applied sciences

MDPI

Article

Soil and Sediments in Natural Underground Ecosystems as a Source of Culturable Micromycetes: A Case Study of the Brestovská Cave (Western Tatras, Slovakia)

Rafał Ogórek, Justyna Borzęcka, Klaudyna Spychała, Agata Piecuch and Jakub Suchodolski *

Department of Mycology and Genetics, University of Wrocław, Przybyszewskiego Street 63-77, 51-148 Wrocław, Poland; rafal.ogorek@uwr.edu.pl (R.O.); justyna.borzecka@uwr.edu.pl (J.B.); klaudyna.spychala@uwr.edu.pl (K.S.); agata.piecuch@uwr.edu.pl (A.P.)
* Correspondence: jakub.suchodolski@uwr.edu.pl; Tel.: +48-71-375-6291

Abstract: Soil and sediment host microorganisms are able to survive in extremely resource-limited environments. Therefore, more and more attention is being paid to cave sediments as a reservoir of microbiota. The aim of this study is the speleomycological evaluation of the culturable soil and sediment fungal communities in the Brestovská Cave. To explore the origins of fungi, speleomycological studies were conducted both inside and outside the cave under investigation. Additionally, two incubation temperatures (5 and 24 °C) were used to increase the species spectrum of isolated fungi. To achieve the most accurate species identification, we combined an assessment of morphological characteristics of the isolates with molecular sequencing (ITS, internal transcribed spacer). Twenty different species were found and the most frequent was *Penicillium commune*, followed by *Trichosporiella cerebriformis* and *Pseudogymnoascus pannorum*. To our knowledge, our study has enabled the first identification of fungal species such as *Penicillium swiecicki*, *Cephalotrichum hinnuleum*, *Cosmpospora berkeleyana*, *Lecythophora hoffmannii*, *Ambomucor seriatoinflatus,* and *Mortierella minutissima* in underground sites. Our data showed that the abundance and composition of the fungal community varied between the indoor and outdoor samples and thus from the entrance and less visited sites deeper in the cave.

Keywords: caves mycology; Slovakian geoheritage; cave protection; new species

Citation: Ogórek, R.; Borzęcka, J.; Spychała, K.; Piecuch, A.; Suchodolski, J. Soil and Sediments in Natural Underground Ecosystems as a Source of Culturable Micromycetes: A Case Study of the Brestovská Cave (Western Tatras, Slovakia). *Appl. Sci.* **2024**, *14*, 3517. https://doi.org/10.3390/app14083517

Academic Editor: Daniela Isola

Received: 21 March 2024
Revised: 17 April 2024
Accepted: 18 April 2024
Published: 22 April 2024

1. Introduction

Cave ecosystems display unique environmental conditions that are unfavorable for the life and development of most living organisms [1]. They appear as highly zonal environments with characteristics defined by the ground waters, surrounding rocks, and karst morphology [2–4]. The conditions of subterranean facilities significantly differ from the surface and include, i.e., a constant temperature through the year (oscillating between 6 to 10 °C), high humidity (often close to saturation) [5], minimal to no light, restricted or absent air flow with the outside environment, oligotrophy, and sometimes elevated levels of CO_2 [6,7]. All of the above occur especially in the deeper parts of the underground sites [8–11]. As a result, only a few microorganisms possess specific adaptations to cope with such living conditions [9,12]. Thus, the extreme microclimate within the underground makes it a unique source of extremophilic bacterial or fungal isolates [7].

Fungi in the cave environments play important roles—mainly as parasites/pathogens, saprotrophs (decomposers), or mutualistic fungi (e.g., mycorrhizae), but also participants in geological processes [4,13,14]. Some fungal species are known to parasitize insects [15–17], while others may contribute to the inorganic nutrient pool replenishment by dissolving the bedrock [18]. Another unique feature of underground fungi is their ability to take part in the formation of speleothems [19].

The majority of fungal taxa frequently found in caves are cosmopolitan species and might inhabit various environments associated with soils, plant material, or insects [14].

In general, cave environments are nutrient-poor [13,20,21], and hence, they are extreme habitats providing specific ecological niches only for highly specialized microorganisms. However, not every underground site is oligotrophic, as some caves receive a significant input of soil and organic matter [22]. They originate mainly from dripping waters and animal feces [22–24]. Soil and organic substrates can be also brought inside due to external air currents, as well as accidentally by tourists [25,26].

Ascomycota is the dominant fungal phylum in underground ecosystems, representing ~69% of all cultivated fungi [27]. The Basidiomycota phylum makes up 20%, Zygomycota 6.6%, Mycetozoa 2.6%, Oomycota 1%, and other phyla (namely, Amoebozoa, Chytridiomycota, Microsporidiomycota, and Percolozoa) 0.8% [14]. Some studies have suggested that the most commonly found taxa might be a result of the specific study methodology rather than biological patterns in cave mycology. For instance, the use of nutrient-rich media and incubation at room temperature (above 20 °C) support the growth of the commonly reported fungi that belong to Ascomycota [14,25].

In general, fungi have a high plasticity and their capacity to adopt various forms (filaments, yeasts and yeast-like fungi) in response to adverse conditions allows them to inhabit various environments, including soil [28]. In addition, fungi are capable of producing a wide variety of extracellular enzymes which decompose the underground organic residues (such as soil components) [29–31]. Fungi usually dominate in highly or slightly acidic soils [32]. However, as shown by speleomycological research by Naga et al., fungi can also inhabit alkaline calcareous soils [33]. Additionally, many fungal species are able to act as an effective biosorbent of toxic metals such as cadmium, copper, mercury, lead, and zinc [34].

It should be noted that assessing fungal diversity within underground sites is a relatively new discipline, as in the past, only the emergence of selected fungal species was evaluated [14]. Among them, *Pseudogymnoascus destructans* (*Pd*), causing a bat disease called white-nose syndrome, brought growing attention to this matter [35]. *Pd* is able to persist between hibernation cycles of bats within cave soil before infecting bat roosts. Moreover, it can also propagate in cave sediments, especially in keratin-rich areas due to shed bat hair [36].

The main goal of the present research was to conduct the speleomycological evaluation of the culturable soil and sediment fungal communities in the Brestovská Cave (Western Tatras Mts., Slovakia), which is open to tourists. In order to discuss the possible origin of the fungal species, we determined the number and species composition of fungi in the outdoor and indoor soil and sediments samples.

2. Materials and Methods

2.1. Study Area

The Brestovská Cave, situated near Zuberec village in Slovakia's Tatra National Park (Western Tatra Mountains) [37], stands at an elevation of 867 m asl. Its total length spans 1890 m, of which only 217 m are accessible to the public, making it the largest tourist-accessible cave in Orava and the sole one available for exploration. This cave is part of a vast hydrological system that has formed at the contact of karstic and non-karstic rocks, featuring a river that flows through it. With a temperature ranging between 4 and 6 °C and with a flowing river within, the cave supports mammalian life. Within the Brestovská Cave, nine species of bats have been identified, among which the greater mouse-eared bat (*Myotis myotis*) is the most prevalent [38].

2.2. Sample Collection

On 24 August 2017, during the absence of tourists, soil and sediment samples were collected from six sites—one outside (near the cave's entrance) and five within the cave (Figure 1). The collection involved the upper layer of soil/sediment (to a depth of 10 cm) using sterile plastic tools and storing the samples in sterile bags. From each location under study, around 1000 g of material was collected in three replicates. These samples were then

transported to the lab under cooled conditions (10 ± 2.0 °C) and preserved at 5 ± 0.5 °C until the mycological analysis was conducted, which occurred within 7 days of collection.

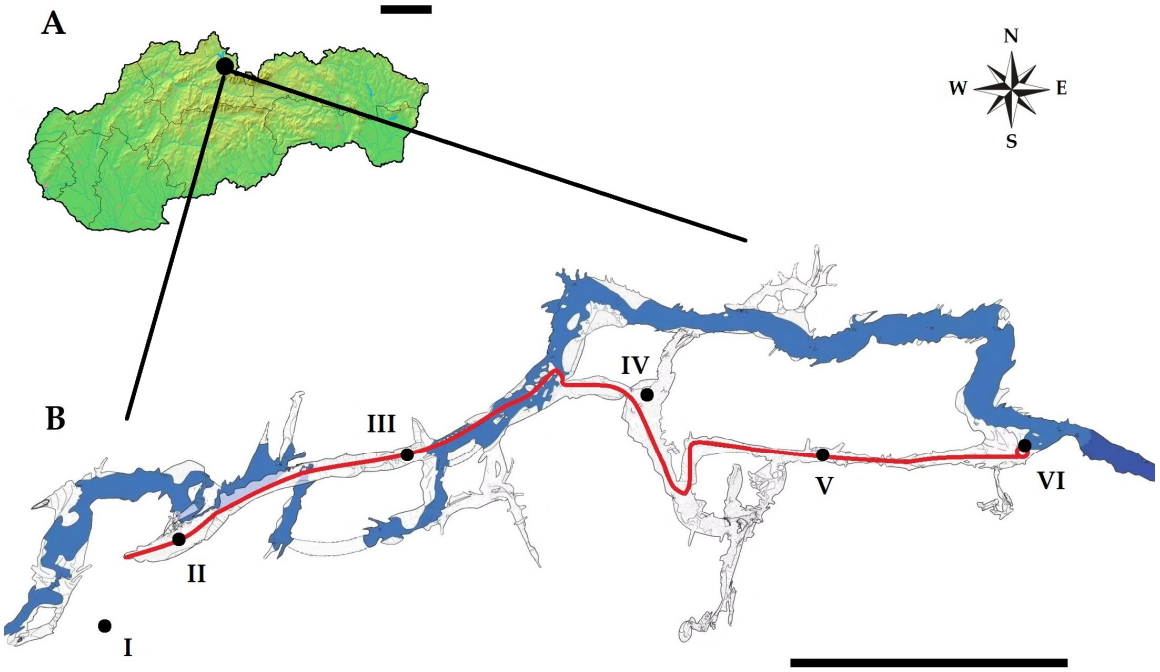

Figure 1. Geographic location of the Brestovská Cave in Slovakia (**A**). Subterranean passages of the cave (**B**) with marked underground stream (in blue) sampling points along the tourist path (indicated by a red line): site I outside the cave, and sites from II to VI within the cave. The cave entrance and exit are located next to study site I. Scale bars: A = 50 km; B = 50 m.

