RESEARCH ARTICLE



Evolution of coprophagy and nutrient absorption in a Cave Salamander

Daphne Soares¹, Rachel Adams², Shea Hammond³, Michael E. Slay⁴, Danté B. Fenolio⁵, Matthew L. Niemiller⁶

I Biological Sciences, 100 summit street, New Jersey Institute of Technology, Newark NJ 07102, USA 2 Department of Ecosystem Science and Management, 400 Bizzell St, Texas A&M University, College Station, TX 77843, USA 3 Ozark Plateau National Wildlife Refuge, Ozarks plateau, 9014 East 21st Street, Tulsa, OK 74129, USA 4 The Nature Conservancy, 601 North University Avenue, Arkansas Field Office, Little Rock, AR 72205, USA 5 Department of Conservation & Research, 3903 North, St. Mary's St. San Antonio Zoological Park, San Antonio TX 78212, USA 6 Department of Biological Sciences, The University of Alabama in Huntsville, 301 Sparkman Drive, Huntsville, AL 35899, USA

Corresponding author: Daphne Soares (soares@njit.edu)

Academic editor: O. Moldov	an Received 11 July 2017 Accepted 17 October 2017 Published 3 November 2017

Citation: Soares D, Adams R, Hammond S, Slay ME, Fenolio DB, Niemiller ML (2017) Evolution of coprophagy and nutrient absorption in a Cave Salamander. Subterranean Biology 24: 1–9. https://doi.org/10.3897/subtbiol.24.15013

Abstract

The transition from carnivory to omnivory is poorly understood. The ability to feed at more than one trophic level theoretically increases an animal's fitness in a novel environment. Because of the absence of light and photosynthesis, most subterranean ecosystems are characterized by very few trophic levels, such that food scarcity is a challenge in many subterranean habitats. One strategy against starvation is to expand diet breadth. Grotto Salamanders (*Eurycea spelaea* (Stejneger, 1892)) are known to ingest bat guano deliberately, challenging the general understanding that salamanders are strictly carnivorous. Here we tested the hypothesis that grotto salamanders have broadened their diet related to cave adaptation and found that, although coprophagous behavior is present, salamanders are unable to acquire sufficient nutrition from bat guano alone. Our results suggest that the coprophagic behavior has emerged prior to physiological or gut biome adaptations.

Keywords

Guano feeding, salamander, evolution, physiology, behavior

Introduction

Coprophagy is a feeding strategy commonly found in invertebrates (Weiss 2006), but much less so in vertebrates. Coprophagy sometimes exists in mammals such as rodents and lagomorphs, and to a lesser degree in pigs, horses, dogs and nonhuman primates (Thacker and Brandt 1955; Soave and Brand 1991; Marinier and Alexander 1995; Aitken 2003; Krief and Hladik 2004). In amphibians, coprophagy is rare but when present may influence larval development of some species with herbivorous larvae. For example, herbivorous tadpoles regularly feed on feces of conspecifics in captivity (Gromko et al. 1973; Steinwascher 1978; Pryor and Bjorndal 2005), even when other food sources are available ad libitum (Pryor and Bjorndal 2005). Herbivorous tadpoles have digestive morphologies and physiologies similar to other herbivorous vertebrates that rely on hindgut fermentative digestion (Pryor and Bjorndal 2005) and ingest feces to inoculate their digestive tracts with beneficial microbes (Steinwascher 1978; Beebee 1991; Beebee and Wong 1992). Growth rates are slower when feces are removed from the diet (Steinwascher 1978) suggesting that herbivorous tadpoles benefit nutritionally from coprophagy even though feces are lower in energy (Gromko et al. 1973).

The literature is scarce when it comes to coprophagy in predatory amphibians (Fenolio et al. 2006). However, faces consist of a readily available food resource for animals living in energy-limited environments, such as caves. Food and nutritional resources in caves are derived from surface inputs and can be limited both temporarily and spatially within these systems (Culver and Pipan 2014). Likewise, foraging in aphotic habitats of caves presents significant challenges for animals that potentially may go weeks to months between feeding bouts. Guano produced by seasonally roosting bats represents an important food source for both terrestrial and aquatic invertebrates (Howarth 1983; Poulson and Lavoie 2000), which in turn are prey for fishes and salamanders (Poulson and Lavoie 2000; Graening 2005; Niemiller and Poulson 2010; Fenolio et al. 2006, 2014). Salamanders have been known to be strictly carnivorous but Fenolio et al. (2006) showed that obligate cave-dwelling Grotto Salamander larvae (Eurycea spelaea (Stejneger, 1892), Fig. 1) ingests bat guano. This behavior is not incidental to the capture of aquatic invertebrate prey. Stable isotope signatures suggest nutrients from bat guano could be incorporated into salamander tissues, and nutritional analyses revealed that bat guano is comparable to potential prey items in nutritional and energy content, suggesting that bat guano could be a viable alternative food source in some energy-poor cave systems. Since the relative importance of guano in the diet of subterranean salamanders is unknown, the aim of this study was to determine whether subterranean salamander larvae could persist on an exclusive guano diet compared to the typical carnivorous diet of salamanders.

Materials and methods

All experiments were conducted under the approval of animal protocol #15022 by the Rutgers Newark Institutional Animal Care and Use Committee that handles NJIT re-



Figure 1. *Eurycea spelaea* showing troglobitic characters, lack of pigmentation and microphthalmy. Scale bar: 0.5 cm.

search. We collected 46 specimens of *Eurycea spelaea* from January-Stansbury Cave located in the Ozark Plateau National Wildlife Refuge in Delaware County, Oklahoma. Salamanders were housed individually in mesocosms submerged in the cave stream. Each mesocosm consisted of a 500 ml plastic bottle with small holes so that the salamanders had continuous access to fresh cave water. All lids were connected to a central line via a short string. Salamanders were collected June 6th but the study did not until June 22nd, salamanders were fed amphipods until the start of the study. During the study, salamanders were fed every four days either a strict diet of live amphipods, bat guano, or nothing. We collected amphipods and bat guano fresh on the day of feeding from the cave. The cave is inhabited by a maternity colony (ca. 15,000 individuals) of federally endangered Gray bats (*Myotis grisescens* A.H. Howell, 1909) from late April through early November (Fenolio et al. 2006, 2014). Salamanders were randomly assigned to a negative control group, or one of two prey types and one of four feeding treatments based on percentage of initial body mass: 0% (control) 2.5%, 5%, and 10%. Salamanders were massed before feeding to track body mass loss or gain and fed the corresponding percentage of initial body mass of amphipods or guano. We used a milligram- accuracy scale (Ohus, Parsippany, NJ, USA). Care was taken to remove any food remnants before the next feeding, although in most cases salamander ate all the food. Salamanders that lost \geq 30% of initial body mass were removed the study. We used ANCOVA to compare body mass of the different treatments in MatLab with an alpha level of 0.05. Normality of ranked data was verified via the Kolmogorov-Smirnov test. Salamanders were released back into the cave after the study per permitting regulations.

All data associated with this study are available from the figshare digital repository: https://doi.org/10.6084/m9.figshare.4805656

Results

Loss of body mass in treatment groups: All treatment groups lost some body mass during the study (34 days; Fig. 2). Animals in the control group (n = 10) were removed from the study earlier (27 days) than the other groups (34 days) due to body mass loss. Salamanders in the control group experienced the steepest loss of body mass. In general, salamanders fed guano lost more body mass than salamanders fed amphipods, and at 34 days, most guano-fed salamanders had reached the 30% loss limit. Body mass was more variable in amphipod-fed groups with both gains and losses. Salamanders fed 2.5% of initial body mass (IBM) lost an average of 31.3% (\pm 14.3%) body mass eating guano compared to an average of 9.6% (\pm 36.0%) eating amphipods. Salamanders fed 5% IBM lost 30.2% (\pm 4.8%) body mass when eating guano compared to 7.9% (\pm 28.1%) eating amphipods. Body mass loss was least for salamanders fed 10% IBM, and guano-fed salamanders lost 28.3% (\pm 4.4%) body mass compared to 7.6% (\pm 11.5%) for the amphipod group.

Comparisons of weight loss: For salamanders fed 2.5% IBM, body mass loss rates for guano-fed and amphipod-fed groups were slower than the control group (Guanofed: F = 6.82, P = 0.01; Amphipod-fed: F = 12.14, P = 0.0007) but not different from each other (F = 2.86, P = 0.09). For salamanders fed 5% IBM, guano-fed animals lost body mass at a slower rate than amphipod-fed animals (F = 11.05, P = 0.0012) and control animals (F = 14.75, P = 0.0002), while amphipod-fed animals lost body mass similarly to control animals (F = 0.07, P = 0.795). For salamanders fed 10% IBM, the amphipod-fed group lost body mass slower than the guano-fed group (F = 6.4, P = 0.131) and control group (F = 26.26, P = 1.09e-6), while the guano-fed group was similar to the control group (F = 8.02, P = 0.005).

Discussion

All amphipod groups had individuals that lost and gained weight within the period of the study. The variability in body mass was smaller in the 10% amphipod IBM com-



Figure 2. Regression lines based on body mass loss of different diet types and amounts. Salamanders were fed nothing (green), live amphipods (red) or guano (blue). Groups were fed every four days based on their initial body weight, with 2.5% (**A**), 5% (**B**) or 10% (**C**). The calculated regression lines were as follows: Control -1.16x+96.01 R² = 0.54, n = 10; 2.5%_{amphipod} -0.26x+98.49, R² = 0.39, n = 6; 2.5%_{guano} -0.70x+93.58, R² = 0.02, n = 6; 5%_{amphipod} -0.28x+102.22, R² = 0.03, n = 6; 5%_{guano} -1.12x+98.89, R² = 0.77, n = 6; 10%_{amphipod} -0.35x+103.36, R² = 0.21, n = 6; 10%_{guano} -0.70x+96.01, R² = 0.53, n = 6.

pared to the 5% and 2.5%, but a few individuals lost weight making the overall average mass at the end of the study slightly less than the IBM. The large variability in the 5% and 2.5% amphipod groups suggests that individuals may be behaving differently or some individuals may be stressed in the mesocosm. Nonetheless, since guano groups resemble more closely control groups we conclude that amphipods are a better food source for the salamanders.

Shifts in habitat are often linked with dietary shifts, as environmental changes frequently cause organisms to alter foraging behaviors (Rosalino et al. 2005; McMeans et al 2015). The transition from surface to subterranean habitats involves dramatic morphological, physiological, and behavioral changes associated with life in complete darkness and often limited energy resources, including a predicted increase in dietary breadth (Culver 1982, 1994; Holyoak and Sachdev 1998; Fenolio et al. 2006). In subterranean salamanders, the evolution of coprophagy may be an unusual foraging strategy to exploit a nutritious and seasonally abundant resource (i.e., bat guano) in an otherwise food-limited environment. While it has been demonstrated that Grotto salamander larvae will regularly employ coprophagy of calorically-rich bat guano (Fenolio et al. 2006), our study suggests that Grotto salamander larvae are unable to thrive on a guano-exclusive diet for a prolonged period. So in this case the coprophagous behavior has emerged in evolution prior to the necessary physiological changes to gain nutrition from it.

The apparent disagreement between coprophagous behavior in Grotto salamanders and the lack of apparent absorption may have several possible explanations. First, Grotto salamander larvae, and salamanders in general, do not possess the morphological and physiological digestive traits necessary to exploit guano as a food resource. Salamanders in general are strict carnivores with short digestive tracts and have buccal enzymes with low amylolytic activity (Stevens and Hume 2004). In contrast, coprophagy is most often associated with herbivory, which predominately utilize postgastric (hindgut) fermentation and the consumption of feces increases the absorption of nutrients and inoculate the hind gut with microbes (Clauss et al. 2007). The selective consumption of predigested material is a form of omnivory. We know relatively little about the adaptive advantages of and the selective drivers that favor omnivory, and by proxy coprophagy, in vertebrates (but see Diehl 2003). Coprophagy requires the evolution of not only a coprophagous behavior but also the evolution of morphological and physiological digestive traits to process feces. It is unknown whether these traits are linked, but theoretically behavioral evolution can precede physiological and morphological evolution. Second, since Grotto salamanders are ingesting feces with high protein content (54%; Fenolio et al. 2006) of insectivores (bats) rather than feces from herbivores, a vastly different gut microbiome is needed to efficiently digest feces. So in addition to lacking the morphological and physiological traits, Grotto salamanders may not possess the necessary gut flora to digest and fully process the contents of bat guano. Ley et al. (2009) found that diet can impact gut microbiome diversity in mammals, which increases with evolution from carnivory to omnivory. Digestive evolution in amphibians, as well as their gut biomes and the gut's propensity for evolution, is yet to be examined in detail. Finally, coprophagy may reflect mistaken identity due to an innate feeding response for moving prey. In subterranean habitats, aquatic salamanders and cavefishes rely heavily on mechanosensation to detect and capture moving prey. Guano falling into a pool and settling on the substrate may elicit a similar feeding response as crustaceans and other aquatic invertebrates. Guano may not be immediately rejected but ingested instead because of the high protein and fat content of the insectivorous guano. Alternatively, guano may possess a micronutrient, vitamin or mineral otherwise scarce in the subterranean habitat (see Fenolio et al. 2006). While guano may not prevent a loss in mass, it may still offer some nutritional benefit.

Acknowledgements

We thank Ozarks Plateau National Wildlife Refuge for lodging and cave access, Gal Haspel for help with the analysis, Sheilah Roenfeldt for field assistance, and Oklahoma Department of Wildlife Conservation for collection and research permits. This work was in part a product of Project E-22 entitled "Management and Protection for the Ozark Big-eared Bat, Gray Bat, and Stygobitic Fauna in Oklahoma," and was funded by the Oklahoma Department of Wildlife Conservation.

References

Aitken MM (2003) Coprophagy in dogs. Veterinary record 153(3): 96-96.

- Beebee TJC (1991) Purification of an agent causing growth inhibition in anuran larvae and its identification as a unicellular unpigmented alga. Canadian Journal of Zoology 69: 2146–2153. https://doi.org/10.1139/z91-300
- Beebee TJC, Wong ALC (1992) Prototheca-mediated interference competition between anuran larvae operates by resource diversion. Physical Zoology 65: 815–831. https://doi. org/10.1086/physzool.65.4.30158541
- Belgrad BA, Griffen BD (2016) The Influence of Diet Composition on Fitness of the Blue Crab, *Callinectes sapidus*. PLoS ONE 11(1): 1–15. https://doi.org/10.1371/journal. pone.0145481
- Clauss M, Schwarm A, Ortmann S, Streich WJ, Hummel J (2007) A case of non-scaling in mammalian physiology? Body size, digestive capacity, food intake, and ingesta passage in mammalian herbivores. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 148(2): 249–65. https://doi.org/10.1016/j.cbpa.2007.05.024
- Cohen AC (1996) Plant Feeding by Predatory Heteroptera: Evolutionary and Adaptational Aspects of Trophic Switching. In: Alomar O, Wiedenmann RN (Eds) Zoophytophagus Heteroptera: Implications for Life History and Integrated Pest Management. Entomological Society of America. Lanham, Maryland, USA, 1–7.

- Culver DC, Pipan T (2014) Shallow Subterranean Habitats: Ecology, Evolution, and Conservation (1st edn). Oxford University press, New York, USA. https://doi.org/10.1093/acprof:oso/9780199646173.001.0001
- Diehl S (2003) The Evolution and Maintenance of Omnivory: Dynamic Constraints and the Role of Food Quality. Ecology 84(10): 2557–2567. https://doi.org/10.1890/02-0399
- Eubanks MD, Denno RF (1999) The Ecological Consequences of Variation in Plants and Prey for an Omnivorous Insect. Ecology 80: 1253–66. https://doi.org/10.1890/0012-9658(1999)080[1253:TECOVI]2.0.CO;2
- Eubanks MD, Styrsky JD, Denno RF (2003) The Evolution of Omnivory in Heteropteran Insects. Ecology 84: 2549–2446. https://doi.org/10.1890/02-0396
- Fenolio DB, Graening GO, Collier BA, Stout JF (2006) Coprophagy in a Cave-Adapted Salamander; the Importance of Bat Guano Examined through Nutritional and Stable Isotope Analyses. Proceedings of the Royal Society of London B: Biological Sciences 273(1585): 439–43. https://doi.org/10.1098/rspb.2005.3341
- Fenolio DB, Niemiller ML, Bonett RM, Graening GO, Collier BA, Stout JF (2014) Life history, demography, and the influence of cave-roosting bats on a population of the Grotto salamander (*Eurycea spelaea*) from the Ozark Plateaus of Oklahoma (Caudata: Plethodontidae). Herpetological Conservation and Biology 9: 394–405.
- Graening GO (2005) Trophic structure of Ozark cave streams containing endangered species. Journal of Oceanological and Hydrobiological Studies 34: 3–17.
- Gromko MH, Mason FS, Smith-Gill SJ (1973) Analysis of the crowding effect in *Rana pipi-ens* tadpoles. Journal of Experimental Zoology 186: 63–72. https://doi.org/10.1002/ jez.1401860109
- Howarth FG (1983) Ecology of cave arthropods. Annual Review of Entomology 28: 365–389. https://doi.org/10.1146/annurev.en.28.010183.002053
- Kitaysky AS, Kitaiskaia EV, Piatt JF, Wingfield JC (2006) A Mechanistic Link between Chick Diet and Decline in Seabirds? Proceedings. Biological Sciences/The Royal Society 273(1585): 445–50. https://doi.org/10.1098/rspb.2005.3351
- Krief S, Jamart A, Hladik CM (2004) On the possible adaptive value of coprophagy in free-ranging chimpanzees. Primates 45(2): 141–145. https://doi.org/10.1007/s10329-003-0074-4
- Marinier SL, Alexander AJ (1995) Coprophagy as an avenue for foals of the domestic horse to learn food preferences from their dams. Journal of Theoretical Biology 173(2): 121–124. https://doi.org/10.1006/jtbi.1995.0049
- Martin SD, Shepard DB, Steffen MA, Phillips JG, Bonett RM (2016) Biogeography and Colonization History of Plethodontid Salamanders from the Interior Highlands of Eastern North America 12625: 410–22. https://doi.org/10.1111/jbi.12625
- McMeans BC, McCann KS, Humphries M, Rooney N, Fisk AT (2015) Food Web Structure in Temporally-Forced Ecosystems. Trends in Ecology & Evolution 30(11): 662–72. https:// doi.org/10.1016/j.tree.2015.09.001
- Nalepa CA, Bignell DE, Bandi C (2001) Detritivory, Coprophagy, and the Evolution of Digestive Mutualisms in Dictyoptera. Insectes Sociaux 48(3): 194–201. https://doi.org/10.1007/ PL00001767

