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ORIGINAL ARTICLE

Fungal heat shock proteins: molecular phylogenetic insights into the host takeover

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Abstract

Heat shock proteins are constitutively expressed chaperones induced by cellular stress, such as changes in temperature, pH, and osmolarity. These proteins, present in all organisms, are highly conserved and are recruited for the assembly of protein complexes, transport, and compartmentalization of molecules. In fungi, these proteins are related to their adaptation to the environment, their evolutionary success in acquiring new hosts, and regulation of virulence and resistance factors. These characteristics are interesting for assessment of the host adaptability and ecological transitions, given the emergence of infections by these microorganisms. Based on phylogenetic inferences, we compared the sequences of HSP9, HSP12, HSP30, HSP40, HSP70, HSP90, and HSP110 to elucidate the evolutionary relationships of different fungal organisms to suggest evolutionary patterns employing the maximum likelihood method. By the different reconstructions, our inference supports the hypothesis that these classes of proteins are associated with pathogenic gains against endothermic hosts, as well as adaptations for phytopathogenic fungi.

Keywords Heat shock proteins · Fungi · Evolutionary adaptation · Molecular phylogeny

Abbreviations

HSP	Heat shock protein
HSF	Heat shock factors
NBD	Nucleotide-biding domain
SBD	Substrate-binding domain
sHSPs	Small HSPs
cAMP	Cyclic AMP

Introduction

Fungi are crucial ecosystem components and play essential roles in environmental processes, such as biotransformation and maintaining nutrient and CO_2 cycling (Case et al. 2022) They have been shown to inhabit diverse niches, having direct or indirect involvement in the health disease processes of all organisms, acting as both pathogen and protector during infections (Zhang et al. 2020). However, this conquest scenario is commonly overlooked.

Since the appearance of the ancestors of these organisms about 1200 million years ago in aquatic bodies, there has been an improvement in characteristics that helped the predatory, parasitoid, symbiont, and commensal lifestyles of fungi. Their terrestrialization boosted the development of morphological structures that allowed their dispersion over land in search of substrates and a protein arsenal that allowed them to obtain nutrients (Nagy et al. 2017). The successful mastery of these environments was essential for their propagation, as the selective pressure of the coevolution of plants, invertebrates, and vertebrates boosted their ability to infect different organisms, mainly by the clades Glomeromycota, Mucoromycota, and Dikarya (Naranjo-Ortiz and Gabaldón 2019).

Millions of years later, the fungi, adapted to sauropodomorph, arthropod, and plant parasitism Woodruff et al. 2022), underwent a severe adaptive process. With the cataclysm of the Cretaceous-Paleogene, which caused the extinction of the extensive megafauna of

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the period, there was a global modification of the post-calamity environment, with a decrease in temperature and luminosity, and an increase in humidity and decomposing substrates, allowing greater development of the funga (Vajda and McLoughlin 2004). However, these organisms, which were associated with ectomorphic vertebrates, would face the challenge of transitioning to the animals that would come to rise and dominate the new environment: mammals. The endothermy and immunity of mammals, which allowed the survival of animals in the face of environmental adversities, also became a barrier to the development of pathogens of the time. This characteristic boosted the development of mechanisms already present in fungi, such as the production of toxins, immune system evasion, and mycelium adaptation to survive in these hosts. This proposal is known as the "fungal infection-mammalian selection" (FIMS) hypothesis (Casadevall 2005; Casadevall and Damman 2020).

Heat shock proteins (HSPs) stand out among the mechanisms that helped adapt fungi to new hosts, responding to

Table 1 Heat shock protein (HSP) function in fungal organisms

Protein	Function	Organisms	References
HSP9/HSP12			
	Entry into stationary phase	*Schizossacaromyces pombe*	Fu et al. (2012)
	Glucose repression	*Cryptococcus gatti*	Jang et al. (1996)
	Temperature/osmolarity stress response	*Ustilago maydis*	Mitra et al. (2023)
	Cell adhesion	*Candida albicans*	Motta et al. (2023)
	Germ tube formation		
	Virulence induction		
	Vesicle stability		
HSP30			
	Entry into stationary phase	*Neurospora crassa*	Feder and Hofmann (1999)
	Temperature/osmolarity stress response	*Saccharomyces cerevisiae*	López-Matas et al. (2004)
	Heme-oxygenase	*Paracoccidioides* spp.	Vanittanakom et al. (2009)
	Virulence induction Chaperone activity	*Penicillium marneffei*	De Souza et al. (2021)
HSP40			
	HSP70 cochaperone	*Verticillium dahliae*	Qian et al. (2001)
	Temperature/osmolarity	*Apiotrichum curvatum*	Zhou et al. (2023)
	Stress response	*Aspergillus niger*	
		Sporothrix brasiliensis	
		Candida albicans	
HSP70			
	Temperature/osmolarity stress response	*Histoplasma capsulatum*	Caruso et al. (1987)
	Protein folding	*Candida albicans*	Eroles et al. (1997)
	Morphological transition Antifungal resistance	*Cryptococcus neoformans*	Jung et al. (2013)
		Aspergillus fumigatus	Jia et al. (2020)
HSP90			
	Chaperone	*Cryptococcus neoformans*	Bui et al. (2016)
	Temperature/osmolarity stress response	*Aspergillus fumigatus*	De Aguiar Cordeiro et al. (2016)
	Antifungal resistance	*Fusarium graminearum*	Neves-da-Rocha et al. (2019)
	Virulence induction	*Trichphyton rubrum*	Rocha et al. (2020)
	Cell wall integrity		
	Conidiation		
	Cellular proteostasis		
HSP110			
	Cellular proteostasis	*Candida albicans*	Dragovic et al. (2006)
	HSP70 cochaperone	*Saccharomyces cerevisiae*	Raviol et al. (2006)
	Protein folding		Li et al. (2021)

