A world review of fungi, yeasts, and slime molds in caves

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Abstract: We provide a review of fungi, yeasts, and slime molds that have been found in natural solution caves and mines worldwide. Such habitats provide frequent roost sites for bats, and in eastern North America the environmental conditions that support white-nose syndrome, a lethal fungal disease currently devastating bat populations. A list of 1029 species of fungi, slime molds, and yeasts in 518 genera have been documented from caves and mines worldwide in 225 articles. Ascomycota dominate the cave environment. Most research has been conducted in temperate climates, especially in Europe. A mean of 17.9±24.4SD fungal species are reported per study. Questions remain about the origin and ecological roles of fungi in caves, and which, if any, are cave-specialists. In the northern hemisphere, caves are generally characterized by relatively stable, low temperatures and a lack of organic substrates. This environment favors communities of oligotrophic, psychrotolerant fungi. Data that may help explain how cave environmental features and faunas influence the introduction and transmission of cave fungi remains scant.

Keywords: cave; fungi; yeast; mine; Geomyces destructans; bats

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INTRODUCTION

The sudden and catastrophic appearance of a lethal fungal disease, White Nose Syndrome (WNS), in cave-dwelling North American bats in 2006 has stimulated increased interest in the fungi that occur in caves (Wibbelt et al., 2010; Lindner et al., 2011). WNS is caused by the recently described fungus Geomyces destructans Blehert & Gargas 2009 (Lorch et al., 2011), but it is still unclear if G. destructans is native to North America or a newly arrived exotic (Gargas et al., 2009; Puechmaille et al., 2011). The current limited information suggests the latter and points to Europe as the origin for this infection (Puechmaille et al., 2011); an infection now estimated to have killed 5.7-6.7 million North American bats and one predicted to result in North American agricultural losses of $3.7 billion/year (Boyles et al., 2011; U.S. Fish and Wildlife Service news release, January 17, 2012).

This paper reviews information on fungi, yeasts, and slime molds that have been reported from caves and cave-like habitats (i.e. mines) worldwide. Much of the published literature dealing with fungi, yeasts, and slime molds in caves is found in sources that are scattered, obscure, or not readily available. Rutherford and Huang (1994) presented a short review of the literature on cave fungi (127 species in 59 genera) but did not include all relevant papers available at that time, and many articles have since been published. Landolt et al. (1992) summarized the few records of slime molds found in caves, but otherwise there has been no comprehensive review of the microflora in cave habitats. Here we organize data from 225 studies dealing with fungi, yeasts, and slime molds occurring in caves, with 130 of these allowing some exploration of broader patterns of occurrence in relation to the ecology of the cave habitat. We also identify gaps in information, but most importantly, we note the need for adequately applied and consistently reported methodologies. Our hope is that the review of cave fungi presented here will prove timely and useful to both speleologists and WNS researchers.

METHODS

This review summarizes data on the fungi, yeasts, and slime molds of caves from 225 papers published in the peer-reviewed literature of 149 journals and
10 books issued in 14 languages (English, French, German, Dutch, Russian, Spanish, Italian, Czech, Croatian, Romanian, Slovene, Polish, Japanese, and Greek). Although we have included much non-English scientific literature, additional literature likely exists. We have restricted our review to solution caves (caves formed naturally in limestone and gypsum bedrock) and mines, sites most frequently used as hibernacula by bats. Thus, we do not cover fungi, yeasts, or slime molds reported from sea caves, ice caves, lava tubes, tectonic rift caves, or other natural or human-made subterranean habitats (e.g. tombs and temples, Agrawal et al., 1988; Saarelta et al., 2004; Simonovicova et al., 2004; Kiyuna et al., 2008), some of which may infrequently support roosting bats. All mine types except open-pit were included. Mine types studied in the relevant literature include gold, asbestos, coal, iron, clay, copper, nickel, phosphate, aluminum, uranium, and manganese. *Histoplasma capsulatum*, one of the best studied fungal species associated with caves, has often been targeted exclusively by researchers, and of late, *Geomyces destructans* has become a fungal species of great interest. The numerous reports of these species are examined separately. We located papers using the internet search engines Thomson Reuters’ ISI Web of Science and Google Scholar, as well as by scanning bibliographies, reading books on cave science, and drawing on two previous compilations (Rutherford & Huang, 1994; Landolt et al., 1992). A fungal species list was extracted from each paper and where reported, we recorded details of the methodologies followed in each study, including location, the number of samples collected, the number and type of caves or mines sampled, whether bats or other fauna were present, the presence of standing or running water, the length of the cave or mine, cave temperature and humidity, the substrates sampled, whether culture or genetic methods were used, culture media used, incubation temperature and length of incubation period. As used here, a ‘location’ is a country with two exceptions: Canada has been divided into eastern and western halves, and the United States has been divided into 4 quadrants, although no studies could be found for the northwest quadrant. Spearman rank correlation analysis (r) was used to explore the relationship of cave temperature, incubation temperature, and length of incubation with the number of fungal taxa reported. Other variables were not analyzed due to lack of data. Analysis was conducted on a subset of 130 out of a total 225 papers. Excluded papers lacked detail on methodology, and generally were published before 1965. The earliest published study of fungi in caves was by Humboldt (1794) as described in Dobat (1967); unfortunately the data could not be used due to outdated and untraceable nomenclature.

