

Metabolic responses to long-term food deprivation in subterranean and surface amphipods

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Abstract

A long-standing hypothesis in subterranean biology posits that organisms living in poor resource subsurface habitats can withstand long periods of bioenergetic shortages due to an innate reduced metabolic rate when compared to their epigeal counterparts. However, previous studies have proposed that caves with ample energy resources may not evolve organisms with reduced metabolic rate. The equivocal nature of previous findings suggests that there is a need to compare food deprivation responses of subterranean and surface species in order to elucidate whether there are widespread adaptations to low energy systems in subterranean taxa. The purpose of the study was to examine patterns in basal metabolism and the effects of food deprivation in closely related subterranean- and epigeal- amphipods, *Stygobromus pecki* and *Synurella* sp. from central and east Texas, USA, respectively. Basal metabolic rates (measured as O₂ consumption) differed between species, with *S. pecki* having substantially lower rates than *Synurella*. Individuals of both species were food deprived for a pre-determined time interval and changes in total body protein, lipids, and carbohydrates were measured throughout food deprivation experiments. *Stygobromus pecki* had larger initial energy stores than *Synurella* and were more conservative in the use of energetic reserves over a prolonged period of food deprivation. Thus, it appears that although *S. pecki* are currently found in shallow phreatic and spring opening environments, they have maintained more efficient metabolic adaptations to deal with prolonged periods of food deprivation.

Keywords

biochemical composition, hypogean, karst, metabolic rate, physiological adaptation, *Stygobromus pecki*, *Synurella*

Introduction

Subterranean habitats are thought to be energy-limited ecosystems characterized by spatiotemporal patchiness of food resources (Culver et al. 1995; Juan et al. 2010). This is due to a lack of *in situ* autotrophic production and intermittent allochthonous (surface-generated organic matter) inputs of organic matter (OM) (Poulson 1964; Hüppop 1985; Venarsky et al. 2014; but see Hutchins et al. 2016). It is widely reported that hypogean (subterranean) organisms living in such low energy habitats have evolved a variety of behavioral, physiological, and metabolic adaptations that allow them to withstand long periods of resource shortage and show high starvation resistance (Hervant et al. 1997, 1999; Hervant and Renault 2002; Issartel et al. 2010). For example, some hypogean organisms have greater energetic reserves (e.g., greater glycogen, triglycerides, and protein content) and utilize these reserves at slower rates than related epigean taxa (Hervant et al. 1997, 1999; Hervant and Renault 2002; Mezek et al. 2010). Several studies have demonstrated that hypogean invertebrates exhibit physiological responses to prolonged food deprivation that differ from related epigean species. Hypogean amphipods and isopods respond to prolonged food deprivation in successive phases, with an immediate but slower rate of glycogen depletion, followed by utilization of proteins, and then use of lipids (Hervant et al. 1999; Hervant and Renault 2002). In contrast, epigean amphipods and isopods exhibit immediate, linear, and simultaneous declines in all energy reserve forms when exposed to periods of prolonged food deprivation (Hervant et al. 1999; Hervant and Renault 2002). However, it is unclear how conserved these metabolic responses to prolonged food deprivation is across hypogean fauna from different subterranean conditions.

Although organisms in many subterranean systems face low-energy conditions and rely heavily on infrequent inputs of surface-generated OM, there is growing recognition that some systems do not align with this paradigm. Some subterranean systems are relatively open and receive frequent and/or sustained inputs of allochthonous terrestrial OM (Culver and Poulson 1971; Schneider et al. 2011) or receive large inputs of high quality OM in the form of guano (Ferreira et al. 2000; Gnaspini 2005). In addition, some subterranean systems exhibit *in situ* chemoautotrophic production by microbial communities (Engel 2007; Porter et al. 2009), which can increase overall resource availability for organisms and lead to increased organismal diversity and greater food web complexity (Hutchins et al. 2016). It has been hypothesized that selection pressures to exhibit reduced metabolic rates or other adaptations to low energy conditions would be relaxed in these relatively energy-rich subterranean environments (Culver and Poulson 1971; Spicer 1998; Riesch et al. 2011). Thus, there is potential for subterranean obligate fauna to exhibit metabolism and physiological responses to prolonged food deprivation similar to those found in related epigean taxa, but there has been limited experimental examination of this hypothesis.

In addition to variation in resource availability among subterranean systems, many groundwater systems also exhibit vertical gradients in resource availability. Specifically, shallow phreatic habitats within aquifers, such as spring opening ecotones (i.e., transi-

tion zones between groundwater and surface–water habitats) are more likely to have greater access to terrestrial OM sources and therefore greater resource availability for faunal assemblages (Nair et al., in review). Spring ecotones often contain unique and diverse assemblages composed of surface, crenic (spring obligates) and hypogean taxa (Cantonati et al. 2012). Indeed, spring ecotone environments are considered “windows” to the subterranean world and present unique environments to investigate the ecology and evolution of groundwater organisms (Galassi et al. 2014). Although it has been hypothesized that subterranean fauna living in more energy-rich subterranean environments do not experience strong selection pressures to exhibit stygomorphic adaptations and should exhibit metabolic responses more akin to epigean species, this question has not been assessed for subterranean fauna that occupy shallow phreatic zones and spring opening ecotones.

The purpose of this study was to examine metabolic and food deprivation responses of a subterranean adapted organism that exists in a more energy-rich environment (i.e., shallow phreatic habitats and spring openings) in order to assess the hypothesis that subterranean organisms in more energy-rich environments experience relaxation of selective pressures on stygomorphic metabolic adaptations. These responses to prolonged food deprivation were then compared to food deprivation responses of a related surface species. Specifically, we examined the energy utilization (use of proteins, carbohydrates and lipids) and metabolic responses (O_2 consumption rates) to long-term food deprivation of two crangonyctid amphipods: the subterranean amphipod *Stygobromus pecki* and the largely epigean amphipod *Synurella* sp. *Stygobromus pecki* (Peck's cave amphipod) (Holsinger, 1967) is a federally endangered species endemic to two spring systems in the Edwards Aquifer of central Texas (i.e., Comal and Hueco springs) (United States Fish and Wildlife Service 1997; Gibson et al. 2008). *Stygobromus pecki* is found in shallow phreatic areas and at spring ecotones. It feeds largely on surface derived OM sources at its occurrence sites (Nair et al., in review), but is morphologically adapted for subterranean existence (e.g., eyeless, long appendages) (Holsinger 1967). In contrast, *Synurella* is widely distributed mostly epigean genus found in a variety of habitat types across the southeastern United States (including portions of Texas) and Europe (Holsinger 1977). The examination of metabolic responses of *S. pecki* to prolonged food deprivation and the comparison of these responses to a known epigean close relative presents a unique opportunity to examine the physiological responses and potential adaptations of a subterranean organism living in a more energy-rich environment.

Material and methods

Site description and organism collection

Live individuals of *S. pecki* were collected from spring openings at Comal Springs (29°43.0887'N, 98°7.8823'W). Comal Springs (city of New Braunfels, Comal County) is the largest spring complex in Texas and is located along the eastern edge of the

Edwards Plateau. The Comal Springs system discharges groundwater from the Edwards Aquifer from more than 400 spring openings and is the principle location for *S. pecki*. Live individuals of this species were collected from the immediate vicinity of spring openings by a combination of hand picking and sweeps of small aquarium nets. *Synurella* were collected using the same techniques from perennially flowing surface streams (30°35.8967'N, 95°7.71'W) near the city of Coldspring, Texas (San Jacinto County). Coldspring is located ~300 km to the northeast of Comal Springs. All known *Synurella* species in the southeastern United States are epigeal (Holsinger 1977) and individuals collected at Coldspring are morphologically consistent with the epigeal species *Synurella bifurca* (O.P. Hay, 1882). However, there is potentially substantial cryptic diversity within the genus that has yet to be resolved (J.R. Gibson, USFWS, personal communication). Thus, we hereafter have elected to refer to the species collected at Coldspring as *Synurella*. Live animals of both species were placed in high-quality coolers filled with site water and were brought back to the Freeman Aquatic Biology building at Texas State University (San Marcos, Texas).

Animals were acclimated to laboratory conditions in species-specific large plastic flow-through chambers with untreated Edwards Aquifer groundwater approximating the conditions found at collection sites [water temperature = 23 °C, dissolved oxygen (DO) concentration > 6 mg /L]. *Synurella* were exposed to a 12:12 light: dark cycle during housing, but *S. pecki* individuals were maintained in 24h darkness (Nowlin et al. 2015). Flow-through chambers contained pre-cleaned limestone river cobbles, polyester mesh, and well-conditioned conditioned wood and leaf material. Both species were fed *ad libitum* (typically weekly) with dense culture fish food (Pentair Dense Culture Food, F2A) prior to the start of experiments (Nowlin et al. 2015). Animals used in experiments were not differentiated based on sex due to the potential stress and mortality associated with sex determination. However, gravid females were excluded from the study in order to omit issues related to variation in biochemical composition. Individuals were held for a minimum of two weeks in the laboratory to acclimate to conditions before they were used for experiments.

Estimation of basal metabolic rates

We estimated basal metabolic rates of both species by measuring O₂ consumption. Oxygen consumption rates of well-fed individuals were estimated using Qubit systems OX1LP-30 DO cuvettes with Clark cell type polarographic oxygen sensor (Qubit Systems, Kingston, ON, Canada). The respirometric cuvette chamber had a small magnetic stirring bar positioned at the bottom of the chamber (but physically separated from the experimental animal) to continuously mix chamber water and ensure accurate O₂ concentrations in the chamber. The stirring rate was set to minimal speed in order to adequately mix chamber water but not induce stress on experimental animals. An individual test subject of either species was placed in a cuvette filled with 5mL of Edwards Aquifer water and was allowed to acclimate to the chamber for 30 minutes

prior to recording O₂ changes (in mg/L) of the chamber. After acclimation, DO concentration was recorded at 30-second intervals for 15 minutes. Cuvettes are externally jacketed with a water flow through system in order to maintain thermal stability at 23 °C. All experiments were carried out in a dark room. Per capita O₂ consumption rates were calculated by the dividing the change in DO concentration by 15 min. Mass-specific O₂ consumption rates (μmol O₂/g/h) were calculated by dividing O₂ consumption by wet weight of each individual (g). Oxygen consumption rates were estimated for n = 5 fed individuals of each species.

Food deprivation and metabolic responses

To assess biochemical changes and use of potential energy reserves in amphipods during extended periods of food deprivation, we experimentally examined change in whole-body metabolites of both species. Changes in metabolites during food deprivation was estimated at regular sampling intervals during food deprivation over 90 days for *S. pecki* (metabolites measured on days 0, 15, 30, 60, and 90) and over 30 days for *Synurella* (metabolites measured on days 0, 15, and 30). These species-specific food deprivation time periods were based on the literature and our own pilot experiments. Previous studies (Hervant et al. 1999; Hervant and Renault 2002) that compared metabolic responses to prolonged food deprivation in epigeal and hypogean invertebrates found that hypogean species could withstand longer periods of starvation. Our pilot study that assessed mortality during an extended food deprivation period in the laboratory for both *S. pecki* and *Synurella* and found that *S. pecki* exhibited could withstand a substantially longer food deprivation period of time before death (~120 days) than *Synurella* (~45 days).

Prior the start of food deprivation experiments, animals were acclimated to laboratory conditions, maintained, and fed as above for ~1 month. At the start of experiments, individuals of each species were separated into two treatments: fed (receiving weekly dense culture fish food pellets) and unfed (food deprived). For both species, each treatment contained n = 75 individuals. Individual animals were housed in 4 cm long and 1.91 cm diameter PVC flow-through holding chambers. Amphipods were segregated from each other during experiments in order to prevent cannibalism (Nowlin et al. 2015). Edward Aquifer groundwater was continuously fed through holding chambers to ensure volume was replaced every 2–3 minutes. On each sampling date, randomly selected individual amphipods from each of the four treatment groups (*Synurella* fed or food deprived and *S. pecki* fed or food deprived) were removed for metabolite analysis. Because of sample mass requirements for biochemical analysis, three individuals of each treatment were pooled prior to analysis (a pilot study was conducted and determined that n = 3 individuals were required to exceed metabolite analytical detection limits). Thus, on each sampling date each treatment had n = 5 analytical replicates (each replicate consisting of three randomly selected individuals). Animals were checked weekly for mortality; animals were not replaced in cases of mortality.

Metabolite analysis

Whole-body metabolites were estimated on each sampling date (Day 0, 15, 30, 60 and 90 days for *S. pecki* and day 0, 15, and 30 for *Synurella*). In order to minimize the inclusion of food materials in the guts of animals in biochemical analyses, animals were removed from chambers prior to weekly feedings; no leftover food was observed in holding chambers when animals were removed for analysis. In addition, once animals were removed, they were held for ~2 hours to clear gut contents and then frozen at -80 °C in clean 2 mL microcentrifuge tubes. Before metabolites were assayed, animals were thawed and wet weight of each individual was determined (mg). For each sample (composed of $n = 3$ individuals), tissue was homogenized and one-third of the tissue was further homogenized in phosphate buffer solution (pH = 7.4) for protein and total carbohydrate analysis. Proteins were analyzed using a Coomassie (Bradford) protein assay kit (Thermo Fisher Scientific) and total carbohydrates were analyzed using a total carbohydrate assay kit (Cell Biolabs, STA-682). Remaining tissue for each sample was homogenized in 2:1 chloroform-methanol (v/v) and lipids were extracted using a modified procedure by Folch et al. (1957), and quantified (unsaturated fatty acids only) using a lipid quantification kit (Cell Biolabs, STA-613). All assays were performed using a spectrophotometer at 25 °C.

Data analysis

For analysis of basal metabolic rates, difference in mass-specific O₂ consumption rates between species was assessed using one-way ANOVA. For the food deprivation experiment, body composition (protein, carbohydrate, and lipid content) between species was compared on Day 0 (immediately prior to the start of food deprivation) using one-way ANOVA. The effect of food deprivation on body composition within each species was then assessed by comparing treatments (fed *versus* unfed) with repeated measures ANOVA, which provides the treatment effect (fed *versus* unfed), time effect (Days 15 and Day 30 dates for *Synurella*, Days 15, 30, 60, and 90 for *S. pecki*), and the treatment x time interaction. Prior to analyses, data were examined for normality, homoscedasticity, and sphericity (for the repeated measures ANOVA). If data did not meet assumptions, they were either *ln*- or square root-transformed. Significance for all tests was inferred at $P \leq 0.05$ and analyses were performed in R (version 3.5.0, R Core Team 2018).

Results

O₂ consumption rates

Basal metabolic rates differed between *S. pecki* and *Synurella* ($F_{1,8} = 15.99$, $P < 0.004$; Fig. 1). Mass-specific O₂ consumption rates of *S. pecki* ($3.3 \pm 0.9 \mu\text{mol/g/h}$) were significantly lower (by an order of magnitude) than those of *Synurella* ($32.5 \pm 7.23 \mu\text{mol/g/h}$).

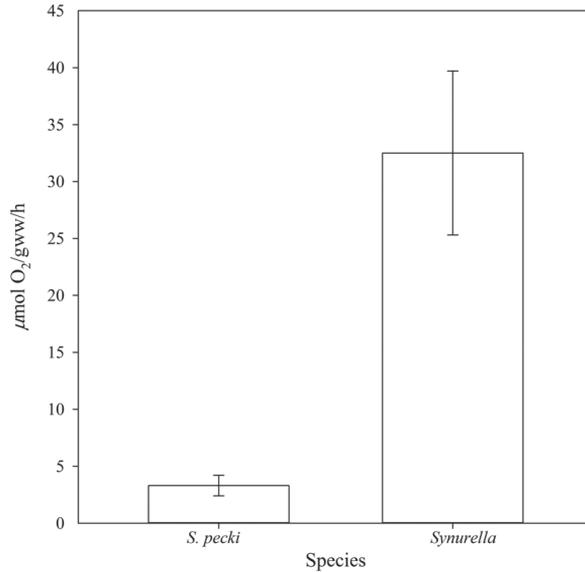


Figure 1. Oxygen consumption in darkness for *Stygobromus pecki* and *Synurella* at 23 °C. Values are means \pm Standard Error Means (SEM) for $n = 5$ animals.

Effects of food deprivation on metabolite content

Prior to the start of experiments (Day 0), the content of some metabolites differed between the two species (Fig. 2A–C). Total carbohydrate content was higher in *S. pecki* ($F_{1,8} = 43.83$, $P < 0.001$). However, protein content did not differ between species ($F_{1,8} = 1.46$, $P = 0.261$), but lipid content was higher in *Synurella* ($F_{1,8} = 7.40$, $P = 0.026$).

During the period of food deprivation, total carbohydrate content of *S. pecki* did not differ between fed and unfed treatments ($F_{1,4} = 0.356$, $P = 0.583$; Fig. 2A). However, in both treatments, total carbohydrate content of animals declined with time ($F_{3,12} = 19.89$, $P < 0.001$), but the rate of carbohydrate reduction did not vary with treatment (Time \times Treatment: $F_{3,12} = 0.456$, $P = 0.718$). In contrast, food deprivation in *Synurella* led to an immediate reduction in total carbohydrates ($F_{1,8} = 17.44$, $P = 0.003$; Fig. 2A). Total carbohydrate content in *Synurella* varied temporally (Time: $F_{1,8} = 15.61$, $P = 0.004$), but this temporal variation was dependent upon whether *Synurella* were fed or deprived of food (Time \times Treatment: $F_{1,8} = 10.11$, $P = 0.013$).

Protein content of *S. pecki* differed between fed and unfed treatments, with fed animals having higher protein content ($F_{1,8} = 19.28$, $P = 0.023$; Fig. 2B). Protein content varied through time in both treatments ($F_{3,24} = 3.17$, $P = 0.043$), but there was no interdependence between treatment and time ($F_{3,24} = 0.316$, $P = 0.813$). Food deprivation of *Synurella* led to an immediate reduction in protein content ($F_{1,8} = 8.07$, $P = 0.022$; Fig. 2B), but content did not vary temporally after 15 days of food deprivation (Time: $F_{1,8} = 5.17$, $P = 0.0517$; Time \times Treatment: $F_{1,8} = 2.55$, $P = 0.149$).

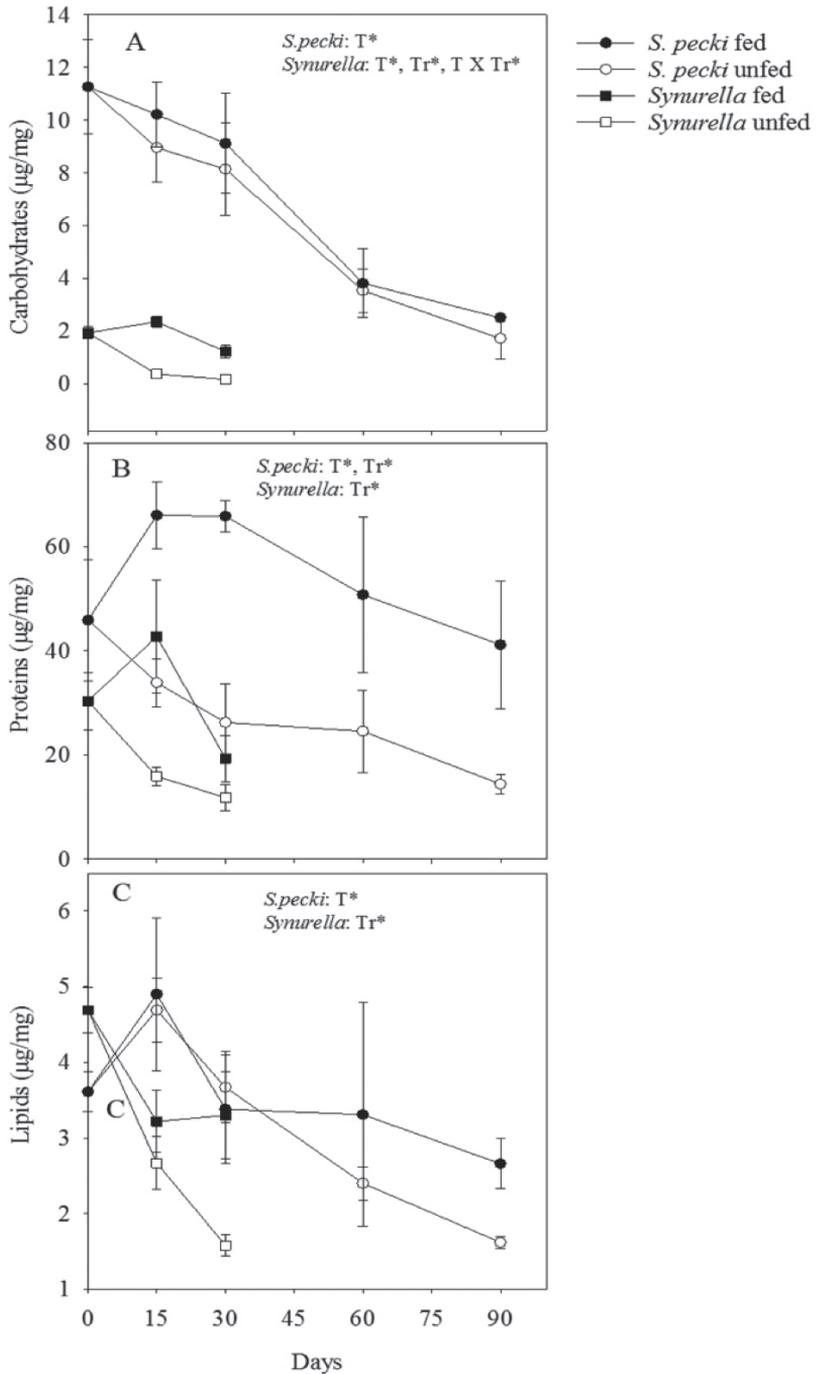


Figure 2. Changes in the levels of body metabolites in *Stygobromus pecki* and *Synurella* sp. **A** Carbohydrates **B** proteins **C** lipids concentrations during long-term food deprivation at 23 °C in darkness. Values are means ± SEM for n = 5 replicates. (*) indicates significance at P < 0.05 for the main effects of Treatment, Time and the Time × Treatment interaction.

Lipid content of *S. pecki* did not differ between treatments during the food deprivation period ($F_{1,8} = 2.51$, $P = 0.152$; Fig. 2C). Lipid content of *S. pecki* varied through time in both treatments ($F_{3,24} = 4.43$, $P = 0.01$), but this variation did not depend upon treatment (Time \times Treatment: $F_{3,24} = 0.481$, $P = 0.698$). For *Synurella*, lipid content was higher in the fed treatment during the starvation period ($F_{1,8} = 15.66$, $P = 0.004$; Fig. 2C). However, once the period of food deprivation started, *Synurella* lipid content did not vary temporally (Time: $F_{1,8} = 2.95$, $P = 0.124$; Time \times Treatment: $F_{1,8} = 2.91$, $P = 0.127$).

Over the entire experimental period, mortality of *Synurella* was 24% for fed and 38% for unfed treatments. *S. pecki* mortality was 18% for fed and 37% for unfed treatments.

Discussion

Capacity to withstand periods of low to no food supply depends on the presence of endogenous reserves and metabolic responses that ensure efficient utilization of stored metabolites (Hervant et al. 1997, 1999, 2001; Fuglei et al. 2000; Issartel et al. 2010). Previous studies have reported species-specific differences in storage and utilization of metabolites in epigeal and hypogeal crustaceans during periods of food deprivation (Hervant et al. 1999, 2001; Hervant and Renault 2002; Sacristán et al. 2016). In the current study, related hypogeal and epigeal amphipods exhibited markedly different physiological strategies to deal with food deprivation. Lower overall metabolic rates observed in *S. pecki* (in comparison to *Synurella*) are in accordance with studies that have found that hypogeal organisms have lower metabolic activity when compared to surface relatives (Hervant et al. 1997, 1998; Spicer 1998; Hervant and Renault 2002; Simčič et al. 2005; Mezek et al. 2010). *Stygobromus pecki* used in experiments were substantially larger than *Synurella* (*S. pecki* mean wet mass = 13.4 mg, *Synurella* mean wet mass = 7.1 mg) and metabolic scaling indicates that mass-specific metabolic rates decline with increasing body size (Brown and Sibly 2012). However, when we express O_2 consumption rates on a per capita basis ($\mu\text{mol } O_2$ consumed/h per individual), *Synurella* still exhibits a substantially greater basal metabolic rate than *S. pecki* ($\sim 5\times$ greater), further indicating that *S. pecki* has lower basal metabolic rates even when body size differences are considered. In addition, presence of greater total carbohydrate stores in *S. pecki* (relative to *Synurella*) on Day 0 prior to start of food deprivation is in line with previous studies that have compared metabolic responses of epigeal and hypogeal taxa to food deprivation (Hervant et al. 1999, 2001; Hervant and Renault 2002). Presence of greater energy stores in hypogeal species is thought to be indicative of adaptation to low energy subterranean conditions so that individuals can continuously fuel metabolic needs for longer periods of low food availability. Thus, results from this study indicate that despite the shallow subterranean existence of *S. pecki* (and its presumed greater access to food resources), basal metabolism and initial energy reserves in this species seem to follow the paradigm that subterranean species have relatively depressed metabolic rates and different energy reserve concentrations when compared to epigeal fauna.

In the present study, lipid reserves were substantially higher in *Synurella*, when compared to *S. pecki*. In contrast, Hervant et al. (1999) found that that the subterranean amphipods, *Niphargus virei* and *N. rhenorhodanensis* had higher stored lipids and carbohydrates (as glycogen) than surface amphipods (*Gammarus fossarum*). Contrasting results from Hervant et al. (1999) and this study could be due to differences in species-specific adaptations to food limitation resulting from different ways of life and metabolic needs (Danielopol and Rouch 1991). In addition, species-specific differences in storage and utilization of body reserves may occur due to differences in thermal adaptation, type and quality of food consumed, feeding history and life-cycle strategy, as well as their biosynthesis from either dietary fatty acids or dietary proteins, carbohydrates or lipids (Lahdes et al. 2010; Pond 2012). The two species which Hervant et al. (1999) studied (*N. virei* and *N. rhenorhodanensis*) are typically found in deep phreatic habitats, whereas *S. pecki* is a shallow phreatic zone specialist. Thus, it is possible that differences between the findings of this study and that of Hervant et al. (1999) could come from the evolutionary history, specific habitat associations, and access to food resources of the specific fauna used in experiments.

Although *S. pecki* did not have higher lipid stores than *Synurella*, the present study found differences between epigeal and hypogean species in terms of utilization and depletion of various energy reserves during starvation. Starvation leads to changes in the body composition (Gibert and Mathieu 1980; Barclay et al. 1983), and the relative importance of different metabolite reserves depends on the duration of starvation as well as species-specific differences in metabolism and regulation (Hervant et al. 1999; Caruso et al. 2008). During starvation, crustaceans meet energy demands by regulating enzymatic activities to access energy reserves (i.e., hydrolysis of proteins to amino acids, glycogen to glucose, and triglycerides to free fatty acids), while ensuring cell integrity (Sánchez-Paz et al. 2006). In the present study, *Synurella* demonstrated a monophasic response to food deprivation, characterized by an immediate linear decrease in all energy reserves, including lipids. In contrast, the only metabolite that differed between fed and unfed treatments during starvation of *S. pecki* were proteins. Thus, it appears that *S. pecki* accessed proteins as an energy reserve during the 90-day starvation period. Hervant et al. (1999) observed that glycogen stores were first utilized by food deprived hypogean amphipods (*Niphargus* sp.) and that protein reserves were sparingly utilized after 30 days of food deprivation. Thus, initial utilization of protein energy reserves during periods of starvation may not be a cosmopolitan feature of hypogean amphipod metabolism, but further comparative studies are required.

Our study found that the use of bulk energy reserves differed between the two crangonyctid species we examined. *Synurella* exhibited monophasic declines in all bulk energy reserves during food deprivation, whereas *S. pecki* only exhibited differences in protein content in fed and food deprived animals. However, it is likely that our analysis of bulk energy reserves (i.e., total lipids and carbohydrates) may have obscured differences between the two study species in the utilization of specific energy reserve constituents during periods of food deprivation. Within crustaceans, neutral lipids (mainly triglycerides) are preferentially catabolized during food deprivation, but po-

lar lipids (i.e., phospholipids and cholesterol) are conserved because of they serve as structural components of cell membranes (Hervant et al. 1999). Similarly, crustaceans access carbohydrate reserves stored mainly as glycogen when they are food deprived (Hervant et al. 1999; Hervant and Renault 2002; Sánchez-Paz et al. 2006). Given the differential use of energy reserve constituents (i.e., triglycerides versus phospholipids, glycogen versus glucose) during food deprivation periods, future studies should attempt to examine these constituents in order to better elucidate patterns of energy use within and among our study species.

In this study, carbohydrate reserves in *S. pecki* were depleted in both fed and unfed populations in the lab throughout the experimental period. *Stygobromus pecki* in the fed treatment were supplied food at similar rates to other *S. pecki* individuals we have maintained in the laboratory and it appeared as though they were consuming the added food items (P. Nair, personal observation). The reason or mechanisms for carbohydrate depletion in fed *S. pecki* in the current experiments is not known, but it may be due to stress associated with being held in captivity in a for a relatively long period of time. Some hypogean species can be sensitive to being held in captivity for extended time periods; Gibert and Mathieu (1980) found that individuals of the hypogean amphipod *N. rhenorhodanensis* that were fed and kept under conditions close to their natural environment in the lab showed significant reduction in carbohydrates reserves within a month of being held in captivity. These authors hypothesized that this was due to captive stress, but this hypothesis requires further study. The USFWS maintains captive populations of *S. pecki* at the San Marcos Aquatic Resource Center facility (SMARC; San Marcos, Texas), but it is unclear if these individuals similarly experience carbohydrate reduction or any stress associated with being held in a captive setting. Clearly, there is a need to further explore these results from an applied and captive breeding perspective as it applies to *S. pecki* and its endangered conservation status.