2.3. Isolation of Fungi from Samples

Before analysis, the samples were aseptically homogenized, and 3 g of the soil/sediment was transferred into sterile, individually sealed conical polypropylene tubes (50 mL) with screw-on lids (FL Medical, Padova, Italy), along with 12 mL of a saline solution (0.85% NaCl). These mixtures were then agitated at an ambient temperature (20 min; 24 ± 1 °C). Subsequently, the samples were diluted at ratios of 50, 500, or 5000 times and vortexed, and 100 µL aliquots were evenly spread onto triple replicate plates containing potato dextrose agar (PDA, Bio-maxima, Lublin, Poland). The plates were incubated at temperatures of 5 ± 0.5 °C and 24 ± 0.5 °C for durations ranging from 5 to 42 days in darkness, from the emergence to the final appearance of colonies. Post-incubation, fungal colonies were enumerated, and the average number of colony-forming units, represented as CFU per g of soil/sediment, were calculated. Fungal colonies were then transferred onto fresh plates with the same agar for subculturing and incubated in darkness at 5 ± 0.5 °C and 24 ± 0.5 °C for periods between 4 and 35 days. Following this incubation period, fungi were isolated using the single-spore technique and subsequently grown on PDA slants for further morphological and molecular identification.

2.4. Fungal Identification

For fungal identification, a dual approach encompassing both phenotypic and genotypic techniques was employed. Pure cultures underwent examination through microscopic and macroscopic analyses. The initial phenotypic classification utilized several mediums including PDA, Czapek yeast autolysate agar (CYA, 30.0 g·L^{-1} sucrose, 15 g·L^{-1} agar, 5.0 g·L^{-1} yeast extract, 3.0 g·L^{-1} $NaNO_3$, 1.0 g·L^{-1} K_2HPO_4, 0.5 g·L^{-1} KCl, 0.5 g·L^{-1} $MgSO_4 \cdot 7H_2O$, and 0.01 g·L^{-1} $FeSO_4 \cdot 7H_2O$), Czapek–Doxagar (1.2% agar, BioMaxima, Poland), and malt extract agar (MEA, BioMaxima, Lublin, Poland) in the case of *Penicillium* spp., focusing on characteristics like colony hue, expansion, and the presence

of distinctive morphological features such as spores, following established diagnostic keys and monographs [39–61].

Species confirmation involved sequencing the internal transcribed spacer (ITS) regions of fungal rDNA. DNA extraction from PDA-cultivated fungal colonies was performed using the Bead-Beat Micro AX Gravity kit (A&A Biotechnology, Gdańsk, Polska), with ITS regions amplified through PCR using specific primers (ITS1, 5′-TCCGTAGGTGAACCTGCGG-3′ and ITS4, 5′-TCCTCCGCTTATTGATATGC-3′) [62]. PCR was performed using a T100 Thermal Cycler (Bio-Rad, Berkeley, CA, USA), according to our previous protocol [63]. The PCR products underwent agarose gel electrophoresis on a 1.2% agarose gel, purification (Clean-Up kit, A&A Biotechnology), and sequencing (Macrogen Europe, Amsterdam, The Netherlands).

2.5. Data Analyses

The sequences of PCR products were examined using the BioEdit Sequence Alignment Editor (http://www.mbio.ncsu.edu/bioedit/bioedit.html, accessed on 10 December 2022). Fungal ITS sequences underwent comparison with those in the NCBI's GenBank through the BLAST algorithm (http://www.ncbi.nlm.nih.gov/; accessed on 14 December 2022). Criteria established by Zhang et al. [64] guided the interpretation of sequence similarities: a match of $\geq$97% confirmed the genus and species, a match between 95% and 97% identified the genus only, and a match <95% led to classification at higher taxonomic levels or as 'unassigned'. These sequences were then uploaded to the GenBank database, accessed on 19 December 2022.

To analyze the count of fungal colonies, the Statistica 12.0 software (StatSoft Polska Sp. z o.o., Kraków, Poland) was utilized, incorporating a one-way analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) test with a significance level of $\alpha \leq 0.05$. To assess the diversity of fungal species at various sites, the Shannon Diversity Index (H) was employed, calculated with the formula $H = -\sum Pi(\ln Pi)$, where Pi represents the proportion of each species within the sample [65].

3. Results

The study of micromycetes in sediments/soil of the Brestovská Cave was carried out at one outdoor and five indoor sites using the culturing procedure (PDA medium, incubation at 5 and 24 $\pm$ 0.5 °C) (Figure 1). The phenotypic analysis of the obtained fungi allowed for their classification into 20 different species, and molecular studies confirmed those observations. The obtained fungi belong to four phyla (Ascomycota—75% of isolates, Mortierellomycota—15%, Mucoromycota—5%, and Basidiomycota—5% isolates) and thirteen families (*Aspergillaceae*—represent 20% of isolates, *Mortierellaceae*—15% of isolates, *Coniochaetaceae* and *Microascaceae*—each represent 10% of isolates, and each of the following represent 5% of isolates—*Cordycipitaceae*, *Dermateaceae*, *Hypocreaceae*, *Mrakiaceae*, *Mucoraceae*, *Myxotrichaceae*, *Nectriaceae*, *Onygenaceae*, and *Pseudeurotiaceae*). Most of the species belong to filamentous fungi (*Ambomucor seriatoinflatus*, *Cephalotrichum hinnuleum*, *Chrysosporium merdarium*, *Cosmospora berkeleyana*, *Doratomyces stemonitis*, *Lecanicillium muscarium*, *Lecythophora hoffmannii*, *Mortierella elongata*, *Mortierella hyalina*, *Mortierella minutissima*, *Oidiodendron truncatum*, *Paecilomyces farinosus*, *Penicillium camemberti*, *Penicillium chrysogenum*, *Penicillium commune*, *Penicillium swiecickii*, *Pseudogymnoascus pannorum*, and *Trichoderma atroviride*), although two species belong to basidiomycetous and ascomycetous yeast (*Tausonia pullulans* and *Trichosporiella cerebriformis*, respectively). The nucleotide sequences of fungal ITS rDNA acquired during this study were deposited in GenBank and assigned accession numbers from OQ073897 to OQ073916. BLAST analysis of these sequences revealed E values of zero, indicating highly significant matches, with identity percentages ranging from 98.98% to 100% and query coverage spanning from 99% to 100%, as detailed in Table 1.

Table 1. Cultured fungi detected in soil and sediment samples of the Brestovská Cave and the results of BLAST analysis (all E values were zero): [1]—current name.

Fungi Isolated from Soil and Sediment Samples						Identity with Sequence from GenBank		
Isolate Number	**Identified Species**	**Phylum**	**Family**	**GenBank Accession No.**	**The Sequence Length (bp)**	**Query Cover %**	**Identity %**	**Accession**
UWR_314	*Ambomucor seriatoinflatus*	Mucoromycota	*Mucoraceae*	OQ073897	494	100	100.00	MG827311.1
UWR_315	*Cephalotrichum hinnuleum*	Ascomycota	*Microascaceae*	OQ073898	503	100	100.00	LC519564.1
UWR_316	*Chrysosporium merdarium*	Ascomycota	*Onygenaceae*	OQ073899	454	100	100.00	MH859164.1
UWR_317	*Cosmospora berkeleyana*	Ascomycota	*Nectriaceae*	OQ073900	428	100	100.00	MH859038.1
UWR_318	*Doratomyces stemonitis*	Ascomycota	*Microascaceae*	OQ073901	506	100	99.60	LN850985.1
UWR_319	*Lecanicillium muscarium*	Ascomycota	*Cordycipitaceae*	OQ073902	526	100	100.00	MF467854.1
UWR_320	*Lecythophora hoffmannii*	Ascomycota	*Coniochaetaceae*	OQ073903	428	100	100.00	FJ903377.1
UWR_321	*Mortierella elongata* (*Linnemannia elongata*) [1]	Mortierellomycota	*Mortierellaceae*	OQ073904	470	100	99.79	MT366011.1
UWR_322	*Mortierella hyalina* (*Linnemannia hyalina*)	Mortierellomycota	*Mortierellaceae*	OQ073905	584	100	100.00	MT003063.1
UWR_323	*Mortierella minutissima* (*Podila minutissima*)	Mortierellomycota	*Mortierellaceae*	OQ073906	552	100	100.00	MK513846.1
UWR_324	*Oidiodendron truncatum*	Ascomycota	*Myxotrichaceae*	OQ073907	398	100	100.00	KF835845.1
UWR_325	*Paecilomyces farinosus*(*Cordyceps farinosa*)	Ascomycota	*Cordycipitaceae*	OQ073908	376	100	100.00	AF368793.1
UWR_326	*Penicillium camemberti*	Ascomycota	*Aspergillaceae*	OQ073909	507	100	100.00	MT530220.1
UWR_327	*Penicillium chrysogenum*	Ascomycota	*Aspergillaceae*	OQ073910	496	100	100.00	MT328526.1
UWR_328	*Penicillium commune*	Ascomycota	*Aspergillaceae*	OQ073911	520	100	100.00	KU936231.1
UWR_329	*Penicillium swiecickii*	Ascomycota	*Aspergillaceae*	OQ073912	493	100	100.00	MH865783.1
UWR_330	*Pseudogymnoascus pannorum*	Ascomycota	*Pseudeurotiaceae*	OQ073913	472	100	100.00	MT573491.1
UWR_231	*Tausonia pullulans*	Basidiomycota	*Mrakiaceae*	OQ073910	392	100	98.98	MK782486.1
UWR_232	*Trichoderma atroviride*	Ascomycota	*Hypocreaceae*	OQ073915	545	100	100.00	MN533771.1
UWR_233	*Trichosporiella cerebriformis*	Ascomycota	*Dermateaceae*	OQ073916	552	99	99.28	MH865134.1

The density of mycobiota in the Brestovská Cave at 5 °C ranged from 8.32 to 49.92 CFU × 10^2 per 1 g of sediment/soil samples inside the underground facility and was 91.65 CFU × 10^2 per 1 g of outdoors samples. In the case of the incubation temperature at 24 °C, the overall concentrations of fungal propagules obtained inside the cave were from 9.16 to 156.66 CFU × 10^2 per 1 g, and outside, the concentration was 177.76 CFU × 10^2 per 1 g (Figure 2). In the case of incubation at 5 °C, the highest value of fungal propagule concentration was recorded at site I, although statistically (Tukey HSD test at $\alpha \leq 0.05$), this location did not differ significantly from location no. II, III, IV, and VI. In turn, the lowest concentration of fungi was noted at site V, although statistically, this location also did not differ significantly from location no. II, III, IV, and VI. However, significant differences were noted only between locations I and V ($p = 0.004211$). Meanwhile, the highest statistically significant density of mycobiota in the Brestovská Cave grown at 24 °C was recorded at location no. I and II, and the lowest at location no. V ($p_{\text{study sites II, V}} = 0.000197$). Moreover, in three study sites (I, II, and IV) out of six, statistical differences in the number of obtained fungi between the incubation temperatures of the samples were noted ($p_{\text{study sites I}} = 0.02343$, $p_{\text{study sites II}} = 0.004203$, and $p_{\text{study sites IV}} = 0.034669$)—Figure 2.