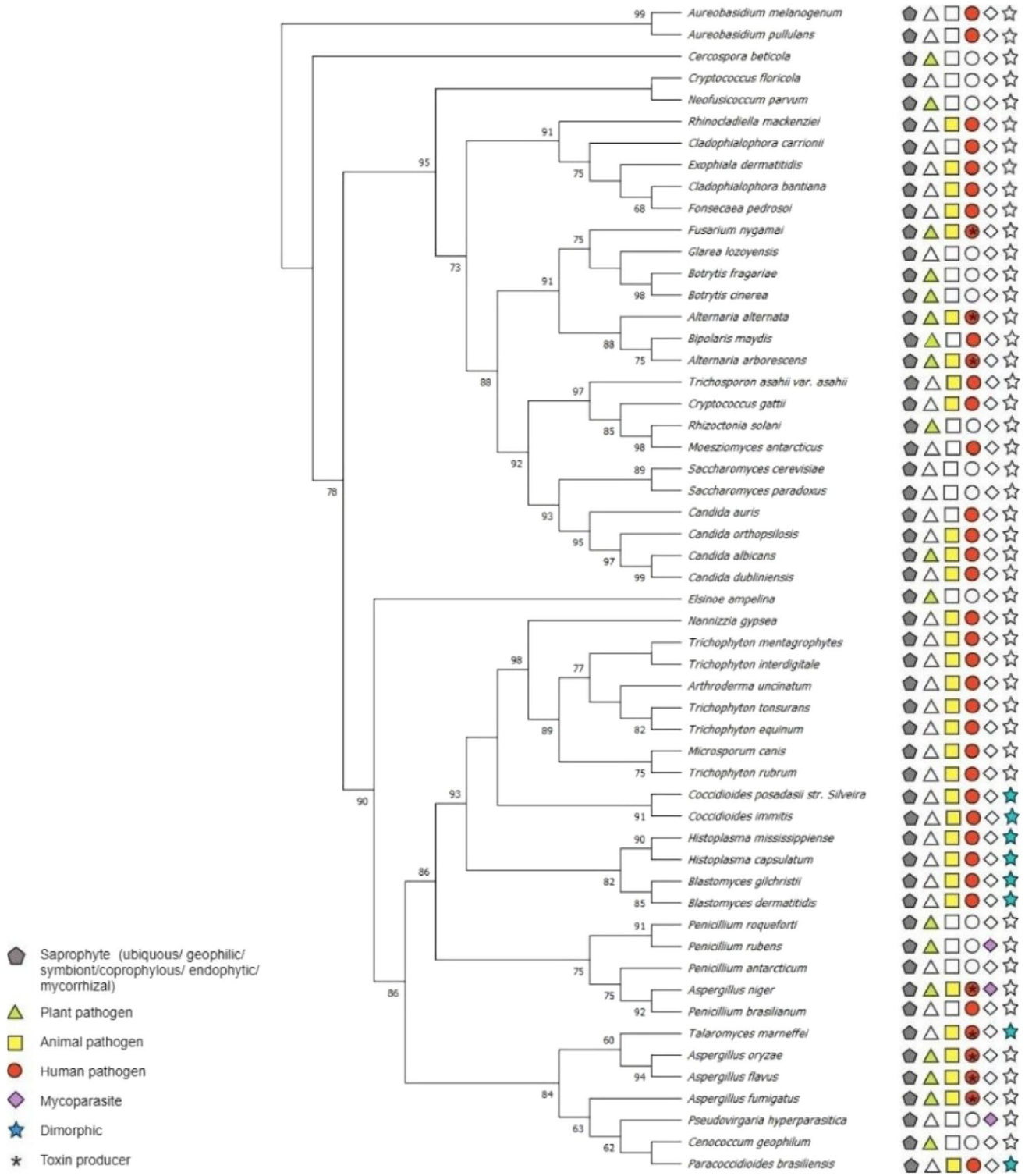


Fig. 1 Phylogenetic reconstruction based on the heat shock proteins 9 kDa and 12 kDa (HSP9/HSP12), using the maximum likelihood method (Q.pfam model). Confidence scores ≥ 60% are shown along each branch. Classification is shown following each corresponding OTU, according to the colors: saprophytes/ubiquitous (gray), plant pathogens (green), animal pathogens (yellow), human pathogens (orange), mycoparasites (purple), dimorphic fungi (blue), and toxin producers (asterisk)

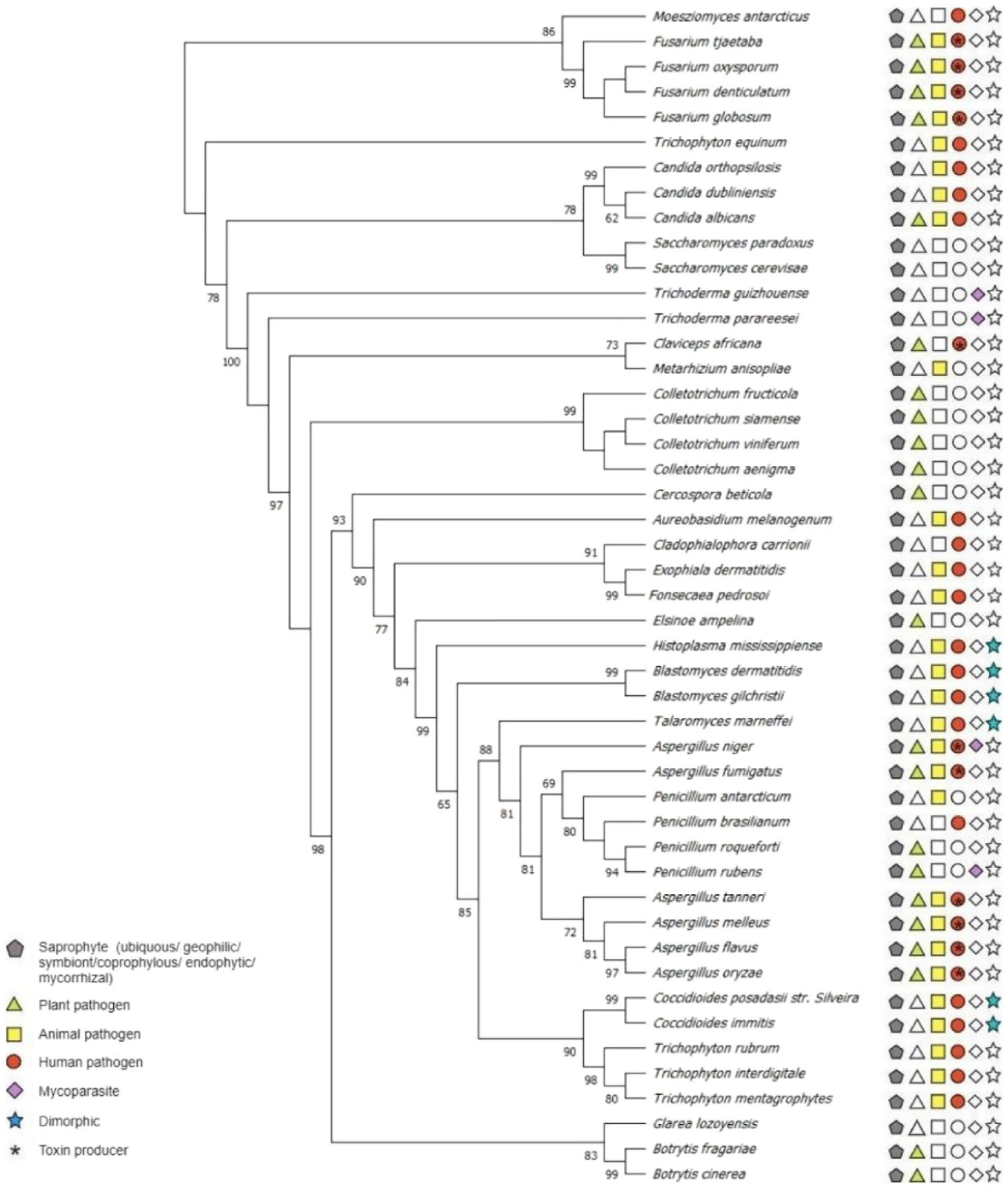


Fig. 2 Phylogenetic reconstruction based on the heat shock protein 30 kDa (HSP30), using the maximum likelihood method (Q.pfam model). Confidence scores > 60% are shown along each branch. Classification is shown following each corresponding OTU, according to the colors: saprophytes/ubiquitous (gray), plant pathogens (green), animal pathogens (yellow), human pathogens (orange), mycoparasites (purple), dimorphic fungi (blue), and toxin producers (asterisk)

