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Microscopic fungi on cadavers and skeletons from cave and mine environments

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During long-term studies of microscopic fungi in 80 European caves and mine environments many cadavers and skeletons of animals inhabiting these environments and various animal visitors were found, some of them with visible microfungal growth. Direct isolation, the dilution plate method and various types of isolation media were used. The resulting spectrum of isolated fungi is presented and compared with records about their previous isolation.

Compared to former studies focused mainly on bat mycobiota, this paper contributes to a wider knowledge of fungal assemblages colonising various animal bodies in underground environments. The most interesting findings include ascocarps of *Acaulium caviariforme* found abundant on mammals cadavers, while *Botryosporium longibrachiatum* isolated from frogs, *Chaetocladium jonesiae* from bats and *Penicillium vulpinum* from spiders represent the first records of these species from cadavers or skeletons.

Key words: European caves, abandoned mines, dead bodies, bones, mammals, frogs, spiders, isopods, micromycetes.

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V průběhu dlouhodobého studia mikroskopických hub v osmdesáti evropských podzemních prostorách (jeskyně, opuštěné doly) byly nalezeny také mrtvá těla a kostry živočichů žijících v tomto prostředí či nahodilých návštěvníků, některé s viditelnými nárosty mikroskopických hub. Přímá izolace, zředovací metoda a různá média byly použity pro izolaci konkrétních druhů. Získané spektrum hub je prezentováno a srovnáno s předchozími nálezy.

Oproti dosavadním studiím, zaměřeným hlavně na houby napadající netopýry, je tato práce příspěvkem k širšímu poznání společenstev hub, kolonizujících těla různých živočichů v podzemním prostředí. Mezi nejzajímavější nálezy lze zařadit *Acaulium caviariforme*, jehož plodnice byly hojně nacházeny na mrtvých tělech savců, zatímco druhy *Botryosporium longibrachiatum* (izolované z žab), *Chaetocladium jonesiae* (z netopýrů) a také *Penicillium vulpinum* (z pavouků) představují první nálezy těchto druhů na mrtvých tělech nebo kostrách živočichů.

INTRODUCTION

Hitherto, the majority of studies into microfungal assemblages in underground spaces have been predominantly focused on cave air and sediments or on rock and speleothem surfaces. This seemingly nutrient-poor ecosystem can however also include many spots with organic matter, such as plant material (e.g. seeds, twigs, detritus) washed from the environment above as well as various deposits of animal inhabitants, excretions of cave-inhabiting invertebrates and vertebrates, bat droppings and guano.

Underground environments including caves are often visited by various animals which seek refuge from unfavourable weather conditions or search for food, and some of them also fall into the underground (frogs, lizards). All of these animals may die after some time as a consequence of age, food absence or collapse from exhaustion. Cadavers of cave-inhabiting animals, mainly bats, are utilised as food for cave-inhabiting terrestrial invertebrates such as earthworms, isopods, diplopods, spring-tails, mites etc. which, together with cave microbiota, gradually decompose dead organic matter. Cadavers in various stage of decomposition are found on cave sediment or speleothems, from fresh dead bodies to skeletons or particular bones. Given the high air humidity and constant air temperature in the cave environment, successional decomposition is not limited to dry periods, as in above-ground conditions, but goes on continually. Some cadavers are covered by visible mycelia of microscopic fungi, other ones are without visible microfungal infection.

During long-term studies of cave mycobiota in various underground environments, from 2002 up to the present, mainly focused on microscopic fungi in cave air, cave sediments and bat guano, we found a number of recently deceased animals as well as skeletons and separate bones. We used various isolation techniques to elucidate which microfungal species colonise these substrates and are responsible for their decomposition. This report provides an overview of microfungal species which have been found on skeletons/bones and dead animals (with the exception of insects) inhabiting underground environments.

MATERIAL AND METHODS

Sampling sites. Studies of microfungal assemblages have been carried out since 2002, in a total of 80 underground environments of several European countries, mainly in the Czech Republic, Slovakia, Spain and Romania. Their original and English names and other characteristics are given in Tab. 1. Studied caves are karstic, oligotrophic or eutrophic, one is chemoautotrophic (Movile Cave), one is of marine origin (Treasure Cave), and two sites belong to non-karstic environments (Na Rozhraní Cave and Simon and Jude Mines).

Tab. 1. Overview of the studied caves. Caves in which cadavers or skeletons were found are in bold.

