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Review

Fungi Affecting Wall Paintings of Historical Value: A Worldwide Meta-Analysis of Their Detected Diversity

Laura Zucconi [1,†], Fabiana Canini [1,†], Daniela Isola [1,2,*] and Giulia Caneva [2]

[1] Department of Ecological and Biological Sciences (DEB), University of Tuscia, 01100 Viterbo, Italy; zucconi@unitus.it (L.Z.); canini.fabiana@unitus.it (F.C.)
[2] Department of Sciences, Roma Tre University, 00146 Rome, Italy; giulia.caneva@uniroma3.it
* Correspondence: daniela.isola@uniroma3.it or isola@unitus.it
† These authors contributed equally to this work.

Abstract: Wall paintings have been a cultural expression of human creativity throughout history. Their degradation or destruction represents a loss to the world's cultural heritage, and fungi have been identified as a major contributor to their decay. We provide a critical review of fungi isolated from worldwide wall paintings between 1961–2021. One-hundred three scientific papers were reviewed focusing on fungal diversity, isolation protocols, and spatial distribution of data. The study sites were grouped into five environmental categories on the basis of the expected major microclimatic conditions (temperature, relative humidity, ventilation), and the possible relationship with the species found was investigated. The highest number of records were localized in Europe, with 38 sites on a total of 74, 20 of which were from Italy. A total of 378 fungal entries were obtained, consisting of 1209 records, belonging to 260 different species and 173 genera. The accuracy level in taxa determination was highly variable among different papers analyzed. Data showed a dominance of Ascomycota, mainly of orders Eurotiales and Hypocreales probably due to their wide distribution and easily air dispersed spores and due to the possible pitfalls linked to the isolation methods, favoring rapidly growing taxa. Statistical analyses revealed that fungal communities were not strictly linked to environmental categories with different ventilation, temperature, and humidity. Such findings may be due to the wide geographical area, the wide heterogeneity of the data, and/or the absence of standardized sampling and analyses protocols. They could also be the result of the dominance of some prevailing factors in the various sites that mask the influence one of each other.

Keywords: frescoes deterioration; fungal diversity; fungal ecology; hypogean conservation; mural paintings biodeterioration; subterranean cultural heritage deterioration; wall paintings conservation

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1. Introduction

Wall paintings are among the most representative elements of figurative artworks and have been developed by human creativity since prehistoric times [1]. Their technique of execution requires a layered structure consisting of a support, a ground, and a paint layer, which changed over time across different cultures (secco, such as tempera, or frescoes) [2]. In secco technique, which is the earliest, the preparation layers are applied, but the colors remain on the surface, whereas in the frescoes the colors are applied before the mortar dries, allowing their in-depth penetration [2]. The employed colors usually have a mineral origin, but some pigments can also be derived from plants. Organic compounds can later be added during restoration or because of other human activities (e.g., firing candles in the churches) [3].

The observed deterioration phenomena of mural paintings depend largely on the materials used and the environmental conditions [4]. Indeed, mural paintings are subject

to a variety of biodeterioration phenomena, which varies depending on the humidity, lighting, temperature, ventilation, and nutrients, which also select the occurring biological agents [1,5]. Furthermore, many environmental factors may synergistically or antagonistically contribute to the deteriorating actions of microorganisms [6]. Organisms belonging to all domains (bacteria, algae, fungi, animals and sometimes also lichens, mosses, ferns, and higher plants) have been isolated from the surfaces of mural paintings [4,7]. Indeed, given the indoor conditions of most mural paintings, photoautotrophs are highly limited, while fungi and bacteria are more frequent [8]. Bacteria with reduced nutritional needs have been often suggested to be the first colonizers. With their death and lyses, they release organic matter that promotes the growth of secondary colonizers, such as fungi [9–11]. Fungi, instead, can produce a large assortment of enzymes and have the remarkable ability to grow and thrive in a wide variety of environmental conditions, including low water activity [12]. Fungi have been rightly recognized as the most common cause of biodeterioration of painted surfaces and other artworks, causing both physical and chemical deterioration phenomena, with aesthetic and structural consequences [1,13,14].

Generally, damage is due to the mycelial growth on the substrate, hyphal penetration, and fruiting bodies production onto and into the substrate, all of which increases the volume and number of cracks, causing the rupture of the pigment layer and leading to surface fragments detachments [1,15]. Fungal colonization generally starts on the surface and then moves in-depth, up to decreasing painted layer cohesion and cause exfoliations and loss of the paint [9,16]. A study carried out by Dornieden and colleagues demonstrated that some fungi, as the so called microcolonial black fungi, are among the most dangerous for cultural heritages and can influence the resistance to shear and torsion stress of mortar and marble, contributing to the separation of different layers of material in mural paintings [17]. Aesthetic damages are also frequent, due to pigment discolorations, mycelial pigmentation, and/or the release of organic pigments of different colors, depending on the species involved. Moreover, secondary compounds such as extracellular enzymes and/or organic acids are generally released in the substrate from fungal hyphae, and this may cause chemical alterations of the mineral constituents of the surfaces as well as the original pigments [9,16]. The secretion of organic acids (e.g., oxalic, citric, succinic, formic, malic, acetic, fumaric, glyoxylic, gluconic, and tartaric acids) also plays a significant role in chemical attack, causing acidification of the substrate [18,19]. They can cause dissolution of cations and chelation of metal ions from mortar and mineral pigments, leading to the formation of stable metal complexes whose crystallization causes an increase of internal pressure resulting in cracking, peeling, and the eventual loss of mural fragments [20].

Awareness of the considerable role played by microorganisms in the preservation of art objects and historical buildings dates back to the 1950s [21]. Ionita and colleagues provided one of the first detailed descriptions of the mycoflora involved in the deterioration of mural paintings of monasteries in Moldavia, noting that it was favored by the various nutritional sources present in the materials used for the realization of the frescoes and by local environmental parameters [21]. This was perhaps one of the first statements of the importance of interdisciplinary studies to prevent and control deterioration processes and define restoration and preservation strategies. Two interesting mini-reviews were later published by Garg and Ciferri teams [1,16]. Many papers have been published after that, showing a growing awareness of the degradative role of fungi as well as the importance of mycological analyses as an integral part of the state-of-the-art system of wall painting safeguards [22].

Despite the fact that the fungal role in the deterioration of frescoes has been documented by a huge number of papers, a global inventory of fungal diversity and their optimal settlement conditions is not yet available. These paintings are mainly present in confined and semi-confined environments, both hypogean and non-hypogean. A fungal alteration pattern dependent on the environmental conditions of these different sites was

expected. Those present in hypogean environments are often subjected to a constant extreme humidity, promoting fungal spores germination and mycelial growth. The amount and type of available nutrients also affects the fungal growth rate and the type of fungal taxa. Nutrients may arrive from the external environment as airborne particles, and the more confined are the environments, the lower are the air spores dispersion phenomena. With this contribution, we aimed to describe the diversity of fungal colonizers involved in the deterioration of wall paintings, as well as their distribution under different environmental conditions. Additionally, we aimed to determine if a correlation among the different species recorded and the different types of environments-hypogean, non-hypogean, confined, non-confined, and open-exists and to speculate on their preferential habitat and their possible origin. A dataset of all the fungal taxa occurring on wall paintings based on bibliographic references was created for these purposes.

2. Materials and Methods

2.1. The Bibliographic Search

An extensive search was made among peer reviewed literature, proceedings to conferences, and books. The literature was identified using international databases, such as Scopus (https://www.scopus.com, 29 December 2021), Science Direct (https://www.sciencedirect.com, 29 December 2021), Web of Science (http://www.webofknowledge.com, 29 December 2021) and Google Scholar (https://scholar.google.com, 29 December 2021), that were consulted by using keywords such as 'wall paintings', 'mural paintings', 'frescoes', 'fungi', 'biodeterioration', 'microbial deterioration', and 'biodeteriogenic agents'. The thematic databases of ICCROM (International Centre for the Study of the Preservation and Restoration of Cultural Property) and the Italian ISCR (Istituto Superiore per la Conservazione e il Restauro) were also consulted, being important reference institutions in the field. Such sources were fundamental in the search of literature related to congresses and reports, that are not found by the most common scientific reference tools. The search covered more than 50 years, dating back to the first papers published in the 1960s (Figure 1), even if mostly of the papers containing useful taxonomic information were published after the 1980s.

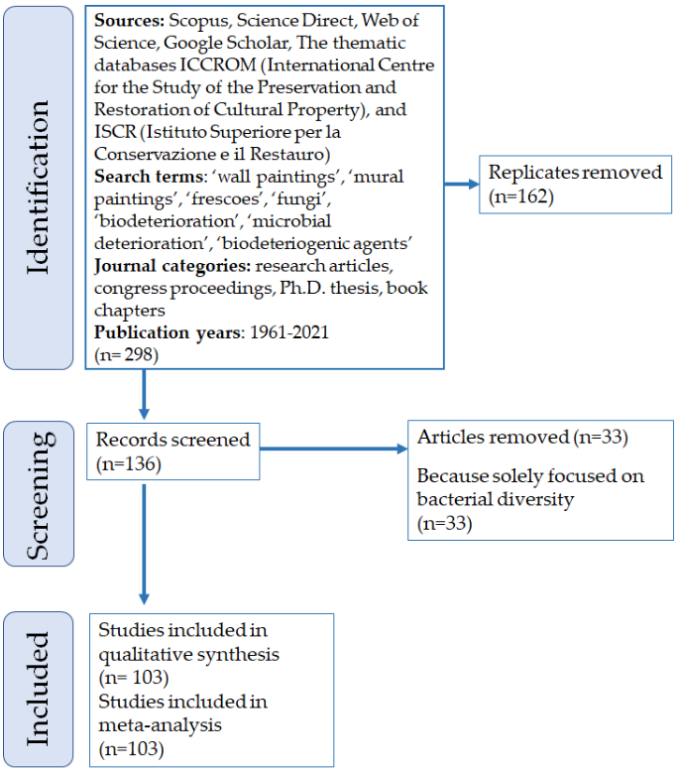

Figure 1. PRISMA flow diagram of the search process.