- Niemiller ML, Poulson TL (2010) Subterranean fishes of North America: Amblyopsidae. In: Trajano E, Bichuette ME, Kapoor BG (Eds) The Biology of Subterranean Fishes. Science Publishers, Enfield, New Hampshire, USA, 169–280. https://doi.org/10.1201/ EBK1578086702-c7
- Poulson TL, Lavoie KH (2000) The trophic basis of subsurface ecosystems. In: Wilkins H, Culver DC, Humphreys WF (Eds) Ecosystems of the World: Subterranean Ecosystems. Elsevier, Amsterdam, 231–249.
- Pryor GS, Bjorndal KA (2005) Symbiotic fermentation, digesta passage, and gastrointestinal morphology in bullfrog tadpoles. Physiology and Biochemical Zoology 78: 201–215. https://doi.org/10.1086/427050
- Rabosky DL, Lovette IJ (2008) "Explosive Evolutionary Radiations: Decreasing Speciation or Increasing Extinction Through Time?" Evolution 62(8): 1866–75. https://doi. org/10.1111/j.1558-5646.2008.00409.x
- Rosalino LM, Loureiro F, Reis MS, Macdonald DW, Santos-Reis M (2005) Dietary Shifts of the Badger (*Meles meles*) in Mediterranean Woodlands: An Opportunistic Forager with Seasonal Specialisms. Mammalian Biology 70(1): 12–23. https://doi.org/10.1078/1616-5047-00172
- Sakamaki T (2010) Coprophagy in wild bonobos (*Pan paniscus*) at Wamba in the Democratic Republic of the Congo: a possibly adaptive strategy? Primates 51(1): 87. https://doi. org/10.1007/s10329-009-0167-9
- Soave O, Brand CD (1991) Coprophagy in animals: a review. The Cornell Veterinarian 81(4): 357–364.
- Steinwascher K (1978) The effect of coprophagy on the growth of *Rana catesbeiana* tadpoles. Copeia 1978: 130–134. https://doi.org/10.2307/1443833
- Stevens CE, Hume ID (2004) Comparative Physiology of the Vertebrate Digestive System (2nd edn). Cambridge University Press, Cambridge, 400 pp.
- Thacker EJ, Brandt CS (1955) Coprophagy in the rabbit. Journal of Nutrition 65: 375–385.
- Weiss MR (2006) Defecation behavior and ecology of insects. Annual Review of Entomology 51: 635–661 https://doi.org/10.1146/annurev.ento.49.061802.123212
- Whitman DW, Blum MS, Slansky Jr F (1994) Carnivory in Phytophagous Insects. In: Ananthakrishnan NT (Ed.) Functional Dynamics of Phytophagous Insects. Science Publications. Lebanon, New Hampshire, 161–205.
- Yoshizawa M, Gorički Š, Soares D, Jeffery WR (2010) Evolution of a Behavioral Shift Mediated by Superficial Neuromasts Helps Cavefish Find Food in Darkness. Current Biology 20(18): 1631–36. https://doi.org/10.1016/j.cub.2010.07.017

RESEARCH ARTICLE



Three new species of the subterranean huntsman spider genus Spariolenus (Araneae, Sparassidae, Heteropodinae) in Iran

Majid Moradmand¹

I Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran

Corresponding author: Majid Moradmand (moradmand.arachnids@gmail.com; m.moradmand@sci.ui.ac.ir)

Academic editor: O. Moldovan Received 14 September 2017 Accepted 15 November 2017 Publis	shed28November2017
http://zoobank.org/7A48B2C0-CCFE-474F-9E95-D1DCEA68D4AC	

Citation: Moradmand M (2017) Three new species of the subterranean huntsman spider genus *Spariolenus* (Araneae, Sparassidae, Heteropodinae) in Iran. Subterranean Biology 24: 11–25. https://doi.org/10.3897/subtbiol.24.20985

Abstract

The taxonomy of the genus *Spariolenus* Simon, 1880 in Iran is revisited by describing three new species: *Spariolenus fathpouri* **sp. n.** (male & female) from Pebdeh cave ecosystem (Khuzestan Province), *S. mansourii* **sp. n.** (male and female) a more widespread species discovered in the Pataveh and Nezel Caves entrances (Kohgiluye-va-Buyer Ahmad Province), and *S. hormozii* **sp. n.** (female) from the Geno Biosphere Reserve (Hormozgan Province). Notes on the current status of the explored caves are given. The recently erected species, *S. khoozestanus* is redescribed based on the examination of the holotype and an additional diagnosis is provided. The Iranian plateau can be considered as the hotspot diversity for this genus in the world by hosting 8 out of 13 known species.

Keywords

New species, Middle East, Cave, taxonomy, huntsman spiders

Introduction

Members of the genus *Spariolenus* Simon, 1880 are medium to large huntsman spiders living underground both in caves and/or other karstic substrates close to active water stream systems (Moradmand and Jäger 2011, per obs.). These spiders are active hunters in caves or nocturnal hunters close to the cave entrances and karstic regions (pers obs.).

Moradmand and Jäger (2011) revised *Spariolenus* and gave extended diagnosis for a better understanding of its taxonomy in addition to describing four new species. Molecular phylogeny recovered *Spariolenus* in the "true" Heteropodinae clade and as the sister taxon of *Heteropoda* Latreille, 1804 (Moradmand et al. 2014). *Spariolenus khoozestanus* Zamani, 2016 was described from a single female specimen from Iran. It is here redescribed and illustrated.

Materials and methods

The specimens new to science in this paper (except *S. hormozii* sp. n.) were recently collected by the author from different subterranean habitats in Iran (2015–2017). The male specimens, collected as immatures, were subsequently reared in captivity, in some cases over one year to get mature and ready for description and a more reliable taxonomic decision over their identity as new species. Morphological characters were studied and illustrated using Leitz Wetzlar and Olympus SZX12 stereomicroscopes equipped with a drawing tube. For this purpose, specimens were submerged in 75% ethanol. The description style follows Moradmand and Jäger (2011) and Moradmand (2013). Measurements are given in millimetres. The size classes of specimens follow Jäger (2001) [small (3–10), medium (10–20), large (20–30)]. The spination pattern is given according to Davies (1994): sum of all spines (prolateral, dorsal, retrolateral, ventral), the latter is only listed if present.

The following abbreviations are used throughout the text: AB – anterior band of epigynal field; ALE – anterior lateral eyes, AME – anterior median eyes; BRB – basal retrolateral bulge; C – conductor; CO – copulatory opening; DE – distal part of embolus; EF – Epigynal field; EP – epigynal pit; ET – embolus tip; FC – first coil of vulva; FD – fertilisation duct; LID – lumen of internal duct system; MEP – margin of epigynal pit; PET – prolateral part of embolus; PLE – posterior lateral eyes; PME – posterior median eyes; RET – retrolateral part of embolus; RTA – retrolateral tibial apophysis; SD – sperm duct, T – tegulum; SC – second coil of vulva; SD – tissue sample for spider DNA catalogue number deposited in ZMUI; SS – slit sensillum; TC – third coil of vulva; I-IV – 1st to 4th leg.

Depositories. **SMF** – Senckenberg Research Institute, Frankfurt am Main, Germany (Julia Altmann, Peter Jäger). **ZMUI** – Zoological Museum, Department of Biology, University of Isfahan, Isfahan, Iran (Majid Moradmand).

Results

Family Sparassidae Bertkau, 1872 Subfamily Heteropodinae Thorell, 1873 Genus *Spariolenus* Simon, 1880

For description and diagnosis see Moradmand and Jäger (2011).

http://zoobank.org/4221312C-BFB7-4B28-9CD8-65E86D04C5EF Figs 1, 2, 3A

Type material. *Holotype*: \Diamond , IRAN: Khuzestan Province: N of Lali, Pebdeh Cave, 32°26.50'N, 42°13.35'E, 11 April 2015, SD 111, M. Moradmand, F. Moin, Sh. Esmailbegi, A. Bagheri leg. (SMF). *Paratypes*: $1 \Diamond$ and $2 \heartsuit \heartsuit$ with same data as for holotype ($1 \Diamond$, $1 \heartsuit$ ZMUI; $1 \heartsuit$ SMF)

Etymology. The species is named in honour of Dr Hossein Fathpour, retired associate professor of Zoology (University of Isfahan), who first perceived and supported the author's enthusiasm for investigating the world of arthropods; genitive case.

Diagnosis. The male can be distinguished from other congeners by its bifurcated ET (similar to *S. zagros* and *S. mansourii* sp. n.) but differ from the two later by the crescent shape of the prolateral ET (PET) having a notch (Fig. 1A–D). The female vulva can be distinguished by having unique expansion in EP anteriorly, constructing a marsupial-like structure (Fig. 2B).

Description. *Male: Measurements.* Medium-sized Sparassidae; holotype: total length 14.0, carapace length 6.7, width 5.5, anterior width 3.6, opisthosoma length 7.3, width 4.3. Anterior eye row slightly recurved, posterior eye row straight (Fig. 1F).

Chelicerae. With 3 anterior and 4 posterior teeth, cheliceral furrow with 10–15 intermarginal denticles; retromargin with two bristles at base of fang (Fig. 1E).

Eyes. AME 0.27, ALE 0.70, PME 0.48, PLE 0.76, eye inter distances: AME-AME 0.17, AME-ALE 0.06, PME-PME 0.28, PME-PLE 0.53, AME-PME 0.34, ALE-PLE 0.52.

Legs. Leg formula: II I IV III. Palp 10.8 [3.6, 1.5, 2.2, 3.5], I 47.0 [12.2, 4.6, 13.5, 13.2, 3.5], II 51.2 [13.8, 4.5, 15.5, 14.2, 3.2], III 38.0 [10.8, 3.7, 11.2, 9.7, 2.6], IV 39.9 [11.1, 3.5, 11.2, 11.3, 2.8].

Spination. Palp 131, 101, 1013; Legs: Femur I–III 323, IV 321; Patella I–IV 101; Tibia I 222(10), II 222(10), III 1218, IV 3236; Metatarsus I 0004, II–III 2024, IV 3036.

Palp. As in diagnosis, with cymbium 1.5 times longer than tibia, BRB present, RTA short, dRTA pointed and vRTA blunt in retrolateral view, both are blunt and the same length in ventral view, PET shorter than RET, PET cover proximal half of RET partially in ventral view. Conductor hyaline and not extending beyond or roughly the same length of ET (Fig. 1A–D).

Female: Habitus as in Fig. 3A. *Measurements*. Large-sized Sparassidae; total length 27.6, carapace length 13.6, width 11.7, anterior width 6.5, opisthosoma length 14.0, width 8.0.

Chelicerae. With 3 anterior and 5 posterior teeth, cheliceral furrow with 15–20 intermarginal denticles.

Eyes. AME 0.51, ALE 1.1, PME 0.70, PLE 1.3, eye inter distances: AME-AME 0.23, AME-ALE 0.11, PME-PME 0.48, PME-PLE 0.97, AME-PME 0.67, ALE-PLE 0.95.

Legs. Leg formula: II I IV III. Palp 19.8 [5.7, 3.0, 4.5, 6.6], I 71.3 [18.2, 8.2, 20.4, 19.5, 5.0], II 79.3 [21.4, 8.9, 22.8, 21.2, 5.0], III 66.6 [18.2, 7.3, 17.2, 17.3, 6.6], IV 68.7 [18.7, 7.3, 17.9, 19.5, 5.3].



Figure 1. *Spariolenus fathpouri* sp. n., male holotype, Khuzestan, Pebdeh Cave, Iran (SMF). **A–C** left palp (**A** prolateral **B** ventral **C** retrolateral) **D** bulbus, ventral **E** chelicerae, ventral **F** eye arrangement, dorsal. Abbreviations: BRB – basal retrolateral bulge, C – conductor, ET – embolus tip, PET – prolateral part of embolus, RET – retrolateral part of embolus, RTA – retrolateral tibial apophysis, SD – sperm duct, T – tegulum. Scale bars: 1 mm (**A–C, E, F**), 0.5 mm (**D**).



Figure 2. *Spariolenus fathpouri* sp. n., female paratype, Khuzestan, Pebdeh Cave, Iran (ZMUI). **A** epigynum, ventral **B** vulva, dorsal **C** right vulva, lateral. Abbreviations: CO – copulatory opening; EF – epigynal field; EP – epigynal pit; FC – first coil of vulva; FD – fertilisation duct; LID – lumen of internal duct system; MEP – margin of epigynal pit; SC – second coil of vulva; SS – slit sensillum; TC – third coil of vulva. Scale bar: 1 mm.

Spination. Palp 131, 101, 2221, 2014; Legs: Femur I–III 323, IV 321; Patella I–IV 101; Tibia I 101(10), II 111(10), III 2228, IV 2226; Metatarsus I– II 0004, III 2024, IV 3036.



Figure 3. A Habitus of *Spariolenus fathpouri* sp. n., paratype female, alive in the type locality **B** Pebdeh Cave, Iran, the type locality, entrance (arrow) **C** ditto, Pebdeh Cave **D** Blattidae **E** dead Bat suffered from fire **F** Coleopteran beetles.

Epigynum. As in diagnosis, with EF as long as wide, AB present but short, MEP extending first half laterad and second half frontad (Fig. 2A–C).

Distribution and habitat preferences. Known only from the type locality, the Pebdeh cave ecosystem (Fig. 3B, C). The cave is rich in biodiversity of arthropods in-

cluding unidentified species of insects (Fig. 3D, F). A relatively large population of bats inhabit the cave and produce huge piles of guano, a source of energy for a potential food chain inside the cave.

Conservation status of the type locality. The Pebdeh cave suffered from a manmade fire just a few years ago which resulted in a decline of the bat population (Fig. 3E) and of the rest of the diversity of inhabitants (pers. ob.). The cave is fortunately under formal registration as national heritage of Iran because of evidences of earliest date of human occupation inside the cave.

Spariolenus mansourii Moradmand, sp. n.

http://zoobank.org/F79FD97F-E8DF-43CF-AB40-4B90A74CFF14 Figs 4, 5A

Type material. *Holotype:* \Diamond , IRAN: Kohgiluyeh and Bouyer-Ahmad Province: Sarfaryab, Choram, Nezel Cave entrance, at night, 30°47'29.47"N, 50°56'52.25"E, 4 June 2016, Naghsh-e-Jahan Caving club, M. Moradmand and M. Saboohi leg. (SMF). *Paratypes:* $1 \Diamond$ and $2 \heartsuit \heartsuit$, $1 \Diamond$ *Kohgiluyeh and Bouyer-Ahmad Province*: Pataveh, Deh-Sheikh Cave, first corridor, 30°57'N, 51°14'E (ZMUI). $2 \heartsuit \heartsuit$ with same data as for holotype ($1 \heartsuit$ ZMUI; $1 \heartsuit$ SMF).

Etymology. The species is named in honour of Mr Mohammad Mansouri (Iran: Isfahan), a highly qualified caving instructor. I experienced my first underground adventure with him and I owe him my caving skills; genitive case.

Diagnosis. The male is distinguished from other congeners by dRTA 1.5 times longer than vRTA and the shape of ET bifurcated (same as *S. zagros* and *S. fathpouri* sp.nov.). It differs from *S. zagros* by the prolateral ET shorter than retrolateral one (same size in *S. zagros*) (Fig. 4A–D). The female differ from other *Spariolenus* spp. by vulva with lateral extension of the first coil continuous to second coil (similar to those of *S. manesht*, but differ from it by dorsal epigynum lacking continuous ridge anterior to the CO) (Fig. 5A–C).

Description. *Male: Measurements.* Small to medium-sized Sparassidae; holotype: total length 11.0, carapace length 5.2, width 4.1, anterior width 2.6, opisthosoma length 5.8, width 3.2.

Chelicerae. With 3 anterior and 4 posterior teeth, cheliceral furrow with 10–15 intermarginal denticles (Fig. 4E).

Eyes. AME 0.27, ALE 0.57, PME 0.36, PLE 0.65, eye inter distances: AME-AME 0.12, AME-ALE 0.03, PME-PME 0.24, PME-PLE 0.48, AME-PME 0.25, ALE-PLE 0.54. Anterior and posterior eye rows slightly recurved (Fig. 4F).

Legs. Leg formula: II I IV III. Palp 5.7 [2.8, 1.3, 1.6], I 32.8 [8.5, 3.4, 9.1, 9.2, 2.6], II 35.8 [9.6, 3.5, 9.9, 10.1, 2.7], III 28.4 [8.2, 2.8, 7.6, 7.5, 2.3], IV 29.4 [8.4, 2.9, 7.3, 8.3, 2.5].

Spination. Palp 131, 101, 1013; Legs: Femur I 223, II–III 323, IV 321; Patella I–IV 101; Tibia I–II 131(10), III 2128, IV 2126; Metatarsus I–III 2024, IV 3036.



Figure 4. *Spariolenus mansourii* sp. n., male holotype, Kohgiluyeh and Bouyer-Ahmad Province, Nezel Cave entrance, Iran (SMF). **A–C** left palp (**A** prolateral **B** ventral **C** retrolateral) **D** bulbus, ventral **E** chelicerae, ventral **F** eye arrangement, dorsal. Scale bars: 1 mm.



Figure 5. *Spariolenus mansourii* sp. n., female paratype, Kohgiluyeh and Bouyer-Ahmad Province, Nezel Cave entrance, Iran (ZMUI). **A** epigynum, ventral **B** vulva, dorsal **C** right vulva, lateral. Scale bar: 1 mm.

Palp. As in diagnosis, with cymbium 1.5 times longer than tibia, BRB present, RTA short, dRTA 2 times longer than vRTA, both distally rounded in ventral view, PET and RET both pointed distado-prolaterad, PET shorter than RET. Conductor hyaline and extending beyond ET in ventral view (4A–D).

Female: Measurements. Medium-sized Sparassidae; total length 15.4, carapace length 7.6, width 6.1, anterior width 3.7, opisthosoma length 7.8, width 5.2.



