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Biodiversity and conservation correlation in the case of a Roman fresco located in a semi-confined environment

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A R T I C L E I N F O

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A B S T R A C T

The subterranean heritage includes both natural and built sites with a strong cultural and historical fingerprint, some of each being enriched with painted surfaces. These semi-confined environments shelter specific and fragile biodiversity. This paper is focused on the case of a Roman painting (2nd-3rd century AD) located in an underground archaeological site in Marino Laziale, near Rome, which was opened to the public for the first time in 2021. The painted Mithraic scene is in a good state of conservation. The methodological approach included on site and laboratory investigations aimed to screen the main biological components associated to this hypogeum monument. The observed biodiversity included heterotrophic and chemolithotrophic microorganisms, and a mesofauna composed of eutroglophile and subtroglophile species, characteristic for many subterranean environments. The ecological mechanisms and the conservation state of the work of art were analyzed for planning the best fruition practices. The aesthetic change, the possible mechanical damages induced by various organisms, and the presence of significant amounts of organic matter, represent the main risks for painting conservation. These aspects, beside the new possible risks associated with the presence of visitors, are under a constant and ongoing conservation surveillance program.

1. Introduction

The subterranean cultural heritage is found both in natural environments, such as caves (Geneste and Mauriac, 2014; Lasheras et al., 2014; Mulec, 2014; Ontañon et al., 2014; Pakhunov et al., 2014), and also in spaces where the original underground morphology was modified by the man by carving or by preparing the walls for painting (Ma et al., 2023; He et al., 2021; Tapete et al., 2014). These last ones include hypogean structures such as catacombs (Cuzman et al., 2014; Gomez-Bolea et al., 2014), necropolis (Fernandez-Cortes et al., 2014), rupestrian churches (Nugari et al., 2009), tomb-chapels (Galan, 2014), Roman cisterns (Bedetti, 2010), and water tunnels (Garcia-Sanchez et al., 2014). Different artworks may be hosted on some walls, such as rock art (Intxaurbe et al., 2020), graffiti (Galan, 2014) or paintings (Nugari et al., 2009; Bedetti, 2010; Pakhunov et al., 2014), used by human beings as places of worship since prehistory until more recent times.

These locations are semi-confined environments influenced by the characteristics of overlying and surrounding terrain, the small variations of temperature, the higher concentration of carbon dioxide compared to the outside due to poor air circulation, and by the high and constant humidity value (RH > 70% and often higher than 90%) that can easily lead to condensation phenomena (Albertano et al., 2007; Sanchez-Moral et al., 2014). These conditions foster biological colonization. The biodiversity of the caves includes trophic webs with a complexity depending on the availability of the nutrients (Venarsky and Huntsma, 2018), hosting organisms that can be considered biodeteriogens if they alter a hypogeum artefact from chemical, physical, aesthetical, or mechanical point of view. The main components of the subterranean communities include microorganisms (mainly actinomycetes, bacteria and fungi), arthropods, and, occasionally, the phototrophic organisms in illuminated areas.

The hypogeum sites are fragile microecosystems (Bastian et al., 2008; Martin-Sanchez et al., 2015) that can be easily disturbed by anthropic presence (Mulec, 2014; Frasca et al., 2020). For example, an increase of CO_2 level due to the presence of visitors may induce carbonation phenomena, while the use of light systems can lead to the

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development of phototrophic biofilms. Moreover, the visitors may bring allochthonous species that may disturb the original biodiversity and the ecological equilibrium of the hypogean environments, by increasing of the air microbial load if no or insufficient air change is present (Saarela et al., 2004).

This work is focused on an old Roman cistern, located in Marino Laziale, near Rome, where a painted Mithraic scene (2nd-3rd century AD) in a good state of conservation was discovered by chance in the 1960's (Bedetti, 2010). An exhibition area, to welcome the visitors, in front of the Mithraic gallery, protects this cultural heritage, which was opened to the public in 2021 for the first time. The hosted biodiversity related with the main biological components of flora, micro and mesofauna was investigated within different diagnostic campaigns through on-site investigations and laboratory investigations. Moreover, the microclimate was continuously monitored. The ecological mechanisms present in this hypogean environment and their possible influence on the conservation state of the Roman painting were analyzed, in order to propose the best fruition practices for the public.

2. Materials and methods

2.1. Hypogean site

The Mithraeum of Marino (Luvidi et al., 2021) is an important sanctuary of the Roman era, with a big wall painting (about 2,25 × 1,75 m) representing a tauroctony scene in the main central frame and other iconographies in the side panels, traditionally associated with the worship of Mithras (Fig. 1a). The main entrance of the gallery is about 2 m under the current street level and the wall painting located at its end is about 8–10 m under the ground level. The area (Fig. S1) was originally used as a cistern and it consists of a narrow corridor (about 30 m length, 3 m width, 3 m height) at the beginning of which, on the left- and right-hand sides, there are other two small frescoes representing the torchbearers Cautes and Cautopates (Fig. 1b and c).

2.2. Past and present investigation campaigns

Several on-site investigation campaigns were performed during the last 17 years with the aim to characterize the whole components of this hypogean site. These studies were performed not only to acquire information about its historical, cultural, and material value of the site, but also to better understand its conservation state and how to valorize it, preventing its equilibrium alteration.

The first scientific investigations in the Mithraic gallery were carried out by Istituto Centrale di Restauro (ICR, Rome, Italy) between 2006 and 2008 to characterize the painted and unpainted surfaces, their state of conservation and the environment in which they are located. The ICR results are summarized in **SM1**.

Since 2018, the Institute of Heritage Science of the National Research Council (ISPC-CNR, Italy) has been in charge of carrying out diagnostic evaluations for updating and defining the conservation state of the site (Luvidi et al., 2021). A continuous monitoring program of microclimatic data is still in progress, allowing the visitors access while maintaining safety conditions for the conservation of the paintings. With the same purpose, several surveys were performed in 2022 to evaluate the biological presence. Two campaigns with sampling were performed in September and October of the same year, while some non-invasive observations (microscopic and microclimatic) were performed monthly directly onsite.

2.3. On site investigations

2.3.1. Portable microscopy

The surface of the painting was observed by a portable digital microscope (Dino Lite Premier Digital Microscope AM7013MZT(R4), at 50× magnification. The microscopic observations were made randomly on surfaces presenting different alteration phenomena.

2.3.2. Microclimatic monitoring

In August 2018, the microclimate monitoring campaign was launched in the main spaces of the Mithraeum: the exhibition room and the Mithraic gallery. Microclimatic monitoring was performed with Delta Ohm sensors for a period of four years in order to analyze the thermo-hygrometric conditions of the environments. This monitoring documented the situation at the end of the restoration campaign and supported the new project and recovery intervention of the exhibition room in front of the gallery, aimed at opening the site to the public.

During 2022, several technical surveys were done to check the

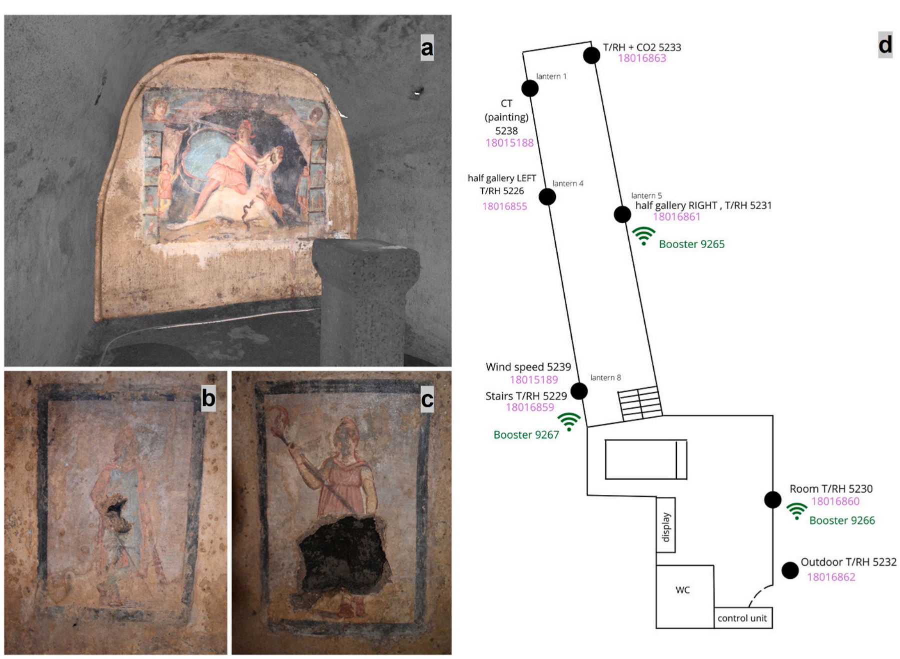

Fig. 1. 3D model of the Mithraic scene (a) located -at the end of the gallery (credits to Andrea Angelini, Roberto Gabrielli and Eleonora Scopinaro - RDR Lab CNR-ISPC, 2021), the images of the torchbearers located at the beginning of the gallery on the left and right walls (b, c) and positioning of the microclimate sensors (d).

correct functioning of microclimate sensors and signal boosters; some of them had to be replaced with new sensors due to malfunctions caused by the extreme humidity conditions (stagnation of water on the sensor and saline crystallizations which have damaged the battery and electronic components) or general blackouts of the power grid that interrupted data acquisition. Following the installation of a UPS system and the maintenance interventions on the Delta Ohm sensors, their current positioning (January 2023) is reported in the map in Fig. 1d: outside the main entrance to the site, in the Mithraic gallery and in the room in front of it; they are installed at different distances starting from the entrance to the gallery (staircase) up to the wall where the Mithraic scene is depicted and at different heights from the floor up to the vault (as the UNI 10829:1999 standard suggests).

