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RESEARCH ARTICLE

Cave Entrance dependent Spore Dispersion of Filamentous Fungi Isolated from Various Sediments of Iron Ore Cave in Brazil: a colloquy on human threats while caving

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Study Area: Nova Lima, Minas Gerais, Brazil
Coordinates: 20°04'39"S, 44°00'64" W

Key words: Iron ore Cave; Iron Quadrangle, Pathogenic fungi, Cave Sediments.

Abstract

Caves are stable environments with characteristics favoring the development of fungi. The fungal community present in a cave also includes pathogenic and opportunistic species out of which some are also served as energy sources in such energy stared ecosystems. Studies on microbial diversity and their role on such energy starved ecosystem are scarce. The present study was aimed to identify the cultivable filamentous fungi present in the various sediment of an iron ore cave and to recognize them as pathogenic and/or opportunistic species. Further the impact of cave entrance on the spore depositions on

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various distances dependent sediments were analyzed. The results suggest a diverse microbial community inhabiting the cave and an influence of cave entrance over spore deposition on various sediments. We counted a total of 4,549 filamentous fungi that included 34 species of 12 genera: *Acremonium, Aspergillus, Cladosporium, Fusarium, Geotrichum, Paecilomyces, Purpureocillium, Penicillium, Torula, Trichoderma, Mucor* and *Rhizopus*. A positive significant relation was observed between spore deposition and distance from cave entrance (p= 0.001). Areas of potential mycoses risks were recognized. This is the first study on microbiological community of an iron ore cave in the country.

Introduction

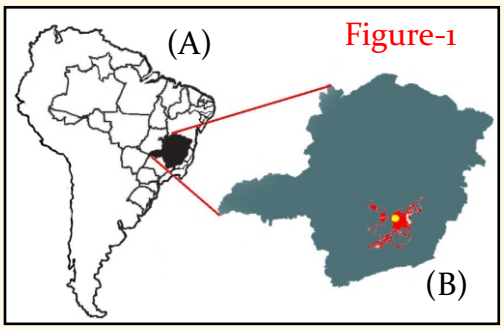
From an angle of national economy and biodiversity, the Iron Quadrangle (Quadrilátero Ferrífero) is an important region of Southeastern Brazil. It is located in the central area of Minas Gerais state and it has been scenario of polemic discussions due to occurring large mining activities and urban development there. Though, the region has one of the greatest iron deposits in the world (Auler & Piló, 2005), it is considered an economically important region not only for the country Brazil but for the entire world too. Further, due to its geographic position between the two important biodiverse hotspot biomes Atlantic Forest and Cerrado (Drummond *et al.,* 2005) it could be also referred as an environmentally potential region.

The occurrences of numerous subterranean caves formed in distinctive lithological types are the additional factor of interest for this particular area (CECAV, 2011). Iron ore caves are the randomly exploring subterranean passages in Brazil since the year 2000, when large mining activities enhanced in the Iron Quadrangle. However till date only a fraction of that has been limelighted (Auler & Piló, 2005) and the biospeleological studies in such caves are still scanty (Ferreira, 2005). The available information on such caves is mostly concentrated to the issues required by the government, prior to the exploitation of that particular region for mining activities. Considering the importance of this region and the increasing environmental impacts where the caves are located (Piló & Auler, 2005), it is alarming the lack of studies on cave microbiota of iron ore caves of the Iron Quadrangle.

Caves have unique environmental characteristics that may favor the development of many microorganisms. The most distinctive features are the absence of light, high humidity and the almost constancy in temperature (Vandel, 1965; Poulson & White, 1969; Gunn 2004; Culver & White 2005;). Fungi have fundamental importance on the decomposing of different substrates, influencing energetic flow through the subterranean food webs and thus playing an important ecological role on the cave community structuring. They itself also serve as food resource to some of the cave-dwelling organisms (Estrada-Bárcenas *et al.*, 2010; Gunn, 2004; Culver & White, 2005) and aid in the population control of other invertebrates by entomopathogenic organisms (Gunde-Cimerman *et al.*, 1998; Yoder *et al.*, 2009).

