Functional properties of extended body representations in the context of kinesthesia

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Parengyodontium album, a frequently reported fungal species in the cultural heritage environment

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Abstract

Fungi are one of the main agents responsible for the biodeterioration of cultural heritage through physical and chemical processes. The frequent isolation of certain fungal species from deteriorated materials indicates that these species appear to have a particular affinity for environmental conditions occurring in cultural heritage. It is important to identify the conditions that favour the development of such species in order to understand how to preserve monuments and collections. Among these fungal species, the involvement of *Parengyodontium album* (Limber) Tsang et al. seems to have been underestimated until now. This species is abundant in many environments and its taxonomic position has changed quite frequently, which may have concealed the effect of this fungus on cultural heritage sites. This review seeks to compile the involvement of *P. album* in cultural heritage biodeterioration phenomena under all the names that it has been attributed in order to effectively determine its occurrence. This paper also aims to determine whether *P. album* is marginal or dominant when detected on materials. Finally, the conditions that favour the development of *P. album* on cultural heritage objects and sites are discussed.

Keywords

Parengyodontium album, Cultural heritage, Halotolerant species, Mesophilic species, Enzymes producer, Entomogenous species

1. Introduction

The risk encountered by cultural heritage when colonized by fungal strains is widely known and recognized. Fungi can cause serious damage not only to buildings but also to their contents in the case of museums or libraries, whatever the nature of the material considered (Sterflinger, 2010; Sterflinger and Piñar, 2013). First, fungi can cause aesthetic damage through staining or pigment discoloration (Ciferri, 1999; Krumbein, 2002), as observed when black fungi grow on historical stones (Cappitelli et al., 2007; Isola et al., 2015), or in the phenomenon known as “foxing” that occurs on historical papers (Sterflinger and Piñar, 2013). This initial aesthetic damage can be followed by more serious damage to the support (Ciferri, 1999). Indeed, fungal growth can also lead to physical and chemical damage (Scheerer et al., 2009; Warscheid and Braams, 2000). Physical damage can occur when fungal hyphae enter the support through stone pores or through decayed stone parts and then by tunnelling into otherwise intact mineral material (Gadd, 2007). In painted surfaces, the growth of fungi through cracks and its migration below the paint layer can cause detachments (Sterflinger, 2010). Finally, the secretion of organic acids by fungi lead to chemical damage to the support, meaning that fungi are able to solubilize minerals (Burford et al., 2003; Gadd, 2007; Warscheid and Braams, 2000).

Among the fungi observed in cultural heritage, we were surprised and above all intrigued by the frequent observation of the fungal species *Parengyodontium album* (Limber) Tsang et al. in several published and unpublished studies (Leplat et al., 2017; Leplat et al., 2019). The articles cited in this introduction provide a wide review of fungal biodeterioration on monuments as well as in museums, yet only Gadd (2007) mentions *P. album* as a fungus that is frequently observed on stone. This review therefore aims to determine the true occurrence of *P. album* in cultural heritage and identify the factors that lead to this occurrence in cultural sites.

2. Brief history of *Parengyodontium album* taxonomy

Like many other fungal species, *Parengyondontium album* has had several successive names and changed fungal genus several times before being referred to by its current name. This species was first described as *Tritirachium album* (Limber, 1940). The new genus *Tritirachium* was created to describe fungal species bearing verticillately branched conidiophores like *Verticillium*, but which differ insofar that they have a final zigzag conidia-bearing portion (Fig. 1). The genus included three species including *Tritirachium album,* which had been separated from a colony of *Penicillium* found on a book in a marine biological laboratory and had been considered as a possible contaminant.

The species was then renamed *Beauveria alba* and moved into the already existing *Beauveria* genus, which is also characterized by zigzag conidia-bearing portions because of the denticulation of its rachis and the absence of pigment in aerial mycelium (Saccas, 1948; Fig. 2), which characterizes *Tritirachium* species.