2.2. The Database

A list of all the entries retrieved, corresponding to taxa identified at both species and genus level, was compiled. Few were the entries referring to taxa above the rank of genus and they are listed at the end of the database. Current names of the taxa were reported according to the Index Fungorum (www.indexfungorum.org, 20 January 2022). The synonyms under which the different species were eventually reported in the analyzed papers were also indicated. Those entries recorded on paintings that have been the subject of multiple studies over the years, such as Takamatsuzuka and Kitora tumuli in Japan [23–25], were reported once accompanied by all bibliographic references.

2.2.1. The Geographic Localization of the Study Sites

The geographical locations and coordinates of the studied monuments were retrieved using Google Maps. The graphical representation on a map of sites distribution was performed using the 3D Map function of the Excel package. Some papers dealt with an unidentified number of monuments, as in the case of 'Monasteries in Romania' [21], 'several churches' in Northern Portugal [26], or 'Ajanta caves' in India [27–32]. In these cases, one or more sites were counted, depending on the details provided by the authors within the studied area and on their geographical distribution. Instead, some papers dealt with different monuments from the same area (e.g., different buildings in the historical site of Herculanum, Italy), that have been considered as a single site for the purpose of this study. This is why the total number of monuments is higher than the total number of geographical sites assessed in the statistical analyses.

2.2.2. Isolation and Identification Methods Used

The following information has been recorded: the type of culture media used, the growth temperature and incubation time, and the methods used for isolates identification.

2.2.3. The Types of Environments

The environments that housed the wall paintings object of the studies considered were grouped into five categories, based on whether they were hypogean or not, as well as the expected ventilation, confinement, and moisture conditions. The following are the definitions of the categories:

1. **C-HE:** Confined Hypogean Environment (isolated, without air circulation, with generally high humidity levels and relatively stable and low temperatures) as tumuli, close tombs, or prehistoric caves. They are often characterized by not negligible organic matter inputs from dripping waters, animals, and their fecal pellets and may be influenced by the presence of visitors, promoting the introduction and movement of airborne particulate.
2. **NC-HE**: Non-Confined Hypogean Environment (hypogean environments with reduced air circulation), such as underground crypts, catacombs, rupestrian churches, or Roman houses. These sub-aerial environments offer semi- or non-confined situations that are partially isolated from the external environment, with humidity and temperature comparatively more stable than the outdoor conditions but influenced by external day-night cycles and seasonal fluctuations.
3. **C-NHE**: Confined Non-Hypogean Environment: the sub-aerial environment of churches, monasteries, temples, refectories, and castle chapels represents a confined condition in which the microbial community is enclosed in a mesocosm. They are partially isolated from the external environment and have relatively more stable humidity and temperature than outdoor conditions, but they are influenced by external day-night cycles, seasonal variations in temperature, and relative humidity values. They are more prone to microbial attacks since they include more visited sites.
4. **SC-NHE**: Semi Confined Non-Hypogean Environment (open towards the outdoor environment but protected by wide changes in environmental parameters), such as ancient archaeological buildings and private homes. They are open to the outdoors but sheltered from rain and ventilation; they all experience seasonal and daily relative humidity and temperature fluctuations.
5. **O-SPE**: Open and Semi-Protected or Protected Environment; they include buildings' walls or collapsed caves, which are among the most exposed sites to biodeterioration risks.

2.3. Statistical Analyses

A data dissimilarity matrix was inferred using the Jaccard's dissimilarity index [33], and a hierarchical cluster analysis was performed on this matrix using the UPGMA method. Two dendrograms relating dissimilarities between sites and entries were plotted. Entries identified at the genus level were considered as diverse elements. The Silhouette index was used to resolve the optimal number of clusters [34]. A contingency table between the obtained clusters and the environmental categories to which they belonged was also constructed to assess the relationship between their data. An indicator species analysis of the individual clusters was performed, which identifies associations between entries or combinations of entries and clusters, using the Indval index [35,36].

All analyses were performed with the R Software with the packages ade4, vegan, gclus, cluster, vegclust, and indicspecies.

3. Results

3.1. The Fungal Data Set

A total of 103 papers dealing with the fungal deterioration of wall paintings were collected, regarding 107 different monuments grouped in 74 sites. A total of 378 fungal entries were obtained, consisting of 1209 records belonging to 173 genera and 260 species (Table 1).

Table 1. List of the fungal entries retrieved from the different papers grouped by genera, in association with the corresponding references and the environmental categories where they have been registered.

Genus	Fungal Name	Reference	Environment
Acremoniella	*Acremoniella atra*	[21]	C-NHE
Acremonium	*Acremonium camptosporum*	[37]	NC-HE
	Acremonium charticola	[38–41]	NC-HE, C-NHE
	Acremonium masseei	[23,25]	C-HE
	Acremonium murorum (syn. *Gliomastix murorum*)	[23–25,28,32]	C-HE, NC-HE
	Acremonium rutilum (syn. *A. roseum*)	[21]	C-NHE
	Acremonium cf. *rutilum*	[39]	C-NHE
	Acremonium sp.	[23,38–47]	C-HE, NC-HE, C-NHE
Acrodontium	*Acrodontium crateriforme*	[48]	C-NHE
Acrophialophora	*Acrophialophora fusispora* (syn. *A. nainiana*)	[28,32]	NC-HE
Acrostalagmus	*Acrostalagmus luteoalbus* (syn. *Verticillium lateritium*)	[49]	C-NHE
Acrothecium	*Acrothecium* sp.	[50]	O-SPE
Actinomucor	*Actinomucor elegans*	[51]	C-HE
Akanthomyces	*Akanthomyces lecanii* (syn. *Verticillium lecanii*)	[39,43,52]	NC-HE, C-NHE
Allophoma	*Allophoma labilis* (syn. *Phoma labilis*)	[53]	SC-NHE
Alternaria	*Alternaria alternata* (syn. *A. tenuis* and *Ulocladium alternariae*)	[21,26,28,29,31,32,40,46,49,51,53–59]	All environments
	Alternaria angustiovoidea	[60]	C-NHE
	Alternaria chartarum (syn. *Ulocladium chartarum*)	[21,61]	C-NHE
	Alternaria dianthi	[31]	C-NHE
	Alternaria longipes	[31]	C-NHE
	Alternaria longissima	[28,31,32]	NC-HE, C-NHE
	Alternaria oudemansii (syn. *Ulocladium oudemansii*)	[62]	NC-HE, C-NHE
	Alternaria tenuissima	[21,22,24,31,46,57,63]	NC-HE, C-NHE, O-SPE
	Alternaria sp. (syn. *Ulocladium* sp.)	[10,11,21,23,26,38,41,46,64,65]	C-HE, NC-HE, C-NHE, O-SPE
Amphinema	*Amphinema* sp.	[66]	C-NHE
Amyloporia	*Amyloporia sinuosa* (syn. *Antrodia sinuosa*)	[11]	C-NHE
Antrodia	*Antrodia* sp.	[66]	C-NHE
Apiotrichum	*Apiotrichum* sp. (syn. *Hyalodendron* sp.)	[43]	C-NHE
Arachnomyces	*Arachnomyces* sp.	[45]	NC-HE
Armillaria	*Armillaria* sp.	[66]	C-NHE
Arthrinium	*Arthrinium arundinis*	[40]	C-NHE

	Arthrinium phaeospermum (syn. *Papularia sphaerosperma*)	[28,32]	NC-HE
	Arthrinium sp.	[46,65]	C-NHE; O-SPE
Arthrobotrys	*Arthrobotrys* sp.	[23]	C-HE
Ascochyta	*Ascochyta medicaginicola* (syn. *Phoma medicaginis*)	[20,56]	O-SPE
	Ascochyta sp.	[63]	C-NHE
Ascotricha	*Ascotricha guamensis*	[32]	NC-HE
Aspergillus	*Aspergillus aeneus*	[67]	SC-NHE
	Aspergillus amstelodami (syn. *Eurotium amstelodami*)	[46,55,63]	C-NHE
	Aspergillus aureolatus	[20,56]	O-SPE
	Aspergillus auricomus	[22]	O-SPE
	Aspergillus candidus	[28,32,39,68]	C-HE, NC-HE, C-NHE
	Aspergillus clavatus	[37]	C-HE
	Aspergillus creber	[20,56]	O-SPE
	Aspergillus echinulatus	[21]	C-NHE
	Aspergillus europaeus	[20,56]	O-SPE
	Aspergillus fischeri (syn. *Neosartorya fischeri*)	[62]	NC-HE; C-NHE
	Aspergillus flavipes	[20,56]	O-SPE
	Aspergillus flavus (syn. *A. oryzae*)	[10,17,20,28,30–32,56,57,68–75]	C-HE, NC-HE, C-NHE, O-SPE
	Aspergillus fumigatus	[30,31,38,46,61,76–79]	C-HE, NC-HE, C-NHE
	Aspergillus glaucus group	[79]	C-NHE
	Aspergillus ivoriensis	[67]	SC-NHE
	Aspergillus japonicus	[78]	C-HE
	Aspergillus melleus	[67]	SC-NHE
	Aspergillus multicolor	[67]	SC-NHE
	Aspergillus nidulans (syn. *Emericella nidulans*)	[28,30–32,46,51,61,70,71,78]	C-HE, NC-HE, C-NHE
	Aspergillus niger	[10,17,19,20,28,30–32,49,50,53–56,72,74,75,77,80–82]	All environments
	Aspergillus niger group	[46]	C-NHE
	Aspergillus ochraceus	[38,49,67]	C-HE, C-NHE, SC-NHE
	Aspergillus ostianus	[20,56,67]	SC-NHE, O-SPE
	Aspergillus pallidofulvus	[20,56]	O-SPE
	Aspergillus parasiticus	[20,56]	O-SPE
	Aspergillus penicilloides	[83]	C-HE
	Aspergillus petrakii	[67]	SC-NHE
	Aspergillus proliferans	[28,32]	NC-HE
	Aspergillus protuberus	[67]	SC-NHE
	Aspergillus puniceus	[67]	SC-NHE
	Aspergillus repens	[21,41]	C-NHE
	Aspergillus restrictus	[63,84]	C-HE, C-NHE