Among the Fungi more frequently found in the subterranean ecosystems are some human opportunistic pathogens (Resende-Stoianoff *et al.,* 2012, Vanderwolf *et al.,* 2013; Taylor *et al.,* 2013, Biswas *et al.,* 2013). The genera *Aspergillus, Paecilomyces, Purpureocillium, Fusarium, Mucor* and *Trichoderma* are filamentous fungi commonly isolated from caves which also reported to be infectious to humans (Armstrong, 1993; Pfaller & Diekema, 2004; Trabulsi & Alterthum, 2004). *Histoplasma capsulatum* is a true human pathogen commonly causing a severe respiratory and systemic infection named histoplasmosis (Gompertz *et al.*, 2004) which is reported to be associated with cave visitation in Brazil (Cury et al., 2001) and also other caves worldwide (Carvajal-Zamora, 1977; Ashford *et al.*, 1999; Lyon *et al.*, 2004).

Studies on cave fungal communities have been increasing since the past few decades, but we have very limited available literature concerning microbial diversity in Brazilian caves. The study of fungal distribution in caves and their role in its complete biosphere are of extreme importance (Vanderwolf *et al.*, 2013). Interestingly, Iron ore caves are recently discovered in the Iron Quadrangle, Brazil and yet no serious attempt has been taken to know its cave microbiota. Nevertheless, Ferreira *et al.* (2005) have reported diversified cave fauna; including a large number of new troglobitic species from the caves of the Iron Quadrangle. Thus, it is plausible to affirm that the region may be harboring an important microbiological biodiversity in caves too. By keeping the same in mind, the present study was aimed to identify the cultivable filamentous fungi present in the sediment of an iron ore cave which is located in an environmentally protected area of Brazil. Further, Fernandez-Cortes *et al.,* (2011) reported a relation between air-borne spore load with opening of a show cave entrance door. Thus it is plausible to state that there may be a gradient of spore-load deposition on sediment associated with distance from entrance. Furthermore, this relation may also be expressing a relation between external micobiota and the colonization process by air flow toward deeper zones of caves. In this study we have also tried to verify the same hypothesis. The specific roles of identified mycospecies as human pathogens have also been discussed to keep alert the visiting tourists.



RM3 cave (Nova Lima, MG). (A) Localization of Minas Gerais state (left) and (B) the Iron Quadrangle (in red) with cave represented by yellow dot.

Materials and Methods:

Study area: for our study, we targeted RM3 an iron ore cave, which is located in the Iron Quadrangle (Quadrilátero Ferrífero) region . It belongs to an environmentally protected area known as Parque Estadual do Rola Moça (PERM-MG), in the municipality of Nova Lima, Minas Gerais state, Brazil. This park is surrounded by areas exposed to impacting human activities such as urban development, agriculture, pasture and mining.

Rm3 is a hollow tubular shaped cave of about 27 meters linear development. The temperature on the sampling day was noted as 19 to 20°C and humidity 94%. There were

apparently no active bat colonies inhabiting the cave. Although the cave is not a show/tourist cave, the human intrusion in the cave was accustomed as a common phenomenon (Figure-2). Furthermore, the area comprises a natural reserve close to a prominent Brazilian city (Belo Horizonte) which attracts many tourists. Conclusively, the cave has potential to receive tourists especially due to the unusual lithology (iron ore) in which it has been formed allied to the scenic beauty of external landscape. Although the access may be difficult for the common tourist (due to proximity of entrance to the clift), it may be a centre of attraction for trained tourists like cavers, park guides, adventurous sports persons and scientists too.