cellular stress, thermotolerance, and changes in pH and osmolarity (Burnie et al. 2006). These proteins are ubiquitous, highly conserved, and play a fundamental role for both parasites and hosts. Alongside the agent, they stimulate the growth phase, change in dimorphic microorganisms, and increase resistance and pathogenicity (Cleare et al. 2017; Martinez-Rossi et al. 2016). On the other hand, in the host, HSPs allow the activation of several defense cells through toll-like receptors and play a role in the activation by presenting antigens in infective diseases. Due to these features, we can characterize them as both targets and therapeutic agents (Fang et al. 2011; Jacob et al. 2015). Other functions include chaperone activity, assembly of protein complexes, and transport and compartmentalization of molecules (Table 1) (Bolhassani and Agi 2019).

The HSPs seem to be related to diverse responses in morphological features of fungi. In *Candida albicans*, a yeast-like fungus involved in both saprophytic and invasive lifestyles, the modulation of heat shock factors (HSFs) plays a role in the regulation of the transition to yeast from hyphae and virulence. Via multiple pathways, particularly in temperature, osmolarity, and CO_2 changes, the overexpression of the HSP70 impacts the filamentation of *C. albicans* and its invasiveness (Robbins and Cowen 2023; Tiwari and Shankar 2018).

These chaperones can be classified according to their molecular weight ranging from 10 to more than 100 kDa. The main known HSPs are HSP10, HSP12, HSP26, HSP30, HSP40, HSP60, HSP70, HSP78, HSP90, and HSP110, and are allocated in families according to their function and location (Lindquist & Craig, 1988; Zara et al. 2002). Their structure generally comprises two subunits: an amino-terminal nucleotide-biding domain (NBD) with ATPase function and a carboxy-terminal substrate-binding domain (SBD). A high percentage of identity between sequences of these proteins is estimated among distantly related eukaryotes (> 50%), with a higher percentage among fungi (> 80%) (Burnie et al. 2006; Ramos et al. 2008). However, there is no data about the evolutionary relationship between fungal organisms, their lifestyle, and these proteins specifically.

Given the increasing adaptation of fungi to different niches and hosts, it is necessary to investigate the evolutionary patterns of the family of HSPs among the fungi that help them adapt to new habitats and select new organisms to infect. Here, we sought to elucidate the evolutionary relationship of these chaperones from different fungal clades with their ecological transition, according to their interaction with other hosts.

Material and methods

Library construction

The nucleotide sequences corresponding to the different HSPs of different fungal organisms were obtained from NCBI Protein, and the coding regions were identified based on the most recent annotation of the genomes. The organisms were selected based on origin/host (saprophytes/symbionts, phytopathogens, animal pathogens, human pathogens, mycoparasites, and toxin producers) and morphology (yeasts, filamentous, and dimorphic). The selected HSPs were based on their molecular weight and classification: small HSPs (sHSPs)—HSP9 kDa, HSP12 kDa, HSP30 kDa; cochaperone HSP40 kDa; and classical HSP70 kDa, HSP90 kDa, and HSP110 kDa. The corresponding amino acid sequences were employed for phylogenetic inference. Protein Data Bank entries and correlated species are shown on Supplementary Table 1.

