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SYSTEMATICS AND EVOLUTION OF *ESCOVOPSIS* AND HYPOCREALEAN RELATIVES ASSOCIATED WITH ATTINE ANT COLONIES

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SYSTEMATICS AND EVOLUTION OF *ESCOVOPSIS* AND HYPOCREALEAN RELATIVES ASSOCIATED WITH ATTINE ANT COLONIES

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To my mother…

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Science is born from ideas, crawls and pronounces its first words on the arms of hypothesis, feeds and nourishes itself on discussion, and makes its way guided by the light of the evidence.

ABSTRACT

Escovopsis is a group of fungi that inhabit the colonies of fungus-growing ants. The study of this fungal genus has been based on several assumptions that became dogmas. For instance, i) many fungi associated with attines were treated as *Escovopsis*, without taxonomic support, ii) the genus was considered a specialized mycoparasite of the attines' mutualistic fungi, and iii) it was thought that the genus coevolved with attines based on the assumption of its parasitic lifestyle. However, for many years the *Escovopsis* taxonomy, its relationship with the ants and its phylogeographic distribution were almost an empty space for the scientific community. In addition, the origin of the genus was never addressed. Therefore, the main objective of this study was to build the systematics of the *Escovopsis* and shed light on its origin, evolution, diversification, and phylogeographic distribution. Three manuscripts are the result of this study and are presented here as chapters. The first manuscript presents the description of two new *Escovopsis* species (published in MycoKeys). The second manuscript brings the reassessment of the *Escovopsis* taxonomy, provides a suitable taxonomic and phylogenetic framework for the systematics of the genus, and describes two new Hypocreaceae genera (submitted to IMA Fungus). The third manuscript shows the origin, evolution, phylogeographic distribution and the trait adaptations experienced by *Escovopsis* since its entry in fungus-growing ant colonies. This study fills an important gap in the taxonomy, systematics, and evolution of *Escovopsis* and related genera which certainly will help researchers to better understand the evolution of the attines' system.

Key-words: Hypocreales, Taxonomy, Phylogeny, Symbiosis, Evolution

RESUMO

Escovopsis é um interessante grupo de fungos que habita as colônias das formigas atíneas. O estudo desse fungo se baseou em diversos pressupostos que, por muito tempo, se tornaram dogmas. Por exemplo, i) vários fungos associados à essas formigas foram tratados como *Escovopsis*, porém sem nenhum suporte taxonômico, ii) o gênero foi considerado um micoparasita especializado da associação formiga - fungo cultivado, e iii) acreditou-se que o gênero co-evoluiu com as atíneas, pois parasita o parceiro fúngico dessas formigas desde a origem da associação. No entanto, a taxonomia de *Escovopsis*, bem como sua relação com as formigas e sua distribuição filogeográfica foram quase um espaço vazio para a comunidade científica. Além disso, a origem do gênero nunca foi abordada. Nesse contexto, o objetivo deste estudo foi construir a sistemática do gênero *Escovopsis* e angariar evidências sobre sua origem, evolução, diversificação e distribuição filogeográfica. Três manuscritos são o resultado deste estudo e são apresentados como capítulos. O primeiro manuscrito traz a descrição de duas novas espécies de *Escovopsis* (publicado na MycoKeys). O segundo manuscrito apresenta a reavaliação da taxonomia de *Escovopsis*, fornece um marco taxonômico e filogenético robusto para a sistemática do gênero e descreve dois novos gêneros dentro da família Hypocreaceae (submetido na IMA Fungus). O terceiro manuscrito mostra a origem, evolução, distribuição filogeográfica e as adaptações experimentadas pelo gênero desde seu ingresso nas colônias das atíneas. Este estudo preenche uma lacuna importante na taxonomia, sistemática e evolução de *Escovopsis* e gêneros próximos e, certamente, ajudará os pesquisadores a compreender melhor a evolução do sistema das formigas atíneas.

Palavras-chave: Hipocreales, Taxonomia, Filogenia, Simbiose, Evolução

SUMMARY

Introduction

Sixty-five million years ago, after the massive extinction of the dinosaurs, fungus-growing ants (subfamily Myrmicinae, Attini tribe, Attina subtribe, "the attines") started producing their own food. Finding food is a constant and demanding struggle to organisms, and sometimes it leads them to certain death. Thus, the emergence of fungus-growing behaviour was not only a safer way to deal with lack of food, but directly influenced the evolutionary success of the attines and established the beginning of one of the most interesting and complex insect-fungus associations we know on earth.

Fungiculture performed by attine ants can be divided into five types: i) the fungiculture practiced by the genera in the basal attines, *Cyphomyrmex*, *Mycetophylax*, *Mycocepurus*, *Apterostigma auriculatum* group that grow fungi from the tribe Leucocoprinae (Agaricaceae); ii) the fungiculture practiced by species of the *Apterostigma pilosum* group that cultivate fungi of the Pterulaceae family, named coral fungi; iii) the fungiculture practiced by ant species of the *Cyphomyrmex rimosus* group, which cultivate yeasts (in Leucocoprinae); iv) the fungiculture practiced by the genera *Trachymyrmex* and *Sericomyrmex* that cultivate other fungi in Leucocoprinae, and v) the fungiculture of leaf-cutting ants, which cultivate *Leucoagaricus gongylophorus*.

Attine ants are restricted to the American continent and comprise 17 genera with more than 250 species phylogenetically divided into Neoattini and Paleoattini. The genera *Atta* and *Acromyrmex*, in the Neoattini group (more derived clades) are known as leaf-cutting ants, and they are important crop pests, because the large amounts of fresh plant material they collect to feed their fungal partner. In return, the fungal cultivar *L. gongylophorus* produces vesicles called gongylidia which are used by the ants as food source. On the other hand, the

less derived attini genera, known as non-leaf-cutting ants (comprising ant genera in both Neoattini and Paleoattini) use other substrates like seeds, insect carcasses, and dry plant material to nourish their fungal partners. In addition, for millions of years attines have been introducing into their colonies a great diversity of microorganisms along with the substrates they collect and also microorganisms attached to their bodies. Consequently, complex symbiotic networks made their way slowly in attine colonies.

Though several symbiotic relationships have been described between the microorganisms living in the attine ant fungus garden, the mutualism between attines and the fungi they cultivate is the backbone of this ecosystem. With the constant entrance of microorganisms into the colonies, attine's environment is prone to parasites that are able to take advantage of the system. Indeed, ecological factors suggest that several parasites could have arrived at the fungus-farming ant colonies. However, the genus *Escovopsis* (Ascomycota: Hypocreales, Hypocreaceae) was proposed as the only and specialized parasite in this system.

Due to the ecological importance that a parasite represents within the attine system, many studies turned their efforts to the study of the *Escovopsis*-mutualistic fungus interaction. However, the study of this diverse and interesting group of fungi has been based on several assumptions that became dogmas, since its discovery. For instance, it was proposed that: i) all fungi producing brown conidia found in attine colonies belong to *Escovopsis*. However, the *Escovopsis* taxonomy was almost an empty space for the scientific community and most that we know about its taxonomy was raised by non-taxonomic studies; ii) the genus is a specific and specialized mycoparasite. Nonetheless, our knowledge on the parasitic mechanisms used by *Escovopsis* to overcome the fungal cultivar defences and the

ant colonies barriers is still limited. The conclusion that *Escovopsis* is a parasite was based on the evaluation of few strains from one of the 26 clades of the genus; and iii) *Escovopsis* had coevolved with the attines and their mutualistic fungi. However, the origin of *Escovopsis* is a complete mystery, as it is its phylogenetic relationship with the attine and the fungal cultivars.

Under this scenario, this study aimed to build the systematics of the *Escovopsis* showing its origin, diversification, and phylogeographic distribution. To reach this goal we used the following approach: i) increase the number of *Escovopsis* strains in our collection, ii) standardize the taxonomy of the genus to access its morphological features, iii) create a phylogenetic framework to perform the *Escovopsis* tree of life, iv) combine the taxonomic and phylogenetic analysis to build the *Escovopsis* systematics, v) estimate the origin of the genus, vi) evaluate its relationship with the ants, and vii) unravel its phylogeographic distribution.

The first two years, we gathered a collection of 365 strains [153 strains already deposited in the Laboratory of Fungal Ecology and Systematics (LESF - UNESP, Rio Claro, SP) and 212 obtained in this study]. In addition, we carried out an in-depth study of the taxonomy of this genus to know more about its taxonomic and to plan a strategy to standardize it. The main issues of the *Escovopsis* taxonomy were presented in the paper "**More pieces to a huge puzzle: Two new *Escovopsis* species from fungus gardens of attine ants**" which is the first chapter of this thesis. This study introduced *Escovopsis clavata* and *E. multiformis* within a well detailed taxonomic and phylogenetic framework, and emphasized the non-standardized taxonomy and the phylogenetic incongruities of the genus.

Some suggestions to reach a better view of the *Escovopsis* systematics were proposed in this paper.

Moving on to which was suggested in that study, we sequenced five molecular markers (ITS, LSU, *tef1*, *rpb*1, and *rpb*2) to start building the phylogenetic tree of life of *Escovopsis*. Our results suggested that the *Escovopsis* clade is composed by more than one genus. Based on these results we raised the hypothesis that: If more than one genus is living in attines fungus garden, then it would suppose the possibility that more than one fungus would have co-evolved with the attines colonies in a multiparasitism relationship. To answer these questions, we aimed to build the most comprehensive phylogenetic tree of *Escovopsis* and infer the divergence time of the genus to access its evolutionary history and to shed light on our hypothesis. This part of the study was developed at Emory University, Department of Biology, O. Wayne Rollins Research Center, Atlanta, USA.

We assembled samples from LESF and from Dr. Nicole M. Gerardo' Laboratory (Emory University, Atlanta, USA) and carried out the broadest phylogenetic analysis of the genus *Escovopsis*. This analysis comprehended a total of 584 strains from different regions across America (Argentina, Brazil, Ecuador, Panama, and Costa Rica) spanning several biomes. The results supported our previous hypothesis that *Escovopsis* comprehends more than one genus. Then, we reassessed the taxonomy of *Escovopsis* by using standardized parameters including a select and informative set of morphological characters and a comprehensive multilocus phylogeny which is explained in the manuscript **"Fungi of the strong conidial form" in attine ant colonies: taxonomic and phylogenetic reassessment of the genus *Escovopsis***". This manuscript was submitted to IMA Fungus and is presented here as the second chapter.

The reassessment of *Escovopsis* and the comprehensive phylogeny of the genus provided the ground to estimate the origin of the genus as well as its phylogenetic relationship with the attines and its phylogeographic distribution. Our findings support that the origin of *Escovopsis* and the beginning of the attine fungus domestication correspond in time. However, *Escovopsis* appears to have reached the ant colonies of just few attine genera during the last 38 million of years, along with the plant material collected to nourish the fungus gardens. These results are presented in the manuscript "**Digging into the past of a fungus-growing ant guest: origin and evolution of *Escovopsis***", which is presented here as the third chapter. Interesting fungal trait adaptations and hypothesis about the origin and the evolutionary history of the genus are discussed in this manuscript.

Objectives

General

To build the systematics of the genus *Escovopsis* showing its origin, diversification, and phylogeographic distribution

Specific

- To standardize the conditions to assess the taxonomy of *Escovopsis*
- To infer the phylogenetic tree of life of *Escovopsis* and associated fungal genera
- To infer the *Escovopsis*-attine ant co-cladogenesis
- To infer the evolutionary history of *Escovopsis* within the attine ant-fungus system

Chapter I

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More pieces to a huge puzzle: Two new *Escovopsis* species from fungus gardens of attine ants

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Abstract

Escovopsis (Ascomycota: Hypocreales, Hypocreaceae) is the only known parasite of the mutualistic fungi cultivated by fungus-growing ants (Formicidae: Myrmicinae: Attini: Attina, the "attines"). Despite its ecological role, the taxonomy and systematics of *Escovopsis* have been poorly addressed. Here, based on morphological and phylogenetic analyses with three molecular markers (internal transcribed spacer, large subunit ribosomal RNA and the translation elongation factor 1-alpha), we describe *Escovopsis clavatus* and *E. multiformis* as new species isolated from fungus gardens of *Apterostigma* ant species. Our analysis shows that *E. clavatus* and *E. multiformis* belong to the most derived *Escovopsis* clade, whose main character is the presence of conidiophores with vesicles. Nevertheless, the most outstanding feature of both new species is the presence of a swollen region in the central hypha of the conidiophore named swollen cell, which is absent in all previously described *Escovopsis* species. The less derived *Escovopsis* clades lack vesicles and their phylogenetic position in the Hypocreaceae still remains unclear. Considering the high genetic diversity in *Escovopsis*, the description of these new species adds barely two new pieces to a huge taxonomic puzzle; however, this discovery is an important piece to the building of the systematics of this group of fungi.

Key-words: Hypocreales, Taxonomy, Phylogeny, Parasitic fungi, Symbiosis.

Introduction

Microorganisms play important roles in the stability of social insect colonies (Hughes et al. 2008, Joop and Vilcinskas 2016, Vanderpool et al. 2018). The environment of these insects has a high potential to harbour unique fungal species (Attili-Angelis et al. 2014, Harrington et al. 2014, Menezes et al. 2015, Montoya et al. 2016). The evolutionary success of the fungus garden of the fungus-farming ants (Formicidae: Myrmicinae: Attini: Attina, the "attines") depends on complex symbiotic interactions amongst bacteria, fungi and the ants (Currie et al. 2003, Gerardo et al. 2006a, Kost et al. 2007). The association between attine ants and their mutualistic fungi (Basidiomycota: Agaricales) is the core of the attine colonies; however, *Escovopsis* (Ascomycota: Hypocreales: Hypocreaceae) can exploit this association. Although no specialised parasitic structures were found, studies showed that this parasite is able to kill its native host as well as the ants and its mutualistic bacteria by chemical compounds (Currie 2001, Varanda-Haifig et al. 2017, Custodio and Rodrigues 2018, Dhodary et al. 2018, Heine et al. 2018). Despite the ecological relevance of *Escovopsis* as parasites of attine ant colonies, the taxonomy of this genus has been neglected.

Attine ants are classified in two sister clades: the Palaeoattina and Neoattina (Branstetter et al. 2017). Leafcutter ants (*Atta* and *Acromyrmex*) are considered the most derived attines within the Neoattina. Their behaviour is characterised by collecting fresh leaves and flowers to feed several cultivars from two clades of fungi in the Agaricaceae (Mueller et al. 2017, 2018). On the other hand, non-leafcutter ants also occur in both the Neoattina and Palaeoattina clades. Distinct from *Atta* and *Acromyrmex*, non-leafcutter ants forage on seeds, insect frass and dry leaves to nourish a wide range of fungal cultivars in the Agaricaceae and Pterulaceae (Villesen et al. 2004, Schultz and Brady 2008).

The attine ant-fungus cultivar-*Escovopsis* symbiosis has been widely studied in leafcutter ants (Mueller and Gerardo 2002, Currie et al. 2003, Gerardo et al. 2004, 2006a,b). In addition to their contributions on the biology of *Escovopsis*, these studies also revealed considerable diversity of the parasite. Considering the variety of mutualistic fungi that non-leafcutter ants may cultivate, as well as the different substrates used for that purpose, a high diversity of *Escovopsis* species is unsurprising. This is especially true for *Apterostigma* (Gerardo et al. 2006b), a genus of non-leafcutter attine with species that cultivate different cultivars including *Leucoagaricus gongylophorus*, the domesticated fungus cultivated by many higher attine ant species, mostly leafcutter ants (Sosa-Calvo and Schultz 2010, Schultz et al. 2015, Ješovnik et al. 2016, Sosa-Calvo et al. 2017, Mueller et al. 2017, 2018).

While *Escovopsis* species exploiting gardens of *Atta*, *Acromyrmex*, *Trachymyrmex* and *Mycetophylax* were formally described, the morphological characters of the species associated with *Apterostigma* are unknown. A previous study associated clades of the parasite with the colour pattern of *Escovopsis* colonies (brown, yellow, white and pink; Gerardo et al. 2006b). However, no taxonomic studies were undertaken to formally describe these clades. Here, we describe *Escovopsis clavatus* and *E. multiformis* as new species isolated from the fungus garden of *Apterostigma*. The distinctive feature of these lineages is the presence of swollen cells at the base of the conidiophore branches. This phenotype differentiates these two new species from previously described *Escovopsis*. Considering that previous studies showed a high genetic diversity within *Escovopsis*, the description of these species adds two pieces to the enormous taxonomic puzzle which is *Escovopsis*.

Material and Methods

Sampling sites and *Escovopsis* isolation

Five *Escovopsis* isolates were obtained from fungus gardens of five different colonies of *Apterostigma* spp. (Table S1). The isolates LESF 847, LESF 853, LESF 854 and LESF 855 were obtained from colonies found in the Atlantic Rain Forest in Florianópolis, State of Santa Catarina, Brazil (October 2015). The isolate LESF 1136 was obtained from a single colony found in the Amazon Forest in Cotriguaçu, State of Mato Grosso, Brazil (October 2017). The nests were found inside or under rotten logs. Fungus gardens, along with tending workers and brood, were collected in UV-sterilised plastic containers using sterilised spoon and forceps. Samples were taken to the Laboratory of Fungal Ecology and Systematics (LESF) at the UNESP – São Paulo State University, Rio Claro, Brazil.

For fungal isolation, seven garden fragments (0.5–1 mm^3) were inoculated on plates (three plates per colony) containing potato dextrose agar (PDA, Neogen Culture Media, Neogen®) supplemented with the antibacterial antibiotic chloramphenicol [150 µg ml^{-1}, Sigma] and incubated at 25°C in darkness. Plates were monitored daily for fungal growth and, when *Escovopsis* mycelia sprouted from garden fragments, they were transferred to new PDA plates. All isolates were prepared as axenic (monosporic) cultures and stored under sterile distilled water kept at 8°C (Castellani 1963) and at −80°C (as conidia suspensions in 10% glycerol).

Morphological analysis

The morphological characters of the five isolates (LESF 847, LESF 853, LESF 854, LESF 855 and LESF 1136) were examined. Due to the lack of standardisation of culture conditions for *Escovopsis*, the macroscopic characters of the colonies, i.e. radial growth,

mycelium colour, morphology and presence of soluble pigments, were evaluated on eight different media: PDA, malt agar 2% [MA2%: 2% of malt extract (Neogen Culture Media) and 1.5% of agar (Neogen Culture Media)], cornmeal agar (CMD, Neogen Culture Media), synthetic nutrient agar [SNA: 1 g of KH_2PO_4 (Labsynth®), 1 g of KNO_3 (Labsynth®), 0.5 g of $MgSO_4(7H_2O)$ (Labsynth®), 0.5 g of KCl (Labsynth®), 0.2 g of Glucose (Labsynth®), 0.2 g of Sucrose (Labsynth®) and 15 g of Agar (Neogen Culture Media)], oatmeal agar (OA), potato carrot agar (PCA, HiMedia®), malt extract agar 2% [MEA: 30 g l^{-1} of malt extract (Neogen Culture Media), 5 g l^{-1} of bacteriological peptone (Neogen Culture Media), 20 g l^{-1} of glucose (Labsynth®) and 15 g l^{-1} of Agar (Neogen Culture Media)] and Czapek yeast extract agar [CYA; 30 g of Sucrose (Labsynth®), 5 g of Yeast extract (Neogen Culture Media), 1 g of KH_2PO_4 (Labsynth®), 0.3 g of $NaNO_3$ (Synth), 0.05 g of KCl (Labsynth®), 0.05 g of $MgSO_4(7H_2O)$ (Labsynth®), 0.001 g of $FeSO_4$ (Labsynth®), 0.001 g of $ZnSO_4$ (Labsynth®), 0.0005 g of $CuSO_4$ (Labsynth®), 15 g of Agar (Neogen Culture Media)] at five temperatures (10°C, 20°C, 25°C, 30°C and 35°C). These temperatures correspond to the conditions used in previous studies that described *Escovopsis* species (Seifert et al. 1995, Augustin et al. 2013, Masiulionis et al. 2015, Meirelles et al. 2015a). For this purpose, 200 µl of conidia were spread on plates with water-agar (WA) and incubated for seven days at 25°C in darkness. Then, mycelium fragments of 0.5 cm diameter were cut from the WA plates and inoculated in the centre of the plates (90 x 15 mm) containing the eight culture media. All the strains examined showed better development in the dark and with unsealed Petri dishes to allow air passage; therefore, incubation was carried out in the darkness and without sealing the plates, for 14 days. Three replicate plates were inoculated for each media and for each incubation temperature.

To examine the microscopic characters, i.e. the morphology, size, branching patterns, vesicles and swollen cells of the conidiophores, as well as phialides and conidia, slide cultures on PDA and MEA were performed. Briefly, we placed a 5 mm^2 fragment of culture medium on a microscopic slide and then we inoculated the fungus at the centre of the fragment. Then, the inoculated medium was covered with a coverslip and incubated at 25°C for 4-7 days in the dark. After that, the coverslips, where the fungus grew, were removed and placed in new slides with a drop of lactophenol. Finally, the slides were examined under a light microscope (DM750, Leica, Germany). Fungal microscopic structures were photographed and measured (with 30 measurements per structure) in LAS EZ v.4.0 (Leica Application Suite).

Microscopic structures were also examined under scanning electron microscopy (SEM). Fungal samples (five days old cultures on PDA) were fixed in osmium tetroxide vapour for 72 h. Then, samples were dehydrated using a series of acetone concentrations (50, 75, 90, 95 and 100%) and dried to critical point using liquid CO_2 (Balzers CPD030). The dried material was sputtered with gold (Balzers SCD050) and examined under the scanning electron microscope (TM3000, Hitachi).

DNA extraction, PCR and sequencing

DNA extraction of the five strains was performed, following the steps published in Meirelles et al. (2015a). Three molecular markers were amplified: the internal transcribed spacer (ITS) region (White et al. 1990, Schoch et al. 2012); translation elongation factor 1-alpha (*tef*1) (Taerum et al. 2007); and the large subunit ribosomal RNA (LSU) (White et al. 1990, Haugland and Heckman 1998, Currie et al. 2003) (Table S2).

PCR and sequence reaction conditions followed the steps published in Meirelles et al. (2015b) for the ITS region, Meirelles et al. (2015a) for *tef*1 and Augustin et al. (2013) for LSU. The final amplicons were cleaned up with Wizard SV Gel and PCR Clean-up System kit (Promega), following the manufacturer's protocol. Sequences (forward and reverse) were generated in ABI3500 (Life Technologies). The LSU of 29 strains previously used in Meirelles et al. (2015b) also was amplified and sequenced for this study (Table S1). The sequences were assembled in contigs in BioEdit v. 7.1.3 (Hall 1999) and deposited in GenBank (Table S1 for accession numbers).

Phylogenetic analyses

To infer the phylogenetic position of the new species in the *Escovopsis* clade, sequences from previous studies were retrieved from the GenBank and aligned with our new sequences in a dataset for each marker (Chaverri et al. 2003, Spatafora et al. 2007, Jaklitsch and Samuels 2011, Põldmaa 2011, Meirelles et al. 2015b). This data included sequences from the seven *Escovopsis* ex-type strains, from *Escovopsioides nivea* and some species from *Hypomyces* and *Trichoderma*, as the phylogenetic closest relatives of *Escovopsis*. First, the three datasets [46 sequences of ITS (621 bp), LSU (531 bp) and *tef*1 (758 bp)] were aligned separately in MAFFT v.7 (Katoh and Standley 2013). The end parts of each alignment were removed manually by considering a point where the sequences presented greater homogeneity (all alignments are deposited in Treebase: http://purl.org/phylo/treebase/phylows/study/TB2:S23689). Then, a phylogenetic tree was inferred using each dataset separately (Fig. 6). The nucleotide substitution model was selected by independent runs in jModelTest 2 (Darriba et al. 2012) using the Akaike Information Criterion (AIC) with a 95% confidence interval. Second, the three datasets were

concatenated using Winclada v.1.00.08 (Nixon 2002). The final file comprised 40 sequences totalling 1911 bp. All phylogenetic trees were reconstructed using maximum likelihood (ML) in RAxML v.8 (Stamatakis 2014 with 1000 independent trees and 1000 bootstrap replicates; MLB) and Bayesian Inference (BI) in MrBayes v.3.2.2. (Ronquist et al. 2012). The ML phylogenetic trees were reconstructed using the GTR+ G substitution model and the BI phylogenetic trees were performed with the GTR + I + G substitution model. In the case of BI, two separate runs were carried out, each consisting of three hot chains and one cold chain and a Markov Chain Monte Carlo (MCMC) sampling for two million generations to obtain Bayesian posterior probability (PP) values for the clades. Convergence occurred when the standard deviation (SD) of split frequencies fell below 0.01 and the first 25% of the generations of MCMC sampling were discarded as burn-in. The final phylogenetic trees were edited in FigTree v.1.4 and in Adobe Illustrator CC v.17.1. *Lecanicillium antillanum* CBS 350.85 was used as the outgroup in all trees, because it belongs to a family phylogenetically close to Hypocreaceae (Spatafora et al. 2007).

Results

Taxonomy

Escovopsis clavatus Q.V. Montoya, M.J.S. Martiarena, D.A. Polezel, S. Kakazu & A. Rodrigues sp. nov. Figs. 1-3

MycoBank: MB828328

Etymology: "*clavatus*" in reference to the predominantly clavate shape of vesicles.

Typification: BRAZIL. Santa Catarina, Florianópolis, (27°44'39.6"S, 48°31'10.14"W), elev. 46 m, Fungus garden, 08, 2015. *A. Rodrigues*. Holotype: CBS H-23845 (dried culture on PDA). Ex-type strain LESF 853 (= CBS 145326).

Sequences: ITS (MH715096), *tef*1 (MH724270) and LSU (MH715110).

Colonies grow only at 20 and 25°C (Fig. 1). At both temperatures, growth starts on the third day on CMD, CYA, MA 2%, MEA, OA, PCA, PDA; and on the sixth day on SNA. Colonies have floccose aerial mycelia with a pale-brown colour after seven days. Faster growth was observed on MA2% and heavy sporulation was identified on MA2%, PDA and OA. At 20°C, colonies reached 0.5 – 0.7 cm, 1.5 – 2.5 cm and 0.5 – 1 cm on CMD, CYA and SNA, respectively. At this temperature, colonies reached the edge of the plate after 10 days on MA 2% and PCA; after 12 days on OA and MEA; and after 14 days on PDA and CYA. At 25°C, colonies reach 2 cm, 3 – 3.2 cm and 2 cm on CMD, CYA and SNA, respectively, after 14 days. At this temperature, colonies reached the plate edge after seven days on OA and PCA; and after 10 days on MA2%, MEA and PDA. Concentric rings were observed only on PCA at 20°C (Fig. 1). No pustule-like structures were observed.

Conidiophores arising from aerial hypha alternated or opposite (Fig. 3A), with the main axis of 50 – 780 µm in length, some without branching and often with 1 – 2 levels of branching (Figs. 2A and E, 3A and E). Branches arise from the main axis of the conidiophore in an alternated or opposite pattern, with a septum near to the central axis and before the vesicle, usually with 1-2 branches at each branching point (16 – 138 µm long) or 2-4 branches arising from swollen cells (28 – 35 µm long), mostly forming angles less than 90º and less frequently right angles, usually straight and sometimes slightly curved up or down. Each branch terminates in a vesicle, with 1-8 fertile heads per conidiophore. Swollen cells are present in the 15% of the total of conidiophores examined (Figs. 2C-D and 3E) and can

measure 10 – 18 μm long x 7 – 9 μm wide. *Vesicles* with only a septum at the base, in various shapes: globose (8%), subglobose (24%), broadly ellipsoidal (33%), ellipsoidal (27%), cylindrical (8%) (Figs. 2E-G and 3F-G); and reaching 9 – 27 μm long x 7 – 20 μm wide. *Phialides* lageniform formed on vesicles (Fig. 3H), with 5 – 8 μm in total length, elongated base (0.5 – 1.5 μm x 0.5 – 1 μm), followed by a swollen section (1.5 – 2.5 μm x 1 – 3 μm) and a thin neck (1.5 – 4 μm x 0.5 μm). *Conidia* with 1.5 μm – 2.5 μm long x 0.5 μm – 1.5 μm wide, in various shapes: broadly ellipsoidal (5%), ellipsoidal (43.3%), cylindrical (51.7%); brown, with smooth and slightly thickened walls and in chains (Figs. 2H and 3I).

Habitat: Isolated from fungus gardens of *Apterostigma* sp.

Additional specimens examined: BRAZIL. Santa Catarina, Florianópolis, (27°44'38.94"S, 48°31'9.3"W), elev. 32 m, fungus garden, 08, 2015. *A. Rodrigues*. LESF 854 (ITS - MH715097, *tef*1 - MH724271 and LSU - MH715111). Santa Catarina, Florianópolis, (27°44'39.49"S; 48°31'9.72"W), elev. 38 m, fungus garden, 08, 2015. *A. Rodrigues*. LESF 855 (ITS - MH71509), *tef*1 - MH724272 and LSU - MH715112).

Notes: *Escovopsis clavatus* is phylogenetically closely related to *E. multiformis* and its most distinctive characters are its growth temperatures, the conidiophore branching and the swollen cells. It grows at 20 and 25°C; nevertheless, *E. multiformis* grows at 10, 20, 25 and 30°C. The conidiophore of *E. clavatus* is larger and more branched than the conidiophore of *E. multiformis*. In addition, the swollen cells of *E. clavatus* are less frequent and shorter than in *E. multiformis*. The character distinguishing *E. clavatus* from other species of *Escovopsis* is the swollen cell on the conidiophores.

Escovopsis multiformis Q.V. Montoya, M.J.S. Martiarena, D.A. Polezel, S. Kakazu & A. Rodrigues sp. nov. Figs. 1, 4 and 5

Mycobank: MB828329

Etymology: "*multiformis*" in relation to the different vesicle shapes found in the same isolate.

Typification: BRAZIL. Santa Catarina, Florianópolis, (27°28'11.28"S, 48°22'39.48"W), elev. 119 m, Fungus garden, 08, 2015. *A. Rodrigues*. Holotype: CBS H-23846 (dried culture on PDA). Ex-type strain LESF 847 (= CBS 145327).

Sequences: ITS (MH715091), *tef*1 (MH724265) and LSU (MH715105).

Colonies grow at 10, 20, 25 and 30°C (Fig. 1). The best growth temperature was 30°C. At this temperature, colonies reached 1.2 – 1.4 cm, 2.7 – 3 cm, 2.6 – 3 cm, 3.3 – 3.5 cm, 2.5 – 2.8 cm, 2.7 – 2.9 cm and 1.9 – 2.5 cm in radius on CMD, CYA, MA2%, MEA, OA, PCA and PDA, after 14 days, respectively. Colonies exhibit light-brown floccose mycelia (colony edge usually lighter or white). The colour shades and the character of the aerial mycelium vary on each culture medium (Fig. 1). Colonies present concentric rings with a hardened ring similar to a crust in the centre of the colony on CYA (Fig. 1) and the sporulation is more abundant on PCA and PDA. At 20°C, on CMD, CYA, MA2%, MEA, OA, PCA, PDA and SNA, colonies attain 0.5 - 0.8 cm, 1.1 - 2.2 cm, 2 - 2.5 cm, 2.1 - 2.3 cm, 2 - 2.5 cm, 2.8 cm, 1.9 - 2.4 cm and 0 - 0.1 cm in radius, respectively. At 25°C, colonies reach 1 cm, 2.1 - 2.3 cm, 2 - 2.4 cm, 2.5 - 2.6 cm, 2.2 - 2.7 cm, 2.8 - 3 cm, 1.8 - 2 cm and 0.1 - 0.2 cm in radius on CMD, CYA, MA2%, MEA, OA, PCA, PDA and SNA, respectively. Pustule-like structures are observed on OA and CMD at 20, 25 and 30°C. At 10°C, the colony growth was inconspicuous, reaching 0.2 – 0.3 cm, 0.2 – 0.4 cm, 0.3 cm, 0.6 – 0.8 cm, 0.8 cm and 0.3 – 0.5 cm in radius on CYA, MA2%, MEA, OA, PCA and PDA, respectively, after 14 days. At this temperature, growth started in these culture media after seven days and sporulation occurred only after the 12th day. No growth was observed at 35°C.

Conidiophores arising from aerial hypha alternated or opposite (Fig. 3A), with the main axis of 41 – 293 μm in length, some without branching and most of them with one level of branching. Rarely, branches form two levels branching (Figs. 4A-C and 5A-B). Branches arise from the main axis of the conidiophore alternated, with a septum near the central axis and before the vesicle, usually with one branch at each branching point (32 – 84 μm long) or 2-4 branches arising from swollen cells (17 – 86 μm long), mostly forming right angles, usually slightly curved up. Each branch terminates in a vesicle, with 1-4 fertile heads per conidiophore. Swollen cells are present in 27% of the total of conidiophores examined (Figs. 4D-G and 5C-F) and can measure 16 – 34 μm long x 9 – 20 μm wide. Sometimes, one swollen cells' branch gives rise to another swollen cell with more branches (Figs. 2F and 3C). *Vesicles* with only a septum at the base, in various shapes: globose (22%), subglobose (37%), broadly ellipsoidal (26%), ellipsoidal (10%), cylindrical (5%) (Figs. 4H-I and 5G-H); and reaching 12 – 27 μm x 9 – 17 μm wide. *Phialides* lageniform formed on vesicles (Fig. 5I), with 6 – 10 μm in total length, elongated base (1– 2.5 μm x 0.5 – 1μm), followed by a swollen section (2.5 – 4.5 μm x 2 – 3.5 μm) and a thin neck (1– 4.5 μm x 0.5 – 1 μm). *Conidia* are 2.5 – 3.5 μm long x 1.5 – 2.5 μm wide, in various shapes: globose (2%), subglobose (3%), broadly ellipsoidal (33%), ellipsoidal (47%), cylindrical (15%); brown, with smooth and slightly thickened walls and in chains (Figs. 4 and 5J).

Habitat: Isolated from fungus garden of *Apterostigma* sp.

Additional specimens examined: BRAZIL. Mato Grosso, Cotriguaçu, (09°49'22.74"S, 58°15'32.04"W), elev. 252 m, Fungus garden, 10, 2017. *Q. V. Montoya*. LESF 1136 (ITS - MH715092, *tef*1 - MH724266 and LSU - MH715106).

Notes: *Escovopsis multiformis* is closely related to *E. clavatus*. Different from *E. clavatus* that grow at 20 and 25°C, *E. multiformis* grow at 10, 20, 25 and 30°C. The optimum growth

temperature of *E. multiformis* is 30°C and that of *E. clavatus* is 25°C. The conidiophores of *E. multiformis* are smaller and less branched than *E. clavatus* and the swollen cells are more frequent and larger than those found in *E. clavatus*. *E. multiformis* differs from other described species by the presence of conidiophores with a swollen cell, the presence of different vesicles shapes and because it is phylogenetically placed in a distinct clade.

Morphological analyses

The isolates LESF 853 (*Escovopsis clavatus*, Figs. 1-3) and LESF 847 (*Escovopsis multiformis*, Figs. 1, 4 and 5) differed from the seven previously described *Escovopsis* species, mainly in micro-morphological structures. All isolates had white colonies with a floccose appearance on all culture media, but *E. clavatus* had the most floccose colonies. After 5-7 days incubation, the centre of the colonies turned pale brown and, after 7 days, the entire colony gradually turned from white to light brown (not always from the middle to the edge in *E. multiformis*).

Escovopsis multiformis showed growth at wide ranges of temperature (from 10-30°C); nonetheless, *E. clavatus* showed growth only at 20 and 25°C (Fig. 1). None of the isolates grew at 35°C. On all culture media, the best growth was obtained at 25°C for *E. clavatus* and at 30°C for *E. multiformis*. In all cases where growth was observed, it started between 24 to 36 hours and sporulation started on the third day.

All strains of both species have a unique type of conidiophore with a swollen cell, from which branches emerge (Figs. 2C-D, 3E, 4D-F and 5C-F). These conidiophores were more frequent in *E. multiformis* than *E. clavatus* (27% and 15%, respectively). Mono or polycephalous conidiophores, without the swollen cells, that were described in the other *Escovopsis* species, were also present but with some differences in the size and branching

pattern (Figs. 2A-B, 3A-D, 4A-C and 5A-B). Conidiophores with cruciform or opposed branches were rarely observed. On the other hand, the two new species had basipetal and smooth-walled conidia with slightly thickened walls, formed from phialides. No chlamydospores were observed in the aerial or submersed mycelia of the three strains.

Phylogenetic analyses

Separate phylogenetic analyses with the three molecular markers showed topological differences because of the incongruity placement of the formal described *Escovopsis* species and some strains that form new phylogenetic clades within the genus (Fig. 6). The phylogenetic placement of *E. multiformis* and *E. clavatus* also presented conflicts amongst the three molecular markers; however, the position of each strain that made up both new species was concordant through the three genealogies (PP= 1; MBL= 100%, Fig. 6).

The combined analysis also confirmed *E. multiformis* and *E. clavatus* as two new phylogenetic species in *Escovopsis* (PP= 1; MLB= 100%, Fig. 7) and showed the strain LESF018 (a vesiculated *Escovopsis* species) as the closest relative of both. Nevertheless, the concatenated BI and ML trees also presented few differences between them with respect to the position of the *E. aspergilloides* and *E. lentecrescens*. The BI analysis placed *E. aspergilloides* and *E. lentecrescens* separate from *E. multiformis* and *E. clavatus* (Fig. 7); however, the ML analysis showed the former species as sister clades of *E. multiformis* and *E. clavatus*.

It is important to highlight that the concatenated analysis, as well as the trees inferred with ITS and LSU, showed the vesiculated *Escovopsis* (*E. aspergilloides, E. clavatus, E. lentecrescens, E. microspora, E. moelleri, E. multiformis, E. weberi*) as the most derived group, separated from the non-vesiculated *Escovopsis* (*E. kreiselii* and *E. trichodermoides*).

In addition, both the combined and the analysis performed with ITS and *tef*1 showed some *Escovopsis* species (*E. aspergilloides, E. kreiselii, E. lentecrescens* and *E. trichodermoides*) often clustering with other Hypocreaceae genera or falling outside the *Escovopsis* clade, which reveals that *Escovopsis* is apparently paraphyletic (Figs. 6 and 7).

Discussion

The attine ants have persisted for millions of years because of the biological relationships that these insects maintain with the beneficial microorganisms that inhabit their colonies. Several studies tried to understand how these biological relationships sculptured the evolutionary history of the attine ants (Mueller et al. 1998, Currie et al. 2003, Gerardo et al. 2006ab, Nygaard et al. 2016, Mueller et al. 2018). Nevertheless, the taxonomy of *Escovopsis*, the only known parasite in the attine's environment, has been poorly addressed. Considering that *Escovopsis* co-evolved with the attine ants' cultivar, improved knowledge about the taxonomy and systematics of this genus could shed light on the evolutionary success of these insects. Therefore, the discovery and description of new *Escovopsis* species is an important advance in understanding this system.

Subsequent to the formal description of *Escovopsis* (Muchovej and Della Lucia 1990), several studies showed a high genetic diversity of this genus in the colonies of both leafcutter and non-leafcutter attine ants (Gerardo et al. 2006a, Meirelles et al. 2015b). Only seven species of the parasite have been described so far (Seifert et al. 1995, Augustin et al. 2013, Masiulionis et al. 2015, Meirelles et al. 2015a) and the morphological diversity and physiology of the parasite remain unknown. In addition, a lack of standardised conditions for describing the morphology of *Escovopsis* hinders researchers from identifying morphological characters that might help to distinguish *Escovopsis* species from one another

and from the other related genera from the Hypocreaceae. Unfortunately, this fact made it difficult to describe new species of the parasite. Studies showed that the expressed phenotypic characters (phenotypic plasticity) of fungi are directly influenced by growth conditions (Slepecky and Starmer 2009, Sharma and Pandey 2010, Wrzosek et al. 2017, Kim et al. 2017). As the morphological plasticity of *Escovopsis* species is still poorly understood, the standardisation of cultivation conditions is imperative. The strains described as new species here were evaluated on eight different culture media (those used in the description of the seven previous species) and at five temperatures (to establish cardinal growth temperatures). Due to the lack of standard culture conditions, the comparison with each species previously described was only partial. Nonetheless, we are providing characters of these two new species in all the conditions previously used, to help future researchers to standardise the taxonomy of the genus.

Recent attempts to expand the morphological concept of *Escovopsis* generated inconsistencies in the taxonomy and systematics of this genus (Masiulionis et al. 2015, Meirelles et al. 2015a). The morphologic characters that initially gave rise to the concept of *Escovopsis* (presence of terminal vesicles and phialidic conidiogenesis, see Muchovej and Della Lucia 1990) are distinctive to delineate *Escovopsis*, because no other genus in the Hypocreaceae family has such combined characters. However, some *Escovopsis* species described recently, namely *E. trichodermoides* and *E. kreiselii*, lack vesicles and each has a different kind of conidiogenesis (synchronous and sympodial, respectively). Besides, the results of the phylogenetic analysis performed in previous studies (Meirelles et al. 2015b, Masiulionis et al. 2015, Augustin et al. 2013), as well as the results from our analysis, reveals that *Escovopsis* is paraphyletic (Figs. 6 and 7). Therefore, future studies will have to reconsider if both species indeed belong to *Escovopsis*. For this purpose, the taxonomic

conditions need to be delimited and additional molecular markers will have to be included to help us resolve those phylogenetic incongruities. Then the generic concept of *Escovopsis* species should be revisited.

Our study shows that the ex-type strains LESF 853 (*E. clavatus*) and LESF 847 (*E. multiformis*) form a monophyletic clade within most derived *Escovopsis* (vesiculated *Escovopsis*) (PP = 1, BML = 100%). Most interesting, unlike the other *Escovopsis* species, the two new species present a unique type of conidiophore with a swollen cell, from which one to four branches arise. The newly described species also possess smooth conidia with slightly thickened walls. A recent study suggests the possibility that conidia ornamentation could be associated with the mechanism for horizontal transmission of *Escovopsis* between ant colonies and with the latency of the parasite conidia. This hypothesis was based on observations of some conidia adhering to the ant legs and in spore dormancy in vitro bioassays (Augustin et al. 2017). The same authors also argued that such character could be used as morphological markers for the taxonomy of the genus. However, because of scarce knowledge of the morphological features of the *Escovopsis* species, it is difficult to decipher which phenotypic character could be considered diagnostic for this genus. Therefore, future researchers need to carefully evaluate the phenotypic characters of each *Escovopsis* clade to determine which of characters are homologous versus those that are homoplasious to build taxonomic keys.

Considering the high genetic diversity of *Escovopsis* and the poor knowledge of its taxonomic diversity, the description of these new species are merely two small pieces of a complex puzzle. Nonetheless, our work should help future researchers to build the framework for the systematics of this parasitic fungus. In addition, our study also suggests that the

fungus gardens of attine ants could host a high diversity of *Escovopsis* that has yet to be discovered.

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Figure legends

Fig 1. Colony macroscopic characters of *Escovopsis clavatus* and *Escovopsis multiformis* on CMD, CYA, MA2%, MEA, OA, PCA, PDA and SNA media after 14 days at 10, 20, 25 and 30°C.

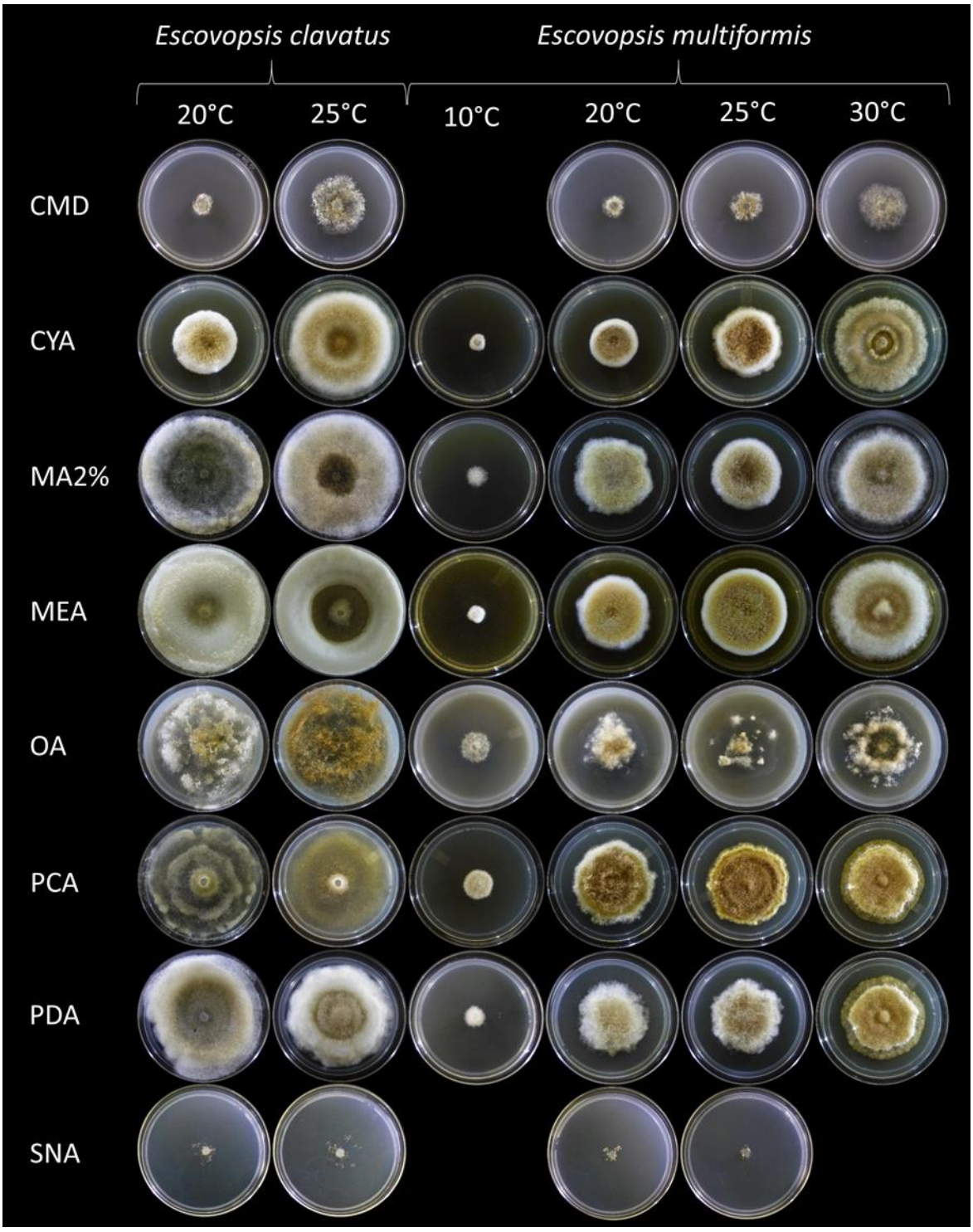


Fig 2. *Escovopsis clavatus*. A-B: Conidiophores without "swollen cells". C-D: Conidiophores with "swollen cells" (red arrow). E-G: Vesicles in various shapes with phialides pattern. G: Conidia.

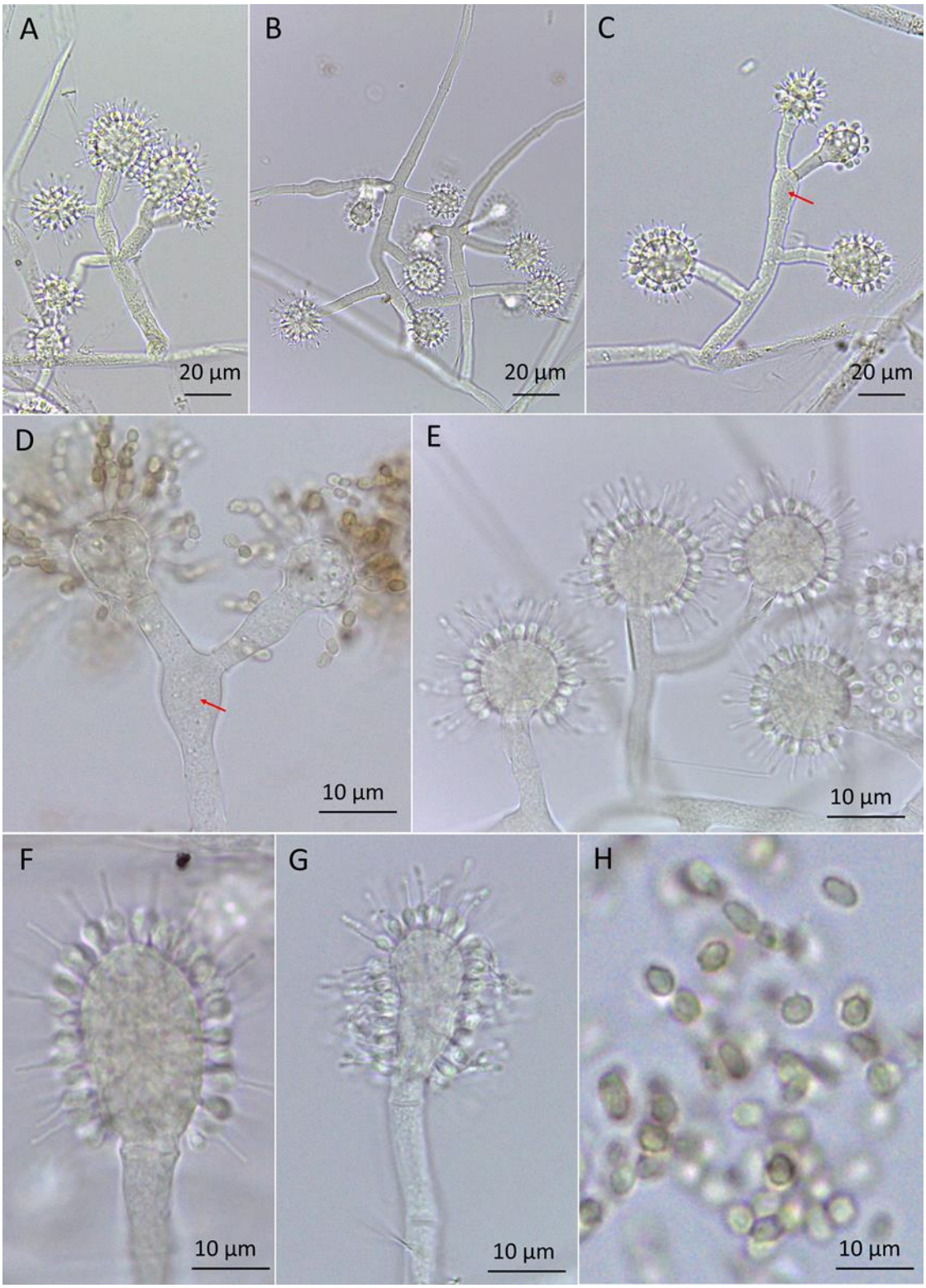


Fig 3. *Escovopsis clavatus*. SEM images A-D: Conidiophores without "swollen cells". E: Conidiophore with "swollen cells" (red arrow). F-G: Vesicles. H: Phialides. G: Conidia.