Conclusions

Our study shows that *S. pecki* has lower energetic requirements (i.e., basal metabolic rates), greater total carbohydrate reserves, and lower rates of lipid use during starvation when compared to a surface relative. Cumulatively, these findings suggest that *S. pecki* maintains a stygomorphic metabolic strategy for survival in environments with low or sporadic food availability, despite its occurrence in shallow phreatic and spring opening environments. *S. pecki* is closely related to amphipod species which occur in deeper phreatic environments (Ethridge et al. 2013) and it is hypothesized that ancestors of these taxa invaded freshwater subterranean systems during the late Cretaceous or early Cenozoic (Holsinger 1967). Thus, present-day metabolic adaptations to persistence in a deeper phreatic low-energy environments observed in *S. pecki* is consistent with their longer-term evolutionary history, rather than their current distribution in and around surface spring opening ecotones. *Stygobromus pecki* serves as the top invertebrate predator in spring opening ecotones, feeding on surface invertebrates, including the epigeal

hyaellid amphipod *Hyalella azteca* (Nair et al., in review) and *S. pecki* has the ability to detect and actively avoid light, presumably to avoid predation (Nowlin et al. 2015; Worsham et al., in preparation). The juxtaposition of some surface-adapted traits (i.e., surface foraging, light avoidance) and subterranean metabolic characteristics in *S. pecki* suggests that metabolic traits are not easily modified even though *S. pecki* now lives in shallow groundwater – surface water interfaces. However, future studies focused on comparing metabolic requirements and response of *S. pecki* with their deep phreatic relatives are needed to further evaluate the hypothesis that *S. pecki* exhibits deeper phreatic metabolic characteristics despite its current shallow phreatic distribution.

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Notes on the predation of an assassin bug by a spider in a Neotropical cave

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Abstract

Intraguild predation have rarely been documented in scientific literature, even though this type of interaction can affect population dynamics and competition. In this study we present an intraguild predation event that occurred at different times, but in the same cave, where we observed spiders of the species *Enoploctenus cyclotorax* preying on specimens of assassin bug *Zelurus diasi*. Inside the studied caves, food resources are scarce and populations can be fairly small in size. It is possible, therefore, that these events are the result of ecological pressures imposed by the hypogean environment.

Keywords

Brazil, intraguild, invertebrates, *Zelurus*, *Enoploctenus*

The study of ecological interactions is essential to understand how subterranean communities are assembled, however, few studies addressing this theme have been conducted so far. In the Neotropical region, research that aimed to elucidate the relationships among cave species are even scarcer and little is known about how such interactions can influence the communities present in the hypogean environment (Ferreira and Martins 1999; Bernardi et al. 2010; Souza-Silva and Ferreira 2014; Resende and Bichuette 2016; Vasconcelos et al. 2017). Even more unusual are reports about intraguild predation interactions (Souza-Silva and Ferreira 2014; Resende and Bichuette

2016). Although rare, this type of interaction is potentially important, since it may decrease competition between apex predators, affecting population dynamics more strongly than the commonly observed predation and competition interactions (Polis et al. 1989; Finke and Denno 2006; Moeller et al. 2019). Thus, in this study, we report the occurrence of intraguild predation events involving a spider species (Araneae: Ctenidae: *Enoploctenus cyclotorax*) (Bertkau 1880) and an assassin bug species (Hemiptera: Reduviidae: *Zelurus diasi*) (Costa Lima 1940) (Figure 1), in a limestone cave located in the Sete Lagoas municipality, Minas Gerais state, Brazil (Figure 2).

We observed the intraguild predation events during a cave fauna monitoring project involving 26 caves near a mining area. We have been monitoring those caves since 2016, visiting each cave seasonally. Six visits have been made so far (September 2016, January 2017, January and June 2018 and May 2019). During data collection, we have sampled the distributions of cave species larger than 1 cm, counting these species through visual sense and spatial plotting individuals on the cave map. The time we spent on the monitoring varied for each cave. Because every cave environment is different and unique, the necessary sampling time may increase or decrease. However, the average sampling time was 12 minutes per 10 m² per cave. The team was always composed by three biologists with experience in cave fauna collection, as recommended by Weinstein and Slaney (1995).

The studied caves are in the area of the Cerrado biome, but in a very anthropized region. The caves are located in a small patch of vegetation, surrounded by planted pastures. The karst relief where the caves are located is part of the Bambuí Group, which consists of gray limestone interspersed with marble and slate. The climate is Humid subtropical climate (Cwa) (Peel et al. 2007) with an annual average tempera-

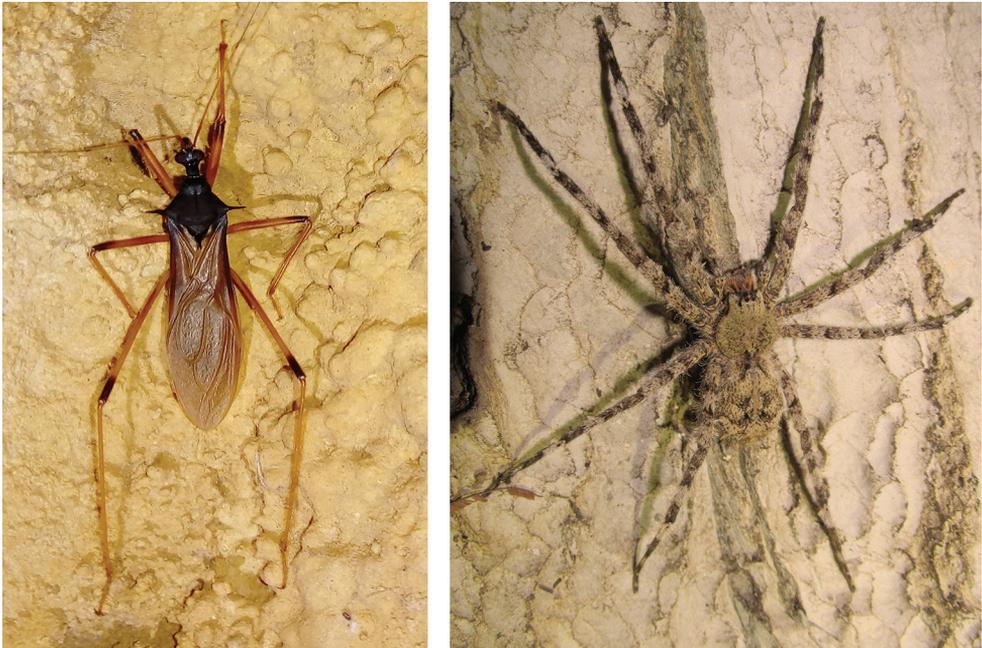


Figure 1. Adult specimens of *Zelurus diasi* (left) and *Enoploctenus cyclotorax* (right) observed in the study area.



Figure 2. Location of the cave where we observed the intraguild predation events in Sete Lagoas, Minas Gerais, Brazil.

ture of 21.5 °C and the distribution of average rainfall throughout the year for the region is 1328.7 mm, but marked by a conspicuous annual variation (drought and rainy periods) (INMET 2019).

We observed the intraguild predation in one of the studied caves (WGS84 – 19°29'45"S, 44°13'12"W). This cave presents a small underground space with a horizontal projection of 5.2m, volume of 2.2 m³ and area of 5.3 m². The access to the underground is a small entrance. The main portion of the cave is composed of a single passage that presents a strongly ascending floor, towards the end of the cave. Despite the relatively small size, it is possible to distinguish two zonation inside the cave: (i) entrance, which is photic and (ii) middle/distal portion, which is dysphotic. The entrance of the cave is located approximately one meter above the epigeal floor. Such morphology combined with the cave slope make it difficult for organic matter to enter from the epigeal to the hypogean environment. The main organic resource observed inside the cave is mid-sized mammal feces.

We observed intraguild predation events at two different times. The first record was during the dry season, in September 2016, when a female of *Enoploctenus cyclotorax* was spotted preying on an adult *Zelurus diasi* (we could not identify the sex because the specimen had the abdomen smashed, probably by the spider). We observed the second event in January 2017, during the rainy season. In this occasion we found an adult female spider *E. cyclotorax* preying upon a juvenile *Z. diasi* (Figure 3).

Zelurus and *Enoploctenus* are voracious predators with a wide distribution in caves and epigeal environment of Brazilian territory (Pinto-da-Rocha 1995; Pellegatti-Franco 2004; Ferreira et al. 2016). Both species have a similar diet, eating a wide variety of invertebrates, such as crickets, cockroaches, scorpions, beetles, velvet worms, wasps, harvestmen and pseudoscorpions (Pellegatti-Franco 2004; Willemart and Pellegatti-Franco 2006; Fischer et al. 2006; Franco 2006; Franco and Monge-Nájera 2016; Grossi et al. 2012; Lira et al. 2016; Stevenson and Stohlgren 2015; Westcott et al. 2016). Intraguild predation is a potentially dangerous strategy, because the prey (in this case, *Z. diasi*) is also a predator,



Figure 3. Intraguild predation between female *Enoploctenus cyclotorax* and adult *Zelurus diasi* observed during the study.

armed and capable of killing. In addition, some Reduviidae produce a repulsive substance that may deter other predators (Evangelin et al. 2014). However, in the cave environment, the ability to catch and feed on other predators may be an advantageous behavior, since caves are notoriously known for their scarcity of food resources (Poulson and White 1969, Simon et al. 2007). The studied cave is known by its food scarcity and low density of potential prey for the *Zelurus* and *Enoploctenus* species, with only a few large-sized invertebrate species observed during the monitoring. During the study, we noticed that the cave where the events of intraguild predation occurred had a reduced number of prey. In this cave, we sampled only two other invertebrate species that could be potential prey for *Enoploctenus* and *Zelurus*.

Furthermore, these potential prey species have a body size compatible with other prey used by these species on previous studies. The potential prey are Lepidoptera from the genus *Hypena* (Noctuidae), which had an observed abundance ranging from 0 to 3 adult individuals. Although juvenile Lepidoptera from this genus are also potential prey of *Zelurus*, they were observed, but not accounted for the study. We also observed a small number of individuals of *Endecous* crickets (Phalangopsidae) (zero to one adult) during the visits to the cave. The *E. cyclotorax* spiders had their abundance ranging between 0 and 2 specimens during the monitoring, and *Z. diasi* was the only species observed in all six visits, showing an abundance of 4 to 8 individuals during the monitoring. The other species observed in the cave were spiders (*Mesabolivar* sp., *Plato* sp. and *Isoctenus* sp.), ants (*Solenopsis* sp.), booklice (not identified) and mites (*Erythracarus* sp.). We considered those invertebrate species as potential preys to *Zelurus* and *Enoploctenus*, because we have previously observed these invertebrates' species being preyed by *Zelurus* and *Enoploctenus* in other caves of the same area.

It is noteworthy mentioning that *Zelurus* is apparently not a frequent prey for spider species. Thus, it may be that they use specimens of this group as a resource only in very specific situations. In the study conducted by Pellegatti-Franco (2004) regarding the feeding behavior of *Ctenus fasciatus*, several species were offered as prey to this spider. Some of them, such as crickets, cockroaches, small vertebrates and even other spiders were accepted and used as a resource. However, species of the genus *Zelurus* were not preyed by *C. fasciatus* spiders.

Even though intraguild predation may be a rare phenomenon, it can be beneficial for predators, for example decreasing competition for prey (Polis et al. 1989). In caves where resource and prey are scarce, this may be an important factor, maintaining the species in that challenging environment. In conclusion, we suggest that unfavourable circumstances may have led the spiders to prey upon an unconventional type of prey.

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Invertebrates diversity in mountain Neotropical quartzite caves: which factors can influence the composition, richness, and distribution of the cave communities?

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Abstract

Twenty caves located in a high altitudinal quartzite area in Brazil were examined for invertebrate richness and composition and in terms of environmental factors that determine community structure. We evaluate how distance, altitude, cave extension, environmental stability, number and size of cave entrances and stream presence can act on species composition and richness. The caves presented a high richness of troglomorphic (463 spp.) and troglobitic species (6 spp.) in relation to other siliciclastic caves around the world. The average richness was 39.55 species per cave (sd = 21.87), the quantitative similarity among caves was 41% and turnover was $\beta_{\text{repl.}} = 0.769$. Araneae (20% of the sampled species), Diptera (18%) and Coleoptera (14%) were the dominant orders regarding species richness. Only twenty percent of the caves were placed out of the confidence interval of the average taxonomic distinctness ($\Delta+$); however, the $\Delta+$ decreased with the increase of environmental stability. Cave extension and stream presence were the main factors determining the variation of species composition among caves. Cave extension also influenced species richness variations. Furthermore, the total richness and richness of troglobitic species increased with cave extension. The threats to these habitats further revealed that the fauna is at risk due to tourism, trampling and natural soil erosion that can promote microhabitat alterations. Therefore, quartzite caves also require special attention regarding conservation actions in order to keep their natural biological dynamics.

Keywords

Cave fauna, Neotropics; quartzite rocks; troglobitic species

Introduction

Studies related to ecology and conservation of subterranean fauna have been receiving increasing attention in recent years (Mammola 2018, Mammola and Isaia 2018, Pellegrini et al. 2018, Moldovan et al. 2018). However, most of the knowledge regarding subterranean fauna has been historically focused on limestone caves so that other kinds of rocks, such as siliciclastic, have received less attention (Sharratt et al. 2000, Souza-Silva et al. 2011). Despite quartzite caves being less frequent than caves in other lithologies, studies with fauna from these habitats have been conducted in some regions of the world, as in Cape Peninsula in South Africa (Sharratt et al. 2000), in the Northwestern United States (Peck and Peck 1982), in the Amazon Tepuis in South America (Galán and Herrera 2006, Aubrecht et al. 2012), in the Brazilian Atlantic Rain Forest (Zeppelini et al. 2003, Gallão and Bichuette 2015) and in Brazilian Savannah (Guadanucci et al. 2015). Some of those studies have shown that quartzite and sandstone caves are important habitats for troglomorphic and troglobitic fauna in Neotropics, thus deserving attention for conservation (Gallão and Bichuette 2015, Souza-Silva et al. 2015).

In Brazil, the cave fauna started to be systematically studied in the 1980s (Dessen et al. 1980), but only a few studies assessed ecological aspects of cave community structure. Furthermore, most of the knowledge on cave ecology in Brazil comes from works conducted in limestone caves (Bento et al. 2016, Pellegrini et al. 2016, Simões et al. 2015, Rabelo et al. 2018).

In Brazil, there are at least 2,300 known sandstone and quartzite caves, but they represent only 1% of the potential occurrence (Nascimento and Mantesso-Neto 2013, Cecav 2019). Only a small part of them have been sampled (about 50 caves), which revealed more than 500 invertebrate species. Furthermore, 20 caves presented at least two troglobitic species (Dessen et al. 1980, Trajano and Gnaspini-Netto 1991, Trajano and Moreira 1991, Gnaspini-Netto and Trajano 1994, Pinto-da-Rocha 1995, Lourenço et al. 2004, Zeppelini et al. 2003, Bichuette et al. 2008, Alves et al. 2011, Souza-Silva et al. 2011, Bertani et al. 2013, Fernandes and Bichuette 2013, Gallão and Bichuette 2015, Pellegrini et al. 2018).

Previous ecological studies conducted in 14 Brazilian quartzite caves in the South of Minas Gerais state found 400 species (44.85 species/cave, $sd = 24.54$), for an average cave extension of 231 m ($sd = 219$) (Souza-Silva et al. 2011). Gallão and Bichuette (2015) sampled 11 quartzite caves from Chapada Diamantina, a central region of the state of Bahia, Brazil, and found 160 morphotypes (29.72 species/cave, $sd = 19.6$). They did not present the extension of caves. Overall, 23 obligate cave species were mentioned (plus the other 14 species with some troglomorphic traits) (Gallão and Bichuette 2015). Accordingly, the authors found 37 species with troglomorphic traits in 11 caves in the area, which represent 23% of the total invertebrates sampled (160 species).

Zeppelini et al. (2003) studied nine sandstone caves in the Northern region of the state of São Paulo, Brazil, and found 83 morphotypes (10 species/cave, $sd= 10$) for an average cave extension of 373 m ($sd= 246$).

However, a study performed on 33 temperate sandstone caves of the Cape Peninsula in South Africa (approximately 20–90 m long), found only 85 species (Sharratt et al. 2000). They did not present the number of species per caves, which also precludes further comparisons.

However, the previous studies did not present information on how composition, richness, and distribution cave communities are related to some cave attributes (distance between caves, cave size, number and size of entrances and altitudinal position among others). Such features were proven to be determinant for local and regional species richness and dissimilarity, mainly in the tropics (Souza-Silva et al. 2011, Simões et al. 2015, Jaffé et al. 2016). This study aimed to evaluate how some local environmental factors (distance, altitude, cave extension, environmental stability, number and size of entrances and stream presence) can act on invertebrate composition and richness. In addition, we described the human uses and alterations inside and surrounding the caves.

Material and methods

Study area

The study was conducted in the Ibitipoca mountain quartzite province, south of Minas Gerais state, Brazil. The caves in this region were formed and modeled by hierarchically organized drainages influenced by differences between the local water table and the regional base level. The Ibitipoca Mountain belongs to the Andrelândia geological group mainly composed of quartzite rocks of Mesoproterozoic lithostratigraphic age (Auler and Sauro 2019).

The mountain is located within a protected area, with 1,488 ha of extension and altitude ranging from 1,200 to 1,784 m asl. This reserve was created in 1973 and protects epigeal fauna and flora and quartzite caves and their fauna (Nobre et al. 2013). The dominant vegetation is grasslands on hilltops and rainforest in the valleys (Figure 1). The climate is tropical (Cwb of Köppen) with mild summers, with dry (May to August) and rainy (August to January) well defined seasons (Alvares et al. 2013). The external temperatures range from 2 to 20 °C and internal caves temperatures range from 12 to 20 °C (Souza-Silva et al. 2013).

Environmental features of quartzite caves

Most of the caves in this study were mapped by Silva (2004) using a standardized mapping methodology with a British Cave Research Association (BCRA) – 4C survey grade. Additional information related to the cave extension (for those caves that were not mapped), number and area of entrances were determined during the fieldwork (as in Bento et al. 2016).

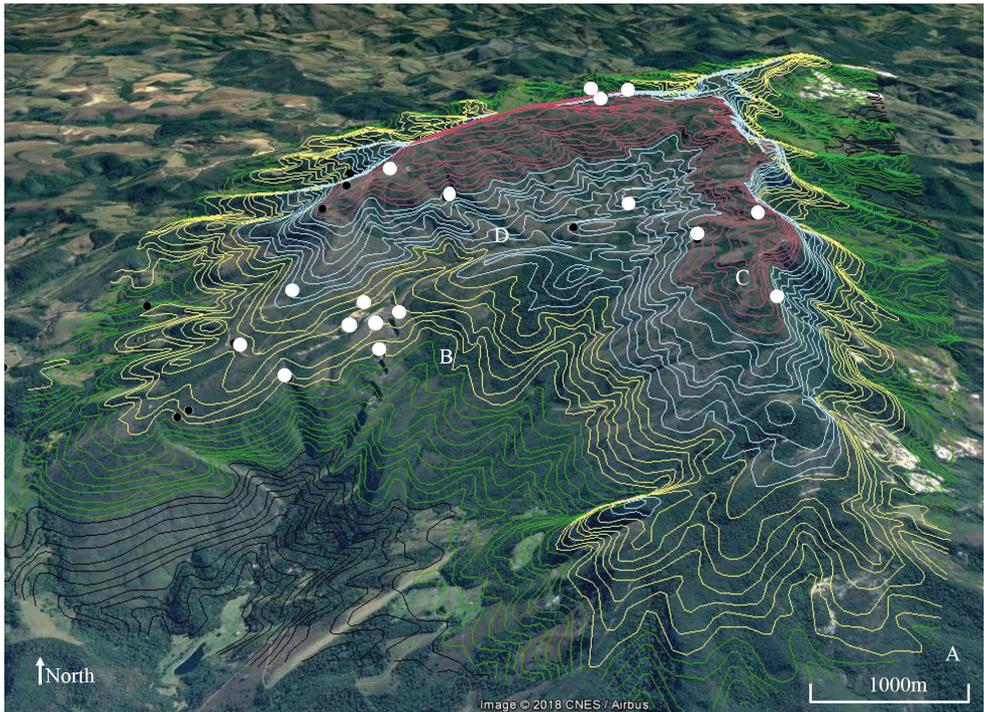


Figure 1. Borders of the Ibitipoca Estadual Park (A), sampled caves (white dots) and altitudinal layers (red lines 1610–1780, blue lines 1460–1600, yellow lines 1310–1450, green lines 1124–1450, black lines 950–1100 meters). Vegetation types vary from slope forest (B) to grasslands (D and C) on the top of the hills.

The altitude above sea level and geographic position of the caves were obtained with a Global Positioning System (GPS) in decimal degrees (Table 1). Moreover, the width of entrances and linear development of the caves were measured using a laser. The greatest horizontal length of an entrance was considered as width, while the greatest vertical length was considered as height. The cave linear development represented the linear development sampled in each cave since some caves were not sampled throughout their total length (Souza-Silva et al. 2011).

Faunal survey

Species richness and composition of the invertebrate communities were assessed in 20 caves (Table 1, Figure 1). Only one visit to each cave was conducted. Sampling was carried out only once by visual search within the accessible parts of each cave, prioritizing places with organic matter (such as plant debris, carcasses, and guano) and humid soils, cracks, speleothems, water bodies and spaces under rocks. Hand collections were made with the aid of tweezers, brushes and entomological nets (Bento et al. 2016, Wynne et al. 2019). Invertebrates were collected from water bodies with the aid of forceps. In the

Table 1. Biotic and abiotic characteristics of the 20 quartzite caves in southeastern Brazil. Cave with stream (S) or dry caves (D), total richness (TS); relative richness (RS), abundance (A), altitude (ALT in meters), geographic coordinates (Lat and Long in decimal degrees), Sampled extension of the caves (SE in meters), Number of morphotypes with troglomorphic traits (ST), entrance number (NE) extension of the entrances (EE) and results of the environmental stability index (IEA). *Caves open to tourist visitation. Numbers above cave names are the morphotypes with troglomorphic traits; 1 – Blattodea, 2 – *Brasilomma enigmatica*, 3 – Hypogastruridae, 4 – Projapygidae, 5 – Palpigradi, 6 – Pselaphidae.

Cave names	S/D	TS	RS	A	ALT	Latitude, Longitude	SE	ST	NE	EE	IEA
Catedral III	S	22	0.13	145	1634	-21.701486, -43.872046	170	0	5	60	9.28
Bichana II	D	19	0.63	580	1350	-21.171357, -43.389859	30	0	1	4	2.01
Bichana I	D	31	1.55	132	1360	-21.712851, -43.898126	20	0	1	1	1.61
Catedral I	S	14	0.54	89	1646	-21.701486, -43.872046	26	0	5	1	1.65
Manequinho	S	61	0.1	940	1270	-21.719923, -43.903194	160	0	4	40	8.76
*Cruz	D	61	1.45	344	1632	-21.694923, -43.896249	50	0	3	20	10.93
Dobras ¹	D	26	0.19	220	1600	-21.696294, -43.896608	138	1	2	15	8.32
*Ponte de Pedra	S	30	0.44	103	1283	-21.171659, -43.898472	54	0	2	20	7.68
*Gnomos	S	23	0.38	137	1363	-21.171159, -43.389486	32	0	2	15	6.46
Lagarto Teiú	S	40	0.4	266	1349	-21.712168, -43.893929	40	0	1	10	1.40
*Monjolinhos	D	22	1.05	39	1428	-21.169659, -43.880138	21	0	1	5	1.44
Martiniano	D	35	0.16	270	1360	-21.715316, -43.900316	240	0	4	40	2.3
Martiniano II	D	18	0.36	96	1340	-21.710917, -43.894719	50	0	4	50	2.53
*Viajantes	D	33	0.2	333	1660	-21.704646, -43.876249	166	0	2	50	9.5
Fugitivos	D	33	0.08	842	1669	-21.677731, -43.883096	440	0	4	80	7.45
*Pião ¹	D	34	0.27	270	1643	-21.701868, -43.874027	126	1	1	4	3.45
*Coelhos	D	67	0.84	488	1358	-21.709646, -43.895972	80	0	2	10	9.46
Bromélias ²	D	96	0.19	2869	1450	-21.704923, -43.899583	500	1	3	10	5.11
Moreiras ^{3,4}	D	75	0.13	3735	1651	-21.676595, -43.388241	600	2	6	80	9.33
Casas ^{1,4,5,6}	S	47	0.07	249	1340	-21.700479, -43.883749	650	4	1	7	4.53

laboratory, invertebrates were separated into morphotypes (Oliver and Beattie 1996). The sampling time spent in each cave was dependent on the extension of the cave.

The determination of potentially obligate cave species was conducted by identifying the specimens with troglomorphic traits (Christiansen 2012). However, for some specific groups (e.g., Palpigradi, Diplura), other traits were considered, such as the increased number of sensory receptors (lateral organs) (Souza and Ferreira 2012) and an increased number of antennomeres and cercal articles (Sendra et al. 2012). Furthermore, experts on Diplura, Blattodea, and Collembola were consulted to evaluate the degree of troglomorphisms.

Descriptions of human uses and impacts

Human modifications were determined in relation to uses and impacts. Tourist and religious activities were considered uses while real impacts were trampling, illumination and construction resulting from these activities (Souza-Silva et al. 2015). Impacts were determined for each cave as a function of the presence or absence of visual modifications inside the cave.

Data analyses

The components of beta diversity were calculated using the BAT package developed by Cardoso et al. (2015) in the software R to assess β_{total} (overall beta diversity), β_{repl} (the replacement component) and β_{rich} (the richness differences component) (R Development Core Team 2008).

The average taxonomic distinctness ($\Delta+$) analysis was conducted with the software Primer 7 using 297 from the 463 morphotypes because they were identified up to family level (Anderson et al. 2008). We used Phyla (weight 100), Class (weight 80), Order (weight 60), Family (weight 40) and morphotypes (weight 20) as variables for a matrix of morphotypes distribution among caves (Anderson et al. 2008). Previous analysis comparing the group of sampled morphotypes and the above mentioned 297 morphotypes were carried out using a Spearman Rank Order Correlations to test the representativeness of this sub-sample (of species identified until the family level), which revealed regression $R_s = 0.93$ and $p \leq 0.05$.

The Environmental stability of each cave was determined using the Environmental Stability Index (IEA) proposed by Ferreira (2004) (Pellegrini et al. 2016, Bento et al. 2016), which considers the degree of isolation between the cave and epigeal environments through a mathematical ratio, calculated as follows. For caves having just one entrance: $IEA = \ln(AT/EE)$ and for caves having more than one entrance: $IEA = \ln((AT/\sum EE)/((NE)*(\bar{x} DEE)/AT))$, in which: AT (total size of each cave), EE (sum of the highest and longest measurements of cave entrances using perpendicular lines), NE (number of entrances), DEE (average distance between entrances, taken from one reference entrance).

A non-parametric multivariate analysis (DistLM – Distance-based Linear Model) was used to evaluate the influences of the distance between caves (dist), the extension of the sampled cave (SE), environmental stability (IEA), number (NE) and size of entrances (EE) and altitude (Alt) over invertebrate composition, total richness and average taxonomic distinctness with AICc as selection criteria and Forward as selection procedure (Anderson et al. 2008). The similarity measure based on Bray-Curtis Index was used for fauna composition and Euclidean distance was used for total richness and average taxonomic distinctness in DistLM analysis (Anderson et al. 2008). Jaccard similarity based on presence/absence, specifically as a measure of β diversity and tested against predictor variables (dist, SE, IEA, NE, EE, Alt) using DistLM analysis. Once the definition of β -diversity is based on variation in the identities of species (Whittaker 1972), the similarity measures with abundance do not provide a β diversity measure (Anderson et al. 2008).

The distance-based redundancy analysis (dbRDA) was performed to determine the strength and direction (- or +) of the predictor variables relationship selected by the DistLM routine. A metric multidimensional scaling (MDS) using bootstrap-average analysis was performed to determine the level of variation in species composition within sampled caves with and without stream and to produce two 95% bootstrap regions (Clarke et al. 2014).

Similarity analysis (ANOSIM) one-way layout with pairwise analysis was used to select faunal group formation based on Bray-Curtis similarity using caves with and without streams, frequency classes of cave sampled extension (SE), environmental stability (IEA), number (NE) and extension of entrances (EE) and altitude (Alt) as selected factors. The Similarity Percentages analysis (SIMPER) was used to determine species responsible for sample groupings using Bray-Curtis dissimilarities (Clarke 1993). These analyses were performed in the Plymouth routine in Multivariate ecological research – Primer 7 (<http://www.primer-e.com>). We also used the Spearman correlation test (R_s) (Zar 1984) to evaluate the relationship between cave physical attributes (SE, IEA, NE, EE, and Alt) and total richness and average taxonomic distinctness. Significant differences of average richness, diversity and taxonomic distinctness among caves with and without stream were evaluated using Mann-Whitney U Test (Sprent and Smeeton 2000).

Results

Environmental factors of the caves

Caves are located at altitudes between 1270 and 1669 m asl (sd = 150 m). Sampled cave extension varied from 20 to 650 m in length (sd = 202 m), presenting one to six entrances. Surface of entrances varied from 1 to 80 m² (sd = 26 m). Environmental stability varied from 1.4 to 10.93 (Table 1).

Faunal composition, abundance, and richness

We found a total of 12,123 individuals distributed in 463 morphotypes and at least 117 families. The composition considering higher taxa is presented in Figure 2A and Table 2.

The richest higher taxa in the sampled caves were the orders Araneae (100 spp., 21.6% of the total richness) and Diptera (85 spp., 18.35% of the total richness), while Dermaptera, Neuroptera, Gastropoda, and Nematomorpha presented a single species each (0.2% of the total richness). The most abundant higher taxa were the orders Diptera (3,322 individuals, 27% of the total abundance), Araneae (2,164 individuals, 18% of the total abundance), Opiliones (1,462 individuals, 12% of the total abundance) and Coleoptera (1,396 individuals, 11% of the total abundance). The less abundant higher taxa were Odonata, Megaloptera, Dermaptera, Diplura, Ephemeroptera, Zygentoma, Mollusca and Nematomorpha with less than 10 individuals each (0.3% of the total abundance) (Figure 2A).

The average richness of the caves was 39.55 spp. (sd= 21.87). The Bromélias (95 spp.) and Moreiras (75 spp.) caves presented the highest invertebrate richness, contrasting with Martiniano II (18 spp.) and Catedral I (14 spp.) caves, which presented the lowest richness values (Table 1).