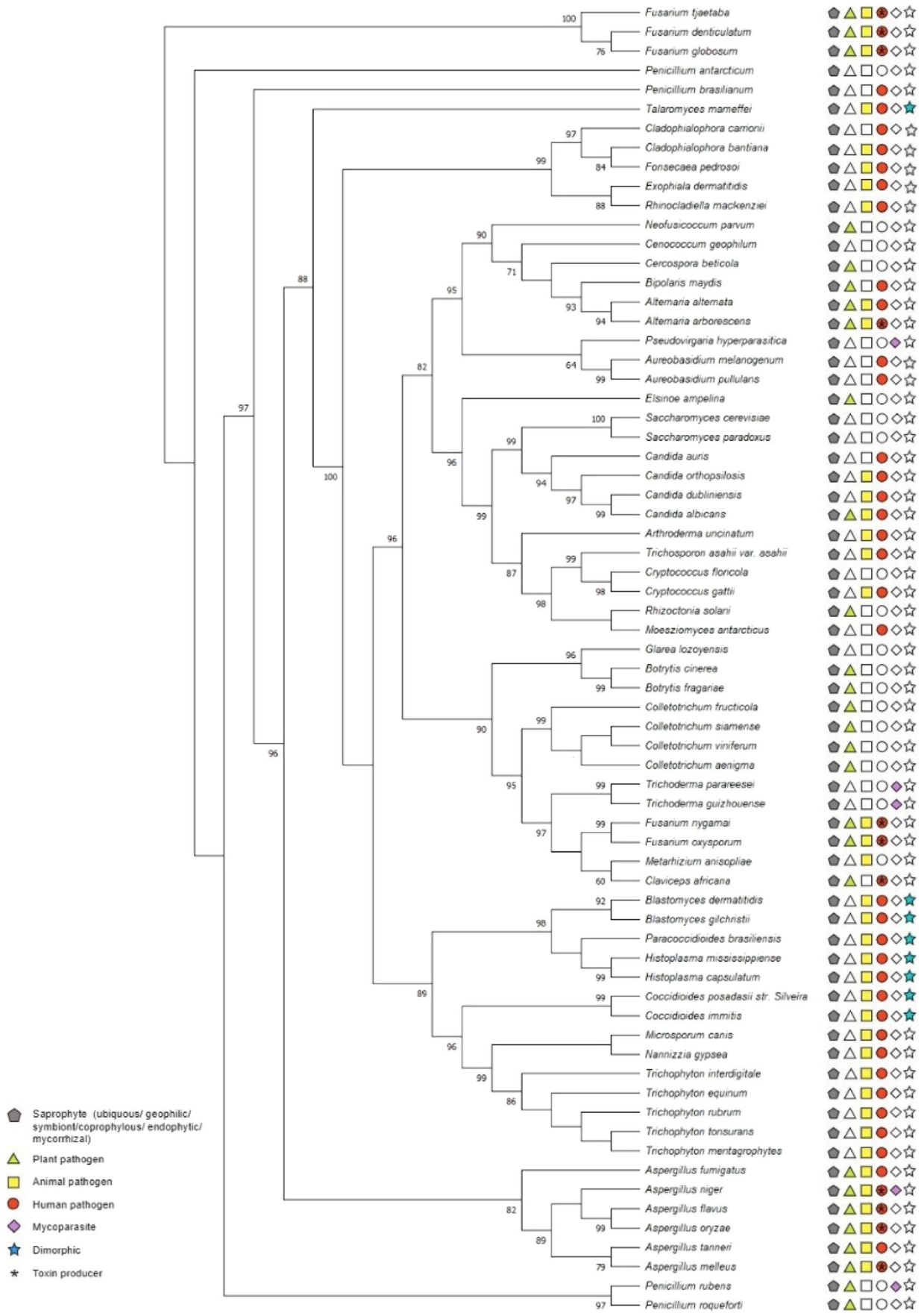
Phylogenetic inference

Amino acid sequences were aligned using the multiple sequence alignment program MAFFT (Katoh et al. 2017) filtered for hypervariable regions with GUIDANCE2 (Sela et al. 2015) using default parameters of the online platform. The following thresholds were employed for each set: HSP9/HSP12 (0.8349), HSP30 (0.6166), HSP40 (0.8446), HSP70 (0.5681), HSP90 (0.5506), and HSP110 (0.9617). The resulting alignments were used for phylogenetic inference, employing the maximum likelihood method via default parameters of the online suite PhyML (Guindon et al. 2010), choosing the best evolutionary model being with Smart Model Selection (SMS) (Lefort et al. 2017). The statistical support for the ramifications was estimated by the approximate likelihood-ratio test (aLRT), requiring a minimum confidence threshold of ≥ 60% (Anisimova and Gascuel 2006).

Results and discussion

HSP9 and HSP12

This analysis evaluated 54 NCBI Protein fungal sequences corresponding to HSP9/HSP12. The sequences containing at least one of these two proteins were annotated, and the tree was constructed using both. Figure 1 shows the phylogenetic inference of these sHSPs in fungi of different families.


Fusarium tjaetaba
Fusarium denticulatum
Fusarium globosum
Penicillium antarcticum
Penicillium brasilianum
Talaromyces mameffei
Cladophialophora carrionii
Cladophialophora bantiana
Fonsecaea pedrosoi
Exophiala dermatitidis
Rhinocladiella mackenziei
Neofusicoccum parvum
Cenococcum geophilum
Cercospora beticola
Bipolaris maydis
Alternaria alternata
Alternaria arborescens
Pseudovirgaria hyperparasitica
Aureobasidium melanogenum
Aureobasidium pullulans
Elsinoe ampelina
Saccharomyces cerevisiae
Saccharomyces paradoxus
Candida auris
Candida orthopsilosis
Candida dubliniensis
Candida albicans
Arthroderma uncinatum
Trichosporon asahii var. asahii
Cryptococcus floricola
Cryptococcus gattii
Rhizoctonia solani
Moesziomyces antarcticus
Glarea lozoyensis
Botrytis cinerea
Botrytis fragariae
Colletotrichum fructicola
Colletotrichum siamense
Colletotrichum viniferum
Colletotrichum aenigma
Trichoderma parareesei
Trichoderma guizhouense
Fusarium nygamai
Fusarium oxysporum
Metarhizium anisopliae
Claviceps africana
Blastomyces dermatitidis
Blastomyces gilchristii
Paracoccidioides brasiliensis
Histoplasma mississippiense
Histoplasma capsulatum
Coccidioides posadasii str. Silveira
Coccidioides immitis
Microsporum canis
Nannizzia gypsea
Trichophyton interdigitale
Trichophyton equinum
Trichophyton rubrum
Trichophyton tonsurans
Trichophyton mentagrophytes
Aspergillus fumigatus
Aspergillus niger
Aspergillus flavus
Aspergillus oryzae
Aspergillus tanneri
Aspergillus melleus
Penicillium rubens
Penicillium roqueforti
Saprophyte (ubiquous/ geophilic/ symbiont/coprophylous/ endophytic/ mycorrhizal)
Plant pathogen
Animal pathogen
Human pathogen
Mycoparasite
Dimorphic
Toxin producer

◂**Fig. 3** Phylogenetic reconstruction based on the heat shock protein 40 kDa (HSP40), using the maximum likelihood method (Q.pfam model, +G, +I). Confidence scores > 60% are shown along each branch. Classification is shown following each corresponding OTU, according to the colors: saprophytes/ubiquitous (gray), plant pathogens (green), animal pathogens (yellow), human pathogens (orange), mycoparasites (purple), dimorphic fungi (blue), and toxin producers (asterisk)

All phylogenies have identifying icons based on the literature for the following correspondences: saprophytes/ubiquitous (gray), plant pathogens (green), animal pathogens (yellow), human pathogens (orange), mycoparasites (purple), dimorphic fungi (blue), and toxin producers (asterisk). The evolutionary history was inferred by using the maximum likelihood method based on the Q.pfam model (Minh et al. 2021).

From the maximum likelihood analysis, it was possible to observe several monophyletic groups with their particularities in the consensus tree. The first group, formed by fungi present in nature, diversifies into 2 clades with specific adaptations: the group of environmental fungi of the Herpotrichiellaceae family known for having pleomorfism and etiological agents of chromoblastomycosis (*Fonsecaea pedrosoi*, *Cladophialophora* spp., *Exophiala dermatitidis*, and *Rhinocladiella mackenziei*) and the group formed by those who cause diseases in plant hosts (*Fusarium nygamai*, *Botrytis* spp., *Alternaria* spp., *Bipolaris maydis*).

Heat shock proteins of less than 43 kDa are known as sHSPs. Their structure contains α/β-crystalline domains and a conserved site at the C-terminal up to 100 amino acids (Gusev et al. 2002). The 9 kDa and 12 kDa HSPs are present in the cytoplasm and membrane of the fungal cell and are involved, in addition to the temperature variance, with barotolerance and response to stress associated with nutrients, being demonstrated their role in the biofilm formation in *Saccharomyces cerevisiae* and oxidative stress protection in *Candida albicans* (de Groot et al. 2000; Zara et al. 2002; Chauhan et al. 2003).

The other group is made up of primary human pathogens known to cause disease in other animals. This clade is composed of the monophyletic group of dermatophytes, adapted for infection with keratinolytic enzymes (*Trichophyton* spp., *Microsporum canis*, *Nannizzia gypsea*) as well as the paraphyletic group of endemic dimorphic fungi, adapted to cross the endothermy barrier of mammals (*Histoplasma* spp., *Blastomyces* spp., *Coccidioides* spp.). This could be related to the role of these sHSPs in *C. albicans* in yeast-to-hyphal transition and tissue invasion (Fu et al. 2012)

