Collecting eco-evolutionary data in the dark: Impediments to subterranean research and how to overcome them

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ABSTRACT

(1) Caves and other subterranean habitats fulfil the requirements of experimental model systems to address general questions in ecology and evolution. Yet, the harsh working conditions of these environments and the uniqueness of the subterranean organisms have challenged most attempts to pursuit standardized research.

(2) Two main obstacles have synergistically hampered previous attempts. First, there is a *habitat impediment* related to the objective difficulties of exploring subterranean habitats and our inability to access the network of fissures that represent the elective habitat for the so-called "cave species." Second, there is a *biological impediment* illustrated by the rarity of most subterranean species and their low physiological tolerance, often limiting sample size and complicating lab experiments.

(3) We explore the advantages and disadvantages of four general experimental setups (*in-situ, quasi in-situ*, *ex-situ*, and *in-silico*) in the light of habitat and biological impediments. We also discuss the potential of indirect approaches to research. Furthermore, using bibliometric data, we provide a quantitative overview of the model organisms that scientists have exploited in the study of subterranean life.

(4) Our over-arching goal is to promote caves as model systems where one can perform standardised scientific research. This is important not only to achieve an in-depth understanding of the functioning of subterranean ecosystems but also to fully exploit their long-discussed potential in addressing general scientific questions with implications beyond the boundaries of this discipline.

Keywords: Anchialine; *Asellus aquaticus*; *Astyanax*; Cave laboratory; Computer simulations; Experimental design; Groundwater; Model system; Non-model organisms; Natural laboratory; Sampling strategy; Stygobite; Troglobite

INTRODUCTION

For a *Homo sapiens*—a clumsy vertebrate inhabiting a primarily lighted world—to enter a cave is enterprising. As the sunlight fades, the air becomes moist, and a maze of passages opens in front of us, our first instinct as humans is to dismiss the subsurface world as one of the most inhospitable environments on Earth. Mentions to this apparent extremeness emerge in most caving stories (MacNeil & Brcic, 2017) insofar as speleology is indeed physically demanding and potentially hazardous (Zagmajster, Culver, Christman, & Sket, 2010). However, by over-emphasizing this anthropocentric view of caves, we tend to dismiss a different reality: caves are not so extreme from the perspective of the eyeless and depigmented organisms that have adapted to living in darkness, which in contrast experience the exposure to sunlight and the wide climatic fluctuation of the outside world as harmful threats (Mammola, 2020). Interestingly, this dichotomous interpretation has framed the two main approaches followed by researchers over recent years: those who have studied caves as unique entities versus those who, in search of a deeper understanding, have established them as general model systems to answer broad scientific questions (Martinez & Mammola, 2020).

Scientists across several generations have been aware of the potential of caves as eco-evolutionary models (Poulson & White, 1969), developing innovative methodologies and creative experimental designs to face the challenges associated with subterranean exploration. Thanks to these efforts, we have been able to tackle important subjects in ecology (Mammola, 2019), ethology (Parzefall, 1982), and evolution (Juan, Guzik, Jaume, & Cooper, 2010), ultimately reaching conclusions relevant to disciplines as diverse as medicine (Riddle et al., 2018; Stockdale et al., 2018; Yoshizawa et al., 2018), engineering (Lepore, Marchioro, Isaia, Buehler, & Pugno, 2012), and exobiology (Northup et al., 2011). Under this perspective, and despite the numerous obstacles to research, subterranean habitats may well qualify as frontiers for modern scientific research (Mammola et al., 2020).

In this work, we discuss the main impediments that we must address to standardize research in subterranean ecosystems and, subsequently, we illustrate old solutions, recommend best practices, and advance new frontiers to approach subterranean-based studies (Figure 1). By further elaborating on the established model organisms in subterranean biology, we seek to promote caves and other subterranean ecosystems (Box 1) as experimental arenas for asking general questions in ecology, ethology, evolution, and beyond.

RATIONALE FOR THIS WORK AND COMPLEMENTARY REVIEWS

It is impossible to cover all methods in subterranean biology while keeping this review tight and comprehensible. Therefore, we decided to focus on the main challenges related to subterranean research and the philosophy underlying the different experimental designs suited to overcome these: two aspects only marginally discussed in the recent literature. Readers interested in other aspects of cave research are referred to the classic review on bio-monitoring (Culver & Sket, 2002) and published syntheses on sampling approaches (Weinstein & Slaney, 1995; Dole-Olivier et al., 2009; Wynne, Sommer, Howarth, Dickson, & Voyles, 2018; Wynne, Howarth, Sommer, & Dickson, 2019; Lunghi, Corti, et al., 2020), species distribution modelling (Mammola & Leroy, 2018), and best practices in experimental trials (Di Lorenzo et al., 2019). Sampling techniques in non-cave subterranean habitats (see Box 1) have also been reviewed elsewhere—e.g., boreholes (Hancock & Boulton, 2009), epikarst (Brancelj, 2004), subaquatic caves (Iliffe & Bowen, 2001; Iliffe, 2018), *Milieu Souterrain Superficiel* (Mammola et al., 2016), hyporheic (Fraser & Williams, 1997), interstitial habitats in coastal marine and lotic environments (Schmidt-Rhaesa, 2020).

Box 1. A modern definition of subterranean habitats and implications for subterranean studies.

The term “subterranean habitat” is often used as a synonym for “cave” (Poulson & White, 1969; Mammola, 2019). However, scientists have become aware that terrestrial caves represent only a small fraction of the total habitat available to the subterranean fauna. More precisely, subterranean habitats comprise the breadth of underground voids of different sizes, either dry or filled with water, sharing two main ecological features: darkness and buffered climatic conditions (Culver & Pipan, 2019). These voids may open a few centimetres below ground level (Culver & Pipan, 2014) or descend several kilometres towards areas where the environmental conditions exceed the limits of life (Fišer, Pipan, & Culver, 2014). They are widespread on all continents, having been documented from different geological substrates, mostly limestone but also lava fields and unconsolidated sediments. In summary, the cavities that we can access and explore by entering them represent just the tip of the iceberg of what lies below our feet (Ficetola, Canedoli, & Stoch, 2019; Mammola, Cardoso, et al., 2019).

Even though subterranean habitats are more widespread and diversified than it is usually recognised, subterranean research started with field observations in human-accessible habitats (different types of terrestrial caves, artificial subterranean habitats such as mines and bunkers, lava tubes, cenotes, etc.), later encompassed pumped water (e.g. from drinking water wells), and only then extended to other difficult-to-access voids. Even today, there is still a significant research bias towards human-accessible habitats, which should always be kept in mind. In a nutshell, it implies that we may have to relativize part of the information available to date, i.e. being aware that we have mostly documented how animals behave in cave-like environments, rather than in the extended network of fissures.

CHALLENGES TO SUBTERRANEAN RESEARCH

Habitat impediment

Whereas several different habitats have been categorized as subterranean (Box 1), most in-field research takes place in caves, mines, and other human-accessible voids. These are always dark, often muddy and humid, and sometimes even very cold, hence not offering favourable conditions to perform extensive, standardized observations (MacNeil & Brcic, 2017). There are often high ceilings, narrow fissures, and other geomorphological features that hamper the task of approaching and observing target animals without them being disturbed by light or by the presence of the researcher (CO_2, heat, vibrations, or even diver's bubbles in the case of submerged passages). Furthermore, cave exploration requires well trained researchers mastering the use of speleological equipment (Zagmajster et al., 2010). Even more challenging, in this sense, are those studies set in submerged passages of freshwater and marine caves (Exley, 1983; Iliffe & Bowen, 2001), as testified by the frequent fatalities associated with cave diving (Buzzacott, Zeigler, Denoble, & Vann, 2009).

Given our human size, we can directly access only a small fraction of the habitats available to the subterranean fauna. As noted by Howarth (1983; p. 380), this is a significant obstacle to scientific research because, more often than not, we cannot directly inspect the extended network of fissures "[...] *where probably the major drama in the cave ecosystem occurs"*. In a way, caves and other human-accessible habitats may act as surrogates of the subterranean world in its entirety, windows allowing us to glimpse what usually happens away from human sight (Wilkens, Parzefall, & Iliffe, 1986; Uéno, 1987; Polak, 1997; Mammola et al., 2016). Yet, in this case the existence of a habitat bias should be clearly acknowledged. For instance, we must be aware that the foraging behaviour of a centipede that we have observed in a large chamber of a cave may not replicate in the same way—or may not even take place at all!—when the exact same centipede is dwelling in the millimetric fissures connected with the chamber. Not to mention certain typically benthic aquatic animals that have been spotted in the water column of flooded caves only after the disturbance produced by the divers (Humphreys, Poole, Eberhard, & Warren, 1999).

As a corollary, however, it must be noted that a number of organisms primarily belong to human-accessible cavities (Moseley, 2009) and *ipso facto* are more readily studied (Mammola, 2019). Classic examples are vertebrates with a centimetric body size, such as cave-roosting bats and groundwater fishes, but also the parasites and commensals associated with them (Lunghi, Ficetola, et al., 2018) or the scavengers that feed upon their carcasses and faeces (Ferreira & Martins, 1999). There are also subterranean invertebrates constrained to human-sized voids by their extended phenotypes; notably, different species of orb spiders needing larger voids for web construction (Mammola & Isaia, 2017) or aquatic suspension feeders adapted to drift in the still water column of anchialine caves (Koenemann, Schram, Iliffe, Hinderstein, & Bloechl, 2007; Martínez, Kvindebjerg, Iliffe, & Worsaae, 2017).

Biological impediment

In several cases, the biology of subterranean species represents a further impediment to research. In general, food-deprived subterranean environments select for long-lived species with low metabolism and small numbers of offspring. As a consequence, the density of individuals of subterranean adapted species is often low—it is not unusual that such species were observed once at the time of their description, and never recorded thereafter (Martínez, Di Domenico, & Worsaae, 2013; Delić & Sket, 2015; Manenti et al., 2018). Also, specialized subterranean species are often unevenly distributed in space and time, mostly because they aggregate around the scarce and heterogeneously distributed food sources (Culver & Sket, 2002). These difficulties in finding sufficient individuals for experiments or *in-situ* observations may result in studies with a reduced sample size and less robust data. This may explain why the ecology and behaviour of many subterranean organisms is documented, at best, anecdotally thanks to casual observations.

