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IDENTIFICATION OF FUNGI FROM SOIL AND SEDIMENT IN JEFRIZ CAVE; THE FIRST SURVEY IN A CAVE FROM IRAN

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Abstract

The aim of this study was to characterize the mycobiota in soil and sediment samples of Jefriz cave in Kerman, Iran. During 2018−2019, the culturable mycobiota from several sites within the Jefriz cave, resulted in 82 fungal isolates. Morphological characteristics of the isolates, as well as molecular sequence data, were used for species identifications. The fungi were identified as species of *Fusarium*, *Fusicolla*, *Geomyces* (*Pseudogymnoascus*), *Humicola*, *Chalastospora*, *Penicillium*, *Aspergillus*, *Epiciccum*, *Podospora* and *Mucor*. The most prevalent was *Aspergillus spelunceus*, followed by *Geomyces pannorum* and *Humicola grisea*. The majority of these species have been reported as cave residents in previous studies of cave environments. Our data showed that the fungal community composition varied between the samples from the entrance and less visited sites deeper in the cave. This study is the first cave mycological investigation in Iran, and one of the identified species is reported for the first time from a cave.

INTRODUCTION

Caves are considered as extreme environments characterized by darkness, low temperatures, high humidity, and limited organic matter (Barton and Northup, 2007). Microbial communities in caves are uniquely adapted to oligotrophic conditions (Jurado et al., 2009). Fungi are an important group of cave microflora that play critical roles as decomposers, mutualists, and pathogens (Shapiro and Pringle, 2010) and they interact with cave fauna. Some fungal species are involved in processes of disintegration and formation of cave speleothems by providing nucleation sites for precipitation of calcium carbonate (Barton and Northup, 2007), while some other species play roles in biodeterioration of cave structures via physical penetration, metabolite secretion, and pigmentation (Cañveras et al., 2001; Wang et al., 2011). These facts make the caves microorganisms interesting to microbiologists.

A number of studies have explored the fungal community occurring in caves soil and sediments and have proved that caves harbor a high diversity of fungi (Vanderwolf et al., 2013). In July 2001, an outbreak of *Fusarium solani* in the prehistoric painted Lascaux Cave in France was discovered and biocide treatments were applied (Dupont et al., 2007; Bastian et al., 2009b). *Pseudogymnoascus destructans*, the causal agent of white-nose syndrome emerged in North America and spread in 2006, drawing more attention to fungal cave communities (Meteyer et al., 2009). Vanderwolf et al. (2013) published a list of species and genera of fungi, slime molds, and yeasts reported from caves worldwide. The fungal community composition is affected by factors such as sampling site, isolation method, time of year (Wang et al., 2011), human activity, and presence of nutrient sources such as bat guano and droppings (Nováková, 2009; Shapiro and Pringle, 2010).

No data have been reported on metabolic properties and the mechanisms of adaptation of the fungal flora in caves in Iran. Jefriz cave, located in Baft, Kerman province, Iran (Fig. 1), is an attractive limestone cave with unique speleothems. This cave is important since there are reports of bat species residing in the cave (personal communication with local people). In this study, we aimed to investigate fungal flora of Jefriz cave based on culture-dependent methods to determine the diversity of fungi and thereby enhance our understanding of the evolution of adaptations to the extreme ecosystems in caves.

MATERIALS AND METHODS

Sampling Site

Jefriz is a limestone cave located 3 km east of Jefriz village, Baft, Kerman province, Iran (Fig. 1). This cave has a vertical, eight-meter deep entrance located on a hillside. A gate has been installed over the entrance (Fig. 2). Exploration into the cave is by rappelling through a shaft that requires the use of specialized equipment such as a rope or cable ladder. The cave length is about 332 m (personal communications from the explorers). The cave is linear with few side passages, but there are two main tunnels. After entry, there is a big chamber with passageways covered with stalactites and stalagmites. The main passage in some parts is only accessible through narrow tunnels that requires the explorer to crawl to pass. The cave floor is covered with wet debris and tiny ponds are present. Jefriz cave is not scientifically

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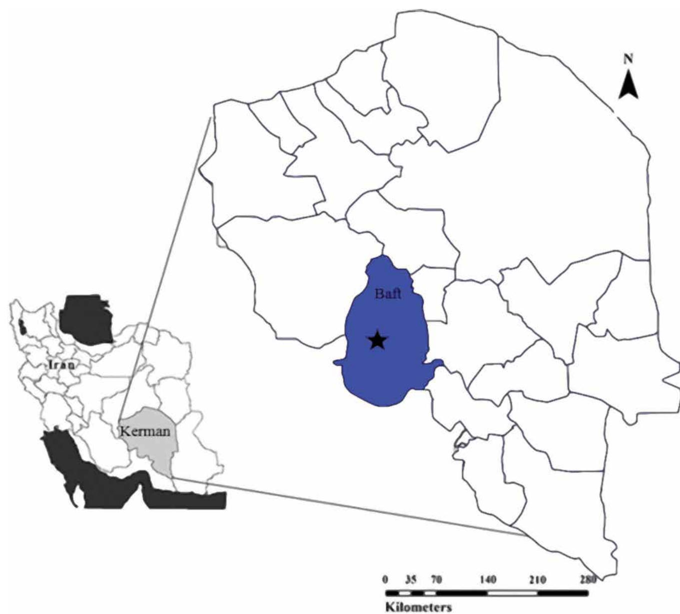