De Hoog (1978) created the genus *Engyodontium* and incorporated *Beauveria alba* under the name *Engyodontium album* (or *Engyodontium albus*). This change made the genus *Beauveria* more homogeneous by limiting it to species with dense clusters of short branches and fertile cells on undifferentiated hyphae, whereas *Beauveria alba* did not have a dense subverticillate to verticillate branching pattern.

Finally, Tsang et al. (2016) found that *Engyodontium album* was phylogenetically and chemotaxonomically different to the three other species making up the genus *Engyodontium* and created the new genus *Parengyodontium* for the sole species *Parengyodontium album*. It was the first time that the species was renamed for other reasons than morphological features.

The study of this new genus also revealed that the species *Parengyondontium album* probably includes several subclades. This article will exclusively use the name *Parengyodontium album* to describe this fungus.

3. Occurrence of *Parengyodontium album* in cultural heritage

3.1 *Parengyodontium album*: a worldwide distributed species

Whatever the name used to describe it, *Parengyodontium album* has frequently been reported in historical buildings, museums, libraries and touristic sites. Its detection around the world shows that it is a ubiquitous fungal species (Fig. 3). It has been found on a Chilean world heritage site in southern America (Ortiz et al., 2014), in a Cuban museum in central America (Borrego and Molina, 2019), and in a show cave in north America (Vaughan et al., 2011). The fungus has also been isolated across Europe from Portugal to Russia, with observations of its presence in England, Spain, France, Germany, Italy and Poland (Jeffries, 1986; Liñán et al., 2018; Nugari et al., 2009; Pfendler et al., 2018; Piotrowska et al., 2014; Ponizovskaya et al., 2019; Schabereiter-Gurtner et al., 2001; Trovão et al., 2019). Finally, *P. album* has also been detected in Africa and in Asia, but not in a cultural heritage-related environment (Jaouani et al., 2014; Zhang et al., 2013).

3.2. *Parengyodontium album*: a species which develops on a great diversity of cultural sites and materials

The most commonly reported occurrences of *Parengyodontium album* are in religious monuments such as churches, cathedrals or monasteries (Table 1). A number of different materials are affected by the presence of the fungus in these monuments. The first of these materials is wall paintings: the fungus has been isolated on wall paintings located in the “aerial parts” of the monuments (Jeffries, 1986; Sáiz-Jiménez and Samson, 1981; Sampo and Mosca, 1989) and also in the underground parts of the monuments such as the crypts (Gorbushina and Petersen, 2000; Leplat et al., 2017; Fig. 4). The stone structure of buildings is also affected, as shown by the isolation of the fungus from the bare stone inside (Karpovich-Tate and Rebrikova, 1991; Pfendler et al., 2018; Pinheiro et al., 2018) and outside the monument (Saiz-Jimenez, 1993; Trovão et al., 2019). Historical glasswork is also affected, as shown by the isolation of *P. album* from the windows of Stockkämpfen church (Schabereiter-Gurtner et al., 2001).

Caves are the second kind of cultural sites in which *P. album* have frequently been reported. The term “cave” covers a wide variety of different geological cases that vary from large caves that are several hundred meters deep to small sites that are around ten-meters deep or even simple rock shelters. The caves in which *P. album* has been isolated represent different aspects of cultural interest, ranging from caves known for their Paleolithic ornamentation, such as Lascaux cave in France (Bastian et al., 2010) to more recent Byzantine-style ornamentation, such as the crypt of the Original Sin in Italy (Nugari et al., 2009) and show caves visited for their geological interest (Vaughan et al., 2011). Like in religious monuments, *P. album* was isolated in caves from wall paintings (Nugari et al., 2009), or directly from the bare rock (Bastian et al., 2010; Lepinay et al., 2017; Mihajlovski et al., 2019; Nováková, 2009; Vaughan et al., 2011). The novel difference observed in comparison with religious sites is that *P. album* has been abundantly detected not only on surfaces but also in the atmosphere of the caves (Leplat et al., 2019; Liñán et al., 2018; Marvanová et al., 1992; Nováková, 2009). Finally, *P. album* was reported on a historical wood staircase located in a mine that dates back to the bronze and iron age in Austria (Piñar et al., 2016), an environment that is comparable to that of caves. After wall paintings, stone, and glasswork, wood is therefore another kind of support that can be colonized by *P. album*.