Furthermore, many specialised subterranean organisms live in environments showing constant and buffered conditions and, over evolutionary time, have reduced their resilience against environmental fluctuations. For example, some terrestrial subterranean species are threatened by the smallest variations in air moisture content (Howarth, 1983), whereas aquatic animals may perish upon changes in pH driven by the water exposure to the air (Carpenter, 1999). Similarly, many terrestrial and aquatic obligate subterranean species survive only within narrow temperature ranges (Mermillod-Blondin et al., 2013; Mammola, Piano, Malard, Vernon, & Isaia, 2019; Pallarés, Colado, et al., 2020; Pallarés, Sanchez-Hernandez, et al., 2020). This limited physiological plasticity may pose a real challenge when a researcher is aiming to conduct experiments in the unnatural conditions of a typical laboratory. Maintaining living individuals of most of these animals is not a trivial task: breeding them requires skill and experience, in-depth knowledge of their biology and, often, none negligible doses of luck.

EXPERIMENTAL SETUPS

The most classical and intuitive way to learn about subterranean organisms lies in quantitative observational studies, either in the field (*in-situ*), under laboratory conditions (*ex-situ*) or, when available, in laboratories set within caves (hereafter *quasi in-situ*). An experimental setup entirely based on simulations—*in silico*—could also be adopted. The choice amongst these setups is not always straightforward. In general, choosing between alternative options is a trade-off between the biological realism of the observations and either the ease or the extensiveness of study (Figure 2). More detailed pros and cons of each setup are discussed in the followings.

In-situ

The *in-situ* approach provides the least artefactual representation of the ecology, physiology, and behaviour of the target species. Yet, this approach forces the researcher to comply with both the habitat (harsh working conditions and impossibility of exploring inaccessible habitats) and the biological (low density of most subterranean species) impediments. To minimise these impediments, a careful selection of the study site is critical. If possible, one should favour cavities with a linear development and reduced habitat complexity, thereby facilitating standardised observations (Smithers, 2005; Mammola & Isaia, 2018; Lunghi, Corti, et al., 2020) while maximising detectability of the animals (Lunghi, 2018). In the same vein, studying aquatic target species in a semi-submerged or shallow passageway not only increases permanence times and minimizes decompression procedures, but also maximizes safety (Iliffe, 2018).

It must be noted that *in-situ* studies can be carried out exclusively in cavities the researcher can enter herself or at least insert instruments into. There are different types of traps and sampling devices that allow us to indirectly collect the fauna in inaccessible and interstitial habitats or even tools for detecting the presence of a species indirectly (see section 'Indirect means of research'). Conversely, *in-situ* observations are virtually impossible for porous groundwater, forcing researchers to heavily rely on laboratories studies (e.g., Di Lorenzo et al., 2014).

Ex-situ

The use of a meso- or micro-cosmos replicating the species' natural habitat allow us to bypass the habitat impediment in its entirety. In general, obtaining standardised observations in controlled conditions enhance a greater replicability of the results. Furthermore, *ex-situ* approaches permit to explore the life history of those animals that prefer inaccessible habitat, or that are too small to be observed with the naked eye. Yet, by choosing an *ex-situ* approach, the researcher needs to comply with the biological impediment of maintaining specialised and delicate organisms in the laboratory (Di Lorenzo et al., 2019), as well as with the local conservation policies for endangered species. This can be circumvented by selecting certain model organisms, often not legally protected and relatively easy to keep in the lab (see section "Model organisms in subterranean biology").

As a drawback, laboratory observations may not accurately reflect the natural traits, especially behavioural and physiological, as shown in the cave (Silva, Oliveira, Bastos-Pereira, & Ferreira, 2018). Although laboratory studies are useful, the *ex-situ* conditions rarely resemble those found in the natural habitat (e.g., higher density, different environmental conditions). For well established model organisms, a prolonged *ex-situ* breeding may even produce unwelcome effects such as artificial selection or adaptation to the laboratory conditions (Ross, Endersby-Harshman, & Hoffmann, 2019). This is why observations obtained from studies in the laboratory must be carefully interpreted and preferably confirmed by *in-situ* approaches (Blin et al., 2020). For example, by surveying semi-natural replicas of the sheltered reproductive sites of *Hydromantes* with infrared cameras, Oneto et al. (2010) were able to provide some of the first observational data on their complex reproductive behaviour and parental care. Subsequent observations performed under natural conditions (Lunghi et al., 2014, 2015; Lunghi, Corti, et al., 2018) confirmed the validity of these observations.

Quasi in-situ

The history of subterranean biology teaches us that a *quasi in-situ* approach—*i.e.* to bring the laboratory into the target species' natural habitat—eases many of the problem associated with experimental studies in the lab. Establishing a laboratory within the cave itself not only spare living animals from long transportation away from the cave, but also facilitates fine-regulation of ambient parameters within a microcosmos. The most famous example is probably the *Laboratoire Souterrain de Moulis*, a cave-based laboratory established in the French Pyrenees by René Jeannel (1879–1965) and Albert Vandel (1894–1980). Since its foundation in 1948, this semi-natural experimental setting has aided generations of subterranean biologists in the challenging task of shedding light on the natural history and behaviour of a wide range of elusive subterranean life forms (Durand, 1970; Clergue-Gazeau, 1974; Juberthie, 1985; Juberthie, Durand, & Dupuy, 1996; Manenti, Melotto, Guillaume, Ficetola, & Lunghi, 2020). For aquifers, the equivalent would be to lower sediment, substrate cages, bags into groundwater wells (Schmidt, Hahn, Watson, Woodbury, & Hatton, 2004), which, however, would still have to be retrieved every time to study the organisms.

In-silico

As a consequence of the habitat and biological impediments, cave studies often rely on data that is far from ideal. In a complex subterranean setting, we may lack information on environmental seasonal fluctuations, species abundances across space or time, their physiological rates and life-history traits, or the species they interact with. Not to mention the dependency between observations and the correlation amongst traits (body size and trophic guild, fecundity with longevity, etc.), which often confounds with putative drivers for the process that we aim to disentangle. In those scenarios, simulations, such as agent-based models and cellular automata, are increasingly used to explore the dynamics of natural ecosystems and trigger novel ideas for further exploration in real-world settings (DeAngelis & Grimm, 2014). These mechanistic models rely on so-called ‘first principles’, such as energy budgets, physiology, or fitness seeking (Grimm & Berger, 2016), which define the initial conditions of the simulation so that behaviour and interactions emerge rather than being imposed by the modeller. Given robust enough assumptions, simulations are thus able to realistically replicate sets of empirical patterns without restricting them to a single deterministic scenario (Grimm et al., 2005). For example, the use of eco-evolutionary agent-based models, which include heritable traits and the use of genetic algorithms, provides insights on the evolution of certain morphological, physiological, and behavioural traits (Ayllón et al., 2018).

Surprisingly, however, simulations have rarely been applied in cave biology. Applications to subsurface systems so far have been restricted to porous groundwater, with the focus being mainly on contaminant degradation (Tang, Valocchi, Werth, & Liu, 2013; Benioug, Golfier, Tinet, Buès, & Oltéan, 2015; Benioug et al., 2017; Schmidt, Kreft, Mackay, Picioreanu, & Thullner, 2018; Jung & Meile, 2019), and to soils (Banitz et al., 2013; Kim & Or, 2016; Borer, Ataman, Hatzimanikatis, & Or, 2019). It is easy to see how the simulation of a virtual cave would be an interesting aid to research. Caves may represent ideal model systems for *in-silico* studies due to their constant environmental conditions, which can be easily and predictably simulated, and their simple community structure with few species and limited interactions. For example, these models would allow us to achieve a mechanistic understanding of the processes behind interactions between species within a typical subterranean community, to explore pathways of subterranean evolution, and even to elucidate the impact of climate change on underground biodiversity.

The applicability of these theoretical models to the real biological world, however, still depends on the quality and availability of data. Parametrization of simulations might be relatively simple for broad questions in spatial or temporal scope, but quite complex for very specific systems, often implying the need for possessing detailed information. Thus, and this is true for other methods as well, the necessity to parametrise theoretical models with the real-world biological observations may require combining simulation approaches with actual fieldwork. Importantly, models may single out those parameters that warrant the most attention, and may thus steer experiments towards focussing on sensitive and critical parameters. A complementary avenue is combining qualitative observations, for example that state changes are confined within a certain interval, for parameterization. Even if a single observation does not contain much information, a combination of several qualitative observations can be as distinctive as a single high-precision observation. This inverse, “pattern-oriented” parameterization (Wiegand, Revilla, & Knauer, 2004; Grimm et al., 2005) has been proven to be a powerful approach, and overlaps with the more formal Approximate Bayesian Computing approach (Hartig, Calabrese, Reineking, Wiegand, & Huth, 2011).

Indirect means of research

A plethora of indirect methodologies can be used to overcome both the habitat and the biological impediments (Figure 1). These approaches are mostly species- and system-specific, and it is impossible to provide widely general recommendations. Therefore, we here discuss examples chosen to illustrate the concept of ‘indirect research’.

Information about the ecology and behaviour of large-sized animals can be acquired via infra-red video surveillance. This represents a low-cost and low-personnel effort methodology, which has a long tradition in ethological research and biomonitoring (Swann, Hass, Dalton, & Wolf, 2004). In caves, thermal-infra red imaging and laser scanning have been extensively applied to study the swarming and roosting behaviours of bats (Elliott, Kaufmann, Samoray, & Gardner, 2005; Azmy et al., 2012), but could potentially be used for other vertebrates as well, such as cave salamanders (Lunghi et al., 2016). In at least one case, camera trapping has even been used to quantifying wildlife use of cave entrances (Baker, 2015).

Recently, there has also been a great deal of discussion on the use of molecular tools to obtain indirect evidence of the presence and behaviour of species, especially in difficult-to-access habitats. For example, environmental DNA was successfully used to detect the presence of focal subterranean species, such as amphibians (Gorički et al., 2017) and crustaceans (Niemiller et al., 2018; Boyd, Niemiller, Dooley, Nix, & Niemiller, 2020; DiStefano, Ashley, Brewer, Mouser, & Niemiller, 2020). The analysis of gut or stomach content of species inhabiting both human-accessible and interstitial environments provides information on dietary requirements and trophic behaviours taking place in both these compartments (Lunghi, Cianferoni, et al., 2018; Lunghi, Manenti, et al., 2020), but also trophic web studies with aquatic subterranean species (Saccò et al., 2019). These analyses can be done visually, but also through massive sequencing techniques, allowing the identification of the gut content using DNA (Rastorgueff, Rocher, Selva, & Chevaldonné, 2015). Similarly, stable isotopes proved useful to understand species interactions and niche partitioning (Chávez-Solís, Solís, Simões, & Mascaró, 2020), as well as identifying potential carbon sources through space (Brankovits et al., 2017) and time (Saccò et al., 2020).