**Taxa Documented from Caves**

A list of 1029 species in 518 genera of fungi, slime moulds, and yeasts documented from caves is given in Table 1, with an average of 17.9±24.4SD species reported per study (n=130, range 1-195, mode 1, median 10). Table 1 should be interpreted with caution; apparent geographic differences may be a reflection of different methodologies, variations in the focus of the studies, or the amount of effort expended, rather than demonstrating underlying biological patterns. Some studies consist of one visit while other studies span several years. Of the 225 papers, 45 were conducted in North America (including Mexico), 18 in Central and South America, 132 in Europe, 25 in Asia, 10 in Africa, and 6 in Oceania (Fig. 1). The countries with the greatest number of cave mycological papers are: United States (37, especially the northeast with 25), France (25), Italy (23), Spain (19), and Great Britain (17).

Of the fungal taxa reported (species and genera combined) from caves and mines, 69.1% are Ascomycota, 20% Basidiomycota, 6.6% Zygomycota, 2.6% Mycetozoa, 1% Oomycota, and 0.8% other (Amoebozoa, Chytridiomycota, Microsporidiomycota, and Percolozoa). Only to a very limited degree do these percentages reflect the number of species described for each phylum. Kirk et al. (2008) report 64,163 described species of Ascomycota, 31,515 Basidiomycota, 1,065 Zygomycota, Mycetozoa (there are 1019 spp. in kingdom Amoebozoa, to which this phylum belongs), and 956 Oomycota. Basidiomycota are often associated with nutrient rich substrates such as wood and dung in caves (Table 1 available online at http://dx.doi.org/10.5038/1827-806X.42.1.9). Wood and dung are generally not significant elements of cave environments, although wooden support timbers may be prevalent in old mines and some caves contain massive guano piles. Nonetheless, the general lack of large, nutrient rich substrates in caves may explain the comparative rarity of Basidiomycota versus Ascomycota. However, Basidiomycota are difficult to culture, and particularly to identify in culture, so the methods used by the majority of cave fungal studies (Table 2) are biased to detecting Ascomycota. Zygomycota are comparatively easy to detect due to abundant spore production and fast growth, so the relative abundance of such taxa in the cave environment may be overestimated. The disproportionate abundance of Mycetozoa is largely a reflection of the studies of Landolt et al. (1992), Landolt & Stihler (1998), and Landolt et al. (2006).

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(2004) found a core population of 9 fungal species present during all three sampling periods. This pattern is frequent in fungal studies of both cave and non-cave environments (Marletto, 1966; Christensen, 1989; Kuzmina et al., 2012; Vanderwolf et al., 2013).

Geomyces destructans

Geomyces destructans, only recently described (Gargas et al., 2009), has been reported from caves in the northeastern United States, eastern Canada, and multiple European countries (Martinkova et al., 2010; Puechmaille et al., 2010; Wibbelt et al., 2010; Kubatova et al., 2011; Turner et al., 2011). Knowledge of the distribution of this pathogenic fungus is expanding rapidly and has generated increased interest in cave mycology generally. Attempts to document the presence of Geomyces destructans in caves have resulted in the inadvertent isolation of other fungi. While searching for Geomyces destructans in cave sediments in the

Table 2: The percentage of studies reporting selected environmental features of cave study sites and the methodologies employed. A study was considered to use genetic methods if genetics were employed for initial environmental sampling or for identification of fungal cultures.

<table>
<thead>
<tr>
<th>Feature</th>
<th>% of studies (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td># of samples taken</td>
<td>34.6 (130)</td>
</tr>
<tr>
<td># of caves sampled</td>
<td>93.1 (130)</td>
</tr>
<tr>
<td>Type of cave/mine</td>
<td>56.2 (130)</td>
</tr>
<tr>
<td>Presence/absence of bats</td>
<td>29.2 (130)</td>
</tr>
<tr>
<td>Presence of other fauna</td>
<td>23.1 (130)</td>
</tr>
<tr>
<td>Presence of standing or running water</td>
<td>16.2 (130)</td>
</tr>
<tr>
<td>Size of cave</td>
<td>29.2 (130)</td>
</tr>
<tr>
<td>Cave temperature</td>
<td>41.5 (130)</td>
</tr>
<tr>
<td>Cave humidity</td>
<td>21.5 (130)</td>
</tr>
<tr>
<td>Substrate sampled</td>
<td>96.9 (130)</td>
</tr>
<tr>
<td>Using genetics</td>
<td>30.8 (130)</td>
</tr>
<tr>
<td>Using culture methods</td>
<td>91.5 (130)</td>
</tr>
<tr>
<td>Incubation temperature(s) of samples</td>
<td>67.2 (119)</td>
</tr>
<tr>
<td>Length of incubation</td>
<td>48.7 (119)</td>
</tr>
<tr>
<td>Agar type(s) used</td>
<td>84.9 (119)</td>
</tr>
</tbody>
</table>
United States, Lindner et al. (2011) documented many Geomyces spp. distinct from G. destructans. Given Geomyces destructans temperature requirements for optimal growth and its known distribution (Blehert et al., 2009; Gargas et al., 2009), it appears to be adapted to the cave environment. Multiple fungal species can grow on dead bats in caves (Voyron et al., 2011; Vanderwolf et al., 2013), but G. destructans has the unique ability to utilize live, hibernating bats as a nutrient source. Although fungi in addition to G. destructans have been cultured from live bats in caves (Larcher et al., 2003; Disalvo et al., 1970; Johnson et al., 1970; Tesh & Schneidau, 1967), it appears to be adapted to the cave environment. Multiple fungal species can grow on dead bats in caves (Voyron et al., 2011; Vanderwolf et al., 2013), but G. destructans has the unique ability to utilize live, hibernating bats as a nutrient source. Although fungi in addition to G. destructans have been cultured from live bats in caves (Larcher et al., 2003; Voyron et al., 2011; Vanderwolf et al., 2013), it is not known if these fungi are able to utilize live bats as a substrate for growth. Geomyces destructans has the ability to grow on other cave substrates (e.g. raccoon dung, see McAlpine et al., 2011) but faster growing fungi seem to outcompete G. destructans in these microhabitats. Bats are not known to have any innate defenses against this fungal infection during the winter, although the impact of G. destructans varies among bat species (Turner et al., 2011). Krutzsch & Watson (1978) found that in the laboratory two bat species (Antrozous pallidus and Macrotrus californicus) had different susceptibilities to the fungal pathogen Coccidioides immitis. They attributed the resistance of A. pallidus to the presence of the bacterium Serratia marcescens in the tissues of the bat host.

