Taxonomy and systematics of the fungus-growing ant associate *Escovopsis* (*Hypocreaceae*)

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Abstract: *Escovopsis* is a symbiont of fungus-growing ant colonies. Unstandardised taxonomy prevented the evaluation of the morphological diversity of *Escovopsis* for more than a century. The aim of this study is to create a standardised taxonomic framework to assess the morphological and phylogenetic diversity of *Escovopsis*. Therefore, to set the foundation for *Escovopsis* taxonomy and allow interspecific comparisons within the genus, we redescribe the ex-type cultures of *Escovopsis aspergilloides*, *E. clavata*, *E. lentecrescens*, *E. microspora*, *E. moelleri*, *E. multiformis*, and *E. weberi*. Thus, based on the parameters adopted in this study combined with phylogenetic analyses using five molecular markers, we synonymize *E. microspora* with *E. weberi*, and introduce 13 new species isolated from attine nests collected in Argentina, Brazil, Costa Rica, Mexico, and Panama: *E. breviramosa*, *E. chlamydosporosa*, *E. diminuta*, *E. elongatistipitata*, *E. gracilis*, *E. maculosa*, *E. papillata*, *E. peniculiformis*, *E. phialicopiosa*, *E. pseudocylindrica*, *E. rectangula*, *E. rosisimilis*, and *E. spicaticlavata*. Our results revealed a great interspecific morphological diversity throughout *Escovopsis*. Notwithstanding, colony growth rates at different temperatures, as well as vesicle shape, appear to be the most outstanding features distinguishing species in the genus. This study fills an important gap in the systematics of *Escovopsis* that will allow future researchers to unravel the genetic and morphological diversity and species diversification of these attine ant symbionts.

Key words: fungus-growing ants, *Hypocreaceae*, new taxa, symbiosis, systematics, taxonomic diversity.

Taxonomic novelties: New species: *Escovopsis breviramosa* Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, *E. chlamydosporosa* Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, *E. diminuta* Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, *E. elongatistipitata* Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, *E. gracilis* Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, *E. maculosa* Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, *E. papillata* Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, *E. peniculiformis* Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, *E. phialicopiosa* Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, *E. pseudocylindrica* Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, *E. rectangula* Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, *E. rosisimilis* Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, *E. spicaticlavata* Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues.

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INTRODUCTION

The genus *Escovopsis* (*Ascomycota*: *Sordariomycetes*, *Hypocreales*, *Hypocreaceae*) is a common inhabitant of fungus-growing ant colonies (*Formicidae*: *Myrmicinae*: *Attini*: *Attina*, often known as the "attines") that have co-evolved with these insects (Currie *et al.* 2003, Yek *et al.* 2012, Gotting *et al.* 2022). Attine colonies are a model system for studies on symbiosis and evolution (Mueller *et al.* 1998, Mueller & Gerardo 2002, Caldera *et al.* 2009) because the evolutionary success of these ants is directly influenced by microorganisms. Therefore, establishing *Escovopsis* taxonomy is necessary to interpret the impact of species diversity in the genus and roles played by these fungi in the symbiotic network that enabled the evolutionary success of attines. However, since the discovery of *Escovopsis* by Möller (1893), the assessment of the morphological diversity of the genus has been almost completely neglected, and the absence of a taxonomic framework continues to limit the description of new species (Montoya *et al.* 2019, 2021).

Thus far, 12 *Escovopsis* species have been described (Muchovej & Della Lucia 1990, Seifert *et al.* 1995, Augustin *et al.* 2013, Marfetán *et al.* 2019, Montoya *et al.* 2019). However, an evaluation of morphological characters in the genus is extremely difficult because the cultivation media used to assess cultural and microscopic morphology differ in each study, and most descriptions are based on only one or a few isolates of each species. For example, in the case of *E. weberi*, the type species of the genus (Muchovej & Della Lucia 1990), features of colonies grown on culture media are still unknown, and its microscopic morphology is still not fully described (Augustin *et al.* 2013).

The first step towards standardization of parameters to describe *Escovopsis* species was provided by Seifert *et al.* (1995), who described colonies of *E. aspergilloides* on malt extract agar (MEA) and Czapek yeast agar (CYA), typically used in descriptions of *Penicillium* and *Aspergillus* species (Samson *et al.* 2014, Visagie *et al.* 2014), together with potato dextrose agar (PDA), widely used for other fungi in *Hypocreaceae*. Micromorphology was described from colonies grown on MEA, the standard then used for *Penicillium* and *Aspergillus.* However, the conditions used to assess the morphology of subsequent species descriptions of *Escovopsis lentecrescens*, *E. microspora*, and *E. moelleri* (Augustin *et al.* 2013); as well as *E. atlas*, *E. catenulata*, *E. longivesica*, *E. primorosea*, and *E. pseudoweberi* (Marfetán *et al.* 2019) varied from those used by Seifert *et al.* (1995). Although *E. clavata* and *E. multiformis* were described according to Seifert *et al.* (1995) and

Augustin *et al.* (2013), morphological comparisons of these species with the other described are confounded by the use of different media (Montoya *et al.* 2019).

The value of standardizing cultivation media, a uniform set of diagnostic and descriptive characters, accompanied by a comprehensive reference set of diagnostic DNA sequences, has been demonstrated, for example, by the adoption of such protocols by the communities of taxonomists working on the taxonomy of *Penicillium* and *Aspergillus* (Samson *et al.* 2014, Visagie *et al.* 2014). In these genera, the standardised approach has facilitated the description of many new species by taxonomists from laboratories all around the world.

This study proposes a standardised taxonomic framework for *Escovopsis* species delimitation, based on the combination of macroscopic characters using three different media and a consistent set of micromorphological characters, with phylogenetic analyses using a set of five molecular markers, following Genealogical Concordance Phylogenetic Species Recognition (GCPSR) (Taylor *et al.* 2000). This approach will provide a solid ground for identifying species in the genus. In addition, following these standards, thirteen new *Escovopsis* species are proposed here.