Figure 1. Location of the study area in Iran.

explored and a map has not been published. A gate at the entrance (Figure 2b) of the cave was installed by local people to prevent entering of unprofessional cavers, to preserve the cave from damages produced by some careless visitors, and to protect the untrained visitors because the exploration needs training and special equipment.

Sample Collection and Isolation oOf Culturable Fungi

Soil and sediment samples were collected using a sterilized spatula from 0-4 cm of the cave floor, placed in sterile bags, transferred to the laboratory, and stored at 4 °C. Four visits were carried out for sampling and four samples were taken during each visit. Sample collections took place in all four seasons, on January 11, 2018, and May 14, 2019, August 19, 2019, and October 22, 2019. For each trip, one sample was collected from sites close to the entrance corridor in the first large chamber, and three samples were collected randomly throughout the cave from deeper sites. Isolations were carried out by direct plating and plate dilution methods (Garrett, 1981). Soil samples (1 g) were suspended in nine mL sterile distilled water, vortexed vigorously for 1 min and allowed to stand for 3 min. Two hundred µL of 10^{-2} to 10^{-4}-fold diluted suspensions was inoculated onto potato dextrose agar (PDA; Merck, Germany) and dichloran rose bengal chloramphenicol (DRBC) agar (peptone 5 g, KH2 PO4 1 g, MgSO4 0.5 g, glucose 10 g, dichloran solution (0.2% (w/v) in ethanol, Rose Bengal 0.025 g, chloramphenicol 0.1 g, agar 15 g, and distilled water to 1 L; King et al., 1979). All ingredients of culture media were obtained from Merck, Germany. Chloramphenicol (Sigma–Aldrich, Germany) was added to eliminate bacteria. Cultures were incubated at 25 °C for four weeks in darkness. Plates were examined for fungal growth every three days. Colonies that appeared on cultures were transferred to new plates for further examination. All the isolates were deposited in the KGUT Fungal Culture Collection at Kerman Graduate University of Advanced Technology, Kerman, Iran.

Figure 2. Entrance to Jefriz cave.

Morphological Studies

The morphological identification of fungal isolates was based on the macroscopic and microscopic characteristics of the isolates, such as colony morphology, conidia, and conidiophores according to reliable identification guides (Domsch et al., 1980; Barnett and Hunter, 1998; Hanlin et al., 1998; Leslie and Summerell, 2006; Seifert et al., 2011). All cultures were done in triplicate. Media used were Malt Extract Agar (MEA: malt 20 g/L, glucose 20 g/L, peptone 1 g/L, agar 16 g/L), Czapek Yeast autolysate Agar (CYA: sucrose 30 g/L, powdered yeast extract 5 g/L, K2HPO4 1 g/L, NaNO3 2 g/L, KCl 0.5 g/L, MgSO4·7H2O 0.5 g/L, FeSO4·7H2O 0.01 g/L, ZnSO4·7H2O 0.01 g/L, CuSO4·5H2O 0.005 g/L, agar 20 g/L) and Czapek–Dox agar (CZ: sucrose 30 g/L, K2HPO4 1 g/L, NaNO3 2 g/L, KCl 0.5 g/L,MgSO4·7H2O0.5 g/L, FeSO4·7H2O 0.01 g/L, ZnSO4·7H2O 0.01 g/L, CuSO4·5H2O 0.005 g/L, agar 20 g/L) (Hubka et al., 2016) to observe colony characteristics. All ingredients of culture media were obtained from Merck, Germany. Lactophenol, lactophenol cotton blue, and 50% lactic acid (Merck, Germany) solutions were used to prepare microscope slides. The measurements and microphotographs of fungal features were taken using Dino-eye microscope camera USB lens (The Microscope Store, LLC., USA) from agar plates and slides. Colony diameter was assessed on plates after 7–28 days at 25 °C. The mean diameter was obtained from three replicates.

