difficult to gather the necessary information to classify most subterranean invertebrate species under the IUCN Red List criteria. These same shortfalls apply not only globally, but also to most subterranean species in most karst regions of Europe, North America, Central America, and South America. To address these issues, recommendations have been suggested to modify current IUCN Red List criteria to increase the feasibility and adequacy of the assessment process, in hopes of achieving greater representation of invertebrates on priority species lists (Martin et al. 2010; Cardoso et al. 2011a).

IUCN Red List Categories and Criteria also are applied at smaller spatial scales—including regions and countries—which are more practical for management and conservation planning. To date, Regional and National Red Lists have been developed in 26 regions, 113 countries, and 45 subnational entities. A database of these Regional and National Red Lists is maintained by the IUCN National Red List project (http://www.nationalredlist.org). As is the case for the global IUCN Red List, few subterranean species are included in Regional and National Red Lists. An exception is the subterranean fauna of Croatia, which includes over 450 cave obligate species from caves in the richest cave biodiversity region in the world, the Dinaric Karst (Culver and Sket 2000; Gottstein-Matocec 2002; Ozimec 2011). The Croatian Red Book of Cave Fauna is the first Red List assessment of troglobionts and stygobionts of its kind in the world, covering 186 taxa spanning 16 classes, 29 orders, and 54 families. Thirty-five percent (60 taxa) of the taxa included were assessed as Critically Endangered. Several specific threats to Croatian cave fauna have been identified, such as threats associated with urbanization and heavy tourism, groundwater pollution and exploitation, and illegal collection (Ozimec 2011).

Brazil has 158 described troglobionts distributed in 13 states, with the greatest diversity in Bahia (Serra do Ramalho karst area and São Desidério region—part of Bambuí group, Una-Irecê and Rio Pardo groups, Canudos supergroup, and sandstone Chapada Diamantina) and São Paulo (part of Açungui group) (Gallão and Bichuette 2018). Only 33 troglobionts were included in the Brazilian Red List of 2004 and another 30 species known as of 2003 were not evaluated. From 2004 to 2014, the number of troglobionts included in the Brazilian Red List increased to 83 species. Many of these species are listed as Endangered or Critically Endangered. Mining and hydroelectric projects are the main threats to subterranean biodiversity in Brazil, but other impacts, such as pollution of aquifers by pesticides and deforestation for agriculture and pastureland, also represent significant threats (Gallão and Bichuette 2018).

There is a pressing need to address higher levels of biodiversity, such as habitats, communities, and ecosystems (Nicholson et al. 2009; Rodriguez et al. 2011, 2012;

Keith et al. 2015). IUCN is currently developing categories and criteria for a Red List of Ecosystems (RLE) as a global standard for ecosystem risk assessment at multiple scales (Rodriguez et al. 2011, 2012; Keith et al. 2015). Much like the IUCN Red List of Threatened Species, RLE has eight categories of risk for each ecosystem based on five quantitative criteria designed to evaluate symptoms of risk in terrestrial, subterranean, freshwater, and marine ecosystems (Keith et al. 2013). The RLE will establish an objective, robust, and repeatable international standard for ecosystem risk assessment and losses of ecosystem functions and services that will allow for comparisons among regions and time periods. Subterranean ecosystems are being considered in the development of RLE criteria, which has a target date of 2025 to achieve global coverage of ecosystems.

NatureServe is a nonprofit organization that provides scientific expertise, resources, and data for conservation action. The NatureServe Network includes over 80 Natural Heritage programs and conservation data centers throughout the Western Hemisphere, including the United States, Canada, Mexico, Brazil, and several other countries and territories in the Caribbean, Central America, and South America. NatureServe offers several tools and services for conservation science and biodiversity assessment, which includes standards and methods for collecting, managing, mapping, and sharing biodiversity data and conducting assessments of species conservation status, ecosystem ecological integrity, and climate change vulnerability, among others. NatureServe maintains biodiversity data for over 77,000 species and 870 ecosystems in the United States and Canada, which can be accessed through a web-based product, NatureServe Explorer (http://explorer.natureserve.org). A similar product, NatureServe Infonatura (http://infonatura.natureserve.org), provides information for some 8700 species of birds, mammals, and amphibians in the Caribbean, Central America, and South America. NatureServe also partners with IUCN by providing data and coordination of their own assessments to IUCN Red List assessments, as well as working together on conservation assessments.

Of particular usefulness are standards and methods for conservation status rank assessment to determine relative extinction risk of a species or ecosystem (Faber-Langendoen et al. 2009; Master et al. 2009). NatureServe conservation status ranks are based on a one to five scale, from most to least at risk of extinction: G1 (Critically Imperiled), G2 (Imperiled), G3 (Vulnerable), G4 (Apparently Secure), and G5 (Secure). Two additional ranks associated with extinction exist: GH (Possibly Extinct) and GX (Presumed Extinct). Status ranks can be assessed at three geographic scales: global (G), national (N), and state (S). Conservation ranks are based on ten primary factors grouped into three main categories: rarity, trends, and threats (Master et al. 2009). Rarity factors include range extent of occurrence (EOO), area of occupancy (AOO), number of occurrences, number of occurrences with good viability or ecological integrity, population size, and environmental specificity. Trend factors include both short-term and long-term trends in population size, EOO, AOO, number of occurrences, and viability or ecological integrity of occurrences. Threat factors include threat impact and intrinsic vulnerability to threats.

The comprehensive and rigorous biodiversity information data maintained and the tools and services developed by NatureServe have made NatureServe Conservation Status Assessment, like the IUCN Red List, a key tool in conservation from regional to global scales in the Western Hemisphere. Approximately 12% of troglobiotic taxa in the United States and Canada remain to be evaluated, which is a large improvement over taxa that remain to be evaluated under IUCN Red List criteria. Seventy-four percent of taxa have been assessed at an elevated risk of extinction (G1 to G3). Two species, *Bactrurus cellulanus* and *Pseudanophthalmus krekeleri*, may have already been lost and are presumed extinct (GX) (Elliott 2000; Taylor and Niemiller 2016), while ten additional species may possibly be extinct (GH). This conservation tool, however, is also not without its shortcomings with respect to subterranean biodiversity. The conservation status of most subterranean fauna has not been reviewed in 10+ years, and much subterranean biodiversity remains to be assessed in many regions, including Mexico, the Caribbean, Central America, and South America. Only mammals, birds, and amphibians have been assessed to date in these regions. Moreover, biological surveys are likely needed for many taxa assessed as possibly (GH) or presumed extinct (GX) in the wild, as there is hope that some of these taxa have been lost already. For example, a recent study by Niemiller et al. (2017) rediscovered two species of cave carabid beetles, *Pseudanophthalmus insularis* and *P. paulus*, which were classified as Possibly Extinct (GH), as they had not been observed in 60 and 50 years, respectively.

22.4 National Legislation

Most countries in Europe, North America, Central America, and South America are member parties to the conventions mentioned at Sect. 22.2, although there are notable exceptions (Table 22.1). Relatively few countries have enacted national endangered species legislation, despite worldwide threats and decline in biodiversity. Even when enacted, few subterranean taxa are directly protected under endangered species legislation. Important legislation related to the conservation and protection of subterranean biodiversity and ecosystems in Europe, North America, Central America, and South America are listed in Table 22.1. Several of these new pieces of national legislation are related to specific provisions of international conventions listed above. Unfortunately, there is considerable variation among countries in the level of protection enacted for subterranean biodiversity and ecosystems. Numerous local and regional ordinances and regulations exist in several countries in Europe, North America, Central America, and South America that may also protect cave systems and cave and groundwater fauna (Juberthie 1995; Lamoreaux et al. 1997; Tercafs 2001; Huppert 2006). This includes the establishment of city parks or ordinances to protect groundwater from karst groundwater pollution.

22.4.1 European Legislation

European bats were protected under the Bonn Convention by the Agreement on the Conservation of Populations of European Bats (EUROBATS). However, with notable exceptions, troglobiotic invertebrates receive limited if any specific protection in most European countries. For example, no subterranean invertebrates are specifically protected under the Biodiversity Diversity Act in Bulgaria, while in Slovenia, which possesses one of the most diversity subterranean faunas in the world (Culver and Sket 2000; Sket and Zagmajster 2004), the subterranean environment in total, including biodiversity, is protected by the national Cave Protection Act and other legislation. Many stygobionts and troglobionts are also strictly protected under the Regulation on Protection of Wild Species in Croatia.

Although some cave systems and associated fauna are locally protected in their respective countries, in general no national conservation programs directed specifically for subterranean habitats and diversity have been established in most European countries. In contrast, subterranean waters—and by proxy their biodiversity—are universally protected, mainly for reasons associated with public health (e.g., Belgium). Many European cave systems are directly protected by national legislation and the establishment of protected areas such as national parks, nature reserves, and Natura 2000 sites (reviewed in Juberthie 1995). In Belgium, several nature reserves have been established by the Royal Commission of Monuments and Sites to protect karst environments, including caves, and in Greece, caves are considered natural and cultural heritage monuments and therefore protected. Similar cave protection legislation exists in other European countries such as Hungary and Italy.

The *Council of Europe's Convention on the Conservation of European Wildlife and Natural Habitats* of 1979, also known as the Bern Convention, was the first international treaty to protect species and habitats in Europe. To date, 50 European countries, as well as some African countries, and the European Union have acceded to the convention, which came into force in 1982. The Convention aims to ensure conservation and protection of species and habitats by imposing restrictions on the take and trade of protected flora and fauna and constitutes a commitment to protect habitats of imperiled species. The Convention imposes legal obligations of contracting parties for the protection of over 500 plants and over 1000 animal species and their habitats, listed in Appendices I, II, and III. The only troglobiont listed on the Bern Convention is *Proteus anguinus* (Appendix II); however, some non-troglobiotic vertebrates that rely on caves are listed, such as *Atylodes genei*, *Speleomantes* spp., and most species of European cave-dwelling bats.

The Convention on the Conservation of Migratory Species of Wild Animals, also called the Bonn Convention or CMS, aims to conserve species that regularly move across national borders. The Bonn Convention was adopted in 1972 and came into force in 1985. Some 122 parties, including the European Union, work cooperatively to conserve endangered migratory species and their habitats. The Agreement on the Conservation of Populations of European Bats (EUROBATS) under the Bonn Convention came into force in 1994. This agreement aims to conserve all 53 European bat species through legislation, education, and other conservation actions with international cooperation of the 36 parties of EUROBATS.

The *European Union Habitats Directive* (EUHD) is a primary piece of legislation adopted in 1992 to protect habitats and species in freshwater, terrestrial, and marine habitats in Europe as a European Union response to the Bern Convention. Many provisions of the Ramsar Convention and Bonn Convention also have been incorporated in the EUHD. The EUHD contains a series of annexes that identify species and habitats of conservation concern in the 27 Member States of the European Union. In addition, the EUHD provides for the creation of a network of Special Areas of Conservation (SACs), called the Natura 2000 Network. The Natura 2000 Network also includes Species Protected Areas designated under the Birds Directive. Over 26,000 Natura 2000 sites have been designated across Europe to date, including SACs designated for subterranean biodiversity. For example, 55 localities for the olm (*Proteus anguinus*) have been protected within 26 SACs in the Slovenian Natura 2000 Network (Hudoklin 2011).

The EUHD aims to protect some 230 habitats and over 1000 species listed in the Directive's five annexes. Annex I contains a list of priority habitat types. In relation to subterranean biodiversity and ecosystems, the following habitat types are listed: caves not open to the public (Natura 2000 code 8310), fields of lava and natural excavations (Natura 2000 code 8320), and submerged or partially submerged sea caves (Natura 2000 code 8330). Annex II is a list of species whose core areas of habitats are designated as Sites of Community Importance (SCIs) that ultimately must be protected under the Natura 2000 Network as SACs. A strict protection protocol must be implemented for species listed in Annex IV across their entire distribution both within and outside of Natura 2000 sites. Annex V includes a list of species whose exploitation and taking in the wild may be subject to management actions. Some species appear on multiple annex lists.

Very few troglobionts and stygobionts are included in Annexes II, IV, or V but include the salamander *Proteus anguinus* (II and IV), the beetle *Leptodirus hochenwartii* (II and IV), and the bivalve *Congeria kusceri* (II and IV). All European bats species as well as six species of the salamander genus *Speleomantes* are listed on Annex II and/or IV. Several biases and criticisms have been noted in the list of species protected under the EUHD (Cardoso 2012). As in many aspects of conservation (Clark and May 2002), vertebrates dominate over invertebrate taxa on the list. Even among the list of arthropod taxa that are included in Annexes II, IV, and V, 94% of taxa belong to the more "charismatic" insect orders Lepidoptera, Coleoptera, Odonata, and Orthoptera (Cardoso 2012). Cardoso (2012) also noted other bias in the listing of arthropod species related to taxonomy, geography, range size, body size, and aesthetic value. These biases in the species lists have significant legal and financial implications. Species that are not listed in the Annexes cannot be used as justification for support for LIFE Nature sub-program conservation projects (Cardoso 2012). Consequently, non-listed species are neglected in conservation policy and funding.

The *EU Groundwater Directive* of 2006 (GWD) establishes a regime that sets groundwater quality standards and introduces measures to prevent or limit inputs of pollutants into groundwater, which complement the EU Water Framework Directive. The GWD emphasizes the importance of protective measures for groundwater ecosystems by proposing increased monitoring and research to provide better standards for groundwater quality (Griebler et al. 2010; Stein et al. 2012). This directive has led to new research on ascertaining and evaluating biological criteria as indicators for groundwater quality assessment (Griebler et al. 2010; Korbel and Hose 2011; Stein et al. 2010, 2012).

22.4.2 North, Central, and South American Legislation

In North and Central America, the primary legislation for species protection is the Endangered Species Act of 1973 (ESA) in the United States and similar legislation, the Species at Risk Act of 2002 (SARA), in Canada. In contrast to the European Union Habitats Directive (see above), which focuses more on habitats, species are the primary focus of the ESA and SARA. Similar endangered species legislation has been passed in Mexico (General Wildlife Act of 2000), Costa Rica (Biodiversity Law of 1998), and other countries (Table 22.1). In Bermuda, 24 species of cave invertebrates are protected under the Protected Species Act (2003). As in Europe, several additional pieces of legislation afford at least indirect protection of cave and karst landscapes and, in turn, subterranean biodiversity in North America, the Caribbean, and Central America (reviewed in Kueny and Day 2002). However, little legislation directly targets the conservation and protection of caves. In Central America, 18% of karst is afforded some protection, with the highest level of karst protection in Belize (Kueny and Day 2002).

Brazil is the only country with a government agency devoted specifically to the study, protection, and management of caves, the National Cave Research and Conservation Center (CECAV), created in 1997. However, no current legislation specifically protects caves and karst areas in Brazil. In 1996, a Decree (99556) was published that effectively offered some protection to Brazilian caves by stating that the “the use of caves is restricted to speleological activities, tourism, education, scientific, and preserving its physical integrity.” In the past decade, however, changes to existing laws, due to pressure from the mineral and other sectors, have significantly weakened protections. Decree 99556 was replaced by Decree 6640 in 2008, which requires that caves be classified according to criteria proposed in the Instruction Normative no. 2 of 2009, and in its revised form of 2017, as low, medium, high, and maximum relevance. Caves of maximum relevance are completely protected, but caves of lesser relevance are subject to lesser protection and can possibly be destroyed. However, other existing legislation can sometimes be applied to protect caves and cave biodiversity, such as laws related to groundwater aquifer and fauna protection.

The *Endangered Species Act* (ESA) of 1973 is the foremost tool for the protection of biodiversity in the United States. The primary purpose of the ESA is to protect and recover imperiled species and their associated habitats and ecosystems. Under the ESA, species may be listed as either endangered or threatened. “Endangered” status is warranted when a species is at high risk of extinction throughout all or a significant portion of its range, whereas "Threatened" status is warranted when a species is likely to become endangered within the foreseeable future. The U.S. Fish and Wildlife Service (USFWS) oversees the listing and protection of all terrestrial animals and plants and freshwater animals, while the National Marine Fisheries Service oversees marine animals. The ESA prohibits "take" of listed species as well as interstate and international trade. Take includes such actions as harassing, harming, hunting, trapping, capturing, trapping, killing, or collecting. Protections also include prohibition of acts that result in significant habitat modification or degradation that may result in the death or injury of wildlife. The ESA also requires federal agencies to use their legal authorities to conserve listed species and consult with USFWS to ensure that any actions authorized, funded, or implemented by a federal agency do not jeopardize the continued existence of a listed species. USFWS is required to develop recovery plans for each listed species unless it is determined that such a plan will not promote the conservation of a species. Recovery plans serve as essential guides in the management and recovery of listed species. The ESA also requires the designation of critical habitat of some listed species. Critical habitat is defined as specific geographic areas that contain features critical to the conservation of a listed species that may require special management and protection. The designation of critical habitat provides protections of listed species by prohibiting federal agencies (but not private landowners) from activities that adversely modify designated areas.

