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LACK OF HOST FIDELITY AND LOW VIRULENCE OF THE FILAMENTOUS FUNGUS *Escovopsis trichodermoides*

Dissertação apresentada ao Instituto de Biociências, do Câmpus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciências Biológicas (Microbiologia Aplicada).

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TÍTULO DA DISSERTAÇÃO: LACK OF HOST PREFERENCE AND LOW INFECTION OF THE FILAMENTOUS FUNGUS *Escovopsis trichodermoides*

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“I've seen things you people wouldn't believe. Attack ships on fire off the shoulder of Orion. I watched C-beams glitter in the dark near the Tannhäuser Gate. All those moments will be lost in time, like tears in rain. Time to die”

Roy Batty, replicant (Blade Runner)

RESUMO

Uma das associações simbióticas fascinantes na natureza é o mutualismo entre as formigas da tribo Attini (atíneas) e fungos que cultivam como alimento. Fungos do gênero *Escovopsis* são considerados parasitas dos fungos cultivados por esses insetos. Tais parasitas são especializados a determinados fungos das formigas, embora eventuais trocas de hospedeiros ocorreram durante a evolução. Recentemente, nosso grupo de pesquisa descreveu o fungo *Escovopsis trichodermoides* em colônias de atíneas basais. Entretanto, a especificidade e infectividade frente aos fungos cultivados por esses insetos ainda não foram abordados. Evidências provenientes de ensaios *in vitro* e em colônias sugerem um padrão generalista de infecção de *E. trichodermoides*, com ausência de fidelidade frente a diferentes fungos mutualistas. A produção de metabólitos inibitórios está envolvida no antagonismo de *E. trichodermoides*, caracterizando a competição por interferência como mecanismo frente aos diferentes hospedeiros. Além disso, foi observada baixa suscetibilidade de colônias de *Mycocepurus goeldii* (uma atínea basal) à infecção de *E. trichodermoides*, com alta porcentagem de sobrevivência. No geral, os resultados confirmam o antagonismo de *E. trichodermoides*, entretanto, com baixa virulência e ausência de fidelidade frente a diferentes cultivares, padrão ainda não observado na fungicultura das formigas atíneas.

Palavras-chave: Fungicultura. Antibiose. Antagonismo. Formigas cultivadoras de fungos. Simbiose.

ABSTRACT

A fascinating symbiotic association in nature is the mutualism between ants in the tribe Attini (attine) and fungi they cultivate for food. Fungi in the genus *Escovopsis* are parasites of the ant fungal cultivars. Such parasites are specialized to certain fungal cultivars, although host-switching events occurred during the evolution of this parasite. Recently, our research group described *E. trichodermoides* associated with lower attine ant colonies. However, the specificity and infectivity towards the ant fungal cultivars are still elusive. Evidence from *in vitro* assays as well as experiments in live ant colonies indicates a generalist pattern of infection of *E. trichodermoides*, with lack of host fidelity. The production of inhibitory metabolites is implicated in the antagonism of *E. trichodermoides*, characterizing interference competition as a mechanism towards the different hosts. In addition, colonies of the ant species *Mycocepurus goeldii* (a lower attine ant) showed high survival rates after exposure to conidia of *E. trichodermoides*. Collectively, our results confirm the antagonism of *E. trichodermoides*, with low virulence and absence of fidelity towards different fungal cultivars, a pattern first reported in the fungiculture of attine ants.

Keywords: Fungiculture. Antibiosis. Antagonism. Fungus-growing ants. Symbiosis.

SUMÁRIO

INTRODUÇÃO

Simbiose pode ser definida como uma estreita interação entre organismos, tendo como resultado associações benéficas ou não. Associações entre plantas e endófitos, leguminosas e bactérias fixadoras de nitrogênio, algas e fungos formando líquens são alguns exemplos de tais interações. Uma simbiose fascinante é o mutualismo obrigatório entre formigas da subtribo Attina[1] e fungos basidiomicetos (ordem Agaricales, gêneros *Leucocoprinus* e *Leucoagaricus*), originado há cerca de 50 milhões de anos. Esses insetos cultivam o parceiro fúngico como única fonte de alimento para as larvas e a rainha; em contrapartida, a formiga providencia: substrato para o crescimento do fungo, meios para sua dispersão e proteção contra antagonistas.

Diferentes tipos de fungicultura são praticados pelas atíneas e são definidos pelo fungo associado e pelos hábitos de forrageamento das formigas. Cinco fungiculturas são descritas, sendo: (i) fungicultura das atíneas basais (i.e. na filogenia da subtribo), que cultivam diferentes espécies de fungos do gênero *Leucocoprinus* sp. (família Agaricaceae), e forrageiam detritos vegetais, fezes e carcaças de insetos para nutrição do simbionte; (ii) Fungicultura de fungos-coral, realizado por formigas do gênero *Apterostigma* que cultivam fungos da família Pterulaceae; (iii) Fungicultura praticada por formigas do gênero *Cyphomyrmex* gr. *rimosus*, que cultivam o fungo em forma de levedura; (iv) Fungicultura das atíneas derivadas (i.e. formigas mais derivadas na filogenia), praticada pelos gêneros *Trachmymyrmex* e *Sericomyrmex*, consideradas não cortadeiras de folhas; e (v) Fungicultura das formigas cortadeiras, uma subdivisão das derivadas (gêneros *Atta* e *Acromyrmex*) que cultivam o fungo *Leucoagaricus gongylophorus* e cortam folhas e flores frescas para nutrição do fungo.

Nessa relação, outro simbionte descrito é o fungo do gênero *Escovopsis*, considerado micoparasita que infecta os jardins de fungo[2] e que potencialmente pode devastar as colônias das formigas atíneas. Esse parasita apresenta adaptações que o auxiliam sobrepujar as defesas do fungo cultivado e das próprias formigas. Encontrado unicamente associado às colônias desses insetos, *Escovopsis* causa diminuição do crescimento do jardim, o qual não acumula biomassa fúngica para suprir a colônia. Os mecanismos do parasitismo estão associados à produção de enzimas, reconhecimento de metabólitos produzidos pelo fungo mutualista e de mecanismos estruturais, com degeneração das hifas do hospedeiro pelo contato direto com hifas de *Escovopsis*. Apesar dos estudos que sugeriram tais mecanismos, ainda não está claro como *Escovopsis* parasita o fungo cultivado pelas formigas.

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[1] **Formigas da subtribo Attina.** Conhecidas também como "atíneas", são informalmente divididas em derivadas e basais, segundo características morfológicas, ecológicas, filogenia e fungicultura.

[2] **Jardim de fungo.** Estrutura elaborada pelas formigas que compreende o fungo mutualista e o substrato (vegetal ou restos e fezes de insetos) coletado pelas operárias.

A co-evolução entre o parasita e o hospedeiro pode resultar em co-cladogênese e especialização dos simbiontes. Nesse contexto, há superação das defesas do hospedeiro pelo parasita e a intensificação dos mecanismos defensivos pelo hospedeiro, responsáveis por manter os padrões de especificidade da interação, fenômeno previsto pela hipótese evolutiva da Rainha Vermelha (*Red Queen hypothesis*). Existem linhagens de *Escovopsis* que apresentam padrões de especificidade para com os tipos de fungiculturas praticados pelas atíneas. Entretanto, alguns estudos indicam incongruências na co-cladogênese entre *Escovopsis* e o fungo mutualista. Eventos de troca de hospedeiro por *Escovopsis* foram relatados, os quais podem estar associados às incongruências observadas.

O requerimento para que haja troca de hospedeiro na natureza, é que um parasita seja capaz de superar as defesas do novo hospedeiro e estabelecer com sucesso a infecção. Parasitas com arsenal diverso para infecção e mecanismos eficientes de transmissão possuem vantagem na infecção de hospedeiros diferentes. Entretanto, a diversidade de espécies de *Escovopsis* é ainda desconhecida, assim como seus modos de transmissão entre colônias. Nosso grupo de pesquisa descreveu *Escovopsis trichodermoides*, um fungo associado à colônias de diferentes espécies de atíneas basais. O estilo de vida desse fungo, bem como aspectos de sua infectividade e preferência de hospedeiros ainda são desconhecidos.

Baseado no papel descrito para espécies conhecidas de *Escovopsis* que infectam jardins de formigas atíneas derivadas e basais, o estudo teve como objetivo: (i) Descrever os padrões de interação de *E. trichodermoides* frente a diferentes fungos mutualistas; (ii) Descrever os padrões de preferência e fidelidade do fungo frente aos hospedeiros; (iii) Determinar se a infecção está associada a mecanismos químicos de ação e (iv) Determinar a infectividade de *E. trichodermoides*.

Para atingir os objetivos, ensaios de cultivo pareado entre *E. trichodermoides* e diferentes fungos mutualistas foram realizados, assim como ensaios com chance de escolha de hospedeiros. Também foi avaliada a interação entre os fungos mutualistas e os metabólitos produzidos por *E. trichodermoides*. Colônias da formiga atínea basal *Mycocepurus goeldii* foram coletadas e utilizadas como modelo de estudo para avaliar a infectividade de *E. trichodermoides*.

Ainda existem lacunas sobre a evolução do parasitismo em *Escovopsis* e sobre aspectos ecológicos na interação com as formigas atíneas. Utilizando *E. trichodermoides* como modelo de estudo, pretende-se com o presente trabalho, adicionar novos elementos para esse campo de estudo.

LACK OF HOST FIDELITY AND LOW VIRULENCE OF THE FILAMENTOUS FUNGUS *ESCOVOPSIS TRICHODERMOIDES*

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ABSTRACT

Symbioses are widespread in several forms of life, with ecological and evolutionary implications for the organisms involved. Several insects maintain symbioses with microorganisms and a paramount example is the fungus-growing ants, known to practice fungiculture for food. Their basidiomycetous fungal cultivars are threatened by fungal parasites in the genus *Escovopsis* (Ascomycota: Hypocreales) that shows patterns of specificity towards its host. *Escovopsis trichodermoides* was recently described to be associated with colonies of the lower attine *Mycocepurus goeldii*, however its ecological role is still unknown. Here we provide clues of the generalist nature of *E. trichodermoides*, with lack of fidelity to fungal hosts and low infection in ant colonies. Our results also indicate the production of inhibitory soluble compounds by *E. trichodermoides* as a mechanism of interference competition. The generalist lifestyle of *E. trichodermoides* may have allowed host-switching events towards different fungal cultivars of the lower attine ants. Interestingly this lifestyle is not a common trait of *Escovopsis* species, which usually shows partner fidelity. Thus, our study indicates that *Escovopsis* has adicional lifestyles then previously thought, prompting further investigations on the evolution of *Escovopsis* in the attine ant-fungal symbiosis.

Keywords: Fungiculture, antibiosis, antagonism, fungus-growing ants, symbiosis.

1. INTRODUCTION

Symbiosis implies in close relationships between organisms, establishing beneficial or detrimental associations, such interactions are important models for studying evolution. In nature, different organisms live in symbiosis with fungi (BATRA 1963; DEJEAN et al., 2005; HELGASON et al., 1998; KÄMPER et al., 2006; LUTZONI et al., 2001; MARTIN et al., 2017; SIMARD et al., 1997; SPRIBILLE et al., 2016; WEBB 1945; WEBER 1972). The ecological success of these interactions involves many factors, related to the health of organisms as well as the environment they live.

Some social insects maintain symbiotic associations with fungi (MUELLER; GERARDO, 2002). Fungus-growing ants (Hymenoptera: Attini: Attina, hereafter named "attine ants"), have grown basidiomycetous fungi (Agaricales: Agaricaceae: *Leucoagaricus gongylophorus* or *Leucocoprinus* spp.) over the last 50 million of years in an obligatory

mutualism, as the main food source for the larvae and queen (SCHULTZ, BRADY; 2008). Meanwhile, the ants disperse the fungus and provide a stable environment for its development, providing substrate and protection against competitors.

Among the several undesirable microbes found in attine ant colonies, the fungal genus *Escovopsis* (Ascomycota: Hypocreales) has been reported as a specialized parasite of the ants' cultivars (CURRIE; MUELLER; MALLOCH, 1999; CURRIE, 2001). Infections with this parasite weaken the fungus garden, consequently decreasing the ant workforce (CURRIE, 2001). *Escovopsis* shows host fidelity (i.e., one strain of the parasite associated with phylogenetically related hosts) and production of inhibitory compounds towards its hosts (CURRIE; MUELLER; MALLOCH, 1999; CURRIE, 2001; DHODARY et al., 2018; GERARDO et al., 2006a; HEINE et al., 2018; REYNOLDS; CURRIE, 2004).

The fungiculture of attine ants has been described as an ancient tripartite coevolution, with phylogenetic congruence between the ants, their cultivars, and *Escovopsis* (CURRIE et al., 2003; GERARDO et al., 2006b). Such congruence is maintained by host or parasite adaptations and counter-adaptations, which drive a coevolutionary arms race (ANTONOVICS et al., 2013; BIRNBAUM; GERARDO, 2016). As a result of this coevolution some traits can be noted such as *Escovopsis* host fidelity at in finer and broader phylogenetic scales (BIRNBAUM; GERARDO, 2016; CUSTODIO; RODRIGUES, 2019; GERARDO et al., 2006a). However, host-switching events may have occurred during the evolution of these organisms. Some *Escovopsis* strains can overcome the defenses from phylogenetically distantly related fungal hosts (BIRNBAUM; GERARDO, 2016; GERARDO et al., 2004, 2006b; MEIRELLES et al., 2015).

Fungus-growing ants are usually and informally classified in higher and lower attines, according to their morphological characteristics, social aspects, phylogeny and type of fungiculture (MUELLER et al., 2018). The cultivars are vertically transmitted (from parental to offspring colonies) during the establishment of a new colony (CHAPELA et al., 1994). In the case of lower attines, they may also recruit new fungi from free-living stocks, increasing the genetic variability in the symbiosis (KELLNER et al., 2013; MEHDIABADI, SCHULTZ, 2009; MUELLER et. al., 1998). Such aquisitions might provide protective mechanisms towards infections (KELLNER et al., 2018). Althought horizontal transmission of *Escovopsis* has been proposed (CURRIE et al., 1999; AUGUSTIN et al., 2017), the amplitude of this mechanism and its ecological role in ant colonies are still poorly addressed.

Escovopsis trichodermoides is a recently described fungus associated with different lower attine ant species (MASIULIONIS et al., 2015; AR personal observation). This fungus

differs from other species of *Escovopsis* by the highly branched conidiophores and absence of vesicles, in addition to verrucose conidia. The patterns of interaction and the mechanisms involved towards its hosts are still unknown. To reveal new ecological traits in *Escovopsis*, we provide clues about the generalist nature of *E. trichodermoides*, with lack of host fidelity and low infection towards cultivars of lower attine ant colonies. This lifestyle of *E. trichodermoides* is uncommon and reported for the first time in the attine ant-fungal symbiosis.

2. MATERIAL AND METHODS

2.1. Preservation and maintenance of fungal cultures

Fungi examined in this study are kept in the collection of the Laboratory of Fungal Ecology and Systematics (LESF), UNESP - Rio Claro, State of São Paulo, Brazil (Table 1). *Escovopsis* fungi (n = 6 strains) were maintained as conidial suspensions (in glycerol 10%) at -80 °C and in sterile distilled water at 10 °C. Strains were revived on Potato Dextrose Agar medium (PDA; Acumedia, final pH: 5.6 ± 0.2) supplemented with 150 μg mL^{-1} of chloramphenicol, and incubated at 25 °C, in the dark, for 7 days. *Escovopsis* strains were maintained in agar slants with PDA and stored at 10 °C (working stocks). Cultures of the mutualistic fungi (n = 4 strains) were maintained by successive transfers on PDA every 20 days and kept at 25 °C in darkness.

All fungi were evaluated periodically to confirm the purity and maintenance of vigor of strains. Before each assay, fungal strains were previously grown on PDA at 25 °C in darkness until the 7^{th} (for Ascomycetes) or the 20^{th} days (for the mutualistic fungi).

2.2. Molecular characterization of fungal cultivars

The mutualistic fungi used in this study were characterized. Mycelium of fungal isolates, previously grown on PDA, was harvested for genomic DNA extraction. This extraction was conducted by physical-chemical lysis following Lacerda et al. (2018).

The internal transcribed spacer (ITS) region, a fragment of the ribosomal large subunit (LSU) gene, and a segment of the translation elongation factor-1 alpha gene (*tef*1) were used in the molecular analyses (Table S1 for primers and conditions). The amplicons were purified with ExoSAP-IT™ PCR Product Cleanup kit (Thermo Fisher Scientific), and sequenced

using BigDye Terminator® v. 3.1 kit (Thermo Fisher Scientific). Forward and reverse sequences were generated on ABI 3500 sequencer (Thermo Fisher Scientific) and assembled in BioEdit v.7.0.5.3 (HALL, 1999). The consensus sequences were compared with homologous sequences deposited in GenBank. For the phylogenetic analyzes each gene/region were aligned with the dataset available in Mueller et al. (2018) in MAFFT v.7. (KATOH et al., 2013), followed by edition in GBLOCKS (CASTRESANA, 2000). All sequences used and their information are available in Table S2.

The datasets were concatenated in Winclada v. 1.00.08 (NIXON, 2002). The final alignment comprised 168 sequences and a total of 1511 bp (characters 1-417, 418-950 and, 951-1511 for *tef*1, ITS and LSU, respectively). Phylogenetic trees were reconstructed in MrBayes v.3.2.2 (RONQUIST et al. 2012) under the Bayesian inference. Nucleotide substitution models were selected in JModelTest 2 (DARRIBA et al., 2012), using Bayesian Information Criterion with 95% of confidence interval. The selected models were: HKY+I+G for *tef*1 and ITS partitions, and GTR+I+G for LSU. Analyzes were carried out with 55.600.000 Markov Chain Monte Carlo (MCMC) generations, until the standard deviation of split frequencies was below 0.015. The first 25% of the MCMC generations were discarded, and the final tree was edited in FigTree v.1.4.3 (RAMBAUT, 2016). *Chlorophyllum agaricoides* (AFTOL 440) was used as outgroup of the analysis, according to Mueller et al. (2018).