Molecular Examination Of Isolates

Genomic DNA of fungal isolates was extracted using a CTAB extraction procedure (Doyle and Doyle, 1987; Zhang et al., 2010) from mycelia grown on PDA for 7−15 days at 25 °C. The DNA concentrations were estimated by a NanoDrop spectrophotometer (NanoDrop Technologies, USA). ITS-rDNA regions were amplified using two primers, ITS1 and ITS4 (White et al. 1990). A ~700 bp portion of the *TEF1-α* gene (Translation Elongation Factor 1α) was amplified from *Fusarium* isolates using the primers, EF1 (5'-ATG GG TAA GGA RGA CAA GAC-3') and EF2 (5' GGA RGT ACC AGT SAT CAT G-3') (O'Donnell et al., 1998; Geiser et al., 2004; Edel-Hermann et al., 2012). Primers were synthesized by Macrogen, Inc. (South Korea). Twenty-five µL polymerase chain reaction (PCR) contained 1X reaction buffer, 2.5 mM $MgCl_2$, 200 mM dNTPs, 0.4 mM of each primer, 20 ng of DNA, and 1 unit of Taq polymerase (Ampliqon, Denmark). A Biometra TAdvanced Thermal Cycler (Biometra, Göttingen, Germany) were used to perform PCRs. The cycling conditions for ITS1 and ITS 4 consisted of 95 °C for 5 min, followed by 30 cycles of 95 °C for 45 s, 60 °C for 30 s, and 72 °C for 1 min, and then 5 min at 72 °C. The annealing temperature of 54 °C was used for *TEF1-α*. The sequencing was carried out by Macrogen (Macrogen Inc., South Korea). The sequences were edited by Geneious (Biomatters Inc., USA) when needed and compared to the sequences in the NCBI (GenBank) database using BLAST (Basic Local Alignment Search Tool) (Altschul et al., 1990) to find the most likely taxonomic designation of each isolate. All nucleotide sequences obtained in this study were deposited in GenBank and accession numbers were obtained as MN643060 to MN643071.

RESULTS

Fungal Isolates

Figure 3. Colony morphology of fungal species from Jefriz cave incubated on Potato Dextrose Agar (PDA) a, *Fusarium solani*. b, *Fusicolla septimanifiniscientiae*. c, *Pseudogymnoascus pannorum*. d, *Humicola grisea*. e, *Chalastospora gossypii*. f, *Penicillium brevicompactum*. g, *Penicillium expansum*. h, *Aspergillus spelunceus*. i, *Epiciccum nigrum*.

In our current investigation, 16 samples of soil and sediments from Jefriz cave were collected in 4 visits and a total of 82 fungal isolates were isolated. The morphological and molecular investigations led to the identification of *Fusarium solani*, *Fusicolla septimanifiniscientiae*, *Pseudogymnoascus* (*Geomyces*) *pannorum*, *Humicola grisea*, *Chalastospora gossypii* (formerly *Alternaria malorum*), *Penicillium expansum*, *P. brevicompactum*, *Aspergillus spelunceus*, *Epiciccum nigrum*, *Podospora* sp., *Ochroconis* sp. and *Mucor* spp. (Figs. 3 and 4). Fifteen isolates were mycelia sterilia and were not identified to species level. The most prevalent genera were *Aspergillus spelunceus* (21%), *Pseudogymnoascus pannorum* (13%) and *Humicola grisea* (10%) (Table 1). Species diversity in this cave were not attempted to estimate due to the method of isolation that was limited to culture-dependent methods. These methods give an estimate of the fungi present but is biased toward isolation of spore-forming species. Fungal species in cave air were not attempted because the air, especially in the entrance corridor, is in contact with outside air resulting in biased isolation toward common airborne fungi from outside of the cave.

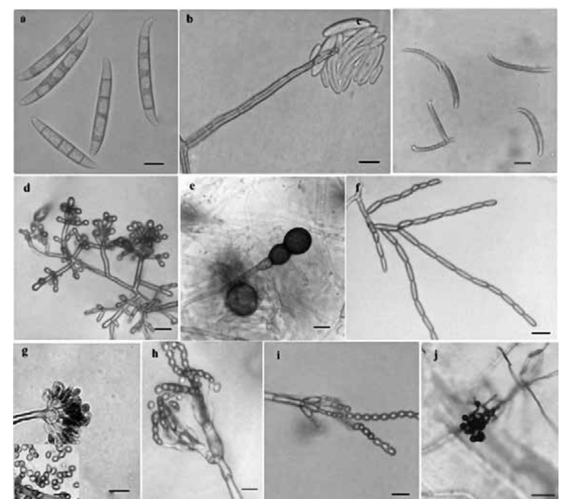

Figure 4. a-b, *Fusarium solani*: Macroconidia (a), false head and microconidia (b). c, *Fusicolla septimanifiniscientiae*: Macroconidia. d, *Pseudogymnoascus pannorum*: conidiophores and conidia. e, *Humicola grisea*: Aleurioconidia. f, *Chalastospora gossypii*: Ellipsoid conidia. g, *Aspergillus spelunceus*: Conidial head. h, *Penicillium brevicompactum*: Conidia and conidiophore. i, *Penicillium expansum*: Conidia and conidiophore. j, *Epicoccum nigrum*: Conidia. Scale bars = 10 µm, j = 50 µm.