Of the 1200+ troglobionts in the United States, only 35 species are listed as federally endangered or threatened under the ESA, including seven species of beetles, seven spiders, three harvestmen, one pseudoscorpion, two crayfish, three shrimps, four amphipods, two isopods, one snail, three fish, and two salamanders. Six additional taxa are Candidate species, which are taxa under consideration for listing under the ESA. Candidate species are not afforded protection under the ESA. Seven bats that regularly use caves, either as summer roosts or winter hibernacula, are listed under the ESA. In addition to species native to the United States, the ESA offers protections for foreign species, which includes bans on the importation and sale in the United States of foreign species listed. The only foreign troglobiont listed on the U.S. ESA is the Mexican Blindcat (*Prietella phreatophila*), a catfish from Mexico.

The small percentage of troglobionts listed or considered for listing under the ESA is somewhat surprising, given that most troglobionts have restricted distributions or are known from just a few occurrence records (Culver et al. 2000; Elliott 2007; Niemiller and Zigler 2013). Rarity, as well as presumed low reproductive rates, poor dispersal ability, and susceptibility to environmental change, renders these species extremely vulnerable to anthropogenic threats (Culver and Pipan 2009). There are marked biases in diversity and geography of listed species (Fig. 22.2). Five of the 19 vertebrate troglobionts (26.3%) are listed, despite comprising just 1.4% of all troglobionts in the United States. In contrast, just 2.2% of invertebrate troglobionts, which comprise 98.6% of troglobionts in the United States, are listed. In fact, the only invertebrate group with >5% of species listed are decapods (crayfish and shrimp) at 11.6% (Fig. 22.2b). Some critics have argued that the ESA and USFWS are biased against insects and other invertebrates in the listing of species (Greenwald et al. 2005) and in the amount of money spent on conservation when compared to listings and expenditures for vertebrate species (Bossart and Carlton 2002; Male and Bean 2005). It has also been suggested that the paucity of invertebrates listed under the ESA may be attributed more to a lack of scientific data and lack of qualified biologists to file and review listing petitions (Lugo 2007). This may also hold true for some groups of invertebrate troglobionts for which few experts exist.

Geographically, 57% (20 of the 35) of the troglobionts listed under the ESA occur in central Texas karst, including the Edwards Aquifer. The high number of listed species from this karst region does not reflect greater diversity and endemism relative to other karst regions in the United States. Rather, it reflects greater vulnerability and risk of extinction from higher levels of development and demands for groundwater associated with continued growth of the cities of San Antonio, Austin, and areas in between relative to other karst regions (Culver and Pipan 2009).

Other U.S. federal laws have been passed that provide varying degrees of protection to caves and karst on federal and private lands, and—directly or indirectly—subterranean biodiversity. This legislation has been reviewed in depth by Huppert (1995, 2006), Lera (2002), Seiser (2013), and others. Caves designated as "significant caves" that occur on federally owned lands in the United States are protected by the Federal Cave Resources Protection Act of 1988. This act requires federal land managers to account for all cave resources under their jurisdiction (Huppert 2006). However, only significant caves under the Department of Agriculture and Department of the Interior are covered by the act. Federal lands under the jurisdiction of the Department of Defense and other extensive tracts of land are not included under provisions of this act (Huppert 1995, 2006). In addition, there is considerable debate regarding the definition of a "significant cave" and levels of protection for caves not deemed significant. The U.S. National Park Service Act of 1916 protects caves that occur on National Park Service lands, including prominent national parks where the protection of specific caves is the primary focus, such as Mammoth Cave National Park in Kentucky, Carlsbad Caverns National Park in New Mexico, and Wind Cave National Park in South Dakota. This act also protects caves that occur in national parks and other lands managed by the U.S. National Park Service (national monuments, national scenic rivers, etc.).

The Wilderness Act of 1964 established a means to designate wilderness areas where activities such as logging, mining, and even road development are prohibited and created the National Wilderness Preservation System. This system now protects some 110 million acres of wilderness areas in the United States. Caves within the boundaries of these designated wilderness areas are protected. However, this legislation has largely focused on the surface environment and does not specifically define which ecosystems are and are not to be considered (Seiser 2013). Consequently, there have been efforts to designate select cave systems as Cave Wilderness. Unfortunately, despite four attempts since 1967 to create a Cave Wilderness designation, including two by the Cave Research Foundation, one by the National

Speleological Society, and by a unit within the National Park Service, no Wilderness designation has been made to date to protect and preserve a specific cave or area within a karst region in the United States (Seiser 2013).

Some caves that occur along rivers designated as Scenic Rivers are protected by the Wild and Scenic Rivers Act of 1968. Notable Scenic Rivers important for cave conservation include the Buffalo National River in Arkansas and the Ozarks National Science River in Missouri (Huppert 2006). A bill, the Oregon Caves Revitalization Act, was introduced in the United States Senate in 2013, which included a Scenic River designation for the subterranean section of Cave Creek, also known as River Styx. Unfortunately, this bill passed the Senate but was never passed by the House of Representatives. If passed, this legislation would have represented the first time that the Wild and Scenic Rivers Act was specifically applied to a subterranean stream or river (Seiser 2013).

Several other federal acts exist that may have a bearing on the protection of cave resources in the United States, including biodiversity (reviewed in Huppert 2006). These include the Archaeological Resources Protection Act of 1979, Antiquities Act of 1906, Eastern Wilderness Act of 1975, Endangered American Wilderness Act of 1978, Historical Sites Act of 1935, National Environmental Policy Act of 1969, National Forest Management Act of 1976, National Historic Preservation Acts of 1966 and 1976, National Parks and Recreation Act of 1978, National Wildlife Refuge System Administration Act of 1966, and several others. Moreover, several "clean water acts" may be used to help protect karst aquifers, and, in turn, groundwater fauna (Jones et al. 2003), such as the Resource Conservation Recovery Act, the Comprehensive Environmental Resource Compensation Liability Act, and the Safe Drinking Water Act.

In the United States, cave-dwelling species may be afforded protection under state endangered species acts. For example, some species are protected under the Virginia Endangered Species Act of 1972 and the Virginia Endangered Plant and Insect Species Act of 1979. Species that are listed under the federal U.S. Endangered Species Act of 1973 are automatically listed under state law in Virginia. Both state and federal agencies have regulatory authority, while the Virginia Natural Heritage Program in the Virginia Department of Conservation and Recreation provides technical expertise to aid in the recovery and long-term protection of a species culminating in its removal from the list of protected species (Orndorff 2005). Although scope, details, enforcement, and punishments vary by state, in general it is unlawful to kill, harm, collect, possess, or traffic imperiled species listed as protected in their respective states. Most of the existing state endangered acts simply provide a measure for listing and prohibition of taking and trafficking of listed species, but lack mechanisms for the recovery, consultation, or critical habitat designation (George and Snape 2010). Just six US states have a provision requiring critical habitat designation, and only five states require recovery plans for listed species. Penalties for violation of state endangered species acts range from a misdemeanor with fines up to $1000 and/or 90 days imprisonment to up to $10,000, 180 days imprisonment, and possible seizure and forfeiture of property in some states.

Twenty-eight US states have laws aimed at the protection of caves and their resources, with most passed since 1976 (Huppert 1995, 2006; LaMoreaux et al. 1997; Lera 2002). Puerto Rico and the Cherokee Nation also have similar legislation. Other US states, in addition to the 28 states that have cave protection legislation, have passed resource protection legislation that mentions cave resources. These laws vary in their definition of a cave, application, and effectiveness in cave protection. Many state cave protection laws only apply to caves on state lands, while those that also cover caves occurring on private lands usually require landowner consent to seek prosecution (Huppert 1995, 2006). Caves fall under the definition of wilderness in some states and may be afforded varying levels of protection under state wilderness acts. Caves may be protected in some states if they occur on specific state-owned or managed lands, such as state parks, wildlife management, or natural areas. In addition, several states have passed legislation prohibiting the contamination and pollution of surface waters and groundwater. Similar legislation has been passed in some Canadian provinces to protect caves and cave resources, such as the Cave Protection Act in British Columbia.

The Nature Conservancy (TNC) is a US-based nonprofit organization dedicated to the conservation of lands and waters of ecological importance. TNC is the largest environmental nonprofit organization in terms of assets and revenue in the Western Hemisphere. TNC has helped protect almost 48 ha of land and 8000 km of rivers in all 50 US states and more than 35 countries worldwide. TNC owns or manages several properties with significant cave and karst resources, including some where the primary features are cave resources. An example is Hubbard's Cave in Tennessee, which contains one of the largest hibernacula of the federally endangered Gray Bat (*Myotis grisescens*). TNC also works with state, federal, and other organizations and researchers to monitor bat populations, to document subterranean biodiversity, and to develop strategies to protect endangered species and subterranean ecosystems. For example, TNC staff and Chiquibul National Park officials in Belize developed a 5-year management plan for the Chiquibul Cave system, the largest cave in Belize and longest in all of Central America. TNC in Tennessee constructed an artificial cave to assist in bat and White-Nose Syndrome research.

22.4.3 Regional and Local Conservation Organizations

There are organizations that work at national and regional scales to assist government agencies and other conservation organizations on many biodiversity conservation issues, setting priorities for funding, research, and conservation efforts and developing sound conservation policies, legislation, and practices. The *National Speleological Society* (NSS) is the world's largest organization dedicated to the exploration, study, and conservation of caves and their environments, with over 10,000 members and 250 grottos. NSS and affiliated cave conservancies own or manage over 100 caves in the United States, which includes several biologically significant caves. NSS has also been actively involved in response to White-Nose

Syndrome since its discovery in New York in 2007, including raising grant money to support WNS research (e.g., NSS White-Nose Syndrome Rapid Response Fund), participating and organizing WNS conferences and symposia, and participating and planning in WNS-related research and state and federal planning. The *Brazilian Speleological Society* (SBE—http://www.cavernas.org.br), founded in 1969, has ca. 1800 members, including speleological organizations. SBE and other independent speleological organizations in Brazil (e.g., Grupo Bambuí de Pesquisas Espeleológicas/GBPE and Grupo Pierre Martin de Pesquisas Espeleológicas/GPME) conduct projects related to cave discovery and documentation, as well as cave conservation. SBE maintains a catalog of more than 10,000 caves in Brazil. Recent conservation-based projects involving SBE include the creation of Conservation Units, such as Natural Heritages and Parks to protect caves and cave fauna, such as the karst of Mambaí, central Brazil). Another Brazilian organization is the *Boticario Foundation of Nature Protection* (FBPN), which provides grants for conservation projects to project many environments and biodiversity, including projects related to caves and karst.

22.5 Conclusions

A variety of legislation has been proposed and enacted worldwide that may directly (e.g., endangered species acts) or indirectly (e.g., water quality legislation) provide some protection for subterranean fauna. Conservation efforts have largely focused on protecting sites of exceptional species richness or phylogenetically unique biodiversity (Culver and Sket 2000; Danielopol et al. 2009; Gibert et al. 2009). Levels of protection for subterranean life offered by international and national and regional legislation vary greatly throughout Europe and the Americas. At the international level, subterranean fauna appearing on lists and in appendices as protected species, including CITES, IUCN Red List, Bern Convention, and EU Habitats Directive, are largely biases toward vertebrates, particularly bats. This is despite the fact that the overwhelming biodiversity of subterranean ecosystems comprises invertebrates. The importance of and threats to subterranean invertebrate fauna in Europe have been known for some time (Juberthie 1995), specifically recognized in Recommendation no. 36 of the Bern Convention (1992) in which it is recommended that national inventories of subterranean invertebrates and subterranean habitats to protect be compiled and that species of conservation concern be identified. Unfortunately, this recommendation remains to be completed (Haslett 2007). This marked taxonomic bias is also observed in national and regional legislation, such as the list of species protected under the United States Endangered Species Act. The taxonomic biases favoring conservation and protection of vertebrates over invertebrates are not limited just to subterranean ecosystems (Cardoso et al. 2011a, b). The four main shortfalls in invertebrate conservation identified by Cardoso et al. (2011b), as well as other impediments, apply to the conservation of subterranean biodiversity and ecosystems. In particular, the Linnean shortfall (i.e., much subterranean biodiversity remains to be described) is the primary driver behind the other three shortfalls: the Wallacean, Prestonian, and Hutchinsonian shortfalls in the conservation of subterranean invertebrate faunas. In some regions, these shortfalls also apply to vertebrate subterranean biodiversity. Gallão and Bichuette (2012) emphasized the importance of the IUCN Red List for protection of cavefishes in Brazil and highlighted limitations of some criteria in the inclusion of taxa on the IUCN Red List. These shortfalls also can have significant political implications, as the presence of IUCN Red Listed species is one of the most important tools to prioritize caves based on biological criteria for protection in Brazil, for example.

A major factor behind taxonomic bias in subterranean biodiversity conservation is the lack of biologists qualified to study subterranean taxa and ecosystems. This is particularly evident in the continuing decrease in taxonomic experts that can describe and properly identify often difficult groups, such as springtails, flatworms, and annelids. Correct species identification, generally requiring a high degree of familiarity with taxon-specific morphological characters, is paramount to addressing the other major shortfalls in subterranean biodiversity conservation, including elucidating accurate species distributions and habitats, determining abundance and population size, and predicting species responses to habitat change. Some progress has been made in recent years to address the Wallacean shortfall in subterranean conservation of Europe and the Americas, highlighted by the PASCALIS project (Gibert 2005). The PASCALIS (Protocols for the Assessment and Conservation of Aquatic Life in the Subsurface) project was a 3-year (2002–2004) large-scale groundwater bioinventory initiative that developed standard sampling protocols for comparing stygobiont biodiversity in six European regions in five countries: Belgium, France, Italy, Slovenia, and Spain. Unfortunately, limited prospects for securing employment for newly trained taxonomic experts, reflecting a lack of value society places on invertebrate taxonomy, continues to discourage young scientists from placing emphasis on taxonomy during their graduate training, especially in the United States.

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Chapter 23
Conservation and Impact Assessment of Subterranean Fauna in Australia

Stuart Halse

23.1 Introduction

This chapter examines some of the difficulties encountered when trying to protect subterranean species occurring in the broad landscape, rather than in caves, in Western Australia. We refer to these species as *landscape subterranean fauna*. A particular focus of the chapter is the protection of landscape subterranean fauna in areas outside nature reserves in situations where land development is likely (e.g. Henle et al. 2008), particularly as part of the environmental impact assessment process. Assessments usually deal with mining and water supply developments (e.g. Sheppard et al. 2009; Mudd 2010).

Box 23.1
Subterranean fauna can be protected by legislation in two ways. Often the focus of the legislation is maintaining their subterranean habitat, as happens for many subterranean species occurring in caves that have high aesthetic and tourism values and a relatively easily defined spatial extent. In other cases, the legislation is directly concerned with protecting the subterranean species themselves. This usually happens for species with strong public appeal, such as cave nesting birds and bats (although, ironically, almost all high profile subterranean species occur in caves). Most Australian subterranean fauna species occur outside caves in the groundwater aquifers or in the vadose zone deep below the ground surface. While standard biodiversity legislation has a theoretical capacity to protect subterranean species in these habitats, their

(continued)

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Box 23.1 (continued)
unique ecology and the complexities associated with protecting their habitat present significant logistical challenges for both species and habitat-based protection policies.

The classical method of protecting surface fauna, at least in Australia, is to create nature reserves where populations of all (or most) species can be conserved (e.g. Margules and Pressey 2000; Scott et al. 2001). This can work well for cave fauna, although in most cases the motivation for creating reserves has been a strong public desire to protect the cave itself, rather than its subterranean fauna (Bryan 2002). It is frequently only when protected caves are used for tourism (as commonly happens; see Chap. 22) that there is focus on the protection of the subterranean fauna within the cave system as visitor use threatens to alter cave conditions (Russell and MacLean 2008; Pellegrini and Ferreira 2012). Most of the few exceptions to cave reservation being based on the aesthetic values of caves are those caves supporting large colonies of bats or birds (Hall 1990).

Achieving protection of landscape subterranean fauna, whether by reservation or by constraining threatening or inappropriate land use, is more challenging than protecting cave fauna. There has been no reservation of land in Western Australia for the explicit protection of landscape subterranean fauna, despite there probably being more focus on landscape subterranean fauna in Western Australia than anywhere else in the world (see Chap. 20 and references therein). However, landscape subterranean fauna has received a unique level of attention in the environmental impact assessment process in Western Australia since the mid-1990s.

The original reasons for subterranean fauna being included in environmental impact assessments in Western Australia are threefold. First, considerable work had been done showing the occurrence of rich stygofauna communities in groundwater aquifers of north-western Australia (e.g. Humphreys 1993, 1999; Pesce and de Laurentiis 1996). Second, several submissions lodged during the public comment period of several development proposals, both for mining and water supply in north-western Australia, suggesting there might be significant impacts on stygofauna, caused re-scoping of the assessments (e.g. EPA 1996, 1998). Third, and related to the second point, the object of *Environment Protection Act 1986* is “to protect the environment of [Western Australia], having regard to [five] principles”, one of which is that “Conservation of biological diversity and ecological integrity should be a fundamental consideration”, and accordingly the Environmental Protection Authority has recognized that subterranean fauna constitutes a part of Western Australia’s biodiversity to be included in their assessment of projects. Ultimately, however, all environmental assessment decisions are based on a consideration of social and economic issues as well as environmental ones. As such, there have been cases where the recommendations of the Environmental Protection Authority against approval of a project because of threats to subterranean fauna have been overturned by the Minister for the Environment for social or economic reasons, but with conditions set to minimize environmental harm (EPA 2006, 2007, 2016a).