Country	Underground site	Original name	Underground type	Air temperature (°C)*
Czech Republic	Chýnov Cave	Chýnovská jeskyně	Karstic cave, show cave	5.0–9.0
	Koněprusy Caves	Koněpruské jeskyně	Karstic cave, show cave	10.6
	Bozkov Dolomite Caves	Bozkovské dolomitové jeskyně	Karstic cave, show cave	7.5–9.0
	Na Pomezí Caves	Jeskyně Na Pomezí	Karstic cave, show cave	7.0–8.0
	Na Špičáku Cave	Jeskyně Na Špičáku	Karstic cave, show cave	7.0–9.0
	Mladeč Caves	Mladečské jeskyně	Karstic cave, show cave	8.0
	Javoříčko Caves	Javoříčské jeskyně	Karstic cave, show cave	7.0–8.0
	Zbrašov Aragonite Caves	Zbrašovské aragonitové jeskyně	Hydrothermal cave, show cave	14.0–16.0
	Hranická Abyss – Rotunda	Hranická propast – Rotunda	Hydrothermal abyss	14.3–18.0
	Na Tuřidu Cave	Jeskyně Na Tuřidu	Karstic cave, show cave	7.1–9.1
	Punkva Caves	Punkvní jeskyně	Karstic cave, show cave	7.0–8.0
	(Catherine's) Kateřina's Cave	Kateřinská jeskyně	Karstic cave, show cave	7.0–8.0
	Balcarka Cave	Jeskyně Balcarka	Karstic cave, show cave	7.0–8.0
	Sloup-Šošůvka Caves	Sloupsko-šošůvské jeskyně	Karstic cave, show cave	7.0–8.0
	Vypustek Cave	Jeskyně Vypustek	Karstic cave, show cave	7.0–9.0
	Pustoželebská zazděná Cave	Pustoželebská zazděná jeskyně	Karstic cave, closed to the public	7.0–9.0
	New Amateur Cave	Nová Amatérská jeskyně	Karstic cave, closed to the public	7.0–9.0
	Býčí skála Cave	Jeskyně Býčí skála	Karstic cave, closed to the public	7.0–9.0
	Harbešská Cave	Harbešská jeskyně	Karstic cave, closed to the public	7.0–9.0
	Marželský Sinkhole	Marželský závrť	Karstic cave, closed to the public	7.0–9.0
	Králova Cave	Králova jeskyně	Karstic cave, closed to the public	9.0–10.0
	Pod křížem Cave	Jeskyně Pod křížem	Karstic cave, closed to the public	9.0–10.0
	Na Rozhraní Cave	Jeskyně Na Rozhraní	Non-karstic cave, closed to the public	9.0–10.0
	Ochozská Cave	Ochozská jeskyně	Karstic cave, closed to the public	8.0
	Slánova sluj Cave	Jeskyně Slánova sluj	Karstic cave, closed to the public	8.0–10.0
	Simon and Jude Mines	Doly Šimona a Judy	Abandoned iron ore mines	5.0–10.0

Country	Underground site	Original name	Underground type	Air temperature (°C)*
Slovakia	Domica Cave	Jaskyňa Domica	Karstic cave, show cave	10.2–11.4
	Čertova diera Cave	Jaskyňa Čertova diera	Karstic cave, closed to the public	10.2–11.4
	Aršovská Cave	Aršovská jaskyňa	Karstic cave, closed to the public	7.9–11.5
	Gombasecká Cave	Gombasecká jaskyňa	Karstic cave, show cave	9.0–9.4
	Krásnohorská Cave	Krásnohorská jaskyňa	Karstic cave, show cave	8.7–8.9
	Hrušovská Cave	Hrušovská jaskyňa	Karstic cave, closed to the public	9.0
	Jasovská Cave	Jasovská jaskyňa	Karstic cave, show cave	8.8–9.4
	Šingliarova Abyss	Šingliarova priepasť	Karstic cave, closed to the public	9.0–10.0
	Stará Brzotínska Cave	Stará Brzotínska jaskyňa	Karstic cave, closed to the public	9.5–10.4
	Drienovská Cave	Drienovská jaskyňa	Karstic cave, closed to the public	9.3–11.3
	Silická Ice Cave	Silická ľadová jaskyňa	Karstic cave, closed to the public	4.0–10.0 (–1.0 to –11.0)**
	Ochtinská Aragonite Cave	Ochtinská aragonitová jaskyňa	Karstic cave, show cave	7.6–8.4
	Dobšinská Ice Cave	Dobšinská ľadová jaskyňa	Karstic cave, show cave	–0.4 to –1.0 (0.8–3.5)**
	Demänovská Ice Cave	Demänovská ľadová jaskyňa	Karstic cave, show cave	0 (1.3–5.7)**
	Demänovská Peace Cave	Demänovská jaskyňa Mieru	Karstic cave, closed to the public	6.5–7.0
	Demänovská Cave of Liberty	Demänovská jaskyňa Slobody	Karstic cave, show cave	6.1–7.0
	Dead Bats Cave	Jaskyňa mŕtvych netopierov	Karstic cave, show cave	3.0–3.5
	Bobačka Cave	Jaskyňa Bobačka	Karstic cave, closed to the public	7.3–9.1
	Harmanecká Cave	Harmanecká jaskyňa	Karstic cave, show cave	5.8–6.4
	Modrovská Cave	Modrovská jaskyňa	Karstic cave, closed to the public	8.9–9.1
	Suchá Cave	Suchá jaskyňa	Karstic cave, closed to the public	3.4–9.5
	Pružinská Dúpná Cave	Pružinská Dúpná jaskyňa	Karstic cave, show cave	7.4–8.9
	Belianska Cave	Belianska jaskyňa	Karstic cave, show cave	5.0–5.8
	Milada Cave	Jaskyňa Milada	Karstic cave, closed to the public	9.0–10.0
	Michňová Abyss	Michňová priepasť	Karstic cave, closed to the public	8.0–10.0
	Brestovská Cave	Brestovská jaskyňa	Karstic cave, show cave	4.9–5.4
	Perlová Cave	Perlová jaskyňa	Karstic cave, closed to the public	9.0–10.0
Spain	Altamira Cave	Cueva de Altamira	Karstic cave, closed to the public	18.0
	Ardales Cave	Cueva de Doña Trinidad	Karstic cave, show cave	17.6