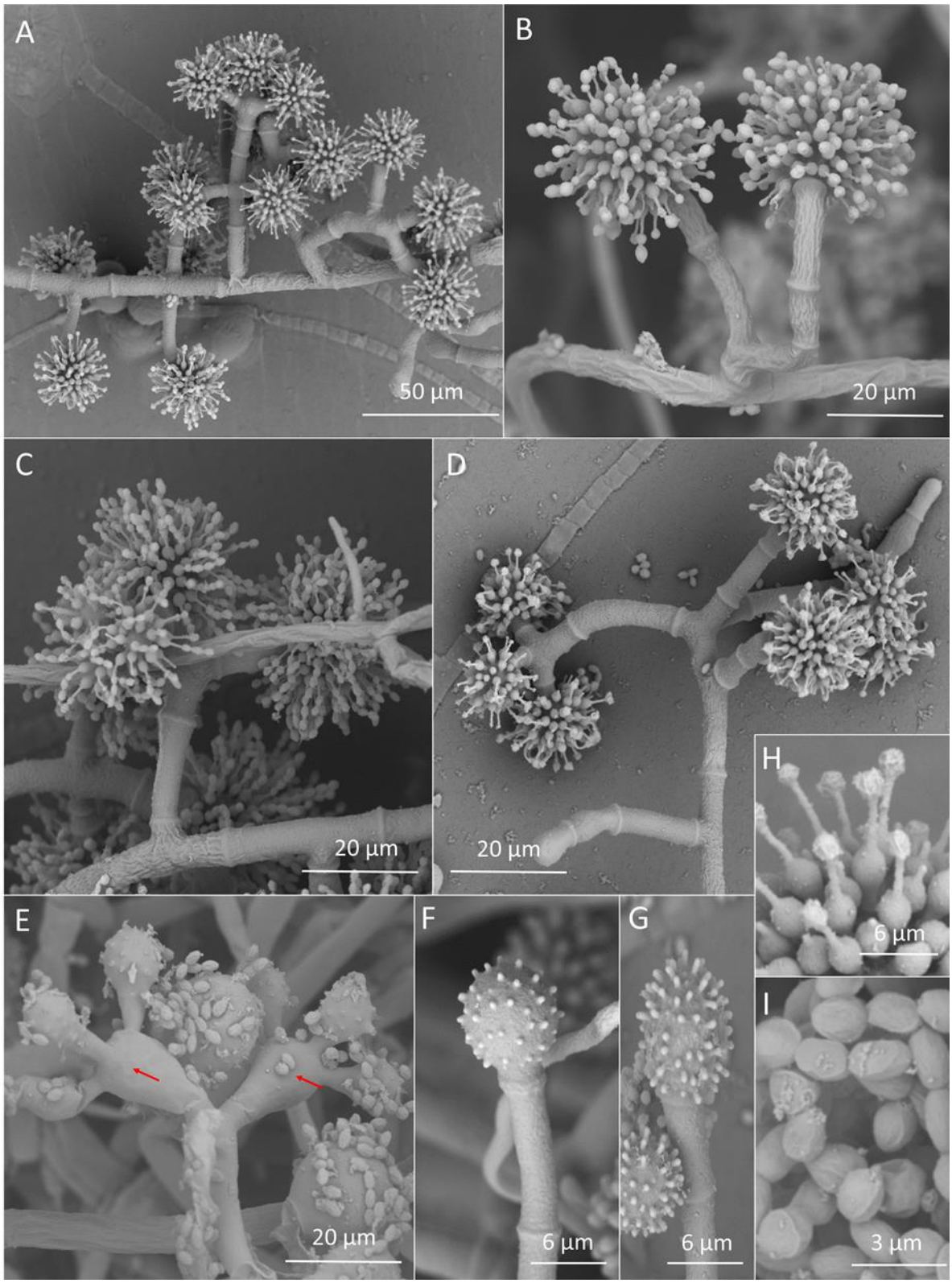

A
50 µm
B
20 µm
C
20 µm
D
20 µm
H
6 µm
I
3 µm
E
20 µm
F
6 µm
G
6 µm

Fig 4. *Escovopsis multiformis*. A-C: Conidiophores mono- and polycephalous without "swollen cells". D-G: Conidiophores mono and polycephalous with "swollen cells" (red arrow). H-I: Vesicles in various shapes. J: Conidia.

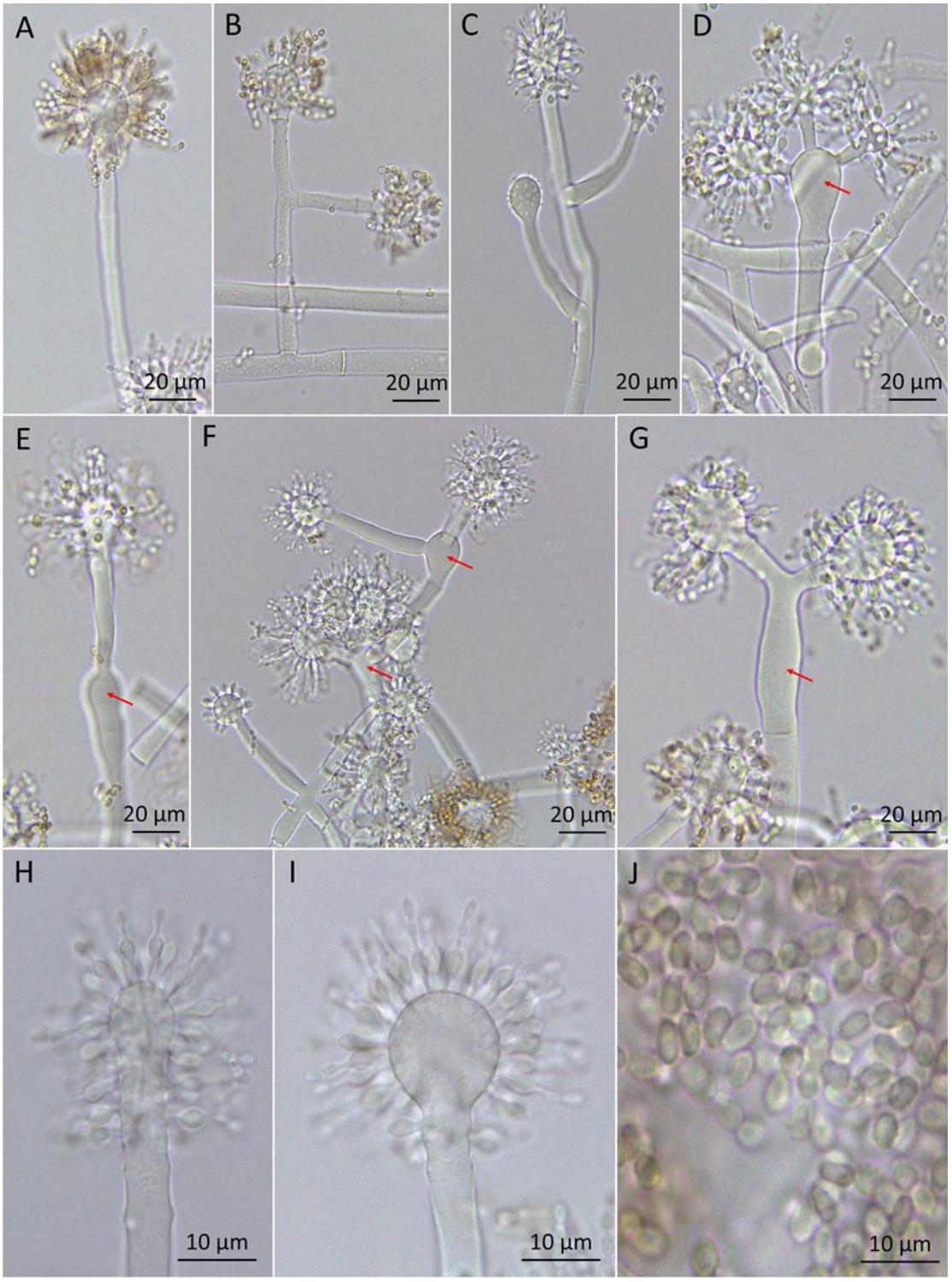


Fig 5. *Escovopsis multiformis*. SEM images A-B: Conidiophores mono- and polycephalous without “swollen cells”. C-F: Conidiophores mono- and polycephalous with “swollen cells” (red arrow). G-H: Vesicles. I: Phialides. J: Conidia.

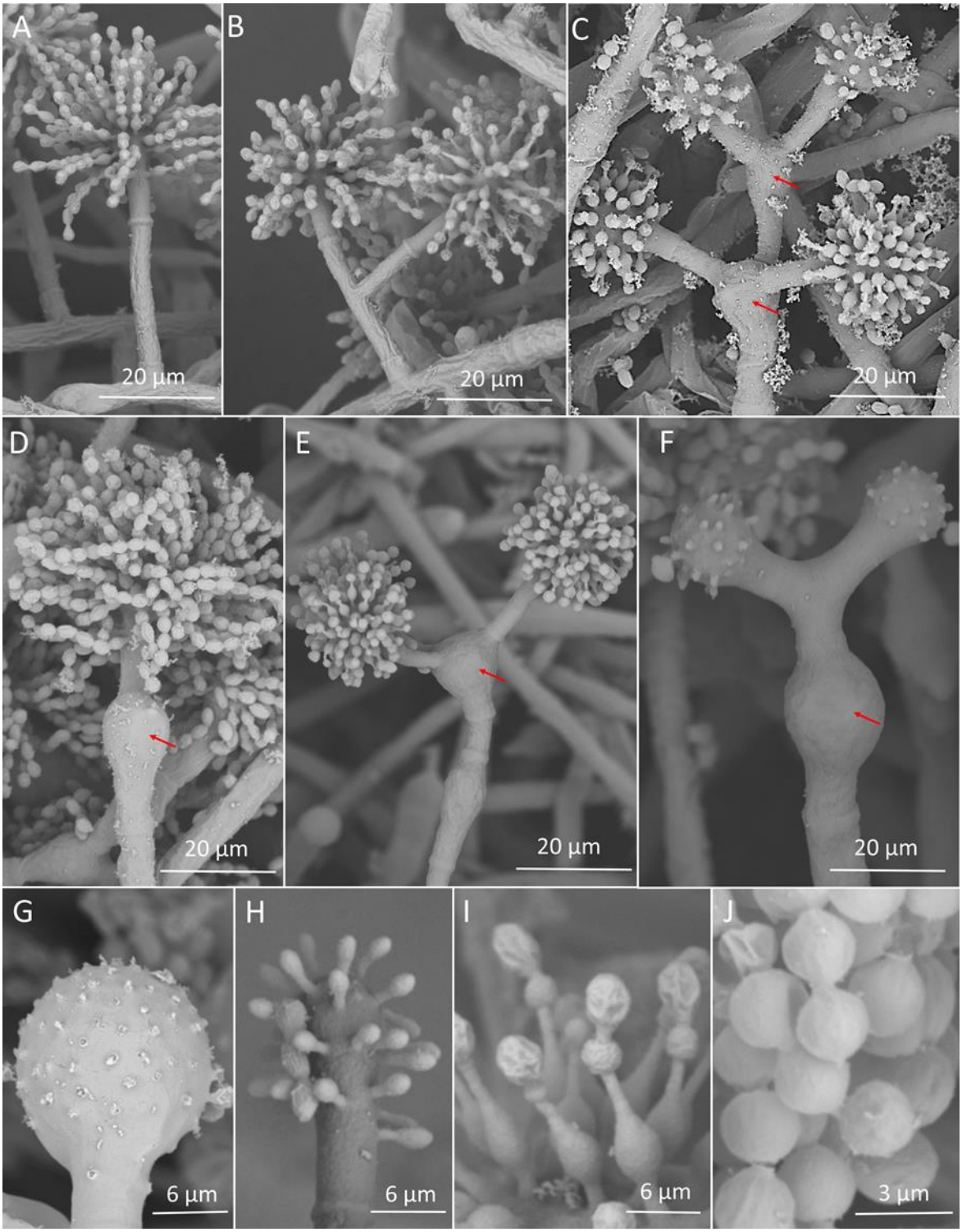


Fig 6. Phylogenetic position of *Escovopsis clavatus* and *Escovopsis multiformis* considering each molecular marker separately (ITS, LSU and *tef1*). The trees were reconstructed under Bayesian and Maximum Likelihood inferences. The numbers on branches indicate the posterior probabilities and the bootstrap support values, respectively. The seven *Escovopsis* ex-type strains are denoted in bold and the new species are highlighted in green (*E. clavatus*) and light brown (*E. multiformis*). The trees include a total of 40 *Escovopsis* sequences of each marker (ITS - 621 bp, LSU - 531 bp and *tef*1 - 758 bp) and *Escovopsioides*, *Hypomyces*, *Sphaerostilbella*, *Trichoderma* and *Protocrea* were included as the closest phylogenetic relatives of *Escovopsis*. *Lecanicillium antillanum* CBS 350.85 was used as the outgroup. ET: ex-type.

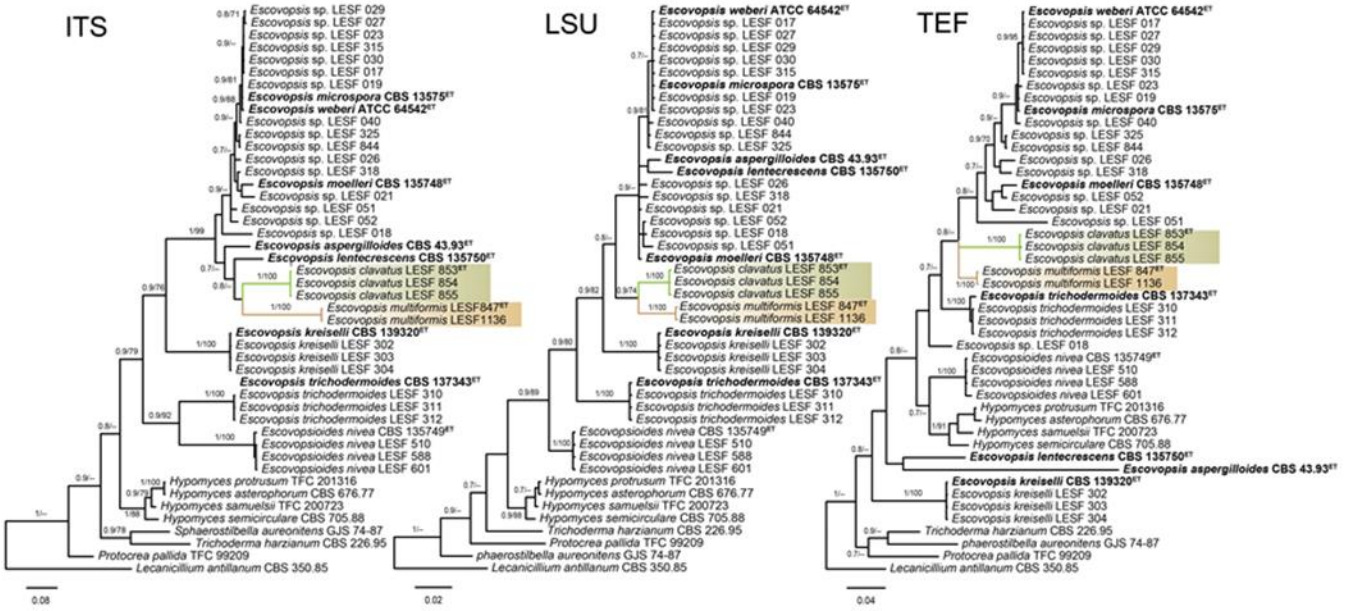
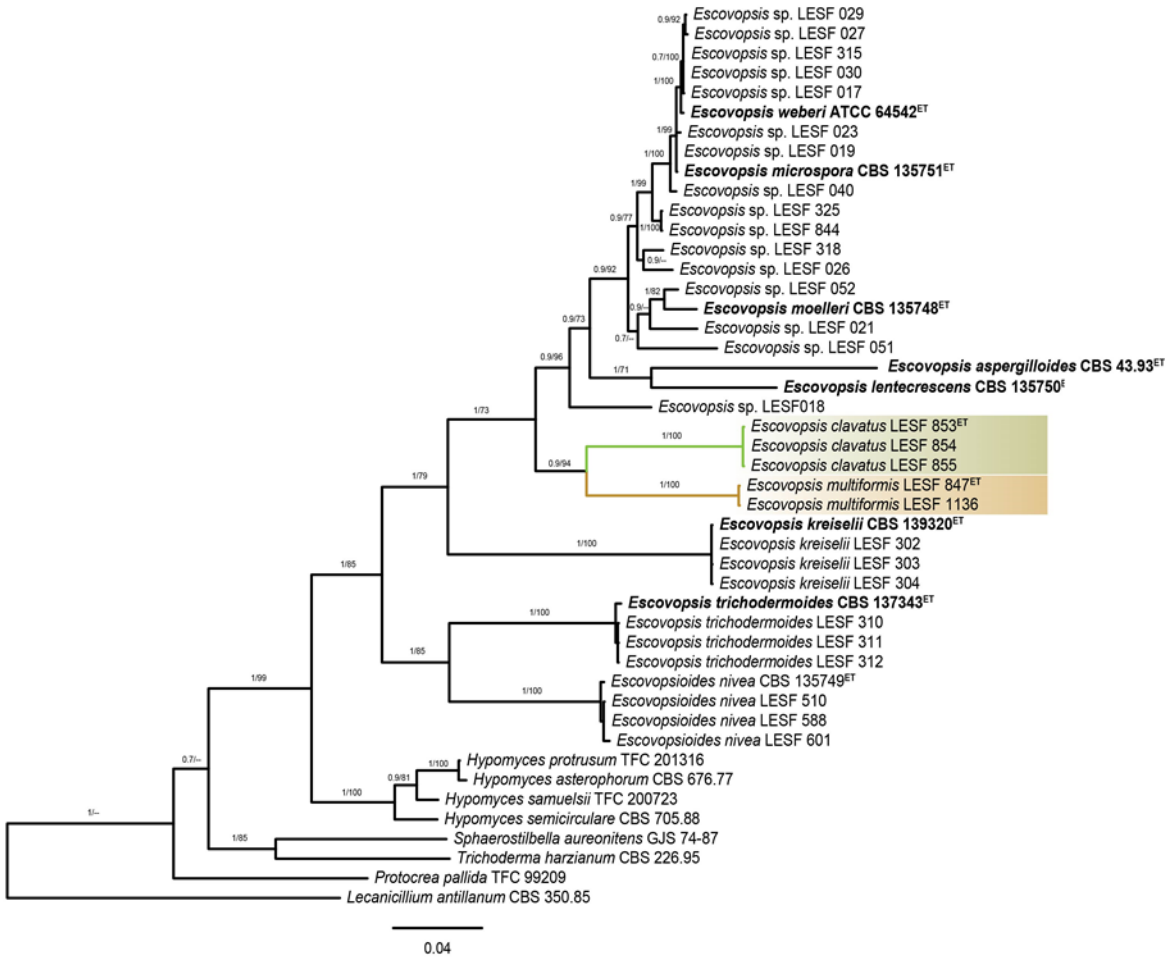


Fig 7. Phylogenetic position of *Escovopsis clavatus* and *Escovopsis multiformis*. The phylogenetic analysis is based on the concatenated sequences of ITS, LSU and *tef1*; and the tree was reconstructed using Bayesian and Maximum Likelihood inferences. Numbers on branches indicate the posterior probabilities and the bootstrap support values, respectively. All *Escovopsis* species previously described are denoted in bold and the new species are highlighted in green for *E. clavatus* and light brown for *E. multiformis*. The tree includes a total of 40 *Escovopsis* sequences with 1911 bp (ITS - 621 bp, LSU - 531 bp and *tef*1 - 758 bp). The data also included sequences from *Escovopsioides*, *Hypomyces*, *Sphaerostilbella*, *Trichoderma* and *Protocrea* as the closest phylogenetic relatives of the parasite. *Lecanicillium antillanum* CBS 350.85 was used as the outgroup. ET: ex-type strains. Bar: 0.04 substitutions per nucleotide position.



Supporting information

Table S1. *Escovopsis* strains used in the phylogenetic analyses and their associated metadata.

Fungal species	Strain ID	Specimen voucher	City, State, Country	GPS	Habitat	Ant colony ID	GenBank accession numbers			References
							ITS	LSU	*tef*1	
Escovopsis microspora	CBS 135751 ET	VIC:31756	Viçosa, Minas Gerais, Brazil	20°44'31.71"S 42°52'43.83"W	Fungus garden of *Acromyrmex subterraneus molestans*	---	JQ815076	KF293284	KJ935030&	Augustin et al. (2013); Meirelles et al. (2015a)
Escovopsis weberi	ATCC 64542 ET	---	Viçosa, Minas Gerais, Brazil	---	Carpenter ant fungal mass	---	KF293285	KF293281	AY172623*	Augustin et al. (2013); Currie et al. (2003)
Escovopsis moelleri	CBS 135748 ET	VIC:31753	Viçosa, Minas Gerais, Brazil	20°44'31.71"S 42°52'43.83"W	Fungus garden of *Acromyrmex subterraneus molestans*	---	JQ815077	JQ855715	JQ855712	Augustin et al. (2013)
Escovopsis aspergilloides	CBS 423.93 ET	DAOM:216382	Trinidad and Tobago: Trinidad	---	Nest of *Trachymyrmex ruthae*	---	NR_137160	KF293283	AY172632*	Augustin et al. (2013); Currie et al. (2003)
Escovopsis lentecrescens	CBS 135750 ET	VIC:31755	Viçosa, Minas Gerais, Brazil	20°44'31.71"S 42°52'43.83"W	Fungus garden of *Acromyrmex subterraneus subterraneus*	---	JQ815079	JQ855717	JQ855714	Augustin et al. (2013)
Escovopsis multiformis	CBS 145327 ET	1606w	Florianópolis, Santa Catarina, Brazil	27°28'11.28"S 48°22'39.48"W	Fungus garden of *Apterostigma* sp.	AR150816-06	MH715091	MH715105	MH724265	This study
Escovopsis multiformis	LESF 1136	QVM277	Alta Floresta, Mato Grosso, Brazil	09°49'22.74"S 58°15'32.04"W	Fungus garden of *Apterostigma* sp.	QVM171004-02	MH715092	MH715106	MH724266	This study
Escovopsis clavatus	CBS 145326 ET	1707	Florianópolis, Santa Catarina, Brazil	27°44'39.6"S 48°31'10.14"W	Fungus garden of *Apterostigma* sp.	AR150817-07	MH715096	MH715110	MH724270	This study
Escovopsis clavatus	LESF 854	1704A	Florianópolis, Santa Catarina, Brazil	27°44'38.94"S 48°31'9.3"W	Fungus garden of *Apterostigma* sp.	AR150817-04A	MH715097	MH715111	MH724271	This study
Escovopsis clavatus	LESF 855	1705B	Florianópolis, Santa Catarina, Brazil	27°44'39.49"S 48°31'9.72"W	Fungus garden of *Apterostigma* sp.	AR150817-05B	MH715098	MH715112	MH724272	This study
Escovopsis kreiselii	CBS 139320 ET	LESF 053	Florianópolis, Santa Catarina, Brazil	27°37'50.01"S 48°27'03.64"W	Fungus garden of *Mycetophylax morchi*	AR090306–01	KJ808767	HJ808765	KJ 808766	Meirelles et al. (2015a)

Escovopsis kreiselii	LESF 302	AR14022705	Florianópolis, Santa Catarina, Brazil	27°31'24.96"S 48°25'3.78"W	Fungus garden of *Mycetophylax morchi*	AR140227–05	MH715085	MH715099	MH724259	This study
Escovopsis kreiselii	LESF 303	AR14022705B	Florianópolis, Santa Catarina, Brazil	27°31'24.96"S 48°25'3.78"W	Fungus garden of *Mycetophylax morchi*	AR140227–05	MH715086	MH715100	MH724260	This study
Escovopsis kreiselii	LESF 304	AR14022705T2D	Florianópolis, Santa Catarina, Brazil	27°31'24.96"S 48°25'3.78"W	Fungus garden of *Mycetophylax morchi*	AR140227–05	MH715087	MH715101	MH724261	This study
Escovopsis trichodermoides	CBS 137343 ET	VEM-2014	Florianópolis, Santa Catarina, Brazil	22°23'46.93"S, 47°32'40.12"W	Fungus garden of *Mycocepurus goeldii*	---	KJ485699	MF116052$	KF033128	Masiulionis et al. (2015); Osti and Rodrigues (2018)
Escovopsis trichodermoides	LESF 310	AR14022604A1	Florianópolis, Santa Catarina, Brazil	27°37'49.62"S, 48°27'3.6"W	Fungus garden of *Mycetophylax morchi*	AR140226-04A	MH715088	MH715102	MH724262	This study
Escovopsis trichodermoides	LESF 311	AR14022604A2	Florianópolis, Santa Catarina, Brazil	27°37'49.62"S, 48°27'3.6"W	Fungus garden of *Mycetophylax morchi*	AR140226-04A	MH715089	MH715103	MH724263	This study
Escovopsis trichodermoides	LESF 312	AR14022604ALA	Florianópolis, Santa Catarina, Brazil	27°37'49.62"S, 48°27'3.6"W	Fungus garden of *Mycetophylax morchi*	AR140226-04A	MH715090	MH715104	MH724264	This study
Escovopsis sp.	LESF 017	NL001	Botucatu, São Paulo, Brazil	22° 50.774' S 48° 26.160'W	Midden of *Atta capiguara*	N66	KM817072	MH715113#	KM817142	Meirelles et al. (2015b)
Escovopsis sp.	LESF 018	NL002	Botucatu, São Paulo, Brazil	22° 50.774' S 48° 26.160'W	Fungus garden of *Atta capiguara*	N66	KM817073	MH715114#	KM817143	Meirelles et al. (2015b)
Escovopsis sp.	LESF 019	NL005	Botucatu, São Paulo, Brazil	---	Fungus garden of *Atta sexdens rubropilosa*	N68	KM817074	MH715115#	KM817144	Meirelles et al. (2015b)
Escovopsis sp.	LESF 021	ES002	Rio Claro, São Paulo, Brazil	---	Fungus garden of *Atta sexdens rubropilosa*	N-Ale	KM817053	MH715116#	KM817123	Meirelles et al. (2015b)
Escovopsis sp.	LESF 023	ES005	Alta Floresta, Mato Grosso, Brazil	---	Fungus garden of *Atta cephalotes*	SES040129-06	KM817056	MH715117#	KM817126	Meirelles et al. (2015b)
Escovopsis sp.	LESF 026	ES009	Carreiro da Várzea, Amazonas, Brazil	---	Fungus garden of *Atta cephalotes*	SES040220-01	KM817060	MH715118#	KM817130	Meirelles et al. (2015b)
Escovopsis sp.	LESF 027	ES010	Rio Claro, São Paulo, Brazil	---	Fungus garden of *Acromyrmex landolti*	---	KM817061	MH715119#	KM817131	Meirelles et al. (2015b)
Escovopsis sp.	LESF 029	ES012	Corumbataí, São Paulo, Brazil	---	Fungus garden of *Atta sexdens*	13B	KM817063	MH715120#	KM817133	Meirelles et al. (2015b)
Escovopsis sp.	LESF 030	ES013	Corumbataí, São Paulo, Brazil	---	Fungus garden of *Atta sexdens*	1	KM817064	MH715121#	KM817134	Meirelles et al. (2015b)

Escovopsis sp.	LESF 040	RS020	Nova Petrópolis, Rio Grande do Sul, Brazil	---	Fungus garden of *Acromyrmex laticeps*	AOMB060904-05	KM817077	MH715122[#]	EU082803	Meirelles et al. (2015b)
Escovopsis sp.	LESF 051	SES009	Palmeiras, Bahia, Brazil	---	Fungus garden of *Trachymyrmex* sp.	SES081108-04	KM817092	MH715123[#]	KM817153	Meirelles et al. (2015b)
Escovopsis sp.	LESF 052	SES010	Camp 41, Manaus, Amazonas, Brazil	---	Fungus garden of *Trachymyrmex diversus*	SES090109-04	KM817093	MH715124[#]	KM817154	Meirelles et al. (2015b)
Escovopsis sp.	LESF 315	NL007	Botucatu, São Paulo, Brazil	---	Fungus garden of *Atta sexdens rubropilosa*	N68	KM817075	MH715125[#]	KF240730	Meirelles et al. (2015b)
Escovopsis sp.	LESF 318	ES029	Palmas, Tocantins, Brazil	---	Fungus garden of *Trachymyrmex* sp.	WGPM091021-01	KM817069	MH715126[#]	KM817139	Meirelles et al. (2015b)
Escovopsis sp.	LESF 325	BA004	Camacan, Bahia, Brazil	14°47'56.8"S, 39°10'16.4"W	Fungus garden of *Atta cephalotes*	BMSR120703-01(FL5)	KM817049	MH715127[#]	KM817119	Meirelles et al. (2015b)
Escovopsis sp.	LESF 844	BA005	Camacan, Bahia, Brazil	14°47'56.8"S, 39°10'16.4"W	Fungus garden of *Atta cephalotes*	BMSR120703-01(FL5)	KM817050	MH715128[#]	KM817120	Meirelles et al. (2015b)
Escovopsioides nivea	CBS 135749[ET]	AUJ6	Viçosa, Minas Gerais, Brazil	20°44'31.71"S, 42°52'43.83"W	Fungus garden of *Acromyrmex subterraneus subterraneus*	---	JQ815078	JQ855716	JQ855713	Augustin et al. (2013)
Hypomyces protrusum	TFC 201316	---	Madagascar	---	Eucalyptus forest	---	FN859414	FN859414	FN868732	Põldmaa (2011)
Hypomyces semicirculare	CBS 705.88	---	Cuba	---	On old polypore	---	NR_121425	FN859417	FN868735	Põldmaa (2011)
Hypomyces asterophorum	CBS 676.77	---	Japan	---	---	---	FN859395	AJ583469	FN868712	Põldmaa (2011)
Hypomyces samuelsii	TFC 2007-23	---	Peru	---	on basidioma of an agaricoid basidiomycete on a stem of a palm	---	FN859451	FN859451	FN868769	Põldmaa (2011)
Hypomyces samuelsii	C.L.L. 7259	---	West Indies	---	on *Auricularia* cf. *polytricha* on bark of Cyathea	---	FN859445	FN859445	FN868764	Põldmaa (2011)
Trichoderma harzianum	CBS 226.95	---	England	---	---	---	AY605713	HM466680	AF534621	Chaverri et al. (2003)
Protocrea pallida	TFC 99-209	---	New York, Cleaveland	---	---	---	NR_111329	EU710769	EU703903	Jaklitsch and Samuels (2011)
Sphaerostilbella aureonitens	GJS 74-87	---	---	---	---	---	FJ442633	HM466683	FJ467644	unpublished
Lecanicillium antillanum	CBS 350.85	---	Cuba	---	on basidioma of an agaricoid	---	NR_111097	AF339536	DQ522350	Spatafora et al. (2007)

[ET] Ex-type; [&] *tef*1 sequences obtained by Meirelles et al. (2015a); [*] *tef*1 sequences obtained by Currie et al. (2003); [$] LSU sequences obtained by Osti and Rodrigues (2018); [#] LSU sequences obtained in this study.

Table S2. Molecular markers, primers and PCR conditions used in this study.

Marker	Primers	Conditions	References
ITS	ITS4 (5'TCCTCCGCTTATTGATATGC3') ITS5 (5'GGAAGTAAAAGTCGTAACAAGG3')	96°C for 3 min, 35 cycles at 94°C for 1 min, 55°C for 1 min and a final extension step at 72°C for 2 min	White et al. (1990); Schoch et al. (2012)
*tef*1	EF6–20F (5'AAGAACATGATCACTGGTACCT3') EF6–1000R (5'CGCATGTCRCGGACGGC3')	96°C for 3 min, 35 cycles at 96°C for 30 s, 61°C for 45 s and a final extension step at 72°C for 1 min	Taerum et al. (2007)
LSU	CLA-F (5'GCATATCAATAAGCGGAGGA3') CLA-R (5'GACTCCTTGGTCCGTGTTTCA3')	96°C for 3 min, 35 cycles at 94°C for 1 min, 55°C for 1 min and a final extension step at 72°C for 2 min	White et al. (1990); Haugland and Heckman (1998); Currie et al. (2003)

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Chapter II

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"Fungi of the strong conidial form" in attine ant colonies: taxonomic and phylogenetic reassessment of the genus *Escovopsis*

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Abstract

Escovopsis (Ascomycota: Hypocreales, Hypocreaceae) is a fascinating group of fungi, members of which are considered specialized parasites of the fungal cultivars of fungus-growing ants (Formicidae: Myrmicinae: Attini: Attina, the "attines"). The lack of a suitable taxonomic framework and phylogenetic inconsistencies have long hampered *Escovopsis* research. The aim of this study was to create a standardized taxonomic framework and provide a comprehensive multilocus phylogenetic analysis, in order to set the basis of the *Escovopsis* systematics, and the stage for future *Escovopsis* research. Morphological and phylogenetic analyses support the separation of *Escovopsis* into three distinct genera. In light of this, we redefine *Escovopsis* and place *E. kreiselii* and *E. trichodermoides* into two new genera (*Sympodiorosea* and *Luteomyces*, respectively). Furthermore, we propose *Escovopsis microspora* as a synonym of *E. weberi*, and we recombined *E. kreiselii* and *E. trichodermoides* as *Sympodiorosea kreiselii* and *Luteomyces trichodermoides*, respectively. This study significantly expands our understanding of the systematics of *Escovopsis* and related genera, thereby facilitating future research on the evolutionary history, taxonomic diversity, and ecological roles of *Escovopsis*, *Sympodiorosea*, and *Luteomyces*.

Keywords: *Hypocreaceae*, Phylogenetics, Attines, Symbiosis, Evolution, fungus-growing ants

Introduction

Fungi can exhibit bewildering morphological and genetic diversity (Mueller and Schmit 2007; Hawksworth and Lücking 2017). Through systematic approaches, taxonomists have developed mechanisms to categorize organisms based on their phenotypic and genetic characters (Komarek and Beutel 2006; Pavlinov 2018). Classification of organism demands that a selected set of morphological characters be evaluated under standardized parameters (Komarek and Beutel 2006). The lack of standardization precludes detailed study of many fungal groups, as is the case for the genus *Escovopsis*. *Escovopsis* is a diverse group of Hypocrealean fungi (Ascomycota: Hypocreales, Hypocreaceae), members of which are presumed to have evolved parasitizing the mutualistic fungus of fungus-growing ants (Formicidae: Myrmicinae: Attini: Attina, the "attines") (Yek et al. 2012). Besides, *Escovopsis* has only been found associated with fungus-growing ant colonies, suggesting that the genus has evolved in relation to these ants' system, potentially for millions of years. Despite their fascinating biology in relation to a canonical system for the study of coevolution and symbiosis, the paucity of taxonomic studies and unresolved phylogenetic inconsistencies have prevented the development of a comprehensive understanding of the systematics, ecology, and evolution of these fungi.

More than a century has passed since Möller (1893) observed a group of "fungi of the strong conidial form" in attine gardens, which 80 years later would be named *Phialocladus* (Kreisel 1972). Almost two decades later, *Phialocladus* was considered an invalid name because of the lack of the type specimen of the genus, and consequently, it was renamed as *Escovopsis* (*Escova*- Portuguese for brush, *-opsis* Greek for like), with *Escovopsis weberi* designated as the type species of the genus (Muchovej and Della Lucia 1990). Although the

macroscopic characters of *E. weberi* were not fully described by the authors, the description of this species established the foundation for *Escovopsis* taxonomy. In 1995, Seifert et al. (1995) described *Escovopsis aspergilloides* in a detailed taxonomic study, providing a suitable framework to standardize the conditions for assessment of the the morphological features of *Escovopsis*. However, after this study, the taxonomy of *Escovopsis* was set aside for eighteen years.

Unlike taxonomic studies, the relationship between *Escovopsis* and the attine ants' mutualistic fungi has been the topic of numerous studies (Currie et al. 2003; Gerardo et al. 2006a,b; Taerum et al. 2007; Folgarait et al. 2011; Elizondo Wallace et al. 2014; Marfetán et al. 2015; Birnbaum and Gerardo 2016; de Man et al. 2016; Heine et al. 2018). Through these studies, a great diversity of *Escovopsis* was revealed and the first ideas about the phylogenetic position of this fungal group were gradually emerging (Gerardo et al. 2006b; Augustin et al. 2013; Masiulionis et al. 2015; Meirelles et al. 2015a,b). Initially, some authors suggested that *Escovopsis* belonged to Hypocreales, although, at the time no phylogenetic evidence was provided to support that hypothesis (Currie et al. 1999a,b). The first phylogenetic analysis of *Escovopsis* confirmed the genus to be within Hypocreales and place it as a sister clade of the Hypocreaceae (Currie et al. 2003). However, a more extensive phylogenetic analysis of *Escovopsis* strains associated with fungus gardens of *Apterostigma* ants indicated that the genus belonged to the Hypocreaceae (Gerardo et al. 2006b).

Augustin et al. (2013) were the first authors to combine morphological and phylogenetic approaches to study *Escovopsis*. Based on internal transcribed spacer (ITS) and large subunit ribosomal RNA (LSU) sequences, the authors described *Escovopsis lentecrescens*, *E. microspora*, and *E. moelleri*, which formed a monophyletic clade with *E.*

weberi and *E. aspergilloides*. The most remarkable character of these newly named species was the presence of conidiophores with vesicles, as previously described by Muchovej and Della Lucia (1990) and Seifert et al. (1995). Nonetheless, for the description of the new species, Augustin et al. (2013) used different culture media from those used by Seifert et al. (1995), hindering a clear comparison of interspecific morphological differences. Furthermore, while phylogenetic analyses of Augustin et al. (2013) based ITS and LSU sequences suggested that *Escovopsis* formed a monophyletic clade, the analysis based on translation elongation factor 1-alpha (*tef*1) (where the new species were not included) suggested that *Escovopsis* may not be monophyletic. Furthermore, the authors also introduced the genus *Escovopsioides* (Ascomycota: Hypocreales, Hypocreaceae) which formed a polytomic clade with all Hypocreaceae.

For almost thirty years, the genus *Escovopsis* was morphologically defined by the presence of conidiophores with vesicles that support the phialides (i.e., determinate growth conidiogenous cells), from which conidia are produced (phialidic conidiogenesis). This changed with the introduction of *Escovopsis trichodermoides* (Masiulionis et al. 2015) and *Escovopsis kreiselii* (Meirelles et al. 2015a). These species have conidiophores without vesicles and with synchronous (*E. trichodermoides*) and sympodial (*E. kreiselii*) conidiogenous cells instead of phialides. Therefore, Meirelles et al. (2015a) amended the morphological description of *Escovopsis* to insert the morphological features of *E. kreiselii*. However, because Masiulionis et al. (2015) and Meirelles et al. (2015a) were published at nearly the same time, the new definition did not include *E. trichodermoides*. Furthermore, as with Augustin et al. (2013), the parameters used to describe *E. kreiselii* and *E.*

trichodermoides were different from those used in previous studies, making comparisons with other *Escovopsis* species difficult.

The insertion of *E. trichodermoides* and *E. kreiselii* within *Escovopsis* not only meant changes to the morphological description of the genus but also intensified the phylogenetic uncertainties. In the phylogenies produced by Masiulionis et al. (2015) and Meirelles et al. (2015a,b), it was clear that vesiculate *Escovopsis* were closely related to *Escovopsioides* (Augustin et al. 2013) than to *E. trichodermoides* and *E. kreiselii*. Nonetheless, both Masiulionis et al. (2015) and Meirelles et al. (2015a,b) preferred to maintain *E. trichodermoides*, *E. kreiselii* and the vesiculate *Escovopsis* as being in the same genus.

Recently, Montoya et al. (2019) used the ITS, LSU, and *tef*1 markers in a multilocus phylogenetic approach to describe *Escovopsis clavata* and *E. multiformis*. The authors noticed that disagreements in *Escovopsis* taxonomy occurred between vesiculate *Escovopsis*, *E. trichodermoides* and *E. kreiselii*. Therefore, they highlighted the need to establish a standardized taxonomic framework and to utilize new molecular markers to resolve the phylogeny of the genus. Nevertheless, subsequent description of five new *Escovopsis* species (Marfetán et al. 2019) further complicated *Escovopsis* taxonomy because the phenotypic characters of these species were assessed under different conditions than those used in previous studies. Moreover, interpretation of the phylogenetic analyses carried out by Marfetán et al. (2019) had several limitations: i) it was based on the LSU and *tef*1 genes separately; ii) the *tef*1 sequences obtained in the study do not align with the *tef*1 sequences in previously published studies; and, iii) some of the new species (*Escovopsis atlas*, *E. catenulata*, *E. longivesica* and *E. pseudoweberi*) fall in the same clade yet strains of *E. atlas* fall in different, non-monophyletic clades.

Given this complicated and piecemeal research history, the aim of this study is to reassess the taxonomy of *Escovopsis* by using standardized taxonomic parameters, a select and informative set of morphological characters, and a comprehensive multilocus phylogeny. Our results fill an important gap in mycology and will help future researchers to access the diversity and the evolutionary history of *Escovopsis* and related genera that inhabit the colonies of fungus-growing ants.

Methods

Phylogenetic placement of all strains previously named as *Escovopsis*

The target of this first analysis was to show the phylogenetic placement of the strains currently treated as *Escovpsis*. It is difficult to performe a multilocus analysis because there are few strains on the literature that were sequenced for more than one molecular marker. Nonetheless, the *tef* gene was the one used in most of the studies already published (Currie et al. 2003; Gerardo et al. 2006; Taerum et al. 2007, 2010; Augustin et al. 2013; Meirelles et al. 2015a, b; Masiulionis et al. 2015; Montoya et al. 2019). Accordingly, we gather all *tef* sequences from the literature and combined it with our *tef* data set to reconstruct a phylogenetic tree. The final data set contained a total of 415 *tef* sequences (754 bp), that included vesiculate-*Escovopsis* (n= 281), non-vesiculate *Escovopsis* (n= 70), 63 strains from five *Hypocreaceae* genera (*Escovopsioides*, *Hypomyces*, *Protocrea*, *Sphaerostilbella*, and *Trichoderma*); and *Lecanicillium antillanum* CBS 35085 as the outgroup (Table S1). The *tef*1 sequences of species described in Marfetán et al. (2019) were not included in our analysis because they do not align with the *Escovopsis* sequences from the previous studies.

The data set was first aligned in MAFFT v.7 (Katoh and Standley 2013), and phylogenetic trees were reconstructed using maximum likelihood (ML) and Bayesian inferences (BI) in RAxML (Stamatakis 2014) and MrBayes v.3.2.2 (Ronquist et al. 2012), respectively. The nucleotide substitution model was GTR for ML and K80 + G for BI and was calculated in jModelTest 2 (Darriba et al. 2012), using the Akaike Information Criterion (AIC) with 95% confidence intervals. For ML analysis, 1000 independent trees and 1000 bootstrap replicates were performed, while for BI two separate runs (each consisting of three hot chains and one cold chain) were carried out. In last case, five million generations of the Markov Chain Monte Carlo (MCMC) were enough to reach convergence (standard deviation (SD) of split frequencies fell below 0.01). To generate final BI tree, the first 25% of trees were discarded as burn-in. The final tree was edited in FigTree v.1.4 (http://tree.bio.ed.ac.uk/software/figtree/) and Adobe Illustrator CC v.17.1.

Phylogenetic and taxonomic framework for *Escovopsis*' systematics

Isolates

A total of 103 strains (vesiculate [n= 65], and non-vesiculate [n= 38]) were included in this study (Table S2). Of these, 31 strains were obtained from previous studies (Augustin et al. 2013, Masiulionis et al. 2015, Meirelles et al. 2015b, Montoya et al. 2019), and the remaining (n= 72) were isolated from three regions in Brazil (Anavilhanas, state of Amazonas; Camp 41, state of Amazonas; and Botucatu, state of São Paulo - Table S2). The process of isolation, purification, and preservation of the strains followed methods outlined in Montoya et al. (2019). Briefly, from each attine colony, 21 garden fragments (0.5–1 mm^3) were inoculated on potato dextrose agar (PDA, Neogen Culture Media, Neogen®) plates

supplemented with chloramphenicol [150 µg mL-1, Sigma] (seven garden fragments per plate). The plates were incubated at 25 °C in darkness. Plates were monitored daily for seven days, and, when *Escovopsis* mycelia sprouted out, it was transferred to new PDA plates without chloramphenicol. Axenic cultures were prepared (monosporic cultures) and stored in sterile distilled water kept at 8 – 10 °C (Castellani 1963), and in 10% glycerol at -80 °C.

DNA extraction, PCR and sequencing

The genomic DNA of the strains was extracted using a modified CTAB method (Möller et al. 1992). Briefly, fungal aerial mycelia, grown for seven days at 25 ° C on PDA, was crushed with the aid of glass microspheres (Sigma) in lysis solution and incubated at 65 ° C for 30 minutes. The organic phase was separated using a solution of chloroform-isoamyl alcohol (24: 1). Then, the material was centrifuged (10,000 g for 10 minutes), and the supernatant with the genomic DNA was collected. This extract was precipitated with 3M sodium acetate and isopropanol and purified with two successive washes of 70% ethanol. The DNA was suspended in 30 µL of Tris-EDTA solution and stored at -20 °C.

Five molecular markers were amplified for all newly isolated *Escovopsis* strains: the internal transcribed spacer (ITS), the large subunit ribosomal RNA (LSU), the translation elongation factor 1-alpha (*tef*1), and the RNA polymerase II protein-coding genes (*rpb*1 and *rpb*2, Table S3). For strains from previous publications, we utilized previously published ITS, LSU and *tef*1 sequences, when available, and generated missing sequences for other molecular markers (Table S2). Sequences of *rpb*1 and *rpb*2 for 23 strains in the genus *Escovopsioides* were also generated in this study to complete our dataset (Table S2).

PCR reactions were carried out following Meirelles et al. (2015b) for ITS, Meirelles et al. (2015a) for *tef*1 and Augustin et al. (2013) for LSU (Table S3). PCRs for the *rpb*1 and *rpb*2 genes (Table S3) were performed in a final volume of 25 µL (4 µL of dNTPs [1.25 mM each]; 5 µL of 5X buffer; 1 µL of BSA [1 mg mL^{-1}]; 2 µL of $MgCl_2$ [25 mM]; 1 µL of each primer [10 µM]; 0.5 µl of Taq polymerase [5 U μL^{-1}], 2 µL of diluted genomic DNA [1:100] and 8.5 µL of sterile ultrapure water). When amplification was difficult, we added 1.5 µL of dimethyl sulfoxide (DMSO), decreasing the volume of sterile ultrapure water to 7.0 µL, or we used the PuReTaq™ Ready-to-Go™ PCR kit (illustra™) following the manufacturer's protocol. Touchdown PCR conditions were used for *rpb*1 and *rpb2*: 1) 96 °C for 5 min; 2) 15 cycles of 94 °C for 30s, 65 °C for 1.5 min for *rpb*1 and for 1 min for *rpb*2 (the annealing temperature gradually decreased 1 °C per cycle) and 72 °C for 1.5 min for *rpb*1 and for 1 min for *rpb*2; and then 3) 35 cycles of 94°C for 30s, 50 °C for 1min and 72 °C for 1 min (Table S3).

Final amplicons were purified with the Wizard SV Gel and PCR Clean-up System (Promega) following the manufacturer's protocol. Sequences (forward and reverse) were generated on an ABI3500 (Life Technologies), and the consensus sequences were assembled in BioEdit v. 7.1.3 (Hall 1999) and Geneious (Kearse et al. 2012). All sequences are deposited in GenBank (Table S2 for accession numbers).

Phylogenetic analyses

Phylogenetic analyses were performed at family, genus and species levels. The objective of the analyses at the family level was to investigate whether all clades formally

described as *Escovopsis* belong to *Hypocreaceae*. The objective of the analyses at the genus level was to investigate whether vesiculate *Escovopsis* forms a monophyletic clade, separating them from the non-vesiculate *Escovopsis,* as previously observed by Montoya et al. (2019). Besides, we wanted to know if the monophyly of all those clades remains constant considering the analysis using the five molecular markers separately as well as combined. The analyses at the species level aimed to clarify whether *E. weberi* and *E. microspora* are two different species based on the phylogenetic species concept.

Species described in Marfetán et al. (2019) were not included in our analyses because of the inavailability of ITS, *rpb*1, and *rpb*2 sequences, and because the *tef*1 sequences provided by the authors do not align with the *Escovopsis* sequences from other studies (Augustin et al. 2013; Masiulionis et al. 2015; Meirelles et al. 2015a, b; Montoya et al. 2019). Nonetheless, the LSU sequences generated by Marfetán et al. (2019) were combined with our LSU data to show phylogenetic placement of these strains (Fig. S1).

For the 'family-level', we used a concatenated data set that included 145 sequences for LSU (625 bp), 143 for *rpb*1 (851 bp), 144 for *rpb*2 (980 bp), and 145 for *tef*1 (849 bp) from six families of the Hypocreales order. For this analysis, we generated the *rpb*1 and *rpb*2 sequences for *Escovopsis* (including the nine ex-type strains) and *Escovopsioides* (including the ex-type strain). All other sequences were obtained from the NCBI GenBank database (Currie et al. 2003; Sung et al. 2008; Augustin et al. 2013; Masiulionis et al. 2015; Meirelles et al. 2015a, b; Montoya et al. 2019, Table S4). The *Stachybotrys* clade was used to root the tree (Sung et al. 2008).

Multiple loci were used to address the 'genus-level' questions, and all possible combinations of ITS, LSU, *tef*1, and *rpb*2 (25 combinations) were analyzed. Data sets included 134 sequences of ITS (661 bp), LSU (593 bp), *tef*1 (758 bp), and *rpb*2 (1023 bp); and 132 sequences of *rpb*1 (766 bp). The sequences represented 103 strains from the *Escovopsis* clade (vesiculate (n = 65) and non-vesiculate (n = 38), including the nine ex-type strains), 31 strains from five *Hypocreaceae* genera (*Escovopsioides*, *Hypomyces*, *Protocrea*, *Sphaerostilbella,* and *Trichoderma*); and *Lecanicillium antillanum* CBS 35085 as the outgroup (Table S2).

For the 'species-level', we used a concatenated data set that consisted of only sequences from vesiculate *Escovopsis* (65 sequences of ITS [661 bp], LSU [593 bp], *tef*1 [758 bp], *rpb*1 [766 bp], and *rpb*2 [1023 bp]). *E. kreiselii* (CBS 139320) was used to root the tree (Table S2).

For all analyses, data sets were first aligned separately for each gene in MAFFT v.7 (Katoh and Standley 2013). The nucleotide substitution model for each alignment was calculated in jModelTest 2 (Darriba et al. 2012), using the Akaike Information Criterion (AIC) with 95% confidence intervals. Then, the data sets were concatenated in Winclada v.1.00.08 (Nixon 2002). All phylogenetic trees were constructed using Maximum Likelihood (ML) in RAxML v.8 (Stamatakis 2014) and Bayesian Inference (BI) in MrBayes v.3.2.2 (Ronquist et al. 2012). For ML, we estimated 1000 independent trees and performed 1000 bootstrap replicates using the GTR model for each partition independently. For BI analyses, we carried out two separate runs (each consisting of three hot chains and one cold chain) using the GTR+I+G model for each partition independently; for all analyses, two million generations of the Markov Chain Monte Carlo (MCMC) were enough to reach convergence

(standard deviation (SD) of split frequencies fell below 0.01). To generate BI trees, the first 25% of trees were discarded as burn-in. Trees were edited in FigTree v.1.4 (http://tree.bio.ed.ac.uk/software/figtree/) and Adobe Illustrator CC v.17.1.

Morphology and Growth

Here, we used nine *Escovopsis* ex-type species (*E. aspergilloides*, *E. clavata*, *E. kreiselii*, *E. lentecrescens*, *E. microspora*, *E. moelleri*, *E. multiformis*, *E. trichodermoides*, and *E. weberi*), representing the known diversity of the genus, to i) develop standardized conditions for evaluating the macroscopic and microscopic features of *Escovopsis*; ii) determine if morphological features support the results observed in phylogenetic analyses; and iii) create a taxonomic key for the genus, including illustrations of key microscopic structures.

Macroscopic characters (i.e. radial growth, mycelium colour, morphology and presence of soluble pigments) of the nine *Escovopsis* species were evaluated on eight different media (Table S5) at five temperatures (10 °C, 20 °C, 25 °C, 30 °C and 35 °C). These media and temperatures correspond with those previously used for the description of the known *Escovopsis* species (Seifert et al. 1995; Augustin et al. 2013; Masiulionis et al. 2015; Meirelles et al. 2015a, b; Montoya et al. 2019). After evaluation under these conditions, a set of media was selected based on the i) ability to evaluate growth rate; ii) expression of unique phenotypic characters of each species, iii) feasibility of comparison of morphological features of *Escovopsis* with other *Hypocreaceae*, and iv) ease of access to media in most laboratories. To obtain the inoculum, 200 µL of 10^6 conidia per 1 uL sterile water from

colonies with seventh days of growth were homogeneously spread on Petri dishes (90 x 15 mm) with water-agar (WA) and incubated for seven days at 25 °C in darkness. Then, for all assays, 0.5 cm diameter fragments of WA with mycelia were inoculated in the center of Petri dishes (90 x 15 mm) containing each culture media. Dishes were not sealed and were incubated in the darkness following Montoya et al. (2019), which documented better development of *Escovopsis* under these conditions. We performed three replicates on each media and temperature, and we examined the morphological characters of all species every 24 hours for 14 days. After this evaluation, the optimal time for measuring growth and evaluating macroscopic characters was determined. Both parameters were standardized based on the point at which we were able to observe the most significant differences in growth and morphological characters between species.

Microscopic structures (i.e., conidiophores, conidiophores' branches, conidiophores' swollen cells, vesicles, conidiogenous cells, chlamydospores, and conidia) and their features (i.e., shape, size, colour, and pattern) were evaluated on PDA because *Escovopsis* species form such structures in greater numbers and much faster than in other media. To assess the microscopic structures, we carried out slide culture preparations. To do so, we placed three fragments of 0.5 mm^3 PDA on a microscopic slide, and we then inoculated each fragment with conidia of the fungus. Each inoculated fragment was covered with a coverslip and incubated at 25°C for 4-7 days in the dark. After that, the fragments of PDA were removed and the coverslips with fungal mycelia were placed on slides with a drop of lactophenol. The slides were examined under a light microscope (DM750, Leica, Germany), and the microscopic fungal structures were photographed and measured (30 measurements per structure) using LAS EZ v.4.0 (Leica Application Suite).