While the focus of actions to protect landscape subterranean fauna species has been based on the Environment Protection Act, more specific biodiversity legislation provides broad support for the actions of the Environmental Protection Authority. The *Wildlife Conservation Act 1950*, section 14, states "Except to the extent which the Minister declares by notice . . . all fauna is wholly protected throughout the whole of the State at all times". This section is usually interpreted to mean that actions (including removal of habitat by proposed development projects) that would reasonably be considered likely to result in the extinction of a species cannot be legally approved. The critical issue is that the action being considered must threaten the persistence of the whole species. Protection is stronger for the 258 species that are listed as endangered or vulnerable under the Wildlife Conservation Act. For these species, both individual animals and recognized habitats of the species receive protection. Forty-one of these listed species are subterranean, with three of them being fish and 38 being invertebrates (Government of Western Australia 2017).

Currently, there is a transfer occurring from the Wildlife Conservation Act to the *Biodiversity Conservation Act 2016*. The objects of the new Act are "(a) to conserve and protect biodiversity and biodiversity components in [Western Australia]; and (b) to promote the ecologically sustainable use of biodiversity components in the State. In the pursuit of the objects of this Act, regard must be had to the principles of ecologically sustainable development . . .".

23.2 Complexities of Subterranean Fauna Assessment

Box 23.2

The key issue in relation to environmental impact assessments is that both stygofauna and, more particularly, troglofauna species may have ranges that are smaller than the footprints of proposed developments. Furthermore, there is usually a high level of uncertainty associated with the estimated ranges of species and a bias towards underestimation, so that many apparently restricted species probably occur beyond the project footprint. Given that the economic and social costs of refusing development approval may be very high, decision-making is complex. The difficulties of accurately estimating species ranges are unsurprising, given that landscape subterranean fauna began to be studied in Western Australia only about 20 years ago and little is understood about the ecology of these species.

While agricultural operations in Australia (horticulture, broad-acre cropping, animal feedlots and, probably to a lesser extent, grazing) are likely to have cumulative impacts on the habitat of many subterranean fauna species (Korbel and Hose 2011), in most of these situations the species would be expected to persist at reduced densities rather than being sent extinct (Di Lorenzo and Galassi 2013; Di Lorenzo et al. 2014). The more threatening impacts come from excavation of open-cut mine pits and large-scale water abstraction, especially the dewatering of mines to allow safe mining below the water table, abstraction to provide large volumes of water for ore processing and abstraction for irrigation and urban water supply. Some mine pits extend more or less continuously for many tens of kilometres (EPA 2014, 2016b), some have depths below the ground surface (and water table) of several hundreds of metres (e.g. EPA 2002), and many borefields that dewater mine pits or supply mine process water result in groundwater drawdown over distances of many tens of kilometres (EPA 2014, 2016b, c). In contrast, many troglofauna species have linear ranges of only a few kilometres at most (Halse and Pearson 2014; see also Chap. 20). While stygofauna species usually have larger ranges than troglofauna in the Pilbara, sampling suggests that approximately 10% of species have linear ranges smaller than 25 km (Halse et al. 2014), and some species in catchment headwaters appear to have linear ranges of less than 5 km. Many stygofauna species in calcretes of the Yilgarn region, in inland central and southern Western Australia, appear to have even smaller ranges (Karanovic and Cooper 2011, 2012; Karanovic et al. 2015), as probably do troglofauna species. Thus, the entire distributions of some troglofauna and stygofauna species are likely to lie within large mine pits or areas where the water table has been drawn down significantly by groundwater abstraction. However, defining the ranges of individual species and determining which species are actually confined to these impact areas is difficult.

The main challenges to defining species' ranges are the inefficiency of subterranean fauna sampling, lack of information about the microhabitats used by species and the very limited information about the pattern of occurrence of these microhabitats in the landscape, especially outside proposed areas of impact (where there has usually been little geological work as well as little subterranean fauna sampling). A more simplistic way of looking at the problem is that most of the difficulty in defining ranges results from the species living underground and sampling being a blind, below-ground process. A useful analogy is to think of subterranean fauna as occurring in a forest below ground, where soil faunas inhabit the understorey (near the surface) and stygofauna and troglofauna occur in the canopy at depth (see Hose et al. 2017). These canopy species are difficult to observe and are underrepresented in the data collected. Furthermore, there is spatial variation in the structure of this forest that often results in the occurrence of different stygofauna and troglofauna species in adjacent areas. Currently, sampling is usually undertaken without any understanding of variation in the forest structure or the species preferences for particular forest types. Sampling is done blindly through bores or drill holes that run from the forest floor (close to the surface) to outer sections of canopy (at deep depths). A further complication is that in many geological formations the subterranean forest is a patchy one and many drill holes intersect clearings in the forest rather than the subterranean voids or spaces constituting the subterranean canopy in which stygofauna and troglofauna occur (Fig. 23.1).

Fig. 23.1 Drill hole being sampled for troglofauna by scraping. Hole has short PVC collar to prevent collapse at the surface and a metal "sleeve" has been inserted in the collar to reduce friction when retrieving the sample. Deeper sections of the hole are uncased

However, while the technical challenges involved in undertaking impact assessment of subterranean fauna are considerable, perhaps the biggest challenge to the continued inclusion of subterranean fauna in environmental impact assessment is the low level of empathy for this group of animals amongst policymakers, the public at large and many zoologists working on surface fauna. This is principally related to a low level of knowledge, but, nevertheless, it should be recognized that there is no impetus to protect microscopic species that have poorly documented, and sometimes not obvious, ecological roles.

23.2.1 Sampling Framework

Effective impact assessment requires a well-defined framework. The Environmental Protection Authority in Western Australia has issued a series of guidelines for subterranean fauna assessment since 2003, with the most recent describing the principles of assessment (EPA 2016d). Elsewhere in Australia, the Queensland Government released a subterranean fauna assessment guideline in 2014 (DSITI 2015), while South Australia released a discussion document in 2015 (Goonan et al.

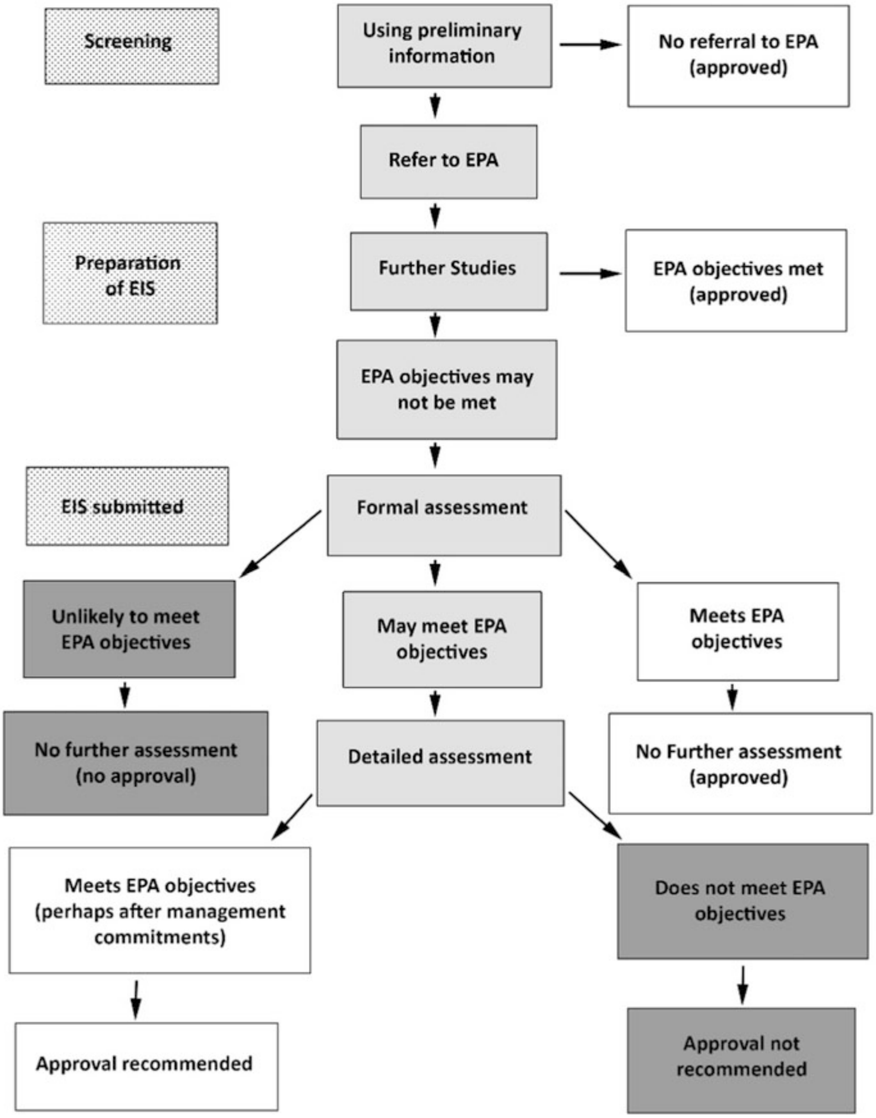

Fig. 23.2 Framework for environmental impact assessment in Western Australia, based on EPA (2015). Subterranean fauna is a factor that may lead to recommendation that a project should not receive environmental approval. EPA, Environmental Protection Authority

2015). Subterranean fauna has been identified as an issue requiring assessment in many development projects in New South Wales, but the framework for assessment is less formal than in states such as Western Australia and Queensland.

The main elements of the Western Australian framework for assessment are shown in Fig. 23.2. It consists of an initial desktop investigation to determine whether subterranean fauna is likely to occur in the vicinity of the project and whether the project may potentially have a deleterious impact on any subterranean fauna present. This may be followed by limited fieldwork to confirm the conclusions of the desktop investigation or more intensive field study aimed at identifying the species in the impact area(s) of the project and whether or not they are likely to be restricted to the impact area(s). This stage may also include geological habitat mapping to help define the likely ranges of species and the finer scale distributions of species within the project impact area(s). Based on the lateral and vertical distribution of species in relation to project impacts, the likely effect of the project on subterranean fauna conservation values can be determined. If persistence of some species may be threatened, the potential effectiveness of management measures to mitigate impacts is investigated before a final assessment is made of threat to subterranean fauna. Some of the details of the assessment approach have been criticized (e.g. Karanovic et al. 2013) or supported (e.g. Harvey et al. 2011) elsewhere, and further discussion of technical aspects of the framework is provided below.

23.2.2 Sampling Efficiency

As described in Chap. 20, sampling subterranean fauna in the broad landscape occurs via various types of drill holes. While this sampling is often considered to be inefficient because relatively few animals are collected and repeatability is quite low, there is in fact relatively little information about the relationship between capture rates and how completely the species present in the holes and surrounding matrix are documented.

Repeated sampling of stygofauna at selected bores in the Pilbara region in the north-west of Western Australia has shown that the first sample from a bore captures 46% of species occurring in high abundance and 23% of species found in low abundance (Eberhard et al. 2009). Based on species richness estimates generated by Chao 2 or ICE, six samples collected over three to four years captured more than 80% of all species present at the bore holes sampled and more than 90% of the abundant species. These results suggest that, contrary to general perceptions, it is possible to document most of the stygofauna species at a site through conventional sampling techniques. It is also realistic to expect to show through sampling that all (or nearly all) species known from the impact area of a project occur outside this area, provided of course that they actually do so. However, the level of sampling effort required (at least six sampling events) represents a cost and period of time that is frequently seen as unaffordable in impact assessments, especially if the project is in a remote area.

There is no reliable information on the number of samples required to collect most of the troglofauna species occurring in a drill hole or its immediate vicinity. While assessment results suggest it is relatively straightforward to show by sampling that the abundant species known from the impact area of a project also occur outside

Table 23.1 Numbers of troglofauna species collected from 150 drill holes sampled three times in relation to maximum capture abundance of the species in any sampling event and how often the species was collected at each bore (SA Halse, unpublished data)

Maximum abundance	No. of times the species collected from a bore			Probability of being collected twice (or more) (%)
	1	2	3	
≥3 animals	12	15	4	61
2 animals	15	3	0	17
1 animal	76	5	0	6

it, the effort required to show wider occurrence of low abundance species will be considerable. For example, preliminary results from 150 drill holes in the Pilbara sampled three times each show that while abundant species (defined as those for which at least three animals were collected in one of the sampling events) had 61% probability of being collected at least twice, the species always collected as single animals had only 6% probability of being collected twice (Table 23.1). A further challenge is that in most survey programmes many of the holes sampled for troglofauna yield no animals, even if repeatedly sampled in areas that generally yield troglofauna. The most likely reason for this is that the holes do not intersect the vugs and other subterranean spaces that house animals. This is a consequence of the relatively small volume of troglofauna habitat within even troglofauna-rich landscapes (Fig. 23.3).

Karanovic et al.'s (2013) contention that the levels of sampling recommended by the Environmental Protection Authority (40 stygofauna and 60 troglofauna samples from impact areas) are too low, especially for troglofauna (see Halse and Pearson 2014), is correct in most situations, as can be seen in the collectors' curves shown in environmental impact reports. However, notwithstanding the need for greater sampling effort, the assessment of impacts of development in Western Australia is probably more thorough than practised anywhere else.

23.2.3 *Inferring Distributions*

While assessments of the potential impacts of development on subterranean fauna usually focus on threats to species, another important aspect of the conservation impact is the richness and biological interest of the subterranean fauna assemblage in the development area. Regional surveys provide a framework of information that enables the characteristics of the assemblage to be assessed. They also enable the likely values of an area to be predicted with moderate confidence in advance of survey, based on geological information and broad patterns of subterranean fauna occurrence. Another benefit of regional surveys is that they provide information about the characteristic ranges of species of various taxonomic groups (especially

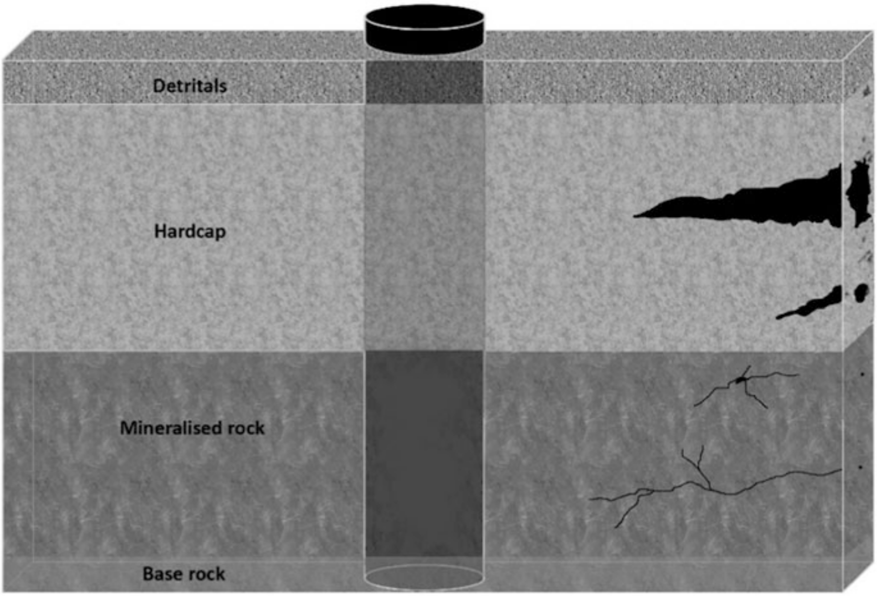

Fig. 23.3 Schematic illustration of the location of a drill hole in relation to the subterranean meso- and microcaverns providing habitat for troglofauna. Hardcap is a ferricrete duricrust found above many rock formations in the Pilbara. It is usually vuggy and often contains mesocaverns and even caves

families and genera) in different geologies, and the extent to which a species' range can be predicted (or not) from that of related species. This information will be better refined over time as general ecological and life history studies of species are undertaken.

The main factors affecting the occurrence of stygofauna species in the Pilbara and Yilgarn regions of Western Australia are hydrological connectivity (including to the surface), salinity, dissolved oxygen levels and geology/lithology (Halse et al. 2014), but factors other than hydrological connectivity and salinity do not predict occurrence well. A major issue when trying to predict species ranges from environmental characteristics is that these characteristics often vary substantially within an aquifer over vertical and spatial scales of a few metres as a result of differences in the size and spacing of voids that affect transmissivity (see Larned 2012; Bradford et al. 2013) or stratification of the aquifer if groundwater is moderately saline (e.g. Halse et al. 2014). Fine-scale changes can rarely be mapped well from the surface. However, it may be said that in general both calcrete and alluvial aquifers are likely to contain areas of high transmissivity and calcrete aquifers usually contain haloclines. Alluvial habitats often occur within the dewatered zone around mine pits, and both calcrete and alluvial habitats are potential sources of mine processing water.