Country	Underground site	Original name	Underground type	Air temperature (°C)*
	Castañar de Ibor Cave	Cueva de Castañar	Karstic cave, show cave	16.9
	Treasure Cave	Cueva del Tesoro	Cave of marine origin, show cave	17.6–19.2
	Grotto of the Marvels	Grua de las Maravillas	Karstic cave, show cave	18.1–19.0
	Nerja Cave	Cueva de Nerja	Karstic cave, show cave	19.5–20.5
Romania	Scărișoara Ice Cave	Peștera ghetarului Scărișoara	Karstic cave, show cave	0–1.0 (–7.0 in winter)
	Vărtop Ice Cave	Peștera ghetarului Vărtop	Karstic cave, show cave	0–3.0
	Coiba Mare Cave	Peștera Coiba Mare	Karstic cave, closed to the public	9.0–10.0
	Poarta lui Ionele Cave	Peștera Poarta lui Ionele	Karstic cave, show cave	9.0–10.0
	Fănațe Cave	Peștera de la Fănațe	Karstic cave, closed to the public	9.0–10.0
	Ferice Cave	Peștera de la Ferice	Karstic cave, closed to the public	9.0–10.0
	Măgura Cave	Peștera Măgura	Karstic cave, closed to the public	11.0–12.0
	Urșilor Cave (Bears Cave)	Peștera Urșilor	Karstic cave, show cave	9.8
	Meziad Cave	Peștera Meziad	Karstic cave, show cave	9.0–10.0
	Limanu Cave	Peștera Limanu	Karstic cave, closed to the public	15.0–18.0
	Liliecilor de la Gura Dobrogei Cave	Peștera Liliecilor de la Gura Dobrogei	Karstic cave, closed to the public	18.0–19.0
	Mobile Cave	Peștera Mobile	Chemoautotrophic cave, closed to the public	21.5
Hungary	Zidita Cave	Peștera Zidita	Karstic cave, closed to the public	9.0–10.0
	Dracoaia Cave	Peștera Dracoaia	Karstic cave, closed to the public	9.0–10.0
	Ungurului Cave	Peștera Ungurului	Karstic cave, closed to the public	9.0–10.0
	Coliboaia Cave	Peștera Coliboaia	Karstic cave, closed to the public	9.0–10.0
	Ungureasca Cave	Peștera Ungureasca	Karstic cave, show cave	9.0–10.0
Hungary	Baradla Cave	Baradla barlang	Karstic cave, show cave	10.2
Croatia	Hrustovača Cave	Hrustovača pećina	Karstic cave, closed to the public	10.0–12.0
Slovenia	Škocjan Caves	Škocjanske jame	Karstic cave, show cave	12.0
France	Lascaux Cave	Grotte de Lascaux	Karstic cave, closed to the public	12.7

* Temperature values were taken from official websites of the caves or the Czech and Slovak Caves Administration. Temperatures measured by the authors (digital Multi-thermometer, Guangdong, China) are in bold.

** Different values indicate temperatures in different parts of the cave (with or without ice deposition).

All studied sites are characterised by high air humidity (c. 98–99%) but air temperature differed according to their geographical location as well as to cave type. While Czech, Slovak, and some Romanian caves in the Apuseni Mts. are characterised by an air temperature of 7 to 11 °C, most of the studied Spanish caves and Romanian ones in the Dobrogea region are warmer cave systems with an air temperature of 16–21 °C. Some of the studied caves are abundantly inhabited by bats (e.g. the Czech Javoříčko Caves or Na Turoldu Cave, the Slovak Domica Cave, Drienovská Cave, Dead Bats Cave, Jasovská Cave, and Ardovská Cave, the Romanian Liliecilor de la Gura Dobrogei Cave, Meziad Cave, Ferice Cave and Fânațe Cave), in others bats are less common (e.g. the Slovak Demänovská Peace Cave, Demänovská Cave of Liberty, Gombasecká Cave and Krásnohorská Cave, the Romanian Limanu Cave, the Spanish Nerja Cave, the Czech Kateřina's Cave and Sloup-Šošůvka Caves). In contrast, bats are absent from the Romanian Movile Cave, the Spanish Grotto of the Marvels, Ardales Cave, Altamira Cave, and the Slovak Ochtinská Aragonite Cave. Several caves, such as Altamira Cave, Grotto of the Marvels, and Ardales Cave (Spain), are regularly visited by various species of rodents.