	Aspergillus sclerotiorum	[76]	C-NHE
	Aspergillus spectabilis (syn. *Emericella spectabilis*)	[67]	SC-NHE
	Aspergillus stellatus (syn. *Emericella variecolor*)	[67]	SC-NHE
	Aspergillus sydowii	[28,32,39,51,52,62,69]	C-HE, NC-HE, C-NHE
	Aspergillus terreus	[29–32,78]	C-HE, NC-HE, C-NHE
	Aspergillus unguis	[76]	C-NHE
	Aspergillus ustus	[46,67]	C-NHE, SC-NHE
	Aspergillus versicolor	[17,28,31,32,39,41,43,46,49,51,55,61,67–71,78,82,84–86]	C-HE, NC-HE, C-NHE, SC-NHE
	Aspergillus wentii	[28,31,32]	NC-HE, C-NHE
	Aspergillus sp.	[11,18,19,22–24,27,44–46,50,64,66,69,75,77,82,87–95]	C-HE, NC-HE, C-NHE, O-SPE
Aureobasidium	*Aureobasidium pullulans*	[31,41,43,45,49,52,57]	NC-HE, C-NHE
Beauveria	*Beauveria bassiana*	[48]	C-NHE
	Beauveria sp.	[45,52]	NC-HE, C-NHE
Bispora	*Bispora* sp.	[65]	O-SPE
Bjerkandera	*Bjerkandera adusta*	[77]	C-NHE
Blastobotrys	*Blastobotrys aristatus*	[39]	C-NHE
Blastomyces	*Blastomyces* sp.	[84]	C-HE
Botryotrichum	*Botryotrichum atrogriseum*	[17,55,59]	NC-HE, C-NHE
	Botryotricum domesticum	[60]	C-NHE
	Botryotrichum murorum (syn. *Chaetomium murorum*)	[20,21,46,55,56]	C-NHE, O-SPE
Botrytis	*Botrytis cinerea*	[40,41,43,46,69]	C-NHE
Brunneochlamydosporium	*Brunneochlamydosporium nepalense* (syn. *Acremonium nepalense*)	[38,85]	C-HE
Burgoa	*Burgoa* sp.	[23]	C-HE
Candida	*Candida takamatsuzukensis*	[23,96]	C-HE
	Candida tumulicola	[23,96]	C-HE
	Candida sp.	[23,24,65]	C-HE, NC-HE
Capronia	*Capronia coronata*	[85]	C-HE
Cephalotrichum	*Cephalotrichum verrucisporum* (syn. *Doratomyces verrucisporus*)	[23,24]	C-HE
	Cephalotrichum sp. (syn. *Doratomyces* sp.)	[23,24]	C-HE
Cephalosporium	*Cephalosporium* sp.	[88,90]	C-NHE
Chaetomium	*Chaetomium ancistrocladum*	[20,56]	O-SPE
	Chaetomium elatum	[40]	C-NHE
	Chaetomium globosum	[21,22,26,31,32,38,41,46,51,55]	C-HE, NC-HE, C-NHE, O-SPE
	Chaetomium piluliferum	[21,55]	C-NHE

	(syn. *Botryotrichum piluliferum*)		
	Chaetomium sp.	[9,22,27,31,39,40,43,46, 65,70–72]	NC-HE, C-NHE, O-SPE
Chondrostereum	*Chondrostereum* sp.	[66]	C-NHE
Chrysosporium	*Chrysosporium pseudomerdarium*	[85]	C-HE
	Chrysosporium sp.	[47,52,62,97]	C-HE, NC-HE, C-NHE, O-SPE
Circinella	*Circinella muscae* (syn. *Circinella sydowii*)	[55]	C-NHE
Cladophialophora	*Cladophialophora tumulicola*	[24,98]	C-HE
Cladosporium	*Cladosporium cladosporioides*	[22,28–32,38,41,46,49,51,57,59 ,68,69,85,99,100]	C-HE, NC-HE, C-NHE, O-SPE
	Cladosporium cucumerinum	[46,49,51]	C-HE, C-NHE
	Cladosporium herbarum	[21,28,31,32,39,46,51,5 5,58,63,82]	C-HE, NC-HE, C-NHE
	Cladosporium macrocarpum	[60]	NC-HE
	Cladosporium sphaerospermum	[5,7,9,22,28,31,32,39–43,46,48,51,52,62,63,69]	C-HE, NC-HE, C-NHE, O-SPE
	Cladosporium uredinicola	[20,56]	O-SPE
	Cladosporium xylophilum	[60]	C-NHE
	Cladosporium sp.	[15,18,19,22,23,27,42–45,52,58,62,64–66,71,75,77,80,82,86,87 ,90,91,94,101–103]	All environments
Clonostachys	*Clonostachys rosea* (syn. *Gliocladium roseum)*	[58]	C-HE
Cochliobolus	*Cochliobolus geniculatus* (syn. *Curvulata geniculata*)	[32,76]	NC-HE, C-NHE
Collariella	*Collariella bostrychodes* (syn. *Chaetomium bostrychodes*)	[28,32]	NC-HE
Coltricia	*Coltricia* sp.	[66]	C-NHE
Coprinellus	*Coprinellus aokii* (syn. *Coprinus aokii*)	[67]	SC-NHE
Coprinopsis	*Coprinopsis atramentaria*	[38]	C-HE
	Coprinopsis cothurnata (syn. *Coprinus cothurnatus*)	[63]	C-NHE
Cordyceps	*Cordyceps farinosa* (syn. *Isaria farinosa*)	[68]	C-HE
Coriolopsis	*Coriolopsis* sp.	[66]	C-NHE
Cunninghamella	*Cunninghamella echinulata*	[9,28,32,40,55]	NC-HE, C-NHE
	Cunninghamella elegans	[38]	C-HE
	Cunninghamella sp.	[23,24]	C-HE
Curvularia	*Curvularia australiensis* (syn. *Drechslera australiensis*)	[28–32]	NC-HE, C-NHE
	Curvularia hawaiiensis (syn. *Drechslera hawaiiensis*)	[28–32]	NC-HE, C-NHE

	Curvularia lunata	[28,30–32,76,83]	NC-HE, C-NHE
	Curvularia pallescens	[29–32]	NC-HE, C-NHE
	Curvularia spicifera (syn. *Drechslera spicifera*)	[46]	C-NHE
	Curvularia sp.	[75]	C-NHE
Cutaneotrichosporon	*Cutaneotrichosporon mucoides* (syn. *Trichosporon mucoides*)	[63]	C-NHE
Cylindrocarpon	*Cylindrocarpon* sp.	[23,24]	C-HE
Cyphellophora	*Cyphellophora olivacea*	[42]	C-HE
	Cyphellophora sp.	[42]	C-HE
Cyphellostereum	*Cyphellostereum* sp.	[66]	C-NHE
Cystoderma	*Cystoderma* sp.	[66]	C-NHE
Devriesia	*Devriesia* sp.	[45]	NC-HE
Dichotomophilus	*Dichotomophilus indicus* (syn. *Chaetomium indicum*)	[55]	C-NHE
Didymella	*Didymella glomerata* (syn. *Phoma glomerata*)	[21,40]	C-NHE
Dipodascus	*Dipodascus geotrichum*	[55]	C-NHE
	Dipodascus sp. (syn. *Geotrichum* sp.)	[47,75]	NC-HE, C-NHE
Discostroma	*Discostroma corticola* (syn. *Seimatosporium lichenicola*)	[20,56]	O-SPE
Drechslera	*Drechslera* sp.	[65]	O-SPE
Emericella	*Emericella ruber*	[31]	C-NHE
	Emericella sp.	[49,75]	C-HE, C-NHE
Engyodontium	*Engyodontium* sp.	[45,69]	C-HE, NC-HE, C-NHE
Epicoccum	*Epicoccum nigrum* (syn. *Epicoccum purpurascens*)	[20,28,31,32,46,49,56,58]	C-HE, NC-HE, C-NHE, O-SPE
	Epicoccum sp.	[27,65,87,90]	C-HE, NC-HE, C-NHE, O-SPE
Eurotium	*Eurotium halophilicum*	[104]	C-NHE
	Eurotium herbariorum	[68]	C-HE
	Eurotium sp.	[47,65,89,92]	C-HE, NC-HE, C-NHE
Exophiala	*Exophiala angulospora*	[42,98]	C-HE
	Exophiala moniliae	[85]	C-HE
	Exophiala sp.	[23,42]	C-HE
Fomitopsis	*Fomitopsis vinosa*	[63]	C-NHE
Fusarium	*Fusarium chlamydosporum*	[51]	C-HE
	Fusarium culmorum	[31]	C-NHE
	Fusarium equiseti	[53]	SC-NHE
	Fusarium fujikuroi (syn. *F. moniliforme*)	[29,30,32,74]	C-HE, NC-HE
	Fusarium oxysporum	[23,28,31,32,38,46,49,58,67,73,105]	C-HE, NC-HE, C-NHE, SC-NHE
	Fusarium proliferatum	[77]	C-NHE
	Fusarium sporotrichioides	[38]	C-HE