While G. destructans causes mass mortality of bats in North America, it has not been documented to cause significant mortality or morbidity among European bats (Puechmaille et al., 2010; Puechmaille et al., 2011). The lack of European mortality may be due to differences in host immune response or other aspects of physiology or behaviour, differences in the cave environment, or variation in associated microflora or fauna (Cryan et al., 2010; Wibbelt et al., 2010). Unfortunately, not enough is currently known about caves or bats in relation to G. destructans such that intercontinental comparisons can be made that might explain the dramatic difference in bat mortality between North America and Europe.

It is interesting to note that although 132 of the 225 papers on cave mycology (58.7%) were conducted in Europe, G. destructans was not documented until it was targeted by researchers after WNS appeared in North America. It is now believed that G. destructans is widespread and native in Europe (Puechmaille et al., 2010; Wibbelt et al., 2010). This event illustrates the importance of methodology and the inherent difficulties of studying cave fungi.

### Histoplasma capsulatum

Recent molecular data suggests that Histoplasma capsulatum consists of at least eight clades and multiple geographically isolated species (Kasuga et al., 2003). However, much of the literature does not reflect this recent finding and we therefore report on H. capsulatum sensu lato. As the etiological agent of histoplasmosis, a potentially fatal mammalian disease acquired by the inhalation of spores, H. capsulatum is certainly the most widely studied fungus found in caves. The fungus is most commonly found in soil enriched with bird or bat guano, both inside and outside of caves. Histoplasmosis occurs worldwide but is considered endemic to Southeast Asia, India, Australia, Africa, and parts of South America and North America, particularly the Mississippi-Ohio River Valley in the United States. Histoplasma capsulatum has been isolated inside caves from guano, sediment, air, water, and bats from the following locations:

- South/Central America: Argentina (Gonzalez-Gonzalez et al., 2012), Belize (Quinones et al., 1978),

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Family</th>
<th>Abundance</th>
<th>Ecology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascomycota</td>
<td>Trichocomaceae</td>
<td>620</td>
<td>Saprotrophic, rarely on living plants or animals</td>
</tr>
<tr>
<td>Zygomycota</td>
<td>Mucoraceae</td>
<td>148</td>
<td>Saprotrophic in soil and organic materials, many species proteolytic</td>
</tr>
<tr>
<td>Ascomycota</td>
<td>Nectriaceae</td>
<td>106</td>
<td>Saprotrophic, mycoparasitic and phytoparasitic</td>
</tr>
<tr>
<td>Ascomycota</td>
<td>Laboulbeniaceae</td>
<td>105</td>
<td>Entomoparasitic</td>
</tr>
<tr>
<td>Mycetozoa</td>
<td>Dichtyostelidiaceae</td>
<td>87</td>
<td>Phagotrophic on organic debris and other microorganisms</td>
</tr>
<tr>
<td>Ascomycota</td>
<td>Pleosporaceae</td>
<td>86</td>
<td>Saprotrophic or neotrophic on living and dead plants</td>
</tr>
<tr>
<td>Ascomycota</td>
<td>Microasaceae</td>
<td>77</td>
<td>Saprotrophic on organic materials</td>
</tr>
<tr>
<td>Ascomycota</td>
<td>Hypocreaceae</td>
<td>77</td>
<td>Saprotrophic on plant materials or mycoparasitic</td>
</tr>
<tr>
<td>Ascomycota</td>
<td>Cordycipitaceae</td>
<td>76</td>
<td>Entomoparasitic</td>
</tr>
<tr>
<td>Ascomycota</td>
<td>Chaetomiaceae</td>
<td>72</td>
<td>Saprotrophic, most species strongly cellulolytic</td>
</tr>
<tr>
<td>Ascomycota</td>
<td>Biocaracteraceae</td>
<td>70</td>
<td>Saprotrophic, mycoparasitic, or phytoparasitic</td>
</tr>
<tr>
<td>Ascomycota</td>
<td>Davidiellaceae</td>
<td>64</td>
<td>Saprotrophic and phytoparasitic</td>
</tr>
<tr>
<td>Basidiomycota</td>
<td>Polyporaceae</td>
<td>50</td>
<td>Saprotrophic on wood</td>
</tr>
<tr>
<td>Ascomycota</td>
<td>Myxotrichaceae</td>
<td>50</td>
<td>Saprotrophic or mycorrhaliz</td>
</tr>
<tr>
<td>Ascomycota</td>
<td>Arthrodermataceae</td>
<td>48</td>
<td>Saprotrophic, often keratinolytic</td>
</tr>
<tr>
<td>Zygomycota</td>
<td>Mortierellaceae</td>
<td>44</td>
<td>Saprotrophic, often chitinolytic</td>
</tr>
<tr>
<td>Ascomycota</td>
<td>Clavicipitaceae</td>
<td>33</td>
<td>Entomoparasitic, phytoparasitic or endomycotrophic on plants</td>
</tr>
<tr>
<td>Basidiomycota</td>
<td>Fomitipodaceae</td>
<td>30</td>
<td>Saprotrophic, causing brown rot of wood</td>
</tr>
<tr>
<td>Basidiomycota</td>
<td>Psathyrellaceae</td>
<td>29</td>
<td>Saprotrophic in soil, dung, wood</td>
</tr>
<tr>
<td>Basidiomycota</td>
<td>Mycenaceae</td>
<td>28</td>
<td>Saprotrophic in wood and leaf litter</td>
</tr>
</tbody>
</table>
Columbia (Lewis, 1974; Castaneda et al., 1981), Costa Rica (Lyons et al., 2004; Neuhauser et al., 2008), Cuba (Nocedo Pous et al., 1965; Font D’Escoubet & Macola Olano, 1976; Fernandez, 1988), El Salvador (Klite, 1965), Guatemala (Dreux, 1974), Jamaica (Finchman, 1978), Panama (Taylor et al., 1962; Klite & Diercks, 1965; Klite & Young, 1965; Hasenclever et al., 1967; Shacklette et al., 1967), Peru (Lazarus & Ajello, 1955), Puerto Rico (Carvajal-Zamora, 1977a, b, c; Nieves-Rivera, 2003), Trinidad (Ajello et al., 1962), Venezuela (Campins et al., 1956; Montemayor et al., 1958; Ajello et al., 1960a), and the West Indies (Magnaval et al., 1984).