Isolation and identification. Samples of cadavers – dead bodies – with visible microfungal growth and degrading organic matter were collected using sterile forceps into sterile microtubes or directly on agar isolation media in Petri dishes. In the laboratory, isolations were carried out by transferring the collected material on agar discs in Petri dishes or preparing a dilution in sterile water. Dichloran rose Bengal chloramphenicol agar (DRBC), Sabouraud's glucose agar and beer wort agar, both with rose Bengal (0.1 g/l) and chloramphenicol (0.1 g/l) (Atlas 2010) were used as isolation media. Cultivation lasted for 7 days at 25 °C in the dark.

Microfungal identification was performed according to the macro- and micromorphological characters using relevant taxonomic literature focused on particular genera (e.g. de Hoog 2000, Samson & Varga 2004, 2007, Samson & Houbraken 2011, Seifert et al. 2011, Samson et al. 2011, 2013, Bensch et al. 2012, Guarro et al. 2012, Dijksterhuis et al. 2013, Hubka et al. 2016, Sandoval-Denis et al. 2016, Chen et al. 2016, 2017, etc.). Malt extract agar (MEA), Czapek yeast autolysate agar (CYA) and Czapek-Dox agar (CZA) were used for microfungal identification (Atlas 2010).

Molecular analysis. Some isolates were also identified with molecular methods. DNA was extracted from 7-day-old colonies with the ArchivePure DNA yeast and Gram2 +kit (5PRIME Inc., Gaithersburg, Maryland, USA) with modified incubation times: lytic enzyme solution (2 h, 37 °C) and cell lysis solution (4 h, 64 °C). Partial *caM* gene encoding calmodulin was amplified using forward primers CF1M or CF1L and reverse primer CF4 (Peterson 2008). The PCR mixture

(total volume 25 µl) contained 0.1 µl MyTaq DNA polymerase (Bioline GmbH, Luckenwalde, Germany), 5 µl MyTaq Reaction Buffer, 1 µl of each primer (10 µM stock concentration), and 1 µl (50 ng) genomic DNA. The standard thermal cycle profile was 93 °C for 2 min; 38 cycles of 93 °C for 30 s, 55 °C for 30 s, 72 °C for 60 s, and final extension 72 °C for 10 min. Automated sequencing was performed at MacroGen Sequencing Service (Amsterdam, The Netherlands) using the forward and reverse primers. The obtained sequences were inspected and assembled with BioEdit version 7.1.8 (www.mbio.ncsu.edu/BioEdit/bioedit.html) and then compared with those derived from ex-type strains deposited in the GenBank database in order to identify the isolates at the species level.

Representative strains of the sequenced species are deposited in the Culture Collection of Fungi at the Department of Botany, Charles University in Prague (CCF). Obtained DNA sequences were deposited in the European Nucleotide Archive (ENA) database (Tab. 2).

Tab. 2. GenBank database accession numbers of the sequences obtained in this study.

Species	Locality	Strain number / culture collection code*	ITS rDNA	<i>caM</i>
<i>Aspergillus aureolatus</i>	Fânațe Cave	S193		LS974072
<i>Aspergillus baeticus</i>	Movile Cave	S349 = CCF 5046		LT558749
<i>Aspergillus baeticus</i>	Demănovská Peace Cave	CMF ISB 2181 = CCF 4231		HE615119
<i>Aspergillus creber</i>	Demănovská Peace Cave	S321		LS974073
<i>Aspergillus movilensis</i>	Movile Cave	Mo10 = CCF 4410 = CMF ISB 2614 = NRRL 62819 = CBS 134395		HG916740
<i>Aspergillus parasiticus</i>	Harmanecká Cave	S146		LS974074
<i>Aspergillus tennesseensis</i>	Movile Cave	S135		LS974075
<i>Aspergillus thesauricus</i>	Movile Cave	S552 = CCF 4968		LT558753
<i>Aspergillus thesauricus</i>	Movile Cave	S741		LS974076
<i>Botryosporium longibrachiatum</i>	Domica-Baradla cave system	CCF 5732	LS974068	

* CCF – Culture Collection of Fungi, Prague; CMF – Collection of Microscopic Fungi ISB, České Budějovice; S and Mo – strains from A. Nováková's working collection.

RESULTS AND DISCUSSION

Fungi on bat cadavers

Bats belong to the most commonly known inhabitants in caves and other underground environments. Unfortunately, until recently studies were focused on the occurrence of microfungi on bat fur (Larcher et al. 2003, Beguin et al. 2005) and later mainly on *Pseudogymnoascus (Geomyces) destructans* as an infection agent of WNS (white nose syndrome) (Blehert et al. 2008, Gargas et al. 2009,

Tab. 3. Overview of microfungal taxa isolated from dead animal bodies or bones.