The third group of cultural sites in which the presence of *P. album* has been reported is composed of all types of sepulchral site, *i.e.* from hypogean tombs dating from Etruscan and Roman times (Saarela et al., 2004; Sprocati et al., 2008) to the tombstones of an historical Jewish cemetery (Krumbein et al., 1996). In environmental terms, hypogean tombs are cut in the rock and are thus are similar to caves, while tombstones show similar features to the external face of religious monuments, both being made of stone that is typically exposed to an urban environment. The phenomenon observed in caves has also been reported in the Christian catacomb of St. Domitillus in Rome, where *P. album* isolated from the air as well as from the wall paintings on marble, limestone and plaster supports (Saarela et al., 2004).

More recent historical buildings erected during the 20th century are also affected by the presence of the fungus, which has been found in the barracks of the Auschwitz-Birkenau camp in Poland and in buildings at the Humberstone and Santa Laura saltpeter works in Chile (Koziróg et al., 2014; Ortiz et al., 2014; Piotrowska et al., 2014). In Auschwitz, *P. album* was isolated from mineral components such as plaster and brick and also from wooden parts. Similarly, *P. album* was isolated from wood in a building at Santa Laura saltpeter works; this is surprising given the location of the buildings in the Atacama Desert, an extreme environment characterized by dry conditions.

Finally, museums and libraries are also affected by the presence of the fungus, as shown by the isolation of *P. album* inside several of these buildings (Borrego and Molina, 2019; Micheluz et al., 2017; Ponizovskaya et al., 2019; Principi et al., 2011; Trovão et al., 2013). In some cases, the fungus has been detected on the internal stone or plaster walls of the museums (Ponizovskaya et al., 2019), while it has been detected directly on artworks in other cases (Borrego and Molina, 2019). These include samples of remarkable artworks such as Leonardo da Vinci's Atlantic Codex (Principi et al., 2011), indicating that *P. album* can also be detected on paper. The fungus was indirectly detected in the Archives of the University of Coimbra, where it was isolated from an Araneae during a survey about arthropod-disseminated fungi (Trovão et al., 2013), and was also detected in an airborne form in the Library of Humanities of the Ca' Foscari University of Venice (Micheluz et al., 2017).

To resume, *Parengyodontium album* has been detected on a wide variety of cultural heritage supports ranging from wall paintings, stone, plaster and brick to glass, wood and paper, and is even observed in an airborne form.

3.3 *Parengyodontium album*: associated symptoms and relative abundance on cultural heritage

Parengyodontium album has been associated with several different symptoms of deterioration in cultural heritage, without necessarily being considered to play a determining role in their development. Amongst these symptoms, the presence of soluble salt efflorescence has been the most frequently cited in relation to the fungus (Ponizovskaya et al., 2019; Trovão et al., 2019). The most commonly observed is gypsum, which can occur alone or be associated with organic compounds. However, *P. album* has also been isolated from calcium salts of oxalate, carbonate and chloride (Lepinay et al., 2017; Mihajlovski et al., 2019; Saiz-Jimenez, 1993; Sáiz-Jiménez and Samson, 1981). The second kind of symptoms associated with *P. album* is discoloration and patina (modification of the support colour) of various colors, including pink patina (Berner et al., 1997; Nugari et al., 2009), ochre discoloration (Berner et al., 1997), dark stains or black spots (Bastian et al., 2009; Sáiz-Jiménez and Samson, 1981) and green-black patina (Principi et al., 2011; Trovão et al., 2019). The fungus has also been associated with white covering that is visibly of fungal origin (Bastian et al., 2009; Leplat et al., 2017).