Figure 6. A Habitat of *Spariolenus mansourii* sp. n., Nezel pit cave entrance (arrow) B Nezel pit cave entrance, the type locality, Naghsh-e-Jahan caving club entering the cave.

Chelicerae. With 3 anterior and 4 posterior teeth, cheliceral furrow with 10–15 intermarginal denticles.

Eyes. AME 0.28, ALE 0.78, PME 0.47, PLE 0.77, eye inter distances: AME-AME 0.19, AME-ALE 0.05, PME-PME 0.28, PME-PLE 0.69, AME-PME 0.57, ALE-PLE 0.63.

Legs. Leg formula: II I IV III. Palp 8.2 [2.6, 1.4, 1.8, 2.9], I 25.4 [7.2, 3.1, 6.8, 6.5, 1.8], II 27.7 [8.2, 3.3, 7.5, 6.7, 2.0], III 23.6 [7.1, 2.8, 6.2, 5.8, 1.7], IV 25.2 [7.4, 2.9, 6.5, 6.6, 1.8].

Spination. Palp 131, 101, 2121, 1013; Legs: Femur I–III 323, IV 321; Patella I–IV 101 (000); Tibia I–II 101(10), III 2028, IV 2026; Metatarsus I–III 2024, IV 3036.

Epigynum. As in diagnosis, with EF wider than long, AB present and elongated, MEP extending in anterior half in posteriorly and posterior half in laterally.

Remarks. This is the smallest *Spariolenus* species ever described. Both male and female are small to medium sized (11–15 mm). On the other side, *S. iranomaximus* Moradmand and Jäger, 2011 is the largest species, , with 18–31 mm body length.

Distribution and habitat preferences. Known from the type locality, the Nezel cave (Fig. 6A, B) and Pataveh (or Deh-Sheikh) cave. The specimens were observed in relatively large population around the karstic regions and entrances of the Nezel cave at night. The Nezel Cave is composed of four deep pits connected by horizontal corridors. The first pit is 43 meters deep (Fig. 6B).

The Pataveh cave has three entrances. Specimens were observed inside the entrance corridors during daytime. In both caves, the more humid parts inside where the walls were covered with a layer of condensed water, no *Spariolenus* specimens were observed.

Conservation status of the type locality. The Pataveh Cave was recently transformed into a tourist attraction and the corridors suffered from man-made constructions, a serious alert for its biodiversity.

Spariolenus hormozii Moradmand, sp. n.

http://zoobank.org/120A6B4D-1B1D-42B1-8C13-335A5997D8DD Fig. 7

Type material. *Holotype:* female, IRAN: Hormozgan Province: Hamag Protected area, Southern Zagros, Kuhe Fareghan, Hamag-e-Paeen, 27°51'52.00"N, 56°28'31.00"E, June 2015, S. Sami leg. (SMF).

Etymology. The species is named in honour of Mr Parwiz Hormozi who with his colleague Mr Mohammad Dehghani sacrificed their lives and were killed by poachers in 2016 while carrying out their duties as park rangers protecting the Wildlife in the Geno Biosphere Reserve, Hormozgan Province; genitive case.

Diagnosis. This species is distinguished from all other congeners by CO and EP largely widened (CO 1/2 EP width and EP 1/2 EF length) (Fig. 7A).

Male. Unknown.

Female. *Measurements*. large Sparassidae; total length 23.6, carapace length 11.1, width 10.0, anterior width 5.7, opisthosoma length 12.5, width 8.5.

Legs. Leg formula: II I IV III. Palp 14.2 [4.6, 2.5, 3.3, 4.8], I 49.4 [13.6, 5.7, 13.3, 13.7, 3.1], II 53.7 [15.3, 6.2, 15.1, 14.0, 3.1], III 45.1 [13.3, 5.2, 12.2, 11.6, 2.8], IV 48.1 [13.7, 5.2, 12.6, 13.5, 3.1].

Chelicerae. With 3 anterior and 4 posterior teeth, cheliceral furrow with 10–15 intermarginal denticles (Fig. 7D).

Eyes. AME 0.48, ALE 1.1, PME 0.67, PLE 1.4, eye inter distances: AME-AME 0.37, AME-ALE 0.12, PME-PME 0.53, PME-PLE 0.83, AME-PME 0.57, ALE-PLE 1.0.



Figure 7. *Spariolenus hormozii* sp. n., female holotype, Hormozgan Province, Hamag Protected area, Iran (SMF). **A** epigynum, ventral **B** vulva, dorsal **C** right vulva, lateral **D** chelicerae, ventral **E** eye arrangement, dorsal. Scale bars: 1 mm.

Eyes as Fig. 7E.

Spination. Palp 131, 101, 2121, 2013; Legs: Femur I–III 323, IV 321; Patella I–IV 101; Tibia I–II 101(10), III 1018, IV 2026 (2126); Metatarsus I–III 2024, IV 3036.

Epigynum. As in diagnosis, with EF as wide as long, EF quadrate in shape, AB present, MEP extend anterior half posteriorly and posterior half laterally, CO large and partitioned half of EP area (Fig. 7A–C)

Spariolenus khoozestanus Zamani, 2016

Fig. 8

Spariolenus khoozestanus Zamani, 2016: 421, figs 1–5 [holotype female (SMF) examined and illustrated]

Extended diagnosis. This single female differs from those of other species in having the anterior half of the FC extend transversally while in other species extend more longitudinally (Fig. 8A–C). The only exception is *S. iranomaximus* Moradmand and Jäger, 2011 but this species is unique in having wide spread HGO in SC and TC (Moradmand and Jäger 2011: figs 12–13).



Figure 8. *Spariolenus khoozestanus* Zamani, 2016, female holotype (SMF). **A** epigynum, ventral **B** vulva, dorsal **C** right vulva. Scale bar: 1 mm.

Comments. This species is erected on the basis of a single female specimen collected in Lali city, close to the type locality of *S. fathpouri* sp. n. In the original diagnosis, this species was compared with *S. tigris* Simon, 1880 from India, occurring far away from the type locality. Nevertheless its closest similar species seems to be *S. iranomaximus* which is more widespread in Southwest Zagros (per. obs). Both species share the character of widened FC of vulva, the shape of the CO, EP and the pattern of MEP. Since there are variations in the females copulatory structures (in particular the vulva of *S. iranomaximus* (see Moradmand and Jäger 2011: figs 12, 18, 19), *S. khoozestanus* is probably a junior synonym of the former species, but until the male is discovered from the type locality the taxonomic decision cannot be made confidently.

Discussion

Before this study ten species of *Spariolenus* were known worldwide, half of them described from Iran. This study increased the known species to 13. The distribution pattern of *Spariolenus* is currently known from Iran (eight species), Oman in the Arabian Peninsula (one species), and far South Indian Peninsula (four species). The diversity of *Spariolenus* spp. in the Iranian plateau seems to be higher than what is known today. Since Iranian species are discovered along the Zagros Mountain Range, thus their evolutionary history may be connected with the orogeny of these mountains (Moradmand and Jäger 2011).

Five out of 13 known species of *Spariolenus* are described from both sexes. Among known males, the shape of bifurcated ET, previously observed only in *S. zagros* can be seen in two other males herein described. This could mean that bifurcated ET is more common than simple ones and probably a plesiomorphic character.

In a recent checklist on cavernicolous arthropods in Iran, Malek-Hosseini and Zamani (2017) listed 89 taxa from only 47 subterranean habitats. Since the number of explored caves in Iran is more than 2000 (Raeisi et al. 2012) and the global diversity of cavernicolus species is estimated at 100,000 (Culver and Holsinger 1992). Thus the species richness of Iranian caves is expected to be much higher. The discovery of the three new species herein described supports this assumption.

Acknowledgements

I would like to thanks my colleagues at the University of Isfahan, Dr A. Bagheri, Ms Sh. Esmailbegi, Ms F. Moin and Ms M. Ahmadi for their companionship during sampling in Khuzestan Province. I thank Mr Mahmoodi and Mr Moradi from Lali city for their guidance to the Pebdeh cave location. I acknowledge the Naghsh-e-Jahan caving club members (Isfahan) and special thanks to Mrs Modaresi and Mr Mansouri for their supports of this study. I am thankful to Dr P. Jäger (SMF) and Dr C.A. Rheims (Universidade de São Paulo) for their constructive comments. Mr S. Sami (collector)

and Mr A. Zamani are acknowledged for providing the specimen from Hormozgan Province. Dr Y. M. Marusik (Turku) kindly sent the holotype of *S. khoozestanus*, for this, I am very thankful.

References

- Culver D, Holsinger JR (1992) How many species of troglobites are there? National Speleological Society Bulletin 54: 79–80.
- Davies VT (1994) The huntsman spiders *Heteropoda* Latreille and *Yiinthi* gen. nov. (Araneae: Heteropodidae) in Australia. Memoirs of the Queensland Museum 35: 75–122.
- Jäger P (2001) Diversität der Riesenkrabbenspinnen im Himalaya. Über eine Radiation zweier Gattungen in den Schneetropen (Araneae: Sparassidae: Heteropodinae). Courier Forschungsinstitut Senckenberg 232: 1–136.
- Malek-Hosseini MJ, Zamani A (2017) A checklist of subterranean arthropods of Iran. Subterranean Biology 21: 19–46. https://doi.org/10.3897/subtbiol.21.10573
- Moradmand M (2013) The stone huntsman spider genus *Eusparassus* (Araneae: Sparassidae): systematics and zoogeography with revision of the African and Arabian species. Zootaxa 3675: 1–108. http://dx.doi.org/10.11646/zootaxa.3675.1.1
- Moradmand M, Jäger P (2011) A review on the huntsman spider genus *Spariolenus* Simon, 1880 (Araneae: Sparassidae: Heteropodinae) in Iran with description of four new species. Zootaxa 2910: 46–62. http://dx.doi.org/10.11646/%25x
- Moradmand M, Schönhofer AL, Jäger P (2014) Molecular phylogeny of the huntsman spider family Sparassidae with focus on the genus *Eusparassus* and notes on the RTA-clade and "Laterigradae". Molecular Phylogenetics and Evolution 74: 48–65. http://dx.doi. org/10.1016/j.ympev.2014.01.021
- Raeisi E, Ghazy SH, Laumanns M (2012) Iran Cave Directory (3rd edn). Berliner Höhlenkundliche Berichte 45–46: 315.
- World Spider Catalog (2017) World Spider Catalog, version 17, Natural History Museum, Bern. http://wsc.nmbe.ch [accessed 9th Sep. 2017]
- Zamani A (2016) A new species of *Spariolenus* from South Iran (Aranei: Sparassidae). Arthropoda Selecta 25(4): 421–422.
- Zamani A, Mirshamsi O, Marusik YM, Moradmand M (2017) The Checklist of the Spiders of Iran. Version 2017. http://www.spiders.ir [accessed 9th Sep. 2017]

RESEARCH ARTICLE



An ecological survey of the invertebrate community at the epigean/hypogean interface

Stefano Mammola¹, Elena Piano¹, Pier Mauro Giachino², Marco Isaia¹

I Department of Life Sciences and Systems Biology, University of Torino, Via Accademia Albertina, 13-10123 Torino, Italy 2 Settore Fitosanitario Regionale, Environment Park, Palazzina A2, Via Livorno, 60-10144 Torino, Italy

Corresponding author: Stefano Mammola (stefanomammola@gmail.com); Marco Isaia (marco.isaia@unito.it)

cademic editor: O. Moldovan Received 11 October 2017 Accepted 27 November 2017 Published 11 December 2017	17
http://zoobank.org/899734E8-2028-4815-B3E7-316D251E47E9	

Citation: Mammola S, Piano E, Giachino PM, Isaia M (2017) An ecological survey of the invertebrate community at the epigean/hypogean interface. Subterranean Biology 24: 27–52. https://doi.org/10.3897/subtbiol.24.21585

Abstract

We studied the ecological continuum between caves and the associated network of fissures – *Milieu Souterrain Superficiel* (MSS) – in an hypogean site in the Graian Alps, Italy. Over one year, we surveyed the faunal assemblages by means of pitfall traps placed in the caves and specific subterranean sampling devices (SSD) buried in the MSS. We used generalized linear mixed models (GLMMs) and generalized additive mixed models (GAMMs) to compare the spatial and temporal dynamics of the subterranean invertebrates inhabiting the two environments. As expected, arthropod communities occurring near the surface were characterized by minor level of subterranean adaptations, and conversely, subterranean species were more abundant and diversified at higher depths, both in the caves and in the MSS. Diversity and abundance of external elements in the superficial layers were found to be highly seasonal dependent, with minor values in winter compared to the other seasons. We provided information about the faunal assemblages dwelling in the two hypogean compartments, and we characterized the microclimatic conditions therein. We discussed the existence of an ecological gradient of specialization extending from the surface to the deep hypogean layers, which can be interpreted in light of the microclimatic changes occurring at increasing depths and the parallel decrease in available organic matter.

Keywords

Mesovoid Shallow Substratum, Cave fauna, Superficial Subterranean Habitats, Subterranean biology, Subterranean Sampling Device, Ecological gradient, Troglobionts

Introduction

28

According to the modern view of subterranean biology, subterranean organisms do not exclusively inhabit underground vacuums of wide dimensions (i.e. caves), but also naturally occupy the network of fissures the size of which is not commensurable to the human scale (Racovitza 1907, Jeannel 1926, 1942, 1943, Juberthie et al. 1980a, 1980b, 1981, Uéno 1987). Culver and Pipan (2009a, 2014) recently categorised the most superficial subterranean habitats colonized by a strictly subterranean fauna as "Shallow Subterranean Habitats (SSHs)". Among the variety of SSHs listed by Culver and Pipan, the "Milieu Souterrain Superficiel (MSS)" is possibly one of the most inventively studied (Mammola et al. 2016). As a general definition, the MSS consists of a labyrinth of air-filled voids within rocky fragments that have accumulated for various morphogenetic reason on the bedrock, harbouring organisms showing adaptation to the subterranean conditions. In a hypothetical multilayer-structure, the MSS is generally found between the edaphic area - soil and rhizosphere - and the deep hypogean domain resulting, as a whole, in a "gradient from soil to cave" (Gers 1998). The climatic isolation of the MSS from the surface is usually achieved when the rocky layers are progressively covered by evolving soils with edaphic horizons (Giachino and Vailati 2010, Pipan et al. 2011), but other insulation mechanisms may be involved (Mammola et al. 2016). As a general rule, the external climatic variations are buffered in the MSS according to increasing depth (Nitzu et al. 2010, 2014), i.e. approaching a cave-like climate at higher distances from the surface (Badino 2010, Mammola et al. 2016).

Since MSS is not accessible to men unless by indirect means (see, e.g., López and Oromí 2010), biological studies focusing on the subterranean fauna are usually set in caves or in more accessible SSHs, such as lava tubes (e.g., Howarth 1972, Aschmole and Ashmole 1997, Arnedo et al. 2007). As a matter of fact, the great majority of the published papers investigating the MSS dealt with single model taxa, and were usually strictly taxonomical (Mammola et al. 2016). The focus of the MSS studies was rarely set on the whole community and/or on ecological processes (but see, e.g., Gers 1998, Nitzu et al. 2010, Pipan et al. 2011, Rendoš et al. 2012, Ortuño et al. 2013, 2014, Langourov et al. 2014, Jimenéz-Valverde et al. 2015). As an example, all the studies conducted so far in Italy focused on taxonomic descriptions of new species, sampled in the MSS by means of buried pitfall traps or sight-collected after excavation (e.g. Monguzzi 1982, 2011, Casale and Rondolini 1983, Casale and Giachino 1988, Vailati 2008, Magrini et al. 2012, Monzini 2013).

We conducted a one-year ecological study in an alpine hypogean site, aiming at investigating simultaneously the cave environment and the surrounding MSS. Our aims were to 1) compare the faunal assemblages characteristic of the two subterranean compartments; 2) investigate whether a temporal (seasonal) and/or a spatial gradient of specialization exists in the MSS – i.e. higher richness and abundance of specialized elements at increasing depth; 3) investigate whether the same gradients exist in the cave – i.e. variation in richness and abundance related to season and/or to vertical distance from the surface (subjacency) or to distance from the cave entrance.

Study area

The study was set in the Pugnetto hypogean complex, in the nearby to the hamlet of Pugnetto, municipality of Mezzenile, Lanzo Valley, Graian Alps, Piedmont (NW-Italy). The site is protected under the European Habitat Directive 43/92 (S.C.I. IT 1110048) and hosts five natural caves classified as "Caves not open to the public" (H 8310), namely the Borna di Pugnetto (cadastre number Pi 1501, entrance at N45°16'19", E5°02'26"; altitude 820 m a.s.l.), the Tana del Lupo (Pi 1502, N45°16'19", E5°02'22"; 813 m a.s.l.), the Creusa d'le Tampe (Pi 1503, N45°16'12", E5°02'33"; 870 m a.s.l.), the Tana della Volpe (Pi 1504, N45°16'13", E5°02'34", 885 m a.s.l.) and the Cavernetta (N45°16'17", E5°02'36", 895 m a.s.l.) which still lacks a cadastre number but was mentioned in the original description of the hypogean site by Muratore (1946).

Our study was conducted in the Borna di Pugnetto (hereinafter Borna) and in the Creusa d'le Tampe (hereinafter Creusa) caves (Figs 1a, b, d; 2), as well as in the surrounding MSS. The Borna has a planimetric development of 765 m. It consists of a main gallery of 300 m directed north-south, which splits at the end into two main branches – "Ramo della Madonna" and "Ramo della Fontana". The Creusa has a planimetric development of 47 m. It consists of a single tunnel, which leads to the final chamber after a narrow passage. The area surrounding the caves is characterized by a well-developed colluvial MSS made of fragmented rocks (calcschist, marble and phyllite) which form a network of habitat spaces with sizes between 0.1 and 10–20 cm, covered by 30–40 cm of leaf litter and soil (Fig. 1c, e). Surface conditions are characterized by a close and shaded *Luzulo-Fagetum* beech forest (Sindaco et al. 2009).