Sites of origin: 1 – Javoříčko Caves, 2 – Sloup-Šošůvka Caves, 3 – Simon and Jude Mines, 4 – Domic Cave, 5 – Gombasecká Cave, 6 – Demánovská Peace Cave, 7 – Dead Bats Cave, 8 – Harmanecká Cave, 9 – Krásnohorská Cave, 10 – Nerja Cave, 11 – Fânațe Cave, 12 – Liliecilor de la Gura Dobrogei Cave, 13 – Movile Cave, 14 – Baradla Cave.

Dead animals			bat												spider			isopod	frog
Caves	6	5	1	2	3	4	6	7	8	10	11	12	5	9	13	13	4	14	
Mucoromycota																			
<i>Chaetocladium jonesiae</i> (Berk. & Broome) Fresen.			+		+														
<i>Mortierella horticola</i> Linnem.								+											
<i>Mortierella humilis</i> Linnem. ex W. Gams				+		+		+											
<i>Mortierella</i> sp.										+		+							
<i>Mucor hiemalis</i> Wehmer f. <i>hiemalis</i>								+											
<i>Mucor hiemalis</i> f. <i>luteus</i> (Linnem.) Schipper				+															
<i>Mucor hiemalis</i> f. <i>silvaticus</i> (Hagem) Schipper								+											
<i>Mucor mucedo</i> P. Micheli ex St.-Amans								+											
<i>Mucor racemosus</i> Fresen. var. <i>racemosus</i>				+			+	+											
<i>Mucor wosnessenskii</i> Schostak.							+	+											
<i>Rhizomucor pusillus</i> (Lindt) Schipper								+											
<i>Thamnidium elegans</i> Link				+															
Ascomycota																			
<i>Acaulium caviariforme</i> (Malloch & Hubart) Sandoval-Denis, Guarro & Gené*		+					+		+										
<i>Aspergillus aureolatus</i> Munt.-Cvetk. & Bata											+								
<i>Aspergillus baeticus</i> A. Nováková & Hubka							+								+				
<i>Aspergillus creber</i> Jurjević, S.W. Peterson & B.W. Horn	+																		
<i>Aspergillus movilensis</i> Nováková, Hubka, M. Kolařík & S.W. Peterson																+			
<i>Aspergillus parasiticus</i> Speare									+										
<i>Aspergillus tennesseensis</i> Jurjević, S.W. Peterson & B.W. Horn															+				
<i>Aspergillus thesauricus</i> Hubka & A. Nováková																+			
<i>Botryosporium longibrachiatum</i> (Oudem.) Maire																	+	+	
<i>Cephalotrichum stemonitis</i> (Pers.) Nees							+												
<i>Chrysosporium</i> sp.								+											
<i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries							+												
<i>Cladosporium herbarum</i> (Pers.) Link								+											
<i>Cladosporium sphaerospermum</i> Penz.								+											
<i>Cylindrocarpon obtusiusculum</i> (Sacc.) U. Braun							+												
<i>Fusarium merismoides</i> Corda							+												
<i>Gliomastix chartarum</i> (Corda) S. Hughes								+											
<i>Oidiodendron griseum</i> Robak							+	+											
<i>Penicillium aurantiogriseum</i> Dierckx							+												

Dead animals	marten		dormouse		bat												spider			isopod	frog	
Caves	6	5	1	2	3	4	6	7	8	10	11	12	5	9	13	13	4	14				
<i>Penicillium chrysogenum</i> Thom							+															
<i>Penicillium corylophilum</i> Dierckx							+															
<i>Penicillium olsonii</i> Bainier & Sartory								+														
<i>Penicillium vulpinum</i> (Cooke & Massee) Seifert & Samson													+	+								
<i>Pseudogymnoascus pannorum</i> complex							+															
Undetermined strains																						
Sterile dark pigmented mycelium								+														
Undetermined yeast							+															
Undetermined basidiomycetes							+															

* The identification was carried out only on microscopic features of collected material, not isolated.

Martínková et al. 2010, Wibbelt et al. 2010, Puechmaille et al. 2011, Johnson et al. 2013, Zukal et al. 2014, Vanderwolf et al. 2016 etc.) and microfungal colonisation of bat cadavers was neglected.

At the place of their roosting or hibernation as well as in places where they move through, their cadavers can be found in various stages of decomposition and with progressive microfungal colonisation. Bat cadavers with white cottony overgrowth (mostly of *Myotis myotis*, but in some case unidentifiable due to disintegration) were found in several caves during 2005–2011, e.g. Domica Cave, Dobšinská Ice Cave and Dead Bats Cave (Slovakia), Nerja Cave (Spain), and Liliecilor de la Gura Dobrogei Cave (Romania) (Fig. 1a–c). From all these cadavers, non-sporulating strains of the genus *Mortierella* were isolated, which were identified on the genus level according to their characteristic colony growth (typical lobate colonies) and garlic odour. A hanging dead bat (*Myotis myotis*) with white mycelial overgrowth (Fig. 1d) identified according to its macro- and micromorphological properties as *Mortierella humilis* (Fig. 1f–g) was found in Sloup-Šošůvka Caves (Moravian Karst, Czech Republic). This species was also isolated from fine mycelium growing on bat bones (Fig. 1e) on display in the Dead Bats Cave (Slovakia). *Aspergillus aureolatus* was isolated from bat cadavers found in the entrance corridor of the Liliecilor de la Gura Dobrogei Cave in Romania. During our sampling in galleries of the Simon and Jude Mines near the village of Malá Morávka (northern Moravia, Czech Republic) in spring 2015 we found several fresh bat cadavers. These bats had been killed by marten (*Martes foina*), after which their cadavers in an early stage of decomposition were colonised by predominantly zygomycetous fungi (Fig. 2), especially by *Mucor* spp. and *Chaetocladium jonesiae*. *Chaetocladium* sp. was also found on a bat cadaver collected in Javoříčko Caves (Czech Republic). Species of the genus