MATERIALS AND METHODS

Sampling and strains

We used 138 *Escovopsis* strains, including ex-type cultures of *E. aspergilloides*, *E. clavata*, *E. lentecrescens*, *E. microspora*, *E. moelleri*, *E. multiformis*, and *E. weberi* (Table 1). The ex-type material of the five species from Argentina described by Marfetán *et al.* (2019) were unavailable and we did not obtain any isolates that we could identify as these species, so it was not possible to include them in our study. Of the 138 isolates, 86 were obtained from previous studies (Currie *et al.* 2003, Augustin *et al.* 2013, Meirelles *et al.* 2015a, b, Montoya *et al.* 2019, 2021) whereas the remaining 52 isolates were obtained from attine nests collected in Argentina, Brazil, Costa Rica, Mexico, and Panama (Table 1). For isolating cultures in this study, we followed the methods published in Montoya *et al.* (2019). Briefly, ant garden fragments (0.5–1 mm^3) were inoculated onto PDA (Neogen® Culture Media, Lansing, USA) supplemented with 150 µg/mL of chloramphenicol (Sigma-Aldrich, St. Louis, USA). We inoculated three plates for each ant fungus garden and seven garden fragments per plate. The plates were incubated at 25 °C in darkness and monitored daily for seven days. When *Escovopsis* mycelia were grown, they were transferred to new PDA plates without chloramphenicol and finally axenic cultures were obtained by single conidial isolation.

All *Escovopsis* isolates used in this study (except those that have the specimen vouchers QVM281–QVM289) are stored at the Laboratory of Fungal Ecology and Systematics [LESF–Department of General and Applied Biology, São Paulo State University (UNESP), Rio Claro, SP, Brazil] in sterile distilled water at 8–10 °C (Castellani 1963), in 10 % aqueous solution of glycerol at -80 °C (cryopreservation), and as freeze-dried in 10 % Skim Milk. Isolates that have the specimen vouchers QVM281–QVM289 were sequenced while still viable, but later they lost viability, so we were unable to preserve them (Table 1). Holotypes (metabolically inactive, freeze-dried cultures) and the ex-type cultures of the new species were also deposited at the culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands (CBS) (Table 1).

Adjusting the parameters to evaluate the morphology of *Escovopsis*

To enable reliable comparison of cultural and micromorphological characters, we selected a set of media and optimal cultivation conditions for *Escovopsis* species. To do so, we recorded characters of colonies in culture, *i.e.*, mycelium colour, morphology and presence of soluble pigments, in 21 isolates of seven *Escovopsis* species (including the ex-type cultures), *i.e.*, *E. aspergilloides* (n = 1), *E. clavata* (n = 3), *E. lentecrescens* (n = 1), *E. microspora* (n = 1), *E. moelleri* (n = 1), *E. multiformis* (n = 4), and *E. weberi* (n = 10), using the combination of all conditions, *i.e.*, eight media [cornmeal agar dextrose (CMD), CYA, PDA, malt agar 2 % (MA2 %), MEA, oatmeal agar (OA), potato carrot agar (PCA), and synthetic nutrient-poor agar (SNA) — Supplementary Table S1] and five temperatures (10, 20, 25, 30, 35 °C), used in previous studies (Seifert *et al.* 1995, Augustin *et al.* 2013, Masiulionis *et al.* 2015, Meirelles *et al.* 2015a, b, Montoya *et al.* 2019).

To standardize the inoculum (make the number of conidia in all inoculum approximately the same), we homogeneously spread 200 µL of 10^6 conidia/mL (from 7-d-old colonies), on Petri dishes (90 × 15 mm) with water agar (WA) (Montoya *et al.* 2019). These Petri dishes were incubated for 7 d at 25 °C in darkness (Montoya *et al.* 2019). An agar plug (*ca*. 5 mm diam × 5 mm height) of WA with mycelium was cut and inoculated in the centre of Petri dishes (90 × 15 mm) containing each test medium, and incubated at 10, 20, 25, 30, and 35 °C. All Petri dishes were incubated unsealed to allow air exchange and better development of fungal colonies (Montoya *et al.* 2019). We performed three replicates for each ex-type culture at each media and temperature. Morphological characters were examined every 24 h for 14 d. From this experiment, we selected CMD (Neogen® Culture Media, Lansing, USA), MEA [30 g/L of malt extract (Neogen® Culture Media, Lansing, USA), 5 g/L of bacteriological peptone (Neogen® Culture Media, Lansing, USA), 20 g/L of glucose (Labsynth, Diadema, Brazil), and 15 g/L of Agar (Neogen® Culture Media, Lansing, USA], and PDA as the most suitable media to evaluate the macroscopic features of *Escovopsis* species. These media were selected based on the (i) ease to evaluate the growth rate; (ii) expression of unique phenotypic characters of each species, (iii) feasibility of comparison of morphological features of *Escovopsis* with other genera in the *Hypocreaceae*, and (iv) ease of access to media in most laboratories. To describe the colony colours, we used the standard names and codes provided by Ridgway (1912). Likewise, the optimal time for measuring the growth and evaluating the macroscopic characters of colonies were standardised based on the point at which we were able to observe the most significant differences in growth rate and morphological characters between species.

To evaluate the growth rate of the *Escovopsis* ex-type cultures, and the new described species, on the selected media, we grew the colonies, in quadruplicate, on each medium at 20, 25 and 30 °C, on four separate time periods for 1 wk. Measurements of the colony radius (Supplementary Table S2) were carried out on the fourth day (see the results section for details on the selected temperatures and time for growth measurements). The growth measurements shown in the descriptions of the species (taxonomy section) represent the minimum and maximum values observed among the 16 values obtained. Statistical analyses were performed in R Studio using one-way ANOVA, followed by Duncan's multiple range test. Differences were considered significant when $P \leq 0.05$.

Table 1. Isolates and their associated metadata used in the phylogenetic analysis of *Escovopsis* based on five gene markers (Figs 3, S1). In addition to 138 isolates of *Escovopsis* spp., *Sympodiorosea kreiselii* CBS 139320 was used as the outgroup.