	Fusarium sp.	[10,23,24,27,31,39,43,47,62,64,82,91,94,106]	C-HE, NC-HE, C-NHE
Fuscoporia	*Fuscoporia* sp.	[66]	C-NHE
Fusidium	*Fusidium viride*	[48]	C-NHE
Ganoderma	*Ganoderma* sp.	[66]	C-NHE
Gliomastix	*Gliomastix tumulicola* (syn. *Acremonium tumulicola*)	[23,25]	C-HE
	Gliomastix sp.	[58,97]	C-HE
Gloiothele	*Gloiothele* sp.	[66]	C-NHE
Helminthosporium	*Helminthosporium* sp.	[65,75,82]	C-NHE, O-SPE
Humicola	*Humicola fuscoatra*	[49]	C-NHE
	Humicola udagawae	[38]	C-HE
	Humicola sp.	[31]	C-NHE
Hyphodontia	*Hyphodontia alutaria*	[66]	C-NHE
	Hyphodontia sp.	[66]	C-NHE
Hyphodontiella	*Hyphodontiella* sp.	[66]	C-NHE
Hypholoma	*Hypholoma* sp.	[66]	C-NHE
Idriella	*Idriella* sp.	[58]	C-HE
Kendrickiella	*Kendrickiella phycomyces*	[23,24,107]	C-HE
Kernia	*Kernia geniculotricha*	[67]	SC-NHE
	Kernia hippocrepida	[67]	SC-NHE
Lactarius	*Lactarius* sp.	[66]	C-NHE
Lecanicillium	*Lecanicillium psalliotae*	[38,59]	C-HE, NC-HE
	Lecanicillium sp.	[68]	C-HE
Leptobacillium	*Leptobacillium muralicola*	[37]	NC-HE
Leptosphaeria	*Leptosphaeria* sp.	[65]	O-SPE
Leptosphaerulina	*Leptosphaerulina* sp.	[64]	NC-HE
Macrophomina	*Macrophomina phaseolina*	[28,31,32]	NC-HE
Malbranchea	*Malbranchea* sp.	[46]	C-NHE
Mammaria	*Mammaria echinobotryoides*	[83]	C-HE
Memnoniella	*Memnoniella* sp.	[31]	C-NHE
Metapochonia	*Metapochonia bulbillosa* (syn.*Verticillium bulbillosum*)	[47]	C-HE, NC-HE
	Metapochonia suchlasporia (syn.*Verticillium suchlasporium*)	[52]	NC-HE, C-NHE
Meyerozyma	*Meyerozyma guilliermondii*	[60]	NC-HE
Microascus	*Microascus brevicaulis* (syn. *Scopulariopsis brevicaulis*)	[21,52,68]	C-HE, NC-HE, C-NHE
	Microascus chartarum (syn. *Scopulariopsis chartarum*)	[62]	NC-HE, C-NHE
	Microascus cirrosus	[67]	SC-NHE
	Microascus sp.	[68]	C-HE
Microdochium	*Microdochium lycopodinum*	[38]	C-HE
Monilinia	*Monilinia* sp. (syn. *Monilia* sp.)	[82]	C-NHE
Monocillium	*Monocillium*-like	[23]	C-HE

Monodictys	*Monodictys castaneae* (syn. *Stemphylium macrosporoideum*)	[21]	C-NHE
	Monodictys sp.	[31,58]	C-HE, C-NHE
Mortierell	*Mortierella alpina*	[47]	NC-HE
	Mortierella ambigua	[38]	C-HE
	Mortierella parvispora (syn. *M. gracilis*)	[31]	C-NHE
	Mortierella sp.	[47,58]	C-HE, NC-HE
Mucor	*Mucor plumbeus* (syn. *M. spinosus*)	[21]	C-NHE
	Mucor racemosus (syn. *M. globosus*)	[28,32,47,60]	NC-HE
	Mucor silvaticus	[28,32]	NC-HE
	Mucor sp.	[23,46,58,64,82,93]	C-HE, NC-HE, C-NHE
Myxotrichum	*Myxotrichum stipitatum*	[46]	C-NHE
	Myxotrichum sp.	[46]	C-NHE
Nectria	*Nectria* sp.	[15]	C-NHE
Neocosmospora	*Neocosmospora solani* (syn. *Fusarium solani*)	[23,28,31,76,97,105]	C-HE, NC-HE, C-NHE
Neodevriesia	*Neodevriesia modesta* (syn. *Devriesia modesta*)	[99,108]	O-SPE
	Neodevriesia simplex (syn. *Devriesia simplex*)	[99,108]	O-SPE
	Neodevriesia sp.	[45]	NC-HE
Neosartorya	*Neosartorya* sp.	[62]	NC-HE, C-NHE
Neosetophoma	*Neosetophoma cerealis* (syn. *Coniothyrium cerealis*)	[69]	C-NHE
Neurospora	*Neurospora intermedia*	[76]	C-NHE
	Neurospora sp.	[82]	C-NHE
Nigrospora	*Nigrospora oryzae* (syn. *N. sphaerica*)	[28,32,82]	C-HE, NC-HE, C-NHE
	Nigrospora sp.	[28,82]	NC-HE, C-NHE
Oidiodendron	*Oidiodendron cereale*	[69]	C-NHE
	Oidiodendron tenuissimum	[49]	C-NHE
Ophiostoma	*Ophiostoma* sp.	[23]	C-HE
Paecilomyces	*Paecilomyces variotii*	[28–32,61]	NC-HE, C-NHE
	Paecilomyces sp.	[27,46,68,75,82,97]	C-HE, NC-HE, C-NHE
Parengyodontium	*Parengyodontium album* (syn. *Beauveria alba, Tritirachium album*, and *Engyodontium album*)	[9,21,39–43,48,52,59,68,94,103,109–111]	C-HE, NC-HE, C-NHE
Penicillium	*Penicillium aethiopicum*	[67]	SC-NHE
	Penicillium albicans	[93]	C-NHE
	Penicillium aurantiogriseum (syn. *P. verrucosum* var. *cyclopium*)	[9,41,43,51]	C-HE, C-NHE

Penicillium brevicompactum	[39,40,46,68,69,94]	C-HE, NC-HE, C-NHE
Penicillium camemberti	[93]	C-NHE
Penicillium canescens (syn. *P. raciborski*)	[40,93]	C-NHE
Penicillium carneum	[67]	SC-NHE
Penicillium chrysogenum (syn. *P. notatum*)	[9,21,40,41,46,51,54,60, 67,69,81,87,93,112,113]	C-HE, NC-HE, C-NHE, SC-NHE
Penicillium citreonigrum (syn. *P. citreoviride*)	[22,58]	C-HE, O-SPE
Penicillium citrinum	[31,32,40,58,76,93]	C-HE, NC-HE, C-NHE
Penicillium commune	[51,53,68,93]	C-HE, C-NHE, SC-NHE
Penicillium concentricum	[67]	SC-NHE
Penicillium coprobium	[67]	SC-NHE
Penicillium corylophilum	[44,91]	C-NHE
Penicillium daleae	[91]	C-NHE
Penicillium decumbens	[9,40,93]	C-NHE
Penicillium dierckxii (syn. *P. fellutanum*)	[46]	C-NHE
Penicillium digitatum	[66]	C-NHE
Penicillium dipodomyicola	[67]	SC-NHE
Penicillium expansum	[46,49]	C-NHE
Penicillium fuscoglaucum	[60]	C-NHE
Penicillium glabrum (syn. *P. frequentans*)	[9,39,46,49,66,93,94]	NC-HE, C-NHE
Penicillium granulatum	[31]	C-NHE
Penicillium griseofulvum	[46,56,57,67]	C-NHE, SC-NHE
Penicillium herquei	[46]	C-NHE
Penicillium italicum	[53]	SC-NHE
Penicillium javanicum (syn. *Eupenicillium javanicum*)	[62]	NC-HE, C-NHE
Penicillium lanosum	[20,56,100]	NC-HE, O-SPE
Penicillium lilacinum	[21,55]	C-NHE
Penicillium meleagrinum	[38,69]	C-HE, C-NHE
Penicillium miczynskii	[47]	SC-NHE
Penicillium olsonii	[51]	C-HE
Penicillium oxalicum	[49,58]	C-HE, C-NHE
Penicillium pancosmium	[38]	C-HE
Penicillium paneum	[23,67,114]	C-HE, SC-NHE
Penicillium polonicum	[51]	C-HE
Penicillium purpurescens	[31]	C-NHE
Penicillium purpurogenum	[69,93]	C-NHE
Penicillium restrictum	[93]	C-NHE
Penicillium simplicissimum (syn. *P. janthinellum*)	[58,93]	C-HE, C-NHE
Penicillium spinulosum (syn. *P. nigricans*)	[9,40,84]	C-HE, C-NHE

	Penicillium thomii	[93]	C-NHE
	Penicillium turbatum	[53]	SC-NHE
	Penicillium verrucosum	[39,46]	C-NHE
	Penicillium vulpinum	[67]	SC-NHE
	Penicillium sp.	[11,15,18,19,23,24,26,28,31,43–48,58,61,64,66,70,75,77,78,80,82,86,88–91,94,101]	C-HE, NC-HE, C-NHE
Pestalotia	*Pestalotia* sp.	[26]	C-NHE
Phialophora	*Phialophora* sp.	[23,24,43,47]	C-HE, C-NHE
Phlebia	*Phlebia* sp.	[66]	C-NHE
Pholiota	*Pholiota* sp.	[66]	C-NHE
Phoma	*Phoma* sp.	[23,31]	C-HE, C-NHE
Physalacria	*Physalacria* sp.	[66]	C-NHE
Pleospora	*Pleospora* sp.	[65]	O-SPE
Postia	*Postia* sp.	[66]	C-NHE
Preussia	*Preussia terricola*	[68]	C-HE
	Preussia sp.	[68]	C-HE
Pseudogymnoascus	*Pseudogymnoascus pannorum* (syn. *Geomyces pannorum* and *Chrysosporium pannorum*)	[39,43,48]	C-NHE
Pseudozyma	*Pseudozyma prolifica*	[51]	C-HE
Purpureocillium	*Purpureocillium lilacinus* (syn. *Paecilomyces lilacinus*)	[19,38,49]	C-HE, C-NHE
Pyrenophora	*Pyrenophora biseptata* (syn. *Drechslera biseptata*)	[28,32]	NC-HE
Radulomyces	*Radulomyces* sp.	[66]	C-NHE
Rhinocladiella	*Rhinocladiella*-like	[23]	C-HE
Rhizoctonia	*Rhizoctonia solani* (syn. *Thanatephorus cucumeris*)	[20,28,32,56]	NC-HE, O-SPE
Rhizopus	*Rhizopus stolonifer* (syn. *R. nigricans*)	[28,31,32,53,57,73,74]	C-HE, NC-HE, C-NHE
	Rhizopus sp.	[27,101]	NC-HE, C-NHE
Rhodotorula	*Rhodotorula glutinis*	[49]	C-NHE
	Rhodotorula mucilaginosa	[66]	C-NHE
	Rhodotorula sp.	[15,18,66,69,80,91]	C-NHE
Russula	*Russula* sp.	[66]	C-NHE
Sagenomella	*Sagenomella griseoviridis*	[24]	C-HE
	Sagenomella striatispora	[24]	C-HE
	Sagenomella sp.	[44]	NC-HE
Sarocladium	*Sarocladium bacillisporum* (syn. *Acremonium bacillisporum)*	[94]	NC-HE
	Sarocladium kiliense (syn. *Acremonium kiliense*)	[21,52]	NC-HE, C-NHE
	Sarocladium strictum (syn. *Acremonium* cfr. *strictum*)	[23,52]	C-HE, NC-HE, C-NHE