Other: Australia (Hunt et al., 1984), Israel (Ajello et al., 1977), Malaysia (Ponnampalam, 1963), New Guinea (Quilici et al., 1984), Nigeria (Gugnani et al., 1994; Muotoe-Okafor & Gugnani, 1997), Tanzania (Manson-Bahr, 1958; Ajello et al., 1960b), and Romania (Alteras, 1966).

Due to its detrimental affect on humans, some effort has been made to control *H. capsulatum* in the environment. Tosh et al. (1966) found that solutions of formaldehyde and cresol were effective decontaminating agents against *H. capsulatum* in soil, both in the field and laboratory, although the fungus was again detected after several weeks in field experiments. Increasing the quantity of solution used in the field resulted in more permanent decontamination (Tosh et al., 1967; Bartlett et al., 1982). Morehart and Larsh (1967) found that *H. capsulatum* was vulnerable to multiple organic fungicides in the laboratory and Emmons and Piggott (1963) found that covering contaminated soil with 15-20cm of fresh soil suppressed the spread and growth of the pathogen. However, none of these experiments involved caves, nor would these methods likely be practical or effective in caves. To that end, Muotoe-Okafor and Gugnani (1997) investigated antibiosis between *H. capsulatum var. duboisii* and other fungi found in cave environments. Most of the fungi tested were neither antagonistic nor parasitic, and although there was overgrowth and intermingling growth by some species, this did not affect the viability of *Histoplasma* strains (Muotoe-Okafor & Gugnani, 1997). Only *Chrysosporium indicum* inhibited growth of *Histoplasma* strains and Muotoe-Okafor and Gugnani (1997) mention its potential as a biocontrol. Flooding may provide a natural control. Shacklette and Hasenclever (1968) found that *H. capsulatum* could not be isolated from cave soil for 2-3 months following a flooding event, although previously it had been easily obtainable. Whether any of these observations have applicability for the control of *G. destructans* has yet to be examined. Taylor et al. (1999) suggested that caves with small ceiling to floor distances and large guano deposits favor the infection of bats with *H. capsulatum*. Since infection with *H. capsulatum* occurs through the inhalation of spores, this pattern is likely not applicable to *G. destructans*, which has a very different etiology.

**Mycological cave ecology**

Fungi in the cave environment generally function as parasites or decomposers, although these roles may not be mutually exclusive. Mycorrhizal fungi have been documented, though not identified, from plant roots that penetrate into shallow caves (Lamont & Lange, 1976; Jasinska et al., 1996). Many fungi that can be cultured from caves may not grow in the cave environment, but are present regularly or rarely as spores, carried in by water, air currents, or animals. In many caves, fungi and bacteria probably constitute the major food sources for nondredigotic invertebrates, such as isopods and collemboans, as well as protozoa (Dickson & Kirk, 1976; Sustr et al., 2005; Walochnik & Mulec, 2009; Bastian et al., 2010). Several fungal species in caves are known to parasitize cave insects (Benoit et al., 2004; Santamarina & Faille, 2007; Yoder et al., 2009), and cave mycota can contribute to the inorganic nutrient pool by solubilizing bedrock (Cubbon, 1976). Fungi may also contribute to the formation of speleothems, such as secondary calcium carbonate deposits (needle fiber calcite) (Bindschedler et al., 2012).

Cave microbiota are sensitive to changes in organic inputs from external sources (Chelius et al., 2009); several fungal species found in caves will proliferate opportunistically when suddenly presented with a food source (e.g. vegetation inputs, carcasses; Cubbon, 1976). Min (1988) noted that organic debris in caves is often rapidly covered with conidia of *Aspergillus* sp., *Penicillium* sp., and *Mucor* sp. Due to the scarcity of organic matter in caves, fungi are generally nutrient limited and dependent on inputs of organic matter from the outside environment (Feldhake, 1986; Jurado et al., 2010). However, in the Lechuguilla Caves, New Mexico, interdependency was observed between bacteria and fungi, with chemolithotrophic bacteria supporting populations of chemoheterotrophic bacteria and many varieties of fungi (Cunningham et al., 1995). Gherman et al. (2007) documented a similar situation in a Romanian mine. In a Mexican cave, abundant chemovetrotrophic microbes appear to act as primary producers in the cave ecosystem which includes fungi, multiple insect species, and cave fish (Hose & Pisarowicz, 1999).

