Cyanobacteria and associated invertebrates in Leontari Cave, Attica (Greece)

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Abstract: The present paper deals with biocommunities of cave Leontari, Attica (Greece) focusing on its lithophytic cyanobacteria, and the associated microfauna. The cave is of archaeological importance, not touristically exploited, naturally lighted through the entrance; it consists of one chamber with poor stalactite and stalagmite limestone decoration. During a survey in three campaigns, samples of cyanobacteria and soil invertebrates were collected from four sites (I-IV) along a light(PAR)-temperature-humidity gradient. Light microscopic observations of natural and cultured material have shown that epilithic and endolithic cyanobacteria were almost the exclusive component of cave photosynthetic microflora. Twenty two taxa were identified including the taxonomically interesting morphotypes Chroococcus spelaeus, Asterocapsa sp. and Chlorogloea sp. Arthropods were found as dominant soil invertebrates represented by nine taxa.

Key words: Karstic environment, caves, cyanobacteria, soil invertebrates, Greece

Introduction

Caves are the most typical and well known of the various karstic formations geologically created on limestone substrates. Cave ecosystems belong to hypogean environments with rather stable conditions throughout the year, thus providing refuges for the existing microflora and microfauna. Cave communities are generally characterized by relatively low species richness, biomass and density (Albertano 1993, Bruno & Albertano 1999, Albertano & Urzi 1999, Ducarme et al. 2004, Poulíčková & Hasler 2007).


The specific caves’ environment may favour speciation, and up to date, some new cyanobacteria taxa have been established, such as Spelaeopogon sommierii Borzi (1917), Geitleria calcarea Friedmann (1955), Chroococcidiopsis kashaii Friedmann (1961), Herpyzonema pulverulentum Hernandez-Marine & Canals (1994), Chlorogloea novacekii Komárek & Montejano (1994), Symphyonema cavernicolum Ascencio, Aboal & Hoffmann (1996). Furthermore, morphotypes found in caves and not yet established as new species of cyanobacteria [e.g. Gloeethece sp. (Sant’Anna et al. 1991), Loriella sp. (Hernandez-Marine & Aboal et al. 1999)] exhibit characteristics in morphology and ecology distinct from those of
the corresponding type species. However, it is noted that representatives of other groups of plants (microalgae such as chlorophytes and diatoms, as well as mosses, lichens, pteridophytes and even phanerogams), mainly thriving at cave entrances or surviving even deeper in lower light supply, show modifications in structure or propagation which were considered only as ecological adaptations, thus restricting the number of species which could be defined as genuine ‘cave plants’ (see Racovitza 1907).

On the contrary, the cavernicolous fauna may be classified into four categories based on the degree of cave adaptation, according to Barr (1963): a) troglobites (species exhibiting a high degree of anatomic modification and depending upon caves), b) troglophiles (species facultative cavernicolous since they can complete life cycle in a cave but they may also live in epigean conditions ecologically similar to the casual epigean visitors), c) trogloxenes (species frequent thriving in caves and unable to complete their life cycle there but not exhibiting some kind of cave modification), and d) epigeans (surface organisms whose presence in caves is accidental). Invertebrates, especially arthropods, make up the majority of the existing cave organisms in terms of species variability and abundance (Wellborn 1999).

Despite the extended karstic environment in Greece with a high number of cave formations, a few biological inventories have been compiled (e.g. Paragamian et al. 1987, Kollaros et al. 1987, 1991). These inventories are restricted to a simple record of some endemic animal species without any estimation either of species abundance and population dynamics or any other ecological approach of the cave ecosystems; there is a complete lack of knowledge regarding the structure of subterranean communities as well as the trophic relationships among microflora, invertebrates and vertebrates inside the cave ecosystems and, finally, the interactions between cave inhabitants and cave microclimate.

The present study focuses on the taxonomy of cyanobacteria in one Greek cave (cave ‘Leontari’) and their distribution in relation to the main abiotic variables, as well as on the associated invertebrate soil communities.

Materials and Methods

The cave ‘Leontari’ is located on the eastern slopes of Korakovouni, Hymmetos mountain, in Attica (Greece; coordinates 37° 59' 12” N and 23° 49' 47” E; altitude 550 m a.s.l.). It consists of one chamber of approx. 50 m long, 20 m wide and 11 m high (see scheme) with poor stalactite and stalagmite limestone decoration, and it is touristically unexploited. The cave was named after a marble statue of a lion (‘leon’ in ancient Greek, ‘leontari’ in modern Greek) found there, dated back to the 5th-4th century BC (Chatzilazaridis 1979).