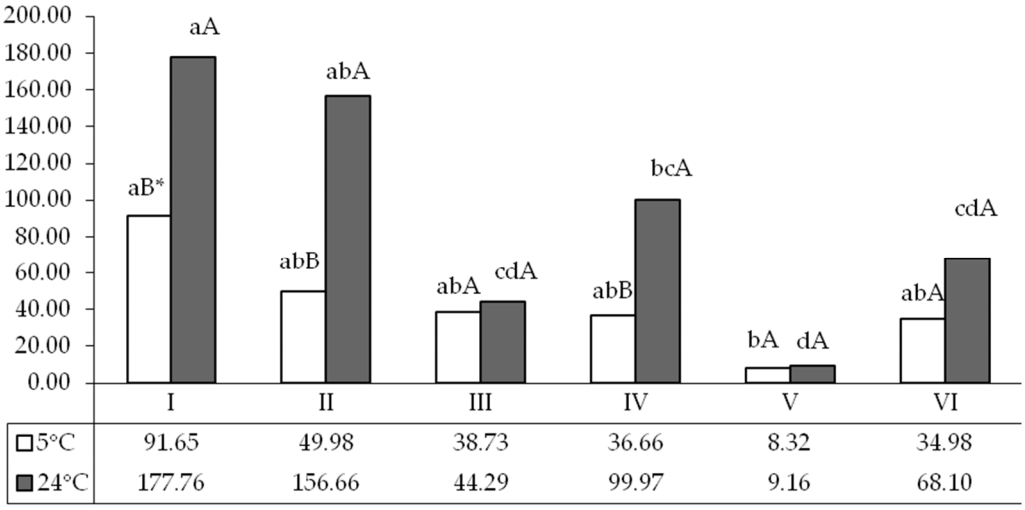

Figure 2. The average number (CFU × 10^2 per 1 g) of fungi from the soil and sediment samples of the Brestovská Cave and incubated at 5 or 24 °C. * For each location, the number of fungi followed by the same letter are not statistically different, and others are (Tukey HSD test, $\alpha \leq 0.05$). Small letters are used to denote the variations in the number of fungi cultured at a specific incubation temperature across different study sites. Capital letters highlight the differences in the number of fungi cultured at different incubation temperatures within a particular study site.

In total, all 20 fungal species identified in this study were discovered within the Brestovská Cave, with only 8 of them (*M. elongata*, *M. hyalina*, *M. minutissima*, *O. truncatum*, *P. commune*, *P. pannorum*, *T. atroviride*, and *T. cerebriformis*) isolated from the indoor soil/sediment samples considering both incubation temperatures. In turn, separating the results according to the incubation temperature, in both cases, outside the cave, 4 fungal species were isolated from the sediment and soil samples, and in the case of samples taken inside the cave, 12 species were obtained at 5 °C, and only 9 species at 24 °C (Figure 3, Table A1). *Cephalotrichum hinnuleum*, *Ch. merdarium*, *C. berkeleyana*, *D. stemonitis*, *L. hoffmannii*, *M. elongata*, *M. minutissima*, *O. truncatum*, *P. camemberti*, *T. pullulans*, and *T. cerebriformis* were cultured only at a temperature of incubation of 5 °C (Figure 4, Table A1). In turn, *A. seriatoinflatus*, *L. muscarium*, *M. hyalina*, *P. farinosus*, *P. chrysogenum*, *P. commune*, *P. swiecickii*, and *T. atroviride* were isolated only by using an incubation temperature of 24 °C (Figure 5, Table A1).

Penicillium commune dominated overall in the outside and inside samples (45.37% and 42.31% of all cultured fungi in the study, respectively) of the Brestovská Cave at 5 °C as well as at 24 °C (68.76% of all cultured fungi in the study for the outside samples and 61.18% for the inside samples). In the case of the samples incubated at 5 °C, *T. cerebriformis* dominated (45.46% of all cultured fungi in the study) in the outside of the cave and *P. pannorum* (28.89% of all cultured fungi in the study) in the inside of the cave (Figure 3).

Similar tendencies regarding the most frequently isolated fungal species were noted in individual locations inside the underground facility, as in the case without division into individual locations of the study, with the exception of study site no. II at 5 °C. Namely, *P. pannorum* also dominated at 5 °C in study sites no. III, IV, V, and VI (from 33.31% to 58.76%, which corresponded to 2.78 to 20.55 CFU × 10^2 per 1 g of sediment/soil samples), but *O. truncatum* was most abundant in location no II at 5 °C and accounted for 33.33% of all fungi obtained in this location (16.66 CFU × 10^2 per 1 g)—Figure 4, Table A1. In turn, the most frequently isolated species at 24 °C from all five surveyed locations inside the Brestovská Cave was also *P. commune*, which accounted for 31.92% to 91.70% of all fungi cultured at individual study sites, which corresponded to 6.66 to 91.67 CFU × 10^2 per 1 g (Figure 5, Table A1).

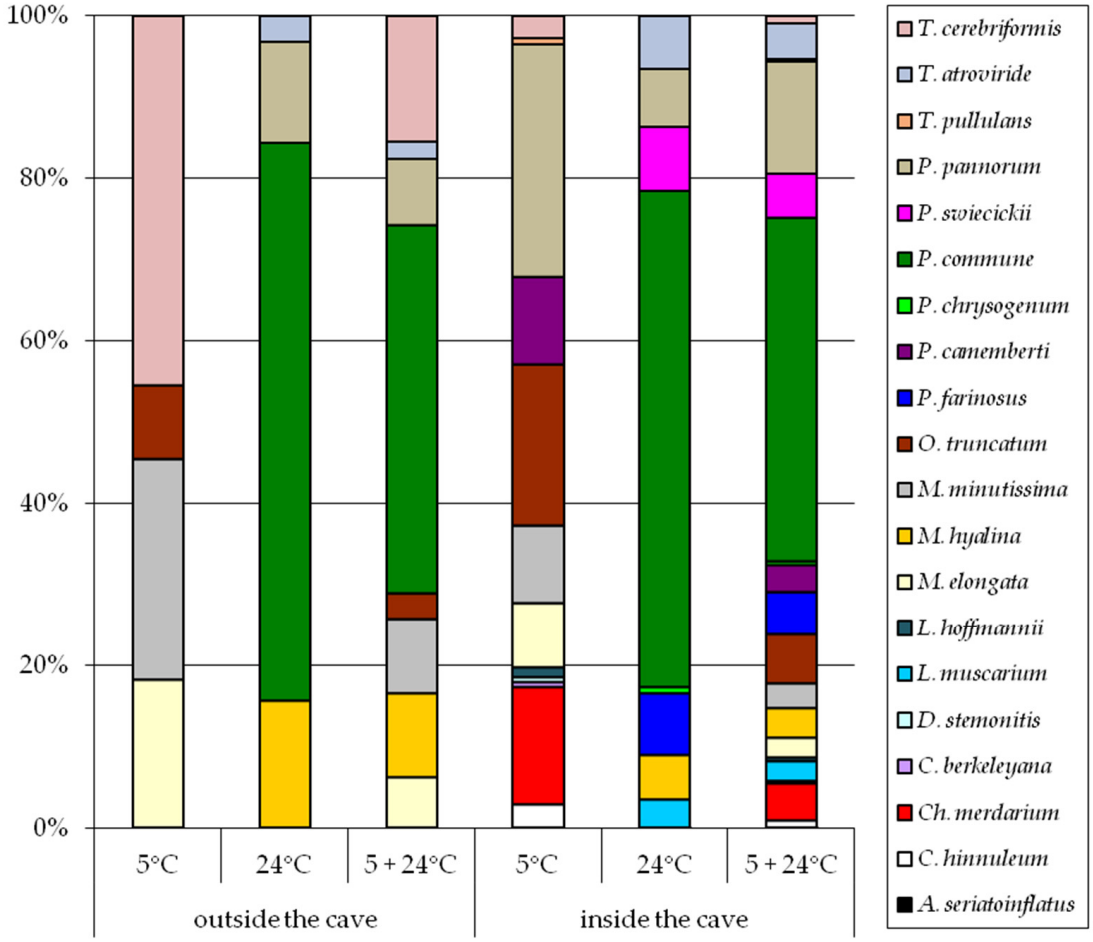

Figure 3. The percentage of each fungal species contributing to the total fungi cultured from all study sites of soil and sediment samples of the Brestovská Cave.