The factors affecting troglofauna occurrence in the Pilbara and Yilgarn are poorly documented, but the most important factor is probably the frequency of subterranean spaces over a threshold size (this is analogous to hydrological connectivity being important for stygofauna). Troglofauna are common in vuggy habitats, such as those occurring in weathered or mineralized iron ore formations and calcretes. Mineralized iron ore formations are areas of potential mine development. The limited information available suggests that while broad geological categories are important determinants of the occurrence of troglofauna, subtle variations in geological classification (in the sense of mineral composition and origin) do not affect troglofauna occurrence greatly if lithology (general physical characteristics including texture) remains similar.

23.2.4 Identifying Species

Another challenge in determining species ranges and, more particularly, whether a species has been collected both inside and outside a project impact area is the species identification process. The key issue is usually whether animals found inside and outside the impact area represent a single species or two sister species. Identification of subterranean fauna is especially challenging because of the very short history of study (approximately 20 years for stygofauna and 10 years for troglofauna in north-western Western Australia). It is often suggested that morphological convergence towards a subterranean life form (loss of pigment and eyes, elongated appendages and sensory structures) contributes to difficulty in distinguishing species morphologically, but the high degree of plasticity in many subterranean species is also an issue, whereby the numbers of setae and segments in structures such as antennae can vary between left and right side.

Use of DNA sequencing to identify species in the assessment process is becoming routine, but, just as with morphology, there are many situations when it is unclear from sequence results whether one or two species are present. The main reason for this is that the potential for dispersal and intermingling of animals in the subterranean environment is limited. This applies even to alluvial aquifers where dispersal would be expected to be comparatively easy, but genetic differentiation may be observed over distances as small as 50 m (Asmyhr et al. 2014). Genetic differentiation also occurs within calcrete aquifers in the absence of obvious barriers, perhaps as a result of areas within the calcrete becoming isolated during (geologically) dry periods and reuniting with the remainder of the calcrete during wetter periods (Guzik et al. 2011; see also Chap. 20). In some cases, sequence divergence in mitochondrial DNA can be high while nuclear DNA shows little difference (Javidkar et al. 2016), and it has been suggested the important units for conservation may be the genetic lineages that have at least some morphological support to suggest they are separate species (De Queiroz 1998; Carstens et al. 2013). However, the likely high future emphasis on the use of environmental DNA (e.g. Jerde et al. 2011) may lead to greater use of a genetic species concept, with a consequent increase in the numbers of restricted species recognized. It is interesting to note that, given the patterns of diversity revealed by morphologically and genetically defined species are similar

(Eme et al. 2017), many of the objectives of conserving subterranean fauna, especially at the assemblage level, can probably be achieved by morphological species identifications alone. However, if environmental DNA can improve the detection rate of species (and there are challenges to be faced—see Korbel et al. 2017), the level of information about species ranges will be improved and there will be a consequent increase in confidence that environmental impact assessments are accurate.

23.2.5 Determining Impacts

In situations such as a mine pit being excavated to the water table (or below) throughout the range of a restricted troglofauna species, it is clear that all of the species' habitat will be lost and that extinction is highly likely. Similarly, if groundwater drawdown occurs to bedrock (or substantially greater than 30 m below the ground surface) across the range of a restricted stygofauna species, extinction would be expected to occur. In many cases, however, possibly suitable habitat will remain in parts of the aquifer. This is particularly the case at the edges of the groundwater drawdown cone, where only a small part of the aquifer will be drawn down. Assessing the degree of impact in such situations is complex because of limited information about species' vertical distributions and the vertical distribution of the different habitats the species may occupy.

The issue of impact being unlikely at the margin of the zone of dewatering can be partly addressed by setting a threshold of drawdown (often 2 m) that must be exceeded before groundwater abstraction is considered potentially threatening. In uniform alluvial aquifers, the safe drawdown may be much greater than 2 m because there will be a considerable depth of similar habitat remaining unaffected by drawdown. Many borefields designed to produce sustainable yields of processing water will probably contain significant remaining stygofauna habitat across the borefields. However, in calcrete aquifers the preferred habitat of at least some species may be the more karstic habitat associated with water table fluctuations and drawdowns of 2 m from current level will result is significant loss of habitat for species.

It is often suggested another reason to think stygofauna will usually survive groundwater drawdown is that pockets of water remain in the subterranean matrix after drawdown. This is easy to conceptualize in rock aquifers but may be less likely in calcrete and alluvial aquifers where the matrix is more porous and may drain more completely over time. Hose et al. (2017) have examined the likelihood of stygofauna surviving in water films in more detail.

This very brief review of determining impacts in environmental assessment has focused on mining. Some other potential impacts are considered in the next section, but the main message is that understanding likely impacts and, more importantly, how they might be mitigated through management actions requires an understanding of the biology of the species involved or, at the very least, improved information about the ecology of troglofauna or stygofauna in general.

23.3 Broad Threats

The broad threats to subterranean fauna across the general landscape have not been considered in Western Australia outside environmnetal impact assessments. However, there is more information on the threats to stygofauna than troglofauna. Reviews have examined the possible threats from mining and the water chemistry changes associated with mining, especially increased salinity, and reduced carbon and nutrient inputs (Hancock et al. 2005; Humphreys 2009; Nevill et al. 2010). Elsewhere, it has been shown that nutrient overload, from sewerage treatment works, can substantially alter the abundances of some stygofauna species (Scarsbrook and Fenwick 2003). However, studies of the impacts of agriculture on stygofauna have shown mixed results, with not all impacts being deleterious (Korbel et al. 2013); just as the effects of metals and pesticides on stygofauna appear to be similar or less than on surface aquatic invertebrates (Hose 2005; Hose et al. 2016), although Humphreys (2007) has cautioned against this conclusion. In line with Hose's findings, Marmonier et al. (2013) also showed similar sensitivities of surface and groundwater species to pollution. Thus, it is suggested here that the main differences between stygofauna and surface aquatic invertebrates in their responses to pollution perhaps relate mostly to the time and method of exposure rather than innate physiological differences.

In the absence of other information, it is tentatively suggested that the situation for troglofauna is analogous to stygofauna. Some papers have suggested that troglofauna will be sensitive to many impacts, such as pesticides (Souza Silva and Ferreira 2015), but there is currently little empirical support for these conclusions in troglofauna across the broad landscape.

23.4 Awareness of Subterranean Fauna

Box 23.3

Other than a few fish species, the subterranean fauna of the broad landscape in Australia consists of microscopic invertebrate species about which relatively little is known. The subterranean environment itself is out of sight and subterranean species will always be low on the list of environmental concerns unless it can be clearly shown that they are important to maintaining ecosystem function in a way that benefits the human population. This work is well advanced for stygofauna but needs to be a focus of troglofauna research. It would also be useful if the role of subterranean fauna species in the landscape, and the ecological services they provide, were better understood.

It is difficult to envisage strong community support in Western Australia for subterranean fauna conservation without a greater appreciation of the role of subterranean fauna in the ecosystem. This includes a scientific consensus that support is warranted. At present, while authors such as Tomlinson et al. (2007) argue strongly for greater inclusion of subterranean fauna in environmental monitoring and management systems, others such as Majer (2009) question the existing level of funding for subterranean research and argue it should be spent on surface systems. This view was rebutted by Harvey et al. (2011), but it illustrates the need to justify the protection of subterranean fauna beyond the oft-cited requirement of the Wildlife Conservation Act to ensure the persistence of all species. While there are strong administrative, as well as conservation, reasons for retaining the position that all species should be protected, rather than finding a rationale for protecting some species and not others, it would be much easier if ecological justifications for the protection of subterranean fauna could be provided.

As with the understanding of threats, identifying ecological reasons for protection is more advanced for stygofauna than troglofauna, especially in relation to the ecosystem services they provide (e.g. Danielopol et al. 2003; Steube et al. 2009; Griebler and Avramov 2015). It is anticipated, however, that further study over the next decade is likely to provide compelling justification for the preservation of both troglofauna and stygofauna across the broad landscape and around development projects.

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Part VI
Cave Ecology for the Twenty-First Century

Chapter 24
Where Angels Fear to Tread: Developments in Cave Ecology

William F. Humphreys

24.1 Introduction

The preceding chapters describe the state of knowledge of cave ecology across a wide range of habitats and geographies. They address the substantial advances made over the last several decades in our understanding of subterranean biology generally and of subterranean ecology in particular, and many identify outstanding areas of research specific to their subject that need to be addressed. However, we are still far from understanding such fundamental issues as adaptive mechanisms, species distribution patterns, trophic interactions and ecosystem functioning.

The advances have been made, variously, by fundamental reappraisal of accepted theory (Pipan and Culver 2012) and paradigms (Culver and Pipan 2014) or as a result of novel applications of established methods in both laboratory and field experiments; new experimental approaches; large-scale, interdisciplinary collaborative studies; and the application of molecular genetics (Griebler et al. 2014). The latter, especially, has benefitted hugely from a culture of open-access data—often driven by funding agencies and journal publication guidelines—such as GenBank and others within the International Nucleotide Sequence Database Collaboration—and by ProteomeXchange for proteomics data (Vizcaíno et al. 2014). This trend of increasing openness in scientific culture is seen also in the numerous biodiversity data bases such as Global Biodiversity Information Facility (GBIF; Robertson et al. 2014) and numerous specialised data bases (https://en.wikipedia.org/wiki/List_of_biodiversity_databases) including the IUCN Red List for conservation status. Likewise, the availability of standardised procedures and shared data storage of ecological data will also accelerate ecological understanding

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"Where angels fear to tread" was first written by Alexander Pope in his 1711 poem "An Essay on Criticism"

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although its broader scope presents much greater challenges (Reichman et al. 2011; Thomas et al. 2012). This progress will build upon itself with further application of current methodological, technological and analytical advances to test hypotheses in subterranean ecology, but we will also see the application of emerging techniques to the same end.

In this chapter, I have selected some emerging or recently emerged techniques and equipment and suggest how they may be applied to advance subterranean ecology. The choice is, of course, idiosyncratic, but I hope by so doing to encourage multidisciplinary collaborative studies (Wicks and Humphreys 2011) using a wider variety of techniques to advance understanding of subterranean ecology.

24.2 Methodological and Technological Innovations

A few methods and technologies have been game changers for subterranean ecology, as they have in other areas, but some have addressed issues specifically pertaining to subterranean ecology, to single out a few amongst the former: stable isotope ratio analysis permitting both the source and pathways of energy supporting subterranean ecosystems (Martínez del Rio et al. 2009), the analysis of continental- and regional-scale distributions (Christman and Culver 2001; Culver et al. 2003, 2006) and molecular methods assisting the recognition of cryptic species and informing about the ecological and biogeographic history of the species present (Juan et al. 2010; Jurado-Rivera et al. 2017). Those techniques specifically addressing subterranean studies include sampling the MSS (Juberthie et al. 1980; see also Chap. 3) which promoted extensive development of research in shallow subterranean systems (Culver and Pipan 2014), the invention of the Bou-Rouch pump for sampling hyporheic and stygofauna habitats in sediments (Bou and Rouch 1967; Hunt and Stanley 2000), sampling fauna from drips in caves to study the biogeography of the fauna of epikarst (Culver et al. 2012a; Pipan 2005) and cave diving, especially in anchialine ecosystems, later enhanced by the use of rebreathing apparatus (Iliffe and Bowen 2001).

24.3 Natural History: Basic Biology

The natural history of many European and some North American and Japanese troglobionts has been studied by generations of scientists and amateur naturalists—citizen scientists. Together, they have established a broad background to the natural history of troglobionts, knowledge well beyond that of the diversity and distributional information lately being recorded in other parts of the world. It is hard to envisage such natural history information emerging elsewhere in the context of contemporary social and scientific funding mores. For example, the enormous increase in knowledge of the biodiversity and phylogeography of the Australian subterranean fauna has not been accompanied by any great increase in natural history knowledge. Indeed, there is probably not a single subterranean species in Australia where the basic life history parameters have been well established—unlike in Brazil where single-species studies are still pursued (Gnaspini 1996; Moracchioli 2002; Bichuette and Trajano 2003; Trajano and Bichuette 2008). In consequence, the general paradigms drawn from the Northern Hemisphere are widely applied to the Australian subterranean fauna despite so doing with other fauna, such as birds, having shown to be totally misleading (Christidis and Boles 2008). Indeed, the late blossoming of subterranean biology in Australia may be attributed to the application of the contemporary northern paradigms to the Southern Continent leading to attention being focussed on karst and cooler, wetter areas and those most recently influenced by glaciation, rather than arid and tropical regions now known for their globally exceptional subterranean biodiversity. The lack of basic natural history is a serious impediment to the development of ecological research in those areas where subterranean biological research has developed more recently. The compilation of information on subterranean natural history and its formal publication remains a critical issue in developing subterranean ecology across much of the globe. While there may be few outlets in mainstream publications, it is facilitated by dedicated journals such as Subterranean Biology and online publications such as Speleobiology Notes.

24.4 Enumerating Numbers and Movements of the Fauna

Emerging and improved technologies that permit individual animals to be identified and tracked are important aids to ecological studies and biodiversity conservation (Pimm et al. 2015), but they are often inappropriate, largely due to size, for application to research on the invertebrates typical of subterranean ecosystems.

Mark-release-recapture is a routine method of gathering data on population size and movement of animals, and marking individuals, rather than batches, provides data of much greater resolution. The method is limited by the ability to unambiguously mark individuals, and this becomes increasingly difficult with smaller individuals. Bee tags have been used to mark queen bees by apiarists, and the scope of this method has been greatly extended by the availability of individual microdots (0.5 mm diameter) containing up to 26 characters of information (Whitehead and Peakall 2012) and which have been successfully applied to 9 mm parasitoid wasps. This inexpensive method is widely applicable if there is a suitable non-toxic adhesive—due to the slight sclerotisation and greater permeability of troglobionts than epigean species (Hadley et al. 1981; Robinson 2005; Vittori et al. 2012), toxin penetration is potentially a greater issue for cave studies.

Progressive improvement in the size, cost and diversity of microsensor technology is making available a range of sensors that will allow for the identification and movement of individuals; indeed, it may also extend to the detailed recording of microenvironments. Currently the size (2.5 mm^2) and passive response means the use of microsensors is restricted to species the size of honeybees and depends on the carrier returning in close proximity to an RFID (radio frequency identification) reader. Research is scoped to greatly reduce the size of the chips to permit the tagging of mosquitoes, to monitor environmental conditions such as gases or temperature and to derive power from the locomotion of the arthropod carrier to transmit information allowing monitoring from a greater distance (https://blog.csiro.au/tiny-technology-creates-a-buzz/). Amongst the questions such technology could resolve is the degree to which cave animals utilise the meso- and microvoids and the attributes of these environments. The application of any of these techniques to troglobionts is likely to be more favourable than when used on epigean species because the marks have the potential to persist for longer, and recaptured rates should be much greater and represent a greater proportion of the population. This is based on the expectation that cave animals are long-lived and slow growing (but see Vernarski et al. 2012), have modest population sizes and live in clearly bounded habitats (but see Trontelj et al. 2009).

Population size and structure are fundamental attributes affecting the ecology of species and also their evolution because both genetic drift and the phenotypic effect for efficient selection are inversely related to population size (Koonin 2015). Subterranean habitats, however, are often difficult, even impossible, to access and sample to obtain estimates of population size, and consequently such estimates in aquatic and terrestrial subterranean habitats are few (Jugovic et al. 2015). This is rather surprising given the oft repeated claim of rarity in subterranean animals and the relationship between the size and variation in populations and risk of extinction (Legendre et al. 2008). Conversely, the utility of population size as a measure of endangerment against Red List criteria (Cardoso et al. 2011) has been challenged as inappropriate and impractical for invertebrates.

Jugovic et al. (2015) estimated *Troglocaris anophthalmus* populations of above 1000 individuals in a 180 m^2 lake in Vipavska jama using mark-release-recapture (MRC, marking by amputation) but with wide confidence intervals and, unsurprisingly, detected no seasonal variation. MRC (marked using fluorescent pigments) was used to determine if cave populations of millipedes (*Stygiochiropus communis*) and schizomids (*Draculoides vinei*) were sufficient to sustain experiments; analysis using Bayesian statistics indicated that the apparently modest populations of both species in two small caves in Australia proved to have unexpectedly large populations (Humphreys and Shear 1993; Humphreys et al. 1989) indicating exchange between the cave and the mesovoids in the limestone. Simon and Buikema (1997) reported densities of the isopod *Caecidotea recurvata* (marked by staining) of up to 74.6 m^{-2} in moderately polluted cave pools. Knapp and Fong (1999) estimated a population of amphipods (marked with ink) in a headwater cave stream of about 3000–4200 individuals. However, many, perhaps most populations in subterranean systems are more rarely encountered leaving too few individuals to achieve adequate recaptures following marking, or else the species has attributes making it inappropriate or impossible to mark. This can potentially be resolved using genetic criteria to establish the effective population size (Ne) of the subterranean populations using a sample of the individuals and achievable using a number of estimators (Do et al. 2014). However, owing to the effects on gene flow of the patchy nature of populations and subterranean habitat (Bradford et al. 2013), such analyses could be enhanced, as in another context (Hillary et al. 2018), through the application of a close-kin mark-recapture framework and demographic models to estimate actual populations.