Fauna in caves can represent a considerable, though often inaccessible, nutrient source for fungi. There are no published studies on the fungi found on fish, reptiles, birds, invertebrates other than insects, or mammals other than bats, occurring in caves. Only a single study of the fungi present on amphibians in caves has been published. Rimer & Briggler (2010) tested amphibians in a Missouri cave for the presence of *Batrachochytrium dendrobatidis*, the cause of chytridiomycosis; species of *Eurycea* and *Lithobates* tested positive while *Plethodon* spp. were negative. There are multiple fungal species that can utilize insects in caves as nutrient sources (e.g. species of *Isaria, Beaaueria, Rhachomyces*) and insects are the best studied host-taxon in caves mycologically (Table 1). These infectious fungal spores can be transmitted among and between insect species, or be acquired from the cave environment (Boyer-Lefevre, 1966). However, unlike *Geomyces destructans*, which is usually restricted to bats in caves, active insect fungal infections are well known both inside and outside cave environments.
Bats can affect the diversity of fungi found in caves by transporting spores and through the deposition of guano and carcasses. Bats may be vectors for fungal spores in and out of the cave environment (Vanderwolf et al., 2013). Novakova (2009a) found that the greatest diversity of fungal species in a Slovakian cave occurred on bat guano as compared to other cave substrates. Min (1988) reported that guano can be a dominant factor in determining the mycota of caves. Sustr et al. (2005) found that fungi on bat guano had a higher biomass compared to the rest of the cave. Several papers have assumed that bat guano fuels cave food webs (Beck et al., 1976) but the available evidence is contradictory (Horst, 1972; Poulson, 1972). In tropical caves, especially dry ones, bat guano is the most common source of organic matter and may form the trophic base for many invertebrate communities (Decu, 1986; Ferreira & Martins, 1999; Fenolfo et al., 2006). Invertebrates may eat the guano directly or the fungi that grow on the guano (Moulds, 2006; Ribeiro et al., 2012). Other types of dung, such as that from birds (e.g. Steatornis caripensis in Venezuela) and rodents (e.g. Erethizon dorsatum in eastern North America), can also influence the fauna (Calder & Bleakney, 1965; Herrera, 1995; Moseley, 2007) and mycoflora of caves (Vanderwolf et al., 2013).

Many different types of insects have been associated with dung in caves (Calder & Bleakney, 1965; Estrada-Barcenas et al., 2010), and this can affect the mycoflora. Estrada-Barcenas et al. (2010) found that mites were the most diverse invertebrate taxa in bat guano, with Sancassania sp. representing the most abundant microarthropod. These mites eat microfungi, including Histoplasma capsulatum, and may provide a natural biological control for this pathogen, as well as regulating numbers of cave microfungi in general (Estrada-Barcenas et al., 2010). Insects on dung in caves are exposed to a high diversity and quantity of fungi, and some species are known to produce antimicrobial substances to prevent infection (Ribeiro et al., 2012). Insects may introduce fungi into caves by transporting spores externally and internally. Dickson (1975) noted that populations of invertebrates were positively correlated with populations of fungi in Virginia cave sediments. Cave Crickets (Ceuthophilus gracilipes gracilipes) are believed to be vectors of dictyostelid cellular slime molds into and within caves (Stephenson et al., 2007).

A number of studies have investigated whether visits to caves by humans has an impact on the fungal diversity of subterranean habitats. Vaughan-Martini et al. (2000) found that an Italian cave with high human visitation (~400,000 visitors/year) had more yeast strains than two other caves with low human visitation (a few speleologists/year). Mosca & Campanino (1962) reported the same pattern for cave fungi and Somavilla et al. (1978) for cave bacteria. The quantity of airborne fungal spores in Chinese caves was positively correlated with human visitor numbers (Wang et al., 2010), while Fernandez-Cortes et al. (2011) and Adetutu et al. (2011) found that the microfungal species composition was altered with increasing human visitation. Kuzmina et al. (2012) recorded the highest number of heterotrophic bacteria and fungi in areas of the study cave with the most human traffic. Conversely, Shapiro & Pringle (2010) reported that caves with high human visitation had lower fungal diversity compared to caves with low visitation. This pattern was also found in Australian caves when samples were cultured, but genetic methods showed the reverse (Adetutu et al., 2011). Most of these studies compared high-use show caves to caves closed to the public. When caves with a few hundred human visitors per year were compared to caves with <100 visits, no effect was detected (Vanderwolf et al., 2013). Clearly, human visitors can influence the quantity and diversity of microbiota in caves, but the threshold(s) at which these influences occur is unknown. Changes in the quantity and diversity of cave mycota are likely due to the organic inputs accompanying human visitation (lint, hair, dander), as well as the introduction of new spores (Chelius et al., 2009). The introduction of organic matter often leads to a proliferation of fast-growing species that can mask slow-growing oligotrophic species. Northup et al. (2000) found that organic inputs from human visitation supported the growth of bacteria and fungi alien to the oligotrophic cave environment; this eliminated native species and reduced biodiversity. It is also possible that microclimate changes due to human visitation and lighting systems (Pulido-Bosch et al., 1997) could impact fungi in caves.