All the microclimate sensors were provided by Delta Ohm company. Six sensors (model HD 35EDW N/3 TC) installed in the following positions: outdoor (at the entrance of the site), in the exhibition room and in the gallery hosting the wall paintings, record the hourly values of Temperature (°C) and Relative Humidity (RH%). One sensor (model HD 35EDW 1NBTV) was installed at the end of the gallery, close to the Mithraic scene, to detect hourly concentration of CO_2 (ppm) and one sonic anemometer (model HD52.3D) was installed in front of the stairs connecting the exhibition room with the gallery. Finally, three signal amplifiers have been positioned respectively in the middle of the gallery, next to the sonic anemometer and in the exhibition room to ensure proper data streaming from the sensors to the control unit that transmits the data to the cloud storage.

2.3.3. ATP measurements

The ATP (adenosine triphosphate) was measured in relative light units (RLU) by using a bioluminometer (3M™ Clean-Trace™ NG Luminometer) and its specific swabs (3M Clean-Trace Surface3M™) (Spada et al., 2021). The read values were expressed as RLU/cm^2. The selected areas for the ATP measurements are indicated in Table 1, while details of the technique are given in **SM2**.

2.4. Laboratory investigations

Sampling (Table 1) was performed in different ways in order to analyze the biotic component present in the area of the painting, avoiding overlapping on the same area as well as on the ones previously analyzed with the bioluminometer. Details are given in **SM3**. The samples for the faunistical investigations were collected with soft brushes, stored in ethanol 70% v/v.

2.4.1. Microscopy observations

For the microscopic observations, different microscopes (Nikon Eclipse E600, Axioskope Zeiss, Wild M5 and Wild M20 microscopes and a Dino-Lite digital microscope) were used. Details are given in **SM3**.

2.4.2. Isolation of bacterial and fungal strains

Bacteria isolation was performed on nutrient agar (VWR Chemicals) amended with glucose (0.25%) (NGA) and tryptic soy agar (Fluka analytical) media, both amended with cycloheximide (250 ppm), while potato dextrose agar medium (PDA) (VWR Chemicals) amended with streptomycin (100 ppm) was used for the fungi isolation. Details of the isolation procedures are reported in **SM4.**

2.4.3. Molecular analyses

One strain representative of each bacterial and fungal morphology was chosen to perform the DNA extraction. Details of the procedures are reported in **SM5**. The primer pairs and thermal conditions used for DNA amplification are described in Table SM1. The bacterial and fungal amplicons were purified by using the PureLink Quick PCR Purification Kit, Invitrogen. The purity and quantity of the extracted DNA were checked at NanoDrop ND-1000. DNA sequencing was performed by BMR Genomics srl (Italy).

Table 1
Investigated areas and the type of analysis performed on samples. In bold are indicated the main typologies of alteration that have been investigated, while FT = Fungi Tape, CS = Cotton Swab, ATP = adenosine triphosphate measurement, IFB = isolation fungi and bacteria, MA = molecular analysis of the isolates, MO = microscopic observations in laboratory.

Acronym of area	Sampling site and description of the alteration phenomena	Type of sampling	Type of analysis
1	outside of the fresco on the left-hand side, with various **dark stains difficult to remove**, presumable vermiculations	FT, CS	ATP, IFB, MA, MO
2	area painted with dark blue color, in the lower left part of the painting, with small and diffuse **white spots easy to remove**	FT, CS	ATP, IFB, MA, MO
3	light blue area in the second and third box (from top to bottom) on the left, with **dark stains difficult to remove**	FT, CS	ATP, IFB, MA, MO
4	white area in the bottom of the Mithraic scene with **small and diffuse deposits** of a dark color very visible in raking light, **easy to remove**	FT, CS	ATP, IFB, MA, MO
5	area of the coat, with **yellow, non-removable stains**	FT, CS	ATP, IFB, MA, MO
6	light blue area in the second box on the left (from top to bottom) of the Mithraic scene, with **dark stains difficult to remove**, similar to area 3	–	ATP
Ddx A	right torchbearer with respect to the site entrance, on the lower left part of the black frame, with small island-shaped **white spots**, some with a crystallization aspect, quite **easy to remove**	FT, CS	ATP, IFB, MA, MO
Ddx B	torchbearer on the right wall with respect to the site entrance, on the upper part of the background, with extensive **whitish stains**, **difficult to remove**	CS	ATP, IFB, MA, MO
Dsx A	torchbearer on the left wall with respect to the site entrance, on the lower left part of the frame, with small island-shaped **white spots** similar to DdxA, quite **easy to remove**	–	ATP
Dsx B	torchbearer on the left wall with respect to the site entrance, on the upper part of the background, with extended **whitish stains** similar to Ddx B, **difficult to remove**	–	ATP

Similarities of partial 16S rRNA and ITS rRNA sequences of the bacterial and fungal strains with known sequences in the NCBI GenBanK database, were determined by BLASTn (http://blast. ncbi.nlm.nih.gov/). BLAST results were obtained by only comparing sequences from type material. Sequences have been deposited in the EMBL/GenBank/DDBJ nucleotide sequence data libraries.

2.4.4. Fauna observations

For the mesofauna observations in situ a Dino-Lite digital microscope was used. During the survey in October 2022 some specimens of each species were also collected for taxonomic identifications under microscopes in the laboratory following Vandel (1960, 1962), Schmalfuss (2003), Mammola et al. (2018). The microfauna was observed on the FT tape samples (Table 1) through microscopic observations in the laboratory with the help of Nikon Eclipse E600 and Axioskope Zeiss microscopes.

2.4.5. Nucleotide sequence accession numbers

The partial 16S rRNA bacterial and the ITS rRNA fungal gene sequences were deposited in the EMBL/GenBank/DDBJ nucleotide sequence data libraries with the following accession numbers: A =

OQ247888; B=OQ248031; G = OQ248063; H=OQ248064; I=OQ248065; M = OQ248067; S=OQ248079 for bacteria and OA1 = OQ257345; OA2 = OQ257346; OA4 = OQ257370; OA10 = OQ257371 for fungi.

3. Results

3.1. On site investigations

3.1.1. Portable microscopy

The on-site observations evidenced the presence of different visible alteration phenomena (Table SM2), some of them being randomly and widely extended on large areas of the site walls, independently if these surfaces are painted or not. This is the case of the black stains attributed by previous investigations to vermiculation phenomena (§2.2) and of the light-brown deposits attributed by this work to the fecal pellets belonging to mesofauna. The microscopic analysis performed on site revealed evidence of changing morphology of the fecal pellets (Fig. 2a, f) induced by colonization and degradation of microorganisms (Fig. 2c–e). This phenomenon occurs not only on the top of the surfaces but also inside fissures and holes present on the painted surfaces (Fig. 2b), being widely distributed as well. An attentive observation of the surfaces revealed the presence of plentiful arthropods moving all around the walls and investigation details are reported below in §3.3.

3.1.2. Microclimatic monitoring

Compared with the external environment, a substantially stable microclimatic frame of constant temperature and relative humidity emerged through the monitoring campaign.

In the warmer months the temperature in the Mithraic gallery is lower than the external one (between 16 and 17 °C) while in the cold months the opposite occurs (8–13 °C) (Fig. 3a). The phenomenon that needs to be monitored is surface water condensation on the wall paintings, especially on the Mithraic scene. In fact, in the gallery dew temperature varies between 15 and 17 °C in the summer period and between 12 and 15 °C in the autumn period. These values, together with the year-round high relative humidity (100%) (Fig. 3b), cause the phenomena of water vapor condensation and its following drying of the painted surface, causing the formation of salt efflorescence. The daily variations of T(°C) and RH(%) are minimal and therefore negligible. By analyzing the variation and trend of CO_2 levels (Fig. 3c), it emerged that the natural average concentration of the gas on the site without visitors is around 500 ppm. Consequently, regular peaks high up to twice this value testify the presence of visitors. In fact they are mostly recorded on Saturdays and Sundays, the opening days of the Mithraeum dedicated to two small groups of 15 visitors per day, staying maximum 30 min in the site. It takes around 6 h to return to normal levels of concentration after the visits in the summertime and 8–12 h in the cold season. Other details of microclimatic monitoring are given in Fig. 3 and **SM6**.