Sampling was carried out in four visits, one in each season of the year. In each visit, samples were collected from the sites close to the entrance corridor and from sites located deep in the cave. Comparisons showed that the composition of fungal species was different among sampling sites. *Penicillium expansum* and *Aspergillus spelunceus* were the most abundant species detected from samples close to the entrance of the cave in the first large room while the most abundant species in remote and out of reach parts were *Pseudogymnoascus pannorum* and *Humicola grisea*. In addition, there was no observed difference in the composition of fungal species isolated in different seasons of the year.

Table 1. Fungal species isolated from Jefriz cave. The relative frequency is calculated by dividing the frequency of each species by the total number of all isolates.

Species	Relative frequency	Isolate used for sequencing	Accession numbers obtained from GenBank
Pseudogymnoascus pannorum	13	J2	MN643060
Humicola grisea	10	J4	MN643061
Chalastospora gossypii	5	J10	MN643062
Penicillium brevicompactum	5	J11	MN643063
Penicillium expansum	7	J17	MN643064
Aspergillus spelunceus	21	J14	MN643065
Epicoccum nigrum	4	J20	MN643066
Podospora sp.	2	J12	MN643067
Ochroconis sp.	1	J21	MN643068
Fusicolla septimanifiniscientiae	2	J0	MN643069
Fusicolla septimanifiniscientiae	...	J0	MN643070
Fusarium solani	2	J22	MN643071
Mucor spp.	7	...	...
Mycelia sterilia	20	...	...

DISCUSSION

Our goal was to provide preliminary data on fungal communities of caves to study biological interactions in subterranean and extreme environments. The present study is the first one studying the mycobiota of a cave in Iran. We were able to successfully identify eleven fungal genera from soil and sediment samples of Jefriz cave on the basis of macroscopic, microscopic, and molecular analyses.

Literature suggests that most of these species are commonly associated with cave habitats around the world (Cunningham et al., 1995; Dupont et al., 2007; Jurado et al., 2009; Bastian et al., 2010; Docampo et al., 2010; Wang et al., 2011; Jacobs et al., 2017). Some of the isolated species such as *Fusarium* spp. are common soil inhabitants (Leslie and Summerell, 2006) and it is possible that they have been carried in by the water streams that run from outside through the walls of Jefriz cave. A similar scenario was suggested for *F. solani* entering Lascaux Cave by Dupont et al. (2007). Human visitors, small animals, and arthropods are other vectors that may have contributed to the fungal flora composition of Jefriz cave. The relationship between fungi and arthropods in caves is reported in several studies. Greif and Currah (2007) and Dromph (2003) isolated several fungal species from collembolans. *Isaria farinosa* (syn: *Paecilomyces farinosus*) is reported as the one of frequent parasites occurring on insects in underground environments in the Czech Republic by Kubátová and Dvořák (2005), and also as a parasite of *Stenophylax* (syn: *Micropterna*) *fissus* in Spanish caves by Jurado et al. (2008). Arthropod roles as host and vectors of fungal species (Bastian et al., 2009a) in Jefriz cave remain to be studied in further research by entomologists.

Water films resulting from condensation cover Jefriz walls and speleothems. These wet surfaces are a suitable niche for fungal growth (Barton and Jurado, 2007). *Lecanicillium psalliotae*, *L. aranearum*, *Engyodontium album* and *Torrubiella* spp. in biofilm samples of Roman catacombs are reported (Saarela et al., 2004; Jurado et al., 2008). Another factor influencing the cave mycobiota is availability of potential food sources. Droppings and guano especially of bats may serve as possible source of nutrients and energy input for many fungal species as well (Zhang et al., 2017).

The predominant species in Jefriz cave were *Aspergillus spelunceus* (21%), *Pseudogymnoascus pannorum* (13%), and *Humicola grisea* (10%). *Aspergillus spelunceus* (sect. *Nidulantes*) have been frequently isolated from caves (Vanderwolf et al., 2013; Hubka et al., 2016). Species related to *A. nidulans* may have medical importance (Hubka et al., 2016); the prevalence of this species should receive more attention from cavers. *Aspergillus* spp. are known to be allergenic, and causal agents of aspergillosis and mycotoxin producers (IARC 2002; Perrone et al., 2007). Mycotoxins are associated with immune deficiency and cancer (Shephard, 2008). *Pseudogymnoascus pannorum* was one of the dominant species in Jefriz cave. This species expresses keratinolytic enzymes and can cause skin infections and nail geomycosis (Gianni et al., 2003; Zelenková; 2006; Reynolds and Barton, 2014) and may be dangerous to humans. *P. pannorum* tolerates low temperatures and higher salinity than seawater (Poole and Price, 1971; Leushkin et al., 2015). Characteristics such as tolerating low temperatures justifies its high prevalence in extreme environments such as caves. This species is reported from caves, arctic, and low temperature soils worldwide (Out et al., 2016). It should be noted that its close relative, *P. destructans*, is one of the commonly reported fungi from caves all over the world

(Martinkova et al., 2010; Turner et al., 2011) causing white nose syndrome in bats (Shelley et al., 2013). Another commonly-occurring species isolated from the cave samples was *Humicola grisea*. This species has high potential in starch saccharification (Maheshwari et al. 2000), which makes it a promising species in the process of producing bioethanol by microbial fermentation (Pervez et al., 2014). Several studies have reported bio-deterioration of structures in caves associated with fungal species extracellular metabolites. Gargani (1968) studied the microbial floras of damaged wall paintings in Florence after a flood and associated the damage with the growth of microorganisms. The deteriorations are the result of extracellular metabolites secreted while hyphae are penetrating inside the wall painting and cave speleothems (Strzelczyk, 1981; Garg et al., 1995).