In some circumstances, the species' extended phenotype also informs indirectly on specific behaviours and ecological needs. The web in web-building spiders, for example, can be viewed as an extended phenotype that enlarges the sensory world of its builder in interaction with the environment (Blamires, 2010). The web also provides a record frozen in time of the spider’s foraging behaviour, as spiders modify their webs in response to a large range of biotic and abiotic stimuli, including previous prey experiences, climatic variables, and the structural complexity of the habitat (Vollrath & Selden, 2007; Hesselberg, 2015). The easily quantifiable two-dimensional orb-web, in particular, is highly suitable for behavioural studies, as orb spiders can easily be maintained in the laboratory (Zschokke & Herberstein, 2005) or their webs measured in the field (Hesselberg, 2010). The ubiquity of orb web spiders near the entrance of temperate caves, makes this approach especially promising (Hesselberg, Simonsen, & Juan, 2019). Likewise, the calcified tubes of several hard-bodied aquatic organisms, such as tube-building polychaetes, bring us information on the evolution of aquatic caves communities and paleoclimate from past geological eras (Moldovan et al., 2011).

The living world has long been used as a source for developing biologically-inspired robots using biomimetics design principles to provide innovative technical solutions (Vincent, Bogatyreva, Bogatyrev, Bowyer, & Pahl, 2006; Pfeifer, Lungarella, & Iida, 2007; Lenau, Metze, & Hesselberg, 2018). In recent years, the use of biorobotic models to test and generate biological hypotheses has been gaining ground (Gravish & Lauder, 2018). Following this recent trend, we propose that the use of small, agile biorobots to explore, record, and interact with subterranean animals in their natural habitats might overcome many of the habitat and biological impediments previously discussed (Woodward & Sitti, 2014). For example, the use of a simple biomimetic robot fish has been successfully used to highlight similarities and differences in social behaviour between surface and cave-dwelling populations of *Poecilia mexicana* (Bierbach et al., 2018).

MODEL ORGANISMS IN SUBTERRANEAN BIOLOGY

Model organisms represent only a small part of Earth’s biodiversity and yet have largely contributed to our knowledge on many fields within the biological sciences (Hedges, 2002).

The earliest models, such as flies, mice, or roundworms, were selected for the task simply because they were small, proliferative, and easy to culture and manipulate; they were, however, quite limiting in advancing many aspects in ecology and evolution. Luckily, the growth of modern molecular methods, staining and imaging techniques, and gene editing, have facilitated choosing more appropriates models for the biological question at hand rather than enforcing the ones that can be easily grown and manipulated (Müller & Grossniklaus, 2010). Consequently, the number of model species has diversified along with the number scientific questions, and now includes representatives of many animal phyla (as well as plants and fungi). This exciting transition in contemporary biology is embodied by the term ‘non-model’ organism, which reflects that the diversity of model species has grown nearly parallel with the diversity of problems addressed (Sullivan, 2015; Goldstein & King, 2016; Russell et al., 2017).

The trend of diversification of model systems and research question is evident in cave biology as well. To comprehend it, we have compiled a list of those subterranean animals that can be considered as model organisms (Table S1). We selected models based on two criteria: i) organisms/groups with accumulated at least 20 papers in the Web of Science (accessed on 25 November 2020); ii) and organisms/groups with at least two independent research labs focusing on them. Our list of model organisms in cave biology includes representatives of three phyla, but it is dominated by Teleostei fish and Crustacea (Figure 3). This reflects the traditional research bias in subterranean biology towards these groups, only partially justified by their dominance across subterranean environments. Only a few of these species satisfy the traditional requirement of a model—successful culturing in the lab and keeping long-standing laboratory breeds (e.g., *Astyanax mexicanus*, *Asellus aquaticus*, and *Poecillia mexicana*). The most famous and studied amongst these is by far the cavefish *Astyanax mexicanus* (Torres-Paz, Hyacinthe, Pierre, & Rétaux, 2018; Jeffery, 2020), which has been kept in captivity for many generations (Wilkens, 1971) and is increasingly used and recognized as suitable for tackling problems beyond the typical cave biology realm (Maher, 2009; McGaugh et al., 2020). Other models thrive in laboratory conditions, but are unable to complete their life cycle therein (e.g., *Gammarus minus,* Australian calcrete Dytiscidae, *Proasellus* spp.). Most models in subterranean biology are lineages with both surface and subterranean populations, or species whose populations exhibit different degrees of subterranean specialization. Among those, *Astyanax mexicanus* and *Asellus aquaticus* are even able to form hybrid offspring between cave and surface morphs (Protas & Jeffery, 2012; Jeffery, 2020).

Alongside every other biological discipline, cave biology research has now entered the genomics era (Friedrich, 2013; Pérez-Moreno, Iliffe, & Bracken-Grissom, 2016). Already half of cave models listed in the Table 3 have been included in genome (transcriptome) sequencing projects, becoming theoretical windows into the molecular basis of adaptation (Barbosa et al., 2017; Berning, Adams, Luc, & Gross, 2019). With the decreasing prices and the development of more user friendly bioinformatic recourses, so-called *-omics* tools will soon be at the forefront of cave research and exploited in the remaining model systems. Such tools may enable overcoming traditional restrictions on the use of cave species as models and we predict that the peculiar, and even bizarre, traits of subterranean animals are going to draw attention from an increasingly wider audience, and possibly attract new researchers into the field (Mammola et al., 2020).

In subterranean biology, the concept of model organism has also been applied to supra-specific lineages widely used to investigate evolutionary processes associated to cave colonization or to answer biogeographic and macroecological questions. Similar studies typically rely on comparative methods within explicit phylogenetic frameworks, allowing us to distinguish the role played by ecological adaptations and evolutionary history on the observed ecological and distribution patterns (Juan et al., 2010). Some of these models account for lineages including both surface and subterranean species exhibiting different degrees of adaptations and ecological preferences, such as *Asellus* (Verovnik, Sket, & Trontelj, 2004), *Niphargus* (Fišer, 2009), *Trechus* (Möst, Donabauer, Arthofer, Schlick-Steiner, & Steiner, 2020), and *Dysdera* (Arnedo, Oromí, Múrria, Macías-Hernández, & Ribera, 2007). Others exclusively consist of subterranean species, such as atyd shrimps of the genera *Typhlatya, Stygiocaris, Speleocaris,* and *Troglocaris* (Zakšek, Sket, Gottstein, Franjević, & Trontejl, 2009; Jurado-Rivera et al., 2017). While lineages in the first group are useful to understand different mechanisms for ecological speciation and habitat shift, subterranean-exclusive lineages allow us to understand the role of historical stochastic processes in cave diversity and biogeography (Juan et al., 2010). In addition, in certain cases a distant surface-dwelling species has been used as a comparison to exclusively subterranean species (e.g., zebrafish for *Phreatichthys andruzzii*, *Gammarus* for *Niphargus*). Although intuitively less ideal, this approach has yielded some important insights, such as the impacts of life in darkness on the circadian clock or DNA repair mechanisms (Cavallari et al., 2011).

Finally, some subterranean species with unique features have been established as models to investigate scientific questions not necessarily related to the classic subterranean research agenda. This is the case of the carnivorous sponge *Lycopodina hypogea,* used as a model for early nervous system evolution and developmental biology (Godefroy et al., 2019);

the crustaceans in the class Remipedia, key to understanding the evolution of terrestrial arthropods (Lozano-Fernandez et al., 2016), as well as the evolution of the nervous system (Stemme et al., 2013) and venoms toxins (von Reumont et al., 2014); or the “forever young” aquatic salamander *Proteus anguinus*, whose progenetic origin and long lifespan has triggered fruitful research on the molecular mechanisms of aging (Voituron, De Fraipont, Issartel, Guillaume, & Clobert, 2011). While those are indeed not the questions that have inspired most cave-based researchers over the years, they serve to illustrate the general idea of our review here: to emphasize that caves, in their uniqueness for humans, still hold the secrets for understanding broad scientific questions (Martinez & Mammola, 2020).

CONCLUSIONS

In this work, we discussed the philosophy of performing research in subterranean ecosystems, by focusing on key impediments, experimental ideas, and model systems. Some take-home messages emerge from this exercise:

1) *Be aware of the many options out there.* Insofar as each subterranean system and organism is unique to some extent, and in light of the impediments to subterranean research, scientists must be creative in designing their experiments. Research in subterranean biology often implies combining traditional *in-situ* field observations with standardised studies in a laboratory setting, either within a cave (*quasi in-situ*) or outside the cave (*ex-situ*). It is also important to be aware of the potential of novel tools, especially simulations, artificial intelligence methods, and biorobotics (Figure 1).

2) *Choose the right model*. Many impediments to subterranean research can be overcome by focusing on model organisms, which have been established owing to their specific traits and/or their broad availability in subterranean environments. Whereas model systems in subterranean biology are probably not as developed as in other disciplines, there are options across the animal tree of life offering great potential for tackling specific research questions (Figure 3). Since a major challenge before fully exploiting a given model is to breed it in the laboratory, it would be worthwhile endeavour to run a wider screening amongst candidate organisms. In this way, a model suitable to answer a given set of questions and able to complete its lifecycle in the lab can be identified.

(3) *Be aware of the taxonomic bias*. As a corollary of the previous point, it is important to remember that our knowledge of subterranean species is still strongly biased in its taxonomical coverage. Even today, the natural history information on subterranean species remains largely fragmented, rarely standardised, and often biased towards the few well-studied model organisms. We stress the importance of broadening eco-evolutionary studies to incorporate a larger range of organisms and subterranean habitats, to explore hypotheses about the emergence of convergent traits and behaviours across distant taxa while accounting for phylogenetic effects.

(4) *Embrace multidisciplinarity*. In light of the habitat and biological impediments, combining ecological and behavioural observations with evolutionary approaches, genetic

tools, and simulations is a critical premise. In the *-omics* era, integrative studies are expected to grow, allowing us to understand which molecular adjustments (including epigenetic effects) occur during the surface-subterranean transitions. This is required, for example, to disentangle the role of standing genetic variation and phenotypic plasticity in driving the evolution of subterranean populations (Bilandžija et al., 2020).