Schizophyllum	*Schizophyllum commune*	[64]	C-NHE
	Schizophyllum sp.	[67]	C-NHE
Schizopora	*Schizopora paradoxa* (syn. *Hyphodontia paradoxa*)	[64]	C-NHE
Scolecobasidium	*Scolecobasidium anomalum* (syn. *Ochroconis anomala*)	[115]	C-HE
	Scolecobasidium lascauxensis	[85,115]	C-HE
	Scolecobasidium tshawytschae (syn. *Ochroconis tshawytschae*	[46]	C-NHE
Scopulariopsis	*Scopulariopsis brevicaulis*	[21,55]	C-NHE
	Scopulariopsis fusca	[39]	C-NHE
	Scopulariopsis sp.	[46,47,81,90]	NC-HE, C-NHE
Scytalidium	*Scytalidium* sp.	[70,71]	C-NHE
Simplicillium	*Simplicillium lamellicola* (syn. *Verticillium lamellicola*)	[48]	C-NHE
Skeletocutis	*Skeletocutis* sp.	[67]	C-NHE
Sordaria	*Sordaria humana*	[28,32]	NC-HE
Sphaerostilbella	*Sphaerostilbella* sp. (syn. *Gliocladium sp.)*	[23,93,97]	C-HE, C-NHE
Sporothrix	*Sporothrix* sp.	[44,91]	NC-HE, C-NHE
Sporotrichum	*Sporotrichum* sp.	[41,43]	C-NHE
Stachybotrys	*Stachybotrys chartarum* (syn. *S. atra*)	[21,31,48,54,84]	NC-HE, C-NHE
	Stachybotrys cylindrosporus	[21]	C-NHE
	Stachybotrys echinatus (syn. *Memnoniella echinata*)	[28,31,32]	NC-HE
	Stachybotrys sp.	[9,27,31]	NC-HE, C-NHE
Stagonosporopsis	*Stagonosporopsis lupini*	[60]	C-NHE
Stemphylium	*Stemphylium botryosum*	[41]	C-NHE
	Stemphylium pyriforme	[55]	C-NHE
	Stemphylium sp.	[93]	C-NHE
Stereum	*Stereum* sp.	[66]	C-NHE
Syncephalastrum	*Syncephalastrum* sp.	[75]	C-NHE
Talaromyces	*Talaromyces aculeatus*	[51]	C-HE
	Talaromyces flavus	[38,69]	C-HE
	Talaromyces pinophilus (syn. *Penicillium pinophilum)*	[54,94]	C-HE, NC-HE
	Talaromyces rugulosus (syn. *Penicillium rugulosum*)	[68,69,113]	C-HE, C-NHE
	Talaromyces variabilis (syn. *Penicillium variabile*)	[69]	C-NHE
Thysanorea	*Thysanorea papuana*	[85]	C-HE
Tilletiopsis	*Tilletiopsis* sp.	[69]	C-NHE
Torrubiella	*Torrubiella alba* (syn. *Lecanicillium aranearum*)	[94]	NC-HE
	Torrubiella sp.	[68,94]	C-HE, NC-HE
Torula	*Torula herbarum*	[55]	C-NHE
	Torula sp.	[46]	C-NHE

Tricharina	*Tricharina* sp.	[64]	NC-HE
Trichocladium	*Trichocladium asperum*	[68]	C-HE
Trichoderma	*Trichoderma harzianum*	[29–32,69]	NC-HE, C-NHE
	Trichoderma sect. L*ongibrachiatum*	[23,105]	C-HE
	Trichoderma virens (syn. *Gliocladium virens*)	[58]	C-HE
	Trichoderma viride	[23,55,58]	C-HE, C-NHE
	Trichoderma sp.	[19,23,24,31,44,47,75,82,85,95,97]	C-HE, NC-HE, C-NHE
Trichothecium	*Trichothecium indicum* (syn. *Acremonium indicum*)	[28,31,32]	NC-HE
	Trichothecium roseum	[21,92]	C-NHE
Tritirachium	*Tritirachium* sp.	[67,82]	C-HE, C-NHE
Tubaria	*Tubaria* sp.	[66]	C-NHE
Tyromyces	*Tyromyces* sp.	[66]	C-NHE
Umbelopsis	*Umbelopsis ramanniana* (syn. *Mortierella ramanniana*)	[39,47]	NC-HE, C-NHE
Venturia	*Venturia carpophila* (syn. *Cladosporium carpophilum*)	[32]	NC-HE
Verticillium	*Verticillium alboatrum*	[32]	NC-HE
	Verticillium sp.	[23,48,52,58,97]	C-HE, NC-HE, C-NHE
Wallemia	*Wallemia sebi*	[63]	C-NHE
	Wallemia sp.	[92]	C-NHE
Westerdykella	*Westerdykella* sp.	[64]	NC-HE
Xylodon	*Xylodon nespoli*	[66]	C-NHE
	Xylodon nothofagi	[66]	C-NHE
	Xylodon raduloides	[66]	C-NHE
Zygosporium	*Zygosporium masoni*	[23]	C-HE
	Basidiomycota (Phylum)	[64]	NC-HE
	Black meristematic fungi	[5,99,116]	NC-HE, O-SPE
	Chaetomiaceae (Family)	[64]	NC-HE
	Filobasidiales	[64]	NC-HE
	Hyaline sterile mycelia	[62]	NC-HE, C-NHE
	Melanized sterile mycelia	[62]	NC-HE, C-NHE
	Pezizomycotina (Subphylum)	[64]	NC-HE
	Undetermined dark pigmented fungi	[17]	O-SPE
	Undetermined yeasts	[46]	C-NHE
	Uredinales (Order)	[65]	O-SPE
	Ustilaginales (Order)	[65]	O-SPE

The taxonomic distribution of the total fungal diversity and within different types of environments, at phylum and order level, is reported in Figure 2. Ascomycota was the dominant phylum, ranging from 89 to 97% (except for the environment O-SPE where a great proportion of undetermined taxa was retrieved) and accounting for 100% of the fungal entries in 40 out of 74 sites. The other two phyla identified were Basidiomycota (1–

6%), more abundant in C-NHE, and Mucoromycota, reaching a maximum value (2.35%) in C-NHE (Figure 2A).

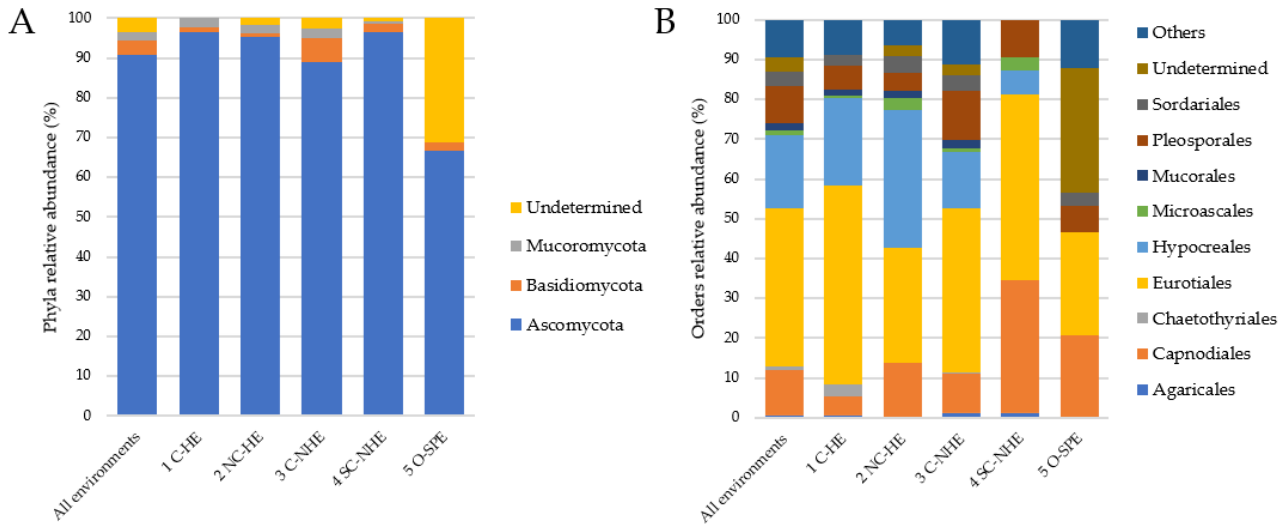

Figure 2. Taxonomic composition of the fungal diversity recorded on wall paintings on the total dataset and in relation to the different environments, at (**A**) phylum and (**B**) order level.