2.3. Pairwise culture assays

To determine the antagonism of *E. trichodermoides* towards different strains of *Leucocoprinus* sp. (i.e. the ant fungal cultivars), dual-culture assays were performed following the method by Silva et al. (2006), and herein referred as Dual-culture type 1. Mycelial fragments (0.5 cm²) of the mutualistic fungus were placed 1.5 cm from the edge of a Petri dish containing PDA. This system was incubated for 14 days at 25 °C in darkness. Then, a mycelial fragment (0.5 cm²) from each of the six *E. trichodermoides* strains was placed 3 cm from the mycelium of the mutualistic fungus (Figure S1). Two controls were prepared: (i) *Leucocoprinus* sp. strains growing alone, and (ii) *E. trichodermoides* strains growing alone.

The dual-cultures as well as the controls were incubated for 10 days at 25 °C in darkness, and plates were scanned on the days 1, 2, 3, 5, 7 and 10. Growth areas of both fungi were measured (in cm²) in ImageJ v.1.8.0_112 (SCHNEIDER; RASBAND; ELICEIRI, 2012). Each of the six *E. trichodermoides* strains were considered as a replicate in these

experiments (using the mean of eight plates per strain). Each control was prepared containing six plates as replicates.

2.4. Bioassays with multiple host possibilities

To determine the host fidelity by *E. trichodermoides* towards different host possibilities, bioassays with chance of choice were performed (herein referred as Dual-culture type 2). These bioassays were conducted according to Gerardo et al. (2006a) and an experimental design was performed with Petri dishes (150 x 15 mm) containing 60 mL of PDA (Figure S1). The culture medium was cut with a sterile scalpel to create six equidistant tracks. Two sets were carried out to provide different host possibilities: in the first set (i) mycelium fragments of the four *Leucocoprinus* strains (AR01, AR02, QVM2 and QVM12) were placed at the end of the four tracks, a mycelium fragment of *Moniliophthora perniciosa* (LESF1140) was placed on the fifth end, and the sixth end was left blank (control). In the second set (ii) mycelium fragments of *Leucocoprinus* strains (AR01, QVM2, QVM11 and RB03) were placed at the end of the four tracks, a mycelium fragment of *Leucoagaricus gongylophorus* (RB02) was placed on the fifth end, and the sixth end was left blank (control). The bioassays were incubated for seven days at 25 °C in darkness. The selection of *M. perniciosa* as a distant group was based on its phylogenetic distance, its distinct ecological role (i.e. plant pathogen, MONDEGO et al., 2008), and also the non-related lifestyle with attine ant gardens. In addition, this fungus was also selected as a comparative group in another study (AUGUSTIN et al., 2017).

Afterwards, a mycelium fragment (0.5 cm^2) of each *E. trichodermoides* strain or *T. atroviride* (LESF118) as placed at the center of the plate. This system was incubated at 25 °C in the dark for 28 and 14 days for the first and second bioassays, respectively. Growth distances (in cm) towards each end of the tracks were measured as described (item 2.3). *Trichoderma atroviride* (LESF 118) was used as a comparative group for *E. trichodermoides*, due to its ecological role (i.e. mycoparasite) and because it belongs to Hypocreaceae but did not coevolve with the ants (DE MAN et al., 2016; DRUZHININA et al., 2009; KUBICEK et al., 2011). Each assay was conducted with ten plates, and each *E. trichodermoides* strain was considered a replicate (using the mean of ten plates per strain).

2.5. Production of soluble antifungal metabolites

To evaluate if the antagonism of *E. trichodermoides* could be mediated by interference competition, the production of metabolites was assessed following the method by Varanda-Haifig et al. (2017) with modifications. Two types of *E. trichodermoides* filtrates were obtained: (i) in the absence of the mutualistic fungi (Et1) and (ii) in the presence of the mutualistic fungi (Et2). For the production of both filtrates, *E. trichodermoides* strains were previously grown on PDA at 25 °C for 10 days. Conidial suspensions were prepared according to Newmeyer (1990) in 0.05% Tween 80 solution and adjusted to 10^6 conidia mL^{-1} in a NeuBauer chamber.

Two Erlenmeyer flasks (125 mL) with 90 mL of Potato Dextrose Broth medium (PDB; Acumedia, final pH: 5.1 ± 0.2) were used for production of filtrates. To prepare the Et1 filtrates, 1 mL of the conidia suspension was inoculated in flasks and then incubated at 25 °C at 120 rpm for 14 days. To prepare the Et2 filtrates, five fragments (0.5 cm^2) of each mutualistic fungus were inoculated, and the flasks incubated at 25 °C at 120 rpm for 3 days. Then, 1 mL of conidia suspension was inoculated, and the flasks incubated under the same conditions for 14 days. After incubation the medium was filtrated in a 0.45 µm membrane (MF-Millipore, MCE membrane) and mixtured with double-strengthened PDA medium in a 1:1 ratio (v/v). For the control, PDB was added in a 1:1 ratio (v/v) with double-strengthened PDA, simulating absence of metabolic production.

Then, a mycelium fragment (0.5 cm^2) of each mutualistic fungus was placed at the center of a Petri plate with the respective prepared media. Plates were incubated at 25 °C in darkness, and growth areas (in cm^2) were recorded at the 3, 7, 10, 14, 21, 28 and 35 days of incubation (item 2.3). Each of the six *E. trichodermoides* strain was considered a biological replicate (using the mean of eight plates per strain). The control consisted in six plates.

2.6. Assays in live colonies of *Mycocepurus goeldii*

We performed assays in colonies of *Mycocepurus goeldii* to characterize the effects of *E. trichodermoides* infections. A total of twenty queen-less colonies were collected in Anhembi (State of São Paulo, Brazil), from March 18th to 20th, 2018. After excavation, fungus gardens along with tending workers and brood were collected in plastic containers with a fine layer of gypsum at the base. The containers were previously submitted to UV exposure for 30 minutes. Fungal isolation from colonies was conducted following Rodrigues et al. (2008a) transferring seven gardens fragments to PDA plates supplemented with 150 µg mL^{-1} of chloramphenicol (see details in the Supplementary Material). Colonies were transferred to

new containers of 250 mL or 500 mL depending on the size of the fungus gardens. These containers had one or two holes (1.0 cm in diameter) for ant mobility. Finally, these containers with fungus gardens were placed in a larger container (1000 mL) with a hole to insert or remove cornmeal flour as substrate for ant foraging (Figure S1). Such system was kept for acclimation in darkness for three days.

The experimental design comprised 20 colonies distributed in groups of five, considering the size and age for homogeneity between treatments. Conidial suspensions of the *E. trichodermoides* LESF 003, LESF 895 and LESF 927 (selected for these experiments because they were isolated from *M. goeldii* colonies) were prepared in 0.05 % Tween 80 solution and adjusted to 10^6, 10^7 and 10^8 conidia mL^{-1} in a NeuBauer chamber. Using a hand spray (previously exposed to UV light for 30 minutes) 2 mL of each suspension were distributed in the fungus garden starting from 10^6 conidia mL^{-1} and increasing the concentrations in intervals of seven days, for a total of 21 days. The sham-treated group consisted of 0.05 % of sterile Tween 80 solution only. Each conidial suspension was also spread on PDA plates to check for conidia viability. Every two days, 0.2 g of cornmeal flakes was offered as food and the gypsum humidified with 1 mL of sterile deionized water.

The colonies were evaluated daily regarding the (i) survivalship, (ii) food incorporation on fungus gardens, (iii) presence of fungal infection indicated by fungal mycelium overgrowing the fungus gardens, (iv) final aspect of the fungus garden after consecutive exposures, and (v) accumulated garden weight relative to waste weight (considering the sum of the ratio between waste and garden weight for each exposure).

2.7. Statistical analyses

Mycelial growth areas of mutualistic fungi in the Dual-culture type 1 assays were compared to the control after 3, 5 and 10 days of experiment by: (i) Two Sample T-test with an alpha threshold of 0.05, or Welch Two Sample T-test for treatments that violated the parametric assumptions. We selected these days since they represent the first contact between fungi (day 3); the complete overgrowth of mutualistic fungi by *E. trichodermoides* (day 5); and the last day of experiment (day 10); (ii) One-way ANOVA followed by Tukey posthoc test with an alpha threshold of 0.05 for multiple comparisons, using the model available in Agricolae package (DE MENDIBURU, 2014). In this analysis we compared the relative growth of each mutualistic fungus (ratio of treatment by the respective control); (iii) Inhibition percentage (I %) of each mutualistic fungi with the formula: $I = [(C - Et)/C] * 100$,

where C indicates mean growth of control group, and Et the growth in the presence of *E. trichodermoides* on the tenth day of incubation. Shapiro-wilk and Bartlett tests were applied to check the normality and homoscedasticity assumptions of the data. Analyses were conducted in R v. 3.3.3 (R CORE TEAM, 2017).

The growth of *E. trichodermoides* towards the different mutualistic fungi in the Dual-culture type 1 was compared to the control using: (i) Mixed-ANOVA using treatments (between-subjects) and the days of culture (within-subjects) as factors. Multiple comparisons were conducted with Two-Sample T-test with an alpha threshold of 0.05 with Bonferroni correction. Data were transformed to log (x) for validation of parametric assumptions; (ii) The mycelial growth over time was also analyzed with non-parametric test for longitudinal data for repeated measures (nparLD with an alpha threshold of 0.05), using the same factors. The nparLD analysis was conducted using the "F1-LD-F1" model. Wald-type and ANOVA-type analyses were used, followed by paired comparisons between curves with model available in package nparLD (NOGUCHI et al., 2012). Both analyses were conducted during five days of growth, since the mycelium of *E. trichodermoides* completely covered the mycelium of *Leucocoprinus* at this time. Analyses were conducted in R v. 3.3.3 (R CORE TEAM, 2017).

Fidelity patterns on the Dual-culture type 2 were evaluated in radar charts disposing length values (in cm) over time, with each track of the Petri dish as the axis of the chart. Growth data were compared daily with Friedman test with an alpha threshold of 0.05, followed by Wilcoxon signed-rank test with an alpha threshold of 0.05 for multiple comparisons. Analyses were conducted separately for each day of growth, and were conducted in R.

The final growth of the mutualistic fungus in the presence of metabolites of *E. trichodermoides* was evaluated by: (i) One-way ANOVA followed by Tukey posthoc test with an alpha threshold of 0.05 for multiple comparisons. For treatments that violated the parametric assumptions, we applied Kruskal-Wallis, followed by Mann-Whitney U tests both with an alpha threshold of 0.05; (ii) Relative growth of each mutualistic fungus after 35 days of culture with One-way ANOVA, followed by Tukey posthoc test with an alpha threshold of 0.05 for multiple comparisons; (iii) Inhibition percentage (I %) using the growth values of control and treatments in the presence of metabolites (type 1 or 2) after 35 days of incubation. Analyzes were conducted in R.

For the *in vivo* assays, the colony survival percentage was expressed in a survival chart over time (Kaplan-Meier curve) with survival package (LUMLEY; THERNEAU, 2004) in R. The effect of successive exposures was evaluated by non-parametric multidimensional scaling

(NMDS) using Bray-Curtis index as the dissimilarity measure. Binary values were used to indicate survival and presence of fungal infection; absolute values for the amount of waste produced and number of times that food was incorporated; as well as ordinal data for final aspect of the fungus gardens (Figure S2). Charts were computed in PAST v.3.22 (HAMMER, HARPER, RYAN; 2001), in two-dimensions with the first two coordinates.

3. RESULTS

3.1. Inhibition of different hosts by *Escovopsis trichodermoides*

Escovopsis trichodermoides inhibited different strains of *Leucocoprinus* (Figure 1 and Table S3; Two Sample T-test and Welch Two Sample T-test, $P< 0.05$) with the lowest values of relative growth for QVM12 (Table 2; Tukey posthoc test, $P< 0.05$). The *Leucocoprinus* used in assays clustered in two distint clades within clade-2 of the lower attine ant fungiculture (Figure 1). Mycelial area was reduced at least 1.8 times for all strains towards *E. trichodermoides* compared to the control. In addition, we observed a darkening pattern in the colony of the mutualistic fungi at the contact zones with the antagonist (Figure 2), followed by host mycelial degeneration (Table S3). Inhibition was observed on the fifth day when cultivars were overlapped by *E. trichodermoides* mycelium, except for *Leucocoprinus* sp. QVM12 on the third day (Table 2 and Table S3; Two Sample T-test and Welch Two Sample T-test, $P< 0.05$). High inhibition percentage was observed in the tenth day (48.0%, 43.8%, 46.2%, 57.5% for AR01, AR02, QVM2 and QVM12, respectively).

Contrary to what as expected, we did not observe growth maximization of *E. trichodermoides* towards the different hosts (Figure 2). No statistical differences were observed between treatments and the control group (Figure 2; Mixed-ANOVA, $P> 0.05$), with a general pattern of growth.

3.2. Lack of host fidelity by *Escovopsis trichodermoides*

Absence of host preference was observed for *E. trichodermoides* towards the different mutualistic fungi (Figure 1 and 3). Growth until the end of the track was observed towards all mutualistic fungi (Tables S4 and S5). The growth pattern was similar to other fungi that did not coevolve with the ant cultivars such as *T. atroviridae* (Figure S3). On the other hand, *E. trichodermoides* was inhibited by *M. perniciosa* (Fig. S4), and was not inhibited by *L.*

gongylophorus (RB02), the fungus cultived by some leafcutter ant species (Tables S4 and S5). Thus, the absence of efficient defensive barriers towards *E. trichodermoides* was observed even for fungal cultivars that are phylogenetically distantly related (Figure 1 and 3; Wilcoxon signed-rank test, $P< 0.05$).

3.3. Interference competition by *Escovopsis trichodermoides*

Soluble metabolites produced by *E. trichodermoides* inhibited all *Leucocoprinus* strains in culture (Figure 4). Chemical compounds produced in both Et1 and Et2 filtrates had interference on the mutualistic fungi and reduced the mycelial growth area of the mutualistic fungi (Table S6; Tukey posthoc test, $P< 0.05$; Mann-Whitney U test, $P< 0.05$).

No statistical differences were observed between both methods by the end of the assays, however, in some cases, inhibition above 50% was observed in relation to control group (Table S6). The fungal cultivar QVM2 was the least inhibited (Tukey posthoc test, $P< 0.05$). Curiously, the mutualistic fungus AR02 presented initial basidiome formation in the presence of metabolites of *E. trichodermoides* (Figure 4 and Figure S5). Overall, the results indicated no differences in inhibition between the two methods used to produce metabolites.

3.4. Low virulence of *Escovopsis trichodermoides* in ant colonies

The experiments using queen-less colonies showed that *E. trichodermoides* does not present a destructive profile *in vivo* (Figure 5). The effects observed on infected colonies of *M. goeldii* indicated that the arsenal of *E. trichodermoides* is insufficient to overcome the colony defenses (i.e., mutualistic fungus, the ants and associated microbiome from the fungus garden). The first colony died after 12 days of experiment, despite the large amount of conidia inoculated in the second exposure (Figure 5). Colony death by fungal infection and total removal of the fungus garden by the ants were observed only in five out of twenty colonies (Figure 5 and S2). Thus, colony viability was stable until the second exposure, when the first death was recorded (Figure 5). Food incorporation was also observed along the experiments for two colonies exposed to *E. trichodermoides* LESF 927 (Fig. S2), and may have contributed for colony stability.

Phylogenetic analyses indicated that the majority of fungi maintained by *M. goeldii* colonies used in the experiment clustered with the same fungal strains used the dual-culture bioassays (Figure S6). Thus, essentially the fungal hosts from these colonies were similar to

the ones used in the *in vitro* experiments. Therefore, the generalist trait of *E. trichodermoides* is not efficient to overcome the colony defenses.

Colonies exposed to *E. trichodermoides* show stability towards the large number of viable conidia sprayed. Only colonies exposed to strain LESF 895 presented different spatial dispersion of the data for the evaluated parameters (Figure 6). After the third and final exposure, healthy colonies had viable conidia of *E. trichodermoides* isolated from the garden surface (Figure S7), indicating that the fungus remained in the system.

4. DISCUSSION

Symbiotic interactions are mediated by chemical metabolites for recognition, interference, and nutrition of symbiotic partners (AKIYAMA et al., 2005; GERARDO et al., 2006a; HEINE et al., 2018). *Escovopsis* fungi show host fidelity mediated in part by chemical interaction between the parasite and its host (BIRNBAUM; GERARDO, 2016; GERARDO et al., 2006a). However, occasional host-switching events occurred over the evolution of this interaction (GERARDO et al., 2004, 2006b; MEIRELLES et al., 2015; TAERUM et al., 2007). Here we showed that *E. trichodermoides* presents a generalist pattern with no host fidelity to different strains of *Leucocoprinus*. Such pattern is reported for the first time for the *Escovopsis*-ant cultivar association.

Escovopsis trichodermoides caused high growth inhibition towards the different cultivars tested *in vitro*. The defensive barriers of ant cultivars were insufficient to prevent infection by the antagonist. However, the mechanisms of infection of *E. trichodermoides* were insufficient towards queen-less colonies of *M. goeldii*, and damage was only observed after three successive exposures, with increased conidia dosages. Such patterns differ from other *Escovopsis* species, which were described to have high virulence and high host fidelity (CURRIE; MUELLER; MALLOCH, 1999; CURRIE, 2001; CUSTODIO; RODRIGUES, 2019; GERARDO et al., 2006a). Towards *L. gongylophorus*, the cultivar of some leafcutter ant species (MUELLER et al., 2018), we observed no defensive barriers that prevented *E. trichodermoides* to overgrow this cultivar (even considering the large phylogenetic distance from the lower attine ant cultivars). This is not the case for *Escovopsis kreiselii*, which is also associated with lower attine ants, but it could not overgrow the *L. gongylophorus* mycelium (CUSTODIO; RODRIGUES, 2019). These observations support the generalist pattern of *E. trichodermoides*.

Escovopsis trichodermoides has been found in low frequency only associated with lower attines (AR personal observation). Additional defensive barriers and complexity of colonies can play a role to prevent a successful infection in other fungicultures of attine ants in nature, since, not only the host defenses account for the host-parasite interaction (CURRIE et al., 1999; CURRIE; STUART, 2001; FERNÁNDEZ-MARÍN et al., 2006; RODRIGUES et al., 2008b). Despite the generalist pattern, there is a trade-off between the overcome of a new host (exhibiting new defenses) and the performance of the infection (ANTONOVICS et al., 2013). No differences in the growth of *E. trichodermoides* towards cultivars of attine ants were observed, but inhibition of the parasite was only observed against *M. perniciosa* (a fungus phylogenetic distant of the ant fungal cultivars). The phylogenetic distances between *Leucocoprinus* hosts did not prevent the infection by *E. trichodermoides*, different from the parasitism described for other *Escovopsis* (BIRNBAUM; GERARDO, 2016; GERARDO et al., 2006a), and in other symbiotic systems (GILBERT; WEBB, 2007).