Population estimates could also potentially be complimented by the use of (e)DNA, such as being trialled in lake fisheries (http://dna-barcoding.blogspot.com.au/2016/01/edna-to-measure-fish-abundance-in-lake.html), and which proved useful for the olm (*Proteus*) in confined but inaccessible subterranean systems (Gorički et al. 2017).

24.5 Sampling of Aquatic and Terrestrial Animals

Laser scanning (Azmy et al. 2012), thermal imaging (Betke et al. 2008) and remote infrared cameras can be useful for sampling undisturbed populations in caves, and the latter have been successfully used for a prolonged period both for public education and for ecological studies of the southern bent-wing bat *Miniopterus schreibersii bassanii*, in Australia (Codd et al. 2003). Mini-video cameras have been used to describe groundwater habitats (Niederreiter and Danielopol 1991), but high resolution of digital cameras and low-cost mass storage of digital data now make possible the observation of even quite small species over prolonged periods. Provided with a suitable power source, such cameras could be a useful adjunct to other methods in behavioural ecology, for example, category and periodicity of behaviours and the frequency of interactions within and between species.

Considerable research into subterranean faunas now occurs in habitats only accessible through bores (tube wells) (Danielopol 1989; Humphreys 2001, 2008; Eberhard et al. 2009). Although dedicated shallow bores may be established for a research project (Danielopol 1984), deep bores used to sample subterranean fauna—with notable exceptions (Christner et al. 2014) owing to their high cost—have typically been established for other purposes (water supply, seismic survey, mineral exploration or orebody delineation), and they may or may not be wholly or partly cased (lined). The natural vertical location in the substrate of individuals sampled from bores is typically uncertain because sampling is often conducted by hauling a phreatobiological net through the water column or by traps. In both cases individuals may have moved naturally or accidentally down the tubes, else swum up the bore from a deeper aquifer. While a number of methods have been developed to accurately locate samples in relatively superficial bores, both for aquatic (Hahn 2005) and terrestrial samples (Lopez and Oromí 2010; Rendoš et al. 2016), accurate location in deep bores has rarely been achieved. More recently, techniques such as nested bores and Snap Samplers® (ProHydro, Inc., Fairport, NY) allow accurate discrete interval sampling (Parker and Clark 2002), while pneumatic syringe sampling can provide simultaneous sampling at 5 cm vertical intervals (Seymour et al. 2007) (Fig. 24.1). Sorensen et al. (2013) deployed packers in dedicated boreholes to isolate segments of the bore at defined geological features to investigate both the type, location and density of stygobiont populations in Great Britain. This allowed them to determine that invertebrates and microbes were more abundant in boreholes than in the aquifer that bores were sites of enhanced biogeochemical cycling and that in the chalk aquifer, the ecosystem extended from the surface to a water depth of 70 m. Such collaborative research mobilising the resources of a major agency (in this case the British Geological Survey) comprising at least hydrogeologists and ecologists and the

Fig. 24.1 Snap Samplers® can be chained to take simultaneous samples of undisturbed water from different depths. Image courtesy of Sandy Britt, ProHydro, Inc. (left); pneumatic sampler taking simultaneous samples at 5 cm intervals over a depth of 3 m (Seymour et al. 2007). Photo by © William F Humphreys (right)

use of packers, or bores to dedicated depths, will greatly enhance understanding of the ecology of deep aquifers.

24.6 Sampling of Fauna in Anchialine Systems

Stygobionts have long been collected from emergent streams and springs in continental waters (Rouch and Carlier 1985; Spangler and Barr 1995; Danielopol et al. 1997; Halse et al. 2002; Galassi et al. 2014), including from submerged springs (Hutchins et al. 2013). The recent collection of stygobionts from surface anchialine springs, which are possibly rare, revealed several species of decapod larvae, identified using the CO1 gene, as well as unexpected genetic diversity and biogeography (Weese et al. 2016). The revelation of this obligate hypogean component of anchialine pool-dwelling shrimps will prompt further efforts to sample comparable habitats that should provide new insights on the ecology of anchialine systems globally. Anchialine systems have been characterised as subterranean estuaries (Bishop et al. 2015) which loosely link them with the chemical estuaries discharging as submarine groundwater discharge (SGD) (Moore 1999). Discharge from the latter can be substantial, being estimated to average 4.5 m^3 m^{-1} day^{-1} along the 86 km shoreline of Christmas Island, Indian Ocean (Humphreys 2014), a carbonate-cover island (sensu Mylroie et al. 2001). Where discharge occurs through submarine springs, rather than diffused through sediments, there is potential to sample adults and larvae of any metazoan that may be associated with subterranean estuaries. The suitability is unknown for sampling of submarine groundwater discharge at springs, known as wonky holes, arising in drowned palaeochannels in the Great Barrier Reef, Australia, up to 60 km offshore (Stieglitz 2005, 2016).

24.7 Remotely Operated Vehicles (ROVs) in Air, on Land and in Water

Although ROVs—drones, dare I mention them—may seem inapplicable to cave ecology, they do have potential utility in caves both to gain access and reduce disturbance to cave ecosystems, especially compaction due to the passage of people, and to be guided remotely, by tether, or autonomously. Starting as a novelty and becoming progressively smaller, more sophisticated and customised, drones will no doubt be exploited in caves in a number of applications, such as accessing otherwise inaccessible areas—variously too small, dangerous, difficult or time consuming—to observe, enumerate or sample fauna, substrates or the environment. Indeed, 3D point cloud maps within a cave generated by 2D laser scanner mounted in a micro-quadcopter have already demonstrated their utility and precision (Kaul et al. 2016). Drones moving across surfaces by means of appendages, inching, tracks or wheels have similar potential and could be especially useful for confined space photography and manipulations such as setting up experiments in inaccessible sites to protect them from interference. Propeller-driven drones are applicable to both aerial sites, for which a plethora of models is available, and aquatic sites for which drones are still few and under development. Control of the latter is typically via tethers (e.g. trident www.openrov.com; http://fathomdrone.com/) which is challenging in confined systems, but they do have great potential for recording and sampling, and they still possess limited mission capability (video and depth) (Fig. 24.2). However, the autonomy (https://ibubble.camera/) and environmental recording capability (http://www.hydromea.com/) of underwater drones are being developed rapidly, and they will especially facilitate sampling in highly stratified water columns, such as anchialine systems, that may be disturbed by divers and at depths too great to dive. A video and depth recording drone was deployed in Hranicka Abyss, Czech Republic, to a depth of 404 m, well beyond the depth limit of the diver (K. Starnawski at 265 m) (http://phys.org/news/2016-09-explorer-deepest-underwater-cave.html).

24.8 Diversity of Subterranean Fauna

There is a profuse literature on the diversity of troglobionts, and, as recently as the turn of the millennium, subterranean sites were considered as biodiversity ‘hotspots’ having as few as 20 troglobiont plus stygobiont species (range 20–84, $n = 18$ caves

Fig. 24.2 Clockwise from top left. *1* TILT Ranger (http://inkonova.se/tilt-ranger/) is rugged, flies, climbs and swims and can carry a payload (https://www.youtube.com/watch?v=X5TfsJWz7RY); *2* Trident underwater remotely operated vehicle (ROV) is compact, 410 mm long, and dives to a depth of 100 m with a 3–4 h duration (https://www.youtube.com/watch?v=rnVpXb_1RhA; https://www.openrov.com/products/trident/#specs); *3* Small underwater ROVs are being fitted with equipment racks that will increase their task diversity (https://www.fathomdrone.com/); *4* Aerial drones, such as Micro Drone 3.0, can be tiny, avoid collisions and fly into fine spaces to take stabilised HD video (https://microdrone.co.uk/products/micro-drone-3plus, https://www.google.com.au/search?q=Micro+Drone+3.0.+Stabilised+HD+video.&rlz=1C1CAFB_enAU610AU610&oq=Micro+Drone+3.0.+Stabilised+HD+video.&aqs=chrome..69i57.647j0j8&sourceid=chrome&ie=UTF-8)

and wells—sites, Culver and Sket 2000). Of these only seven caves contained more than 25 troglobionts (26–36 species) and nine with >25 stygobionts (27–48 species) (Culver and Pipan 2013) at sites that encompassed carbonate karst, lava caves and chemoautotrophic systems. Many other types of subterranean environments, not always shallow subterranean systems (Culver and Pipan 2014), also support high, even exceptional, levels of troglobiont diversity. Ten lava caves on the Canary archipelago have more than ten cave-adapted species (range 11–37 species) (see Chap. 17). For perspective, Mammoth Cave (Kentucky, USA) has 26 troglobiont species in more than 540 km passages.

The diversity of subterranean fauna has been considered to be the greatest in the temperate regions, particularly the Northern Hemisphere on account of the presence of extensive limestone formations, especially the Dinaric karst region. It has been proposed that a mid-(northern) latitudinal ridge of biodiversity exists (at about 34° N in North America and 42–46° in Europe) and that this pattern of distribution reflects something fundamental about cave ecology—reflecting long-term surface productivity at these latitudes (Culver et al. 2006)—rather than merely the distribution of favourable habitat.

Indeed, Culver and Sket (2000) were puzzled by the scarcity of high diversity caves in the tropics, and this issue was partly addressed by Deharveng and Bedos (2012) who showed that the tropical subterranean fauna can be modestly diverse, reporting between 3 and 28 obligate subterranean species from 17 caves in Southeast Asia. Similarly, hotspots of cave species from the Neotropical region have been reported from Brazilian limestone caves containing 28 and 22 species (Silva and Ferreira 2016), a much greater diversity than found in Brazilian iron ore caves (Souza-Silva et al. 2011), although the extent of the latter means they contain a high number of troglobionts.

Recently sampling of highly diverse subterranean faunas in atypical, non-carbonate substrates lacking karst and surface access, located in the arid tropics and subtropics, has raised fundamental questions about the biogeographic patterns described above and the ecological requirements for subterranean ecosystems. On the Western Shield of Australia, subterranean habitats are only accessible through boreholes, but rich subterranean faunas occur in a wide range of unconventional habitats, such as groundwater calcretes, and Precambrian-banded iron formations (BIF) and its derivatives (Halse and Pearson 2014) such as fractured rock, scree, valley fill, river gravels and goethite pisolites. Investigations for the development of a proposed uranium mine at Yeelirrie, Western Australia (27° 18′ S), recorded at least 70 species of stygofauna and 45 species of troglofauna (115 subterranean species in total) mostly from groundwater calcretes, with one bore contained 27 stygobiont species with another 13 bores contained 10 or more species (Bennelongia 2015b; Subterranean Ecology Pty Ltd. 2011). Seventy-eight species of stygobionts are reported from the Ethel Gorge calcrete (23°18′ S, within the tropics) (Bennelongia 2015a), now a threatened ecological community that was originally assessed during the development of an iron ore mine in the Pilbara region of Western Australia (Eberhard and Humphreys 1999). Elsewhere in the Pilbara, 54 stygobiont species have been collected from a single borehole (known as PSS016, ~21°18′ S; Halse et al. 2014). Halse and Pearson (2014) claimed to have collected 549 troglofauna species or morphospecies in the Pilbara working in 65 sample areas mostly smaller than 10 km^2 and 90% located within the tropical Pilbara. The efforts of numerous other environmental consultancy firms have certainly produced many additional species to those collected by Halse and Pearson (2014) [e.g. 21 species of troglobionts were described by Harvey et al. (2008), Baehr et al. (2012) and Smith et al. (2012)]. In the same area, Halse et al. (2014) recorded about 350 species of stygofauna. Hence, in the space of several years, sampling a small fraction of the arid tropical Pilbara has yielded approaching 1000 species of subterranean fauna.

The generality of the Australian findings to other continents, especially the Gondwanan terranes, remains to be established. Iron ore bodies in Brazil contain fauna in caves (above), but whether the fauna is more deeply infused within the orebodies and detrital fields remains open. Similarly, although groundwater calcretes are widespread in arid areas, especially in South Africa, Namibia and Botswana (Shaw and de Vries 1988; Weaver et al. 1993; Pickford et al. 1999), there have been no reports there of diverse subterranean faunas either below or above the water table. Globally, comparison will only be made by the widespread sampling of subterranean habitats accessed through boreholes while having an open mind as to which substrates are suitable subterranean habitats. The consensus that the main subterranean habitats are in carbonate karst, lava fields and submerged granular sediments (Sket 2008) is much too restrictive. On the Western Shield of Australia, connected voids in any substrate are significant habitats of subterranean biodiversity.

24.9 Metagenomics and Environmental DNA (eDNA)

Metagenomics has emerged as a powerful tool that can be used to analyse genetic material recovered directly from environmental samples. The field is most fully developed as microbial metagenomics on account of its utility to identify microbial lineages regardless of the ability of member organisms to be cultured in the laboratory (Allen and Banfield 2005; Thomas et al. 2012), but it is increasingly being developed as a tool for general biological survey and monitoring. An excellent primer on the use of eDNA for aquatic biodiversity and biomonitoring surveys is provided by Shaw et al. (2017). However, the full impact of eDNA on subterranean ecology is yet to be developed; when eDNA-derived data are combined with compound-specific stable isotopes and stable isotope probing, it has the potential to progress from community genomic data sets to the knowledge of gene expression and proteomics to reveal how species contribute to the net activity of the community (Allen and Banfield 2005; Dumont and Murrell 2005).

Environmental DNA—eDNA—is a DNA shed into the environment by its living or recently dead inhabitants. It is normally present at extremely low concentrations but can be detected using PCR amplification and then characterised. Conversely, both detection and identification can be performed directly from the water using microarrays containing the appropriate molecular probes. While eDNA of aquatic systems is most commonly analysed using samples of water, it is more concentrated, sometimes by several orders of magnitude, and more persistent in sediments (fish: Turner et al. 2015). Consequently, there may be potential to detect cave fauna from DNA in soil samples. While eDNA from sediments is more detectable, it is probably inappropriate to determine contemporary occupancy from this source.

eDNA is potentially useful in subterranean ecology in characterising the taxonomic composition of the ecosystem, particularly owing to the rarity of the fauna (or low abundance), or where the system is only remotely and sparsely accessible, such as aquifer ecosystems. In parallel with species richness estimates, this could provide a measure of how completely the taxonomic assemblage in an ecosystem/habitat has been sampled and how many of the indicated taxa are known species and the taxonomic load remaining to be addressed in that ecosystem. At the community level, even if a ‘taxon’ has been placed only at an unknown terminal in a molecular phylogeny, generated say from GenBank, it may provide information pertinent to the trophic structure of the community. However, the utility of eDNA in initial broad-scale biological survey is limited owing to the difficulties in characterising the species involved. In addition to providing information on species diversity and species composition in subterranean systems, when combined with metabarcoding, eDNA has the potential to provide information, at low cost and from the same samples, on nutrient cycling, ecosystem health and connectivity (Thomsen and Willerslev 2015).

Perhaps the greatest potential for eDNA is for ecological monitoring, especially in groundwater systems, both in terms of efficacy and efficiency, permitting considerable improvements in confidence of the results. Let me expand on one such circumstance that associated with stygofauna in the Pilbara region of Western Australia where, if there are likely to be 'significant stygofauna or troglofauna values', subterranean fauna surveys are required to be undertaken associated with proposed major resource developments (EPA 2013). Forty stygofauna samples from at least 10 bores (and 60 troglofauna samples from at least 30 bores) are required to be sampled across the impact zone of the development—it includes an interim recommendation for troglofauna sampling from at least 60 samples from 30 bores (EPA 2007). The aim of the process is minimal, namely, to ascertain if any species are restricted to the developmental footprint in order to guide the environmental review process. If subterranean fauna is present and species are found to be restricted to the footprint of the development, then the project may not gain approval, or else some method of mitigation may be required to limit the threat to those subterranean species, and 'a monitoring process may be enshrined' under the ministerial conditions allowing the project to proceed.

Without going into the problems associated with arriving at the requirement to undertake monitoring (e.g. Karanovic et al. 2013; Halse et al. 2014), which needs a lot more ecological understanding, subsequently there are at least three parties with an interest in the veracity of the monitoring, namely, the regulators (Environmental Protection Authority and public; EPA 2013), the mining tenement holder (owner) and any environmental consultants engaged to do the monitoring. Each would benefit in their own way by having accurate information from the monitoring as it would reduce risks as they affect each party. As stygofauna are mostly rare and sparsely accessible, direct monitoring, as outlined below, poses possibly insurmountable difficulties.