Ecological Survey: each and every nook and corner of the RM3 cave was minutely surveyed by our team members to trace and identify all sorts of organic materials available inside the cave. During survey, the apparent cavernicolous species were also tried to recognize and their possible role in the cave ecosystem was tried to understand.

Sample collections and mycoflora identification: for sample collections we selected six fixed stations inside the cave, right from entrance to deeper zone (S1 to S6; Figure-2). Single sample sediment fro the floor was collected from each sampling station and kept in sterilized bags. Further, the bags were sealed in situ, transported to laboratory and were refrigerated at 4°C until their processing (within five days). Each sediment sample was diluted in sterilized distilled water with saline solution (0.85% NaCl) (1:10) and the isolates obtained through the 10-fold dilution technique (10-1 to 10-5). The solutions were inoculated in triplicates on Dichloram Rose Bengal Cloramphenicol Agar (DRBC, Acumedia Laboratories, Lansing, MI USA) and Sabouraud Dextrose Agar (SDA, Difco Laboratories, Detroit, MI USA) with cloramphenicol (0.1%). After inoculation, the Petri dishes were incubated at 25°C for seven days as standardized for filamentous fungi and considering the cave temperature. Colonies were then counted and total fungal abundance (CFU/g of sediment) was noted for further comparison and analysis.

All isolates were purified on Malt Extract Agar (MEA, Difco) and incubated for at least seven days at 25°C. The purified colonies were first taxonomically identified to at least the genus level and clustered according to their macro and microscopic morphological similarities. After this first triage, isolates of each genus were incubated on specific media for morphological identification (macroscopic and microscopic) following the identification keys, specified for each taxonomic group (Pitt, 2000; Klich, 2002; Samson & Frisvad, 2004; Domsch et al., 2007).

This morphological identification method for each identical genus required incubation of isolates at different temperatures and some specific solid media. Following the same, we used the following media (1) Creatine Sucrose Agar (CREA, according to Samson & Frisvad, 2004) (2) Czapeck Yeast Extract Agar (CYA, Labsynth, Diadema, SP Brazil), (3) Malt Extract Agar (MEA, Difco), (4) Potato Dextrose Agar (PDA, Acumedia) and (5) Yeast Extract Sucrose Agar (YES, according with Samson & Frisvad, 2004). We used the mentioned media to identify the following genera (solid media used represented by number): *Acremonium*$_{(2,3)}$ *Aspergillus*$_{(2,3)}$, *Cladosporium*$_{(3)}$, *Fusarium*$_{(2,3,4,5)}$, *Geotrichum*$_{(2,3)}$, *Mucor*$_{(2,3)}$, *Paecilomyces*$_{(2,3)}$, *Purpureocillium*$_{(2,3)}$,

Penicillium$_{(1,2,3)}$, *Torula*$_{(3)}$ and *Trichoderma*$_{(2,3)}$(Pitt, 2000; Klich, 2002; Samson & Frisvad, 2004; Domsch *et al.,* 2007). The fungal isolates are currently deposited at the mycological collection of our Mycological Laboratory, Federal University of Minas Gerais (Laboratório de Micologia da Universidade Federal de Minas Gerais-LM/UFMG) (Belo Horizonte, Minas Gerais state - Brazil).