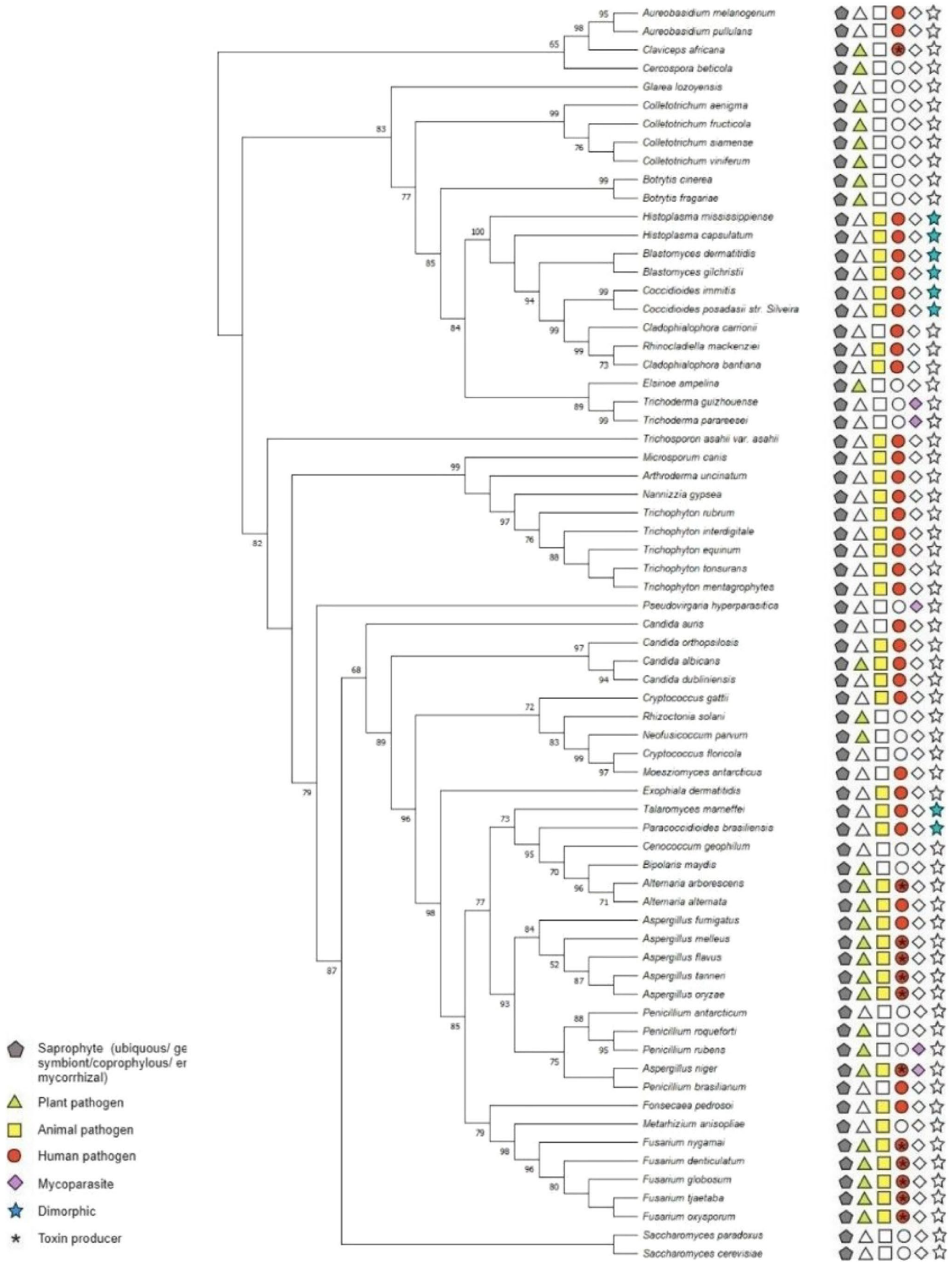
In addition, we can observe that the sHSPs of the species *Cryptococcus floricola* and *C. gattii* are found in different branches of the tree. While the former is in an external clade group, along with the plant pathogen *Neofusicoccum parvum*, the latter is more closely related to the human and animal pathogenic species *Trichosporon asahii*. In *C. neoformans*, the homologous protein HSP12 is involved in cell survival in the face of increased antifungal temperatures via cyclic AMP (cAMP) pathway (Maeng et al. 2010). This could suggest that these proteins may be involved in the ecological adaptation of this species to hosts with higher body temperatures.

HSP30

Unlike previous sHSPs, we performed the analysis with 48 sequences, due to the fewer availability compared to classic HSPs. Despite this, we were able to obtain a consensus tree with the same diversity of fungi in relation to their hosts (Fig. 2). The evolutionary history was inferred by using the maximum likelihood method based on the Q.pfam model (Minh et al. 2021).

Differently from HSP9/HSP12, in this analysis, we observed a large clade representative of the primary and opportunistic pathogens of animals and humans, having an ancestral sequence in common with other phytopathogenic fungi (*Botrytis* spp. and *Cercospora beticola*). Within this clade, we have a paraphyletic group of dimorphic fungi and a monophyletic group of the Trichocomaceae family, which contains the well-known molds of the genera Aspergillus and Penicillium.

HSP30s are proteins with hydrophobic characteristics present in the plasma membrane, and it is suggested that the major inducers of the production of these proteins are factors that affect the fluidity of the membrane, such as high temperatures and substances that cause a change in solubility in the lipid bilayer, such as ethanol (Seymour and Piper 1999). Those chaperones also assist in the stabilization of unfolded proteins in yeasts, forming a complex with HSP80 and HSP70 (Tiwari et al. 2015). Since HSP30s are modulated with the alteration of the pH of the environment and their expression is induced by extracellular acidification, we can insinuate their involvement in the invasion of epithelial tissue in dermatophytosis and dermatomycosis (Behzadi et al. 2009; Freitas et al. 2011).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Alternaria arborescens
Alternaria alternata
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Aspergillus melleus
Aspergillus flavus
Aspergillus tanneri
Aspergillus oryzae
Penicillium antarcticum
Penicillium roqueforti
Penicillium rubens
Aspergillus niger
Penicillium brasilianum
Fonsecaea pedrosoi
Metarhizium anisopliae
Fusarium nygamai
Fusarium denticulatum
Fusarium globosum
Fusarium tjaetaba
Fusarium oxysporum
Saccharomyces paradoxus
Saccharomyces cerevisiae
Saprophyte (ubiquous/ ge
symbiont/coprophylous/ er
mycorrhizal)
Plant pathogen
Animal pathogen
Human pathogen
Mycoparasite
Dimorphic
Toxin producer

◂**Fig. 4** Phylogenetic reconstruction based on the heat shock protein 70 kDa (HSP70), using the maximum likelihood method (LG+R). Confidence scores > 60% are shown along each branch. Classification is shown following each corresponding OTU, according to the colors: saprophytes/ubiquitous (gray), plant pathogens (green), animal pathogens (yellow), human pathogens (orange), mycoparasites (purple), dimorphic fungi (blue), and toxin producers (asterisk)

HSP40

Present in the cytosol, plasma membrane, and endoplasmic reticulum of cells, HSP40s act together with HSP104 as cochaperones to HSP70, binding to unfolded peptides to be delivered to HSP70 (Burnie et al. 2005). Due to their molecular composition, these cochaperones are classified into two categories: class I, containing three conserved domains (cysteine-rich, zinc finger, and carboxy-terminal domains), and class II, containing two conserved domains (glycine-rich and phenylalanine-rich domains). Also, their sequences have similarities with the J domain of the *Escherichia coli* DnaJ, modulating the conformation of the HSP70 in order to facilitate its ATP-hydrolytic activity (Lee et al. 2002, Burnie et al. 2005).