Taxonomic Key to Escovopsis species, Luteomyces, and Sympodiorosea

Thirty-six morphological features (Table S6) from *E. aspergilloides*, *E. clavata*, *E. lentecrescens*, *E. microspora*, *E. moelleri*, *E. multiformis*, *E. weberi*, *E. kreiselii* and *E. trichodermoide*s were analyzed using the "rpart" library (Therneau and Atkinson 2019) in R 3.6.3 . Seven out of the 36 characters were selected using a recursive partitioning algorithm (with the information gain as a measure for deciding between alternative splits) as the most informative features to build the dichotomous key (Williams 2011). Finally, a dichotomous key (in cladogram format) was constructed using a decision tree that started with a single node root that split into multiple branches to end in the leaves corresponding to each species. The final cladogram was manually edited using Adobe Illustrator CC v.17.1, and the information on branches were used to perform the Taxonomic Key.

Results

Phylogenetic placement of all strains previously named as *Escovopsis*

The *tef* phylogenetic tree showed *Escovopsis* as a paraphyletic clade (Fig. 1, S2). While vesiculate *Escovopsis* and *E. trichodermoides* are sister clades, they are away from *E. kreiselii*. *E. kreiselii* formes a monophyletic clade close to *Hypomyces* (Fig. 1, and S2). It is important to note that the strains from the brown clade of the phylogeny performed by Gerardo et al. (2006b) are placed withing the vesiculate *Escovopsis* clade (mostly close to *E. clavata* and *E. multiformis*). On the other hand, the pink and yellow clades formed well supported monophyletic clades separated from vesiculate *Escovopsis*, *E. trichodermoides* and *E. kreiselii* (Fig. 1, and S2).

Phylogenetic and taxonomic framework for *Escovopsis*' systematics

Phylogenetic analyses

Phylogenetic analyses (BI and ML) at the family level placed both the vesiculate and non-vesiculate *Escovopsis* within the *Hypocreaceae* (Fig. 2). Vesiculate *Escovopsis* (*E. aspergilloides*, *E. clavata*, *E. lentecrescens*, *E. microspora*, *E. molleri*, *E. multiformis* and *E. weberi*) formed a monophyletic clade in both the BI and ML analysis (BI Posterior Probability (PP) = 1; ML bootstrap value (MLB) = 100) (Fig. 2). Furthermore, non-vesiculate *Escovopsis* (*E. kreiselii* and *E. trichodermoides*) formed well-supported, monophyletic clades (PP = 1, MLB = 100, each one) outside of the vesiculate *Escovopsis* and separate from all other *Hypocreaceae* genera (Fig. 2).

Analyses at the genus level revealed that *Hypomyces*, *Escovopsioides*, *E. kreiselii*, *E. trichodermoides*, and vesiculate-*Escovopsis* each form separate, monophyletic clades (Fig. 3). The phylogenetic placement of the five clades varies depending on the molecular marker used for analysis. The analyses made separately with ITS, LSU, and *rpb*1 (Fig. 3A, B, D), as well as the concatenated analysis based on the five markers (Fig. 3F) indicate vesiculate-*Escovopsis*, and *E. kreiselii* as sister clades. The analyses made separately with *tef*1 and *rpb*2 (Fig. 3C, E), and the concatenated analysis based on four markers (LSU, *tef*1, *rpb*1, and *rpb*2 - Fig. 3G) indicate vesiculate-*Escovopsis* and *E. trichodermoides* as forming sister clades. In addition, the analyses performed with ITS and the five concatenated-markers showed *Escovopsioides* and *E. trichodermoides* forming a monophyletic clade (PP = 1, MLB = 100 - Fig. 3A). The analysis based on *rpb*1, however, indicated *Escovopsioides* forming a monophyletic clade with *Hypomyces*, while analysis based on *tef*1 placed *Hypomyces* between *Escovopsioides* and *E. kreiselii* (Fig. 3C). Finally, the analyses carried out with LSU,

*tef*1, and *rpb*2 separately (Fig. 3B, C, E), and the concatenated analysis (LSU, *tef*1, *rpb1* and *rpb*2 - Fig. 3G, 3A) placed *Hypomyces*, *Escovopsioides*, *E. kreiselii*, *E. trichodermoides*, and vesiculate-*Escovopsis* in well-supported, monophyletic clades (clearly separated from each other).

At the species level, *E. moelleri*, *E. aspergilloides*, *E. lentecrescens*, *E. clavata*, and *E. multiformis* formed well-supported clades that were clearly separated from each other. However, the ex-type strains of *E. weberi* and *E. microspora* grouped together with 45 other isolates in the same well-supported clade (Fig. 4A, B). This result was also observed at both the family and genus level trees.

Regarding the five species described by Marfetán et al. (2019), our analysis of the available LSU data indicates that they form two different clades closely related to *E. aspergilloides* and *E. lentecrescens* (Fig. S1). Specifically, the four strains of *E. primorosea* formed a well-supported monophyletic clade (Fig. S1), however *E. atlas*, *E. catenulata*, *E. longivesica*, *E. pseudoweberi*, and five strains named by the authors as *E. weberi* formed a single well-supported monophyletic clade (Fig. S1). Future research will need to reassess the morphology of these strains under standardized conditions and construct phylogenies based on additional markers.

Morphology and growth

Macroscopic features and conditions

Vesiculate *Escovopsis* spp. were able to grow between 10 °C and 30 °C. In isolates that grew at 10 °C (*E. clavata*, *E. microspora*, *E. moelleri*, and *E. weberi*), growth was limited and inconspicuous, so at this temperature, growth was only reported as, present or absent. Strains grew better at 20, 25, and 30 °C. No strains grew at 35 °C. Between 20 to 30 °C, colonies started growing between the first (*E. lentecrescens*, *E. microspora*, *E. moelleri*, and *E. weberi*) and fourth day (*E. aspergilloides*, *E. clavata*, *E. multiformis*). Some fast-growing species (*E. weberi*, *E. microspora*, and *E. moelleri*) covered the entirety of the Petri dishes by the third day. Based on these results, we selected the fourth day as the best time to record the presence or absence of growth at 10 °C and to measure the radius of growth at 20, 25, and 30 °C.

Evaluation on the eight media types (Fig. 5) demonstrated that the media influence growth rate and growth form. Growth on CYA, PCA, and PDA resulted in similar morphology for both *E. weberi* and *E. microspora*. Likewise, MA resulted in similar growth patterns as CYA, MEA, OA, and PDA for *E. moelleri*, CYA for *E. aspergilloides*, and PCA and PDA for *E. lentecrescens*, *E. clavata*, *E. multiformis*, *E. kreiselii* and *E. trichodermoides*. In contrast, none of the isolates grew well on SNA (all strains exhibited inconspicuous aerial mycelia, and the morphological patterns were difficult to observe until 14 days post inoculation). OA was also not ideal because the growth of strains was difficult to measure as a result of the medium's opacity. Except to *E. aspergilloides*, all strains exhibited vigorous growth and good expression of colour on MEA. Importantly, differences in growth rate were most apparent on CMD, and, on this medium, some species (e.g., *E. weberi* and *E. microspora*) produced hyphal agglomerations (pustule-like) on which conidiophores were

often produced (similar to the pustules produced by *Trichoderma*). Based on these results, we selected CMD, MEA, and PDA as the most suitable media to evaluate macroscopic features of *Escovopsis* species. It is important to highlight that these media are also used for the morphological assessment of other genera in the *Hypocreaceae.*

On CMD, MEA, and PDA, most of the *Escovopsis* strains grew best at 25 °C (Fig. 5). At this temperature, the differences in macroscopic characters, especially colony colour, were most apparent on day seven. For instance, white to light and dark-brown (*E. clavata*, *E. lentecrescens*, *E. microspora*, *E. moelleri*, *E. multiformis*, and *E. weberi*), and light-yellow to light-brown colony colours (*E. aspergilloides*) were clearly distinguishable. Although less clear, other colours (greenish-brown, pink, pinkish-brown, and reddish-brown) were observed on colonies of *E. weberi* and *E. microspora* on PDA and MEA. Between the seventh and tenth day, most of species became dark-brown on the three media, and the distintion between them became less clear (except for *E. lentecrescens*). After the tenth day, the aerial mycelia of all species started to deteriorate. Therefore, seven days of growth at 25 °C appears to be the most suitable condition to evaluate the macroscopic characters of these species.

E. kreiselii and *E. trichodermoides* (non-vesiculate *Escovopsis*) exhibited slower growth than vesiculate *Escovopsis*. These species start growing between the second and fourth day, reaching the edge of Petri dishes after ten (*E. kreiselii*) and seven days (*E. trichodermoides*), respectively, with variation depending on the media and temperature. Furthermore, *E. kreiselii* and *E. trichodermoides* exhibit the clearest distinguishable growth forms and colony colour (light brown, pink, pinkish-brown, dark-brown, for *E. kreiselii*; and beige, pale-yellow, yellow, yellowish-brown, for *E. trichodermoides*) after 14 days (Fig. 5). Before this time, colonies are usually white to pale-pink (*E. kreiselii*) or white to pale-yellow

(*E. trichodermoides*). Therefore, for both *E. kreiselii* and *E. trichodermoides,* we suggest day seven as the best time to evaluate the colonies' growth radius and day 14 for colony morphology assessment.

Microscopic features and conditions

Two well separated groups were observed based on microscopic characters: i) the vesiculate group, composed of *E. weberi, E. microspora, E. moelleri, E. aspergilloides, E. lentecrescens, E. clavata*, and *E. multiformis*; and ii) the non-vesiculate group, composed of *E. kreiselii*, and *E. trichodermoides*.

Vesiculate *Escovopsis* spp. present conidiophores with one vesicle (mono-vesiculate) or 2 - 53 vesicles (poly-vesiculate) (Fig. 6). Mono-vesiculate conidiophores (Fig. 6A-B) emerge from aerial mycelia in an alternating and opposite pattern in all species of this group. Poly-vesiculate conidiophores (Fig. 6C-H) also emerge from the aerial mycelia and can present short branches composed of one or two cells (most frequent for *E. moelleri*, *E. aspergilloides*, and *E. lentecrescens*) or long branches with multiple cells (most frequent for *E. clavata, E. microspora, E. multiformis*, and *E. weberi*). *E. weberi* and *E. microspora* have the largest and most branched conidiophores of the genus (Fig. 6G). In these clades, it is fairly common to find up to three levels of conidiophore branching. Some poly-vesiculate conidiophores have a swollen cell from were branches emerge (*E. clavata*, and *E. multiformis* – Fig. 6F). Vesicles, mostly composed of a single cell (non-septate vesicles - Fig. 6I-S), and rarely of two cells (septate vesicles - Fig. 6U-V), can emerge from the conidiophore's apex, the apex of the conidiophore’s branches (usually only one vesicle in the short branches and

two or more in long branches), as well as directly from the main axis of both the conidiophores and conidiophore's branches. Vesicles emerge directly separated by a septum (most common for *E. weberi* and *E. microspora*) or form a peduncle made up of one or two cells (most common for *E. moelleri*, *E. aspergilloides*, *E. lentecrescens*, *E. clavate* and *E. multiformis*). Vesicles can exhibit different shapes (globose, sub-globose, capitate, obovoid, prolate, spatulate, clavate, cymbiform, oblanceolate, subulate, cylindric, filiform, clavate-septate, cylindric-septate) (Fig. 6I-V) and sizes, depending of the species. Phialides (conidiogenous cells in the vesiculate *Escovopsis* – Fig. 6W-Z) emerge mainly from the vesicles and less frequently from the aerial mycelia (only observed in *E. weberi* and *E. microspora*). These structures give rise to the conidia and present a lageniform and ampulliform shape. The main differences between the phialides of the different species are mostly related to the sizes of the base, the widened part and the neck (Fig. 6V-Z). Conidia are single cells produced in chains in the phialides (enteroblastic conidiogenesis) and can vary in shape (globose, sub-globose, ellipsoidal, oblong, and oblong-ornamented) (Fig. 6a-e). Most of the species have smooth conidia, but *E. moelleri* have conidia with ornamentations on cell walls (Fig. 6e). Of the vesiculate *Escovopsis* spp., only *E. weberi* and *E. microspora* have chlamydospores, but these are rarely observed.

The non-vesiculate groups form conidiophores remarkably different from those of the vesiculate group. Conidiophores of *E. kreiselii* lack vesicles and are formed on the aerial mycelium in an alternated or opposite pattern, and are more branched (with irregular branching conformation) than those of vesiculate *Escovopsis* (Fig. 23A, B). Conidiogenous cells of *E. kreiselii* are holoblastic sympodial (i.e., have indeterminate growth; Fig. 23C-H), and they are formed at the apex and at both the main and branch axis of the conidiophore

(Fig. 23B). Conidia of *E. kreiselii* are solitary, globose to sub-globose, smooth but with thick-walled, light brown to dark brown, and sometimes with a denticle (Fig. 23I) or with a lesion (when the denticle remains in the conidiogenous cell – Fig. 23J). In contrast to *E. kreiselii*, the conidiophores of *E. trichodermoides* are pyramidal (*Trichoderma*-like), with one to six short levels of branches arising at more or less right angles from the conidiophore axis. They also lack vesicles (Fig. 25 A, B). This species has synchronous conidiogenous cells (Fig. 25B-F) with ampulliform shapes producing either solitary (Fig. 25C) or up to three conidia (Fig. 25D-F). The conidia are sub-globose to ovate, yellow-brown and ornamented (verrucose – Fig. 25G). Unlike the vesiculate *Escovopsis* spp., *E. kreiselii* and *E. trichodermoides* regularly form chlamydospores on all culture media and at all temperature at which they grow (Fig. 23K and 14I).

Taxonomy

Phylogenetic analyses in this study unambiguously demonstrate that species previously treated as *Escovopsis* form three distinct clades. These groups also differ significantly from each other both in colony morphology and in microscopic characters. The first clade includes the ex-type strains of *E. aspergilloides*, *E. clavata*, *E. lentecrescens*, *E. microspora*, *E. moelleri*, *E. multiformis*, and *E. weberi*, which are all vesiculate species. Based on the similarity of their sequences, and their morphological characters, we synonymise *E. microspora* with *E. weberi*. The five other species in this group can regularly be distinguished from *E. weberi* and each other (Fig. S3). We treat these five species, together with *E. weberi*, as *Escovopsis*. Therefore, we restrict, and redefine *Escovopsis* to include only vesiculate species.

The two remaining clades (*E. kreiselii* and *E. trichodermoides*) included only non-vesiculate *Escovopsis* species. Below, we reassign these two clades into two, new genera, *Sympodiorosea* and *Luteomyces*. The descriptions of all the species are emended and aligned with each other based on the criteria established in this study. A taxonomic key is provided to facilitate the recognition of these genera and species.

***Escovopsis* J. J. Muchovej & Della Lucia, Mycotaxon 37: 192 (1990) Fig. 6**

MycoBank: MB11249

Etymology: *Escova*- Portuguese for brush, *-opsis* Greek for like (brush like due to the bottle-brush appearance of the sporophore)

Previous genus description: vesiculate; vesicles cylindrical to globose, evanescent; phialides hyaline, swollen at base, extending to a narrow neck; conidia in short basipetal chains, aseptate, hyaline at first becoming pigmented with an ornamented or mucilaginous brown outer coat or sheath, phoretic. Non-vesiculate conidiophores are present in less derived strains, with non-phialidic conidigenous cells producing solitary conidia.

Genus redescription: Monophyletic Hypocreaceae genus that presents mono- and poly-vesiculate conidiophores formed on hyaline aerial mycelia (Fig. 6A-H). Vesicles are terminal, mostly non-septate (Fig. 6I-S) and rarely with one septum (Fig. 6T-U), and of different shapes (globose, sub-globose, capitate, obovoid, prolate, spatulate, clavate, cymbiform, oblanceolate, subulate, cylindric, filiform, clavate-septate, cylindric-septate – Fig. 6I-U). Phialides hyaline, with a thin base, a swollen section and a thin neck (Fig. 6V-Z), are formed on vesicles. Conidia in chains, smooth or ornamented, aseptate, hyaline to brown,

and in different shapes (globose, sub-globose, ellipsoidal, cylindric), are produced in phialides (Fig. 6a-e).

Notes: *Escovopsis* is phylogenetically placed within Hypocreaceae as a sister clade of *Luteomyces*. *Escovopsis* exhibits faster growth and different colony colour (brown) than *Luteomyces* (yellow). Unlike *Luteomyces*, which presents synchronous conidiogenous cells, *Escovopsis* forms phialides. The main feature of this genus is the presence of conidiophores with terminal vesicles that differentiate it from its sister clade and from all other known Hypocreaceae.

Escovopsis weberi J.J. Muchovej and Della Lucia, Mycotaxon 37: 192 (1990). Fig. *7*

MycoBank: MB127786

= *Escovopsis microspora* H.C. Evans and J.O. Augustin, PLoS One 8 (12): e82265, 5 (2013)

MycoBank: MB800442

Etymology: "*weberi*" in honour of Neal A. Weber, American entomologist who found the fungus in the 1960's.

Typification: BRAZIL. Minas Gerais: Viçosa, T. M. Dela Lucia, isolated from ant colony (ex-type culture, ATCC 64542).

Sequences: ITS (KF293285), *tef*1 (AY172623), LSU (KF293281), *rpb*1 (MT305412), and *rpb*2 (MT305537).

Colonies growing at 10, 20, 25, and 30 °C on CMD, PDA, and MEA. After four days at 10 °C, inconspicuous growth only on inoculum, on the three media. At 20, 25, and 30 °C, growth starts on the first day on all media. After four days at 20 °C, radial growth of colonies reaches 2 – 2.4 cm, 3 – 4 cm, and 4 cm, on CMD, MEA, and PDA, respectively. At 25 °C, colonies reach plate edge on third day on PDA, and after four days radial growth of colonies reaches 1.7 – 3.6 cm, and 2.4 – 4 cm, on CMD and MEA, respectively. After four days at 30 °C, radial growth of colonies reaches 3.7 – 4 cm, 1.2 – 1.5 cm, and 3.7 – 4 cm on CMD, MEA and PDA, respectively. After seven days at 25 °C on CMD, colonies exhibit diffuse aerial mycelia spread by stolon and forming short pustules-like structures with white to brown conidia (Fig. 7C). On MEA, colonies form abundant aerial mycelia and different conidia colours (usually light-brown to brown in centre, followed by pinkish to light-brown and beige to greenish-brown regions, and white to pale - yellow on edge - Fig. 7B). On MEA, colonies can also display other colours, like yellow and reddish-brown, and eventually submerged mycelium forming dense circular zones and white to brown pustules-like. On PDA, colonies form abundant aerial mycelia spread by stolons, and white to brown conidia (Fig. 7A), and can also display other colours, like yellow to greenish-brown and pinkish to reddish-brown, and eventually pustule-like formations. Soluble pigments are not observed.

Conidiophores mono and poly-vesiculate (2 – 20 vesicles), hyaline, usually pyramidal, less frequent with irregular shape, smooth-walled, and arising alternated or opposite (less frequent) from aerial mycelia (Fig. 7D-E). Mono-vesiculate conidiophores reach 20 – 60 μm, and poly-vesiculate ones 30 – 577 μm, in length. Conidiophore stipes 45 – 90 μm long x 5 – 7 μm wide, separated by septum 2 – 5 μm from food cell (basal cell). Conidiophore branches, 34 – 127 μm long, mostly alternated and less opposite, at more or

less right angles and slightly curved up and in one to two levels. Stipes on branches 10 – 36 µm long, separated by septum 1.5 – 3 µm from conidiophore axis. *Vesicles* in various shapes (cylindrical [predominant], clavate, and filiform – Fig. 9F-H), 21 – 63 µm long x 6 – 12 µm wide, predominantly non-septate and less frequent septate (Fig. 7F), separated from conidiophore axis or branch axis by stipe 1 – 12 µm long with one to two septa. *Phialides* formed on vesicles (Fig. 7F-I), total length 7 – 9 µm, lageniform with wide base (0.5 – 1 µm long x 1 – 1.5 µm wide), followed by swollen section (2.5 – 3 µm x 2.5 – 3 µm) and long thin neck (3 – 4 µm x 0.5 – 0.8 µm). *Conidia* formed in chains on phialides, ellipsoidal to oblong (2,5 µm – 3 µm long x 1,5 µm – 2.5 µm wide), brown, with smooth and thickened walls (Fig. 7J).

Notes: *E. weberi* is the most derived clade of the genus and is phylogenetically close to *E. moelleri*. *E. weberi* grows faster than *E. moelleri* and other known *Escovopsis* species and exhibits the most variable colony colours in the genus. Unlike the strains of its sister clade, *E. weberi* forms smooth conidia in long chains, without ornamentations, and presents cylindric, filiform, and septated vesicles.

Escovopsis aspergilloides Seifert, Samson and Chapela, Mycologia 87: 408 (1995). Fig. 8

MycoBank: MB413060

Etymology: *Aspergillus*-like. The etymology of this species was not clearly specified by Seifert et al. (1995). However, the authors implied that the name of the species was chosen because of its similarity with the vesicles of *Aspergillus*, previously observed by Möller 1893.

Typification: Trinidad, near ASA Wright Nature Center, in wet, dense, secondary tropical rainforest, in a nest of *Trachymyrmex ruthae*, from a nest chamber 15-20 cm below the soil

surface, T. R. Schultz, 9 Nov. 1992. Isolated in June 1993 from mycelium in the living nest in Ithaca, New York by I. H. Chapela, no. 92110905C (holotype, DAOM 216382; ex-type culture, CBS 423.93 and DAOM 216382).

Sequences: ITS (NR_137160), *tef*1 (AY172632), LSU (KF293283), *rpb*1 (MT305421), and *rpb*2 (MT305546).

Colonies growing at 20 and 25 °C on CMD, PDA, and MEA. At 20 °C, growth starts on second day on PDA, on third day on MEA, and on fourth day on CMD. After four days at 20 °C, radial growth of colonies reaches 0 – 0.1 cm, 0.2 – 0.5 cm, and 0.5 – 0.8 cm, on CMD, MEA, and PDA, respectively. At 25 °C, growth starts on second day on MEA and PDA, and on third day on CMD. After four days at 25 °C, radial growth of colonies reaches 0.1 – 0.3 cm, 0.5 – 0.7 cm, and 0.5 – 1 cm on CMD, MEA, and PDA, respectively. Colonies do not grow at 10 and 30 °C. After seven days at 25 °C, colonies exhibit diffuse aerial mycelia spread by stolons, and pale-yellow to brown conidia on CMD (Fig. 8C). On MEA, submerged mycelia form a dense circular zone, and diffuse aerial mycelia in circular-sector shapes form white to yellow and yellowish-brown conidia (white on edge and yellow-brown in centre - Fig. 8B). On PDA, abundant aerial mycelia, spread by stolons, form pale-yellow to light-brown conidia (Fig. 8A). Pustule-like formations and soluble pigments are not observed.

Conidiophores mono and poly-vesiculate (1-15 vesicles), hyaline, with irregular shape, smooth-walled and arising alternated or opposite from aerial mycelia (Fig. 8D-F). Mono-vesiculate conidiophores reach 40 – 72 μm, and poly-vesiculate ones 80 – 300 μm, in length. Conidiophore stipes 24 – 101 μm long x 5 – 6 μm wide, separated by septum 4 – 6 μm from food cell (basal cell). Conidiophore branches, 32 – 161 μm long, alternated, at more

or less right angles, and from one to three levels. Second branching level usually longer than other branching levels. Stipes on branches 10 – 49 µm long, separated by septum 2 – 4 µm from conidiophore axis. *Vesicles* in various shapes (globose [predominant], sub-globose, capitate, ovoboid, prolate and spatulate - Fig. 8D-G), 14 – 22 µm long x 10 – 22 µm wide, usually separated from conidiophore axis or branch axis by stipe 10 – 40 µm long with one to two septa. *Phialides* formed on vesicles (Fig. 8G-H), total length 6 – 10 µm, ampulliform, with truncated pyramid shape base (1– 2 µm long x 0.5 – 1,5 µm wide) followed by swollen section (3.5 – 4 µm long x 2 – 3 µm wide) and long thin neck (4 µm long x 2 µm wide). *Conidia* formed in chains on phialides, globose to oblong (2,5 – 3 µm long x 2 – 2,5 µm wide), brown, with smooth and slightly thickened walls (Fig. 8H-I). *Chlamydospores* intercalar, hyaline, 11 – 22 µm long x 8 – 14 µm wide (Fig. 8J).

Notes: *E. aspergilloides* is phylogenetically close to *E. lentecrescens*. *E. aspergilloides* grows faster than *E. lentecrescens*. However, the most remarkable feature of *E. aspergilloides* is its pale-yellow to yellowish-brown colonies, which differentiate it from the brown colonies of *E. lentecrescens*.

Escovopsis clavata Q.V. Montoya, M.J.S. Martiarena, D.A. Polezel, S. Kakazu and A. Rodrigues, Mycokeys 46: 102 (2019). Fig. 9

MycoBank: MB828328

Etymology: "*clavata*" in reference to the predominantly clavate shape of vesicles.

Typification: BRAZIL. Santa Catarina, Florianópolis, GPS: 27°44'39.6"S; 48°31'10.14"W, elev. 46 m, fungus garden of *Apterostigma* sp, 08, 2015. *A. Rodrigues*. Holotype: CBS H-23845 (dried culture on PDA). Ex-type strain LESF 853 (= CBS 145326).

Sequences: ITS (MH715096), *tef*1 (MH724270) and LSU (MH715110), *rpb*1 (MT305419), and *rpb*2 (MT305544).

Colonies growing at 20 and 25 °C on CMD, PDA, and MEA. At both temperatures, growth starts on third day on all media. After four days at 20 °C, radial growth of colonies reaches 0 – 0.3 cm, 0.1 – 0.4 cm, and 0.3 – 0.5 cm on CMD, MEA, and PDA, respectively. After four days at 25 °C, radial growth of colonies reaches 0.2 – 0.8 cm, 0.4 – 0.6 cm, and 0.6 – 1.1 cm on CMD, MEA, and PDA, respectively. No growth is observed at 10 and 30 °C. After seven days at 25 °C, colonies exhibit diffuse aerial mycelia and form beige to pale-brown conidia on CMD (Fig. 9C). On MEA (Fig. 9B) and PDA (Fig. 9A), colonies grow in circular shape with dense, floccose aerial mycelia, with beige conidia near the edge and beige to pale brown conidia in the center. Stolons are rare. Pustule-like formations and soluble pigments are not observed.

Conidiophores mono and poly-vesiculate (2 – 8 vesicles), hyaline, with irregular shape, smooth-walled, and arising alternated or opposite from aerial mycelia (Fig. 9D-G). Mono-vesiculate conidiophores reach 10 – 50 µm, and poly-vesiculate ones up to 780 µm, in length. Conidiophore stipes 10 – 40 µm long x 5 – 8 µm wide, separated by septum 2 – 9 µm from food cell (basal cell). Conidiophore axis usually ends in terminal vesicle (Fig. 9D-E), sometimes in elongated, infertile hypha (Fig. 9F) and less frequently in terminal swollen cell

10 – 18 µm long x 7 – 9 µm wide (Fig. 9G). Conidiophore branches, usually short and sometimes as long as conidiophore axis, 16 – 138 µm long, alternated or opposite, usually at right angles and sometimes slightly curved up or down, and with one to two branching levels. Conidiophores with swollen cells from two to four branches only on swollen cell, 28 – 35 µm long, mostly curved up and less frequently at right angles (Fig. 9G). Stipes on branches 9 – 38 µm long, separated by septum 2 – 6 µm from conidiophore axis. *Vesicles* in various shapes (globose, sub-globose, capitate, obovoid, prolate, spatulate, clavate [predominant-Fig. 9H], cymbiform, and cylindric), 9 – 27 µm long x 7 – 20 µm wide, separated from conidiophore axis or branch axis by stipe 10 – 30 µm long with two to six septa. *Phialides* formed on vesicles (Fig. 9H-I), total length 5 – 8 µm, lageniform with elongated base (0.5 – 1.5 µm long x 0.5 – 1 µm wide) followed by swollen section (1.5 – 2.5 µm long x 1 – 3 µm wide) and thin neck (1.5 – 4 µm long x 0.5 µm wide). *Conidia* formed in chains on phialides, ellipsoidal to oblong (1.5 µm – 2.5 µm long x 0.5 µm – 1.5 µm wide), brown, with smooth and slightly thickened walls (Fig. 9J).

Notes: *E. clavata* is phylogenetically close to *E. multiformis*. Unlike strains of *E. multiformis*, which grows at 10, 20, 25 and 30 °C, *E. clavata* grows only at 20 and 25 °C. Conidiophores of *E. clavata* are usually larger and more branched than those of *E. multiformis*. *E. clavata* and *E. multiformis* are the only species in the genus that form conidiophores with swollen cells, though these structures are less frequent and shorter in *E. clavata* than in *E. multiformis*.

Escovopsis lentecrescens H.C. Evans and J.O. Augustin, PloS ONE 8 (12): e82265, 5 (2013). *Fig. 10*

MycoBank: MB800441

Etymology: Based on the markedly slow growth rate *in vitro* compared to all other described species.

Typification: BRAZIL. Minas Gerais: Viçosa, Mata do Paraíso, 700 m, Apr 2010, J.O. Augustin & H.C. Evans, isolated from fungal garden of *Acromyrmex subterraneus subterraneus* (holotype IMI 501179; isotype DOA628-VIC 31755, CBS 135750).

Sequences: ITS (JQ815079), *tef*1 (JQ855714), LSU (JQ855717), *rpb*1 (MT305415), and *rpb*2 (MT305540).

Colonies growing at 20, and 25 °C on CMD, PDA, and MEA. At 20 °C, growth starts on third day on all media. After four days at 20 °C, radial growth of colonies reaches 0 – 0.2 cm on CMD, and 0 – 0.1 cm on both MEA and PDA. At 25 °C, growth starts on the second day on all three media. After four days at 25 °C, radial growth of colonies reaches 0.2 – 0.3 cm, 0.2 – 0.5 cm, and 0.2 – 0.3 cm on CMD, MEA and PDA, respectively. No growth is observed at 10 and 30 °C. After seven days at 25 °C on CMD, colonies with diffuse aerial mycelia spread by stolons, and pinkish to pale-brown conidia (Fig. 10C). On MEA, colonies form circular shape, with dense cottony aerial mycelia, and white to beige conidia (Fig. 20B). On PDA, colonies form circular shape, with dense cottony aerial mycelia, and white to dark-brown conidia (white on edge and pale to dark-brown in center - Fig. 10A). Conidia are more abundant on PDA than on CMD and MEA. Stolons are rare. Pustule-like formations and soluble pigments are not observed.

Conidiophores mono and poly-vesiculate (1-10 vesicles), hyaline, with irregular shape, smooth-walled and arising alternated or opposite from aerial mycelia (Fig. 10D-E). Mono-vesiculate conidiophores reach 36 – 150 um, and poly-vesiculate ones 57 – 200 μm, in length. Conidiophore stipes 28 – 49 μm long x 5 – 7 μm wide, separated by septum 2 – 6 μm from food cell (basal cell). Conidiophore branches, 20 – 80 μm long, alternated at more or less right angles, and from one to three levels. The second branching level is usually much longer than other branching levels. Stipes on branches 7 – 31 μm long, separated by septum up to 9 μm from conidiophore axis. *Vesicles* in various shapes (globose [predominant], sub-globose, spathulate, oblanceolate and cylindric - Fig. 10D-H), 14 – 27 μm long x 13 – 27 μm wide, usually separated from the conidiophore axis or branch axis by stipe 10 – 94 μm long with one to six septa. *Phialides* formed on vesicles (Fig. 10F-I), total length 6 – 8.5 μm, ampulliform, with truncated pyramid shape base (0.5– 1 μm long x 1 – 1.5 μm wide) followed by swollen section (4 – 5 μm long x 2.5 – 3 μm wide) and long thin neck (2 – 3 μm long x 0.5 – 0.6 μm wide). *Conidia* formed in chains on phialides, globose to oblong (2 – 3.5 μm long x 1.5 – 2 μm wide), brown, with smooth and slightly thickened walls (Fig. 10J). *Chlamydospores* intercalary, hyaline, 9.5 – 23 μm long x 8.5 – 16 μm wide (Fig. 10K).

Notes: *E. lentecrescens* is phylogenetically close to *E. aspergilloides*. *E. lentecrescens* grows slower than *E. aspergilloides* and the other known species in the genus. The feature that differenciates *E. lentecrescens* from *E. aspergilloides* is the brown colour of its colonies in comparison to the pale-yellow to yellowish-brown colonies of *E. aspergilloides*.

Escovopsis moelleri H.C. Evans and J.O. Augustin, PloS ONE 8 (12): e82265, 5 (2013). Fig. 11.

MycoBank: MB800440

Etymology: Named in honour of A.F.W. Möller, who pioneered work on fungal gardens of ants and provided the first illustrations of *Escovopsis*.

Typification: BRAZIL. Minas Gerais: Viçosa, Mata do Paraíso, 700 m, Apr 2010, J.O. Augustin & H.C. Evans, isolated from fungal garden of *Acromyrmex subterraneus molestans* Forel (holotype IMI 501176; isotype DOA626-VIC 31753, CBS 135748).

Sequences: ITS (JQ815077), tef1 (JQ855712), LSU (JQ855715), *rpb*1 (MT305413), and *rpb*2 (MT305538).

Colonies growing at 10, 20, and 25 °C on CMD, PDA and MEA. After fourth days at 10 °C on all three media, growth inconspicuous and only on inoculum. At 20 °C, growth starts between first and second day on all three media. After four days at 20 °C, radial growth of colonies reaches 2.3 – 3.8 cm, 3.5 – 4 cm and 3.1 – 4 cm on CMD, MEA and PDA, respectively. At 25 °C, growth starts on the first day, and reaches the plate edge between the third and fourth day on all three media. No growth is observed at 30 °C. After seven days at 25 °C on CMD, colonies form thin aerial mycelia spread by stolon and submerged mycelia, and beige to pale-brown conidia (Fig. 11C). On MEA, colonies form short aerial mycelia, mostly spread by submerged mycelia, and white to beige conidia (Fig. 21B). On PDA, form cottony aerial mycelia, spread predominantly by submerged mycelia and less by stolons, and form beige to brown conidia (brown conidia on plate edge - Fig. 11A). Pustule-like formations and soluble pigments are not observed.

Conidiophores mono and poly-vesiculate (2 – 9 vesicles), hyaline, usually pyramidal and less frequent with irregular shape, smooth-walled, and arising mostly alternated and less frequent opposite from aerial mycelium (Fig. 11D-E). Mono-vesiculate conidiophores are rare and reach 34 – 54 μm, and poly-vesiculate ones reach 70 – 236 μm, in length. Mono-vesiculate conidiophores stipes with only one septum (2 – 23 μm long x 6 – 10 μm wide) or up to five septa (up to 144 μm long), separated by septum 2 – 7 μm from food cell (basal cell). Poly-vesiculate conidiophores stipes 8 – 52 μm long x 6 – 10 μm wide, separated by septum 2 – 5 μm from the basal cell. Conidiophore branches 30 – 89 μm long, mostly alternated and less opposite, at more or less right angles and slightly curved up, and from one to two levels. Stipes on branches 2 – 20 μm long, separated by septum 2 – 8.5 μm from conidiophore axis. *Vesicles* in various shapes (Subulate, oblanceolate, and clavate - Fig. 11F-H), 22 – 60 μm long x 5 – 10 μm wide, mostly non-septate and rarely with one septum (clavate-septate), separated from conidiophore axis or branch axis by stipe 1.5 – 17 μm long with one to three septa. *Phialides* formed on vesicles (Fig. 11I), total length 5 – 7 μm, ampulliform, with short and wide base (1.7 – 3 μm long x 0.5 – 1 μm wide), followed by swollen section (4.4 – 5.6 μm long x 3.6 – 4.8 μm wide) and short thin neck (1 – 1.7 μm long x 1 – 2 μm wide). *Conidia* formed on phialides, mostly solitary and less in short chains, oblong-ornamented (6 – 7 μm long x 3.0 – 3.8 μm wide), brown, with ornamentation and thickened walls (Fig. 11J).

Notes: *E. moelleri* is phylogenetically close to *E. weberi*. *E. moelleri* grows slower than *E. weberi*. Unlike strains of its sister clade, *E. moelleri* forms mainly clavate vesicles. However,

the most remarkable feature that differentiates *E. moelleri* from all known *Escovopsis* species is the ornamented conidia formed in short chains.

Escovopsis multiformis Q.V. Montoya, M.J.S. Martiarena, D.A. Polezel, S. Kakazu and A. Rodrigues, Mycokeys 46: 106 (2019). Fig. 12.

Mycobank: MB828329

Etymology: "*multiformis*" in relation to the different vesicle shapes found in the same isolate.

Typification: BRAZIL. Santa Catarina, Florianópolis, GPS: 27°28'11.28"S; 48°22'39.48"W, elev. 119 m, Fungus garden of *Apterostigma* sp, 08, 2015. *A. Rodrigues*. Holotype: CBS H-23846 (dried culture on PDA). Ex-type strain LESF 847 (= CBS 145327).

Sequences: ITS (MH715091), *tef*1 (MH724265) and LSU (MH715105), *rpb*1 (MT305420), and *rpb*2 (MT305545).

Colonies growing at 10 °C on PDA and MEA, and at 20, 25 and 30 °C on CMD, PDA and MEA. After four days at 10 °C, inconspicuous growth only on inoculum, on both PDA and MEA. At 20 °C, growth starts on second day on CMD and on third day on MEA and PDA. After four days at 20 °C, radial growth of colonies reaches 0.4 – 1 cm, 0.3 – 0.6 cm, and 0.2 – 0.5 cm on CMD, MEA and PDA, respectively. At 25 ºC, growth starts on second day on all media. After four days at 25 °C, radial growth of colonies reaches 0.6 – 1 cm on CMD, and 0.4 – 0.7 cm on MEA and PDA. At 30 ºC, growth starts on second day on CMD and on third day on MEA and PDA. After four days, at 30 °C, radial growth of colonies reaches 0.4 – 0.7 cm, 0 – 0.3 cm and 0 – 0.2 cm on CMD, MEA and PDA, respectively. After seven days at 25 °C on CMD, colonies exhibit submerged mycelia forming a dense circular

zone, diffuse aerial mycelia spread by stolons, and beige to pale-brown conidia (Fig. 12C). On MEA, mycelia form dense, cottony colonies with beige exudates and white to beige conidia (Fig. 12B). On PDA, colonies exhibit cottony raised growth, with white to brown conidia (white on edge and brown in center - Fig. 12A). Stolons are rare on MEA and PDA. Pustule-like formations on CMD at 20, 25, and 30 °C. Soluble pigments are not observed.

Conidiophores mono and poly-vesiculate (2 – 9 vesicles), hyaline, usually with irregular shape, smooth-walled, and arising alternated or opposite from aerial mycelia (Fig. 12D-G). Mono-vesiculate conidiophores reach 66 – 136 µm, and poly-vesiculate ones 82 – 293 µm, in length. Conidiophore stipes 16 – 56 µm long x 7 – 9 µm wide, with one to three septa, separated by septum 1 – 2 µm from food cell (basal cell). Conidiophore axis usually ends in terminal vesicle, and sometimes in terminal swollen cell 16 – 34 µm long x 9 – 20 µm wide (Fig. 12G). Conidiophore branches, usually short and sometimes as long as conidiophore axis, 32 – 84 µm long, alternated, usually at right angles and sometimes slightly curved up, and from one to three branching levels. Conidiophores with swollen cells from two to six branches only on swollen cell, 28 – 35 µm long, mostly curved up and less frequently at right angles (Fig. 12G). Each swollen cell branch usually ends in one vesicle, but sometimes it forms another swollen cell with two to four new branches. Stipes on branches 22 – 70 µm long, separated by septum 1 – 2 µm from conidiophore axis. *Vesicles* in various shapes (globose, sub-globose [predominant], capitate, obovoid, prolate, spatulate, cymbiform, and cylindric), 12 – 27 µm long x 9 – 17 µm wide, separated from conidiophore axis or branch axis by stipe 22 – 70 µm long with one to four septa. *Phialides* formed on vesicles (Fig. 12H-I), total length 6 – 10 µm, lageniform with elongated base (1– 2.5 µm x 0.5 – 1µm), followed by swollen section (2.5 – 4.5 µm x 2 – 3.5 µm) and thin neck (1– 4.5

μm x 0.5 – 1 μm). *Conidia* formed in chains on phialides, globose to oblong (2.5 – 3.5 μm long x 1.5 – 2.5 μm wide), brown, with smooth and slightly thickened walls (Fig. 12J).

Notes: *E. multiformis* is phylogenetically close to *E. clavata*. Unlike strains of *E. clavata*, which grows only at 20 and 25 °C, *E. multiformis* can grow at 10, 20, 25, and 30 °C. Conidiophores of *E. multiformis* are usually shorter and less branched than those of *E. clavata*. *E. multiformis* and *E. clavata* are the only species in the genus that form conidiophores with swollen cells, though these structures are more frequent and larger in *E. multiformis* than in *E. clavata*.

Sympodiorosea Q.V. Montoya and A. Rodrigues, gen. *nov*. MycoBank: MB 835147. Fig. 13

Etymology: "*Sympodio*" refers to the sympodial conidiogenous cells, and "*rosea*" to the colony colour.

Colonies form inconspicuous mycelia at 10 and 30 °C, and floccose, white, pale-beige, pink, and brown aerial mycelia at 20 and 25 °C. *Conidiophores* formed on aerial mycelia, alternated or opposite, usually at right angles, and with irregular branching conformation. Sympodial conidiogenous cells solitary, in pairs or in verticils at conidiophore and branch apices, and solitary, alternated and opposite, at both conidiophore and branch axes. *Conidia* solitary, globose to sub-globose, smooth or rough (thick-walled), light-brown to dark-brown and with denticles or lesions (like holes). Chlamydospores are common.

Habitat: isolated from fungus gardens of *Mycetophylax morchi* (Hymenoptera: Formicidae: tribe Attini).

Notes: *Sympodiorosea* is phylogenetically placed within Hypocreaceae near *Luteomyces* and *Escovopsis*. *Sympodiorosea* presents slower growth and different colony colour (pink) than *Luteomyces* (yellow) and *Escovopsis* (brown). The main feature of this genus is the presence of conidiophores with sympodial conidiogenous cells that differentiate it from all known Hypocreaceae.

Sympodiorosea kreiselii (Meirelles, Montoya, Solomon and Rodrigues) Montoya et al. Journal. XX: XX. 2020, comb. nov. Fig. 14.

MycoBank: MB 835148.

Etymology: "*kreiselii*" in honour of Hanns Kreisel, who first described the *Phialocladus* currently known as *Escovopsis* (Meirelles et al. 2015a).

Typification: BRAZIL. Santa Catarina, Florianópolis, Praia da Joaquina, GPS: 27°37' 50.01" S; 48° 27' 3.64" W, elev. 1 m, Fungus garden of *Mycetophylax morchi*, 03, 2009. A. Rodrigues. Ex-type strain LESF53 (= CBS 139320, = CBMAI 1691). Holotype: CBS H-22062 (dried culture on PDA).

Basionym: *Escovopsis kreiselii* L.A. Meirelles, Q.V. Montoya, S.E. Solomon and A. Rodrigues, PLoS One. 10: 1–14, 2015.

Sequences: ITS (KJ808767), *tef*1 (KJ 808766), LSU (HJ808765), *rpb*1 (MT305418), and *rpb*2 (MT305543).

Colonies growing at 10, 20, 25 and 30 °C on CMD, PDA and MEA. After seven days at 10 °C, inconspicuous growth, and sometimes no growth is observed. At 20 °C, growth starts on fourth day on all media. After seven days at 20 °C, radial growth of colonies reaches 0.7 – 1.5 cm, 0.4 – 0.8 cm, and 1 – 1.5 cm on CMD, MEA and PDA, respectively. At 25 ºC, growth starts on second day on all media. After seven days at 25 °C, radial growth of colonies reaches 1 – 1.5 cm, 0.9 – 1.5 cm, and 1.2 – 1.6 cm on CMD, MEA and PDA, respectively. At 30 ºC, growth only on CMD, starting on second day. After seven days at 30 °C, radial growth of colonies reaches 0.8 cm. After 14 days at 25 °C, colonies floccose, and pale-beige to -pink, on CMD (Fig. 14C); cottony, beige to pinkish-brown in centre and white to light yellow on edge, and eventually forming concentric rings on MEA (Fig. 14B); and floccose, light-pink to pinkish-brown in center and white to light-pink on edge, usually forming concentric rings on PDA (Fig. 14A). Pustule-like, stolons, and soluble pigments are not observed.

Conidiophores 29 – 229 µm long, hyaline, with irregular shape, smooth-walled, alternated or opposite, at more or less right angles (Fig. 24D-F). Conidiophore stipes 6 – 40 µm long x 3.5 – 5.5 µm wide, separated by septum 0.5 – 2 µm from the food cell (basal cell). Conidiophore branches 14 – 40 µm long, solitary, opposite (two branches) or in verticils (three to four branches), at more or less right angles, and from one to three levels. Stipes on branches 6 – 14 µm long, separated by septum 1.5 – 2.5 µm from conidiophore axis. Second level branches always formed by stipe (similar to the supporting cells in *Trichoderma*) and conidiogenous cells on apex (Fig. 14D). *Sympodial* conidiogenous cells 5.3–13.4 µm long x 2.5–3.5 µm wide, formed at conidiophore apex or at axis and apex from branches (Fig. 14F), hyaline, ampulliform to lageniform, solitary or in verticils (up to 6 cells), and with numerous

denticles (Fig. 14G). *Conidia* globose to sub-globose (2.4 – 3.2 µm long x 2 – 2.7 µm wide), solitary (Fig. 14G), light-brown to dark-brown, smooth or rough surface, thick-walled, and with one denticle (coming from conidiogenous cell) or one hole (when denticle remains on conidiogenous cell) (Fig. 14I). *Chlamydospores* globose to sub-globose (3 – 3.5 µm long x 2.5 – 3 µm wide), hyaline, and with smooth walls (Fig. 14H).

Luteomyces Q.V. Montoya and A. Rodrigues, gen. nov. MycoBank: MB 835150. Fig. 15

Etymology: "*Luteomyces*" in relation to the colour exhibited by the colonies of the strains that make up the genus.

Colonies form surface mycelia at 10 and 30 °C; and floccose, white, beige and yellow aerial mycelia at 20 and 25 ºC. Stolons, and beige to yellow soluble pigments are exhibit at all temperatures. *Conidiophores* formed on aerial mycelia, alternated, usually arising at right angles, smooth-walled, and pyramidal shape. *Synchronous* conidiogenous cells on apex and axis from conidiophore and branches, solitary, ampulliform to lageniform. *Conidia* solitary, dry, ornamented, and yellow to light-brown. *Chlamydospores* abundant, hyaline, and smooth.

Habitat: Isolated from fungus gardens of *Mycocepurus goeldii* (Hymenoptera: Formicidae: tribe Attini).

Notes: *Luteomyces* is phylogenetically placed within Hypocreaceae as a sister clade of *Escovopsis* (Fig. 2, 2G). *Luteomyces* grows more slowly and has different colony colour (mainly yellow) than *Escovopsis* (mainly brown). The main feature of this genus is the presence of conidiophores with synchronous conidiogenous cells that differentiate it from its sister clade and all other known Hypocreaceae.

Luteomyces trichodermoides (Cabello, Masiulionis, Seifert, Rodrigues and Pagnocca) Montoya et al. Journal. XX: XX. 2020, comb. nov. Fig. 16.

MycoBank: MB 835152.

Etymology: "*trichodermoides*" referring to the branching pattern, which resembles that of *Trichoderma* spp.

Typification: Brazil. Rio Claro, Sao Paulo, isolated from the upper part of a fungus garden of *M. goeldii* (Hymenoptera: Formicidae: tribe Attini). Isolation number VEM001, ex-type CBS 137343 and CBMAI 1620 and LPSC 1176.

Basionym: *Escovopsis trichodermoides* Cabello, Masiulionis, Seifert, Rodrigues and Pagnocca, Antonie van Leeuwenhoek, Int J Gen Mol Microbiol. 107: 731–740, 2015.

Sequences: ITS (KJ485699), *tef*1 (KF033128), LSU (MF116052), *rpb*1 (MT305417), and *rpb*2 (MT305542).

Colonies can grow at 10, 20, 25 and 30 °C on CMD, PDA and MEA. After seven days at 10 °C, inconspicuous growth, only on inoculum. At 20 ºC, growth starts on third day on all media. After seven days at 20 °C, radial growth of colonies reaches 0.3 – 1.1 cm, 0.3 – 0.5 cm, and 0.5 – 1.1 cm on CMD, MEA and PDA, respectively. At 25 ºC, growth starts on second day on all media. After seven days at 25 °C, radial growth of colonies reaches 2.1 – 4 cm, 2.9 – 4 cm, and 2.7 – 4 cm on CMD, MEA and PDA, respectively. At 30 ºC, growth starts on second day on all media and reaches the plate edge between the fifth and sixth day on PDA and MEA. After seven days at 30 °C, radial growth of colonies reaches 1.3 – 3.3 cm on CMD. After fourteen days at 25 °C, colonies floccose, and white to beige, on CMD (Fig.

16C); dense cottony, white to pale-yellow in center and yellow on the edge, and forming concentric rings and diffuse yellow pigment on MEA (Fig. 16B); and floccose, yellow, with stolons and diffuse yellow pigment on PDA.

Conidiophores 16 – 300 μm long, hyaline, with pyramidal or irregular shape, smooth-walled, alternated or opposite and at right angles (Fig. 16D-E). Conidiophore stipes measuring 5 – 10 μm long x 2 – 3 μm wide, separated by septum 2 – 3 μm from the food cell (basal cell). Conidiophore branches, 7 – 56 μm long, alternated, at right angles**,** longer at the base and shorter towards the apex, and from one to four levels. Stipes on branches 2 – 7 μm long x 1.5 – 2 μm wide, separated by septum 1.5 – 2 μm from conidiophore axis. *Synchronous* conidiogenous cells 10 – 20 μm long x 7 – 8.5 μm wide, formed at apex and axis from conidiophore and branches (Fig. 16F), hyaline, ampulliform to lageniform. *Conidia* globose to sub-globose (2.5 – 3.5 μm long x 2–3 μm wide), formed on conidiogenous cells and directly from conidiophore and branch axes (Fig. 16E-F), solitary, yellow to yellowish-brown, and with verrucose cell wall (Fig. 16H). *Chlamydospores* globose to sub-globose (11.5 – 18 μm long and 10 – 18 μm wide), hyaline, and with smooth walls (Fig. 16G).

Taxonomic key to species of *Escovopsis*, *Luteomyces*, and *Sympodiorosea*

Morphological characters described at each node of the dichotomous cladogram clearly distinguish the six *Escovopsis* species in just seven steps (Fig. S3). Interestingly, topologies of the phylogenetic tree of the genus based on DNA sequence markers (Fig. 4A, B) and the cladogram performed based on morphological features (Fig. S3) are congruent.

1a. Pink or Yellow colonies with conidiophores without terminal vesicles……..……...2

1b Brown colonies and conidiophores with terminal vesicles where phialides are emerging……..……3

2a Pink colonies and conidiophores with sympodial conidiogenous cells……..…… *Sympodiorosea*

2b Yellow colonies and conidiophores with synchronous conidiogenous cells…….*Luteomyces*

3a Pyramidal and irregular conidiophores with greater presence of clavate to cylindrical vesicles…….……...7

3b Only irregular conidiophores with more globose than spatulate vesicles…...……...4

4a Yellowish-brown to brown coloured colonies at seven days on PDA…….……*Escovopsis aspergilloides*

4b Light-brown to brown coloured colonies at seven days on PDA…….....5

5a Conidiophores without swollen cells…..……..……*Escovopsis lentecrescens*

5b Conidiophores with swollen cells.…….……6

6a Colonies grow at 10°C, and conidiophore axis terminates in vesicles…….…...*Escovopsis multiformis*

6b Colonies do not grow at 10°C, and some conidiophore axes terminate in an infertile hypha…….…..... *Escovopsis clavata*

7a Colonies grow at 30 °C, and ornamented conidia form on phialides that emerge only on vesicles……..…….…..*Escovopsis moelleri*

7b Colonies do not grow at 30 °C, and smooth conidia form on phialides that can emerge on the vesicles and in the aerial mycelia……..……...…*Escovopsis weberi*

Discussion

Here, we provide a starting point for the systematics of *Escovopsis* and related genera using a standardized taxonomic framework, a set of morphological characters, and comprehensive multilocus phylogenetic analyses. Our results supported the separation of

species previously treated as *Escovopsis* into three distinct genera. Accordingly, we redefine and restrict *Escovopsis* to vesiculate species, and we describe *Sympodiorosea* to accommodate *E. kreiselii* and *Luteomyces* to accomodate *E. trichodermoides*. This study provides a long-awaited revision of *Escovopsis* systematics and related genera, thus helping future researchers to assess the diversity and evolutionary history of these fungus-growing ant associates.