The entries were distributed in 39 orders. Eurotiales and Hypocreales were the most represented ones, together accounting for more than half of total fungal diversity. Pleosporales, Capnodiales, Sordariales, and Chaetothyriales were also well represented orders within Ascomycota. Basidiomycota accounted for a maximum of around 6% of all taxa, with Agaricales, Helotiales, and Saccharomycetales being the most abundant ones accordingly. Mucorales was the most abundant order within the phylum Mucoromycota.

The accuracy in taxa determination varied among the different papers analyzed, with many entries identified only at genus level. In fact, a total of 107 out of 378 entries (28.3%) referred to genera not determined at the species level, and it was not possible to quantify the number of possible different species belonging to these genera within different papers. A total of 61 genera (35.2%) were represented by a single species. *Aspergillus* and *Penicillium* (order Eurotiales) were the most frequently recorded genera and were represented by a greater number of species, 40 and 46, respectively, present in 44 (59.4%) and 32 (43.2%) sites, which increased to 54 (73%) and 51 (68.9%) when those sites where the genera were reported as undetermined at the species level were also considered. Their contribution to the total number of records was significant, with 219 (18.1%) and 154 (12.7%) records, respectively. Other genera frequently recorded were *Alternaria* and *Fusarium* with seven species each; A*cremonium*, *Cladosporium,* and *Trichoderma* with six species; *Curvularia* and *Talaromyces* with five species; and *Chaetomium* with four species.

3.2. The Geographic Distribution of the Study Sites

The data came from 107 monuments, grouped in 74 sites and distributed among 19 different countries. The countries where they were reported as well as the different number of sites were graphically represented in Figure 3.

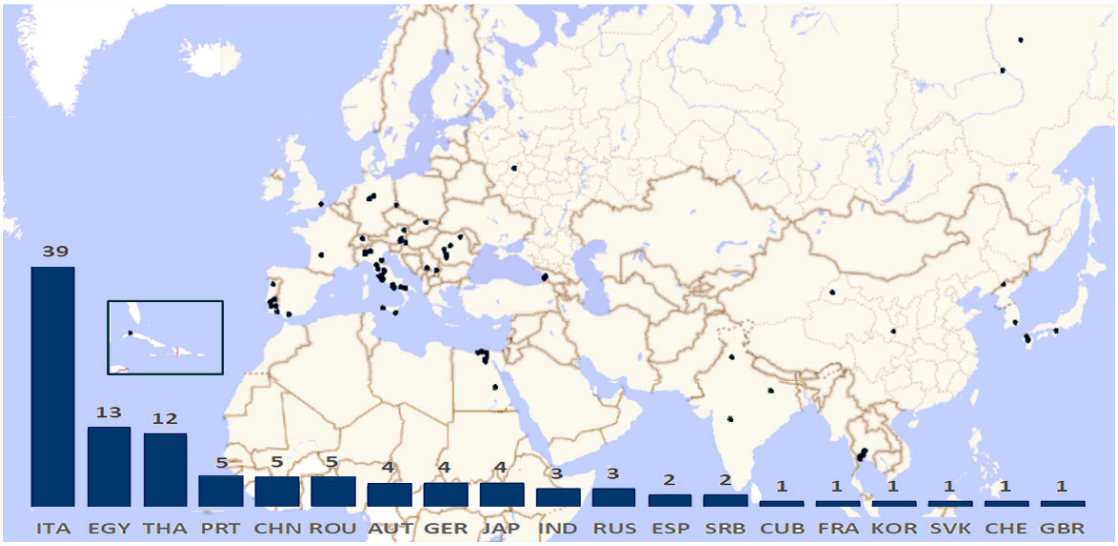

Figure 3. Geographic distribution of the reviewed study's 107 monuments. Nations are indicated with the international alpha-3 code: ITA: Italy, EGY: Egypt, THA: Thailand, PRT: Portugal, CHN: China, ROU: Romania, AUT: Austria, GER: Germany, JAP: Japan, IND: India, RUS: Russian Federation, ESP: Spain, SRB: Republic of Serbia, CUB: Cuba, FRA: France, KOR: Republic of Korea, SVK: Slovak Republic, CHE: Swiss, GBR: Great Britain. In the blue rectangle Cuba.

3.3. Isolation and Identification Methods

A dominance of culture-based methods associated with morphological identification (58%) and target regions sequencing (31%) was recorded, the latter has become progressively dominant since the 2000s. The culture media used varied depending on the research purposes and included Czapek Dox agar (CZ), malt extract agar (MEA), malt agar (MA), potato dextrose agar (PDA), Sabouraud dextrose agar (SAB), and oatmeal agar (OA) among the most frequently used. The use of mycological agar (MYC), Cook's Rose Bengal (CRB), and yeast peptone dextrose (YPD) was sporadic. Data on growth temperature and incubation time were frequently missing, accounting for 31.25% (Figure 4A, green) and 54.12% (Figure 4B, green) of all papers, respectively. This trend was particularly evident in the earliest papers where more attention was paid to fungal species than to the conditions used to isolate them (e.g., [40,47,84,88]). The most frequent temperature settings were 25 °C (35%) and within the range 27–32 °C (21.25%). When reported, the incubation frequently corresponded to 7 days (31.76%) (Figure 4B). When isolated strains were identified by molecular approaches, the identification was performed targeting different barcoding regions, such as the complete internal transcribed spacer (ITS), a part of it (ITS1), portions of 18S (SSU), 26S (LSU), and β-tubulin.

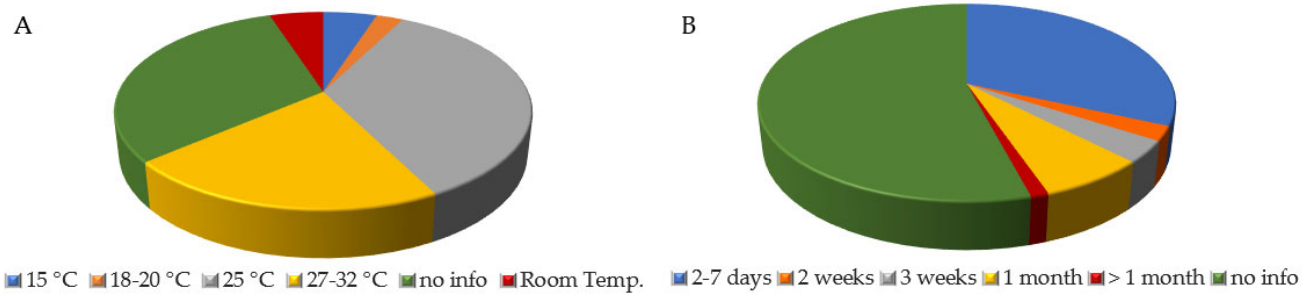

Figure 4. Values/ranges of (**A**) growth temperature and (**B**) incubation time recorded in culture-based protocols.

3.4. Distribution of Fungal Entries in Different Environments

The distribution of the sites among the environmental categories revealed a predominance of wall painting recorded in C-NHE, which alone accounted for 54% of the sites. The hypogean environments accounted for 36.5% of the sites, distributed between C-HE (18.9%) and NC-HE (17.6%). The remaining two categories, SC-NHE and O-SPE, were less represented, comprising 4.1% and 5.4% of the sites, respectively.

The cluster analysis at the level of different sites resulted in a general dispersion, with no distinct clusters retrieved. Several clusters consisted of one or few sites highly different one to each other (13-11-14-3-15-10-9-7-12). Other clusters (4-5-6) were slightly more similar to each other and contained many entries (Figure 5).

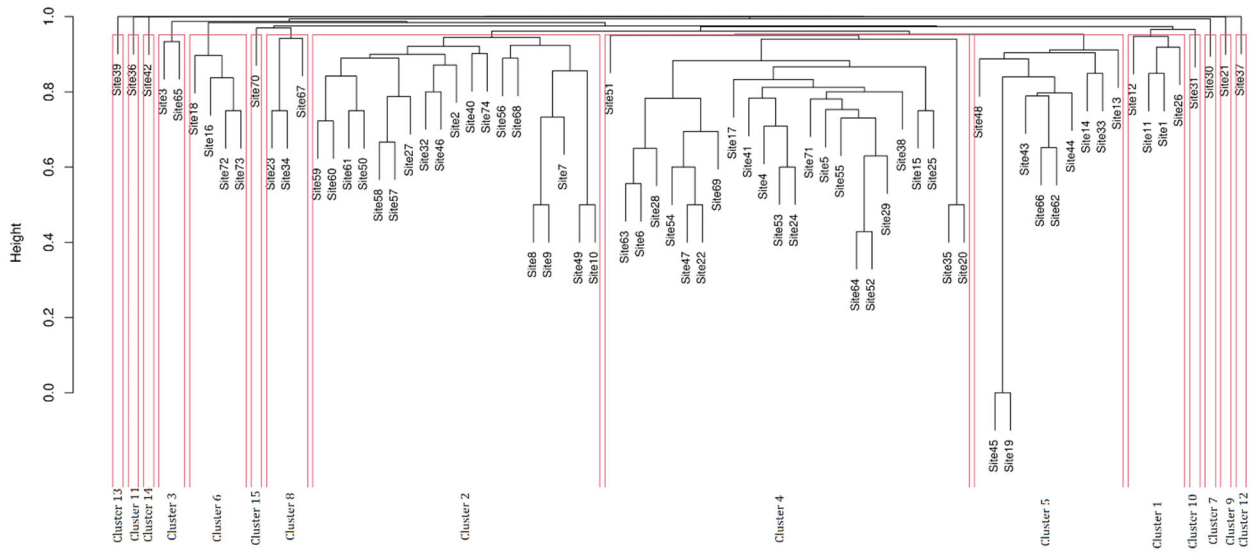

Figure 5. Cluster dendrogram. Cluster analysis on the Jaccard distance of the composition at the level of the different sites. Sites belonged to the following categories: Site1–Site14 C-HE; Site15–Site27 NC-HE; Site 28–Site67 C-NHE; Site 68–Site70 SC-NHE; Site 71–Site74 O-SPE. The references associated to each site are reported in Supplementary Table S1.