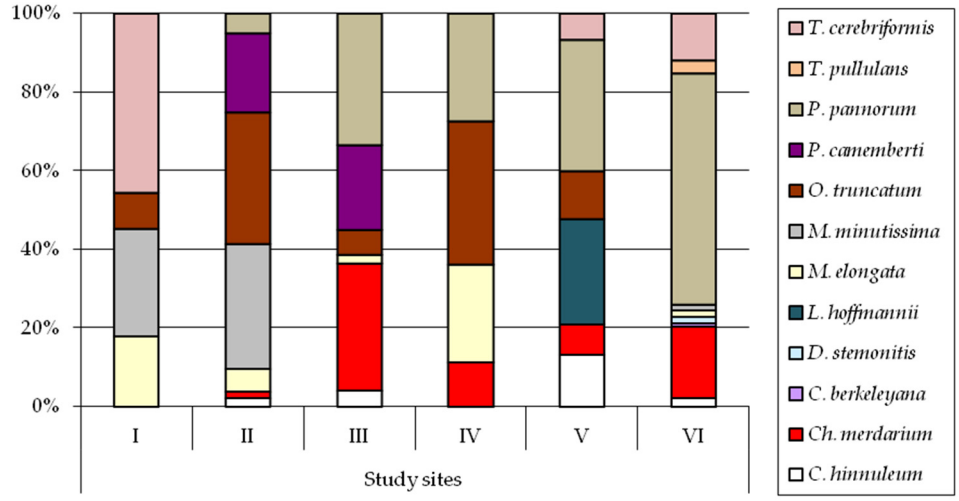

Figure 4. The percentage contribution of each fungal species to the overall fungi cultured at 5 °C from specific study sites within the soil and sediment samples of the Brestovská Cave.

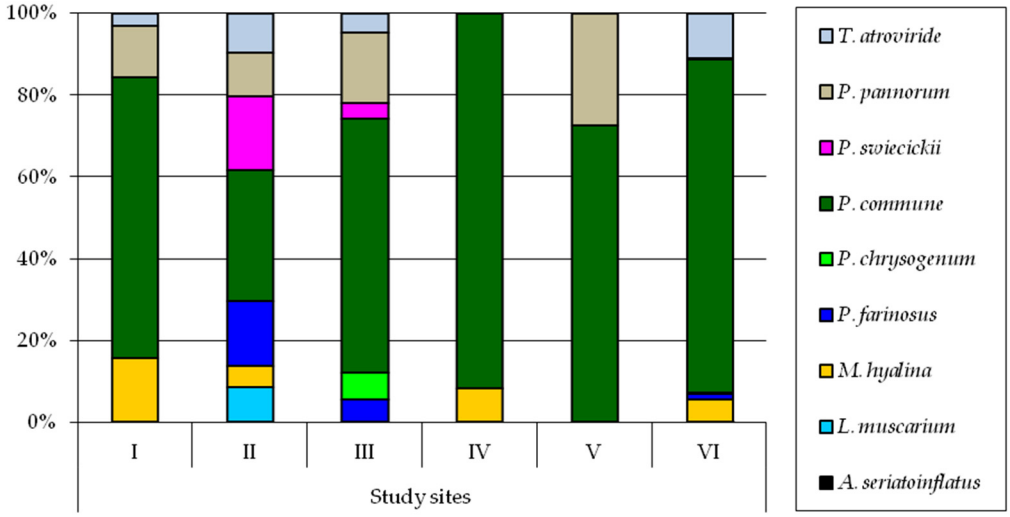

Figure 5. The percentage contribution of each fungal species to the overall fungi cultured at 24 °C from specific study sites within the soil and sediment samples of the Brestovská Cave.

The diversity of fungal species varied across the studied sites, as reflected in the Shannon index values for samples incubated in both temperature conditions. In the case of incubation at 5 °C, the species diversity of fungi outside the cave (0.539) was lower compared to inside the cave (from 0.569 to 0.705). In turn, in the case of fungi incubated at 24 °C, as many as two study sites inside the cave (no. II and no. III) were characterized by a higher index value (0.780 and 0.524, respectively) than the outdoor location (0.398). A similar situation was also noted for the general Shannon index calculated for both incubation temperatures (0.724 for outside samples and 0.837 for no. II and 0.804 for no. III). Moreover, there was positive correlation between the Shannon Diversity Index and the concentrations of fungal propagules for samples incubated at 24 °C and overall for samples incubated at both temperatures ($p < 0.05$; r = 0.87 and r = 0.41 for 24 °C, and r = 0.20, respectively). However, in the case of incubation at 5 °C, the above correlation was negative ($p < 0.05$; r = 0.67) (Figure 6).

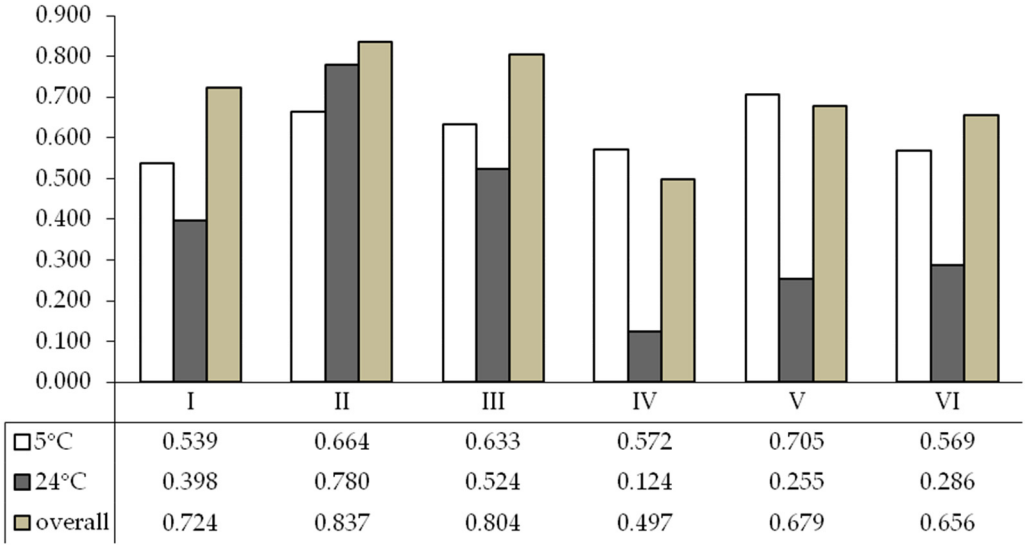

Figure 6. The Shannon Diversity Index values were calculated to assess the diversity of fungal communities within soil and sediment samples from the Brestovská Cave at designated research locations, labeled as study sites I–VI.

4. Discussion

Numerous speleomycological studies conducted in recent years have indicated differences in fungal communities depending on the geographic location of the tested object, the location of the study within a given object, the season of the year, the external environment, and the presence of visitors and animals living in them. The presence of animals and the different seasons are among the most important determinants of the occurrence of fungi in underground ecosystems [14,26,66–69]. This was partly confirmed by our previous study in the Brestovská Cave, which investigated the occurrence of psychrophilic and psychrotolerant airborne fungi in air samples [1] as well as the presence of keratinophilic and keratinolytic fungi [7]. This underground ecosystem is distinguished by its unique aeromycota composition, as highlighted by our research findings. For the first time, we identified the presence of fungal species such as *Coniothyrium pyrinum*, *Cystobasidium laryngis*, *Filobasidium wieringae*, *Leucosporidium drummii*, *Mortierella parvispora*, *Mrakia blollopis*, *Nakazawaea holstii*, and *Vishniacozyma victoriae* in the air of underground locations. Notably, *C. pyrinum*, *C. laryngis*, *L. drummii*, *M. blollopis*, and *N. holstii* were previously undetected in any part of underground ecosystems [1]. During the study of keratinophilic and keratinolytic species in the Brestovská Cave, we discovered for the first time the presence of two species in underground sites: *Chrysosporium europae* and *Penicillium charlesii* [7]. Additionally, in that case, we discovered that study sites located inside the cave displayed much more fungal species than on the outside [7]. This conclusion was in contrary to the belief that cave microorganisms are usually carried in the dust from the outside. Taking into account that soil microorganisms are responsible for critical ecosystem processes (biogeochemical cycle of carbon and other nutrients, soil aggregation etc., Acosta-Martínez et al. [70]), investigation of their biodiversity in deeper cave parts seems crucial. Considering the above, we decided to revisit the previously investigated Brestovská Cave and study the fungi inhabiting the soil/sediments in this cave.

Additionally, in our opinion, such analyses are important not only because of the possibility of discovering new fungal species [7], but above all, because the underground object is a popular touristic attraction and contact with potentially pathogenic fungal matter (e.g., spores) [24] might cause serious health issues, especially in individuals with impaired immune systems [71].

Identification revealed the presence of microscopic fungi belonging to various species of distinct environmental interactions, with the highest prevalence of Ascomycota phylum, which is consistent with previous studies [14]. Four *Penicillium* spp. were found in tested samples: *P. chrysogenum*, *P. camemberti*, *P. commune,* and *P. swiecicki*. The genus is commonly found in soil, especially in the rhizosphere [72] where it is usually beneficial for plants. Underground ecosystems are also enriched with other *Penicillium* spp., such as *P. chrysogenum*, *P. camemberti*, and *P. commune,* which are common in European caves [7,24,68,73], but they are also isolated in other parts of the world, e.g., *P. chrysogenum* was identified in cave soil samples in Russia, Korea, and Thailand, *P. camemberti* in Australia, and *P. commune* in northeastern USA [14]. *Penicillium chrysogenum* was isolated in our previous aeromycological studies of the Brestovská Cave from the air inside and outside of the cave [1]. Moreover, to the best of our knowledge, *P. swiecicki* was never previously isolated from caves or other underground sites. Overall, *Penicillium* spp. are known for their adaptation to cold environments due to the cold-active enzymes like endo-1,4-β-glucanasesor phosphatases [74,75]. They have been frequently isolated not only from caves but also from glaciers or Arctic soil [76]. Among *Penicillium* spp., *P. commune* was the most abundant taxa isolated, which is commonly associated with cheese spoilage [77]. However, this species is also a known producer of mycotoxins, such as cyclopiazonic acid, penitrem A and roquefortine [78,79]. Despite the fact that roquefortine and penitrem A are not volatile [80] and should not pose a threat to tourists, both display highly neurotoxic effects if accidently ingested [78]. They might be harmful to animals and critters feeding on cave sediments.