Here, we evaluated the sequence of 68 fungal organisms, as illustrated in the phylogeny in Fig. 3. The homologous sequences mainly involved the proteins Caj1, Sis1, Psi1, Xdj1, and bpa. As with sHSPs, we can suggest the presence of a common ancestor for the ecological transition of animal pathogenic fungi. In addition, we can observe a divergence between the sequences of yeast, dimorphic, and filamentous fungi. SMS (Lefort et al. 2017) suggested the Q.pfam model (Minh et al. 2021) to account for evolutionary rate differences among sites (four categories, +G), considering some sites to be evolutionarily invariable (+I).

It is possible to observe a divergence between the species of the genus Fusarium. This genus is highly disseminated and known for its transkingdom infections, affecting plants, animals, and humans, and can even produce toxins known as fumonisins. In our analysis, *Fusarium oxysporum* and *Fusarium nygamai* species form a polyphyletic group along with the phytopathogens of the genus Colletotrichum. In the species *F. oxysporum*, it has been shown that post-translational modifications involving the HSP40-HSP70 complexes modulate the formation of conidia, which are crucial for their dispersal and reproduction (Lv et al. 2022).

HSP70

The results of the phylogenetic analysis of 68 sequences of HSP70 are shown in Fig. 4. SMS (Lefort et al. 2017) suggested the Legascuel (LG) (Le and Gascuel 2008) considering rate variation (+R).

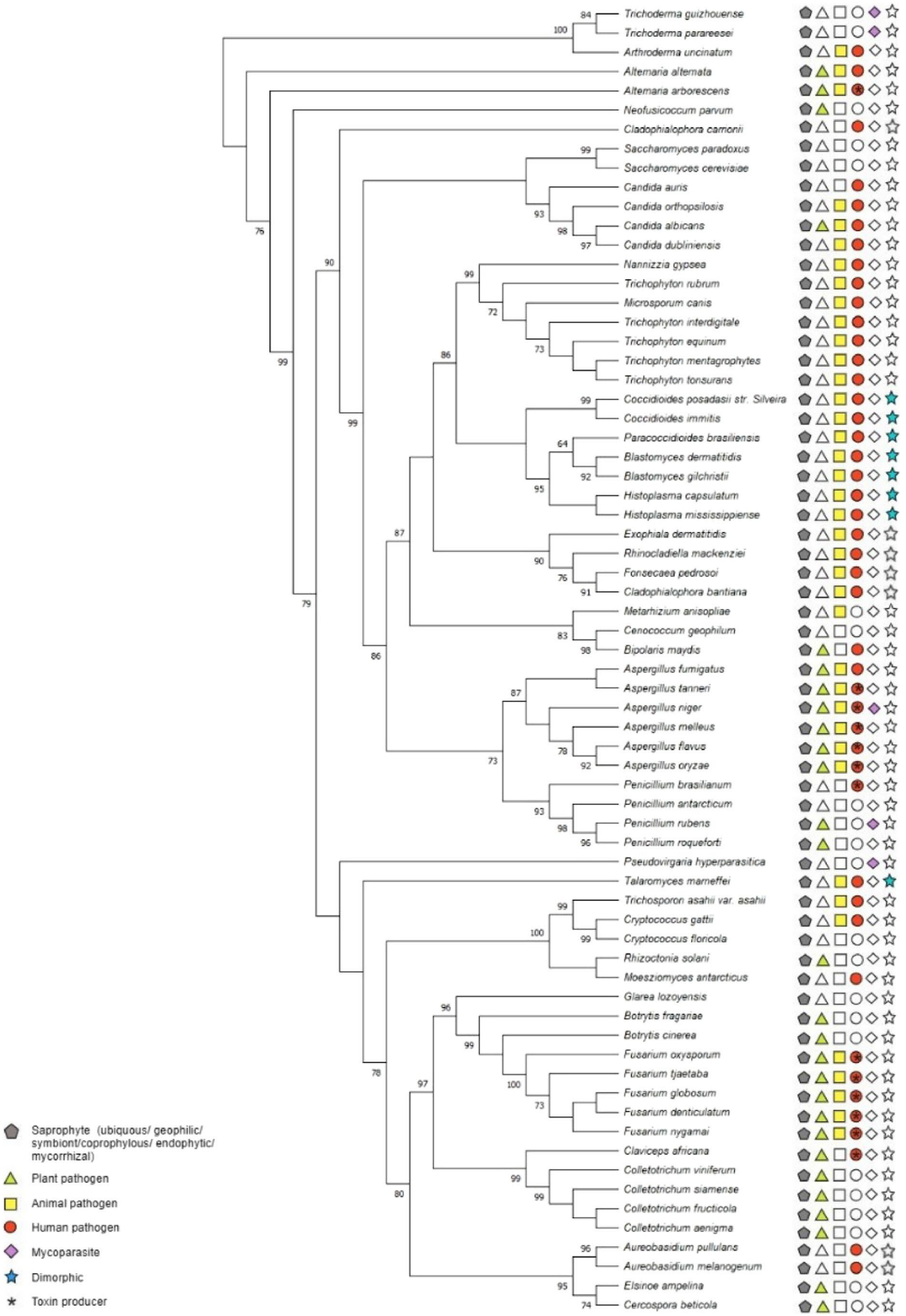
Both the genera Aspergillus and Fusarium are known to produce mycotoxins linked to grain cultivation. Aflatoxins, produced by species of the genus Aspergillus, as an example of the section Flavus of this genus, are a worrying group of compounds capable of poisoning animals and humans. Aflatoxins may be related to about 30% of hepatocellular cancers, given their great hepatotoxic and carcinogenic potential. Here, we can observe the formation of a polyphyletic group involving the toxin-producing fungi linked to agriculture between the heat shock protein sequences. This relationship is particularly interesting since the HSP70 seems to be involved in aflatoxin production at different temperatures in both toxigenic and atoxigenic isolates. The increase in temperature in toxigenic isolates of *A. flavus* decreased the expression of HSP70, impairing the production of aflatoxins. On the other hand, in non-toxigenic isolates, the overexpression of HSP70 seems to be related to the inhibition of aflatoxin biosynthesis (Thakur et al., 2016; Ting et al. 2020).

Interestingly, we can observe that the species of the genus Cryptococcus are present in distinct sister clades, with *C. floricola* present in a clade together with fungi that do not have pathogenic potential for animals. In addition, we can suggest an adaptation in relation to the proteins of the sister clades of environmental fungi in two groups: (i) dimorphic clade that infects vertebrates and (ii) clade that composes the species of the genus Trichoderma, known to be mycoparasites, widely used in biological control.