The contingency table highlighted that the obtained clusters did not have a strong correlation with the suggested environmental categories (Table 2). However, some clusters showed some affinities: cluster 15 with cat. 4 (SC-NHE) (aff. index 17); cluster 6 with cat. 5 (O-SPE) (aff. index 12.8); clusters 2,4,5 with cat. 3 (C-NHE) (aff. indexes 8.1, 9, and 6.2, respectively); cluster 4 with cat. 2 (NC-HE) (aff. index 5.9); cluster 1 with cat. 1 (C-HE) (aff. index 8.2).

Table 2. Contingency table and affinity indices. (**A**) Contingency table between the obtained clusters and the environmental categories to which they are correlated and (**B**) their relative affinity indices.

(**A**)	**Contingency Table**															
		Clusters														
		13	**11**	**14**	**3**	**6**	**15**	**8**	**2**	**4**	**5**	**1**	**10**	**9**	**7**	**12**
Environmental categories	**C-HE**				1				5	3	2	**3**				
	NC-HE					2		1	1	**6**	1	1			1	
	C-NHE	1	1	1	1			2	**11**	**13**	**7**		1	1		1
	SC-NHE						**1**		1	1						
	O-SPE					**2**			1	1						
(**B**)	**Affinity Indices**															
		Clusters														
		13	**11**	**14**	**3**	**6**	**15**	**8**	**2**	**4**	**5**	**1**	**10**	**9**	**7**	**12**

Environmental categories	**C-HE**				1.82				4.8	1.37	1.46	**8.2**					
	NC-HE					3.93		1.31	0.21	**5.89**	0.39	0.98			3.93		
	C-NHE	1.28	1.28	1.28	0.64			1.7	**11**	**8.98**	**6.25**		1.28	1.28		1.28	
	SC-NHE						**17**		1	0.71							
	O-SPE					**12.8**			0.67	0.53							

When the correlation among different entries was analyzed, very scattered results were retrieved (Figure S1). However, some entries or combinations of them showed some affinities (specificity and fidelity above 0.5) with certain clusters such as *Alternaria alternata* with cluster 2 (Indval Index = 0.75); *Acrothecium* sp. and *Penicillium* sp. with cluster 4 (Indval Index = 0.70 and 0.66, respectively); *Parengyodontium album* and *Rhinocladiella*-like with cluster 5 (Indval Index = 0.70 and 0. 64); *Actinomucor elegans*, *Penicillium pancosmium*, *Acremoniella atra*, *Alternaria angustiovoidea*, *Scolecobasidium anomalum*, *Scolecobasidium lascauxensis*, when considered in combination, the entries *Trichoderma* sp. and *Verticillium* sp. with cluster 1 (Indval Index = 0.70, 0.70, 0.70, 0.70, 0.70, and 0.70, respectively); and *Akanthomyces lecanii* with cluster 9 (Indval Index = 0.93).

4. Discussion

4.1. The Fungal Data Set

Even if fungi have been suggested as secondary colonizers of painted mural substrates, they are among the most common microbial life-forms present in these environments and the primary cause of their biodeterioration [1,13,14]. The wide biodiversity observed confirms the potential key role of fungi in such colonization process and suggests a combination of causes that can favor their growth. Their broad enzymatic activities allow them to grow on every type of material, or wherever they find organic matter. Fungi recorded belonged to species generally reported from natural environments like soils, plants, and air where they live as saprotrophs, as well as plant and animal parasites and pathogens. A detailed survey of their diversity and distribution should become a prerequisite before any restoration measures in order to prevent further damages [39]. Most records belonged to Ascomycota, with Eurotiales being the most common order, due to the prevalence of *Aspergillus* and *Penicillium* genera. The former was one of the most frequently isolated genera, with *A. flavus* and *A. niger* among the more frequently recorded species. As reported in the literature, even from the first older papers in this field, these two genera, along with *Alternaria*, *Fusarium*, *Cladosporium*, *Mortierella*, *Chaetomium*, and *Acremonium*, are among the most common deteriogens of such paintings [1,11,15,31,39,46,62,78,87,91]. These taxa are ubiquitous, and their frequent occurrence is due to the production of numerous conidia, which are widespread in the environment because they are easily dispersed by air. A diversity of filamentous fungi, with the most predominant genera *Penicillium*, *Cladosporium*, *Aspergillus*, and *Trichoderma*, were also isolated from mural paintings of the Parish Church of Santo Aleixo (Portugal). Their dehydrogenase activity was determined, as an indicator of the presence of metabolic active cells to allow a deeper insight on the deteriogenic role of the isolates [19].

Species of these genera were recorded on indoor frescoes in numerous monasteries in Romania, possibly favored by the organic components and vegetal pigments used, as well as high moisture levels caused by frequent rainwater penetration, which also resulted in the formation of efflorescences [55]. *Cladosporium* species can cope in a variety of harsh environmental conditions thanks to their low nutritional requirements (i.e., in oligotrophic conditions). Otherwise, *Chaetomium* species are proteolytic and cellulolytic ascomycetes, favored by nutrient-rich substrates [22,65,117]. They were reported as the most frequent microfungi on the frescoes of the St. Damian Monastery in Assisi (Italy) [46] and on frescoes in a Serbian church [22]. Furthermore, a community of *Aspergillus*, *Penicillium*, *Cladosporium*, and *Chaetomium* species was recorded from Medieval wall paintings in Styria (Austria), forming spots of different colors [39]. This group of genera

was dominant on two deteriorating frescos in St Clare's Refectory of the Monastery of St Damian in Assisi [46].

Hypocreales was the second most abundant order, accounting for 18% of total fungal diversity, within which *Acremonium*, *Trichoderma*, and *Fusarium* were among the most common genera. Hypocreales is one of the largest orders of filamentous ascomycetes and exhibits a broad range of ecologies, ranging from plant-associated nutritional modes to animal pathogens (e.g., insect pathogens) and mycoparasites [118]. *Neocosmospora solani*, recorded in Thailand, India, Japan, and France; *Simplicillium lamellicola*, recorded in Russia; and *Clonostachys rosea* [48,58], recorded in Japan, are examples of mycoparasitic species, while *Parengiodontium album* is an insect parasite and was recorded in several countries (Germany, Russia, Romania, Austria, Italy, and England) [109]. The recurrent presence of mites and insects pointed out their possible role in spreading fungi on painted surfaces [46,119]

Finally, the plant pathogen species *Fusarium oxysporum* has been shown to produce an extracellular pinkish pigment that disfigures and aesthetically damages colonized mural paintings and stone surfaces with permanent stains [78].

Phylum Basidiomycota was present with several occasional species, mostly represented by one or two records, and comprises litter, soil, and wood-saprotrophs, ectomycorrhizal, epiphyte, and plant-pathogen species. Their occurrence must be regarded as sporadic, potentially aided by root penetration. The possible role of roots as a carrier for rhizosphere microorganisms, like a dripping line for water condensation, and as an organic carbon source by root exudates has been hypothesized [57,120]. In any case, a Basidiomycete was also recorded at the entrance of Roman catacombs [121], possibly due to spores carried by water infiltrations and germinating using organic nutrients from the soil and/or the phototrophic biofilm.

Mucoromycota was present with few species and records, and black meristematic fungi were rarely recorded as well. These latter may grow on a wide range of substrates and are resistant to a variety of environmental stresses, as well as being widely distributed epi- and endolithically on monuments [122,123]. Although the biodiversity of black fungi on historical monuments is not fully elucidated, recent samplings indicate that they are also present on wall paintings and that their rare finding could be linked to the isolation protocols used, generally favoring fast-growing species [124]. Two new species of the genus *Neodevrisia* have been found in the restricted sampling area of the Vallerano cave and another, still undescribed, from Maijishan grottoes [45,99,108]. *Scolecobasidium lascauxensis* and *S. anomalus* were isolated and described from black stains in Lascaux Cave, France [85,115], while the chaetothyrialean black fungi *Cladophialophora*, *Exophiala*, and *Phialophora* have been reported from different sites [23,24,42,98].

Yeasts have been rarely reported, such as Saccharomycetales (Ascomycota) that usually grow by individual yeast cells or *Rhodotorula* spp. (Basidiomycota) often linked to pink/orange stains due to the release of carotenoids [19,93].

Among those more commonly reported, some species such as those belonging to the genera *Alternaria*, *Fusarium*, *Aspergillus*, *Penicillium*, and *Cladosporium* may be responsible for annoying allergic and toxic reactions suffered by conservationists and visitors [81,125,126]. *Alternaria alternata* is a very common fungal species, frequently recorded on frescoes. Its spores are recognized as common powerful aeroallergens, and indoor environments offer higher levels of exposure to this risk than open-air [59,127]. Records of *Fusarium* species have also been reported, such as *F. solani* in the Lascaux caves [97] and *F. oxysporum* in many sites. They are mainly plant pathogens, but they can also be the causal agents of human mycoses [97,128,129]. Some *Aspergillus* species are pathogenic to humans and animals and are responsible for clinical manifestations (https://www.aspergillus.org.uk/species-archive/, 29 December 2021). Among these, *A. fumigatus* is a human pathogenic fungus recorded on frescoes within different environments (3,11,22,29,42,49,70,71), causing infections in humans which can be fatal in immunocompromised patients (61). *Aspergillus flavus* has been frequently recorded in

monasteries, churches, temples, caves, and tombs, mostly due to their numerous aerosolized spores. It mainly grows in the soil, but it is also a facultative and opportunistic pathogen of both animals and plants, producing mycotoxins that are highly harmful to humans [130].

In light of the above, the identification of the species deteriorating wall painting is needed for the protection of restorers and visitors. However, the temperature values characterizing confined and semi-confined sites are generally too low for potential pathogenic fungi. In fact, truly thermophilic fungi which cannot grow at temperatures below 20 °C are not active in these environments, at least during winter in temperate regions.