Another fungus with high prevalence in extreme environments and also present in the soil of the Brestovská Cave is *Pseudogymnoascus pannorum,* closely related to *P. destructans*

which causes white-nose syndrome in bats. Although *P. pannorum* (basionym: *Sporotrichum pannorum* Link 1824; obligate synonyms: *Chrysosporium pannorum* Hughes 1958 and *Geomyces pannorum* Sigler and Carmichael 1976) [53] is not known for its pathogenicity toward bats, it can on rare occasions cause disease in humans and dogs [81,82]. This fungus was also detected in our previous study in the Brestovská Cave—it was isolated from external and internal air samples [1] and internal soil/sediment samples during a study on keratinophilic and keratinolytic fungi [7]. Most likely, its presence in underground locations is attributed to the utilization of various substrates like keratin or complex carbohydrates but also to its tolerance to high salinity [82,83]. Another keratinophilic fungus isolated in the present study is *Chrysosporium merdarium* (basionym: *Sporotrichum merdarium* Link 1818) [46] which was also isolated from internal air samples in our previous study in the Brestovská Cave [1]. However, it had also been isolated from caves in other countries, like Hungary, Russia, and the Czech Republic [14]. Like other keratinophilic fungi, *Chrysosporium* spp. can cause the breakdown of keratinous substrates and potentially infect skin or nails. *Chrysosporium* spp. have been occasionally associated with human disease; however, there is no evidence of *C. merdarium* infections [84–86].

Other new records of species found in cave soil and sediment are *Cephalotrichum hinnuleum*, which is usually isolated from the rhizosphere, decaying plants and dung [87], and *Cosmpospora berkeleyana* (basionym: *Verticillium berkeleyanum* P. Karst. 1891; obligate synonyms: *Acremonium berkeleyanum* (P. Karst.) Gams 1982) [52], which is usually found on fruiting bodies of fungi, but also from soil and rabbit dung [88]. *Lecytophora* (now transferred to *Cioniochaeta*) *hoffmannii* (basionym: *Margarinomyces hoffmannii* J.F.H. Beyma 1938; obligate synonyms: *Phialophora hoffmannii* (J.F.H. Beyma) Schol-Schwarz. 1970) [50], also never before isolated from caves, is an intriguing discovery in our study, since it is a potential pathogen of humans and animals. Although a saprophyte in the environment, *L. hoffmannii* has been associated with human infections resulting in keratitis or subcutaneous abscesses, particularly in immunocompromised patients [89,90].

Among isolated Ascomycota, *Doratomyces stemonitis* (basionym: *Isaria stemonitis* Pers. 1797; obligate synonyms: *Stysanus stemonitis* (Pers.) Corda 1837 and *Periconia stemonitis* (Pers.) Pers. 1801) [47,49] was previously found in caves in the USA, Russia, Slovakia, Japan, and Korea [14]. It is not surprising since this species belongs to coprophilous fungi, inhabiting feces mainly of herbivores. A set of secreted enzymes, like those engaged in the degradation of cellulose, facilitates the degradation of indigestible parts of plants and enables *D. stemonitis* to inhabit this specific environment [91].

Lecanicillium muscarium (basionym: *Cephalosporium muscarium* Petch 1931; taxon synonyms: *Cephalosporium aphidicola* Petch 1931 and *Verticillium hemileiae* Bouriquet [51]) is an entomopathogenic fungus, frequently isolated from whitefly (*Trialeurodes vaporariorum*). Although it has been found previously in Slovakian caves, there are no reports on its occurrence in other regions of the world [14,92]. *L. muscarium* presence in the caves may be supported by its psychrotolerance, with the ability of some strains isolated from Antarctica to propagate even at 0 °C. Facilitation of the development in harsh conditions may be also due to the activity of lytic enzymes, degrading various substrates like chitin [93]. Another cold-adapted species isolated in the present study is *Oidiodendron truncatum*. We also found its presence during our earlier aeromycological study in the Brestovská Cave [1]. According to some reports, this species was also found in cave soil in Russia and in air samples from Czech Republic caves [14,94].

An important entomopathogen that can inhabit the cave environment is *Paecilomyces farinosus* (current name: *Cordyceps farinosa* (Holmsk.) Kepler, B. Shrestha and Spatafora 2017; basionym: *Ramaria farinosa* Holmsk. 1781; obligate synonyms: *Isaria farinosa* (Holmsk.) Fr., 1832, *Spicaria farinosa* (Holmsk.) Vuill., 1911, *Clavaria farinosa* (Holmsk.) Dicks., 1790, *Corynoides farinosa* (Holmsk.) Gray 1821 and *Penicillium farinosum* (Holmsk.) Biourge 1923) [44,59]. Although it is distributed globally, the cave isolates were found mainly in Europe, and cave soil samples that contained this species were obtained from Slovakia and Italy [14,95].

Among soilborne fungi, *Trichoderma atroviridae* was isolated from the soil and sediment of the Brestovská Cave, which is consistent with previous information about fungi inhabiting other Slovak caves [14]. *Trichosporella cerebriformis* (basionym: *Sporotrichum cerebriforme* de Vries and Kleine-Natrop 1957) [48] is also a species that could be found in soil, with some adaptations to cold environments since it had been isolated from Arctic tundra [96]. Cave soil isolates of this species have been found in the Brestovská Cave but also in caves of the Czech Republic and Russia [14].

Four isolates of Mucormycota phylum were obtained from the soil and sediment of the Brestovská Cave. *Ambomucor seriatoinflatus* was previously isolated from soil samples from Mongolia, China, and Alaska (with low prevalence); however, this is its first occurrence in the cave ecosystems [97,98]. Moreover, three species of the *Mortierella* genus were found in our study. *Mortielella elongate* (current name: *Linnemannia elongata* (Linnem.) Vandepol and Bonito 2020) [43,61] is one of the most prevalent fungi found in soil, with various adaptations to harsh environmental conditions, like low temperatures [99,100]. On the other hand, cave ecosystems are rarely inhabited by this species. Besides the Brestovská Cave, it was isolated only from caves in Belgium and from insect samples [14]. *M. hyalina* (current name: *Linnemannia hyalina* (Harz) Vandepol and Bonito 2020; basionym: *Hydrophora hyalina* Harz 1871) [61] exhibits higher prevalence in cave ecosystems, having been previously isolated from caves in the Czech Republic and Great Britain [14]. On the other hand, the third species—*M. minutissima* (current name: *Podila minutissima* (Tiegh.) Vandepol and Bonito 2020) [39]—had never been reported in caves until now. This species was frequently isolated from soil, decaying plant and animal tissues, or fecal matter; however, due to its psychrotolerant nature, it could be adapted to the cold cave environment [101,102].

The sole species of the Basidiomycota phylum isolated from Brestovská Cave soil and sediment samples is *Tausonia pullulans* (basionym: *Oidium pullulans* Lindner 1901; obligate synonyms: *Basidiotrichosporon pullulans* (Lindner) 1977, *Monilia pullulans* (Lindner) Kloecker 1924, *Oospora pullulans* (Lindner) Sacc. 1906, *Trichosporon pullulans* (Lindner) 1942 and *Guehomyces pullulans* (Lindner) Fell and Scorzetti 2004) [57]. This yeast is commonly found in plant material and soil samples and is considered a psychrotolerant, with a set of produced cold-active enzymes, such as lignin-degrading enzymes or lipases. It also harbors a collection of hydrolytic enzymes, whose activities provide nutrients for fungal growth from various substrates [103,104]. Basidiomycota fungi are generally found in association with substrates rich in nutrients, such as wood and manure, within underground settings. However, these types of substrates are not common components of cave environments, although wooden beams can often be found in old mines, and some caves may house substantial guano deposits [14]. The scarcity of large, nutrient-dense substrates within caves could account for the comparative rarity of Basidiomycota, especially when contrasted with Ascomycota.

A huge role in the effectiveness of the isolation of individual fungal species from samples using culture methods is played by the types of substrates used and the temperature during incubation [83,105]. On the one hand, the Basidiomycota phylum is difficult to culture and identify [69,106], so the methods used in most studies are directed toward the detection of Ascomycota. Zygomycota fungi, characterized by their prolific spore production and swift growth rates, are relatively easy to identify, which could lead to an overestimation of their abundance within cave environments [14]. On the other hand, using specific culture media and temperatures, it is possible to target the isolations toward given functional groups of fungi [24]. This is evidenced, among others, by our previous study in the Brestovská Cave. From the same samples, we carried out isolation targeted at keratinophilic and keratinolytic fungi using Vanbreuseghem hair bait [7]. This resulted in acquiring fungal species that were not detected during the study using PDA medium and the two incubation temperatures. Nevertheless, culture-based analysis is still the most popular method for speleomycological research [1,6,10,14], which is effective for detecting even the spores of slow-growing fungi such as Basidiomycota when the proper medium, incubation temperature, and low sample concentration are used [69].

5. Conclusions

This study sheds new light on the diversity of fungi residing in the soil and sediments of the Brestovská Cave in Slovakia, enriching our understanding of these ecosystems. We successfully isolated 20 fungal species, with 12 unique to the cave's interior and 8 found both inside and outside. Among the most frequently isolated was *P. commune*, a species known for mycotoxin production. Remarkably, our study marks the first identification of six fungal species in underground ecosystems: *P. swiecicki*, *C. hinnuleum*, *C. berkeleyana*, *L. hoffmannii*, *A. seriatoinflatus*, and *M. minutissima*. This not only enhances our biological knowledge of these species but also highlights the potential risks they pose to immunocompromised visitors and cave-dwelling fauna. The density of mycobiota decreased with distance from the cave entrance and was primarily influenced by the external environment. In turn, the observation of greater species diversity inside the cave compared to outside it can most likely be attributed to its unique ecosystem and suggests a need for further investigation into spore dispersion mechanisms, such as insect vectors, to develop protective measures for both cave preservation and visitor safety.

Author Contributions: Conceptualization, R.O.; methodology, R.O.; software, R.O.; validation, R.O. and J.S.; formal analysis, R.O.; investigation, R.O. and J.B.; resources, R.O.; data curation, R.O. and J.S.; writing—original draft preparation, R.O., J.B., K.S., A.P. and J.S.; writing—review and editing, R.O., J.B., K.S., A.P. and J.S.; visualization, R.O.; supervision, R.O.; project administration, R.O.; funding acquisition, J.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Acknowledgments: The authors would like to thank Zuzana Višňovská (biospeleologist of Slovak caves) for her help in the study.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Table A1. The average number (CFU $\times 10^2$ per 1 g) of fungi cultured from soil and sediment samples of the Brestovská Cave: [1]—not detected.