3.1.3. ATP measurements

ATP measurements performed on-site confirmed the presence of important amounts of microorganisms on investigated surfaces, and the further laboratory investigations (§3.2) helped to identify the main cultivable bacterial and fungal strains through molecular techniques. The ATP levels (Fig. 4) ranged between 14 327 RLU/cm^2 (area 6) and 24 010 RLU/cm^2 (area 3), with the lowest ATP amount (5035 RLU/cm^2) registered for area 4 which contains mainly fecal pellets. The measurements made on the black stain alterations revealed similar values for area 1 (outside of the fresco) and area 6 (on the fresco), while area 3 showed the highest values. Greater values were also observed for the areas containing the white spots with crystallization like morphology (areas Dsx A and Ddx A), followed by the yellow stains alteration and the other two types of white alteration (areas 2, Ddx B and Dsx B). The high values obtained for the areas containing the white spots with crystallization like morphology can be correlated with the microbial degradation process of the organic material observed on the surface (Fig. 2c–e). Nevertheless, the lowest ATP value was registered for the area 4 (Fig. 4), this result is not neglectable, indicating the presence of active microorganisms both on the surface and inside the fecal pellets (these ones being probably quite fresh due to their well-defined morphology).

3.2. Isolation and molecular identification of microbial strains

Several bacterial and fungal colony types were observed on the agar plates after the appropriate period of incubation. Twenty-six bacterial and twelve fungal isolates showing various colony morphologies were picked-up and purified. Examples of the morphology of the pure microbial cultures representative of the strains obtained in this study are shown in Fig. S2. Isolates were separated in different groups characterized by identical colony morphology (Table 2). Based on the non-staining KOH test, twenty bacterial strains were Gram positive and the remaining six were Gram negative (Table 2). One strain of each type of bacterial and fungal colony morphology was selected to be identified by the 16S rDNA gene (for bacteria) and ITS (for fungi) sequence analyses. All remaining isolates were arbitrarily affiliated to the genus or species of the strain representative each of group. Results regarding the identification of the selected seven bacterial and four fungal isolates are

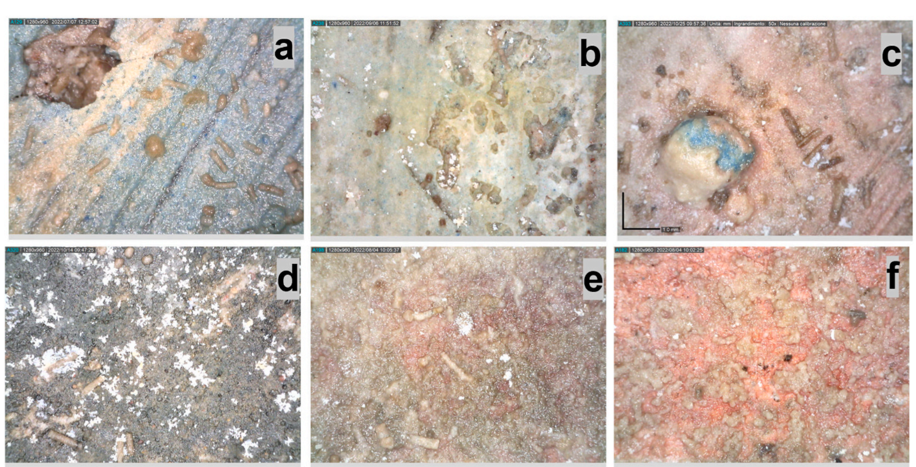

Fig. 2. Morphological aspect of the arthropods fecal pellets dropped both on the surface and inside the irregularities of the fresco painting (a–b), their colonization and further degradation by white color microorganisms (c–e) with the result of a complete changing of the initial morphology of the pellets (f). Scale bar 1 mm for all pictures, as indicated in Fig. 2c.

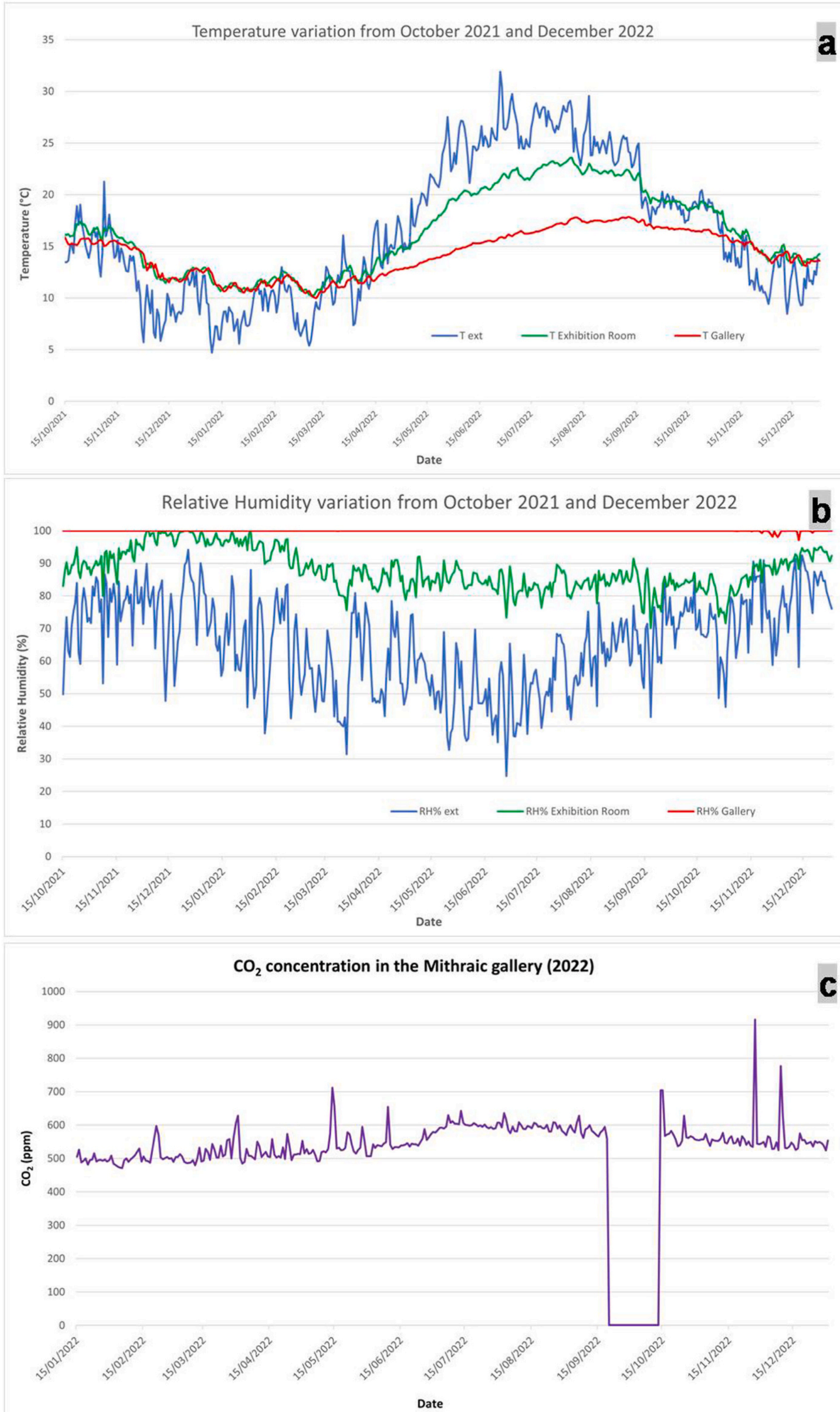

Fig. 3. Values of Temperature (°C) (a) and Relative Humidity (RH%) (b) hourly registered (from October 2021 to December 2022), and values of CO2 concentration (ppm) (c) hourly registered in the Mithraic Gallery from January to December 2022. Between September 20th and October 13th CO2 data are missing due to electrical fault interesting the sensor.

shown in Table 2. Analysis of partial sequence of 16S rDNA gene allowed to identify three bacterial strains at species level (*Bacillus mycoides*, *Stenotrophomonas rhizophila* and *Lysobacter prati*) while the remaining four strains were identified only at genus level (*Streptomyces*, *Rhodococcus* and *Microbacterium*) (Table 2). *Gamszarea microspora* and *Purpureocillium lavendulum* fungal species were identified by the analysis of ITS region, while for the other two strains the identification was only at genus level (*Talaromyces* sp. and *Podila* sp.) (Table 2). All bacterial and fungal genera identified in this study include species known as common soil inhabitants and this finding is obviously related to the subterranean ubication of the painting.

Some genera or species were retrieved from different sampling sites.