Genera of Hypocreaceae have morphological features that differentiate them from each other (Jaklitsch and Samuels 2011; Põldmaa 2011). Due to the morphological plasticity of fungi (Slepecky and Starmer 2009; Wrzosek et al. 2017), variations in the shades of colours expressed by these organisms are highly expected. Nonetheless, the prevalent brown colour of *Escovopsis* colonies is a unique feature of this genus within the family. Curiously, other genera in the same family also exhibit unique colours, as is the case for the genus *Trichoderma,* which is characterized by its mostly green colonies (Jaklitsch 2009), and *Escovopsioides,* which is characterized by its white colour (Augusting et al. 2013). Colours exhibited by *Sympodiorosea* (pink) and *Luteomyces* (yellow) are also unique within Hypocreaceae. Interestingly, the separation of these clades by the colour patterns was previously observed by other authors (Gerardo et al. 2006b; Meirelles et al. 2015b), but the lack of a deep morphological analysis prevented reaching the conclusion that they were different genera.

Microscopic features also differentiate *Escovopsis* from other genera in the Hypocreaceae. Conidiophores with terminal-vesicles producing phialides, present in *Escovopsis,* are a unique feature for this family. *Escovopsioides* also presents vesicles, however they are formed intercalated on aerial mycelia and solitary on the apex of the

conidiophores. Furthermore, the vesicles of this genus are smaller and have fewer phialides than those of *Escovopsis* (Augustin et al. 2013). On the other hand, *Sympodiorosea* and *Luteomyces* are the only groups within Hypocreaceae that present sympodial and synchronous conidiogenous cells, respectively. Interestingly, only a distant group of entomopathogenic fungi, as is the case of *Beauveria* (Hypocreales, Cordicipetaceae), has sympodial conidiogenesis (Rehner et al. 2011), and there are no other groups of fungi within the Hypocreales that form synchronous conidiogenous cells. Future studies will hopefully shed light on the evolutionary pressures that led *Escovopsis*, *Sympodiorosea*, and *Luteomyces* to form these unique microscopic characters.

The consideration of all fungi producing brown conidia in the attine ant's colonies as *Escovopsis* (without taxonomic and phylogenetic analyses) made of *Escovopsis* a paraphyletic clade (Fig. 1, S2). Lack of a comprehensive phylogenetic analysis has precluded resolving the phylogenetic uncertainties of *Escovopsis* (Montoya et al. 2019). In light of our results, we considered two hypotheses to disentangle the phylogenetic disagreements of this group of fungi: First, *Escovopsis*, *Luteomyces,* and *Sympodiorosea* belong to the same genus. In this case, *Escovopsioides* (sister clade of *Luteomyces*, Fig. 3A, F) and *Hypomyces* (sister clade of *Sympodiorosea*, Fig. 3 C, D) would have to belong to the same genus to enforce monophyly (Baun and Smith, 2013). Nonetheless, both *Escovopsioides* and *Hypomyces* are well supported, separate monophyletic clades (Fig. 2,2) and present unique morphological characters that differentiate them from other Hypocreaceae. *Escovopsioides*, for instance, is the only Hypocreaceae that forms one type of conidia on phialides and another direct from aerial mycelia (without conidiogenous cells). In contrast, *Hypomyces* forms septate conidia and sexual structures, which is not observed in *Escovopsis*, *Luteomyces*, *Sympodiorosea* or

Escovopsioides. Second, *Escovopsis, Luteomyces,* and *Sympodiorosea* represent taxa within different genera. In this case, considering that the same genes, in different genera, follow different evolutionary paths (Gompel and Prud'homme 2009), the variation of the phylogenetic position of the three clades (Fig. 3) could be better explained if they were diffent genera. Most important, regardless of the molecular markers used in this study, each clade preserves its monophyly within Hypocreaceae. In light of this evidence, and the unique derived morphological characters expressed by *Escovopsis, Luteomyces*, and *Sympodiorosea* (Fig. 4-7), we propose the second hypothesis as the most parsimonious. Future research, using genome-based phylogenetic methods, may resolve the relationship of these genera to each other.

The circumscription of *Escovopsis* raises important questions for the genus. How diverse is *Escovopsis*? What is its host range? How is the genus phylogeographically distributed? And, what is its role in attine gardens? For many years, *Escovopsis* was considered a diverse group of fungi (Gerardo et al. 2006a,b; Rodrigues et al. 2008, 2011; Caldera et al. 2009; Pagnocca et al. 2012; Yek et al. 2012; Meirelles et al. 2015a,b). However, that assumption was based on considering *Sympodiorosea*, *Luteomyces* and other clades (Fig. 1, and S2) within *Escovopsis*. Currently, *E. weberi*, *E. aspergilloides*, *E. clavata*, *E. lentecrescens*, *E. microspora* (synonym of *E. weberi*), *E. moelleri*, and *E. multiformis* are the only species described under standardized taxonomical conditions. The species introduced by Marfetán et al. (2019) also belong to *Escovopsis* (Fig. S1), nonetheless, the taxonomy and phylogeny of these species are still unclear. Therefore, future studies should consider that both the genetic diversity and the number of known species of the genus were overestimated. Likewise, future studies will unveil the geographical distribution of *Escovopsis* and its

phylogenetic correspondence with the ants, and the mutualistic fungi. Finally, several studies suggested that *Escovopsis* is a mycoparasite (Currie et al. 1999a, 2003; Currie 2001; Gerardo et al. 2004; Little and Currie 2007; de Man et al. 2016). However, the parasitic mechanisms of *Escovopsis* species are poorly understood, and this hypothesis was also raised considering *Sympodiorosea* and *Luteomyces* within the same genus. Therefore, the mechanisms of the parasitism of *Escovopsis* should be carefully addressed.

Similar questions as those raised for *Escovopsis* must be addressed in future studies for *Sympodiorosea*, *Luteomyces* and the other clades (Fig.1, and S2). The genetic and morphological diversity, as well as the geographical distribution of both genera, are still a mystery. Some authors suggested that *Sympodiorosea* and *Luteomyces* are more likely to be associated with the colonies of lower attine ants than higher attine (e.g., leaf-cutting) ants (Gerardo et al. 2006b), but more evidence is necessary to confirm this hypothesis. Recent studies have shown that some strains of *Sympodiorosea* behave as antagonists of the mutualistic fungus of *Mycetophylax morschi* being able to kill it under laboratory conditions (Custodio and Rodrigues 2019). However, the mechanisms by which it manages to kill the cultivars are completely unknown. On the other hand, Bizarria et al. (2020) demonstrated that *Luteomyces* had little negative impact on the mutualistic fungus of *Mycocepurus goeldii*, being just able to inhibit the fungus cultivars *in-vitro* and unable to overcome defenses of the ant colonies. The ecology and lifestyle of the other clades previously treated as *Escovopsis* (Fig. 1, and S2) is a completely mistery. Therefore, the assumption of a parasitic lifestyle for these groups of fungi should be reconsidered and examined carefully.

Conclusion

Since Alfred Möller (1893) observed the "fungi of the strong conidial form" within the fungus gardens of fungus-growing ants, several groups of fungi that share the same niche were classified as *Escovopsis*. Many taxonomic and phylogenetic incongruities have been reported in the last two decades, and the lack of taxonomic studies for this genus have hampered scientists to recognize the root of the problem. After a detailed systematic study, we conclude that taxonomic disagreements in the *Escovopsis* genus were caused due to the inclusion of two groups of fungi that belong to different genera (*Luteomyces* and *Sympodiorosea*) within the same genus. This discovery not only solves the taxonomic and phylogenetic disagreements of the genus but significantly expands our understanding of the systematics of *Escovopsis*, and related genera, and provides a stable foundation from which to build future research on the evolutionary history, taxonomic diversity, and ecological roles of these unique fungi.

Declarations

Ethics approval and consent to participate

Not applicable

Adherence to national and international regulations

Not applicable

Consent for publication

Not applicable

Availability of data and material

The data set used in this study will available in the NCBI-GenBank (Table S1, S2, and S3). The alignments used to perform the phylogenetic trees will be deposited in Treebase as soon as the reviewers' comments were addressed and the final trees were generated.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

QVM and AR designed the study. QVM carried out the morphological and phylogenetic analyses. QVM, MJSM, RBJ carried out *in vitro* growth experiments. QVM, AR, MSJM and NMG wrote the manuscript. All authors read and approved the final manuscript.

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Fig. 1.

Colapsed phylogenetic tree (see the whole tree in Fig. S2) based on Bayesian Inference of the *tef*1, indicating the placement of all strains previously named as *Escovopsis*. The tree gathers all available *tef*1 sequences found in the literature with the data set used in this study (including the sequences of the nine *Escovopsis* ex-type strains evaluated here). The tree contains a total of 415 sequences which include: 281vesiculate-*Escovopsis* (Clade A), 70 non-vesiculate *Escovopsis* (Clades B, C, D, E) and 63 strains from five Hypocreaceae genera (*Escovopsioides*, *Hypomyces*, *Protocrea*, *Sphaerostilbella*, *Trichoderma*). *Lecanicillium antillanum* CBS 35085 is the outgroup of the tree. There is no information on literature about the morphological feature of the clades C and D.

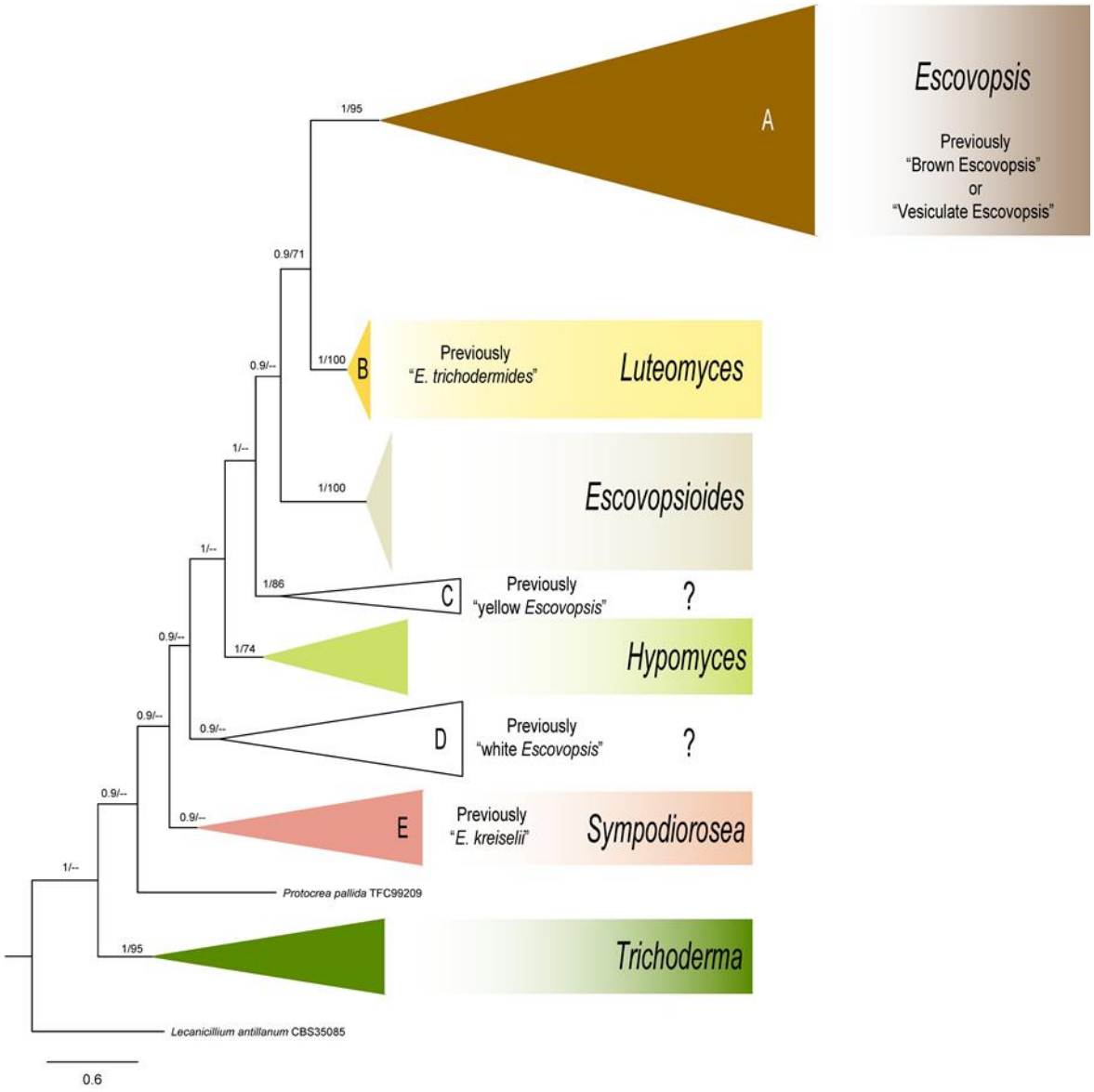
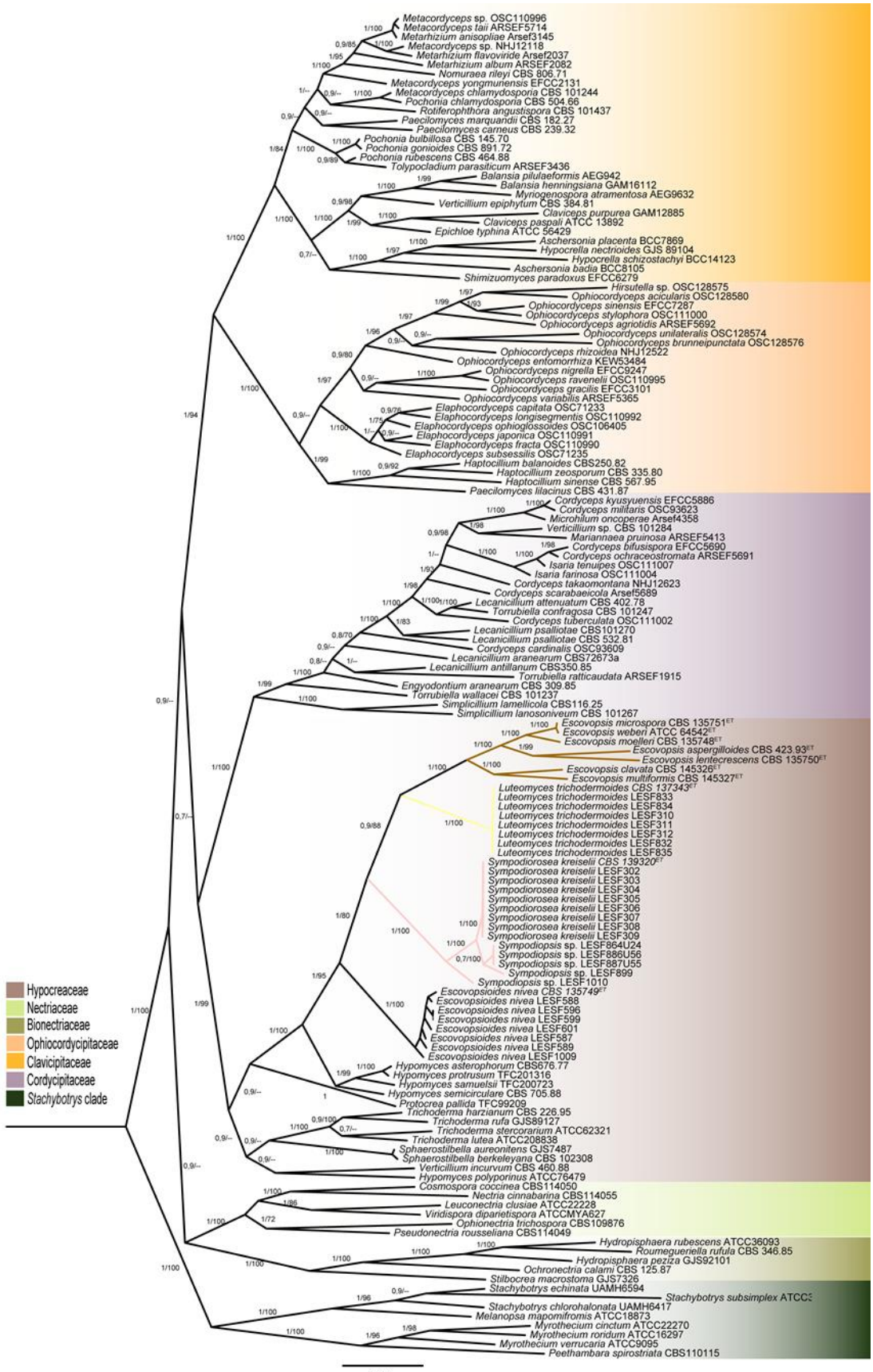


Fig. 2.

Phylogenetic tree, based on Bayesian Inference, indicating the placement of *Escovopsis* (brown branches), *Sympodiorosea* (pink branches) and *Luteomyces* (yellow branches) within Hypocreales. Highlighted clades in different colours represent six different Hypocreales families (Clavicipitaceae, Cordycipietaceae, Hypocreaceae, Nectriaceae, and Ophiocordycipitaceae). The analysis was based on concatenated sequences of LSU, *tef*1, *rpb*1 and *rpb*2. The final alignment of 3305 characters consisted of sequences from 145 strains. Numbers on branches indicate BI posterior probabilities and ML bootstrap support values. The *Stachybotrys* clade (dark green) was used to root the tree. ET indicates ex-type isolates.


Metacordyceps sp. OSC110996
Metacordyceps taii ARSEF5714
Metarhizium anisopliae Arsef3145
Metacordyceps sp. NHJ12118
Metarhizium flavoviride Arsef2037
Metarhizium album ARSEF2082
Nomuraea rileyi CBS 806.71
Metacordyceps yongmuniensis EFCC2131
Metacordyceps chlamydosporia CBS 101244
Pochonia chlamydosporia CBS 504.66
Rotiferophthora angustispora CBS 101437
Paecilomyces marquandii CBS 182.27
Paecilomyces carneus CBS 239.32
Pochonia bulbillosa CBS 145.70
Pochonia gonioides CBS 891.72
Pochonia rubescens CBS 464.88
Tolypocladium parasiticum ARSEF3436
Balansia pilulaeformis AEG942
Balansia henningsiana GAM16112
Myriogenospora atramentosa AEG9632
Verticillium epiphytum CBS 384.81
Claviceps purpurea GAM12885
Claviceps paspali ATCC 13892
Epichloe typhina ATCC 56429
Aschersonia placenta BCC7869
Hypocrella nectrioides GJS 89104
Hypocrella schizostachyi BCC14123
Aschersonia badia BCC8105
Shimizuomyces paradoxus EFCC6279
Hirsutella sp. OSC128575
Ophiocordyceps acicularis OSC128580
Ophiocordyceps sinensis EFCC7287
Ophiocordyceps stylophora OSC111000
Ophiocordyceps agriotidis ARSEF5692
Ophiocordyceps unilateralis OSC128574
Ophiocordyceps brunneipunctata OSC128576
Ophiocordyceps rhizoidea NHJ12522
Ophiocordyceps entomorrhiza KEW53484
Ophiocordyceps nigrella EFCC9247
Ophiocordyceps ravenelii OSC110995
Ophiocordyceps gracilis EFCC3101
Ophiocordyceps variabilis ARSEF5365
Elaphocordyceps capitata OSC71233
Elaphocordyceps longisegmentis OSC110992
Elaphocordyceps ophioglossoides OSC106405
Elaphocordyceps japonica OSC110991
Elaphocordyceps fracta OSC110990
Elaphocordyceps subsessilis OSC71235
Haptocillium balanoides CBS250.82
Haptocillium zeosporum CBS 335.80
Haptocillium sinense CBS 567.95
Paecilomyces lilacinus CBS 431.87
Cordyceps kyusyuensis EFCC5886
Cordyceps militaris OSC93623
Microhilum oncoperae Arsef4358
Verticillium sp. CBS 101284
Mariannaea pruinosa ARSEF5413
Cordyceps bifusispora EFCC5690
Cordyceps ochraceostromata ARSEF5691
Isaria tenuipes OSC111007
Isaria farinosa OSC111004
Cordyceps takaomontana NHJ12623
Cordyceps scarabaeicola Arsef5689
Lecanicillium attenuatum CBS 402.78
Torrubiella confragosa CBS 101247
Cordyceps tuberculata OSC111002
Lecanicillium psalliotae CBS101270
Lecanicillium psalliotae CBS 532.81
Cordyceps cardinalis OSC93609
Lecanicillium aranearum CBS72673a
Lecanicillium antillanum CBS350.85
Torrubiella ratticaudata ARSEF1915
Engyodontium aranearum CBS 309.85
Torrubiella wallacei CBS 101237
Simplicillium lamellicola CBS116.25
Simplicillium lanosoniveum CBS 101267
Escovopsis microspora CBS 135751ET
Escovopsis weberi ATCC 64542ET
Escovopsis moelleri CBS 135748ET
Escovopsis aspergilloides CBS 423.93ET
Escovopsis lentecrescens CBS 135750ET
Escovopsis clavata CBS 145326ET
Escovopsis multiformis CBS 145327ET
Luteomyces trichodermoides CBS 137345ET
Luteomyces trichodermoides LESF833
Luteomyces trichodermoides LESF834
Luteomyces trichodermoides LESF310
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Luteomyces trichodermoides LESF312
Luteomyces trichodermoides LESF832
Luteomyces trichodermoides LESF835
Sympodiorosea kreiselii CBS 139320ET
Sympodiorosea kreiselii LESF302
Sympodiorosea kreiselii LESF303
Sympodiorosea kreiselii LESF304
Sympodiorosea kreiselii LESF305
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Sympodiopsis sp. LESF886U56
Sympodiopsis sp. LESF887U55
Sympodiopsis sp. LESF899
Sympodiopsis sp. LESF1010
Escovopsioides nivea CBS 135749ET
Escovopsioides nivea LESF588
Escovopsioides nivea LESF596
Escovopsioides nivea LESF599
Escovopsioides nivea LESF601
Escovopsioides nivea LESF587
Escovopsioides nivea LESF589
Escovopsioides nivea LESF1009
Hypomyces asterophorum CBS676.77
Hypomyces protrusum TFC201316
Hypomyces samuelsii TFC200723
Hypomyces semicirculare CBS 705.88
Protocrea pallida TFC99209
Trichoderma harzianum CBS 226.95
Trichoderma rufa GJS89127
Trichoderma stercorarium ATCC62321
Trichoderma lutea ATCC208838
Sphaerostilbella aureonitens GJS7487
Sphaerostilbella berkeleyana CBS 102308
Verticillium incurvum CBS 460.88
Hypomyces polyporinus ATCC76479
Cosmospora coccinea CBS114050
Nectria cinnabarina CBS114055
Leuconectria clusiae ATCC22228
Viridispora diparietispora ATCCMYA627
Ophionectria trichospora CBS109876
Pseudonectria rousseliana CBS114049
Hydropisphaera rubescens ATCC36093
Roumegueriella rufula CBS 346.85
Hydropisphaera peziza GJS92101
Ochronectria calami CBS 125.87
Stilbocrea macrostoma GJS7326
Stachybotrys echinata UAMH6594
Stachybotrys subsimplex ATCC
Stachybotrys chlorohalonata UAMH6417
Melanopsa mapomiformis ATCC18873
Myrothecium cinctum ATCC22270
Myrothecium roridum ATCC16297
Myrothecium verrucaria ATCC9095
Peethambara spirostriata CBS110115
Hypocreaceae
Nectriaceae
Bionectriaceae
Ophiocordycipitaceae
Clavicipitaceae
Cordycipitaceae
Stachybotrys clade
0.07

Fig. 3.

Phylogenetic placement of *Escovopsis*, *Sympodiorosea* and *Luteomyces* within *Hypocreaceae*. Phylogenies were inferred using BI analysis and are separated for each molecular marker: (A) ITS, B) LSU, C) *tef*1, D) *rpb*1, and E) *rpb*2). The remaining two trees are based on concatenated datasets of (F) all five markers, and (G) four of the five markers (LSU, *rpb*1, *rpb*2, and *tef*1). Numbers on branches indicate BI posterior probabilities and ML bootstrap support values. *Lecanicillium antillanum* CBS35085 was used as the outgroup. ET indicates ex-type isolates.

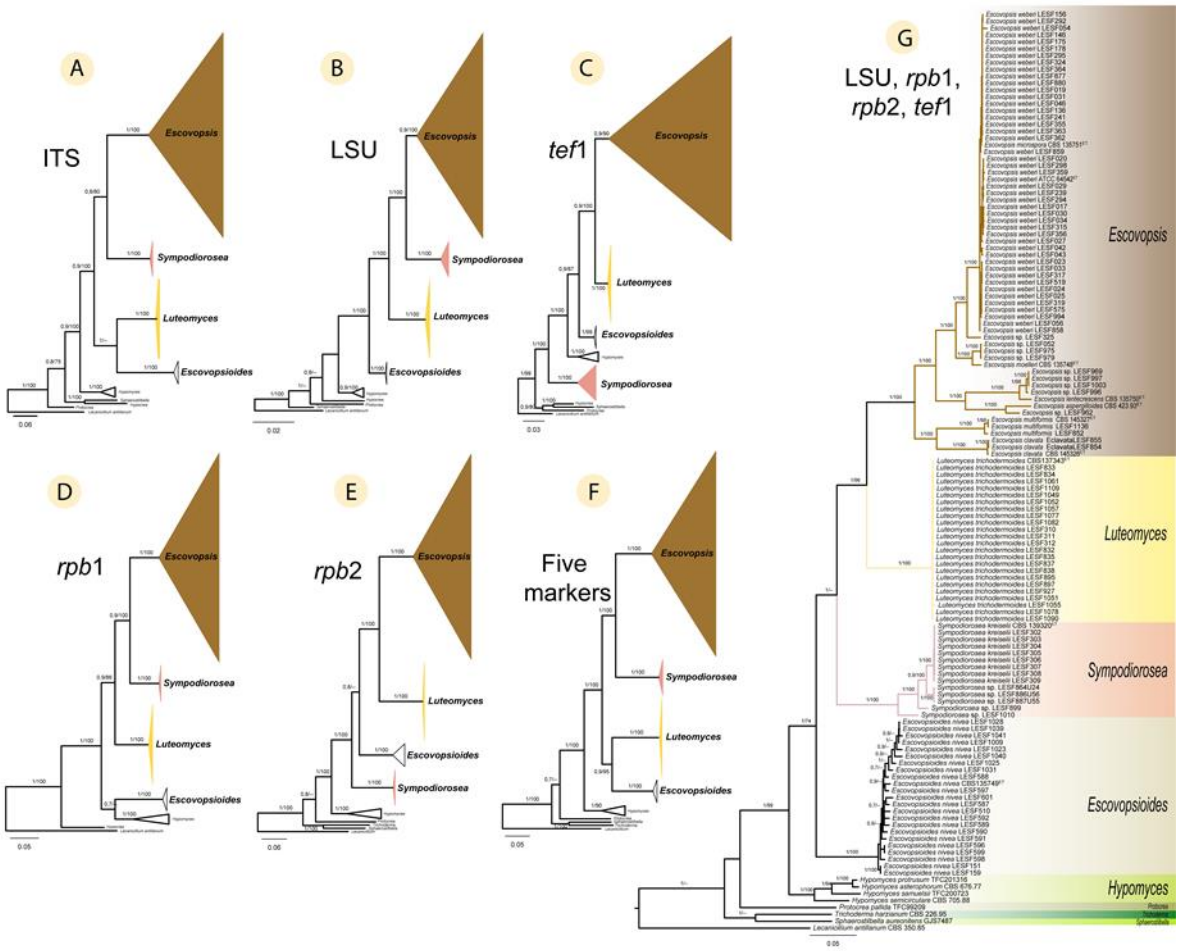


Fig. 4.

Phylogenetic placement of *Escovopsis* species. Phylogenies were inferred using BI analysis: A) Collapsed tree obtained from a combined dataset of LSU, *rpb*1, *rpb*2, and *tef*1 markers. B) Concatenated tree obtained combining ITS, LSU, *rpb*1, *rpb*2, and *tef*1. C) Conidiophores of each ex-type species. The numbers on branches indicate the BI posterior probabilities and ML bootstrap support. ET indicates ex-type isolates.

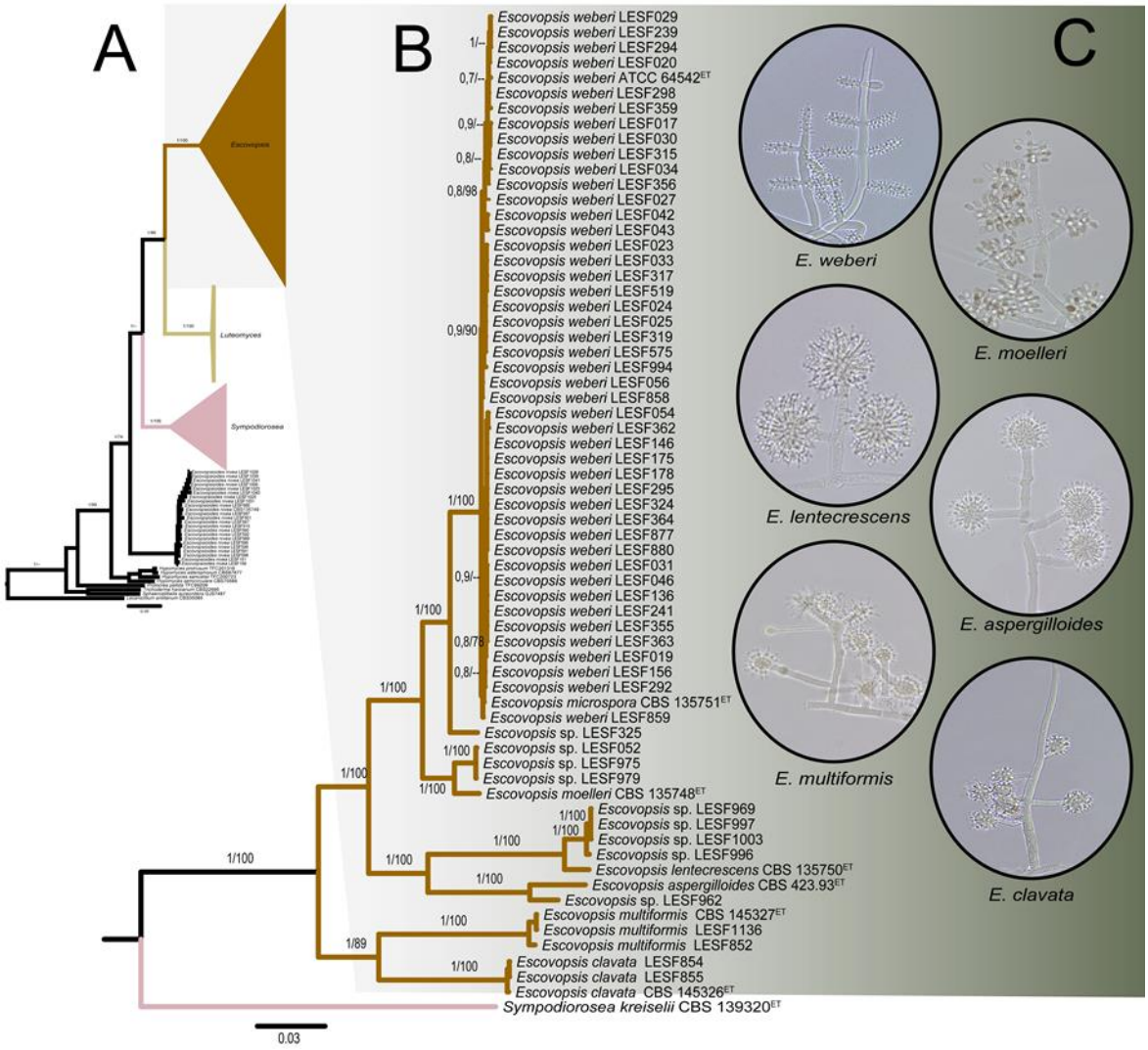


Fig. 5.

Colony morphology of the seven *Escovopsis* type species (*Escovopsis senso stricto*), *E. trichodermoides* (*Luteomyces trichodermoides*) and *E. kreiselii* (*Sympodiorosea kreiselii*). All cultures were evaluated at 25 °C in the dark, after seven days for *Escovopsis* and 15 days for *L. trichodermoides* and *S. kreiselii*.

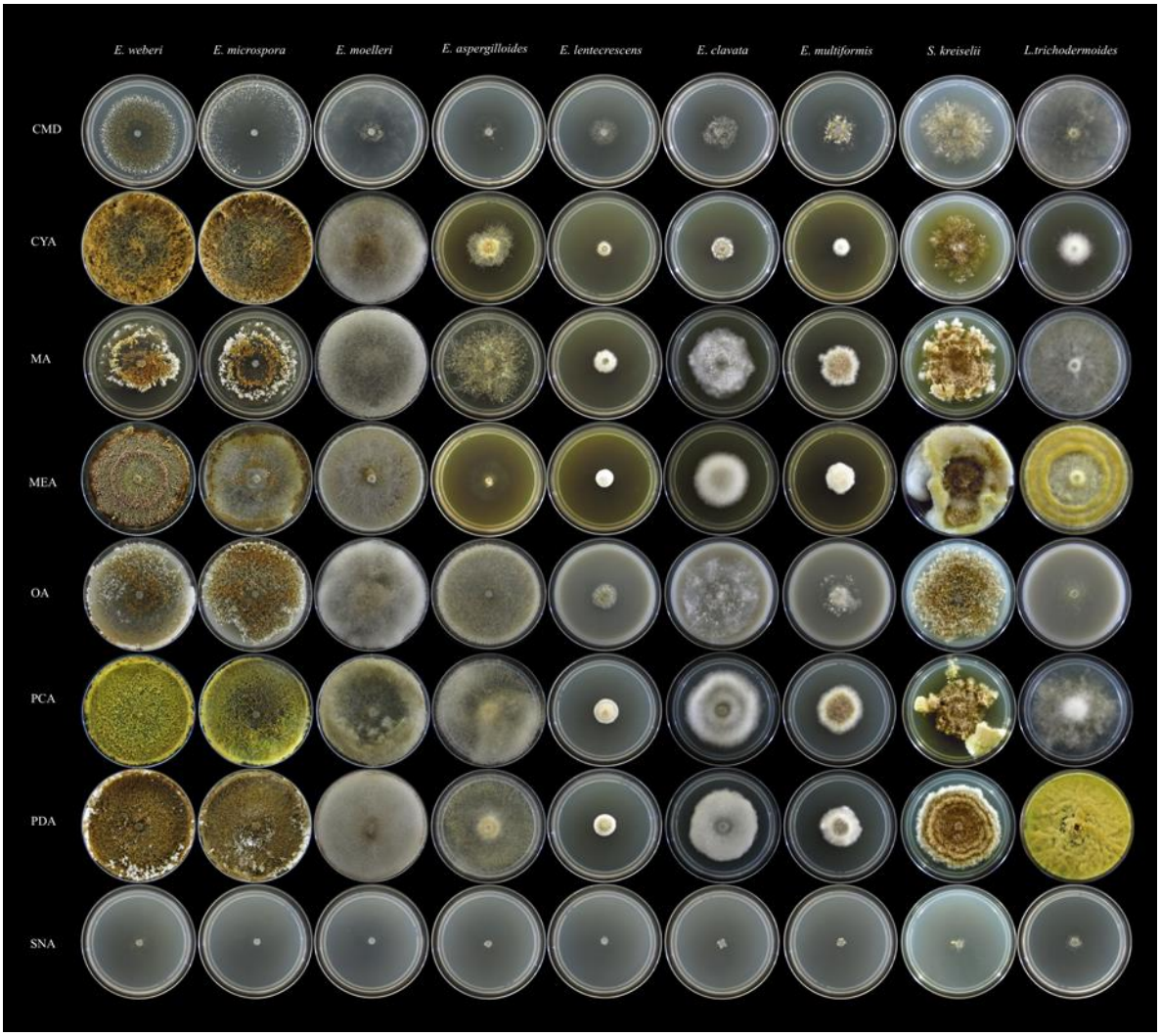


Fig. 6.

Illustration of the microscopic structures of the genus *Escovopsis*. A-B: Mono-vesiculate conidiophores. C-H: Poly-vesiculate conidiophores. F: Conidiophore with "swollen cell". I-U: Different shapes of vesicles (I: Globose, J: Sub-globose, K: Capitate, L: Obovoid, M: Prolate, N: Spatulate, O: Clavate, P: Cymbiform, Q: Oblanceolate, R: Subulate, S: Cylindric, T: Filiform, U: Clavate-septate, V: Cylindric-septate). W-Z: Different shapes of the phialides. Different shapes of conidia (a: Globose, b: Sub-globose, c: Ellipsoidal, d: Oblong, e: Oblong-ornamented).

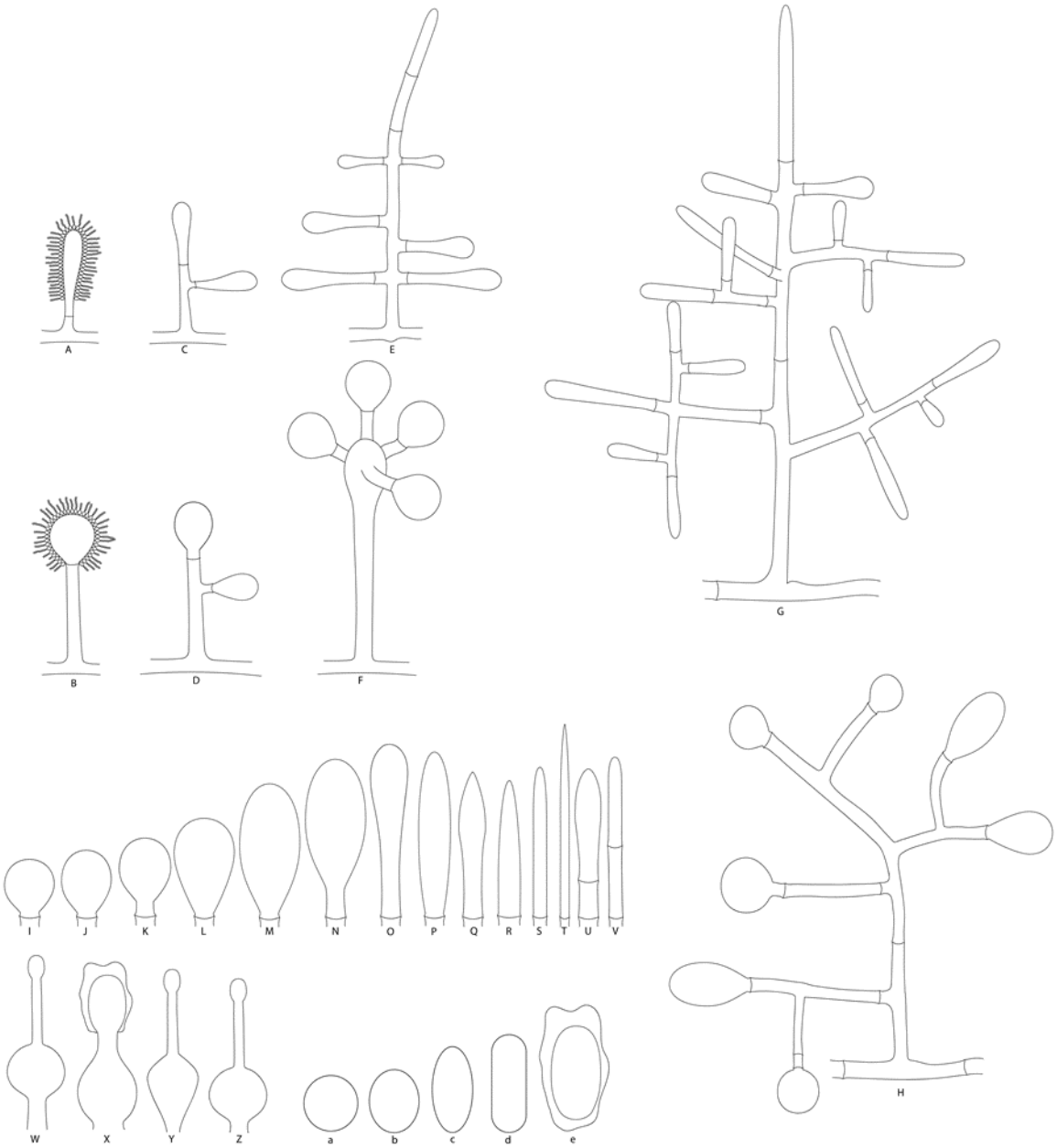


Fig. 7. Morphological characters of *Escovopsis weberi*. A-C: Cultures on PDA, MEA and CMD, respectively, after 7 days of growth at 25 °C. D: Mono-vesiculate conidiophores on aerial mycelium. E. Poly-vesiculate conidiophores. F: Septate-cylindric vesicle. G: Cylindric vesicle. H: Clavate vesicle. I: Phialides. J: Conidia.

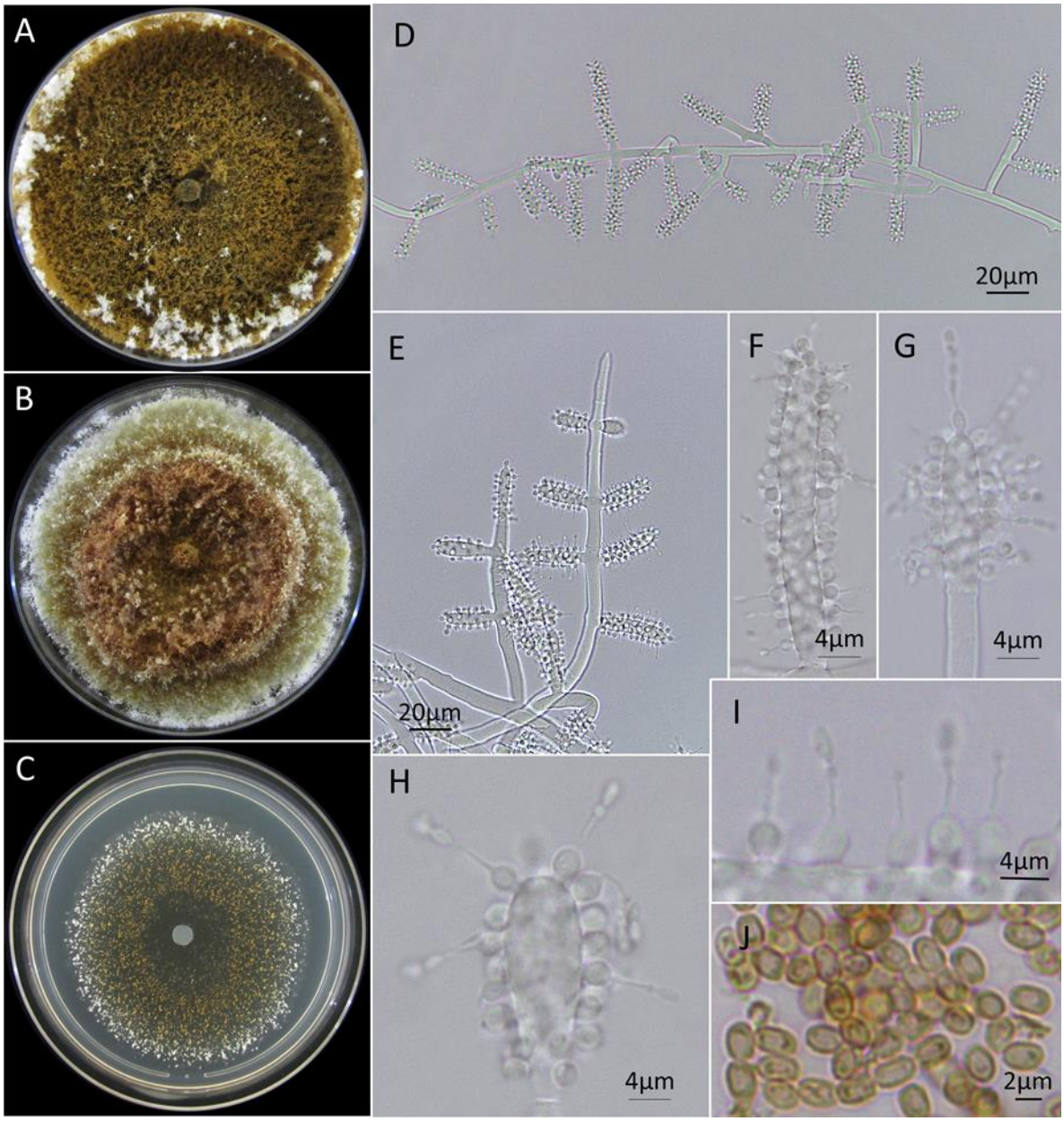


Fig. 8.

Morphological characters of *Escovopsis aspergilloides*. A-C: Cultures on PDA, MEA and CMD, respectively, after 7 days of growth at 25 °C. D. Mono-vesiculate conidiophore. E-F: Poly-vesiculate conidiophores. G: Globose vesicle with phialides. H: Phialides. I: Conidia. J: Chlamydospore.

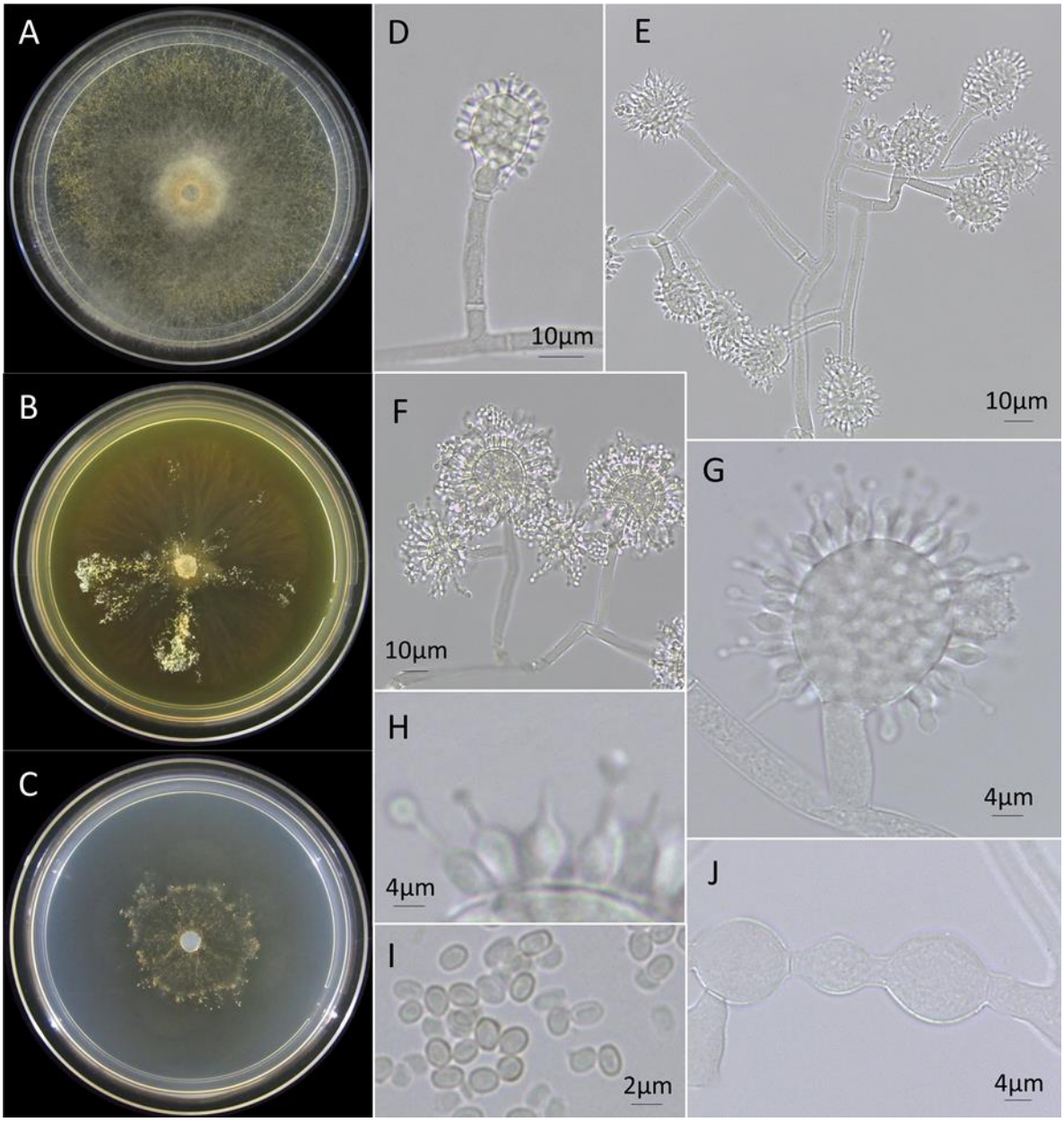


Fig. 9.

Morphological characters of *Escovopsis clavata*. A-C: Cultures on PDA, MEA and CMD, respectively, after 7 days of growth at 25 °C. D: Mono-vesiculate conidiophores. E. Poly-vesiculate conidiophore. F: Conidiophore with infertile hypha at the apex. G: conidiophore with swollen cell. H: Clavate vesicle with phialides. I: Phialides. J: Conidia.

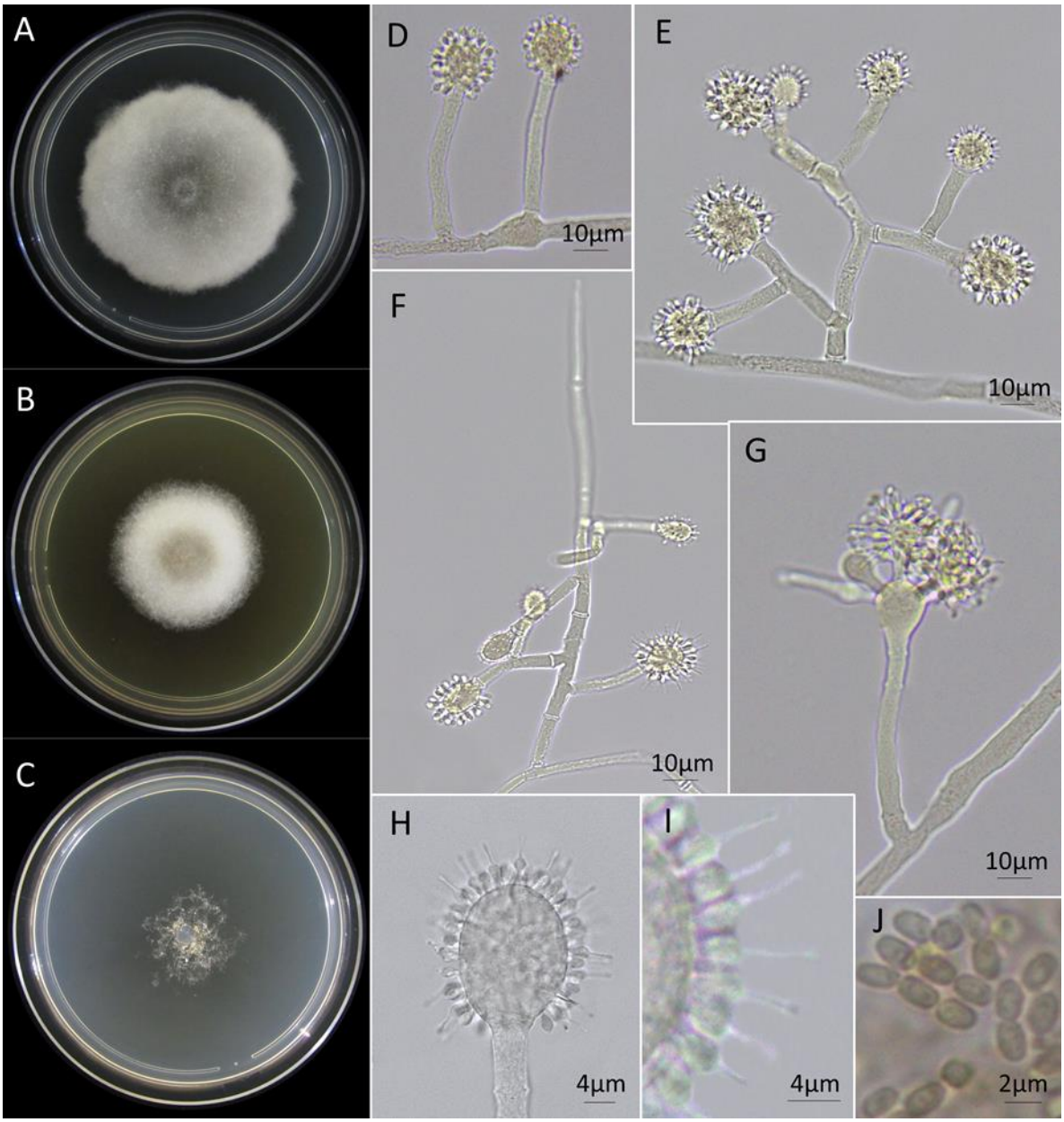


Fig 10.

Morphological characters of *Escovopsis lentecrescens*. A-C: Cultures on PDA, MEA and CMD, respectively, after 7 days of growth at 25 °C. D: Mono-vesiculate conidiophores. E. Poly-vesiculate conidiophore. F: Globose vesicle with phialides. G: Sub-globose vesicle with phialides. H: Cylindric vesicle with phialides. I: Phialides. J: Conidia. K: Chlamydospores.