Fungal species name	Isolate ID	Specimen voucher	City, State, Country	Geographical coordinates	Habitat	GenBank accessions					References
						ITS	LSU	*tef*1	*rpb*1	*rpb*2	
E. 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); Montoya *et al.* (2021)
E. breviramosa	CBS 149741[T]	LESF 055; AR022	Camacan, Bahia, Brazil	15°23'43.0"S 39°33'49.1"W	Fungus garden of *Acromyrmex* sp.	KM817044	OQ589727	KM817114	OQ596350	OQ603820	Meirelles *et al.* (2015b); This study
	LESF 039[$]	RS019	Nova Petrópolis, Rio Grande do Sul, Brazil	29°22'38.2"S 50°57'18.1"W	Fungus garden of *Acromyrmex ambiguus*	KM817076	OQ589725	EU082802	OQ596348	OQ603818	Meirelles *et al.* (2015b); This study
	LESF 040	RS020	Nova Petrópolis, Rio Grande do Sul, Brazil	29°19'05.9"S 51°10'13.6"W	Fungus garden of *Acromyrmex laticeps*	KM817077	OQ589721	EU082803	OQ596344	OQ603814	Meirelles *et al.* (2015b); This study
	LESF 041	RS030	São Marcos, Rio Grande do Sul, Brazil	28°58'02.8"S 51°08'08.8"W	Fungus garden of *Acromyrmex lundii*	KM817078	OQ589728	EU082795	OQ596351	OQ603821	Meirelles *et al.* (2015b); This study
	LESF 045	RS076	Vacaria, Rio Grande do Sul, Brazil	28°27'51.7"S 50°53'07.0"W	Fungus garden of *Acromyrmex coronatus*	KM817082	OQ589726	EU082801	OQ596349	OQ603819	Meirelles *et al.* (2015b); This study
	LESF 316	ES001	Rio Claro, São Paulo, Brazil	22°23'46.0"S 47°32'43.2"W	Fungus garden of *Mycetomoellerius* sp.	KM817052	OQ589720	KM817122	OQ596343	OQ603813	Meirelles *et al.* (2015b); This study
E. chlamydosporosa	CBS 149748[T]	LESF 984; QVM71	Novo Airão, Amazonas, Brazil	2°31'25.6"S 60°49'32.4"W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	OQ589809	OQ589759	OQ603902	OQ596382	OQ603852	This study
	LESF 1000	QVM87	Novo Airão, Amazonas, Brazil	–	Fungus garden of *Acromyrmex* sp.	OQ589814	OQ589764	OQ603907	OQ596387	OQ603857	This study
	LESF 1001	QVM88	Novo Airão, Amazonas, Brazil	2°32'01.4"S 60°50'00.4"W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	OQ589799	OQ589749	OQ603892	OQ596372	OQ603842	This study
	LESF 1002	QVM89	Novo Airão, Amazonas, Brazil	2°32'01.4"S 60°50'00.4"W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	OQ589800	OQ589750	OQ603893	OQ596373	OQ603843	This study
	LESF 1026[$]	QVM154	Manaus, Amazonas, Brazil	2°26'52.56"S 59°45'53.4"W	Fungus garden of *Trachymyrmex sp. sensu lato*	OQ589807	OQ589757	OQ603900	OQ596380	OQ603850	This study
	LESF 961[$]	QVM48	Novo Airão, Amazonas, Brazil	2°16'15.7"S 61°01'8.46"W	Fungus garden of *Acromyrmex* sp.	OQ589801	OQ589751	OQ603894	OQ596374	OQ603844	This study
	LESF 963[$]	QVM50	Novo Airão, Amazonas, Brazil	2°16'15.7"S 61°01'8.46"W	Fungus garden of *Acromyrmex* sp.	OQ589802	OQ589752	OQ603895	OQ596375	OQ603845	This study
	LESF 966	QVM53	Novo Airão, Amazonas, Brazil	2°16'14.5"S 61°01'6.8"W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	OQ589803	OQ589753	OQ603896	OQ596376	OQ603846	This study
	LESF 967	QVM54	Novo Airão, Amazonas, Brazil	2°16'14.5"S 61°01'6.8"W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	OQ589804	OQ589754	OQ603897	OQ596377	OQ603847	This study
	LESF 970	QVM57	Novo Airão, Amazonas, Brazil	2°31'23.4"S 60°49'31.9"W	Fungus garden of *Apterostigma* sp.	OQ589794	OQ589744	OQ603887	OQ596367	OQ603837	This study
	LESF 971	QVM58	Novo Airão, Amazonas, Brazil	–	Fungus garden of *Acromyrmex* sp.	OQ589795	OQ589745	OQ603888	OQ596368	OQ603838	This study

Table 1. (Continued).

Fungal species name	Isolate ID	Specimen voucher	City, State, Country	Geographical coordinates	Habitat	GenBank accessions					References
						ITS	LSU	*tef*1	*rpb*1	*rpb*2	
	LESF 972	QVM59	Novo Airão, Amazonas, Brazil	–	Fungus garden of *Apterostigma* sp.	OQ589796	OQ589746	OQ603889	OQ596369	OQ603839	This study
	LESF 974	QVM61	Novo Airão, Amazonas, Brazil	–	–	OQ589797	OQ589747	OQ603890	OQ596370	OQ603840	This study
	LESF 976	QVM63	Novo Airão, Amazonas, Brazil	–	–	OQ589808	OQ589758	OQ603901	OQ596381	OQ603851	This study
	LESF 977	QVM64	Novo Airão, Amazonas, Brazil	–	–	OQ589811	OQ589761	OQ603904	OQ596384	OQ603854	This study
	LESF 978	QVM65	Novo Airão, Amazonas, Brazil	–	–	OQ589812	OQ589762	OQ603905	OQ596385	OQ603855	This study
	LESF 981	QVM68	Novo Airão, Amazonas, Brazil	–	Fungus garden of attini	OQ589805	OQ589755	OQ603898	OQ596378	OQ603848	This study
	LESF 982	QVM69	Novo Airão, Amazonas, Brazil	–	Fungus garden of attini	OQ589806	OQ589756	OQ603899	OQ596379	OQ603849	This study
	LESF 986	QVM73	Novo Airão, Amazonas, Brazil	2°31'.25.3''S 60°49'33.1''W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	OQ589798	OQ589748	OQ603891	OQ596371	OQ603841	This study
	LESF 991[$]	QVM78	Novo Airão, Amazonas, Brazil	2°31'26.04"S 60°49'31.62"W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	OQ589813	OQ589763	OQ603906	OQ596386	OQ603856	This study
	LESF 995	QVM82	Novo Airão, Amazonas, Brazil	–	Fungus garden of *Acromyrmex* sp.	OQ589810	OQ589760	OQ603903	OQ596383	OQ603853	This study
E. clavata	CBS 145326 [ET]	LESF 853; 1707	Florianópolis, Santa Catarina, Brazil	27°44'39.6''S 48°31'10.14''W	Fungus garden of *Apterostigma* sp.	MH715096	MH715110	MH724270	MT305419	MT305544	Montoya *et al.* (2019, 2021)
	LESF 854[$]	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, 2021)
	LESF 855[$]	1705B	Florianópolis, Santa Catarina, Brazil	27°44'39.49''S 48°31'9.72''W	Fungus garden of *Apterostigma* sp.	MH715098	MH715112	MH724272	MT305496	MT305621	Montoya *et al.* (2019, 2021)
E. diminuta	CBS 149747[T]	LESF 969; QVM56	Novo Airão, Amazonas, Brazil	2°31'23.4"S 60°49'31.9''W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	MT273476	MT273565	MT305385	MT305509	MT305634	Montoya *et al.* (2021)
	LESF 1003[$]	QVM90	Novo Airão, Amazonas, Brazil	2°32'1.4"S 60°50'0.4"W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	MT273482	MT273571	MT305391	MT305515	MT305640	Montoya *et al.* (2021)
	LESF 996[$]	QVM83	Novo Airão, Amazonas, Brazil	2°32'02.7"S 60°50'11.7"W	Fungus garden of *Apterostigma* sp.	MT273480	MT273569	MT305389	MT305513	MT305638	Montoya *et al.* (2021)
	LESF 997	QVM84	Novo Airão, Amazonas, Brazil	2°31'23.4"S 60°49'31.9''W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	MT273481	MT273570	MT305390	MT305514	MT305639	Montoya *et al.* (2021)
E. elongatistipitata	CBS 149750[T]	LESF 999; QVM86	Novo Airão, Amazonas, Brazil	2°31'23.4"S 60°49'31.9''W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	OQ589831	OQ589781	OQ603924	OQ596404	OQ603874	This study
	LESF 1021[$]	QVM149	Manaus, Amazonas, Brazil	2°26"54.3''S 59°45'53.9"W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	OQ589829	OQ589779	OQ603922	OQ596402	OQ603872	This study