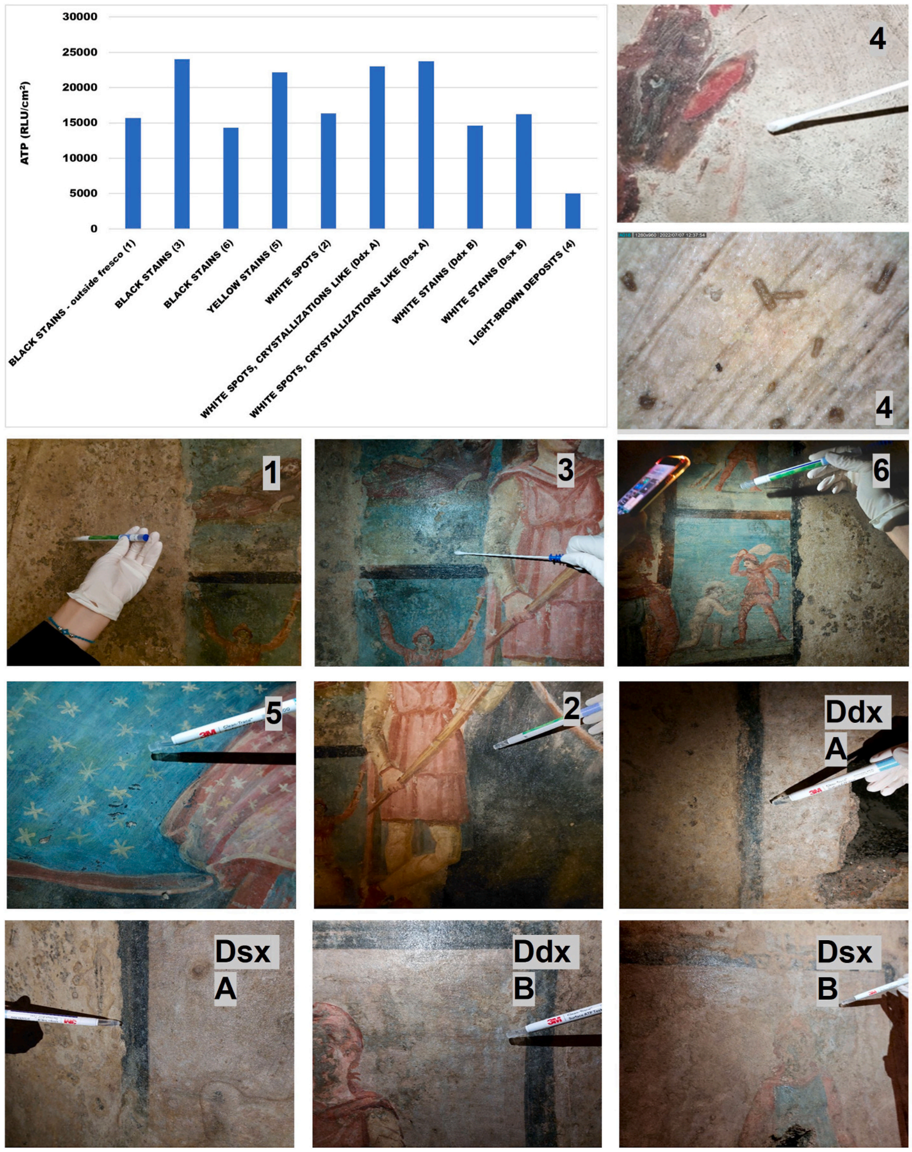

Fig. 4. ATP values measured on the investigated areas described in Tables 1 and 2, according to the following alteration phenomena: light-brown deposits (4, investigated area (up) and a detail of it (down)), black stains similar to vermiculations (1, 3 and 6), yellow stains (5), white spots easily removable (2), white spots similar to crystallization phenomena, easily removable (Ddx A and Dsx A), white irregular stains difficult to remove (Ddx B and Dsx B).

In fact, *Bacillus mycoides* was isolated from all samples, the actinomycete *Streptomyces* sp. strain B and the fungus *Podila* sp. were isolated from six out of seven sampling sites, the bacterium *Stenotrophomonas rhizophila* from four sites while *Streptomyces* sp. strain S and *Rhodococcus* sp. from three out of the seven sampling sites. The samples showing the highest biodiversity of cultivable microflora were those collected from the right torch-bearer fresco located at the entrance of the corridor (Ddx A and DdxB), followed by area 1 (showing black stains, located at the end of the corridor, near fresco on the wall, see Table 1). In these three locations the presence of the *Gamszarea microspora* fungus was also observed. From sample 4, where fecal pellets were found, two species of bacteria (*Microbacterium* sp. And *B. mycoides*) and one fungus (*Podila* sp.) were recovered. This sample is the only one from which the actinomycete *Microbacterium* sp. was isolated.

3.3. Micro and mesofauna investigations

The attentive observation of the fresco with ranking light (Fig. 5a) evidenced the presence of different deposits attributed to mesofauna's fecal pellets (mainly isopods) with shapes from cylindrical to spherical until formless, as confirmed by the microscopic observations (Figs. 2, Fig. 5b and c). The visual inspection revealed mainly the presence of terrestrial isopods, followed by single species of springtails, mosquitoes, diplopods and spiders (Fig. 6). Most of the identified species are considered to be troglophiles (*Androniscus dentiger*, *Trichoniscus matulici*, *Chaetophiloscia cellaria*, *Kryptonesticus eremita* and *Limonia nubeculosa*), widely distributed in Europe and mainly occurring in subterranean habitats with a high degree of humidity, like caves and cellars. The species composition did not vary on the different observation days, due to the small variation in the microclimatic conditions of the site throughout the year.

The microscopic observation (in visible and UV light) of the samples taken with the aid of FungiTape (Scientific Device Laboratory) revealed the presence of a microfauna composed of mites, millipedes, rotifers and other unidentified invertebrates (Fig. S3).

Table 2
Identification of bacteria and fungi isolated from the painted surface located in the Mithraeum Temple of Marino Laziale, Rome.

Identified strains		Description of colony morphology	Species identified (%ID) (Phylum; Order)	Accession number	Sample acronym
BACTERIA	A	Gram +, whitish creamy	*Bacillus mycoides* (98.1) (*Firmicutes*; *Bacillales*)	OQ247888	1, 2, 3, 4, 5, Ddx A, Ddx B
	B	Gram +, whitish; brown exopigment	*Streptomyces* sp. (98.7) (*Actinobacteria*; *Actinomycetales*)	OQ248031	1, 2, 3, 5, Ddx A, Ddx B
	G	Gram +, light pink	*Rhodococcus* sp. (98.3) (*Actinobacteria*; *Actinomycetales*)	OQ248063	3, 5, Ddx B
	H	Gram -, light yellow and transparent	*Stenotrophomonas rhizophila* (99.3) (*Proteobacteria*; *Xanthomonadales*)	OQ248064	1, 2, 3, Ddx B
	I	Gram +, white-gray	*Microbacterium* sp.(98.8) (*Actinobacteria*; *Micrococcales*)	OQ248065	4
	M	Gram -, light yellow	*Lysobacter prati* (98.3) (*Proteobacteria*; *Xanthomonadales*)	OQ248067	5, Ddx A
	S	Gram +, white; orange/brown exopigment	*Streptomyces* sp. (98.2) (*Actinobacteria*; *Actinomycetales*)	OQ248079	3, Ddx A, Ddx B
FUNGI	OA1	green, white border, yellow on the back	*Talaromyces* sp. (99.1) (*Ascomycota*; *Eurotiales*)	OQ257345	Ddx A
	OA2	white thin spreading mycelium	*Podila* sp. (99.8) (*Mortierellomycota*; *Mortierellales*)	OQ257346	1, 2, 4, 5, Ddx A, Ddx B
	OA4	white, slight yellow on the back	*Gamszarea microspora* (99.6) (*Ascomycota*; *Hypocreales*)	OQ257370	1, Ddx A, Ddx B
	OA10	pink, white border, yellow on the back	*Purpureocillium lavendulum* (99.1) (*Ascomycota; Hypocreales*)	OQ257371	1, Ddx B

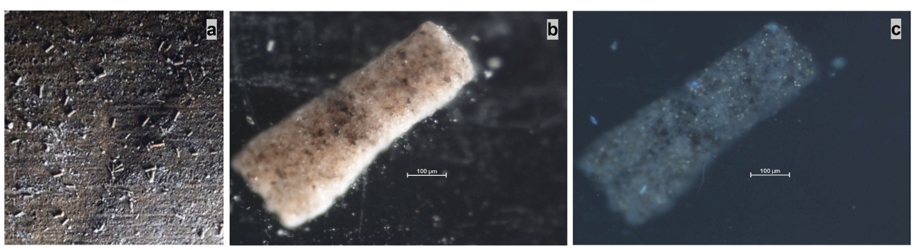

Fig. 5. The fecal pellets observed on site on the fresco surface under raking light (a) and in laboratory at a higher magnification under visible (b) and UV fluorescent light (c). Scale bar 100 μm.

4. Discussions

4.1. Biodiversity and ecological observations

The Mithraeum site is a hypogeum site carved in peperino stone to a depth of about 10 m underground the current urban settlement. The environmental and ecological features are interconnected with the geomorphological conditions above the site (Fig. S1). This heritage site acts as a good habitat for different organisms able to adapt to dark conditions, high levels of humidity and little air circulation. They are living in an ecological equilibrium with the substrate in such specific environmental conditions, by creating a virtuous food web (Fig. S4). The most abundant groups were the primary producers and the primary consumers. The microorganisms identified in this site, belonging to the first group, are decomposers and chemolitotrophs, with a key role in the organic and inorganic C flux. The detritivores or grazers, as primary consumers, are consequently abundant due to the high availability of the nutrients. As predators, spiders were observed in the case of the Mithraeum location.