Fungal Species	Fungi Cultured at 5 °C						Fungi Cultured at 24 °C					
	I	II	III	IV	V	VI	I	II	III	IV	V	VI
Ambomucor seriatoinflatus	—[1]	—	—	—	—	—	—	—	—	—	—	0.03
Cephalotrichum hinnuleum	—	1.25	1.67	—	1.11	0.83	—	—	—	—	—	—
Chrysosporium merdarium	—	0.83	12.50	4.17	0.66	6.39	—	—	—	—	—	—
Cosmospora berkeleyana	—	—	—	—	—	0.28	—	—	—	—	—	—
Doratomyces stemonitis	—	—	—	—	—	0.55	—	—	—	—	—	—
Lecanicillium muscarium	—	—	—	—	—	—	—	13.33	—	—	—	—
Lecythophora hoffmannii	—	—	—	—	2.22	—	—	—	—	—	—	—
Mortierella elongata	16.66	2.91	0.83	9.16	—	0.64	—	—	—	—	—	—
Mortierella hyalina	—	—	—	—	—		27.77	8.33	—	8.33	—	3.83
Mortierella minutissima	25.00	15.83	—	—	—	0.47	—	—	—	—	—	—
Oidiodendron truncatum	8.33	16.66	2.50	13.33	1.00	—	—	—	—	—	—	—
Paecilomyces farinosus	—	—	—	—	—	—	—	25.00	2.50	—	—	0.87
Penicillium camemberti	—	10.00	8.33	—	—	—	—	—	—	—	—	—
Penicillium chrysogenum	—	—	—	—	—	—	—	—	2.91	—	—	0.14
Penicillium commune	—	—	—	—	—	—	122.22	50.00	27.50	91.67	6.66	55.55
Penicillium swiecickii	—	—	—	—	—	—	—	28.33	1.66	—	—	—
Pseudogymnoascus pannorum	—	2.50	12.9	10.00	2.78	20.55	22.22	16.67	7.64	—	2.50	0.18
Tausonia pullulans	—	—	—	—	—	1.11	—	—	—	—	—	—
Trichoderma atroviride	—	—	—	—	—	—	5.55	15.00	2.08	—	—	7.50
Trichosporiella cerebriformis	41.66	—	—	—	0.55	4.16	—	—	—	—	—	—

References

1. Ogórek, R.; Speruda, M.; Borzęcka, J.; Piecuch, A.; Cal, M. First Speleomycological Study on the Occurrence of Psychrophilic and Psychrotolerant Aeromycota in the Brestovská Cave (Western Tatras Mts., Slovakia) and First Reports for Some Species at Underground Sites. *Biology* **2021**, *10*, 497. [CrossRef]
2. Kuzmina, L.Y.; Galimzianova, N.F.; Abdullin, S.R.; Ryabova, A.S. Microbiota of the Kinderlinskaya Cave (South Urals, Russia). *Microbiology* **2012**, *81*, 251–258. [CrossRef]
3. Gabriel, C.R.; Northup, D.E. Microbial ecology: Caves as an extreme habitat. In *Cave Microbiomes: A Novel Resource for Drug Discovery*; Cheeptham, N., Ed.; Springer Press: New York, NY, USA, 2013; pp. 85–108. [CrossRef]
4. Zhang, Z.F.; Zhao, P.; Cai, L. Origin of Cave Fungi. *Front. Microbiol.* **2018**, *9*, 1407. [CrossRef]
5. Cigna, A.A. Modern trend(s) in cave monitoring. *Acta Carsologica* **2002**, *31*, 35–54. [CrossRef]
6. Ogórek, R.; Pusz, W.; Zagożdżon, P.P. Abundance and diversity of psychrotolerant cultivable mycobiota in winter of a former aluminous shale mine. *Geomicrobiol. J.* **2017**, *34*, 823–833. [CrossRef]
7. Ogórek, R.; Suchodolski, J.; Piecuch, A.; Przywara, K.; Višňovská, Z. Keratinophilic and Keratinolytic Fungi in Cave Ecosystems: A Culture-Based Study of Brestovská Cave and Demänovská Ľadová and Slobody Caves (Slovakia). *Appl. Sci.* **2022**, *12*, 1455. [CrossRef]
8. Pusz, W.; Ogórek, R.; Knapik, R.; Kozak, B.; Bujak, H. The Occurrence of Fungi in the Recently Discovered Jarkowicka Cave in the Karkonosze Mts. (Poland). *Geomicrobiol. J.* **2014**, *32*, 59–67. [CrossRef]
9. Ghosh, S.; Kuisiene, N.; Cheeptham, N. The cave microbiome as a source for drug discovery: Reality or pipe dream? *Biochem. Pharmacol.* **2017**, *134*, 18–34. [CrossRef] [PubMed]
10. Nováková, A.; Hubka, V.; Valinová, Š.; Kolařík, M.; Hillebrand-Voiculescu, A.M. Cultivable microscopic fungi from an underground chemosynthesis-based ecosystem: A preliminary study. *Folia Microbiol.* **2018**, *63*, 43–55. [CrossRef]
11. Burow, K.; Grawunder, A.; Harpke, M.; Pietschmann, S.; Ehrhardt, R.; Wagner, L.; Voigt, K.; Merten, D.; Büchel, G.; Kothe, E. Microbiomes in an acidic rock–water cave system. *FEMS Microbiol. Lett.* **2019**, *366*, fnz167. [CrossRef]
12. Poulson, T.L.; White, W.B. The cave environment. *Science* **1969**, *165*, 71–981. [CrossRef] [PubMed]
13. Barton, H.A.; Northup, D.E. Geomicrobiology in cave environments: Past, current and future perspectives. *J. Cave Karst Stud.* **2007**, *69*, 163–178.
14. Vanderwolf, K.J.; Malloch, D.; McAlpine, D.F.; Forbes, G. A world review of fungi, yeasts, and slime molds in caves. *Int. J. Speleol.* **2013**, *142*, 77–96. [CrossRef]
15. Benoit, J.B.; Yoder, J.A.; Zettler, L.W.; Hobbs, H.H. Mycoflora of a trogloxenic Cave Cricket, *Hadenoecus cumberlandicus* (Orthoptera: Rhaphidophoridae), from two small caves in northeastern Kentucky. *Ann. Entomol. Soc. Am.* **2004**, *97*, 989–993. [CrossRef]
16. Santamaria, S.; Faille, A. *Rhachomyces* (Ascomycota, Laboulbeniales) parasites on cave inhabiting Carabid beetles from the Pyrenees. *Nova Hedwig.* **2007**, *85*, 159–186. [CrossRef]
17. Yoder, J.A.; Benoit, J.B.; Christensen, B.S.; Croxall, T.J.; Hobbs, H.H. Entomopathogenic fungi carried by the cave orb weaver spider, *Meta ovalis* (Araneae, Tetragnathidae), with implications for mycoflora transfer to cave crickets. *J. Cave Karst Stud.* **2009**, *71*, 116–120.
18. Cubbon, B.D. Cave flora. In *The Science of Speleology*; Ford, T.D., Cullingford, C.H.D., Eds.; Academic Press: London, UK, 1976; pp. 423–452.
19. Bindschedler, S.; Milliere, L.; Cailleau, G.; Job, D.; Verrecchia, E.P. An ultrastructural approach to analogies between fungal structures and needle fiber calcite. *Geomicrobiol. J.* **2012**, *29*, 301–313. [CrossRef]
20. Engel, A.S. Microbial Diversity of Cave Ecosystems. In *Geomicrobiology: Molecular and Environmental Perspective*; Barton, L., Mandl, M., Loy, A., Eds.; Springer: Dordrecht, The Netherlands, 2010; pp. 219–238. [CrossRef]
21. Barton, H.A.; Jurado, V. What's up down there? Microbial Diversity in Caves Microorganisms in caves survive under nutrient-poor conditions and are metabolically versatile and unexpectedly diverse. *Microbe* **2007**, *2*, 132–138.
22. Jurado, V.; Sanchez-Moral, S.; Saiz-Jimenez, C. Entomogenous fungi and the conservation of the cultural heritage: A review. *Int. Biodeterior. Biodegrad.* **2008**, *62*, 325–330. [CrossRef]
23. Ogórek, R.; Dyląg, M.; Kozak, B.; Višňovská, Z.; Tancinova, D.; Lejman, A. Fungi isolated and quantified from bat guano and air in Harmanecka' and Driny Caves (Slovakia). *J. Cave Karst Stud.* **2016**, *78*, 41–49. [CrossRef]
24. Borzęcka, J.; Piecuch, A.; Kokurewicz, T.; Lavoie, K.H.; Ogórek, R. Greater Mouse-Eared Bats (*Myotis myotis*) Hibernating in the Nietoperek Bat Reserve (Poland) as a Vector of Airborne Culturable Fungi. *Biology* **2021**, *10*, 593. [CrossRef] [PubMed]
25. Ogórek, R.; Pusz, W.; Lejman, A.; Uklańska-Pusz, C. Microclimate effects on number and distribution of fungi in the Włodarz undeground complex in the Owl Mountains (Góry Sowie), Poland. *J. Cave Karst Stud.* **2014**, *76*, 146–153. [CrossRef]
26. Ogórek, R.; Lejman, A.; Matkowski, K. Influence of the external environment on airborne fungi isolated from a cave. *Pol. J. Environ. Stud.* **2014**, *23*, 435–440.
27. Shapiro, J.; Pringle, A. Anthropogenic Influences on the Diversity of Fungi Isolated from Caves in Kentucky and Tennessee. *Am. Midl. Nat.* **2010**, *163*, 76–86. [CrossRef]
28. Sun, J.M.; Irzykowski, W.; Jędryczka, M.; Han, F.H. Analysis of the genetic structure of *Sclerotinia sclerotiorum* (Lib.) de Bary populations from different regions and host plants by Random Amplified Polymorphic DNA markers. *J. Integr. Plant. Biol.* **2005**, *47*, 385–395. [CrossRef]