Table 1. (Continued).

Fungal species name	Isolate ID	Specimen voucher	City, State, Country	Geographical coordinates	Habitat	GenBank accessions					References
						ITS	LSU	*tef*1	*rpb*1	*rpb*2	
	LESF 985[§]	QVM72	Novo Airão, Amazonas, Brazil	2°31'26.8"S 60°49'28.4"W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	OQ589830	OQ589780	OQ603923	OQ596403	OQ603873	This study
	–	QVM285[+]	Novo Airão, Amazonas, Brazil	2°31'23,4"S 60°49'31.9"W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	OQ708429	OQ708420	OQ709465	OQ709447	OQ709456	This study
	–	QVM286[+]	Novo Airão, Amazonas, Brazil	2°31'25.3"S 60°49'33.1"W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	OQ708430	OQ708421	OQ709466	OQ709448	OQ709457	This study
E. gracilis	CBS 149743[T]	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, 2021)
	LESF 843[§]	B120301; BA003	Camacan, Bahia, Brazil	14°47'51.18"S 39°10'17.4"W	Fungus garden of *Atta cephalotes*	KM817048	OQ589722	KM817118	OQ596345	OQ603815	Meirelles *et al.* (2015b); This study
	LESF 844[§]	B410301; BA005	Camacan, Bahia, Brazil	15°23'14.82"S 39°33'28.38"W	Fungus garden of *Atta cephalotes*	KM817050	OQ589723	KM817120	OQ596346	OQ603816	Meirelles *et al.* (2015b); This study
E. lentecrescens	CBS 135750[T]	AUJ9	Viçosa, Minas Gerais, Brazil	–	Fungus garden of *Acromyrmex subterraneus molestans*	JQ815079	JQ855717	JQ855714	MT305415	MT305540	Augustin *et al.* (2013); Montoya *et al.* (2021)
E. maculosa	CBS 149746[T]	LESF 962; QVM49	Novo Airão, Amazonas, Brazil	2°16'15.7"S 61°01'8.5"W	Fungus garden of *Acromyrmex* sp.	MT273475	MT273564	MT305384	MT305508	MT305633	Montoya *et al.* (2021)
	–	QVM281[+]	Novo Airão, Amazonas, Brazil	2°31'32.2"S 60°49'35.5"W	Fungus garden of *Acromyrmex* sp.	OQ708425	OQ708416	OQ709461	OQ709443	OQ709452	This study
	–	QVM282[+]	Novo Airão, Amazonas, Brazil	–	Fungus garden of *Acromyrmex* sp.	OQ708426	OQ708417	OQ709462	OQ709444	OQ709453	This study
	–	QVM283[+]	Novo Airão, Amazonas, Brazil	2°36'37.9"S 60°52'34.4"W	Fungus garden of *Acromyrmex* sp.	OQ708427	OQ708418	OQ709463	OQ709445	OQ709454	This study
	–	QVM284[+]	Novo Airão, Amazonas, Brazil	2°32'2.1"S 60°50'6.1"W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	OQ708428	OQ708419	OQ709464	OQ709446	OQ709455	This study
E. microspora (now *E. 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); Montoya *et al.* (2021)
E. 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); Meirelles *et al.* (2015a); Montoya *et al.* (2021)
E. multiformis	CBS 145327 [ET]	LESF 847	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, 2021)
	LESF 1007	QVM135	Florianópolis, Santa Catarina, Brazil	27°35'27.8"S 48°28'27.4"W	Fungus garden of attine ant	OQ589837	OQ589787	OQ603930	OQ596410	OQ603880	This study
	LESF 1134	QVM275	Cotrigaçu, Mato Grosso, Brazil	9°49'25.1"S 58°15'22.1"W	Fungus garden of *Apterostigma* sp	OQ589833	OQ589783	OQ603926	OQ596406	OQ603876	This study

Table 1. (Continued).