4.2. Geographic Distribution

Considering the geographic distribution of the data, just one site among the studied paintings comes from the Americas (the Cathedral of Havana at Cuba) [82]. The highest number of records was from Europe, with 70 monuments, mostly representative of hypogean environments and of churches and historical buildings, with a considerable prevalence of Italian monuments (39). A total of 26 monuments were from Asia, while the 13 African ones were all from Egypt.

This distribution arises from the old tradition of people of the Euro-Mediterranean area of using such artistic expression, starting from the old prehistoric caves to the Etruscan and Greek-Roman traditions until the consolidated use both in the decoration of Christian churches and historical buildings [2]. In the case of the Egyptian area, the recorded taxa derived from the old tombs of the Pharaohs [54,72,73,77,78], and similarly in East Asia, the tradition is mainly found in kings' and Emperors' tombs [23–25,38]. Most of the ancient paintings in buildings do not exist anymore, due to frequent rebuilt or remake of the materials [131].

Our results suggest that the monuments studied were often confined to restricted geographic areas. In any case a wider geographic distribution than that recorded may be possible, as a number of sites may have escaped the search. In fact, even if formally available on the web and on the major repositories, because of the language barriers, some studies could not be taken into account.

4.3. Isolation and Identification Methods

Culture-based methods favor the growth of microorganisms best fitting with the laboratory conditions used (namely, culture media, temperatures, and incubation times). In this study, we found that the most frequent experimental settings were favorable to fast-growing, highly-sporulating fungi, with the use of culture media rich in easily accessible carbon sources, alongside short incubation times and optimal growth temperatures favoring their sporulation. Otherwise, lower growth temperatures (≤20 °C), wide temperature ranges, different isolation media, and a longer incubation time could enlarge the detectable culturable fraction.

Since the early 2000s, molecular phylogenetic methods have highlighted the limitations of morphological identification, allowing us to gain a better understanding of the kingdom of Fungi [132]. Nowadays, the identification by barcode regions sequencing is a common practice. Even if the nuclear ITS region has been recognized as a fungal barcode, its discriminating power changes within the taxonomical groups, and other/more barcode regions are often necessary to have a reliable identification [133]. This is the case of the identification of species within large groups, as *Fusarium*, *Penicillium*, *Aspergillus*, and *Cladosporium* genera, where cryptic species can be detected only by sequencing multiple molecular markers [134].

In detail, *Fusarium* species determination has been best made with the combined phylogeny of protein coding genes such as elongation factor (TEF1), RNA polymerase (RPB2) and the partial β-tubulin (BT2) gene [134]. To discriminate between *Penicillium* and *Aspergillus* species, β-tubulin (BT2) and calmodulin (cmdA) genes have been proposed as

secondary barcodes, respectively [135,136]. While the most phylogenetic informative markers for *Cladosporium* were TEF1 and actin gene (actA), ITS sequences being identical for species of the same complex [137,138].

The correct identification of strains is required in order to provide restorers more information about strains' ecology and degradative potential. In this light, standardized identification protocols should be implemented.

High throughput sequencing methods have recently been applied to cultural heritage purposes. These methods represent a powerful tool to define the whole fungal diversity present but not necessarily to deepen the mechanisms and the main actors of the deterioration phenomena [139]. The combination of culture-based and molecular methods should be used for a better understanding of deterioration processes. Indeed, pure cultured microorganisms represent the key to uncover settlers' physiological and ecological traits, as well as representing a resource for many in silico applications and barcoded identifications [123,124].

4.4. Distribution of Taxa in the Different Environments

The most prevalent sites were confined non-hypogean environments, which are characterized by varied thermo-hygrometric temperatures and air movement. Hypogean (both confined and non-confined), where nutrients and humidity can favor fungal growth were represented as well.

Temperature and relative humidity are among the environmental parameters most important to microbial colonization capability, and in the case of heterotrophs, a certain amount of nutrients is also needed [4,140]. It is well known that fungi rapidly grow when relative humidity is higher than 65% and when a certain quantity of nutrients is available. The low values of temperatures, even if are not favorable for microbial growth by themselves, have a positive effect in contributing to increase in humidity, favoring water condensation on surfaces. Walls, especially in hypogean environments, generally provide these requirements [1]. Temperatures in confined environments are generally more stable than in non-confined environments, where daily and seasonal changes may occur, with ranges that have effects on microbial settlement. Elevated moisture values and stable temperatures have been reported as ideally suited to promote microbial growth on surfaces in catacombs sites [7,94,141]. Indeed, the highest risk occurs when high humidity is coupled with high temperature values, and negative effects of rising temperatures arise only if their highest values can strongly influence the humidity values [142]. In the case of hypogea, the underground conditions favor the maintaining of humidity.

Air movement differences between confined, semi-confined, and non-confined environments were expected to alter the number and type of fungal species recorded as well as incoming nutrients from the outside environment. A great proportion of entries in the database belonged to soil and litter dwellers such as saprotrophs, producing numerous spores that are well adapted to air-borne dispersal, and therefore, air ventilation may have a significant impact on the risk of contamination [143]. The more limited air volume movement of confined mural paintings compared to semi-confined ones was suggested to decrease the number of air-borne dust particles, with biofilm communities relying more on internal interactions between different microorganisms than on the external organic inputs [17]. Among the first aerobiological studies, Savulescu and Ionita reported a greater number of isolates inside the studied monasteries than outside of them, probably due to a more favorable microclimate inside the church, which favors the development of microorganisms [55]. Pangallo and colleagues proposed for the first time a comparative analysis of the microbial component of paintings and the surrounding air to gather information on the origin of fungal contamination [70]. Aside from the importance of aerobiological studies for the conservation and prevention of microbial attacks on indoor painted surfaces [144], these studies have received little attention. In light of the large number of fungal species potentially harmful for restorers and visitors, constant monitoring of air spore quality and concentration, as well as the use

of air filters to reduce fungal spores concentrations, would be required for site conservation [62,101,144,145].

Significant correlations between the different taxa and the various environmental categories have not been recorded. Indeed, such data is not the result of the absence of a correlation between fungal growths and environmental conditions but can be consequence of several other influencing conditions that hide it. In fact, many are the ecological requirements that shape the ecological niches of the different species (i.e., the limiting factors), but the most conditioning factors are those that result in a quantity proximal to the upper or lower tolerance limit of an organism [146]. Then, for the various sites examined, some factors may become more relevant if their values are closer to the tolerance limit of certain organism, but this does not mean that other parameters do not play a role [147].

Indeed, our results may be influenced by the wide number of taxa in the wide geographic distribution of sites and by the different methodologies used to characterize the fungal diversity. In fact, different sampling techniques and isolation conditions were used within the studies we analyzed. Other factors that allow fungi to thrive and/or survive in a variety of conditions are their wide nutritional versatility and range of adaptations. The presence of numerous genera that are widespread and highly sporulating and hence present in all the environmental categories must also be considered. The absence of evident correlations could have been determined by the absence of distinct boundaries between the categories identified, with overlapping microclimatic conditions which could have resulted in overlaps within their respective microbial communities. Finally, the heterogeneity of the data, with taxa identified at the genus or species level, may have resulted in dispersed clusters and hampered the ability to demonstrate any relationship.

This result seems to be in line with other studies. The influence of environmental factors such as temperature, relative humidity, and the opening or closure of the temples was not evident for fungal growths on wall paintings of 12 archaeological sites in the central and western parts of Thailand [76]. Furthermore, a stronger relationship with the age of five caves in China than with the environmental conditions, such as temperature and relative humidity, was proposed to explain the observed differences in fungal communities [64]. In two distinct mural paintings, instead, the differences recorded in the microbial communities were associated to the different organic input origin (i.e., wine cellar evaporation, and insect exuvia/excrements) and the microclimatic conditions. The more humid conditions favored the growth of actinomycetes, bacteria, and dark-pigmented fungi, while the other showed a biofilm, mainly dominated by xerotolerant and patchy growing sporulating fungi [17]. Differences in fungal communities were also recorded on mural paintings of two subterranean ancient Chinese tombs dating back over 1700 years, mostly due to variations in interior temperature and relative humidity as well as to their history and drawing techniques used [51].

Other significant concerns could be related to the identification of the isolated species, which was initially based solely on morphological observation. Indeed, phylogenetic molecular approaches are nowadays routinely applied, providing a universal tool for accurately identifying fungal species.

New methodologies such as omics techniques are now available; however, they rarely provide information at species or genus level, and there is no guarantee that the recorded taxa are actively growing. Moreover, culture-dependent approaches may not provide a real picture of the microbial diversity actively growing at the sampling time. This is because not all fungi actively growing on the deteriorated substrates can grow under laboratory conditions, and fungi growing under laboratory conditions may not actively grow on the sampled surfaces. Therefore, a combination of culture-based and molecular approaches may be needed to gain a clear picture of the actual biodiversity present on the painted surfaces as well as to have strains to investigate their potential degradative roles.

5. Conclusions

This study contributes to illustrate the high fungal diversity on wall paintings and raises awareness about the fungal threat on the deterioration of such artworks. Ascomycota was the most common phylum, with Eurotiales and Hypocreales as the most common orders. Statistical analyses did not enhance core communities that can be considered characteristics of different environmental categories of sites hosting wall paintings. Our results were likely due to the heterogeneity and fragmentation of the data in the databases, the dispersed geographical area considered, and the complexity of factors which can condition the biological growths. It is therefore crucial to cover the knowledge gaps through (i) international collaborations, (ii) enlarging the isolation and cultivation protocols as to easily detect also strains different from fast growing ones, and (iii) standardizing the identification protocols. Standardizing and improving the site descriptions (e.g., repeated microclimatic data) could allow for possible relations between site and their settlers and for further comparisons among different environmental conditions.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/article/10.3390/app12062988/s1, Figure S1: Cluster analysis on the Jaccard distance of the distribution of the different entries retrieved from the papers analyzed; Table S1: List of all the references associated to each site defined in the analyses.

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