Fusicolla septimanifiniscientiae is reported here from a cave for the first time. *Fusarium solani* was one of the especially interesting microorganisms that was identified. An aggressive isolate of this species was discovered in 2001 threatening the Paleolithic paintings of Lascaux cave, France, and resulted in multiple applications of biocides (Dupont et al., 2007). Arthropods have symbiotic relationships with *Fusarium* spp. (Morales-Ramos et al., 2000; Sharma and Marques, 2018) and are suggested as possible vectors of some species (Jurado et al., 2008). The pathogenicity of *Fusarium verticillioides* on grasshoppers (Pelizza et al., 2011) and *F. keratoplasticum* and *F. proliferatum* on *Tribolium* species (Chehri, 2017) have been reported.

Penicillium expansum, followed by *Aspergillus spelunceus*, prevailed in samples collected near the cave entrance, while *Humicola grisea* was the most abundant species in the samples collected in remote parts of the cave. Air exchange with the outside environment and the activities of cave explorers are possibly impacting on the cave airborne fungal community at the entrance. The number of visitors is not officially reported, however, the number is recently reduced because of the door implemented by locals. The impact of human activity on fungal diversity in caves is documented by Shapiro and Pringle, (2010). Their investigation showed complex associations between levels of human disturbance and the diversity of fungal species in four caves in Kentucky and Tennessee. They showed that the fungal diversity was higher in sites with moderate levels of disturbance than highly visited sites.

CONCLUSIONS

This survey was conducted to determine the major components of the mycoflora present in Jefriz cave to understand the complex biological interactions in subterranean habitats. In conclusion, 12 fungal species associated with Jefriz cave soil and sediments were identified in this study. Our data showed that the biological and climatic condition of this cave is selective. The fungal species were composed of both cosmopolitan species and common cave residents. Fungal species that can be harmful to the human health were found in Jefriz cave that evidenced the need to further investigate the hygienic safety of this place for cavers, although no illnesses have been reported. Work on unculturable species is needed to provide a thorough perspective of the fungal community in this cave. Furthermore, studies on metabolic activity and bioactive compounds of fungi, such as *Humicola grisea,* help our understanding of the biological interactions in a subterranean environment. We suggest studying the possible agents contributing to the dispersion of spores (*i.e*. arthropod vectors) in order to better take preventive actions, if needed, about the preservation of the cave and the safety of the explorers.

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REFERENCES

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J., 1990, Basic local alignment search tool: Journal of Molecular Biology, v. 215, p. 403−410. https://doi.org/10.1016/s0022-2836(05)80360-2

Barnett, H.L., and Hunter, B.B., 1998, Illustrated Genera of Imperfect Fungi: 4th. Ed, APS Press, St. Paul, MN, USA, 218 p.

Barton, H.A., and Jurado, V., 2007, What's up down there? Microbial diversity in caves: Microbe, v. 2, p. 132–138.

Barton, H.A., and Northup, D.E., 2007, Geomicrobiology in cave environments: past current and future perspectives: Journal of Cave and Karst Studies, v. 69, no. 1, p. 163−178.

Bastian, F., Alabouvette, C., and Saiz-Jimenez, C., 2009a, The impact of arthropods on fungal community structure in Lascaux cave: Journal of Applied Microbiology, v. 106, p. 1456−1462. https://doi.org/10.1111/j.1365-2672.2008.04121.x

Bastian, F., Alabouvette, C., and Saiz-Jimenez, C., 2009b, Impact of biocide treatments on the bacterial communities of the Lascaux cave: Naturwissenschaften, v. 106, p. 863–868. https://doi.org/10.1007/s00114-009-0540-y

Bastian, F., Jurado, V., Nováková, A., Alabouvette, C., and Saiz-Jimenez, C., 2010, The microbiology of Lascaux cave: Microbiology, v. 156, p. 644−652. https://doi.org/10.1099/mic.0.036160-0

Cañveras, J.C. Sanchez-moral, S., Soler, V., and Saiz-Jimenez, C., 2001, Microorganisms and microbially induced fabrics in cave walls: Geomicrobiology Journal, v. 18, no. 3, p. 223−240. https://doi.org/10.1080/01490450152467769

Chehri, K., 2017, Molecular identification of entomopathogenic *Fusarium* species associated with *Tribolium* species in stored grains: Journal of Invertebrate Pathology, v. 144, p. 1−6. https://doi.org/10.1016/j.jip.2017.01.003