Fig. 1. *Mortierella* spp. on dead bat bodies. **a** – Domica Cave (Slovakia); **b** – Nerja Cave (Spain); **c** – Lilecilor de la Gura Dobrogei Cave (Romania); **d** – growth of *Mortierella humilis* on dead *Myotis myotis* (Sloup-Šošůvka Caves, Czech Republic); **e** – exhibition of bones in Dead Bats Cave (Slovakia); **f** – 14-day old colony of *M. humilis* on malt extract agar; **g** – *M. humilis*, sporangiophore with young sporangium. Scale bar = 20 μ m. Photo A. Nováková (a–c, e–g), P. Zajíček (d).

Fig. 2. *Chaetocladium jonesiae*. **a** – colonies on dead bats in Simon and Jude Mines, Malá Morávka (Czech Republic); **b** – 10-day old colony on MEA; **c**, **d** – branched fertile hyphae with unispored sporangia and sterile spines (c – Nomarski interference contrast, d – SEM). Scale bars = 20 μ m. Photo A. Kubátová.

Chaetocladium mainly belong to facultative, gall-forming parasites on other *Mucorales* (Benny 2005) and strains of this genus were earlier isolated from marten dung in Ardovská Cave as well as from cave sediment of the Domica Cave (Nováková, unpublished).

On the contrary, older bat cadavers found in Demänovská Peace Cave and Harmanecká Cave (Fig. 3) as well as dormouse cadavers found in Gombasecká Cave (Slovak Karst, Slovakia) were found to be covered by a mass of black ascomycetes of *Acaulium caviariforme*. The isolation of this species was unsuccessful but its typical morphology enabled reliable identification. In the case of two dormouse (*Dryomys nitedula*) cadavers found in Gombasecká Cave after summer floods, we could observe a fungal succession on dead bodies: the occurrence of zygomycetous fungi (*Mucor* spp. and *Mortierella* spp. in an early stage) was fol-

Fig. 3. *Acaulium caviariforme*. **a** – black ascocarps on dead dormouse body; **b** – detail of dead dormouse body with black ascocarps of *A. caviariforme* and white *Mortierella* sp. growth; **c–f** – a mass of black ascocarps on bat skeletons from Slovak caves: Guličková Passage in Demänovská Peace Cave (**c**, **d**), Ruins Passage in Demänovská Peace Cave (**e**), Stray Dome in Harmanecká Cave (**f**). Photo A. Nováková.

lowed by large numbers of ascocarps of *Acaulium caviariforme* after several months. This fungus was first found in the Cave of Ramioul (Belgium) and described as *Microascus caviariformis* (Malloch & Hubart 1987) and recently transferred to the genus *Acaulium* (Sandoval-Denis et al. 2016). Vanderwolf et al.

Fig. 4. *Aspergillus baeticus*. **a** – bat skeleton with a mass of black material from which this species was isolated (Guličková Passage in Demänovská Peace Cave, Slovakia); **b** – 7-day old colonies on CYA (top), MEA (bottom left) and CZA (bottom right); **c** – conidiophore; **d** – Hülle cells; **e** – conidia. Scale bars = 20 µm (c, d), 10 µm (e). Photo A. Nováková (a, b), A. Kubátová (c–e).

(2013a) isolated this species from fur of hibernating bats in three New Brunswick caves, Canada. The occurrence of *A. caviariforme* ascomata on bat carcasses in an advanced stage of decomposition, just consisting of remnant fur and bone, was observed in the New Brunswick hibernacula (Vanderwolf et al. 2016a). In spite of the fact that this species has been rarely reported from underground environments, it is probably a common microfungal species participating in the decomposition of dead animals in caves and mines.

Using the dilution isolation method (Garrett 1981), several filamentous fungi were isolated during our surveys (Tab. 3). *Aspergillus baeticus*, *Cephalotrichum stemonitis*, *Cylindrocarpon obtusiusculum*, *Fusarium merismoides*, *Mucor racemosus* var. *racemosus*, *Mucor wosnessenskii*, *Oidiodendron griseum* and *Pseudogymnoascus pannorum* s.l. were isolated from dead bats collected in the Demänovská Peace Cave. *Aspergillus baeticus* (Fig. 4) had already been isolated from Spanish Treasure Cave, Grotto of the Marvels (Nováková et al. 2012) and

Tab. 4. An overview of microfungal taxa isolated from dead bats, reported in previous studies.