Finally, *P. album* has been isolated from powdered historical stones (Karpovich-Tate and Rebrikova, 1991).

The abundance of *P. album* when detected on cultural heritage varied as much as the symptoms encountered or the materials affected. In some cases, *P. album* was the most abundant species isolated from deterioration symptoms, while in other cases only traces of the fungus were detected. In other studies, *P. album* was detected at varying levels of abundance along with other species. For example, *P. album* has often been the most abundant detected species when salt efflorescence (a coating caused by the migration of soluble salt contained in a porous material to the surface) was present (Ponizovskaya et al., 2019; Trovão et al., 2019), but in other cases this species was noted simply as a relatively abundant secondary species (Lepinay et al., 2017; Mihajlovski et al., 2019; Sáiz-Jiménez and Samson, 1981). Nugari et al. (2009) failed to isolate the fungus on culture medium during their study of the pink patina on the Crypt of the Original Sin and finally identified it through SEM, yet it was the most abundant fungal species isolated by Berner et al. (1997) from a pink patina sample of the chapel of Herberstein Castle. This result does not suffice to confirm a role of *P. album* in the development of the pink patina, as the fungus was also abundantly isolated from ochre discoloration in the same study. However, these elements can indicate that this environment is globally favourable to the growth of *P. album*. The fungus has been abundantly isolated from the air of monuments when it was tested, and this was especially true of hypogean environments: *P. album* was one of the most abundant airborne fungi isolated in Roman catacombs of St. Domitilla and in the Nerja cave (Liñán et al., 2018; Saarela et al., 2004), while it was only a secondary species in an air sample taken from a library in Venice (Micheluz et al., 2017).

4. Particularities of cultural heritage environments that favour the development of *Parengyodontium album*

The microbial colonization of cultural heritage depends on environmental factors such as water availability, pH, climatic exposure, nutrient sources and the capacity of microorganisms to withstand possible sources of stress such as desiccation or UV radiation (Scheerer et al., 2009; Warscheid and Braams, 2000). *Parengyodontium album* is a chemoorganotrophic organism, and its development is dependent on its capacity to obtain organic compounds from an environment which is sometimes poor in nutrients, such as stone (Gadd, 2007; Sterflinger, 2010). The central necessity is therefore to identify the common factors in the identified cultural heritage sites that can lead to the development of *P. album*.

4.1 *Parengyodontium album*: a species adapted to salty, moist environments

One of the principle factors in the growth of *P. album* seems to be the presence of salts on the supports it colonizes. Although the preceding paragraph notes that the fungus has been abundantly isolated directly from salt efflorescences, salts were also often present but not necessarily visible in other sites where the fungus grew. For example, the phenomenon of pink patina on historical stones and wall painting has been well documented to date. *Parengyodontium album* is not the main agent of these discolorations, which are often related to bacterial biofilms (Piñar et al., 2014). Sites affected by pink patina all have problems of water infiltration and salt crystallization, two phenomena that often occur simultaneously. Problems of moisture and of water infiltration have also been widely observed on the walls of monuments from which *P. album* has been isolated (Berner et al., 1997; Karpovich-Tate and

Rebrikova, 1991; Leplat et al., 2017; Pfendler et al., 2018; Simonovicova et al., 2003). Moreover, airborne *P. album* has been repeatedly reported in caves, an environment known for relative humidity values that can sometimes approach 100 % (Liñán et al., 2018; Nováková, 2009). This affinity of *P. album* for humid, salty environments is not a surprise since the fungus has often been isolated from the marine environment, including marine sediments (Chellappan et al., 2006; Kirichuk et al., 2012; Yao et al., 2014; Zhang et al., 2013), marine sponges (Wu et al., 2016), or directly from sea water (Pindi, 2012). *P. album* is therefore regarded as a halotolerant fungus that can grow on synthetic media supplemented with NaCl 20 % (Ali et al., 2013; Jaouani et al., 2014).