Chemical mechanisms are associated with inhibition by *E. trichodermoides*. We observed a darkening pattern and mycelium degeneration in the mutualistic fungi at the contact zones with the antagonist, pattern also observed in other studies that performed similar experiments (SILVA et al., 2006; VARANDA-HAIFIG et al., 2017). It is believed this darkening of the colony might be associated with cell degeneration or antibiosis as a response by the host (FOLGARAIT; MARFETÁN; CAFARO, 2011**;** SAVOIE; MATA; BILLETTE, 1998; SILVA et al., 2006; VARANDA-HAIFIG et al., 2017). The production of soluble compounds as a mechanism of interference competition (WICKLOW 1992) was observed for *E. trichodermoides*, and also for other *Escovopsis* strains (DHODARY et al., 2018; HEINE et al., 2018; VARANDA-HAIFIG et al., 2017). This feature can be essential for colony infection. Interestingly, a strain of *Leucocoprinus* initiated the formation of a basidiome only in the presence of metabolites of *E. trichodermoides*. The event of a basidiome formation was previously reported in laboratory conditions for *Leucocoprinus* fungi associated with lower attines (MUELLER, 2002). The event we observed in this study might be associated with stress conditions or activation of metabolic patways for basidiome formation, by the soluble compounds of *E. trichodermoides*.

Escovopsis trichodermoides is so far found only associated with healthy colonies of lower attine ants (AR personal observation). Low infection was observed on queen-less colonies even when experimentally infected with high amounts of conidia. Susceptibility can be understood by the equilibrium of the interaction between the ants, *Escovopsis* and the fungal cultivar (KELLNER et al., 2018). In our assays, the ants were not highly affected by *E.*

trichodermoides, and the majority of colonies resisted to three successive exposures of conidia. Although *in vitro* assays with isolated cultivars showed high inhibition of the mutualistic fungus, *in vivo* assays indicated the role of the ants in maintaining the stability of the system. Here, towards *E. trichodermoides*, the queen-less colonies showed high survival percentage, besides the interference of not having a queen in this symbiotic system (KELLER; NONACS, 1993).

In the lower attine fungiculture, acquisition of free-living fungal cultivars by ants promotes the genetic diversity of the association (KELLNER et al., 2013; MEHDIABADI, SCHULTZ, 2009; MUELLER et. al., 1998). Such diversity can provide a better defense for the colonies against specialized pathogens (KELLNER et al., 2018). On the other hand, generalist antagonists may increase their own fitness by host-switching events. Our study revealed new ecological traits in the *Escovopsis*-fungal cultivar interaction, with low infection and lack of host fidelity, an antagonistic lifestyle that may have allowd host-switching events over the evolutionary time.

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6. CONFLICT OF INTEREST

The authors have declared no conflicts of interest.

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Tables

Table 1. Fungi examined in the present study.

Fungal ID[1]	Fungi	Isolation source	Ant colony ID	City, State
LESF 003	*Escovopsis trichodermoides*	*Mycocepurus goeldii*	VM1	Rio Claro, SP
LESF 310	*Escovopsis trichodermoides*	*Mycetophylax morschi*	AR14022604A1	Florianópolis, SC
LESF 311	*Escovopsis trichodermoides*	*Mycetophylax morschi*	AR14022604A2	Florianópolis, SC
LESF 312	*Escovopsis trichodermoides*	*Mycetophylax morschi*	AR14022604ALA	Florianópolis, SC
LESF 895	*Escovopsis trichodermoides*	*Mycocepurus goeldii*	QVM160527-03	Anhembi, SP
LESF 927	*Escovopsis trichodermoides*	*Mycocepurus goeldii*	QVM160528-07	Anhembi, SP
LESF 118	*Trichoderma atroviride*	*Atta sexdens rubropilosa*	Nest 39	Corumbataí, SP
AR01	*Leucocoprinus* sp.	*Mycetophylax morschi*	AR140227-01	Florianópolis, SC
AR02	*Leucocoprinus* sp.	*Mycetophylax morschi*	AR140227-02	Florianópolis, SC
QVM2	*Leucocoprinus* sp.	*Mycocepurus goeldii*	QVM160527-03	Anhembi, SP
QVM12	*Leucocoprinus* sp.	*Mycocepurus goeldii*	QVM160528-01	Anhembi, SP
QVM11	*Leucocoprinus* sp.	*Mycocepurus goeldii*	QVM160527-15	Anhembi, SP
RB03	*Leucocoprinus* sp.	*Mycocepurus goeldii*	RB180518-03	Anhembi, SP
RB02	*Leucoagaricus gongylophorus*	*Acromyrmex coronatus*	BLS170701-01	Rio Claro, SP
LESF 1140	*Moniliophthora perniciosa*	*Theobroma cacao*	CP44	-

[1] LESF: Laboratory of Fungal Ecology and Systematics (UNESP, Rio Claro, SP).

Table 2. *Leucocoprinus* growth in the dual-culture assays. Figures indicate the mean of relative mycelial area (± SD) between the control group and towards *Escovopsis trichodermoides* (Et). Different letters indicate significant statistical differences between groups on each day (Tukey test at 5%).

Days	AR01[1]	AR02[1]	QVM2[2]	QVM12[2]
3	0,94 ± 0,07a	0,98 ± 0,06a	0,96 ± 0,03a	0,84 ± 0,03b
5	0,75 ± 0,05ab	0,82 ± 0,06a	0,81 ± 0,04a	0,69 ± 0,02b
10	0,52 ± 0,05a	0,56 ± 0,04a	0,54 ± 0,03a	0,43 ± 0,02b

[1] Mutualistic fungi of *Mycetophylax morshi*.
[2] Mutualistic fungi of *Mycocepurus goeldii*.

Figures

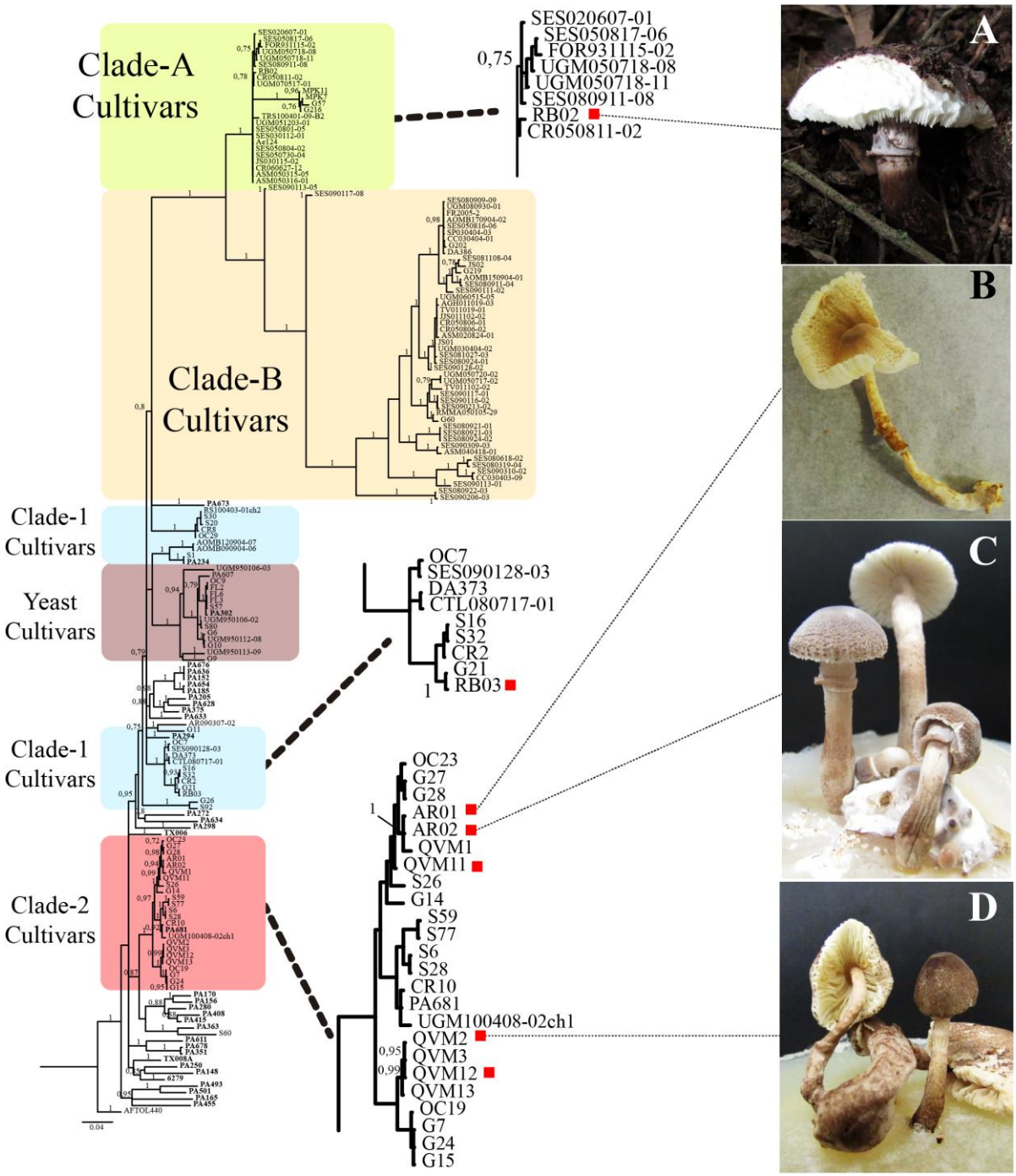

Figure 1. Phylogeny of fungal cultivars based on *tef*1, ITS and LSU markers (with 1511 bp in the final alignment). To characterize the position of strains from this study, the sequences were aligned with sequences from cultivars from Mueller et al. (2018). Free-living fungi (not in association with ant colonies) are shown in bold. *Chlorophyllum agaricoides* (AFTOL 440) were used as outgroup. Red squares on each clade indicate the position of strains from this study. The analysis was conduced using the Bayersian inference algorithm and the numbers on branches indicate posterior probabilities greater than or equal to 0.7. Information on the strains is available in Table S2. Each strain is indicated by the Sample ID code. Pictures of basidiomes of cultivars produced in culture. **A**: *Leucoagaricus gongylophorus* (RB02) associated with *Acromyrmex coronatus*. **B** and **C**: *Leucocoprinus* sp. associated with *Mycetophylax morschi* (AR01 and AR02, respectively). **D**: *Leucocoprinus* sp. associated with *Mycocepurus goeldii* (QVM2). Photos by Salomé Urrea Valencia (AR01), and Rodolfo Bizarria Jr. (RB02, AR02, and QVM2).

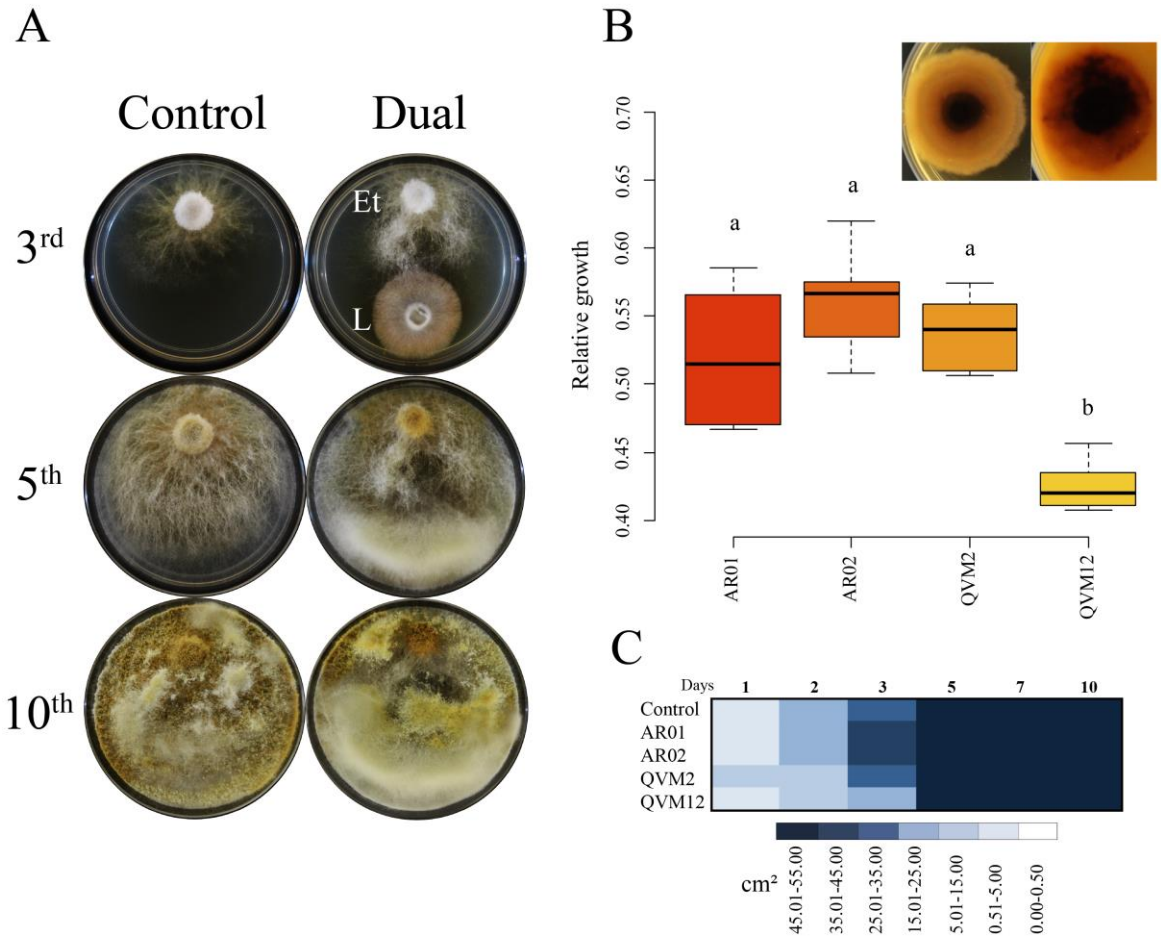

Figure 2. *Escovopsis trichodermoides* shows a generalist pattern of inhibition. A. Mycelial growth pattern in the absence (Control) and in interaction (dual-culture) with *Leucocoprinus* fungi. Photos indicate the 3rd, 5rd and 10th days of interaction between *E. trichodermoides* (Et) LESF 927 and *Leucocoprinus* (L) QVM2. B. Boxplot of relative growth of mutualistic fungi after 10 days of culture, different letters indicate significant differences (Tukey test, $P< 0.05$). Dual-culture plate after 10 days of assay showing darkening of the mutualistic fungus. Right and left indicate: mutualistic fungus in the absence and in the presence of *E. trichodermoides*, respectively. C. Heat maps of *E. trichodermoides* growth in dual-culture (values in cm²). No significant differences with the control group (Mixed-ANOVA, $P> 0.05$ and nparLD, $P> 0.05$).

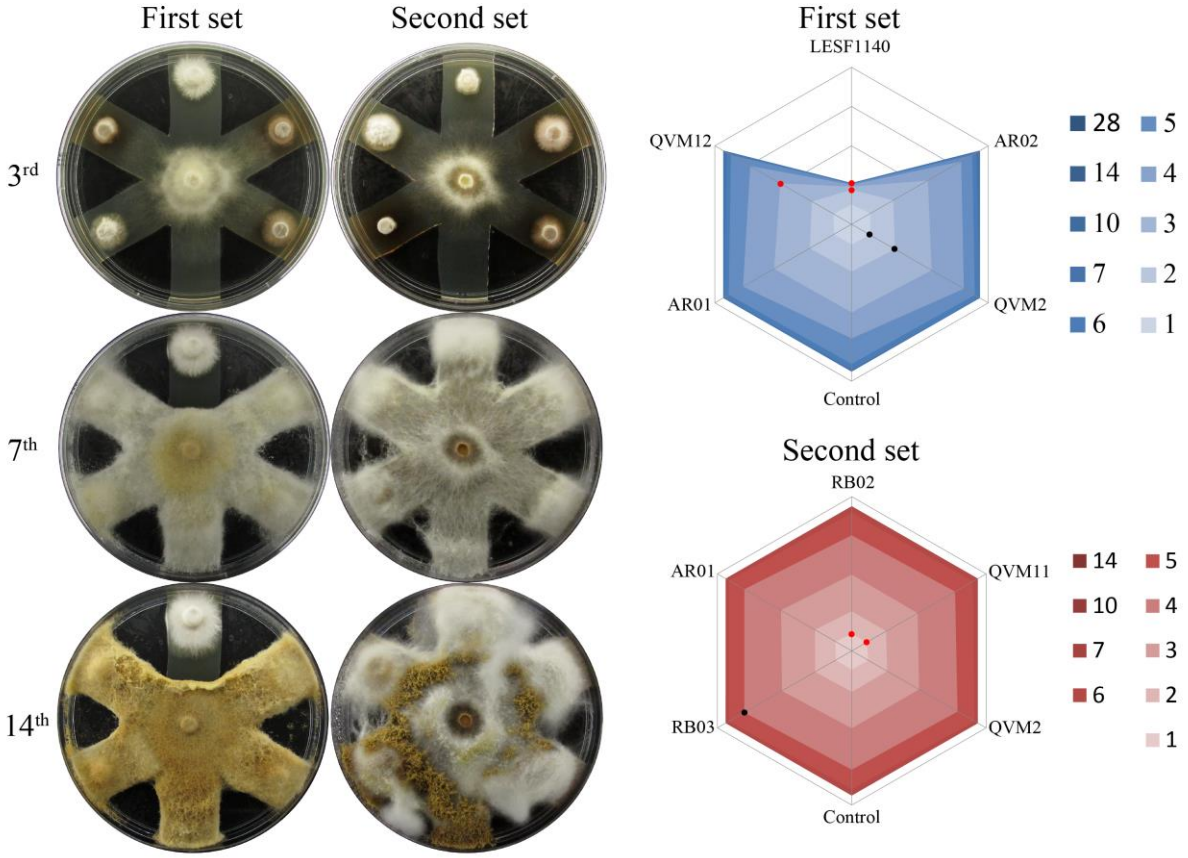

Figure 3. Lack of host fidelity by *Escovopsis trichodermoides*. Growth pattern of *E. trichodermoides* on PDA towards multiple fungal cultivars (hosts) in two sets, after the 3rd, 7th and 14th days of incubation. Information regarding the sets was described on session 2.4 of Material and Methods. Pictures show the growth of *E. trichodermoides* LESF 311 (First set) and LESF 895 (Second set). Radar charts on the right show the growth of *E. trichodermoides* over time (squares indicate each day of growth). The fungal cultivar strains IDs are indicated on the vertices of each chart. LESF 1140 and RB02 stand for *Moniliophthora perniciosa* and *Leucoagaricus gongylophorus*, respectively. The others IDs stand for *Leucocoprinus* sp. strains. Red dots on axis inficates lower values in relation to control group, while black dots indicate higher values (Wilcoxon signed-rank test, $P < 0.05$).