The Pilbara has an extraordinarily diverse subterranean fauna (Eberhard et al. 2009; Halse et al. 2014), and, as elsewhere, additional species continue to be collected with increased sampling effort. For example, one sampling event (six net hauls in one bore) collected 33% and six samples 82% of the species known to be present. However, as only about 60% of the species in the region were known—based on rarefaction statistics—these initial captures represent about 20% and 50%, respectively, of the species probably present in the system. 'Currently, it is unusual for bores in Australia to be sampled more than twice in … environmental assessment programmes …' (Eberhard et al. 2009). Sampling bores at a rate to detect even half the species would deplete the fauna while providing no party with the confidence they need in the veracity of the data. Indeed, it is worthless as a method of detecting temporal change in the stygofauna—detecting risk of extinction—the purpose of monitoring.

Conversely, eDNA taken from pumped water samples potentially could detect the entire suite of species in the community at a single pass without depleting the fauna. Should the method prove suitable, it would be most effective if used to monitor only those species known to be present using custom microarrays—additional molecular probes could be added as additional species are detected. The successful development of eDNA methodology in this context could provide a high degree of certainty to all parties involved in the rather limited ambition of the monitoring but one that currently imposes considerable costs but yields no effective conservation outcome. Owing to the potential for greatly increased certainty, efficacy and efficiency, the development of eDNA methods for monitoring groundwater systems is likely to attract considerable research effort that could lead to substantial improvements in the technique and would be applicable across a broad range of ecological research and of global import.

24.10 Determining Predator-Prey Relationships

Bradford et al. (2013) used molecular methods to detect prey items in the guts of three different sized sympatric species of subterranean diving beetles in a simple aquifer ecosystem in the Australian arid zone and showed differential prey choice between the species. In more complex systems, in which the target prey may be unknown, PCR amplification may be undesirable owing to amplification biases and cross reactivity with predators and with related species genomes. These potential problems may be overcome using alternative methods, for example, those applied to epigean beetles by Paula et al. (2014) who used PCR-free direct shotgun sequencing of total DNA isolated as a time series from harlequin beetle guts and then identified prey items by matching sequences to reference data bases. By this means, they were able to reveal the dynamics of the complex interactions amongst predators, prey and their symbionts. Methods such as these, especially when combined with various stable isotope methods, such as the emerging compound-specific stable isotope techniques, can do much to inform on food webs, food selectivity and niche segregation in subterranean systems in which direct observation is typically not possible.

Despite the elegance of these approaches, it may be still being problematic to distinguish predation from scavenging on carcasses, owing to the long duration that DNA derived from cadavers may be detected in gut contents, as was found in soil insect predator-prey systems (Juen and Traugott 2005). This is an issue of special relevance to subterranean ecology because many troglobiont predators opportunistically scavenge, as pointed out by Racovitza (1907) and widely reiterated (Culver 2012). However, the transition to other feeding types in epigean and troglobiontic lineages may occur in other ways (Poulson 2012); various trogloxenic opilionid species typically scavenge, while the troglobiotic forms are more commonly predators (Hobbs 2012).

The truncated nature of subterranean biodiversity in both functional and evolutionary aspects results from the typical absence of primary production (in the absence of chemoautotrophy) and the scarcity of top predators (Gibert and Deharveng 2002). However, many authors note the high prevalence of predators amongst troglobiont communities, a disparity that may have resulted from (Gibert and Deharveng 2002) emphasis on aquatic systems. Oromí (Chap. 17) points to the 'remarkably high diversity' of predators amongst the troglobiont community in Tenerife, a total of 37 predatory species, nearly three quarters of the 50 troglobionts recorded. In addition, he notes the diversification there of lineages, especially the nine species of *Dysdera*, with up to five species per cave. This is analogous to the stygobiont diving beetles in Australian calcrete aquifers which have up to four species in a single borehole (Watts and Humphreys 2009), even three sister species (Guzik et al. 2009).

Not only is the main taxonomic composition of subterranean systems fundamentally different from the epigean systems, but the trophic and biodiversity structure may differ between aquatic and terrestrial subterranean systems. Such differences may have profound or subtle influence on the sensitivity of subterranean ecosystems to, for example, the dynamics of trophic cascades (sensu Ripple et al. 2016) which may be dampened (Finke and Denno 2004).

24.11 Are Parasites and Commensals of Subterranean Fauna So Rare?

These two categories have rarely been reported for subterranean species (Culver 2012), and this may be an accurate reflection of the rarity of these interactions for ecological reasons pertaining to the subterranean habitat. It may to some extent, however, be the result of under-sampling owing to the relative scarcity of individuals of subterranean species. A sulphur-oxidising filamentous bacteria—a *Thiothrix* phylotype—lives only as an epibiont on *Niphargus* amphipods in the sulphide-rich Frasassi Cave complex in Italy (Dattagupta et al. 2009). Surprisingly, there is a possible evidence of chemosynthetic ectosymbiont microbes on *Xibalbanus tulumensis*, a member of the Remipedia which are considered to be top predators (Pakes and Mejia-Ortiz 2014). Firmer evidence has been presented for the atyid shrimp, *Typhlatya pearsei*, which harbour endosymbiont mutualistic bacteria and show host-mediated adaptations both to the symbionts and to sulphide toxicity in their extreme anchialine environment (Pakes et al. 2014). The latter example is especially interesting for both current and palaeoecology because some clades within *Typhlatya* diverged more than 100 Mya (Jurado-Rivera et al. 2017). It is unknown whether such endosymbiosis is characteristic of the genus or else an ecotype induced in response to sulphidic water present in some anchialine systems. If such endosymbiosis is a characteristic of *Typhlatya*, then the phylogeography of the microbial consortium may be concordant with that of the host or else driven by host ecology in response to sulphidic or other ecosystem inhabited by a given species of *Typhlatya*.

24.12 Ecotone and the Transition to Subterranean Life

The adaptation of an epigean lineage to subterranean life, which I will refer to as trogloneogenesis, must involve the ecotone between the epigean and subterranean realm. The photic environment across this ecotone was used as the basis of the earliest classification of cave animals (Schiödte 1849; Racovitza 1907).

Caves and other subterranean habitats may come to be occupied by species through active or passive means (Danielopol and Rouch 2012), but there have been minimal studies of ecological processes in the entrance (twilight) zone of caves (Hobbs 2012), and there are remarkably few studies examining the ecological relationships between epigean and troglobiont species, such as the study of amphipods by Luštrik et al. (2011). This type of study could be extended to examine interactions between species of trogloxenes, troglophiles and troglobites.

The hypothesis that light is the prime environmental factor that separates the surface from the subsurface realm (Pipan and Culver 2012), and the comprehensive review of the wide variety of shallow subterranean habitats and their faunas (Culver and Pipan 2014), has refocused attention on that ecotone. This has the added advantage of facilitating experimental research owing to the diversity and accessibility of shallow subterranean habitats and greater population densities. These factors will lead to a proliferation of research into the ecological and evolutionary processes that lead to the separation of surface and subsurface populations, of which the ecology of light may make a significant contribution (Tierney et al. 2016). For example, Fišer et al. (2014) reviewed limits to the vertical extent of groundwater metazoans. Subsequently, they investigated how the response to light of ten co-occurring pairs of surface and stygobiont amphipods in springs influenced habitat choice and ultimately the geographic extent of subterranean amphipods (Fišer et al. 2016); light triggered habitat choice of the eyeless subterranean species but not of the eyed surface species. No comparable multispecies study has been conducted on troglobionts; however, millipedes of the highly troglomorphic genus *Stygiochiropus* (Paradoxosomatidae) may be found in full tropical sunlight if the outflowing air results in a condensing atmosphere, as in Papillon Cave in Cape Range, Australia (Humphreys and Shear 1993). This indicates, as was suggested for surface amphipods by Fišer et al. (2016), that habitat choice by troglobionts may also be affected by factors besides light, in this case by humidity.

The extreme ecotone marking the transition from epigean to subterranean life has always suggested a zone of marked selective pressure gradients. Yet there is mounting evidence that the development of many troglomorphies arises by neutral processes not selection (Wilkens and Strecker 2017). The common occurrence of shallow subterranean environments will facilitate research in the evolutionary process involved, a long contentious area of subterranean biology (Culver and Wilkens 2000).

24.13 Invasive Species in Subterranean Systems

As climate change is considered the major driver of trogloneogenesis, the current anthropogenic climate change is expected to increase the rate of species crossing the ecotone between surface and subterranean realms. As we have seen above (Fišer et al. 2014), the maintenance of ecotonal separation between surface and subterranean amphipods was achieved by their response to light, the major signal at the ecotone. But this tells us little about those fundamental questions in the discipline: what are the drivers of trogloneogenesis, and how do species colonise already inhabited subterranean spaces? The structural and numerical simplicity of subterranean ecosystems would seem to provide a good model from which to explore the broader ecological questions of ecotonal and niche transition, hampered perhaps by the sensitivity and slow life of subterranean species. Although cave animals are considered to have been derived from surface lineages which must have undergone trogloneogenesis, examples are rare of even epigean species impacting on subterranean lineages. Romero et al. (2002) recorded the replacement of troglomorphic populations of the silver catfish fish, *Rhamdia quelen*, by epigean populations of the same species in Trinidad. Some species have invaded extremophile environments such as *Poecilia* spp. that are prolific in the sulphide spring complexes at the Baños del Azufre in Mexico (Plath et al. 2007). This has raised concerns about the ability of *P. reticulata*, established in a karst window in Cape Range, Western Australia, to adapt to subterranean life, including the sulphidic parts, and threaten the rich anchialine fauna of the region. These examples show that invasive species may possibly have direct ecological implications to subterranean fauna and ecological studies are needed in order to inform potential mitigation measures. One such study showed that invasive fish in anchialine pools in Hawaii preyed on ‘ōpae‘ula’, the atyid shrimp *Halocaridina rubra*, and displaced the survivors to deeper more cryptic depths (Havird et al. 2013).

24.14 Is Carbon Dioxide Concentration Significant to Cave Ecology?

The thesis that areas with elevated levels of carbon dioxide—bad-air zones—in high humidity cave passages would ‘be found to harbour unique communities of obligate cave species’ (Howarth and Stone 1990) and that this zone may be the principal zone present in mesocavernous cracks and voids (Howarth 1993) has been sustained in the literature (Deharveng 1988; Deharveng and Bedos 2000; Stone et al. 2012, Chap. 3), questioned (Humphreys 2012) but never formally been tested or refuted despite its standing for more than a quarter of a century. This is surprising given the implications that such a widespread zone would have on the ecology of subterranean animals, affecting the evolution, habitat selection, physiology and energetics. It is notable that the original paper does not provide strong support for the thesis as the data from Bayliss Cave shows a marked increase in troglomorphic species coincident with a rise to near-saturated relative humidity before the region of elevated CO_2 occurs. Thereafter, the number of troglobiont species decreases with increasing concentration of CO_2 (see Fig. 23 in Stone (2010) and Howarth (1988)). The high-stress environment thesis proposes, amongst others, that elevated CO_2 levels may be a common feature of habitats in mesocavernous cracks and voids (Howarth 1993) although no additional evidence is provided of the role of elevated CO_2 in this formal presentation. Few biologists routinely measure CO_2 in caves, other than in show caves for the purpose of conservation and public safety (Šebela 2011), and when measured (Deharveng 1988), it is rarely associated with other parameters, such as oxygen, to determine the source. Further, the carbon dioxide level of soil atmospheres is typically 0.3–3% (Lavelle and Spain 2001); soils on karst may not be very elevated (Czech Republic, annual variation of CO_2 ~0.1–0.43%; Faimon et al. 2012) but are similar to those in some species-rich tropical caves such as in Cape Range, which had mean carbon dioxide concentrations of 0.18% (range 0.06–0.5%; s.d. $= 0.16$, $n = 7$) (Humphreys 1989). In a single entrance cave, Trinh et al. (2018) found seasonal variation in CO_2 between 1000 and 8000 ppm by volume. The CO_2 concentration resulted from mixing background atmospheric CO_2 with CO_2 produced from soil, water and cave biota including humans in show-cave sections. As in most such studies, the focus of the research is on the influence of CO_2 concentration on karst processes. If sufficient data are available, a meta-analysis may help to resolve whether carbon dioxide concentration is a significant issue in subterranean ecology.

24.15 Understanding the Dynamics of Cave Climate

Although cave environments are often considered the epitome of stability, there are regions within caves that are profoundly variable, and these relate to the size of the openings, the distance from entrances and the weather outside. Caves breathe largely owing to changes in barometric pressure and temperature, and the resulting mass movement of air transfers heat and moisture into and out of a cave depending on the vectors of the pressure differential. Even in the absence of air movement, caves may lose or gain moisture owing to water vapour pressure gradients that cause excessive drying of caves in the temperate winter and at night in the tropics when external temperature falls below cave temperature (the tropical winter effect; Howarth 1980). Recording cave climate is especially difficult in remote areas where changes are frequent (diurnal) and remote (deep into cave in distance or time) or the magnitude of change is great (closer to entrances). Cave climate is an important ecological factor, but the recording of an adequate number of parameters at sufficient spatial and temporal scales is challenged by the harsh environment and difficulty of access.

Recent advances in fibre-optical communication and sensors, mainly designed for extreme subterranean environments, offer new opportunities (Ahuja and Parande 2012) which, integrated with wireless communications, could facilitate real-time distributed monitoring throughout caves. This would enable recording of cave climate and enable researchers to take advantage of rare events signalled from any of a wide range of parameters (e.g. temperature, pressure, density, flow rate, etc.), trace gases (De Freitas et al. 1982) or potential metabolic signals using biomedical sensors (simultaneous measurement of CO_2, O_2, pH and temperature: Ferguson et al. 1997). Such information could provide the climatological context, at a range of spatial scales, to fundamental cave ecology and applied ecology, especially microbial ecology, for cave management of tourist caves and the preservation of archaeological sites, especially sensitive Palaeolithic art sites such as Niaux Cave, Ariège (Andrieux 1990).

24.16 Cave Faunas as Indicators of Climate Change

Climate change has been predicted to have profound effects on karst systems generally (Day 2011), and limestone caves themselves have been a major focus of climate change studies, particularly using sediments and speleothems as retrospective proxies for climate change (Denniston et al. 2015, 2016) and correlative species distribution models to the same end (Mammola and Leroy 2017). Climate change is often invoked to explain the colonisation by epigean species of subterranean habitats and their distribution, variously, due to the associated aridity, glaciation and changes in sea level (e.g. Leys et al. 2003; Faille et al. 2013; Stock 1980; Moritsch et al. 2014, respectively). While prospective studies of climate change on subterranean systems are lacking, they are likely to be established in relation to subterranean biology as they have been for other disciplines. However, it is important to recognise that the entire subterranean communities may be at risk from climate change because subterranean species have minimal chance of relocating and characteristically forming communities comprising small-range species, precisely those species that are at greatest risk from climate change (Ohlemüller et al. 2008).

Conversely, it has been posited that subterranean systems are exceptionally resilient to climate change, and the evidence for this comes from the age of subterranean lineages and their restricted dispersal (Faille et al. 2015) in addition to the presumed property of being confined to their geological context by dint of their adaptation to subterranean life (Humphreys 2009). Proposed for the anchialine assemblage in Cape Range, Australia (Humphreys 2000a, b, c), the persistence of subterranean lineages has been established more firmly by molecular clock dating for numerous old subterranean lineages with small-range endemicity (Leys et al. 2003; Abrams et al. 2012; Humphreys 2017). In addition, some stygobiont lineages have apparently persisted from the fragmentation of Gondwana (Poore and

Humphreys 1998; Wilson 2008) and would have endured extreme changes in climate from rainforest to desert.

Temperature is relatively stable in subterranean habitats, and subterranean species are presumed to be stenothermal (adapted to a narrow range of temperature). However, some subterranean lineages adapt to a wide range of thermal conditions, and there is no reason to posit that the ecophysiology of subterranean lineages is evolutionarily fixed. Subterranean invertebrates occur at temperatures ranging from ice caves (Coleoptera Leptodirini; Racovita 2000) to hot springs (48 °C, Thermosbaenacea; Barker 1959), and a given species may complete its life at constant temperature in caves covering a temperature range of 11 °C (18–29 °C; Humphreys and Shear 1993). Cave temperatures are commonly close to the mean annual surface temperature (Wigley and Brown 1976), and so, although buffered from short-term temperature variation, the cave temperature will ultimately track the climatic mean temperature. Consequently, old subterranean lineages will have already been subjected to repeated and sometimes severe change in climate and tracked profound millennial-scale changes in mean annual temperature (Humphreys 2000b). In the context of mitigation, the presumption may be unfounded that protection from variation in temperature may be beneficial because local adaptation may result from a complex interplay of ecological trade-offs depending on both the absolute temperature and the temperature range as has been shown for rock-pool copepods by Hong and Shurin (2015). Indeed, owing to their ubiquity and density in subterranean waters and epikarst (Pipan 2005; Galassi et al. 2009), copepods are likely to be good models for climate change adaptation in stygobionts.