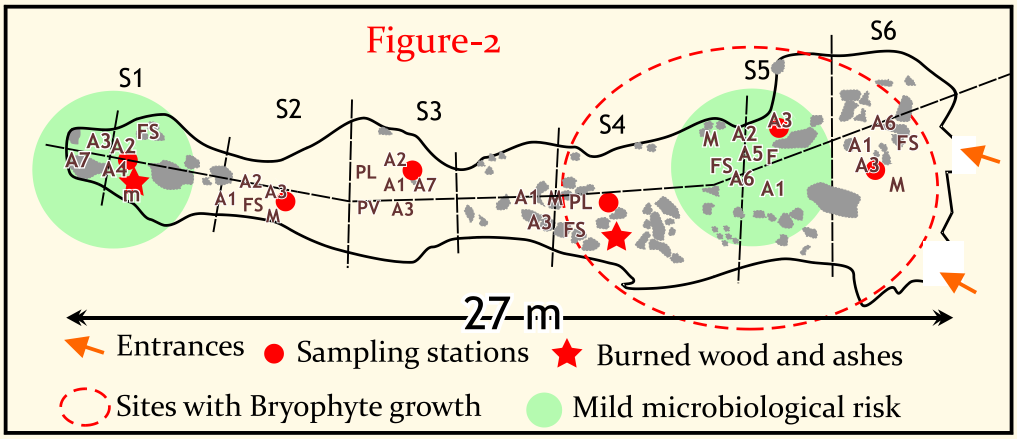
Impact of Cave entrance on spore distribution inside the cave: we verified the relation between distances from the entrance and sediment spore-load by comparing total abundance, expressed by colony forming units (CFU) per gram of sediment (CFU/g), obtained after seven days of incubation at 25°C. This data was extracted from the Petri dishes used in the serial dilution process (10^{-1}-10^{-5}). These dishes contained DRBC solid media, which was selected due to its nutrient-rich nature and bacterial inhibition by cloramphenicol.

The analysis was performed in triplicates for a more statistically reliable result. The statistical significance was determined by a linear regression explained below.

Statistical analysis: a Linear Regression analysis between fungal total abundance (H) and distance from cave entrance (m) was applied to determine the influence of cave entrance on spore deposition on sediment. Total abundance was determined by total CFU/g of sediment. The Ordinary Least Sum of Squares method (OLS) was used. The data was logarithymized for more reliable results. Significance of results was determined as $p< 0.05$. The programs Statistica 9.0 and PAST 2.13 were used for statistical analysis.

Pathogenic and other opportunistic species: presence of pathogenic and other opportunistic fungal species was investigated by processing the material obtained from the serial dilution on SDA and incubated for 7-42 days at 37°C. Fungi capable of growing at this temperature were identified and an extensive search for published medical reports relating these species to human opportunistic infections was investigated. When a relation was found, the related species was classified as medically relevant and areas with high fungal concentrations (>30% of total CFU) as areas of microbiological risk.

Special attention was given to one particular species, *H. capsulatum* due to its critical medical importance and close relation with subterranean environments. We have attempted to isolate the same from the sediment using the previously mentioned serial dilution method (in duplicates) on SDA (25°C) and Brain Heart Infusion Agar (BHIA, Acumedia) (37°C) (Carvajal-Zamora, 1977; Gompertz et al., 2004; Guimarães et al., 2006) with chloramphenicol (0.1%) to inhibit bacteriological growth. The Petri dishes were observed until 42 days after inoculation. Conversion of suspicious yeast into mycelial form (and vice-versa), as well as slide preparation for observation of microscopic morphology were performed to confirm or refute the presence of *H. capsulatum* (Carvajal-Zamora, 1977; Gompertz *et al.,* 2004; Guimarães *et al.,* 2006). Finally, all the identified opportunistic and pathogenic species were plotted in the cave map and microbiological risk was indicated.



Distribution of opportunistic filamentous fungi are also represented: A1 (*A. caespitosus*), A2 (*A. candidus*), A3 (*A. flavus*), A4 (*A. fumigatus*), A5 (*A. japonicus*), A6 (*A. niger*), FS (*F. solani*), PL (*P. lilacinum*), PV (*P. variotii*), M (*Mucor* spp), and F (*Fusarium* sp). Although *Fusarium* sp and *Mucor* spp were not confirmed as opportunistic species, they were included in the map due to the risk posed by the genera.