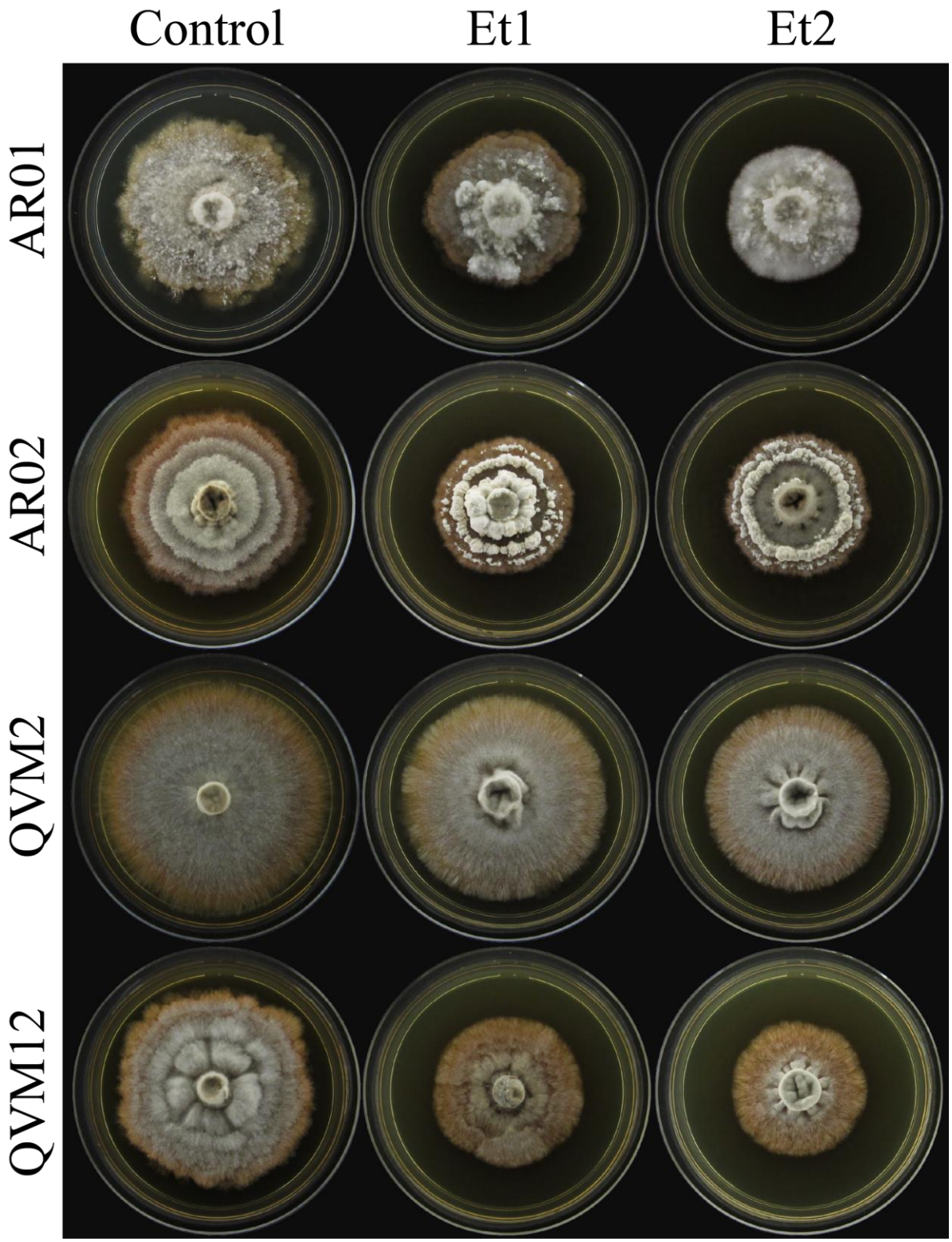

Figure 4. Interference competition by *Escovopsis trichodermoides* via metabolites production. Mycelial growth pattern of fungal cultivar *Leucocoprinus* spp. (AR01, AR02, QVM2 and QVM12) in the presence of metabolites of *E. trichodermoides* obtained from isolated culture (Et1), in dual-culture (Et2), and absence of metabolites (Control). Plates represent 35 days-old cultures. Note the presence of initial basidiome formation (for AR02 in Et1 and Et2) in the presence of metabolites.

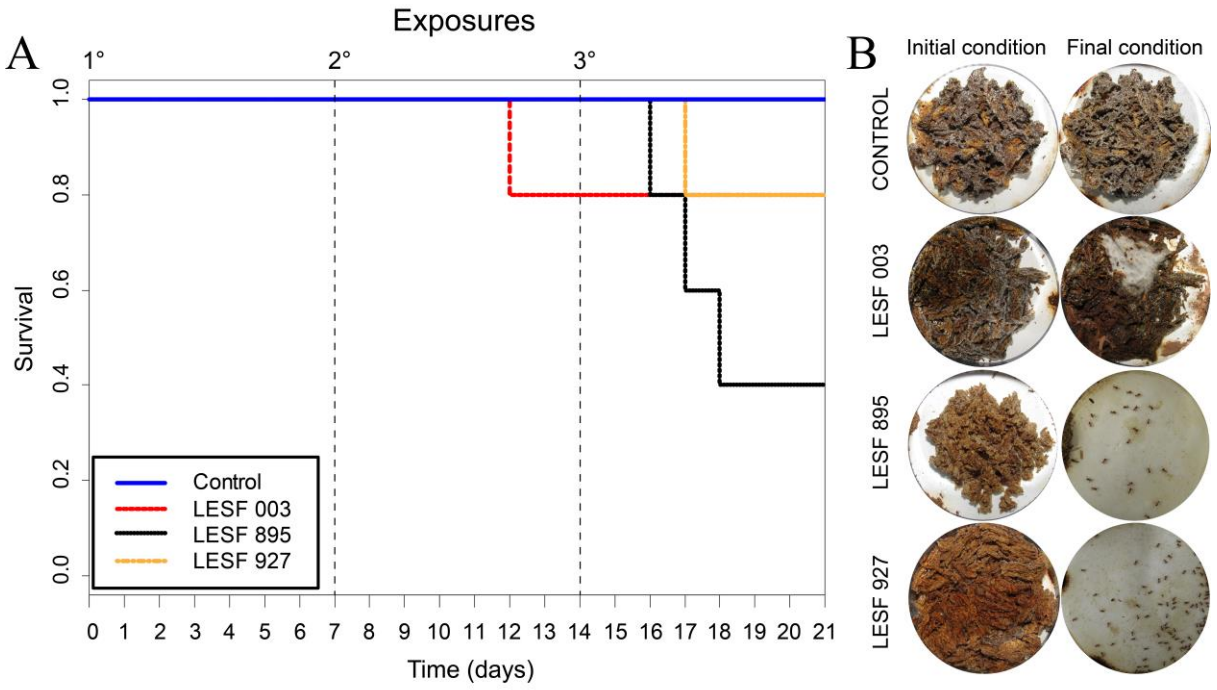

Figure 5. Low infection of *Escovopsis trichodermoides* in colonies of the lower attine ant *Mycocepurus goeldii*. A. Colony survival after three successive exposures with increased concentrations of conidia over time (days). Vertical dashed lines indicate the second and third exposures. Concentrations of 10^6, 10^7 and 10^8 conidia mL^{-1} were used for the first, second and third exposures, respectively. B. Initial and final condition (after the third exposure) of colonies on trials with each *E. trichodermoides* strain.

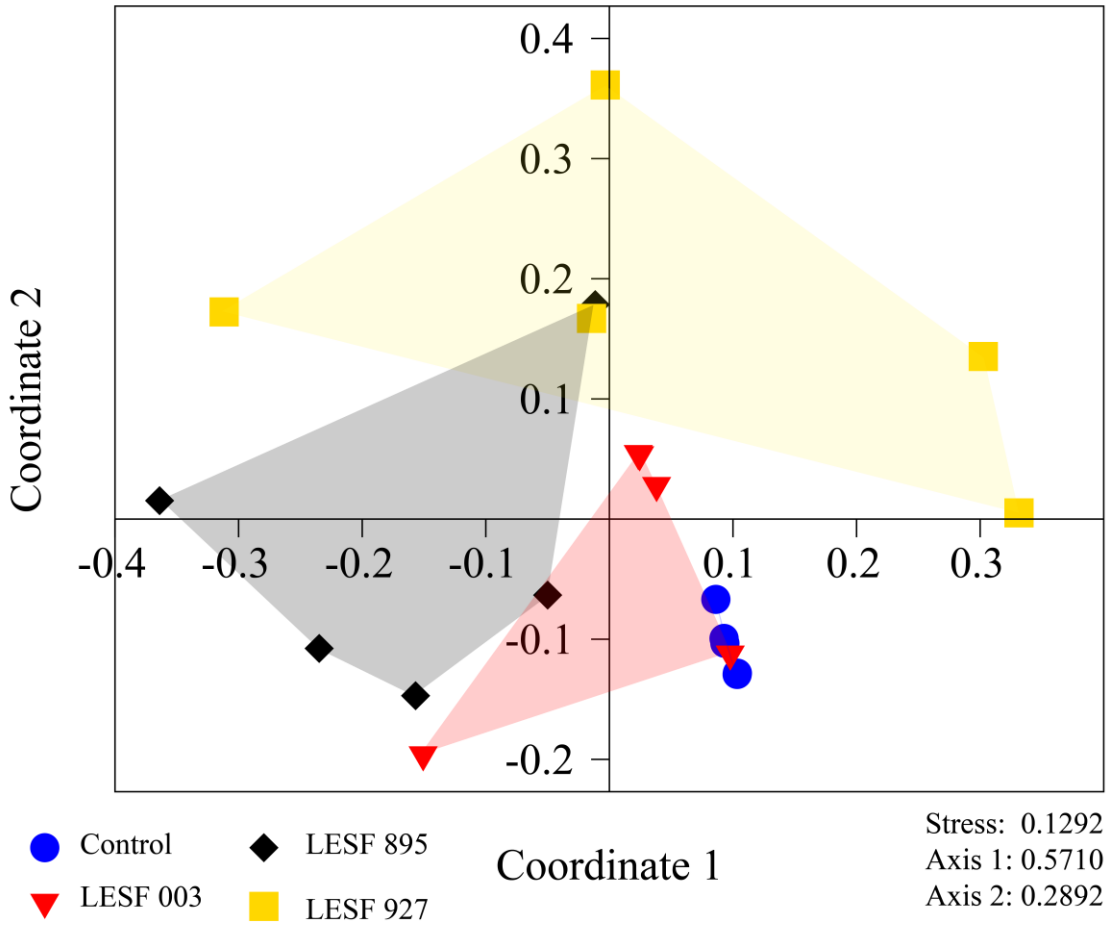

Figure 6. Effects of conidia exposure of *Escovopsis trichodermoides* on *Mycocepurus goeldii* colonies. Non-metric multidimensional scaling (NMDS) using Bray-Curtis dissimilarity index. NMDS analyses clearly discriminates differences between control and exposure with *E. trichodermoides* LESF 895.

8. SUPPLEMENTARY MATERIAL

8.1. MATERIAL AND METHODS

8.1.1. Basidiome formation by *Leucocoprinus* sp.

To access the morphological differences between cultivars, we induced basidiome formation of *Leucocoprinus* sp. isolates. Mycelium fragments of a 20 day-old culture were transferred to 500 mL Erlenmeyers containing 200 mL of oatmeal agar (OA: 50 g of oat flakes boiled in 1 L of deionized water and 18 g L^{-1} agar). Flasks were incubated for approximately 2 months at 25 °C in darkness, until the formation of fruiting bodies. For isolate AR02, basidiome formation was observed only in the presence of *Escovopsis trichodermoides* metabolites in Petri dishes (item 2.6). We also evaluated metabolites produced by *Trichoderma atroviride*, however, no basidiome formation was not induced (data not shown). After growth in the presence of *E. trichodermoides* metabolites, a mycelium fragment of the colony of isolate AR02 was transferred to PDA. After vigorous growth, a fragment of the mycelium was transferred to Erlenmeyers containing OA and incubated in the same conditions.

8.1.2. *Escovopsis trichodermoides* growth towards a fungus not related to the attine ant-fungus symbiosis

To determine the antagonism of *E. trichodermoides* towards a fungus not related to the attine ant-fungus symbiosis, we carried out the Dual-culture type 1 bioassays (item 2.3). Each of the six *E. trichodermoides* strains was challenged with *Moniliophthora perniciosa* (Basidiomycota: Agaricales: Marasmiaceae), a phylogenetic distant group from the cultivars of attine ants, with non-related lifestyle and distinct ecological role (i.e. plant pathogen). Each strain was considered a replicate (mean of eight plates). The control group of *M. perniciosa* was performed with six plates. Growth areas (in cm^2) were analyzed after 10 days of incubation at 25 °C in darkness.

8.1.3. Isolation, purification and preservation of fungi obtained in assays using live colonies

To evaluate the presence of *E. trichodermoides* and the cultivar associated to the colonies of *Mycocepurus goeldii* used in the *in vivo* experiments (item 2.6), we performed isolation according to Rodrigues et al. (2008) with modifications. Seven gardens fragments were transferred to PDA plates supplemented with 150 μg mL^{-1} of chloramphenicol (Sigma). Two PDA plates were used for each colony. Once growth of *Escovopsis* and *Leucocoprinus* was observed, we transferred these fungi to new PDA plates to obtain axenic cultures. Isolates were purified by monosporic culture, with serial dilution of conidia or mycelial fragments in sterile deionized water. An amount of 100 μL of suspensions was surface-spread on PDA supplemented with 150 μg mL^{-1} of chloramphenicol, and incubated at 25 °C in darkness until vigorous growth was obtained. Then, cultures were transferred to PDA slants and are maintained at 10 °C. Cultures of *Leucocoprinus* spp. are maintained by successive transfer every 30 days on PDA plates.

To evaluate the viability of *E. trichodermoides* in ant colonies after exposure to conidia suspensions, we selected *Mycocepurus goeldii* colonies that survived the treatments for fungal isolation. Isolation, purification and preservation of fungi were conducted as described.

8.1.4. Fungal identification

For morphological identification of *Escovopsis* isolates from colonies of *Mycocepurus goeldii*, fungal colonies were separated into morphotypes, and the traits of *Escovopsis trichodermoides* were evaluated in PDA at 25°C after seven days in the dark. Morphological characteristics such as colony surface, coloration, aerial mycelial growth, and the presence of pigments on agar were evaluated with a stereomicroscope. Slides were prepared with water to evaluate the microscopic characteristics (conidiophores, conidia and chlamydospores). Structures were observed under optical microscopy (LEICA DM 500).

For molecular identification of fungi isolates from colonies of *Mycocepurus goeldii*, *Leucocoprinus* sp. and *Escovopsis trichodermoides* isolates were submitted to DNA extraction (item 2.2). The internal transcribed spacer region (ITS) and elongation factor 1-alpha (*tef1*) were used for *Leucocoprinus* spp. and *E. trichodermoides*, respectively. PCR conditions and the primer pairs used are detailed in Table S1. The amplicons were purified with ExoSAP-IT™ PCR Product Cleanup kit (Thermo Fisher Scientific), and sequenced

using BigDye Terminator® v. 3.1 kit (Thermo Fisher Scientific). Forward and reverse sequences were generated on ABI 3500 sequencer (Thermo Fisher Scientific), and assembled in BioEdit v. 7.0.5.3 (HALL, 1999).

The consensus sequences were compared with homologous sequences deposited in GenBank. For phylogenetic analyzes, sequences were aligned with the sequences found in the dataset of Muller et al. (2018) for *Leucocoprinus* fungi (dataset 1), and with sequences from different studies (GERARDO et al., 2006; MASIULIONIS et al., 2015; MEIRELLES et al., 2015) for *E. trichodermoides* (database 2). Alignments were conducted in MAFFT v.7 (KATOH et al., 2013). In the final alignment, dataset 1 had 107 sequences with 727 bp, and dataset 2 had 139 sequences with 731 bp. All sequences and their respective information are available in Tables S7 and S8.

Phylogenetic inference was performed using Bayersian inference algorithm in MrBayes v.3.2.2 (RONQUIST et al., 2012). Models of nucleotide substitution were selected in jModelTest 2 (DARRIBA et al., 2012), using Akaike information criterion with 95% of confidence interval. GTR+I+G was used as model for nucleotide substitution for dataset 1, and HKY+I+G was used for dataset 2. Analyzes occurred with 2.2 and 1.5 million of Markov Chain Monte Carlo generations for database 1 and 2, respectively, until the standard deviation of split frequencies reached values below 0.01. Twenty-five percent of the first generations were discarded as burn-in, and final trees were generated and edited in FigTree v. 1.4. (RAMBAUT, 2016).