The 70-kDa heat shock proteins are among the HSPs most induced in relation to temperature increase, being directed to several compartments: cytoplasm, cell membrane, endoplasmic reticulum, and mitochondria. It is estimated that these proteins have more than 60% similarity between members of the Eukarya domain (Boorstein et al. 1994). In fungi, different homologous proteins are expressed constitutively, such as the Ssa1 and Ssa2 proteins, as well as facultative proteins, such as the Ssa3 and Ssa4 proteins, produced in response to stress.

In its structure, HSP70 has an NBD domain of 44 kDa and an SBD domain of 18 kDa. Thus, like HSP40, its cochaperone is similar to the DnaK protein of *Escherichia coli*. Ssa1, the most studied cytosolic protein in *Saccharomyces cerevisiae*, is induced by the expression of HSP40 Sis1, whose activity involves the direction of proteins in the assembly process for conformation modification by the ATPase domain of Ssa1 (Qian et al. 2001; Burnie et al. 2006).

The signaling and induction pathway between these proteins is well established and involves different mechanisms, including pH change and phosphate levels. This pathway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Alternaria arborescens
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Botrytis cinerea
Fusarium oxysporum
Fusarium tjaetaba
Fusarium globosum
Fusarium denticulatum
Fusarium nygamai
Claviceps africana
Colletotrichum viniferum
Colletotrichum siamense
Colletotrichum fructicola
Colletotrichum aenigma
Aureobasidium pullulans
Aureobasidium melanogenum
Elsinoe ampelina
Cercospora beticola
Saprophyte (ubiquous/ geophilic/ symbiont/coprophylous/ endophytic/ mycorrhizal)
Plant pathogen
Animal pathogen
Human pathogen
Mycoparasite
Dimorphic
Toxin producer

◂**Fig. 5** Phylogenetic reconstruction based on the heat shock protein 90 kDa (HSP90), using the maximum likelihood method (LG+R). Confidence scores > 60% are shown along each branch. Classification is shown following each corresponding OTU, according to the colors: saprophytes/ubiquitous (gray), plant pathogens (green), animal pathogens (yellow), human pathogens (orange), mycoparasites (purple), dimorphic fungi (blue), and toxin producers (asterisk)

involves heat shock factor 1 (HSF1), heat shock elements (HSEs), a species-dependent variable set of genes. Among the transcription factors involved with the highest homology is PacC, which responds to increased temperature in the physiological environment by the *pal* genes cascade, modulating the expression of HSP70 genes and increasing glycosylation and enzyme secretion. In addition, these factors are related to a higher gene expression in dermatophyte cultures in response to heat and the presence of keratin (Nozawa et al. 2003; Ferreira-Nozawa et al. 2006; Martinez-Rossi et al. 2016).

In dimorphic fungi, it is known that this class of proteins is responsible for cellular adaptation during the change from filamentous to yeast-like stage in *Histoplasma capsulatum*. Its heat-inducive response may involve this adaptation, hitting its maximum expression at higher temperatures in more virulent strains (Caruso et al. 1987; Allendoerfer et al. 1996). The same was observed for *Paracoccidioides brasiliensis* HSP80, with about 90% of identity in relation to *H. capsulatum*. In this case, it had a lower expression when transitioning to the mycelial state (Díez et al. 2002).

HSP90

The tree reconstruction of 68 sequences corresponding to HSP90 of fungal organisms suggests an involvement of these proteins in the morphology of the species (Fig. 5). The evolutionary history was inferred by using the maximum likelihood method based on the LG (Le and Gascuel 2008) + R model.

HSP90 chaperones are the largest representatives of this class, as they correspond to about 2% of cytosolic proteins in different eukaryotes. These proteins have a structural difference in relation to the other HSPs, since they have a dimerization domain associated with the NBD and SBD domains. This is reflected in their activity, being recruited to aid in assembling proteins that are difficult to fold, as well as in preventing aggregate formation and intermediate formations (Finkelstein and Strausberg 1983; Taipale et al. 2010).

Among pathogenic fungi, its modulation is related to the gain of invasive characteristics. In the dermatophyte *T. rubrum*, its expression is related to increased keratinolytic activity at human body temperature (37 °C) via metabolic pathways after alternative splicing (Neves-da-Rocha et al. 2019). It is also possible that these proteins are acting in the adaptive process to gain resistance to azoles and echinocandins in *C. albicans* (Cowen and Lindquist 2005). The role of HSP90 in the virulence of *C. albicans* was suggested by the overexpression of its homologous in *S. cerevisiae* (84% identity) in a mice model (Hodgetts et al. 1996). This protein is also present in the cell wall of *C. albicans* and plays a key role in hyphal elongation and protein secretion (Hube and Naglik 2001).

Our inference supports the hypothesis that this class of proteins is associated with pathogenic gains against endothermic hosts. What reinforces this suggestion is the presence of four sister groups of pathogens with a common ancestral sequence, especially with the presence of primary pathogens and endemic dimorphic fungi of different origins.

Following this perspective, we can justify the presence of an exclusive clade of phytopathogenic fungi. The crucial role of HSP90 in the modulation of genes involved in vegetative growth, virulence, and conidiation of *Fusarium graminearum* has been demonstrated. The repression of this gene prevents the formation of phialides, and most of the conidia are produced directly by the hyphae, modifying the phenotype (Bui et al. 2016).