In other semi-confined environments with more or less similar extreme conditions (natural caves with paintings or graffiti, catacombs, necropolis, etc.) (Bastian et al., 2008; Fernandez-Cortes et al., 2014), the living organisms are often highly specialized and the biodiversity is mainly composed of: (i) chemolitoautotrophs as primary producers as well, organized or not in biofilms, (ii) detritivores as primary consumers, and (iii) few predators as secondary consumers. The trophic linkages of these biocenosis are similar to those of the soil food webs (Parimuchová et al., 2021).

However, the ecological homeostasis inside these environments can be easily disrupted through anthropic presence and activity (Mulec, 2014; Martin-Sanchez et al., 2015), especially in the case of visitable heritage sites, inducing irreversible changes on both biotope and biocenosis. In the Altamira cave (Spain), due to the anthropization phenomena it was observed a predominance of epigean or epigeomorphic species with respect to the troglobiomorphic species, the last ones being at risk due to their low reproduction rate and metabolism (Luque and Labrada, 2016). The Mithraeum site presents different eutroglophiles or subtroglophiles species, indicating little anthropic disturbance.

Considering that this site has recently been open to the public, the micro-ecosystem conservation should be ensured together with the health safety of visitors. For this reason, the study of composition of the main biodiversity components was one of the main tasks for the conservation program of this hypogean cultural heritage site.

Among the microorganisms that were isolated from the Mithraeum of Marino Laziale, seven bacterial and four fungal strains were identified by molecular analysis. The identified bacterial strains resulted to be ubiquitous and commonly found as soil or plant inhabitants. The *Streptomyces* spp. is one of the most common genera found in the caves. These microorganisms have a high capability of survival, ubiquity and metabolic versatility, widespread all over the world and show a high abundance within the bacterial community (Farda et al., 2022; Dominguez-Moñino et al., 2021). Colonies showing the characteristics of *Streptomyces* sp. were very common in the Mithraeum hypogeum site, since they were found in almost all the investigated samples. *Streptomyces* sp. plays active roles in geomicrobiological interactions,

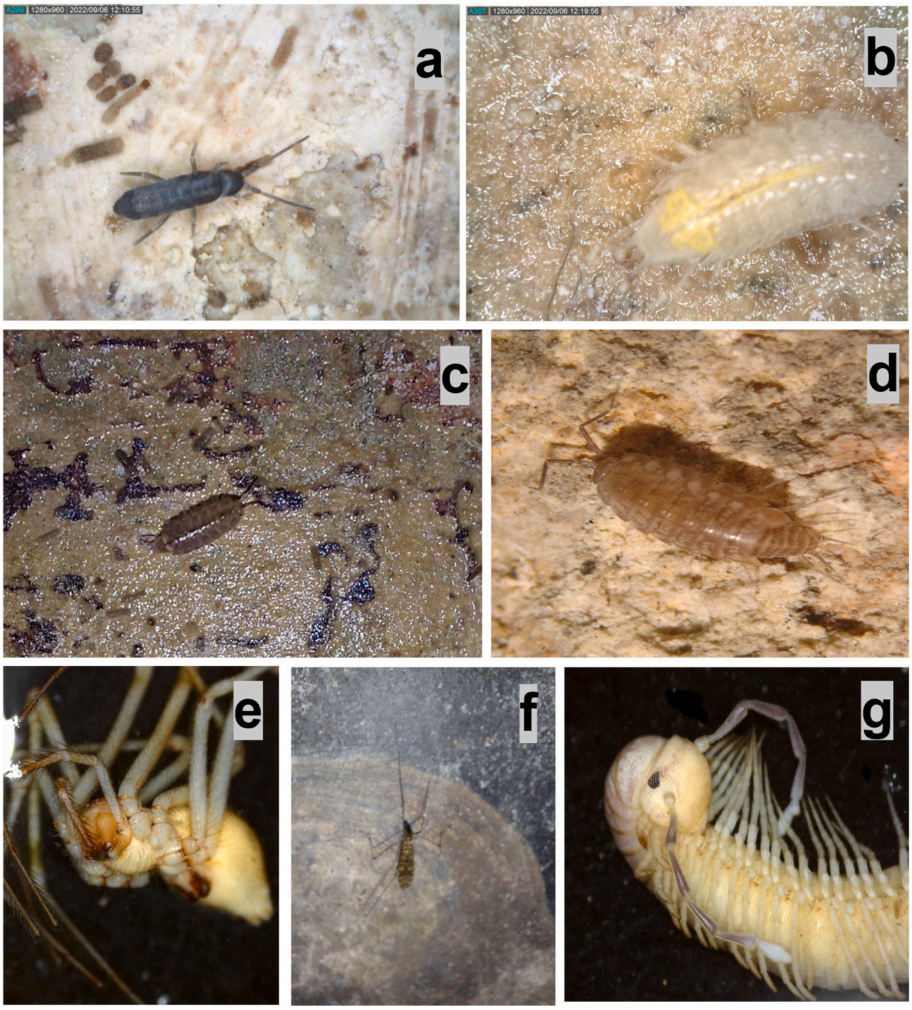

Fig. 6. The mesofauna identified for the Mithraeum Temple of Marino Laziale containing different types of arthropods: *Tomocerus*sp., Insecta, Collembola, Tomoceridae (a), *Androniscusdentiger,* Crustacea, Oniscidea, Trichoniscidae (b), *Trichoniscusmatulici,* Crustacea, Oniscidea, Trichoniscidae (c), *Chaetophiloscia cellaria,* Crustacea, Oniscidea, Philosciidae (d), *Kryptonesticus eremita,* Arachnida. Araneae, Nesticidae (e), *Limonia nubeculosa,* Insecta, Diptera, Limoniidae (f), and Diplopoda, Julida, Julidae (g).

especially in volcanic caves (Riquelme et al., 2015) and may have significant biodeterioration effects on the mural paintings located in these kinds of environments (Giaeobini et al., 1988; Elhagrassy, 2018). *Streptomyces* sp. could also be involved in the vermiculation phenomena (Addesso et al., 2021), alterations that are present in the Mithraeum as well. *Microbacterium* sp., another actinobacteria, was found in caves (Dominguez-Moñino et al., 2021), in guano (Newman et al., 2018) and in water springs (Lukač et al., 2022). This bacterium was isolated only from the sample 4 which was collected from a site containing fecal pellets. *Rhodococcus* sp. are commonly isolated from soil as plant colonizer or pathogen (Putnam and Miller, 2007). *Rhodococcus* sp., *Streptomyces* spp. and other actinomycetes seem to be involved in the formation of mineral deposits in caves (Miller et al., 2013; Enyedi et al., 2020).

Bacillus mycoides has already been retrieved in other hypogean monuments, sometimes associated with a phototrophic biofilm (Urzì et al., 2010). In the case of Mithraeum temple, the presence of evident phototrophic biofilms was not observed by the naked eye. Recent studies attribute chitinolytic activity to this species (Drewnowska et al., 2020) and confer to it a very high adaptability potential with a good functionality in various ecological niches (Fiedoruk et al., 2021). The results obtained in our research confirm a high ubiquity of this species, as it was present in all the investigated samples.

Stenotrophomonas rhizophila is a bacterium often associated with the plant roots, playing beneficial interactions with the rhizosphere and showing antimycotic properties (Ryan et al., 2009). *Lysobacter prati* belongs to the same order as *S. rhizophila* (Xanthomonadales) and it has been isolated for the first time from a plateau meadow in China (Fang et al., 2020) evidencing that the soil is its main habitat as well.

All of the four fungi isolated during the present case study were reported as components of the mycobiota of some Chinese caves (Jiang et al., 2017; Zhang et al., 2021). The identified species can be correlated with the heritage location, being known as common species of soil habitats. *Purpureocillium lavendulum* is a nematophagous fungus (Liu et al., 2019) while *Talaromyces* spp. have been often associated with insects (Nicoletti and Becchimanzi, 2022), especially with troglophile ones, being found on cadavers of larvae and adults of the cave cricket *Troglophilus neglectus* (Gunde-Cimerman et al., 1988). Different species belonging to *Podila* were previously named *Mortierella* (Vandepol et al., 2020). The *Mortierella* species colonize plant roots, and many of them are able to live on fecal pellets, animal remains in contact with soil or on exoskeletons of arthropods (Deacon, 2006; Webster and Weber, 2007). *Gamszarea microspora* was recently found inside the Tianliang Cave, Sichuan, Xingwen, China (Zhang et al., 2021) and in the Cave Church of Sts. Peter and Paul in Serbia (Ljaljević Grbić et al., 2022). The four fungi identified in the present work may have similar roles (Fig. S4, Fig. S5) as the ones indicated above by different authors.