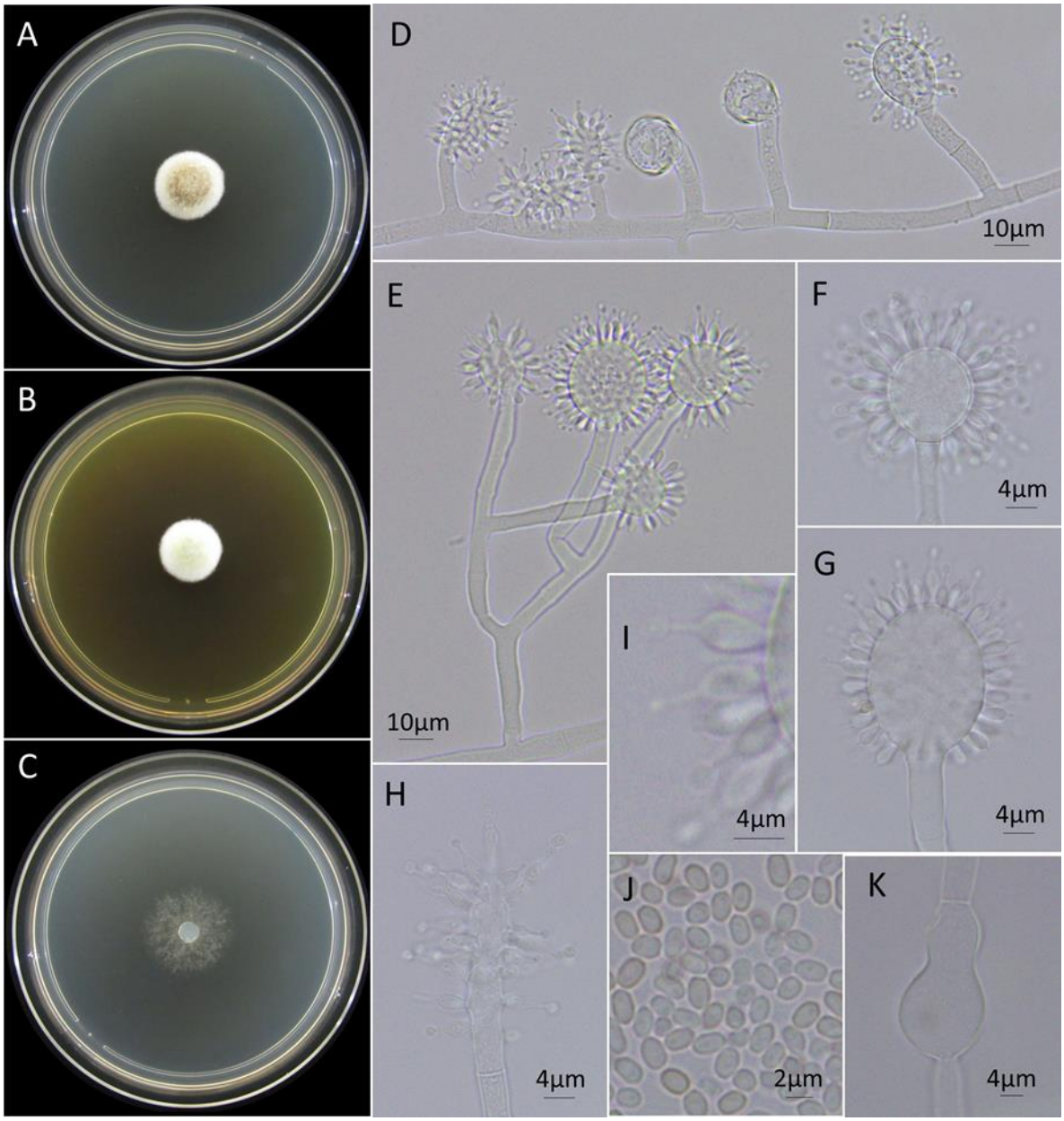


Fig. 11.

Morphological characters of *Escovopsis moelleri*. A-C: Cultures on PDA, MEA and CMD, respectively, after 7 days of growth at 25 °C. D: Mono-vesiculate conidiophore. E: Poly-vesiculate conidiophore. F: Subulate vesicle. G: Oblanceolate vesicle. H: Clavate vesicle. I: Phialides with conidia. J: Ornamented conidia.

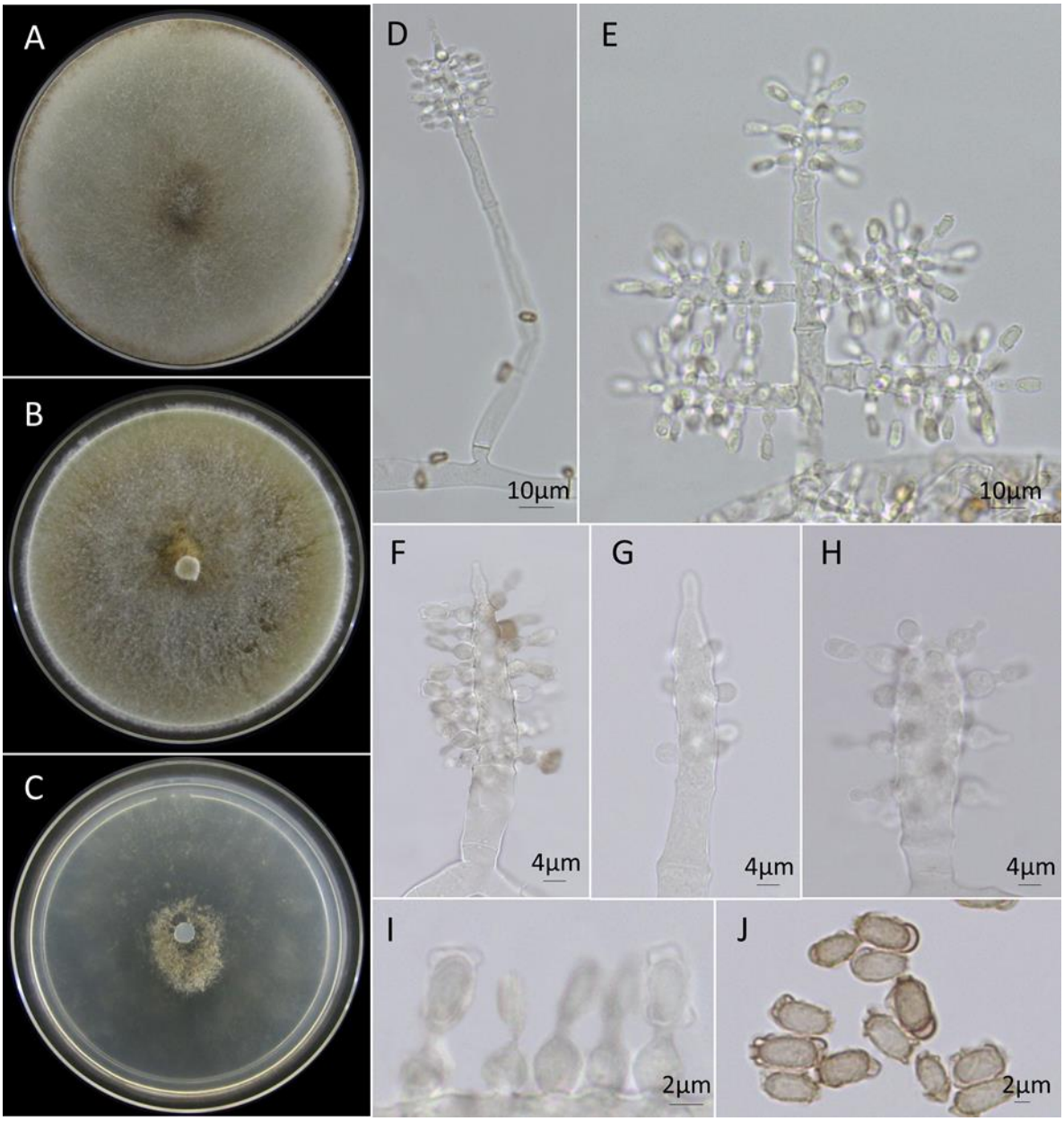


Fig. 12.

Morphological characters of *Escovopsis multiformis*. A-C: Cultures on PDA, MEA and CMD, respectively, after 7 days of growth at 25 °C. D: Mono-vesiculate conidiophore. E-F: Poly-vesiculate conidiophores. G. Conidiophores with swollen cell. H: Clavate vesicle with phialides. I: Phialides. J: Conidia.

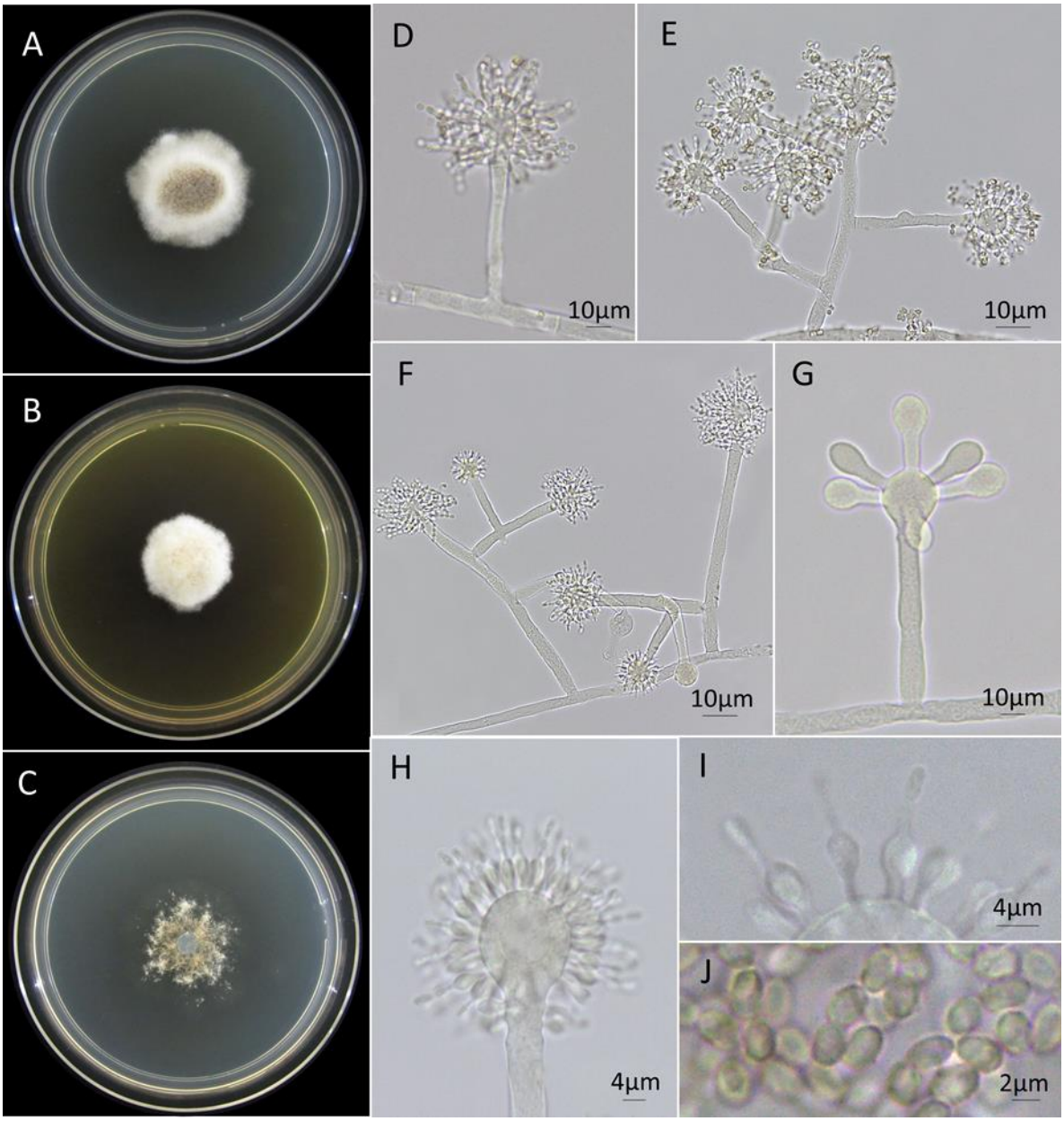


Fig. 13.

Illustration of the microscopic structures of the genus *Sympodiorosea*. A: Conidiophore pattern on aerial mycelium. B. Conidiophore shape. C-H: Shapes of the sympodial conidiogenous cells. I: Conidia with denticle (coming from the conidiogenous cell), J: Conidia with a hole (when the denticle remains on the conidiogenous cell). K: Chlamydospores in chain.

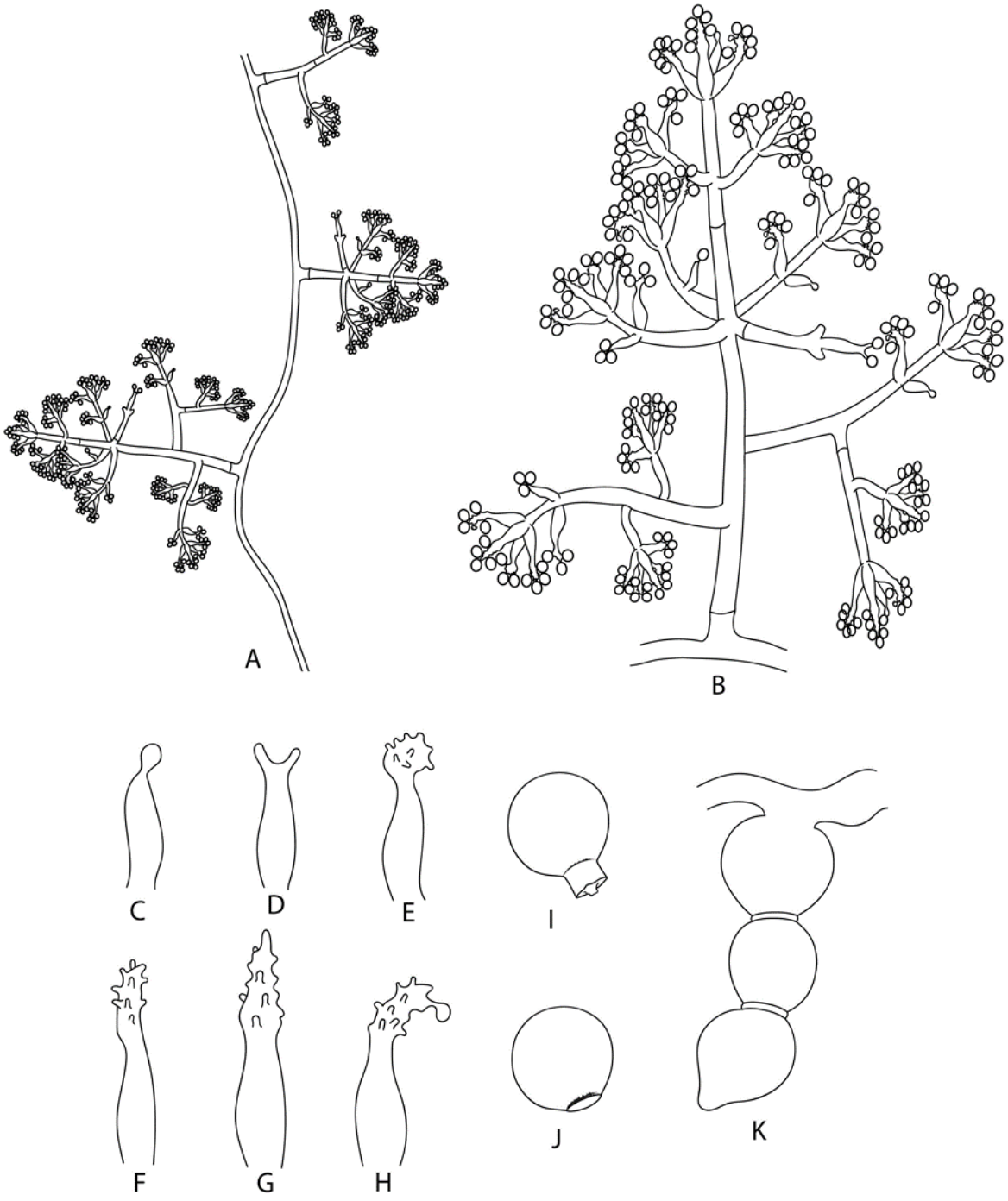


Fig. 14.

Morphological characters of *Sympodiorosea kreiselii*. A-C: Cultures on PDA, MEA and CMD, respectively, after 7 days of growth at 25 °C. D-F. Conidiophores. G: Sympodial conidiogenous cell with conidia. H: Chlamydospores. I: Conidia.

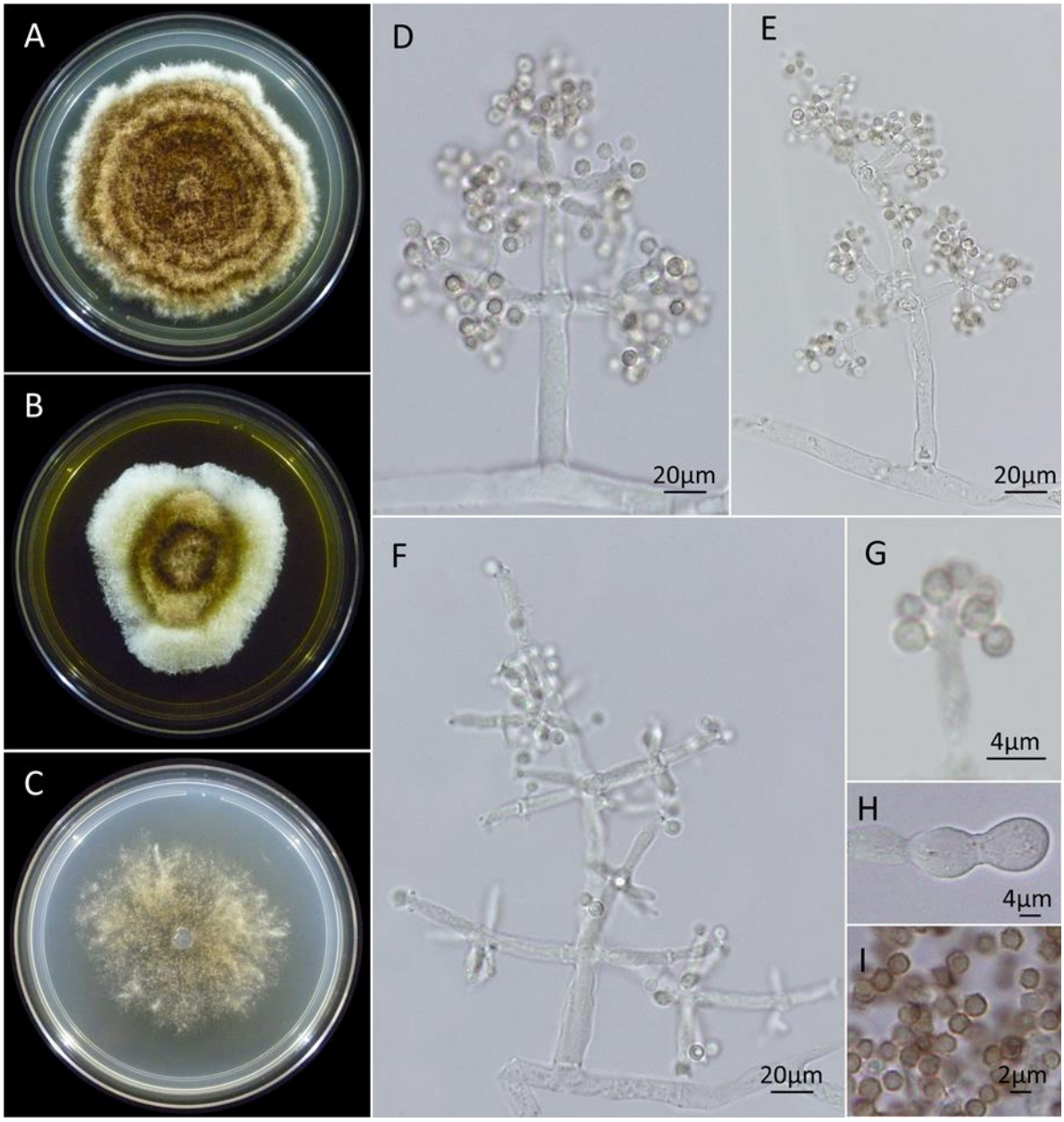


Fig. 15.

Illustration of the microscopic structures of the genus *Luteomyces*. A: Conidiophore pattern on aerial mycelium. B. Conidiophore shape. C-F: Shapes of the syncronous conidiogenous cells. G: Conidia with ornamentation. H: Smooth conidia. I: Chlamydospores.

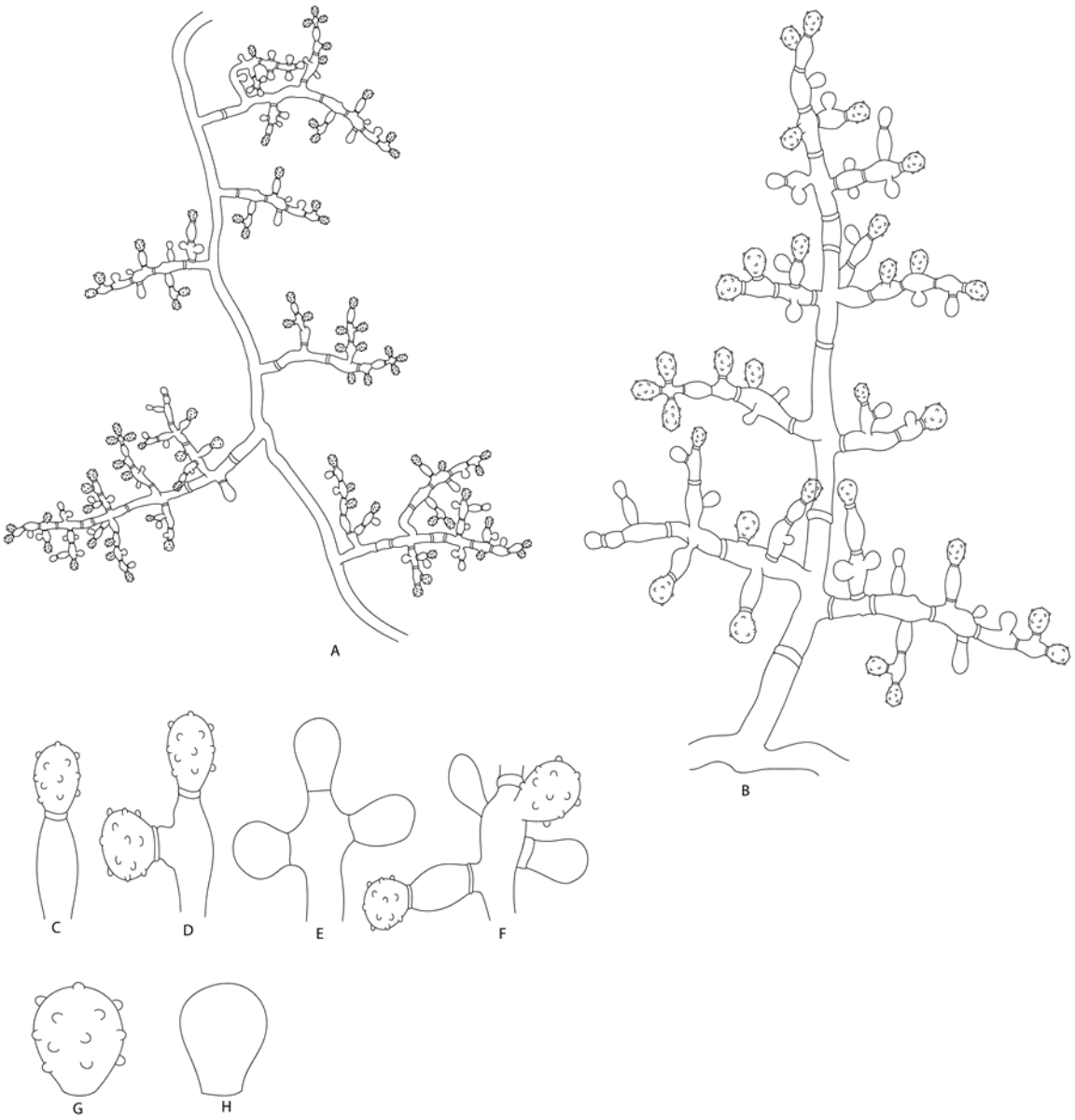
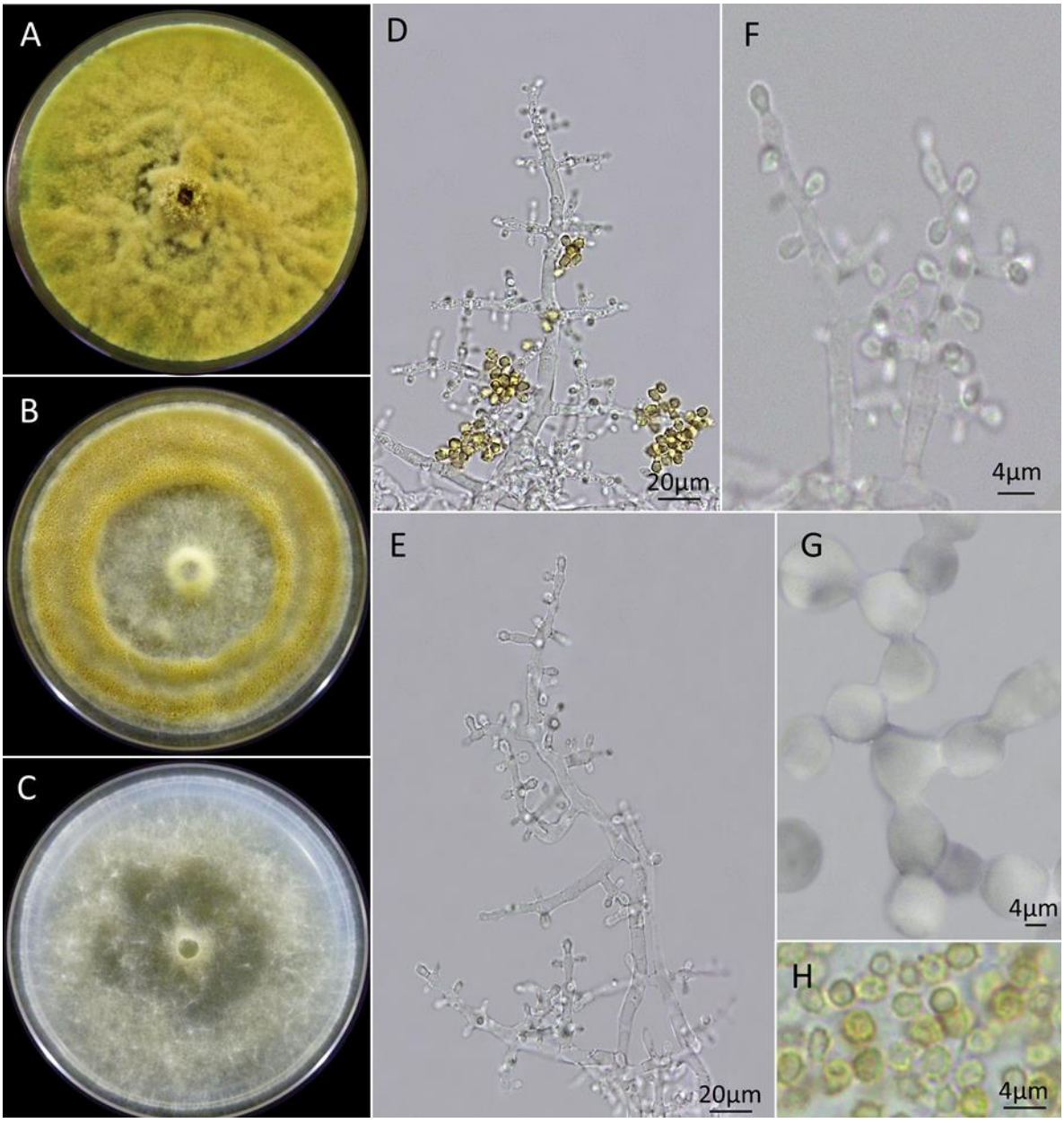


Fig. 16.

Morphological characters of *Luteomyces trichodermoides*. A-C: Cultures on PDA, MEA, and CMD, respectively, after 7 days of growth at 25 °C. D-E. Conidiophores. F: Conidiogenous cells. G: Chlamydospores. H: Conidia.



Supporting information

Fig. S1.

Phylogenetic placement of *Escovopsis* species described in Marfetán et al. (2019). The phylogenetic tree was reconstructed in order to include the 24 LSU sequences (in the green box on the tree) generated in Marfetán et al. (2019). The tree was reconstructed using Maximum Likelihood (ML) in RAxML v.8 (Stamatakis 2014) and Bayesian Inference (BI) in MrBayes v.3.2.2 (Ronquist et al. 2012) using the GTR model. For ML analyses, 1000 independent trees and 1000 bootstrap replicates were generated; one million generations of the Markov Chain Monte Carlo (MCMC) were enough to reach convergence. The numbers on branches indicate the BI posterior probabilities and ML bootstrap support. *Lecanicillium antillanum* CBS35085 was used as the outgroup. The five described species formed two clades (green box) within *Escovopsis* and close to *E. aspergilloides* and *E. lentecrescens*. *E. atlas*, *E. catenulate*. *E. longivesica*, *E. pseudoweberi*, were placed into the same clade. Some strains identified as *E. weberi* are in the same clade. The four strains of *E. primorosea* formed a monophyletic clade.

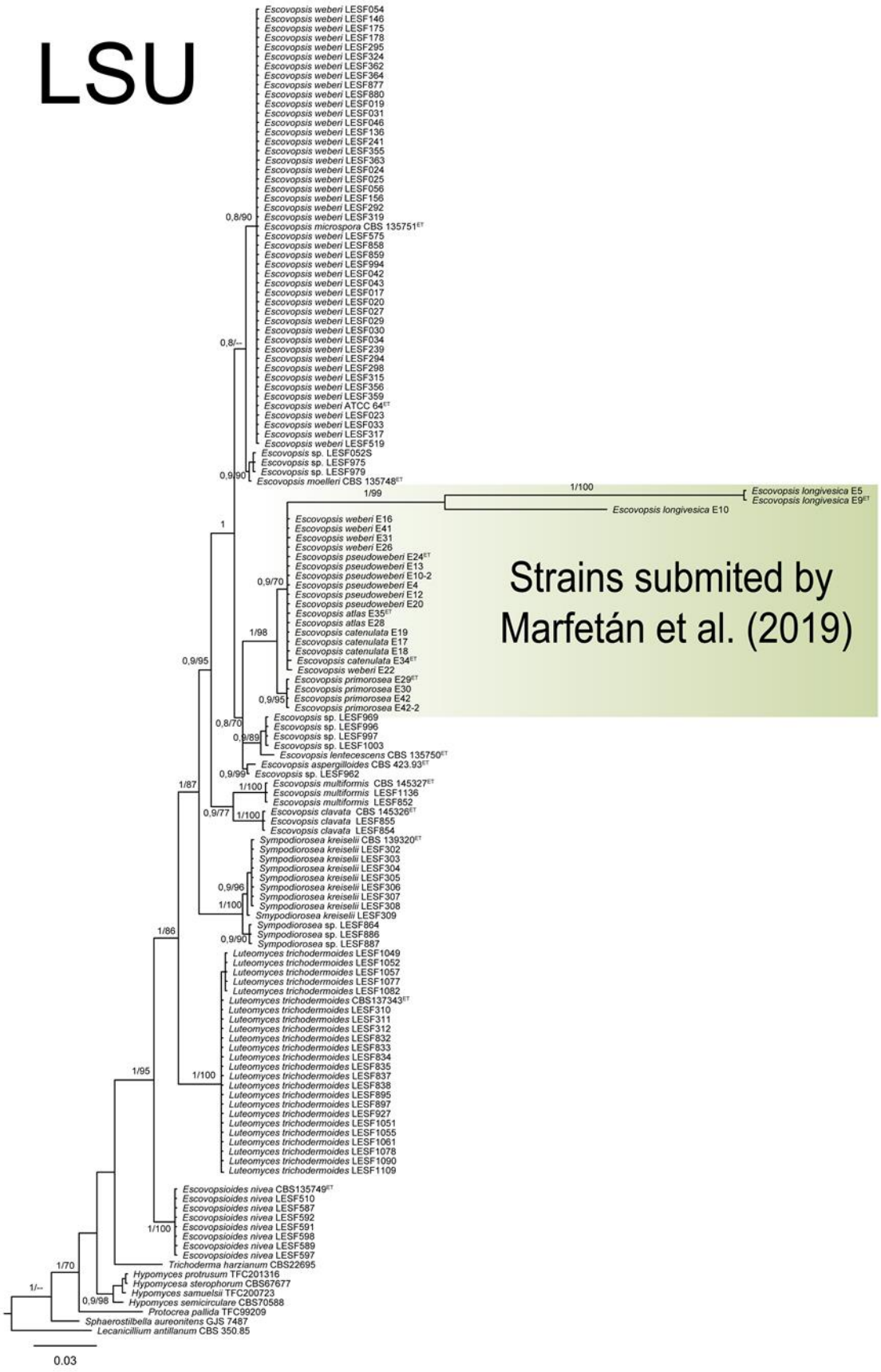

LSU
Escovopsis weberi LESF054
Escovopsis weberi LESF146
Escovopsis weberi LESF175
Escovopsis weberi LESF178
Escovopsis weberi LESF295
Escovopsis weberi LESF324
Escovopsis weberi LESF362
Escovopsis weberi LESF364
Escovopsis weberi LESF877
Escovopsis weberi LESF880
Escovopsis weberi LESF019
Escovopsis weberi LESF031
Escovopsis weberi LESF046
Escovopsis weberi LESF136
Escovopsis weberi LESF241
Escovopsis weberi LESF355
Escovopsis weberi LESF363
Escovopsis weberi LESF024
Escovopsis weberi LESF025
Escovopsis weberi LESF056
Escovopsis weberi LESF156
Escovopsis weberi LESF292
Escovopsis weberi LESF319
Escovopsis microspora CBS 135751ET
Escovopsis weberi LESF575
Escovopsis weberi LESF858
Escovopsis weberi LESF859
Escovopsis weberi LESF994
Escovopsis weberi LESF042
Escovopsis weberi LESF043
Escovopsis weberi LESF017
Escovopsis weberi LESF020
Escovopsis weberi LESF027
Escovopsis weberi LESF029
Escovopsis weberi LESF030
Escovopsis weberi LESF034
Escovopsis weberi LESF239
Escovopsis weberi LESF294
Escovopsis weberi LESF298
Escovopsis weberi LESF315
Escovopsis weberi LESF356
Escovopsis weberi LESF359
Escovopsis weberi ATCC 64ET
Escovopsis weberi LESF023
Escovopsis weberi LESF033
Escovopsis weberi LESF317
Escovopsis weberi LESF519
Escovopsis sp. LESF052S
Escovopsis sp. LESF975
Escovopsis sp. LESF979
Escovopsis moelleri CBS 135748ET
Escovopsis longivesica E5
Escovopsis longivesica E9ET
Escovopsis longivesica E10
Escovopsis weberi E16
Escovopsis weberi E41
Escovopsis weberi E31
Escovopsis weberi E26
Escovopsis pseudoweberi E24ET
Escovopsis pseudoweberi E13
Escovopsis pseudoweberi E10-2
Escovopsis pseudoweberi E4
Escovopsis pseudoweberi E12
Escovopsis pseudoweberi E20
Escovopsis atlas E35ET
Escovopsis atlas E28
Escovopsis catenulata E19
Escovopsis catenulata E17
Escovopsis catenulata E18
Escovopsis catenulata E34ET
Escovopsis weberi E22
Escovopsis primorosea E29ET
Escovopsis primorosea E30
Escovopsis primorosea E42
Escovopsis primorosea E42-2
Escovopsis sp. LESF969
Escovopsis sp. LESF996
Escovopsis sp. LESF997
Escovopsis sp. LESF1003
Escovopsis lentecrescens CBS 135750ET
Escovopsis aspergillodes CBS 423.93ET
Escovopsis sp. LESF962
Escovopsis multiformis CBS 145327ET
Escovopsis multiformis LESF1136
Escovopsis multiformis LESF852
Escovopsis clavata CBS 145326ET
Escovopsis clavata LESF855
Escovopsis clavata LESF854
Sympodiorosea kreiselii CBS 139320ET
Sympodiorosea kreiselii LESF302
Sympodiorosea kreiselii LESF303
Sympodiorosea kreiselii LESF304
Sympodiorosea kreiselii LESF305
Sympodiorosea kreiselii LESF306
Sympodiorosea kreiselii LESF307
Sympodiorosea kreiselii LESF308
Smypodiorosea kreiselii LESF309
Sympodiorosea sp. LESF864
Sympodiorosea sp. LESF886
Sympodiorosea sp. LESF887
Luteomyces trichodermoides LESF1049
Luteomyces trichodermoides LESF1052
Luteomyces trichodermoides LESF1057
Luteomyces trichodermoides LESF1077
Luteomyces trichodermoides LESF1082
Luteomyces trichodermoides CBS137343ET
Luteomyces trichodermoides LESF310
Luteomyces trichodermoides LESF311
Luteomyces trichodermoides LESF312
Luteomyces trichodermoides LESF832
Luteomyces trichodermoides LESF833
Luteomyces trichodermoides LESF834
Luteomyces trichodermoides LESF835
Luteomyces trichodermoides LESF837
Luteomyces trichodermoides LESF838
Luteomyces trichodermoides LESF895
Luteomyces trichodermoides LESF897
Luteomyces trichodermoides LESF927
Luteomyces trichodermoides LESF1051
Luteomyces trichodermoides LESF1055
Luteomyces trichodermoides LESF1061
Luteomyces trichodermoides LESF1078
Luteomyces trichodermoides LESF1090
Luteomyces trichodermoides LESF1109
Escovopsioides nivea CBS135749ET
Escovopsioides nivea LESF510
Escovopsioides nivea LESF587
Escovopsioides nivea LESF592
Escovopsioides nivea LESF591
Escovopsioides nivea LESF598
Escovopsioides nivea LESF589
Escovopsioides nivea LESF597
Trichoderma harzianum CBS22695
Hypomyces protrusum TFC201316
Hypomycesa sterophorum CBS67677
Hypomyces samuelsii TFC200723
Hypomyces semicirculare CBS70588
Protocrea pallida TFC99209
Sphaerostilbella aureonitens GJS 7487
Lecanicillium antillanum CBS 350.85
0.03
Strains submited by Marfetán et al. (2019)

Fig. S2

Extended phylogenetic tree, based on Bayesian Inference of the *tef*1, indicating the placement of all strains previously named as *Escovopsis*. The tree gathers all available *tef*1 sequences found in the literature with the data set used in this study (including the sequences of the nine *Escovopsis* ex-type strains evaluated here). The tree contains a total of 415 sequences which include: 281vesiculate-*Escovopsis* (Clade A), 70 non-vesiculate *Escovopsis* (Clades B, C, D, E) and 63 strains from five Hypocreaceae genera (*Escovopsioides*, *Hypomyces*, *Protocrea*, *Sphaerostilbella*, *Trichoderma*). *Lecanicillium antillanum* CBS35085 is the outgroup of the tree. There is no information on literature about the morphological feature of the clades C and D.

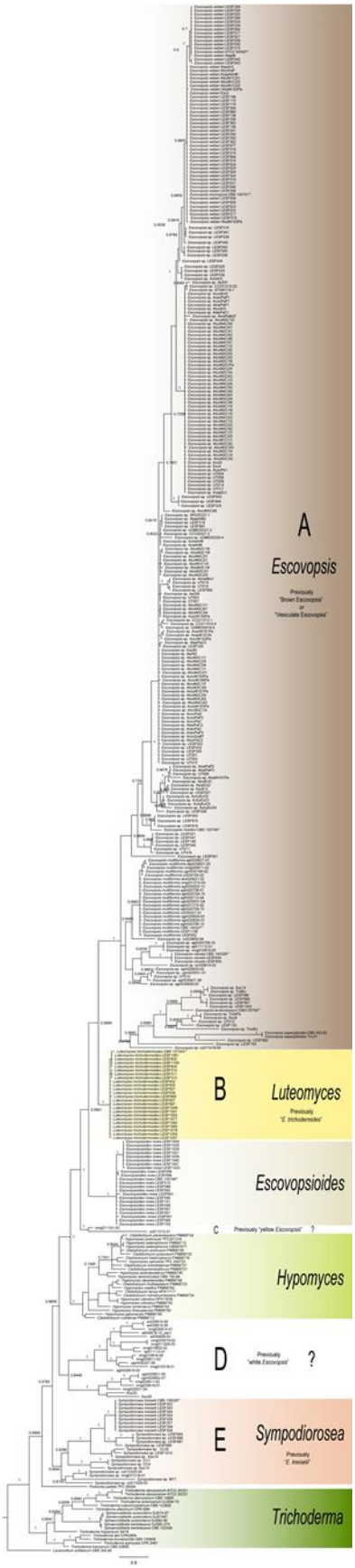
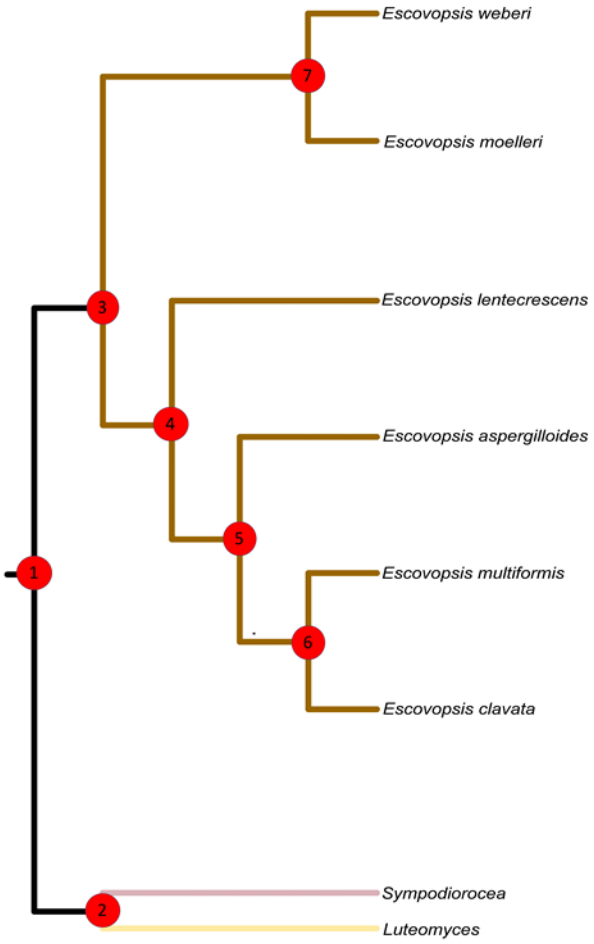

A
Escovopsis
Previously
"Brown Escovopsis"
or
"Vesiculate Escovopsis"
B
Luteomyces
Previously
"E. trichodermoides"
Escovopsioides
C
Previously "yellow Escovopsis"
?
Hypomyces
D
Previously
"white Escovopsis"
?
E
Sympodiorosea
Previously
"E. insolens"
Trichoderma

Fig. S3.

Cladogram and dichotomous key to identify *Escovopsis* species. A) Cladogram performed using a recursive partitioning algorithm in R 3.6.3 "rpart" library. The nodes numbered in red correspond to the steps (morphological features) needed to identify the species. B) dichotomous key reconstructed following the steps (nodes in red) of the cladogram.



1a. Pink or Yellow colonies with conidiophores without terminal vesicles……..2

1b Brown colonies and conidiophores with terminal vesicles where phialides are emerging……..3

2a Pink colonies and conidiophores with sympodial conidiogenous cells……*Sympodiorosea*

2b Yellow colonies and conidiophores with synchronous conidiogenous cells……*Luteomyces*

3a Pyramidal and irregular conidiophore with greater presence of clavate to cylindric vesicles……7

3b Only irregular conidiophores with greater presence of globose to spatulate vesicles……4

4a Yellowish-brown to brown colour on Colonies at seven days on PDA……*Escovopsis aspergilloides*

4b Light-brown to brown colour on Colonies at seven days on PDA……5

5a Conidiophore without swollen cell……*Escovopsis lentecrescens*

5b Conidiophore with swollen cell……6

6a Colonies growing at 10°C, and conidiophore axis ending only in vesicles……*Escovopsis multiformis*

6b Colonies do not grow at 10°C and some conidiophores axis ending in an infertile hypha……*Escovopsis clavata*

7a Colonies growing at 30 °C and ornamented conidia formed on phialides that emerge only on vesicles……*Escovopsis molleri*

7b Colonies do not grow at 30 °C and smooth conidia formed on phialides that can emerge on both in the vesicles and in the aerial mycelium……*Escovopsis weberi*

Table S1. Metadata used to know the phylogenetic placement of all strains previously named as *Escovopsis*.

Previous fungal species name	**Current fungal species name**	**Strain ID**	**Specimen voucher**	**City, State, Country**	**Habitat**	**GenBank accession numbers *tef*1**	**References**
Cladobotryum asterophorum	*Cladobotryum asterophorum*	CBS 676.77	---	---	---	FN868712	Poldmaa,K. (2011)
Cladobotryum cubitense	*Cladobotryum cubitense*	CBS 416.85	---	---	---	FN868713	Poldmaa,K. (2011)
Cladobotryum heterosporum	*Cladobotryum heterosporum*	CBS 719.88	---	---	---	FN868716	Poldmaa,K. (2011)
Cladobotryum multiseptatum	*Cladobotryum multiseptatum*	CBS 472.71	---	---	---	FN868723	Poldmaa,K. (2011)
Cladobotryum paravirescens	*Cladobotryum paravirescens*	TFC 97-23	---	---	---	FN868724	Poldmaa,K. (2011)
Cladobotryum penicillatum	*Cladobotryum penicillatum*	CBS 407.80	---	---	---	FN868725	Poldmaa,K. (2011)
Cladobotryum protrusum	*Cladobotryum protrusum*	CBS 118999	---	---	---	FN868726	Poldmaa,K. (2011)
Cladobotryum purpureum	*Cladobotryum purpureum*	CBS 154.78	---	---	---	FN868733	Poldmaa,K. (2011)
Cladobotryum rubrobrunnescens	*Cladobotryum rubrobrunnescens*	CBS 176.92	---	---	---	FN868734	Poldmaa,K. (2011)
Cladobotryum sp. KP-2010b	*Cladobotryum* sp. KP-2010b	TFC 201295	---	---	---	FN868721	Poldmaa,K. (2011)
Cladobotryum tchimbelense	*Cladobotryum tchimbelense*	TFC 201146	---	---	---	FN868737	Poldmaa,K. (2011)
Cladobotryum tenue	*Cladobotryum tenue*	G.A. 05/54.K	TFC:05-91	Germany	*Russula* sp.	HF911717	Poldmaa,K. (2011)
Escovopsioides nivea	*Escovopsioides nivea*	CBS 135749^{ET}	AUJ6	Viçosa, Minas Gerais, Brazil	Fungus garden of *Acromyrmex subterraneus subterraneus*	JQ855713	Augustin et al. (2013)
Escovopsioides sp.	*Escovopsioides nivea*	LESF159	J08	Corumbataí, São Paulo, Brazil	Fungus garden of *Atta sexdens rubropilosa*	MF140949	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF601	J09	Rio Claro - SP, Brazil	Fungus garden of *Trachymyrmex* sp.	MF140964	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 151	J02	Corumbataí, São Paulo, Brazil	Fungus garden of *Atta sexdens rubropilosa*	MF140948	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 510	J10	Botucatu, São Paulo, Brazil	Fungus garden of *Atta sexdens rubropilosa*	MF140950	Osti and Rodrigues (2018)

Escovopsioides sp.	*Escovopsioides nivea*	LESF 587	J01	Camacan, Bahia, Brazil	Fungus garden of *Atta cephalotes*	MF140951	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 588	J03	Camacan, Bahia, Brazil	Fungus garden of *Atta cephalotes*	MF140952	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 589	J05	Camacan, Bahia, Brazil	Fungus garden of *Atta cephalotes*	MF140953	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 590	J06	Camacan, Bahia, Brazil	Fungus garden of *Atta cephalotes*	MF140954	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 591	J04	Botucatu, São Paulo, Brazil	Fungus garden of *Atta capiguara*	MF140955	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 592	J12	Camacan, Bahia, Brazil	Fungus garden of *Acromyrmex* sp.	MF140956	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 596	J16	Chuvisca - RS, Brazil	Fungus garden of *Acromyrmex* sp.	MF140960	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 597	J17	Camacan, Bahia, Brazil	Fungus garden of *Atta cephalotes*	MF140961	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 598	J18	Camacan, Bahia, Brazil	Fungus garden of *Atta cephalotes*	MF140962	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 599	J19	Sentinela do Sul - RS, Brazil	Fungus garden of *Acromyrmex heyeri*	MF140963	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 1009	QVM137	Camp 41, Manaus, Amazonas, Brazil	Fungus garden of *Apterostigma* sp.	MT305392	This study
Escovopsioides sp.	*Escovopsioides nivea*	LESF 1023	QVM151	Camp 41, Manaus, Amazonas, Brazil	Fungus garden of *Trachymyrmex* sp.	MT305394	This study
Escovopsioides sp.	*Escovopsioides nivea*	LESF 1025	QVM153	Camp 41, Manaus, Amazonas, Brazil	Fungus garden of *Trachymyrmex* sp.	MT305395	This study
Escovopsioides sp.	*Escovopsioides nivea*	LESF 1028	QVM156	Camp 41, Manaus, Amazonas, Brazil	Fungus garden of *Trachymyrmex* sp.	MT305396	This study
Escovopsioides sp.	*Escovopsioides nivea*	LESF 1031	QVM159	Camp 41, Manaus, Amazonas, Brazil	Fungus garden of *Trachymyrmex* sp.	MT305397	This study
Escovopsioides sp.	*Escovopsioides nivea*	LESF 1039	QVM167	Camp 41, Manaus, Amazonas, Brazil	Fungus garden of *Cyphomyrmex* sp.	MT305398	This study
Escovopsioides sp.	*Escovopsioides nivea*	LESF 1040	QVM168	Camp 41, Manaus, Amazonas, Brazil	Fungus garden of *Apterostigma* sp.	MT305399	This study
Escovopsioides sp.	*Escovopsioides nivea*	LESF 1041	QVM169	Camp 41, Manaus, Amazonas, Brazil	Fungus garden of *Apterostigma* sp.	MT305400	This study
Escovopsis aspergilloides	*Escovopsis aspergilloides*	CBS 423.93 ET	DAOM:216382	Trinidad and Tobago: Trinidad	Fungus garden of *Trachymyrmex ruthae*	AY172632	Augustin et al. (2013), Currie et al. (2003)
Escovopsis clavata	*Escovopsis clavata*	LESF 854	1704A	Florianópolis, Santa Catarina, Brazil	Fungus garden of *Apterostigma* sp.	MH724271	Montoya et al. (2019)
Escovopsis clavata	*Escovopsis clavata*	LESF 855	1705B	Florianópolis, Santa Catarina, Brazil	Fungus garden of *Apterostigma* sp.	MH724272	Montoya et al. (2019)

Escovopsis clavata	*Escovopsis clavata*	CBS 145326 ET	1707	Florianópolis, Santa Catarina, Brazil	Fungus garden of *Apterostigma* sp.	MH724270	Montoya et al. (2019)
Escovopsis lentecrescens	*Escovopsis lentecrescens*	CBS 135750 ET	VIC:31755	Viçosa, Minas Gerais, Brazil	Fungus garden of *Acromyrmex subterraneus subterraneus*	JQ855714	Augustin et al. (2013)
Escovopsis moelleri	*Escovopsis moelleri*	CBS 135748 ET	VIC:31753	Viçosa, Minas Gerais, Brazil	Fungus garden of *Acromyrmex subterraneus molestans*	JQ855712	Augustin et al. (2013)
Escovopsis sp. agh030627-01 esc1	*Escovopsis multiformis*	agh030627-01 esc1	---	Ecuador	Fungus garden of *Apterostigma* cf. *dentigerum*	DQ848196	Gerardo et al. (2006)
Escovopsis sp.	*Escovopsis multiformis*	LESF 852	1706B	Florianópolis, Santa Catarina, Brazil	Fungus garden of *Apterostigma* sp.	MT305372	This study
Escovopsis sp.	*Escovopsis multiformis*	LESF 1136	QVM277	Alta Floresta, Mato Grosso, Brazil	Fungus garden of *Apterostigma* sp.	MH724266	Montoya et al. (2019)
Escovopsis multiformis	*Escovopsis multiformis*	CBS 145327 ET	1606w	Florianópolis, Santa Catarina, Brazil	Fungus garden of *Apterostigma* sp.	MH724265	Montoya et al. (2019)
Escovopsis sp. agh030627-03 esc2	*Escovopsis multiformis*	agh030627-03 esc2	---	Ecuador	Fungus garden of *Apterostigma* sp.	DQ848197	Gerardo et al. (2006)
Escovopsis sp. nmg020611-02 esc2	*Escovopsis multiformis*	nmg020611-02 esc2	---	Panama	Fungus garden of *Apterostigma dentigerum*	DQ848164	Gerardo et al. (2006)
Escovopsis sp. ugm030106-02 escc	*Escovopsis multiformis*	ugm030106-02 escc	---	Panama	Fungus garden of *Apterostigma* sp.	DQ848188	Gerardo et al. (2006)
Escovopsis sp. cc030106-02 escb	*Escovopsis multiformis*	cc030106-02 escb	---	Panama	Fungus garden of *Apterostigma* sp.	DQ848207	Gerardo et al. (2006)
Escovopsis sp. abs020621-02 esc1	*Escovopsis multiformis*	abs020621-02 esc1	---	Costa Rica	Fungus garden of *Apterostigma dentigerum*	DQ848166	Gerardo et al. (2006)
Escovopsis sp. nmg031215-04	*Escovopsis multiformis*	nmg031215-04	---	Panama	Fungus garden of *Apterostigma* sp.	DQ848204	Gerardo et al. (2006)
Escovopsis sp. agh030222-12	*Escovopsis multiformis*	agh030222-12	---	Costa Rica	Fungus garden of *Apterostigma dentigerum*	DQ848187	Gerardo et al. (2006)
Escovopsis sp. agh020709-10 esc1	*Escovopsis multiformis*	agh020709-10 esc1	---	Costa Rica	Fungus garden of *Apterostigma dentigerum*	DQ848169	Gerardo et al. (2006)