Samplings were made on the 25th of March 2005, 28th of October 2005, and 27th of July 2006. Four sampling sites (I–IV) were selected inside the cave, at different distances from the cave entrance, representing distinct environmental conditions and growth habits (Figs 2–6). Temperature (T °C), relative humidity (RH%) and photosynthetically active radiation (PAR μmol.s⁻¹.m⁻²) were measured at each sampling site and sampling date by a LI-1400 data logger (LI-COR Biosciences, USA).

Four subsamples of cave microflora were collected from the cave decoration (stalactites, stalagmites) and limestone walls in each site under sterile conditions, and material was partly fixed with formaldehyde solution 2.5% and partly kept alive for culturing. Invertebrates were extracted from two randomly chosen soil sample units of 150 cm³ in each site using a Berlese-Tullgren apparatus, were kept there for 10 days and finally were preserved in 75% ethanol solution with 5% glycerin.

Enrichment cultures were obtained in flasks and petri-dishes with BG11 liquid medium (Stanier
Figs 1–9. Cave ‘Leontari’, Sampling sites: (1) Entrance of the cave; (2, 3) Sampling site I; (4) Sampling site II; (5) Sampling site III, (a, b) extensive growths of Chlorogloea sp.; (6) Sampling site IV; (7) Acarina, scale bar 2mm; (8) Collembola, scale bar 1mm; (9) a member of Acarina (left) and a larva of Diptera, scale bar 1mm.
Figs 10–19. Cyanobacteria found in cave ‘Leontari’ (LM): (10) Epilithic and chasmoendolithic growths of Chlorogloea sp. (stereomicrograph); (11–13) Chlorogloea sp., scale bars 5μm, 10μm, 15μm correspondingly; (14, 15) Asterocapsa sp., scale bars 5μm, indicating envelopes with wart-like projections; (16) Chroococcus turgidus, scale bar 15μm; (17) Chroococcus spelaeus, scale bar 15μm; (18) Chroococcus turgidus mixed with Chroococcus spelaeus, scale bar 15μm; (19) Chroococcus tenax with the characteristic lamellated sheath, scale bar 15μm.
Table 2: List of the identified Cyanobacteria from the sampling sites (I–IV) of Cave ‘Leontari’.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>sampling site</th>
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<tr>
<td>Aphanocapsa muscicola (Meneghini) Wille 1919</td>
<td>I, II</td>
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<tr>
<td>Asterocapsa sp.</td>
<td>I</td>
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<td>Chlorogloea sp.</td>
<td>II, III</td>
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<td>Chroococcidiopsis doonensis Singh 1968</td>
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<td>Chroococcus cohaerens (Brébisson) Nägeli 1849</td>
<td>II</td>
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<tr>
<td>Chroococcus minor (Kützing) Nägeli 1849</td>
<td>I, II, III</td>
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<td>Chroococcus minutus (Kützing) Nägeli 1849</td>
<td>II</td>
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<td>Chroococcus spelaeus Ercegović 1925</td>
<td>I, II</td>
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<td>Chroococcus tenax (Kirchner) Hieronymous 1892</td>
<td>I</td>
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<tr>
<td>Chroococcus turgidus (Kützing) Nägeli 1849</td>
<td>I, II</td>
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<td>Gloeocapsa biformis Ercegovič 1925</td>
<td>I</td>
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<td>Gloeothece palea (Kützing) Rabenhorst 1865</td>
<td>I</td>
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<td>Hassalia byssoida Hassal ex Bornet et Flahault 1888</td>
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<td>Leptolyngbya boryana Anagnostidis et Komárek 1988</td>
<td>I</td>
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<td>Leptolyngbya gracillima (Zopp ex Hangirg) Anagnostidis et Komárek 1988</td>
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<td>Leptolyngbya lurida (Gomont) Anagnostidis et Komárek 1988</td>
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<td>Leptolyngbya tenuis (Gomont) Anagnostidis et Komárek 1988</td>
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<td>Phormidium cf. molle Gomont 1892</td>
<td>I, II</td>
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<td>Pseudocapsa dubia Ercegović 1925</td>
<td>I, II</td>
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<tr>
<td>Pseudophormidium hollerbachianum (Elkenkin) Anagnostidis 2001</td>
<td>I, II</td>
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<tr>
<td>Schizothrix cf. lardacea Gomont 1892</td>
<td>II</td>
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<tr>
<td>Scytonema julianum (Kützing) Meneghini 1847</td>
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et al. 1971). Cultures were maintained under daylight (north facing window) at room temperature. For the microscopic investigation of endolithic cyanobacteria the overgrowth was removed and the calcareous substrate was dissolved using Pereny’s solution (10% HNO$_3$, 0.5% Cr$_2$O$_3$, 95% C$_2$H$_5$OH in proportion 4:3:3) (Börnet & Flahaut 1889), and the extracted individuals were observed on glass slides under a high-resolution light microscope (Photomicroscope III, Zeiss, Germany). Invertebrates were identified to the order level and counted using a stereo-microscope (Stemi 2000-C, Zeiss, Germany); the specimens were recorded as individuals.m$^{-3}$.