Beside the heterotrophic biofilm developed on the damp walls of the Mithraeum Temple site, the most spread and evident signs of biological colonization were related with the fecal pellets deposited all over on the surfaces. Such by-products indicate the presence of detritivores and active processes of organic matter turnover (Coq et al., 2022). The amount of organic debris is huge, a fact that induces not only coprophagy phenomena (Zimmer, 2002; Pezzi et al., 2019) but mainly confirms their role as a nutrient source for other microorganisms, within a virtuous food chain cycling. The renewal of the organic supply is done also through the water flux coming from the ground above. In the Mithraeum site, the meso- and microfauna were abundant (Fig. 6, Fig. S3): three different species of terrestrial isopods, one species of diplopoda, one of collembola, one of arachnida, and one of diptera. The presence of at least four species of not identified microinvertebrates (such as mites, nematodes, rotifers) were noticed. The main components of the mesofauna were represented by terrestrial isopods (*Androniscus dentiger*, *Trichoniscus matulici* and *Chaetophiloscia cellaria*) and springtails (*Tomocerus* sp.), which are important vectors of the microorganisms (Bastian et al., 2008), as they are feeding both with biofilm, fungi, and even their own fecal pellets (Knight and Angel, 1967; Zimmer, 2002). The cultivable microorganisms isolated in this work from the sample containing fecal pellets, consisted of two types of bacteria (*Microbacterium* sp. and *B. mycoides*) and one type of fungus (*Podila* sp.), suggesting that these species may be preferred as a food source by the arthropods, or they could be involved in degradation of the dejections. At the end of consumer and predator life, some microorganisms will use the remains as a nutritional source (Fig. S5).

Some of the microbes dwelling in subterranean environments may be opportunistic pathogens for enfeebled people (Jurado et al., 2010), while other species may have allergenic potential, especially the sporulating ones. Inside the Mithraeum at Marino no noticed pathogenic species were identified, but species belonging to the genera *Streptomyces*, *Rhodococcus* and *Microbacterium* may show a potential risk even for humans in favorable conditions (Jurado et al., 2014). However, for a two-sided protection of both human health and subterranean natural equilibrium, it is recommended to wear protective masks, gloves and clothing (overshoes and similar protection devices), and to be aware that visiting natural subterranean environments is not a risk-free tourist activity (Jurado et al., 2014).

4.2. Conservation issues and valorization aspects

The site, due to its particular location in a subterranean environment, was often affected by abundant infiltrations and percolation of water through the rock from the above terrain in the rainy seasons with the consequent presence of stagnant water in the hypogeum for months. These events induced very critical microclimatic and biological conditions. The water presence is correlated with life and possible biodeteriogens activity but also with the non-living processes involved in the degradation of organic materials (Li and Gu, 2022). Moreover, water fluctuations inside materials increase the occurrence of their damage events. In spite of these conditions, it is worth to say that the good state of conservation of the Mithraeum painting (about 2000 years) is certainly due to their stability in an undisturbed semi-confined environment. Community of organisms were surely present in this site even before its discovery, being in an ecological equilibrium with the biotope (Liu et al., 2022a). Attention on study of biological component was only paid at beginning of 2000, when it was decided to open the Mithraeum, 50 years after its discovery. Important hydraulic engineering interventions were only recently done, solving the problem of the water percolation without altering the microclimatic stability of the site. In fact, the main characteristics of the site (e.g. presence of salts efflorescence, of crystallization and vermiculation phenomena, high level of microbial growth on the surfaces, abundant presence of isopods) remained stable in time, being observed by both ICR (before interventions, in 2006) and CNR groups (after interventions, in 2022) (**SM1** and Luvidi et al., 2021). The CNR group added knowledge with the taxonomic identification of the main biotic components present in this subterranean monument, observing significant similarities with the biodiversity of other similar environments.

However, heritage sites have to constantly pursue a difficult equilibrium between conservation and fruition but in some cases, as the one of Marino's Mithreum, a virtuous result from this challenge can be achieved. In September 2021, after a complex project of restoration, the site was finally opened to the public and the microclimatic monitoring program was enhanced with new investigations due to a growing number of visitors.

The activity of the organisms present in the site (mainly bacteria, fungi, arthropods, and nematodes) may induce mechanical stress on the painting surface stability, representing a critical point of the management plan aimed to preserve both the paintings and biocenosis. Moreover, the significant presence of the actinomycetes (*Streptomyces* spp., *Rhodococcus* sp.; *Microbacterium* sp.), able to induce biomineralization, exo-pigment and other metabolic products production, could have important consequences in painting alteration (He et al., 2021; Laiz et al., 2003). The microbiota present inside the Mithraeum could be responsible for the vermiculation and crystallization phenomena, but further studies are needed for confirming it.

Therefore, regular maintenance of the surfaces should be foreseen by using soft mechanical methods and avoiding the use of organic substances. A programmed monitoring of both the airflora and the biological presence on the surface of the paintings is compulsory to control the possible risks of the biocenosis changing (such as development of cyanobacteria due to the use of light sources, dwelling of new microorganisms coming from outside, etc.), as well as to timely select suitable cleaning solutions (He et al., 2022). The use of multidimensional culture-independent technologies (high-throughput sequencing, multi-omics) can enhance the understanding of community interrelationships, functional and biodeterioration mechanisms (Liu et al., 2022b). The frequence of visits and the number of users should be limited to not destabilize the environment inside the hypogeum site, aiming to keep the natural balance of this autochthon biocenosis. All these actions should be flanked by a continuous monitoring of the microclimatic parameters.

5. Conclusions

The biological presence inside the Mithraeum site is not induced by the painting characteristics made mainly of inorganic materials, but surely by its specific environmental characteristics. An ecological and microclimatic equilibrium is present, with a high functional trophic chain composed mainly by decomposers and heterotrophic organisms. They widely colonize all the site surfaces, including the roughness of the frescoes, without, up to date, compromising the aesthetic and surface integrity of the paintings. A careful evaluation of the microclimatic parameters, the qualitative and quantitative control of biocenosis is essential to avoid frescoes damage. A continuous monitoring supports the development of a correct fruition plan, allowing the heritage to be passed onto future generations.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ibiod.2023.105605.

References

Addesso, R., Gonzalez-Pimentel, J.L., D'Angeli, I.M., De Waele, J., Saiz-Jimenez, C., Jurado, V., Miller, A.Z., Cubero, B., Vigliotta, G., Baldantoni, D., 2021. Microbial community characterizing vermiculations from karst caves and its role in their formation. Microb. Ecol. 81, 884–896. https://doi.org/10.1007/s00248-020-01623-5.

Albertano, P., Urzì, C., Caneva, G., 2007. Tombe, catacombe e altri ipogei. In: Caneva, G., Nugari, M.P., Salvadori, O. (Eds.), La Biologia Vegetale Per I Beni Culturali, vol. I. Nardini Editore, Florence, Italy, pp. 184–189.

Bastian, F., Alabouvette, C., Saiz-Jimenez, C., 2008. The impact of arthropods on fungal community structure in Lascaux Cave. J. Appl. Microbiol. 106, 1456–1462. https://doi.org/10.1111/j.1365-2672.2008.04121.x.

Bedetti, A., 2010. Il Mitreo di Marino - una scoperta eccezionale alle porte di Roma. Archeologia sotterranea 3, 21–29.

Coq, S., Ganault, P., Le Mer, G., Nahmani, J., Capowiez, Y., Dignac, M.F., Rumpel, C., Joly, F.-X., 2022. Faeces traits as unifying predictors of detritivore effects on organic matter turnover. Geoderma 422, 115940. https://doi.org/10.1016/j.geoderma.2022.115940.

Cuzman, O.A., Tapete, D., Fratini, F., Mazzei, B., Riminesi, C., Tiano, P., 2014. Assessing and facing the biodeteriogenic presence developed in the Roman catacombs of Santi Marco, Marcelliano e Damaso, Italy. Eur. J. Sci. Theol. 10 (3), 185–197.

Deacon, J., 2006. Fungal ecology: saprotrophs. In: Fungal Biology, fourth ed. Blackwell Publishing Ltd, England TJ International, Padstow, Cornwall, pp. 213–236.

Dominguez-Moñino, I., Jurado, V., Rogerio-Candelera, M.A., Hermosin, B., Saiz-Jimenez, C., 2021. Airborne bacteria in show caves from Southern Spain. Microb. Cell. 8 (10), 247–255. https://doi.org/10.15698/mic2021.10.762.

Drewnowska, J.M., Fiodor, A., Barboza-Corona, J.E., Swiecicka, I., 2020. Chitinolytic activity of phylogenetically diverse *Bacillus cereus* sensu lato from natural environments. Syst. Appl. Microbiol. 43, 126075 https://doi.org/10.1016/j.syapm.2020.126075.

Elhagrassy, A.F., 2018. Isolation and characterization of actinomycetes from mural paintings of Snu-Sert-Ankh tomb, their antimicrobial activity, and their biodeterioration. Microbiol. Res. 2016, 47–55. https://doi.org/10.1016/j.micres.2018.08.005.

Enyedi, N.T., Makk, J., Kótai, L., Berényi, B., Klébert, S., Sebestyén, Z., Molnár, Z., Borsodi, A.K., Leél-Őssy, S., Demény, A., Németh, P., 2020. Cave bacteria-induced amorphous calcium carbonate formation. Sci. Rep. 10, 8696. https://doi.org/10.1038/s41598-020-65667-w.