Traps and sampling design

We used twenty-four pitfall traps (diameter 9 cm, volume 40 ml) to collect invertebrates in caves. The pitfall traps were arranged in groups of three (hereinafter cavetriplets), at a distance of ~5 m to one another. Six cave-triplets (code: C1–C6) were placed in the Borna at 4, 30, 90, 150, 230 ("Ramo della Madonna") and 350 ("Ramo della Fontana") meters from the main entrance. Two cave-triplets (code: C7, C8) were placed in the Creusa at 5 and 25 meters from the main entrance (Fig. 2). We derived the subjacency (DEPTH; i.e., the direct vertical distance from the surface) of each cave triplet from the geological survey of Motta and Motta (2015). Specifically, we created three categories of subjacency (Fig. 2): 0–20 m (triplets C1, C5, C7, C8), 20–40 m (C2, C6), and 60–80 m (C3, C4) [see Mammola et al. (2015) for details].

For collecting invertebrates in the MSS, we utilized twenty-four Subterranean Sampling Devices (SSD; after López and Oromí 2010). Since the description of the MSS by Juberthie et al. (1980a, 1980b, 1981) and Uéno (1980), there has been considerable effort to construct and improve effective sampling techniques in this habitat



Figure I. a Main entrance of the Borna di Pugnetto (photo credit: Alberto Chiarle and Mauro Paschetta, 2014) **b** Main entrance of the Creusa d'le Tampe (photo credit: Elena Piano 2013) **c** exposed soil/MSS profile in a fresh-cut along a slope in the vicinity of the Borna di Pugnetto (photo credit: Jacopo Orlandini, 2014) **d** the typical cave geo-morphology within the Borna di Pugnetto (photo credit: Alberto Chiarle and Mauro Paschetta, 2014) **e** detail of the MSS geo-morphological structure (photo credit: Jacopo Orlandini, 2014).

(reviewed in López and Oromí 2010, Domingo-Quero and Alonso-Zarangara 2010, Mammola et al. 2016, Růžička and Dolanský 2016). In this study, we constructed SSDs relying on the prototypes of Nitzu et al. (2010, 2014) and Deltshev et al. (2011). SSDs consisted of PVC pipe (diameter 12.5 cm) with a silicone cap closing the top. A straight row of 25 holes (diameter: ~1.5 cm) was pierced at a distance of 8 cm from the lower-end of each pipe (Fig. 3a). At the same height, in correspondence of the level of holes row, we placed a standard pitfall trap. The holes drilled along the surface of the PVC pipe allowed the fauna to access to the inside and eventually fall into the pitfall trap; at the same time, our SSD allowed to recover, empty, refill and replace the pitfall trap trough the closing cap, ensuring that the substrate was not mechanically excavated at each sampling session (Fig. 3f, g). In addition, we inserted a styrofoam cylinder within the PVC tube, filling completely the pipe and insulating the trap from the external meteorological fluctuations. A screw was placed just above the sampling holes, to prevent the styrofoam cylinder from sliding down (Fig. 3b).

Three adjacent SSDs of three different length (40, 60 and 80 cm) were buried vertically in the ground (hereinafter MSS-triplet; Fig. 3c, d, e). The 40 cm SSD intercepted the substrate at the interface soil/MSS, while the 60 and 80 cm SSDs inter-



Figure 2. Map of the study area. The shape and the topographic position of the four caves (Borna Maggiore di Pugnetto, Tana del Lupo, Creusa d'le Tampe, Tana della Volpe) was obtained from the original planimetric drawings of Muratore (1946). The position of the sampling plots in caves ("cave triplets", C1–C8), in the MSS ("MSS triplets", M1–M8) and in the leaf litter ("epigean", L1–L6) are represented by coloured dots. The different sectors of the cave are coloured with different shades of grey representing the subjacency – i.e., vertical distance from the surface – according to Motta and Motta (2015).

cepted the MSS. Six MSS-triplets (18 SSDs; code: M1–M6) were set in the nearby of the Borna, and two MSS-triplets (6 SSDs; code: M7, M8) were placed in the nearby of the Creusa (Fig. 2). We installed the traps in the MSS following the advices reported in literature (Domingo-Quero and Alonso-Zarangara 2010, Giachino and Vailati 2010, Lopez and Oromì 2010, Mammola et al. 2016; see Fig. 3). Pitfall traps in caves and in the MSS were baited with chicken meat and filled with brine (supersaturated preserving solution of water and NaCl; Giachino and Vailati 2010).

Six pitfall traps (with brine, not baited) were also placed in leaf litter habitat (epigean; code: L1–L6), as a reference to discriminate correctly between the epigean fauna and the specialized fauna – see paragraph "Specimens sorting and ecological classification" for details.

Figure 2 shows the position of the traps in the area. We replaced the traps approximately once a month from June 2012 to June 2013. However, the access to the Borna is forbidden from 1st November to 31th March in order to protect the roosting bats inhabiting the cave. In order to reduce disturbance, in this period we accessed



Figure 3. a sampling holes (details) **b** Blocking screw **c** installation of an MSS-triplet **d** SSD of three different length **e** MSS-triplet buried in the ground **f**, **g** renewing the pitfall trap inside the SSD. Photo credits: Elena Piano.

the Borna only twice (December and March), resulting in ten total sampling sessions over the year. In order to evaluate the effect of the bait on the sampling probability, we replaced it every two sampling sessions, thus resulting in 5 sampling sessions with fresh and 5 with exhausted baits.

To characterize the subterranean microclimate, we placed one Hygrochron temperature and humidity datalogger in correspondence of each pitfall trap in the cave and at the lower-end of each SSD. Hygrochron were programmed to sample temperature (T) and relative humidity (RH) every three hours for the whole sampling period (accuracy of $\pm 0.5^{\circ}$ C and $\pm 1\%$, respectively). We also derived the mean daily outside temperature to the same periods from the nearest thermo-hygro-pluviometric weather station (Fua, Lanzo Torinese, Cod. 111; N45°17'23", E7°29'38"; 550 m a.s.l.). The temperature values recorded by the weather station were corrected with the standard environmental lapse rate – the change of temperature with altitude for the stationary atmosphere. In all analyses relating the abundance and species richness of external and adapted elements with the explanatory parameters (see later sections), we used the pseudo-replicates of each trap as basic sample units.

Specimens sorting and ecological classification

Trapped individual were sorted, identified and classified either as epigean (category: "external") or subterranean elements (category: "adapted"). According to our expertise (MI and SM: Araneae; PMG: Coleoptera and some other orders of Insecta) and the availability of specialists for additional *taxa* (see Acknowledgments), identification of the species levels was possible for the orders Araneae, Opiliones, Pseudoscorpiones, Chilopoda, Isopoda (one species), and for most orders of insects (especially Coleoptera; see Appendix 1).

In subterranean biology, species are often classified into ecological categories (e.g. trogloxenes, troglophiles, troglobionts) according to their preferred habitat and general association with the subterranean domain (Sket 2008, Trajano and Carvalho 2017). However, most ecological classifications, such as the Schiner-Racovitza system, are deemed to oversimplify real cases given that boundaries between the categories are often vague and difficult to attribute (Martìn et al. 2001, Giachino and Vailati 2016). Alternatively, in this work, to categorise our samples we followed the general idea based on a source-sink population model recently proposed by Trajano (2012). By examining the prevalence of individuals either in the leaf litter traps or in the traps installed in the subterranean habitats, we were able to classify species as "external" (species likely having source populations in the epigean environment) or "adapted" (species likely having source populations in the hypogean environment). Following this criterion, we calculated the abundance and species richness of external and subterranean elements (Next, Rext, Nad, Rad, respectively) for each trap in each sampling survey. Species represented by less than three individuals were not classified, and thus excluded from the analysis, unless if we possessed a solid literature background testifying the species ecological requirements - e.g., Lana (2001) for a few subterranean species in Piedmont and Mammola et al. (2017) for most spider species.

Statistical analysis

We performed regression-type analysis following the general advices of Zuur and Ieno (2016). All analysis were conducted in R (R development core team 2015). In order to test statistically our working hypothesis, we computed three distinct sets of regression models. Firstly, we compared the cave and the MSS habitats in term of species richness

and abundance of external and adapted organisms (aim 1). Subsequently, we analysed the spatial and temporal gradients in the MSS and cave environments separately, aiming at investigate the relevant abiotic factors driving richness and abundance in these two hypogean compartments (aims 2 and 3).

The first set of models was computed through mixed-design analysis of variance with Poisson distributed data (i.e. generalized linear mixed models, GLMMs; equation 1), whereas for the second (equation 2) and third (equation 3) sets, we primarily relied on Poisson generalized additive mixed models (GAMMs). The mixed part of both GLMMs and GAMMs was introduced in order to account for multiple observations from the same triplet over time, by specifying the triplet as random factor. The latter variable was included as random factor in order to account for the variation it introduced in our samples – and thus to correctly estimate the regression coefficients, – rather than to test for its direct effect on the dependent variables.

Prior to model fitting, we explored the three datasets following the standard protocol for data exploration proposed by Zuur et al. (2010). According to Zuur et al. (2009, 2010), the inclusion of outliers and highly correlated predictors in the regression analysis leads to misleading results – type I and II statistical errors. We thus used Cleveland's dotplots to assess the presence of outliers in dependent and independent variables and we investigated multi-collinearity among covariates. For each basic sample unit and for each set of models, we chose the abundance of external elements (Next), the species richness of external elements (Rext) and the abundance of adapted elements (Nad) as dependent variables. Such variables were selected aiming at investigate the gradient of specialization trough the hypogean environment (aims 2 and 3). Instead, considering the generally low species richness of adapted elements per trap (Rad), we were forced to exclude this latter variable from our analysis. For both abundance (Next, Nad) and richness (Rext) count data we assumed a Poisson distribution, but we tested for overdispersion after model fitting and we switched to a negative binomial distribution when the over-dispersion parameter was higher than 1.2.

Comparing MSS and cave communities (aim 1)

Species richness of external elements and abundance of adapted and external elements were analysed in relation to the explanatory two-levels factor HABITAT (levels: "Cave" and "MSS") using the *glmer* R command in the package 'lme4' (Bates et al. 2013).

The fixed structure of the model was:

(1) y ~ HABITAT

Where y is one of Next, Rext and Nad. The random part of the model allowed us to deal with repeated observations and measurements of the same triplet (temporal dependence) and the clumped distribution of the traps within the triplet (spatial dependence).

Spatial and temporal gradients in MSS (aim 2) and cave (3) communities

Species richness of external elements and abundance of adapted and external elements were analysed in relation to the explanatory variables using the *gamm* R command in the package 'mgcv' (Wood 2015). Generalized additive mixed model were used in order to account for possible non-linear trends of the sampling period (Serie_i; continuous variable). The optimum amount of smoothing was estimated trough generalized cross-validation (GCV). However, whenever the effect was linear and/or not significant, we dropped the smoothed term and fitted a new model including only parametric terms, thus adopting a linear approach (Poisson or negative binomial GLMM).

For both the cave and the MSS compartments, we also included the two-levels factor BAIT (level= "Fresh" and "Exhausted") as covariates and the two-levels factor SITE (levels= "Borna" and "Creusa"). The first variable was included to evaluate the effect of the ageing of the bait on our dependent variables. The second variable was introduced to take into account for possible local effects, since we pooled together records from two different caves and associated MSS.

For the analysis of the MSS compartment, in addition to the above mentioned variables we also included in the models the three-level categorical variable sampling depth (DEPTH; levels: "0.4m", "0.6m" and "0.8m"). For the cave habitat, in addition to the aforementioned variables, we also included the distance from the cave entrance (Dst_i; continuous variable) and the three-levels categorical variable subjacency (SUBJ; levels: "0–20m", "20–40m" and "60–80m"). We excluded from the regression analysis the microclimatic variables, given that due to malfunctioning, several dataloggers did not recorded reliable measurements of temperature and relative humidity during the sampling period (see results, further details in Mammola et al. 2015).

The (fixed) structures of the initial models were (aims 2 and 3, respectively):

- (2) $y \sim DEPTH + s(Serie_i) + BAIT + SITE$
- (3) y ~ Dst_i + SUBJ + s(Serie_i) + BAIT + SITE

Where y is one of Next, Rext and Nad, and s(Serie_i) indicate the smoothing term. The random part of the model is equal to the previous model (1). For the 2^{nd} and 3^{rd} models, we adopted a statistical hypothesis testing framework, whereby model reduction was carried out on the full model by sequentially deleting non-significant terms and potential interactions according to AIC values (Zuur et al. 2009), until a minimum adequate model of significant fixed effects remained (i.e. best model supported by observations). For all models, p-values for parametric term were based on z-tests; t-test in the case of GAMMs and GLMM with negative binomial distribution. P-values for smoother terms represent approximate significance based on F statistic. Model validation was carried out on the final models, following the approach of Zuur et al. (2009).

Results

General considerations

Approximately 15,700 Arthropoda (20 Orders), 117 Mollusca (order Pulmonata), 868 Crustacea (order Isopoda), and 14 Anellida (*Lumbricus* sp.) were collected. We report the complete list of the taxa and number of specimen collected in each habitat in Appendix 1. Diptera were the most represented, with 7,469 individuals collected (adults and larvae), followed by Coleoptera (3,543 individuals; adults and larvae), Collembola (1,755 individuals), Hymenoptera (1,046 individuals, mostly Formicidae) and Acari (631 individuals). MSS showed higher values of order diversity compared to caves, in which, abundance of specimens was higher.

Cave and MSS microclimate

Due to malfunction, several dataloggers did not sample either temperature or relative humidity during the sampling period. Specifically, nine out of eighteen in the Borna, two out of six in the Creusa and ten out of twenty-four in the MSS. Due to this significant loss of data, it was not possible to include climatic data in the regression analyses.

Relative humidity in the caves proved to be almost constantly close to saturation, with mean monthly values ranging from 85 to 100 %. However, in the vicinity of the entrance the relative humidity dropped down to 70–75 % in winter. In the MSS the mean monthly relative humidity was always above 90 %. With regard to temperature, changes and max–min ranges were attenuated with increasing distance from the entrance and delayed in respect to the outside values. The mean annual temperature values deep inside the two caves was comparable (Tmean \pm SD: Borna = 9.0 \pm 0.4 °C; Creusa = 8.9 \pm 0.8 °C) and presented little variations over the year. In the outermost sections, temperature was nearly stable in summer, spring and autumn, with a max–min range around 4.5 °C in the vicinity of the entrance for both caves. However, the microclimate at the entrance zone drastically changed during winter, when we observed a drop in the mean temperature values (mean values always below 6 °C). The coldest temperature values were recorded in December and January (Tmin: Borna = -2.0 °C and Creusa = -0.9 °C).

The range of temperature variation, both daily and monthly, was lower in the MSS in respect to surface (Fig. 4). In the MSS the thermal variability at the surface seemed to moderately affect the microclimatic conditions of MSS, resulting in seasonal variations in temperature at all sampled depths. The maximum temperature in the MSS did not exceed 19 °C even though air temperatures reached as high as 32.3 °C (June). Minimum temperature values rarely fell below 3 °C, with occasional drops below 0 °C (lower Tmin recorded in the MSS in December: –2.0 °C).



Figure 4. Annual trends of temperatures in the Pugnetto hypogean complex. The shade of blues indicate the relative position of the dataloggers at each cave-triplet, from the outermost (lighter blues) to the innermost sections (darker blues). Records from only one MSS-triplet are shown.

Cave and MSS comparison and faunal dynamics

Initial data exploration revealed the presence of a few outlying values (mostly due to higher prevalence of Diptera and Hymenoptera in certain traps), which were removed from the dataset. Both abundance and species richness of external elements were lower in caves in respect to MSS (Next: t = -4.37, p < 0.001; Rext: z = -4.93, p < 0.001). Conversely, the abundance of subterranean elements was higher in cave (Nad: t = 5.39, p < 0.001; Table 1).

The best model structures resulting from model selection concerning the analysis of MSS and caves (equations 2 and 3) are reported in Table 1. The analysis of the MSS data revealed contrasting patterns of species richness and abundance of external and subterranean elements according to sampling depth. The abundance and species richness of external elements was lower at -0.80 m (test relative to "0.40 m"; Next: t = -3.217, p = 0.001; Rext: t = -3.283, p = 0.001), whereas no significant effect was detected in respect to -0.60 m (Fig. 5a, b). The abundance of specialized elements was higher at -0.60 m (test relative to "0.40m"; Nad: z = 2.481, p = 0.013) and -0.80 m (test relative to "0.40m"; Nad: z = 4.333, p < 0.001; Fig. 5c). The ageing of the bait negatively influenced the abundance and richness of external elements, with higher values with fresh bait (test relative to "Exhausted"; Next: t = 4.489, p < 0.001; Rext: t = 3.005, p = 0.002). No effect was detected in respect to the abundance of adapted elements (Nad: z = 4.333, p = 0.507).

Abundance and richness of external elements showed a significant non-linear Ushaped trend in respect to the sampling series (Next: F = 9.02, p < 0.001; Rext: F =10.68, p < 0.001), with higher values in summer and early autumn, followed by a drastic decline in winter and an uprising in spring (Fig. 6 a, b). There was no significant relationship between the sampling series and the abundance of adapted elements.

Concerning the cave habitat, we detected an higher abundance of external and adapted elements in the Creusa cave (test relative to "Borna"; Next: t = 3.693, p < 0.001;

Table 1. Estimated regression parameters and approximate significance of smooth terms according to GLMMs and GAMMs, respectively, obtained from the 3 sets of models and the 3 dependent variables considered (Next, Rext, Nad, see text). The final model structures resulting from model selection are reported (only fixed terms are shown).