Fungi are not distributed evenly throughout caves. The distribution of cave microbiota colonies is influenced by susceptibility of the host material to be colonized, by microenvironmental conditions (water availability, temperature, pH, and nutrient sources), and by parameters such as mineral composition, porosity and rock permeability (Gorbushina, 2007). Dickson and Kirk (1976) noted that more fungi and bacteria were found on the cave floor than on the walls or ceiling, likely because organic matter accumulates on the floor. Bacteria are more uniformly distributed than fungi, which are often associated with invertebrates and organic matter (Dickson & Kirk, 1976; Khizhnyak et al., 2003). Cave bacterial diversity and biomass is much higher than that of fungi, while yeasts and amoeba are the least common (Dickson & Kirk, 1976; Fitzalis et al., 1991; Vaughan-Martini et al., 2000; Khizhnyak et al., 2003; Urzi et al., 2010). However, Mulec et al. (2012) found that the quantity of fungi in cave air exceeded that of bacteria, except in areas frequented by tourists, where bacteria dominated. Bacteria are more numerous in dry than in wet cave passages, while fungi show the reverse pattern (Dickson & Kirk, 1976).

Caves generally lack the rich fungal diversity commonly found elsewhere in nature. An exception to this is the high diversity of fungi found on dung in caves (Dickson & Kirk, 1976). Dickson and Kirk (1976), Volz and Yao (1991), and Hsu and Agoramoorthy (2001) found a greater diversity of fungal species in soils outside of caves compared to inside. Hsu and Agoramoorthy (2001), Urzi et al. (2010), Kuzmina et al. (2012), and Mulec et al. (2012) noted that microorganism biodiversity and biomass decreased
from the entrance to deep zones in caves. Conversely, in a South African gold mine, Pohl et al. (2007) determined that the diversity of filamentous fungal genera in the air increased from outside to deeper into the mine, whereas the diversity of yeast genera showed the opposite trend. In Russia, mesophilic species dominate close to the cave entrance while the ratio of psychrotolerant micromycetes increased with depth (Kuzmina et al., 2012). Koirraj et al. (1999) found that some fungal genera occurring in Indian caves were more abundant in the sediment at the entrance compared to the interior (e.g. *Aspergillus, Penicillium*), while other genera were more prevalent in the interior of the cave (e.g. *Absidia, Rhizopus, Mucor, Chaetomium, Sepedonium*). Fernandez-Cortes et al. (2011) found that *Cladosporium* spores were more abundant in the air at the cave entrance compared to the interior, while *Penicillium* showed the opposite pattern. These contradictory results of fungal abundance patterns demonstrate the need for more detailed studies.

It appears that the majority of fungi documented from caves originate from the non-subterranean environment. The fungal taxa most commonly reported from caves (Table 1) are also commonly found in the environment above ground. Mason-Williams and Benson-Evans (1967) found that the bacteria in the air of many smaller British caves were directly related to the frequency of disturbance of the cave air by outside influences. Air currents entering and circulating within caves can distribute spores from the surface environment, suggesting that the study of cave structure and air flow is important when determining the origin of cave micro-organisms. Seasonal patterns in the presence of cave microfungi confirm the importance of outside influences. Wang et al. (2010) found a greater diversity and quantity of microfungi in Chinese cave air in the summer when compared to the winter and Docampo et al. (2010) documented the same pattern in Spanish cave air. Borda et al. (2004) found more colony forming units of bacteria and fungi in the air of Romanian caves in the summer than in the winter. This seasonal pattern is also seen among microfungi outside caves. In a Spanish cave Docampo et al. (2011) found a significant positive correlation between outside rainfall and concentrations of certain airborne spore types inside the cave. These spore types have a proven association between the rainy season and increases in their airborne concentrations in the outside environment (Docampo et al., 2011). Wang et al. (2010) found the quantity of airborne fungal spores in Chinese caves was positively correlated with temperature (inside and outside the cave) and negatively correlated with relative humidity and outside rainfall (water can wash away spores that may otherwise be carried into the cave via air currents). The composition of fungal genera in these caves was identical to the genera found outside (Wang et al., 2010).

Many of the patterns described above are similar to those found by investigators studying fungi occurring in human homes versus the outside environment (Sterling & Lewis, 1998; Shelton et al., 2002). The types of fungal spores found indoors are dependent on geographic location, season, and outdoor climate (Sterling & Lewis, 1998). Amend et al. (2010) found that as one moved away from the equator fungal diversity inside buildings increased (contrary to the pattern found in nearly all other organisms), and that distance from the equator was the strongest predictor of fungal assemblage similarity between buildings, followed by mean annual rainfall and mean annual temperature. Amend et al. (2010) states that the fungal composition inside buildings is most strongly influenced by local outdoor environmental factors. However, Sterling & Lewis (1998) found indoor microclimates are important in the secondary release and growth of spores within homes, which can alter the fungal assemblage from that found outside. Microclimates and substrates found in caves often differ from the outside environment, and likely these are major factors that drive the formation of unique cave fungal assemblages. The absence of sunlight may also be a factor. The lack of sunlight is often cited as the cause of deformities that mushrooms growing in mines occasionally exhibit (Saric-Sabados, 1957; Lohwag, 1961; Georgescu & Tutunaru, 1966; Dobat, 1970; Rutherford, 1977).