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AUTHOR CONTRIBUTION

SM wrote the first draft. AM and SIS provided most arguments on aquatic habitats. EL and HB provided most arguments on vertebrates. AM, SM, and HB developed the section on model organisms. PC, SIS, and VG developed the *in-silico* section. TH provided expert opinion on different sections, especially on behavioural topics. AM and SM prepared figures. All authors contributed to the writing with comments and additions.

SUPPLEMENTARY MATERIAL

Table S1. **Example of model organisms in subterranean biology.** We selected models based on two criteria: i) Organisms/groups with at least 20 papers listed in the Web Of Science; ii) Organisms/groups with at least two research labs focusing on them.

REFERENCES

Arnedo, M. A., Oromí, P., Múrria, C., Macías-Hernández, N., & Ribera, C. (2007). The dark side of an island radiation<bold>:</bold> systematics and evolution of troglobitic spiders of the genus Dysdera Latreille (Araneae:Dysderidae) in the Canary Islands. *Invertebrate Systematics*, *21*(6), 623–660. Retrieved from https://doi.org/10.1071/IS07015

Ayllón, D., Railsback, S. F., Almodóvar, A., Nicola, G. G., Vincenzi, S., Elvira, B., & Grimm, V. (2018). Eco-evolutionary responses to recreational fishing under different harvest regulations. *Ecology and Evolution*, *8*(19), 9600–9613. doi:10.1002/ece3.4270

Azmy, S. N., Sah, S. A. M., Shafie, N. J., Ariffin, A., Majid, Z., Ismail, M. N. A., & Shamsir, M. S. (2012). Counting in the dark: Non-intrusive laser scanning for population counting and identifying roosting bats. *Scientific Reports*, *2*, 524. doi:10.1038/srep00524

Baker, G. M. (2015). Quantifying wildlife use of cave entrances using remote camera traps. *Journal of Cave and Karst Studies*, *77*, 200–210. doi:10.4311/2015ES0101

Banitz, T., Johst, K., Wick, L. Y., Schamfuß, S., Harms, H., & Frank, K. (2013). Highways versus pipelines: contributions of two fungal transport mechanisms to efficient bioremediation. *Environmental Microbiology Reports*, *5*(2), 211–218. doi:https://doi.org/10.1111/1758-2229.12002

Barbosa, P., Leal, E. V., da Silva, M., de Almeida, M. C., Moreira-Filho, O., & Artoni, R. F. (2017). Variability and evolutionary implications of repetitive DNA dynamics in genome of Astyanax scabripinnis (Teleostei, Characidae). *Comparative Cytogenetics*, *11*(1), 143–162. doi:10.3897/CompCytogen.v11i1.11149

Benioug, M., Golfier, F., Oltéan, C., Buès, M. A., Bahar, T., & Cuny, J. (2017). An immersed boundary-lattice Boltzmann model for biofilm growth in porous media. *Advances in Water Resources*, *107*, 65–82. doi:https://doi.org/10.1016/j.advwatres.2017.06.009

Benioug, M., Golfier, F., Tinet, A.-J., Buès, M. A., & Oltéan, C. (2015). Numerical Efficiency Assessment of IB–LB Method for 3D Pore-Scale Modeling of Flow and Transport. *Transport in Porous Media*, *109*(1), 1–23. doi:10.1007/s11242-015-0497-6

Berning, D., Adams, H., Luc, H., & Gross, J. B. (2019). In-Frame Indel Mutations in the Genome of the Blind Mexican Cavefish, Astyanax mexicanus. *Genome Biology and Evolution*, *11*(9), 2563–2573. doi:10.1093/gbe/evz180

Bierbach, D., Lukas, J., Bergmann, A., Elsner, K., Höhne, L., Weber, C., … Krause, J. (2018). Insights into the social behavior of surface and Cave-Dwelling Fish (Poecilia mexicana) in light and darkness through the use of a Biomimetic robot. *Frontiers Robotics AI*, *5*, 3. doi:10.3389/frobt.2018.00003

Bilandžija, H., Hollifield, B., Steck, M., Meng, G., Ng, M., Koch, A. D., Gračan, R., Ćetković, H., Porter, M.L., Renner, Kenneth J., Jeffery, W. (2020). Phenotypic plasticity as a mechanism of cave colonization and adaptation. *ELife*, *9*, e51830. doi:10.7554/eLife.51830

Blamires, S. J. (2010). Plasticity in extended phenotypes: Orb web architectural responses to variations in prey parameters. *Journal of Experimental Biology*, *213*, 3207–3212. doi:10.1242/jeb.045583

Blin, M., Fumey, J., Lejeune, C., Policarpo, M., Leclercq, J., Père, S., Torres-Paz, J., Pierre, C., Imarazene, B., Rétaux, S. (2020). Diversity of olfactory responses and skills in *Astyanax mexicanus* cavefish populations inhabiting different caves. *Diversity*, *12*, 395. doi:10.3390/d12100395

Borer, B., Ataman, M., Hatzimanikatis, V., & Or, D. (2019). Modeling metabolic networks of individual bacterial agents in heterogeneous and dynamic soil habitats (IndiMeSH). *PLOS Computational Biology*, *15*(6), e1007127. Retrieved from https://doi.org/10.1371/journal.pcbi.1007127

Boyd, S. H., Niemiller, K. D. K., Dooley, K. E., Nix, J., & Niemiller, M. L. (2020). Using environmental DNA methods to survey for rare groundwater fauna: Detection of an endangered endemic cave crayfish in northern Alabama. *PLOS ONE*, *15*(12), e0242741. Retrieved from https://doi.org/10.1371/journal.pone.0242741

Brancelj, A. (2004). Biological sampling methods for epikarst water. In W. K. Jones & et al (Eds.), *Epikarst.* (pp. 99–103). Sheperdstown, WV, USA: Karst Waters Inst. Special Publ.

Brankovits, D., Pohlman, J. W., Niemann, H., Leigh, M. B., Leewis, M. C., Becker, K. W., … Phillips, B. (2017). Methane-and dissolved organic carbon-fueled microbial loop supports a tropical subterranean estuary ecosystem. *Nature Communications*, *8*(1), 1–12. doi:10.1038/s41467-017-01776-x

Buzzacott, P. L., Zeigler, E., Denoble, P., & Vann, R. (2009). American Cave Diving Fatalities 1969-2007. *International Journal of Aquatic Research and Education*, *3*, 162–177. doi:10.25035/ijare.03.02.07

Carpenter, J. H. (1999). Behavior and ecology of Speleonectes epilimnius (Remipedia, Speleonectidae) from surface water of an anchialine cave on San Salvador Island, Bahamas. *Crustaceana*, *72*(8), 979–991. doi:10.1163/156854099503889

Cavallari, N., Frigato, E., Vallone, D., Fröhlich, N., Lopez-Olmeda, J. F., Foà, A., Berti, R., Sánchez-Vázquez, F., Bertolucci, C., Foulkes, N. S. (2011). A blind circadian clock in cavefish reveals that opsins mediate peripheral clock photoreception. *PLoS Biology*, *9*(9), e1001142. doi:10.1371/journal.pbio.1001142

Chávez-Solís, E. M., Solís, C., Simões, N., & Mascaró, M. (2020). Distribution patterns, carbon sources and niche partitioning in cave shrimps (Atyidae: Typhlatya). *Scientific Reports*, *10*(1), 1–16. doi:10.1038/s41598-020-69562-2

Clergue-Gazeau, M. (1974). Reproduction des Urodèles: perturbation du cycle sexuel des Euproctes males en élevage à la Grotte de Moulis. *Memoires de Biospeologie*, *29*, 137–141.

Culver, D. C., & Pipan, T. (2014). *Shallow Subterranean Habitats: Ecology, Evolution, and Convervation*. *Journal of Cave and Karst Studies*. Oxford University Press, USA. doi:10.4311/2014br0127

Culver, D. C., & Pipan, T. (2019). *The biology of caves and other subterranean habitats* (Second edi). Oxford University Press, USA.

Culver, D. C., & Sket, B. (2002). Biological monitoring in caves. *Acta Carsologica*, *31*, 55–64. doi:10.3986/ac.v31i1.403

DeAngelis, D. L., & Grimm, V. (2014). Individual-based models in ecology after four decades. *F1000Prime Reports*, *6*, 39. doi:10.12703/P6-39

Delić, T., & Sket, B. (2015). Found after 60 years: the hows and whys of Sphaeromides virei montenigrina (Crustacea: Isopoda: Cirolanidae) rediscovery in Obodska pećina, Montenegro. *Natura Sloveniae*, *17*, 59–65.

Di Lorenzo, T., Di Marzio, W. D., Fiasca, B., Galassi, D. M. P., Korbel, K., Iepure, S., Pereira, J.L., Reboleira, A.S.P.S., Schmidt, S., Hose, G. C. (2019). Recommendations for ecotoxicity testing with stygobiotic species in the framework of groundwater environmental risk assessment. *Science of The Total Environment*, *681*, 292–304. doi:10.1016/J.SCITOTENV.2019.05.030

Di Lorenzo, T., Di Marzio, W. D., Sáenz, M. E., Baratti, M., Dedonno, A. A., Iannucci, A., Cannicci, S., Messana, G., Galassi, D. M. P. (2014). Sensitivity of hypogean and epigean freshwater copepods to agricultural pollutants. *Environmental Science and Pollution Research*, *21*, 4643–4655. doi:10.1007/s11356-013-2390-6

DiStefano, R. J., Ashley, D., Brewer, S. K., Mouser, J. B., & Niemiller, M. (2020). Preliminary investigation of the critically imperiled Caney Mountain cave crayfish

Orconectes stygocaneyi (Hobbs III, 2001) (Decapoda: Cambaridae) in Missouri, USA. *Freshwater Crayfish*, *25*, 47–57. doi:10.5869/fc.2020.v25-1.047

Dole-Olivier, M. J., Castellarini, F., Coineau, N., Galassi, D. M. P., Martin, P., Mori, N., García-Valdecasas, A., Gibert, J. (2009). Towards an optimal sampling strategy to assess groundwater biodiversity: Comparison across six European regions. *Freshwater Biology*, *54*, 777–796. doi:10.1111/j.1365-2427.2008.02133.x

Durand, P. J. (1970). Fortpflanzung und Entwicklung von Hydromantes, dem Höhlenmolch. *Aqua Terra*, *7*, 42–48.

Elliott, R. W., Kaufmann, J. E., Samoray, S. T., & Gardner, S. E. (2005). The MDC method: counting bats with infrared video. In G. T. Rea (Ed.), *Proceedings of the 2005 National Cave and Karst Managment Symposium* (pp. 147–153). Albany, New York: The NCKMS Steering Commitee.