Results:

Trophic characterization of RM3 cave: few debris were the only visible organic resources present in the deeper stations of the cave, left either by human visitors or animals. Burned wood (charcoal pieces) and ashes were observed in S1 and S4 stations. Few bryophyte growths are apparent in S4 station. Mammalian dung was recorded in S3 station. Finally S5 and S6 stations of deeper zone were characterized by large Bryophyte growth and other vegetal organic matter. An expressive fauna was observed at these last two stations (S5 and S6), which includes fungivorous insects, Psocoptera and Collembola (systematic identification is under process). Hazardous litter was observed in S1 (PET plastic bottle), S2 (newspaper and plastic bag) and S4 (aluminum can). Additionally, high concentration of Actinomycetes was observed on the walls and sediment of the cave, especially near sampling station S4 (Figure 2).

Filamentous Fungi Inventory: total 4,549 filamentous fungi were counted from the sediment processed by serial dilution and incubated on DRBC (3,331) and SDA with cloramphenicol (1,218) out of which, 3,856 were purified and identified. This mycobiota included at least 34 species distributed among 12 genera: *Acremonium* (1), *Aspergillus* (10), *Cladosporium* (1), *Fusarium* (2), *Geotrichum* (1), *Paecilomyces* (1), *Purpureocillium* (1), *Penicillium* (11), *Torula* (1), *Trichoderma* (1), *Mucor* (3) and *Rhizopus* (1). The most diverse genus was *Penicilium* (11 species) followed by *Aspergillus* (10 species) (Table 1).

The species *Aspergillus flavus* and *Penicillium chrysogenum* were found abundantly distributed, being isolated from all the sampling stations. Further, *A. caespistosus, A. versicolor, F. solani* and *Mucor* sp3 were also found to be well distributed as isolated from at least four stations each carrying 66.7%. The *Penicillium* genera were exhibited more limited frequency per species. *Penicillium chrysogenum* performed the best distribution (100%), followed by *P. decumbens* (50%) (Table 1).

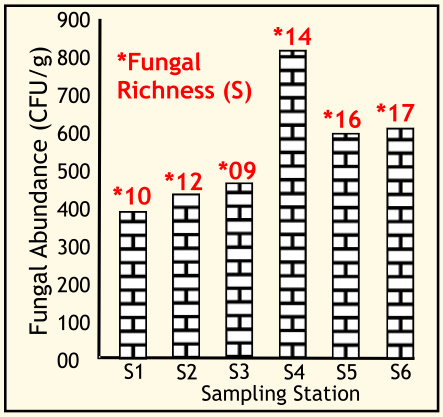


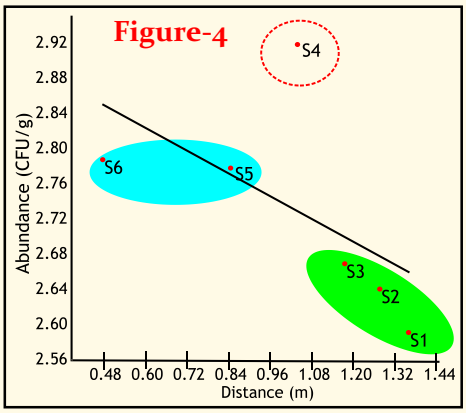
Figure-3: Abundance and Richness of filamentous fungi isolated from sediment in RM3 cave

The highest abundance was observed in sampling station S4 (827), which was in the disphotic (twilight) zone. The second and third highest values were registered in S5 (598) and S6 (613), both the stations were also nearer to the entrance. The lowest value was registered in the deepest station S1 (390). Fungal richness was determined by number of species. Although, in general, the richness inside the cave did not vary considerably, the highest values were observed near the entrance (S5 and S6). The lowest value was observed in S3 (Figure-3).