**Results**

Environmental variables measured in the cave under study were found more or less stable during sampling periods as given in Table 1. The highest variations have been observed during the hot and dry period of sampling, July 2006.

Nine taxa of invertebrates were found in ‘Leontari’ cave, the arthropods being the majority (Table 3). The highest densities of Diptera, Acarina and Collembola were recorded in March 2005 near the entrance of the cave; in the hot and dry period (July 2006) no invertebrate was found. The morphological features in all individuals found correspond to the epigean mode of life without specific cave adaptation (Figs 7–9).

Natural and cultured materials were dominated by cyanobacteria, although some coccal and colonial chlorophytes and also bryophytes were observed both on the calcareous walls and in soil samples of the cave (not listed here). A total number of 22 taxa of cyanobacteria were identified: 13 chroococcalean, 7 oscillatorialean and 2 nostocalean (Table 2).

Deviations from the types in various morphological features were noted in certain cyanobacteria (i.e. Phormidium cf. molle, Gloeocapsa biformis, Hassalia byssoida, Chroococcus tenax; Fig. 19). The unusual morphological features ascertained are small and considered as being within the limits of the microorganismsms’ variability. However, the following three morphotypes (identified as Chroococcus spelaeus, Asterocapsa sp. and Chlorogloea sp. both in fresh and cultured material) require further taxonomical interest.

**Chroococcus spelaeus** Ercegović 1925

(Figs 17, 18)


Taxonomic remarks: According to Ercegović (1925, p.76–77), Chroococcus spelaeus is distinguished in two varieties, var. aerugineo and var. violacescens, differing only in cell colour. Our material due to the violaceous cell colour could be identified as C. spelaeus var. violacescens. However the taxonomical value of this feature is controversial (Cohen-Bazire & Bryant 1982, Coyler et al. 2005) and need molecular or experimental verification. It is noted that Komárek & Anagnostidis (1998) do not mention varieties under the type species considering it with unknown distribution.

Distribution: C. spelaeus was found as epilithic and cryptoendolithic at the sampling sites I, and II in association with Schizothrix sp., Aphanocapsa montana and Leptolyngbya gracillima.

**Chlorogloea sp.** (Figs 10–13)

Colonies mucilaginous in both macroscopic and microscopic observation. Colonies usually gelatinous, irregularly hemispherical, or composed of numerous sub-sphaerical, amorphously agglomerated sub-colonies; linear sub-colonies are also observed. Sub-colonies consisting of rather densely packed, usually unsheathed cells. Cells spherical or sub-spherical, irregular, polygonal-rounded or sometimes elongated, (2)–3–4–(5) μm in diameter (average±standard deviation: 3.18 ±0.62 μm).

Taxonomic remarks: The specimens found correspond to the diacretical features of the little known genus Chlorogloea Wille (1900) with 18 species (see Komárek & Anagnostidis 1998). The only species of the genus established from cave environments is Chlorogloea novacekii (Komárek & Montejan 1994), which differs from our material in cell dimensions and the
general thallus morphology. On the other hand, our material corresponds to the morphology of the freshwater species *Chlorogloea microcystoides* Geitler (1925), a species however with completely different ecology (KOMÁREK & MONTEJANO 1994). Therefore, further investigation with molecular tools is needed for clarifying the taxonomic position of the observed morphotype.

**Distribution:** The taxon was found to have cryptoendolithic mode of life in sampling sites II and III; it retains its morphological characteristics in cultures growing well at least for a period of six months. In sampling site III (characterized by very low PAR) it forms extensive, almost monospecific grayish, pale blue-green to pale olive-green endolithic growths.

*Asterocapsa* sp. (Figs 14, 15)

Colonies spherical, consisting of 2–8 cells. Cells or colonies enveloped by distinct, delimited, usually firm mucilage. Cells 3–5 μm (average: 3.8 μm), spherical or subspherical, oval or irregular in outline, sometimes slightly elongated or polygonal rounded. Common envelopes thin or thick with a surface covered by minute or stout, wart-like projections of different length.

**Taxonomic remarks:** Our material corresponds to the diacritical features of genus *Asterocapsa* CHU (1952). Two stages of lifecycle were observed: a) cells solitary or in small, 2–4 to few-celled colonies, enveloped only by a firm sheath which splits after cell division (‘status arthrocytosus’ sensu LEDERER 2000) and b) cells in 4–8 celled clusters, sometimes with nanocyte-like cell division within colonies and daughter cells liberated with their own spiny sheaths (early stages of ‘status nanocytosus’). The third stage (i.e. irregular-shaped cells enclosed in spiny sheaths and localized in an amorphous gelatinous mass sensu KOMÁREK & ANAGNOSTIDIS 1998, or ‘status familiaris’ sensu LEDERER 2000), was not observed in our material.