Order	Family	TS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Lepidoptera	Noctuidae	5			+	+	+		+			+	+									+	
	Pyralidae	1		+	+		+					+											
	Tineidae	10		+			+								+	+			+	+	+	+	
	Not identified	2			+	+																+	
Megaloptera	Corydalidae	2	+				+																
Neuroptera	Myrmeleontidae	1						+	+													+	
Odonata	Not identified	2	+																			+	
Psocoptera	Pseudocaciliidae	3													+			+				+	
	Psyllipsocidae	1																				+	
	Ptiloneuridae	3	+		+	+					+	+											
	Not identified	8			+			+	+				+	+	+	+						+	
Trichoptera	Hydropsychidae	7	+		+		+				+	+	+									+	
Zygentoma	Nicoletiidae	2							+				+										
Geophilomorpha	Geophilidae	2													+							+	
	Not identified	1																				+	
Lithobiomorpha	Not identified	5								+							+	+	+	+	+	+	
Scolopendromorpha	Not identified	1																				+	
Polydesmida	Chelodesmidae	1											+									+	
	Cryptodesmidae	1																				+	
	Pyrgodesmidae	1													+							+	
	Not identified	1																				+	
Spirostreptida	Pseudonannolenidae	2		+	+		+					+	+	+		+					+	+	

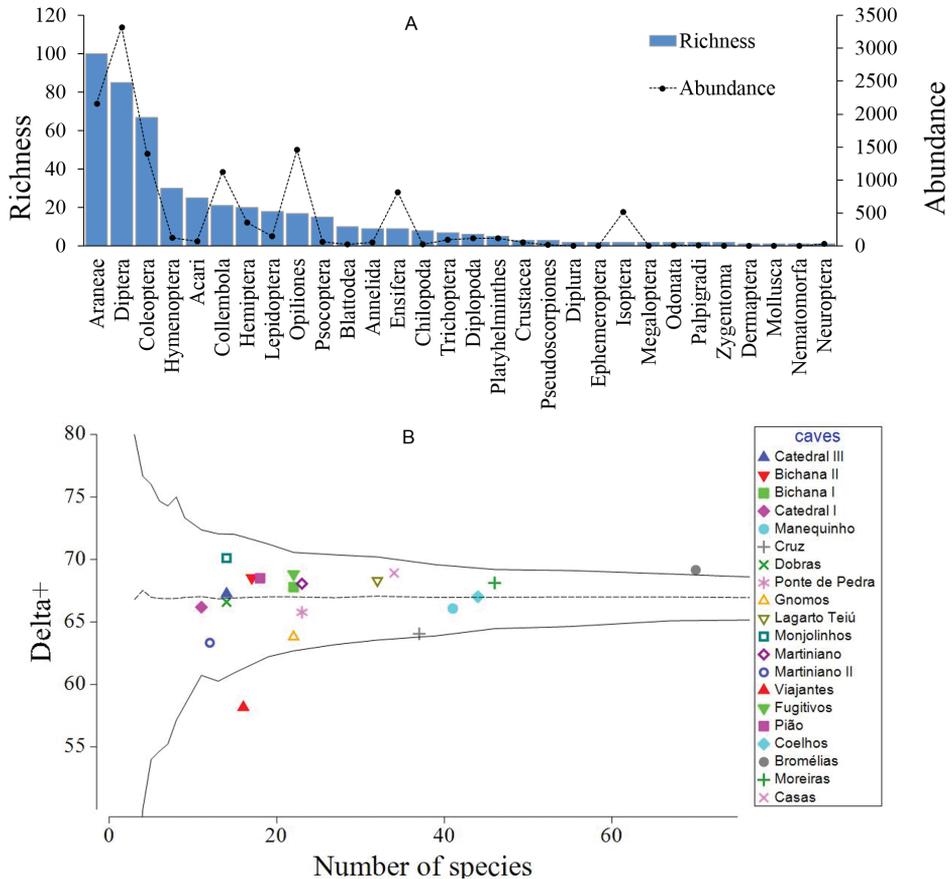


Figure 2. Higher taxa invertebrate abundance, taxonomic diversity (richness) (A) and average taxonomic distinctness ($\Delta+$) (B) in all 20 quartzite caves placed above 1200 m high in Minas Gerais (Brazil).

Obligate cave species richness

Six invertebrate species, distributed in five caves, presented troglomorphic traits. Casas cave had four troglobitic species ((Blattodea (Figure 3B), Projapygidae (Diplura) (Figure 3D), Pselaphinae (Coleoptera: Staphylinidae), and *Eukoenenia ibitipoca* Souza & Ferreira, 2019 (Palpigradi: Eukoeniidae) (Figure 3E)), Moreiras cave had two species ((Hypogastruridae (Collembola) species (Figure 3A) and one Projapygidae species (Diplura)). Three other caves had each only one troglomorphic species. Pião and Dobras caves had the same Blattodea species that occurred in Casas cave. Bromélias cave had the Prodidomidae spider *Brasilomma enigmatica* Brescovit, Ferreira & Rheims, 2012 (Figure 3C).

Invertebrate diversity and distribution and their relationships with abiotic factors

The quantitative similarity among cave communities was low ($< 41\%$) and the β_{total} was 0.952, $\beta_{\text{repl}} = 0.769$ and $\beta_{\text{rich}} = 0.178$.

Average taxonomic distinctness $\Delta+$ varied between 58 to 70, and 18 caves were placed within the 95% confidence interval of the average taxonomic distinctness ($\Delta+$) (Figure 2, Table 3). However, $\Delta+$ decreased with environmental stability ($R_s = -0.46$, $p \leq 0.05$).

The Distance-based linear models (DistLM) revealed in marginal tests that the sampled extension (SE) of the caves ($R^2 = 0.08$, AICc = 168.7, Pseudo-F = 1.5071; $p = 0.01$) was the only predictor determining the similarity of cave communities, both for Bray-Curtis and Jaccard similarities. The two axes of the distance-based redundancy analysis (dbRDA) graphic model captured nearly 52.6% of the variability in the fitted



Figure 3. Obligate cave species found in the Ibitipoca Estadual Park, Brazil. **A** Hypogastruridae **B** Blattodea **C** *Brasilomma enigmatica* (Prodidomidae) **D** Projapygidae **E** *Eukoenenia ibitipoca* (Palpigradi).

Table 3. Average taxonomic distinctness with respective lambda and significance values (% sig) of the 20 mountain quartzite caves in southeastern Brazil and the 297 morphotypes that were identified to family level.

Cave names	Richness	Delta+	Delta+ Sig %	Lambda+	Lambda+ Sig %
Catedral III	14	67.25	94.1	197.95	57.1
Bichana II	17	68.53	47	215.48	77.1
Bichana I	22	67.79	67.1	233.65	91.5
Catedral I	11	66.18	75.9	187.24	56.1
Manequinho	41	66.07	45	249.46	57.3
Cruz	37	64.05	5.8	280.86	12.2
Dobras	14	66.59	84.9	255.43	53.3
Ponte de Pedra	23	65.77	52.3	271.84	30.2
Gnomos	22	63.81	15.4	317.96	5.6
Lagarto Teiú	32	68.31	36	208.42	39.4
Monjolinhos	14	70.11	21.8	240.65	72.3
Martiniano	23	68.06	55.9	279.65	22
Martiniano II	12	63.33	24.2	261.62	50.5
Viajantes	16	58.17	1	333.31	6.6
Fugitivos	22	68.83	31.4	233.7	93.3
Piã	18	68.5	49.8	191.86	34.4
Coelhos	44	67.02	99.5	238.26	84.9
Bromélias	70	69.16	1	234.78	96.9
Moreiras	46	68.12	35.2	228.62	85.7
Casas	34	68.91	17	210.05	36.4

model and 16.4% of the total variation in the data cloud. The first overlay showed that the first dbRDA axis is strongly related to cave sampled extension (SE) (Figure 4). The Distance-based linear models (DistLM) revealed that the sampled cave extension (SE) was also the only predictor influencing variations over species richness ($R^2 = 0.335$, $AICc = 117.45$, $Pseudo-F = 9.1066$, $p = 0.009$) of the cave communities. Furthermore, only total richness ($R_s = 0.58$, $p \leq 0.05$) and richness of obligate cave species ($R_s = 0.58$, $p \leq 0.05$) increased with cave extension.

Finally, Figure 5 shows the two groups formed in ANOSIM between caves with and without a stream ($R = 0.262$, $p = 0.02$) and the bootstrap data variation within the 95% confidence interval. Araneae (*Mesabolivar* sp., Ochyroceratidae spp., Theridiidae sp.), Opiliones (*Mitogoniella indistincta* Mello-Leitão, 1936), Diplopoda (*Pseudonanolene* sp.), Ensifera (*Endecous* sp.), Psocoptera (Ptiloneuridae sp.), Collembola sp., Diptera (Tipulidae sp.), Isopoda sp., Hemiptera (Emesinae sp.) and Trichoptera sp., were the taxa that most contributed for dissimilarity between the two types of caves.

Trophic resources for the cave invertebrates

Organic resources were composed of plant debris deposited close to vertical or horizontal entrances, as well as sparse roots, root stalagmites, termite galleries, guano of carnivorous bats (*Chrotopterus auritus* Peters, 1856), hematophagous bats (*Desmodus rotundus* (É. Geoffroy, 1810)) and swifts (*Streptoprocne biscutata* (Sclater, 1865)), a very abundant bird in these caves. In some caves, bacterial and fungal biofilms were

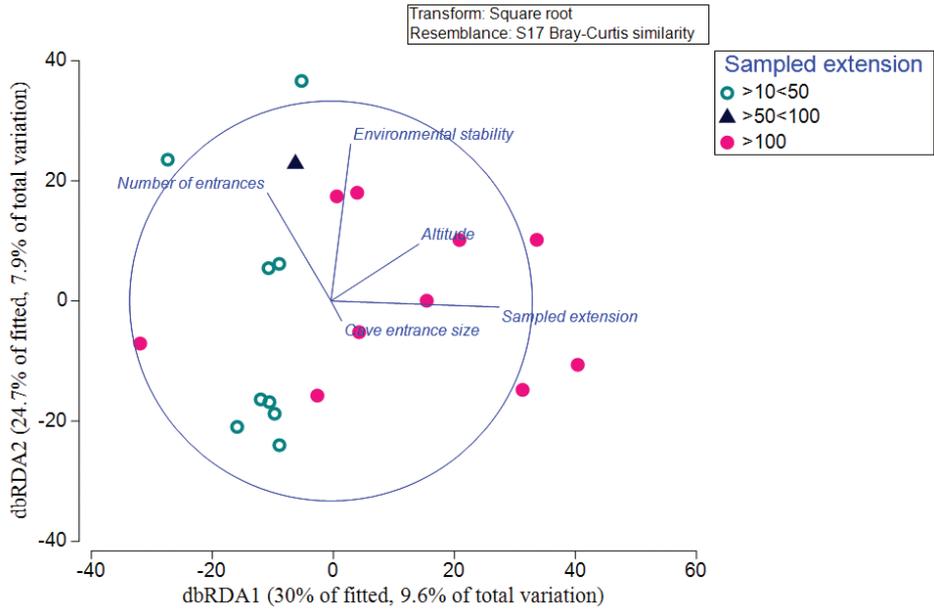


Figure 4. Distance-based redundancy analysis (dbRDA) showing the influences of the environmental factors on cave fauna composition in the 20 studied caves. The two axes explained nearly 55% of the variability in the fitted model and nearly 17% of the total variation in the data cloud. The first overlay shows how the first dbRDA axis is strongly related to cave sampled extension.

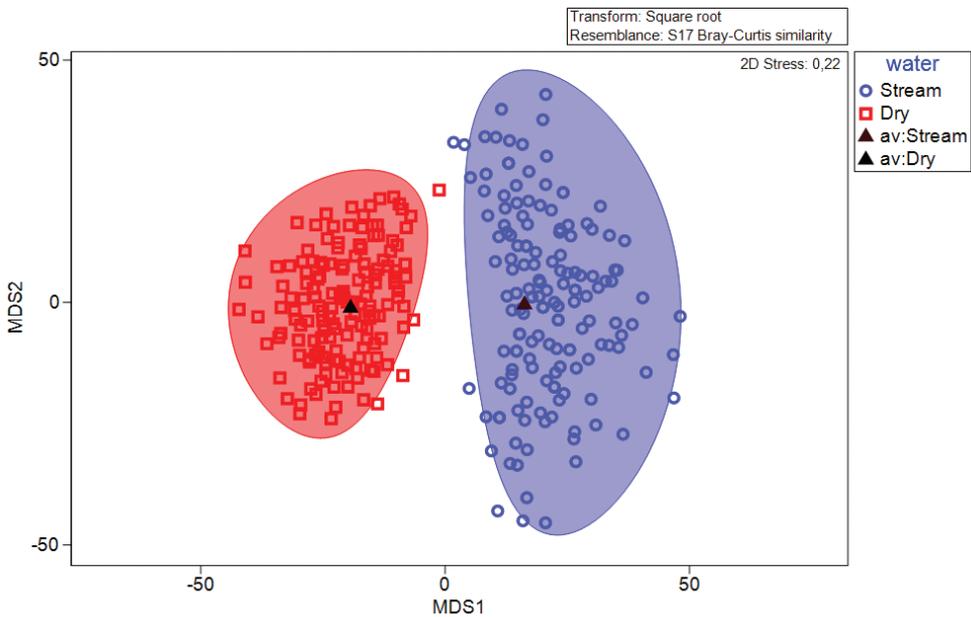


Figure 5. Metric multidimensional scaling (MDS) ordination plot of the 20 quartzite caves with and without a stream using bootstrap regions for group means around their centroids (triangles). Average (Av).

seen on different substrates, which were identified as an alternative organic resource for invertebrates.

The bat guano piles were always small and scarce, but were colonized by Diptera larvae (1500 specimens in total), Collembola (300 specimens in total), Staphylinidae (250 specimens) and Leiodidae (300 specimens), while guano deposits of swifts harbored Acari (50 specimens), Diptera larvae (300 specimens, crickets and spiders. The bat species *Desmodus rotundus* was the most frequent in caves on the park periphery, quite close to cattles. A high diversity of invertebrates, such as Ensífera, Acari, Coleoptera and Diptera larvae, Annelida (Haplotaxida) were found in the guano of these bat. In Casas cave, Collembola, Acari, and Blattodea were observed associated with termite galleries or abandoned nests, the only macroscopic organic matter observed inside this cave. Top predator taxa were Opiliones (about 1000 counted *Mitogoniela* sp.), Reduviidae (about 200 counted *Zelurus* sp.), Pholcidae (about 500 counted *Mesabolivar* sp.) among others.

Description of human uses and impacts

All impacts observed in the caves were the consequences of tourism. Impacts like graffiti on the cave walls, trails and trampled soil were the most common, which were observed in almost all the studied caves. In some caves, the entrances located near the touristic trails garbage (organic and plastics) was found. In the Cruz cave, a wooden ladder was installed to facilitate the access of visitors. Currently, only three caves in the Park are open to tourist visitation: Pião, Coelhos and Monjolinhos caves. Other caves, like Bichana I and II, showed no signs of visitation, although they are located near the access road. Although few impacts were found inside the Park boundaries, the surrounding forests were removed for pastures and monocultures (such as *Eucalyptus*).

Discussion

Besides the influence of potential epigeal colonizers, the higher average richness found in the present work (39.55 spp., SD = 21.87 spp.) may have been influenced by the size of the studied caves (average: 180 m in length), which can promote a greater heterogeneity of habitats and support richer fauna. Furthermore, the dissimilarity and turnover may also have been determined by a higher variation in the heterogeneity of caves in terms of number and size of entrances, microhabitats and trophic conditions, which can promote diverse and heterogeneous communities (Howarth 1993, Souza-Silva et al. 2011, Lunghi et al. 2014, Simões et al. 2015). The environmental structural complexity by habitat variation and resource amount may increase the niche availability and thus allow more species to coexist (Tews et al. 2004, Schneider et al. 2011, Stein et al. 2014, Busse et al. 2018).

Many troglophilic species do not occur randomly in caves, being preferentially found in humid, deep and dark areas (Lunghi et al. 2014). Furthermore, some studies

in limestone Brazilian caves have suggested that factors such as cave extension, number, size and position of entrances, besides their trophic conditions and human impacts, may influence the invertebrate species richness and replacement (Ferreira and Horta 2001, Souza-Silva et al. 2011, Simões et al. 2015, Rabelo et al. 2018).

It is known that cave extension represents an important environmental component determining species richness, since it can eventually provide more habitat heterogeneity (Culver and Pipan 2009, Lunghi et al. 2014, Manenti et al. 2015) and availability of organic resources (specially guano), since larger caves may support larger bats colonies (Brunet and Medellín 2001, Souza-Silva et al. 2011).

The influence of big cave entrances is well known since cave entrances act as ecotones between epigeal and hypogean sites, sheltering rich communities, with representatives of both epigeal and hypogean fauna (Prous et al. 2015).

The presence of streams increase humidity and bring organic matter inside caves, and can also transport epigeal species inside caves (Waters 1981, Souza Silva et al. 2011a and 2013). All these characteristics provided by the presence of streams can lead to distinct community compositions in wet and dry quartzite caves, as found by Simões et al. (2015) for some limestone caves in Brazil.

Obligate cave species diversity

In Brazil, with exception of the iron ore cavities (Souza-Silva et al. 2015; Ferreira et al. 2018), non-carbonate caves usually have a low richness of obligate cave species (one to three species) in comparison to limestone (Souza-Silva et al. 2011). However, for the Ibitipoca quartzite landscape, a higher number of obligate cave species was found. Two factors may be determinant for this richness. It is known that variations in environmental conditions associated with a complex geological history are leading causes for the colonization of the subterranean environment in some areas of the world, *e. g.* western Balkans (Deharveng et al. 2012, Mammola 2017), which can be also the cause of cave colonization in Ibitipoca caves.

However the 37 species with troglomorphic traits sampled by Gallão and Bichuette (2015) in 11 caves from Chapada Diamantina, This constitutes an extremely atypical scenario for Neotropical caves. It is important to highlight that, from this total, only 5 species were described and considered as true troglobites (*Tmesiphantes hypogeus* Bertani et al., 2013, *Scolopocryptops troglocaudatus* Chagas-Jr & Bichuette, 2015, *Troglo-rhopalurus translucidus*, Baptista & Giupponi, 2004 *Discocyrtus pedrosoi* Kury 2008, *Glaphyropoma spinosum* Bichuette, Pinna & Trajano, 2008).

Sharratt et al. (2000) reviewed the fauna of 31 sandstone caves placed between 450–750 m asl on the Cape Peninsula, South Africa, and found 85 invertebrate morphotypes (13 of them troglobites). Peck and Peck (1982) observed 15 trogloniles and 2 obligate cave species in Devils Den cave, USA. Although these studies used different sampling methodologies, these caves showed a lower average richness when compared to the quartzite caves from this study. The smaller size, lower number of entrances

might play a role in the richness in siliciclastic caves. To these features, the latitude of caves can be added possibly influencing the richness, since tropical regions have greater species richness (and diversity) compared to temperate regions (Gaston and Blackburn 2000) and thus, the pool of potential cave colonizers is richer.

Threats to the cave fauna

The use of caves for tourism is not uncommon and can be extremely important for the local economy worldwide (Bočić et al. 2006, Polak and Pipan 2011). This practice can be conducted with direct alterations to the cave structure, with the installation of stairs, railings and lighting equipment or without such modifications, characterizing an option for sporting nature (Polak and Pipan 2011). In fact, such modifications can cause intense instantaneous or cumulative pressures to the fauna (Gillienon 2011), directly influencing the species richness and abundance. In some cases, the touristic impact may focus on alterations in entrance zones, affecting both the flow of animals to the environment (*e.g.* bats) and the availability of organic resources (Polak and Pipan 2011). However, it is important to emphasize that depending on the cave structure (like the size of the chambers), the impacts can be different for the cave biodiversity (Faille et al. 2015).

Ibitipoca is a State Park and represents one of the most visited areas in the state of Minas Gerais. Nevertheless, its caves are not arranged for tourists. The management plan of the Park included caves, but cave species were not considered for planning conservation strategies (Trajano et al. 2007).

Areas deforested for cattle ranching were observed in the surroundings of Ibitipoca Mountain and may have directly influenced the abundance of *Desmodus rotundus* (hematophagous bat) in peripheral caves. Therefore, further attention to the preservation of forests surrounding the Park is also required for the conservation of cave invertebrates because obligate cave species depends on the bat and swift guano.

Final considerations

The present study revealed a rich and diverse invertebrate community sheltered in Ibitipoca quartzite caves, influenced mainly by the extension of the caves and presence of streams. This relationship has been recurrent in several other studies conducted with caves associated with different lithologies in Brazil and can be considered a keystone element for the maintenance of cave biodiversity.

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Terrestrial invertebrate fauna of Polish caves – a summary of 100 years of research

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Abstract

The year 2018 is particularly important in the history of zoological research in Poland. A hundred years ago, Kazimierz Demel published the first work concerning the terrestrial cave fauna of caves in the Ojców area. In this paper we present the extent of research on the terrestrial invertebrate fauna of Polish caves in the last 100 years. All accessible research papers that have been published during this period were analysed. Based on published literature, 593 species of terrestrial invertebrate were recorded in Polish caves. Additionally, detailed list of species of individual taxonomic groups was provided.

Keywords

subterranean fauna, terrestrial, invertebrate, checklist, caves, Poland

Introduction

The year 2018 is particularly important in the history of zoological research conducted in our country as it closes a period of 100 years of research on terrestrial invertebrates inhabiting the caves of Poland. A hundred years ago, Kazimierz Demel published the first groundbreaking work concerning the fauna of caves in the Ojców area. Although many works regarding Poland's cave fauna have been published in the last 100 years, it is still of marginal research interest to Polish zoologists, ecologists or general biologists. The aim of this paper is to present the entire range of research on the terrestrial invertebrate fauna of Polish caves in the last 100 years.

Materials and methods

All accessible research papers regarding the terrestrial invertebrate fauna of Polish caves that were published in the last 100 years were analysed in this article. These include the accessible papers published as monographs, original research papers, notes and conference materials. The results were presented here as a historical overview of the research, a list of references and a list of proven invertebrate species. The generic names have been provided regarding taxonomic indexes Fauna Europaea <https://fauna-eu.org> and World Spider Catalog <https://wsc.nmbe.ch>.

Results

Historical overview of the research

The beginning of interest in the terrestrial fauna of Polish caves dates back to the first years of the 20th century when Kazimierz Demel conducted his pioneer research in 1914. Unfortunately, the First World War prevented him from processing and issuing the results, which were actually published in 1918. Demel presented the fauna inhabiting eight caves of the Ojców area (southern part of Kraków-Częstochowa Upland), i.e. Jaskinia Koziarnia, Jaskinia Pustelnia, Jaskinia Biała, Jaskinia Złodziejska, Jaskinia Zbójecka, Jaskinia Łokietka, Jaskinia Ciemna and Jaskinia Jerzmanowska. Despite the period devoted to the research being relatively short (two and a half months), Demel collected a vast number of invertebrates, and in the presented results listed 30 species of invertebrates, 11 of which were described as cave forms, although they could be observed outside of caves (contemporarily named as troglöphiles). In his works, Demel compared his results to the ones already presented by the cave fauna researchers from Germany, Belgium, France and Austria, giving attention to faunal similarities with other caves. In the papers of the above-mentioned author, one can find both: a list of species and data concerning cave environment observations, i.e. temperature, humidity, light coverage and access to degrading organic matter, which enabled the author to perform ecological analyses.

Based on the material compiled by Demel, Stach (1919) proved the presence of five species of springtails, including description of one species new to science, i.e. *Mesochorutes ojcovensis*. The author also compared the Polish springtail fauna to that of other European countries and confirmed Demel's assumptions that troglöbionts are scarce in Polish caves. Furthermore, the data concerning springtail species found in Polish caves were summarised by Stach (1947–1963).

In the 1920s, Arndt (1921, 1923) published two papers based on his research in a few caves and mines (e.g. Złota Sztolnia) in Sudety Mountains. Nowadays most of these mines are located in the Czech Republic, while others, i.e. Niedźwiedzia Jama or Solna Jama, in Poland. Despite the research was conducted only from March to October 1920, Arndt proved the presence of 42 invertebrate species (including stygobiotic

ones) and recognised the *Schaefferia emucronata* springtail as a troglobiont species. He noticed that the fauna of the Sudety Mountains lacks the species typical to the caves of Harz Mountains, Central Alps and Urals, i.e. blind beetles (Coleoptera), Orthoptera and Acari. In the second paper, Arndt (1923) supplemented the information on ecological groups and faunal genesis in the examined subterranean sites.

Other papers devoted to the cave-dwelling fauna of the Sudety Mountains were published by Pax and Maschke (1935) and Pax (1937). The object of the research was both terrestrial and aquatic fauna in the Rogóżka Cave. The research, which began in 1933, was focused not only on the cave fauna but also on the environmental conditions, i.e. the physical and chemical properties of the lake water, and the air temperature. Nowadays the Rogóżka Cave is the non-existent cave, in 1962 it was partly destroyed by mining in the crystalline limestones quarry, and next the entrance was covered by stones (Bieroński et al. 2009).

The Second World War delayed the faunal research in Polish caves for a few years. The first post-war researcher who was interested in cave-dwelling invertebrate fauna was Kazimierz Kowalski, who presented the results of his biological observations and the research project on the Tatra Mountains caves for the years 1951–1952 in the “Wierchy” annual. In 1955, he published a paper with the results of the above-mentioned research. During his research, he visited all known Tatra caves, but his main research interest was in six caves, i.e. Jaskinia Dziura Wyżnia, Jaskinia Zimna, Jaskinia Groby, Jaskinia Magurska, Jaskinia Lodowa and Jaskinia Dziura. His methodology included not only the faunal collection by means of Barber traps or direct search methods, but also microclimate analyses, i.e. light influence and air movement. Kowalski did not limit his research to the invertebrate fauna, as it included also bats. In his work, the characteristics of the research area and detailed descriptions of microclimate conditions can be found.

In the following decades, i.e. in the 60s and 70s of the 20th century, Skalski presented exceptional research activity in the field of cave-dwelling fauna. He published his first work together with Wójcik (Skalski and Wójcik 1968), where they presented faunal genesis and microclimate characteristics of selected caves of the Częstochowa area. They proved the presence of six troglobiont species i.e. *Mesachorutes ojcowiensis*, *Catops tristis infernus*, *Choleva lederiana gracilentia*, *Porrhomma egeria*, *Arrhopalites pygmeus*, *Onychiurus alborufescens* and also determined the geographical distribution of these species.

The subsequent works by Skalski (1967, 1969) were dedicated to invertebrate fauna in the Tatra Mountains. In addition to the presented list of species, he also considered the relationship between the age of the cave and the number of troglobionts as an important factor influencing the species variety. Furthermore, he presented detailed information about the occurrence of *Onychiurus armatus* springtails in the Mylna and Czarna caves, *Hypena obsitalis* butterflies in the Mylna cave and *Exephanes ischioxanthus* hymenoptera in Szczelina Chochołowska.

In 1973, Skalski published an extensive paper on the research and observations conducted in the years 1957–1970 in 19 caves of the Częstochowa Upland. Using a

wide range of entomological methods (Barber traps, Tullgren funnels, entomological sieves and direct searches), he detected 83 species belonging to a few arthropod and molluscan orders. He extensively discussed ecological issues, also concerning troglonexenes, for example he paid special attention to describing the associations of arthropods living or resting on cave walls. The paper includes valuable data on reproduction, position and spread of individual species in the caves like characteristics of massive occurrence of *Choleva lederiana gracilenta* if there is high availability of food.

Apart from the above mentioned research published in the form of papers, presentations at Speleological Conferences, which have been taking place since 1965, give an update on the invertebrate cave fauna research. The first information can be found in the materials from 3rd/4th Speleological Conference (1971), where Skalski (1971) presented overview of terrestrial and aquatic fauna, named all the known species and compared the knowledge from various Polish cave regions.

In his subsequent paper, Skalski (1981) focused on known data of invertebrate fauna again. However, he put particular emphasis on dominant groups and tried to determine the reasons for underground sites colonisation. He emphasised the primary dominance of not only insects and springtails in the caves but also spiders, and analysed the conditions favourable to cave organisms, i.e. food accessibility and cave habitat as a shelter for fauna during the winter.

In 1975, Baranek and Powichrowski published an article about the cave fauna in Dolina Wodącej. Three caves of the valley, Jaskinia Źródłana (Psia), Jasknia Zegarowa and Jaskinia Mroczna, were discussed in the paper. The authors observed the associations of arthropods troglonexenes like numerous fly community, spiders (particularly *Meta* sp.) and other arachnids on the cave walls. Springtails and beetles of families Staphylinidae and Catopidae (Leiodidae) were recognised among the regular cave-dwelling species. The only troglobiont species recorded was *Porhomma moravicum* (currently *P. egeria*), which was observed in Jaskinia Mroczna.

Sanocka-Wołoszynowa (1981) on Kraków – Częstochowa Upland arachnids study added great value to the state of knowledge of invertebrate fauna exploration. The author examined 184 caves and shelters; 40 of them were examined in all four seasons. Samples were collected by means of Barber traps or direct search. As a result, 13000 specimens representing 186 species of spiders, arachnids and pseudoscorpions were collected. Two species of spiders recorded deserve special attention: troglobiontic *Porhomma egeria* (in the paper *P. moravicum*) and troglophilic *Meta menardi*. Among arachnids also harvestman *Ischyropsalis helwigii*, known earlier from the Sudety Mountains area, the observation of which in Kraków – Częstochowa Upland widened the range of its occurrence. The author in addition to the detailed list of species, carried out an ecological and zoocoenological analysis, for example, she distinguished communities of arachnids living in the litter, associations of wall arachnids and a deep-cave group of species with dominant species *Porrhomma egeria*.

At the 15th Speleological Conference, Sobiepanek (1985) described the results of the research conducted during Tatra Mountains caves inventory. During the research, the samples were collected by direct search or by means of Barber traps. As a result,

many taxa belonging to dipterans, beetles, trichopterans, millipedes, spiders, gastropods, springtails and earthworms were observed. During the 30th Speleological Conference, Gubała (1996) presented his paper about the results of an inventory of 131 caves and shelters in świętokrzyskie region.

The end of the 20th century provided new data to particular systematic groups. Pomorski (1992) focused on the springtail fauna in the Sudety Mountains caves and mines. In his study, Pomorski detected 34 species, seven of which were classified as troglobionts i.e. *Bonetogastrura cavicola*, *Schaefferia emucronata*, *Oligaphorura schoetti*, *Onychiurus ambulans*, *Onychiurus cebennarius*, *Oncopodura reyersdorfensis*, *Arrhopalites bifidus* and 10 as troglaphiles. Skalski (1994) noted the successful introduction of *Speonomus hydrophilus* to Jaskinia Dzwonnicza in Wyzyna Częstochowska.