Pathogenic and opportunistic filamentous fungi: total nine species were such identified which could be suspected to play roles in human opportunistic infections, such as Pulmonary Aspergilosis, caused by *A. fumigatus, A. flavus, A. japonicus, A. niger* and *A. candidus* (Ribeiro *et al.,* 2005); Onicomycosis, caused by *A. versicolor, A. caespitosus, A. candidus* and *Fusarium solani* (Bahram et al., 2012); Keratomycosis, caused by *A. flavus, A. fumigatus, Fusarium solani* and *P. lilacinum* and few other systemic opportunistic infections caused by *F. solani.* (Takayasu *et al.*, 1977; Boutati & Anaissie, 1997; Lacaz *et al.*, 2002; Nucci & Anaissie, 2002 Ribeiro *et al.*, 2005; Kredics et al., 2008; Veraldi et al., 2010; Agudo *et al.,* 2011; Bahram *et al.*, 2012).

Although *H. capsulatum* was not isolated and no guano patches were observed in RM3, other fungal risks were pointed. A high concentration of *A. flavus* was observed in S5 (32%) and a low concentration of *A. fumigatus* in S1 station (1.02%).

Impact of Cave entrance on spore distribution inside the cave: fungal spore-loads on various sediments were analyzed considering the total abundance (UFC/g) in each sampling station and the relation between them and their respective distances from the cave entrance were statistically calculated. The Linear Regression analysis showed a negative statistically significant relation between total abundance of filamentous fungi and distance from the entrance (R=0.64, R2=-0.41, p=0.001). It was also possible to observe a clear differentiation between stations. These clustered in three groups: 1) composed of the most external stations and presenting similar total abundance (S6 and S5); 2) one station in the



Linear Regression analysis (OLS method) correlating total fungal abundance (CFU/g) with distance(m) from entrance. The sampling stations grouped in three distinct groups and represented in graphic by: light grey blue (more external stations); green ellipsis line (deeper stations in aphotic station); and red dotted line circle (station in disphotic station) (p=<0.001)

twilight zone and presenting higher abundance(S4); and 3)stations grouped in the dark stations with lower results and smaller total abundance (S1, S2 and S3) (Figure -4). However, no statistically significant result was observed between richness and distance from the entrance.

Discussion:

Most of the species isolated from RM3 have also been reported from the caves lying in the other parts of Brazil (Casirillión *et al.,* 1976; Resende-Stoianoff *et al.,* 2012; Vanderwolf *et al.,* 2013; Taylor *et al.,* 2013) and also elsewhere around the world (Carvajal-Zamora & Nieves Rivera, 1998; Koilraj *et al.,* 1999; Nieves-Rivera, 2003; Ulloa *et al.,* 2006; Nieves-Rivera *et al.,* 2009, Nováková, 2009; Jurado *et al.,* 2010; Vanderwolf e*t al.,* 2013). Our results strengthened the worldwide distribution and occurrence of fungi in subterranean environments with special reference to an unusual lithological biosphere (iron sediments).

Aspergillus and *Penicillium* are the most common genera isolated from caves (Vanderwolf *et al.,* 2013). In our study, we found *Penicillium* and *Aspergillus* in almost similar proportion (Table-1). *Aspergillus flavus* and *Penicillium chrysogenum* were found in all the sampled stations. These fungi usually occurs in various habitats and are well distributed in the soil and the air of epigean and hypogean environments (Pitt, 2000; Klich, 2002; Hedayati *et al.,* 2007; Vanderwolf *et al.,* 2013; Taylor *et al.,* 2013; Biswas *et al.,* 2013).

While isolation, we identified few fungi from the sediments of this cave which are will established opportunistic human pathogens. *Aspergillus fumigatus* is the main causative agent of Pulmonary Aspergollosis, followed by *A. flavus, A. niger* and *A. candidus* (Lacaz et al., 2002; Kousha et al., 2011). Pulmonary Aspegillosis is a fungal infection that starts with spore inhalation and develops into a more serious pulmonary infection. This opportunistic infection is strongly related with the host immunologic response and also occasionally proven fatal. *A. fumigatus* was found in RM3 cave, though its concentration was low (1.02%) at S1, we only indicate this station as demanding further monitoring of spore levels. Although A. flavus is not considered as the main causative agent of serious opportunistic Pulmonary Aspergillosis, S5 zone was considered of mild risk due to high levels of this fungus (Figure- 2). Both S1 and S5 were classified as mild microbiological risk.