Escovopsis sp. agh020706-01	*Escovopsis multiformis*	agh020706-01	---	Costa Rica	Fungus garden of *Apterostigma dentigerum*	DQ848168	Gerardo et al. (2006)
Escovopsis sp. agh020712-04 esc1	*Escovopsis multiformis*	agh020712-04 esc1	---	Costa Rica	Fungus garden of *Apterostigma dentigerum*	DQ848173	Gerardo et al. (2006)
Escovopsis sp. agh020629-02 esc6	*Escovopsis multiformis*	agh020629-02 esc6	---	Costa Rica	Fungus garden of *Apterostigma dentigerum*	DQ848167	Gerardo et al. (2006)
Escovopsis sp. ugm020531-04 esc1	*Escovopsis multiformis*	ugm020531-04 esc1	---	Panama	Fungus garden of *Apterostigma* sp.	DQ848192	Gerardo et al. (2006)
Escovopsis sp. agh031215-02	*Escovopsis multiformis*	agh031215-02	---	Panama	Fungus garden of *Apterostigma* sp.	DQ848203	Gerardo et al. (2006)
Escovopsis sp. agh020709-10 esc3	*Escovopsis multiformis*	agh020709-10 esc3	---	Costa Rica	Fungus garden of *Apterostigma dentigerum*	DQ848171	Gerardo et al. (2006)
Escovopsis sp. agh020709-10 esc8	*Escovopsis multiformis*	agh020709-10 esc8	---	Costa Rica	Fungus garden of *Apterostigma dentigerum*	DQ848172	Gerardo et al. (2006)
Escovopsis sp. cc030327-01 esc4	*Escovopsis multiformis*	cc030327-01 esc4	---	Argentina	Fungus garden of *Apterostigma* sp.	DQ848201	Gerardo et al. (2006)
Escovopsis sp. agh020630-01 esc1	*Escovopsis multiformis*	agh020630-01 esc1	---	Costa Rica	Fungus garden of *Apterostigma dentigerum*	DQ848176	Gerardo et al. (2006)
Escovopsis sp.	*Escovopsis* sp.	LESF 052	SES010	Camp 41, Manaus, Amazonas, Brazil	Fungus garden of *Trachymyrmex diversus*	KM817154	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF 325	BA004	Camacan, Bahia, Brazil	Fungus garden of *Atta cephalotes*	KM817119	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF 962	QVM49	Anavilhanas, Manaus, Amazonas, Brazil	Fungus garden of *Acromyrmex* sp.	MT305384	This study
Escovopsis sp.	*Escovopsis* sp.	LESF 969	QVM56	Anavilhanas, Manaus, Amazonas, Brazil	Fungus garden of *Apterostigma* sp.	MT305385	This study
Escovopsis sp.	*Escovopsis* sp.	LESF 975	QVM62	Anavilhanas, Manaus, Amazonas, Brazil	Fungus garden of *Trachymyrmex* sp.	MT305386	This study
Escovopsis sp.	*Escovopsis* sp.	LESF 979	QVM66	Anavilhanas, Manaus, Amazonas, Brazil	Fungus garden of *Trachymyrmex* sp	MT305387	This study
Escovopsis sp.	*Escovopsis* sp.	LESF 996	QVM83	Anavilhanas, Manaus, Amazonas, Brazil	Fungus garden of *Apterostigma* sp.	MT305389	This study

Escovopsis sp.	*Escovopsis* sp.	LESF 997	QVM84	Anavilhanas, Manaus, Amazonas, Brazil	Fungus garden of *Trachymyrmex* sp	MT305390	This study
Escovopsis sp.	*Escovopsis* sp.	LESF 1003	QVM90	Anavilhanas, Manaus, Amazonas, Brazil	Fungus garden of *Trachymyrmex* sp.	MT305391	This study
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AccoEcO	AGH030518-14 Acro Esc	Ecuador	Fungus garden of *Acromyrmex coronatus*	EF589915	Taerum et al. (2007)
Escovopsis weberi	*Escovopsis* sp.	AtcoN3C1I2	---	Panama	Fungus garden of *Atta colombica*	GQ240733	Taerum et al. (2010)
Escovopsis sp.	*Escovopsis* sp.	Acol2	---	Panama	---	AY172622	Currie et al. (2003)
Escovopsis sp.	*Escovopsis* sp.	Esc4	---	Panama	Fungus garden of *Acromyrmex octospinosus*	AY172617	Currie et al. (2003)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AtsePaC1	CC020529-01	Panama	Fungus garden of *Atta sexdens*	EF589919	Taerum et al. (2007)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AcecPaP1	CC020610-02 Acro Esc 5	Panama	Fungus garden of *Acromyrmex echinatior*	EF589920	Taerum et al. (2007)
Escovopsis weberi	*Escovopsis* sp.	AtcoN4C3I5	---	Panama	Fungus garden of *Atta colombica*	GQ240765	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN4C4I1	---	Panama	Fungus garden of *Atta colombica*	GQ240767	Taerum et al. (2010)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AcocPaP1	Esc 4 #78	Panama	Fungus garden of *Acromyrmex octospinosus*	EF589918	Taerum et al. (2007)
Escovopsis weberi	*Escovopsis* sp.	AtcoN3C1I4	---	Panama	Fungus garden of *Atta colombica*	GQ240735	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN2C3I1	---	Panama	Fungus garden of *Atta colombica*	GQ240728	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN4C4I2	---	Panama	Fungus garden of *Atta colombica*	GQ240768	Taerum et al. (2010)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AtcePaP1	CC031208-10 A ceph	Panama	Fungus garden of *Atta cephalotes*	EF589917	Taerum et al. (2007)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AcspEc1	AGH030518-14 Acro Esc	Ecuador	Fungus garden of *Acromyrmex coronatus*	EF589948	Taerum et al. (2007)
Escovopsis weberi	*Escovopsis* sp.	AtcoN4C3I6	---	Panama	Fungus garden of *Atta colombica*	GQ240766	Taerum et al. (2010)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AtcePaBdT	ST041021-01 CI	Panama	Fungus garden of *Atta cephalotes*	EF589914	Taerum et al. (2007)

Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AcecPa1	CC020610-02 Acro Esc 5	Panama	Fungus garden of *Acromyrmex echinatior*	EF589949	Taerum et al. (2007)
Escovopsis weberi	*Escovopsis* sp.	AtcoN4C3I1	---	Panama	Fungus garden of *Atta colombica*	GQ240761	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN4C1I2	---	Panama	Fungus garden of *Atta colombica*	GQ240753	Taerum et al. (2010)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AtvoArC	SES030404-03 Atta col Esc E	Argentina	Fungus garden of Atta vollenweirderi	EF589921	Taerum et al. (2007)
Escovopsis weberi	*Escovopsis* sp.	AtcoN2C3I2	---	Panama	Fungus garden of *Atta colombica*	GQ240729	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN3C2I2	---	Panama	Fungus garden of *Atta colombica*	GQ240739	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN3C1I3	---	Panama	Fungus garden of *Atta colombica*	GQ240734	Taerum et al. (2010)
Escovopsis cf. *weberi* Ae291	*Escovopsis* sp.	Ae291	---	---	Fungus garden of *Acromyrmex echinatior*	EU283878	---
Escovopsis cf. *weberi* CC031210-221	*Escovopsis* sp.	CC031210-22	---	---	Nets of *Acromyrmex octospinosus* CC031210-22	EU283880	---
Escovopsis cf. *weberi* ST040116-01	*Escovopsis* sp.	ST040116-1	---	---	Nets of *Acromyrmex octospinosus* ST040116-01	EU283870	---
Escovopsis weberi	*Escovopsis* sp.	AtcoN4C4I3	---	Panama	Fungus garden of *Atta colombica*	GQ240769	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN2C2I3	---	Panama	Fungus garden of *Atta colombica*	GQ240724	
Escovopsis weberi	*Escovopsis* sp.	AtcoN3C1I6	---	Panama	Fungus garden of *Atta colombica*	GQ240737	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtceN2S1Pa	---	Panama	Fungus garden of Atta cephalotes	GQ240705	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN2C2I5	---	Panama	Fungus garden of *Atta colombica*	GQ240726	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN2C3I4	---	Panama	Fungus garden of *Atta colombica*	GQ240731	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN3C4I3	---	Panama	Fungus garden of *Atta colombica*	GQ240751	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN2C1I3	---	Panama	Fungus garden of *Atta colombica*	GQ240715	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN4C2I4	---	Panama	Fungus garden of *Atta colombica*	GQ240759	Taerum et al. (2010)

Escovopsis weberi	*Escovopsis* sp.	AtcoN4C3I3	---	Panama	Fungus garden of *Atta colombica*	GQ240763	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN3C2I4	---	Panama	Fungus garden of *Atta colombica*	GQ240741	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN3C3I6	---	Panama	Fungus garden of *Atta colombica*	GQ240748	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN4C1I4	---	Panama	Fungus garden of *Atta colombica*	GQ240755	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN2C1I6	---	Panama	Fungus garden of *Atta colombica*	GQ240718	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN3C3I2	---	Panama	Fungus garden of *Atta colombica*	GQ240744	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN3C3I3	---	Panama	Fungus garden of *Atta colombica*	GQ240745	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN2C1I5	---	Panama	Fungus garden of *Atta colombica*	GQ240717	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN4C4I4	---	Panama	Fungus garden of *Atta colombica*	GQ240770	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN4C2I3	---	Panama	Fungus garden of *Atta colombica*	GQ240758	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN4C1I3	---	Panama	Fungus garden of *Atta colombica*	GQ240754	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN3C2I3	---	Panama	Fungus garden of *Atta colombica*	GQ240740	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN4C3I2	---	Panama	Fungus garden of *Atta colombica*	GQ240762	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN2C1I2	---	Panama	Fungus garden of *Atta colombica*	GQ240714	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN2C3I3	---	Panama	Fungus garden of *Atta colombica*	GQ240730	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN1C1I1	---	Panama	Fungus garden of *Atta colombica*	GQ240707	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN2C2I2	---	Panama	Fungus garden of *Atta colombica*	GQ240723	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtceN2S2Pa	---	Panama	Fungus garden of *Atta colombica*	GQ240706	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AcspN1S2Ar	---	Panama	Fungus garden of *Acromyrmex* sp.	GQ240700	Taerum et al. (2010)
Escovopsis sp.	*Escovopsis* sp.	LESF040	RS020	Brazil, Nova Petropolis	Fungus garden of *Acromyrmex laticeps* AOMB060904-05	EU082803	Meirelles et al. (2015b)
	Escovopsis sp.	LESF041	RS030	Brazil	Fungus garden of *Acromyrmex lundi*	EU082795	Meirelles et al. (2015b)

Escovopsis weberi	*Escovopsis* sp.	RS004	---	Brazil	Fungus garden of *Acromyrmex coronatus*	EU082798	Meirelles et al. (2015b)
Escovopsis weberi	*Escovopsis* sp.	RS087	---	Brazil	Fungus garden of *Acromyrmex disciger*	EU082800	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF045	RS076	Brazil	Fungus garden of *Acromyrmex coronatus*	EU082801	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF039	RS019	Brazil	Fungus garden of *Acromyrmex ambiguus*	EU082802	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF044	RS061	Brazil	Fungus garden of *Acromyrmex heyeri*	EU082799	Meirelles et al. (2015b)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AchiArC	UGM030406-02 Acro hisp Esc B	Argentina	Fungus garden of *Acromyrmex hispidus*	EF589913	Taerum et al. (2007)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AcocPaD	CC030106-15 Acro Darien Esc G fast	Panama	Fungus garden of *Acromyrmex octospinosus*	EF589942	Taerum et al. (2007)
Escovopsis sp.	*Escovopsis* sp.	Esc90	---	Panama	Fungus garden of *Acromyrmex echinatior*	AY172616	Currie et al. (2003)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AcocPaP2	CC020602-02 Acro Esc Y	Panama	Fungus garden of *Acromyrmex octospinosus*	EF589937	Taerum et al. (2007)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AtcoPaC2	CC020529 A col fast	Panama	Fungus garden of *Atta colombica*	EF589936	Taerum et al. (2007)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AcocPaC	ST041019-03 I	Panama	Fungus garden of *Acromyrmex octospinosus*	EF589933	Taerum et al. (2007)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AcocGuBT	ASM031226-05 Acro octospinosus	Panama	Fungus garden of *Acromyrmex octospinosus*	EF589935	Taerum et al. (2007)
Escovopsis cf. *weberi* Ae292	*Escovopsis* sp.	Ae292	---	---	Fungus garden of *Acromyrmex echinatior*	EU283879	---
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AtsePaC3	ST041014-01	Panama	Fungus garden of *Atta sexdens*	EF589939	Taerum et al. (2007)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AcecPaC	ST041019-01 III	Panama	Fungus garden of *Acromyrmex echinatior*	EF589932	Taerum et al. (2007)

Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AcecPaP2	CC020610-02 Acro Esc 2	Panama	Fungus garden of *Acromyrmex echinatior*	EF589943	Taerum et al. (2007)
Escovopsis cf. *weberi* CC031212-1	*Escovopsis* sp.	CC031212-1	---	---	Fungus garden of *Acromyrmex echinatior*	EU283875	---
Escovopsis cf. *weberi* UGM020518-5	*Escovopsis* sp.	UGM020518-5	---	---	Fungus garden of *Acromyrmex octospinosus*	EU283876	---
Escovopsis cf. *weberi CC011010-4*	*Escovopsis* sp.	CC011010-4	---	---	Fungus garden of *Acromyrmex octospinosus* CC011010-04	EU283871	---
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AtmeMxV	SES030113-01 Esc 1 Atta Mexico	Mexico	Fungus garden of *Atta mexicana*	EF589938	Taerum et al. (2007)
Escovopsis cf. *weberi* Ae295	*Escovopsis* sp.	Ae295	---	---	Fungus garden of *Acromyrmex echinatior*	EU283869	---
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AtsePaC2	ST041013-01 I	Panama	Fungus garden of Atta sexdens	EF589931	Taerum et al. (2007)
Escovopsis cf. *weberi UGM030330-4*	*Escovopsis* sp.	UGM030330-4	---	---	Fungus garden of *Acromyrmex laticeps*	EU283873	---
Escovopsis cf. *weberi* UGM030327-2	*Escovopsis* sp.	UGM030327-2	---	---	Fungus garden of *Acromyrmex hispidus fallax* UGM030327-02	EU283877	---
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AcheArM	SP030401-01 Acro Esc B	Argentina	Fungus garden of *Acromyrmex heyeri*	EF589940	Taerum et al. (2007)
Escovopsis cf. *weberi* CC030327-2	*Escovopsis* sp.	CC030327-2	---	---	*Acromyrmex niger* CC030327-02	EU283872	---
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AclaArM	UGM030330-05 Acro Esc B	Argentina	Fungus garden of *Acromyrmex laticeps*	EF589934	Taerum et al. (2007)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AtseArM2	CC030328-05 Atta Esc A	Argentina	Fungus garden of *Atta sexdens*	EF589941	Taerum et al. (2007)
Escovopsis cf. *weberi* SP030327-1	*Escovopsis* sp.	SP030327-1	---	---	Fungus garden of *Acromyrmex hispidus fallax* SP030327-01	EU283874	---
Escovopsis weberi	*Escovopsis* sp.	AtcoN2C1I1	---	Panama	Fungus garden of *Atta colombica*	GQ240713	Taerum et al. (2010)

Escovopsis weberi	*Escovopsis* sp.	AtcoN3C4I1	---	Panama	Fungus garden of *Atta colombica*	GQ240749	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AcocN1S5Pa	---	Panama	Fungus garden of *Acromyrmex octospinosus*	GQ240697	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN3C3I4	---	Panama	Fungus garden of *Atta colombica*	GQ240746	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AcocN1S4Pa	---	Panama	Fungus garden of *Acromyrmex octospinosus*	GQ240696	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN2C1I7	---	Panama	Fungus garden of *Atta colombica*	GQ240719	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AcocN1S3Pa	---	Panama	Fungus garden of *Acromyrmex octospinosus*	GQ240695	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN2C2I1	---	Panama	Fungus garden of *Atta colombica*	GQ240722	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN3C1I1	---	Panama	Fungus garden of *Atta colombica*	GQ240732	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN4C2I5	---	Panama	Fungus garden of *Atta colombica*	GQ240760	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN4C3I4	---	Panama	Fungus garden of *Atta colombica*	GQ240764	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN4C1I1	---	Panama	Fungus garden of *Atta colombica*	GQ240752	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN4C2I2	---	Panama	Fungus garden of *Atta colombica*	GQ240757	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AcocN1S6Pa	---	Panama	Fungus garden of *Atta colombica*	GQ240698	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN3C3I5	---	Panama	Fungus garden of *Atta colombica*	GQ240747	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AcocN1S1Pa	---	Panama	Fungus garden of *Atta octospinosus*	GQ240693	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN2C2I4	---	Panama	Fungus garden of *Atta colombica*	GQ240725	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN3C4I2	---	Panama	Fungus garden of *Atta colombica*	GQ240750	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN3C1I5	---	Panama	Fungus garden of *Atta colombica*	GQ240736	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN2C1I8	---	Panama	Fungus garden of *Atta colombica*	GQ240720	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN2C1I9	---	Panama	Fungus garden of *Atta colombica*	GQ240721	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN3C2I5	---	Panama	Fungus garden of *Atta colombica*	GQ240742	Taerum et al. (2010)

Escovopsis weberi	*Escovopsis* sp.	AtcoN4C2I1	---	Panama	Fungus garden of *Atta colombica*	GQ240756	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN1C1I3	---	Panama	Fungus garden of *Atta colombica*	GQ240709	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN3C3I1	---	Panama	Fungus garden of *Atta colombica*	GQ240743	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN2C1I4	---	Panama	Fungus garden of *Atta colombica*	GQ240716	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN3C2I	---	Panama	Fungus garden of *Atta colombica*	GQ240738	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AcspN1S3Ar	---	Panama	Fungus garden of *Acromyrmex* sp.	GQ240701	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AcecN1S1Pa	---	Panama	Fungus garden of *Acromyrmex echinatior*	GQ240691	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AcspN1S1Ar	---	Argentina	Fungus garden of *Acromyrmex* sp.	GQ240699	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AcocN1S2Pa	---	Panama	Fungus garden of *Acromyrmex octospinosus*	GQ240694	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis* sp.	AtcoN2C2I6	---	Panama	Fungus garden of *Atta colombica*	GQ240727	Taerum et al. (2010)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AtcePaP2	AL031210-29 A ceph	Panama	Fungus garden of *Atta cephalotes*	EF589923	Taerum et al. (2007)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AtcePaP3	AL031210-29 A ceph	Panama	Fungus garden of *Atta cephalotes*	EF589929	Taerum et al. (2007)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AtcoEcO	NMG030611-01 Atta Esc	Ecuador	Fungus garden of *Atta colombica*	EF589926	Taerum et al. (2007)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AtceEcO	---	Ecuador	Fungus garden of *Atta cephalotes*	EF589925	Taerum et al. (2007)
Escovopsis sp. Esc612	*Escovopsis* sp.	Esc612	---	Ecuador	Fungus garden of *Atta* cf. *cephalotes*	AY172621	Taerum et al. (2007)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AchyEcO2	NMG030616-01 Atta Esc	Ecuador	Fungus garden of *Acromyrmex hystrix*	EF589927	Taerum et al. (2007)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AchyEcO3	CC030615-12 Acro Esc	Ecuador	Fungus garden of *Acromyrmex hystrix*	EF589928	Taerum et al. (2007)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AchyEcO1	AL030615-05 Acro Esc	Ecuador	Fungus garden of *Acromyrmex hystrix*	EF589924	Taerum et al. (2007)

Escovopsis cf. *weberi* CRC-2007a	*Escovopsis* sp.	AchyEcO4	AL030515-06 Acro Esc	Ecuador	Fungus garden of *Acromyrmex hystrix*	EF589930	Taerum et al. (2007)
Escovopsis weberi	*Escovopsis* sp.	AtceN1S1Pa	---	Panama	Fungus garden of *Atta cephalote*	GQ240702	Taerum et al. (2010)
Escovopsis sp. cc020605-04 esc4	*Escovopsis* sp.	cc020605-04 esc4	---	Panama	Fungus garden of *Apterostigma* sp.	DQ848163	Gerardo et al. (2006)
Escovopsis sp. agh020629-02 esc4	*Escovopsis* sp.	agh020629-02 esc4	---	Costa Rica	Fungus garden of *Apterostigma dentigerum*	DQ848161	Gerardo et al. (2006)
Escovopsis sp. ugm020531-01 esc2	*Escovopsis* sp.	ugm020531-01 esc2	---	Panama	Fungus garden of *Apterostigma dentigerum*	DQ848183	Gerardo et al. (2006)
Escovopsis sp. agh030627-08 esc1	*Escovopsis* sp.	agh030627-08 esc1	---	Ecuador	Fungus garden of *Apterostigma* sp.	DQ848182	Gerardo et al. (2006)
Escovopsis sp. agh030609-03 esc1	*Escovopsis* sp.	agh030609-03 esc1	---	Ecuador	Fungus garden of *Apterostigma* sp.	DQ848194	Gerardo et al. (2006)
Escovopsis sp. agh020709-10 esc11	*Escovopsis* sp.	agh020709-10 esc11	---	Costa Rica	Fungus garden of *Apterostigma dentigerum*	DQ848170	Gerardo et al. (2006)
Escovopsis sp. sp011112-01 esc11	*Escovopsis* sp.	sp011112-01 esc11	---	Panama	Fungus garden of *Apterostigma dentigerum*	DQ848180	Gerardo et al. (2006)
Escovopsis sp. nmg010816-05 esc1	*Escovopsis* sp.	nmg010816-05 esc1	---	Panama	Fungus garden of *Apterostigma dentigerum*	DQ848157	Gerardo et al. (2006)
Escovopsis sp. cc011018-04 esc1	*Escovopsis* sp.	cc011018-04 esc1	---	Panama	Fungus garden of *Apterostigma* sp.	DQ848177	Gerardo et al. (2006)
Escovopsis sp. sv030614-02 esc1	*Escovopsis* sp.	sv030614-02 esc1	---	Ecuador	Fungus garden of *Apterostigma* cf. *pilosum*	DQ848186	Gerardo et al. (2006)
Escovopsis cf. weberi CRC-2007a	*Escovopsis* sp.	TrZePa	Esc 8 97T27	Panama	Fungus garden of *Trachymyrmex zeteki*	EF589947	Taerum et al. (2007)
Escovopsis sp. Esc8	*Escovopsis* sp.	Esc8	---	Panama	Fungus garden of *Trachymyrmex* cf. *zeteki*	AY172630	Currie et al.(2003)
Escovopsis cf. weberi CRC-2007a	*Escovopsis* sp.	TrcoEc	AGH030609-07 Trachy Esc	Ecuador	Fungus garden of *Trachymyrmex cornetzi*	EF589944	Taerum et al. (2007)
Escovopsis cf. weberi CRC-2007a	*Escovopsis* sp.	TrdiEc	Esc 14 980218-06	Ecuador	Fungus garden of *Trachymyrmex diversus*	EF589945	Taerum et al. (2007)

Escovopsis sp. Esc14	*Escovopsis* sp.	Esc14	---	Ecuador	Fungus garden of *Trachymyrmex diversus*	AY172631	Currie et al.(2003)
Escovopsis cf. weberi CRC-2007a	*Escovopsis* sp.	TrruTr	Esc 16 E. asperg.	Trinidad and Tobago	Fungus garden of *Trachymyrmex ruthae*	EF589946	Taerum et al. (2007)
Escovopsis sp.	*Escovopsis* sp.	LESF021	ES002	Rio Claro - SP, Brazil	Fungus garden of *Atta sexdens*	KM817123	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF028	ES011	Corumbatai - SP, Brazil	Fungus garden of *Atta sexdens*	KM817132	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF022	ES003	Frei Caneca - PE, Brazil	Fungus garden of *Atta cephalotes*	KM817124	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF026	ES009	Carreiro da Varzea - AM, Brazil	Fungus garden of *Atta cephalotes*	KM817130	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF037	ES033	Paraupebas – PA, Brazil	Fungus garden of *Atta cephalotes*	KM817141	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF843	BA003	Camacan - BA, Brazil	Fungus garden of *Atta cephalotes*	KM817118	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF325	BA004	Camacan - BA, Brazil	Fungus garden of *Atta cephalotes*	KM817119	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF844	BA005	Camacan - BA, Brazil	Fungus garden of *Atta cephalotes*	KM817120	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF326	BA006	Camacan - BA, Brazil	Fungus garden of *Atta cephalotes*	KM817121	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF032	ES008	Santarem - PA, Brazil	Fungus garden of *Acromyrmex sp.*	KM817129	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF035	ES025	Botucatu - SP, Brazil	Fungus garden of *Acromyrmex balzani*	KM817136	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF036	ES027	Rio Claro - SP, Brazil	Fungus garden of *Acromyrmex rugosus rugosus*	KM817138	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF050	SES008	Fazenda Sao Sebastiao - RO, Brazil	Fungus garden of *Acromyrmex* sp.	KM817152	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF055	AR022	Camacan - BA, Brazil	Fungus garden of *Acromyrmex* sp.	KM817114	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF316	ES001	Rio Claro - SP, Brazil	Fungus garden of *Trachymyrmex sp.*	KM817122	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF318	ES029	Palmas – TO, Brazil	Fungus garden of *Trachymyrmex* sp.	KM817139	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF047	SES002	Fazenda Pau Brasil – GO, Brazil	Fungus garden of *Trachymyrmex* sp.	KM817147	Meirelles et al. (2015b)

Escovopsis sp.	*Escovopsis* sp.	LESF135	SES003	Uberlandia – MG, Brazil	Fungus garden of *Trachymyrmex* sp.	KM817148	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF048	SES005	Uberlandia – MG, Brazil	Fungus garden of *Trachymyrmex* sp.	KF240731	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF051	SES009	Palmeiras – BA, Brazil	Fungus garden of *Trachymyrmex* sp.	KM817153	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF052	SES010	Manaus – AM, Brazil	Fungus garden of *Trachymyrmex diversus*	KM817154	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF105	SES004	Uberlandia – MG, Brazil	Fungus garden of *Sericomyrmex luederwaldti*	KM817149	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	UT001	---	Guadeloupe	Fungus garden of *Acromyrmex octospinosus*	KM817155	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	UT002	---	Guadeloupe	Fungus garden of *Acromyrmex* sp.	KM817156	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	UT003	---	Panama	Fungus garden of *Acromyrmex* sp.	KM817157	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	UT004	---	Panama	Fungus garden of *Acromyrmex colombica*	KM817158	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	UT005	---	Argentina	Fungus garden of *Acromyrmex* sp.	KM817159	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	UT006	---	Panama	Fungus garden of *Atta cephalotes*	KM817160	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	UT007	---	Panama	Fungus garden of *Acromyrmex colombica*	KM817161	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	UT008	---	Panama	Fungus garden of *Acromyrmex colombica*	KM817162	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	UT009	---	Panama	Fungus garden of *Acromyrmex colombica*	KM817163	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	UT010	---	Panama	Fungus garden of *Atta sexdens*	KM817164	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	UT011	---	Panama	Fungus garden of *Trachymyrmex* sp.	KM817165	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	UT012	---	Panama	Fungus garden of *Trachymyrmex* sp.	KM817166	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	UT014	---	Panama	Fungus garden of *Acromyrmex colombica*	KM817167	Meirelles et al. (2015b)

Escovopsis sp.	*Escovopsis* sp.	UT015	---	Panama	Fungus garden of *Acromyrmex colombica*	KM817168	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	UT016	---	Mexico	Fungus garden of *Trachymyrmex* sp.	KM817169	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	UT017	---	Panama	Fungus garden of *Acromyrmex colombica*	KM817170	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	UT018	---	Panama	Fungus garden of *Trachymyrmex* sp.	KM817171	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	UT019	---	Mexico	Fungus garden of *Atta cephalotes*	KM817172	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis* sp.	LESF892	UT020	Mexico	Fungus garden of *Trachymyrmex* sp.	KM817173	Meirelles et al. (2015b)
Escovopsis microspora	*Escovopsis weberi*	CBS 135751[ET]	VIC:31756	Viçosa, Minas Gerais, Brazil	Fungus garden of *Acromyrmex subterraneus molestans*	KJ935030[&]	Augustin et al. (2013), Meirelles et al. (2015a)
Escovopsis sp.	*Escovopsis weberi*	LESF046	SES001	Rio Claro, São Paulo, Brazil	Fungus garden of *Trachymyrmex* sp.	KM817146	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis weberi*	LESF355	ES021	Corumbataí, São Paulo, Brazil	Fungus garden of *Atta sexdens rubropilosa*	MT305358	This study
Escovopsis sp.	*Escovopsis weberi*	LESF 017	NL001	Botucatu, São Paulo, Brazil	Midden of *Atta capiguara*	KM817142	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis weberi*	LESF 019	NL005	Botucatu, São Paulo, Brazil	Fungus garden of *Atta sexdens rubropilosa*	KM817144	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis weberi*	LESF 020	NL006	Botucatu, São Paulo, Brazil	Fungus garden of *Atta sexdens rubropilosa*	MT305340	This study
Escovopsis sp.	*Escovopsis weberi*	LESF 023	ES005	Alta Floresta, Mato Grosso, Brazil	Fungus garden of *Atta cephalotes*	KM817126	Meirelles et al. (2015b), Montoya et al. (2019)
Escovopsis sp.	*Escovopsis weberi*	LESF 024	ES006	Alta Floresta, Mato Grosso, Brazil	Fungus garden of *Acromyrmex coronatus*	KM817127	Montoya et al. (2019)
Escovopsis sp.	*Escovopsis weberi*	LESF 025	ES007	Alta Floresta, Mato Grosso, Brazil	Fungus garden of *Acromyrmex coronatus*	KM817128	Montoya et al. (2019)
Escovopsis sp.	*Escovopsis weberi*	LESF 027	ES010	Rio Claro, São Paulo, Brazil	Fungus garden of *Acromyrmex landolti*	KM817131	Meirelles et al. (2015b), Montoya et al. (2019)
Escovopsis sp.	*Escovopsis weberi*	LESF 029	ES012	Corumbataí, São Paulo, Brazil	Fungus garden of *Atta sexdens*	KM817133	Meirelles et al. (2015b),

							Montoya et al. (2019)
Escovopsis sp.	*Escovopsis weberi*	LESF 030	ES013	Corumbataí, São Paulo, Brazil	Fungus garden of *Atta sexdens*	KM817134	Meirelles et al. (2015b), Montoya et al. (2019)
Escovopsis sp.	*Escovopsis weberi*	LESF 031	ES014	Corumbataí, São Paulo, Brazil	Fungus garden of *Atta sexdens*	MT305341	This study
Escovopsis sp.	*Escovopsis weberi*	LESF 033	ES004	Bahia, Brazil	Fungus garden of *Acromyrmex* sp.	KM817125	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis weberi*	LESF 034	ES024	Botucatu, São Paulo, Brazil	Fungus garden of *Acromyrmex balzanii*	MT305342	This study
Escovopsis sp.	*Escovopsis weberi*	LESF042	RS053	Chuvisca, Rio Grande do Sul, Brazil	Fungus garden of *Acromyrmex lundii*	EU082797	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis weberi*	LESF043	RS055	Chuvisca, Rio Grande do Sul, Brazil	Fungus garden of *Acromyrmex heyeri*	EU082796	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis weberi*	LESF054	AR003	Ilhéus, Bahia, Brazil	Fungus garden of *Acromyrmex balzanii*	KM817113	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis weberi*	LESF056	AR033	Camacan, Bahia, Brazil	Fungus garden of *Acromyrmex* sp.	KM817115	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis weberi*	LESF136	4a	Corumbataí, São Paulo, Brazil	Fungus garden of *Atta sexdens rubropilosa*	MT305343	This study
Escovopsis sp.	*Escovopsis weberi*	LESF146	1cT4	Corumbataí, São Paulo, Brazil	Fungus garden of *Atta sexdens rubropilosa*	MT305344	This study
Escovopsis sp.	*Escovopsis weberi*	LESF156	A088	Corumbataí, São Paulo, Brazil	Fungus garden of *Atta sexdens rubropilosa*	MT305345	This study
Escovopsis sp.	*Escovopsis weberi*	LESF178	A086a	Corumbataí, São Paulo, Brazil	Fungus garden of *Atta sexdens rubropilosa*	MT305346	This study
Escovopsis sp.	*Escovopsis weberi*	LESF239	13B	Corumbataí, São Paulo, Brazil	Fungus garden of *Atta sexdens rubropilosa*	MT305347	This study
Escovopsis sp.	*Escovopsis weberi*	LESF241	H1b	Corumbataí, São Paulo, Brazil	Fungus garden of *Atta sexdens rubropilosa*	MT305348	This study
Escovopsis sp.	*Escovopsis weberi*	LESF292	NL003	Botucatu, São Paulo, Brazil	Fungus garden of *Atta capiguara*	MT305349	This study

Escovopsis sp.	*Escovopsis weberi*	LESF294	H33	Corumbataí, São Paulo, Brazil	Fungus garden of *Atta sexdens rubropilosa*	MT305350	This study
Escovopsis sp.	*Escovopsis weberi*	LESF295	NL009	Botucatu, São Paulo, Brazil	Fungus garden of *Atta sexdens rubropilosa*	MT305351	This study
Escovopsis sp.	*Escovopsis weberi*	LESF298	NL004	Botucatu, São Paulo, Brazil	Fungus garden of *Atta capiguara*	MT305352	This study
Escovopsis sp.	*Escovopsis weberi*	LESF315	NL007	Botucatu, São Paulo, Brazil	Fungus garden of *Atta sexdens rubropilosa*	KF240730	Meirelles et al. (2015b), Montoya et al. (2019)
Escovopsis sp.	*Escovopsis weberi*	LESF317	ES026	Rio Claro, São Paulo, Brazil	Fungus garden of *Trachymyrmex* sp.	KM817137	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis weberi*	LESF319	ES030	Palmas, Tocantins, Brazil	Fungus garden of *Trachymyrmex* sp.	KM817140	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis weberi*	LESF324	RS105	Thermas de Santa Bárbara, São Paulo, Brazil	Fungus garden of *Atta laevigata*	KM817145	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis weberi*	LESF356	ES032	Botucatu, São Paulo, Brazil	Fungus garden of *Atta laevigata*	MT305359	This study
Escovopsis sp.	*Escovopsis weberi*	LESF359	ES019	Corumbataí, São Paulo, Brazil	Fungus garden of *Atta sexdens*	MT305360	This study
Escovopsis sp.	*Escovopsis weberi*	LESF362	ES028	Corumbataí, São Paulo, Brazil	Fungus garden of *Atta sexdens*	MT305361	This study
Escovopsis sp.	*Escovopsis weberi*	LESF363	ES023	Corumbataí, São Paulo, Brazil	Fungus garden of *Atta sexdens*	MT305362	This study
Escovopsis sp.	*Escovopsis weberi*	LESF364	ES015	Corumbataí, São Paulo, Brazil	Fungus garden of *Atta sexdens*	MT305363	This study
Escovopsis sp.	*Escovopsis weberi*	LESF519	ES016	---	Fungus garden of *Atta sexdens rubropilosa*	MT305364	This study
Escovopsis sp.	*Escovopsis weberi*	LESF575	RS087	Indaial, Santa Catarina, Brazil	Fungus garden of *Acromyrmex diciger*	MT305365	This study
Escovopsis sp.	*Escovopsis weberi*	LESF858	A210201	Camacan, Bahia, Brazil	Fungus garden of *Atta cephalotes*	MT305373	This study
Escovopsis sp.	*Escovopsis weberi*	LESF859	B110302	Camacan, Bahia, Brazil	Fungus garden of *Atta cephalotes*	MT305374	This study
Escovopsis sp.	*Escovopsis weberi*	LESF877	NL010	---	---	MT305376	This study
Escovopsis sp.	*Escovopsis weberi*	LESF880	2aT=3	---	---	MT305377	This study
Escovopsis sp.	*Escovopsis weberi*	LESF 994	QVM81	Anavilhanas, Manaus, Amazonas, Brazil	Fungus garden of *Acromyrmex* sp.	MT305388	This study

Escovopsis weberi	*Escovopsis weberi*	ATCC 64542 ET	---	Viçosa, Minas Gerais, Brazil	Carpenter ant fungal mass	AY172623	Augustin et al. (2013), Currie et al. (2003)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis weberi*	AtspAr2	UGM030330-07 Atta Esc B	Argentina	Fungus garden of *Atta sexdens*	EF589910	Taerum et al. (2007)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis weberi*	AtcoPaP	SP020523-01 A col Esc B slow	Panama	Fungus garden of *Atta colombica*	EF589912	Taerum et al. (2007)
Escovopsis weberi	*Escovopsis weberi*	AtcoN1C2I1	---	Panama	Fungus garden of *Atta colombica*	GQ240710	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis weberi*	AtceN1S3Pa	---	Panama	Fungus garden of *Atta cephalotes*	GQ240704	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis weberi*	AtceN1S2Pa	---	Panama	Fungus garden of *Atta cephalotes*	GQ240703	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis weberi*	AtcoN1C2I2	---	Panama	Fungus garden of *Atta colombica*	GQ240711	Taerum et al. (2010)
Escovopsis weberi	*Escovopsis weberi*	AtcoN1C2I3	---	Panama	Fungus garden of *Atta colombica*	GQ240712	Taerum et al. (2010)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis weberi*	AcspAArM	CC030402-02 Acro Esc A	Argentina	Fungus garden of *Acromyrmex* sp.	EF589922	Taerum et al. (2007)
Escovopsis sp.	*Escovopsis weberi*	Esc2	---	Ecuador	Fungus garden of *Atta* cf. *cephalotes*	AY172620	Currie et al. (2003)
Escovopsis cf. *weberi* CRC-2007a	*Escovopsis weberi*	AtspBr	---	Brazil	Fungus garden of *Atta* sp.	EF589916	Taerum et al. (2007)
Escovopsis sp.	*Escovopsis weberi*	LESF025	ES007	Alta Floresta – MT, Brazil	Fungus garden of *Acromyrmex coronatus*	KM817128	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis weberi*	LESF106	SES006	Uberlandia – MG, Brazil	Fungus garden of *Trachymyrmex dichrou*	KM817150	Meirelles et al. (2015b)
Hypomyces armeniacus	*Hypomyces armeniacus*	TFC 02-86/2	---	---	---	FN868742	Poldmaa,K. (2011)
Hypomyces asterophorum	*Hypomyces asterophorum*	CBS 676.77	---	Japan	---	FN868712	Põldmaa (2011)
Hypomyces aurantius	*Hypomyces aurantius*	TFC 95-171	---	---	---	FN868743	Poldmaa,K. (2011)
Hypomyces australasiaticus	*Hypomyces australasiaticus*	TFC 99-95	---	---	---	FN868745	Poldmaa,K. (2011)
Hypomyces dactylarioide	*Hypomyces dactylarioide*	CBS 141.78	---	---	---	FN868748	Poldmaa,K. (2011)
Hypomyces gabonensis	*Hypomyces gabonensis*	TFC 201156	---	---	---	FN868749	Poldmaa,K. (2011)

Hypomyces khaoyaiensis	*Hypomyces khaoyaiensis*	G.J.S. 01-304	---	---	---	FN868750	Poldmaa,K. (2011)
Hypomyces odoratus	*Hypomyces odoratus*	Grogan 192B1	IMI:372795	United Kingdom	*Agaricus bisporus*	HF911639	Tamm and Poldmaa (2013)
Hypomyces odoratus	*Hypomyces odoratus*	C.T.R. 72-23	---	---	---	FN868752	Poldmaa,K. (2011)
Hypomyces protrusum	*Hypomyces protrusum*	TFC 201316	---	Madagascar	Eucalyptus forest	FN868732	Põldmaa (2011)
Hypomyces rosellus	*Hypomyces rosellus*	TFC 01-25	---	---	---	FN868760	Poldmaa,K. (2011)
Hypomyces samuelsii	*Hypomyces samuelsii*	TFC 2007-23	---	Peru	on basidioma of an agaricoid basidiomycete on a stem of a palm	FN868769	Põldmaa (2011)
Hypomyces semicirculare	*Hypomyces semicirculare*	CBS 705.88	---	Cuba	On old polypore	FN868735	Põldmaa (2011)
Lecanicillium antillanum	*Lecanicillium antillanum*	CBS 350.85	---	Cuba	on basidioma of an agaricoid	DQ522350	Spatafora et al. (2007)
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 310	AR14022604 A1	Florianópolis, Santa Catarina, Brazil	Fungus garden of *Mycetophylax morchi*	MH724262	Montoya et al. (2019)
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 311	AR14022604 A2	Florianópolis, Santa Catarina, Brazil	Fungus garden of *Mycetophylax morchi*	MH724263	Montoya et al. (2019)
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 312	AR14022604 ALA	Florianópolis, Santa Catarina, Brazil	Fungus garden of *Mycetophylax morchi*	MH724264	Montoya et al. (2019)
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 832	13I3	Rio Claro, São Paulo, Brazil	Fungus garden of *Mycocepurus smithii*	MT305366	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 833	13I1	Rio Claro, São Paulo, Brazil	Fungus garden of *Mycocepurus smithii*	MT305367	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 834	13I2	Rio Claro, São Paulo, Brazil	Fungus garden of *Mycocepurus smithii*	MT305368	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 835	3I1	Rio Claro, São Paulo, Brazil	Midden of *Mycocepurus smithii*	MT305369	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 837	2I2	Rio Claro, São Paulo, Brazil	Fungus garden of *Mycocepurus smithii*	MT305370	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 838	2I3	Rio Claro, São Paulo, Brazil	Fungus garden of *Mycocepurus smithii*	MT305371	This study

Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 895	Q03I	Botucatu, São Paulo, Brazil	Fungus garden of *Mycocepurus goeldii*	MT305380	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 927	Q23III	Botucatu, São Paulo, Brazil	Fungus garden of *Mycocepurus goeldii*	MT305383	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 1049	QVM177	Botucatu, São Paulo, Brazil	Fungus garden of *Mycocepurus goeldii*	MT305401	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 1051	QVM179	Botucatu, São Paulo, Brazil	Fungus garden of *Mycocepurus goeldii*	MT305402	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 1052	QVM180	Botucatu, São Paulo, Brazil	Fungus garden of *Mycocepurus goeldii*	MT305403	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 1055	QVM183	Botucatu, São Paulo, Brazil	Fungus garden of *Mycocepurus goeldii*	MT305404	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 1057	QVM185	Botucatu, São Paulo, Brazil	Fungus garden of *Mycocepurus goeldii*	MT305405	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 1061	QVM189	Botucatu, São Paulo, Brazil	Fungus garden of *Mycocepurus goeldii*	MT305406	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 1077	QVM205	Botucatu, São Paulo, Brazil	Fungus garden of *Mycocepurus goeldii*	MT305407	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 1078	QVM206	Botucatu, São Paulo, Brazil	Fungus garden of *Mycocepurus goeldii*	MT305408	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 1082	QVM210	Botucatu, São Paulo, Brazil	Fungus garden of *Mycocepurus goeldii*	MT305409	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 1090	QVM218	Botucatu, São Paulo, Brazil	Fungus garden of *Mycocepurus goeldii*	MT305410	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 1109	QVM237	Botucatu, São Paulo, Brazil	Fungus garden of *Mycocepurus goeldii*	MT305411	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	CBS 137343 ET	VEM001	Rio Claro, Sao paulo, Brazil	Fungus garden of *Mycocepurus goeldii*	KF033128	Masiulionis et al. (2015), Osti and Rodrigues (2018)
Protocrea pallida	*Protocrea pallida*	TFC 99-209	---	New York, Cleaveland	---	EU703903	Jaklitsch and Samuels (2011)
Sphaerostilbella aureonitens	*Sphaerostilbella aureonitens*	GJS 74-87	---	---	---	FJ467644	unpublished

Sphaerostilbella berkeleyana	*Sphaerostilbella berkeleyana*	GJS 82-274	---	---	---	AF543783	Sung,G.-H. and Spatafora,J.W. (2003)
Sphaerostilbella berkeleyana	*Sphaerostilbella berkeleyana*	GJS 82-274	---	---	---	FJ860648	Currie et al. (2003)
Sphaerostilbella cf. *aureonitens*	*Sphaerostilbella* cf. *aureonitens*	G.J.S. 74-87	---	New Zealand	---	DQ834452	---
Sphaerostilbella cf. *aureonitens*	*Sphaerostilbella* cf. *aureonitens*	G.J.S. 82-40	---	---	---	DQ834453	---
Escovopsis sp. cc010325-06 esc2	*Sympodiorosea*	cc010325-06 esc2	---	Panama	Fungus garden of *Apterostigma auriculatum*	DQ848181	Gerardo et al. (2006)
Escovopsis sp. nmg031218-01 esc2	*Sympodiorosea*	nmg031218-01 esc2	---	Panama	Fungus garden of *Apterostigma auriculatum*	DQ848206	Gerardo et al. (2006)
Escovopsis sp. cc011029-02 esc1	*Sympodiorosea*	cc011029-02 esc1	---	Panama	Fungus garden of *Apterostigma auriculatum*	DQ848178	Gerardo et al. (2006)
Escovopsis sp. MT1	*Sympodiorosea*	MT1	---	Panama	Fungus garden of *Mycocepurus tardus*	AY172627	Currie et al. (2003)
Escovopsis sp. CC1	*Sympodiorosea*	CC1	---	Panama	Fungus garden of *Cyphomyrmex costatus*	AY172624	Currie et al. (2003)
Escovopsis sp. CC4	*Sympodiorosea*	CC4	---	Panama	Fungus garden of *Cyphomyrmex costatus*	AY172625	Currie et al. (2003)
Escovopsis sp. Esc10	*Sympodiorosea*	Esc10	---	Panama	Fungus garden of *Myrmicocrypta ednaella*	AY172628	Currie et al. (2003)
Escovopsis sp. Esc19	*Sympodiorosea*	Esc19	---	Ecuador	Fungus garden of *Cyphomyrmex faunulus*	AY172626	Currie et al. (2003)
Escovopsis sp. 10-20	*Sympodiorosea*	10-20	---	Guyana	Fungus garden of *Myrmicocrypta* sp.	AY172629	Currie et al. (2003)
Escovopsis kreiselii	*Sympodiorosea kreiselii*	LESF 302	AR14022705	Florianópolis, Santa Catarina, Brazil	Fungus garden of *Mycetophylax morchi*	MH724259	Montoya et al. (2019)
Escovopsis kreiselii	*Sympodiorosea kreiselii*	LESF 303	AR14022705 B	Florianópolis, Santa Catarina, Brazil	Fungus garden of *Mycetophylax morchi*	MH724260	Montoya et al. (2019)
Escovopsis kreiselii	*Sympodiorosea kreiselii*	LESF 304	AR14022705 T2D	Florianópolis, Santa Catarina, Brazil	Fungus garden of *Mycetophylax morchi*	MH724261	Montoya et al. (2019)

Escovopsis kreiselii	*Sympodiorosea kreiselii*	LESF 305	AR14022601	Florianópolis, Santa Catarina, Brazil	Fungus garden of *Mycetophylax morchi*	MT305353	This study
Escovopsis kreiselii	*Sympodiorosea kreiselii*	LESF 306	AR14022705 A	Florianópolis, Santa Catarina, Brazil	Fungus garden of *Mycetophylax morchi*	MT305354	This study
Escovopsis kreiselii	*Sympodiorosea kreiselii*	LESF 307	AR14022705 T2	Florianópolis, Santa Catarina, Brazil	Fungus garden of *Mycetophylax morchi*	MT305355	This study
Escovopsis kreiselii	*Sympodiorosea kreiselii*	LESF 308	AR14022604 AL	Florianópolis, Santa Catarina, Brazil	Fungus garden of *Mycetophylax morchi*	MT305356	This study
Escovopsis kreiselii	*Sympodiorosea kreiselii*	LESF 309	AR14022605 T2	Florianópolis, Santa Catarina, Brazil	Fungus garden of *Mycetophylax morchi*	MT305357	This study
Escovopsis kreiselii	*Sympodiorosea kreiselii*	CBS 139320 ET	LESF 053	Florianópolis, Santa Catarina, Brazil	Fungus garden of *Mycetophylax morchi*	KJ 808766	Meirelles et al. (2015a)
Sympodiorosea sp.	*Sympodiorosea* sp.	LESF 864	SES030331-05	---	---	MT305375	This study
Sympodiorosea sp.	*Sympodiorosea* sp.	LESF 886	UGM23	---	---	MT305378	This study
Sympodiorosea sp.	*Sympodiorosea* sp.	LESF 887	UGM26(C)	---	---	MT305379	This study
Sympodiorosea sp.	*Sympodiorosea* sp.	LESF 897	Q03III	Botucatu, São Paulo, Brazil	Fungus garden of *Mycocepurus goeldii*	MT305381	This study
Sympodiorosea sp.	*Sympodiorosea* sp.	LESF 899	Q03V	Botucatu, São Paulo, Brazil	Fungus garden of *Mycocepurus goeldii*	MT305382	This study
Sympodiorosea sp.	*Sympodiorosea* sp.	LESF 1010	QVM138	Camp 41, Manaus, Amazonas, Brazil	Fungus garden of *Apterostigma* sp.	MT305393	This study
Trichoderma alni	*Trichoderma alni*	CPK2854	---	---	---	EU498314	Jaklitsch et al. (2008)
Trichoderma americanum	*Trichoderma americanum*	G.J.S. 94-79	---	---	---	DQ835491	---
Trichoderma brunneoviride	*Trichoderma brunneoviride*	CBS120928	---	---	---	EU498318	Jaklitsch et al. (2008)
Trichoderma epimyces	*Trichoderma epimyces*	CPK2487	---	---	---	EU498322	Jaklitsch et al. (2008)
Trichoderma harzianum	*Trichoderma harzianum*	CBS 226.95	---	England	---	AF534621	Chaverri et al. (2003)
Trichoderma hispanicum	*Trichoderma hispanicum*	S419	---	---	---	JN715657	Jaklitsch et al. (2012)

Trichoderma luteocrystallinum	*Trichoderma luteocrystallinum*	CBS 123828	---	---	---	FJ860646	Jaklitsch et al. (2011)
Trichoderma stercorarium	*Trichoderma stercorarium*	CBS 148.85	---	---	---	FJ860607	Jaklitsch,W.M. (2011)
Trichoderma stercorarium	*Trichoderma stercorarium*	ATCC 62321	---	---	---	AF543782	Sung,G.-H. and Spatafora,J.W. (2003)
Escovopsis sp. sv030615-04 esc1	?	sv030615-04 esc1	---	Ecuador	Fungus garden of *Apterostigma* sp.	DQ848199	Gerardo et al. (2006)
Escovopsis sp. sv030615-05 esc1	?	sv030615-05 esc1	---	Ecuador	Fungus garden of *Apterostigma* sp.	DQ848200	Gerardo et al. (2006)
Escovopsis sp. nmg030614-01 esc1	?	nmg030614-01 esc1	---	Ecuador	Fungus garden of *Apterostigma* cf. *pilosum*	DQ848202	Gerardo et al. (2006)
Escovopsis sp. al030618-10 esc1	?	al030618-10 esc1	---	Ecuador	Fungus garden of *Apterostigma* sp.	DQ848189	Gerardo et al. (2006)
Escovopsis sp. al030609-03 esc1	?	al030609-03 esc1	---	Ecuador	Fungus garden of *Apterostigma* sp.	DQ848191	Gerardo et al. (2006)
Escovopsis sp. nmg020519-02 esc2	?	nmg020519-02 esc2	---	Panama	Fungus garden of *Apterostigma* cf. *pilosum*	DQ848185	Gerardo et al. (2006)
Escovopsis sp. nmg011029-03 esc1	?	nmg011029-03 esc1	---	Panama	Fungus garden of *Apterostigma* sp.	DQ848193	Gerardo et al. (2006)
Escovopsis sp. nmg010802-02 esc1	?	nmg010802-02 esc1	---	Panama	Fungus garden of *Apterostigma dentigerum*.	DQ848156	Gerardo et al. (2006)
Escovopsis sp. sp011112-01 esc1	?	sp011112-01 esc1	---	Panama	Fungus garden of *Apterostigma dentigerum*.	DQ848162	Gerardo et al. (2006)
Escovopsis sp. nmg010816-05 esc19	?	nmg010816-05 esc19	---	Panama	Fungus garden of *Apterostigma dentigerum*.	DQ848158	Gerardo et al. (2006)
Escovopsis sp. nmg020611-02 esc7	?	nmg020611-02 esc7	---	Panama	Fungus garden of *Apterostigma dentigerum*.	DQ848175	Gerardo et al. (2006)
Escovopsis sp. ugm030327-05 esc4	?	ugm030327-05 esc4	---	Argentina	Fungus garden of *Apterostigma* sp.	DQ848208	Gerardo et al. (2006)
Escovopsis sp. nmg010318-21 esc2	?	nmg010318-21 esc	---	Panama	Fungus garden of *Apterostigma dentigerum*.	DQ848159	Gerardo et al. (2006)

Escovopsis sp. agh030618-02 esc1	?	agh030618-02 esc1	---	Ecuador	Fungus garden of *Apterostigma* sp.	DQ848195	Gerardo et al. (2006)
Escovopsis sp. cc011213-31 esc1	?	cc011213-31 esc1	---	Panama	Fungus garden of *Apterostigma auriculatum*	DQ848179	Gerardo et al. (2006)
Escovopsis sp. nmg011101-03	?	nmg011101-03	---	Panama	Fungus garden of *Cyphomyrmex longiscapus*	DQ848209	Gerardo et al. (2006)
Escovopsis sp. agh020621-05 esc2	?	agh020621-05 esc2	---	Costa Rica	Fungus garden of *Apterostigma dentigerum*	DQ848160	Gerardo et al. (2006)
Escovopsis sp. ugm020602-07 esc1	?	ugm020602-07 esc1	---	Panama	Fungus garden of *Apterostigma* sp.	DQ848165	Gerardo et al. (2006)
Escovopsis sp. nmg020611-02 esc6	?	nmg020611-02 esc6	---	Panama	Fungus garden of *Apterostigma dentigerum*	DQ848174	Gerardo et al. (2006)
Escovopsis sp. nmg020521-04 esc1	?	nmg020521-04 esc1	---	Panama	Fungus garden of *Apterostigma dentigerum*	DQ848190	Gerardo et al. (2006)
Escovopsis sp. nmg030618-01 esc1	?	nmg030618-01 esc1	---	Ecuador	Fungus garden of *Apterostigma* cf. *pilosum*	DQ848184	Gerardo et al. (2006)
Escovopsis sp. Esc26	?	Esc26	---	Guyana	Fungus garden of *Apterostigma dorothea*	AY172619	Currie et al. (2003)
Escovopsis sp. Esc20	?	Esc20	---	Ecuador	Fungus garden of *Apterostigma* cf. *pilosum*	AY172618	Currie et al. (2003)

Table S2. *Escovopsis* strains used in the phylogenetic analyses and their associated metadata.