4.2 *Parengyodontium album*: a mesophilic species

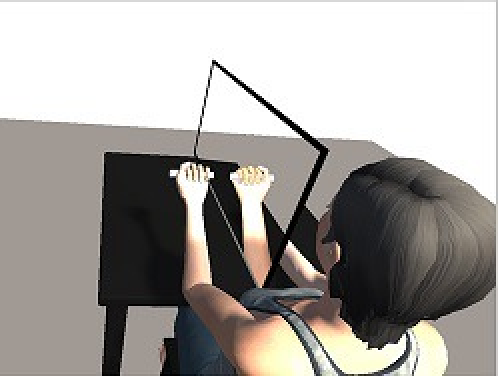
Little data is available concerning the effect of temperature on the growth of *Parengyodontium album*. The fungus is generally considered mesophilic (Airaudi and Marchisio, 1996; Xia et al., 2018). Although *P. album* is capable of surviving at sub-zero temperatures, as proved by multiple observations of this species in Arctic and Antarctic environments, it is unable to grow at 0 °C (Bergero et al., 1999; Caretta et al., 1994; Zucconi et al., 2012). The lowest temperatures at which this fungus can grow seem to be around 4 or 5 °C, temperatures at which *P. album* developed in some cases but not in others (Fernandes et al., 2008; Jaouani et al., 2014). The *P. album* samples that had not grown at low temperatures, germinated when temperatures were increased to 25 °C, suggesting that the fungus was inhibited at low temperatures without being killed. *Parengyodontium album* is generally not capable of growing at temperatures above 31 °C. The only exception is the strains belonging to subclade 2 of the three subclades defined by Tsang et al. (2016), which can grow at 35 °C. This temperature range for growth ranging from approximately 5 to 30 °C and the capacity of the fugus to survive at low temperatures make *P. album* a species that is well adapted to the environmental conditions of cultural heritage monuments. Notably, *P. album* survives on monuments subjected to cold winters, then grows when weather becomes milder (Piotrowska et al., 2014), or grows in sites such as caves that remain relatively cool throughout the year (Nováková, 2009).

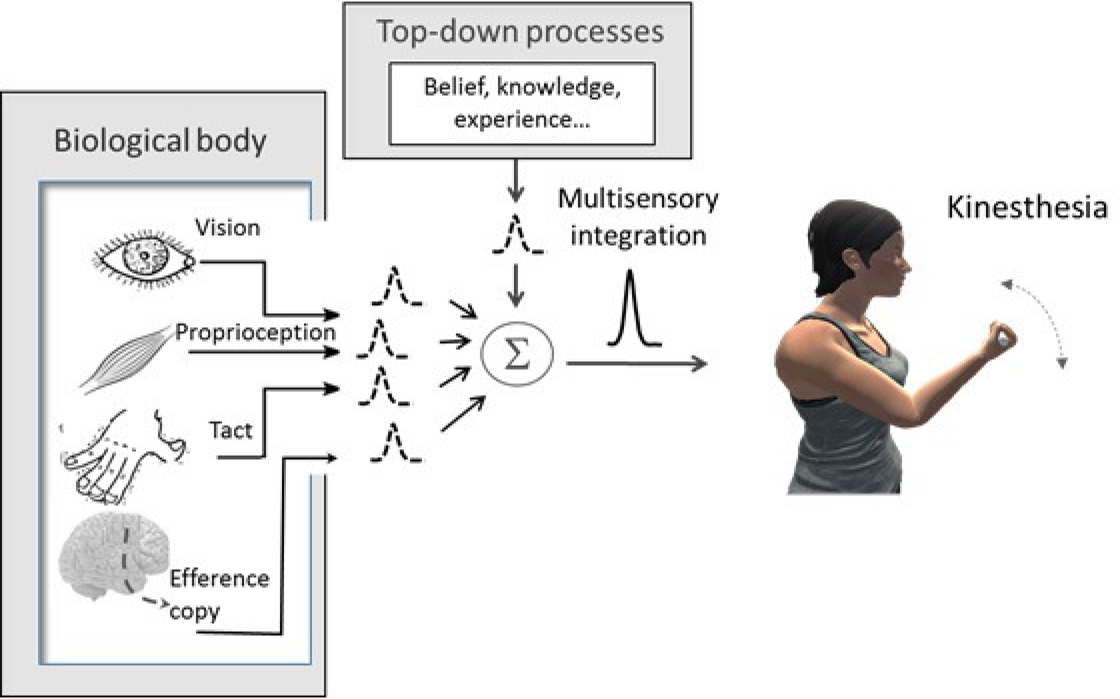
4.3 *Parengyodontium album*: a species capable of feeding on various nutrient sources