References	Country	Species
Zeller (1966)	Hungary	<i>Chrysosporium merdarium</i>
Wibbelt et al. (2010)	United Kingdom	<i>Penicillium</i> sp.
Voyron et al. (2011)	Italy	<i>Alternaria</i> sp., <i>Aspergillus</i> sp., <i>Candida palmioleophila</i> , <i>Chrysosporium/Gymnoascus</i> , <i>Chrysosporium merdarium</i> , <i>Cladosporium cladosporioides</i> , <i>Fusarium dimerum</i> , <i>Fusarium equiseti</i> , <i>Gymnoascaceae</i> , <i>Lecanicillium lecanii</i> , <i>Mortierella gamsii</i> , <i>Mortierella polycephala</i> , <i>Mucor hiemalis</i> f. <i>hiemalis</i> , <i>Mucor plumbeus</i> , <i>Mucor racemosus</i> , <i>Ophiostomataceae</i> , <i>Penicillium griseofulvum</i> , <i>Penicillium</i> sp., <i>Thielavia</i> sp., <i>Trichosporon chiropterorum</i>
Nováková (2012)	Slovakia	<i>Mortierella humilis</i>
Vanderwolf et al. (2013b)	world	<i>Acremonium implicatum</i> , <i>Alternaria</i> sp., <i>Arthroderma silverae</i> , <i>Aspergillus baeticus</i> , <i>Aspergillus</i> sp., <i>Candida palmioleophila</i> , <i>Chrysosporium merdarium</i> , <i>Chrysosporium</i> sp., <i>Cladosporium cladosporioides</i> , <i>Clonostachys rosea</i> f. <i>catenulata</i> , <i>Fusarium dimerum</i> , <i>Fusarium equiseti</i> , <i>Gliocladium atrum</i> , <i>Gymnoascus</i> sp., <i>Gymnoascaceae</i> unidentified, <i>Lecanicillium lecanii</i> , <i>Mortierella gamsii</i> , <i>Mortierella polycephala</i> , <i>Mucor hiemalis</i> f. <i>hiemalis</i> , <i>Mucor piriformis</i> , <i>Mucor plumbeus</i> , <i>Mucor racemosus</i> , <i>Ochroconis constricta</i> , <i>Ophiostomataceae</i> unidentified, <i>Penicillium glandicola</i> , <i>Penicillium restrictum</i> , <i>Penicillium</i> sp., <i>Scolecobasidium tenerum</i> , <i>Sporobolomyces pruinosum</i> , <i>Thielavia terricola</i> , <i>Thielavia</i> sp., <i>Trichosporon chiropterorum</i>
Vanderwolf et al. (2016)	Canada	<i>Acaulium caviariforme</i> , <i>Acremonium</i> spp., <i>Arachniotus</i> sp., <i>Arthroderma silverae</i> , <i>Cephalotrichum stemonitis</i> , <i>Chrysosporium merdarium</i> , <i>Chrysosporium</i> sp., <i>Fusarium</i> sp., <i>Leuconeurospora capsici</i> , <i>Mortierella</i> sp., <i>Mucor</i> sp., <i>Oidiodendron truncatum</i> , <i>Pseudogymnoascus destructans</i> , <i>Pseudogymnoascus pannorum</i> s.l., <i>Trichoderma</i> sp., <i>Trichosporon dulcitum</i>

Nerja Cave (Nováková, unpublished) and was later isolated from the Romanian Movile Cave (Nováková et al. 2018). *Aspergillus parasiticus*, *Gliomastix chartarum*, *Mortierella humilis*, *M. horticola*, *Mucor hiemalis* var. *hiemalis*, *M. hiemalis* var. *silvaticus*, *M. mucedo*, *M. wosnessenskii*, *Cladosporium herbarum* s.l., *Rhizomucor pusillus* and a sterile dark pigmented fungus were isolated from a dead bat collected in Harmanecká Cave and *Clonostachys rosea* f. *rosea*, *Mortierella* sp. and *Mucor mucedo* were isolated from dead dormouse bodies found in the Gombasecká Cave.

Previously published records of micromycetes including yeasts isolated from dead bats in underground environments are given in Tab. 4. A total of 48 fungal species were isolated from bat cadavers. Twenty microfungal taxa are reported in the study on dead bats in the Grotta del Palummaro located in southern Italy and in the Grotta delle Vene, Piedmont, Italy (Voyron et al. 2011), while 16 micro-fungal species were isolated from dead bats in New Brunswick hibernacula (Vanderwolf et al. 2016). A world review of Canadian researchers (Vanderwolf et al. 2013b) reported a survey of published records of fungi, yeasts and slime

Fig. 5. *Aspergillus creber*. **a** – marten skeleton in Guličková Passage, Demänovská Peace Cave (Slovakia) from which the species was isolated; **b** – 7-day old colonies on CYA (top), MEA (bottom left) and CZA (bottom right); **c** – conidiophore; **d** – conidia. Scale bars = 20 μ m (c), 10 μ m (d). Photo A. Nováková (a, b), A. Kubátová (c, d).

moulds in caves including 32 microfungal species isolated from bat cadavers. On the contrary, Zeller (1966) and Wibbelt et al. (2010) presented only one isolated species. The most frequently reported species from dead bats is *Chrysosporium merdarium*. Nováková (2009) isolated a species identified as *Mortierella humilis* only once (Nováková 2012).