Exley, S. (1983). Lanzarote volcanic cave expedition 1983. *Explorers Journal*, 118–123.

Ferreira, R. L., & Martins, R. P. (1999). Trophic structure and natural history of bat guano invertebrate communities, with special reference to Brazilian caves. *Tropical Zoology*, *12*, 231–252. doi:10.1080/03946975.1999.10539391

Ficetola, G. F., Canedoli, C., & Stoch, F. (2019). The Racovitzan impediment and the hidden biodiversity of unexplored environments. *Conservation Biology*, *33*(1), 214–216. doi:10.1111/cobi.13179

Fišer, C. (2009). Niphargus—A model system for evolution and ecology. In *Encyclopedia of caves* (Academic P, pp. 746–755).

Fišer, C., Pipan, T., & Culver, D. C. (2014). The Vertical Extent of Groundwater Metazoans: An Ecological and Evolutionary Perspective. *BioScience*, *64*(11), 971–979. doi:10.1093/biosci/biu148

Fraser, B. G., & Williams, D. D. (1997). Accuracy and precision in sampling hyporheic fauna. *Canadian Journal of Fisheries and Aquatic Sciences*, *54*(5), 1135–1141. doi:10.1139/f97-024

Friedrich, M. (2013). Biological Clocks and Visual Systems in Cave-Adapted Animals at the Dawn of Speleogenomics. *Integrative and Comparative Biology*, *53*(1), 50–67. doi:10.1093/icb/ict058

Godefroy, N., Le Goff, E., Martinand-Mari, C., Belkhir, K., Vacelet, J., & Baghdiguian, S. (2019). Sponge digestive system diversity and evolution: filter feeding to carnivory. *Cell and Tissue Research*, *377*(3), 341–351. doi:10.1007/s00441-019-03032-8

Goldstein, B., & King, N. (2016). The Future of Cell Biology: Emerging Model Organisms. *Trends in Cell Biology*, *26*(11), 818–824. doi:https://doi.org/10.1016/j.tcb.2016.08.005

Gorički, Š., Stankovic, D., Snoj, A., Kuntner, M., Jeffery, W. R., Trontelj, P., Pavićević, M., Grizelj, Z., Năpăruş-Aljančič, M., Aljancic, G. (2017). Environmental DNA in subterranean biology: Range extension and taxonomic implications for *Proteus*. *Scientific Reports*, *7*, 91–93. doi:10.1038/srep45054

Gravish, N., & Lauder, G. V. (2018). Robotics-inspired biology. *Journal of Experimental Biology*, *221*, jeb138438. doi:10.1242/jeb.138438

Grimm, V., & Berger, U. (2016). Structural realism, emergence, and predictions in next-generation ecological modelling: Synthesis from a special issue. *Ecological Modelling*, *326*, 177–187. doi:https://doi.org/10.1016/j.ecolmodel.2016.01.001

Grimm, V., Revilla, E., Berger, U., Jeltsch, F., Mooij, W. M., Railsback, S. F., Thulke, H.H., Weiner, J., Wiegand, T., DeAngelis, D. L. (2005). Pattern-Oriented Modeling of Agent-Based Complex Systems: Lessons from Ecology. *Science*, *310*(5750), 987 LP – 991. doi:10.1126/science.1116681

Hancock, P. J., & Boulton, A. J. (2009). Sampling groundwater fauna: Efficiency of rapid assessment methods tested in bores in eastern Australia. *Freshwater Biology*, *54*, 902–914. doi:10.1111/j.1365-2427.2007.01878.x

Hartig, F., Calabrese, J. M., Reineking, B., Wiegand, T., & Huth, A. (2011). Statistical inference for stochastic simulation models - theory and application. *Ecology Letters*, *14*(8), 816–827. doi:10.1111/j.1461-0248.2011.01640.x

Hedges, S. B. (2002). The origin and evolution of model organisms. *Nature Reviews Genetics*, *3*(11), 838–849. doi:10.1038/nrg929

Hesselberg, T. (2010). Ontogenetic changes in web design in two orb-web spiders. *Ethology*, *116*, 535–545. doi:10.1111/j.1439-0310.2010.01760.x

Hesselberg, T. (2015). Exploration behaviour and behavioural flexibility in orb-web spiders: A review. *Current Zoology*, *61*(2), 313–327. doi:10.1093/czoolo/61.2.313

Hesselberg, T., Simonsen, D., & Juan, C. (2019). Do cave orb spiders show unique behavioural adaptations to subterranean life? A review of the evidence. *Behaviour*, 1–28. doi:https://doi.org/10.1163/1568539X-00003564

Howarth, F. G. (1983). Ecology of Cave Arthropods. *Annual Review of Entomology*, *28*(1), 365–389. doi:10.1146/annurev.en.28.010183.002053

Humphreys, W. F., Poole, A., Eberhard, S. M., & Warren, D. (1999). Effects of research diving on the physico-chemical profile of Bundera Sinkhole, an anchialine remiped habitat at Cape Range, Western Australia. *Journal of the Royal Society of Western Australia, 82*, 99–108.

Iliffe, T. M. (2018). Collecting and processing crustaceans from anchialine and marine caves. *Journal of Crustacean Biology, 38*(3), 374–379. doi:10.1093/jcbiol/ruy011

Iliffe, T. M., & Bowen, C. (2001). Scientific cave diving. *Marine Technology Society Journal, Marine Tec*(45), 36–41. doi:10.4031/002533201788001901

Jeffery, W. R. (2020). Astyanax surface and cave fish morphs. *EvoDevo, 11*(1), 14. doi:10.1186/s13227-020-00159-6

Juan, C., Guzik, M. T., Jaume, D., & Cooper, S. J. B. (2010). Evolution in caves: Darwin's 'wrecks of ancient life' in the molecular era. *Molecular Ecology, 19*(18), 3865–3880. doi:10.1111/j.1365-294X.2010.04759.x

Juberthie, C. (1985). Cycle vital de Telema tenella dans la Grotte-Laboratoire de Moulis et strategies de reproduction chez les Araignees cavernicoles. *Memoires de Biospeologie, 12*, 77–89.

Juberthie, C., Durand, J., & Dupuy, M. (1996). La reproduction des Protées (Proteus anguinus): bilan de 35 ans d'élevage dans les grottes-laboratoires de Moulis et d'Aulignac. *Memoires de Biospeologie, 23*, 53–56.

Jung, H., & Meile, C. (2019). Upscaling of microbially driven first-order reactions in heterogeneous porous media. *Journal of Contaminant Hydrology, 224*, 103483. doi:https://doi.org/10.1016/j.jconhyd.2019.04.006

Jurado-Rivera, J. A., Pons, J., Alvarez, F., Botello, A., Humphreys, W. F., Page, T. J., Iliffe, T.M., Willassen, E., Meland, K., Juan, C., Jaume, D. (2017). Phylogenetic evidence that both ancient vicariance and dispersal have contributed to the biogeographic patterns of anchialine cave shrimps. *Scientific Reports*, *7*(1), 2852. doi:10.1038/s41598-017-03107-y

Kim, M., & Or, D. (2016). Individual-Based Model of Microbial Life on Hydrated Rough Soil Surfaces. *PLOS ONE, 11*(1), e0147394. Retrieved from https://doi.org/10.1371/journal.pone.0147394

Koenemann, S., Schram, F. R., Iliffe, T. M., Hinderstein, L. M., & Bloechl, A. (2007). Behavior of remipedia in the laboratory, with supporting field observations. *Journal of Crustacean Biology*, *27*(4), 534–540. doi:10.1651/S-2809A.1

Lenau, T. A., Metze, A.-L., & Hesselberg, T. (2018). Paradigms for biologically inspired design. In *Paradigms for biologically inspired design* (p. 1059302). Proceedings Volume 10593, Bioinspiration, Biomimetics, and Bioreplication VIII. doi:10.1117/12.2296560

Lepore, E., Marchioro, A., Isaia, M., Buehler, M. J., & Pugno, N. M. (2012). Evidence of the Most Stretchable Egg Sac Silk Stalk, of the European Spider of the Year Meta menardi. *PLOS ONE*, *7*(2), e30500. Retrieved from https://doi.org/10.1371/journal.pone.0030500

Lozano-Fernandez, J., Carton, R., Tanner, A. R., Puttick, M. N., Blaxter, M., Vinther, J., Olsen, J., Giribet, G., Edgecombe, G., Pisani, D. (2016). A molecular palaeobiological exploration of arthropod terrestrialization. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1699), 20150133. doi:10.1098/rstb.2015.0133

Lunghi, E. (2018). Ecology and life history of *Meta bourneti* (Araneae: Tetragnathidae) from Monte Albo (Sardinia, Italy). *PeerJ*, *6*, e6049. doi:10.7717/peerj.6049

Lunghi, E., Cianferoni, F., Ceccolini, F., Veith, M., Manenti, R., Mancinelli, G., Corti, C., Ficetola, G. F. (2018). What shapes the trophic niche of European plethodontid salamanders? *PLoS ONE*, *13*, e0205672. doi:10.1371/journal.pone.0205672

Lunghi, E., Corti, C., Manenti, R., Barzaghi, B., Buschettu, S., Canedoli, C., Cogoni, R., De Falco, G., Fais, F., Manca, A., Ficetola, G. F. (2018). Comparative reproductive biology of European cave salamanders (genus Hydromantes): nesting selection and multiple annual breeding. *Salamandra*, *54*, 101–108.

Lunghi, E., Corti, C., Mulargia, M., Zhao, Y., Manenti, R., Ficetola, G. F., & Veith, M. (2020). Cave morphology, microclimate and abundance of five cave predators from the Monte Albo (Sardinia, Italy). *Biodiversity Data Journal*, *8*, e48623. doi:10.3897/BDJ.8.e48623

Lunghi, E., Ficetola, G. F., Mulargia, M., Cogoni, R., Veith, M., Corti, C., & Manenti, R. (2018). Batracobdella leeches, environmental features and Hydromantes salamanders. *International Journal for Parasitology: Parasites and Wildlife*, *7*, 48–53. doi:10.1016/j.ijppaw.2018.01.003

Lunghi, E., Manenti, R., Canciani, G., Scarì, G., Pennati, R., & Ficetola, G. F. (2016). Thermal equilibrium and temperature differences among body regions in European plethodontid salamanders. *Journal of Thermal Biology*, *60*, 79–85. doi:10.1016/j.jtherbio.2016.06.010

Lunghi, E., Manenti, R., Cianferoni, F., Ceccolini, F., Veith, M., Corti, C., Ficetola, F.G., Mancinelli G. (2020). Interspecific and interpopulation variation in individual diet specialization: Do environmental factors have a role? *Ecology*, *101*(8), e03088. doi:10.1002/ecy.3088

Lunghi, E., Manenti, R., Manca, S., Mulargia, M., Pennati, R., & Ficetola, G. F. (2014). Nesting of cave salamanders (Hydromantes flavus and H. italicus) in natural environments. *Salamandra*, *50*, 105–109.