Whether true troglobitic fungi exist is currently unknown. Certainly there are species that have been exclusively isolated from caves (e.g. *Aspergillus baeticus* (Novakova et al., 2012), *Aspergillus speluneus* (Raper & Fennell, 1965; Marvanova et al., 1992), *Aspergillus thesaurosus* (Novakova et al., 2012), *Chrysosporium chiropterorum* (Beguin et al., 2005), *Chrysosporium speluncarum* (Novakova & Kolarik, 2010), *Microascus caviariformis* (Malloch & Hubart, 1987; Vanderwolf et al., 2013), *Mucor troglophilus* (Gunde-Cimerman et al., 1998), *Ochroconis anomala* (Martin-Sanchez et al., 2012), *Ochroconis lascauxensis* (Martin-Sanchez et al, 2012), *Ombrophila speluncarum* (Lagarde, 1913), *Trichosporon akiyoshidainum* (Sugita et al., 2005), *Trichosporon cavernicola* (Sugita et al., 2005), *Trichosporon chiroterorum* (Sugita et al., 2005)), but this may reflect insufficient sampling outside the cave habitat. *Geomyces destructans*, the best known ‘cave fungus’, has been isolated from bats outside caves (Dobony, 2012).

### Sampling and culturing cave fungi

The most common method of detecting cave microfungi is by cultivating spores from cave substrates on agar plates (Table 2). Studies that do not use this method either employ genetic environmental sampling or directly sample visible fungal growth in the cave (e.g. mushrooms). Studies have exposed plates to cave air (gravity settling method), placed cave sediment or other substrates directly onto the plates (usually diluted), or used swabs. A multitude of agar types have been used, with variations of Malt agar (31.7% of 101 studies reporting agar type used), Sabouraud agar (21.8%), Potatoe Dextrose agar (20.8%), and Czapek (18.8%) being the most common. The choice of media can significantly affect results since different fungal taxa have different physiological requirements. For
example, Semikolenykh et al. (2004), in a study of Russian caves, used two media, Wort agar and Czapek’s agar. They found that Wort agar supported a more diverse population of fungi (particularly rare species) than Czapek agar, while the opposite pattern was found for soils outside of their study cave. Cave mycological studies should use more than one type of medium; in our experience, low nutrient agar that mimics the oligotrophic cave environment, such as dextrose-peptone-yeast extract agar, yield the greatest diversity of genera (Papavizas & Davey, 1958; Vanderwolf et al., 2013).

The incubation temperature of agar plates should also be carefully chosen. As a general guideline, samples should be incubated close to the temperature of the cave they originated from. Of 35 cave mycology studies that reported both incubation temperature and cave temperature, 21 (60%) studies cultured samples within 4°C of ambient cave temperature. Many investigators cultured samples at room temperature which may exclude some psychrophilic and psychrotrophic fungal species. Of 81 cave mycology papers that reported incubation temperature, 49 (60.5%) studies cultured their samples ±25°C, 19 (23.5%) at 20°C − <25°C, 11 (13.6%) at >10°C − <20°C, 12 (14.8%) at ≤10°C, and 5 (6.2%) at ‘room temperature’ (note: a given study may fall into more than one category). Although a greater diversity of fungal species seemed to be isolated at lower incubation temperatures, the relationship was not significant (r = 0.201 p=0.08; 68 studies listed fungal taxa isolated by temperature). Very different microfungal species spectra were obtained from forest soils in Rhode Island when two different isolation temperatures (0°C, 25°C) were used (Carreiro & Koske, 1992). Ivarson (1973) found that the isolation frequency of the psychrophilic Geomyces pannorum increased as incubation of the soil and agar plates approached 0°C. The number of microfungi species isolated from a Czech Republic cave wall was 1.5 times higher when plates were incubated at 15°C vs. 25°C (Marvanova et al., 1992). A similar pattern was found for cave bacteria, with fewer colonies growing at 37°C vs. 30°C vs. 18°C after the plates had been exposed to cave air in England (Mason-Williams & Benson-Evans, 1967). Khizhnyak et al. (2003) and Kuzmina et al. (2012) found that psychrophilic and psychrotolerant strains of bacteria and fungi outnumbered mesophilic species in Russian caves. Conversely, Semikolenykh et al. (2004) found the opposite pattern for fungi in Russian caves (more species at 25°C vs. 15°C vs. 5°C) and attributed this to the stress situation induced by the atypical temperature stimulating the growth of dormant species.

Cave mycological studies should also incubate samples for an appropriate length of time. The average length of incubation was 18.2±20.4SD days, range 2-90 days (for the 57 studies where reported), but we have found it can take >30 days for some slow growing fungal species to become visible (Vanderwolf et al., 2013). However, length of incubation for the 57 studies was not significantly correlated with the number of fungal species documented (r = 0.236, p=0.078). Several fungal species found in caves are slow growing (Vanderwolf et al., 2013), perhaps an adaptation to the oligotrophic environment. Culturing is therefore a time consuming approach, and even with long incubation periods and multiple agar types, may underestimate the number of taxa present. Some fungal taxa may not grow in culture (Bass & Richards, 2011), or may be present in numbers too small to detect by culturing methods.

There are other methods that do not involve culturing. One method targets fungi in cave air by trapping spores using sampling machines (Wang et al., 2010; Docampo et al., 2011; Porca et al., 2011). Baiting is also used, such as the To-Ka-Va hair baiting method which targets keratinophilic fungi and dermatophytes. A traditional method for the isolation of Histoplasma capsulatum is to mix sediment or guano from caves with saline solution and then inject it into small mammals (usually mice or bats). After a period of time the mice are euthanized and their organs cultivated for fungi (Taylor et al., 1962). However, this procedure has largely been abandoned in favor of genetic methods (Neuhauser et al., 2008).

Genetic methods are now widely used to identify cave fungi as they are more accurate and less time consuming than culturing. Direct genetic sampling of the environment may yield many new fungal taxa from caves (Lindner et al., 2011). Shapiro and Pringle (2010) found that 5 sets of morphologically identical species of fungi in caves each had different ITS locus sequences, suggesting they may have been different genetic species. However, genetic methods have limitations as cultures are required for describing new species, studying ecology, and determining physiological requirements. As well, genetic identifications require a pre-existing bank of sequences for comparison. Given the large number of undescribed fungal taxa (Hawsworth, 2001; Bass & Richards, 2011), and the apparent rarity of many fungal species encountered in caves and mines, existing gene banks may lack the required sequences.