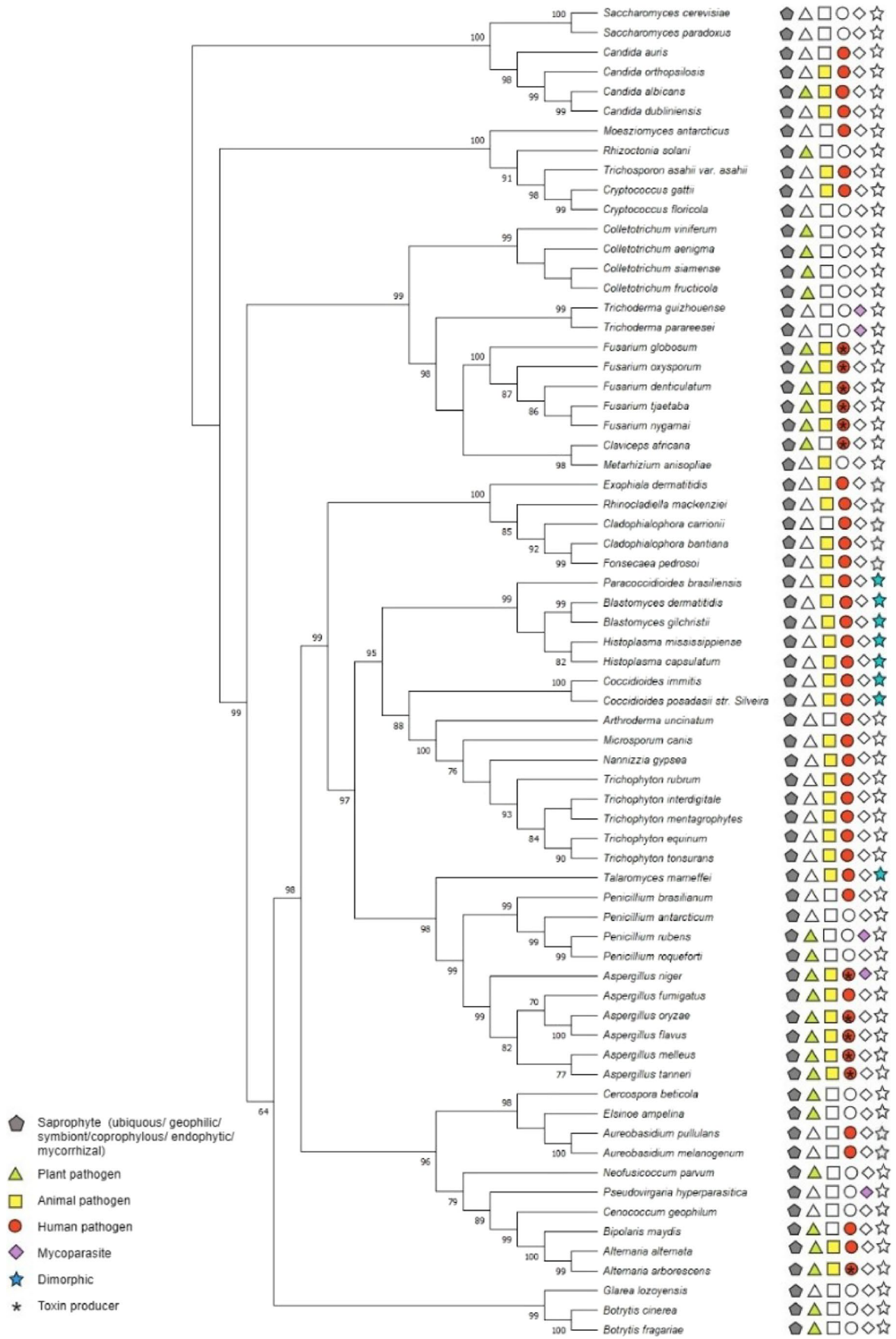
HSP110

HSP110s are highly similar to HSP70s and have been suggested as a subgroup of them. However, they have an additional holdase activity and act like cochaperones, facilitating their activity as nucleotide exchange factors (Oh et al. 1997; Andréasson et al. 2008; Polier et al. 2008).

One of the members of this class, HSP104, which has a role in the recovery of denatured proteins, is known for having two NBDs. This is relevant in a scenario of temperature increase, in which it is expressed for the maintenance of the stationary stage (Hattendorf 2002).

As in previous reconstructions, there is the formation of monophyletic groups related to endothermic and plant hosts. The evolutionary transition to warm hosts may be associated with a change in mycelial to yeast structures (Fig. 6). SMS (Lefort et al. 2017) suggested the Q.yeast model to account for evolutionary rate differences among sites (four categories, +G), considering some sites to be evolutionarily invariable (+I) (Minh et al. 2021). Nevertheless, further studies are needed to evaluate the role of this HSP in the infective process.

The investigation of HSPs and their role in host-pathogen interaction, morphological features, and production


Saccharomyces cerevisiae
Saccharomyces paradoxus
Candida auris
Candida orthopsilosis
Candida albicans
Candida dubliniensis
Moesziomyces antarcticus
Rhizoctonia solani
Trichosporon asahii var. asahii
Cryptococcus gattii
Cryptococcus floricola
Colletotrichum viniferum
Colletotrichum aenigma
Colletotrichum siamense
Colletotrichum fructicola
Trichoderma guizhouense
Trichoderma parareesei
Fusarium globosum
Fusarium oxysporum
Fusarium denticulatum
Fusarium tjaetaba
Fusarium nygamai
Claviceps africana
Metarhizium anisopliae
Exophiala dermatitidis
Rhinocladiella mackenziei
Cladophialophora carrionii
Cladophialophora bantiana
Fonsecaea pedrosoi
Paracoccidioides brasiliensis
Blastomyces dermatitidis
Blastomyces gilchristii
Histoplasma mississippiense
Histoplasma capsulatum
Coccidioides immitis
Coccidioides posadasii str. Silveira
Arthroderma uncinatum
Microsporum canis
Nannizzia gypsea
Trichophyton rubrum
Trichophyton interdigitale
Trichophyton mentagrophytes
Trichophyton equinum
Trichophyton tonsurans
Talaromyces marneffei
Penicillium brasilianum
Penicillium antarcticum
Penicillium rubens
Penicillium roqueforti
Aspergillus niger
Aspergillus fumigatus
Aspergillus oryzae
Aspergillus flavus
Aspergillus melleus
Aspergillus tanneri
Cercospora beticola
Elsinoe ampelina
Aureobasidium pullulans
Aureobasidium melanogenum
Neofusicoccum parvum
Pseudovirgaria hyperparasitica
Cenococcum geophilum
Bipolaris maydis
Alternaria alternata
Alternaria arborescens
Glarea lozoyensis
Botrytis cinerea
Botrytis fragariae
Saprophyte (ubiquous/ geophilic/ symbiont/coprophylous/ endophytic/ mycorrhizal)
Plant pathogen
Animal pathogen
Human pathogen
Mycoparasite
Dimorphic
Toxin producer

◂**Fig. 6** Phylogenetic reconstruction based on the heat shock protein 110 kDa (HSP110), using the maximum likelihood method (Q.yeast model, +G, +I). Confidence scores > 60% are shown along each branch. Classification is shown following each corresponding OTU, according to the colors: saprophytes/ubiquitous (gray), plant pathogens (green), animal pathogens (yellow), human pathogens (orange), mycoparasites (purple), dimorphic fungi (blue), and toxin producers (asterisk)

of compounds can provide new perspectives in health, agriculture, and industry (Tiwari and Shankar 2018). The application of these proteins as molecular targets to new vaccines and treatments could improve the assistance in invasive fungemia and another systemic mycosis (De Souza et al. 2019; Baltazar et al. 2021; Hu et al. 2023). In addition, this could prevent crop loss due to anthracnosis and benefit production via biological control development (Montero-Barrientos et al. 2010; Dong et al. 2023).

Conclusion

In this article, we evaluated by phylogenetic inferences the hypothesis that the heat shock proteins may be involved in the fungal host range by its temperature and ecological adaptation. Since these proteins seem to be related to different mechanisms of protection against unfavorable environments, it is possible that it led to transitions in order to predate under specific substrates, such as keratin and cellulose.

This taxonomically diverse investigation of HSP evolutionary relationships highlights the relevance of these stress-related proteins for fungal environment and host plasticity. Regardless of their high sequence conservation, it is tempting to consider them as suitable targets for novel antifungal treatments. Still, further studies could be able to determine if the post-translational changes promoted by HSPs aided in adapting to the new hosts, pinpointing how these chaperones were able to influence fungal dispersion and environmental conquests.

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Declarations

Conflict of interest The authors declare no competing interests.

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