Our records are rather poor in comparison with the results of studies by Voyron et al. (2011) and Vanderwolf et al. (2016) only targeted at mycobiota of dead bats, because they originate from random finds during studies focused on

Fig. 7. Spiders. **a** – *Agraeina cristianii*; **b** – dead *A. cristianii* with synnematal microfungal growth, cave wall (Lake Room, Movile Cave, Romania); **c** – collected sample on Petri dish with DRBC; **d** – colonies of *Penicillium vulpinum* on dead spider body (*Meta menardi*) in entrance corridor of Krásnohorská Cave (Slovakia). Photo A. Nováková.

◀ **Fig. 6.** *Botryosporium longibrachiatum*. **a, b** – frog skeletons with visible white growth (Domica-Baradla cave system, Slovakia); **c, d** – 14-day old colonies of *B. longibrachiatum* on MEA; **e, f** – detail of colony margin with visible conidiophores; **g–i** – conidiophore details: branches with conidiogenous cells and conidia. Scale bars = 50 μm (g), 20 μm (h, i). Photo A. Nováková (a–f), A. Kubátová (g–i).

cave mycobiota including fungal occurrence in cave air, sediments, bat guano, and animal excretions. Nevertheless, some of our records are very interesting and include rarely isolated species and first reports from this substrate.

Fungi on other animals

Underground environments are also visited by other animals, e.g. martens (*Martes foina*), dormice (*Dryomys nitedula*), frogs (*Pelophylax* sp.), and spiders (*Meta menardi* in entrance sections but also some true cave-inhabitants such as the endemic *Agracina cristianii* in Movile Cave). Besides the above-mentioned dormice, martens are very frequent visitors to caves and abandoned mines. Some of them enter underground sites to protect themselves from bad weather conditions or in order to search for food. Therefore marten cadavers or skeletons were also occasionally found in caves – e.g. a well-preserved marten skeleton was found in the Guličková Passage of the Demänovská Peace Cave (Fig. 5). Bones lacked visible microfungal growth, but cultivation yielded *Aspergillus creber* (identified using the *caM* gene, see Tab. 2).

Other animals such as frogs and lizards visit cave environments occasionally after summer floods or storms, because of downfall or during searching for a colder site during a hot summer. Their long-term stay in the underground mostly ends in death because of starvation. Frog skeletons with visible white microfungal growth identified as *Botryosporium longibrachiatum* were discovered in the Domica-Baradla cave system (Fig. 6). Our find is the first record of this fungus from an animal substrate as well as from a cave environment. Thus far, this species had been recorded predominantly from plant material including *Curcuma rubricaulis* leaves (Tribe & Weber 2001) and later from a wide variety of dead and decaying plant material (Tribe & Weber 2001, Park & Park 2013) or as an airborne species (Anonymus 2016).

During our study of the mycobiota of the Movile Cave (Romania), we isolated microscopic fungi from dead invertebrate bodies. Several species of the genus *Aspergillus* were isolated from a dead endemic spider, *Agracina cristianii*, i.e. *Aspergillus baeticus* and *A. tennesseensis*, whilst *A. movilensis* and *A. thesauricus* were isolated from a dead diplopod, *Trachelipus troglolobius* (Nováková et al. 2018).

Synnematal microfungal colonies (Fig. 7b) were found on some cadavers of the spider *Agracina cristianii* in the Lake Room of Movile Cave in 2013. It was impossible to identify this fungus based on morphological characters and isolation was unsuccessful. Unfortunately, similar dead spider bodies with synnematal colonies were not found during sampling in the following years. Dead spider bodies (*Meta menardi*) were also found in Gombasecká Cave and Krásnohorská Cave (Slovak Karst) (Fig. 7d) and in both cases, *Penicillium vulpinum* was isolated from them. This species is a coprophilous fungus frequently found in caves on various sorts of dung.

CONCLUSIONS

During our studies, a total of 39 fungal taxa (classified as species, forms or identified at a supraspecific level) on various cadavers and skeletons found in underground environment were identified in several European caves and mines. Thirty-eight species were isolated in pure cultures – 12 of them belonging to Mucoromycota, 23 species to Ascomycota, while 3 strains were not identified to the species level. Species of the genera *Mucor* and *Aspergillus* were isolated most abundantly. The richest microfungal spectrum was found in the Demänovská Peace Cave and Dead Bats Cave, from which 14 and 16 microfungal species were isolated, respectively. The ascomycete *Acaulium caviariforme* was reported repeatedly from several Slovak caves, but unfortunately the isolation of these strains failed. However, this fungus is probably a common fungal species participating in the decomposition process of dead animals in underground environments.

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