Further papers were published after the year 2000. The studies by Maślak and Barczyk (2011) and Barczyk and Madej (2014) were devoted to cave mites. The first article refers to *Oribatida* dwelling in five Jurassic caves, i.e. Jaskinia Nietoperzowa, Jaskinia Wierna, Jaskinia Studnisko, Jaskinia Błotna and Jaskinia Pod Porzeczką, and their immediate surroundings. The study presented an analysis of faunistic similarities between the species and caves. Altogether 65 Orbatida species, including one typically cave dwelling species, were recorded. It was proven that the faunal composition of larger caves significantly differs from smaller ones. Communities of small caves, such as Jaskinia Błotna, Jaskinia Pod Porzeczką were similar to soil fauna communities. The species composition was mainly influenced by the accessibility of particular food sources (leaves, guano, wood) (Maślak and Barczyk 2011). The second paper refers to eight caves of the Kraków – Częstochowa Upland (Jaskinia Lodowa, Jaskinia poniżej Lodowej, Jaskinia Pod Porzeczką, Jaskinia Studnisko, Jaskinia Pod Sokołą, Jaskinia Jasna, Jaskinia Błotna and Jaskinia Schronisko koło Jaskini Lodowej), where 316 samples were collected. There were 270 samples collected from the immediate surroundings of the caves. As a result, the author identified 106 species from 13 families, including four species new in Polish fauna i.e. *Paragamasus arcuatus*, *Parasitus hortivagus*, *Pachylaelaps sublongisetis*, *Pachyglobolaelaps hallidayi* and troglobiont species recognised earlier in other caves. Significant differences in species composition between Acari communities inhabiting caves and cave surroundings were described.

Among the contemporary studies devoted to invertebrate fauna, Dumnicka and Płotek (2013) discussed the differences in invertebrate fauna between the caves Jaskinia Towarna and Jaskinia Dzwonnicza, with regards to microclimate conditions, organic matter content in the cave deposits and tourism intensity.

During the 49th Speleological Conference, Kocot – Zalewska (2015) presented the preliminary results of the research on Jaskinia Kroczycka fauna. The presentation included general data however indicated seven orders: butterflies, dipterans, spiders, beetles, trichopterans, mites and springtails. At the next Speleological Conference, Kocot – Zalewska (2016) presented the results of the observation of Jaskinia Towarna colonisation by *Speonomus normandi hydrophilus* of Leiodidae family. The beetle was introduced in the caves in 1982 and its natural place of occurrence is Arize Massif in Pyrenees.

In a contemporary paper, Kur et al. (2016) characterised the fauna of Jaskinia Szeptunów (Szmaragdowa). Based on the samples collected in the years 2005, 2006, 2010, 2014, 2015, 5 species of springtails, 2 species of Myriapoda, 1 spider species, 2 species of butterflies and mites were observed. The cave was opened in 1990 and the colonisation rate by living organisms was monitored.

At the 51st Speleological Conference, Kocot – Zalewska and Słupińska (2017) presented the discovery of a great population of *Choleva lederiana gracilenta*, which is endemic to Sokole Mountain caves, and presented detailed information about its quantity, sex ratio and cave positioning. During the same Speleological Conference, Dumnicka (2017) discussed the state of knowledge of invertebrate cave fauna of Polish Tatra Mountains based on literature data, mainly the works by Stach (1934, 1954) and Kowalski (1955).

Several short articles were devoted to interesting discoveries of invertebrate species found in caves (Dylewska and Błoszyk 2006, Kocot-Zalewska and Rozwałka 2018, Kasprzak 1973, Weiner 1990), however, several synthetic articles were also written, summarizing the state of knowledge and valorization of a valuable studied area (Błoszyk and Rozwałka 2008, Ochman 2004, Wołoszyn and Wójcik 1964).

Checklist of terrestrial invertebrate record in Polish caves

Based on published literature, 593 species of terrestrial invertebrate are recorded in Polish caves.

Insects are represented by 146 species. Respectively: Coleoptera 50 species, Diptera 60 species, Hymenoptera 18 species, Lepidoptera 10 species, Trichoptera 4 species, Siphonaptera 3 species, Thysanoptera 1 species. Detailed list of recorded species is presented in Table 1.

Table 1. The checklist of insects recorded in Polish caves.

Species	References
Coleoptera:	
<i>Acidota cruentata</i> Mannerheim, 1830	Ochman 2004, Skalski 1973a
<i>Acrotrichis intermedia</i> (Gillmeister, 1845) [= <i>Trichopteryx intermedia</i> ‡]	Pax and Maschke 1935
<i>Aleochara diversa</i> (J. Sahlberg, 1876)	Pax and Maschke 1935
<i>Aphodius fimetarius</i> (Linnaeus, 1758)	Pax and Maschke 1935
<i>Apocatops nigrita</i> (W.F. Erichson, 1837) [= <i>Catops nigrita</i> W.F. Erichson, 1837‡]	Pax and Maschke 1935
<i>Atheta fungi</i> (J.L.C. Gravenhorst, 1806)	Pax and Maschke 1935
<i>Atheta trinotata</i> (E.G. Kraatz, 1856)	Pax and Maschke 1935
<i>Cantharis rustica</i> C.F. Fallén, 1807	Pax and Maschke 1935
<i>Catops alpinus</i> Gyllenhal, 1827	Pax and Maschke 1935
<i>Catops fuliginosus</i> Erichson, 1837	Kowalski 1955
<i>Catops fuscus</i> (Panzer, 1794)	Pax and Maschke 1935
<i>Catops longulus</i> Kellner, 1846	Kowalski 1955, Pax and Maschke 1935
<i>Catops subfuscus</i> Kellner, 1846	Kowalski 1955, Pax and Maschke 1935, Skalski 1973a

Species	References
<i>Catops tristis tristis</i> (Panzer, 1794)	Kowalski 1955, Pax and Maschke 1935
<i>Catops tristis infernus</i> Szymczakowski, 1957	Ochman 2004, Skalski 1971, Skalski 1973a, Skalski 1973b, Skalski 1981, Skalski 1994-1995, Skalski 1994a, Skalski and Wójcik 1968, Szymczakowski 1957
<i>Choleva agilis</i> (Illiger, 1798)	Gubała 1996, Wołoszyn and Wójcik 1964
<i>Choleva angustata</i> (Fabricius, 1781)	Gubała 1996
<i>Choleva cisteloides</i> Frölich, 1799	Ochman 2004, Skalski 1973a
<i>Choleva elongata</i> (Paykull, 1798)	Pax and Maschke 1935
<i>Choleva glauca</i> Britten, 1918	Skalski 1967
<i>Choleva lederiana gracilentia</i> Szymczakowski, 1957	Kocot-Zalewska and Shupirńska 2017, Ochman 2004, Skalski 1971, Skalski 1973a, Skalski 1973b, Skalski 1981, Skalski 1994-1995, Skalski 1994a, Skalski and Wójcik 1968, Szymczakowski 1957
<i>Choleva nivalis</i> Kraatz, 1856 [= <i>Choleva bicolor</i> Jeannel, 1923 †]	Kowalski 1955, Skalski 1967
<i>Choleva (Cholevopsis) spadicea</i> (J. Sturm, 1839)	Pax and Maschke 1935
<i>Cryptophagus pilosus</i> Gyllenhal, 1827	Pax and Maschke 1935
<i>Duvaliopsis pilosella stobieckii</i> (Csiki, 1907)	Kowalski 1955, Skalski 1971
<i>Enicmus minutus</i> (Linnaeus, 1767)	Ochman 2004, Skalski 1973a
<i>Lathrobium pallidum</i> Nordmann, 1837	Pax and Maschke 1935
<i>Lesteva monticola</i> Kiesenwetter, 1847	Kowalski 1955
<i>Nebria tatraica</i> Miller, 1859	Kowalski 1955
<i>Ocalea badia</i> W.F. Erichson, 1837	Pax and Maschke 1935
<i>Omalium excavatum</i> Stephens, 1834	Kowalski 1955
<i>Omalium rivulare</i> Paykull, 1789	Kowalski 1955, Pax and Maschke 1935
<i>Omalium septentrionis</i> C.G. Thomson, 1857	Pax and Maschke 1935
<i>Othius myrmecophilus</i> Kiesenwetter, 1843	Ochman 2004, Skalski 1973a
<i>Otiorhynchus scaber</i> (Linnaeus, 1758)	Kowalski 1955
<i>Philonthus (Philonthus) carbonarius</i> (Gravenhorst, 1802) [= <i>Philonthus varius</i> (Gyllenhal, 1810) †]	Skalski 1973a
<i>Poecilus lepidus lepidus</i> (Leske, 1785) [= <i>Pterostichus virens</i> (Müller, 1776) †]	Sobiepanek 1985
<i>Pselaphus beisei</i> J.F.W. Herbst, 1792	Pax and Maschke 1935
<i>Pterostichus niger</i> (Schaller, 1783)	Pax and Maschke 1935
<i>Quedius humeralis</i> Stephens, 1832	Kowalski 1955
<i>Quedius maurus</i> (Sahlberg, 1830)	Ochman 2004, Skalski 1973a
<i>Quedius mesomelinus</i> (Marsham, 1802)	Demel 1918, Gubała 1996, Kowalski 1955, Ochman 2004, Pax and Maschke 1935, Skalski 1973a, Skalski 1981, Skalski and Wójcik 1968
<i>Quedius ochropterus</i> Erichson, 1840	Kowalski 1955
<i>Speonomus normandi hydrophilus</i> Jeannel, 1907	Dumnicka and Plotek 2013, Kłasiński 2006, Kocot-Zalewska 2016, Skalski 1994a, Skalski 1994b
<i>Sphodrus leucophthalmus</i> (Linnaeus, 1758)	Gubała 1996
<i>Tachinus subterraneus</i> (Linnaeus, 1758)	Kowalski 1955
<i>Trechus latus</i> Putzeys, 1847	Kowalski 1955
<i>Trechus quadristriatus</i> (Schrank von Paula, 1781)	Pax and Maschke 1935
<i>Trichophya pilicornis</i> (L. Gyllenhal, 1810)	Pax and Maschke 1935
<i>Xylodromus concinnus</i> (Th. Marsham, 1802)	Pax and Maschke 1935
Diptera:	
<i>Amoebaleria spectabilis</i> (Loew, 1862)	Kowalski 1955
<i>Aphiochaeta rufipes</i> Meigen, 1804	Arndt 1921
<i>Apteromyia claviventris</i> (Strobl, 1909) [= <i>Limosina claviventris</i> Strobl, 1909 †]	Pax and Maschke 1935
<i>Bradysia forficulata</i> (Bezzi, 1914) [= <i>Neosciara forficulata</i> Bezzi, 1914 †]	Pax and Maschke 1935

Species	References
<i>Bolitophila ofnerea</i>	Sobiepanek 1985
<i>Calliphora vicina</i> Robineau-Desvoidy, 1830 [= <i>Calliphora erythrocephala</i> Meigen, 1826 ‡]	Pax and Maschke 1935
<i>Calliphora vomitoria</i> (Linnaeus, 1758)	Pax and Maschke 1935, Skalski 1973a, Skalski 1981
<i>Copromyza glacialis</i> (Meigen, 1830)	Sobiepanek 1985
<i>Copromyza nigra</i> (Meigen, 1830)	Sobiepanek 1985
<i>Copromyza nitida</i> (Meigen, 1830)	Sobiepanek 1985
<i>Crumomyia nitida</i> (Meigen, 1830)	Kowalski 1955
<i>Crumomyia nigra</i> (Meigen, 1830) [= <i>Borborus nigra</i> Meigen, 1830 ‡]	Pax and Maschke 1935
<i>Crumomyia glacialis</i> (Meigen, 1830)	Hajduk and Ogorzałek 1970, Skalski 1967
<i>Culex pipiens</i> Linnaeus, 1758	Arndt 1921, Hajduk and Ogorzałek 1970, Kowalski 1955, Pax and Maschke 1935, Skalski 1967, Skalski 1973a, Skalski 1981,
<i>Culiseta annulata</i> (Schrank, 1776)	Hajduk and Ogorzałek 1970, Pax and Maschke 1935, Skalski 1973a, Skalski 1981, Skalski and Wójcik 1968
<i>Cypselia nigra</i> Meigen †	Arndt 1921
<i>Cypselia suillina</i> †	Arndt 1921
<i>Diploneura florescens</i> (Turton, 1801) [= <i>Diploneura versicolor</i> Schmitz, 1929 ‡ = <i>Diploneura florea</i> (Fabricius, 1794) ‡]	Pax and Maschke 1935
<i>Drosophila (Sophophora) melanogaster</i> Meigen, 1830 [= <i>Drosophila fasciata</i> ‡]	Skalski 1973a
<i>Ecoptomera emarginata</i> Loew, 1862	Hajduk and Ogorzałek 1970, Kowalski 1955, Pax and Maschke 1935, Sobiepanek 1985
<i>Ecoptomera obscura</i> (Meigen, 1830)	Sobiepanek 1985
<i>Ecoptomera pallescens</i> (Meigen, 1830)	Hajduk and Ogorzałek 1970, Kowalski 1955, Skalski 1967
<i>Exechia indecisa</i> (Walker, 1856) [= <i>Exechiopsis indecisa</i> ‡]	Ochman 2004, Skalski 1973a, Skalski 1981, Skalski and Wójcik 1968
<i>Gymnomus caesioides</i> (Meigen, 1830) [= <i>Scolioecentra caesis</i> = <i>Heleomyza caesia</i> (Meigen, 1830) = <i>Amoebaleria caesia</i> (Meigen, 1830) ‡]	Arndt 1921, Hajduk and Ogorzałek 1970, Kowalski 1955, Pax and Maschke 1935, Sobiepanek 1985
<i>Gymnomus amplicornis</i> (Czerny, 1924) [= <i>Amoebaleria amplicornis</i> Czerny, 1924 ‡]	Skalski 1967
<i>Heleomyza captiosa</i> (Gorodkov, 1962)	Dumnicka and Płotek 2013, Sobiepanek 1985
<i>Heleomyza modesta</i> (Meigen, 1835)	Arndt 1921, Hajduk and Ogorzałek 1970, Kowalski 1955, Pax and Maschke 1935, Sobiepanek 1985, Skalski 1967
<i>Heleomyza serrata</i> (Linnaeus, 1758)	Arndt 1921, Hajduk and Ogorzałek 1970, Kowalski 1955, Ochman 2004, Pax and Maschke 1935, Skalski 1967, Skalski 1973a, Skalski 1981, Skalski and Wójcik 1968, Sobiepanek 1985,
<i>Heteromyza atricornis</i> Meigen, 1830 [= <i>Theleida atricornis</i> ‡]	Kowalski 1955
<i>Hydrophorus bipunctatus</i> (Lehmann, 1822)	Pax and Maschke 1935
<i>Hydrotaea dentipes</i> (Fabricius, 1805)	Pax and Maschke 1935
<i>Limonia nubeculosa</i> Meigen, 1804	Arndt 1921, Hajduk and Ogorzałek 1970
<i>Limonia silvatica</i> (Meigen, 1830) [= <i>Limosina silvatica</i> ‡]	Arndt 1921
<i>Megaselia albicaudata</i> (Wood, 1910)	Pax and Maschke 1935
<i>Megaselia angusta</i> (Wood, 1909)	Pax and Maschke 1935
<i>Megaselia pulicaria</i> (Fallen, 1823)	Pax and Maschke 1935
<i>Megaselia rufipes</i> (Meigen, 1804)	Pax and Maschke 1935
<i>Meiosimyza rorida</i> (Fallén, 1820) [= <i>Sapromyza rorida</i> Fallén, 1820 ‡]	Pax and Maschke 1935
<i>Mycetophila ruficollis</i> Meigen, 1818	Sobiepanek 1985
<i>Neuroctena anilis</i> Fallen, 1820 [= <i>Dryomyza anilis</i> Fallén, 1820 ‡]	Pax and Maschke 1935
<i>Oecothea praecox</i> Loew, 1862	Arndt 1921

Species	References
<i>Phaonia populi</i> (Meigen, 1826)	Ochman 2004, Skalski 1973a, Skalski 1981, Skalski and Wójcik 1968
<i>Sarcophaga alpina</i> Zetterstedt, 1838 [= <i>Acrophaga alpina</i> Zetterstedt, 1838‡]	Pax and Maschke 1935
<i>Sciara atomaria</i> (Lynch Arribalzaga, 1892) [= <i>Neosciara vivida</i> Winnertz, 1867‡]	Pax and Maschke 1935
<i>Scoliocentra villosa</i> (Meigen, 1830)	Dumnicka and Plotek 2013, Ochman 2004, Skalski 1973a, Skalski 1981, Skalski and Wójcik 1968,
<i>Spelobia pseudosetaria</i> (Duda, 1918) [= <i>Limosina penetralis</i> Collin, 1925‡]	Pax and Maschke 1935
<i>Spelepta leptogaster</i> (Winnertz, 1863)	Pax and Maschke 1935, Sobiepanek 1985
<i>Sphaerophoria scripta</i> (Linnaeus, 1758)	Kowalski 1955
<i>Sylvicola fenestralis</i> (Scopoli, 1763) [= <i>Anisopus fenestralis</i> (Scopoli 1763) ‡]	Hajduk and Ogorzałek 1970
<i>Theobaldia alascaensis</i> Ludlow †	Kowalski 1955
<i>Tipula scripta</i> Meigen, 1830	Arndt 1921
<i>Trichocera hiemalis</i> (De Geer, 1776)	Pax and Maschke 1935
<i>Trichocera maculipennis</i> Meigen, 1818	Arndt 1923, Demel 1918, Ochman 2004, Pax and Maschke 1935, Skalski 1967, Skalski 1973a, Skalski 1981, Skalski and Wójcik 1968, Sobiepanek 1985
<i>Trichocera regelationis</i> (Linnaeus, 1758)	Pax and Maschke 1935
<i>Triphleba antricola</i> (Schmitz, 1918)	Pax and Maschke 1935
<i>Triphleba distinguenda</i> (Strobl, 1892) [= <i>Triphleba unicalcarata</i> (Becker, 1901) ‡]	Pax and Maschke 1935
<i>Triphleba hyalinata</i> (Meigen, 1830)	Pax and Maschke 1935
<i>Triphleba lyria</i> Schmitz, 1935	Pax and Maschke 1935
<i>Triphleba uncinata</i> Schmitz †	Pax and Maschke 1935
<i>Thoracocheata brachystoma</i> (Stenhammar, 1855)	Pax and Maschke 1935
Hymenoptera:	
<i>Amblytelus atratorius</i> (Villers, 1789) [= <i>Coelichneumon atratorius</i> ‡]	Arndt 1921
<i>Amblytelus quadripunctorius</i> Muller †	Kowalski 1955
<i>Aspilota nervosa</i> (Haliday, 1833)	Pax and Maschke 1935
<i>Blacus instabilis</i> Ruthe, 1861	Pax and Maschke 1935
<i>Diphyus monitorius</i> (Panzer, 1801)	Skalski 1973a
<i>Diphyus fossorius</i> (Linnaeus, 1758)	Skalski 1973a
<i>Exallonyx ater</i> (Gravenhorst, 1807) [= <i>Serphus filicornis</i> Kieffer, 1908 ‡]	Pax and Maschke 1935
<i>Exallonyx ligatus</i> Nees, 1834 [= <i>Serphus ligatus</i> Nees, 1834‡]	Pax and Maschke 1935
<i>Exephantes hilaris</i> Gravenhorst †	Hajduk and Ogorzałek 1970, Kowalski 1955, Pax and Maschke 1935
<i>Exephantes ischioxanthus</i> (Gravenhorst, 1829)	Skalski 1969, Skalski 1973a, Skalski 1981
<i>Formica fusca</i> Linnaeus, 1758	Pax and Maschke 1935
<i>Hemiteles fulvipes</i> Gravenhorst, 1929 †	Pax and Maschke 1935
<i>Ichneumon extensorius</i> Linnaeus, 1758	Pax and Maschke 1935
<i>Ichneumon gracilicornis</i> Gravenhorst, 1829	Pax and Maschke 1935
<i>Megastylus cruentator</i> Schiodte, 1839	Pax and Maschke 1935
<i>Mutilla europaea</i> Linnaeus, 1758	Hajduk and Ogorzałek 1970
<i>Proctotrypes gravidator</i> (Linnaeus, 1758)	Skalski 1981
<i>Tretoserphus laricis</i> (Haliday, 1839) [= <i>Cryptoserphus laricis</i> (Haliday, 1839) ‡]	Pax and Maschke 1935
Lepidoptera:	
<i>Aglais urticae</i> (Linnaeus, 1758) [= <i>Vanessa urticae</i> (Linnaeus, 1758) ‡]	Kowalski 1955, Pax and Maschke 1935, Skalski 1967, Skalski 1973a

Species	References
<i>Bomolocha obesalis</i> Treitschke 1828	Kowalski 1955, Skalski 1967
<i>Canadrina simulans</i> (Hufnagel, 1766) [= <i>Rhyacia simulans</i> (Hufnagel, 1766) ‡]	Skalski 1973a
<i>Hypena obsitalis</i> (Hübner, 1813)	Skalski 1969
<i>Hypena rostralis</i> (Linnaeus, 1758)	Skalski 1973a
<i>Monopis laevigella</i> (Denis & Schiffermüller, 1775) [= <i>Monopis rusticella</i> (Hübner, 1813) ‡]	Skalski 1973a
<i>Nudaria mundana</i> (Linnaeus, 1760)	Skalski 1973a
<i>Scoliopteryx libatrix</i> (Linnaeus, 1758)	Baranek and Powichrowski 1975, Demel 1918, Dumnicka and Plotek 2013, Hajduk and Ogorzałek 1970, Kowalski 1955, Kur et al. 2016, Pax and Maschke 1935, Skalski 1967, Skalski 1973a, Skalski 1981, Skalski 1994-1995, Skalski and Wójcik 1968, Sobiepanek 1985, Wołoszyn and Wójcik 1964
<i>Triphosa dubitata</i> (Linnaeus, 1758)	Arndt 1921, Baranek and Powichrowski 1975, Demel 1918, Dumnicka and Plotek 2013, Hajduk and Ogorzałek 1970, Kowalski 1955, Kur et al. 2016, Pax and Maschke 1935, Pax 1937, Skalski 1967, Skalski 1973a, Skalski 1981, Skalski 1994-1995, Skalski and Wójcik 1968, Sobiepanek 1985, Wołoszyn and Wójcik 1964,
<i>Vanessa io</i> (Linnaeus, 1758)	Arndt 1921, Dumnicka and Plotek 2013, Kowalski 1955, Pax and Maschke 1935, Skalski 1967, Skalski 1973a, Wołoszyn and Wójcik 1964,
Trichoptera:	
<i>Micropterna nycterobia</i> McLachlan, 1875	Arndt 1921, Hajduk and Ogorzałek 1970
<i>Micropterna sequax</i> McLachlan, 1875	Arndt 1921
<i>Micropterna testacea</i> (Gmelin, 1789)	Arndt 1921, Hajduk and Ogorzałek 1970
<i>Stenophylax permistus</i> McLachlan, 1895	Arndt 1921, Baranek and Powichrowski 1975, Demel 1918, Hajduk and Ogorzałek 1970, Kowalski 1955, Ochman 2004, Skalski 1973a, Skalski 1981, Skalski and Wójcik 1968, Sobiepanek 1985,
Siphonaptera:	
<i>Ctenophthalmus solutus</i> Jordan & Rothschild, 1920	Skalski 1981
<i>Ischnopsyllus hexactenus</i> (Kolenati, 1856)	Pax and Maschke 1935
<i>Nosopsyllus fasciatus</i> (Bosc, 1800)	Skalski 1981
Thysanoptera	
<i>Chirothrips manicatus</i> Haliday, 1836	Pax and Maschke 1935

† – status of the species name is unclear, ‡ – name published in original paper

Arachnids are the most often represented group of terrestrial invertebrate with 317 recorded species. Among the arachnids the mites (Acari) are represented by 189 species, spiders (Araneae) by 103 species. Remaining orders of Arachnids (Opiliones and Pseudoscorpionida) are represented by 20 and 5 species respectively. Detailed list of arachnids recorded in Polish caves is presented in Table 2.

Table 2. The checklist of arachnids recorded in Polish caves.

Species	References
Acari	
<i>Adoristes ovatus</i> (Koch, 1839)	Maślak and Barczyk 2011
<i>Ameroseius furcatus</i> Karg, 1971	Barczyk and Madej 2014
<i>Ameroseius plumea</i> Oudemans, 1930	Barczyk and Madej 2014
<i>Ameroseius plumigerus</i> Oudemans, 1930	Barczyk and Madej 2014

Species	References
<i>Anachipteria deficiens</i> Grandjean, 1932	Maślak and Barczyk 2011
<i>Antemoseius bacatus</i> Athias-Henriot, 1961	Barczyk and Madej 2014
<i>Arctoseius brevicheles</i> Karg, 1969	Barczyk and Madej 2014
<i>Arctoseius cetratus</i> (Sellnick, 1940)	Barczyk and Madej 2014
<i>Arctoseius magnanalis</i> Evans, 1958	Barczyk and Madej 2014
<i>Arctoseius venustus</i> (Berlese, 1917)	Barczyk and Madej 2014
<i>Asca aphidioides</i> (Linnaeus, 1758)	Barczyk and Madej 2014
<i>Atropacarus striculus</i> (Koch, 1835)	Maślak and Barczyk 2011
<i>Autogneta longilamellata</i> (Michael, 1885)	Maślak and Barczyk 2011
<i>Berniniella bicarinata</i> (Paoli, 1908)	Maślak and Barczyk 2011
<i>Carabodes femoralis</i> (Nicolet, 1855)	Maślak and Barczyk 2011
<i>Carabodes ornatus</i> Štokán, 1925	Maślak and Barczyk 2011
<i>Cepheus dentatus</i> (Michael, 1888)	Maślak and Barczyk 2011
<i>Ceratozetes gracilis gracilis</i> (Michael, 1884)	Maślak and Barczyk 2011
<i>Ceratozetes mediocris</i> Berlese, 1908	Maślak and Barczyk 2011
<i>Chamobates (Xiphobates) voigsi</i> (Oudemans, 1902)	Maślak and Barczyk 2011
<i>Chamobates cuspidatus</i> (Michael, 1884)	Maślak and Barczyk 2011, Pax and Maschke 1935
<i>Chamobates pusillus</i> (Berlese, 1895)	Maślak and Barczyk 2011
<i>Chamobates subglobulus</i> (Oudemans, 1900)	Maślak and Barczyk 2011
<i>Coproglyphus stammeri</i> (Türk & Türk, 1957)	Skalski 1994a
<i>Cultroribula bicultrata</i> (Berlese, 1905)	Maślak and Barczyk 2011
<i>Cyrtolaelaps chiropterae</i> Karg, 1971	Barczyk and Madej 2014
<i>Cyrtolaelaps mucronatus</i> (G. & R. Canestrini, 1881)	Barczyk and Madej 2014
<i>Cyta latirostris</i> (Hermann, 1804)	Pax and Maschke 1935
<i>Damaeus gracilipes</i> (Kulczyński, 1902) [= <i>Belba gracilipes</i> Kulczyński‡]	Pax and Maschke 1935
<i>Dendrolaelaps (Punctodendrolaelaps) arvicolus</i> (Leitner, 1949)	Barczyk and Madej 2014
<i>Dendrolaelaps (Punctodendrolaelaps) rotundus</i> (Hirschm, 1960)	Barczyk and Madej 2014
<i>Dissorhina ornata</i> (Oudemans, 1900)	Maślak and Barczyk 2011, Skalski 1973a
<i>Eugamasus cavernicola</i> Trägårdh, 1912	Barczyk and Madej 2014
<i>Eugamasus furcatus</i> (G. et R. Canestrini, 1882)	Barczyk and Madej 2014
<i>Eupelops acromios</i> (Hermann, 1804)	Maślak and Barczyk 2011
<i>Eupelops plicatus</i> (Koch, 1835)	Maślak and Barczyk 2011
<i>Euphthiracarus cribnarius</i> (Berlese, 1904)	Maślak and Barczyk 2011
<i>Euryparasitus emarginatus</i> (Koch, 1839)	Barczyk and Madej 2014
<i>Eviphis ostrinus</i> (Koch, 1836)	Barczyk and Madej 2014
<i>Fosseremus laciniatus</i> (Berlese, 1905)	Maślak and Barczyk 2011
<i>Foveacheles (Proxistella) terricola</i> (Koch, 1835) [= <i>Rhagidia terricola</i> Koch, 1935 ‡]	Pax and Maschke 1935, Pax 1937
<i>Gaeolaelaps aculeifer</i> (Canestrini, 1883)	Barczyk and Madej 2014
<i>Gaeolaelaps brevipilis</i> (Bernhard, 1969)	Barczyk and Madej 2014
<i>Gaeolaelaps nollii</i> (Karg, 1962)	Barczyk and Madej 2014
<i>Gamasellodes bicolor</i> (Berlese, 1918)	Barczyk and Madej 2014
<i>Gamasellus montanus</i> (Willmann, 1936)	Barczyk and Madej 2014
<i>Gamasellus spiricornis</i> (G. & R. Canestrini, 1882)	Barczyk and Madej 2014
<i>Gamasodes spiniger</i> (Trägårdh, 1910)	Barczyk and Madej 2014
<i>Geholaspis (Geholaspis) longispinosus</i> (Kramer, 1876)	Barczyk and Madej 2014
<i>Geholaspis (Longicheles) mandibularis</i> (Berlese, 1904)	Barczyk and Madej 2014
<i>Globozetes birulai</i> (Kulczynski, 1902)	Maślak and Barczyk 2011
<i>Haemogamasus nidi</i> Michael, 1892	Barczyk and Madej 2014
<i>Hemileius initialis</i> (Berlese, 1908)	Maślak and Barczyk 2011
<i>Holoparasitus calcaratus</i> (Koch, 1839)	Barczyk and Madej 2014