Cunningham, K.I., Northup, D.E., Pollastro, R.M., Wright, W.G., and LaRock, E.J., 1995, Bacteria, fungi and biokarst in Lechuguilla Cave, Carlsbad Caverns National Park, New Mexico: Environmental Geology, v. 25, p. 2–8. https://doi.org/10.1007/bf01061824

Docampo, S., Trigo, M., Recio, M., Melgar, M., Garcia-Sanchez, J., Calderon-Ezquerro, M., and Cabezudo, B., 2010, High incidence of *Aspergillus* and *Penicillium* spores in the atmosphere of the cave of Nerja (Malaga, southern Spain): Aerobiologia, v. 26, no. 2, p. 89−98. https://doi.org/10.1007/s10453-009-9146-2

Domsch, K.H., Gams, W. and Anderson, T.H. 1980, Compendium of Soil Fungi: London, Academic Press, 860 p.

Doyle, J.J., and Doyle, J.L., 1987, A rapid DNA isolation procedure for small quantities of fresh leaf tissue: Phytochemical Bulletin, v. 19, p. 11–15.

Dromph, K.M., 2003, Collembolans as vectors of entomopathogenic fungi: Pedobiologia, v. 47, p. 245–256. https://doi.org/10.1078/0031-4056-00188

Dupont, J., Jacquet, C., Dennetière, B., Lacoste, S., Bousta, F., Orial, G., Cruaud, C., Couloux, A., and Roquebert, M.F., 2007, Invasion of the French Paleolithic painted cave of Lascaux by members of the *Fusarium solani* species complex: Mycologia, v. 99, p. 526–533. https://doi.org/10.3852/mycologia.99.4.526

Edel-Hermann, V., Gautheron, N., and Steinberg, C., 2012, Genetic diversity of *Fusarium oxysporum* and related species pathogenic on tomato in Algeria and other Mediterranean countries: Plant Pathology, v. 61, no. 4, p. 787−800. https://doi.org/10.1111/j.1365-3059.2011.02551.x

Garg, K., Jain, K.K., and Mishra, A., 1995, Role of fungi in the deterioration of wall paintings: Science of the Total Environment, v. 167, no. 1-3, p. 255−271. https://doi.org/10.1016/0048-9697(95)04587-q

Gargani, G., 1968, Fungus contamination of Florence art masterpieces before and after the 1966 disaster: *In* Walters, A.H, and Elphick, J.J, eds., Biodeterioration of Materials. Amsterdam, The Netherlands, Elsevier, P.C. p. 252–257.

Garrett, S.D., 1981, Soil Fungi And Soil Fertility: An Introduction to Soil Mycology: 2nd edn. Pergamon Press, Oxford, 160 p.

Geiser, D.M., Jiménez-Gasc, M., Kang, S., Makalowska, I., Veeraraghavan, N., Ward, T., Zhang, N., Kuldau, G.A., and O'Donnell, K., 2004, FUSARIUM-ID v.1.0: a DNA sequence database for identifying *Fusarium*: European Journal of Plant Pathology, v. 110 p. 473−479. https://doi.org/10.1023/b:ejpp.0000032386.75915.a0

Gianni, C., Caretta, G,. Romano, C., 2003, Skin infection due to *Geomyces pannorum* var. *pannorum*: Mycoses, v. 46, p. 430−432. https://doi.org/10.1046/j.1439-0507.2003.00897.x

Greif, M.D. and Currah, R.S. 2007, Patterns in the occurrence of saprophytic fungi carried by arthropods caught in traps baited with rotted wood and dung: Mycologia, v. 99, p. 7–19. https://doi.org/10.3852/mycologia.99.1.7

Hanlin, R.T., 1998, Combined Keys to Illustrated Genera of Ascomycetes, Volumes I and II: American Phytopathological Society, St. Paul, Minn. USA.

Hubka, V., Nováková, A., Peterson, S. W., Frisvad, J. C., Sklenář, F., Matsuzawa, T., Kubátová, A., and Kolařík, M. 2016, A reappraisal of *Aspergillus* section *Nidulantes* with descriptions of two new sterigmatocystin-producing species: Plant Systematics and Evolution, v. 302, no. 9, p. 1267−1299. https://doi.org/10.1007/s00606-016-1331-5

IARC, 2002, Aflatoxins. International Agency for Research on Cancer (IARC) Monograph, http://monographs.iarc.fr/ENG/Monographs/vol82/volume82.pdf.