Fungal species name	Isolate ID	Specimen voucher	City, State, Country	Geographical coordinates	Habitat	GenBank accessions					References
						ITS	LSU	*tef*1	*rpb*1	*rpb*2	
	LESF 1135	QVM276	Cotrigaçu, Mato Grosso, Brazil	9°50'32.0"S 58°15'12.7"W	Fungus garden of *Apterostigma* sp.	OQ589834	OQ589784	OQ603927	OQ596407	OQ603877	This study
	LESF 1136[$]	QVM277	Alta Floresta, Mato Grosso, Brazil	09°49'22.7"S 58°15'32.0"W	Fungus garden of *Apterostigma* sp.	MH715092	MH715106	MH724266	MT305536	MT305661	Montoya *et al.* (2019, 2021)
	LESF 849	1612	Florianópolis, Santa Catarina, Brazil	27°35'18.6"S 48°22'20.8"W	Fungus garden of *Apterostigma* sp.	OQ589832	OQ589782	OQ603925	OQ596405	OQ603875	This study
	LESF 850	1703	Florianópolis, Santa Catarina, Brazil	277°44'38.9"S 48°31'9.3"W	Fungus garden of *Apterostigma* sp.	OQ589835	OQ589785	OQ603928	OQ596408	OQ603878	This study
	LESF 852	1706B	Florianópolis, Santa Catarina, Brazil	27°44'39.4"S 48°31'10.0"W	Fungus garden of *Apterostigma* sp.	MT273460	MT273549	MT305372	MT305494	MT305619	Montoya *et al.* (2021)
	LESF 884	U42	Argentina	–	Fungus garden of *Apterostigma* sp.	OQ589838	OQ589788	OQ603931	OQ596411	OQ603881	This study
E. papillata	CBS 149745[T]	LESF 960; QVM47	Novo Airão, Amazonas, Brazil	2°31'25.8"S 60°49'28.62"W	Fungus garden of *Apterostigma* sp.	OQ589840	OQ589790	OQ603933	OQ596413	OQ603883	This study
	LESF 959	QVM46	Novo Airão, Amazonas, Brazil	2°31'25.8"S 60°49'28.62"W	Fungus garden of *Apterostigma* sp.	OQ589839	OQ589789	OQ603932	OQ596412	OQ603882	This study
E. peniculiformis	CBS 149744[T]	LESF 876; UT008	Gamboa - Panama	–	Fungus garden of *Atta colombica*	KM817101	OQ589724	KM817162	OQ596347	OQ603817	Meirelles *et al.* (2015b); This study
	LESF 297[$]	RC005	Austin, Texas, USA	30°22'9.9"S 97°47'49.8"W	Fungus garden of *Trachymyrmex turrifex*	OQ589792	OQ589742	OQ603885	OQ596365	OQ603835	This study
	LESF 878[$]	U59	Panama	–	Fungus garden of *Apterostigma* sp. G4	OQ589793	OQ589743	OQ603886	OQ596366	OQ603836	This study
	LESF 881	U51	Panama	–	Fungus garden of attine ant	OQ589836	OQ589786	OQ603929	OQ596409	OQ603879	This study
E. phialicopiosa	CBS 149738[T]	LESF 048; SES005	Uberlândia, Minas Gerais, Brazil	19°17'17.5"S 48°39'40.2"W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	KM817088	OQ589739	KF240731	OQ596362	OQ603832	Meirelles *et al.* (2015b); This study
	LESF 021[$]	ES002	Rio Claro, São Paulo, Brazil	–	Fungus garden of *Atta sexdens rubropilos*	OQ589828	OQ589778	OQ603921	OQ596401	OQ603871	This study
	LESF 047[$]	SES002	Fazenda Pau, Goias, Brazil	–	Fungus garden of *Trachymyrmex sp. sensu lato*	KM817085	OQ589737	KM817147	OQ596360	OQ603830	Meirelles *et al.* (2015b); This study
	LESF 106[$]	SES006	Uberlândia, Minas Gerais, Brazil	19°17'17.5"S 48°39'40.2"W	Fungus garden of *Mycetomoellerius dichrous*	KM817089	OQ589738	KM817150	OQ596361	OQ603831	Meirelles *et al.* (2015b); This study
E. pseudocylindrica	CBS 149749[T]	LESF 993; QVM80	Novo Airão, Amazonas, Brazil	2°31'29.64"S 60°49'28.92"W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	OQ589819	OQ589769	OQ603912	OQ596392	OQ603862	This study
	LESF 1018	QVM146	Manaus, Amazonas, Brazil	2°26'55.1"S 59°46'16.38W	Fungus garden of *Apterostigma* sp.	OQ589820	OQ589770	OQ603913	OQ596393	OQ603863	This study
	LESF 1024	QVM152	Manaus, Amazonas, Brazil	2°26'52.6"S 59°45'53.4"W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	OQ589821	OQ589771	OQ603914	OQ596394	OQ603864	This study
	LESF 1029[$]	QVM157	Manaus, Amazonas, Brazil	2°26'55.5"S 59°45'54.2"W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	OQ589818	OQ589768	OQ603911	OQ596391	OQ603861	This study

Table 1. (Continued).