8.2. Tables

Table S1. Primers and PCR conditions used in the molecular analyses

Marker	Foward (5' → 3')	Reverse (5' → 3')	PCR conditions	Reference (Primer)
ITS (ITS5, ITS4)	GGAAGTAAAAG TCGTAACAAGG	TCCTCCGCTTA TTGATATGC	96 °C for 3 min; 35 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min; and 10 °C on hold	White et al. (1990)
LSU (LR0R, LR5 or LR7)[1]	ACCCGCTGAACT TAAGC	TCCTGAGGGAA ACTTCG TACTACCACCA AGATCT	96 °C for 3 min; 35 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min; and 10 °C on hold	Rehner and Samuels (1994) and Vilgalys and Hester (1990)
EF-1α (EF-1α-F, EF-1α-R)	GTTGCTGTCAAC AAGATGGACACT AC	GCCTTGATGAT ACCAGTCTCGA CACG	94°C for 3 min; 35 cycles of 94°C for 45 s, 51°C for 45 s; and 72°C for 1 s; 72°C for 10 min; and 10°C on hold	Mikheyev et al. (2006)
*tef*1 (EF6–20F, EF6A-1000R)	AAGAACATGATC ACTGGTACCT	CGCATGTCACG GACGGC	96°C for 3 min; 35 cycles of 96°C for 30 s, 61°C for 45 s; and 72°C for 1 min; and 10°C on hold	Meirelles et al. (2015)

[1]For the systematics analyses a region for a segment of LSU rDNA gene were used that corresponde to the first bases of LSU gene (regions amplified by LR0R-LR3 primers) according to Mueller et al. (1998) and Mueller et al. (2018).

Table S2. Metadata of sequences from fungal strains used in the molecular identification.

GenBank accessions			**Specimens information**		
ITS	***tef1***	**LSU**	**Sample ID**	**Ant host/Fungus**	**Location**
DQ779958	GQ854056		ASM050316-01	*Atta insularis*	Cuba: Mayabeque Province, Batabanó
JX259044	GQ854104		CR060627-12	*Trachymyrmex saussurei*	Mexico: Tamaulipas, El Encino, Gomes Farias
JX259045	GQ854358		UGM051203-01	*Trachymyrmex desertorum*	USA: Arizona, Gila County, 6.3 miles NNW Salt River on Rt288
JX259046	GQ854117		JS030115-02	*Trachymyrmex saussurei*	Mexico: Chiapas, Palenque
JX259051	JX258948	KT898385	CR050811-02	*Trachymyrmex arizonensis*	USA: Arizona, Cochise County, Chiricahua Mountains
DQ779956	GQ853928		Ae124	*Acromyrmex echinatior*	Panamá: Canal Zone, Gamboa
JX259047	GQ854260		SES050730-04	*Atta cephalotes*	Venezuela: Aragua, Rancho Grande Biological Station
EU561492	DQ767904		MPK11	*Atta colombica*	Panamá: Canal Zone, Gamboa
EU561491	DQ767905		MPK7	*Atta cephalotes*	Panamá: Canal Zone, Gamboa
EU561489	DQ767911		G216 = CC01110-06	*Acromyrmex* sp.	Panamá: Canal Zone, Parque Soberanía, Pipeline Road km2
		KP406344	TRS100401-09-B2	*Apterostigma megacephala*	Brazil: Pará, Carajás National Forest
EU561490	DQ767906		G57 = UGM960411-05	*Acromyrmex* cf. *hystrix*	Guyana: Kurupukari
JX259048	GQ854055		ASM050315-05	*Atta insularis*	Cuba: Mayabeque Province, Batabanó
JX259049	GQ854270		SES050804-02	*Atta cephalotes*	Venezuela: Merida, Parque Recreacional La Palmita
DQ779959	GQ854243		SES020607-01	*Atta cephalotes*	Panamá: Bocas del Toro, Isla Colon
DQ779960	GQ854170		SES030112-01	*Atta cephalotes*	Mexico: Veracruz, Sierra de los Tuxtlas
JX259050	JX258949		UGM070517-01 = UGM060511-01	*Atta texana*	USA: Texas, Travis County, Hornsby Bend, Center for Environmental Research
JX259052	GQ854261		SES050801-05	*Atta cephalotes*	Venezuela: Aragua, Parque Nacional

					Henri Pittier
JX259053	JX258950		SES090113-05	*Trachymyrmex wheeleri*	Brazil: Amazonas, Manaus, Fazenda Dimona
JX259054	JX258951	KT898386	SES080911-08	*Acromyrmex* cf. *balzani*	Brazil: Minas Gerais, Base de Estudos do Pantanal
JX259055	GQ854291		SES050817-06	*Acromyrmex hystrix*	Venezuela: Delta Amacuro, Campamento Rio Grande
	GQ854325		UGM050718-08	*Trachymyrmex intermedius*	French Guiana: Arrondissement of Cayenne, Kaw
	GQ854326		UGM050718-11	*Trachymyrmex intermedius*	French Guiana: Arrondissement of Cayenne, Kaw
JX259056	GQ854108		FOR931115-02	*Atta sexdens*	Brazil: São Paulo, Botocatu
JX259062	JX258952		SES080921-01	*Trachymyrmex* species L	Brazil: Minas Gerais, Uberlândia
JX259057	JX258953		SES080909-09	*Trachymyrmex* sp.	Brazil: Mato Grosso do Sul, Fazenda Sao Bento
GU202430	DQ767909		G202 = UGM960816-01	*Sericomyrmex* cf. *amabilis*	Panamá: Chiriqui Province, Tole
JX259058	JX258954		UGM080930-01	*Atta laevigata*	Brazil: Goias, Jussara, Fazenda Pau, Reserve 19
JX259059	Footnote1		SES090111-02	*Trachymyrmex* sp.	Brazil: Amazonas, Manaus
GU202429	DQ767910		G219 = NMG011030-02	*Sericomyrmex* sp.	Panamá: Canal Zone, Parque Soberanía, Pipeline Road
EU561500	EU561426		JS02	*Trachymyrmex septentrionalis*	USA: Louisiana, Beauregard Parish, DeRidder
JX259088	JX258955		DA386	*Trachymyrmex papulatus*	Argentina: Tucumán Province, Tucumán, Ruta 340 between Las Tipas & San Javier
JX259060	JX258956		SES080911-04	*Trachymyrmex* sp.	Brazil: Mato Grosso do Sul, Fazenda Sao Bento, Capao
KT898377	GQ854036		AOMB150904-01	*Acromyrmex crassispinus*	Brazil: Paraná, Tibagi
JX259061	JX258957	KT898387	SES081108-04	*Trachymyrmex fuscus*	Brazil: Bahia, Palmeiras
JX259063	Footnote2		SES080924-01	*Trachymyrmex* species AV	Brazil: Minas Gerais,

					Uberlândia
JX259064	GQ854361		UGM060515-05	*Trachymyrmex septentrionalis*	USA: Texas, Baylor County, Round Timber, River Road
EU561499	EU561425		JS01	*Trachymyrmexseptentrionalis*	USA: Illinois, Madison County, Highland
JX259065	GQ854098	KT898388	CR050806-01	*Trachymyrmex arizonensis*	USA: Arizona, Cochise County, Chiricahua Mountains
JX259066	GQ854099		CR050806-02	*Trachymyrmex carinatus*	USA: Arizona, Cochise County, Chiricahua Mountains
EU561497	EU561413		AGH011019-03	*Trachymyrmex septentrionalis*	USA: Texas, Bastrop County, Stengl Biological Station
EU561493	EU561411		TV011019-01	*Trachymyrmex septentrionalis*	USA: Texas, Bastrop County, Stengl Biological Station
EU561498	EU561415		JJS011102-02	*Trachymyrmex septentrionalis*	USA: Texas, Bastrop County, Stengl Biological Station
JX259067	JX258958		SES081027-03	*Trachymyrmex* species BI	Brazil: Piauí, São Felix
EU561501	EU561433		ASM020824-01	*Trachymyrmex septentrionalis*	USA: Illinois, Pope County, Dixon Springs
JX259068	GQ854329	KT898389	UGM050720-02	*Trachymyrmex intermedius*	French Guiana: Arrondissement of Cayenne, Kaw
JX259069	JX258959		UGM050717-02	*Trachymyrmex intermedius*	French Guiana: Arrondissement of Cayenne, Kaw
KT898378	GQ854042		AOMB170904-02	*Atta laevigata*	Brazil: São Paulo, Thermas de Santa Barbara
KT898379	GQ854287		SES050816-06	*Atta laevigata*	Venezuela: Ciudad Guyana, Bolívar
KT898380	GQ854079		CC030404-01	*Atta vollenweideri*	Argentina: Chaco Province, Resistencia
KT898381	GQ854301		SP030404-03	*Atta vollenweideri*	Argentina: Chaco Province, Resistencia
KT898382	GQ853939		FR2005-2	*Atta vollenweideri*	Argentina: Formosa Province, Reserva

					Ecólogica El Bagual
KT898383	GQ854318		UGM030404-02	*Acromyrmex striatus*	Argentina: Chaco Province, Resistencia
EU561494	EU561412		TV011102-02	*Trachymyrmex septentrionalis*	USA: Texas, Bastrop County, Stengl Biological Station
JX259070	JX258960		SES090117-01	*Trachymyrmex relictus*	Brazil: Amazonas, Manaus, Fazenda Dimona
JX259071	GQ854146		RMMA050105-29	*Trachymyrmex* cf. *zeteki*	Panamá: Canal Zone, Gamboa
GU202428	DQ767907		G60 = UGM960412-13	*Trachymyrmex* sp.	Guyana: Upper Takutu-Upper Essequibo Region, Annai
JX259072	JX258961		SES090116-02	*Trachymyrmex* species AG	Brazil: Amazonas, Manaus, Fazenda Dimona
JX259073	JX258962		SES090213-02	*Trachymyrmex* species C	Brazil: Pará, Parauapebas
JX259074	JX258963		SES090128-02	*Trachymyrmex* species AK	Brazil: Pará, Alter do Chao
EU561495	EU561427		ASM040418-01	*Trachymyrmex septentrionalis*	USA: Texas, Jasper County, at Martin Dies State Park
JX259075	JX258964	KT898390	SES090309-03	*Trachymyrmex* cf. *iheringi*	Brazil: Rio Grande do Sul, Taquara
JX259076	JX258965		SES080921-03	*Trachymyrmex* species E	Brazil: Minas Gerais, Uberlândia
JX259077	JX258966		SES080924-02	*Trachymyrmex* species AC	Brazil: Minas Gerais, Uberlândia
JX259078	JX258967		SES080618-02	*Trachymyrmex* sp.	Brazil: São Paulo, Rio Claro, UNESP Campus
KT898384	GQ854077		CC030403-09	*Acromyrmex laticeps*	Argentina: Misiones Province, Parque Salto Encantado
JX259079	JX258968		SES080319-04	*Trachymyrmex* species G	Brazil: São Paulo, Rio Claro, UNESP Campus
JX259080	JX258969		SES090113-01	*Trachymyrmex diversus*	Brazil: Amazonas, Manaus, Fazenda Dimona
JX259081	JX258970	KT898391	SES090310-02	*Trachymyrmex* species F	Brazil: Rio Grande do Sul, Gramado
JX259082	JX258971		SES080922-03	*Trachymyrmex* species C	Brazil: Minas Gerais, Uberlândia
JX259083	JX258972		SES090206-03	*Sericomyrmex* sp.	Brazil: Pará, Belem
JX259084	JX258973		SES090117-08	*Trachymyrmex* sp.	Brazil: Amazonas, Manaus; Fazenda

					Dimona
AF079725	JX258974	AF079643	OC7 = TRS920824-01	*Mycetarotes paralellus*	Brazil: Amazonas, São Gabriel
AF079754	JX258975	AF079672	DA373	*Trachymyrmex papulatus*	Argentina: Tucumán Province, Tucumán, Instituto Lillo Garden
JX259085	JX258976		SES090128-03	*Trachymyrmex* species BG	Brazil: Pará, Alter do Chao
JX259086	JX258977		CTL080717-01	*Trachymyrmex* species BF	Brazil: São Paulo, Corumbatai
AF079673	DQ767912.2	AF079591	S1 = UGM951124-02	*Apterostigma auriculatum*	Panamá: Canal Zone, Gamboa
AF079715	DQ767915.2	AF079633	S16 = UGM951222-01	*Myrmicocrypta ednaella*	Panamá: Canal Zone, Gamboa
AF079710	JX258978	AF079628	S32 = UGM960120-02	*Mycocepurus tardus*	Panamá: Canal Zone, Parque Soberanía, Pipeline Road km6
AF079697	JX258979	AF079615	G21 = UGM960415-12	*Mycocepurus goeldii*	Guyana: Potaro-Siparuni Region, Paramaketoi
AF079698	JX258980	AF079616	CR2 = UGM950616-01	*Mycocepurus smithii*	Costa Rica: Limón Province, Puerto Limón, Playa Vizcaya
AF079737	GQ854136	AF079655	PA234	*Leucocoprinus* sp.	Panamá: Canal Zone, Parque Soberanía, Pipeline Road km6
	GQ854021		AOMB120904-07	*Trachymyrmex iheringi*	Santana da Boa Vista, Rio Grande do Sul, Brazil
	GQ854002		AOMB090904-06	*Trachymyrmex iheringi*	Brazil: Rio Grande do Sul, Taquara
JQ405705	JX258981		RS100403-01 ch2	*Mycocepurus smithii*	Panamá: Canal Zone, Gamboa
AF079728	JX258982	AF079646	CR8 = UGM950613-02	*Mycetosoritis vinsoni*	Costa Rica: Guanacaste, Parque Nacional Santa Rosa
AF079699	JX258983	AF079617	S30 = UGM960116-01	*Mycocepurus smithii*	Panamá: Canal Zone, Gamboa
AF079727	JX258984	AF079645	OC29	*Mycetosoritis hartmanni*	USA: Texas, Walker County, Sam Houston National Forest
EU561488	DQ767916		S20 = UGM951229-02	*Mycocepurus smithii*	Panamá: Canal Zone, Gamboa
AF079740	JX258985	AF079658	PA272	*Leucocoprinus* sp.	Panamá: Canal Zone, Parque Soberanía, Pipeline Road
AF079692	JX258986	AF079610	G6 = UGM960408-14	*Cyphomyrmex rimosus*	Guyana: Upper Demerara-Berbice Region, Kurupukari
AF079683	JX258987	AF079601	UGM950112-08	*Cyphomyrmex minutus*	Trinidad: Mansanillo – Mayaro Road,

					km44
AF079688	JX258988	AF079606	G10 = UGM960408-19	*Cyphomyrmex minutus*	Guyana: Upper Demerara-Berbice Region, Kurupukari
AF079696	DQ767913	AF079614	S80 = UGM960104-08	*Cyphomyrmex salvini*	Panamá: Canal Zone, Parque Soberanía, Pipeline Road km6
AF079681	JX258989	AF079599	UGM950106-02	*Cyphomyrmex minutus*	Trinidad: Simla Biological Station
EF527344	JX258990		PA607	*Leucocoprinus* sp.	Panamá: Panamá Province, El Llano – Cartí Road
AF079695	JX258991	AF079613	OC9 = UGM930224-05	*Cyphomyrmex rimosus*	Costa Rica: Heredia Province, La Selva Biological Station
AF079686	JX258992	AF079604	FL2 = UGM930317-05	*Cyphomyrmex minutus*	USA: Florida, Archbold Biological Station Location
AF079745	JX258993	AF079663	PA302	*Leucocoprinus* sp.	Panamá: Canal Zone, Parque Soberanía, Pipeline Road
AF079691	JX258994	AF079609	FL6 = UGM930813-01	*Cyphomyrmex rimosus*	USA: Florida, Archbold Biological Station
AF079687	JX258995	AF079605	FL3 = UGM930801-01	*Cyphomyrmex minutus*	USA: Florida, Orlando
AF079689	JX258996	AF079607	S57 = UGM951216-02	*Cyphomyrmex minutus*	Panamá: Canal Zone, Gamboa
AF079693	JX258997	AF079611	G9 = UGM960408-18	*Cyphomyrmex rimosus*	Guyana: Upper Demerara-Berbice Region, Kurupukari
AF079684	DQ767914	AF079602	UGM950113-09	*Cyphomyrmex minutus*	Trinidad: Simla Biological Station, radiotower road
AF079682	JX258998	AF079600	UGM950106-03	*Cyphomyrmex minutus*	Trinidad: Simla Biological Station
JX259087	JX258999		AR090307-02	*Mycetophylax cf. simplex*	Brazil: Santa Catarina, Florianópolis
EF527365	JX259000		PA634	*Leucocoprinus* sp.	Panamá: Canal Zone, Gamboa
AF079743	JX259001	AF079661	PA294	*Leucocoprinus* sp.	Panamá: Canal Zone, Parque Soberanía, Pipeline Road
AF079721	JX259002	AF079639	G26 = UGM960421-02	*Mycetophylax conformis*	Guyana: Georgetown, Timehri Airport (now Cheddi Jagan)
AF079676	JX259003	AF079594	S92 = UGM960619-01	*Cyphomyrmex costatus*	Panamá: Canal Zone, Parque Soberanía, Pipeline Road km6

AF079716	JX259004	AF079634	G11 = UGM960410-14	*Myrmicocrypta* cf. *infuscata*	Guyana: Upper Demerara-Berbice Region, Kurupukari
EF527323	JX259005		PA298	*Leucocoprinus* sp.	Panamá: Canal Zone, Parque Soberanía, Pipeline Road
EF527392	JX259006		PA673	*Leucocoprinus* sp.	Panamá: Canal Zone, Parque Soberanía, Pipeline Road
AF079736	JX259007	AF079654	PA205	*Leucocoprinus* sp.	Panamá: Canal Zone, Parque Soberanía, Pipeline Road
EF527360	JX259008		PA628	*Leucocoprinus* sp.	Panamá: Canal Zone, Gamboa
AF079751	JX259009	AF079669	PA375	*Leucocoprinus* sp.	Panamá: Canal Zone, Parque Soberanía, Pipeline Road
EF527364	JX259010		PA633	*Leucocoprinus* sp.	Panamá: Canal Zone, Gamboa
EF527400	JX259011		TX008A	*Leucocoprinus* sp.	USA: Texas, Travis County, Austin, Bridle Path 2400
AF482859	HM488931		6279	*Leucocoprinus brebissonii*	Netherlands
AF079738	DQ767922	AF079656	PA250	*Leucocoprinus* cf. *fragilissimus*	Panamá: Canal Zone, Parque Soberanía, Pipeline Road
AF079730	JX259012	AF079648	PA148	*Leucocoprinus* sp.	Panamá: Canal Zone, Parque Soberanía, Pipeline Road
EF527396	JX259013		PA678	*Leucocoprinus* sp.	Panamá: Canal Zone, Barro Colorado Island
AF079748	JX259014	AF079666	PA351	*Leucocoprinus* sp.	Panamá: Canal Zone, Parque Soberanía, Pipeline Road
AF079678	JX259015	AF079596	OC19 = TRS920823-01	*Mycetophylax faunulus*	Brazil: Amazonas, São Gabriel
AF079714	JX259016	AF079632	G7 = UGM960408-15	*Myrmicocrypta* cf. *buenzlii*	Guyana: Upper Demerara-Berbice Region, Kurupukari
AF079713	JX259017	AF079631	G24 = UGM960416-05	*Myrmicocrypta* cf. *buenzlii*	Guyana: Potaro-Siparuni Region, Paramaketoi
AF079677	JX259018	AF079595	G15 = UGM960414-16	*Mycetophylax faunulus*	Guyana: Potaro-Siparuni Region, Paramaketoi
JQ405710	JX259019		UGM100408-02ch1	*Mycocepurus smithii*	Panamá: Panamá Oeste Province, Corozales Afuera