As chemoorganotrophic organisms, *P. album* cannot develop without the presence of organic matter as a nutrient in the environment. For example, it has been proposed that *P. album* can feed on the organic pollutants in black crusts (Sáiz-Jiménez, 1997). Phototrophic organisms such as algae, cyanobacteria or lichens are generally considered as the primary colonizers in environments that are poor in nutrients, such as stone (Dakal and Cameotra, 2012; Scheerer et al., 2009; Warscheid and Braams, 2000). The accumulation of the photosynthetic biomass created by the development of theses primary colonizers provides a suitable environment for the growth of heterotrophic organisms. *Parengyodontium album* appears to be one such recipient of this microbial succession (Karpovich-Tate and Rebrikova, 1991), and is able to recolonize supports on remaining dead biofilms following UVc treatments (Pfendler et al., 2018).

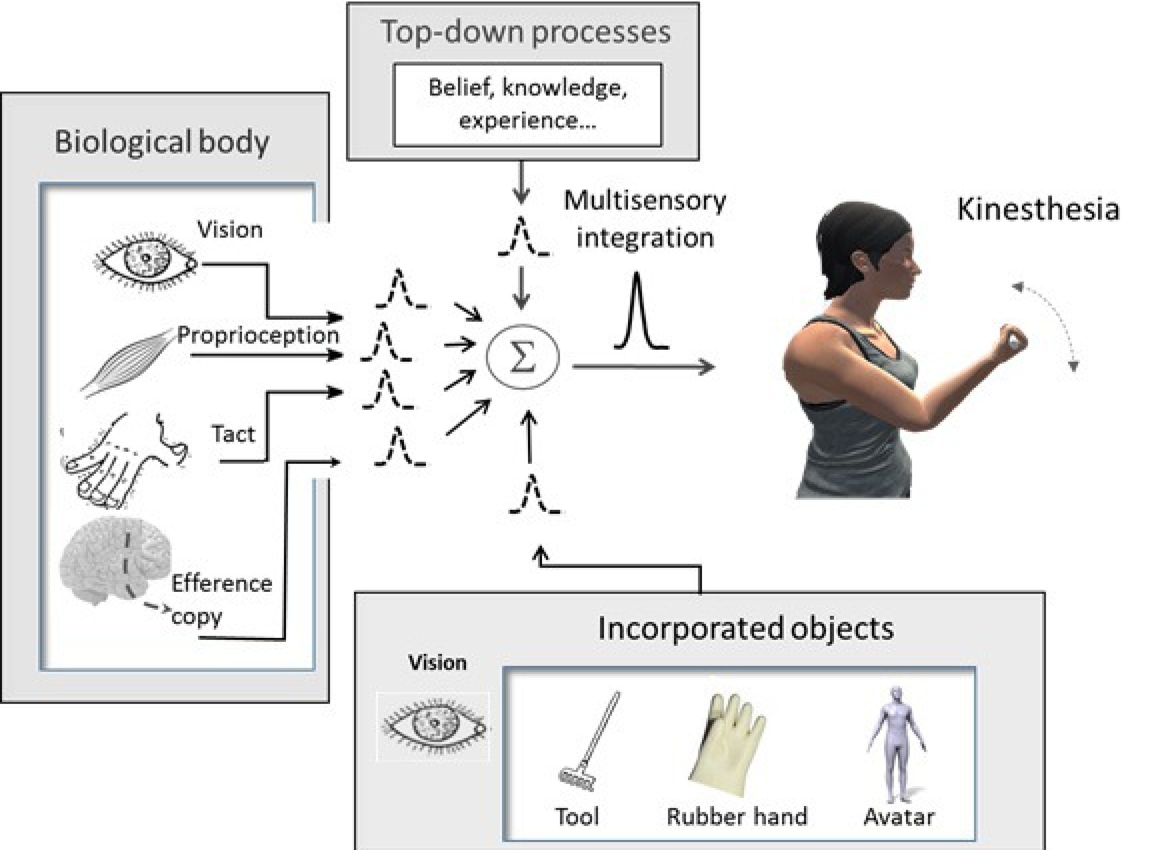
Parengyodontium album has also been shown capable of feeding on products used in restoration of wall paintings. Cappitelli et al. (2004) noted that the fungus could develop on several synthetic resins, and Sampo and Mosca (1989) isolated a fungal species that probably belonged to *P. album* and was capable of growing on calcium caseinate and liquifying animal glue. Indeed, it is well known that *P. album* is an enzyme producer. It is used for the industrial production of proteinase K, which acts as serine endopeptidase and induces a keratin hydrolysis activity (Ebeling et al., 1974; Jany et al., 1986). The use of proteinase K produced by *P. album* to facilitate the hydrolysis of animal glue on paper artwork during a restoration process is somewhat ironic given the otherwise damaging effect of this fungus in the cultural heritage domain (Micheli et al., 2018). This keratinolytic activity and the capacity of the fungus to produce cytotoxic polyketides led to the recognition of *P. album* as an emerging opportunistic pathogen (Tsang et al., 2016). The keratinolytic activity could also become an issue for the conservation of antique textiles (Błyskal, 2009). Besides the production of