Lunghi, E., Murgia, R., De Falco, G., Buschettu, S., Mulas, C., Mulargia, M., Canedoli, C., Manenti, R., Ficetola, G. F. (2015). First data on nesting ecology and behaviour in the imperial cave salamander Hydromantes imperialis. *North-Western Journal of Zoology*, *11*, 324–330.

MacNeil, R. R., & Brcic, J. (2017). Coping With the Subterranean Environment: A Thematic Content Analysis of the Narratives of Cave Explorers. *Journal of Human Performance in Extreme Environments*, *13*(1), 6. doi:10.7771/2327-2937.1089

Maher, B. (2009). Evolution: Biology's next top model? *Nature*, *458*, 695–699.

Mammola, S. (2019). Finding answers in the dark: caves as models in ecology fifty years after Poulson and White. *Ecography*, *42*(7), 1331–1351. doi:10.1111/ecog.03905

Mammola, S. (2020). On deepest caves, extreme habitats, and ecological superlatives. *Trends in Ecology & Evolution*, *35*(6), 469–472. doi:10.1016/j.tree.2020.02.011

Mammola, S., Amorim, I. R., Bichuette, M. E., Borges, P. A. V, Cheeptham, N., Cooper, S. J. B., Culver, D.C., Deharveng, L., Eme, D., Ferreira, R.L., Fišer, C., Fišer, Ž., Fong, D.W., Griebler, C., Jeffery. W.R., Kowalko, J.E., Jugovic, J., Lilley, T.M., Malard, F., Raoul Manenti, Martínez, A., Meierhofer, M.B., Niemiller, M., Northup, D.E., Pellegrini, T.G., Pipan, T., Protas, M., Reboleira, A.S.P.S., Venarsky, M.P., Wynne, J.J., Zagmajster, M., Cardoso, P. (2020). Fundamental research questions in subterranean biology. *Biological Reviews*,. doi:10.1111/brv.12642

Mammola, S., Cardoso, P., Culver, D. C., Deharveng, L., Ferreira, R. L., Fišer, C., Galassi, D.M.P., Griebler, C., Halse, S., Humphreys, W.F., Isaia, M., Malard, F., Martínez, A., Moldovan, O.T., Niemiller, M.L., Pavlek, M., Reboleira, A.S.P.S., Souza-Silva, M., Teeling, E.C., Wynne, J.J., Zagmajster, M. (2019). Scientists' Warning on the Conservation of Subterranean Ecosystems. *BioScience*, *69*(8), 641–650. doi:10.1093/biosci/biz064

Mammola, S., Giachino, P. M., Piano, E., Jones, A., Barberis, M., Badino, G., & Isaia, M. (2016). Ecology and sampling techniques of an understudied subterranean habitat: the Milieu Souterrain Superficiel (MSS). *The Science of Nature*, *103*(11–12), 88. doi:10.1007/s00114-016-1413-9

Mammola, S., & Isaia, M. (2017). Spiders in caves. *Proceedings of the Royal Society B: Biological Sciences*. doi:10.1098/rspb.2017.0193

Mammola, S., & Isaia, M. (2018). Day-night and seasonal variations of a subterranean invertebrate community in the twilight zone. *Subterranean Biology, 27*, 31–51. doi:10.3897/SUBTBIOL.27.28909

Mammola, S., & Leroy, B. (2018). Applying species distribution models to caves and other subterranean habitats. *Ecography, 41*(7), 1194–1208. doi:10.1111/ecog.03464

Mammola, S., Piano, E., Malard, F., Vernon, P., & Isaia, M. (2019). Extending Janzen's hypothesis to temperate regions: a test using subterranean ecosystems. *Functional Ecology, 33*(9), 1638–1650. doi:10.1111/1365-2435.13382

Manenti, R., Barzaghi, B., Lana, E., Stocchino, G. A., Manconi, R., & Lunghi, E. (2018). The stenoendemic cave-dwelling planarians (Platyhelminthes, Tricladida) of the Italian Alps and Apennines: Conservation issues. *Journal for Nature Conservation, 45*, 90–97. doi:10.1016/j.jnc.2018.08.001

Manenti, R., Melotto, A., Guillaume, O., Ficetola, G. F., & Lunghi, E. (2020). Switching from mesopredator to apex predator: how do responses vary in amphibians adapted to cave living? *Behavioral Ecology and Sociobiology, 74*, 126. doi:10.1007/s00265-020-02909-x

Martínez, A., Di Domenico, M., & Worsaae, K. (2013). Evolution of cave *Axiokebuita* and *Speleobregma* (Scalibregmatidae, Annelida). *Zoologica Scripta, 42*(6), 623–636. doi:10.1111/zsc.12024

Martínez, A., Kvindebjerg, K., Iliffe, T. M., & Worsaae, K. (2017). Evolution of cave suspension feeding in Protodrilidae (Annelida). *Zoologica Scripta, 46*(2), 214–226. doi:10.1111/zsc.12198

Martínez, A., & Mammola, S. (2020). Let research on subterranean habitats resonate! *Subterranean Biology, 36*, 63–71. doi:10.3897/subtbiol.36.59960

McGaugh, S. E., Kowalko, J. E., Duboué, E., Lewis, P., Franz-Odendaal, T. A., Rohner, N., Gross, J.B., Keene, A.C. (2020). Dark world rises: The emergence of cavefish as a model for the study of evolution, development, behavior, and disease. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution, n/a*(n/a). doi:https://doi.org/10.1002/jez.b.22978

Mermillod-Blondin, F., Lefour, C., Lalouette, L., Renault, D., Malard, F., Simon, L., & Douady, C. J. (2013). Thermal tolerance breadths among groundwater crustaceans living in a thermally constant environment. *The Journal of Experimental Biology*, *216*(9), 1683–1694. doi:10.1242/jeb.081232

Moldovan, O. T., Mihevc, A., Miko, L., Constantin, S., Meleg, I. N., Petculescu, A., & Bosák, P. (2011). Invertebrate fossils from cave sediments: A new proxy for pre-Quaternary paleoenvironments. *Biogeosciences*, *8*(7), 1825–1837. doi:10.5194/bg-8-1825-2011

Moseley, M. (2009). Size matters: Scalar phenomena and a proposal for an ecological definition of 'cave'. *Cave and Karst Science, 35*, 89–94.

Möst, M. H., Donabauer, M., Arthofer, W., Schlick-Steiner, B. C., & Steiner, F. M. (2020). Towards an evolutionary history of European-Alpine Trechus ground beetles: Species groups and wing reduction. *Molecular Phylogenetics and Evolution*, *149*, 106822. doi:https://doi.org/10.1016/j.ympev.2020.106822

Müller, B., & Grossniklaus, U. (2010). Model organisms — A historical perspective. *Journal of Proteomics*, *73*(11), 2054–2063. doi:https://doi.org/10.1016/j.jprot.2010.08.002

Niemiller, M. L., Porter, M. L., Keany, J., Gilbert, H., Fong, D. W., Culver, D. C., Hobson, C.S., Kendall, K.D., Davis, M.A., Taylor, S. J. (2018). Evaluation of eDNA for groundwater invertebrate detection and monitoring: a case study with endangered Stygobromus (Amphipoda: Crangonyctidae). *Conservation Genetics Resources*, *10*, 247–257. doi:10.1007/s12686-017-0785-2

Northup, D. E., Melim, L. A., Spilde, M. N., Hathaway, J. J. M., Garcia, M. G., Moya, M., Stone, F.D., Boston, P.J., Dapkevicius, M.L.N.E., Riquelme, C. (2011). Lava Cave Microbial Communities Within Mats and Secondary Mineral Deposits: Implications for Life Detection on Other Planets. *Astrobiology*, *11*, 601–618. doi:10.1089/ast.2010.0562

Oneto, F., Ottonello, D., Pastorino, M. V., & Salvidio, S. (2010). Posthatching parental care in salamanders revealed by infrared video surveillance. *Journal of Herpetology*, *44*, 649–653. doi:10.1670/09-181.1

Pallarés, S., Colado, R., Botella-Cruz, M., Montes, A., Balart-García, P., Bilton, D. T., Millán, A., Ribera, I., Sánchez-Fernández, D. (2020). Loss of heat acclimation capacity could leave subterranean specialists highly sensitive to climate change. *Animal Conservation*, *n/a*(n/a). doi:10.1111/acv.12654

Pallarés, S., Sanchez-Hernandez, J. C., Colado, R., Balart-García, P., Comas, J., & Sánchez-Fernández, D. (2020). Beyond survival experiments: using biomarkers of oxidative stress and neurotoxicity to assess vulnerability of subterranean fauna to climate change. *Conservation Physiology*, *8*(1). doi:10.1093/conphys/coaa067

Parzefall, J. (1982). Changement of behaviour during the evolution of cave animals. *Memoires de Biospeologie*, *8*, 55–62.

Pérez-Moreno, J. L., Iliffe, T. M., & Bracken-Grissom, H. D. (2016). Life in the Underworld: Anchialine cave biology in the era of speleogenomics. *International Journal of Speleology*, *49*, 149–170. doi:10.5038/1827-806X.45.2.1954

Pfeifer, R., Lungarella, M., & Iida, F. (2007). Self-organization, embodiment, and biologically inspired robotics. *Science*, *318*, 1088–1093. doi:10.1126/science.1145803

Polak, S. (1997). A classification of the subterranean environment and cave fauna. *Acta Carsologica*, *26*(2), 351–359.