Species	References
<i>Holoparasitus excisus</i> (Berlese, 1906)	Barczyk and Madej 2014
<i>Holoparasitus intermedius</i> (Holzmann, 1969)	Barczyk and Madej 2014
<i>Hypoaspis (Alloparasitus) oblonga</i> (Halbert, 1915)	Barczyk and Madej 2014
<i>Hypoaspis (Alloparasitus) sardoa</i> (Berlese, 1911)	Barczyk and Madej 2014
<i>Hypochthonius luteus</i> Oudemans, 1917	Maślak and Barczyk 2011
<i>Ixodes ricinus</i> Linnaeus, 1758	Skalski 1973a, Skalski 1981
<i>Ixodes vespertilionis</i> Koch, 1844	Skalski 1973a, Skalski 1981
<i>Kunstdamaeus tecticola</i> (Michael, 1888)	Maślak and Barczyk 2011
<i>Lasioseius lawrencei</i> Evans, 1957	Barczyk and Madej 2014
<i>Lasioseius muricatus</i> (Koch, 1839)	Barczyk and Madej 2014
<i>Lauroppia beskidyiensis</i> (Niemi et Skubala, 1993)	Maślak and Barczyk 2011
<i>Lauroppia falcata marginedentata</i> (Strenzke, 1951)	Maślak and Barczyk 2011
<i>Lauroppia maritima</i> (Willmann, 1928)	Maślak and Barczyk 2011
<i>Leptogamasus alstoni</i> (Bhattacharyya, 1963)	Barczyk and Madej 2014
<i>Leptogamasus parvulus</i> (Berlese, 1903)	Barczyk and Madej 2014
<i>Leptogamasus tectegynellus</i> (Athias-Henriot, 1967)	Barczyk and Madej 2014
<i>Licneremaeus lichophorus</i> (Michael, 1882)	Maślak and Barczyk 2011
<i>Liebstadia longior</i> (Berlese, 1908)	Maślak and Barczyk 2011
<i>Liochthonius hystericinus</i> (Forslund, 1942)	Maślak and Barczyk 2011
<i>Macrocheles (Macrholaspis) dentatus</i> (Evans & Browning, 1956)	Barczyk and Madej 2014
<i>Macrocheles (Macrholaspis) recki</i> (Bregetova & Koroleva, 1960)	Barczyk and Madej 2014
<i>Macrocheles (Macrocheles) tardus</i> (Koch, 1841)	Barczyk and Madej 2014
<i>Macrocheles carinatus</i> (Koch, 1839)	Barczyk and Madej 2014
<i>Macrocheles montanus</i> (Willmann, 1951)	Barczyk and Madej 2014
<i>Macronyssus ellipticus</i> (Kolenati, 1856) [= <i>Liponissus ellipticus</i> Kolenati, 1856‡]	Pax and Maschke 1935
<i>Metabelba (Parametabelba) italica</i> (Sellnick, 1931)	Maślak and Barczyk 2011
<i>Metabelba pulverulenta</i> (Koch, 1839)	Maślak and Barczyk 2011
<i>Minunthozetes pseudofisiger</i> (Schweizer, 1922)	Maślak and Barczyk 2011
<i>Moritzoppia keilbachi</i> (Moritz, 1969)	Maślak and Barczyk 2011
<i>Moritzoppia uncarinata</i> (Paoli, 1908)	Maślak and Barczyk 2011
<i>Multioppia glabra</i> (Mihelčič, 1955)	Maślak and Barczyk 2011
<i>Nicoletiella denticulata</i> (Schränk, 1776)	Pax and Maschke 1935, Pax 1937
<i>Ololaelaps placentula</i> (Berlese, 1887)	Barczyk and Madej 2014
<i>Olopachys suecicus</i> Sellnick, 1950	Barczyk and Madej 2014
<i>Oppiella nova</i> (Oudemans, 1902)	Maślak and Barczyk 2011
<i>Oribatella calcarata</i> (Koch, 1835)	Maślak and Barczyk 2011
<i>Oribatella meridionalis</i> (Berlese, 1908)	Pax and Maschke 1935, Pax 1937
<i>Oribellopsis cavaticus</i> (Kunst, 1962)	Maślak and Barczyk 2011
<i>Pachydellus furcifer</i> (Oudemans, 1903)	Barczyk and Madej 2014
<i>Pachydellus ineptus</i> (Hirschmann & Krauss, 1965)	Barczyk and Madej 2014
<i>Pachydellus sculptus</i> Berlese, 1920	Barczyk and Madej 2014
<i>Pachyglobolaelaps hallidayi</i> Masán, 2014	Barczyk and Madej 2014
<i>Pachylaelaps (Longipachylaelaps) cf. longisetis</i> Halbert, 1915	Barczyk and Madej 2014
<i>Pachylaelaps (Longipachylaelaps) sublongisetis</i> Koroleva, 1977	Barczyk and Madej 2014
<i>Pachylaelaps (Pachylaelaps) imitans</i> Berlese, 1920	Barczyk and Madej 2014
<i>Pachylaelaps (Pachylaelaps) littoralis</i> Halbert, 1915	Barczyk and Madej 2014
<i>Pachylaelaps (Pachylaelaps) pectinifer</i> (G. & R. Canestrini, 1881)	Barczyk and Madej 2014
<i>Pachylaelaps (Pachylaelaps) troglophilus</i> Willmann, 1940	Barczyk and Madej 2014
<i>Pachyseius humeralis</i> Berlese, 1910	Barczyk and Madej 2014

Species	References
<i>Pantelozetes cavaticus</i> (Kunst, 1962) [= <i>Oribella cavatica</i> Kunst, 1962‡]	Skalski 1994a
<i>Pantelozetes paolii</i> (Oudemans, 1913)	Maślak and Barczyk 2011
<i>Paragamasus (Aclerogamasus) alpestris</i> (Berlese, 1904)	Barczyk and Madej 2014
<i>Paragamasus (Aclerogamasus) similis</i> (Willmann, 1953)	Barczyk and Madej 2014
<i>Paragamasus (Anidogamasus) arcuatus</i>	Barczyk and Madej 2014
<i>Paragamasus (Anidogamasus) brevicornis</i>	Barczyk and Madej 2014
<i>Paragamasus (Anidogamasus) brevipes</i> (Berlese, 1905)	Barczyk and Madej 2014
<i>Paragamasus (Anidogamasus) digitulus</i> (Karg, 1963)	Barczyk and Madej 2014
<i>Paragamasus (Anidogamasus) homopodooides</i> (Athias-Henriot, 1967)	Barczyk and Madej 2014
<i>Paragamasus (Anidogamasus) lapponicus</i> (Trägårdh, 1910)	Barczyk and Madej 2014
<i>Paragamasus (Anidogamasus) mediocris</i> Berlese, 1904	Barczyk and Madej 2014
<i>Paragamasus (Anidogamasus) misellus</i> (Berlese, 1903)	Barczyk and Madej 2014
<i>Paragamasus (Anidogamasus) runcatellus</i> (Berlese, 1903)	Barczyk and Madej 2014
<i>Paragamasus (Anidogamasus) runciger</i> (Berlese, 1904)	Barczyk and Madej 2014
<i>Paragamasus (Anidogamasus) truncus</i> Schweizer, 1961	Barczyk and Madej 2014
<i>Paragamasus (Anidogamasus) vagabundus</i> (Karg, 1968)	Barczyk and Madej 2014
<i>Paragamasus (Anidogamasus) wasmanni</i> (Oudemans, 1902)	Barczyk and Madej 2014
<i>Parasitus loricatus</i> (Wankel, 1861) [= <i>Parasitus niveus</i> ‡ = <i>Eugamasus loricatus</i> Wankel, 1861‡]	Barczyk and Madej 2014, Pax and Maschke 1935, Skalski 1967, Skalski 1973a, Skalski 1981
<i>Parasitus hortivagus</i> (Berlese, 1903)	Barczyk and Madej 2014
<i>Parasitus kraepelini</i> Berlese, 1903 [= <i>Eugamasus kraepelini</i> Berlese, 1903‡]	Pax and Maschke 1935
<i>Parasitus maschkeae</i> Willmann, 1936	Skalski 1967
<i>Parazercon radiatus</i> (Berlese, 1910)	Barczyk and Madej 2014
<i>Pergamasus (Thenargamasus) barbarus</i> (Berlese, 1904)	Barczyk and Madej 2014
<i>Pergamasus (Pergamasus) brevicornis</i> Berlese, 1903	Barczyk and Madej 2014
<i>Pergamasus (Pergamasus) crassipes</i> Berlese, 1906	Barczyk and Madej 2014
<i>Pergamasus (Pergamasus) mediocris</i> Berlese, 1904	Barczyk and Madej 2014
<i>Pergamasus robustus</i> (Oudemans, 1902)	Skalski 1967
<i>Phaulodiaspis advena</i> (Trägårdh, 1992)	Dylewska and Błoszyk 2006, Skalski 1981
<i>Phthiracarus (Archiphthiracarus) bryobius</i> Jacot, 1930	Maślak and Barczyk 2011
<i>Pilogalumna crassiclava</i> (Berlese, 1914)	Maślak and Barczyk 2011
<i>Pilogalumna tenuiclava</i> (Berlese, 1908)	Maślak and Barczyk 2011
<i>Poecilochirus carabi</i> G. & R. Caenestrini, 1882	Barczyk and Madej 2014
<i>Porrhostaspis lunulata</i> Muller, 1859	Barczyk and Madej 2014
<i>Proctolaelaps pygmaeus</i> (Muller, 1860)	Barczyk and Madej 2014
<i>Prozercon fimbriatus</i> (Koch, 1839)	Barczyk and Madej 2014
<i>Prozercon kochi</i> Sellnick, 1943	Barczyk and Madej 2014
<i>Prozercon sellnicki</i> Halaskova, 1963	Barczyk and Madej 2014
<i>Prozercon traegardhi</i> (Halbert, 1923)	Barczyk and Madej 2014
<i>Punctoribates punctum</i> (Koch, 1839)	Maślak and Barczyk 2011
<i>Pygmeophorus spinosus</i> Kramer, 1877	Pax and Maschke 1935, Skalski 1981
<i>Quadroppia quadricarinata</i> (Michael, 1885)	Maślak and Barczyk 2011
<i>Ramusella (Insculptoppia) furcata</i> (Willmann, 1928)	Maślak and Barczyk 2011
<i>Rhagidia reflexa</i> var. <i>volmsdorfensis</i> †	Pax and Maschke 1935, Pax 1937
<i>Rhinoppia hygrophila</i> (Mahunka, 1987)	Maślak and Barczyk 2011
<i>Rhinoppia nasuta</i> (Moritz, 1965)	Maślak and Barczyk 2011
<i>Rhinoppia obsoleta</i> (Paoli, 1908)	Maślak and Barczyk 2011
<i>Rhinoppia subpectinata</i> (Oudemans, 1900)	Maślak and Barczyk 2011
<i>Rhodacarellus apophyseus</i> Karg, 1971	Barczyk and Madej 2014
<i>Rhodacarellus silesiacus</i> Willmann, 1935	Barczyk and Madej 2014
<i>Rhodacarus calcurulatus</i> Berlese, 1921	Barczyk and Madej 2014

Species	References
<i>Rhodacarus coronatus</i> Berlese, 1921	Barczyk and Madej 2014
<i>Rhodacarus mandibularis</i> Berlese, 1921	Barczyk and Madej 2014
<i>Scheloberibates laevigatus</i> (Koch, 1835)	Maślak and Barczyk 2011
<i>Sphaerozetes piriformis</i> (Nicolet, 1855)	Maślak and Barczyk 2011
<i>Spinturnix murinus</i> Walckenaer †	Pax and Maschke 1935
<i>Subiasella (Lalmoppia) quadrimaculata</i> (Evans, 1952)	Maślak and Barczyk 2011
<i>Suctobelba altwateri</i> Moritz, 1970	Maślak and Barczyk 2011
<i>Suctobelba lapidaria</i> Moritz, 1970	Maślak and Barczyk 2011
<i>Suctobelba trigona</i> (Michael, 1888)	Maślak and Barczyk 2011
<i>Suctobelbella (Flagrosectobelba) alloenasuta</i> Moritz, 1971	Maślak and Barczyk 2011
<i>Suctobelbella similis</i> (Forsslund, 1941)	Maślak and Barczyk 2011
<i>Tectocephus alatus</i> Berlese, 1913	Maślak and Barczyk 2011
<i>Tectocephus velatus</i> (Michael, 1880)	Maślak and Barczyk 2011
<i>Unduloribates undulates</i> (Berlese, 1914)	Maślak and Barczyk 2011
<i>Veigaia cerva</i> (Kramer, 1876)	Barczyk and Madej 2014
<i>Veigaia exigua</i> (Berlese, 1916)	Barczyk and Madej 2014
<i>Veigaia kochi</i> (Trägårdh, 1901)	Barczyk and Madej 2014
<i>Veigaia nemorensis</i> (Koch, 1839)	Barczyk and Madej 2014
<i>Veigaia planicola</i> (Berlese, 1892)	Barczyk and Madej 2014
<i>Veigaia transisalae</i> (Oudemans, 1902)	Barczyk and Madej 2014
<i>Vulgarogamasus kraepelini</i> (Berlese, 1905)	Barczyk and Madej 2014
<i>Vulgarogamasus maschkeae</i> Willmann, 1936	Barczyk and Madej 2014
<i>Vulgarogamasus remberti</i> (Oudemans, 1912)	Barczyk and Madej 2014
<i>Vulgarogamasus oudemansi</i> (Berlese, 1904)	Barczyk and Madej 2014
<i>Zercon arcuatus</i> Trägårdh, 1931	Barczyk and Madej 2014
<i>Zercon baloghi</i> Sellnick, 1958	Barczyk and Madej 2014
<i>Zercon berleseii</i> Sellnick, 1958	Barczyk and Madej 2014
<i>Zercon curiosus</i> Trägårdh, 1910	Barczyk and Madej 2014
<i>Zercon fageticola</i> Halaskova, 1969	Barczyk and Madej 2014
<i>Zercon peltatus</i> Koch, 1836	Barczyk and Madej 2014
<i>Zercon romagniolus</i> Sellnick, 1944	Barczyk and Madej 2014
<i>Zercon storkani</i> Halaskova, 1969	Barczyk and Madej 2014
<i>Zercon triangularis</i> Koch, 1836	Barczyk and Madej 2014
<i>Zercon vacuus</i> Koch, 1839	Barczyk and Madej 2014
<i>Zerconopsis remiger</i> (Kramer, 1876)	Barczyk and Madej 2014
<i>Zetorchestes micronychus micronychus</i> (Berlese, 1883)	Maślak and Barczyk 2011
Araneae:	
<i>Agroeca brunnea</i> (Blackwall, 1833)	Sanocka-Wołoszynowa 1981
<i>Agyneta rurestris</i> (C. L. Koch, 1836)	Sanocka-Wołoszyn 1963
[= <i>Meioneta rurestris</i> C. L. Koch, 1836 †]	
<i>Agyneta subtilis</i> (Pickard-Cambridge, 1863)	Sanocka-Wołoszynowa 1981
<i>Amaurobius fenestralis</i> (Ström, 1768)	Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981
<i>Anguliphantes angulipalpis</i> (Westring, 1851) [= <i>Lepthyphantes angulipalpis</i> †]	Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981
<i>Anguliphantes monticola</i> (Kulczynski, 1882) [= <i>Lepthyphantes monticola</i> †]	Czajka and Hajduk 1978, Ochman 2004, Rozwałka et al. 2010, Sanocka-Wołoszynowa 1981, Skalski 1973a, Skalski 1981, Skalski 1994-1995, Skalski 1994a
<i>Anyphaena accentuata</i> (Walckenaer, 1802)	Sanocka-Wołoszyn 1963
<i>Araneus diadematus</i> Clerck, 1757	Ochman 2004, Sanocka-Wołoszyn 1963, Skalski 1973a
<i>Bathypantes nigrinus</i> (Westring, 1851)	Sanocka-Wołoszynowa 1981
<i>Callobius claustrarius</i> (Hahn, 1833) [= <i>Amaurobius claustrarium</i> Hahn †]	Sanocka-Wołoszyn 1963

Species	References
<i>Centromerita bicolor</i> (Blackwall, 1833)	Sanocka-Wołoszynowa 1981
<i>Centromerus cavernarum</i> (Koch, 1872) [= <i>Centromerus jacksoni</i> Denis, 1952 = <i>Taranucnus cavernarum</i> Simon, 1884‡]	Ochman 2004, Pax 1937, Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981, Skalski 1981
<i>Centromerus sellarius</i> (Simon, 1884)	Sanocka-Wołoszynowa 1981
<i>Centromerus sylvaticus</i> (Blackwall, 1841)	Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981, Wołoszyn and Wójcik 1964,
<i>Cicurina cicur</i> (Fabricius, 1793) [= <i>Cicurina cicurea</i> ‡]	Arndt 1921, Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981
<i>Clubiona brevipes</i> Blackwall, 1841 [= <i>Microclubiona brevipes</i> ‡]	Sanocka-Wołoszynowa 1981
<i>Clubiona frutetorum</i> L. Koch, 1867	Pax and Maschke 1935
<i>Coelotes terrestris</i> (Wider, 1834)	Ochman 2004, Skalski 1973a
<i>Cryphoea silvicola</i> (Koch, 1834)	Sanocka-Wołoszynowa 1981, Sobiepanek 1985
<i>Cybaeus angustiarum</i> Koch, 1868	Pax and Maschke 1935, Sobiepanek 1985
<i>Dicymbium nigrum brevisetosum</i> (Blackwall, 1834)	Sanocka-Wołoszynowa 1981
<i>Diplocephalus cristatus</i> (Blackwall, 1833)	Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981
<i>Diplocephalus picinus</i> (Blackwall, 1841)	Sanocka-Wołoszynowa 1981
<i>Diplostyla concolor</i> (Wider, 1834)	Sanocka-Wołoszynowa 1981
<i>Drassodes lapidosus</i> (Walcknaer, 1802)	Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981
<i>Drassodes pubescens</i> (Thorell, 1856)	Sanocka-Wołoszynowa 1981
<i>Eratigena atrica</i> (Koch, 1843) [= <i>Tegenaria atrica</i> ‡]	Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981
<i>Erigone atra</i> Blackwall, 1833	Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981
<i>Erigone dentipalpis</i> (Wider, 1834)	Sanocka-Wołoszynowa 1981
<i>Formiphantes lephthyphantiformis</i> (Strand, 1907) [= <i>Lepthyphantes pisai</i> ‡]	Błoszyk and Rozwałka 2008, Sanocka-Wołoszynowa 1981
<i>Gnaphosa bicolor</i> (Hahn, 1833)	Sanocka-Wołoszynowa 1981
<i>Goniatium rubens</i> (Blackwall, 1833)	Sanocka-Wołoszynowa 1981
<i>Gongylidium rufipes</i> (Linnaeus, 1758)	Sanocka-Wołoszynowa 1981
<i>Hahnia pusilla</i> Koch, 1841	Ochman 2004, Sanocka-Wołoszynowa 1981
<i>Harpactea hombergi</i> (Scopoli, 1763)	Błoszyk and Rozwałka 2008, Sanocka-Wołoszynowa 1981
<i>Harpactea rubicunda</i> (Koch, 1838)	Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981
<i>Helophora insignis</i> (Blackwall, 1841)	Sanocka-Wołoszynowa 1981
<i>Histopona torpida</i> (Koch, 1837)	Sanocka-Wołoszynowa 1981
<i>Hypomma bituberculatum</i> (Wider, 1834)	Sanocka-Wołoszynowa 1981
<i>Inermocoelotes inermis</i> (Koch, 1855)	Sanocka-Wołoszynowa 1981
<i>Kaestneria dorsalis</i> (Wider, 1834)	Sanocka-Wołoszynowa 1981
<i>Labulla thoracica</i> (Wider, 1834)	Czajka and Hajduk 1978, Hajduk and Ogorzałek 1970, Sanocka-Wołoszynowa 1981
<i>Lepthyphantes leprosus</i> (Ohlert, 1865)	Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981, Skalski 1973a, Wołoszyn and Wójcik 1964,
<i>Lepthyphantes minutus</i> (Blackwall, 1833)	Sanocka-Wołoszynowa 1981
<i>Lepthyphantes nodifer</i> Simon, 1884	Ochman 2004, Sanocka-Wołoszynowa 1981
<i>Leptorhoptrum robustum</i> (Westring, 1851)	Sanocka-Wołoszynowa 1981
<i>Liocranum rupicola</i> (Walckenaer, 1830)	Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981
<i>Linyphia triangularis</i> (Clerck, 1757)	Sanocka-Wołoszyn 1963
<i>Macrargus rufus</i> (Wider, 1834)	Sanocka-Wołoszynowa 1981
<i>Mansuphantes arciger</i> (Kulczyński, 1882) [= <i>Lepthyphantes arciger</i> ‡]	Błoszyk and Rozwałka 2008, Sanocka-Wołoszynowa 1981
<i>Mansuphantes mansuetus</i> (Thorell, 1875) [= <i>Lepthyphantes mansuetus</i> ‡]	Błoszyk and Rozwałka 2008, Sanocka-Wołoszynowa 1981
<i>Maso sundevalli</i> (Westring, 1851)	Czajka and Hajduk 1978, Sanocka-Wołoszynowa 1981

Species	References
<i>Megalepthyphantes nebulosus</i> (Sundevall, 1830) [= <i>Lepthyphantes nebulosus</i> ‡]	Arndt 1921, Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981, Skalski 1973a, Wołoszyn and Wójcik 1964
<i>Meta menardi</i> (Latreille, 1804)	Błoszyk and Rozwalka 2008, Czajka and Hajduk 1978, Demel 1918, Dumnicka and Płotek 2013, Gubała 1996, Hajduk and Ogorzałek 1970, Kowalski 1955, Kur et al. 2016, Ochman 2004, Pax and Maschke 1935, Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981, Skalski 1967, Skalski 1973a, Skalski 1981, Skalski 1994-1995, Sobiepanek 1985, Wołoszyn and Wójcik 1964
<i>Metellina merianae</i> (Scopoli, 1763)	Arndt 1921, Czajka and Hajduk 1978, Demel 1918, Sanocka-Wołoszyn 1963, Skalski 1981, Wołoszyn and Wójcik 1964
<i>Metellina segmentata</i> (Clerck, 1757) [= <i>Meta reticulata</i> ‡]	Arndt 1921
<i>Micrargus herbigradus</i> (Blackwall, 1854)	Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981,
<i>Microneta viaria</i> (Blackwall, 1841)	Sanocka-Wołoszynowa 1981
<i>Nesticus cellulanus</i> (Clerck, 1757)	Błoszyk and Rozwalka 2008, Dumnicka and Płotek 2013, Ochman 2004, Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981, Skalski 1973a, Wołoszyn and Wójcik 1964
<i>Obscuriphantes obscurus</i> (Blackwall, 1841) [= <i>Lepthyphantes obscurus</i> ‡]	Sobiepanek 1985
<i>Oedothorax agrestis</i> (Blackwall, 1853)	Sanocka-Wołoszynowa 1981
<i>Oedothorax apicatus</i> (Blackwall, 1850)	Sanocka-Wołoszynowa 1981
<i>Oedothorax gibbosus</i> (Blackwall, 1841)	Sanocka-Wołoszynowa 1981
<i>Palliduphantes pallidus</i> (O. Pickard-Cambridge, 1871) [= <i>Lepthyphantes pallidus</i> ‡]	Ochman 2004, Sanocka-Wołoszynowa 1981
<i>Pardosa agrestis</i> (Westring, 1861)	Sanocka-Wołoszyn 1963
<i>Pardosa monticola</i> (Clerck, 1757)	Sanocka-Wołoszynowa 1981
<i>Pardosa nigra</i> (Koch, 1834)	Sobiepanek 1985
<i>Pelecopsis parallela</i> (Wider, 1834)	Sanocka-Wołoszynowa 1981
<i>Pholcus opilionoides</i> (Schrank, 1781)	Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981, Wołoszyn and Wójcik 1964
<i>Phrurolithus festivus</i> (Koch, 1835)	Sanocka-Wołoszynowa 1981
<i>Pocadicnemis pumila</i> (Blackwall, 1841)	Sanocka-Wołoszynowa 1981
<i>Pociloneta variegata</i> (Blackwall, 1841) [= <i>Pociloneta globose</i> ‡]	Sanocka-Wołoszynowa 1981
<i>Porrhomma convexum</i> (Westring, 1851)	Błoszyk and Rozwalka 2008, Czajka and Hajduk 1978, Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981, Wołoszyn and Wójcik 1964
<i>Porrhomma egeria</i> Simon, 1884 [= <i>Porrhomma moravicum</i> Miller & Kratochvíl, 1940 ‡]	Błoszyk and Rozwalka 2008, Demel 1918, Ochman 2004, Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981, Skalski 1971, Skalski 1973a, Skalski 1981, Skalski 1994-1995, Skalski and Wójcik 1968, Wołoszyn and Wójcik 1964
<i>Porrhomma pallidum</i> Jackson, 1913	Ochman 2004, Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981, Skalski 1973a, Wołoszyn and Wójcik 1964
<i>Porrhomma pygmaeum</i> (Blackwall, 1834)	Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981
<i>Porrhomma rosenhaueri</i> (L. Koch, 1872)	Pax and Maschke 1935, Pax 1937
<i>Robertus lividus</i> (Blackwall, 1836)	Sanocka-Wołoszynowa 1981
<i>Scotargus pilosus</i> Simon, 1913 [= <i>Macrargus strandi</i> (Schenkel, 1934) ‡]	Sanocka-Wołoszynowa 1981
<i>Scotophaeus blackwalli</i> (Thorell, 1856)	Sanocka-Wołoszynowa 1981

Species	References
<i>Segestria senoculata</i> (Linnaeus, 1758)	Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981
<i>Sosticus loricatus</i> (L. Koch, 1866) [= <i>Scotophaeus loricatus</i> L. Koch, 1866 ‡]	Sanocka-Wołoszyn 1963
<i>Steatoda bipunctata</i> (Linnaeus, 1758)	Sanocka-Wołoszynowa 1981
<i>Stemonyphantes lineatus</i> (Linnaeus, 1758)	Sanocka-Wołoszyn 1963
<i>Syedra gracilis</i> (Menge, 1869)	Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981
<i>Tegenaria domestica</i> (Clarck, 1757)	Arndt 1921, Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981, Skalski 1973a,
<i>Tegenaria ferruginea</i> (Panzer, 1804)	Ochman 2004, Pax and Maschke 1935, Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981, Skalski 1973a
<i>Tegenaria silvestris</i> Koch 1872	Błoszyk and Rozwałka 2008, Sanocka-Wołoszynowa 1981
<i>Tenuiphantes alacris</i> (Blackwall, 1853) [= <i>Lepthyphantes alacris</i> ‡]	Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981
<i>Tenuiphantes cristatus</i> (Menge, 1866) [= <i>Lepthyphantes cristatus</i> ‡]	Sanocka-Wołoszynowa 1981
<i>Tenuiphantes flavipes</i> (Blackwall, 1854) [= <i>Lepthyphantes flavipes</i> ‡]	Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981, Wołoszyn and Wójcik 1964
<i>Tenuiphantes mendei</i> (Kulczyński, 1887) [= <i>Lepthyphantes mendei</i> ‡]	Sanocka-Wołoszynowa 1981, Sobiepanek 1985
<i>Tenuiphantes tenebricola</i> (Wider, 1834) [= <i>Lepthyphantes tenebricola</i> ‡]	Sanocka-Wołoszyn 1963, Sanocka-Wołoszynowa 1981
<i>Tenuiphantes tenuis</i> (Blackwall, 1852) [= <i>Lepthyphantes tenuis</i> ‡]	Sanocka-Wołoszynowa 1981
<i>Teranucnus cavernarum</i> L. Koch †	Pax and Maschke 1935
<i>Tetragnatha pinicola</i> L. Koch, 1870	Sanocka-Wołoszyn 1963
<i>Textrix denticulata</i> (Olivier, 1789)	Sanocka-Wołoszynowa 1981
<i>Theridion impressum</i> Koch, 1881	Skalski 1973a
<i>Thyreostbenius parasiticus</i> (Westring, 1851)	Sanocka-Wołoszynowa 1981
<i>Trochosa ruricola</i> (De Geer, 1778)	Sanocka-Wołoszynowa 1981
<i>Zodarion germanicum</i> (C. L. Koch, 1837)	Sanocka-Wołoszyn 1963
<i>Zora spinimana</i> (Sundevall, 1833)	Sanocka-Wołoszynowa 1981
<i>Zygiella montana</i> (Koch, 1834)	Sobiepanek 1985
Opiliones:	
<i>Gyas annulatus</i> (Olivier, 1791)	Sobiepanek 1985
<i>Ischyropsalis hellwigi</i> (Panzer, 1794)	Błoszyk and Rozwałka 2008, Kocot-Zalewska and Rozwałka 2018, Sanocka-Wołoszynowa 1981
<i>Ischyropsalis manicata</i> Koch, 1869 [= <i>Ischyropsalis milleri</i> = <i>Ischyropsalis dacica</i> Roewer, 1916‡]	Kowalski 1955, Skalski 1967, Sobiepanek 1985
<i>Lacinius ephippiatus</i> (Koch, 1835)	Sanocka-Wołoszynowa 1981
<i>Leiobunum blackwalli</i> Meade, 1861	Arndt 1921, Sanocka-Wołoszynowa 1981
<i>Leiobunum rotundum</i> (Latreille, 1798)	Błoszyk and Rozwałka 2008, Sanocka-Wołoszynowa 1981
<i>Leiobunum rupestre</i> (Herbst, 1799)	Arndt 1921, Kowalski 1955, Ochman 2004, Sanocka-Wołoszynowa 1981, Skalski 1967, Skalski 1973a, Sobiepanek 1985
<i>Mitopus morio</i> (Fabricius, 1779)	Błoszyk and Rozwałka 2008, Sanocka-Wołoszynowa 1981, Sobiepanek 1985
<i>Mitostoma chrysomelas</i> (Hermann, 1804)	Błoszyk and Rozwałka 2008, Sanocka-Wołoszynowa 1981
<i>Nemastoma lugubre</i> (Müller, 1776)	Sanocka-Wołoszynowa 1981
<i>Oligolophus tridens</i> (Koch, 1836)	Błoszyk and Rozwałka 2008, Sanocka-Wołoszynowa 1981
<i>Opilio parietinus</i> (De Geer, 1778)	Błoszyk and Rozwałka 2008, Pax and Maschke 1935, Sanocka-Wołoszynowa 1981
<i>Opilio saxatilis</i> Koch, 1839	Sanocka-Wołoszynowa 1981
<i>Paranemastoma kochi</i> (Nowicki, 1870)	Sobiepanek 1985

Species	References
<i>Paranemastoma quadripunctatum</i> (Perty, 1833)	Ochman 2004, Sanocka-Wołoszynowa 1981, Skalski 1994-1995, Skalski 1994a,
<i>Phalangium opilio</i> Linnaeus, 1758	Błoszyk and Rozwałka 2008, Pax and Maschke 1935, Sanocka-Wołoszynowa 1981
<i>Platybunus bucephalus</i> (Koch, 1835)	Sobiepanek 1985
<i>Rilaena triangularis</i> (Herbst, 1799)	Sanocka-Wołoszynowa 1981
<i>Siro carpaticus</i> Rafalski, 1956	Skalski 1971
<i>Trogulus tricarinatus</i> (Linnaeus, 1758)	Sanocka-Wołoszynowa 1981
Pseudoscorpionida:	
<i>Chelifer cancroides</i> (Linnaeus, 1758)	Błoszyk and Rozwałka 2008, Sanocka-Wołoszynowa 1981
<i>Chthonius (Ephippiochthonius) tetrachelatus</i> (Preyssler, 1790)	Błoszyk and Rozwałka 2008, Sanocka-Wołoszynowa 1981
<i>Neobisium (Neobisium) carcinoides</i> (Hermann, 1804) [= <i>Neobisium muscorum</i> ‡]	Błoszyk and Rozwałka 2008, Kowalski 1955, Sanocka-Wołoszynowa 1981
<i>Neobisium (Neobisium) erythrodactylum</i> (Koch, 1873)	Błoszyk and Rozwałka 2008, Sanocka-Wołoszynowa 1981
<i>Neobisium (Neobisium) sylvaticum</i> (Koch, 1835)	Kowalski 1955, Sanocka-Wołoszynowa 1981

† – status of the species name is unclear, ‡ – name published in original paper

The remaining Arthropoda groups are represented as follows: Collembola 79 species, Diplura 1 species, Chilopoda 1 species, Diplopoda 8 species, Symphyla 1 species, Isopoda 9 species. Among other invertebrates recorded in Polish caves 11 species are represented by Oligochaeta and 20 species by Mollusca. Detailed list of species is presented in Table 3.