The morphological characteristics of our specimens differ in cell dimensions and details of life cycle to those of *A. divina*, a species reported from calcareous rocks (MONTEJANO et al. 2008), and the only one from caves (ABOAL et al. 2003). We are of the opinion that the *Asterocapsa* found in cave ‘Leontari’ represents a new species, however ultrastructure and molecular data are needed.

**Distribution:** Genus *Asterocapsa* CHU (1952) was described from China, and has been revised by KOMÁREK (1993). All known species were recorded out of Europe, except of the epiphytic *Asterocapsa aerophytica* recorded from Slovenia (LEDERER 2000). The tropical species *Asterocapsa divina*, described from Mexico (KOMÁREK 1993), is the only one reported from caves (from a limestone rock cave in SE Spain, ABOAL et al. 2003).

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**Table 3. Mean density (ind.cm⁻³) of soil Invertebrates found in Cave ‘Leontari’ [(I–IV) sampling sites; (M) March 2005; (J) July 2006; (O) October 2005].**

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* mainly Oribatei

** larvae of Chironomidae
Discussion

Cyanobacteria from field material of cave ‘Leontari’ show an epilithic or endolithic growth habit. Epilithic assemblages form extensive dark-green to olive-green coverings (with *Pseudocapsa dubia* as dominant species) or pale blue-green to whitish coverings (with *Scytonema julianum* as dominant species); among the epilithic cyanobacteria found, *Scytonema julianum* is reported as a typical species of limestone caves (Ascencio & Aboal 1996, Hernández-Mariné et al. 1999, Roldan & Hernández-Mariné 2009).

Endolithic assemblages form a grayish monospecific patina (*Chlorogloea* sp.), or mixed populations consisted of chasmoendolithic and/or cryptoendolithic species, i.e. *Aphanocapsa muscicola, Chroococcus turgidus, Gloeocapsa biformis, Chroococcidiopsis doonensis, Gloeocapsa dubia, Leptolyngbya gracillima* and *Leptolyngbya gracillima*. It is noted that very little is known about the growth and distribution of chasmoendolithic taxa in caves. Ascencio & Aboal (2000) reported a similar biocoenosis of chasmoendolithic taxa from cave-like environments; some of the taxa reported there (*Chroococcidiopsis doonensis*, *Gloeocapsa biformis*, *Phormidium molle*, *Pseudocapsa dubia*, *Leptolyngbya gracillima* and *Schizothrix sp.*) were also identified from the cave under study (see Table 2).

The highest species richness of cyanobacteria (17 taxa) was observed at sampling site I, where PAR was the highest. On the contrary, the lowest one (2 taxa) was observed at sampling site III. Extensive monospecific, epilithic and endolithic growths of *Chlogloea* sp. covered the calcareous surfaces (Fig. 10). No cyanobacteria were found at site IV.

Similarly, the highest invertebrate densities were found near the cave entrance (site I). In addition, the dominance of arthropods in the invertebrate community is in accordance with data from caves (e.g. Wellbourn 1999), whereas the density of microarthropods (Collembola and Acarina) is much lower comparing with that of caves (e.g. Ducarme et al. 2004). Furthermore, the soil invertebrates found in cave ‘Leontari’ show dominant orders (Collembola, Acarina, and larvae of Diptera) and temporal distribution (a density peak in spring with the lowest value during the hot and dry period of the year) similar to the pattern of epigean invertebrates from mediterranean ecosystems such as pine (*Pinus halepensis*) forests, shrublands and phrygana (Geoffroy et al. 1981, Flogaitis 1984, Radea 1989, Karamaouna 1990, Maggioris 1991), and do not exhibit any anatomic modification. These ecological and morphological adaptations characterize the ‘obligate cave dwellers’ sensu Barr (1963). Therefore, the invertebrates found in cave ‘Leontari’ could rather be characterized as ‘trogloxenes’ or even ‘epigean animals’.

Studies on functional feeding types of soil invertebrates have shown that a high proportion of food material for Collembola, Acarina (except Gamasina) and Diptera (larvae of Chironomidae) consists of microalgae (grazers-microphytophages) even in epigean ecosystems where a variety of food sources (such as humus, higher plant material, mycorrhizae, fungal material and pollen) is available (Wallwork 1970, Peterson & Luxton 1982, Ponge 2000). Therefore, it is supposed that the invertebrates collected in cave ‘Leontari’ may use the associated microalgae as food resource, since they were found in abundance at sites characterized by a rich cyanobacterial growth.

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References