A wide variety of substrates in caves have been examined for fungi. The substrate that has yielded the greatest number of isolates is sediment/soil (Fig. 2). Whether this is a true biological pattern cannot be determined because sediment is also the most commonly studied cave substrate (Fig. 2). Precise characterization of what is meant by ‘sediment’ or ‘soil’ should be reported by authors given the variability in definitions (e.g. sediment type, presence of organic debris). Air has also been commonly studied, but in particular it has been intensively studied (high number of samples taken per study), in part due to air quality concerns in show caves and in part due to the ease with which air sampling devices can be employed. The fur and skin of live bats, although infrequently examined, appears to be a surprisingly diverse substrate for fungi (Fig. 2). However, we have done much of the limited work and believe this result is largely a reflection of methodology. Nonetheless, differences in methodology do not seem to explain the lower diversity of fungal genera encountered by Lorch et al. (2012) in cave soils from the northeastern USA when compared to live bats sampled in eastern caves.
A world review of fungi, yeasts, and slime molds in caves

Among fungal species or associated fauna in caves, or their response to fungicides, suggests that the success of spraying will be unpredictable. Both chemical (e.g. Lastanox) and biological control (e.g. use of mycoparasites such as *Trichoderma viride*) have been attempted to preserve wooden supports in mines from degradation by fungi (Prihoda, 1965; Fassatiova et al., 1974). However, the results of such efforts are not quantified. Various chemical compounds and ultraviolet light have been used in caves for fungal control but, again, the microbial response is not quantified (Oba et al., 1985; Garg et al., 1995).

In France, treating prehistoric cave paintings with benzalkonium chloride to control destructive microfungi resulted in the proliferation of resistant bacteria and further fungal colonizations of the cave art (Bastian et al., 2009b; Martin-Sanchez et al., 2012a). It is believed that antifungal compounds

Management of cave fungi

Although there has been little attention paid to the suppression of fungi present in caves generally, fungicides have been suggested as control agents for *Geomyces destructans* (Chaturvedi et al., 2011). However, limited understanding of the interactions among fungal species or associated fauna in caves, or their response to fungicides, suggests that the success of spraying will be unpredictable. Both chemical (e.g. Lastanox) and biological control (e.g. use of mycoparasites such as *Trichoderma viride*) have been attempted to preserve wooden supports in mines from degradation by fungi (Prihoda, 1965; Fassatiova et al., 1974). However, the results of such efforts are not quantified. Various chemical compounds and ultraviolet light have been used in caves for fungal control but, again, the microbial response is not quantified (Oba et al., 1985; Garg et al., 1995). In France, treating prehistoric cave paintings with benzalkonium chloride to control destructive microfungi resulted in the proliferation of resistant bacteria and further fungal colonizations of the cave art (Bastian et al., 2009b; Martin-Sanchez et al., 2012a). It is believed that antifungal compounds

![Fig. 2. The number of fungal taxa recorded from various cave substrates. Each taxon in a study is counted as 1 report; therefore the same species may be reported from the same substrate multiple times by different studies. The percentage of papers sampling that substrate is given above the bars; most papers sampled >1 substrate type. n=225 studies for the bars, n=126 for the percentages. Many of the studies excluded from the percentage calculations did not report the substrate sampled.](image-url)
produced by cave bacteria (Kim et al., 1998; Groth et al., 1999) can have a significant effect on cave fungi. Jurado et al. (2009) found that no fungal growth or fungal DNA could be isolated from cave walls colonized by bacteria. Attempts to eradicate specific cave microorganisms may lead to undesired fungal or bacterial outbreaks since intact communities may interact as biological control agents (Jurado et al., 2009). For this reason, and the largely unknown but more general ecological role fungi may play in caves, management plans that include spraying biocides in caves require caution.

Future work

Clearly there is still much to learn about the identity, distribution, and ecology of fungi in caves. Basic taxonomic studies of cave fungi are required; there are undoubtedly many species new to science still to be discovered in caves. Fungi and bacteria from poorly described ecosystems such as caves may have significant biotechnological and bioremediation potential (Frisch et al., 2003). Questions remain about the origin and ecological roles of fungi in caves, and which, if any, are cave-specialists. In the northern hemisphere, where most cave mycological studies have been conducted, caves are generally characterized by a lack of organic substrates and relatively stable, low temperatures. This environment favors communities of oligotrophic, psychrotolerant fungi. Fungal spores can be transported into caves by many organisms including invertebrates, bats, rodents, humans and other animals, as well as by air circulation and water sources (Northup et al., 1994). And yet, data that might help explain how environmental features of caves and cave faunas influence the introduction and transmission of cave fungi remains scant (Table 2). In addition to better reporting of culture methodologies, we also suggest that those engaged in studies of cave mycology should routinely report physical and ecological characteristics of study caves, including the presence and identity of troglozenes, presence of water bodies and whether they are running or stagnant, temperature, humidity, and the length of main passages. Many previous studies have not reported these variables or other aspects of methodology (Table 2) and this now hinders the recognition and understanding of ecological patterns among fungi found in cave environments.

Additionally, the literature on bacteria found in caves is even more extensive than that for fungi and yeasts, although no review is currently available. Such a review may prove useful to cave microbiologists in general, as well as WNS researchers. As a start, Jurado et al. (2010a) have reviewed bacteria found in caves that may be pathogenic to humans.

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