29. Treseder, K.K.; Lennon, J.T. Fungal traits that drive ecosystem dynamics on land. *Microbiol. Mol. Biol. Rev.* **2015**, *79*, 243–262. [CrossRef] [PubMed]
30. Žifčáková, L.; Vetrovský, T.; Howe, A.; Baldrian, P. Microbial activity in forest soil reflects the changes in ecosystem properties between summer and winter. *Environ. Microbiol.* **2016**, *18*, 288–301. [CrossRef] [PubMed]
31. Frąc, M.; Hannula, S.E.; Bełka, M.; Jędryczka, M. Fungal Biodiversity and Their Role in Soil Health. *Front. Microbiol.* **2018**, *9*, 707. [CrossRef]
32. Lavelle, P.; Spain, A.V. Soil Organisms. In *Soil Ecology*; Springer: Dordrecht, The Netherlands, 2005; pp. 201–356. [CrossRef]
33. Naga, K.; Suzuk, K.; Okada, G. Studies on the distribution of alkalophilic and alkali-tolerant soil fungi II: Fungal flora in two limestone caves in Japan. *Mycoscience* **1998**, *39*, 293–298. [CrossRef]
34. Baldrian, P. Interactions of heavy metals with white-rot fungi. *Enzym. Microb. Technol.* **2003**, *32*, 78–91. [CrossRef]
35. Creecy, J.P.; Caire, W.; Gilcrest, K.A. Examination of several Oklahoma bat hibernacula cave soils for *Pseudogymnoascus destructans*, the causative agent of White-Nose Syndrome. *Southwest. Nat.* **2015**, *60*, 213–217. [CrossRef]
36. Reynolds, H.T.; Ingersoll, T.; Barton, H.A. Modeling the environmental growth of *Pseudogymnoascus destructans* and its impact on the White-Nose Syndrome Epidemic. *J. Wildl. Dis.* **2015**, *51*, 318–331. [CrossRef] [PubMed]
37. Droppa, A. Karst on Sivývrch. *Ceskoslov. Kras* **1972**, *23*, 77–98. (In Slovak)
38. Brestovská Cave, Slovak Caves Administration. Available online: http://www.ssj.sk (accessed on 21 January 2024).
39. van Tieghem, P. Troisième mémoire sur les Mucorinées. *Ann. Sci. Nat.* **1878**, *4*, 312–399. (In French)
40. Karsten, P.A. Finlands mögelsvampar (Hyphomyctes fennici). Bidrag till Kännedom av Finlands Natur och Folk. *Fin. Litt.-Sällskapets Tryckeri* **1892**, *51*, 343–534. (In Swedish)
41. Thom, C. *Fungi in Cheese Ripening; Camembert and Roquefort*; US Department of Agriculture, Bureau of Animal Industry: Washington, DC, USA, 1906; Volume 82, 39p.
42. Thom, C. *Cultural Studies of Species of Penicillium*; US Department of Agriculture, Bureau of Animal Industry: Washington, DC, USA, 1910; Volume 118, 107p.
43. Linnemann, G. Die Mucorineen-Gattung *Mortierella* Coemans. *Pflanzenforschung* **1941**, *23*, 1–64. (In German)
44. Brown, A.H.S.; Smith, G. The genus *Paecilomyces* Bainier and its perfect stage *Byssochlamys* Westling. *Trans. Br. Mycol. Soc.* **1957**, *40*, 17–89. [CrossRef]
45. Barron, G.L. New species and new records of *Oidiodendron*. *Canad. J. Bot.* **1962**, *40*, 589–607. [CrossRef]
46. Carmichael, J.W. *Chrysosporium* and some other aleuriosporic Hyphomycetes. *Canad. J. Bot.* **1962**, *40*, 1137–1173. [CrossRef]
47. Morton, F.J.; Smith, G. The genera *Scopulariopsis* Bainier, *Microascus* Zukal and *Doratomyces* Corda. *Mycol. Pap.* **1963**, *86*, 1–96.
48. von Arx, J.A. Über die Typusart, zwei neue und einige weitere Arten der Gattung *Sporotrichum*. *Persoonia* **1971**, *6*, 179–184. (In German)
49. Matsushima, T. *Icones Microfungorum: A Matsushima Lectorum*; Matsushima: Kobe, Japan, 1975; p. 63.
50. Gams, W.; McGinnis, M.R. *Phialemonium*, a new anamorph genus intermediate between *Phialophora* and *Acremonium*. *Mycologia* **1983**, *75*, 977–987. [CrossRef]
51. Zare, R.; Gams, W. A revision of *Verticillium* section Prostrata. IV. The genera *Lecanicillium* and *Simplicillium*. *Nova Hedwig.* **2001**, *73*, 1–50. [CrossRef]
52. Gräfenhan, T.; Schroers, H.J.; Nirenberg, H.I.; Seifert, K.A. An overview of the taxonomy, phylogeny, and typification of nectriaceous fungi in *Cosmospora*, *Acremonium*, *Fusarium*, *Stilbella*, and *Volutella*. *Stud. Mycol.* **2011**, *68*, 79–113. [CrossRef] [PubMed]
53. Minnis, A.M.; Lindner, D.L. Phylogenetic evaluation of *Geomyces* and allies reveals no close relatives of *Pseudogymnoascus destructans*, comb. nov., in bat hibernacula of eastern North America. *Fungal Biol.* **2013**, *117*, 638–649. [CrossRef] [PubMed]
54. Zheng, R.Y.; Liu, X.Y. *Ambomucor* gen. & spp. nov. from China. *Mycotaxon* **2013**, *126*, 7–108. [CrossRef]
55. Visagie, C.M.; Hirooka, Y.; Tanney, J.B.; Whitfield, E.; Mwange, K.; Meijer, M.; Amend, A.S.; Seifert, K.A.; Samson, R.A. *Aspergillus*, *Penicillium* and *Talaromyces* isolated from house dust samples collected around the world. *Stud. Mycol.* **2014**, *78*, 63–139. [CrossRef] [PubMed]
56. Liu, X.Y.; Zheng, R.Y. New taxa of *Ambomucor* (Mucorales, Mucoromycotina) from China. *Mycotaxon* **2015**, *130*, 165–171. [CrossRef]
57. Liu, X.Z.; Wang, Q.M.; Göker, M. Towards an integrated phylogenetic classification of the *Tremellomycetes*. *Stud. Mycol.* **2015**, *81*, 85–147. [CrossRef]
58. Sandoval-Denis, M.; Guarro, J.; Cano-Lira, J.F.; Sutton, D.A.; Wiederhold, N.P.; de Hoog, G.S.; Abbott, S.P.; Decock, C.; Sigler, L.; Gené, J. Phylogeny and taxonomic revision of *Microascaceae* with emphasis on synnematous fungi. *Stud. Mycol.* **2016**, *83*, 193–233. [CrossRef]
59. Kepler, R.M.; Luangsa-Ard, J.J.; Hywel-Jones, N.L.; Quandt, C.A.; Sung, G.H.; Rehner, S.A.; Aime, M.C.; Henkel, T.W.; Sanjuan, T.; Zare, R.; et al. A phylogenetically-based nomenclature for *Cordycipitaceae* (Hypocreales). *IMA Fungus* **2017**, *8*, 335–353. [CrossRef] [PubMed]
60. Dyląg, M.; Sawicki, A.; Ogórek, R. Diversity of Species and Susceptibility Phenotypes toward Commercially Available Fungicides of Cultivable Fungi Colonizing Bones of *Ursus spelaeus* on Display in Niedźwiedzia Cave (Kletno, Poland). *Diversity* **2019**, *11*, 224. [CrossRef]