AF079726	JX259020	AF079644	OC23 = TRS920807-11	*Mycetarotes acutus*	Brazil: Amazonas, Reserva Ducke
AF079722	JX259021	AF079640	G27 = UGM960421-06	*Mycetophylax conformis*	Guyana: Georgetown, Timehri Airport (now Cheddi Jagan)
AF079724	JX259022	AF079642	G28 = UGM960404-10	*Kalathomyrmex emeryi*	Guyana: Upper Takutu-Upper Essequibo Region, Pirara
AF079717	JX259023	AF079635	S26 = UGM960107-15	*Myrmicocrypta* species 1	Panamá: Panamá Province, El Llano – Cartí Road
AF079711	JX259024	AF079629	G14 = UGM960412-14	*Myrmicocrypta* cf. *buenzlii*	Guyana: Upper Takutu-Upper Essequibo Region, Annai
AF079709	JX259025	AF079627	CR10 = UGM950612-03	*Mycocepurus curvispinosus*	Costa Rica: Guanacaste, Parque Nacional Santa Rosa
EF527398	JX259026		PA681	*Leucocoprinus* sp.	Panamá: Canal Zone, Barro Colorado Island
AF079675	DQ767917	AF079593	S77 = UGM960503-03	*Cyphomyrmex costatus*	Panamá: Parque Soberanía, Pipeline Road km8
AF079680	DQ767918	AF079598	S59 = UGM951227-05	*Cyphomyrmex muelleri*	Panamá: Parque Soberanía, Pipeline Road km14
AF079700	JX259027	AF079618	S6 = UGM951213-01	*Mycocepurus smithii*	Panamá: Canal Zone, Gamboa
AF079708	JX259028	AF079626	S28 = UGM960110-03	*Mycocepurus* cf. *curvispinosus*	Panamá: Canal Zone, Gamboa
AF079741	JX259029	AF079659	PA280	*Leucocoprinus* sp.	Panamá: Canal Zone, Gamboa
EU561487	DQ767923		PA408	*Leucocoprinus* cf. *zamurensis*	Panamá: Canal Zone, Gamboa
AF079753	JX259030	AF079671	PA415	*Leucocoprinus* cf. *zamurensis*	Panamá: Canal Zone, Gamboa
AF079732	JX259031	AF079650	PA156	*Lepiota* cf. *abruptibulba*	Panamá: Canal Zone, Gamboa
AF079734	JX259032	AF079652	PA170	*Leucocoprinus* sp.	Panamá: Canal Zone, Gamboa
AF079750	DQ767919	AF079668	PA363	*Leucocoprinus* sp.	Panamá: Canal Zone, Gamboa
AF079701	DQ767920	AF079619	S60 = UGM951229-01	*Mycocepurus smithii*	Panamá: Canal Zone, Gamboa
EF527399	JX259033		TX006	*Lepiotaceae* sp.	USA: Texas, Travis County, Austin, Bridle Path 2300
EF527348	JX259034		PA611	*Lepiotaceae* sp.	Panamá: Canal Zone, Gamboa
EF527334	JX259035		PA493	*Lepiotaceae* sp.	Panamá: Parque Soberanía, Pipeline Road

EF527335	JX259036		PA501	*Lepiotaceae* sp.	Panamá: Parque Soberanía, Pipeline Road
EF527327	JX259037		PA455	*Lepiotaceae* sp.	Panamá: Parque Soberanía, Pipeline Road
AF079733	JX259038	AF079651	PA165	*Lepiotaceae* sp.	Panamá: Canal Zone, Gamboa
EF527394	JX259039		PA676	*Lepiotaceae* sp.	Panamá: Canal Zone, Gamboa
AF079731	JX259040	AF079649	PA152	*Lepiotaceae* sp.	Panamá: Canal Zone, Gamboa
EF527367	JX259041		PA636	*Lepiotaceae* sp.	Panamá: Canal Zone, Gamboa
AF079735	DQ767921	AF079653	PA185	*Leucocoprinus* cf. *subclypeolaria*	Panamá: Parque Soberanía, Pipeline Road km6
EF527380	JX259042		PA654	*Lepiotaceae* sp.	Panamá: Parque Soberanía, Pipeline Road km6
DQ200928	DQ457631	AY700187	AFTOL 440	*Chlorophyllum agaricoides*	Greece
-	-	-	AR01	*Mycetophylax morschi*	Brazil: Santa Catarina, Florianópolis
-	-	-	AR02	*Mycetophylax morschi*	Brazil: Santa Catarina, Florianópolis
-	-	-	QVM1	Sp.1	Brazil: São Paulo, Anhembi
-	-	-	QVM2	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
-	-	-	QVM3	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
-	-	-	QVM11	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
-	-	-	QVM12	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
-	-	-	QVM13	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
-	-	-	RB01[3]	*Acromyrmex coronatus*	Brazil: São Paulo, Rio Claro
-	-	-	RB02	*Acromyrmex coronatus*	Brazil: São Paulo, Rio Claro
-	-	-	RB03	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi

[1]Sequence for *tef*1 gene for isolate SES090111-02 was used in analyses and are available in the supplementary material of Mueller et al. (2018).
[2]Sequence for *tef*1 gene for isolate SES080924-01 was used in analyses and are available in the supplementary material of Mueller et al. (2018).
[3]Sequences obtained from a basidiome of *Leucoagaricus gongylophorus*.

Table S3. *Leucocoprinus* spp. growth in the absence and presence of *Escovopsis trichodermoides*. Values indicate mycelial area in cm^2 (± SD) of the control group (C), in the presence of *E. trichodermoides* (Et). Growth values in bold are statistically different from respective control group (Two Sample T-test, $P< 0.05$ for AR01 and AR02; and Welch Two Sample T-test, $P< 0.05$ for QVM2 and QVM12).

	AR01[1]		AR02[1]		QVM2[2]		QVM12[2]	
Days	**C**	**Et**	**C**	**Et**	**C**	**Et**	**C**	**Et**
0	8.73 ± 0.8	8.88 ± 0.3	7.67 ± 0.3	7.74 ± 0.2	11.95 ± 1.0	12.31 ± 0.2	4.56 ± 0.3	4.57 ± 0.1
1	10.27 ± 0.7	10.08 ± 0.3	8.43 ± 0.2	8.47 ± 0.4	13.53 ± 1.4	13.84 ± 0.5	5.62 ± 0.2	5.39 ± 0.3
2	11.34 ± 0.9	10.97 ± 0.6	9.21 ± 0.3	9.06 ± 0.6	15.29 ± 1.5	15.25 ± 0.5	6.89 ± 0.7	6.09 ± 0.3
3	12.24 ± 0.9	11.54 ± 0.9	10.05 ± 0.3	9.88 ± 0.6	16.89 ± 1.5	16.28 ± 0.5	7.62 ± 0.7	**6.39 ± 0.2**
5	14.59 ± 1.1	**11.00 ± 0.8**	11.91 ± 0.5	**9.78 ± 0.7**	19.91 ± 1.9	**16.17 ± 0.7**	9.21 ± 0.8	**6.39 ± 0.2**
7	17.09 ± 1.1	10.96 ± 1.1	13.86 ± 0.7	9.67 ± 0.5	23.28 ± 2.1	14.98 ± 0.9	10.98 ± 1.0	6.07 ± 0.3
10	19.90 ± 1.0	**10.35 ± 1.0**	16.72 ± 0.6	**9.40 ± 0.6**	27.71 ± 2.3	**14.92 ± 0.8**	13.73 ± 0.8	**5.84 ± 0.3**

[1] Mutualistic fungi of *Mycetophylax morshi*.
[2] Mutualistic fungi of *Mycocepurus goeldii*.

Table S4. Mycelial growth of *Escovopsis trichodermoides* "assay 1" towards different host possibilities. Figures indicate means in cm (±SD) for each host possibility during 7 days of incubation. * indicates higher values in relation to the control group, while ■ indicates lower values. Statistical differences were assessed by Friedman test with an alpha threshold of 0.05, performed for each day, followed by Wilcoxon signed-rank test for multiple comparisons.

Days	**AR01[1]**	**AR02[1]**	**QVM2[2]**	**QVM12[2]**	**LESF1140[3]**	**Control**
1	1.03 ± 0.1	1.10 ± 0.1	1.08 ± 0.1 *	1.05 ± 0.1	1.01 ± 0.1	1.03 ± 0.1
2	2.44 ± 0.5	2.43 ± 0.5	2.53 ± 0.5 *	2.32 ± 0.6	1.76 ± 0.3 ■	2.41 ± 0.5
3	4.52 ± 1.0	4.46 ± 0.9	4.68 ± 0.9	4.17 ± 1.1 ■	1.91 ± 0.3 ■	4.49 ± 1.0
4	6.35 ± 1.3	6.40 ± 1.5	6.56 ± 1.3	5.92 ± 1.5	2.01 ± 0.2 ■	5.81 ± 1.0
5	7.18 ± 0.8	7.05 ± 1.1	7.20 ± 0.7	7.05 ± 1.1	2.06 ± 0.2 ■	7.11 ± 0.7
6	7.45 ± 0.1	7.28 ± 0.5	7.50 ± 0.0	7.29 ± 0.5	2.10 ± 0.2 ■	7.39 ± 0.3
7	7.50 ± 0.0	7.44 ± 0.1	7.50 ± 0.0	7.50 ± 0.0	2.10 ± 0.2 ■	7.50 ± 0.0

[1] Mutualistic fungi of *Mycetophylax morshi*.
[2] Mutualistic fungi of *Mycocepurus goeldii*.
[3] Comparative fungal group (*Moniliophthora perniciosa*).

Table S5. Mycelial growth of *Escovopsis trichodermoides* "assay 2" towards different host possibilities. Figures indicate means in cm (±SD) for each host possibility during 7 days of incubation. * indicates higher values in relation to the control group, while ■ indicates lower values. Statistical differences were assessed by Friedman test with an alpha threshold of 0.05, performed for each day, followed by Wilcoxon signed-rank test for multiple comparisons.

Days	RB02[1]		QVM11[2]		QVM2[2]	RB03[2]		AR01[3]	Control
1	0.88 ± 0.3	■	0.90 ± 0.3	■	0.98 ± 0.4	0.95 ± 0.4		0.97 ± 0.4	0.97 ± 0.3
2	2.05 ± 0.7		2.04 ± 0.7		2.10 ± 0.7	2.18 ± 0.8		2.19 ± 0.7	2.12 ± 0.8
3	3.95 ± 1.0		3.91 ± 1.0		4.00 ± 0.9	4.15 ± 1.0		4.21 ± 0.8	4.01 ± 0.9
4	5.99 ± 1.2		6.15 ± 1.2		6.30 ± 1.1	6.39 ± 1.0	*	6.39 ± 1.0	6.16 ± 1.1
5	7.15 ± 0.8		7.21 ± 0.7		7.30 ± 0.5	7.31 ± 0.5		7.30 ± 0.5	7.17 ± 0.7
6	7.50 ± 0.0		7.49 ± 0.0		7.50 ± 0.0	7.50 ± 0.0		7.49 ± 0.0	7.50 ± 0.0
7	7.50 ± 0.0		7.50 ± 0.0		7.50 ± 0.0	7.50 ± 0.0		7.50 ± 0.0	7.50 ± 0.0

[1] Mutualistic fungus of *Acromyrmex coronatus*.
[2] Mutualistic fungi of *Mycocepurus goeldii*.
[3] Mutualistic fungus of *Mycetophylax morshi*.

Table S6. *Leucocoprinus* spp. growth in the presence and absence of *Escovopsis trichodermoides* metabolites. Values indicate mycelial area in cm^2 (± SD) of the control group (C), in the presence of metabolites of *E. trichodermoides* grown alone (Et1) and in dual culture (Et2). Bold values are statistically different from respective control group in final day (Tukey test, $P< 0.05$; Mann-Whitney U test, $P< 0.05$ for QVM12). Different letters indicate significant differences between groups of relative growth (RG) after 35 days of culture (Tukey test, $P< 0.05$).

	AR01[1]			**AR02[1]**			**QVM2[2]**			**QVM12[2]**		
Days	**C**	**Et1**	**Et2**	**C**	**Et1**	**Et2**	**C**	**Et1**	**Et2**	**C**	**Et1**	**Et2**
0	0.50 ± 0.0	0.50 ± 0.0	0.50 ± 0.0	0.50 ± 0.0	0.50 ± 0.0	0.50 ± 0.0	0.50 ± 0.0	0.50 ± 0.0	0.50 ± 0.0	0.50 ± 0.0	0.50 ± 0.0	0.50 ± 0.0
3	0.89 ± 0.1	0.87 ± 0.0	0.92 ± 0.1	0.81 ± 0.1	0.79 ± 0.0	0.85 ± 0.0	0.87 ± 0.1	0.79 ± 0.1	0.83 ± 0.1	0.75 ± 0.1	0.71 ± 0.0	0.75 ± 0.1
7	1.79 ± 0.2	1.82 ± 0.3	1.44 ± 0.2	2.02 ± 0.2	1.59 ± 0.2	1.40 ± 0.1	2.08 ± 0.3	1.62 ± 0.3	1.53 ± 0.1	1.40 ± 0.3	1.21 ± 0.1	1.09 ± 0.2
10	4.27 ± 0.3	2.78 ± 0.5	2.24 ± 0.6	3.92 ± 0.7	2.46 ± 0.3	2.56 ± 0.5	4.23 ± 0.5	2.82 ± 0.5	3.22 ± 0.5	4.30 ± 0.4	1.80 ± 0.1	1.98 ± 0.8
14	7.43 ± 0.8	4.63 ± 0.8	3.58 ± 1.2	6.53 ± 0.2	4.27 ± 0.5	4.12 ± 0.4	8.22 ± 0.8	6.03 ± 0.8	5.81 ± 0.6	6.44 ± 0.5	3.24 ± 0.4	3.69 ± 1.7
21	14.71 ± 2.0	8.44 ± 0.8	6.41 ± 2.0	12.56 ± 0.9	7.79 ± 0.6	6.98 ± 0.9	17.70 ± 1.8	14.48 ± 2.1	12.75 ± 2.0	13.56 ± 1.6	6.84 ± 1.0	7.81 ± 3.5
28	25.87 ± 2.5	13.48 ± 1.3	11.37 ± 2.8	22.11 ± 1.7	12.51 ± 1.3	12.04 ± 1.6	32.63 ± 2.3	29.03 ± 3.4	26.04 ± 2.9	24.43 ± 1.4	12.81 ± 1.7	14.74 ± 5.5
35	34.31 ± 2.9	**18.94 ± 1.8**	**15.86 ± 3.8**	30.21 ± 2.5	**17.72 ± 1.6**	**16.47 ± 2.5**	45.98 ± 3.9	41.73 ± 3.2	**38.20 ± 4.3**	33.79 ± 2.4	**19.84 ± 2.3**	**21.23 ± 6.4**
RG[3]		0.55b	0.46b		0.59b	0.55b		0.91a	0.83a		0.59b	0.63b
I%[4]		44.8	53.8		41.3	45.5		9.2	16.9		41.3	37.2

[1] Mutualistic fungi of *Mycetophylax morshi*.
[2] Mutualistic fungi of *Mycocepurus goeldii*.
[3] Relative growth
[4] Inhibition percentage

Table S7. Information of *Leucocoprinus* isolates used on all experiments.

ITS GenBank accessions	Sample ID[1]	Ant colony	Location
X	AR01 (AR140227-01)	*Mycetophylax morschi*	Brazil: Santa Catarina, Florianópolis
X	AR02 (AR140227-02)	*Mycetophylax morschi*	Brazil: Santa Catarina, Florianópolis
X	AR02b (AR140227-02)[2]	*Mycetophylax morschi*	Brazil: Santa Catarina, Florianópolis
X	QVM2 (QVM160527-03)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
X	QVM3 (QVM160527-06)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
X	QVM6 (QVM160527-09)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
X	QVM12 (QVM160528-01)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
X	QVM13 (QVM160528-05)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
X	RB03 (RB180518-03)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
X	RB05 (RB180518-05)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
X	RB06 (RB180518-06)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
X	RB07 (RB180518-07)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
X	RB08 (RB180518-08)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
X	RB09 (RB180518-09)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
X	EB10 (RB180518-10)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
X	EB11 (RB180519-01)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
X	RB12 (RB180519-02)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
X	RB15 (RB180519-06)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
X	RB16 (RB180519-07)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
X	RB17 (RB180519-08)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
X	RB19 (RB180519-10)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
X	RB20 (RB180519-11)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
X	RB21 (RB180519-12)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
X	RB22 (RB180519-13)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
X	RB23 (RB180520-01)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
X	RB24 (RB180520-02)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi
X	RB29 (RB180519-04)	*Mycocepurus goeldii*	Brazil: São Paulo, Anhembi

[1]Sample ID indicate the fungal code ID, followed by the colony code ID in parentheses.
[2] Sequences obtained from Basidiome of *Leucocoprinus* sp.