				Distri		Parametric coefficients:			Smooth terms		
	у	Final model	Model	bution	Variables	Estimate (α or β)	SE	р	edf	F	р
				Negative binomial	Intercept (α)	0.9722	0.4490	-	-	-	-
	Next	- HABITAI	GLMM		HABITAT (CAVE)	-2.9168	0.6669	<0.001 ***	-	-	_
MSS vs CAVE	D	LIADITAT	CLARK	р ·	Intercept (α)	0.1418	0.2975	_	_	_	_
(Equation 1)	Kext	~ HABITAT	GLMM	Poisson	HABITAT (CAVE)	-2.2727	0.4613	<0.001 ***	_	_	_
	Nad	ԱԴԵԼԱՆՆ	CIMM	Negative	Intercept (α)	-1.4751	0.4491	-	-	_	_
	INAU	~ HADITAT	GLMM	binomial	HABITAT (CAVE)	3.2843	0.6087	<0.001 ***	-	-	_
	Next				Intercept (α)	0.5339	0.2475	-	_	-	_
		~ BAIT + DEPTH + s(Serie_i)	GAMM		BAIT (Fresh)	0.9040	0.2014	<0.001 ***	-	-	_
				Poisson	DEPTH (0.6m)	-0.1260	0.1939	0.516	-	-	_
					DEPTH (0.8m)	-0.7835	0.2436	0.001	-	-	_
					s(Serie_i)	_	-	-	3.321	9.02	<0.001 ***
	Rext	~ BAIT + DEPTH + s(Serie_i)	GAMM	Poisson	Intercept (α)	-0.440	0.1813	-	_	-	_
MSS					BAIT (Fresh)	0.4196	0.1396	0.002	_	-	_
(Equation 2)					DEPTH (0.6m)	0.0497	0.1410	0.724	_	-	_
					DEPTH (0.8m)	-0.5601	0.1706	0.001 **	-	-	_
					s(Serie_i)	_	_	-	4.478	10.68	<0.001 ***
					Intercept (α)	-3.4961	0.6420	-	_	-	_
	Nad	~ BAIT +	GIMM	Poisson	BAIT (Fresh)	1.3337	0.2422	<0.001 ***	-	_	_
	INAU	ad DEPTH	GLIVIIVI	1 0122011	DEPTH (0.6m)	0.8145	0.3283	0.013	-	_	_
					DEPTH (0.8m)	1.3131	0.3030	<0.001 ***	-	_	_

			D	D	N	Parametric coefficients:			Smooth terms		
	у	Final model	Model	Distri- bution	Variables	Estimate (α or β)	SE	р	edf	F	р
				Poisson	Intercept (α)	-2.7564	0.5078	_	-	-	-
					SITE (Creusa)	2.9922	0.8183	<0.001 ***	_	_	_
	Next	~ SITE + s(Serie_i) x	GAMM		s(Serie_i) x SUBj (0–20m)	_	_	_	5383	5.544	<0.001 ***
		SUBJ			s(Serie_i) x SUBj (20–40m)	-	-	_	1.000	0.193	0.661
					s(Serie_i) x SUBj (60–80m)	-	_	-	1.000	0.194	0.660
CAVE	Rext	~ SUBJ + Dst_i	GLMM		Intercept (α)	-0.7185	0.2413	-	-	-	_
(Equation 3)				Poisson -	SUBj (20–40m)	-1.0541	0.6363	0.097	-	-	_
					SUBJ (60–80m)	-2.5317	1.1017	0.021 *	-	_	_
					Dst_i	-0.0077	0.0029	0.010 *	-	_	_
		~ SITE			Intercept (α)	1.3087	0.2343	_	-	_	_
					SITE (Creusa)	0.8874	0.2993	0.003 **	-	_	_
	Nad	+ BAIT + SUBJ + Dst_i	GLMM	Negative binomial	SUBj (20–40m)	1.3205	0.2918	<0.001 ***	_	_	-
					SUBJ (60–80m)	0.6406	0.2853	0.024 *	_	_	_
				Dst_i -0.00	-0.0017	0.0009	0.072	_	_	_	

Notes: BAIT = ageing of the bait (Categorical variable; levels: "Exhausted" and "Fresh"); Dst_i = distance from the cave entrance (Continuous variable); DEPTH = MSS sampling depth (Categorical variable; levels: "0.4m", "0.6m" and "0.8m"); HABITAT = habitat type (Categorical variable; levels: "Cave" and "MSS"); Serie_i = sampling series (Continuous variable); SITE = sampling site (Categorical variable; levels: "Borna", "Creusa"); SUBJ = subjacency (Categorical variable; levels: "0–20m", "20–40m", "60–80m"). The notation "s(Variable_i)" indicate continuous variables treated as smoothers. "x" denote statistical interaction. Significance codes: < 0.001 ***; < 0.005 **; < 0.05 *.

Nad: t = 2.965, p = 0.003), whereas the richness of external elements was not significantly influenced by this parameter (Fig. 5d, f). We detected a significant interaction between the sampling series and subjacency, with non-linear effects of the series in respect the abundance of external elements at 0–20m subjacency (Next at "0–20m": F = 5.544, p < 0.001). In the proximity of the surface, higher abundances were predicted during summer, autumn and spring, while a drastic decline was observed in winter (Fig. 6c). There was no significant interaction between the sampling serie and the other subjacency levels.



Figure 5. Boxplots showing the results of the regression analysis of the MSS (**a–c**) and the cave (**d–f**) data. Outlying values are not shown. Significance codes: < 0.001 ***; < 0.005 **; < 0.05 *.



Figure 6. Predicted values (black line) and 95% confidence intervals (grey surface) of the effect of the sampling series (Serie_i) on the abundance of external elements in the MSS (**a**), on the species richness of external elements in the MSS (**b**) and on the abundance of external elements in the cave at subjacency of 0–20m (**c**) derived from GAMM analyses. Only fixed effects are shown.

We observed a decrease in the richness of external elements at increasing distance from the cave entrance (Rext; z = -2.575, p = 0.010). Moreover, the richness of external elements was lower at 60–80m subjacency (test relative to "0–20 m"; Rext: z = -2.298, p = 0.021). No significant effect was detected in respect to the 20–40m subjacency (Fig. 5e). There was also a significant increase in the abundance of subterranean elements at higher subjacency in respect to 0–20m (Nad at "20–40m": t = 4.525, p < 0.001; Nad at "60–80m": t = 2.245, p = 0.024; Fig. 5f). There was no significant relationship between the distance from the entrance and the abundance of adapted elements.

Discussion

Despite subterranean animal communities being relatively simple, their precise characterization still represents an interesting topic in subterranean ecology. This is mostly because spatial boundaries and species composition of the communities are difficult to define, especially when considering MSS and its interconnections with the deep hypogean domain. Relying on the classical definitions (Racovitza 1907, Juberthie 2000, Culver and Pipan 2009b), Sendra et al. (2014) recently defined the subterranean domain as "[...] a network of intercommunicated micro, meso and macro voids, filled or not with meteoric water, extending from the surface towards the subsurface, allowing the movement of fauna communities and nutrients mostly from the surface." Accordingly, the connections between the superficial and the deep subterranean compartments represent an ideal ecological continuum with undefined boundaries, with the MSS acting as an extension of the deep hypogean domain toward the surface.

In this contribution, we aimed to investigate this ecological continuum both spatially and temporally, by comparing the arthropod communities inhabiting caves and the adjacent MSS compartments (Appendix 2). As far as we are aware, aside from this contribution, the only work in which a direct comparison between caves and the MSS is taken into consideration was the seminal study of Gers (1998), set in southwestern France. To some extent, we used a similar approach, by sorting out our samples on the basis of the degree of adaptation to subterranean life. However, we introduced a fresh approach, as we were able to discriminate objectively between subterranean and epigean organisms, thanks to the baseline provided by a series of control pitfall traps placed in the leaf litter (see paragraph "Specimens sorting and ecological classification" in the Materials and methods).

Although several species sampled at the Pugnetto hypogean complex are unique for obvious biogeographical reasons, the composition of the animal community was in general terms quite similar to that reported from other MSS sites in the Canary Islands (Pipan et al. 2011), Carpathians (Nitzu et al. 2010, 2014, Rendoš et al. 2012), Spain (Gilgado et al. 2014, Ortuño et al. 2014 Jiménez-Valverde et al. 2015), France (Juberthie and Decu 2006), and Bulgaria (Langourov et al. 2014). An abundance of individuals belonging to the main taxonomical groups sampled in the MSS, also aligned literature data (Borges 1993, Rendoš et al. 2012, Ortuño et al. 2013). In particular, we mostly collected arthropods, molluscs and anellids. The most represented orders were Diptera, Acari, Collembola and Coleoptera. In the case of Coleoptera, the proportion of individuals per family collected were, however, inverted in respect of the works of Juberthie and Decu (1998) and Moldovan (2005), in which Carabidae were more abundant than Cholevidae. In our study more than 60% of the captured beetles were Cholevidae and only \approx 20% were Carabidae.

We found that the abundance of specialized organisms was higher in the cave compartment, whilst we documented a higher diversity and abundance of epigean species in the MSS. The dominance of epigean species in the superficial layers of the MSS has been reported by several authors (Medina and Oromí 1990, Crouau-Roy et al. 1992, Borges 1993, Gers 1998, Růžička and Thaler 2002, Deltshev et al. 2011, Pipan et al. 2011, Nitzu et al. 2010, 2014, Rendoš et al. 2012, Barranco et al. 2013, Langourov et al. 2014, Ortuño et al. 2013, 2014, Jimenéz-Valverde et al. 2015, among others). This result suggests that our survey actually detected the superficial layers of the MSS, which are intimately connected with the epigean and the edaphic mediums, and thus more easily colonized by external elements (Mammola et al. 2016).

Concerning the cave habitat, we detected a higher diversity and abundance in the Creusa rather than in the Borna (Fig. 5d, f). The same pattern was previously documented by Capra and Conci (1951). The explanation for this result could be twofold. Firstly, the larger entrance of the Creusa – almost four time wider than the Borna; Fig. 1a,b – results in higher energy inputs (especially beech leaves) and a higher occurrence of epigean species (Fig. 5d), which may, in turn, indirectly support higher density and diversity of strictly subterranean species (Fig. 5f). Secondly, the Creusa is characterized by high values of relative humidity, an environmental factor playing a key role for the subterranean fauna (Mammola et al. 2015: 247, f. 7).

When analysing the cave and MSS habitats separately, we detected a gradient of subterranean specialization of the biological community in both compartments. In the cave, regression analyses suggests that there was a gradient of subterranean specialization of the biological community from the entrance zone toward the deepest sectors (see, e.g., Tobin et al. 2013) – or, more generally, from shallow toward deep subterranean spaces (Laška et al. 2011). The richness and abundance of epigean, less adapted species was indeed higher at lower depths in MSS and at lower subjacency in the caves. Conversely, subterranean species were more abundant and diversified at higher depth and subjacency. These results mirror that of Růžička and Klimeš (2005), Giachino and Vailati (2010), Deltshev et al. (2011) and Ortuño et al. (2013). The increase of climatic stability related to depth (Fig. 4; Nitzu et al. 2010, 2014, Rendoš et al. 2016b) and the parallel decrease in available organic matter (Gers 1998, Rendoš et al. 2016b) convincingly explains these patterns.

At the same time, we observed how the presence of external elements was seasonally dependent, their abundance being highly fluctuating during the year. We observed this trend both in the MSS (Fig. 6a, b) and in the cave at reduced subjacency (Fig. 6c). According to the results of the GAMM, the prevalence of external elements dropped significantly during winter. Seasonal dynamics in faunal assemblages were observed by previous authors focusing on MSS (e.g., Crouau-Roy et al. 1992, Nitzu et al. 2011, 2014, Rendoš et al. 2012, 2016a) and on caves (e.g., Tobin et al. 2013, Ferreira et al. 2015, Mammola et al. 2015, Bento et al. 2016, Lunghi et al. 2017). It is possible to argue that, over the year, at the blurry epigean/hypogean interface, there are complex exchanges involving fauna with different levels of subterranean adaptation (Prous et al. 2004, Moseley 2009, Novak et al. 2012), which introduce fundamental trophic resources in the subterranean habitat (Novak et al. 2013) but also cause greater fluctuations in species composition over the year. Ultimately, these results suggest how boundaries between these habitats are very undefined and may vary with the season. In contrast, we did not detect any seasonal variation in respect to the occurrence of subterranean elements both in the cave and in the MSS – i.e., the smoothed terms were not significant and we opted for regression models.

As a side question, with this study we were able to test the effect of the quality of the bait – at least up to two months – on the probability of capturing invertebrates in the two subterranean environments. This was possible since we renewed the bait in the trap every two sampling sessions, so that at each sampling session the condition of the bait changed from fresh to exhausted. According to the regression analysis, we demonstrated that a fresh bait is more effective in capturing individuals in the MSS (Fig. 5a, b, c) (i.e. that the bait is more attractive within the first month), unlike the cave compartment, in which we did not detect any significant effect. A similar trend is also discussed by Mammola et al. (2015).

Acknowledgments

We would like to thank all the taxonomists that helped us to identify specific taxa, namely Axel L. Schönhofer (Opiliones), Giulio Gardini (Pseudoscorpiones), Marzio Zapparoli (Chilopoda) and Massimo Meregalli (Coleoptera, Curculionidae). We are greatful to Paolo Debernardi (Natural Park of La Mandria) for allowing us to access the Borna di Pugnetto during winter, and to all friends and colleagues which helped in the samplings: Michele and Luigi Motta, Massimo Meregalli, Jacopo Orlandini, Mauro Paschetta, Fabio Ferrero, Enrico Lana, Elena Piano Sr., Davide Giuliano and Martina Dalle. We are indebted to Alberto Sendra, Vlastmil Růžička and Oana Moldovan for their help in improving the quality of the manuscript through their useful comments during the review process.

This study was set within the CaveLab project "From microclimate to climate change: caves as laboratories for the study of the effects of temperature on ecosystems and biodiversity", funded by University of Torino and Compagnia di San Paolo [grant number ORTO11T92F].

References

- Arnedo MA, Oromi P, Múrria C, Macías-Hernández N, Ribera C (2007) The dark side of an island radiation: systematics and evolution of troglobitic spiders of the genus Dysdera Latreille (Araneae: Dysderidae) in the Canary Islands. Invertebrate Systematics 21: 623–660. https://doi.org/10.1071/IS07015
- Ashmole PN, Ashmole MJ (1997) The land fauna of Ascension Island: new data from caves and lava flows, and a reconstruction of the prehistoric ecosystem. Journal of Biogeography 24: 549–589. https://doi.org/10.1111/j.1365-2699.1997.tb00070
- Badino G (2010) Underground meteorology. What's the weather underground? Acta Carsologica 39: 427–448. https://doi.org/10.3986/ac.v39i3.74

- Barranco P, Gilgado JD, Ortuño VM (2013) A new mute species of the genus Nemobius Serville (Orthoptera, Gryllidae, Nemobiinae) discovered in colluvial, stony debris in the Iberian Peninsula: A biological, phenological and biometric study. Zootaxa 3691: 201–219. https:// doi.org/10.11646/zootaxa.3691.2.1
- Bates D, Maechler M, Bolker B, Walker S (2013) lme4: Linear mixed-effects models using Eigen and S4. R package version 1.0-5 http://CRAN.R-project.org/package=lme4
- Bento DDM, Ferreira RL, Prous X, Souza-Silva M, Bellini BC, Vasconcellos A (2016) Seasonal variations in cave invertebrate communities in the semi-arid Caatinga, Brazil. Journal of Cave and Karst Studies 78(2): 61–71. https://doi.org/10.4311/2015LSC0111
- Borges P (1993) First records from the mesocavernous shallow stratum (M.S.S.) from the Azores. Mémoires de Biospéleologie 20: 49–53.
- Capra F, Conci C (1951) Nota sulle grotte del Pugnetto in val di Lanzo e sulla loro fauna (Piemonte). Rassegna Speleologica Italiana 3: 73–76.
- Casale A, Rondolini G (1983) Morphologie, distribution, écologie d'une espèce de Bathysciinae en milieu souterrain superficiel au Piémont (Coleoptera, Catopidae). Mémoires de Biospéleologie 10: 73–82.
- Casale A, Giachino PM (1988) Note su Sardaphaenops supramontanus Cerruti and Henrot, 1956 (Col. Carabidae), e descrizione di S. supramontanus grafittii n. subsp. Bollettino del Museo Regionale di Scienze Naturali Torino 3: 585–601.
- Crouau-Roy B, Crouau Y, Source CF (1992) Dynamic and temporal structure of the Troglobitic beetle Speonomus hydrophilus (Coleoptera: Bathysciinae). Ecography 15: 12–18. https:// doi.org/10.1111/j.1600-0587.1992.tb00002
- Culver DC, Pipan T (2009a) Superficial subterranean habitats gateway to the subterranean realm? Cave and Karst Science 35 (1/2): 5–12.
- Culver DC, Pipan T (2009b) The biology of caves and other subterranean habitats. Oxford University Press, Oxford.
- Culver DC, Pipan T (2014) Shallow Subterranean Habitats. Ecology, Evolution and Conservation. Oxford University Press, Oxford.
- Deltshev D, Lazarov D, Naumova M, Stoev P (2011) A survey of spiders (Araneae) inhabiting the euedaphic soil stratum and the superficial underground compartment in Bulgaria. Arachnologische Mitteilungen 40: 33–46. https://doi.org/10.5431/aramit4005
- Domingo-Quero T, Alonso-Zarangara AM (2010) Soil and litter sampling, including MSS. Abc Taxa 8: 173–212.
- Ferreira RL, Martins VM, Paixão EA, Silva MS (2015) Spatial and temporal fluctuations of the abundance of Neotropical cave-dwelling moth *Hypena* sp. (Noctuidae, Lepidoptera) influenced by temperature and humidity. Subterranean Biology 16: 47. https://doi.org/10.3897/ subtbiol.16.5137
- Gers C (1998) Diversity of energy fluxes and interactions between arthropod communities: from soil to cave. Acta Oecologica 19: 205–213. https://doi.org/10.1016/S1146-609X(98)80025-8
- Giachino PM, Vailati D (2008) Ulteriori considerazioni su alcuni aspetti biologici ed ecologici dell'ambiente sotterraneo. Ambiente Carsico: i progressi degli studi in Italia sulla soglia del XXI secolo. Atti Seminario Nazionale, Bossea (21-22 maggio 2005).