Previous fungal species name	Current fungal species name	Strain ID	Specimen voucher	City, State, Country	GPS	Habitat	GenBank accession numbers					References
							ITS	LSU	*tef*1	*rpb*1	*rpb*2	
Escovopsioides nivea	*Escovopsioides nivea*	CBS 135749[ET]	AUJ6	Viçosa, Minas Gerais, Brazil	20°44'31.71"S, 42°52'43.83"W	Fungus garden of *Acromyrmex subterraneus subterraneus*	JQ815078	JQ855716	JQ855713	MT305414[#]	MT305539[#]	Augustin et al. (2013)
Escovopsioides sp.	*Escovopsioides nivea*	LESF159	J08	Corumbataí, São Paulo, Brazil	22° 17' 21.7" S; 47° 39' 22.8" W	Fungus garden of *Atta sexdens rubropilosa*	MF116014	MF116034	MF140949	MT305444	MT305569	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF601	J09	Rio Claro - SP, Brazil	---	Fungus garden of *Trachymyrmex* sp.	MF116029	MF116049	MF140964	MT305487[#]	MT305612[#]	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 151	J02	Corumbataí, São Paulo, Brazil	22° 17' 21.7" S; 47° 39' 22.8" W	Fungus garden of *Atta sexdens rubropilosa*	MF116013	MF116033	MF140948	MT305442[#]	MT305567[#]	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 510	J10	Botucatu, São Paulo, Brazil	22° 54' 28.4" S; 48° 18' 55.7" W	Fungus garden of *Atta sexdens rubropilosa*	MF116015	MF116035	MF140950	MT305474[#]	MT305599[#]	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 587	J01	Camacan, Bahia, Brazil	15° 23' 18.2" S; 39° 33' 30.5" W	Fungus garden of *Atta cephalotes*	MF116016	MF116036	MF140951	MT305477[#]	MT305602[#]	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 588	J03	Camacan, Bahia, Brazil	15° 25' 32.3" S 39° 32' 48.1" W	Fungus garden of *Atta cephalotes*	MF116017	MF116037	MF140952	MT305478[#]	MT305603[#]	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 589	J05	Camacan, Bahia, Brazil	15° 23' 14.8" S; 39° 33' 28.4" W	Fungus garden of *Atta cephalotes*	MF116018	MF116038	MF140953	MT305479[#]	MT305604[#]	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 590	J06	Camacan, Bahia, Brazil	15° 23' 15.2" S; 39° 33' 28.0" W	Fungus garden of *Atta cephalotes*	MF116019	MF116039	MF140954	MT305480[#]	MT305605[#]	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 591	J04	Botucatu, São Paulo, Brazil	22° 54' 26.6" S; 48° 18' 29.2" W	Fungus garden of *Atta capiguara*	MF116020	MF116040	MF140955	MT305481[#]	MT305606[#]	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 592	J12	Camacan, Bahia, Brazil	15° 22' 50.3" S; 39° 34' 03.5" W	Fungus garden of *Acromyrmex* sp.	MF116021	MF116041	MF140956	MT305482[#]	MT305607[#]	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 596	J16	Chuvisca - RS, Brazil	30° 50' 10.2" S; 51° 55' 10.4" W	Fungus garden of *Acromyrmex* sp.	MF116025	MF116045	MF140960	MT305483[#]	MT305608[#]	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 597	J17	Camacan, Bahia, Brazil	15° 23' 29.7" S; 39° 33' 31.3" W	Fungus garden of *Atta cephalotes*	MF116026	MF116046	MF140961	MT305484[#]	MT305609[#]	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 598	J18	Camacan, Bahia, Brazil	15° 23' 17.8" S; 39° 33' 22.3" W	Fungus garden of *Atta cephalotes*	MF116027	MF116047	MF140962	MT305485[#]	MT305610[#]	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 599	J19	Sentinela do Sul - RS, Brazil	---	Fungus garden of *Acromyrmex heyeri*	MF116028	MF116048	MF140963	MT305486[#]	MT305611[#]	Osti and Rodrigues (2018)
Escovopsioides sp.	*Escovopsioides nivea*	LESF 1009	QVM137	Camp 41, Manaus, Amazonas, Brazil	2° 26' 55,3" S; 59° 46' 10.9" W	Fungus garden of *Apterostigma* sp.	MT273483	MT273572	MT305392	MT305516	MT305641	This study
Escovopsioides sp.	*Escovopsioides nivea*	LESF 1023	QVM151	Camp 41, Manaus, Amazonas, Brazil	2° 26 '52.5" S; 59° 45' 53.4" W	Fungus garden of *Trachymyrmex* sp.	MT273485	MT273574	MT305394	MT305518	MT305643	This study

Escovopsioides sp.	*Escovopsioides nivea*	LESF 1025	QVM153	Camp 41, Manaus, Amazonas, Brazil	2° 26' 52.5" S; 59° 45' 53.4" W	Fungus garden of *Trachymyrmex* sp.	MT273486	MT273575	MT305395	MT305519	MT305644	This study
Escovopsioides sp.	*Escovopsioides nivea*	LESF 1028	QVM156	Camp 41, Manaus, Amazonas, Brazil	S 2° 26' 52.6"; 59° 45' 52.4" W	Fungus garden of *Trachymyrmex* sp.	MT273487	MT273576	MT305396	MT305520	MT305645	This study
Escovopsioides sp.	*Escovopsioides nivea*	LESF 1031	QVM159	Camp 41, Manaus, Amazonas, Brazil	2° 26' 55.5" S; 59° 45' 54.2 W	Fungus garden of *Trachymyrmex* sp.	MT273488	MT273577	MT305397	MT305521	MT305646	This study
Escovopsioides sp.	*Escovopsioides nivea*	LESF 1039	QVM167	Camp 41, Manaus, Amazonas, Brazil	2° 26' 52.3' S; 59° 45' 51.9" W	Fungus garden of *Cyphomyrmex* sp.	MT273489	MT273578	MT305398	MT305522	MT305647	This study
Escovopsioides sp.	*Escovopsioides nivea*	LESF 1040	QVM168	Camp 41, Manaus, Amazonas, Brazil	2° 26' 52.6" S; 59° 45' 52.4' W	Fungus garden of *Apterostigma* sp.	MT273490	MT273579	MT305399	MT305523	MT305648	This study
Escovopsioides sp.	*Escovopsioides nivea*	LESF 1041	QVM169	Camp 41, Manaus, Amazonas, Brazil	2° 26' 55.5" S; 59° 45' 54.2 W	Fungus garden of *Apterostigma* sp.	MT273491	MT273580	MT305400	MT305524	MT305649	This study
Escovopsis aspergilloides	*Escovopsis aspergilloides*	CBS 423.93 ET	DAOM:216382	Trinidad and Tobago: Trinidad	---	Fungus garden of *Trachymyrmex ruthae*	NR_137160	KF293283	AY172632	MT305421#	MT305546#	Augustin et al. (2013), Currie et al. (2003)
Escovopsis clavata	*Escovopsis clavata*	LESF 854	1704A	Florianópolis, Santa Catarina, Brazil	27°44'38.94"S 48°31'9.3"W	Fungus garden of *Apterostigma* sp.	MH715097	MH715111	MH724271	MT305495#	MT305620#	Montoya et al. (2019)
Escovopsis clavata	*Escovopsis clavata*	LESF 855	1705B	Florianópolis, Santa Catarina, Brazil	27°44'39.49"S 48°31'9.72"W	Fungus garden of *Apterostigma* sp.	MH715098	MH715112	MH724272	MT305496#	MT305621#	Montoya et al. (2019)
Escovopsis clavata	*Escovopsis clavata*	CBS 145326 ET	1707	Florianópolis, Santa Catarina, Brazil	27°44'39.6"S 48°31'10.14"W	Fungus garden of *Apterostigma* sp.	MH715096	MH715110	MH724270	MT305419#	MT305544#	Montoya et al. (2019)
Escovopsis lentecrescens	*Escovopsis lentecrescens*	CBS 135750 ET	VIC:31755	Viçosa, Minas Gerais, Brazil	20°44'31.71"S 42°52'43.83"W	Fungus garden of *Acromyrmex subterraneus subterraneus*	JQ815079	JQ855717	JQ855714	MT305415#	MT305540#	Augustin et al. (2013)
Escovopsis moelleri	*Escovopsis moelleri*	CBS 135748 ET	VIC:31753	Viçosa, Minas Gerais, Brazil	20°44'31.71"S 42°52'43.83"W	Fungus garden of *Acromyrmex subterraneus molestans*	JQ815077	JQ855715	JQ855712	MT305413#	MT305538#	Augustin et al. (2013)
Escovopsis sp.	*Escovopsis multiformis*	LESF 852	1706B	Florianópolis, Santa Catarina, Brazil	27° 44' 39.4 S; 48° 31' 10.0 W	Fungus garden of *Apterostigma* sp.	MT273460	MT273549	MT305372	MT305494	MT305619	This study
Escovopsis sp.	*Escovopsis multiformis*	LESF 1136	QVM277	Alta Floresta, Mato Grosso, Brazil	09° 49' 22.7"S 58° 15' 32.0"W	Fungus garden of *Apterostigma* sp.	MH715092	MH715106	MH724266	MT305536#	MT305661#	Montoya et al. (2019)
Escovopsis multiformis	*Escovopsis multiformis*	CBS 145327 ET	1606w	Florianópolis, Santa Catarina, Brazil	27°28'11.28"S 48°22'39.48"W	Fungus garden of *Apterostigma* sp.	MH715091	MH715105	MH724265	MT305420#	MT305545#	Montoya et al. (2019)
Escovopsis sp.	*Escovopsis* sp.	LESF 052	SES010	Camp 41, Manaus, Amazonas, Brazil	---	Fungus garden of *Trachymyrmex diversus*	KM817093	MH715124	KM817154	MT305437#	MT305562#	Meirelles et al. (2015b), Montoya et al. (2019)
Escovopsis sp.	*Escovopsis* sp.	LESF 325	BA004	Camacan, Bahia, Brazil	14°47'56.8"S; 39°10'16.4"W	Fungus garden of *Atta cephalotes*	KM817049	MH715127	KM817119	MT305467#	MT305592#	Meirelles et al. (2015b),

												Montoya et al. (2019)
Escovopsis sp.	*Escovopsis* sp.	LESF 962	QVM49	Anavilhanas, Manaus, Amazonas, Brazil	2° 16 ' 15.7" S; 61° 01' 8.5" W	Fungus garden of *Acromyrmex* sp.	MT273475	MT273564	MT305384	MT305508	MT305633	This study
Escovopsis sp.	*Escovopsis* sp.	LESF 969	QVM56	Anavilhanas, Manaus, Amazonas, Brazil	2° 31' 23,4" S; 60° 49' 31,9" W	Fungus garden of *Apterostigma* sp.	MT273476	MT273565	MT305385	MT305509	MT305634	This study
Escovopsis sp.	*Escovopsis* sp.	LESF 975	QVM62	Anavilhanas, Manaus, Amazonas, Brazil	2° 31' 25.3" S; 60° 49' 33.1" W	Fungus garden of *Trachymyrmex* sp.	MT273477	MT273566	MT305386	MT305510	MT305635	This study
Escovopsis sp.	*Escovopsis* sp.	LESF 979	QVM66	Anavilhanas, Manaus, Amazonas, Brazil	---	Fungus garden of *Trachymyrmex* sp	MT273478	MT273567	MT305387	MT305511	MT305636	This study
Escovopsis sp.	*Escovopsis* sp.	LESF 996	QVM83	Anavilhanas, Manaus, Amazonas, Brazil	2° 32' 02.7" S; 60° 50' 11.7" W	Fungus garden of *Apterostigma* sp.	MT273480	MT273569	MT305389	MT305513	MT305638	This study
Escovopsis sp.	*Escovopsis* sp.	LESF 997	QVM84	Anavilhanas, Manaus, Amazonas, Brazil	2° 31' 23,4" S; 60° 49' 31,9" W	Fungus garden of *Trachymyrmex* sp	MT273481	MT273570	MT305390	MT305514	MT305639	This study
Escovopsis sp.	*Escovopsis* sp.	LESF 1003	QVM90	Anavilhanas, Manaus, Amazonas, Brazil	2° 32' 1.4" S; 60° 50' 0.4" W	Fungus garden of *Trachymyrmex* sp.	MT273482	MT273571	MT305391	MT305515	MT305640	This study
Escovopsis microspora	*Escovopsis weberi*	CBS 135751[ET]	VIC:31756	Viçosa, Minas Gerais, Brazil	20°44'31.71"S 42°52'43.83"W	Fungus garden of *Acromyrmex subterraneus molestans*	JQ815076	KF293284	KJ935030[&]	MT305416[#]	MT305541[#]	Augustin et al. (2013), Meirelles et al. (2015a)
Escovopsis sp.	*Escovopsis weberi*	LESF046	SES001	Rio Claro, São Paulo, Brazil	22°23' 45.9"S; 47°32' 43.2"W	Fungus garden of *Trachymyrmex* sp.	KM817084	MT273511[#]	KM817146	MT305436[#]	MT305561[#]	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis weberi*	LESF355	ES021	Corumbataí, São Paulo, Brazil	---	Fungus garden of *Atta sexdens rubropilosa*	MT273445	MT273534	MT305358	MT305468	MT305593	This study
Escovopsis sp.	*Escovopsis weberi*	LESF017	NL001	Botucatu, São Paulo, Brazil	22° 50' 46.44" S 48° 26' 9.6" W	Midden of *Atta capiguara*	KM817072	MH715113	KM817142	MT305422[#]	MT305547[#]	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis weberi*	LESF019	NL005	Botucatu, São Paulo, Brazil	22° 50' 45.8" S; 48° 26' 09.4" W	Fungus garden of *Atta sexdens rubropilosa*	KM817074	MH715115	KM817144	MT305423[#]	MT305548[#]	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis weberi*	LESF020	NL006	Botucatu, São Paulo, Brazil	22° 50' 45.8" S; 48° 26' 09.4" W	Fungus garden of *Atta sexdens rubropilosa*	MT273425	MT273503	MT305340	MT305424	MT305549	This study
Escovopsis sp.	*Escovopsis weberi*	LESF023	ES005	Alta Floresta, Mato Grosso, Brazil	---	Fungus garden of *Atta cephalotes*	KM817056	MH715117	KM817126	MT305425[#]	MT305550[#]	Meirelles et al. (2015b), Montoya et al. (2019)
Escovopsis sp.	*Escovopsis weberi*	LESF 024	ES006	Alta Floresta, Mato Grosso, Brazil	---	Fungus garden of *Acromyrmex coronatus*	KM817057	MT273504[#]	KM817127	MT305426[#]	MT305551[#]	Montoya et al. (2019)

Escovopsis sp.	*Escovopsis weberi*	LESF 025	ES007	Alta Floresta, Mato Grosso, Brazil	---	Fungus garden of *Acromyrmex coronatus*	KM817058	MT273505#	KM817128	MT305427#	MT305552#	Montoya et al. (2019)
Escovopsis sp.	*Escovopsis weberi*	LESF 027	ES010	Rio Claro, São Paulo, Brazil	---	Fungus garden of *Acromyrmex landolti*	KM817061	MH715119	KM817131	MT305428#	MT305553#	Meirelles et al. (2015b), Montoya et al. (2019)
Escovopsis sp.	*Escovopsis weberi*	LESF 029	ES012	Corumbataí, São Paulo, Brazil	22° 17' 22" S; 47° 39' 23" W	Fungus garden of *Atta sexdens*	KM817063	MH715120	KM817133	MT305429#	MT305554#	Meirelles et al. (2015b), Montoya et al. (2019)
Escovopsis sp.	*Escovopsis weberi*	LESF030	ES013	Corumbataí, São Paulo, Brazil	22° 17' 22" S; 47° 39' 23" W	Fungus garden of *Atta sexdens*	KM817064	MH715121	KM817134	MT305430#	MT305555#	Meirelles et al. (2015b), Montoya et al. (2019)
Escovopsis sp.	*Escovopsis weberi*	LESF031	ES014	Corumbataí, São Paulo, Brazil	22° 17' 22" S; 47° 39' 23" W	Fungus garden of *Atta sexdens*	MT273426	MT273506	MT305341	MT305431	MT305556	This study
Escovopsis sp.	*Escovopsis weberi*	LESF033	ES004	Bahia, Brazil	---	Fungus garden of *Acromyrmex* sp.	KM817055	MT273507#	KM817125	MT305432#	MT305557#	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis weberi*	LESF034	ES024	Botucatu, São Paulo, Brazil	---	Fungus garden of *Acromyrmex balzanii*	MT273427	MT273508	MT305342	MT305433	MT305558	This study
Escovopsis sp.	*Escovopsis weberi*	LESF042	RS053	Chuvisca, Rio Grande do Sul, Brazil	30° 50' 10.2" S; 51° 55' 10.4" W	Fungus garden of *Acromyrmex lundii*	KM817079	MT273509#	EU082797	MT305434#	MT305559#	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis weberi*	LESF043	RS055	Chuvisca, Rio Grande do Sul, Brazil	30° 50' 10.2" S; 51° 55' 10.4" W	Fungus garden of *Acromyrmex heyeri*	KM817080	MT273510#	EU082796	MT305435#	MT305560#	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis weberi*	LESF054	AR003	Ilhéus, Bahia, Brazil	14° 47' 56.8" S; 39° 10' 16.4" W	Fungus garden of *Acromyrmex balzanii*	KM817043	MT273512#	KM817113	MT305438#	MT305563#	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis weberi*	LESF056	AR033	Camacan, Bahia, Brazil	15° 22' 50.3" S; 39° 34' 03.5" W	Fungus garden of *Acromyrmex* sp.	KM817045	MT273513#	KM817115	MT305439#	MT305564#	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis weberi*	LESF136	4a	Corumbataí, São Paulo, Brazil	22° 17' 21.7" S; 47° 39' 22.8" W	Fungus garden of *Atta sexdens rubropilosa*	MT273428	MT273514	MT305343	MT305440	MT305565	This study
Escovopsis sp.	*Escovopsis weberi*	LESF146	1cT4	Corumbataí, São Paulo, Brazil	22° 17' 21.7" S; 47° 39' 22.8" W	Fungus garden of *Atta sexdens rubropilosa*	MT273429	MT273515	MT305344	MT305441	MT305566	This study
Escovopsis sp.	*Escovopsis weberi*	LESF156	A088	Corumbataí, São Paulo, Brazil	22° 17' 21.7" S; 47° 39' 22.8" W	Fungus garden of *Atta sexdens rubropilosa*	MT273430	MT273516	MT305345	MT305443	MT305568	This study
Escovopsis sp.	*Escovopsis weberi*	LESF 178	A086a	Corumbataí, São Paulo, Brazil	22° 17' 21.7" S; 47° 39' 22.8" W	Fungus garden of *Atta sexdens rubropilosa*	MT273431	MT273517	MT305346	MT305445	MT305570	This study

Escovopsis sp.	*Escovopsis weberi*	LESF 239	13B	Corumbataí, São Paulo, Brazil	22° 17' 21.7" S; 47° 39' 22.8" W	Fungus garden of *Atta sexdens rubropilosa*	MT273432	MT273518	MT305347	MT305446	MT305571	This study
Escovopsis sp.	*Escovopsis weberi*	LESF 241	H1b	Corumbataí, São Paulo, Brazil	22° 17' 21.7" S; 47° 39' 22.8" W	Fungus garden of *Atta sexdens rubropilosa*	MT273433	MT273519	MT305348	MT305447	MT305572	This study
Escovopsis sp.	*Escovopsis weberi*	LESF 292	NL003	Botucatu, São Paulo, Brazil	22° 50' 46.4" S 48° 26' 09.6" W	Fungus garden of *Atta capiguara*	MT273434	MT273520	MT305349	MT305448	MT305573	This study
Escovopsis sp.	*Escovopsis weberi*	LESF 294	H33	Corumbataí, São Paulo, Brazil	22° 17' 21.7" S; 47° 39' 22.8" W	Fungus garden of *Atta sexdens rubropilosa*	MT273435	MT273521	MT305350	MT305449	MT305574	This study
Escovopsis sp.	*Escovopsis weberi*	LESF 295	NL009	Botucatu, São Paulo, Brazil	22° 50' 45.8" S; 48° 26' 09.4" W	Fungus garden of *Atta sexdens rubropilosa*	MT273436	MT273522	MT305351	MT305450	MT305575	This study
Escovopsis sp.	*Escovopsis weberi*	LESF 298	NL004	Botucatu, São Paulo, Brazil	22° 50' 46.4" S 48° 26' 09.6" W	Fungus garden of *Atta capiguara*	MT273437	MT273523	MT305352	MT305451	MT305576	This study
Escovopsis sp.	*Escovopsis weberi*	LESF 315	NL007	Botucatu, São Paulo, Brazil	22° 50' 45.8" S; 48° 26' 09.4" W	Fungus garden of *Atta sexdens rubropilosa*	KM817075	MH715125	KF240730	MT305463#	MT305588#	Meirelles et al. (2015b), Montoya et al. (2019)
Escovopsis sp.	*Escovopsis weberi*	LESF 317	ES026	Rio Claro, São Paulo, Brazil	---	Fungus garden of *Trachymyrmex* sp.	KM817067	MT273531#	KM817137	MT305464#	MT305589#	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis weberi*	LESF 319	ES030	Palmas, Tocantins, Brazil	10° 10' 52.9" S; 48° 21' 42.0" W	Fungus garden of *Trachymyrmex* sp.	KM817070	MT273532	KM817140	MT305465#	MT305590#	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis weberi*	LESF 324	RS105	Thermas de Santa Bárbara, São Paulo, Brazil	22º 49' 10.6" S; 49º 16' 06.2" W	Fungus garden of *Atta laevigata*	KM817083	MT273533#	KM817145	MT305466#	MT305591#	Meirelles et al. (2015b)
Escovopsis sp.	*Escovopsis weberi*	LESF 356	ES032	Botucatu, São Paulo, Brazil	---	Fungus garden of *Atta laevigata*	MT273446	MT273535	MT305359	MT305469	MT305594	This study
Escovopsis sp.	*Escovopsis weberi*	LESF 359	ES019	Corumbataí, São Paulo, Brazil	---	Fungus garden of *Atta sexdens*	MT273447	MT273536	MT305360	MT305470	MT305595	This study
Escovopsis sp.	*Escovopsis weberi*	LESF 362	ES028	Corumbataí, São Paulo, Brazil	---	Fungus garden of *Atta sexdens*	MT273448	MT273537	MT305361	MT305471	MT305596	This study
Escovopsis sp.	*Escovopsis weberi*	LESF 363	ES023	Corumbataí, São Paulo, Brazil	---	Fungus garden of *Atta sexdens*	MT273449	MT273538	MT305362	MT305472	MT305597	This study
Escovopsis sp.	*Escovopsis weberi*	LESF 364	ES015	Corumbataí, São Paulo, Brazil	---	Fungus garden of *Atta sexdens*	MT273450	MT273539	MT305363	MT305473	MT305598	This study
Escovopsis sp.	*Escovopsis weberi*	LESF 519	ES016	---	---	Fungus garden of *Atta sexdens rubropilosa*	MT273451	MT273540	MT305364	MT305475	MT305600	This study
Escovopsis sp.	*Escovopsis weberi*	LESF 575	RS087	Indaial, Santa Catarina, Brazil	26º 54' 04.9" S; 49º 10' 51.2" W	Fungus garden of *Acromyrmex diciger*	MT273452	MT273541	MT305365	MT305476	MT305601	This study
Escovopsis sp.	*Escovopsis weberi*	LESF858	A210201	Camacan, Bahia, Brazil	---	Fungus garden of *Atta cephalotes*	MT273461	MT273550	MT305373	MT305497	MT305622	This study

Escovopsis sp.	*Escovopsis weberi*	LESF859	B110302	Camacan, Bahia, Brazil	---	Fungus garden of *Atta cephalotes*	MT273462	MT273551	MT305374	MT305498	MT305623	This study
Escovopsis sp.	*Escovopsis weberi*	LESF877	NL010	---	---	---	MT273466	MT273555	MT305376	MT305500	MT305625	This study
Escovopsis sp.	*Escovopsis weberi*	LESF880	2aT=3	---	---	---	MT273467	MT273556	MT305377	MT305501	MT305626	This study
Escovopsis sp.	*Escovopsis weberi*	LESF 994	QVM81	Anavilhanas, Manaus, Amazonas, Brazil	2° 36' 37.9" S; 60° 52' 34.4" W	Fungus garden of *Acromyrmex* sp.	MT273479	MT273568	MT305388	MT305512	MT305637	This study
Escovopsis weberi	*Escovopsis weberi*	ATCC 64542 [ET]	---	Viçosa, Minas Gerais, Brazil	---	Carpenter ant fungal mass	KF293285	KF293281	AY172623	MT305412[#]	MT305537[#]	Augustin et al. (2013), Currie et al. (2003)
Hypomyces asterophorum	*Hypomyces asterophorum*	CBS 676.77	---	Japan	---	---	FN859395	AJ583469	FN868712	FN868776	FN868649	Põldmaa (2011)
Hypomyces protrusum	*Hypomyces protrusum*	TFC 201316	---	Madagascar	---	Eucalyptus forest	FN859414	FN859414	FN868732	FN868795	FN868668	Põldmaa (2011)
Hypomyces samuelsii	*Hypomyces samuelsii*	TFC 2007-23	---	Peru	---	on basidioma of an agaricoid basidiomycete on a stem of a palm	FN859451	FN859451	FN868769	FN868828	FN868705	Põldmaa (2011)
Hypomyces semicirculare	*Hypomyces semicirculare*	CBS 705.88	---	Cuba	---	On old polypore	NR_121425	FN859417	FN868735	FN868671	FN868798	Põldmaa (2011)
Lecanicillium antillanum	*Lecanicillium antillanum*	CBS 350.85	---	Cuba	---	on basidioma of an agaricoid	NR_111097	AF339536	DQ522350	DQ522396[#]	DQ522450[#]	Spatafora et al. (2007)
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 310	AR14022604 A1	Florianópolis, Santa Catarina, Brazil	27°37'49.62"S, 48°27'3.6"W	Fungus garden of *Mycetophylax morchi*	MH715088	MH715102	MH724262	MT305460[#]	MT305585[#]	Montoya et al. (2019)
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 311	AR14022604 A2	Florianópolis, Santa Catarina, Brazil	27°37'49.62"S, 48°27'3.6"W	Fungus garden of *Mycetophylax morchi*	MH715089	MH715103	MH724263	MT305461[#]	MT305586[#]	Montoya et al. (2019)
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 312	AR14022604 ALA	Florianópolis, Santa Catarina, Brazil	27°37'49.62"S, 48°27'3.6"W	Fungus garden of *Mycetophylax morchi*	MH715090	MH715104	MH724264	MT305462[#]	MT305587[#]	Montoya et al. (2019)
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 832	13I3	Rio Claro, São Paulo, Brazil	---	Fungus garden of *Mycocepurus smithii*	MT273453	MT273542	MT305366	MT305488	MT305613	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 833	13I1	Rio Claro, São Paulo, Brazil	---	Fungus garden of *Mycocepurus smithii*	MT273454	MT273543	MT305367	MT305489	MT305614	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 834	13I2	Rio Claro, São Paulo, Brazil	---	Fungus garden of *Mycocepurus smithii*	MT273455	MT273544	MT305368	MT305490	MT305615	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 835	3I1	Rio Claro, São Paulo, Brazil	---	Midden of *Mycocepurus smithii*	MT273456	MT273545	MT305369	MT305491	MT305616	This study

Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 837	2I2	Rio Claro, São Paulo, Brazil	---	Fungus garden of *Mycocepurus smithii*	MT273458	MT273547	MT305370	MT305492	MT305617	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 838	2I3	Rio Claro, São Paulo, Brazil	---	Fungus garden of *Mycocepurus smithii*	MT273459	MT273548	MT305371	MT305493	MT305618	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 895	Q03I	Botucatu, São Paulo, Brazil	22° 54' 19.6" S; 48°14' 33.7" W	Fungus garden of *Mycocepurus goeldii*	MT273470	MT273559	MT305380	MT305504	MT305629	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 927	Q23III	Botucatu, São Paulo, Brazil	22° 54' 41.7" S; 48°14' 49.5" W	Fungus garden of *Mycocepurus goeldii*	MT273474	MT273563	MT305383	MT305507	MT305632	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 1049	QVM177	Botucatu, São Paulo, Brazil	22° 54' 20.6" S; 48°14' 34.2" W	Fungus garden of *Mycocepurus goeldii*	MT273492	MT273581	MT305401	MT305525	MT305650	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 1051	QVM179	Botucatu, São Paulo, Brazil	22° 54' 19.8" S; 48°14' 33.6" W	Fungus garden of *Mycocepurus goeldii*	MT273493	MT273582	MT305402	MT305526	MT305651	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 1052	QVM180	Botucatu, São Paulo, Brazil	22° 54' 19.8" S; 48°14' 33.6" W	Fungus garden of *Mycocepurus goeldii*	MT273494	MT273583	MT305403	MT305527	MT305652	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 1055	QVM183	Botucatu, São Paulo, Brazil	22° 54' 19.6" S; 48°14' 33.6" W	Fungus garden of *Mycocepurus goeldii*	MT273495	MT273584	MT305404	MT305528	MT305653	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 1057	QVM185	Botucatu, São Paulo, Brazil	22° 54' 18.8" S; 48°14' 33.3" W	Fungus garden of *Mycocepurus goeldii*	MT273496	MT273585	MT305405	MT305529	MT305654	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 1061	QVM189	Botucatu, São Paulo, Brazil	22° 54' 19.5" S; 48°14' 32.7" W	Fungus garden of *Mycocepurus goeldii*	MT273497	MT273586	MT305406	MT305530	MT305655	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 1077	QVM205	Botucatu, São Paulo, Brazil	22° 54' 19.2" S; 48°14' 32.3" W	Fungus garden of *Mycocepurus goeldii*	MT273498	MT273587	MT305407	MT305531	MT305656	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 1078	QVM206	Botucatu, São Paulo, Brazil	22° 54' 19.2" S; 48°14' 32.3" W	Fungus garden of *Mycocepurus goeldii*	MT273499	MT273588	MT305408	MT305532	MT305657	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 1082	QVM210	Botucatu, São Paulo, Brazil	22° 54' 19.2" S; 48°14' 32.3" W	Fungus garden of *Mycocepurus goeldii*	MT273500	MT273589	MT305409	MT305533	MT305658	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 1090	QVM218	Botucatu, São Paulo, Brazil	22° 54' 16.7" S; 48°14' 31.3" W	Fungus garden of *Mycocepurus goeldii*	MT273501	MT273590	MT305410	MT305534	MT305659	This study
Escovopsis trichodermoides	*Luteomyces trichodermoides*	LESF 1109	QVM237	Botucatu, São Paulo, Brazil	22° 54' 17.9" S; 48°14' 33.0" W	Fungus garden of *Mycocepurus goeldii*	MT273502	MT273591	MT305411	MT305535	MT305660	This study

Escovopsis trichodermoides	*Luteomyces trichodermoides*	CBS 137343 ET	VEM001	Rio Claro, Sao paulo, Brazil	22°23'46.93"S, 47°32'40.12"W	Fungus garden of *Mycocepurus goeldii*	KJ485699	MF116052$	KF033128	MT305417#	MT305542#	Masiulionis et al. (2015), Osti and Rodrigues (2018)
Protocrea pallida	*Protocrea pallida*	TFC 99-209	---	New York, Cleaveland	---	---	NR_111329	EU710769	EU703903	---	EU703949	Jaklitsch and Samuels (2011)
Sphaerostilbella aureonitens	*Sphaerostilbella aureonitens*	GJS 74-87	---	---	---	---	FJ442633	HM466683	FJ467644	---	FJ442763	unpublished
Escovopsis kreiselii	*Sympodiorosea kreiselii*	LESF 302	AR14022705	Florianópolis, Santa Catarina, Brazil	27°31'24.96"S 48°25'3.78"W	Fungus garden of *Mycetophylax morchi*	MH715085	MH715099	MH724259	MT305452#	MT305577#	Montoya et al. (2019)
Escovopsis kreiselii	*Sympodiorosea kreiselii*	LESF 303	AR14022705 B	Florianópolis, Santa Catarina, Brazil	27°31'24.96"S 48°25'3.78"W	Fungus garden of *Mycetophylax morchi*	MH715086	MH715100	MH724260	MT305453#	MT305578#	Montoya et al. (2019)
Escovopsis kreiselii	*Sympodiorosea kreiselii*	LESF 304	AR14022705 T2D	Florianópolis, Santa Catarina, Brazil	27°31'24.96"S 48°25'3.78"W	Fungus garden of *Mycetophylax morchi*	MH715087	MH715101	MH724261	MT305454#	MT305579#	Montoya et al. (2019)
Escovopsis kreiselii	*Sympodiorosea kreiselii*	LESF 305	AR14022601	Florianópolis, Santa Catarina, Brazil	27° 37' 49.6" S; 48° 27' 03.6" W	Fungus garden of *Mycetophylax morchi*	MT273438	MT273524	MT305353	MT305455	MT305580	This study
Escovopsis kreiselii	*Sympodiorosea kreiselii*	LESF 306	AR14022705 A	Florianópolis, Santa Catarina, Brazil	27° 31' 25.0" S; 48° 25' 03.8" W	Fungus garden of *Mycetophylax morchi*	MT273439	MT273525	MT305354	MT305456	MT305581	This study
Escovopsis kreiselii	*Sympodiorosea kreiselii*	LESF 307	AR14022705 T2	Florianópolis, Santa Catarina, Brazil	27° 31' 25.0" S; 48° 25' 03.8"W	Fungus garden of *Mycetophylax morchi*	MT273440	MT273526	MT305355	MT305457	MT305582	This study
Escovopsis kreiselii	*Sympodiorosea kreiselii*	LESF 308	AR14022604 AL	Florianópolis, Santa Catarina, Brazil	27° 37' 49.6" S; 48° 27' 03.6"W	Fungus garden of *Mycetophylax morchi*	MT273441	MT273527	MT305356	MT305458	MT305583	This study
Escovopsis kreiselii	*Sympodiorosea kreiselii*	LESF 309	AR14022605 T2	Florianópolis, Santa Catarina, Brazil	27° 37' 47.9" S; 48° 27' 04.0" W	Fungus garden of *Mycetophylax morchi*	MT273442	MT273528	MT305357	MT305459	MT305584#	This study
Escovopsis kreiselii	*Sympodiorosea kreiselii*	CBS 139320 ET	LESF 053	Florianópolis, Santa Catarina, Brazil	27°37'50.01"S 48°27'03.64"W	Fungus garden of *Mycetophylax morchi*	KJ808767	HJ808765	KJ 808766	MT305418#	MT305543#	Meirelles et al. (2015a)
Sympodiorosea sp.	*Sympodiorosea* sp.	LESF 864	SES030331-05	---	---	---	MT273464	MT273553	MT305375	MT305499	MT305624	This study
Sympodiorosea sp.	*Sympodiorosea* sp.	LESF 886	UGM23	---	---	---	MT273468	MT273557	MT305378	MT305502	MT305627	This study
Sympodiorosea sp.	*Sympodiorosea* sp.	LESF 887	UGM26(C)	---	---	---	MT273469	MT273558	MT305379	MT305503	MT305628	This study
Sympodiorosea sp.	*Sympodiorosea* sp.	LESF 897	Q03III	Botucatu, São Paulo, Brazil	22° 54' 19.6" S; 48°14' 33.7" W	Fungus garden of *Mycocepurus goeldii*	MT273472	MT273561	MT305381	MT305505	MT305630	This study
Sympodiorosea sp.	*Sympodiorosea* sp.	LESF 899	Q03V	Botucatu, São Paulo, Brazil	22° 54' 19.6" S; 48°14' 33.7" W	Fungus garden of *Mycocepurus goeldii*	MT273473	MT273562	MT305382	MT305506	MT305631	This study

Sympodiorosea sp.	*Sympodiorosea* sp.	LESF 1010	QVM138	Camp 41, Manaus, Amazonas, Brazil	2° 26' 51.6" S; W59° 45' 53.4" W	Fungus garden of *Apterostigma* sp.	MT273484	MT273573	MT305393	MT305517	MT305642	This study
Trichoderma harzianum	*Trichoderma harzianum*	CBS 226.95	---	England	---	---	AY605713	HM466680	AF534621	JQ031082[#]	AF545549[#]	Chaverri et al. (2003)

[ET] Ex-type; [#] sequences obtained in this study.

Table S3. Molecular markers, primers and Polymerase Chain Reaction (PCR) conditions.

Marker	Primers	PCR conditions	References
ITS	ITS4 (5'TCCTCCGCTTATTGATATGC3') ITS5 (5'GGAAGTAAAAGTCGTAACAAGG3')	96°C for 3 min, 35 cycles at 94°C for 1 min, 55°C for 1 min and a final extension step at 72°C for 2 min	White et al. (1990); Schoch et al. (2012)
*tef*1	EF6–20F (5'AAGAACATGATCACTGGTACCT3') EF6–1000R (5'CGCATGTCRCGGACGGC3')	96°C for 3 min, 35 cycles at 96°C for 30 s, 61°C for 45 s and a final extension step at 72°C for 1 min	Taerum et al. (2007)
LSU	CLA-F (5'GCATATCAATAAGCGGAGGA3') CLA-R (5'GACTCCTTGGTCCGTGTTTCA3')	96°C for 3 min, 35 cycles at 94°C for 1 min, 55°C for 1 min and a final extension step at 72°C for 2 min	White et al. (1990), Haugland and Heckman (1998), Currie et al. (2003)
rpb1	RPB1-Af, RPB1Ac (5'GARTGYCCDGGDCAYTTYGG3') RPB1-Cr (5'CCNGCDATNTCRTTRTCCATRTA3')	96 °C for 5 min followed by 15 cycles at 94 °C for 30s, 65 °C for 1.5 min, (the annealing temperature gradually decreased 1 °C by cycle), and 72 °C for 1.5 min; and 35 cycles at 94°C for 30s, 50 °C for 1min and 72 °C for 1 min.	Liu et al. (1999) (primers), This study (conditions)
rpb2	fRPB2-5F (F) (5'GA(T/C)GA(T/C)(A/C)G(A/T)GATCA(T/C)TT(T/C)GG-3') fRPB2-7cR (R) (5'CCCAT(A/G)GCTTG(T/C)TT(A/G)CCCAT3')	96 °C for 5 min followed by 15 cycles at 94 °C for 30s, 65 °C for 1 min, (the annealing temperature gradually decreased 1 °C by cycle), and 72 °C for 1 min; and 35 cycles at 94°C for 30s, 50 °C for 1min and 72 °C for 1 min.	Liu et al. (1999) (primers), This study (conditions)

Table S4. *Hypocreales* strains used in the phylogenetic analyses and their associated metadata.

Taxon	**Specimen voucher**	**Host/substratum**	**GeneBank accession number**			
			LSU	***tef*1**	***rpb*1**	***rpb*2**
Aphysiostroma stercorarium	ATCC 62321	on cow dung	AF543792	AF543782	AY489633	EF469103
Aschersonia badia	BCC 8105	scale insect (Hemiptera)	DQ518752	DQ522317	DQ522363	DQ522411
Aschersonia placenta	BCC 7869	scale insect (Hemiptera)	EF469074	EF469056	EF469085	EF469104
Balansia henningsiana	GAM 16112	*Panicum* sp. (Poaceae)	AY545727	AY489610	AY489643	DQ522413
Balansia pilulaeformis	AEG 94-2	Poaceae	AF543788	DQ522319	DQ522365	DQ522414
Claviceps paspali	ATCC 13892	Poaceae	U47826	DQ522321	DQ522367	DQ522416
Claviceps purpurea	GAM 12885	*Dactylis glomerata* (Poaceae)	AF543789	AF543778	AY489648	DQ522417
Cordyceps bifusispora	EFCC 5690	lepidopteran pupa	EF468806	EF468746	EF468854	EF468909
Cordyceps cardinalis	OSC 93609	lepidopteran larva	AY184962	DQ522325	DQ522370	DQ522422
Cordyceps kyusyuënsis	EFCC 5886	lepidopteran pupa	EF468813	EF468754	EF468863	EF468917
Cordyceps militaris	OSC 93623	lepidopteran pupa	AY184966	DQ522332	DQ522377	AY545732
Cordyceps cf. *ochraceostromata*	ARSEF 5691	Lepidoptera	EF468819	EF468759	EF468867	EF468921
Cordyceps scarabaeicola	ARSEF 5689	scarabaeid adult (Coleoptera)	AF339524	DQ522335	DQ522380	DQ522431
Cordyceps cf. *takaomontana*	NHJ 12623	Lepidoptera	EF468838	EF468778	EF468884	EF468932
Cordyceps tuberculata	OSC 111002	Lepidoptera	DQ518767	DQ522338	DQ522384	DQ522435
Cosmospora coccinea	CBS 114050 AR2741	*Inonotus nodulosus* (Hymenomycetes)	AY489734	AY489629	AY489667	DQ522438
Elaphocordyceps capitata	OSC 71233	*Elaphomyces* sp. (Euascomycetes)	AY489721	AY489615	AY489649	DQ522421
Elaphocordyceps fracta	OSC 110990	*Elaphomyces* sp. (Euascomycetes)	DQ518759	DQ522328	DQ522373	DQ522425
Elaphocordyceps japonica	OSC 110991	*Elaphomyces* sp. (Euascomycetes)	DQ518761	DQ522330	DQ522375	DQ522428
Elaphocordyceps longisegmentis	OSC 110992	*Elaphomyces* sp. (Euascomycetes)	EF468816		EF468864	EF468919
Elaphocordyceps ophioglossoides	OSC 106405	*Elaphomyces* sp. (Euascomycetes)	AY489723	AY489618	AY489652	DQ522429
Elaphocordyceps subsessilis	OSC 71235	scarabaeid larva (Coleoptera)	EF469077	EF469061	EF469090	EF469108
Engyodontium aranearum	CBS 309.85	spider (Arachnida)	AF339526	DQ522341	DQ522387	DQ522439
Epichloë typhina	ATCC 56429	*Festuca rubra* (Poaceae)	U17396	AF543777	AY489653	DQ522440
Haptocillium balanoides	CBS 250.82	nematode	AF339539	DQ522342	DQ522388	DQ522442
Haptocillium sinense	CBS 567.95	nematode	AF339545	DQ522343	DQ522389	DQ522443
Haptocillium zeosporum	CBS 335.80	nematode	AF339540	EF469062	EF469091	EF469109
Hirsutella sp.	OSC 128575	hemipteran adult	EF469079	EF469064	EF469093	EF469110
Hydropisphaera erubescens	ATCC 36093	*Cordyline banksii* (Laxmanniaceae)	AY545726	DQ522344	DQ522390	AY545731
Hydropisphaera peziza	CBS 102038 GJS 92-101	on bark	AY489730	AY489625	AY489661	DQ522444
Hypocrea lutea	ATCC 208838	on decorticated conifer wood	AF543791	AF543781	AY489662	DQ522446
Hypocrea rufa	CBS 114374	on bark	AY489726	AY489621	AY489656	EF692510 c
Hypocrella schizostachyi	BCC 14123	scale insect (Hemiptera)	DQ518771	DQ522346	DQ522392	DQ522447
Hypocrella nectrioides	GJS 89-104	scale insect (Hemiptera)	U47832	DQ522347	DQ522393	DQ522448
Isaria cf. *farinosa*	OSC 111004	lepidopteran pupa	EF468840	EF468780	EF468886	
Isaria tenuipes	OSC 111007	lepidopteran pupa	DQ518773	DQ522349	DQ522395	DQ522449

Lecanicillium antillanum	CBS 350.85	agaric (Hymenomycetes)	AF339536	DQ522350	DQ522396	DQ522450
Lecanicillium aranearum	CBS 726.73a	spider (Arachnida)	AF339537	EF468781	EF468887	EF468934
Lecanicillium attenuatum	CBS 402.78	leaf litter of *Acer saccharum*	AF339565	EF468782	EF468888	EF468935
Lecanicillium psalliotae	CBS 532.81	soil	AF339560	EF469067	EF469096	EF469112
Lecanicillium psalliotae	CBS 101270	soil	EF469081	EF469066	EF469095	EF469113
Leuconectria clusiae	ATCC 22228	soil	AY489732	AY489627	AY489664	EF469114
Mariannaea pruinosa	ARSEF 5413	*Iragoides fasciata* (Lepidoptera)	AY184968	DQ522351	DQ522397	DQ522451
Metacordyceps chlamydosporia	CBS 101244	egg of slug (Diplopoda)	DQ518758	DQ522327	DQ522372	DQ522424
Metacordyceps liangshanensis	EFCC 1523	lepidopteran pupa	EF468814	EF468755		EF468918
Metacordyceps taii	ARSEF 5714	Lepidoptera	AF543787	AF543775	DQ522383	DQ522434
Metacordyceps yongmunensis	EFCC 2131	lepidopteran pupa	EF468833	EF468770	EF468876	
Metacordyceps sp.	OSC 110996	Lepidoptera	EF468832	EF468773	EF468880	EF468928
Metacordyceps sp.	NHJ 12118	Lepidoptera	EF468829	EF468768	EF468878	EF468927
Metarhizium album	ARSEF 2082	*Cofana spectra* (Hemiptera)	DQ518775	DQ522352	DQ522398	DQ522452
Metarhizium anisopliae	ARSEF 3145	*Oryctes rhinoceros* (Coleoptera)	AF339530	AF543774	DQ522399	DQ522453
Metarhizium flavoviride	ARSEF 2037	*Nilaparvata lugens* (Hemiptera)	AF339531	DQ522353	DQ522400	DQ522454
Microhilum oncoperae	AFSEF 4358	*Oncopera intricata* (Lepidoptera)	AF339532	EF468785	EF468891	EF468936
Myriogenospora atramentosa	AEG 96-32	*Andropogon virginicus* (Poaceae)	AY489733	AY489628	AY489665	DQ522455
Myrothecium cinctum	ATCC 22270	soil	AY489710	AY489605	AY489638	EF692512 c
Myrothecium roridum	ATCC 16297	soil	AY489708	AY489603	AY489636	EF692513 c
Myrothecium verrucaria	ATCC 9095	baled cotton	AY489713	AY489608	AY489641	EF692514 c
Nectria cinnabarina	CBS 114055	*Betula* sp. (Betulaceae)	U00748	AF543785	AY489666	DQ522456
Nomuraea rileyi	CBS 806.71	Lepidoptera	AY624250	EF468787	EF468893	EF468937
Ochronectria calami	CBS 125.87	on palm	AY489717	AY489612	AY489644	EF692515 c
Ophiocordyceps cf. *acicularis*	OSC 128580	Coleoptera	DQ518757	DQ522326	DQ522371	DQ522423
Ophiocordyceps agriotidis	ARSEF 5692	Coleoptera	DQ518754	DQ522322	DQ522368	DQ522418
Ophiocordyceps brunneipunctata	OSC 128576	Coleoptera	DQ518756	DQ522324	DQ522369	DQ522420
Ophiocordyceps entomorrhiza	KEW 53484	coleopteran larva	EF468809	EF468749	EF468857	EF468911
Ophiocordyceps gracilis	EFCC 3101	lepidopteran larva	EF468810	EF468750	EF468858	EF468913
Ophiocordyceps nigrella	EFCC 9247	lepidopteran larva	EF468818	EF468758	EF468866	EF468920
Ophiocordyceps ravenelii	OSC 110995	coleopteran larva	DQ518764	DQ522334	DQ522379	DQ522430
Ophiocordyceps rhizoidea	NHJ 12522	Isoptera	EF468825	EF468764	EF468873	EF468923
Ophiocordyceps sinensis	EFCC 7287	lepidopteran pupa	EF468827	EF468767	EF468874	EF468924
Ophiocordyceps stylophora	OSC 111000	elaterid larva (Coleoptera)	DQ518766	DQ522337	DQ522382	DQ522433
Ophiocordyceps unilateralis	OSC 128574	ant (Hymenoptera)	DQ518768	DQ522339	DQ522385	DQ522436
Ophiocordyceps variabilis	ARSEF 5365	dipteran larva	DQ518769	DQ522340	DQ522386	DQ522437
Ophionectria trichospora	CBS 109876	on liana	AF543790	AF543779	AY489669	DQ522457
Paecilomyces carneus	CBS 239.32	sand dune	EF468843	EF468789	EF468894	EF468938
Paecilomyces lilacinus	CBS 431.87	*Meloidogyne* sp. (Nematoda)	EF468844	EF468791	EF468897	EF468940
Paecilomyces marquandii	CBS 182.27	soil	EF468845	EF468793	EF468899	EF468942
Peethambara spirostriata	CBS 110115	on leaves of *Buxus sempervirens*	AY489724	AY489619	AY489654	EF692516 [c]
Pochonia bulbillosa	CBS 145.70	root of *Picea abies*	AF339542	EF468796	EF468902	EF468943

Pochonia chlamydosporia	CBS 504.66	nematode	AF339544	EF469069	EF469098	EF469120
Pochonia gonioides	CBS 891.72	nematode	AF339550	DQ522354	DQ522401	DQ522458
Pochonia rubescens	CBS 464.88	*Heterodera avenae* (Nematoda)	AF339566	EF468797	EF468903	EF468944
Pseudonectria rousseliana	CBS 114049	*Buxus sempervirens* (Buxaceae)	U17416	AF543780	AY489670	DQ522459
Rotiferophthora angustispora	CBS 101437	rotifer (Rotifera)	AF339535	AF543776	DQ522402	DQ522460
Roumegueriella rufula	CBS 346.85	*Globodera rostochiensis* (Nematoda)	DQ518776	DQ522355	DQ522403	DQ522461
Shimizuomyces paradoxus	EFCC 6279	*Smilax sieboldi* (Smilacaceae)	EF469084	EF469071	EF469100	EF469117
Simplicillium lamellicola	CBS 116.25	*Agaricus bisporus* (Hymenomycetes)	AF339552	DQ522356	DQ522404	DQ522462
Simplicillium lanosoniveum	CBS 101267	*Hemileia vastatrix* (Urediales)	AF339554	DQ522357	DQ522405	DQ522463
Sphaerostilbella berkeleyana	CBS 102308	polypore (Hymenomycetes)	U00756	AF543783	AY489671	DQ522465
Stachybotrys echinata	UAMH 6594	indoor air	AY489736	AY489631	AY489672	EF692518 [c]
Stachybotrys chlorohalonata	UAMH6417					
Stachybotrys subsimplex	ATCC 32888	water hyacinth	AY489711	AY489606	AY489639	EF692519 [c]
Stilbocrea macrostoma	CBS 114375 GJS 73-26	*Geniostoma ligustifolia*	AY489725	AY489620	AY489655	EF692520 [c]
Tolypocladium parasiticum	ARSEF 3436	bdelloid rotifer (Rotifera)	EF468848	EF468799	EF468904	EF468945
Torrubiella confragosa	CBS 101247	*Coccus viridis* (Hemiptera)	AF339555	DQ522359	DQ522407	DQ522466
Torrubiella ratticaudata	ARSEF 1915	spider (Arachnida)	DQ518777	DQ522360	DQ522408	DQ522467
Torrubiella wallacei	CBS 101237	Lepidoptera	AY184967	EF469073	EF469102	EF469119
Verticillium epiphytum	CBS 384.81	*Hemileia vastatrix* (Uredinales)	AF339547	EF468802		EF468947
Verticillium incurvum	CBS 460.88	*Ganoderma lipsiense* (Hymenomycetes)	AF339551	DQ522362	DQ522410	DQ522470
Verticillium sp.	CBS 102184	spider (Arachnida)	AF339564	EF468803	EF468907	EF468948
Viridispora diparietispora	ATCC MYA627	*Crataegus crus-galli* (Rosaceae)	AY489735	AY489630	AY489668	DQ522471

Table S5. Culture media used in previous studies to evaluate the morphological characters of *Escovopsis* species.