Table 3. The checklist of other Arthropoda and invertebrates recorded in Polish caves.

Species	References
Collembola	
<i>Anurida granaria</i> (Nicolet, 1847)	Pomorski 1992
<i>Archaphorura serratotuberculata</i> (Stach, 1933) [= <i>Onychiurus serratotuberculata</i> Stach, 1933 ‡]	Stach 1954
<i>Arrhopalites bifidus</i> Stach, 1945	Pomorski 1992, Skalski 1971
<i>Arrhopalites principalis</i> Stach, 1945	Pomorski 1992, Stach 1956
<i>Arrhopalites pygmaeus</i> (Wankel, 1860)	Demel 1918, Kowalski 1955, Pax and Maschke 1935, Ochman 2004, Skalski 1971, Skalski 1973a, Skalski 1973b, Skalski 1981, Skalski and Wójcik 1968, Stach 1919, Stach 1939, Wołoszyn and Wójcik 1964 Stach 1956
<i>Arrhopalites sericus</i> Gisin, 1947	Ochman 2004, Pomorski 1996
<i>Bonetogastrura cavicola</i> (Börner, 1901) [= <i>Hypogastrura cavicola</i> ‡]	Ochman 2004, Pomorski 1992, Skalski 1971, Skalski 1973a, Skalski 1973b, Skalski 1981
<i>Ceratophysella armata</i> (Nicolet, 1841)	Kowalski 1955, Pomorski 1996, Skalski 1967
<i>Ceratophysella bengissoni</i> (Agren, 1904)	Stach 1949
<i>Ceratophysella granulata</i> Stach, 1949	Kowalski 1955, Stach 1949
<i>Ceratophysella michalinae</i> Skarżyński, 2005	Skarżyński 2005
<i>Cryptopygus bipunctatus</i> (Axelson, 1903)	Pomorski 1992
<i>Desoria fennica</i> (Reuter, 1895) [= <i>Isotoma fennica</i> ‡]	Kowalski 1955, Stach 1947
<i>Desoria hiemalis</i> (Schoett, 1893) [= <i>Isotoma hiemalis</i> Schött, 1893 ‡]	Kowalski 1955

Species	References
<i>Desoria olivacea</i> (Tullberg, 1871) [= <i>Isotoma olivacea</i> ‡]	Kowalski 1955, Stach 1939, Stach 1947
<i>Desoria violacea</i> (Tullberg, 1876) [= <i>Isotoma violacea</i> ‡]	Kowalski 1955, Stach 1947
<i>Deuterophorura cebennaria</i> (Gisin, 1956)	Kur et al. 2016
<i>Dicyrtomina ornata</i> (Nicolet, 1842)	Ochman 2004
<i>Entomobrya multifasciata</i> (Tullberg, 1871)	Stach 1963
<i>Folsomia fimetaria</i> (Linnaeus, 1758)	Pomorski 1992, Stach 1939
<i>Folsomia lawrencei</i> Rusek, 1984	Ochman 2004, Pomorski 1992, Pomorski 1996
<i>Folsomia candida</i> Willem, 1902 [= <i>Folsomia listeri</i> Bagnall, 1939 ‡]	Kur et al. 2016, Ochman 2004, Pomorski 1992, Skalski 1973a, Skalski 1981, Skalski and Wójcik 1968, Stach 1947
<i>Folsomia quadrioculata</i> (Tullberg, 1871)	Kowalski 1955, Ochman 2004, Pomorski 1992, Pomorski 1996, Skalski 1973a, Skalski and Wójcik 1968, Stach 1939, Stach 1947
<i>Heteromurus nitidus</i> (Templeton, 1835)	Arndt 1921, Demel 1918, Kur et al. 2016, Pax and Maschke 1935, Pomorski 1992, Skalski 1981, Stach 1919, Wołoszyn and Wójcik 1964
<i>Hymenaphorura creatricis</i> Pomorski, 1990	Pomorski 1992
<i>Hymenaphorura nova</i> Pomorski, 1990	Pomorski 1992
<i>Hymenaphorura polonica</i> Pomorski, 1990	Pomorski 1992
<i>Hypogastrura armata</i> (Nicolet, 1842)	Arndt 1921, Ochman 2004
<i>Hypogastrura purpurascens</i> (Lubbock, 1868)	Ochman 2004, Pax and Maschke 1935, Pomorski 1992, Pomorski 1996
<i>Isotoma notabilis</i> Schäffer, 1896	Kowalski 1955, Pomorski 1992, Pomorski 1996, Skalski 1973a, Skalski 1981, Ochman 2004, Stach 1939, Stach 1947
<i>Isotoma propinqua</i> Axelson, 1902	Skalski 1973a, Ochman 2004
<i>Isotomiella minor</i> (Schaffer, 1896)	Ochman 2004, Pomorski 1992, Skalski 1973a, Stach 1939, Stach 1947
<i>Lepidocyrtus albus</i> Packard, 1873	Demel 1918, Stach 1919,
<i>Lepidocyrtus cyaneus</i> Tullberg, 1871	Pax and Maschke 1935
<i>Lepidocyrtus lanuginosus</i> (Gmelin, 1788)	Pax and Maschke 1935
<i>Megalothorax incertus</i> Börner, 1903	Ochman 2004, Pomorski 1992, Skalski 1973a
<i>Megalothorax minimus</i> Willem, 1900	Ochman 2004, Pomorski 1992, Pomorski 1996, Skalski 1973a, Stach 1939, Stach 1957
<i>Mesachorutes ojcowiensis</i> Stach, 1918	Skalski 1969, Skalski 1971, Skalski 1973a, Skalski 1981, Skalski 1994a, Skalski and Wójcik 1968, Stach 1919
<i>Mesaphorura hylophila</i> Rusek, 1982	Ochman 2004, Pomorski 1996
<i>Mesaphorura krausbaueri</i> Börner, 1901	Pomorski 1992, Skalski 1973a
<i>Mesogastrura ojcowiensis</i> Stach, 1919 [= <i>Troglogastrura ojcowiensis</i> ‡]	Demel 1918, Stach 1919, Stach 1949
<i>Metaphorura affinis</i> (Börner, 1903)	Pomorski 1996, Ochman 2004
<i>Micranurida pygmaea</i> Börner, 1901	Pomorski 1992, Pomorski 1996, Ochman 2004, Stach 1949
<i>Neanura muscorum</i> (Templeton 1835)	Pomorski 1992
<i>Neeelus murinus</i> Folsom, 1896	Stach 1957
<i>Oligaphorura schoetti</i> (Lie-Pettersen, 1896)	Pomorski 1992, Skalski 1971
<i>Oncopodura reyersdorfensis</i> (Stach, 1936)	Pomorski 1992, Skalski 1971
<i>Onychiurus ambulans</i> (Linnaeus, 1758)	Skalski 1971
<i>Onychiurus cebennarius</i> Gisin, 1956	Pomorski 1992
<i>Onychiurus denisi</i> Stach, 1934	Kowalski 1955, Pomorski 1992, Stach 1939, Stach 1954

Species	References
<i>Onychiurus fimetarius</i> (Linnaeus, 1767)	Arndt 1921, Kowalski 1955, Pax and Maschke 1935, Skalski 1967, Stach 1954
<i>Onychiurus granulatus</i> Stach, 1930	Kowalski 1955, Ochman 2004, Pomorski 1992, Skalski 1973a, Skalski 1981, Skalski and Wójcik 1968, Stach 1939, Stach 1954
<i>Onychiurus rectospinatus</i> Stach, 1922	Stach 1954
<i>Onychiurus sibiricus</i> Tullberg, 1876 †	Kowalski 1955, Stach 1954
<i>Onychiurus scotarius</i> Gisin, 1954	Pomorski 1992
<i>Onychiurus tuberculatus</i> Moniez, 1891	Stach 1954
<i>Pogononathellus flavescens</i> (Tullberg, 1871)	Ochman 2004, Pomorski 1992
<i>Pogonognathellus longicornis</i> (Muller, 1776) [= <i>Tomocerus longicornis</i> Lubbock ‡]	Pax and Maschke 1935
<i>Protaphoruna alborufescens</i> (Vogler, 1895) [= <i>Onychiurus alborufescens</i> ‡]	Skalski 1971, Skalski 1973a, Skalski 1973b, Skalski 1981, Skalski and Wójcik 1968
<i>Proptaphoruna armata</i> (Tullberg, 1869) [= <i>Onychiurus armatus</i> ‡]	Arndt 1921, Demel 1918, Gubała 1996, Kowalski 1955, Ochman 2004, Pax and Maschke 1935, Pomorski 1992, Pomorski 1996, Skalski 1967, Skalski 1969, Skalski 1973a, Skalski 1981, Skalski and Wójcik 1968, Sobiepanek 1985, Stach 1919, Stach 1939, Stach 1954, Wołoszyn and Wójcik 1964
<i>Protaphoruna austriaca</i> (Butschek, 1948)	Pomorski 1992
<i>Protaphoruna campata</i> (Gisin, 1952)	Pomorski 1992
<i>Protaphoruna janosik</i> Weiner, 1977	Weiner 1990
<i>Pseudosinella alba</i> (Packard, 1873)	Skalski 1973a
<i>Pseudosinella pettersemi</i> Börner, 1901	Pomorski 1992
<i>Pseudisotoma monochaeta</i> (Kos, 1942)	Stach 1947
<i>Pseudisotoma sensibilis</i> (Tullberg, 1876)	Ochman 2004, Pomorski 1996
<i>Schaefferia emucronata</i> Absolon, 1900	Arndt 1921, Pomorski 1992, Skalski 1971, Stach 1939, Stach 1949
<i>Sinella hoefli</i> Schaeffer, 1896 †	Arndt 1921
<i>Smintburinus elegans</i> (Fitch, 1863)	Ochman 2004, Skalski 1973a
<i>Tetrodontophora bielensis</i> (Waga, 1842)	Arndt 1921, Kowalski 1955, Pax and Maschke 1935, Pomorski 1992
<i>Tomocerus flavescens</i> Tullberg, 1871	Kowalski 1955, Pax and Maschke 1935, Skalski 1973a, Skalski and Wójcik 1968, Stach 1939
<i>Tomocerus minor</i> (Lubbock, 1862)	Pomorski 1992
<i>Tomocerus vulgaris</i> (Tullberg, 1871)	Arndt 1921, Kur et al. 2016
<i>Willemia intermedia</i> Mills, 1934	Pomorski 1992
<i>Willowsia buskii</i> (Lubbock, 1870)	Ochman 2004, Pomorski 1996
<i>Willowsia nigromaculata</i> (Lubbock, 1873)	Ochman 2004, Pomorski 1996
<i>Xenylla boermeri</i> Axelson, 1905	Ochman 2004, Skalski 1973a
<i>Xenylla brevicauda</i> Tullberg, 1869	Stach 1949
Diplura:	
<i>Campodea staphylinus</i> Westwood, 1842	Arndt 1921
Chilopoda:	
<i>Lithobius mutabilis</i> Koch, 1862	Kowalski 1955
<i>Blaniulus guttulatus</i> (Fabricius, 1798)	Kur et al. 2016
<i>Glomeris hexasticha</i> Brandt, 1833	Kowalski 1955
<i>Julus luscus</i> Meinert, 1868	Arndt 1921
<i>Leptoilulus trilobatus</i> (Verhoeff, 1894)	Pax and Maschke 1935, Pax 1937
<i>Mastigophorophyllon saxonicum</i> Verhoeff, 1916	Pax and Maschke 1935
<i>Mycogona germanica</i> (Verhoeff, 1892) [= <i>Orthochordeuma germanica</i> ‡]	Pax and Maschke 1935, Pax 1937

Species	References
<i>Ochogona caroli</i> (Rothenbuhler, 1900) [= <i>Ceratosoma caroli</i> ‡]	Pax and Maschke 1935, Pax 1937
<i>Strongylosoma stigmatosum stigmatosum</i> (Eichwald, 1830)	Sobiepanek 1985
Symphyla:	
<i>Scutigereilla immaculata</i> (Newport, 1845)	Kowalski 1955
Isopoda:	
<i>Cylisticus convexus</i> (De Geer, 1778)	Kur et al. 2016, Pax and Maschke 1935, Pax 1937, Skalski 1973a
<i>Hyloniscus vividus</i> (Koch, 1841)	Pax and Maschke 1935, Pax 1937
<i>Mesoniscus graniger</i> (Frivaldszky, 1865)	Piksa and Farkas 2007
<i>Porcellio spinicornis</i> Say, 1818	Skalski 1973a
<i>Porcellio scaber</i> Latreille, 1804	Woloszyn and Wójcik 1964
<i>Porcellium conspersum</i> (Koch, 1841)	Pax and Maschke 1935
<i>Protracheoniscus politus</i> (Koch, 1841) [= <i>Porcellio politus</i> Koch, 1841 ‡]	Pax and Maschke 1935
<i>Trachoniscus wächleri</i>	Kowalski 1955
<i>Trachelipus affinis</i> (Koch, 1841) [= <i>Trachelipus wächleri</i> (Strouhal, 1951) ‡]	Skalski 1967
Oligochaeta:	
<i>Achaeta eiseni</i> (Vejdovsky, 1878)	Dumnicka 1977
<i>Buchholzia appendiculata</i> (Buchholz, 1862)	Dumnicka 1977, Skalski 1981
<i>Dendrobaena alpina</i> (Rosa, 1884)	Sobiepanek 1985
<i>Dendrodrilus rubidus</i> (Savigny, 1826) [= <i>Dendrobaena rubida</i> (Savigny, 1826) ‡]	Dumnicka 1977
<i>Enchytraeus polonicus</i> Dumnicka, 1977	Dumnicka 1977, Skalski 1981, Skalski 1994a
<i>Enchytraeus buchholzi</i> Vejdovsky, 1878	Dumnicka 1977, Skalski 1981
<i>Enchytraeus dominicae</i> Dumnicka, 1976	Dumnicka 1977, Skalski 1981, Skalski 1994a
<i>Fridericia bulbosa</i> (Rosa, 1887)	Dumnicka 1977, Skalski 1981
<i>Fridericia ratzeli</i> (Eisen, 1872)	Kasprzak 1973
<i>Henlea ventriculosa</i> (d'Udekem, 1854)	Dumnicka 1977
<i>Marionina argentea</i> (Michaelsen, 1889)	Dumnicka 1977
Mollusca:	
<i>Aegopinella nitens</i> (Michaud, 1831)	Sobiepanek 1985
<i>Arion subfuscus</i> (Draparnaud, 1805)	Ochman 2004, Skalski 1973a, Sobiepanek 1985
<i>Arianta arbustorum</i> (Linnaeus, 1758)	Kowalski 1955
<i>Armiger crista</i> (Linnaeus, 1758) [= <i>Gyraulus crista</i> ‡]	Woloszyn and Wójcik 1964
<i>Cepaea hortensis</i> (Müller, 1774)	Skalski 1973a
<i>Chilostoma cingulella</i> Rossmässler, 1837 [= <i>Helicigona cingulella</i> ‡]	Kowalski 1955, Sobiepanek 1985
<i>Chondrina clienta</i> (Westerlund, 1883)	Kowalski 1955, Sobiepanek 1985
<i>Clausilia dubia</i> Draparnaud, 1805	Sobiepanek 1985
<i>Cochlodina laminata</i> (Montagu, 1803)	Skalski 1973a
<i>Discus (Gonyodiscus) rotundatus</i> (Müller, 1774)	Skalski 1973a
<i>Helicella obvia</i> (Menke, 1828)	Skalski 1973a
<i>Helicigona faustina</i> (Rossmässler, 1835)	Skalski 1973a
<i>Helix pomatia</i> Linnaeus, 1758	Pax and Maschke 1935
<i>Isognomostoma isognomostomos</i> (Schroter, 1784)	Skalski 1973a
<i>Laciniaria plicata</i> (Draparnaud, 1801)	Skalski 1973a
<i>Lehmania marginata</i> (Müller, 1774)	Sobiepanek 1985
<i>Limax cinereoniger</i> Wolf, 1803	Ochman 2004, Skalski 1973a
<i>Monachoides incarnatus</i> (Müller, 1774) [= <i>Perforatella incernata</i> ‡]	Skalski 1973a
<i>Oxychilus glaber</i> (Rossmässler, 1835)	Dumnicka and Płotek 2013, Ochman 2004, Skalski 1973a, Skalski 1981
<i>Oxychilus depressus</i> (Sterki, 1880)	Ochman 2004, Skalski 1973a, Skalski and Wójcik 1968

† – status of the species name is unclear, ‡ – name published in original paper

Summary

Based on presented data, it can be noticed that researchers' interest in terrestrial invertebrate fauna, both historically and contemporarily, is relatively low in Poland. In the span of 100 years, just over 50 published papers have been focused on the terrestrial invertebrate fauna of Polish caves although the presence of 593 species has been reported. Such a low interest could be caused by the conviction that it is not worth involving in the research of fauna that lacks spectacular troglobionts. Once the fauna of Polish caves gains greater interest of academics from various groups, we will learn more about this most mysterious world.

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Updates to the sporadic knowledge on microsporidian infections in groundwater amphipods (Crustacea, Amphipoda, Niphargidae)

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Abstract

A set of 69 specimens from 19 groundwater species of the genera *Niphargus*, *Niphargellus*, *Microniphargus* and *Crangonyx* was genetically screened for microsporidian infections. Samples mostly originated from groundwater-dependent spring environments (71%), natural caves (9%) and artificial caverns/tunnels (13%). Amphipod hosts were identified by morphology and/or molecular data, whereas microsporidian parasites were characterised by a genetic screening assay targeting a section of the small subunit rRNA gene.

Five microsporidian species (*Dictyocoela duebenum*; *Nosema* sp.; *Hyperspora aquatica* and two undescribed *Microsporidium* spp.) were revealed from 13 host specimens (*Niphargus schellenbergi*; *N. aquilex* lineages B, F and G; *Niphargellus arndti*). In particular *N. schellenbergi* was frequently infected with *D. duebenum* as well as a new and potentially niphargid-specific *Nosema* sp. identified in *Niphargellus arndti*.

Our results shed further light on the still largely unknown diversity and specificity of microsporidian parasites in groundwater amphipods and subterranean animals in general.

Keywords

parasites, stygobionts, ecological network, transmission pathways, SSU rDNA, COI, 28S

Introduction

Microsporidians are microparasites that belong to the taxon Opisthosporidia, a sister group of the Fungi (Karpov et al. 2014). Depending on the microsporidian species, they can develop in various host tissues where they form spores that are infective for the next host (horizontal transmission). Some microsporidians are transmitted vertically from the mother to the offspring (Dunn and Smith 2001, Smith 2009). Microsporidians can influence the host population by causing mortality of infected individuals, or by modulating the sex ratio towards a female-biased population in the case of vertical transmission (Dunn and Smith 2001).

Studies on microsporidian diversity in freshwater amphipods have a long history and are steadily increasing (see Bulnheim 1975 for review, Ironside et al. 2003, Haine et al. 2004, Terry et al. 2004, Krebs et al. 2010, Wilkinson et al. 2011, Bacela-Spychalska et al. 2012, 2018, Stentiford et al. 2013, Stentiford and Dunn 2014, Grabner et al. 2015, Madyarova et al. 2015, Weigand et al. 2016, Dimova et al. 2018, Quiles et al. 2019), but knowledge on microsporidians in groundwater amphipods is very scarce. Early last century, Poisson (1924) was the first reporting *Niphargus stygius* (today regarded as a species-group) to be infected with *Microsporidium vandeli* (originally referred to as *Mrazekia niphargi*, later *Bacillidium niphargi*) and *Microsporidium niphargi* (former *Thelohania vandeli*). Almost fifty years later, Bulnheim (1971) stated that *Pleistophora mülleri* (described as *Stempellia mülleri*) was detected in *Niphargus ilidzensis*. Since then, it has become more and more clear that the identification and delineation of microsporidian species as well as of groundwater amphipod hosts had been far from consistent. Again, almost 50 years after Bulnheim's publication, Weigand et al. (2016) were the first addressing microsporidian diversity in a *Niphargus* population by genetically analysing the parasites as well as the host species. The authors revealed *Nosema granulosis*, *Orthosomella* sp., *Microsporidium* sp. I and *Microsporidium* sp. BPAR3 as well as some unclassified infections for the target species *Niphargus schellenbergi*. Notably, all microsporidian infections were shared by a sympatrically occurring population of *Gammarus fossarum* lineage 13. This led to the assumption that groundwater amphipods could enable transmission of microsporidians between surface habitats that are only connected by groundwater (Weigand et al. 2016).

In the present study, we intended to take another step in improving our sporadic knowledge on microsporidian diversity in a variety of groundwater-dependent environments in Central Europe using different niphargids (genera *Niphargus*, *Niphargellus*, *Microniphargus*) as target hosts.

Material and methods

Sample material

In total, 58 *Niphargus* specimens, 9 *Niphargellus*, 1 *Microniphargus leruthi* and 1 *Cranogonyx* sp. have been analysed for microsporidian infections (Table 1; for further infor-

mation see Suppl. material 1). Specimens have been collected in the period between 2015–2018, representing the morphospecies *Niphargus aquilex*, *Niphargus glenniei*, *Niphargus irlandicus*, *Niphargus kochianus*, *Niphargus puteanus*, *Niphargus schellenbergi*, *Niphargellus nolli* and *Niphargellus arndti*, as well as some undetermined *Niphargus* sp. Most of the samples originate from Central Europe (here, Germany, Belgium, Luxembourg and the East of France), fewer from surrounding areas (Poland, Great Britain, Ireland, The Netherlands, Czech Republic and the rest of France). The most frequently sampled aquatic habitats are springs, followed by subterranean water bodies in natural caves and artificial caverns (Table 1).

Host barcoding and parasite detection

One to two molecular markers were investigated for molecular species identification of amphipods, thus to a) allow a genetic cross-validation of the often morphologically hard to identify niphargid specimens, b) identify also juvenile specimens and c) enable a more precise taxonomic identification in case of cryptic species complexes (e.g. for *Niphargus aquilex*) (Fišer et al. 2009). The mitochondrial cytochrome *c* oxidase subunit I (COI) marker and the nuclear 28S rDNA marker (28S) were targeted. DNA was extracted from whole specimens according to the DNeasy Blood & Tissue Kit (Qiagen) and the NucleoSpin Tissue Kit (Macherey-Nagel) manufacturers' protocols. The COI marker was amplified using the primer pair LCO1490-JJ (5'-CHA CWA AYC ATA AAG ATA TYG G-3') and HCO2198-JJ (5'-AWA CTT CVG GRT GVC CAA ARA ATC A-3') of Astin and Stüben (2008). The PCR mix contained 1 µL DNA extract of variable concentration, 0.8 µL of each primer (10 pmol/µL), 5 µL of DreamTaq DNA Polymerase Master Mix (Thermo Scientific) and 2.4 µL of ultrapure water. PCR cycling conditions were 3 min denaturation at 94°C, 36 cycles of 20 s denaturation at 94°C, 45 s annealing at 50°C, and 60 s extension at 65°C; final elongation of 2 min at 65°C. Bi-directional Sanger-sequencing was performed at Genoscreen (Lille, France) using the PCR primer pair. The 28S nuclear fragment was amplified using the primer pair Niph15 (5'-CAA GTA CCG TGA GGG AAA GTT-3') and Niph16 (5'-AGG GAA ACT TCG GAG GGA ACC-3') of Verovnik et al. (2005). The PCR mix contained 2 µL of DNA extract of variable concentration, 1 µL of each primer (10 pmol/µL), 0.2 µL of REDTaq Polymerase (Sigma-Aldrich), 5 µL REDTaq reaction buffer and 15.8 µL ultrapure water. PCR cycling conditions for 28S were an initial 3 min denaturation at 95°C, 56 cycles of 30 s denaturation at 94°C, 60 s annealing at 45°C, and 90 s extension at 72°C. Bi-directional Sanger-sequencing was performed using three primers: Niph 15, Niph 20 (5'-AAA CAC GGG CCA AGG AGT AT-3') and Niph 21 (5'-TAT ACT CCT TGG CCC GTG TT-3') (Flot et al. 2010). All PCR results were visualised on a 1.2% agarose gel prior to sequencing. COI-based molecular species identification was performed against the Barcode of Life Data System (BOLD, Ratnasingham and Hebert 2007) and the 28S marker compared to sequences stored in NCBI GenBank. Additional but so far unpublished COI and 28S sequences as part of an ongoing doctoral thesis were integrated for molecular species identification.

Table 1. Overview of host amphipod species and microsporidian infections. Further information can be retrieved from Supplementary material 1.

Taxonomy	Country	Locality	date	habitat	microsporidium infection
<i>Crangonyx</i> sp.	Germany	Bavaria, Mfömlingen, Interstitial Mümling	4/28/2017	interstitial	
<i>Microniphargus leruthi</i>	Ireland	County Clare, Ballyvaghan, Pollidubh Cave	10/21/2017	cave	
<i>Niphargus armati</i>	Czech Republic	Málkov, spring near Málkov	4/13/2018	spring	
	Germany	Bavaria, Münchberg, Formitzquelle	3/14/2018	spring	
	Germany	Bavaria, Hainstetten, Rotbühlquelle	4/15/2018	spring	<i>Hyperspora aquatica</i> (99.8% to KX364284)
	Germany	Bavaria, Hainstetten, Fensterbachquelle	4/15/2018	spring	
	Germany	Bavaria, Hainstetten, Boiwiequelle 1	4/15/2018	spring	
	Poland	Szczawno-Zdrój, Jaskinia Daisy (former Liebichauer Höhle)	7/7/2018	cave	<i>Nosema</i> sp. (97.2% to KM977840)
	Poland	Szczawno-Zdrój, Jaskinia Daisy (former Liebichauer Höhle)	7/7/2018	cave	<i>Microsporidium</i> sp. (97.5% similar to KX137915)
<i>Niphargellus nollii</i> <i>Niphargus aquilex</i> A	Germany	Saxony, Wüstenbrand, Obere Jungferquelle	5/28/2017	spring	
	Germany	Bavaria, Mfömlingen, Interstitial Mümling	4/28/2017	interstitial	
	Germany	Rhineland-Palatinate, Grünstadt, Queckbrunnen	1/10/2017	spring	
	Germany	Rhineland-Palatinate, Waldleiningen, Felsenbrunnen	12/14/2016	spring	
	Germany	Rhineland-Palatinate, Lambrecht, Bürgermeister-Hermann-Schneider-Brunnen	4/17/2016	spring	
<i>Niphargus aquilex</i> B	Germany	Hesse, Quelle Heidtränktal bei Mündung Schellbach	5/5/2018	spring	
	Germany	Hesse, Hanswagnersborn	5/6/2018	spring	
	Germany	Hesse, Quelle 12 im Krofdorfer Forst	5/7/2018	spring	<i>Dicystocoelea duebenum</i> (99.45% similar to MH753359)
<i>Niphargus aquilex</i> F	Germany	Rhineland-Palatinate, Höhn, Trinkwasserquelle Hilpischmühle	8/14/2018	spring	
	Germany	Saarland, Saarhölzbach, Schankbur	12/1/2016	spring	
	Belgium	Wallonia, Stablo, Interstitial l'Eau Rouge	10/20/2018	interstitial	<i>Dicystocoelea duebenum</i> (99.45% similar to MH753359)
	Germany	Saxony, Geising, Barbara-Stollen Geising	3/18/2018	artificial cavern	
Germany	Saxony, Geising, Barbara-Stollen Geising	3/18/2018	artificial cavern		
Germany	Bavaria, Kasendorf, Friesenquelle	3/14/2018	spring		
Germany	Thuringia, Sankt Ganglof, Tesse	3/15/2018	spring		

Taxonomy	Country	Locality	date	habitat	microsporidium infection
<i>Niphargus aquilex</i> -complex lineage H	France	Meurthe-et-Moselle, Haroué, Drainage Haroué	4/5/2018	spring	
<i>Niphargus aquilex</i> -complex lineage I	France	Haut Rhin, Source Mitteleck	1/14/2018	spring	
<i>Niphargus aquilex</i> -complex lineage M	France	Calvados, Saint-Vaast-en-Auge, Carrière souterrain de Saint-Vaast-en-Auge	5/26/2018	artificial cavern	
<i>Niphargus aquilex</i> -complex, lineage G	Germany	Saarland, Nunkirchen, Zillas Keller	1/10/2015	artificial cavern	
	Germany	Saarland, Steinkopfstollen	3/12/2018	artificial cavern	
	Germany	Saarland, Nunkirchen, Zillas Keller	12/30/2017	artificial cavern	<i>Microsporidium</i> sp. (93.1% similarity to FJ755996)
	Germany	Baden-Württemberg, Blaubeuren, Interstitial Blau	8/3/2018	spring	
	Luxembourg	Minette, Esch sur Alzette, Minière Langegronn	1/1/2016	artificial cavern	
<i>Niphargus aquilex</i> -complex, lineage J	France	Loir et Cher, 35 Pleine-Fougères	n.a.	n.a.	
<i>Niphargus aquilex</i> -complex, lineage K	Belgium	Wallonia, Péruwelz, Source Edouard Simon	9/14/2017	spring	
<i>Niphargus aquilex</i> -complex, lineage L	Belgium	Flandres, Kleine Spouwen, Bron in Kleine Spouwen	10/20/2018	spring	
<i>Niphargus</i> cf. <i>aquilex</i>	Germany	Hesse, Wertenberg, Quelle 37 im Krofdorfer Forst	22.07.2016	spring	
<i>Niphargus glenniei</i>	United Kingdom	Devon, Ashburton, Pridhamsleigh Caverth	9/7/2016	cave	
<i>Niphargus irlandicus</i>	Ireland	County Clare, Ballyvaghgan, Aillwee Cave	10/22/2017	cave	
<i>Niphargus kochianus</i> -complex (not A-D)	the Netherlands	Stokhem, Dorpsrietwell	5/20/2017	well	
<i>Niphargus puteanus</i>	Germany	Baden-Württemberg, Schlattstal, Wasserhäuschen Schwarze Lauter	6/13/2017	spring	
<i>Niphargus schellenbergi</i>	Belgium	Wallonia, Felenne, Source abbevoir Felenne	6/10/2017	spring	
	Belgium	Wallonia, Rotheux-Rimière, Source des Amoureux	2/25/2017	spring	
	Belgium	Wallonia, Baionville, Source sous arbre	3/8/2018	spring	
	Belgium	Wallonia, Lomprez, Source près des Dames	3/10/2018	spring	<i>Dictyococla diebenum</i> (99.45% similar to MH753359)