24.17 Biofilms, the Drivers of Cave Ecology?

Knowledge of microbial communities in caves and other subterranean ecosystems is rudimentary (Northup 2011) despite being an important, perhaps the major, source of food available in some subterranean ecosystems. This applies both to aquatic and to subaerial subterranean systems (Simon et al. 2003, 2007) where there is a similar phylogenetic diversity on cave walls around the world (Engel 2012b). Biofilms serve to concentrate organic matter by capturing dissolved organic carbon (Simon and Benfield 2001) to form the foundation of groundwater food webs (Hartland et al. 2011), or else they are formed by sulphur-oxidising bacteria or microbes supported by one or more of a plethora of other chemolithotrophic metabolic pathways (Engel 2012a). Although biofilms are dominated by microbes in both aquatic and subaerial habitats, they can be components of complex and structured ecological communities, comprising archaea, bacteria, protists, viruses and fungi, displaying a full array of ecological processes (see Humphreys (2006) and Engel (2012b)). While much of the energy captured may be consumed within the biofilm ecosystem itself, the biofilm serves as the food base for the stygofauna community, and some taxa are specialised to harvest it directly, such as harpacticoid copepods (Dahms et al. 2007) and some atyid shrimps with their brushlike setae (Page et al. 2007). Hartland et al. (2011) used emission-excitation matrix (EEM) plots of dissolved organic carbon (DOC) fluorescence (Baker and Spencer 2004) to follow changes along the water flow path to explore the ecological significance of biofilms in New Zealand. They demonstrated that changes in biofilm resulting from sewage-enriched groundwater had profound effects on stygofauna communities—there were fewer species, larger populations and greater dominance, greatly changing community structure with perhaps irreversible consequences.

As discussed by Hershey and Barton (Chap. 5), recent advances in molecular biology have profoundly changed the detail in which microbial ecology can be studied in nature. The application of metagenomics—possibly extended into stable isotope probing and the coupling of molecular biological methods with stable isotope abundance in biomarkers (Radajewski et al. 2000)—provides cultivation-independent means of identifying microbial function. Combined with the quantification of gene expression using real-time PCR (qPCR) (Smith and Osborn 2009), these approaches have greatly improved understanding of the diversity, distribution, community structure and function of individual taxa. For example, Suzuki et al. (2000) were able to determine the spatial and temporal quantitative difference in the distributions of bacteria (cocci) and archaea in marine water, while Takai and Horikoshi (2000) quantified archaea in deep-sea hydrothermal vents, hot springs and freshwater sediments. Using 16S rRNA to distinguish the functional part of the microbial community in hyporheic zone biofilms, Wagner et al. (2014) were able to partition the effects of terrestrial dissolved organic matter (allochthonous DOM) and that produced in situ (autochthonous DOM). They found that only allochthonous DOM drove changes in community composition but that neither source of DOM had any clear effect on the function of the hyporheic biofilms, suggesting this conferred stability to the stream ecosystem. Such methods have great potential to contribute to research on the ecology of biofilms in groundwater over a range of spatial scales to elucidate the functional and dynamic role of biofilm in aquifer systems to provide the secure foundation necessary to elucidate the functional role of stygofauna in aquifers (Boulton et al. 2008). Studies of the human microbiome have revealed the structural complexity of biofilms organised in microbial consortia at the micron scale (Welch et al. 2016), an approach quite applicable to aquatic biofilm. The complex interplay amongst the microbiota reveals their effect on human nutrition and susceptibility to disease (Robinson et al. 2010), and, by analogy, such profound interactions are likely to inform on the ecology of subterranean fauna, particularly stygobionts. Prosser et al. (2007) argue that understanding of microbial ecology will be advanced by a much greater application of theory to harness the predictive power from the unprecedented accumulation of biological information from metagenomics and to test the generality of ecological theory.

As a subset of microbial ecology, it is important to note that mycology of subterranean ecosystems, as elsewhere, is sparsely studied, particularly that in groundwater despite the diversity occurring there. Caves support an array of fungi, yeast and slime moulds, with 1029 species recorded from caves and mines, but only 6% of these are from water, and these are mostly sampled from temperate regions (Vanderwolf et al. 2013). Lategan et al. (2012) reported from 14 to 24 taxa per shallow aquifer in eastern Australia, and Kuehn and Koehn (1988) recorded 25 genera from the artesian Edwards Aquifer in Texas and foreshadowed their potential significance in heterotrophic energy conversion. The application of the techniques appropriate to prokaryotes to the fungi present in caves and groundwater will accelerate and start to elucidate their functional role within subterranean ecosystems.

24.18 Biogeochemical Processes as Drivers of Cave Ecology

The finding of rich chemosynthetic microbial populations associated with deep terrestrial systems (Stevens and McKinley 1995) and deep-sea hydrothermal vents ('smokers'; Deming and Baross 1993) has led to a flurry of related work on subterranean systems, for example, in caves, including anchialine systems, in Europe, Mexico and Australia, and deep aquifers in the USA (Engel 2012a). Chemoautotrophy may be aerobic (oxidation of hydrogen, sulphur, iron, manganese, ammonia or methane) or anaerobic (by methanogenesis, acetogenesis, denitrification or the reduction of sulphate, iron or ammonia) with multiple co-occurring chemolithotrophic metabolic processes reported from most systems—between three and seven processes in about half of 23 systems (Engel 2012a, Table 2). Investigations into chemoautotrophy in subterranean ecosystems are expected to develop rapidly and lead to recognition of the diversity of energy sources in subterranean systems and an understanding that energy sources may switch according to the development stage of the individual and the ecosystem or show periodic change according to the state of ecological development or on diurnal, seasonal or climatic factors.

The presence of ^{13}C-depleted biomass in cave-adapted fauna of Ox Bel Ha, an anchialine system in Yucatán (Pohlman et al. 1997), indicated the presence of chemoautotrophic pathways through methane-linked carbon cycling, as found in the Edwards Aquifer, Texas (Bishop et al. 2014). Brankovits et al. (2017) presented strong evidence that methane and DOC are indeed ecologically important parts of the carbon cycle within the Ox Bel Ha anchialine ecosystem. Further, they showed that rain provides external forcing of both the delivery and distribution of methane in the system. Brankovits et al. (2017) achieved this outcome by means of an integrated study combining water chemistry and fauna sampling, compound-specific stable isotope ratio analysis (CSIA) and long-term continuous sampling of water using electrically independent osmotic pumps. This study demonstrated the presence of high concentrations of methane and methane oxidation in the freshwater section of the anchialine system. Further, methanotrophic bacteria were a large part of the diet of atyid shrimps as indicated by ^{13}C-depleted acid biomarkers (^{13}C values as low as -54.1%) and deuterium-depleted δD values of shrimp tissue (Brankovits et al. 2017).

This study highlights the benefit of using CSIA to determine the ^{13}C signature solely of the C16:C17 fatty acid component; phospholipid-derived fatty acids (PLFA) are widely used as chemotaxonomic markers of bacteria. In contrast, the regular SIA data for whole tissue of *Palaemonetes antrorum* from the Edwards Aquifer, which had ^{13}C as low -44 (Bishop et al. 2014), did not allow further partitioning of the stable isotope signals for ^{15}N, ^{13}C and ^{34}S. As such facilities become more readily available, the increasing use of CSIA in subterranean ecology may be expected to refine greatly the understanding of the range of trophic resources and trophic networks within subterranean systems.

Secondly, the application of osmotic pumps (OsmoSamplers: Jannasch et al. 2004) to subterranean ecology signals the way forwards as they can provide continuous sampling for as long as 2 years in hostile environments, even in deep-sea hydrothermal vents (Wheat et al. 2000), independent of mechanical or electrical systems, and achieving a temporal resolution in the order of 1 day. These attributes make the samplers ideal to recover samples associated with rare and episodic climatic events, such as found widely in arid regions (Humphreys 2006) that can have such profound impacts on ecological systems (Davis et al. 2013). The long-term nature and multidisciplinary approach of this exemplar study have allowed profound insight into the functioning of this anchialine system and present a benchmark for further studies on the ecology of subterranean systems.

24.19 Ecosystem Functions (Services)

The ecological functions provided by subterranean ecosystems are poorly characterised and even less quantified in contexts other than microbiota and bioremediation work (Humphreys 2000c; Colwell and D'Hondt 2013). It is largely unknown whether they have a role in hydrogeology and water quality (Herman et al. 2001) or whether groundwater invertebrates alone or in community (Griebler et al. 2014) are 'ecosystem engineers' helping to maintain aquifer hydrodynamics (Boulton et al. 2008; Griebler and Avramov 2015). However, it has been demonstrated that invertebrate bioturbation of sediments serves to maintain hydrologic conductivity in clogged interfaces enhancing biogeochemical and microbial processes (Nogoro et al. 2006). The role of soil perturbation in terrestrial systems in driving ecological processes (Herrick and Jones 2012) is even less studied amongst troglobionts than amongst stygobionts despite the conspicuous tunnelling and soil pellet movement shown by many species, including earthworms, millipedes and schizomids. There is still a dearth of information on these oft discussed but rarely studied issues, and new experimental approaches are required to better understand the contribution of groundwater fauna to ecosystem dynamics.

While bioremediation by microbes can be ascribed as monetary value under ecosystem services models, the same cannot be done for groundwater invertebrates but that does not mean their function has no value (Silvertown 2015). Analysis of the dynamics of macroinvertebrates in moving carbon and chemotrophic energy through subterranean ecosystems will provide the basis for understanding their functional significance and an appraisal of the ecological services they provide for the ecosystem and beyond. To this end, the recent paper by Smith et al. (2016) is enlightening because they examined how stygobionts interact with the microbial community, an especially cryptic area. They calculated that by hitch-hiking on stygobionts—an undescribed neoniphargid amphipod—the numbers of prokaryotes transported were up to five orders of magnitude greater and, most notably, moved 34 times faster than in the surrounding groundwater allowing them potentially to move much further in the bulk groundwater than those travelling by advection alone. How and which microbes are transported within groundwater is crucial to understanding their role in maintaining ecosystem health because prokaryote communities play a crucial role in the turnover of biomass and energy and in purifying groundwater (Danielopol et al. 2003).

24.20 Age of Subterranean Lineages as Indicators of Past Conditions

The age of subterranean lineages is principally of interest in the context of phylogeography and evolution, but it is also pertinent to ecology because it may help to characterise the ancestral ecological conditions. Cave animals have selective similarities that lead to the ecomorph termed troglomorphs that most easily makes them identifiable as cave animals (Culver et al. 1995). That the degree of troglomorphism is related to the age of cave species has been contentious, but Derkarabetian et al. (2010) established that it is possible to predict the taxon age from morphology in troglobiontic *Laniatores* harvestmen (Opiliones) that diverged from surface populations between 8.2 and 2.9 Mya. This finding that morphology can be used to predict the age of subterranean lineages remains to be more widely tested, but it is likely that the information in such character divergence would become 'saturated' in older lineages.

Climatic vicissitudes during the Pleistocene (2.6 million to 11,700 years ago) had long been considered the driving force isolating subterranean lineages underground (Pleistocene effects model; Barr 1968; Barr and Holsinger 1985). In the last decade, largely owing to molecular clock estimates, it is increasingly appreciated that trogloneogenesis of most subterranean lineages occurred several million years ago (see Trontelj (2007)), or even the late Miocene, driven by aridity (Leys et al. 2003), or Oligocene (possibly the Eocene) but continuing into the Plio-Pleistocene (Ribera et al. 2010; Faille et al. 2013). There is even increasing evidence that some stygobiont species have even persisted beneath continental ice sheets (Taylor and Niemiller 2016). Hence, the climatic conditions favourable to trogloneogenesis may have been quite varied.

Despite the advances mentioned above, the age of hypogean fauna remains, as always, 'contentious' (Humphreys 2000a), especially in respect of the hypothesis that intercontinental vicariance results from the migration of tectonic plates. This is despite evidence that microplate dispersal of subterranean lineages occurred in the Miocene between Sardinia and Iberia at ~33 Mya (Juberthie 1988; Sbordoni 1982;

Ribera et al. 2010; Faille et al. 2011, 2013). The contentious area is the proposed Mesozoic vicariance in the Tethys Sea (Wagner 1994), a debate now largely associated with the core anchialine higher taxa of crustaceans such as remipedes, thermosbaenaceans, some atyid shrimps (*Typhlatya* and close relatives) and some thaumatocyprid ostracods (Iglikowska and Boxshall 2013).

Molecular phylogenies of varying coverage, taxon sampling density and gene spread have been proffered as evidence pertinent to the hypothesis of intercontinental vicariance in anchialine stygobionts. Some proponents of intercontinental vicariance (fish: Chakrabarty et al. 2012) have been challenged on the basis of inappropriate calibration (De Bruyn et al. 2013) and molecular evidence (Larson et al. 2013). Others (amphipods: Bauzà-Ribot et al. 2012) on the basis of calibration, modelling rates across branches and substitution saturation (Phillips et al. 2013). These critiques all suggest much shorter estimates of divergence times that are inconsistent with the intercontinental vicariance proposed criticisms that have been counterargued by Bauzà-Ribot et al. (2013). This debate cannot be resolved with current data and has led to a joint project to reappraisal of the entire evidence using greater taxon sampling and genomic methods (Jurado-Rivera et al. 2017). Resolution of the historical biogeography of anchialine faunas requires a multi-taxon approach to seek congruent temporal and spatial patterns such as was started using allozymes in the 1990s by Adams and Humphreys (1993) (Page et al. 2016) to understand local biogeography. Already, the presence of some core anchialine taxa on isolated seamounts in the Indo-Pacific—*Halosbaena* and *Humphreysella* on Christmas Island (Humphreys and Danielopol 2005; Page et al. 2016) and *Halosbaena* on Minamidaitōjima, Ryukyu Islands, Japan (Shimomura and Fujita 2009)—the topology of phylogenies (Iglikowska and Boxshall 2013; Hoenemann et al. 2013) and the age of some taxa (Botello et al. 2012; Page et al. 2008, 2016) indicate that the resolution will be multifactorial. The common ecology of at least part of the 'core' anchialine taxa may prove to be a result of post-drift community assembly over a long period.

24.21 Conservation Ecology

Conservation ecology is dependent on the information about the number and distribution of species to inform, amongst other things, assessment of endangerment (IUCN Red List of Threatened Species listings; Vié et al. 2008; see also Chap. 22), as well as the establishment and management of protected areas to sustain communities and ecosystems (Michel et al. 2009). The Global Biodiversity Information (GBIF) is capturing records and species across the globe for open access (www.gbif.org), while the World Register of Marine Species (WoRMS) (www.marinespecies.org) is developing a subset comprising the fauna of marine caves (WoRCS) (www.marinespecies.org/about.php). Several other areas are progressing that pertain specifically to subterranean ecology.

Regional knowledge has advanced through, for example, the compilation of existing information (Culver et al. 2003) and by its rigorous analysis (Zagmajster et al. 2014). In addition, de novo regional surveys of stygobiont species have been conducted at both regional (Pilbara, Australia; Halse et al. 2014) and international scales (six Western European countries, PASCALIS project; Gibert and Culver 2009), although with very different taxonomic rigour owing to differential maturity of subterranean biology research between the regions. These exemplars can inform researchers planning regional studies elsewhere because in most parts of the world, regional knowledge of subterranean biodiversity is largely lacking, and they would benefit from standardisation to allow comparison (Culver et al. 2012b).

The corollary to regional surveys is what species are present and how comprehensive is taxonomic knowledge for the subterranean fauna in a region. Such knowledge varies massively between regions, especially between hemispheres. In Europe and the USA, there is a high level of knowledge owing to a long history of subterranean biology research although many undescribed species are still being recovered (Zakšek et al. 2009). By comparison, in Australia, where such studies are recent, very large numbers of unknown taxa are being found continually (Guzik et al. 2010; Eberhard and Giachino 2011) often recognised initially from DNA studies (Javidkar et al. 2015, 2018).

Owing to convergent morphologies associated with adaptation to subterranean life, both troglobionts and stygobionts seem to be especially rich in cryptic species, one of the reasons that molecular methods have routinely been used to provide distribution limits of putative troglobiont species for environmental impact assessment in Western Australia. Although the proportion of cryptic species does not seem to vary between the major metazoan taxa (Pfenninger and Schwenk 2007), it is not known whether the proportion of cryptic species is related to habitat, in this context, between epigean and subterranean members of taxonomic lineages or between interstitial and free-swimming members of a lineage. This is significant because it could affect key information in conservation ecology and influence how conservation effort is allocated. However, any such analysis would need to be treated with caution because cryptic species are commonly determined as a result of molecular studies (Zakšek et al. 2009; Zhang and Li 2014), but whether species are considered cryptic (e.g. King et al. 2012; Karanovic et al. 2016), indeed, whether a taxonomic type II error (mistakenly not recognising that more than one species is present) places species in synonymy, is dependent on the adequacy of taxonomic knowledge; where taxonomic knowledge is weak, taxon-focused conservation efforts must prioritise modern species delimitation research (Hedin 2015).