Jacobs, A., Msimang, D., and Venter, E. 2017, First survey of the fungi from the Bakwena Cave in South Africa suggests low human disturbance: Journal of Cave and Karst Studies, v. 79, no. 2, p. 89−94. https://doi.org/10.4311/2016mb0146

Jurado, V., Fernandez-Cortes, A., Cuezva, S., Laiz, L., Cañaveras, C.J., Sanchez- Moral, S., and Saiz-Jimenez, C., 2009, The fungal colonization of rock-art caves: experimental evidence: Naturwissenschaften, v. 96, 1027e1034. https://doi.org/10.1007/s00114-009-0561-6

Jurado, V., Sanchez-Moral, S., and Saiz-Jimenez, C., 2008, Entomogenous fungi and the conservation of the cultural heritage: a review: International Biodeterioration and Biodegradation, v. 62, no. 4, p. 325−330. https://doi.org/10.1016/j.ibiod.2008.05.002

King, D.A., Hocking, A.D., and Pitt, J.J., 1979, Dichloran-Rose Bengal medium for enumeration and isolation of molds from foods: Applied and Environmental Microbiology, v. 37, p. 959−964. https://doi.org/10.1128/aem.37.5.959-964.1979

Kubátová, A., and Dvorák, L., 2005, Entomopathogenic fungi associated with insect hibernating in underground shelters: Czech Mycology, v. 57, no. 3-5, p. 221−237. https://doi.org/10.33585/cmy.57303

Leslie, J.F., and Summerell, B.A., 2006, The Fusarium Laboratory Manual: Blackwell Publishing, Ames, IA, 388 p.

Leushkin, E.V., Logacheva, M.D., Penin, A.A., Sutormin, R.A., Gerasimov, E.S., Kochkina, G. A., Ivanushkina, N.E., Vasilenko, O.V., Kondrashov, A.S., and Ozerskaya, S.M., 2015, Comparative genome analysis of *Pseudogymnoascus* spp. reveals primarily clonal evolution with small genome fragments exchanged between lineages: BMC Genomics, v. 16, no. 1, e400. https://doi.org/10.1186/s12864-015-1570-9

Maheshwari, R., Bharadwaj, G., and Bhat, M.K., 2000, Thermophilic fungi: their physiology and enzymes: Microbiology and Molecular Biology Reviews, v. 64, no. 3, p. 461−488. https://doi.org/10.1128/mmbr.64.3.461-488.2000

Martınkova, N., Backor, P., Bartonicka, T., Blazkova, P., Cerveny, J., Falteisek, L., Gaisler, J., Hanzal, V., Horacek, D., Hubalek, Z., Jahelkova, H., Kolarik, M., Korytar, L., Kubatova, A., Lehotska, B., Lehotsky, R., Lucan, R.K., Majek, O., Mateju, J., Rehak, Z., Safar, J., Tajek, P., Tkadlec, E., Uhrin, M., Wagner, J., Weinfurtova, D., Zima, J., Zukal, J., and Horacek, I., 2010, Increasing incidence of *Geomyces destructans* fungus in bats from the Czech Republic and Slovakia: PLOS ONE, 5: e13853. https://doi.org/10.1371/journal.pone.0013853

Meteyer, C., U. Buckles, E.L., Blehert, D.S., Hicks, A.C., Green, D.E., Shearn-Bochsler, V., Thomas, N.J., Gargas, A., and Behr, M.J., 2009, Histopathologic criteria to confirm white-nose syndrome in bats: Journal of Veterinary Diagnostic Investigation, v. 21, p. 411–414. https://doi.org/10.1177/104063870902100401

Morales-Ramos, J. A., Rojas, M. G., Sittertz-Bhatkar, H., and Saldaña, G. 2000, Symbiotic relationship between *Hypothenemus hampei* (*Coleoptera: Scolytidae*) and *Fusarium solani* (Moniliales: Tuberculariaceae): Annals of the Entomological Society of America, v. 93, no. 3, p. 541−547. https://doi.org/10.1603/0013-8746(2000)093[0541:srbhhc]2.0.co;2

Nováková, A., 2009, Microscopic fungi isolated from the Domica Cave system (Slovak Karst National Park, Slovakia). A review: International Journal of Speleology, v. 38, no. 1, p. 71−82. https://doi.org/10.5038/1827-806x.38.1.8

O'Donnell, K., Kistler, H.C., Cigelink, E., and Ploetz, R.C., 1998, Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies: Proceedings of the National Academy of Sciences of the United States of America, v. 95, no. 5, p. 2044−2049. https://doi.org/10.1073/pnas.95.5.2044

Out, B., Boyle, S., and Cheeptham, N., 2016, Identification of fungi from soil in the Nakimu caves of Glacier National Park: The Journal of Experimental Microbiology and Immunology, v. 2, p. 26−32.