61. Vandepol, N.; Liber, J.; Desiró, A.; Na, H.; Kennedy, M.; Barry, K.; Grigoriev, I.V.; Miller, A.N.; O'Donnell, K.; Stajich, J.E.; et al. Resolving the *Mortierellaceae* phylogeny through synthesis of multi-gene phylogenetics and phylogenomics. *Fungal Divers.* **2020**, *104*, 267–289. [CrossRef] [PubMed]
62. White, T.J.; Bruns, T.; Lee, S.; Taylor, J.W. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*; Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Eds.; Academic Press: New York, NY, USA, 1990; pp. 315–322. [CrossRef]
63. Ogórek, R.; Dyląg, M.; Kozak, B. Dark stains on rock surfaces in Driny Cave (Little Carpathian Mountains, Slovakia). *Extremophiles* **2016**, *20*, 641–652. [CrossRef] [PubMed]
64. Zhang, T.; Wei, X.L.; Zhang, Y.Q.; Liu, H.Y.; Yu, L.Y. Diversity and distribution of lichen-associated fungi in the Ny-Ålesund Region (Svalbard, High Arctic) as revealed by pyrosequencing. *Sci. Rep.* **2015**, *14*, 14850. [CrossRef]
65. Spellerberg, I.F.; Fedor, P. A tribute to Claude Shannon (1916–2001) and a plea for more rigorous use of species richness, species diversity and the 'Shannon–Wiener' Index. *Glob. Ecol. Biogeogr.* **2003**, *12*, 177–179. [CrossRef]
66. Kokurewicz, T.; Ogórek, R.; Pusz, W.; Matkowski, K. Bats increase the number of cultivable airborne fungi in the "Nietoperek" bat reserve in Western Poland. *Microb. Ecol.* **2016**, *72*, 36–48. [CrossRef]
67. Ogórek, R.; Višňovská, Z.; Tančinová, D. Mycobiota of underground habitats: Case study of Harmanecká Cave in Slovakia. *Microb. Ecol.* **2016**, *71*, 87–99. [CrossRef]
68. Ogórek, R. Fungal communities on rock surfaces in Demänovská Ice Cave and Demänovská Cave of Liberty (Slovakia). *Geomicrobiol. J.* **2018**, *35*, 266–276. [CrossRef]
69. Ogórek, R.; Kozak, B.; Višňovská, Z.; Tančinová, D. Phenotypic and genotypic diversity of airborne fungal spores in Demänovská Ice Cave (Low Tatras, Slovakia). *Aerobiologia* **2018**, *34*, 13–28. [CrossRef]
70. Acosta-Martínez, V.; Van Pelt, S.; Moore-Kucera, J.; Baddock, M.C.; Zobeck, T.M. Microbiology of wind-eroded sediments: Current knowledge and future research directions. *Aeolian Res.* **2015**, *18*, 99–113. [CrossRef]
71. Low, C.Y.; Rotstein, C. Emerging fungal infections in immunocompromised patients. *F1000 Med. Rep.* **2011**, *3*, 14. [CrossRef] [PubMed]
72. Park, M.S.; Lee, J.W.; Kim, S.H.; Park, J.H.; You, Y.H.; Lim, Y.W. *Penicillium* from Rhizosphere Soil in Terrestrial and Coastal Environments in South Korea. *Mycobiology* **2020**, *48*, 431–442. [CrossRef] [PubMed]
73. Pusz, W.; Baturo-Cieśniewska, A.; Zagożdżon, P.; Ogórek, R. Mycobiota of the disused ore mine of Marcinków in Śnieżnik Masiff (western Poland). *J. Mt. Sci.* **2017**, *14*, 2448–2457. [CrossRef]
74. Duncan, S.M.; Farrell, R.L.; Thwaites, J.M.; Held, B.W.; Arenz, B.E.; Jurgens, J.A.; Blanchette, R.A. Endoglucanase-producing fungi isolated from Cape Evans historic expedition hut on Ross Island, Antarctica. *Environ. Microbial.* **2006**, *8*, 1212–1219. [CrossRef] [PubMed]
75. Gawas-Sakhalkar, P.; Singh, S.M.; Simantini, N.; Ravindra, R. High-temperature optima phosphatases from the cold-tolerant Arctic fungus *Penicillium citrinum*. *Polar Res.* **2012**, *31*, 11105. [CrossRef]
76. Hassan, N.; Rafiq, M.; Hayat, M.; Shah, A.A.; Hasan, F. Psychrophilic and psychrotrophic fungi: A comprehensive review. *Rev. Environ. Sci. Biotechnol.* **2016**, *15*, 147–172. [CrossRef]
77. Cheong, E.Y.L.; Sandhu, A.; Jayabalan, J.; Le, T.T.; Nhiep, N.T.; Ho, H.T.; Zwielehner, J.; Bansal, N.; Turner, M.S. Isolation of lactic acid bacteria with antifungal activity against the common cheese spoilage mould *Penicillium commune* and their potential as biopreservatives in cheese. *Food Control* **2014**, *46*, 91–97. [CrossRef]
78. Wagener, R.E.; Davis, N.D.; Diener, U.L. Penitrem A and Roquefortine Production by *Penicillium commune*. *Appl. Environ. Microbiol.* **1980**, *39*, 882–887. [CrossRef]
79. Rundberget, T.; Skaar, I.; Flåøyen, A. The presence of *Penicillium* and *Penicillium* mycotoxins in food wastes. *Int. J. Food Microbiol.* **2004**, *90*, 181–188. [CrossRef]
80. Pickard, C.; Fortin, J.S.; Holmes, D.; Buchweitz, J.P.; Lehner, A.F. A novel chemical marker of tremorgenic mycotoxicosis detected by gas-chromatography/mass-spectrometry. *World Mycotoxin J.* **2021**, *15*, 223–240. [CrossRef]
81. Christen-Zaech, S.; Patel, S.; Mancini, A.J. Recurrent cutaneous *Geomyces pannorum* infection in three brothers with ichthyosis. *J. Am. Acad. Dermatol.* **2008**, *58*, 112–113. [CrossRef] [PubMed]
82. Chaturvedi, V.; DeFiglio, H.; Chaturvedi, S. Phenotype profiling of white-nose syndrome pathogen *Pseudogymnoascus destructans* and closely-related *Pseudogymnoascus pannorum* reveals metabolic differences underlying fungal lifestyles. *F1000Research* **2018**, *7*, 665. [CrossRef] [PubMed]
83. Marshall, W.A. Aerial Transport of Keratinaceous Substrate and Distribution of the Fungus *Geomyces pannorum* in Antarctic Soils. *Microb. Ecol.* **1998**, *36*, 212–219. [CrossRef] [PubMed]
84. Chabasse, D.; de Gentile, L.; Bouchara, J.P. Pathogenicity of some *Chrysosporium* species isolated in France. *Mycopathologia* **1989**, *106*, 171–177. [CrossRef] [PubMed]
85. Stebbins, W.G.; Krishtul, A.; Bottone, E.J.; Phelps, R.; Cohen, S. Cutaneous adiaspiromycosis: A distinct dermatologic entity associated with *Chrysosporium* species. *J. Am. Acad. Dermatol.* **2004**, *51*, 185–189. [CrossRef] [PubMed]
86. Guerrero Palma, M.A.; Avila Espín, L.; Fernández Pérez, A.; Moreno León, J.A. Micosis nasosinusal invasiva por *Chrysosporium tropicum* [Invasive sinusal mycosis due to *Chrysosporium tropicum*]. *Acta Otorrinolaringol. Esp.* **2007**, *58*, 164–166. [CrossRef] [PubMed]

87. Das, K.; You, Y.H.; Lee, S.Y.; Jung, H.Y. A New Species of *Thelonectria* and a New Record of *Cephalotrichum hinnuleum* from Gunwi and Ulleungdo in Korea. *Mycobiology* **2020**, *48*, 341–350. [CrossRef] [PubMed]
88. Lechat, C.; Fournier, J. *Cosmospora xylariae* (*Nectriaceae*), a new species from France, Germany and U.K., with notes on *C. berkeleyana*, now *Sphaerostilbella berkeleyana*, and *C. scruposae*. *Ascomycete.Org* **2021**, *13*, 189–196. [CrossRef]
89. Sakaeyama, S.; Sano, A.; Murata, Y.; Kamei, K.; Nishimura, K.; Hatai, K. *Lecythophora hoffmannii* isolated from a case of canine osteomyelitis in Japan. *Med. Mycol.* **2007**, *45*, 267–272. [CrossRef]
90. Kabtani, J.; Militello, M.; Ranque, S. *Coniochaeta massiliensis* sp. nov. Isolated from a Clinical Sampl28. *J. Fungi* **2022**, *8*, 999. [CrossRef]
91. Peterson, R.; Grinyer, J.; Nevalainen, H. Secretome of the Coprophilous Fungus *Doratomyces stemonitis* C8, Isolated from Koala Feces. *Appl. Environ. Microbiol.* **2011**, *77*, 3793–3801. [CrossRef]
92. Chouikhi, S.; Assadi, B.H.; Lebdi, K.G.; Belkadhi, M.S. Efficacy of the entomopathogenic fungus, *Beauveria bassiana* and *Lecanicillium muscarium* against two main pests, *Bemisia tabaci* (Genn.) and *Tetranychus urticae* (Koch), under geothermal greenhouses of Southern Tunisia. *Egypt. J. Biol. Pest Control* **2022**, *32*, 125. [CrossRef]
93. Fenice, M. The Psychrotolerant Antarctic Fungus *Lecanicillium muscarium* CCFEE 5003: A Powerful Producer of Cold-Tolerant Chitinolytic Enzymes. *Molecules* **2016**, *21*, 447. [CrossRef] [PubMed]
94. Li, L.; Li, D.; Luan, Y.; Gu, Q.; Zhu, T. Cytotoxic metabolites from the antarctic psychrophilic fungus *Oidiodendron truncatum*. *J. Nat. Prod.* **2012**, *75*, 920–927. [CrossRef]
95. Weng, Q.; Zhang, X.; Chen, W.; Hu, Q. Secondary Metabolites and the Risks of *Isaria fumosorosea* and *Isaria farinosa*. *Molecules* **2019**, *24*, 664. [CrossRef] [PubMed]
96. Kurek, E.; Kornillowicz-Kowalska, T.; Slomka, A.; Melke, J. Characteristics of soil filamentous fungi communities isolated from various micro-relief forms in the high Arctic tundra (Bellsund region, Spitsbergen). *Pol. Polar Res.* **2007**, *28*, 57–73.
97. Young, J.M.; Liddicoat, C.; van Dijk, K.J.; Tabernero, P.; Caillet, C.; White, N.J.; Linacre, A.; Austin, J.J.; Newton, P.N. Environmental DNA as an innovative technique to identify the origins of falsified antimalarial tablets—A pilot study of the pharmabiome. *Sci. Rep.* **2022**, *12*, 21997. [CrossRef]
98. Miteva-Staleva, J.; Krumova, E.; Stoyancheva, G.; Kostadinova, N.; Grozdanov, P.; Spassova, B.; Angelova, M. Isolation, Identification and Proteolytic Activity of Filamentous Fungi from Alaska. *Acta Microbiol. Bulg.* **2022**, *38*, 26–30.
99. Weinstein, R.N.; Montiel, P.O.; Johnstone, K. Influence of Growth Temperature on Lipid and Soluble Carbohydrate Synthesis by Fungi Isolated from Fellfield Soil in the Maritime Antarctic. *Mycologia* **2000**, *92*, 222–229. [CrossRef]
100. Gams, W.; Chien, C.-Y.; Domsch, K.H. Zygospore Formation by the Heterothallic *Mortierella elongata* and a Related Homothallic Species, *M. epigama* sp. nov. *Trans. Br. Mycol. Soc.* **1972**, *58*, 5–13. [CrossRef]
101. Nguyen, T.T.T.; Park, S.W.; Pangging, M.; Lee, H.B. Molecular and Morphological Confirmation of Three Undescribed Species of *Mortierella* from Korea. *Mycobiology* **2019**, *47*, 31–39. [CrossRef]
102. Trytek, M.; Fiedurek, J. A novel psychrotrophic fungus, *Mortierella minutissima*, for D-limonene biotransformation. *Biotechnol. Lett.* **2005**, *27*, 149–153. [CrossRef]
103. Tsuji, M. Genetic diversity of yeasts from East Ongul Island, East Antarctica and their extracellular enzymes secretion. *Polar Biol.* **2018**, *41*, 249–258. [CrossRef]
104. Trochine, A.; Bellora, N.; Nizovoy, P.; Duran, R.; Greif, G.; de García, V.; Batthyany, C.; Robello, C.; Libkind, D. Genomic and proteomic analysis of *Tausonia pullulans* reveals a key role for a GH15 glucoamylase in starch hydrolysis. *Appl. Microbiol. Biotechnol.* **2022**, *106*, 4655–4667. [CrossRef]
105. Meletiadis, J.; Meis, J.F.G.M.; Mouton, J.W.; Verweij, P.E. Analysis of growth characteristics of filamentous fungi in different nutrient media. *J. Clin. Microbiol.* **2001**, *39*, 478–484. [CrossRef] [PubMed]
106. Fraatz, M.A.; Naeve, S.; Hausherr, V.; Zorn, H.; Blank, L.M. A minimal growth medium for the basidiomycete *Pleurotus sapidus* for metabolic flux analysis. *Fungal Biol. Biotechnol.* **2014**, *1*, 9. [CrossRef]