Fungal species name	Isolate ID	Specimen voucher	City, State, Country	Geographical coordinates	Habitat	GenBank accessions					References
						ITS	LSU	*tef*1	*rpb*1	*rpb*2	
E. rectangula	CBS 149739[T]	LESF 050; SES008	RO	–	Fungus garden of *Acromyrmex* sp.	KM817091	OQ589729	KM817152	OQ596352	OQ603822	Meirelles *et al.* (2015b); This study
	LESF 022[§]	ES003	Frei Caneca, Pernambuco, Brazil	–	Fungus garden of *Atta cephalotes*	KM817054	OQ589736	KM817124	OQ596359	OQ603829	Meirelles *et al.* (2015b); This study
	LESF 032	ES008	Santarém, Pará, Brazil	–	Fungus garden of *Acromyrmex* sp.	KM817059	OQ589734	KM817129	OQ596357	OQ603827	Meirelles *et al.* (2015b); This study
	LESF 038	RS004	Registro, Santa Catarina, Brazil	28°45'52.5"S 49°17'32.2"W	Fungus garden of *Acromyrmex coronatus*	OQ589816	OQ589766	OQ603909	OQ596389	OQ603859	This study
	LESF 318	ES029	Palmas, Tocantins, Brazil	10°10'37.6"S 48°18'23.7"W	Fungus garden of *Acromyrmex* sp.	KM817069	OQ589732	KM817139	OQ596355	OQ603825	Meirelles *et al.* (2015b); This study
	LESF 326[§]	BA006	Ilhéus, Bahia, Brazil	14°47'56.8"S 39°10'16.4"W	Fungus garden of *Atta cephalotes*	KM817051	OQ589733	KM817121	OQ596356	OQ603826	Meirelles *et al.* (2015b); This study
	LESF 860	U35	Panama	–	–	OQ589815	OQ589765	OQ603908	OQ596388	OQ603858	This study
	LESF 863[§]	U31	Panama	–	Fungus garden of *Apterostigma dentigerum*	OQ589791	OQ589741	OQ603884	OQ596364	OQ603834	This study
	LESF 865[§]	UT001	Guadalupe Island, Mexico	–	Fungus garden of *Acromyrmex octospinosus*	KM817094	OQ589735	KM817155	OQ596358	OQ603828	Meirelles *et al.* (2015b); This study
	LESF 883	UT005	Argentina	–	Fungus garden of *Acromyrmex* sp.	KM817098	OQ589730	KM817159	OQ596353	OQ603823	Meirelles *et al.* (2015b); This study
	LESF 892	UT020	México	–	Fungus garden of *Trachymyrmex* sp. *sensu lato*	KM817112	OQ589731	KM817173	OQ596354	OQ603824	Meirelles *et al.* (2015b); This study
E. rosisimilis	CBS 149742[T]	LESF 135; SES003	Uberlândia, Minas Gerais, Brazil	19°17'17.5"S 48°39'40.2"W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	KM817086	OQ589740	KM817148	OQ596363	OQ603833	Meirelles *et al.* (2015b); This study
	–	QVM287[+]	Novo Airão, Amazonas, Brazil	2°34'49.1"S 61°02'2.7"W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	OQ708431	OQ708422	OQ709467	OQ709449	OQ709458	This study
	–	QVM288[+]	Novo Airão, Amazonas, Brazil	2°55'46.3"S 59°58'28.3"W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	OQ708432	OQ708423	OQ709468	OQ709450	OQ709459	This study
	–	QVM289[+]	Novo Airão, Amazonas, Brazil	2°31'32.2S 60°49'35.5"W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	OQ708433	OQ708424	OQ709469	OQ709451	OQ709460	This study
Escovopsis sp.	LESF 049	SES007	Uberlandia, Minas Gerais, Brazil	–	Fungus garden of *Trachymyrmex* sp. *sensu lato*	KM817090	OQ589719	KM817151	OQ596342	OQ603812	Meirelles *et al.* (2015b); This study
E. spicaticlavata	CBS 149740[T]	LESF 052; SES010	Manaus, Amazonas, Brazil	2°26'54.84"S 59°46'10.02"W	Fungus garden of *Paratrachymyrmex diversus*	KM817093	MH715124	KM817154	MT305437	MT305562	Meirelles *et al.* (2015b); Montoya *et al.* (2019)
	LESF 975[§]	QVM62	Novo Airão, Amazonas, Brazil	2°31'25.3"S 60°49'33.1"W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	MT273477	MT273566	MT305386	MT305510	MT305635	Montoya *et al.* (2021)
	LESF 979[§]	QVM66	Novo Airão, Amazonas, Brazil	–	Fungus garden of *Trachymyrmex* sp. *sensu lato*	MT273478	MT273567	MT305387	MT305511	MT305636	Montoya *et al.* (2021)

Table 1. (Continued).

Fungal species name	Isolate ID	Specimen voucher	City, State, Country	Geographical coordinates	Habitat	GenBank accessions					References
						ITS	LSU	*tef*1	*rpb*1	*rpb*2	
E. weberi	ATCC 64542 ET	–	Viçosa, Minas Gerais, Brazil	–	Carpenter ant fungal mass	KF293285	KF293281	MZ170961	MT305412	MT305537	Augustin *et al.* (2013); Montoya *et al.* (2021)
	LESF 017	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); Montoya *et al.* (2021)
	LESF 019§	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); Montoya *et al.* (2021)
	LESF 020	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	Montoya *et al.* (2021)
	LESF 023§	ES005	Alta Floresta, Mato Grosso, Brazil	–	Fungus garden of *Atta cephalotes*	KM817056	MH715117	KM817126	MT305425	MT305550	Meirelles *et al.* (2015b); Montoya *et al.* (2019)
	LESF 024	ES006	Alta Floresta, Mato Grosso, Brazil	–	Fungus garden of *Acromyrmex coronatus*	KM817057	MT273504	KM817127	MT305426	MT305551	Montoya *et al.* (2019, 2021)
	LESF 025	ES007	Alta Floresta, Mato Grosso, Brazil	–	Fungus garden of *Acromyrmex coronatus*	KM817058	MT273505	KM817128	MT305427	MT305552	Montoya *et al.* (2019, 2021)
	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)
	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, 2021)
	LESF 030	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, 2021)
	LESF 031§	ES014	Corumbataí, São Paulo, Brazil	22°17'22"S 47°39'23"W	Fungus garden of *Atta sexdens*	MT273426	MT273506	MT305341	MT305431	MT305556	Montoya *et al.* (2021)
	LESF 033	ES004	Bahia, Brazil	–	Fungus garden of *Acromyrmex* sp.	KM817055	MT273507	KM817125	MT305432	MT305557	Meirelles *et al.* (2015b); Montoya *et al.* (2021)
	LESF 034	ES024	Botucatu, São Paulo, Brazil	–	Fungus garden of *Acromyrmex balzanii*	MT273427	MT273508	MT305342	MT305433	MT305558	Montoya *et al.* (2021)
	LESF 042§	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); Montoya *et al.* (2021)
	LESF 043§	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); Montoya *et al.* (2021)

Table 1. (Continued).