protease, *P. album* has also been recognized as a producer of α-amylase, esterase, phosphatase lipase and chitinase (Ali et al., 2014; Jaouani et al., 2014; Karpovich-Tate and Rebrikova, 1991). A number of studies have shown these enzymes to be effective under high salinity conditions (Ali et al., 2014; Jaouani et al., 2014), which could explain the favourable development of *P. album* on monuments affected by salt crystallization.

The production of chitinase by *P. album* leads us to consider the entomogenous character of this fungus. The exoskeleton of arthropods is mainly composed of chitin (Merzendorfer, 2006). The role of arthropods in the dissemination of fungal species is commonly recognized, especially in environments where insects are numerous such as caves, catacombs and mural paintings (Bastian et al., 2009; Jurado et al., 2008; Trovão et al., 2013). Dead insects also provide a source of nutrients for entomogenous fungi in these nutrient-poor environments. It is well known that some of the successive fungal genera in which *P. album* has been classified contain entomogenous species, *i.e. Beauveria* and *Engyodontium* (Samson et al., 2013). Indeed, *Beauveria bassiana* and *B. brongnartii* are used as commercial biological control agents against various pests (Butt and Copping, 2000), and *Engyodontium parvisporum* and *E. geniculatum* have been isolated from arthropods (Gams et al., 1984). Several authors have linked the development of *P. album* on cultural heritage monuments to the presence of insects (Gorbushina and Petersen, 2000; Jurado et al., 2008; Liñán et al., 2018; Nugari et al., 2009; Principi et al., 2011). Although the association between *P. album* and arthropods has often been suggested rather than proved, the fungus has been isolated from spiders in at least three





Top-down processes
Belief, knowledge, experience...
Biological body
Vision
Proprioception
Tact
Efference copy
Multisensory integration
Σ
Kinesthesia



Top-down processes
Belief, knowledge, experience...
Biological body
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Σ
Kinesthesia
Incorporated objects
Vision
Tool
Rubber hand
Avatar