Fang, B.-Z., Xie, Y.G., Zhou, X.K., Zhang, X.T., Liu, L., Jiao, J.Y., Xiao, M., Li, W.J., 2020. *Lysobacter prati* sp. nov., isolated from a plateau meadow sample. Antonie Leeuwenhoek 113 (6), 763–772. https://doi.org/10.1007/s10482-020-01386-6.

Farda, B., Djebaili, R., Vaccarelli, I., Del Gallo, M., Pellegrini, M., 2022. Actinomycetes from caves: an overview of their diversity, biotechnological properties, and insights for their use in soil environments. Microorganisms 10, 453. https://doi.org/10.3390/microorganisms10020453.

Fernandez-Cortes, A., Elez, J., Cuezva, S., Cañaveras, J.C., Benavente, D., Rogerio, M.A., Saiz-Jimenez, C., Sanchez-Moral, S., 2014. The conservation of the carmona necropolis (sevilla, Spain). In: Saiz-Jimenez (Ed.), The Conservation of Subterranean Cultural Heritage. Taylor & Francis Group, London, pp. 45–50, 978-1-138-02694-0.

Fiedoruk, K., Drewnowska, J.M., Mahillon, J., Zambrzycka, M., Swiecick, I., 2021. Pan-Genome portrait of *Bacillus mycoides* provides insights into the species ecology and evolution. Microbiol. Spectr. 9, e00311–e00321. https://doi.org/10.1128/Spectrum.00311-21.

Frasca, F., Verticchio, E., Caratelli, A., Bertolin, C., Camuffo, D., Siani, A.M., 2020. A comprehensive study of the microclimate-induced conservation risks in hypogeal sites: the Mithraeum of the Baths of Caracalla (Rome). Sensors 20, 3310. https://doi.org/10.3390/s20113310.

Galan, J.M., 2014. The rock-cut tomb-chapel of Hery and Djehuty on the West Bank of Luxor: history, environment and conservation. In: Saiz-Jimenez (Ed.), The Conservation of Subterranean Cultural Heritage. Taylor & Francis Group, London, pp. 3–16, 978-1-138-02694-0.

Garcia-Sanchez, A.M., Miller, A.Z., Jurado, V., Dionisio, A., Muralha, V.S.F., Afonso, M.J., Chamine, H.I., 2014. Is the presence of bacterial communities related to the urban contamination sources of the 16th century Paranhos spring water tunnel? In: Saiz-Jimenez (Ed.), The Conservation of Subterranean Cultural Heritage. Taylor & Francis Group, London, pp. 95–102, 978-1-138-02694-0.

Geneste, J.-M., Mauriac, M., 2014. The conservation of lascaux cave, France. In: Saiz-Jimenez (Ed.), The Conservation of Subterranean Cultural Heritage. Taylor & Francis Group, London, pp. 165–172, 978-1-138-02694-0.

Giaeobini, C., De Cicco, M.A., Tiglie, I., Accardo, G., 1988. Actinomycetes and biodeterioration in the field of fine art. In: Houghton, D.R., Smith, R.N., Eggins, H.O.W. (Eds.), Biodeterioration 7. Springer, Dordrecht, pp. 418–423. https://doi.org/10.1007/978-94-009-1363-9_55.

Gomez-Bolea, A., Alvaro, I., Llop, E., Sammut, S., Hernandez-Marine, M., 2014. Indoors diversity in phototrophic biofilms at St. Paul's Catacombs (Malta). In: Saiz-Jimenez (Ed.), The Conservation of Subterranean Cultural Heritage. Taylor & Francis Group, London, pp. 83–88, 978-1-138-02694-0.

Gunde-Cimerman, N., Zalar, P., Jeram, S., 1988. Mycoflora of cave cricket *Troglophilus neglectus* cadavers. Mycopath 141, 111–114. https://doi.org/10.1023/A:1006947524503.

He, D., Wu, F., Ma, W., Zhang, W., Gu, J.-D., Duan, Y., Xu, R., Feng, H., Wang, W., Li, S.-W., 2021. Insights into the bacterial and fungal communities and microbiome that causes a microbe outbreak on ancient wall paintings in the Maijishan Grottoes. Int. Biodeterior. Biodegrad. 163, 105250 https://doi.org/10.1016/j.ibiod.2021.105250.

He, D., Wu, F., Ma, W., Gu, J.-D., Xu, R., Hu, J., Yue, Y., Ma, Q., Wang, W., Li, S.-W., 2022. Assessment of cleaning techniques and its effectiveness for controlling biodeterioration fungi on wall paintings of Maijishan Grottoes. Int. Biodeterior. Biodegrad. 171, 105406 https://doi.org/10.1016/j.ibiod.2022.105406.

Intxaurbe, I., Rivero, O., Medina-Alcaide, Ma Á., Arriolabengoa, M., Ríos-Garaizar, J., Salazar, S., Ruiz-López, J.F., Ortega-Martínez, P., Garat, D., 2020. Hidden images in Atxurra Cave (Northern Spain): a new proposal for visibility analyses of Palaeolithic rock art in subterranean environments. Quat. Int. 566–567, 163–170.

Jiang, J.R., Cai, L., Liu, F., 2017. Oligotrophic fungi from a carbonate cave, with three new species of *Cephalotrichum*. Mycol. 8 (3), 164–177. https://doi.org/10.1080/21501203.2017.1366370.

Jurado, V., Laiz, L., Rodriguez-Nava, V., Boiron, P., Hermosin, B., Sanchez-Moral, S., Saiz-Jimenez, C., 2010. Pathogenic and opportunistic microorganisms in caves. Int. J. Speleol. 39 (1), 15–24. https://doi.org/10.5038/1827-806X.39.1.2.

Jurado, V., Laiz, L., Sanchez-Moral, S., Saiz-Jimenez, C., 2014. Pathogenic microorganisms related to human visits in Altamira Cave, Spain. In: Saiz-Jimenez (Ed.), The Conservation of Subterranean Cultural Heritage. Taylor & Francis Group, London, pp. 229–238, 978-1-138-02694-0.

Knight, C.B., Angel, R.A., 1967. A preliminary study of the dietary requirements of *Tomocerus* (Collembola). Am. Midl. Nat. 77 (2), 510–517.

Laiz, L., Gonzalez, J., Saiz-Jimenez, C., 2003. Microbial communities in caves: ecology, physiology, and effects on paleolithic paintings. In: Art, Biology, and Conservation: Biodeterioration of Works of Art. MetPublications, New York, NY, USA, pp. 210–215.

Lasheras, J.A., de las Heras, C., Prada, A., 2014. Altamira and its future. In: Saiz-Jimenez (Ed.), The Conservation of Subterranean Cultural Heritage. Taylor & Francis Group, London, pp. 145–164, 978-1-138-02694-0.

Li, Y.-H., Gu, J.-D., 2022. A more accurate definition of water characteristics in stone materials for an improved understanding and effective protection of cultural heritage from biodeterioration. Int. Biodeterior. Biodegrad. 166, 105338 https://doi.org/10.1016/j.ibiod.2021.105338.

Liu, L., Cao, Y.R., Zhang, C.-C., Fan, H.-F., Guo, Z.-Y., Yang, H.-Y., Chen, M., Han, J.-J., Xu, J., Zhang, K.-Q., Liang, L.-M., 2019. An efficient gene disruption system for the nematophagous fungus *Purpureocillium lavendulum*. Fungal Biol. 123 (4), 274–282. https://doi.org/10.1016/j.funbio.2018.10.009.

Liu, X., Qian, Y., Wu, F., Wang, Y., Wang, W., Gu, J.-D., 2022a. Biofilms on stone monuments: biodeterioration or bioprotection? Trends Microbiol. 30 (9), 816–819. https://doi.org/10.1016/j.tim.2022.05.012.

Liu, X., Qian, Y., Wang, Y., Wu, F., Wang, W., Gu, J.-D., 2022b. Innovative approaches for the processes involved in microbial biodeterioration of cultural heritage materials. Curr. Opin. Biotechnol. 75, 102716 https://doi.org/10.1016/j.copbio.2022.102716.

Ljaljević Grbić, M., Dimkić, I., Savković, Ž., Stupar, M., Knežević, A., Jelikić, A., Unković, N., 2022. Mycobiome diversity of the Cave Church of Sts. Peter and Paul in Serbia - risk assessment implication for the conservation of rare cavern habitat housing a peculiar fresco painting. J. Fungi 8, 1263. https://doi.org/10.3390/jof8121263.

Lukač, M., Jelić, D., Mutschmann, F., Tomić, D.H., Cizelj, I., Gottstein, Ž., Prukner-Radovčić, E., 2022. Cultivable microbiota of *Proteus anguinus* from underground habitats and animals accidently washed to the surface in Croatia. Vet. Arh. 92 (4), 497–508. https://doi.org/10.24099/vet.arhiv.1555.