Fungal species name	Isolate ID	Specimen voucher	City, State, Country	Geographical coordinates	Habitat	GenBank accessions					References
						ITS	LSU	*tef*1	*rpb*1	*rpb*2	
	LESF 046	SES001	Rio Claro, São Paulo, Brazil	22°23'45.9"S 47°32'43.2"W	Fungus garden of *Trachymyrmex* sp. *sensu lato*	KM817084	MT273511	KM817146	MT305436	MT305561	Meirelles *et al.* (2015b); Montoya *et al.* (2021)
	LESF 054[$]	AR003	Ilhéus, Bahia, Brazil	14°47'56.8"S 39°10'16.4"W	Fungus garden of *Acromyrmex balzanii*	KM817043	MT273512	KM817113	MT305438	MT305563	Meirelles *et al.* (2015b); Montoya *et al.* (2021)
	LESF 056	AR033	Camacan, Bahia, Brazil	15°22'50.3"S 39°34'03.5"W	Fungus garden of *Acromyrmex* sp.	KM817045	MT273513	KM817115	MT305439	MT305564	Meirelles *et al.* (2015b); Montoya *et al.* (2021)
	LESF 136	4a	Corumbataí, São Paulo, Brazil	22°17'21.7"S 47°39'22.8"W	Fungus garden of *Atta sexdens rubropilosa*	MT273428	MT273514	MT305343	MT305440	MT305565	Montoya *et al.* (2021)
	LESF 146	1cT4	Corumbataí, São Paulo, Brazil	22°17'21.7"S 47°39'22.8"W	Fungus garden of *Atta sexdens rubropilosa*	MT273429	MT273515	MT305344	MT305441	MT305566	Montoya *et al.* (2021)
	LESF 156[$]	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	Montoya *et al.* (2021)
	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	Montoya *et al.* (2021)
	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	Montoya *et al.* (2021)
	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	Montoya *et al.* (2021)
	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	Montoya *et al.* (2021)
	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	Montoya *et al.* (2021)
	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	Montoya *et al.* (2021)
	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	Montoya *et al.* (2021)
	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, 2021)
	LESF 317	ES026	Rio Claro, São Paulo, Brazil	–	Fungus garden of *Trachymyrmex* sp. *sensu lato*	KM817067	MT273531	KM817137	MT305464	MT305589	Meirelles *et al.* (2015b); Montoya *et al.* (2021)
	LESF 319	ES030	Palmas, Tocantins, Brazil	10°10'52.9"S 48°21'42.0"W	Fungus garden of *Acromyrmex* sp.	KM817070	MT273532	KM817140	MT305465	MT305590	Meirelles *et al.* (2015b); Montoya *et al.* (2021)

Table 1. (Continued).

Fungal species name	Isolate ID	Specimen voucher	City, State, Country	Geographical coordinates	Habitat	GenBank accessions					References
						ITS	LSU	*tef*1	*rpb*1	*rpb*2	
	LESF 324[$]	RS105	Thermas de Santa Bárbara, São Paulo, Brazil	22º49'10.6"S 49º16'06.2"W	Fungus garden of *Atta laevigata*	KM817083	MT273533	KM817145	MT305466	MT305591	Meirelles *et al.* (2015b); Montoya *et al.* (2021)
	LESF 355	ES021	Corumbataí, São Paulo, Brazil	–	Fungus garden of *Atta sexdens rubropilosa*	MT273445	MT273534	MT305358	MT305468	MT305593	Montoya *et al.* (2021)
	LESF 356	ES032	Botucatu, São Paulo, Brazil	–	Fungus garden of *Atta laevigata*	MT273446	MT273535	MT305359	MT305469	MT305594	Montoya *et al.* (2021)
	LESF 359	ES019	Corumbataí, São Paulo, Brazil	–	Fungus garden of *Atta sexdens*	MT273447	MT273536	MT305360	MT305470	MT305595	Montoya *et al.* (2021)
	LESF 362	ES028	Corumbataí, São Paulo, Brazil	–	Fungus garden of *Atta sexdens*	MT273448	MT273537	MT305361	MT305471	MT305596	Montoya *et al.* (2021)
	LESF 363	ES023	Corumbataí, São Paulo, Brazil	–	Fungus garden of *Atta sexdens*	MT273449	MT273538	MT305362	MT305472	MT305597	Montoya *et al.* (2021)
	LESF 364	ES015	Corumbataí, São Paulo, Brazil	–	Fungus garden of *Atta sexdens*	MT273450	MT273539	MT305363	MT305473	MT305598	Montoya *et al.* (2021)
	LESF 519	ES016	–	–	Fungus garden of *Atta sexdens rubropilosa*	MT273451	MT273540	MT305364	MT305475	MT305600	Montoya *et al.* (2021)
	LESF 575	RS087	Indaial, Santa Catarina, Brazil	26º54'04.9"S 49º10'51.2"W	Fungus garden of *Acromyrmex diciger*	MT273452	MT273541	MT305365	MT305476	MT305601	Montoya *et al.* (2021)
	LESF 858	A210201	Camacan, Bahia, Brazil	–	Fungus garden of *Atta cephalotes*	MT273461	MT273550	MT305373	MT305497	MT305622	Montoya *et al.* (2021)
	LESF 859	B110302	Camacan, Bahia, Brazil	–	Fungus garden of *Atta cephalotes*	MT273462	MT273551	MT305374	MT305498	MT305623	Montoya *et al.* (2021)
	LESF 877	NL010	–	–	–	MT273466	MT273555	MT305376	MT305500	MT305625	Montoya *et al.* 2021)
	LESF 880	2aT=3	–	–	–	MT273467	MT273556	MT305377	MT305501	MT305626	Montoya *et al.* (2021)
	LESF 994	QVM81	Novo Airão, Amazonas, Brazil	2°36'37.9''S 60°52'34.4''W	Fungus garden of *Acromyrmex* sp.	MT273479	MT273568	MT305388	MT305512	MT305637	Montoya *et al.* (2021)
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 morschi*	KJ808767	KJ808765	KJ 808766	MT305418	MT305543	Meirelles *et al.* (2015a); Montoya *et al.* (2021)

[T]Holotype; [ET] Ex-type cultures; $: strains used to assess the morphological characters of *Escovopsis* species; **+:** Inactive strains; LESF: Laboratory of Fungal Ecology and Systematics (UNESP, Rio Claro, Brazil); QVM: Quimi Vidaurre Montoya.

The microscopic structures of all *Escovopsis* ex-type cultures, and our newly described species, *i.e.*, conidiophores, branches of conidiophores, swollen cells of conidiophores (formed at the apex of the conidiophore and from which branches are formed), vesicles, conidiogenous cells, conidia and chlamydospores (*sensu* Augustin *et al.* 2013), their shape, size, colour, and pattern of formation and aggregation (Montoya *et al.* 2021), were evaluated on PDA following the method used by Montoya *et al.* (2019). Briefly, we carried out slide culture preparations using plugs from PDA 5 mm diam × 5 mm height, placed on microscopic slides. Then, the plugs were inoculated with conidia, covered with coverslips, and incubated at 25 °C for 4–7 d in the dark. Finally, the coverslips were removed and placed on new slides with a drop of lactophenol. The slides were examined using compound light microscope (DM750, Leica, Wetzlar), and microscopic structures were photographed and 30 measurements recorded for each structure using LAS EZ v. 4.0 (Leica Application Suite) and ImageJ2 v. 2.3.0 in Fiji (Schindelin *et al.* 2012). The measurements of the microscopic structures shown in the descriptions of the species (taxonomy section) represent the minimum and maximum values observed among the 30 measurements obtained.