Other opportunistic infections such as keratomycosis, onychomycsis and otomycosis are caused by different species of the filamentous fungi *Aspergillus, Fusarium, Mucor, Purpureocillium* and *Paecilomyces* (Takayasu *et al.,* 1977; Boutati & Anaissie, 1997; Lacaz *et al.,* 2002; Nucci & Anaissie, 2002; Godoy *et al.,* 2004; Ribeiro *et al.,* 2005; Kredics *et al.,* 2008; Veraldi et al., 2010; Agudo *et al.*, 2011; Bahram *et al.,* 2012). We have found some of these species in RM3 cave. Considering the chances of more visitors accessing the cave (e.g. park guides, cavers, scientists and other adventurous tourists), it is essential that more studies are required to monitor spore levels in the cave. The occasional visit allied with the risk of serious fungal infections by opportunistic fungi is enough to suggest the monitoring of fungal community and time to time the upgrading information on the same must be available in the visitors' centre of the park. Visitors and guides should be aware to the risks they are exposed to.

Concerning the distribution of fungi on the sediment of RM3 cave, we found a significant negative relation with distance from the entrance. This means that spore-load on sediment

decreases towards deeper stations of the cave. Fernandez-Cortes *et al.* (2011) reported the influence of entrance by changes on airborne spore load associated with opening of door in a show cave in Southern Spain. Our results corroborate with the same and highlight the importance of understanding the relations between hypogean and epigean ecosystems to serve as a baseline for further conservational actions.

The low coefficient of determination (R^2) value found in the statistical analysis (41%) may be highlighting the influence of other factors on fungal spore abundance on the sediment that were not evaluated in this study (e.g. predation and antagonist interactions with other microorganisms). The station located at the disphotic zone (S4) presented the highest abundance value. This result may be related with the fact that in this station there is less direct exposure to sunlight, as well as less humidity and temperature oscillations compared to the stations closer to the entrance (S5 and S6). In addition, the fungivorous insects Psocoptera and Collembolla were abundant in stations S5 and S6 (taxonomic identifications of such species is yet to be published).

Fungal predation by cave fauna is a well established phenomenon (Estrada-Bárcenas *et al.,* 2010; Gunn, 2004). Food preference for the cave collected Acari (Sancassania) towards a pathogenic fungi *H.capsulatum* in laboratorial conditions is already reported (Estrada-Bárcenas *et al.,* 2010). It is widely known that Psocoptera (barklice) and Collembola (springtail) feed on fungi (Schou & Simmerling, 2004; Kalinovic *et al.,* 2006). Kalinovic *et al.,* (2006) reported a predatory interaction between a group of *Psocoptera* and *A. flavus* (which occurred abundantly near the cave entrance) (S5). We found large populations of Collembola and Psocoptera nearer to the cave entrance (S5 and S6). Therefore, it is plausible to deduce that predation is higher in the more external sampling stations (S5 and S6) due to fungivorous habits, resulting in a decrease of fungal spore-load on sediment (comparing to S4). Furthermore sampling station S4 has visually more vegetal organic matter than S3, S2, and S1, which could favor fungal growth and spore deposition. Finally, we suggest that interactions between fungal communities and cave fauna should be studied more for a better understanding of these relations.

Cavender-Barres *et al.* (2010) discussed the colonization by different organisms and interactions between these organisms as important factors structuring a community. This elucidates questions on how modifications of surrounding epigean landscape (such as deforestation or reforestation) could be disruptive factors affecting the entire cave microbiota. Changes on pristine cave environmental characteristics have already been reported as cause of fungal community disequilibrium, leading to *Fusarium* and black yeasts outbreak in Lascaux cave, France (Bastian *et al.,* 2010).