Poulson, T. L., & White, W. B. (1969). The Cave Environment. *Science*, *165*(3897), 971–981. doi:10.1126/science.165.3897.971

Protas, M., & Jeffery, W. R. (2012). Evolution and development in cave animals: from fish to crustaceans. *Wiley Interdisciplinary Reviews. Developmental Biology*, *1*(6), 823–845. doi:10.1002/wdev.61

Rastorgueff, P. A., Rocher, C., Selva, M., & Chevaldonné, P. (2015). Preliminary DNA-based diet assessment of a gutless carnivore, the sponge Asbestopluma hypogea. *Journal of Experimental Marine Biology and Ecology*, *467*, 108–114. doi:10.1016/j.jembe.2015.02.014

Riddle, M. R., Aspiras, A. C., Gaudenz, K., Peuß, R., Sung, J. Y., Martineau, B., Peavey, M., Box, A.C., Tabin, J.A., McGaugh, S. Rohner, N. (2018). Insulin resistance in cavefish as an adaptation to a nutrient-limited environment. *Nature*, *555*, 647. Retrieved from https://doi.org/10.1038/nature26136

Ross, P. A., Endersby-Harshman, N. M., & Hoffmann, A. A. (2019). A comprehensive assessment of inbreeding and laboratory adaptation in Aedes aegypti mosquitoes. *Evolutionary Applications*, *12*(3), 572–586. doi:https://doi.org/10.1111/eva.12740

Russell, J. J., Theriot, J. A., Sood, P., Marshall, W. F., Landweber, L. F., Fritz-Laylin, L., Polka, J.K., Oliferenko, S., Gerbich, T. Brunet, A. (2017). Non-model model organisms. *BMC Biology*, *15*(1), 1–31. doi:10.1186/s12915-017-0391-5

Saccò, M., Blyth, A., Bateman, P. W., Hua, Q., Mazumder, D., White, N., Humpheys, W.F., Laini, A., Griebler, C., Grice, K. (2019). New light in the dark - a proposed multidisciplinary framework for studying functional ecology of groundwater fauna. *Science of the Total Environment*, *662*, 963–977. doi:10.1016/j.scitotenv.2019.01.296

Saccò, M., Blyth, A. J., Humphreys, W. F., Karasiewicz, S., Meredith, K. T., Laini, A., Cooper, S.J.B., Bateman, P.W., Grice, K. (2020). Stygofaunal community trends along varied rainfall conditions: Deciphering ecological niche dynamics of a shallow calcrete in Western Australia. *Ecohydrology*, *13*(1), e2150. doi:10.1002/eco.2150

Schmidt-Rhaesa, A. (2020). *Guide to the Identification of Marine Meiofauna*. Verlag Dr Friedrich Pfeil.

Schmidt, S. I., Hahn, H. J., Watson, G. D., Woodbury, R. J., & Hatton, T. J. (2004). Sampling Fauna in Stream Sediments as well as Groundwater Using One Net Sampler. *Acta Hydrochimica et Hydrobiologica*, *32*, 131−137. doi:10.1002/aheh.200300522

Schmidt, S. I., Kreft, J.-U., Mackay, R., Picioreanu, C., & Thullner, M. (2018). Elucidating the impact of micro-scale heterogeneous bacterial distribution on biodegradation. *Advances in Water Resources*, *116*, 67–76. doi:https://doi.org/10.1016/j.advwatres.2018.01.013

Silva, A. P. B. da, Oliveira, I. P. M. R., Bastos-Pereira, R., & Ferreira, R. L. (2018). Are laboratory studies on behavior of troglobitic species always trustful? A case study with an isopod from Brazil. *Behavioural Processes*, *153*, 55–65. doi:10.1016/j.beproc.2018.05.009

Smithers, P. (2005). The early life history and dispersal of the cave spider Meta menardi (Latreille, 1804) (Araneae: Tetragnathidae). *Bull. Br. Arachnol. Soc*, *13*(6), 213–216. doi:10.1636/CT-05-2.1

Stemme, T., Iliffe, T. M., von Reumont, B. M., Koenemann, S., Harzsch, S., & Bicker, G. (2013). Serotonin-immunoreactive neurons in the ventral nerve cord of Remipedia (Crustacea): support for a sister group relationship of Remipedia and Hexapoda? *BMC Evolutionary Biology*, *13*(1), 119. doi:10.1186/1471-2148-13-119

Stockdale, W. T., Lemieux, M. E., Killen, A. C., Zhao, J., Hu, Z., Riepsaame, J., Hamilton, N., Kudoh, T., Riley, P.A., van Aerle, R., Mommersteeg, M. T. M. (2018). Heart Regeneration in the Mexican Cavefish. *Cell Reports*, *25*(8), 1997-2007.e7. doi:https://doi.org/10.1016/j.celrep.2018.10.072

Sullivan, W. (2015). The institute for the study of non-model organisms and other fantasies. *Molecular Biology of the Cell*, *26*(3), 387–389. doi:10.1091/mbc.E14-03-0814

Swann, D. E., Hass, C. C., Dalton, D. C., & Wolf, S. A. (2004). Infrared-triggered cameras for detecting wildlife: an evaluation and review. *Wildlife Society Bulletin*, *32*, 357–365. doi:10.2193/0091-7648(2004)32[357:icfdwa]2.0.co;2

Tang, Y., Valocchi, A. J., Werth, C. J., & Liu, H. (2013). An improved pore-scale biofilm model and comparison with a microfluidic flow cell experiment. *Water Resources Research*, *49*(12), 8370–8382. doi:https://doi.org/10.1002/2013WR013843

Torres-Paz, J., Hyacinthe, C., Pierre, C., & Rétaux, S. (2018). Towards an integrated approach to understand Mexican cavefish evolution. *Biology Letters*, *14*(8), 20180101. doi:10.1098/rsbl.2018.0101

Uéno, S.-I. (1987). The derivation of terrestrial cave animals. *Zoological Science*, *4*, 593–606.

Verovnik, R., Sket, B., & Trontelj, P. (2004). Phylogeography of subterranean and surface populations of water lice Asellus aquaticus (Crustacea: Isopoda). *Molecular Ecology*, *13*, 1519–1532. doi:10.1111/j.1365-294X.2004.02171.x

Vincent, J. F. V, Bogatyreva, O. A., Bogatyrev, N. R., Bowyer, A., & Pahl, A.-K. (2006). Biomimetics: its practice and theory. *Journal of The Royal Society Interface*, *3*(9), 471–482. doi:10.1098/rsif.2006.0127

Voituron, Y., De Fraipont, M., Issartel, J., Guillaume, O., & Clobert, J. (2011). Extreme lifespan of the human fish (Proteus anguinus): A challenge for ageing mechanisms. *Biology Letters*, 7. doi:10.1098/rsbl.2010.0539

Vollrath, F., & Selden, P. (2007). The role of behavior in the evolution of spiders, silks, and webs. *Annual Review of Ecology, Evolution, and Systematics*, *38*, 819–846. doi:10.1146/annurev.ecolsys.37.091305.110221

von Reumont, B. M., Blanke, A., Richter, S., Alvarez, F., Bleidorn, C., & Jenner, R. A. (2014). The First Venomous Crustacean Revealed by Transcriptomics and Functional Morphology: Remipede Venom Glands Express a Unique Toxin Cocktail Dominated by Enzymes and a Neurotoxin. *Molecular Biology and Evolution*, *31*(1), 48–58. doi:10.1093/molbev/mst199

Weinstein, P., & Slaney, D. (1995). Invertebrate Faunal Survey of Rope Ladder Cave, Northern Queensland: a Comparative Study of Sampling Methods. *Australian Journal of Entomology*, *34*(3), 233–236. doi:10.1111/j.1440-6055.1995.tb01329.x

Wiegand, T., Revilla, E., & Knauer, F. (2004). Dealing with uncertainty in spatially explicit population models. *Biodiversity and Conservation*, *13*(1), 53–7. doi:10.1023/B:BIOC.0000004313.86836.ab

Wilkens, H. (1971). Genetic Interpretation of Regressive Evolutionary Processes: Studies on Hybrid Eyes of Two Astyanax Cave Populations (Characidae, Pisces). *Evolution*, 530–544. doi:10.2307/2407352

Wilkens, H., Parzefall, J., & Iliffe, T. M. (1986). Origin and age of the marine Stygofauna of Lanzarote, Canary Islands. *Mitt. Hamb. Zool. Mus. Inst.*, *83*, 223–230.

Woodward, M. A., & Sitti, M. (2014). MultiMo-Bat: A biologically inspired integrated jumping-gliding robot. *International Journal of Robotics Research*, *33*, 1511–1529. doi:10.1177/0278364914541301

Wynne, J. J., Howarth, F. G., Sommer, S., & Dickson, B. G. (2019). Fifty years of cave arthropod sampling: Techniques and best practices. *International Journal of Speleology*, *48*(1), 33–48. doi:10.5038/1827-806X.48.1.2231

Wynne, J. J., Sommer, S., Howarth, F. G., Dickson, B. G., & Voyles, K. D. (2018). Capturing arthropod diversity in complex cave systems. *Diversity and Distributions*, *24*, 1478–1491. doi:10.1111/ddi.12772

Yoshizawa, M., Settle, A., Hermosura, M. C., Tuttle, L. J., Cetraro, N., Passow, C. N., & McGaugh, S. E. (2018). The evolution of a series of behavioral traits is associated with autism-risk genes in cavefish. *BMC Evolutionary Biology*, *18*(1), 89. doi:10.1186/s12862-018-1199-9

Zagmajster, M., Culver, D. C., Christman, M. C., & Sket, B. (2010). Evaluating the sampling bias in pattern of subterranean species richness: Combining approaches. *Biodiversity and Conservation*, *19*(11), 3035–3048. doi:10.1007/s10531-010-9873-2

Zakšek, V., Sket, B., Gottstein, S., Franjević, D., & Trontejl, P. (2009). The limits of cryptic diversity in groundwater: phylogeography of the cave shrimp Troglocaris anophthalmus (Crustacea: Decapoda: Atyidae). *Molecular Ecology*, *18*(5), 931–946. doi:10.1111/j.1365-294X.2008.04061.x

Zschokke, S., & Herberstein, M. E. (2005). Laboratory methods for maintaining and studying web-building spiders. *Journal of Arachnology*, *33*, 205–213. doi:10.1636/CT04-72.1