Media	Formula or Brand	Reference
CMD (cornmeal agar)	Neogen Culture Media	Meirelles et al. (2015a), Montoya et al. (2019)
CYA (Czapek yeast extract agar)	30 g L-1 of Sucrose (Labsynth®), 5 g L-1 of Yeast extract (Neogen Culture Media), 1 g L-1 of KH_2PO_4 (Labsynth®), 0.3 g L-1 of $NaNO_3$ (Synth), 0.05 g L-1 of KCl (Labsynth®), 0.05 g L-1 of $MgSO_4(7H_2O)$ (Labsynth®), 0.001 g L-1 of $FeSO_4$ (Labsynth®), 0.001 g L-1 of $ZnSO_4$ (Labsynth®), 0.0005 g L-1 of $CuSO_4$ (Labsynth®), 15 g L-1 of Agar (Neogen Culture Media)]	Seifert et al. (1995), Montoya et al. (2019)
MA2% (malt agar 2%)	20 g L-1 of malt extract (Neogen Culture Media) and 15 g L-1 of agar (Neogen Culture Media)	Augustin et al. (2013), Meirelles et al. (2015a), Montoya et al. (2019)
MEA (malt extract agar 2%)	30 g L-1 of malt extract (Neogen Culture Media), 5 g L-1 of bacteriological peptone (Neogen Culture Media), 20 g L-1 of glucose (Labsynth®), and 15 g L-1 of Agar (Neogen Culture Media)]	Seifert et al. (1995), Masiulionis et al. (2015), Montoya et al. (2019)
OA (oatmeal agar)	60g L-1 oatmeal and 15 g L-1 of Agar (Neogen Culture Media)	Augustin et al. (2013), Masiulionis et al. (2015), Montoya et al. (2019
PCA (potato carrot agar)	HiMedia®	Augustin et al. (2013), Montoya et al. (2019
PDA (potato dextrose Agar)	Neogen Culture Media	Seifert et al. (1995), Augustin et al. (2013), Meirelles et al. (2015a), Masiulionis et al. (2015), Montoya et al. (2019)
SNA (synthetic nutrient agar)	0.1 g L-1 of KH_2PO_4 (Labsynth®), 1 g L-1 of KNO_3 (Labsynth®), 0.5 g L-1 of $MgSO_4(7H_2O)$ (Labsynth®), 0.5 g L-1 of KCl (Labsynth®), 0.2 g L-1 of Glucose (Labsynth®), 0.2 g L-1 of Sucrose (Labsynth®) and 15 g L-1 of Agar (Neogen Culture Media)	Meirelles et al. (2015a), Montoya et al. (2019)

Table S6. Morphological features used to construct the dichotomous key of *Escovopsis*, *Luteomyces*, and *Sympodiorosea* spp.

	Morphological characters	*E. aspergiloides*	*E. clavata*	*E.lentecrescens*	*E. microspora*	*E. moelleri*	*E. multiformis*	*E. weberi*	*Luteomyces*	*Sympodiorosea*
1	Colonies growing at 10°C	no	no	no	yes	yes	yes	yes	yes	yes
2	Colonies growing 30 °C	no	no	no	yes	no	yes	yes	yes	yes
3	Brown colour on Colonies at seven days	yes	yes	yes	yes	yes	yes	yes	no	no
4	Pink colour on Colonies at 14 days	no	no	no	no	no	no	no	no	yes
5	Yellow colour on Colonies at 14 days	no	no	no	no	no	no	no	yes	no
6	Pink to pinkish-Brown colour on Colonies at seven days on MEA and PDA	no	no	no	yes	no	no	yes	no	no
7	Light-yellow to yellowish-brown colour on Colonies at seven days on PDA	yes	no	no	no	no	no	no	no	no
8	Pustules formation	no	no	no	yes	no	yes	yes	no	no
9	Pyramidal and irregular conidiophore	no	no	no	yes	yes	no	yes	yes	no
10	Only irregular conidiophores	yes	yes	yes	no	no	yes	no	no	no
11	Conidiophore with swollen cell	no	yes	no	no	no	yes	no	no	no
12	Conidiophore axis ending in an infertile hypha	no	yes	no	no	no	no	no	no	no
13	Conidiophore branch as long as conidiofore axis	no	yes	no	no	no	yes	no	no	no
14	Vesicle	yes	yes	yes	yes	yes	yes	yes	no	no
15	Vesicle globose	yes	yes	yes	no	no	yes	no	no	no
16	Vesicle capitate	yes	yes	yes	no	no	yes	no	no	no
17	Vesicle obovoid	yes	yes	yes	no	no	yes	no	no	no
18	Vesicle prolate	yes	yes	no	no	no	yes	no	no	no
19	Vesicle spatulate	yes	yes	no	no	no	yes	no	no	no
20	Vesicle clavate	yes	yes	no	yes	yes	yes	yes	no	no
21	Vesicle cymbiform	no	yes	no	yes	yes	yes	yes	no	no
22	Vesicle oblanceolate	no	no	no	yes	yes	no	yes	no	no
23	Vesicle Subulate	no	no	no	yes	yes	no	yes	no	no
24	Vesicle cilindric	no	yes	no	yes	yes	yes	yes	no	no
25	Vesicle filiform	no	no	no	yes	no	no	yes	no	no
26	Vesicle clavate-septate	no	no	no	yes	yes	no	yes	no	no
27	Vesicle cilindric-septate	no	no	no	yes	no	no	yes	no	no
28	Phialide	yes	yes	yes	yes	yes	yes	yes	no	no
29	phialides on aerial mycelia	no	no	no	yes	no	no	yes	no	no

30	Short and wide phialides	no	no	no	no	yes	no	no	no	no
31	Long and thin phialides	yes	yes	yes	yes	no	yes	yes	no	no
32	Conidia in long chains	yes	yes	yes	yes	no	yes	yes	no	no
33	Conidia in short chains	yes	yes	yes	yes	no	yes	yes	no	no
34	Conidia smooth and with thickened walls	yes	yes	yes	yes	no	yes	yes	no	no
35	Conidia with ornamentation	no	no	no	no	yes	no	no	yes	no
36	Conidia alone	no	no	no	no	no	no	no	yes	yes

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Chapter III

Digging into the past of a fungus-growing ant guest: origin and evolution of *Escovopsis*

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Abstract

Host-parasite relationships shape the evolution of organisms. Fungi in the genus *Escovopsis* are thought to have co-evolved with fungus-growing ants parasitizing their fungal cultivars. However, the origin of *Escovopsis*, how and when the genus reached attine colonies, and its geographical distribution were never addressed so far. Here we investigate the origin and trait evolution of *Escovopsis* to shed light on the evolutionary history of the genus, its relationship with the attine ants and the adaptations it experienced throughout its lifetime. Our results showed that *Escovopsis*: i) is distributed across America and has originated approximately 56.9 Mya, ii) lived outside attine colonies for almost 20 Mya before gaining access to this environment, iii) co-evolved only with few attine ant genera, and iv) has experienced several trait adaptations likely related to overcome the barriers of the attine colonies and establish within this environment. Hypotheses on the ecology of the *Escovopsis* ancestor, the possible scenarios of how it reached the colonies, its evolution, and its current ecology are also discussed.

Keywords: Parasite, Fungus-growing ants, Symbiosis, Evolution, trait evolution

Introduction

Host-parasite relationships are one of the major drivers of the evolution of organisms [1–5]. Exploring the origin of host-parasite relationships is a difficult task [6], but it help us to understand the trait adaptations of the organisms involved[7]. *Escovopsis* (Ascomycota: Hypocreales, Hypocreaceae) is a common resident of fungus-growing ant colonies (subfamily Myrmicinae, Attina subtribe, "the attines"), which was suggested to have co-evolved with these ants parasitizing their fungal cultivars [8–14]. Parasites associated with social insect environments are extremely important to the evolutionary success of these organisms because they affect both the host which they rely on and the colony to which they belong [15–17]. Therefore, the discovery of a specialized parasite within attine ant colonies [10,18] was of great importance for the study of the evolution of these insects. Nonetheless, while it is known that attines originated approximately 65 million years ago [19–21], the origin of *Escovopsis* is still a mystery. Accordingly, the evolutionary history of this genus, when it gained access to attine colonies, and when it adopted a parasitic lifestyle remain poorly understood.

The current *Escovopsis* - attine ant co-evolution hypothesis is based on the assumption that *Escovopsis* parasitizes the fungal cultivars and on the co-cladogenesis patterns between *Escovopsis* and attine ants [9–13,22,23]. Nevertheless, besides the unknown origin of *Escovopsis*, taxonomic studies evidenced that the genus is paraphyletic [24–27]. Recently, *Escovopsis* was re-assessed using a detailed taxonomic and phylogenetic approach and the evidence showed more than one genus within *Escovopsis* (Montoya et al. 2020 submitted). Accordingly, the same study showed that *Escovopsis* could not be related to the

entire Attina subtribe but only with few genera. Notwithstanding, to which attine genera and fungal cultivars *Escovopsis* is related to, is still not fully understood. Although the taxonomy and phylogeny of *Escovopsis* moved forward, other important questions still remain open such as: what is the extent of *Escovopsis* diversity? And how is the genus phylogeographically distributed? To answer those and other similar questions, a large collection of *Escovopsis* from all attine ant genera and from different locations across America is necessary.

Parasites develop different mechanisms to overcome the barriers of their hosts in search for nutrients [28–31]. However, hosts are not passive agents but they react to kill or avoid parasites [32]. The reciprocal adaptations of hosts and parasites to the pressures generated by their interactions and the environment promote the evolution of behavioural, physiological, and morphological traits in both organisms [33–36]. Attine ant colonies are extremely complex superorganisms which present many barriers to avoid the entrance of alien microorganisms within their fungus gardens[37]. Consequently, it is expected that *Escovopsis* had undergone different trait adaptations during the process of establishment within the colonies, and to cope to the pressures generated by the immune system of the colonies and the mutualistic fungus responses (which are still unexplored[37]). Nonetheless, evolutionary adaptations of *Escovopsis* whether behavioural, physiological, or morphological are completely unknown.

In this study, we shed light on the origin and evolutionary history of *Escovopsis*. By assessing a large collection of *Escovopsis* strains from several attine species from many countries across America we determined: i) the origin and the time during the evolutionary process when the genus entered attine ant colonies, ii) its geographical distribution, iii) its phylogenetic correlation with the attine ants and iv) several trait adaptations experienced by

the genus throughout its evolution. Our results are not only answering key-questions on the ecology and evolution of *Escovopsis* but it will allow scientists to better understand the evolutionary history of the attine ants' system.

Results

Escovopsis is not associated to all fungus-growing ants, but only to some genera

Our results evidence that *Escovopsis* is only associated with higher attine ants *Atta*, *Acromyrmex*, *Sericomyrmex*, and *Trachymyrmex*, and with the lower attine genus *Apterostigma* (Fig. 1A-B). There are no exclusive co-cladogenesis patterns between the *Escovopsis* and ant clades (Fig. 1A-B). However, the most derived *Escovopsis* clade (clade I) are more related to the most derived attines clades, the leaf-cutting ant genera *Atta* and *Acromyrmex* (Fig. 1A-B). *Escovopsis moelleri*, *E. lentecrescens*, and *E. aspergilloides* clades (Clade II and III) are more related to *Trachymyrmex* (Fig. 1A-B) and the most basal *Escovopsis* clade (*E. multiformis* and *E. clavata* - Clade IV) is more related to *Apterostigma* (Fig. 1A-B). Interestingly, the ant genera *Atta*, *Acromyrmex* and *Trachymyrmex* harbour the greatest diversity of *Escovopsis*. Moreover, while no *Escovopsis* from the most basal clade were found in *Atta* and *Acromyrmex*, some strains isolated from fungus gardens of *Apterostigma* spp. (NGL075, 080, 081, 083 and LESF 878) are placed in the most derived clade of the genus (Fig. 1A-B).

Escovopsis is distributed across America

Our findings revealed that *Escovopsis* is distributed throughout the American continent (Fig. 1B-C). Our results do not evidence a geographic pattern of distribution of the genus, likely influenced by the difference between the attines sampling efforts. For instance,

most of the *Escovopsis* strains where isolated from Panamá and Brazil. However, there are still many countries (Argentina, Paraguay, Uruguay, Bolivia, Chile, Peru, Colombia, Ecuador, Venezuela, Guyana, Surinam, Costa Rica, Nicaragua, Honduras, El Salvador, Guatemala, Belize, Mexico, and United States) from where few samples were obtained or there are no samples at all. This sample gap generates fragmentary information about the phylogeographic distribution of the genus. Despite this, it is important to highlight that samples from a small region in Panama harbours a great diversity of *Escovopsis* placed throughout the genus phylogeny and the most derived *Escovopsis* clade was only found in this country so far. On the other hand, while Brazil also presents a great *Escovopsis* diversity, the genus is spread across the country, in an area much larger than that observed in Panama, but without a specific prevalence. However, these results could be also being influenced by the difference between the attines sampling efforts in both Panama and Brazil. Future studies that fill this gap will shed light on where *Escovopsis* has originated and how was the genus diversification since there.

Escovopsis originated in the same period as fungus-growing ants but entered their colonies 18 Mya later

Divergence time analysis revealed that *Escovopsis* originated 56.9 Mya ago (clade 12 – Fig. 2), a one-time close to the origin of the attine ants and the fungus domestication [21]. *Escovopsis* diversified into four major clades (clades 13, 15, 17, 18 – Fig. 2). The most basal clade (clade 13) originated approximately 39.1 Mya ago, while the most derived clade (clade 18) originated 17.2 Mya ago. These results point that *Escovopsis* had an independent evolutionary history from the attine before these ants introduce it to their colonies at least 18 Mya later of its origin (Fig. 2).

Escovopsis has experienced different trait adaptations since its arrival to the attine ants' colonies

Morphometric, growth rate and character state reconstruction analysis in *Escovopsis* (Fig. 3) revealed that: i) conidiophores of the basal clades in *Escovopsis* usually form terminal swollen cells at the apex (from where the branches are formed – Fig. 3C). However, these structures are absent in the most derived clades (Fig. 3B); ii) vesicles of the conidiophores have been gradually changing from globose to cylindrical as the genus was diversifying, and the most derived clades are losing these structures (Fig. 3A); iii) the number of vesicles per conidiophore has dramatically increased (from 1-2 in the more basal clades to 53 in the more derived once) throughout the genus diversification (Fig. 3L); iv) Despite the modification of the vesicles, the number of phialides per vesicle has remained over the evolution (Fig. 3M); v) the production of phialides on the basal clades of the genus is restricted to the vesicles (Fig. 3F-I), while the most derived strains are also producing them on the aerial mycelia (Fig. 3D-E); vi) the colony growth rate on artificial media has gradually increased as the genus diversified (Fig. 3J-K).

Discussion

This study provides evidence on the evolutionary history of *Escovopsis*, as well as on the association of the genus with fungus-growing ants, its geographical distribution and the trait adaptations it experienced across its existence. Our results supported that *Escovopsis* is widely distributed across America, and while its origin corresponds in time with the origin of the fungus-growing ants, the genus did not co-evolve with all attines, but just with few

genera. Besides, the ancestor of *Escovopsis* lived outside the attine colonies and once it was introduced to this environment it experienced several trait adaptations likely related to overcome the barriers of the ant environment and to thrive within the fungus gardens. These results fill important gaps on the ecology and evolution of *Escovopsis* and give new directions to understand the evolution of the attine ants' system.

Combining the evidence on the origin of *Escovopsis* (Fig. 2), the *Escovopsis*-ant interaction (Fig. 1), the origin of attines[19,21], and the ants' collecting behaviour[37,38] there are four possible scenarios to explain the evolution of *Escovopsis*, its arrival in the attine ant colonies, and the ecological role of its ancestor. First, the ancestor of *Escovopsis* was living outside the attine colonies at least 18 Mya. This scenario is possible if we consider that: i) *Escovopsis* originated ≅ 56.9 Mya ago, its most basal clade has ≅ 39.1 Mya, and the most derived clade ≅ 17.2 Mya, ii) *Escovopsis* is only associated to *Atta*, *Acromyrmex*, *Trachymyrmex*, *Sericomyrmex* and *Apterostigma* (Fig. 1A-B), iii) *Apterostigma* originated approximately 38 Mya ago, *Trachymyrmex* and *Sericomyrmex* 28 Mya ago, and *Atta*, *Acromyrmex* 18 Mya ago[21]. Therefore, *Escovopsis* might have been living, at least, 18 Mya outside the attine's colonies, and reached this environment at most the last 38 Mya. Thus, *Escovopsis* would have reached first the *Apterostigma* colonies (≅ 38 Mya ago), then the *Trachymyrmex* and *Sericomyrmex* colonies (≅ 28 Mya ago), and last the *Atta* and *Acromyrmex* colonies (≅ 18 Mya ago). Second, the *Escovopsis* ancestor had a saprotrophic lifestyle, living in association with dead plant material, dry seeds, insect frass and carcasses. Thus, the ancestor might have been introduced within the attines' colonies along with the substrate collected by the ants. Three facts support this scenario: i) *Escovopsis* clades related to *Apterostigma*, *Trachymyrmex*, and *Sericomyrmex* originated before the *Escovopsis* clades

associated with *Atta* and *Acromyrmex* [21]; ii) The genera *Apterostigma*, *Trachymyrmex*, and *Sericomyrmex* collect dead plant material to feed their mutualists [38]; iii) *Apterostigma* has a preference for collecting insect frass and carcasses. Third, the *Escovopsis* ancestor had an endophytic lifestyle, living mainly in the leaf's mesophyll, and was introduced within the attines' colonies along with the fresh plant material collected by the ants. Three facts support this hypothesis: i) the most derived *Escovopsis* clade (Fig. 2) originated at the same period of time as *Atta*, and *Acromyrmex*[21] and it is mostly related to these ant genera (Fig. 1A-B), ii) the most remarkable collecting behaviour that differs *Atta* and *Acromyrmex* to the other attine genera is the preference for fresh plant material to feed their mutualist [37,38] and, iii) different from the other attine genera these ants evolved complex and careful behaviours to clean the surface of the collected material, before adding them in the fungus gardens [37,39]. Accordingly, it is less likely that fungi that colonize the surface of the collected substrate can reach the gardens of *Atta* and *Acromyrmex*. Meaning that, *Escovopsis* must have been living in the leaf's mesophyll as an endophyte. Fourth, the *Escovopsis* ancestor had symbiotic relationships with other fungi. This scenario could be possible if we consider that attines from the genus *Acromyrmex* can also collect basidiomatas in nature [40]. This behaviour is still not well studied for the other attines; however, future studies should confirm if this scenario is likely.

The current ecological role of *Escovopsis* is of great interest for scientists that study the attine ant-fungal mutualism [10,13,23,41–46]. The life-cycle of the genus is a cornerstone to unravel this issue, yet it is completely unknown. However, there are two important pieces of information that can help us to have a glimpse about how this big puzzle looks like. First, although the ants share some *Escovopsis* strains, the most derived clades of *Escovopsis* are more related with *Atta* and *Acromyrmex* (which harbour the greatest diversity of *Escovopsis*),

the most basal clades of *Escovopsis* are more related with *Apterostigma* and the clades in transition of the genus are more related with *Trachymyrmex* (Fig. 1A-B). Second, while *Atta* and *Acromyrmex* (leaf-cutting ants) collect preferentially fresh plant material [38], and *Sericomyrmex* and *Trachymyrmex* prefer to collect dry plant material, fruits and seeds [38], the preference for those substrates are not exclusive to none of the groups. Sporadically leaf-cutting ants also collect dry plant material, fruits and seeds, and *Sericomyrmex* and *Trachymyrmex* can also collect fresh plant material [38]. On the other hand, *Apterostigma* collects preferentially insects' frass, but they can also collect dry plant material, fruits and seeds [38]. Therefore, it is very likely that *Escovopsis* is still entering the colonies together with the material collected by the ants (as saprotrophs or endophytes). Then, once the genus reaches the fungus gardens it would be using its saprotrophic ability to take advantage of nutrients of that environment[47], competing with other organisms (including the fungal cultivars) and probably using parasitic mechanisms to take advantage of their competitors. Furthermore, considering that *Escovopsis* that forms globose vesicles are mostly associated to *Apterostigma*; those that form sub-globose to clavate vesicles are more related *Trachymyrmex* and *Sericomyrmex*; and those that form cylindric vesicles to filiform (non-vesicles) are mostly associated to *Atta* and *Acromyrmex*; *Escovopsis* strains with globose vesicles could be more related with a saprotrophic lifestyle, while strains that present sub-globose - clavate and cylindrical - non-vesicles could present both saprotrophic and endophytic lifestyles.

Fungi in the Hypocreaceae are widely known as mycoparasites, however, only few species in this family present a true parasitic lifestyle [48,49]. Conversely, the vast majority of members in this family are somehow associated with plants, either as endophytes or as

saprotrophs [50–54]. Furthermore, parasites in this family are not obligate parasites (like biotrophs), but they can also live as endophytes or saprotrophs [53–55]. Because phylogenetic closely related species can exhibit similar traits or behaviours [56], it is expected that some strains in *Escovopsis* could also present a mycoparasitic lifestyle. Indeed, just one strain from one out of 26 clades of *Escovopsis* (Montoya et al. 2020 submitted) was reported as a virulent parasite[9], and most of the strains of the genus only affect fungal cultivars *in vitro* or in dysfunctional colonies in the laboratory but not in natural conditions.

Parasites develop specialized organs, chemical compounds and other mechanisms to adhere, penetrate and avoid host immune systems [57–59]. Our results evidenced that as *Escovopsis* diversified, it lost the swollen cells of their conidiophores, changed the shape of their vesicles, increased the number of the vesicles per conidiophore, start producing phialides on the aerial mycelia and gradually increased its growth rate. By avoiding swollen cells, the fungus was able to produce more branches per conidiophore which in turn increased the number of vesicles per conidiophore. Because phialides (cells responsible for the conidia production) are produced on vesicles, the number of vesicles is directly related to the number conidia produced. Thus, the larger the vesicles, the greater the number of phialides and the greater the amount of conidia produced. If we add the fact that the growth velocity of the species has also increased as the genus derived (Fig.4J-K), then we can conclude that *Escovopsis* is evolving to increase its reproduction efficiency. Considering what was discussed about the ecology of *Escovopsis* it is more likely that the ecosystem of the attine ant colonies is the main factor that influenced trait evolution in *Escovopsis*.

On the other hand, hosts react against parasites by: i) behavioural avoidance [60,61], ii) physical or physiological barriers [62], iii) fighting against within-host development [17,63] and

iv) adjusting their immune systems [28,64]. Nonetheless, the adaptation of both the fungal cultivar and the ecosystem of the attines to avoid or kill *Escovopsis* are completely unknown. Moreover, considering: i) that more than half of the organisms on our planet have a parasitic lifestyle [17,65], ii) the huge diversity of microorganisms introduced by attines into their gardens for tens of millions of years [19], iii) that the presence of different parasites living on the same host at the same time is a fairly common event in nature [66], and iv) that other Hypocreaceae genera like *Trichoderma* and *Cladobotryum* also have the potential to act as mycoparasites [49,54,67]; then, the possibility that other fungi could be also taking advantage of both the fungal cultivar and the ecosystem of the attines is highly expected. Consequently, trait adaptations of both the mutualistic fungus and the attines' ecosystem (whatever they are) must have arisen in response to the combination of the pressures generated by all these organisms instead of just one of them. Future studies should consider to evaluate the trait evolution of the attines colonies and of their fungal cultivar to understand the parasitic dynamics of this ecosystem.

Material and Methods

Isolates

This study amassed a total of 400 colonies from 11 out of 17 attine genera (*Atta*, *Acromyrmex*, *Apterostigma*, *Cyphomyrmex*, *Myrmecocrypta*, *Mycetarotes*, *Mycetophylax*, *Mycetoritis*, *Myrmicocrypta*, *Sericomyrmex*, and *Trachymyrmex*). The ants' colonies were collected in six different countries (Argentina, Brazil, Costa Rica, Ecuador, Panama, and United States). A total of 286 *Escovopsis* strains where isolated from these colonies (Table

S1). A total of 64 out of the 286 strains were isolated in this study and the remaining were obtained from the Gerardo Laboratory (Emory University, Atlanta, USA) and the Laboratory of Fungal Ecology and Systematics (LESF - São Paulo State University, Rio Claro, SP, Brazil).

For *Escovopsis* isolation, we followed the methods performed by Montoya et al. (2019)[68]. Briefly, seven fungus-garden fragments (varying 0.5–1 mm^3) were inoculated on plates containing potato dextrose agar (PDA, Neogen Culture Media, Neogen®) supplemented with 150 µg mL^{-1} chloramphenicol (Sigma). Three plates were inoculated per each ant colony. Once *Escovopsis* was isolated from garden fragments, axenic cultures were prepared and stored in sterile distilled water (Castellani 1963) at 8 – 10 °C, and in 10% glycerol at -80 °C. All strains were deposited at LESF and at the Microbial Resources Center (CRM-UNESP).

DNA extraction, PCR and sequencing

The genomic DNA of the 286 strains was extracted using a modified CTAB method (Möller et al. 1992). Briefly, fungal aerial mycelia, grown for seven days at 25 °C on PDA, was crushed with the aid of glass beads (Sigma) in lysis solution and incubated at 65 °C for 30 minutes. The organic phase was separated using a solution of chloroform-isoamyl alcohol (24:1). Then, the material was centrifuged (10,000 g for 10 minutes), and the supernatant with the genomic DNA was collected. This extract was precipitated with 3M sodium acetate and isopropanol and purified with two successive washes of 70% ethanol. The DNA was suspended in 30 µL of Tris-EDTA solution and stored at -20 °C.

Five molecular markers were amplified for *Escovopsis* strains isolated in this study: the internal transcribed spacer (ITS4 - 5'TCCTCCGCTTATTGATATGC3', ITS5 - 5'GGAAGTAAAAGTCGTAACAAGG3')[69,70], the large subunit ribosomal RNA (CLA-F - 5'GCATATCAATAAGCGGAGGA3', CLA-R - 5'GACTCCTTGGTCCGTGTTTCA3')[10], the translation elongation factor 1-alpha (EF6–20F - 5'AAGAACATGATCACTGGTACCT3', EF6–1000R - 5'CGCATGTCRCGGACGGC3')[71], the RNA polymerase II protein-coding gene *rpb*1 (RPB1-Af, RPB1Ac - 5'GARTGYCCDGGDCAYTTYGG3', RPB1-Cr - 5'CCNGCDATNTCRTTRTCCATRTA3')[72], and the RNA polymerase II protein-coding gene rpb2 (fRPB2-5F (F) - 5'GA(T/C)GA(T/C)(A/C)G(A/T)GATCA(T/C)TT(T/C)GG-3'), fRPB2-7cR (R) - 5'CCCAT(A/G)GCTTG(T/C)TT(A/G)CCCAT3')[72]. From the strains obtained from Gerardo Laboratory and LESF we used previously published ITS, LSU and *tef*1 sequences, when available, and generated the missing sequences.

PCR reactions for ITS, LSU and *tef*1 were performed in a final volume of 25 µL (4 µL of dNTPs [1.25 mM each]; 5 µL of 5X buffer; 1 µL of BSA [1 mg mL^{-1}]; 2 µL of $MgCl_2$ [25 mM]; 1 µL of each primer [10 µM]; 0.5 µL of Taq polymerase [5 U μL^{-1}], 2 µL of diluted genomic DNA [1:100] and 8.5 µL of sterile ultrapure water); and in the case of *rpb*1 and *rpb*2, we added 1.5 µL of dimethyl sulfoxide (DMSO) and decreased the volume of sterile ultrapure water to 7.0 µL. PCR condition for ITS and LSU was: 96 °C for 3 min, 35 cycles at 94 °C for 1 min, 55 °C for 1 min and a final extension step at 72°C for 2 min [10,69,70]. For *tef*1 the conditions were: 96 °C for 3 min, 35 cycles at 96 °C for 30 s, 61 °C for 45 s and a final extension step at 72 °C for 1 min [42]. For *rpb*1 and *rpb*2 a touchdown PCR was used (96 °C for 5 min; 15 cycles of 94 °C for 30s, 65 °C for 1.5 min for *rpb*1 and for 1 min for *rpb*2 (the annealing temperature gradually decreased 1 °C per cycle) and 72 °C for 1.5 min for

*rpb*1 and for 1 min for *rpb*2; and then 35 cycles of 94 °C for 30s, 50 °C for 1 min and 72 °C for 1 min (Montoya et al. 2020 submitted).

Final amplicons were purified with the Wizard SV Gel and PCR Clean-up System (Promega) following the manufacturer's protocol. Sequences (forward and reverse) were generated on an ABI3500 (Life Technologies), and the consensus sequences were assembled in BioEdit v. 7.1.3 [73] and Geneious [74]. All sequences will be deposited in GenBank (Table S2).

Phylogenetic analysis

We reconstructed the *Escovopsis* phylogeny to evaluate the *Escovopsis* – ants co-cladogenesis and geographic distribution of the genus. A multilocus analysis was carried out for this purpose. The final dataset contained 286 sequences of 3709 bp in length [ITS (587 bp in length), LSU (591 bp), *tef*1 (758 bp), *rpb*1 (607 bp), and *rpb*2 (1045 bp)], and *Sympodiorosea kreiselii* CBS 139320^{ET} (Ex-type strain) was used as the outgroup.

Each file was aligned separately in MAFFT (KATOH; STANDLEY, 2013) and the nucleotide substitution model of each alignment was calculated in jModeltest 2 [75]. Then, all files were concatenated using Winclada v.1.00.08 [76] and the phylogenetic tree was inferred using a Bayesian approach in MrBayes v. 3.2.1 [77]. The analysis was carried out with twelve separate runs (each consisting of eleven hot chains and one cold chain) in CIPRES (http://www.phylo.org/). We used GTR+I+G model for ITS, LSU and *tef*1, and GTR for the genes *rpb*1 and *rpb*2. Ten million generations of the Markov Chain Monte Carlo (MCMC) were necessary to reach convergence [standard deviation of split frequencies fell below 0.01]. The first 50% of the generations of MCMC sampling were discarded as burn-in to generate

a consensus tree, and the final tree was edited in FigTree v.1.4 (http://tree.bio.ed.ac.uk/software/figtree/) and Adobe Illustrator CC v.17.1.

Divergence time analysis

To estimate the origin of the genus *Escovopsis* we reconstructed a phylogenetic tree combining the sequences of the representative strains of each clade in *Escovopsis* (n=35), *Escovopsioides* (n=10), *Luteomyces* (n=10), and *Sympodiorosea* (n=12) with the sequences of the Hypocreales provided by Sung et al. (2008). Thus, 172 sequences of LSU (594 bp), *tef*1 (776 bp), *rpb*1 (820 bp), and *rpb*2 (998 bp) were aligned separately in MAFFT [78] and concatenated in Winclada v.1.00.08 [76]. The final phylogenetic tree was inferred using the GTR model (calculated in jModeltest 2) [75] under a Bayesian approach [77].

Then, we carried out a divergence time analysis based on the calibration of the Hypocreales tree made by Sung et al. (2008)[79]. To do so, we uploaded the alignments of LSU, *tef*1, *rpb*1, and *rpb*2 separately into BEAUti [74] to create the xml file with the partitions, the clock model and the priors. We use the nodes of the previous inferred tree as priors of monophyletic clades and the calibration of the tree was based on seven nodes previously calibrated by Sung et al. (2008)[79] [node 1 (193 Mya), node 2 (176 Mya), node 3 (178 Mya), node 4 (170 Mya), node 5 (173 Mya), node 6 (165Mya) and node 7 (158 Mya)]. The analysis was performed in BEAST 2.5 [80]. We used the uncorrelated relaxed clock site model [81] with a GTR substitution model to accommodate the rate heterogeneity across the branches of the tree. The clock model was the relaxed clock log normal with the priors specifying a Birth Death model processes, with the origin height of 193 (158, 232) Mya. We conducted four independent analyses with an MCMC length of 50.000.000 generations each, with sampling of every 100th generation and removing 300.000 of generations as burn in. Finally, 750.000

trees where summarised using TreeAnotator 2.6 in BEAST[74], and the final tree was manually edited using FigTree v.1.4 and Adobe Illustrator CC v.17.1.

Morphometric analysis of vesicles

A total of 22 strains representing all clades of the *Escovopsis* phylogeny were selected to evaluate the morphometry of the vesicles. Therefore, we carried out slide culture preparations to assess these microscopic structures (Montoya et al. 2020 submitted). To achieve this, each strain was inoculated on a 0.5 mm^3 block piece of PDA medium, covered with a coverslip and incubated at 25 °C for 5 days in darkness. Then, the coverslips were removed and placed on slides with a drop of lactophenol. The slides were examined under a light microscope (DM750, Leica, Germany) and the vesicles were photographed using LAS EZ v.4.0 (Leica Application Suite).

The images of each of 22 strains (30 vesicles per strain) were then used to extract vesicles (660 vesicles in total) that were converted into black silhouettes, placed on white backgrounds and saved as jpeg files using GIMP 2.8 [82]. The outlines were then imported into R as a list of coordinates and four landmarks placed on the widest points of each vesicle (top, bottom and sides), to avoid unexpected twisting of specimens and improve their alignment. We estimated in Momocs that 32 harmonics were more than enough to achieve 99% of harmonic power[83].

To extract shape information from *Escovopsis* vesicles we used the morphometric technique of Elliptic Fourier analysis (eFa) as implemented in the library Momocs v1.3.2 [83] for the R statistical environment [84]. eFa is widely used by morphologists to quantify variation in shapes from outline data and has been used with all kinds of structures, including leaves [85], fossil bivalves [86], and fish [87]. The coefficients were summarized using principal

components analysis (PCA) and morphospaces were drawn to visualize and interpret the results.

Ancestral state reconstruction

To better understand the evolution of vesicle shape in *Escovopsis*, we conducted an ancestral state reconstruction of this structure as a continuous variable. We exported the first principal component from the eFa, calculated a mean value for each strain, matched the strains to the chronogram and used the function contMap of the library phytools v0.7-70 [88] to reconstruct overall vesicle shape. This approach takes as input the phylogenetic tree and data for each tip and estimates the ancestral states at internal nodes using Maximum Likelihood and then interpolates the states along each edge [89,90].

Colony growth rate analysis

The same strains used in the morphometric and the ancestral state reconstruction analyses were also evaluated for the growth rate variation across the *Escovopsis* phylogeny. We followed the growth method described in Montoya et al. (2020 submitted). Briefly, 200 μL of 10^6 conidia per 1 uL were surface spread on Petri dishes (90 x 15 mm) containing water-agar (WA) and incubated for seven days at 25 °C in darkness. Then, we obtained fragments of 0.5 cm diameter of WA with mycelia and inoculated (four replicates to each strain) at the center of Petri dishes (90 x 15 mm) containing cornmeal agar (CMD - Neogen Culture Media) and malt extract agar 2% [MEA - 30 g L-1 of malt extract (Neogen Culture Media), 5 g L^{-1} of bacteriological peptone (Neogen Culture Media), 20 g L^{-1} of glucose (Labsynth®), and 15 g L^{-1} of Agar (Neogen Culture Media)]. This experiment was performed using four replicates of each media and for each strain, in three different weeks. The plates

were incubated at 25 ºC for four days in darkness and then we measured the colonies radius. Statistical analysis was performed in R Studio using one-way ANOVA, followed by Duncan's multiple range test, differences were considered significant when $P \leq 0.05$.

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Author Contributions

QVM, MJSM, NMG and AR designed the study. QVM carried out Phylogenetic Analysis. QVM and MJSM carried out the Growth rate and Microscopic experiments. QVM, CLS, RK carried out the morphometric and Ancestral state reconstruction analysis. QVM, AR, MSJM, NMG and AR wrote the manuscript.

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Figure legends

Fig. 1.

Escovopsis - attine ants co-cladogenesis and phylogeographic distribution. A-B) *Escovopsis* - attine ants co-cladogenesis. Lines indicate the *Escovopsis* strains associated to *Atta* (green), *Acromyrmex* (blue), *Trachymyrmex* (purple), *Sericomyrmex* (orange) and *Apterostigma* (red). C-D) Phylogeographic distribution of *Escovopsis* in the American continent. Lines plotted in the map indicate the origin of the ants from where *Escovopsis* strains were isolated.

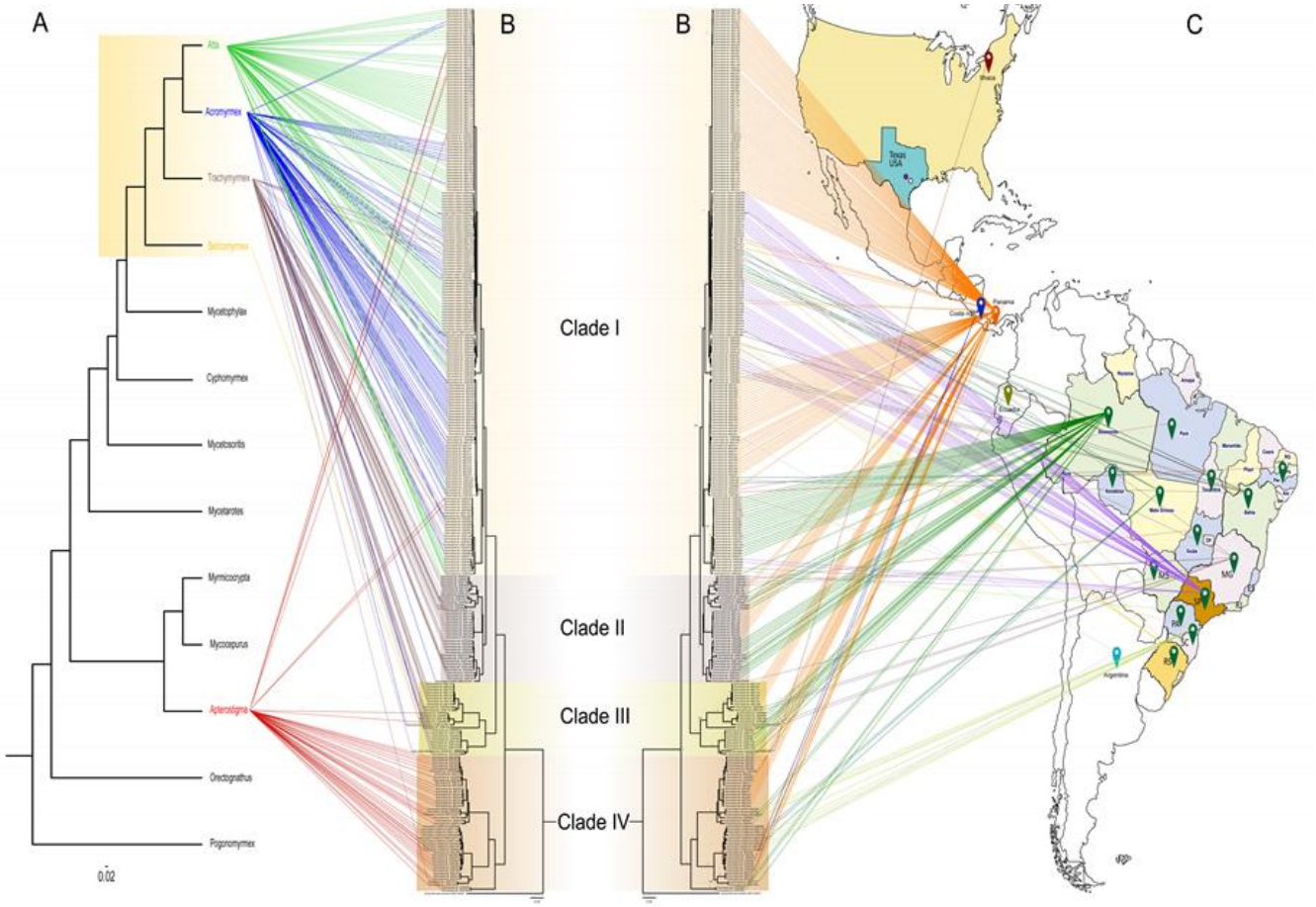


Fig. 2.

Divergence age estimates of *Escovopsis*. Chronogram was constructed based on the tree obtained in the maximum likelihood analyses. A) Calibration was based on seven nodes from Hypocreales previously calibrated by Sung et al. (2008). B) Extended region of the Hypocreales. Calibrated nodes in Hypocreaceae are shown in blue. C) Ages in millions of years (Mya) of each calibrated node. Scales of each Chronogram in Mya.

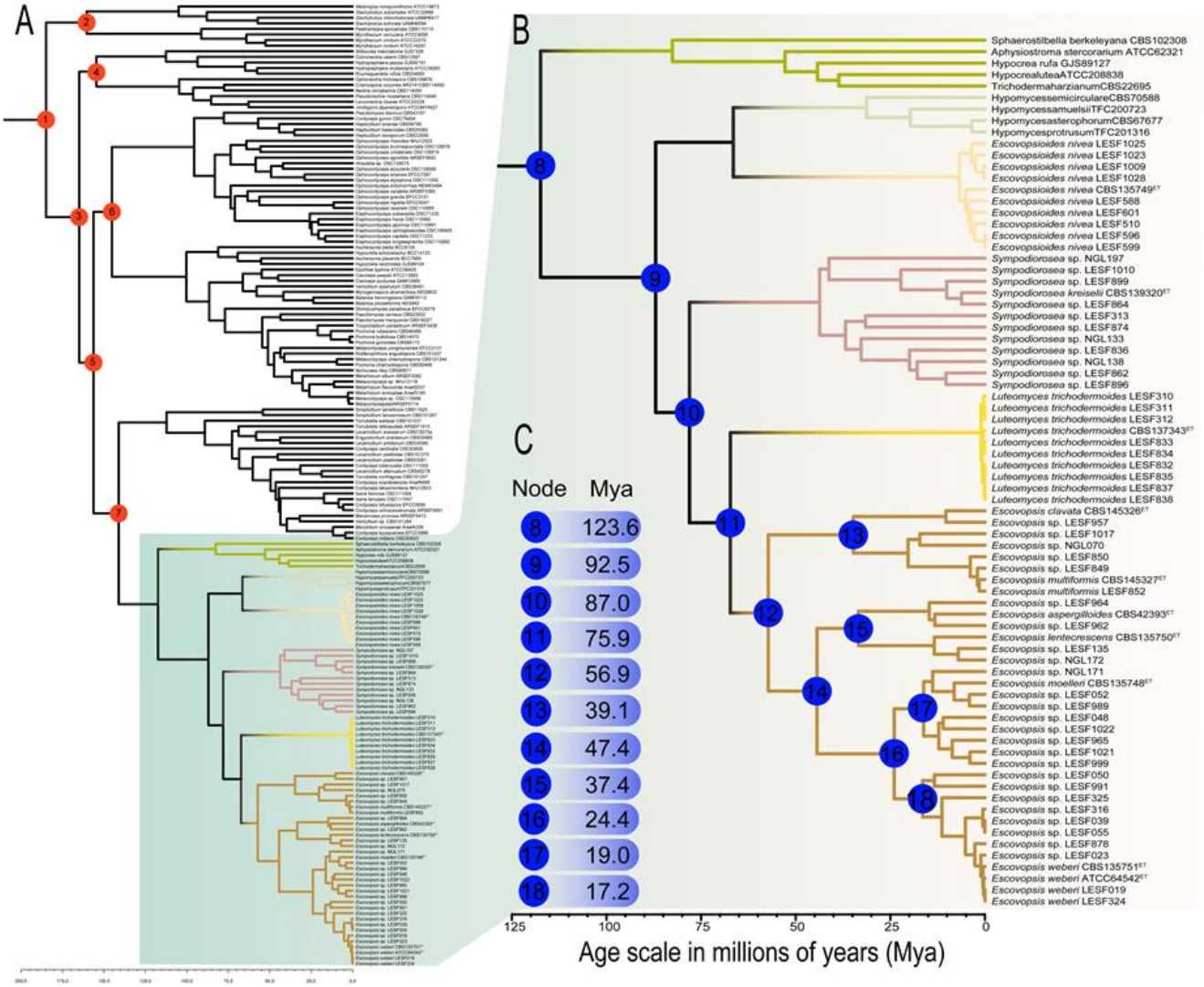
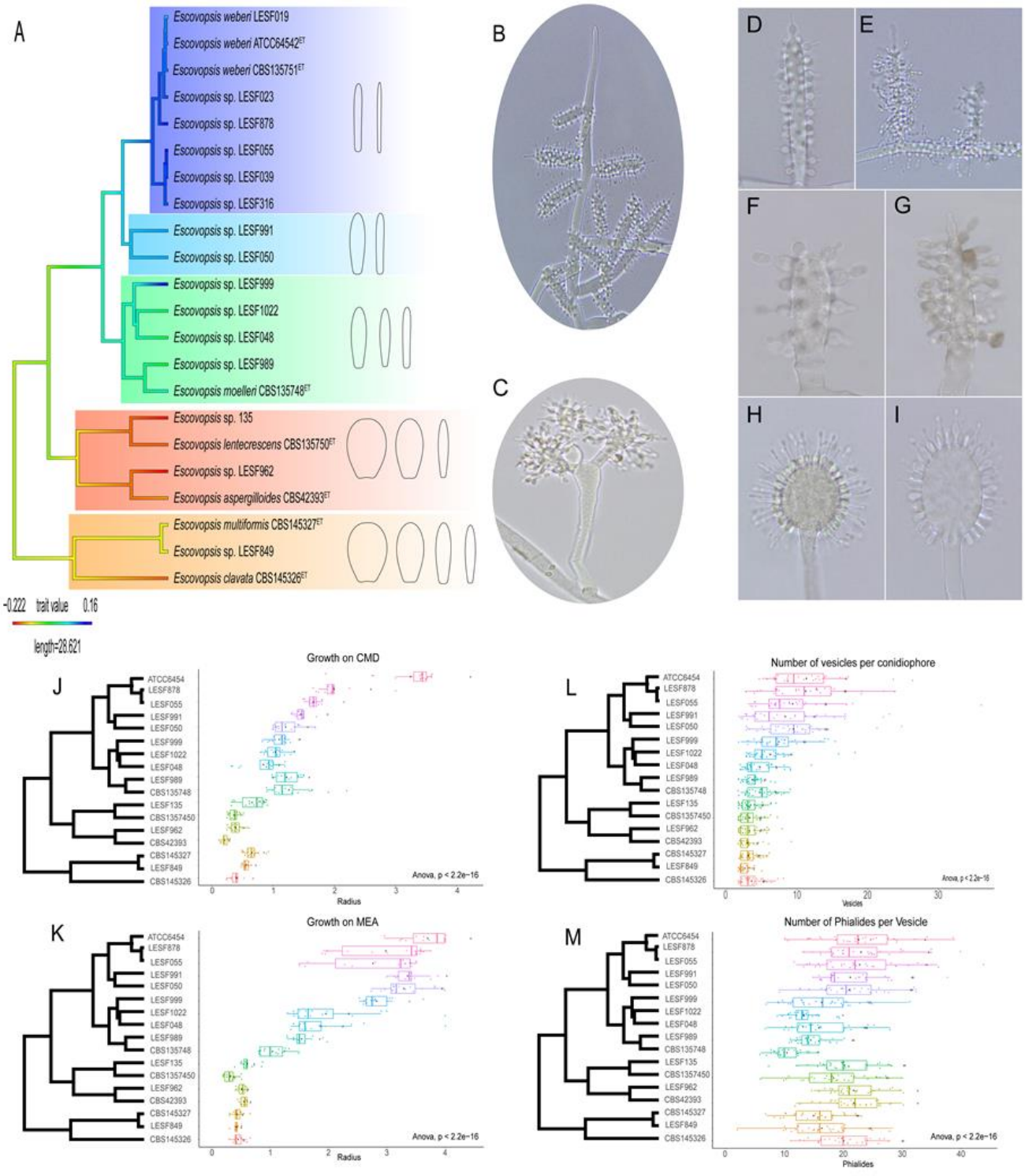


Fig. 3.

Escovopsis trait adaptations over the evolutionary time. A) Ancestral state reconstruction of the vesicles. Strains in the basal clades have globose vesicles that change into cylindric and no-vesicles in strains of the more derived clades. B-C) Types of conidiophores. Strains in the basal clades present conidiophores with swollen cells which appear to be lost in the most derived clades. D-I) Phialides formation. Strains in the basal and in the transitional clades form phialides only on vesicles (F-I – that correspond with the clades in red to light-blue on the ancestral state reconstruction tree) and strains in the most derived clades forms phialides on both vesicles (D) and aerial mycelia (E). J-K) Colony growth rates of the strains from (A). Increased growth rates correlate with the genus diversification. L) Number of vesicles per conidiophore. Increased number of vesicles also correlate with genus diversification. M) Number of phialides per vesicles. *Escovopsis* maintained the number of phialides over the evolution.


A
Escovopsis weberi LESF019
Escovopsis weberi ATCC64542ET
Escovopsis weberi CBS135751ET
Escovopsis sp. LESF023
Escovopsis sp. LESF878
Escovopsis sp. LESF055
Escovopsis sp. LESF039
Escovopsis sp. LESF316
Escovopsis sp. LESF991
Escovopsis sp. LESF050
Escovopsis sp. LESF999
Escovopsis sp. LESF1022
Escovopsis sp. LESF048
Escovopsis sp. LESF989
Escovopsis moelleri CBS135748ET
Escovopsis sp. 135
Escovopsis lentecrescens CBS135750ET
Escovopsis sp. LESF962
Escovopsis aspergilloides CBS42393ET
Escovopsis multiformis CBS145327ET
Escovopsis sp. LESF849
Escovopsis clavata CBS145326ET
-0.222 trait value 0.16
length=28.621
B
C
D
E
F
G
H
I
J
Growth on CMD
ATCC6454
LESF878
LESF055
LESF991
LESF050
LESF999
LESF1022
LESF048
LESF989
CBS135748
LESF135
CBS1357450
LESF962
CBS42393
CBS145327
LESF849
CBS145326
Anova, p < 2.2e−16
Radius
K
Growth on MEA
Anova, p < 2.2e−16
Radius
L
Number of vesicles per conidiophore
Anova, p < 2.2e−16
Vesicles
M
Number of Phialides per Vesicle
Anova, p < 2.2e−16
Phialides

Overall thesis conclusion

Fungiculture by attine ants have fascinated scientists since its discovery about 150 years ago. In addition to the economic importance of some attine species, colonies of these social insects are models to study evolution and symbiosis. Parasites directly influence the evolution of ecosystems, so that the discovery of *Escovopsis* was of great importance to understand the evolution of the fungus-growing ants' environment. The lack of a standardized taxonomy as well as an unresolved phylogeny of *Escovopsis*, studies on its origin, evolution, and ecology (lifestyle) have clouded our understanding of the impact of this fungus in the evolution of fungus-growing ants. By providing grounds for the systematics of *Escovopsis*, inferring its origin and raising hypothesis on its evolution, this study not only provides a stable foundation from which to build future research on the taxonomic diversity, ecology and the evolutionary history of the genus, but raises a different point of view on the evolution of the *Escovopsis*-attine ant symbiosis. We hope to open new windows for discussion to understand the evolution of both *Escovopsis* and the attine ants' ecosystem.