It is important to highlight that opportunistic (e.g. *Aspergillus* spp, *Fusarium* spp, *Mucor* spp, *Paecilomyces* spp, *Purpureocillium* spp, *Trichoderma* spp) and pathogenic (*H. capsulatum*) fungi are always a part of the cave ecosystem and have been constantly isolated from such environments (Casirillión *et al.,* 1976; Silveira, 1985; Koilraj *et al.,* 1999; Cury *et al.,* 2001; Nieves-Rivera, 2003; Ulloa *et al.,* 2006; Nieves-Rivera *et al.,* 2009; Novaková, 2009; Vanderwolf *et al.,* 2013; Taylor *et al.,* 2013) . Perhaps, changes on microbial communities caused

by anthropogenic pressures could misbalance the equilibrium between species and lead to pathogenic and opportunistic fungal outbreaks. This could reach alarming environmental and medical proportions.

Unfortunately, studies on cave microbiota are scarce and have always been neglected in management plans not only in Brazil but throughout the world. Changes of pristine conditions have been a common practice in the Iron Quadrangle. It usually involves deforestation and reforestation of primary vegetation due to urban development, agriculture and mining activities (Jacobi *et al.,* 2007). The post effects scenario regarding ambient epigean environment alterations and its impact on cave mycobiota is still properly not explored. Thus, introduction of new species (pathogenic or non) may be occurring without any acknowledgement of previous community condition. This modification could lead to fungal outbreaks or disruption of fungal community equilibrium, as already discussed by Barton (2006), Barton & Northup (2007) and Vanderwolf *et al.* (2013).

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Table 1. Fungi isolated from sediment in RM3 cave and distribution along the sampling stations.

Taxa	Occurrence in RM3 cave
Ascomycota:	
Acremonium sp	*S3, S6*
Aspergillus caespitosus Raper & Thom 1944	*S2, S3, S4, S5, S6*
A. candidus Link 1809	*S1, S2, S3, S5*
A. flavus Link 1809	*S1, S2, S3, S4, S5, S6*
A. fumigatus Fresenius 1863	*S1*
A. japonicus Saito 1906	*S5*
A. niger Tieghem 1867	**S5, S6**
A. niveus Blochwitz 1929	*S1, S4*
A. ochraceus Wilhelm 1877	*S4, S5, S6*
A. sclerotiorum Hubber 1933	*S5, S6*
A. versicolor (Vuillemin) Tiraboschi 1908	*S1, S3, S5, S6*
Cladosporium cladosporioides (Fresenius) Vries 1952	*S2, S3, S4,*
Fusarium solani (Martius) Saccardo 1881	*S2, S4, S5, S6*
Fusarium sp	*S5*
Geotrichum sp	*S2*
Paecilomyces variotii Bainier 1907	*S6*
Purpureocillium lilacinum (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson 2011	*S3, S4*
P. chrysogenum Thom 1910	*S1, S2, S3, S4, S5, S6*
P. commune Thom 1910	*S6, S2*
P. decumbens Thom 1910	*S1, S4, S6*
P. glabrum (Wehmer) Westling 1911	*S2*
P. griseofulvum Dierckx 1901	*S3, S5*
P. oxalicum Currie & Thom 1915	*S6*
P. purpurogenum Stoll 1904	*S2, S4*
P. restrictum Gilman E Abbott 1927	*S4,*
P. simplicissimum (Oudemans) Thom 1930	*S4, S6*
P. thomii Maire 1917	*S5*
Penicillium sp	*S4*
Torula sp	*S1, S2,*
Trichoderma viride Persoon 1794	*S5, S6*
Zygomycota:	
Mucor sp.1	*S5,S6*
Mucor sp.2	*S5*
Mucor sp.3	*S1, S2, S4,S6*
Rhizopus sp	*S6*