Pelizza, S., Stenglein, S., Cabello, M. N., Dinolfo, M. I., and Lange, C.E., 2011, First record of *Fusarium verticillioides* as an entomopathogenic fungus of grasshoppers: Journal of Insect Science, v. 11, no. 1, e70. https://doi.org/10.1673/031.011.7001

Perrone, G., Susca, A., Cozzi, G., Ehrlich, K., Varga, J., Frisvad, J. C., Meijer, M., Noonim, P., Mahakarnchanakul, W., and Samson, R. A., 2007, Biodiversity of *Aspergillus* species in some important agricultural products: Studies in Mycology, v. 59, p. 53−66. https://doi.org/10.3114/sim.2007.59.07

Pervez, S., Aman, A., Iqbal, S., Siddiqui, N. N., and Qader, S. A. U., 2014, Saccharification and liquefaction of cassava starch: an alternative source for the production of bioethanol using amylolytic enzymes by double fermentation process: BMC Biotechnology, v. 14, no. 1. https://doi.org/10.1186/1472-6750-14-49

Poole, N.J., and Price, P.C., 1971, The occurrence of *Chrysosporium pannorum* in soils receiving incremental cellulose: Soil Biology and Biochemistry, v. 3, p. 161−166. https://doi.org/10.1016/0038-0717(71)90010-1

Reynolds, and H.T., Barton, H.A., 2014, Comparison of the white-nose syndrome agent *Pseudogymnoascus destructans* to cave-dwelling relatives suggests reduced saprotrophic enzyme activity: PLOS One. v. 9, no. 1, e86437. https://doi.org/10.1371/journal.pone.0086437

Saarela, M., Alakomi, H.-L., Suihko, M.-L., Maunuksela, L., Raaska, L., and Mattila-Sandholm, T., 2004, Heterotrophic microorganisms in air and biofilm samples from Roman catacombs, with special emphasis on actinobacteria and fungi: International Biodeterioration and Biodegradation, v. 54, no. 1, p. 27−37. https://doi.org/10.1016/j.ibiod.2003.12.003

Seifert, K., Morgan-Jones, G., Gams, W., and Kendrick, B., 2011, The genera of Hyphomycetes: CBS-KNAW Fungal Biodiversity Centre, Utrecht, 997 p.

Shapiro, J., and Pringle, A., 2010, Anthropogenic influences on the diversity of fungi isolated from caves in Kentucky and Tennessee: The American Midland Naturalist, v. 163, no. 1, 76−87. https://doi.org/10.1674/0003-0031-163.1.76

Sharma, L., and Marques, G., 2018, *Fusarium*, an Entomopathogen—A myth or reality?: Pathogens, v. 7, no. 4, e93. https://doi.org/10.3390/pathogens7040093

Shelley, V., Kaiser, S., Shelley, E., Williams, T., Kramer, M., Haman, K., Keel, M.K., and Barton, H.A., 2013, Evaluation of strategies for the decontamination of equipment for *Geomyces destructans*, the causative agent of white-nose syndrome (WNS): Journal of Cave and Karst Studies, v. 75, no. 1, p. 1−10. https://doi.org/10.4311/2011lsc0249

Shephard, G.S., 2008, Impact of mycotoxins on human health in developing countries: Food Additives and Contaminants, v. 25, p. 146−151. https://doi.org/10.1080/02652030701567442

Strzelczyk, A.B., 1981. Paintings and sculptures, *in* Rose, A.H. ed., Microbial Deterioration, Academic Press, London, United Kingdom, p: 203−234.

Turner, G.G., Reeder, D.M., and Coleman, J.T.H., 2011, A five-year assessment of mortality and geographic spread of white-nose syndrome in North American bats and a look to the future: Bat Research News, v. 52, p. 13–27.

Vanderwolf, K.J., Malloch, D., McAlpine, D.F., and Forbes, G.J., 2013, A world review of fungi, yeasts, and slime molds in caves: International Journal of Speleology, v. 42, no. 1, p. 77−96. https://doi.org/10.5038/1827-806x.42.1.9

Wang, W., Ma, X., Ma, Y., Mao, L., Wu, F., Ma, X., An, L., and Feng, H., 2011, Molecular characterization of airborne fungi in caves of the Mogao Grottoes, Dunhuang, China: International Biodeterioration and Biodegradation, v. 65, no. 5, p. 726−731. https://doi.org/10.1016/j.ibiod.2011.04.006

White, T.J., Bruns, T., Lee, S.J., and Taylor, J.L., 1990, Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics: PCR Protocols, v. 18, no. 1, p. 315−22. https://doi.org/10.1016/b978-0-12-372180-8.50042-1

Zelenková, H., 2006, *Geomyces pannorum* as a possible causative agent of dermatomycosis and onychomycosis in two patients: Acta Dermatovenerologica Croatica, v. 14, n. 1, p. 21–25.

Zhang, Y., Zhang, S., Liu, X., Wen, H., Wang, M., 2010. A simple method of genomic DNA extraction suitable for analysis of bulk fungal strains: Letters in Applied Microbiology, v. 51, no. 1, p. 114−118. https://doi.org/10.1111/j.1472-765x.2010.02867.x

Zhang, Z. F., Liu, F., Zhou, X., Liu, X. Z., Liu, S. J., and Cai, L., 2017, Culturable mycobiota from Karst caves in China, with descriptions of 20 new species: Persoonia, v. 39, p. 1–31. https://doi.org/10.3767/persoonia.2017.39.01