Luque, C.G., Labrada, L., 2016. La fauna subterránea de las cuevas de Altamira (España). Consideraciones para la conservación del arte rupestre clasificado Patrimonio Mundial Subterranean fauna of Altamira Caverns (Spain). Considerations for the conservation of World Heritage Rock-Art. Bol Real Soc Espan Hist Nat. Seccion biologica 110, 93–120.

Luvidi, L., Prestileo, F., De Paoli, M., Riminesi, C., Manganelli del Fà, R., Magrini, D., Fratini, F., 2021. Diagnostics and monitoring to preserve a hypogeum site: the case of the Mithraeum of Marino Laziale (Rome). Heritage 4, 4264–4288.

Ma, W., Wu, F., He, D., Li, J., Zhang, Q., Yang, X., Gu, J.-D., Wang, W., Feng, H., 2023. The biodeterioration outbreak in Dunhuang Mogao Grottoes analyzed for the microbial communities and the occurrence time by C-14 dating. Int. Biodeterior. Biodegrad. 178, 105533 https://doi.org/10.1016/j.ibiod.2022.105533.

Mammola, S., Cardoso, P., Ribera, C., Pavlek, M., Isaia, M., 2018. A synthesis on cave-dwelling spiders in Europe. Zool. Syst. Evol. Res. 56, 301–316.

Martin-Sanchez, P.M., Miller, A.Z., Sáiz-Jiménez, C., 2015. Lascaux Cave, an example of fragile ecological balance in subterranean environments. In: Microbial Life of Cave Systems, de Gruyter. Digital CSIC, pp. 279–302.

Miller, A.Z., Dionísio, A., Jurado, V., Cuezva, S., Sanchez-Moral, S., Cañaveras, J.C., Saiz-Jimenez, C., 2013. Biomineralization by cave dwelling microorganisms. In: Sanjurjo Sanchéz, J. (Ed.), Advances in Geochemistry Research. Nova Science Publishers, pp. 77–105, 978-1-62618-245-5.

Mulec, J., 2014. Lampenflora as an accompaniment of mass cave tourism, problems and solutions for Postojnska jama, Slovenia. In: Saiz-Jimenez (Ed.), The Conservation of Subterranean Cultural Heritage. Taylor & Francis Group, London, pp. 253–256, 978-1-138-02694-0.

Newman, M.M., Kloepper, L.N., Duncan, M., McInroy, J.A., Kloepper, J.W., 2018. Variation in bat guano bacterial community composition with depth. Front. Microbiol. 9, 914. https://doi.org/10.3389/fmicb.2018.00914.

Nicoletti, R., Becchimanzi, A., 2022. *Talaromyces*–insect relationships. Microorganisms 10, 45. https://doi.org/10.3390/microorganisms10010045.

Nugari, M.P., Pietrini, A.M., Caneva, G., Imperi, F., Visca, P., 2009. Biodeterioration of mural paintings in a rocky habitat: the crypt of the original sin (matera, Italy). Int. Biodeterior. Biodegrad. 63, 705–711.

Ontañon, R., Bayarri, V., Herrera, J., Gutierrez, R., 2014. The conservation of prehistoric caves in Cantabria, Spain. In: Saiz-Jimenez (Ed.), The Conservation of Subterranean Cultural Heritage. Taylor & Francis Group, London, pp. 185–192, 978-1-138-02694-0.

Pakhunov, A.S., Brandt, N.N., Chikishev, A.Y., 2014. Raman microscopy and IR imaging of the palaeolithic paintings from kapova cave, southern ural, Russia. 2014. In: Saiz-Jimenez (Ed.), The Conservation of Subterranean Cultural Heritage. Taylor & Francis Group, London, pp. 275–280, 978-1-138-02694-0.

Parimuchová, A., Dušátková, L.P., Kováč, L., Macháčková, T., Slabý, O., Pekár, S., 2021. The food web in a subterranean ecosystem is driven by intraguild predation. Sci. Rep. 11, 4994. https://doi.org/10.1038/s41598-021-84521-1.

Pezzi, P.H., Araujo, P.B., Wood, C.T., 2019. Coprophagy in detritivores: methodological design for feeding studies in terrestrial isopods (Crustacea, Isopoda, Oniscidea). Nauplius 27, e2019010. https://doi.org/10.1590/2358-2936e2019010.

Putnam, M.L., Miller, M.L., 2007. Rhodococcus fascians in Herbaceous Perennials. Plant Dis. 91 (9), 1064–1076. https://doi.org/10.1094/PDIS-91-9-1064.

Riquelme, C., Marshall Hathaway, J.J., Enes Dapkevicius, M.L.N., Miller, A.Z., Kooser, A., Northup, D.E., Jurado, V., Fernandez, O., Saiz-Jimenez, C., Cheeptham, N., 2015. Actinobacterial diversity in volcanic caves and associated geomicrobiological interactions. Front. Microbiol. 6, 1342. https://doi.org/10.3389/fmicb.2015.01342.

Ryan, R.P., Monchy, S., Cardinale, M., Taghavi, S., Crossman, L., Avison, M.B., Berg, G., van der Lelie, D., Dow, J.M., 2009. The versatility and adaptation of bacteria from the genus *Stenotrophomonas*. Nat. Rev. Microbiol. 7 (7), 514–525. https://doi.org/10.1038/nrmicro2163.

Saarela, M., Alakomi, H.L., Suihko, M.L., Maunuksela, L., Raaska, L., Mattila-Sandholm, T., 2004. Heterotrophic microorganisms in air and biofilm samples from Roman catacombs, with special emphasis on actinobacteria and fungi. Int. Biodeterior. Biodegrad. 54, 27–37. https://doi.org/10.1016/j.ibiod.2003.12.003.

Sanchez-Moral, S., Cuezva, S., Garcia-Anton, E., Fernandez-Cortes, A., Elez, J., Benavente, D., Cañaveras, J.C., Jurado, V., Rogiero-Candelera, M.A., Saiz-Jimenez, C., 2014. Microclimatic monitoring in Altamira Cave: two decades of scientific projects. In: Saiz-Jimenez (Ed.), The Conservation of Subterranean Cultural Heritage. Taylor & Francis Group, London, pp. 139–144, 978-1-138-02694-0.

Schmalfuss, H., 2003. World catalog of terrestrial isopods (Isopoda: Oniscidea). Stuttgarter Beiträge zur Naturkunde, Ser. A 654, 1–341.

Spada, M., Sorella, S., Galeotti, M., Tosini, I., Cuzman, O.A., 2021. Non-invasive technologies to timely screen out different application conditions of essential oils on stone. Int. Biodeterior. Biodegrad. 163, 1–11. https://doi.org/10.1016/j.ibiod.2021.105285.

Tapete, D., Piovesan, R., Cantisani, E., Fratini, F., Mazzoli, C., Maritan, L., 2014. Identification of lime-based mural painting techniques in catacombs using well-established criteria of stratigrafic investigation. In: Saiz-Jimenez (Ed.), The Conservation of Subterranean Cultural Heritage. Taylor & Francis Group, London, pp. 59–64, 978-1-138-02694-0.

Urzì, C., De Leo, F., Bruno, L., Albertano, P., 2010. Microbial diversity in paleolithic caves: a study case on the phototrophic biofilms of the Cave of Bats (Zuheros, Spain). Microb. Ecol. 60 (1), 116–129. https://doi.org/10.1007/s00248-010-9710-x.

Vandel, A., 1960. Isopodes terrestres (Première partie). Faune Fr. 64, 1–416.

Vandel, A., 1962. Isopodes terrestres (Deuxième partie). Faune Fr. 66, 417–931.

Vandepol, N., Liber, J., Desirò, A., Na, H., Kennedy, M., Barry, K., Grigoriev, I.V., Miller, A.N., O'Donnell, K., Stajich, J.E., Bonito, G., 2020. Resolving the Mortierellaceae phylogeny through synthesis of multi-gene phylogenetics and phylogenomics. Fungal Divers. https://doi.org/10.1007/s13225-020-00455-5.

Venarsky, M.P., Huntsma, B.M., 2018. Food webs in caves. In: Moldovan, O.T., Kováč, L., Halse, S. (Eds.), Cave Ecology. Part of the Book Series: Ecological Studies Analysis and Synthesis, eds, vol. 235. ECOLSTUD, Springer Nature Switzerland, pp. 309–328. https://doi.org/10.1007/978-3-319-98852-8.

Webster, J., Weber, R.W.S., 2007. Zygomycota. In: Introduction to Fungi, third ed. Cambridge University Press, United States of America, New York, pp. 165–226.

Zhang, Z.-F., Zhou, S.-Y., Eurwilaichitr, L., Ingsriswang, S., Raza, M., Chen, Q., Zhao, P., Liu, F., Cai, L., 2021. Culturable mycobiota from Karst caves in China II, with descriptions of 33 new species. Fungal Divers. 106, 29–136. https://doi.org/10.1007/s13225-020-00453-7.

Zimmer, M., 2002. Nutrition in terrestrial isopods (Isopoda: Oniscidea): an evolutionary-ecological approach. Biol. Rev. 77, 455–493. https://doi.org/10.1017/S1464793102005912.