FIGURES

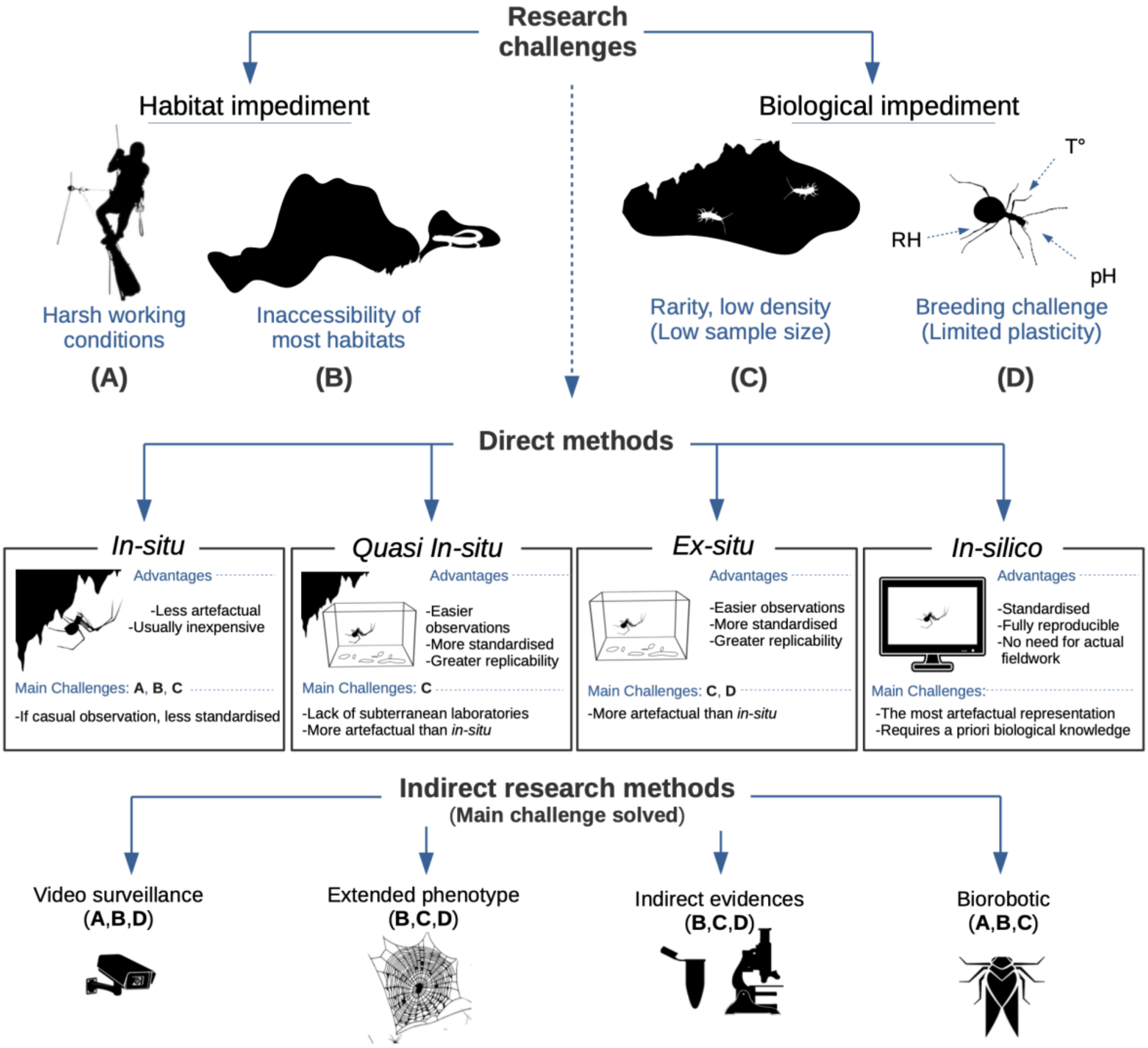

Figure 1. Challenges of subterranean research and experimental designs to avoid these. Schematic representation of the main challenges of subterranean research (coded with capital letters), and main experimental approaches that can be adopted to overcome these impediments.

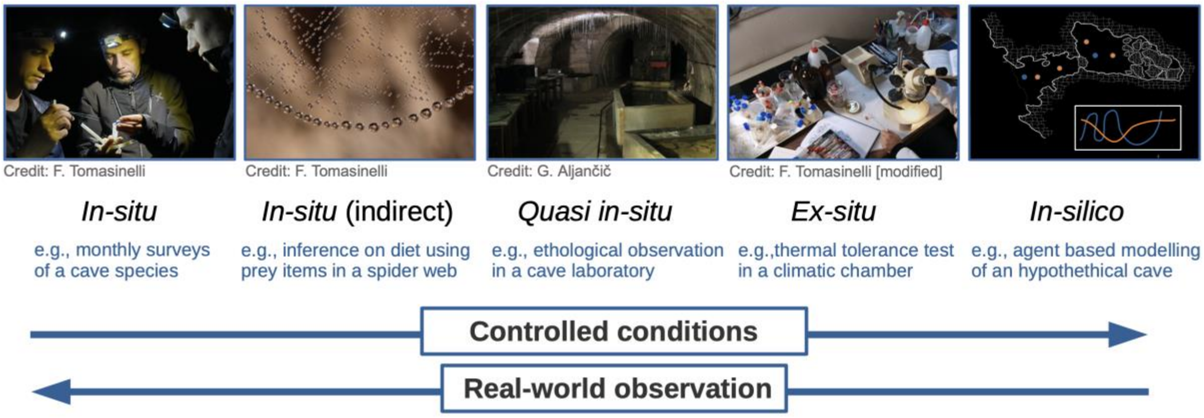

Figure 2. A theoretical trade-off between the ease of study and biological realism of the observations in different experimental setups. On the one hand, exploring a cave is physically demanding and requires specific speleological equipment, whereas it is possible to run a simulation sitting at home in front of a computer in a pyjama—and even during a COVID-19 pandemic. Running a simulation or conducting an experiment in the laboratory also allows us to control for a number of confounding factors. On the other hand, the result obtained in the field are often less artefactual, requiring no abstraction or formulation of *a priori* assumptions. At some point, when studying phenomena in the laboratory or with simulations, one will want to get back to the field to corroborate results using real-world observations.

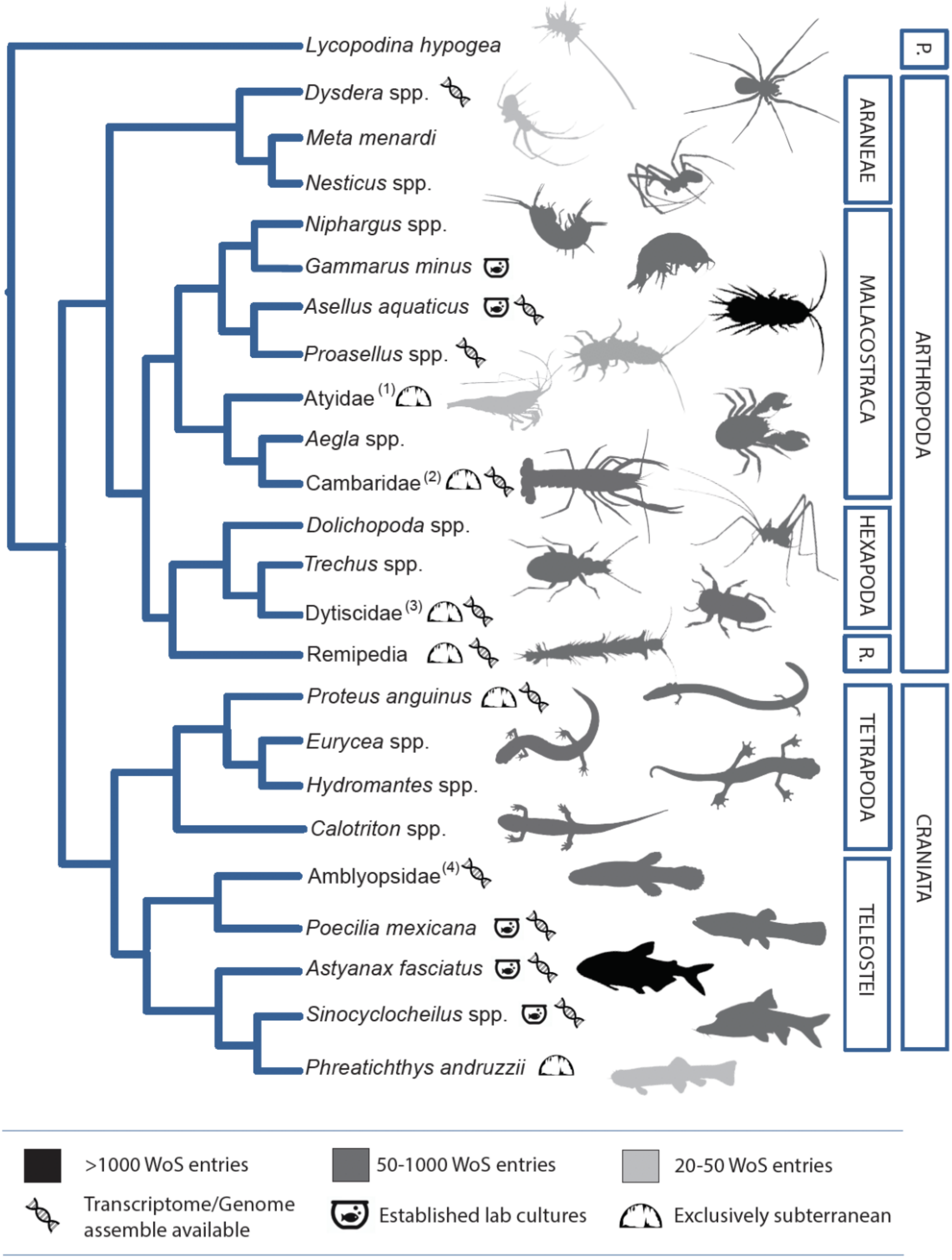

Figure 3. Diversity of model organisms in subterranean biology across the animal Tree of Life. The branch Cambaridae refers to the genera *Cambarus, Orconectes, Procambarus,*

and *Troglocambarus*. Atyidae refers to the exclusively subterranean genera *Speleocaris, Stygiocaris, Troglocaris,* and *Typhlatya.* Dytiscidae indicates the Australian diving beetles of the genera *Limbodesus, Nirridesus, Nirripirti,* and *Paroster.* Amblyopsidae indicates the Northamerican cave fish in the genera *Amblyopsis*, *Chologaster*, *Forbesichthys*, *Speleoplatyrhinus*, and *Typhichthys.* WoS entries = Number of papers focusing on the species in Web of Science (accessed on 25 November 2020). (1–3): The information refers to the genera (1) *Speleocaris*, *Stygiocaris*, *Troglocaris*, and *Typhlatya; (2) Cambarus, Orconectes*, *Procambarus*, and *Troglocambarus;* as well as (3) *Paroster*, *Limbodesus, Nirridesus,* and *Nirripirti*.