- Giachino PM, Vailati D (2010) The subterranean environment. Hypogean life, concepts and collecting techniques. WBA Handbooks, Verona.
- Giachino PM, Vailati D (2016) Riflessioni sulla terminologia biospeleologica: i concetti di troglobio, troglofilo e troglosseno. Atti Convegno Nazionale Bossea (22–23 Giugno 2013), La ricerca carsologica in Italia, 187–192.
- Gilgado JD, Ledesma E, Cuesta E, Arrechea E, Zapata de la Vega JL, Sánchez-Ruiz A, Ortuño VM (2014) *Dima assoi* Pérez Arcas 1872 (Coleoptera: Elateridae): from montane to hypogean life. An example of exaptations to the subterranean environment? Annales de la Société entomologique de France 50(3/4): 264–271. https://doi.org/10.1080/00379271 .2014.981421
- Howarth FG (1972) Cavernicoles in lava tubes on the island of Hawaii. Science 175: 325–326. https://doi.org/10.1126/science.175.4019.325
- Jeannel R (1926) Faune Cavernicole de la France, avec une Etude des Conditions d'Existence dans le Domaine Souterrain. P. Lechevallier, Paris.
- Jeannel R (1942) La genèse des faunes terrestres. Elémentes de biogéograèphie. Presses Universitaires de France éd, Paris.
- Jeannel R (1943) Les fossils vivants des cavernes. Gallimard, Paris.
- Jiménez-Valverde A, Gilgado JD, Sendra A, Pérez-Suárez G, Herrero-Borgoñón JJ, Ortuño VM (2015) Exceptional invertebrate diversity in a scree slope in Eastern Spain, Journal of Insect Conservation 19: 713–728. https://doi.org/10.1007/s10841-015-9794-1
- Juberthie C (2000) The diversity of the karstic and pseudokarstic hypogean habitats in the world. In: Wilkens H, Culver DC, Humphreys EF (Eds) Subterranean Ecosystems. Elsevier, Amsterdam, 17–39.
- Juberthie C, Delay B, Bouillon M (1980a) Sur l'existence d'un milieu souterrain superficiel en zone non calcaire. Comptes Rendus de l'Académie des Sciences Paris 290: 49–52.
- Juberthie C, Delay B, Bouillon M (1980b) Extension du milieu souterrain superficiel en zone non-calcaire: description d'un nouveau milieu et de son peuplement par les coleopteres troglobies. Mémoires de Biospéleologie 7: 19–52.
- Juberthie C, Delay B, Bouillon M (1981) Sur l'existence du milieu souterrain superficiel en zone calcaire. Mémoires de Biospéleologie 8: 77–93.
- Langourov M, Lazarov S, Stoev P, Guéorguiev B, Deltshev C, Petrov B, Andreev S, Simov N, Bekchiev R, Antonova V, Ljubomirov D, Dedov I, Georgiev D (2014) New and interesting records of the MSS and cave fauna of Vitosha Mt., Bulgaria. Proceedings of Balkan Speleological Conference "Sofia 2014", Sofia, Bulgaria, 28–30 March 2014, 66–76.
- Lana E (2001) Biospeleologia del Piemonte. Atlante fotografico sistematico. La grafica nuova, Regione Piemonte, Torino.
- Laška V, Kopecký O, Růžička V, Mikula J, Véle A, Šarapatka B, Tuf IH (2011) Vertical distribution of spiders in soil. Journal of Arachnology 39: 393–398. https://doi.org/10.1636/P09-75.1
- Latella L, Rampini M (1994) *Bathysciolay delayi*. Nuova specie di Leptodirino dei Monti Lepini (Coleoptera, Bathysciole). Fragmenta Entomologica 26: 141–150.
- López H, Oromí P (2010) A pitfall trap for sampling the mesovoid shallow substratum (MSS) fauna. Speleobiology Notes 2 : 7–11. https://doi.org/10.5563/spbn.v2i0.19

- Lunghi E, Manenti R, Ficetola GF (2017) Cave features, seasonality and subterranean distribution of non-obligate cave dwellers. PeerJ 5: e3169. https://doi.org/10.7717/peerj.3169
- Magrini P, Onnis C, Marcia P, Casale A (2012) *Typhloreicheia grafittii*, nuova specie della sardegna centro-orientale (Coleoptera, Carabidae). Fragmenta Entomologica 44: 1–12. https:// doi.org/10.4081/fe.2012.28
- Mammola S, Piano E, Giachino PM, Isaia M (2015). Seasonal dynamics and micro-climatic preference of two Alpine endemic hypogean beetles. International Journal of Speleology 44(3): 239–249. https://doi.org/10.5038/1827-806X.44.3.3
- Mammola S, Giachino PM, 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: 88. https://doi.org/10.1007/ s00114-016-1413-9
- Mammola S, Cardoso C, Ribera C, Pavlek M, Isaia M (2017) A synthesis on cave-dwelling spiders in Europe. Journal of Zoological Systematics and Evolutionary Research, 1-16. https://doi.org/10.1111/jzs.12201
- Martin LJ, Gargia H, Oromí YP (2001) Classification of terrestral subterranean fauna of vulcanic substrate in the Canary Island. International Journal of Speleology 30A(1/4):15–26. https://doi.org/10.5038/1827-806X.30.1.2
- Medina AL, Oromí P (1990) First data on the superficial underground compartment on La Gomera (Canary islands). Mémoires de Biospéleologie 17: 87–91.
- Moldovan O (2005) Beetles. In: Culver D, White WB (Eds) Encyclopedia of Caves. Elsevier Academic Press, NY, 45–51.
- Monguzzi R (1982) Studi sul genere *Boldoriella* Jeannel: sistematica, geonemia, ecologia (Coleoptera Carabidae Trechinae). Atti della Società Italiana di Scienze Naturali e del Museo Civico di Storia Naturale in Milano 123: 189–236.
- Monguzzi R (2011) Sintesi delle attuali conoscenze sul genere Allegrettia Jeannel, 1928 (Coleoptera, Carabidae, Trechinae). Annali Del Museo Civico Di Storia Naturale "Giacomo Doria" 103: 1–70.
- Monguzzi R, Regalin R (2001) Una nuova specie di *Boldoriella* s. str. dell'Alta Brianza (Lombardia) (Coleoptera Carabidae Trechinae). Giornale Italiano di Entomologia 9: 305–311.
- Monzini V (2013) Note su *Boldoriella* del gruppo carminatii: *Boldoriella silvanae* stat. nov. e osservazioni sistematiche sulle specie affini (Coleoptera Carabidae Trechinae). Bollettino della Società Entomologica Italiana 145: 3–8. https://doi.org/10.4081/BollettinoSEI.2013.3
- Moseley M (2009) Are all caves ecotones? Cave and Karst Science 39(2): 53-58.
- Motta L, Motta M (2015) The Climate of the Borna Maggiore di Pugnetto Cave (Lanzo Valley, Western Italian Alps. Universal Journal of Geoscience 3: 90–102. https://doi. org/10.13189/ujg.2015.030303
- Muratore G (1946) Grotte del Pugnetto: Valli di Lanzo-Stura di Ala. Rivista Mensile del C.A.I. 65: 21–29.
- Nitzu E, Nae A, Giurginca A, Popa I (2010) Invertebrate communities from the mesovoid shallow substratum of the Carpatho-Euxinic area: eco-faunistic and zoogeographic analysis. Travaux de l'Institut de Spéologie "Émile Racovitza" 49: 41–79.

- Nitzu E, Popa I, Giurginca A (2011) Invertebrate fauna (Coleoptera, Collembola, Diplopoda, Isopoda) collected in the karst areas of the Aninei-Locvei Mountains, Travaux de l'Institut de Spéologie "Émile Racovitza" 50: 15–35.
- Nitzu E, Nae A, Băncilă R, Popa I, Giurginca A, Plăiaşu R (2014) Scree habitats: ecological function, species conservation and vertical-temporal variation in the arthropod community. Systematics and Biodiversity 12: 65–75. https://doi.org/10.1080/14772000.2013.87876
- Novak T, Janžekovič F, Lipovšek S (2013) Contribution of non-troglobiotic terrestrial invertebrates to carbon input in hypogean habitats. Acta Carsologica 42: 301–309. https://doi. org/10.3986/ac.v42i2-3.669
- Novak T, Perc M, Lipovšek S, Janžekovič F (2012) Duality of terrestrial subterranean fauna. International Journal of Speleology 41(2): 181–188. https://doi.org/10.5038/1827-806X.41.2.5
- Ortuño VM, Gilgado JD, Valverde JA, Sendra A, Pérez-Suárez G, Herrero-Borgoñón JJ (2013) The "Alluvial Mesovoid Shallow Substratum", a new subterranean habitat. PloS ONE 8: e76311. https://doi.org/10.1371/journal.pone.0076311
- Ortuño VM, Cuesta E, Gilgado JD, Ledesma E (2014) A new hypogean Trechus Clairville (Coleoptera, Carabidae, Trechini) discovered in a non-calcareous Superficial Subterranean Habitat of the Iberian System (Central Spain). Zootaxa 3802: 359–372. https://doi. org/10.11646/zootaxa.3802.3.5
- Pipan T, López H, Oromí P, Polak S, Culver DC (2011) Temperature variation and the presence of troglobionts in terrestrial shallow subterranean habitats. Journal of Natural History 45: 253–273. https://doi.org/10.1080/00222933.2010.523797
- Prous X, Ferreira RL, Martins RP (2004) Ecotone delimitation: Epigean-hypogean transition in cave ecosystems. Austral Ecology 29: 374–382. https://doi.org/10.1111 /j.1442-9993.2004.01373
- R Development Core Team (2015) R: A Language and Envi ronment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org
- Racovitza EG (1907) Essai sur les problèmes biospéologiques. Archives de Zoologie Expérimentale et Générale 4(6): 371–488.
- Rendoš M, Mock A, Jászay T (2012) Spatial and temporal dynamics of invertebrates dwelling karstic mesovoid shallow substratum of Sivec National Nature Reserve (Slovakia), with emphasis on Coleoptera. Biologia 67: 1143–1151. https://doi.org/10.2478/s11756-012-0113-y
- Rendoš M, Mock A, Miklisová D (2016a) Terrestrial isopods and myriapods in a forested scree slope: subterranean biodiversity, depth gradient and annual dynamics. Journal of Natural History 50(33-34): 2129–2142. https://doi.org/10.1080/00222933.2016.1193642
- Rendoš M, Raschmanová N, Kováč L, Miklisová D, Mock A, Ľuptáčik P (2016b) Organic carbon content and temperature as substantial factors affecting diversity and vertical distribution of Collembola on forested scree slopes. European Journal of Soil Biology 75: 180–187. https://doi.org/10.1016/j.ejsobi.2016.06.001
- Růžička V, Klimeš L (2005) Spider (Araneae) Communities of Scree Slopes in the Czech Republic. Journal of Arachnology 33: 280–289. https://doi.org/10.1636/04-44.1
- Růžička V, Thaler K (2002) Spiders (Araneae) from deep screes in the Northern Alps (Tyrol, Austria). Berichte des naturwissenschaftlichen-medizinischen Verein Innsbruck 89: 137–141.

- Růžička V, Dolanský J (2016) Catching of spiders in shallow subterranean habitats in the Czech Republic. Arachnologische Mitteilungen 51: 43–48. https://doi.org/10.5431/aramit5106
- Sendra A, Garay P, Ortuño VM, Gilgado JD, Teruel S, Reboleira ASPS (2014) Hypogenic versus epigenic subterranean ecosystem: lessons from eastern Iberian Peninsula. International Journal of Speleology 43: 253–264. https://doi.org/10.5038/1827-806X.43.3.2
- Sket B (2008) Can we agree on an ecological classification of subterranean animals? Journal of Natural History 42: 1549–1563. https://doi.org/10.1080/00222930801995762
- Sindaco R, Savoldelli P, Selvaggi A (2008) La Rete Natura 2000 in Piemonte. I Siti di Importanza Comunitaria, Regione Piemonte.
- Tobin BW, Hutchins BT, Schwartz BF (2013) Spatial and temporal changes in invertebrate assemblage structure from the entrance to deep-cave zone of a temperate marble cave, International Journal of Speleology 42: 203–214.
- Trajano E (2012) Ecological classification of subterranean organisms. In: White WB, Culver DC (Eds) Encyclopedia of caves. Elsevier.
- Trajano E, de Carvalho MR (2017) Towards a biologically meaningful classification of subterranean organisms: a critical analysis of the Schiner-Racovitza system from a historical perspective, difficulties of its application and implications for conservation. Subterranean Biology 22: 1–26. https://doi.org/10.3897/subtbiol.22.9759
- Uéno SI (1980) The anophthalmic trechine beetles of the group of *Trechiama ohshimai*. Bulletin of the National Science Museum Tokyo, Serie A 6: 195–274.
- Uéno SI (1987) The derivation of terrestrial cave animals Zoological Science 4: 593-606.
- Vailati D (1988) Studi su Bathysciinae delle Prealpi centro-occidentali. Revisione sistematica, ecologia, biogeografia della "serie filetica di *Boldoria*" (Coleoptera Catopidae), Museo Civico di Scienze Naturali di Brescia, Brescia.
- Wood S (2015) Mixed GAM Computation Vehicle with GCV/AIC/REML Smoothness Estimation. R package version 1.8-7 http://CRAN.R-project.org/package=mgcv
- Zuur AF, Ieno EN, Walker NJ, Savaliev AA, Smith GM (2009) Mixed effect models and extensions in ecology with R. Springer, Berlin.
- Zuur AF, Ieno EN, C.S. Elphick (2010) A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution 1: 3–14. https://doi.org/10.1111/ j.2041-210X.2009.00001
- Zuur AF, Ieno EN (2016) A protocol for conducting and presenting results of regression type analyses. Methods in Ecology and Evolution 7: 636–645. https://doi.org/10.1111/2041-210X.12577

Appendix I

Taxonomic groups sampled in this study according to the habitat. The total number of sampled individuals in each habitat is reported. The column "adaptation" provides the attribution to the category "external" or "adapted". Classification was based on the criteria defined in the relative paragraph.

Class	Order	Family	Species	Adaptation	Litter	MSS	Cave
Arachnida	Acarina	Fam.	Morphospecies I	Adapted	87	24	137
Arachnida	Acarina	Fam.	Morphospecies II	External	75	-	-
Arachnida	Acarina	Fam.	Morphospecies III	External	18	26	-
Arachnida	Acarina	Fam.	Morphospecies IV	Adapted	-	79	63
Arachnida	Acarina	Fam.	Morphospecies V	Adapted	-	122	-
Arachnida	Araneae	Agelenidae	<i>Histopona leonardoi</i> Pantini & Isaia, 2013	External	17	14	-
Arachnida	Araneae	Agelenidae	Tegenaria silvestris L. Koch, 1872	Adapted	2	8	4
Arachnida	Araneae	Amaurobiidae	Amaurobius sp.	-	2	1	-
Arachnida	Araneae	Dysderidae	Harpactocrates drassoides (Simon, 1882)	External	37	34	1
Arachnida	Araneae	Fam.	Immatures indet.	-	2	3	-
Arachnida	Araneae	Gnaphosidae	Drassodes sp.	-	-	1	-
Arachnida	Araneae	Linyphiidae	Gen. sp.	Adapted	-	6	2
Arachnida	Araneae	Linyphiidae	Mansuphantes aridus (Thorell, 1875)	Adapted	_	1	1
Arachnida	Araneae	Linyphiidae	<i>Micrargus alpinus</i> Relys & Weiss, 1997	Adapted	-	1	-
Arachnida	Araneae	Linyphiidae	Porrhomma convexum (Westring, 1851)	Adapted	_	2	-
Arachnida	Araneae	Linyphiidae	<i>Troglohyphantes bornensis</i> Isaia & Pantini, 2008	Adapted	_	4	15
Arachnida	Araneae	Linyphiidae	<i>Troglohyphantes lucifer</i> Isaia, Mam- mola & Pantini, 2016	Adapted	_	6	1
Arachnida	Araneae	Linyphiidae	Troglohyphantes n. sp.	Adapted	2	6	-
Arachnida	Araneae	Lycosidae	Trochosa hispanica Simon, 1870	External	1	-	-
Arachnida	Araneae	Nesticidae	Kryptoesticus eremita (Simon, 1879)	Adapted	-	3	2
Arachnida	Araneae	Philodromidae	Philodromus sp.	External	-	1	-
Arachnida	Araneae	Phrurolithidae	Phrurolithus festivus (C. L. Koch, 1835)	External	-	1	-
Arachnida	Araneae	Pimoidae	<i>Pimoa graphitica</i> Mammola, Hormi- ga & Isaia, 2016	Adapted	-	-	2
Arachnida	Araneae	Salticidae	Saitis barbipes (Simon, 1868)	External	1	-	-
Arachnida	Araneae	Tetragnathidae	Meta menardi (Latreille, 1804)	Adapted	-	1	2
Arachnida	Araneae	Tetragnathidae	Metellina merianae (Scopoli 1763)	Adapted	-	1	-
Arachnida	Araneae	Theridiidae	Gen. sp.	-	_	1	-
Arachnida	Araneae	Theridiidae	Pholcomma gibbum (Westring, 1851)	External	3	-	-
Arachnida	Opiliones	Dicranolasmatidae	<i>Dicranolasma soerensenii</i> Thorell, 1876	External	35	3	-
Arachnida	Opiliones	Ischyropsalidae	<i>Ischyropsalis dentipalpis</i> Canestrini, 1872	Adapted	_	-	1
Arachnida	Opiliones	Nemastomatidae	Nemastoma dentigerum Canestrini, 1873	External	29	1	_
Arachnida	Opiliones	Nemastomatidae	Paranemastoma quadripunctatum (Perty, 1833)	External	19	4	-
Arachnida	Opiliones	Phalangiidae	Gen. sp.	-	1	-	-