Table S8. Information of *Escovopsis* sequences used in phylogenetic analyses.

tef1 GenBank access	Sample ID	Host	Location
Escovopsis strains from Meirelles et al. (2015) dataset			
KM817142	NL001	*Atta capiguara*	Brazil: São Paulo, Botucatu
KM817143	NL002	*Atta capiguara*	Brazil: São Paulo, Botucatu
KM817144	NL005	*Atta sexdens*	Brazil: São Paulo, Botucatu
KF240730	NL007	*Atta sexdens*	Brazil: São Paulo, Botucatu
KM817123	ES002	*Atta sexdens*	Brazil: São Paulo, Rio Claro
KM817132	ES011	*Atta sexdens*	Brazil: São Paulo, Corumbatai
KM817133	ES012	*Atta sexdens*	Brazil: São Paulo, Corumbatai

KM817134	ES013	*Atta sexdens*	Brazil: São Paulo, Corumbatai
KM817135	ES014	*Atta sexdens*	Brazil: São Paulo, Corumbatai
KM817124	ES003	*Atta cephalotes*	Brazil: Pernambuco, Frei Caneca
KM817126	ES005	*Atta cephalotes*	Brazil: Mato Grosso, Alta Floresta
KM817130	ES009	*Atta cephalotes*	Brazil: Amazonas, Carreio da Varzea
KM817141	ES033	*Atta cephalotes*	Brazil: Pernambuco, Parauapebas
KM817145	RS105	*Atta laevigata*	Brazil: São Paulo, T. de S. Bárbara
KM817116	BA001	*Atta cephalotes*	Brazil: Bahia, Camacan
KM817117	BA002	*Atta cephalotes*	Brazil: Bahia, Camacan
KM817118	BA003	*Atta cephalotes*	Brazil: Bahia, Camacan
KM817119	BA004	*Atta cephalotes*	Brazil: Bahia, Camacan
KM817120	BA005	*Atta cephalotes*	Brazil: Bahia, Camacan
KM817121	BA006	*Atta cephalotes*	Brazil: Bahia, Camacan
KM817125	ES004	*Acromyrmex* sp.	Brazil: Bahia, Camacan
KM817127	ES006	*Acromyrmex coronatus*	Brazil: Mato Grosso, Alta Floresta
KM817128	ES007	*Ac. coronatus*	Brazil: Mato Grosso, Alta Floresta
KM817129	ES008	*Acromyrmex* sp.	Brazil: Pará, Santarem
KM817131	ES010	*Ac. landolti*	Brazil: São Paulo, Rio Claro
KM817136	ES025	*Ac. balzanii*	Brazil: São Paulo, Botucatu
KM817138	ES027	*Ac. rug. rugosus*	Brazil: São Paulo, Rio Claro
EU082802	RS019	*Ac. ambiguus*	Brazil: Rio Grande do Sul, Nova Petropolis
EU082803	RS020	*Ac. laticeps*	Brazil: Rio Grande do Sul, Nova Petropolis
EU082795	RS030	*Ac. lundi*	Brazil: Rio Grande do Sul, São Marcos
EU082797	RS053	*Ac. lundi*	Brazil: Rio Grande do Sul, Chuvisca
EU082796	RS055	*Ac. heyeri*	Brazil: Rio Grande do Sul, Chuvisca
EU082799	RS061	*Ac. heyeri*	Brazil: Rio Grande do Sul, Pelotas
EU082801	RS076	*Ac. coronatus*	Brazil: Rio Grande do Sul, Vacaria
KM817152	SES008	*Acromyrmex* sp.	Brazil: Rondonia, Faz. S. Sebastião
KM817113	AR003	*Ac. balzanii*	Brazil: Bahia, Ilhéus
KM817114	AR022	*Acromyrmex* sp.	Brazil: Bahia, Camacan
KM817115	AR033	*Acromyrmex* sp.	Brazil: Bahia, Camacan
KM817122	ES001	*Trachymyrmex* sp.	Rio Claro - SP, Brazil
KM817137	ES026	*Trachymyrmex* sp.	Rio Claro - SP, Brazil
KM817139	ES029	*Trachymyrmex* sp.	Brazil: Tocantis, Palmas
KM817140	ES030	*Trachymyrmex* sp.	Brazil: Tocantis, Palmas
KM817146	SES001	*Trachymyrmex* sp.	Brazil: São Paulo, Rio Claro
KM817147	SES002	*Trachymyrmex* sp.	Brazil: Goiás, Fazenda Pau Brasil
KM817148	SES003	*Trachymyrmex* sp.	Brazil: Minas Gerais, Uberlândia
KF240731	SES005	*Trachymyrmex* sp.	Brazil: Minas Gerais, Uberlândia
KM817150	SES006	*T. dichrous*	Brazil: Minas Gerais, Uberlândia
KM817151	SES007	*Trachymyrmex* sp.	Brazil: Minas Gerais, Uberlândia
KM817153	SES009	*Trachymyrmex* sp.	Brazil: Bahia, Palmeiras
KM817154	SES010	*T. diversus*	Brazil: Amazonas, Manaus
KM817149	SES004	*S. luederwaldti*	Brazil: Minas Gerais, Uberlândia
AY172623	*Escovopsis weberi*	*Atta* sp.	Brazil

KJ935030	*Escovopsis microspora*	*Ac. sub. molestans.*	Brazil: Minas Gerais, Viçosa
JQ855712	*Escovopsis moelleri*	*Ac. sub. molestans.*	Brazil: Minas Gerais, Viçosa
JQ855714	*Escovopsis lentecrescens*	*Ac. sub. subterraneus*	Brazil: Minas Gerais, Viçosa
KM817155	UT001	*Ac. octospinosus*	Caribbean island of Guadeloupe
KM817156	UT002	*Acromyrmex* sp.	Caribbean island of Guadeloupe
KM817157	UT003	*Acromyrmex* sp.	Panama: Gamboa
KM817158	UT004	*Atta colombica*	Gamboa, Panama
KM817159	UT005	*Acromyrmex* sp.	Argentina: Misiones
KM817160	UT006	*Atta cephalotes*	Panama: Gamboa
KM817161	UT007	*Atta colombica*	Panama: Gamboa
KM817162	UT008	*Atta colombica*	Panama: Gamboa
KM817163	UT009	*Atta colombica*	Panama: Gamboa
KM817164	UT010	*Atta sexdens*	Panama: Coclecito
KM817165	UT011	*Trachymyrmex* sp.	Panama: Gamboa
KM817166	UT012	*Trachymyrmex* sp.	Panama: "Canal Zone"
KM817167	UT014	*Atta colombica*	Panama: Darien
KM817168	UT015	*Atta colombica*	Panama: Darien
KM817169	UT016	*Trachymyrmex* sp.	Mexico: Palenque
KM817170	UT017	*Atta colombica*	Panama: Gamboa
KM817171	UT018	*Trachymyrmex* sp.	Panama: Gamboa
KM817172	UT019	*Atta cephalotes*	Mexico: Palenque
KM817173	UT020	*Trachymyrmex* sp.	Mexico: Palenque
AY172632	*Escovopsis aspergilloides*	*Trachymyrmex ruthae*	Trinidad and Tobago
JQ855713	*Escovopsioides nivea*	*Ac. sub. subterraneus*	Brazil: Minas Gerais, Viçosa
KJ808766	*Escovopsis kreiselii*	*Mycetophylax morschi*	Brasil: Santa Catarina, Florianópolis
Escovopsis strains from Gerardo et al. (2006b) dataset			
DQ848209	nmg011101-03	*Cyphomyrmex longiscapus*	Panama
DQ848208	ugm030327-05 esc4	*Apterostigma* sp.	Argentina
DQ848207	cc030106-02 escb	*Apterostigma* sp.	Panama
DQ848206	nmg031218-01 esc2	*Apterostigma auriculatum*	Panama
DQ848205	nmg031212-06	*Apterostigma* sp.	Panama
DQ848204	nmg031215-04	*Apterostigma* sp.	Panama
DQ848203	agh031215-02	*Apterostigma* sp.	Panama
DQ848202	nmg030614-01 esc1	*Apterostigma* cf. *pilosum*	Ecuador
DQ848201	cc030327-01 esc4	*Apterostigma* sp.	Argentina
DQ848200	sv030615-05 esc1	*Apterostigma* sp.	Ecuador
DQ848199	sv030615-04 esc1	*Apterostigma* sp.	Ecuador
DQ848198	cc030101-01	*Apterostigma* sp.	Panama
DQ848197	agh030627-03 esc2	*Apterostigma* sp.	Ecuador
DQ848196	agh030627-01 esc1	*Apterostigma* cf. *dentigerum*	Ecuador
DQ848195	agh030618-02 esc1	*Apterostigma* sp.	Ecuador
DQ848194	agh030609-03 esc1	*Apterostigma* sp.	Ecuador
DQ848193	nmg011029-03 esc1	*Apterostigma* sp.	Panama
DQ848192	ugm020531-04 esc1	*Apterostigma* sp.	Panama
DQ848191	al030609-03 esc1	*Apterostigma* sp.	Ecuador
DQ848190	nmg020521-04 esc1	*Apterostigma dentigerum*	Panama

DQ848189	al030618-10 esc1	*Apterostigma* sp.	Ecuador
DQ848188	ugm030106-02 escc	*Apterostigma* sp.	Panama
DQ848187	agh030222-12	*Apterostigma dentigerum*	Costa Rica
DQ848186	sv030614-02 esc1	*Apterostigma* cf. *pilosum*	Ecuador
DQ848185	nmg020519-02 esc2	*Apterostigma* cf. *pilosum*	Panama
DQ848184	nmg030618-01 esc1	*Apterostigma* cf. *pilosum*	Ecuador
DQ848183	ugm020531-01 esc2	*Apterostigma dentigerum*	Panama
DQ848182	agh030627-08 esc1	*Apterostigma* sp.	Ecuador
DQ848181	cc010325-06 esc2	*Apterostigma auriculatum*	Panama
DQ848180	sp011112-01 esc11	*Apterostigma dentigerum*	Panama
DQ848179	cc011213-31 esc1	*Apterostigma auriculatum*	Panama
DQ848178	cc011029-02 esc1	*Apterostigma auriculatum*	Panama
DQ848177	cc011018-04 esc1	*Apterostigma* sp.	Panama
DQ848176	agh020630-01 esc1	*Apterostigma dentigerum*	Costa Rica
DQ848175	nmg020611-02 esc7	*Apterostigma dentigerum*	Panama
DQ848174	nmg020611-02 esc6	*Apterostigma dentigerum*	Panama
DQ848173	agh020712-04 esc1	*Apterostigma dentigerum*	Costa Rica
DQ848172	agh020709-10 esc8	*Apterostigma dentigerum*	Costa Rica
DQ848171	agh020709-10 esc3	*Apterostigma dentigerum*	Costa Rica
DQ848170	agh020709-10 esc11	*Apterostigma dentigerum*	Costa Rica
DQ848169	agh020709-10 esc1	*Apterostigma dentigerum*	Costa Rica
DQ848168	agh020706-01	*Apterostigma dentigerum*	Costa Rica
DQ848167	agh020629-02 esc6	*Apterostigma dentigerum*	Costa Rica
DQ848166	abs020621-02 esc1	*Apterostigma dentigerum*	Costa Rica
DQ848165	ugm020602-07 esc1	*Apterostigma* sp.	Panama
DQ848164	nmg020611-02 esc2	*Apterostigma dentigerum*	Panama
DQ848163	cc020605-04 esc4	*Apterostigma* sp.	Panama
DQ848162	sp011112-01 esc1	*Apterostigma dentigerum*	Panama
DQ848161	agh020629-02 esc4	*Apterostigma dentigerum*	Costa Rica
DQ848160	agh020621-05 esc2	*Apterostigma dentigerum*	Costa Rica
DQ848159	nmg010318-21 esc2	*Apterostigma dentigerum*	Panama
DQ848158	nmg010816-05 esc19	*Apterostigma dentigerum*	Panama
DQ848157	nmg010816-05 esc1	*Apterostigma dentigerum*	Panama
DQ848156	nmg010802-02 esc1	*Apterostigma dentigerum*	Panama
Escovopsis derived from Masiulionis et al. (2015)			
KF033128	*Escovopsis trichodermoides*	*Mycocepurus goeldii*	Brazil
Isolates of this study			
-	ESCO13	*Mycocepurus goeldii*	-
-	ESCO20	*Mycocepurus goeldii*	-
-	POS1	*Mycocepurus goeldii*	-
-	POS3	*Mycocepurus goeldii*	-
Other species of Hypocreaceae used as outgroup			
AF534585	*Trichoderma viride*	-	-
AF534620	*Trichoderma hamatum*	-	-
AF534624	*Trichoderma pubescens*	-	-

8.3. Figures

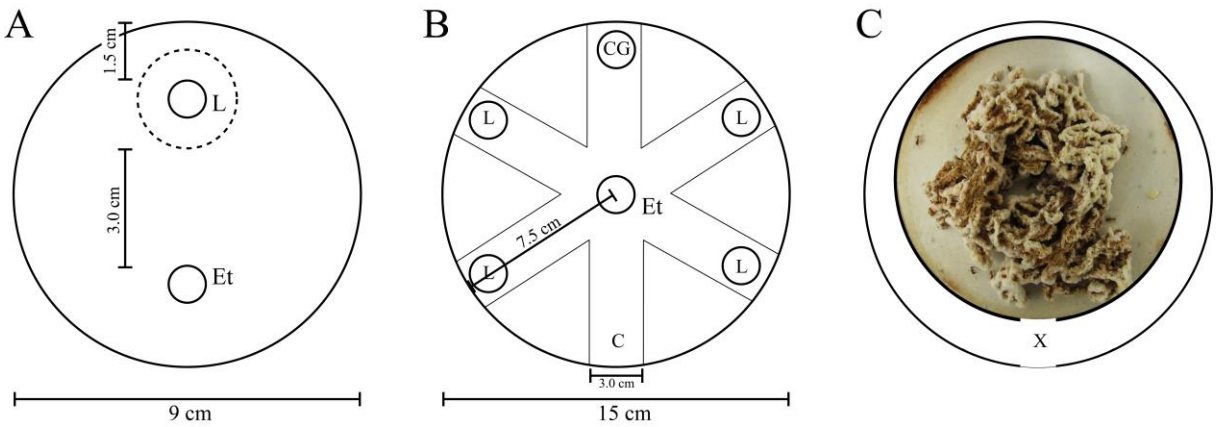

Figure S1. Experimental setup used in the plate assays. A. Pairwise culture assays displaying *Leucocoprinus* strains (L), its growth (after 14 days, dashed lines), and *Escovopsis trichodermoides* (Et). B. Bioassays with multiple host possibilities displaying *Leucocoprinus* strains (L) or a comparative group (CG), *E. trichodermoides* (Et) and Control (C) in the six equidistant tracks. C. Container (with gypsum at the base) used for assays with ant colonies (in the center), and feeding location (x) where cornmeal flakes were offered.

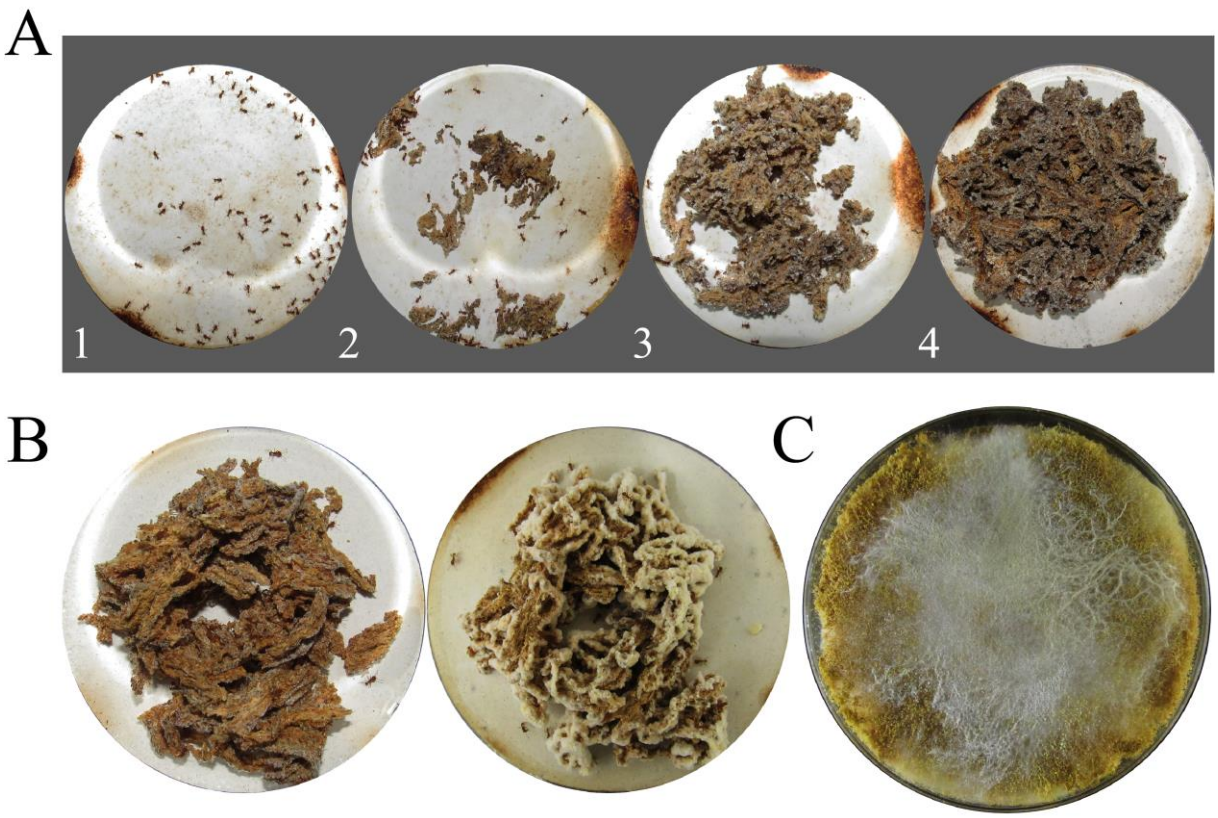

Figure S2. Assays performed in live colonies of *Mycocepurus goeldii*. A. Final aspects of different fungus garden after three successive exposures to *Escovopsis trichodermoides* conidia (the numbers indicate the ordination used for non-parametric multidimensional scaling). B. Initial aspect of the fungus garden (left) and after incorporation of food provided

(right). C. *E. trichodermoides* conidia grown on PDA plates in the viability check of the inoculum.