24.22 Be Alert for Opportunistic Experiments

The information available from background monitoring of subterranean ecosystems allows unexpected or episodic events (Boero 1996) to be used to explore additional attributes of a system, as a ‘natural experiment’ not otherwise possible, such as the

Episodic Events: Great Lakes Experiment (e.g. Chen et al. 2004). I provide a subterranean example from each of an aquatic and terrestrial system. Major earthquakes may markedly affect groundwater, including large and sustained changes to water level and water quality, even a 1000s km distant from the epicentre (Sneed et al. 2003), both of which may impact on groundwater communities. The only detailed study of an earthquake on an aquifer community is from the karstic Gran Sasso Aquifer following the 2009 L'Aquila earthquake, Italy; the aquifer subsequently became almost devoid of stygobiont life, markedly reducing the distribution and abundance of obligate groundwater copepod species, but not of epigean species (Galassi et al. 2014). In a terrestrial context, the monitored decline of populations of several species of tropical troglobionts in an episodically recharged cave in arid Australia provided an opportunity to establish an experiment to determine the effect of water and organic matter on the re-establishment of populations of several species in the cave (Humphreys 1991). These studies were possible only because there had been detailed monitoring of the fauna and its environment prior to the event that provided the opportunity. Such background information would be a natural outcome of the establishment of subterranean biodiversity monitoring systems on permanent plots, as has been widely undertaken on epigean ecosystems worldwide, a component of the implementation of the Convention on Biological Diversity (Dogsé 1998).

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Glossary

Aa lava High-viscosity, lumpy lava flow

Adaptive shift hypothesis Which supports that parapatric speciation occurs in the face of gene flow, with divergent selection between cave and surface habitats driving differentiation

Admixed In population genetics, describing the state of formerly isolated populations that started interbreeding

Aesthetascs A small cuticular process with a chemosensory function placed on the first antennae of most crustaceans

Allele Any kind of variant of the genetic information at a given genetic locus, from single nucleotide to entire chromosomic regions on two homologous chromosomes. Most commonly to denote different genes found at the same position (locus) on homologous chromosomes in a single individual or in a population of individuals

Allochthonous Having an origin distant from the site it was found (here, external to the cave environment)

Allopatric Used of taxa occupying different, mutually exclusive geographical areas

Allozyme Allelic variant of an enzyme, typically distinguished from other such variants by difference in electrophoretic mobility; analogues of alleles at the level of enzymes

Alluvial Comprised of or related to alluvium

Alluvium Unconsolidated sediments deposited by streams, usually consisting of silt, sand, gravel and detrital material

Autotrophy The synthesis of organic carbon from CO_2, using energy from inorganic chemical reactions

Biofilm A microbial community that adheres to itself and surfaces through the formation of a sticky, extracellular substance

Biomass Total quantity or weight of organisms in a given area or volume, at a specific point in time (e.g. mg dry mass m^{-2})

Biospeleology (speleobiology, cave biology) The science that studies living organisms in caves, their origin, phylogeny, adaptations, ecology, distribution, etc.

O. T. Moldovan et al. (eds.), *Cave Ecology*, Ecological Studies 235,
https://doi.org/10.1007/978-3-319-98852-8

Boulder choke A place where the cave passage is blocked by coarse, often breakdown, blocks; similarly, a silt choke consists of very fine-grained material

Breccia Rock formed by predominantly angular clasts of several sizes that predominate on the finer matrix

Breccia pipe A collapsed cave roof that has been extended upwards by stoping, until all of the original cave volume is filled with breccia material; breccia pipes may penetrate through all kinds of overlying rocks and eventually reach the surface

Canga formations Wide coverings of iron ore breccias located on the iron formations and that function as a support element on tops of dissected plateaus

Carbon dioxide equilibria Chemical equilibria between carbon dioxide, water and dissolved carbonate

Cavernicole An organism that lives in caves

Chemolithoautotrophy A metabolic mode of inorganic substrates which gives energy to an organism from light-independent chemical reactions and enables biochemical fixation of carbon dioxide for most or all organism's carbon requirements

Chemolithotrophy Metabolic energy production from electrons obtained from the oxidation of inorganic compounds derived from rocks

Chemosynthesis The synthesis of organic compounds in the absence of sunlight in which the energy is derived from inorganic compounds

Chlorophyll *a* The main photosynthetic pigment in oxygenic phototrophs which has two absorption maxima: in the violet part of the spectrum at 440 nm (γ peak) and in the red part of the spectrum at 680 nm (α peak)

Chloroplast A semi-autonomous and double-membrane organelle known as plastid containing chlorophyll and also various accessory pigments in the cells of eukaryotic photosynthetic organisms

Chlorosis The loss or disintegration of chlorophyll in the tissue of a plant which commonly appears yellowish due to various causes, e.g. microbial infection, activity of phytotoxins, deficiency of light, magnesium or iron

Climatic relict hypothesis Which invokes allopatric speciation after preadapted species become stranded in caves during climatic change

Cloned The process of placing a piece of genetic information (usually a gene) into a vector to allow multiple copies to be made for sequence analysis

Colluvial Comprised of or related to colluvium

Colluvium Unconsolidated sediments deposited at the base of hillslopes or in depressions through sheetwash or downslope creep; they may consist of silt, sand, gravel and rocks

Congelation ice It forms as water freezes on the bottom of the ice cover, and the latent heat of crystallization is conducted upwards through the ice and snow to the atmosphere. It forms as a stable ice sheet with a smooth bottom surface and may develop to massive sheets of different thicknesses that mantle bottoms of ice caves. Ice of this type occurs in caves with dynamic microclimates

Contig A contiguous piece of genetic information assembled from overlapping, smaller pieces of genetic information acquired through DNA sequencing

Convergent evolution As habitually understood, when in two species a character evolves independently from different ancestral states to a similar derived state under the same environmental or ecological conditions. For example, the independent development of an aphaenopsian habitus in two independent lineages of beetles from respective ancestors with different body shapes or the development of fins in cetaceans and fish

Core microbiome The microbial community (along with its genetic information) that remains invariant between ecosystems found in the same conditions

Diastrophism It designates tectonism (orogenic, epirogenetic and tafrogenetic tectonics), affecting the terrestrial crust and resulting in the formation of geological basins, mountain chains, folds, faults, fractures, joints, etc.

Doline From Slavic languages "dolina" = small valley. Technical term for all closed depressions in karst formed through dissolution, suffusion of unconsolidated sediment or collapsing a cave roof. Dolines are important interfaces between surface and underground habitats. The American term sinkhole is used on any kind of hole formed by sinking ground

Ectotherm An animal whose body temperature depends on external sources of heat, in contrast to endotherms

Endoparasite An internal parasite

Epibenthic An organism that lives on the surface of sediments at the bottom of water

Epibiontic An organism that lives attached to another organism without benefit or detriment for the host

Epigean An organism living above the ground, on the surface—in contrast to subterranean or below the ground organisms

Epigenic caves Caves formed by meteoric water, primarily in the vadose zone

Epikarst A weathered zone of enhanced porosity near the surface, at the soil/bedrock contact of many karst landscapes

Ferricrete duricrust Referred to as hardcap in the Pilbara region of Western Australia; it is similar to laterite and consists of a hardened layer where soil particles are cemented by iron oxides released by leaching of underlying iron formations

Genomic sequencing Sequencing of the entire genetic information within a cell, including the chromosomes and extrachromosomal genetic information (such as plasmids)

Georeferencing Defining the spatial position of the locality with coordinates

Glacier caves The caves formed in glaciers by melting water along crevices in the ice and along its contact with bedrock; some glacier caves form by volcanic heat released at fumaroles

Grana (singular: granum) A stack of thylakoids in chloroplasts containing a number of flattened membranous sacks interconnected by membranous bridges to adjacent thylakoids

Hygrophilous An organism living in moist and wet habitats

Hypogean Subterranean, underground

Hypogenic caves Caves formed by rising groundwater or production of deep-seated solutional aggressiveness

Hypogenic speleogenesis The formation of solution-enlarged permeability structures by waters ascending to a cave-forming zone from below, where deeper groundwaters in regional or intermediate flow systems interact with shallower and local groundwater flow systems

Hyporheic Refers to the zone of saturated sediments below the river bed that is usually viewed as an ecotone between the stream and underlying groundwater

Hyporheos The assemblage of organisms living in the hyporheic zone

Internode A section of stem at plants responsible for transport of water, hormones and other necessary compounds to nodes

Interstitial habitat Small spaces filled with water between grains of sand found in sediments below lakes and wetlands, gravel bars in rivers and sand below streams

Itabirites A finely stratified metamorphic rock with iron contents of 50–55%; it has a banded structure composed of silica and iron

Karren and grikes (lapiés or lapiaz) Weathered and exposed limestone surfaces found in karst regions and consisting of different forms of rock pinnacles separated by deep grooves

Karst A terrain formed by the dissolution of soluble rocks such as limestone, dolomite and gypsum and characterized by rocky ground, caves, sinkholes, underground rivers, etc.

Lavicole An organism living exclusively on barren lavas and feeding on fallout of aerial plankton

Macroecology Discipline of ecology that studies patterns and processes on large spatial scales

Macroevolution Major evolutionary change regarding the evolution of whole taxonomic groups over long periods of time

Meiofauna Small benthic invertebrates that live in both marine and fresh water environments; organisms that can pass through a 1 mm mesh but will be retained by a 45 μm mesh

Microarthropods Small invertebrates (<2 mm) in the phylum Arthropoda, the most frequent microarthropods are mites (Acari) and springtails (Collembola)

Microfauna Animals with the body size up to 0.2 mm such as nematodes, rotifers and small arthropods, also covering protozoans (protist kingdom)

Microbivorous An animal eating microorganism, such as bacteria, algae and microfungi

Mesa Flat-topped hill

Metadata Data about data; information on the scope of the data, geographic area, geographic projections, etc.

Metallophilic savannah Type of vegetation occurring in the plateaus of the Amazonian ferruginous formations, represented by a gradient of open brush vegetation with intense deciduousness, resembling semiarid vegetation

Metaproteomics The study of all protein samples recovered directly from environmental samples

Metatranscriptomics The study of the function and activity of the complete set of transcripts (RNA-seq) from environmental samples

Meteoric water Water from the earth surface water cycle, in contrast to endogenic or hypogenic water that originates from the mantle

Microfabrics Structure or arrangement of mineral and organic particles on a microscopic scale

Microphthalmic With reduced eye development, which can be functional or not; in comparison with related species having well-developed, large eyes

Microsatellite A stretch of DNA consisting of a repetition of short nucleotide motifs of up to six nucleotides; the number of repeated motifs is highly variable. Because microsatellites at a given genetic locus behave as alleles that can be distinguished by length, they are used as genetic markers at the level of individual organisms, population or very closely related species

Morphocytological adaptations Changes in a plant's morphology and cytology to enable survival in a certain habitat or a response to a certain stimulus, which includes macroscopic changes such as elongation of stems, leaves and internodes, fewer leaves and microscopic changes such as weakening of cell walls and alterations of chloroplasts, grana and thylakoids

MSS "Milieu souterrain superficiel", superficial underground compartment (also "mesovoid shallow substratum", "upper hypogean zone", or—in part—"subterranean shallow environments"). Originally, the network of empty voids and cracks found at the junction of the soil and rock layer, generally covered by topsoil and with environmental conditions similar to those of deeper fissures or caves

Neoteny Retention of some larval or immature characters by adults in a species

Nivicolous An organism that occurs on snow or in its proximity

Notch A geomorphological term for a V-shaped cut or hole in a surface, which formation probably includes a combination of chemical, physical and biological processes

Oligotrophic Derived from the Greek "little to eat" to describe nutrient-limited environments with less than 0.2 mg/L of organic carbon

Ombrophilous A plant capable of thriving or withstanding in areas with wet and rain conditions

Omnivorous An animal combining different food sources, i.e. decaying organic material, carcasses, microorganisms, living plant tissues and liquids, animals as prey, etc.; feeding on a variety of food of both plant and animal origin

Orthogenesis The development of a fixed evolutionary program independently of the particular environmental or ecological conditions experienced by the species. This fix program can be similar in different lineages, resulting in parallel or convergent evolution

Panmictic Describing the state of unrestricted random mating within a population

Pahoehoe lava Low-viscosity lava that flows like a river ("rope lava")

Parallel evolution As habitually understood, when in two species a character evolves independently from a similar ancestral state to a similar derived state under the same environmental or ecological conditions; for example, the independent development of elongated appendages in Leptodirini beetles from related ancestral species with the same plesiomorphic condition or the development of wings in bats and pterosaurs from the anterior extremities

PCR amplification Amplification of DNA sequences using the polymerase chain reaction

Percolation Slow gravitational movement of water through intergranular pores or rock joints

Phanerophyte A plant with perennating buds well above the surface of the ground, typically referred to trees, shrubs or climbing plants

Photoneutral An organism unaffected by light

Photophilic An organism receptive to, that seeks or thrives in light

Photophobic An organism intolerant to or avoiding light

Phreatic zone The zone below the water table where all spaces are saturated with water

Phylogenetic diversity (PD) Diversity of the species that includes information on phylogenetic relations among them; it can be calculated either per site (alpha PD) or as a measure of comparing species assemblies (beta PD)

Phylogeny A genetic family tree (or phylogram) that describes the evolutionary history between groups of organisms

Phylotype An organism identified (or grouped) by its genetic 16S rRNA gene sequence; phylotypes differ from species in that, while a species has been successfully cultured and classified within the laboratory, a phylotype is only identified through its DNA sequence

Phylum (pl. phyla) A level of classification or taxonomic rank below kingdom and above class

Physiography The study of landforms, synonymous with geomorphology; physiography of a cave refers to its formation, morphology and internal environment

Primer A small fragment of single-stranded DNA that is used to determine the start sites for PCR amplification

Productivity The rate of production of new biomass by an individual, population or community in a given area or volume over a specified time period (e.g. mg dry mass m^{-2} $year^{-1}$)

Protonema (pl. protonemata) A filamentous and branched multicellular structure formed after spore germination which represents the juvenile gametophyte in a life cycle of mosses and liverworts

Pteridophyte In the valid nomenclature, an informal denomination adopted from older literature for flowerless and seedless vascular plant which reproduces with spores referred as Pteridophyta (ferns)

Raphe A longitudinal fissure through the valve in some pennate diatoms (Bacillariophyceae) involved in gliding locomotion

Redox active compounds Compounds with valency states that makes them likely to participate in energetic oxidation/reduction reactions

Regelation The phenomenon of ice melting under pressure and freezing again when the pressure is reduced

Rhizophagous Feeding on roots

Sapsucking Feeding on sap

Sensu lato In taxonomy used to denote the named taxon along with additional groups usually related or confused with the name, in contrast with sensu stricto which refers to only the precise taxon

Sequencing The process of decoding the individual base nucleotide sequence of DNA

Single nucleotide polymorphism (SNP) Allelic variation of a single nucleotide position homologous between chromosomes in an individual or a population of individuals

Speleogenesis The process of cave formation

Streamsink A place where a surface stream enters a cave

Stygal Having the characteristics of stygofauna

Stygobiont/stygobite An animal that lives only in groundwater

Stygofauna A general term covering all animals living in groundwater associated with caves, streams and the broad landscape

Stygophile An animal that lives both on the surface and in groundwater habitats

Stygoxene An animal that is only rarely living in groundwater habitats, accidentally or in search for shelter on short periods of time

Subglacial refuge A habitat under an ice sheet that preserves aquatic subterranean fauna during climate changing periods

Sulphurous ecosystems An ecosystem that exists and functions in sulphurous waters or depends on sulphurous waters

Syphon Section of a cave passage that is completely filled with water

Taxon (pl. taxa) The group of one or more populations that is considered as a taxonomical unit; it can be a species, an order, a class, etc.

Thylakoid A flat, disc-like vesicle resembling a coin, bounded on each side by double-plasma membranes, which run close to each other and contain photosynthetic pigments

Transposon An element of DNA that, through the use of enzymes, can jump around (transpose) within a DNA sequence

Troglobiont/troglobite An animal that lives only in terrestrial subterranean habitats

Troglofauna A general term covering all air-breathing (terrestrial) animals living underground in caves and in the subterranean matrix of the broad landscape

Troglomorphism (troglobiomorphism) The morphological adaptation of an animal characterized by loss of pigment, reduced eye structures or blindness, elongated body and appendages, etc.

Troglophile An animal that can live both on the surface and in subterranean habitats

Trogloxene An animal that is only rarely entering caves, accidentally or in search for shelter on short periods of time

Trophic webs The connection between different food chains (one-way flow of trophic relationships) in a biological community

Vadose zone The unsaturated zone of the groundwater environment between the surface and the phreatic—saturated zone

Venturi effect Pumping effect around a fluid jet

Vicariants Closely related taxa isolated geographically from one another by a biogeographical barrier

Vuggy Refers to rocks containing cavities, voids or large pores

Weathering Set of combined chemical, physical and/or biological processes of disintegration, degradation and decomposition of rocks caused by several agents near the surface of the earth's crust

Index

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