Taxonomy	Country	Locality	date	habitat	microsporidium infection
<i>Niphargus schellenbergi</i>	Belgium	Wallonia, Clermont; Fontaine de Saint-Jean	5/19/2017	spring	
	France	Vosges, Valfroicourt, Lavoir de Valfroicourt	4/7/2018	spring	
	Germany	North Rhine-Westphalia, Behlingen, spring near Behlingen	8/10/2017	spring	<i>Dictyocoela daebenum</i> (99.72% similar to JQ673483)
	Germany	Rhineland-Palatinate, Trier ST Euren, Quelle überm Talbildchen	10/13/2017	spring	
	Germany	Bavaria, Kulmbach, Quelle am Steinernen Gässchen	3/14/2018	spring	<i>Dictyocoela daebenum</i> (99.45% similar to MH753359)
	Germany	Thuringia, Bad Klosterlausnitz, Holzborn	3/15/2018	spring	<i>Dictyocoela daebenum</i> (99.48% similar to MH753359)
	Germany	North Rhine-Westphalia, Behlingen, spring near Behlingen	8/10/2017	spring	<i>Dictyocoela daebenum</i> (99.56% similar to MG063275)
	Germany	North Rhine-Westphalia, Behlingen, spring near Behlingen	8/10/2017	spring	
	Germany	North Rhine-Westphalia, Brilon, Obere Möhnequelle	8/13/2017	spring	
	Germany	Hesse, Martrnhagen, Quelle der Kneippanlage Martinhagen	5/9/2018	spring	
<i>Niphargus cf. schellenbergi</i>	Germany	Hesse, Wärme-Quelle	5/9/2018	spring	
	Germany	North Rhine-Westphalia, Brilon, Obere Möhnequelle	8/13/2017	spring	
	Germany	Rhineland-Palatinate, Trier ST Euren, Quelle überm Talbildchen	10/13/2017	spring	
	Germany	Saarland, Mertlach, Quelle über Mertlach	1/28/2017	spring	
	Germany	Bavaria, Neuschleichach, Aurachquelle	8/23/2017	spring	<i>Dictyocoela daebenum</i> (99.45% similar to MH753359)
	Luxembourg	Gutland, Diekirch, Quelle Diekirch	2/11/2018	spring	
	Luxembourg	Ösling, Urspelt, Quelle am aale Koepchen	5/20/2018	spring	<i>Dictyocoela daebenum</i> (99.45% similar to MH753359)
	Luxembourg	Gutland, Girsterklous, Source de Girsterklous	1/30/2018	spring	
	Luxembourg	Gutland, Osweiler, Wiesenquelle 2 Fromburg	1/31/2018	spring	
	France	Meurthe-et-Moselle, Xirocourt, Fontaine Jevoncourt	4/6/2018	spring	
<i>Niphargus sp.</i>	France	Haute-Saône, Le Thillot, Tunnel du Col des Croix	7/3/2016	tunnel	
	France	Saône-et-Loire, Le Creusot, Le Creusot, tunnel	2/2/2018	tunnel	
	Germany	Bavaria, Steinamwasser, Höhle Ohne Namen	8/24/2017	cave	
	Luxembourg	Gutland, Osweiler, Tümpelquelle Fromburg	1/31/2018	spring	
France	Meurthe-et-Moselle, Chaligny, Lavoir de Chaligny	4/5/2018	spring		

The detection of microsporidians with the primers V1 / Mic-uni3R (targeting a section of about 450 bp of the small subunit (SSU) rRNA gene) was done as described in Weigand et al. (2016). Additionally, selected microsporidian-positive samples were amplified with the primers HG4f and 580r (amplifying a product of about 500 bp) as suggested by Bacela-Spychalska et al. (2018) to obtain additional sequence information from the internal transcribed spacer (ITS) and the large subunit (LSU) of the rDNA gene. The intention was mainly to unambiguously match the isolates of *Dictyocoela* spp. to the respective GenBank entries. PCR products were purified with a Micro Elute Cycle Pure Kit according to manufacturer's instructions (Omega Bio-Tek) and sequenced (Eurofins Genomic Services).

Results

Host diversity and cryptic species

The COI marker was used for DNA barcoding of 57 specimens, the 28S locus analysed for 38 specimens – with a total of 32 specimens being investigated for both markers (Suppl. Table S1). Six specimens were identified by morphology only, as PCR amplification and/or DNA sequencing were not successful. The total groundwater amphipod dataset screened for microsporidian infections comprised 58 *Niphargus* specimens, 9 *Niphargellus* specimens, *Crangonyx* sp. and *Microniphargus leruthi* (Table 1). With 26 specimens *Niphargus* (cf.) *schellenbergi* was the most frequent taxon. Furthermore, the *N. aquilex* morphospecies was revealed to be represented by ten cryptic species in our dataset, which already comprised taxonomic annotations (*N. aquilex* A, B and F sensu McNerney et al. 2014) or were newly named in this study (i.e. *N. aquilex*-complex lineages G to M) using the terminology as introduced by McNerney et al. (2014). The COI sequences can be retrieved from Suppl. material 2.

Microsporidian diversity

A literature review was performed on known microsporidian infections in niphargid amphipods, and our own results added (Table 2).

No microsporidians were detected in the single *M. leruthi* and *Crangonyx* sp. In total, 13 niphargids were tested positive for microsporidians by PCR (19.1%, Table 1). Most of the isolates (9, 13.2%) were identified as *Dictyocoela duebenum* (according to Bacela-Spychalska et al. 2018). This microsporidium was found mainly in *N. schellenbergi* (7 out of 9) as well as in *Niphargus aquilex* lineages B and F. It was found almost exclusively in spring habitats. The sequences of the remaining four microsporidians were clearly different and only one host individual was found infected each (1.4%). One isolate from *Niphargellus arndti* was similar to *Nosema* sp. (97.2% to KM977840) previously isolated from *Eulimnogammarus verrucosus* (Madyarova

Table 2. Overview of microsporidian infections in groundwater amphipods of the family Niphargidae.

Host	Microsporidium	Reference
<i>Niphargellus arndti</i>	<i>Hyperspora aquatica</i> (99.8% similar to KX364284)	this study
	<i>Microsporidium</i> sp. (97.5% to KX137915)*	this study
	<i>Nosema</i> sp. (97.2% to KM977840)	this study
<i>Niphargus aquilex</i> B	<i>Dictyocoela duebenum</i> (99.5% to MH753359)	this study
<i>Niphargus aquilex</i> F	<i>Dictyocoela duebenum</i> (99.5% to MH753359)	this study
<i>Niphargus aquilex</i> G	<i>Microsporidium</i> sp. (93.1% to FJ755996)*	this study
<i>Niphargus ilidzensis</i>	<i>Pleistophora mülleri</i> (probable syn. <i>Stempellia mülleri</i> , <i>Microsporidium giraudi</i> , <i>Thelohania mülleri</i> , <i>T. giraudi</i> , <i>Pleistophora blochmanni</i> , <i>Glugea mülleri</i>)	Bulnheim (1971)
<i>Niphargus schellenbergi</i>	<i>Dictyocoela duebenum</i> (99.5% to MH753359; 99.7% to JQ673483; 99.6% to MG063275)	this study
	<i>Microsporidium</i> sp. BPAR3 (KT633993)*	Weigand et al. (2016)
	<i>Microsporidium</i> sp. I (KT633992)*	Weigand et al. (2016)
	<i>Nosema granulosis</i>	Weigand et al. (2016)
	<i>Orthosomella</i> sp.	Weigand et al. (2016)
<i>Niphargus stygius</i> species group	<i>Microsporidium vandeli</i> (probable syn. <i>Microsporidium niphargi</i> , <i>Mrazekia niphargi</i> , <i>Bacillidium niphargi</i> , <i>Thelohania vandeli</i>)	Poisson (1924)

**Microsporidium* sp. is a transitory genus for genetically identified microsporidian isolates without a link to a morphological description. Therefore, GenBank accession numbers for the isolate or the respective best match are given in these cases.

et al. 2015) and 96.8% to *Nosema granulosis* (MK719384) isolated from *Gammarus roeselii* (Quiles et al. 2019). An isolate obtained from *N. aquilex* lineage G showed a similarity of 93.1% to a microsporidian sequence from the amphipod *Crypturopus tuberculatus* collected in Lake Baikal (FJ755996). Two additional microsporidian isolates were sequenced from *Niphargellus arndti*; one was 97.5% similar to a microsporidian detected in caddisfly larvae (KX137915, Grabner et al. 2017), the other was 99.8% similar to the hyperparasitic microsporidian *Hyperspora aquatica* (KX364284, Stentiford et al. 2017).

Sequencing of the PCR product obtained with the HG4f-580r primers from two *N. schellenbergi*-specimens resulted in two non-overlapping fragments that were between 95.4% (*Pseudocollinia beringensis*; HQ591477) to 98.5% (*Gymnodinioides pitelkae*; EU503534) genetic similarity to sequences of apostome ciliates from krill and marine amphipods. The SSU rDNA sequences can be retrieved from Suppl. material 3.

Discussion

Due to a generally low supply of nutrients and often species-poor local communities, groundwater(-dependent) ecosystems are ecologically particularly sensitive. Therefore, transmission pathways might be ecologically more relevant and effects of parasites might have a stronger regulatory role in these environments. In the present study, five different microsporidian isolates could be obtained from 68 tested niphargid individuals, which correspond to about 0.07 microsporidian species per host individual. This is much lower compared to the study of Weigand et al. (2016) who found four microsporidian species in 21 tested *N. schellenbergi*, therefore a rate

of 0.19 parasite species per host individual. Also, the overall prevalence was much higher in the latter (>80%), compared to the present study (19%). This difference might be explained by the close connection of the investigated *Niphargus*-population to a surface population of *Gammarus fossarum* lineage 13 with a microsporidian prevalence of 90% in the study of Weigand et al. (2016). Transmission from this highly infected surface population might have been the cause for the comparatively high prevalence in *Niphargus* spp. found in their study. Nevertheless, it has to be noted that the majority of niphargid specimens from the present study had been sampled from spring habitats, and as such, also co-exist with epigeal arthropods, including *Gammarus fossarum* (lineage 13). Alternatively, in particular *Niphargus schellenbergi* might be susceptible for microsporidian infections. Further indication might be seen in our study results as well, as out of the 26 specimens identified as *Niphargus* (cf.) *schellenbergi* seven were infected, corresponding to a rate of 0.27 parasite species per host individual.

The most abundant microsporidium found in the present study was *Dictyocoela duebenum*, a common species occurring in a variety of amphipods (Terry et al. 2004, Grabner et al. 2015, Wilkinson et al. 2011, Bacela-Spychalska et al. 2018). This species is generally transmitted vertically (from mother via eggs directly to the offspring) and can feminize the host population (Ironsides et al. 2003). But there is also evidence for phases of horizontal transmission (masses of spores are released after host death and infect other individuals when they ingest the spores) that will cause increased host mortality (Wilkinson et al. 2011). Therefore, *D. duebenum* might be transmitted to *Niphargus* populations when they come in contact with other infected amphipods and persist in the population by vertical transmission. As we tested only few host individuals per site (1-3), we cannot draw conclusions about the actual absence of microsporidians at sites with specimens that were tested negative for microsporidians. Nevertheless, some *Niphargus* populations seem to be free of *D. duebenum*, as this species was not found in the study of Weigand et al. (2016).

In the study by Weigand et al. (2016), *Nosema granulosis* was detected in *N. schellenbergi*. This microsporidium was originally described from *Gammarus duebeni* (Terry et al. 1999) and is the only species of this genus recorded from different species of amphipods (Terry et al. 2004). The *Nosema* isolate from *Niphargellus arndti* detected in the present study shows a sequence divergence of 3.2% to the closest *Nosema granulosis* sequence in GenBank and might be in fact a new *Nosema* species. It is most closely related to a *Nosema* sp. isolate found in the freshwater amphipod *Eulimnogammarus verrucosus* (97.2% to KM977840). This amphipod is endemic to Lake Baikal (Russia) where it inhabits the upper and sub-littoral zones, being commonly sampled in high numbers from water depths between 0.1-15 m (Bazikalova 1945, Rivarola-Duarte et al. 2014). Similar to *D. duebenum*, *Nosema* species might be transmitted to *Niphargus*-populations from other amphipods in phases of horizontal transmission. As the *Nosema* sp. detected here in *Niphargellus arndti* was not revealed by any other genetic study on amphipod microsporidians so far, it might be a niphargid-specific species.

The microsporidian isolate from *N. aquilex* (lineage G) showed only a low similarity (93.1%) to a previously characterized microsporidian isolate from amphipods. Therefore, it should be considered as a new sequence record. Also the isolate from *Niphargellus arndti* with 97.5% similarity to a microsporidian isolate from caddisfly larvae is probably a species that has not been sequenced and described yet.

A puzzling finding is the microsporidium from *Niphargellus arndti* that was genetically 99.8% similar to *Hyperspora aquatica*, a microsporidian hyperparasite of *Marteilia cochillia* (Paramyxida) from cockles (Stentiford et al. 2017). To date, Paramyxia of amphipods were only described from marine species (Ginsburger-Vogel and Desportes 1979, Short et al. 2012), but the sequence of *H. aquatica* shows a close relationship also to other microsporidians of freshwater amphipods. Genetically most similar is a *Microsporidium* sp. (98.9% to HM800853) from a marine parasitic copepod, and Stentiford et al. (2017) discussed a possible involvement of copepods in the life cycle of *H. aquatica*. Based on these assumptions, we can speculate that a related freshwater species utilizes (only) amphipods as hosts.

In this context, it should be mentioned that whole-body homogenates of the hosts were used for DNA extraction, including gut content and organisms associated with the amphipod, e.g. epibiotic ciliates. While *Dictyocoela* and *Nosema* spp. are well characterized parasites of amphipods, we cannot be sure about the location of the other three microsporidian isolates detected in the present study. Therefore, the possibility exists that the microsporidium from the present study with high sequence similarity to *H. aquatica* is actually infecting protists associated with the amphipods (see also discussion in Stentiford et al. 2017) or originates from groundwater copepods ingested by niphargids.

An unexpected finding was the detection of a sequence most similar to an apostome ciliate in two individuals of *N. schellenbergi* from a single site (North Rhine-Westphalia, spring near Behlingen). Apostome ciliates are exuvitrophic or parasitoids of invertebrates, mainly crustaceans, and were described previously from marine and freshwater amphipods (e.g. Bradbury 2005, Chantangsi et al. 2013, Gudmundsdóttir et al. 2018, Lynn and Strüder-Kypke 2019). Apostome ciliates were also detected in groundwater habitats. For example, *Collinia neophargi* was described from *Crangonyx subterraneus* (syn. *Neoniphargus moniezi*, Ginet 1988) (Bradbury 1994). Gudmundsdóttir et al. (2018) isolated five different sequences of apostome ciliates from the groundwater amphipods *Crangonyx islandicus* and *Crymostygius thingvallensis* from Iceland. Interestingly, the ciliate sequence from the present study was most similar (98.5%) to an apostome ciliate from the marine amphipod *Gymnodiniooides pitelkae* (Bradbury 2005), probably due to the lack of related sequence information from freshwater species.

Conclusion

In the present study, *Niphargus schellenbergi* was the most frequent taxon, but also demonstrated a proportionally high infection rate. A total of five different microsporidian species were discovered, with *Dictyocoela duebenum* being the most frequent and

found in different niphargid isolates, but preferably in *N. schellenbergi*. This shows that different populations of groundwater amphipods can be impacted by this feminizing microsporidium. Other single findings of microsporidians give an indication of the diversity but a larger sample size and ultrastructural studies would be desirable to link the genetic data to previous morphological descriptions. We want to conclude that more studies on microsporidians (and other parasites) in groundwater species are needed to improve our understanding on their effect on the host populations and sensitive aquatic communities.

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Supplementary material I

Overview table of all groundwater amphipod specimens screened for microsporidians

Authors: Daniel Grabner, Dieter Weber, Alexander M. Weigand

Data type: specimen metadata information

Explanation note: The table lists specimen ID, taxonomy, collection information, habitat type, microsporidium infection, GenBank Accession numbers for the parasite, identification results via morphology and DNA (COI, 28S), BOLD BINs and comments to the identification method for each specimen analysed.

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Link: <https://doi.org/10.3897/subtbiol.33.48633.suppl1>

Supplementary material 2

COI sequences of groundwater amphipods screened for microsporidians

Authors: Daniel Grabner, Dieter Weber, Alexander M. Weigand

Data type: sequences

Explanation note: Cytochrome C oxidase subunit 1 (COI) sequences for groundwater amphipods screened for microsporidians.

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Link: <https://doi.org/10.3897/subtbiol.33.48633.suppl2>

Supplementary material 3

SSU rDNA sequences for microsporidian parasites of groundwater amphipods

Authors: Daniel Grabner, Dieter Weber, Alexander M. Weigand

Data type: sequences

Explanation note: SSU rDNA sequences for microsporidians of groundwater amphipods

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Trogglomorphism in the brittle star *Ophionereis commutabilis* Bribiesca-Contreras et al., 2019 (Echinodermata, Ophiuroidea, Ophionereididae)

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Abstract

Due to their peculiar and sometimes bizarre morphology, cave fauna (across invertebrates and vertebrates from both aquatic and terrestrial cave habitats) have fascinated researchers throughout history. Despite their success in colonizing most marine ecosystems, the adaptations of cave brittle stars (Ophiuroidea) to a stygobiotic lifestyle have been scarcely examined. Employing comparative methods on a data set of two species belonging to the genus *Ophionereis*, this study addresses whether a cave-dwelling species from Cozumel exhibited similar troglomorphic traits as those of other taxa inhabiting caves. Our work demonstrated that some characters representing potential morphological cave adaptations in *O. commutabilis* were: bigger sizes, elongation of arms and tube feet and the presence of traits potentially paedomorphic. In addition, an element of ophiuroid's photoreceptor system, as well as pigmentation, was observed to be peculiar in this stygobiotic species, plausibly as a result of inhabiting a low light-energy environment. Finally, we add evidence to the statement that *O. commutabilis* is a cave endemic species, already supported by demography, distribution and origin of this species, and now by a typical array of troglomorphisms.

Keywords

adaptation, Aerolito, anchialine system, cave, Cozumel, ophiuroid, stygobiotic

Introduction

Several traits are often associated with cave-dwellers, which are also known as troglomorphisms, defined as a morphological modification in cave fauna (Culver et al. 1995; Romero 2009). These modifications or morphological traits could be expressed as reductive or constructive traits, such as the lack of photoreceptors or depigmentation in the case of the former and as the hypertrophy of appendages for the latter (Mejía-Ortíz et al. 2006, Gonzalez et al. 2018). All these traits allow organisms to survive in caves, environments that constitute isolated and harsh habitats (Culver and Pipan 2009).

According to Bishop et al. (2015), anchialine caves are “a tidally-influenced subterranean estuary located within crevicular and cavernous karst and volcanic terrains that extends inland to the limit of seawater penetration”. These environments are thought to be food-limited, with detritus and dissolved organic matter being the most important nutritional sources (Mejía-Ortíz et al. 2013). However, several caves have an important contribution of food from bacterial activity (Sarbu et al. 1996; Pohlman et al. 1997; Brankovits et al. 2017). The limitation of food is related to the fact that caves are completely devoid of sunlight and lack of sources of primary productivity (Culver et al. 1995; Gonzalez et al. 2018).

Although cave fauna is mainly composed by crustaceans, several studies have reported organisms of other taxa inhabiting these ecosystems (Sket 1996; Gibert and Deharveng 2002; Calderón-Gutiérrez et al. 2017). Concerning echinoderms, 58 species have been reported inhabiting cave environments, distributed into the five classes of the phylum: Crinoidea (2 species), Asteroidea (8), Ophiuroidea (39), Echinoidea (5) and Holothuroidea (4) (Martínez García et al. 2009; Pomory et al. 2011; Bribiesca-Contreras et al. 2013; Tan et al. 2014; Okanishi and Fujita 2019; Okanishi et al. 2019). However, only five of these species have been described as stygobiotic species (restricted aquatic cave-dwelling organism), the starfish *Copidaster cavernicola* Solís-Marín & Laguarda-Figueras, 2010 and the ophiuroids *Amphicutis stygobita* Pomory, Carpenter & Winter, 2011, *Ophiozonella cavernalis* Okanishi & Fujita, 2018, *Ophionereis commutabilis* Bribiesca-Contreras et al., 2019 and *Ophiopsila xmasilluminsans* Okanishi, Oba & Fujita, 2019. This is surprising considering that there are over 7,500 species of echinoderms that live in a wide variety of habitats (Zhang 2011). Also striking is the fact that although Ophiuroidea is the largest class of echinoderms (about 2100 described species according to O’Hara et al. (2018)) and are generally photonegative (Stöhr et al. 2012; O’Hara et al. 2018), only a few (39) species have been found in caves.

Troglomorphism has been investigated mainly on arthropods and vertebrates from both aquatic and terrestrial caves (Poulson and White 1969; Mejía-Ortíz and Hartnoll 2005; White and Culver 2012; Mejía-Ortíz et al. 2013; Rizzato and Bichuette 2017). Despite the existence of some studies about troglomorphism on other taxa (White and Culver 2012; Gonzalez et al. 2018), no extensive study has been conducted on stygobiotic echinoderms. Instead, only a few remarks have been made on the morphology of species of echinoderms and its relation with the cave-habitat (Mejía-Ortíz et al. 2007;

Solís-Marín and Laguarda-Figuera 2010; Pomory et al. 2011; Brom et al. 2015; Carpenter 2016; Okanishi and Fujita 2018; Bribiesca-Contreras et al. 2019).

Thus, the aim of this study is the identification of morphological adaptations of *O. commutabilis*. To test the hypothesis that *O. commutabilis* shows troglomorphisms, we carried out a comparative study between *O. commutabilis* and its epigeal congener *O. reticulata*. A comparison with other epigeal congeners for several traits, as well as the similarities among cave-dwelling brittle stars are discussed. As this is one of the first studies to investigate troglomorphism in brittle stars, our assumptions are mainly based on the analogy to previously identified troglomorphic traits throughout other stygobiotic taxa. Implications for troglomorphism are discussed with respect to concepts of cave biology.

Methods

Species selection

Ophionereis reticulata (Say, 1825) was selected to use as a reference for comparison, based on both morphological and genetic resemblance between Caribbean *Ophionereis* species with *O. commutabilis* (Bribiesca-Contreras et al. 2013, 2019). Therefore, specimens of *O. commutabilis* ($N = 46$) from the anchialine system El Aerolito, Cozumel Island ($20^{\circ}27'58.4''N$, $86^{\circ}58'41.2''W$) (details in Bribiesca-Contreras et al. 2019) were compared with their reef relatives, *O. reticulata* ($N = 59$) from localities in Quintana Roo, Mexico. All the analysed material is deposited in the “Colección Nacional de Equinodermos Dra. María Elena Caso”, at Instituto de Ciencias del Mar y Limnología, of Universidad Nacional Autónoma de México (UNAM).

Selection of morphological traits

Morphological traits were chosen based on the previous studies on other taxa, and from our personal observations. We sampled and compared the morphological traits described below. A detailed discussion of each character is provided.

Arms and tube feet length

Elongation of body appendages are well documented traits in cave fauna, as they affect both sensorial and feeding structures in aquatic and terrestrial environments (Turk et al. 1996; Mejía-Ortiz et al. 2006, 2013). Arms of brittle stars are used mainly for locomotion and present tube feet and spines, both being the only known sensorial organs of this taxa (Hajduk 1992; Zueva et al. 2018). Spines and tube feet also participate in the acquisition of food by suspension feeding (Stöhr et al. 2012). Cave brittle stars show unique patterns of tube feet and arm length, proposed to be the result of differ-

ent forms of locomotion and feeding in these environments (Bribiesca-Contreras et al. 2019; Pomory et al. 2011).

Here we evaluate the elongation of arms and tube feet as a potential morphological adaptation to the cave. Measures of the structure's length were correlated with the disc diameter of each specimen. Length of arms was measured from the first to the last segment, considering only complete arms without regenerating scars. Tube feet and oral tentacle length were measured from the base (at the tentacle pore or tentacle basin, respectively) to the tip of the structure. Tube feet were considered from proximal, middle and distal portions of the arm (based on Munday (1993)), and were measured using image analysis software (ADOBE PHOTOSHOP CC; RRID: SCR_014199) calibrated with scaled photographs of fixed organisms.

Regeneration frequency

Regeneration is a common process on brittle stars, caused by damage of arms through sub-lethal predation. As a result, regeneration rate of arms is often used as an estimate of predation pressure (Sköld and Rosenberg 1996; Dupont and Thorndyke 2006; Yokoyama and Amaral 2010), which in caves decreases in importance or is absent (Gibert and Deharveng 2002). The regeneration frequencies of each specimen were registered by counting the regenerating scars on the oral side. To identify the scars, we compared differences in colour and size patterns between regenerated portions and old sections.

Paedomorphic traits

Morphological juvenile traits retained by sexually mature organisms have been reported for cave-dwellers, also known as paedomorphic features (Culver 1982; Sket 1996; Culver and Pipan 2009). Cave brittle stars could show some paedomorphic traits related to organization, size and calcification of skeletal plates of arms and disc (Pomory et al. 2011). For this reason, external morphology and microstructure of arm plates (dorsal, ventral, lateral, spines, tentacle scales) from proximal, middle and distal portions of the arm of mature and juvenile specimens were analysed. Likewise, radial shields and buccal skeleton (oral and adoral shields; oral and dental plates, as well as teeth) were considered.

The arm ossicles used for microstructure examination were obtained from one arm of each specimen selected. Tissue was removed by soaking two segments of each portion in 5% sodium hypochlorite and later washed with MilliQ water. Ossicles were mounted on a stub and coated with gold for taking micrographs using a Hitachi SU1510 scanning electron microscope (SEM).

Images of the external face of dorsal, ventral and lateral arm plates were analysed using both geometric morphometrics and morphological approaches. For geometric morphometrics, TPS, MAKEFAN8 and MORPHOJ v2.0 software (MorphoJ, RRID: SCR_016483) were used. Landmarks (LM) were digitized to describe the perimeter of the plates (Suppl. materials 1, Fig. S1). Madreporic oral shields, teeth, spines and tentacle scales were examined by comparing the morphology of outer face between

species. Oral, radial and adoral shields, as well as oral and dental plates, were analysed by comparing porosity of external stereom.

Photoreceptors

Brittle stars have a photoreceptor system consisting of nerve bundles, chromatophores and expanded peripheral trabeculae (EPT) (Hendler and Byrne 1987; Aizenberg et al. 2001). EPTs are hemispheres on the outer face of arm plates and have been suggested to be a structural adaptation relating to chromatophore activity (Sumner-Rooney et al. 2018). Considering that caves exhibit total darkness, processes like photoreception and bioluminescence, as well as the involved structures, could be modified. Therefore, we estimated the EPTs density of the central region of the dorsal arm plates by using SEM images.

In addition to morphological traits, spectral transmittance of isolated dorsal arm plates was estimated by coupling a source of white light (LS-1-II Ocean Optics) to an HR4000 spectrophotometer. We used two optical fibres of 50 and 100 μm on different positions to obtain readings of three dorsal-proximal arm plates of each species (*O. reticulata* and *O. commutabilis*). Data were normalized and analysed on ORIGIN PRO 9.1 software (Origin, RRID: SCR_014212).

Statistical analyses

We analyzed the number of arms and specimens regenerating as well as arm and tube feet ratios between the two species considered. Data and R-scripts used throughout this work are available on the supporting information. All analyses were performed under software RSTUDIO v3.5.0. (Team 2016).

Concerning geometric morphometrics analysis, after the Procrustes fit of the sample, canonical variate analysis (CVA) was performed to explore morphological differences among species using geometric morphometric analysis of arm plates. *P* values were calculated using a permutation test based on 100,000 iterations of the Mahalanobis distances for differences. CVA, including estimation of the significance of Mahalanobis distances using a parametric approach (Procrustes ANOVA), was also performed to assess the statistical robustness of the groups delineated in the CVA.

Results

Appendages length

A remarkable arm elongation is observed in *O. commutabilis*, with arms up to 20 times the disc diameter and a mean of 13.2 in comparison to 6.6 of *O. reticulata*. This ratio showed statistical differences between species (ANOVA, $F_{1,105}=559.3$; $p < 0.001$). Furthermore, we found that arms of specimens of equal size from both cave and epigeal

species are integrated by segments of similar length (Suppl. materials 2, Table S1), hence, cave species having more segments. No differences of oral tentacle nor tube feet length were found among species in either proximal or middle portions of arms. Distal tube feet showed statistical differences in length (ANOVA, $F_{1,48}=18.1$; $p < 0.001$), where the stygobiotic specimens had longer tube feet than *O. reticulata*. These results are summarized in Table 1.

After measuring the disc diameter, we observed that stygobiotic specimens showed bigger sizes. For the disc diameter, we observed between mature *O. reticulata* and *O. commutabilis*, statistical differences (ANOVA, $F_{1,88}=4.54$; $p < 0.05$). Cave-dwelling specimens having a disc diameter 14% bigger than its epigeal congener.

Regeneration frequency

Based on recorded observations of specimens of *O. commutabilis*, 32% ($n=46$) showed signs of arm regeneration. Besides, 14% of arms observed ($n=196$) were damaged. In comparison, from the 51 individuals of *O. reticulata* analyzed, 70% showed signs of arm regeneration. Correspondingly, 35% of arms ($n=213$) were damaged in specimens of *O. reticulata*.

The average of arms regenerating per individual in cave-dweller specimens was 0.7 (14.42%) while in reef individuals was 1.6 arms (33.16%). Statistical analysis showed a difference between species for these data (ANOVA, $F_{1,95}=12.52$; $p < 0.001$).