Class	Order	Family	Species	Adaptation	Litter	MSS	Cave
Arachnida	Opiliones	Phalangiidae	Astrobunus bernardinus Simon, 1879	External	22	1	-
Arachnida	Opiliones	Phalangiidae	Leiobunum religiosum (Simon, 1879)	-	-	_	1
Arachnida	Opiliones	Phalangiidae	Odiellus coronatus (Roewer, 1911)	External	4	_	-
Arachnida	Opiliones	Trogulidae	Anelasmocephalus rufitarsis Simon, 1879	External	17	-	_
Arachnida	Opiliones	Trogulidae	Trogulus nepaeformis (Scopoli, 1763)	External	13	1	-
Arachnida	Pseudoscorpiones	Chthoniidae	Chthonius (C.) tenuis L. Koch, 1873	External	10	7	2
Arachnida	Pseudoscorpiones	Chthoniidae	Chthonius (Globochthonius) globifer Simon, 1879	External	6	3	2
Arachnida	Pseudoscorpiones	Chthoniidae	Chthonius sp.	-	-	3	-
Arachnida	Pseudoscorpiones	Neobisiidae	Roncus sp.	External	11	-	-
Chilopoda	Ord.	Fam.	Gen. sp.	External	161	-	-
Chilopoda	Geophilomorpha	Linotaeniidae	Strigamia acuminata (Leach, 1814)	-	-	2	-
Chilopoda	Lithobiomorpha	Lithobiidae	Eupolybothrus tridentinus (Fanzago, 1874)	-	-	1	-
Chilopoda	Lithobiomorpha	Lithobiidae	Lithobius pellicensis Verhoeff, 1935	-	-	-	3
Chilopoda	Lithobiomorpha	Lithobiidae	Lithobius pilicornis Newport, 1844	-	-	1	-
Chilopoda	Scolopendro- morpha	Cryptopidae	Cryptops parisi Brolemann, 1920	-	-	1	-
Clitellata	Haplotaxida	Lumbricidae	Lumbricus sp.	External	12	2	-
Diplopoda	Chordeumatida	Craspedosomatidae	Gen. sp.	External	511	33	4
Diplopoda	Glomerida	Glomeridae	Gen. sp.	External	26	6	
Entognatha	Collembola	Fam.	Morphospecies I	External	45	79	285
Entognatha	Collembola	Fam.	Morphospecies II	Adapted	-	-	323
Entognatha	Collembola	Fam.	Morphospecies III	Adapted	-	321	477
Entognatha	Collembola	Fam.	Morphospecies IV	Adapted	1	127	-
Entognatha	Collembola	Fam.	Morphospecies V	External	22	-	-
Entognatha	Collembola	Fam.	Morphospecies VI	External	75	-	-
Entognatha	Diplura	Fam.	Gen. sp.	External	76	-	-
Gastropoda	Pulmonata	Helicidae	Helix sp.	External	35	_	-
Gastropoda	Pulmonata	Limacidae	<i>Limax</i> sp.	External	22	36	-
Gastropoda	Pulmonata	Oxychilidae	Oxychilus sp.	Adapted	-	22	2
Insecta	Blattodea	Fam.	Larvae indet.	External	-	4	-
Insecta	Blattodea	Blattellidae	<i>Blattella</i> sp.	External	10	2	-
Insecta	Coleoptera	Fam.	Larvae indet.	-	18	42	44
Insecta	Coleoptera	Carabidae	Abax continuus Ganglbauer, 1891	External	163	3	-
Insecta	Coleoptera	Carabidae	Bembidion (Peryphanes) deletum Audinet Serville, 1821	-	-	1	-
Insecta	Coleoptera	Carabidae	<i>Binaghites affinis ovalipennis</i> (Ganglbauer, 1900)	External	1	-	-
Insecta	Coleoptera	Carabidae	Carabus intricatus Linne, 1761	External	3	-	-
Insecta	Coleoptera	Carabidae	Carabus monticola Dejean, 1826	External	26	-	-
Insecta	Coleoptera	Carabidae	Cychrus italicus Bonelli, 1810	External	28	1	-
Insecta	Coleoptera	Carabidae	Limodromus assimilis (Paykull, 1790)	External	1	-	-
Insecta	Coleoptera	Carabidae	Platynus complanatus Dejean, 1828	External	4	2	1
Insecta	Coleoptera	Carabidae	Pterostichus (Oreophilus) externepun- ctatus (Dejean, 1828)	External	106	38	-
Insecta	Coleoptera	Carabidae	Pterostichus (Pterostichus) rutilans (Dejean, 1828)	Adapted	-	5	-
Insecta	Coleoptera	Carabidae	Sphodropsis ghilianii ghilianii (Schaum, 1858)	Adapted	2	189	381

Class	Order	Family	Species Adaptati		Litter	MSS	Cave
Insecta	Coleoptera	Carabidae	<i>Stomis (Stomis) elegans</i> Chaudoir, 1861	External	6	-	-
Insecta	Coleoptera	Carabidae	Trechus modestus Putzeys, 1874	_	-	1	-
Insecta	Coleoptera	Cholevidae	<i>Apocatops monguzzii</i> Giachino & Vailati, 1987	Adapted	-	4	1
Insecta	Coleoptera	Cholevidae	Bathysciola pumilio (Reitter, 1884)	Adapted	49	150	34
Insecta	Coleoptera	Cholevidae	Catops fuliginosus Erichson, 1837	-	-	1	-
Insecta	Coleoptera	Cholevidae	Catops subfuscus Kellner, 1846	Adapted	-	195	175
Insecta	Coleoptera	Cholevidae	Catops tristis (Panzer, 1794)	-	1	2	-
Insecta	Coleoptera	Cholevidae	Catops ventricosus rotundatus Szymczakowski, 1963	External	161	113	2
Insecta	Coleoptera	Cholevidae	Choleva angustata (Fabricius, 1781)	Adapted	-	-	4
Insecta	Coleoptera	Cholevidae	Dellabeffaella roccae (Capra, 1924)	Adapted	-	1	991
Insecta	Coleoptera	Cholevidae	Sciodrepoides watsoni (Spence, 1815)	Adapted	-	38	-
Insecta	Coleoptera	Colonidae	Colon sp.	-	-	4	-
Insecta	Coleoptera	Curculionidae	Otiorhynchus salicicola Heyden, 1908	External	11	3	-
Insecta	Coleoptera	Elateridae	Gen. sp.	-	-	1	-
Insecta	Coleoptera	Latridiidae	Gen. sp.	External	4	_	-
Insecta	Coleoptera	Leiodidae	Gen. sp.	-	2	1	-
Insecta	Coleoptera	Ptinidae	Gen. sp.	-	2	1	-
Insecta	Coleoptera	Scaphydidae	Gen. sp.	-	3	-	-
Insecta	Coleoptera	Scarabeidae	Gen. sp.	External	111	_	1
Insecta	Coleoptera	Scydmaenidae	Gen. sp.	External	_	-	1
Insecta	Coleoptera	Silphidae	Nicrophorus sp.	External	137	14	-
Insecta	Coleoptera	Silphidae	Silpha sp.	External	6	-	-
Insecta	Coleoptera	Staphylinidae	Bryaxis brachati Besuchet, 1980	_	-	2	-
Insecta	Coleoptera	Staphylinidae	Gen. sp.	External	111	133	-
Insecta	Coleoptera	Staphylinidae	Gen. sp. (Pselaphinae)	Adapted	_	4	2
Insecta	Dermaptera	Forficulidae	Forficula sp.	External	2	2	-
Insecta	Diptera	Fam.	Larvae indet.	-	289	421	787
Insecta	Diptera	Limoniidae	Chionea sp.	Adapted	_	37	1
Insecta	Diptera	Limoniidae	Gen. sp.	Adapted	7	570	2085
Insecta	Diptera	Muscidae	Gen. sp.	External	100	79	-
Insecta	Diptera	Phoridae	Gen. sp.	External	1071	1220	802
Insecta	Hymenoptera	Formicidae	Gen. sp.	External	360	678	3
Insecta	Hymenoptera	Vespidae	Gen. sp.	External	4	1	-
Insecta	Orthoptera	Acrididae	Gen. sp.	External	9	_	-
Insecta	Orthoptera	Gryllidae	Gryllus sp.	-	2	_	_
Insecta	Orthoptera	Rhaphidophoridae	Dolichopoda azami septentrionalis Baccetti & Capra, 1958	Adapted	_	1	14
Insecta	Rhyncota	Fam.	Gen. sp.	-	_	3	-
Insecta	Rhyncota	Pentatomidae	Pentatoma sp.	External	7	_	_
Insecta	Rhyncota	Pyrrhocoridae	Pyrrhocoris apterus (Linnaeus, 1758)	External	3	_	-
Malaco- straca	Isopoda	Armadillidiidae	Gen. sp.	External	569	14	-
Malaco- straca	Isopoda	Trichoniscidae	Alpioniscus feneriensis caprae Parona, 1880	Adapted	-	6	279
Zygentoma	Thysanura	Lepismatidae	Lepisma sp.	External	11	1	-

Appendix 2



Graphical abstract.

Subterranean Biology 24:53–61 (2017) doi: 10.3897/subtbiol.24.22905 http://subtbiol.pensoft.net

IN MEMORIAM



Kenneth A. Christiansen (1924-2017)

David C. Culver¹

I Department of Environmental Science, American University, Washington, DC 20016, USA

Corresponding author: David C. Culver (dculver@american.edu)

Received 11 December 2017 Accepted 12 December 2017 Published 22 December 2017
http://zoobank.org/D2D23592-0330-4FEE-AC79-8666664AB9FE4

Citation: Culver DC (2017) Kenneth A. Christiansen (1924–2017). Subterranean Biology 24: 53–61. https://doi. org/10.3897/subtbiol.24.22905



One of the great speleobiologists and Collembola systematists, Ken Christiansen, died on November 26, 2017, at the age of 93. Ken was truly unique; no one who ever came in contact with him ever forgot him. A scholar and intellect of the first order, he always had time and enthusiasm for the work of his students and colleagues. His level of energy and enthusiasm was such that even into his 80's, his colleague and fellow collembologist Louis Deharveng called him "Hurricane Ken" after a visit to Louis at the Paris Museum of Natural History. He touched the lives of generations of students at Grinnell College and the lives of generations of Collembola taxonomists and speleobiologists throughout the world.

One of the most formative influences on Ken's life, surpassed only by his wife Phyllis and their four children, was his service in World War II in the U.S. Army Second Armored Infantry Division as a forward observer in the campaigns in Europe and North Africa. A genuine war hero and fierce anti-fascist, he was awarded a bronze star and an oak leaf cluster for bravery in combat. I once asked Ken what rank he achieved and he told me he was promoted to corporal three times! Anyone who believes the phrase that there are no atheists in a foxhole never met Ken and for many years he was famous at Grinnell for his atheism lecture. Taking advantage of the GI Bill, Ken went to Boston University and Harvard University, and graduated with a Ph.D. from Harvard in 1951. His thesis (Christiansen 1958d) was on a rather large group of Collembola (the genus *Entomobrya*), large both in the sense of numbers of species and in terms of body size, reaching more than 1 mm in the Lilliputian Collembola world. His first job was at American University in Beirut, where he took advantage of his location to study the Collembola of Lebanon and Syria (Christiansen 1956c, 1957, 1958b). Sometime during his stay, probably in the summer of 1954, when he spent time in Switzerland with European collembologists Gisin and Delamare Deboutteville among others, he became interested in cave Collembola, of which there are many. Shortly after that he embarked on the study of adaptation in cave Collembola, especially the convergent evolution of antennal and appendage lengthening, as well as changes in claw structure. In 1955, he accepted a faculty position at Grinnell College in Iowa, where he stayed for the rest of his career.

While the winds of neo-Darwinism were blowing strongly in North America (especially at Harvard, the home institution of the great evolutionist Ernst Mayr), they were at best a faint breeze in continental Europe, the center of research on subterranean biology at the time. In a series of papers that continued for the next six decades (Christiansen 1960a, 1960b, 1965, 1982b, 1992a, 1995, 2003a, 2004, 2012; Christiansen and Culver 1968, 1969, Peck and Christiansen 1990), he offered what was really the first neo-Darwinian explanation for the morphological convergence of not only eye and pigment loss, but also appendage elongation and claw modification, of many lineages of cave Collembola. In 1962, he wrote his only paper in French (of course that is one more than any other cave biologist from the U.S.), coining a new term for the suite of evolutionarily convergent features found in cave organisms—troglomorphy (Christiansen 1962). This short paper, in an obscure journal, is a highly cited papers in cave biology, with over 100 citations. Together with Thomas Barr and Thomas Poulson, he established a North American school of subterranean biology, one with neo-Darwinism at its core. This was the first time since the 19th century that North Americans became a presence in the field. In the 19th century, neo-Lamarckians, especially A.S. Packard, brought North America to prominence in the field.

It was also in the 1960's that he initiated the study of the ecology of Hunters Cave in Iowa (Christiansen 1961b, Christiansen et al. 1961), one of the first ecological studies of a whole cave. Later on, he wrote another paper on cave ecosystems, this time with Michel Bouillon (Christiansen and Bouillon 1978) on caves in the Pyrenees. He spent two sabbatical leaves in 1962, 1967 and 1968 at the Laboratoire Souterrain in Moulis, France, then the leading research institution for subterranean biology. Until at least the mid 1970's, he was the only North American with extensive contacts and collaborations in continental Europe.

His work on evolution of cave animals was more than matched by his work on the taxonomy of cave animals. There are approximately 60 species of cave Collembola known from U.S. caves and he described nearly 50 of them! During the course of his career, he described species from all the major genera of Collembola occupying North American caves—*Onychiurus* (Pomorski et al. 2009), *Pseudosinella* [Christiansen 1960b, 1983; Christiansen and Luther 1986; Christiansen and Moberg 1988; Christiansen and Bellinger 1996b), *Pygmarrhopalites* (Christiansen 1966; Christiansen and Bellinger 1996a; Zeppelini and Christiansen 2003), *Sinella* (Christiansen 1960c), and *Tomocerus* (Christiansen 1964a).

Ken did not limit himself to the taxonomy of cave Collembola. He also described a number of non-cave dwelling species from the U.S. (Christiansen 1956b, 1958d; Christiansen and Bellinger 1973; Christiansen and Tucker 1977), Hungary (Wang et al. 2002c), Ascension Island (Christiansen 1998b), Lebanon and Syria (Christiansen 1956c, 1957, 1958b), Chile (Christiansen 1963), Mexico (Christiansen et al. 1985), and even fossil Collembola (Christiansen 1971d; Christiansen and Pike 2002). Among his international works, that in China stands out. Following at stint as a Visiting Professor at Nanjing University in 1990, he wrote more than 20 papers with Chinese colleagues on the Collembola of China [Chen and Christiansen 1993, 1996, 1997, 1998, 2004; Wu and Christiansen 1997; Ma and Christiansen 1998; Li and Christiansen 1997; Wang and Christiansen 2000; Wang et al. 2002a, 2002b, 2002d, 2003a, 2003b, 2003c, 2003d, 2003e; Chen et al. 2002; Jia et al. 2003; Ma et al. 2003, 2004; Madele et al. 2004). With Peter Bellinger, he wrote two editions of the "The Collembola of North America north of the Rio Grande", a four volume work which is more than 1500 pages in length (Christiansen and Bellinger 1980–1981, 1998).

During his six decades at Grinnell, he introduced countless students to caves and cave biology, often in Hunter Cave. He introduced a number of students to research, and was an enthusiastic mentor to even the most unprepared student. Several of his students went on to get Ph.D's and pursue research careers in ecology and evolutionary biology, including David Culver, Richard Seifert, and Mary Willson, He was also collaborator, mentor, and friend to generations of collembologists, and co-wrote papers with a number of colleagues, including Bellinger, Chen, Culver, de Gama, Li, Palacios-Vargas, Wang, and Zeppelini.

For anyone who has met Ken, a recitation of his academic achievements does not do justice to his influence or his character. Ken was enthusiastic in his support both of intellectual areas of interest, like cave biology and Collembola, and in those of us who shared these interests. Ken never claimed priority or seniority; he was the ideal colleague and mentor. He had an overall joie de vivre which infected those who came in contact with him. He had numerous interests outside of science, including acting in community theater, listening to opera, making wine, and studying history, especially military history. His enthusiasms and overall attitude are all the more remarkable for the many traumatic experiences in his wartime years, in a unit with high mortality. Without complaint or self pity, he kept these stresses and strains under control, with the support and understanding of his loving wife, Phyllis. I had the great fortune to be his student, colleague, and friend for more than 50 years. No one had a greater influence on me as a scientist or a person, and I am grateful to have known him. I am certainly not alone in this, and a little bit of Ken lives on in the best of each of us who knew him.

Partial Bibliography

- Baquero E, Jordana R, Christiansen KA (2004) Redescription of Nothobrya scubarti Arlé 1961 (Collembola Entomobryomorpha) Entomological News 115: 31–34.
- Baquero E, Martinez M, Christiansen K, Jordana R (2004) A new genus and species of Entomobryidae (Collembola Entomobryomorpha) from the Iberian Peninsula. Entomological News 115: 229–235.
- Chen J-X, Christiansen KA (1993) The genus *Sinella* with special reference to *Sinella* s.s. (Collembola Entomobryidae) of China. Oriental Insects 27: 1–54. https://doi.org/10.1080/0030 5316.1993.10432236
- Chen J-X, Christiansen KA (1996) A new species of *Ptenothrix* from China (Collembola: Dicyrtomidae). Florida Entomologist 79: 587–91. https://doi.org/10.2307/3496072
- Chen J-X, Christiansen KA (1997) The subgenus *Cceobrya* of the genus *Sinella* (Collembola Entomobryidae) with special reference to the species of China. Annals Entomological Societyof America 90: 1–19. https://doi.org/10.1093/aesa/90.1.1
- Chen J-X, Christiansen KA (1998) *Tomocerus* (s.s.) *spinulus*, a new species of Chinese springtail. Entomological News 109: 51–55.
- Chen J-X, Wang F, Christiansen KA (2002) A new species of *Pseudosinella* from Guilin China (Collembola: Entomobryidae). Journal of Kansas Entomological Society 75: 80–85
- Chen M, Christiansen KA (2004) Re-examination of three species of *Tomocerus* s.l. (Collembola : Tomoceridae) from China. Journal of Entomological Science 39: 303–310. https://doi. org/10.18474/0749-8004-39.3.303
- Christiansen KA (1950) Massachusetts records of *Cyphoderus assimilus*. Psyche 57: 94. https://doi. org/10.1155/1950/18737
- Christiansen KA (1951a) Notes on Alaskan Collembola I. A new genus and species of the family Isotomidae. Psyche 58: 24–31. https://doi.org/10.1155/1951/70857
- Christiansen KA (1951b) Notes on Alaskan Collembola II. Three new species of Arctic Collembola. Psyche 58: 125–140. https://doi.org/10.1155/1951/13918
- Christiansen KA (1954) Ratios as a means of specific differentiation in Collembola. Entomological News 65: 176–177.
- Christiansen KA (1956a) A recently introduced species of collembolan. Entomological News 67: 129–130.
- Christiansen KA (1956b) The genus *Mesentotoma* (Collembola: Entomobryidae). Psyche 63: 14–20. https://doi.org/10.1155/1956/65185
- Christiansen KA (1956c) The Collembola of Lebanon and western Syria. Part I. General considerations and the Family Onychiuridae. Psyche 64: 119–133. https://doi.org/10.1155/1956/62859
- Christiansen KA (1957) The Collembola of Lebanon and western Syria. Part II. Families Cyphoderidae and Oncopoduridae. Psyche 64: 77–89. https://doi.org/10.1155/1957/32941
- Christiansen KA (1958a) Geographic variation and the subspecies concept in the collembolan Entomobrya guthriei. Systematic Zoology 7: 10–15. https://doi.org/10.2307/2411473
- Christiansen KA (1958b) The Collembola of Lebanon and western Syria. Part III. Family Isotomidae. Psyche 65: 59–80. https://doi.org/10.1155/1958/61256
- Christiansen KA (1958c) The entomobryiform male genital plate. Proceedings of the Iowa Academy of Science 65: 474–476