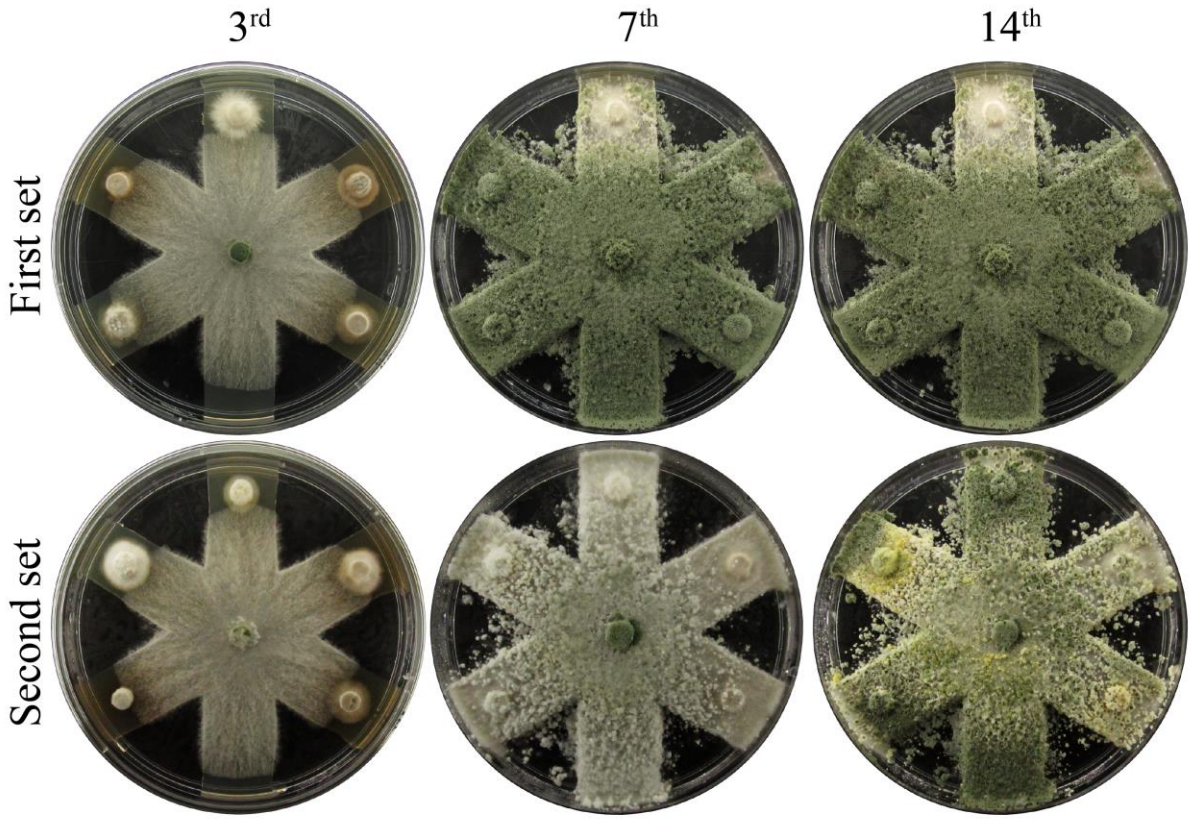

Figure S3. Growth pattern of *Trichoderma atroviride* in the fungal choice assay (Dual-culture type 2). In this assay growth of *T. atroviride* was observed on PDA in two sets. Information about the sets was described on session 2.4 of Material and Methods. Observations were carried out on the 3rd, 7th and 14th day of incubation.

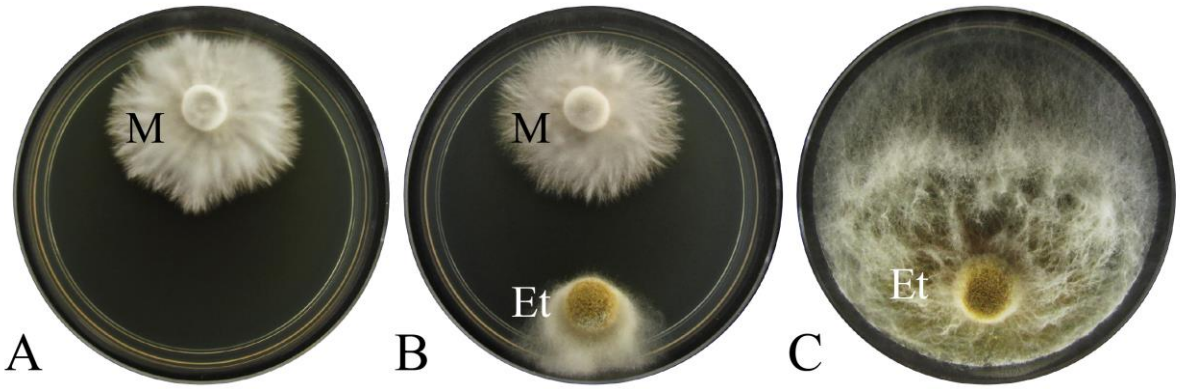

Figure S4. Growth of *Escovopsis trichodermoides* LESF 927 (Et) towards *Moniliophthora perniciosa* LESF 1140 (M) in the dual-culture type 1 experiment. No change in *M. perniciosa* growth was observed after 10 days of incubation (Mann-Whitney test, P = 0.4848). Pictures denote (A) the growth of *M. perniciosa*; (B) the interaction between *E. trichodermoides* and

M. perniciosa and(C) the growth of *E. trichodermoides* on the 5th day of incubation. Note the growth inhibition of *E. trichodermoides* relative to its control.

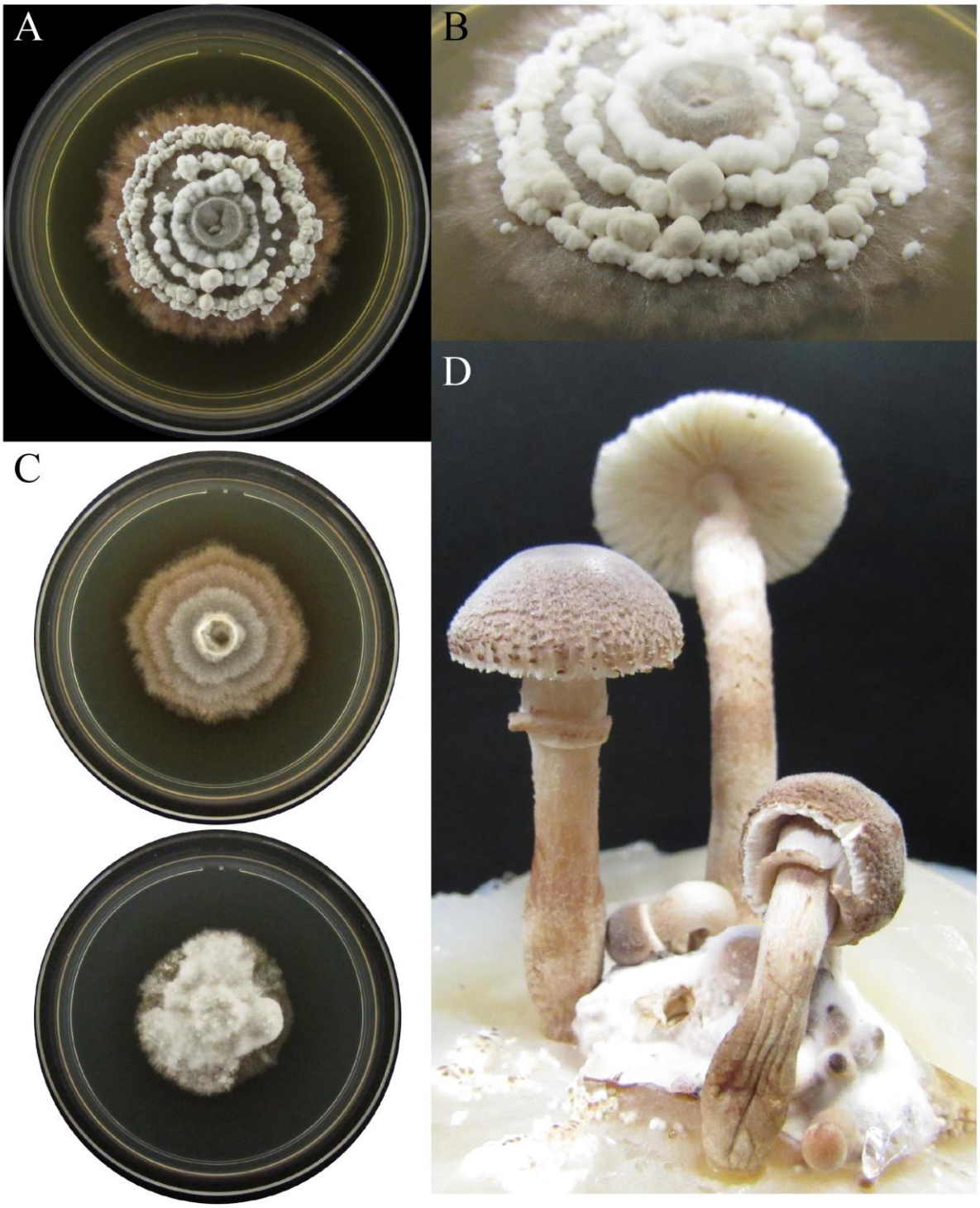

Figure S5. Basidiome formation of the fungal cultivar *Leucocoprinus* sp. AR02. A. Colony aspect after 35 days of incubation on PDA supplemented with metabolites produced by *Escovopsis trichodermoides*. B. Early stages of basidiome formation. C. Macromorphological changes in the colony, before (up) and after exposed to metabolites (bottom). D. Basidiomes after 2 months on OA.

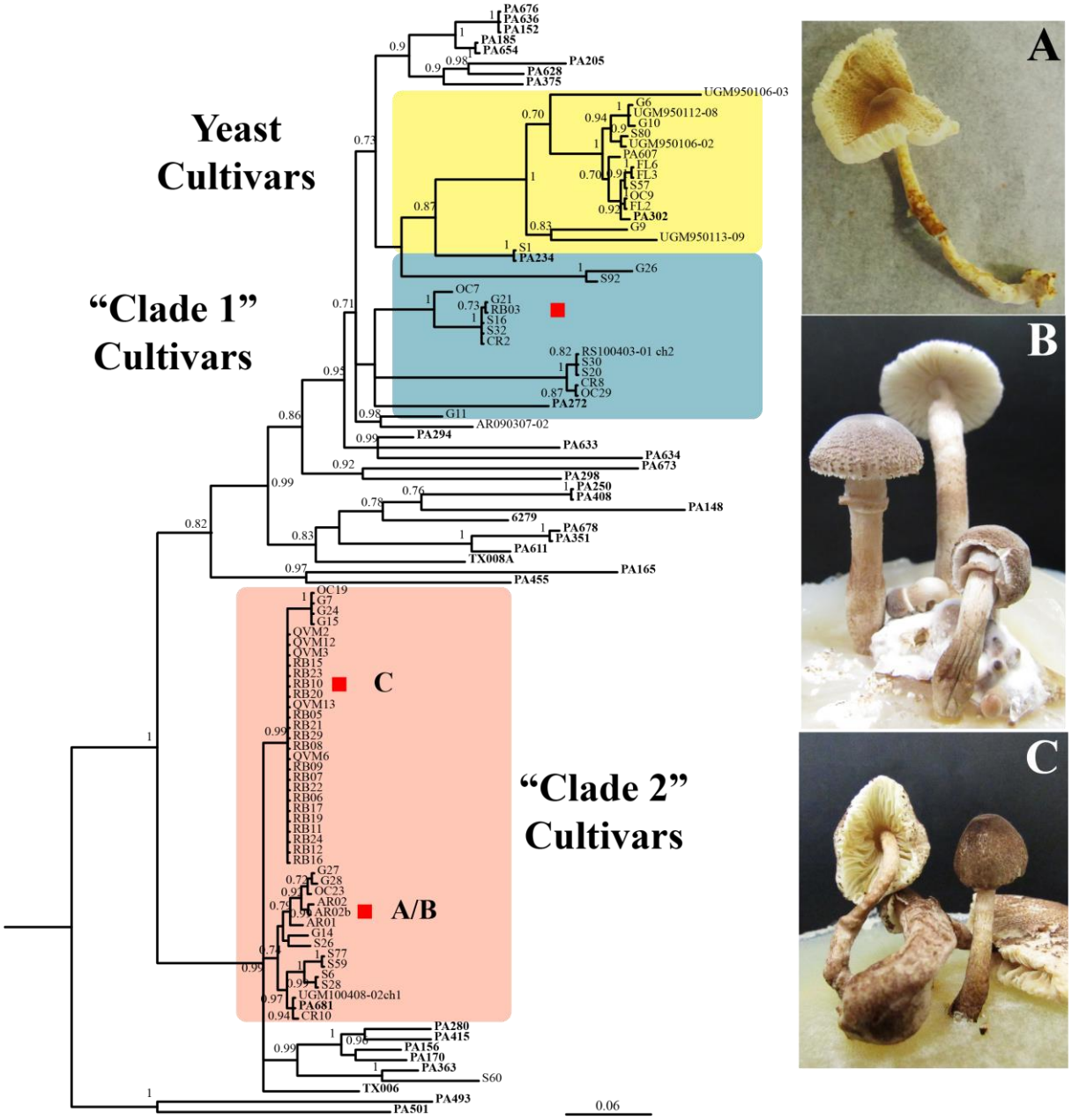

Figure S6. Phylogeny of *Leucocoprinus* fungi based on ITS marker (with 727 pairs of bases in the final alignment). The sequences correspond to lower attine cultivars (from Mueller et al., 2018). Clade-1 and Clade-2 are named according to Mueller et al. (1998). Free-living fungi (not in association with ant colonies) are shown in bold. Free-living fungi (*Leucocoprinus* sp. PA493 and PA501) were used as outgroup. A red squared on each clade indicates the position of strains found in this study. The analysis was conduced using the Bayersian inference algorithm. Figures on branches indicate posterior probabilities greater than or equal to 0.7. Information on the strains are available in Table S2 (ITS sequences of lower attine cultivars and free-living fungi) and Table S7. Each strain was indicated by the Sample ID code. Pictures of basidiomes of *Leucocoprinus* sp. fungi produced in culture. A and B: fungi associated with *Mycetophylax morschi* (AR01 and AR02, respectively). C: fungi associated with *Mycocepurus goeldii* (QVM2). Photos by Salomé Urrea Valencia (AR01), and Rodolfo Bizarria Jr. (AR02, QVM2).

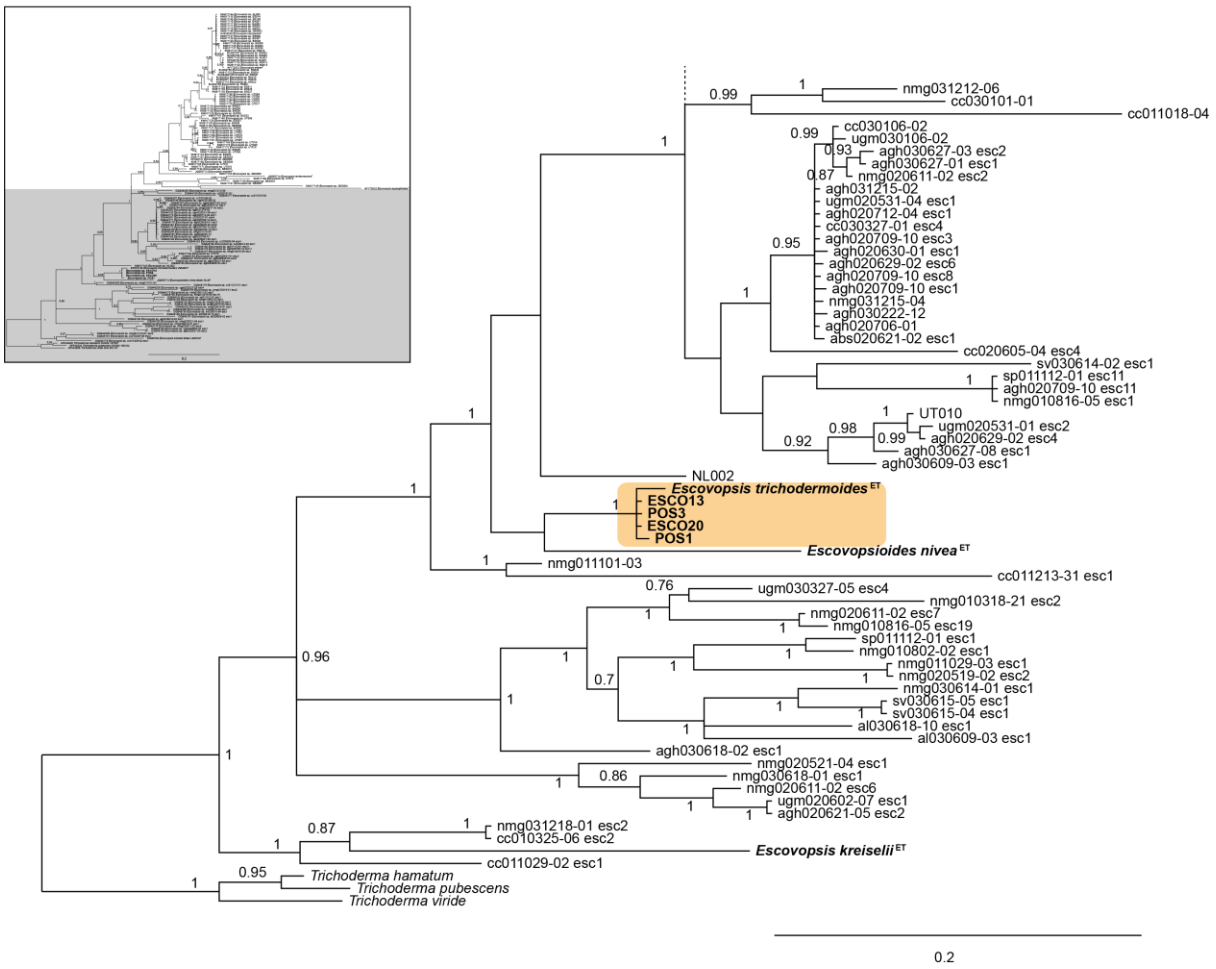

Figure S7. *Escovopsis* phylogeny based on a segment of the translation elongation factor 1-alpha (*tef1*) with 731 bp in the final alignment (tree shown in the upper left inset). *Escovopsis trichodermoides* clade is highlighted in yellow, comprehending all strains from this study. Three species of Hypocreaceae fungi were used as outgroup according to Meirelles et al. (2015). The analysis was conduced using the Bayersian inference algorithm. Figures on branches indicate posterior probabilities greater than or equal to 0.7. Information about strains used is available in Table S8. Each strain was indicated by the isolate ID code. ^{ET}: ex-type strains.

8.4. REFERENCES

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