Microstructures

Dorsal arm plates (DAP) of both species are different in form, being hexagonal in mature *O. commutabilis* and trapezoid in *O. reticulata* (Fig. 1). Meanwhile, DAP of juvenile specimens of *O. reticulata* are hexagonal (Fig. 1c). Geometric morphometric analyses allowed us to confirm the statistical significance of differences in form between plates of mature specimens of both species (Table 2). Deformation grids in the DAP shape showed the mentioned change based on LM displacement vectors (Fig. 1d). Although ventral arm plates (VAP) of *O. reticulata* are slightly wider than those of *O. commutabilis*, the general shape is similar in both species (Fig. 2). Ventral arm plates (VAP) of mature *O. reticulata* specimens are also wider than juveniles of this species (Fig. 2c). Differences in form of VAP between species were confirmed with CVA ($P < 0.001$) and deformation grid exhibited the difference in width among species (Fig. 2d). Both DAP and VAP of juvenile specimens of *O. commutabilis* show the same shape than plates of mature specimens. Finally, lateral arm plates showed significant differences in form between species (Table 4) but no clear pattern associated (Fig. 3). Examination of arm plates allowed us to corroborate the correlation between serial and ontogenetic variation, that is to say, distal plates analogous to plates of juvenile specimens.

Table 1. Summary of morphometric results showing significant (S) and not significant (NS) differences for each trait. For traits with statistically significant differences the result of it with respect to the cave species is expressed as longer/bigger than *O. reticulata* (↑). NT: not statistically tested.

Trait	Differences	Result
Arm length	S	<i>O. commutabilis</i> ↑
Number of arm segments	NT	<i>O. commutabilis</i> ↑
Oral tentacle length	NS	-
Proximal tube feet length	NS	-
Middle tube feet length	NS	-
Distal tube feet length	S	<i>O. commutabilis</i> ↑

Table 2. Geometric morphometrics results for DAP. *Ophionereis* spp. dorsal arm plates Procrustes ANOVA and P values based on permutation test results with environment (Reef for *O. reticulata* and Cave for *O. commutabilis*) as covariate (Mahalanobis distance in parenthesis).

Effect	SS	Procrustes ANOVA			F	P(param.)
		MS	df			
Individual	0.06245164	0.0052043037	12	3.01	0.0005	
Residual	0.53979931	0.0017301260	312			
P value based on permutation test						
Environment				Cave		
Reef				<0.0002(2.39)		

Table 3. Geometric morphometrics results for VAP. *Ophionereis* spp. ventral arm plates Procrustes ANOVA and P values based on permutation test results with environment (Reef for *O. reticulata* and Cave for *O. commutabilis*) as covariate (Mahalanobis distance in parenthesis).

Effect	SS	Procrustes ANOVA			F	P(param.)
		MS	df			
Individual	0.02209517	0.0012275093	18	5.00	<0.0001	
Residual	0.11482214	0.0002453465	468			
P value based on permutation test						
Environment				Cave		
Reef				<0.0001(7.34)		

Table 4. Geometric morphometrics results for LAP. *Ophionereis* spp. lateral arm plates Procrustes ANOVA and P values based on permutation test results with environment (Reef for *O. reticulata* and Cave for *O. commutabilis*) as covariate (Mahalanobis distance in parenthesis).

Effect	SS	Procrustes ANOVA			F	P(param.)
		MS	df			
Individual	0.11434060	0.0043977153	26	7.58	<0.0001	
Residual	0.73910392	0.0005801444	1274			
P value based on permutation test						
Environment				Cave		
Reef				<0.0001(6.27)		

Concerning microstructures qualitatively analyzed, *O. commutabilis* tentacle scales are ovoid and longer than wide, which resembles the same plates of juvenile *O. reticulata* (Fig. 4a–c). On the other hand, spines showed no clear differences in form among species. All examined microstructures exhibited differences in porosity, with

Table 5. Comparison of several traits between a stygobiotic *Ophionereis* species and its closest epigean relatives. dd: disc diameter; al: arm length; DAP: dorsal arm plate; VAP: ventral arm plates; ND: No data.

Species	Habitat	dd (mm) [Max]	Avg ratio (al/dd) [Max]	Avg segment length (mm)	Regeneration frequency (%) [for arms]	DAP shape	VAP shape	Reference
<i>Ophionereis commutabilis</i>	Cave-dwelling	11.4 [17]	13.2 [20]	0.58	32 [14]	Hexagonal	Longer than wide	Present study
<i>O. reticulata</i>	Epigean	11.4 [15]	6.6 [8]	0.55	70 [35]	Trapezoid	Quadrangular	Bribiesca-Contreras et al. 2013, Hendler et al. 1995, Present study
<i>O. vittata</i>	Epigean	6.7 [10]	8 [13]	ND	ND	Rounded hexagonal	Bell shaped	Bribiesca-Contreras et al. 2013, Hendler et al. 1995, Present study
<i>O. squamulosa</i>	Epigean	5 [6]	8 [ND]	ND	ND	Rounded hexagonal	Bell shaped	Bribiesca-Contreras et al. 2013, 2019, Pomory 2007
<i>O. olivacea</i>	Epigean	4.5 [6]	5 [ND]	ND	ND	Roughly hexagonal	Bell shaped	Bribiesca-Contreras et al. 2013, 2019, Hendler et al. 1995

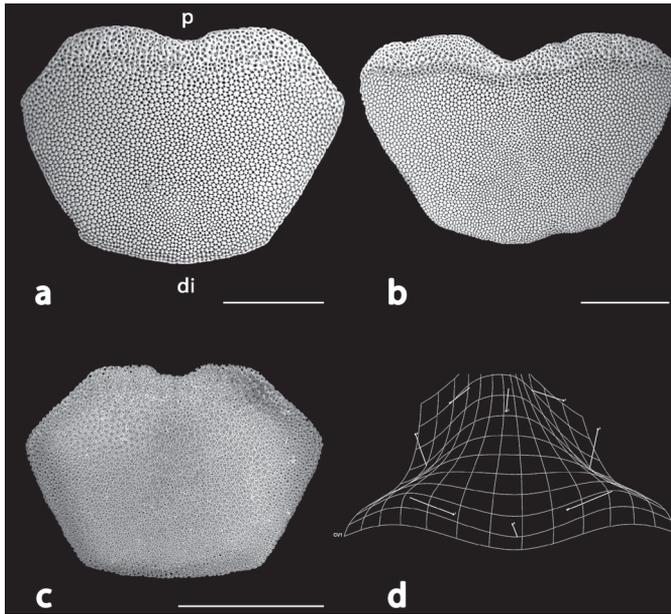


Figure 1. Scanning electron micrograph (SEM) of dorsal arm plates from mature *Ophionereis commutabilis* (a) and *O. reticulata* (b). SEM of dorsal arm plate of juvenile *O. reticulata* (c). Deformation grid of DAP shape showing deformation vectors (d). Orientation (p: proximal, di: distal). Scale bars: 400 μm .

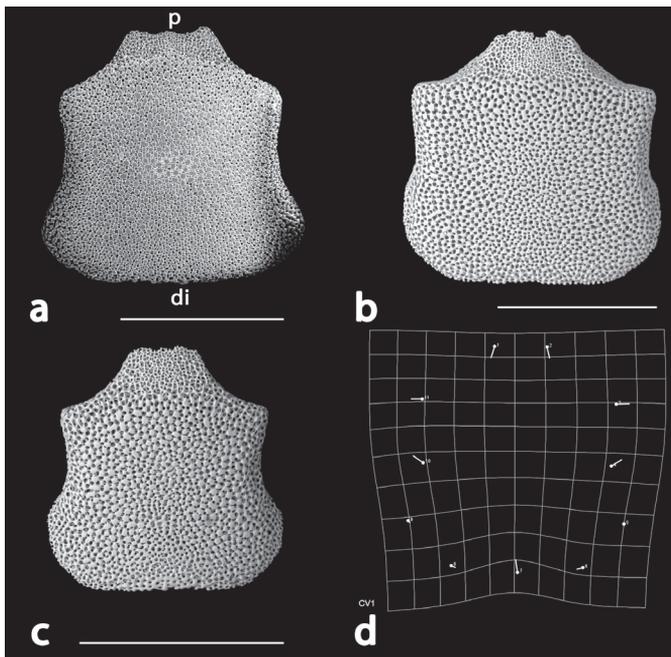


Figure 2. Scanning electron micrograph (SEM) of ventral arm plates from mature *Ophionereis commutabilis* (a) and *O. reticulata* (b). SEM of ventral arm plate of juvenile *O. reticulata* (c). Deformation grid of VAP shape showing deformation vectors (d). Orientation (p: proximal, di: distal). Scale bars: 500 μm .

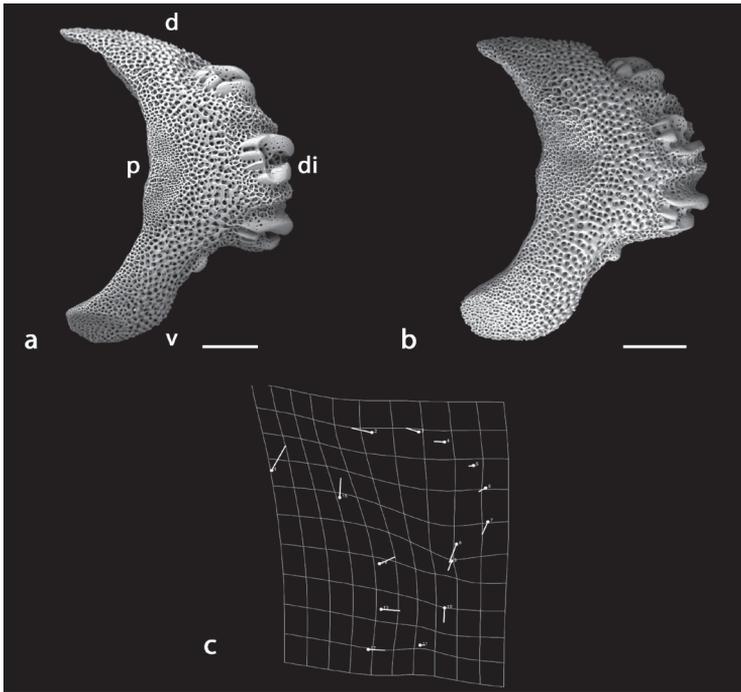


Figure 3. Scanning electron micrograph (SEM) of lateral arm plates from mature *Ophionereis commutabilis* (a) and *O. reticulata* (b). Deformation grid of lateral arm plate shape showing deformation vectors (c). Orientation (p: proximal, di: distal, d: dorsal, v: ventral). Scale bars: 200 μ m.

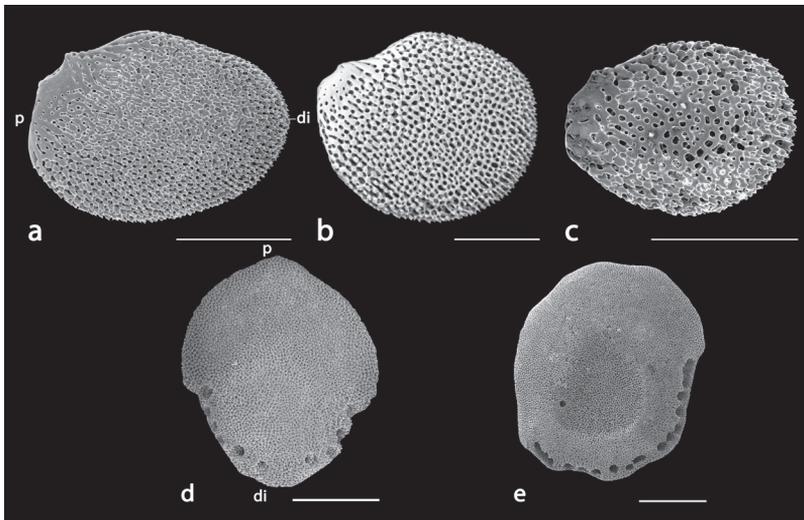


Figure 4. Scanning electron micrograph (SEM) of tentacle scales from mature *Ophionereis commutabilis* (a) and *O. reticulata* (b). SEM of tentacle scale of juvenile *O. reticulata* (c). SEM of madreporite of *O. commutabilis* (d) and *O. reticulata* (e). Orientation (p: proximal, di: distal). Scale bars: 200 μ m (a–c); 500 μ m (d–e).

the ossicles of the stygobiotic specimens being slightly more porous than its epigean congener (e.g. 5 to 9 pores surrounding each EPT in *O. commutabilis* and 4–7 in *O. reticulata* as shown in Figs 1, 5). Although these differences in porosity are observed in all ossicles, it is especially remarkable in distal arm plates, spines, tentacle scales and dental plates (Fig. 4 and Suppl. materials 1, Fig. S2). Madreporites between both species differ in form and in the number of hydropores (around eight in *O. commutabilis* and 17 in *O. reticulata*) (Fig. 4d–e). Lastly, though teeth showed great intraspecific variation, a clear pattern allowed us to distinguish between species. While *O. reticulata* possesses compound teeth, *O. commutabilis* reveals both uniform and compound teeth (Suppl. materials 1, Fig. S3).

Photoreceptors

Dorsal arm plates (DAP) of *O. commutabilis* exhibit a pattern of EPTs agglomeration, however, this pattern is only present on some DAP from the distal portion of the arm of *O. reticulata* (Fig. 5a–c). This pattern decreases the EPT density (increasing its size) on the stygobiotic specimens in comparison to its epigean congener. The EPT density of DAP relativized with the disc diameter in *O. reticulata* (498 EPT/mm²) almost doubled that of *O. commutabilis* (251 EPT/mm²).

Differences in the arrangement and size of the EPT in both species corresponds to a different pattern of the inner stereom through a cross-section. DAP of *O. commuta-*

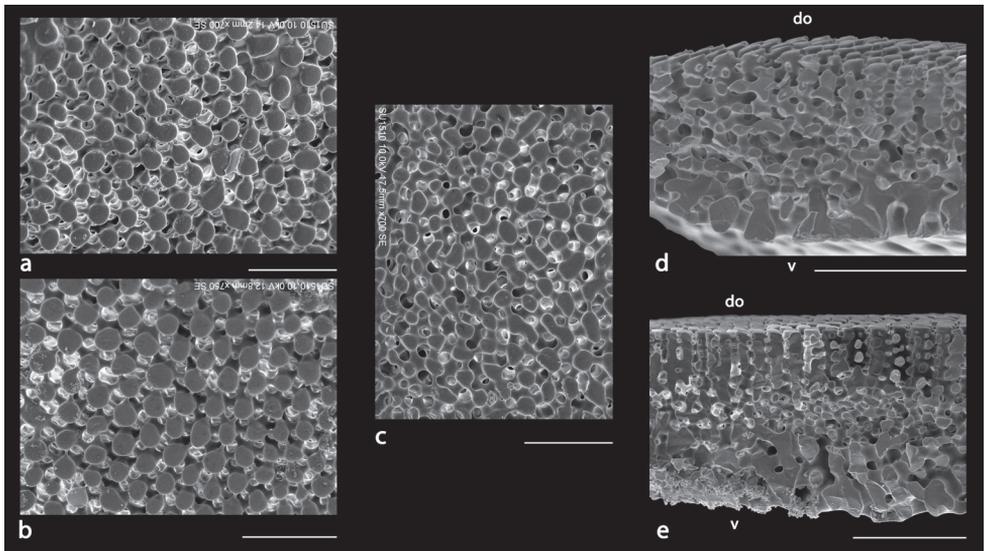


Figure 5. Scanning electron micrographs (SEM) of central region of dorsal arm plates (DAP) of *Ophionereis* brittle stars. SEM of a DAP from mature *Ophionereis commutabilis* (a) and *O. reticulata* (b). SEM of a DAP of juvenile *O. reticulata* (c). SEM of a cross-section of a fractured DAP from mature *O. commutabilis* (d) and *O. reticulata* (e). Orientation (do: dorsal, v: ventral). Scale bars: 50 μm (a–c); 100 μm (d–e).

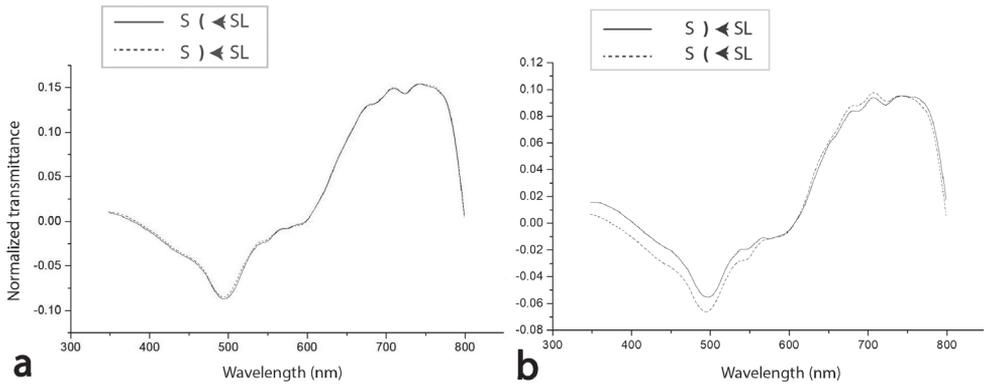


Figure 6. Dorsal arm plates transmittance of *Ophionereis reticulata* (a) and *O. commutabilis* (b). Two different arrangements between spectrophotometer (S), source light (SL) and plates are presented with concave section representing the inner face and convex the outer face)-(.

bilis show a nearly uniform disorganized pattern across the plate, while DAP of the epigeal specimens exhibit two clear patterns depending on the side of the plate: (1) a dorsal half with an organized stereom associated with EPT and (2) a ventral half with unsystematic stereom (Fig. 5d–e).

Correspondingly with the differences on the microstructure of DAP, transmittance values of DAP showed differences among species. The values of epigeal specimens did not differ depending on the position of optical fibres. Meanwhile, differences were observed along the spectra between the two arrangements in the transmittance values of DAP of *O. commutabilis*. For both species, the transmittance was lower around 500 nm and greater for wavelengths between 600 and 780 nm (Fig. 6). In general, plates of stygobionts showed slightly lower values of transmittance than those isolated from epigeal specimens.

Discussion

Using a comparative morphological approach, we provide evidence of cave adaptation in *O. commutabilis*. This study quantified the main morphological differences observed between *O. commutabilis* and its epigeal congener *O. reticulata*. Our findings were similar to those known in arthropods and are characteristic of changes considered as troglomorphy (Culver et al. 1995; Pérez-Moreno et al. 2016; Gonzalez et al. 2018).

Arm length is the most conspicuous trait of *O. commutabilis*, here we provide, for the first time, direct and quantitative evidence of this. As previously mentioned, elongation of body appendages is well documented in cave fauna as a troglomorphic trait, hence, arm elongation represents a potential morphological cave adaptation in *O. commutabilis*. The larger number of segments in *O. commutabilis* suggests that elongation is the result of having more segments than its epigeal congener. Each segment has two

tentacle pores, thus cave-dwelling individuals have more tentacle pores and therefore more tube feet, as well as more spines. It has been proposed that appendage elongation increases the ability of cave fauna to locate food, avoid predation or improve sensory capability (Ilfte and Kornicker 2009; Mejía-Ortíz et al. 2006, 2013). Given that both spines and tube feet are important structures in sensory reception of ophiuroids (Hajduk 1992; Zueva et al. 2018), our results are relevant in giving a plausible explanation about the origin and function of adapted elongated arms. Additionally, these results confirm quantitatively the first report of a species of Ophionereididae having arms that long (Stöhr et al. 2012; Bribiesca-Contreras et al. 2019) in average and as maximum (Table 5). This is valid not only for Caribbean species but also for species from the Pacific Ocean (e.g. *Ophionereis variegata* with an arm length average of five times the size of the disc or *O. amoyensis* with arms ten times the size of the disc) (Clark 1953).

Distal tube feet play an important role on detritus and suspension feeding (Warner 1982), which could explain *O. commutabilis* having longer distal tube feet than *O. reticulata*, suggesting that detritus and suspended particles are also (Mejía-Ortíz et al. 2013) an important source of food in the cave El Aerolito. On the other hand, oral tentacle, as well as proximal and middle tube feet, are commonly less specialized (Keogh and Keegan 2006), which provides an explanation for cave species not having longer structures than epigeal congeners.

The tube feet, as well as their relation with arms in cave ophiuroids, need to be studied more deeply, as these structures probably play an important role in the evolution of cave ophiuroids. This is suggested by the striking differences in length and function among cave species and their epigeal congeners demonstrated in *A. stygobita* (Carpenter 2016; Pomory et al. 2011), *O. xmasillumins* (Okanishi et al. 2019) and *O. commutabilis* (Table 5), and between the cave species itself. Concerning arm length, the results obtained in this study are similar to that observed in *O. xmasillumins* (Okanishi et al. 2019) and contrast with those observed in *A. stygobita* which have shorter arms (Pomory et al. 2011). Given that abiotic conditions are expected to be similar in the three caves, these differences could support the theory that elongation is associated with food-finding abilities or colonization times rather than specific abiotic factors (Delić et al. 2016).

Our results allowed us to confirm that *O. commutabilis* is distinguished by reaching big sizes in comparison to other Caribbean species of the genus (Bribiesca-Contreras et al. 2019) (Table 5). This is striking on a cave species, given that usually this fauna has a reduction in size as a response to a diminished food supply or quality (Culver et al. 1995; Culver and Pipan 2009). This could respond to the low predation rate expected for a cave environment (Gibert and Deharveng 2002) and the high population density of *O. commutabilis* in El Aerolito (Calderón-Gutiérrez and Solís-Marín 2014), factors that have been proposed to favor the survival of bigger organisms (Gage 1990; Bishop and Ilfite 2009). Larger bodies could also be advantageous in terms of energy economy and fecundity, important aspects in a cave (Trontelj et al. 2012). Interestingly, two species inhabiting similar environments show opposite patterns of population densities (low in *A. stygobita* and high in *O. commutabilis*) (Carpenter 2016) and relative sizes (small in *A. stygobita* and big in *O. commutabilis*) with respect to their epigeal congeners.

The contrasting results of this study (*O. commutabilis*) compared with those obtained by Pomory et al. (2011) in *A. stygobita* point out the need for more studies on stygobiotic echinoderms. This will improve our understanding of troglomorphisms in the phylum and it would provide useful information to understand the evolution of marine organisms in caves.

Regeneration frequency was lower in *O. commutabilis* (32% of specimens and 14% of arms) than *O. reticulata* (70% and 35%). *O. commutabilis* shows similar percentages to *A. stygobita* (35% and 14%) (Carpenter 2016), another cave species. On the other hand, the regeneration frequency of *O. reticulata* is similar to that observed in other populations of this species and other epigeal species (Lawrence and Vasquez 1996; Yokoyama and Amaral 2010). Therefore, the low incidence of damage in *O. commutabilis* indicates that sub-lethal predation is low inside El Aerolito as expected. This could be related with specimens inside the cave being usually found exposed rather than hidden (Bribiesca-Contreras et al. 2019: supplementary video 3), corresponding to the reduced escape behaviour observed on cave fauna (White and Culver 2012).

Stereom organization and porosity is the most conspicuous trait potentially considered as paedomorphic, being more porous in all the analysed ossicles of the cave species. Similar patterns have been proposed as an adaptive trait for deep-sea and cave brittle stars, improving feeding and gas exchange mechanisms and enhancing chemo and mechanoreception in harsh environments (Pomory et al. 2011; Carpenter 2016; Stöhr and Martynov 2016). We suggest that tentacle scales, DAP and VAP shape of *O. commutabilis* are paedomorphic traits based on the resemblance between mature cave specimen's ossicles and that of juvenile *O. reticulata*. Moreover, Bribiesca-Contreras et al. (2019) described conspicuous primary plates as a usual trait of *O. commutabilis*, which has been suggested as a paedomorphic trait in ophiuroids (Pomory et al. 2011; Martynov et al. 2015). Therefore, it seems evident that like cave brittle stars from Bahamas (Pomory et al. 2011), *O. commutabilis* specimens show paedomorphic traits on skeletal plates, as previously reported for the size in *O. olivacea* (Byrne 1991). It must be noted that *O. commutabilis* does not show some typical paedomorphic traits such as segment length-to-width ratio close to 10 (Stöhr and Martynov 2016). Moreover, some of the traits here reported as paedomorphic could be the result of other factors affecting the biology of this stygobiotic species (e.g. stereom development could be explained by abiotic factors in the cave). However, it must be considered that there are different levels of heterochronic changes affecting ophiuroids, which implies different levels of difficulty to recognize these characters, as well as different evolutionary effects on the species (Stöhr and Martynov 2016). The resemblance in DAP and VAP shape between *O. commutabilis* and the other Caribbean species (Table 5) should be studied more deeply to understand the evolutionary patterns of these structures in the group as well as the impact of a harsh environment such as caves in this. More species should be compared concerning the potentially paedomorphic traits presented in this work to define whether *O. commutabilis* present paedomorphosis.

The number of hydropores is equal among species of highly different geographical distribution, but inhabiting similar environments as *O. reticulata* and *O. schayeri* (Ezhova et al. 2016). However, that number is very different between closely related

species as *O. reticulata* and *O. commutabilis*, which inhabit very dissimilar environments. This is consistent with the hypothesis claiming that madreporites are ecologically informative (Ezhova et al. 2016). The differences among species in the type of teeth are relevant if we consider that teeth types have been used as an indicator of feeding preferences (uniform teeth for macrophagous feeders and compound for microphagous) (Medeiros-Bergen 1996; Brogger et al. 2015). Five ophionereidids have been reported to possess only compound teeth (Medeiros-Bergen 1996), while *O. commutabilis* shows both compound and uniform teeth, which could represent an adaptation, increasing the importance of omnivorous feeding in this cave system (as suggested by Bribiesca-Contreras et al. (2019) for other food sources). An alternative explanation for the presence of uniform teeth in *O. commutabilis* could be that they represent underdeveloped teeth, either for being ventral (the youngest according to Hendler (2018) or for the slow growth rate of cave organisms (Carpenter 2016). Though all the teeth were obtained from the ventral and dorsal portions of specimens of similar size, this explanation (being underdeveloped teeth) must be considered in further studies.

The presence of EPTs on DAP is conspicuous across the genus *Ophionereis*, not only in Caribbean species like *O. reticulata* but also in the Indo-Pacific species *O. porrecta* and *O. degeneri* (figures 15c and 16c in: Stöhr (2011)). Furthermore, based on figures from available literature, ophionereidids have higher EPT densities than species of the genus *Ophiocoma* (e.g. 15 vs 498) (Hendler and Byrne 1987) and *Ophiopsila* (e.g. 96 vs 498) (Deheyn et al. 2015). Though *O. commutabilis* shows expanded peripheral trabeculae on dorsal arm plates, these structures show a different pattern that decreases in density in contrast to its epigeal congeners. These differences could suggest the effects of low energy and darkness typical of caves, in the photoreceptor system of this species. Moreover, the cave specimens DAP show a disorganized inner mesh, further suggesting stereom organization being reduced as an expensive or dispensable trait, as proposed for cave-dwelling taxa (Klaus et al. 2013). Concerning inner stereom it is especially relevant since it affects chromatophore activity (Hendler and Byrne 1987; Sumner-Rooney et al. 2018), suggesting an important impact of darkness in *O. commutabilis* life as previously discussed for the diversity of colouration of this species (Bribiesca-Contreras et al. 2019).

Stereom of cave specimens are similar to that of deep-sea ophiuroids in having less defined expanded peripheral trabeculae (Hendler and Byrne 1987), both environments having similar conditions (e.g. absence of light and low energy). On the other hand, agglomeration of EPT has been reported for bioluminescent species, particularly conspicuous in *Ophiopsila californica* (Deheyn et al. 2015). In addition, transmittance differences (due to whether the light beam passes through EPT first or last) on DAP of the cave species are similar to differences observed among bioluminescent (*Ophiopsila californica* and *Amphipholis squamata*) and non-bioluminescent (*Amphipholis pugetana*) species (Deheyn et al. 2015). Therefore, our results suggest that both photoreception and bioluminescence processes have affected the evolution of this species and confirm that differences in morphology of DAP between both species have an impact on light transmittance.

The lower transmittance peak observed in the two analyzed species corresponds to wavelengths that activate phototaxis in ophiuroids (Delroisse et al. 2016). This

adds evidence to the hypothesis that this species diverged from a shallow water lineage (Bribiesca-Contreras et al. 2019). On the other hand, the highest peak is usual in ophiuroids, since it corresponds to wavelengths attenuated on the first 10–20 cm depth (Deheyn et al. 2015). Finally, these results highlight the necessity of more studies to understand if the phototaxis in ophiuroids is related to their relative success in caves (in comparison with the other classes of the phylum).

Cave fauna shows particular morphological traits that could be considered to be troglomorphisms if they allow organisms to successfully colonize these harsh environments (Culver and Pipan 2009; Gonzalez et al. 2018). This paper demonstrates that brittle stars inhabiting an anchialine cave in Cozumel show troglomorphic traits such as arm (through the adding of segments) and distal tube feet elongation, increase in size, and possibly paedomorphic traits. This is, to our knowledge, the second study investigating troglomorphic adaptations in a group of echinoderms; our work confirms that *O. commutabilis* can be characterized as a stygobiotic species as demography, distribution and origin of this species suggested (Bribiesca-Contreras et al. 2013, 2019).

Conclusion

In conclusion, troglomorphic traits of *Ophionereis commutabilis* include elongation of arms (as a result of the addition of segments) and increased sizes, similar to those observed for other cave fauna. Additionally, potentially paedomorphic traits are reported for an ophionereidid. Finally, the morphology of *O. commutabilis* confirms it as a stygobiotic species as demography, distribution and origin of this species previously suggested.

The authors have declared that no competing interests exist.

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Supplementary material 1

Supplementary figures S1–S3

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Data type: multimedia

Explanation note: **Figure S1**. Sets of Landmarks (LM) configurations designed to register the shape of the dorsal (A), ventral (B) and lateral (C–D) arm plates. Orientation (p: proximal, di: distal, do: dorsal, v: ventral). **Figure S2**. Scanning electron micrograph (SEM) of dental plates. SEM of dental plates from *Ophionereis commutabilis* (a-b) (modified from Bribiesca-Contreras et al. 2019) and *O. reticulata* (c-d), showing external (a, c) and internal faces (b, d). Scale bars: 500 µm. **Figure S3**. Scanning electron micrograph (SEM) of teeth from *Ophionereis commutabilis*, showing compound (a) and uniform teeth (b). Compound teeth from *O. reticulata* (c-d). Scale bars: a-c, 300 µm; c-d, 400 µm.

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Supplementary material 2

Supplementary table S1

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Data type: table

Explanation note: Arm features of the stygobiotic species (*O. commutabilis*) and its epigeal congener (*O. reticulata*).

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Supplementary material 3

R-scripts

Authors: Francisco Márquez-Borrás, Francisco A. Solís-Marín, Luis M. Mejía-Ortiz

Data type: statistical data

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