- Christiansen KA (1958d) The Nearctic members of the genus *Entomobrya* (Collembola). Bulletin of the Museum of Comparative Zoology 118: 440–545.
- Christiansen KA (1959) The mystery of *Entomobrya duolineata* solved (Collembola). Entomological News 70.
- Christiansen KA (1960a) A preliminary survey of the knowledge of North American cave Collembola. American Midland Naturalist 64: 39–44. https://doi.org/10.2307/2422892
- Christiansen KA (1960b) The genus *Pseudosinella* (Collembola: Entomobryidae) in caves of the United States. Psyche 67: 1–25. https://doi.org/10.1155/1960/25063
- Christiansen KA (1960c) The genus *Sinella* Brook (Collembola: Entomobryidae) in Nearctic caves. Annals of the Entomological Society of America 53: 481–491. https://doi.org/10.1093/aesa/53.4.481
- Christiansen KA (1961a) Convergence and parallelism in cave Entomobryinae. Evolution 15: 288–301. https://doi.org/10.1111/j.1558-5646.1961.tb03156.x
- Christiansen KA (1961b) The Collembola of Hunters Cave. Bulletin of the National Speleological Society 24: 59–62.
- Christiansen KA (1962) Proposition pour la classification des animaux cavernicoles. Spelunca Memoires 2: 76–78.
- Christiansen KA (1963) Preliminary notes on the genus *Entomobrya* in South America with special reference to Patagonia. Bio de l'Amerique Australe 2: 149–168.
- Christiansen KA (1964a) A Revision of the Nearctic members of the genus *Tomocerus* (Collembola: Entomobryidae). Revue d'Ecologie et de Biologie du Sol 1: 639–678.
- Christiansen KA (1964b) Bionomics of Collembola. Annual Review of Entomology 9: 147–178. https://doi.org/10.1146/annurev.en.09.010164.001051
- Christiansen KA (1965) Behavior and form in the evolution of cave Collembola. Evolution 19: 529–537. https://doi.org/10.1111/j.1558-5646.1965.tb03328.x
- Christiansen KA (1966) The genus *Arrhopalites* (Collembola : Sminthuridae) in the United States and Canada. International Journal of Speleology 2: 43–73. https://doi.org/10.5038/1827-806X.2.1.5
- Christiansen KA (1967) Competition between collembolan species in culture jars. Revue d'Ecologie et de Biologie du Sol 4: 438–462.
- Christiansen K (1970a) Scope and direction of contemporary soil arthropod research. Pesticides in the Soil: Ecology, Degradation and Movement.
- Christiansen KA (1970b) Experimental studies on the aggregation and dispersion of Collembola. Pedobiologica 10: 180–198.
- Christiansen KA (1970c) Invertebrate populations in the Moulis Cave. Annales de Speleologie 25: 244–273.
- Christiansen KA (1970d) Survival of Collembola on clay substrates with and without food added. Annales de Speleologie 25: 849–852.
- Christiansen KA (1971a) Factors affecting predation on Collembola by various arthropods. Annales de Speleologie 26: 98–106.
- Christiansen KA (1971d) Notes on Miocene Amber Collembola from Chiapas. University of California Publications in Entomology 63: 45–48.
- Christiansen KA (1973) The genus *Pseudosinella* in Mesoamerican caves. Bulletin of the Association of Mexican Cave Studies. 5: 129–134.

- Christiansen KA (1982a) Notes on Mexican cave *Pseudosinella* with the description of six new species. Folia Entomologica Mexicana 55: 3–25.
- Christiansen KA (1982b)The zoogeography of cave Collembola east of the Great Plains. Bulletin of the National Speleological Society 44: 32–41.
- Christiansen KA (1985) Regressive evolution in cave Collembola. Bulletin of the National Speleological Society 47: 89–100.
- Christiansen KA (1990) Collembola. In: Dindal DL (Ed.) Soil biology guide. Wiley Interscience, New York, 965–995.
- Christiansen KA (1992a) Cave life in light of modern evolutionary theory. In: Camacho AI (Ed.) The natural History of Biospeleology. Museo Nactional de Ciencias Naturales, Madrid, 453–472.
- Christiansen KA (1992b) Springtails. The Kansas School Naturalist 39: 1–16.
- Christiansen KA (1995) La evolucion de la vida cavernícola. Mundos Subterráneos 6: 25–33.
- Christiansen KA (1998a) Las Colas de Resorte. (Spanish translation by Palacios Vargas). Mundos Subterraneos 9: 1–18.
- Christiansen KA (1998b) New species of *Pseudosinella* (Collembola) from Ascension Island. Journal of Natural History 32: 149–156. https://doi.org/10.1080/00222939800770081
- Christiansen KA (2003a) Adaptation: morphological (external). In: Gunn J (Ed.) Encyclopedia of caves and karst science. Fitzroy-Dearborn, New York, 7–9.
- Christiansen KA (2003b) Speciation. In: Gunn J (Ed.) Encyclopedia of caves and karst science. Fitzroy-Dearborn, New York, 665–666.
- Christiansen KA (2004) Morphological adaptations. In: Culver DC, White WB (Eds) Encyclopedia of caves. Academic/Elsevier Press, Amsterdam, 386–397.
- Christiansen KA (2009) The Collembola of Fennoscandia and Denmark (Fauna Entomologica Scandinavica volumes 35 and 42), volume 42 part II: Entomobryomorpha and Symphypleona). Systematic Entomology 34: 401–402. https://doi.org/10.1111/j.1365-3113.2008.00459.x
- Christiansen K (2012) Morphological adaptations. In: White WB, Culver DC (Eds) Encyclopedia of caves. Second edition. Academic/Elsevier Press, Amsterdam, 517–527. https://doi. org/10.1016/B978-0-12-383832-2.00075-X
- Christiansen KA, Barra JA (1975) Experimental study of aggregation during the development of *Pseudosinella impediens* (Collembola, Entomobryidae). Pedobiologia 15: 343–347.
- Christiansen KA, Bellinger PF (1973) Six new Nearctic species of the genus *Friesea*. Pan Pacific Entomologist 49: 390–395.
- Christiansen KA, Bellinger PF (1974) Collembola from Hawaiian caves. Pan Pacific Entomologist 16: 31–40.
- Christiansen KA, Bellinger PF (1980–1981) The Collembola of North America North of the Rio Grande, four volumes. Grinnell College.
- Christiansen KA, Bellinger PF (1988) Marine littoral Collembola of North and Central America. Bulletin of Marine Science 42: 215–245.
- Christiansen KA, Bellinger PF (1992a) The Collembola of Hawaii. University of Hawaii Press, Honolulu, HI.
- Christiansen KA, Bellinger PF (1992b) Update Collembola of North America. Part I families Hypogastruridae. Grinnell College.
- Christiansen KA, Bellinger PF (1994a) Biogeography of Hawaiian Collembola: the simple principles and complex reality. Oriental Insects 28: 307–351.

- Christiansen KA, Bellinger PF (1994b) Biogeography of Hawaiian Collembola: The simple principles and complex reality. Oriental Insects 28: 309–351. https://doi.org/10.1080/00305316 .1994.10432309
- Christiansen KA, Bellinger PF (1995) The biogeography of Collembola. Polskie Pismo Entomologiczne 64: 279–294.
- Christiansen KA, Bellinger PF (1996a) Cave Arrhopalites new to science. Journal of Karst and Cave Studies 58: 168–1890
- Christiansen KA, Bellinger PF (1996b) Cave Pseudosinella and Oncopodura new to science. Journal of Karst and Cave Studies 58: 37–52
- Christiansen KA, Bellinger PF (1998) The Collembola of North America North of The Rio Grande, Revised Edition, Grinnell College.
- Christiansen KA, Bellinger PF (2000a) A survey of the genus Seira (Hexapoda: Collembola: Entomobryidae) in the Americas. Caribbean Journal of science 36: 1–75.
- Christiansen KA, Bellinger PF (2000b) Redescriptions of some of Salmon's isotomid types. Contributions of the Biological Laboratory of Kyoto University 29: 103–115.
- Christiansen KA, Bellinger PF (2003) Collembola. In Encylopedia of Insects. Academic Press/ Elsevier. Amsterdam, 235–239.
- Christiansen KA, Bernard EC (2008) Critique of the article "Collembola (springs) (Arthropoda: Hexapoda: Entognatha) found in scrapings from individuals diagnosed with dulosry pareasitosis. Entomological News 119: 537–540. https://doi.org/10.3157/0013-872X-119.5.537
- Christiansen KA, Bouillon M (1978) An evolutionary and ecological analysis of the terrestrial arthropods of caves in the central Pyrenees with special reference to Collembola. Bulletin of the National Speleological Society 40: 103–117.
- Christiansen KA, Culver DC (1968) Geographical variation and evolution in *Pseudosinella hirsuta*. Evolution 22: 237–255. https://doi.org/10.1111/j.1558-5646.1968.tb05891.x
- Christiansen KA, Culver DC (1969) Geographical variation and evolution in *Pseudosinella violenta*. Evolution 23: 602–621. https://doi.org/10.2307/2406856
- Christiansen KA, Culver DC (1987) Biogeography and the distribution of cave Collembola. Journal of Biogeography 14: 459–477. https://doi.org/10.2307/2844976
- Christiansen KA, Grow AB (1974) Cheatotaxy in the Nearctic member of the genus Friesea. Revue d'Ecologie et de Biologie du Sol 11: 37–99.
- Christiansen KA, Grow AB (1976) Chaetotaxy in Folsomia. Revue d'Ecologie et de Biologie du Sol 13: 611–627.
- Christiansen KA, Luther G (1986) Two new species of Hawaiian *Pseudosinella* (Collembola: Entomobryomorpha: Entomobryidae). Proceedings of the Hawaiian Entomological Society 26: 45–51.
- Christiansen KA, Moberg T (1988) *Pseudosinella* revisited. International Journal of Speleology 17: 1–20. https://doi.org/10.5038/1827-806X.17.1.1
- Christiansen KA, Nascimbene P (2006) Collembola (Arthropoda, Hexapoda) from the mid Cretaceous of Myanmar (Burma). Cretaceous Research. Cretaceous Research 27: 318–363. https:// doi.org/10.1016/j.cretres.2005.07.003
- Christiansen KA, Pike E (2002) A preliminary report on the Cretaceous Collembola. Pedobiologia 46: 267–273. https://doi.org/10.1006/cres.2002.0313

- Christiansen KA, Pike E (2002) Cretaceous Collembola (Arthropoda, Hexapoda) from the Late Cretaceous of Canada. Cretaceous Research 23: 165–188
- Christiansen KA, Reddell JR (1986) The cave Collembola of Mexico, Speleological Monographs 1: 127–164.
- Christiansen KA, Snider RT (1996) Aquatic Collembola. In: Merritt W, Cummins K (Eds) Aquatic insects of North America (3rd edn). Kendall Hunt, 84–96.
- Christiansen KA, Tucker BE (1977) Five new species of *Orchesella*. Proceedings of the Iowa Academy of Science 84: 1–13.
- Christiansen KA, Tucker BE (1977) Four new nearctic species of *Folsomia* (Collembola: Isotommidae). Revue d'Ecologie et de Biologie du Sol 14: 371–382.
- Christiansen KA, Wang H (2006) A revision of the genus *Typhlogastrura* in North American caves with description of five new species. Journal of Cave and Karst Studies 68: 85–98.
- Christiansen KA, Bellinger FP, daGama MM (1983) A catalogue of the species of the genus *Pseudosinella*. Ciencia Biologica 5: 1–31.
- Christiansen KA, Bellinger PF, daGama MM (1991) Computer assisted identification of specimens of *Pseudosinella*. Revue d'Ecologie et de Biologie du Sol 27: 231–246.
- Christiansen KA, Doyle M, Kahlert M, Gobaleza D (1992) Interspecific interactions between collembolan populations in culture. Pedobiologia 36: 274–286.
- Christiansen KA, Lyman S, Johnson D (1972) Contact behaviour in Collembola and the effect of food deprivation, densiry and culture origins. Pedobiologia 12: 222–228.
- Christiansen KA, Palacios-Vargas JG, Ojeda M (1985), Taxonomia y biogeografia de Troglopedetes (Collembola: Paronellidae). Folia Entomologica Mexicana 65: 3–35.
- Christiansen KA, Tecklin J, Willson M (1961) Preliminary study of the microarthropod ecology of Hunters Cave. Bulletin of the National Speleological Society 24: 62–70.
- Jia S, Chen J-X, Christiansen KA (2003). A new collembolan species of the genus *Homidia* (Entomobryidae) from Hubei, China. Journal of the Kansas Entomological Society. 76: 610–615.
- Li L-R, Christiansen KA (1997) A new species of *Homidia* from China (Collembola Entomobryidae). Florida Entomologist 80: 457–460. https://doi.org/10.2307/3495610
- Ma Y, Christiansen KA (1998) A new species of *Tomocerus* (s.s.) from China. Entomological News 109: 47–51.
- Ma Y, Chen J-X, Christiansen KA (2003) A new species of the genus *Tomocerus (Tomocerina)* from Xinjiang (Collembola Tomoceridae) with a discussion of Tomocerina. Journal of Entomological Science 38: 511–518. https://doi.org/10.18474/0749-8004-38.4.511
- Ma Y, Chen J-X, Christiansen KA (2004) New species of the genus *Tomocerus* (*Tomocerua*) from China (Collembola: Tomoceridae) with a discussion of the subgenera of *Tomocerus*. Entomological News. 114: 41–46.
- Madele Y-T, Chen J-X, Christiansen KA (2004) A new record of *Tomocerus baicalensis* from China with its redescription (Collembola: Tomoceridae). Entomological News 114: 47–50.
- Peck SB, Christiansen KA (1990) Evolution and zoogeography of the invertebrate cave faunas of the Driftless Area of the Upper Mississippi Valley. Canadian Journal of Zoology 68: 73–88. https://doi.org/10.1139/z90-012
- Pomorski R, Furgol J, Christiansen KA (2009) Review of North American species of the genus *Onychiurus* (Collembola: Onychiuridae), with a description of four new species From

caves. Annals of the Entomological Society of America 102: 1037–1049. https://doi. org/10.1603/008.102.0612

- Skarzynski D, Christiansen KA (2008) Ceratophysella richardi sp. n. (Collembola: Hypogastruridae) from USA, with synonymization of the genus Mitchellania with Ceratophysella. Annals of the Entomological Society of America 101: 989–992. https://doi.org/10.1603/0013-8746-101.6.989
- Soto-Adames FN, Barr J-A, Christiansen KA, et al. (2008) Suprageneric classification of Collembola Entomobryomorpha. Annals of the Entomological Society of America 101: 501–513. https://doi.org/10.1603/0013-8746(2008)101[501:SCOCE]2.0.CO;2
- Soto-Adames FN, Giordano R, Christiansen KA (2013) *Bellisotoma*, a new genus of Isotomidae from North America (Hexapoda, Collembola). Zookeys 283: 7–13. https://doi.org/10.3897/ zookeys.283.3277
- Wang F, Christiansen KA (2000) A new species of *Sinella* (Collembola: Entomobryidae) from China. Entomological News 111: 332–336.
- Wang F, Chen J-X, Christiansen KA (2002a) A new record of *Pseudosinella* from China with a redescription of *P. sexoculata* (Collembola Entomobryidae). Oriental Insects 36: 51–57. https:// doi.org/10.1080/00305316.2002.10417321
- Wang F, Chen J-X, Christiansen KA (2002b) A new species of the subgenus *Coecobrya* from China (Collembola : Entomobryidae). Journal of Entomological Science 37: 213–218. https://doi. org/10.18474/0749-8004-37.3.213
- Wang F, Chen J-X, Christiansen KA (2002c) A new species of the subgenus *Coecobrya* from Hungary (Collembola: Entomobryidae). Journal of Kansas Entomological Society 75: 43–47.
- Wang F, Christiansen KA, Chen J-X (2002d) A new species of *Pseudosinella* from China (Collembola Entomobryidae). Entomological News 113: 63–67.
- Wang F, Chen J-X, Christiansen KA (2003a) A new species of *Pseudosinella* from Nanjing China (Collembola : Entomobryidae). Entomological News 113: 243–246.
- Wang F, Chen J-X, Christiansen KA (2003b) A survey of the genus *Pseudosinella* (Collembola: Entomobryidae) from East Asia. Annals of the Entomological Society of America 97: 364–385. https://doi.org/10.1603/0013-8746(2004)097[0364:ASOTGP]2.0.CO;2
- Wang F, Chen J-X, Christiansen KA (2003c) Similarity of *Pseudosinella hui* sp. nov. (Collembola: Entomobryidae) to European and North American species. Journal of Entomological Research 38: 240–246. https://doi.org/10.18474/0749-8004-38.2.240
- Wang F, Chen J-X, Christiansen KA (2003d) Taxonomy of the genus *Lepidocyrtus* s.l. (Collembola: Entomobryidae) in East Asia and Southeast Asia and Malatsia with the description of a new species from the People's Republic of China. The Canadian Entomologist 135: 823–837. https://doi.org/10.4039/n02-106
- Wang F, Christiansen KA, Chen J-X (2003e) A new species of *Pseudosinella* from Xinjiang China (Collembola Entomobryidae). Journal Kansas Entomologhical Society 76: 603–609.
- Wu M, Christiansen KA (1997) A new species of *Arrhopalites* from China (Collembola Sminthuridae). Florida Entomologist 80: 266–269. https://doi.org/10.2307/3495560
- Zeppelini D, Christiansen KA (2003) *Arrhopalites* (Collembola: Arrhopalitidae) in U.S. caves with the description of seven new species. Journal of Cave and Karst Studies 65: 30–36.