DNA extraction, PCR and sequencing

We used five molecular markers in this study, *i.e.*, the internal transcribed spacers (ITS), the large subunit nuclear ribosomal RNA gene (28S), the translation elongation factor 1-alpha (*tef*1), and the RNA polymerase II protein-coding largest and second largest subunit genes *rpb*1 and *rpb*2 (Supplementary Table S3). Of the 138 isolates used in this study, 64 were previously sequenced for these five markers in other studies (Augustin *et al.* 2013, Meirelles *et al.* 2015a, b, Montoya *et al.* 2019, 2021; see Table 1). The ITS and *tef*1 regions of 22 isolates were previously sequenced by Meirelles *et al.* (2015b). These were supplemented with our sequences of the 28S, *rpb*1, and *rpb*2 genes. All five markers were sequenced for the remaining 52 isolates (Table 1).

For the isolates sequenced in this study, we first extracted the genomic DNA using a modified CTAB method (Möller *et al.* 1992). Briefly, aerial mycelium, grown for 7 d at 25 °C on PDA, was crushed with the aid of glass beads (Sigma) in a lysis solution. Five µL of Proteinase K were added to this solution and incubated at 65 °C for 30 min. Then, the organic phase of the solution was separated by centrifugation at 10 000 g for 10 min, using chloroform-isoamyl alcohol (24: 1). Four hundred µL of the supernatant were collected, and the genomic DNA was precipitated with 3 M sodium acetate and isopropanol. The genomic DNA was purified with two successive washes of 70 % ethanol and left at room temperature to dry overnight. Finally, the DNA was suspended in 30 µL of Tris-EDTA solution and stored at -20 °C.

Amplification reactions for ITS, 28S, *tef*1, *rpb*1 and *rpb*2 regions were carried out using the primers and conditions published by Meirelles *et al.* (2015a, b), Augustin *et al.* (2013), and Montoya *et al.* (2021) (summarized in Supplementary Table S3). Amplicons were purified with the Wizard SV Gel and PCR Clean-up System (Promega, Madison) following the manufacturer's protocol and sequenced (forward and reverse sequencies) on an ABI 3500 Genetic Analyzer (Life Technologies). Consensus sequences were assembled using Geneious v. 6.0 (Kearse *et al.* 2012) and deposited in GenBank (see Table 1 for accession numbers).

Phylogenetic analyses

We performed a multilocus analysis combining the five molecular markers. First, the data sets were aligned separately for each marker in MAFFT v. 7 (Katoh & Standley 2013). The nucleotide substitution model for each alignment was calculated in jModelTest v. 2 (Darriba *et al.* 2012) using the Akaike Information Criterion (AIC) with 95 % confidence intervals. To determine if all *Escovopsis* species clades remained constant (monophyletic) considering the GCPSR concept (Taylor *et al.* 2000), a phylogenetic tree was inferred using each molecular marker separately (Supplementary Fig. S1). Finally, the datasets were concatenated using Winclada v. 1.00.08 (Nixon 2002). The final data set contained a total of 139 sequences 3 700 bp long [ITS (570 bp), 28S (591 bp), *rpb*1 (751 bp), *rpb*2 (1 030 bp), and *tef*1 (758 bp)]. *Escovopsis* species described by Marfetán *et al.* (2019) were not included in the multilocus analyses of this study because most of the sequences of these species are unavailable (Montoya *et al.* 2021). However, 21 available 28S sequences of those species were combined with our 28S data to reveal their phylogenetic relationships (Supplementary Table S4 and Supplementary Fig. S2).

We reconstructed the final phylogenetic trees using Bayesian Inference (BI) in MrBayes v. 3.2.2 (Ronquist *et al.* 2012) and Maximum Likelihood (ML) in RAxML v. 8 (Stamatakis 2014). For the BI analysis, we carried out two separate runs (each consisting of three hot chains and one cold chain) using the GTR model for each partition independently. Two million generations of the Markov Chain Monte Carlo (MCMC) were enough to reach convergence (standard deviation of split frequencies < 0.01). The first 25 % of trees were discarded as burn-in to generate the best BI tree. For the ML analysis, we estimated 1 000 independent trees and performed 1 000 bootstrap replicates using the GTR model. The final tree was visualized in FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) and edited in Adobe Illustrator CC v. 17.1. The alignments and resulting trees were deposited in TreeBASE (Study Accession URL: http://purl.org/phylo/treebase/phylows/study/TB2:S30754).

Taxonomic key to *Escovopsis* species

Sixty-eight morphological features from species of *Escovopsis* (Supplementary Table S5) were analysed using the "rpart" library (Therneau & Atkinson 2019) in R v. 3.6.3. Nineteen out of the 68 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 reconstructed using a decision tree that started with a single node root that split into 18 (Supplementary Fig. S3; numbered in red) branches to end in the leaves corresponding to each species (Supplementary Fig. S3). The final cladogram was manually edited using Adobe Illustrator CC v. 17.1, and the information on branches was used to construct the taxonomic key. The *Escovopsis* species described by Marfetán *et al.* (2019) could not be included in this key because the morphological features of those species had been described using conditions different to those used in this study.

RESULTS

Adjusting the parameters to evaluate the morphology of *Escovopsis*

Colony growth of *Escovopsis* species was observed on different media at temperatures between 10 and 30 °C. *Escovopsis weberi*, *E. clavata*, *E. microspora*, and *E. moelleri* were able to grow at 10 °C, but their growth was limited and inconspicuous. Thus, 10 °C was selected as the minimum test temperature and growth of all strains at this temperature is reported as present or absent. The seven *Escovopsis* ex-type cultures grew well at 20, 25 and 30 °C, although at 30 °C, colony growth of *E. aspergilloides*, *E. clavata*, *E. lentecrescens*, *E. multiformis* was much slower than that of *E. microspora*, *E. moelleri*, and *E. weberi*. Therefore, we consider these temperatures most appropriate for evaluating macroscopic characters. At temperatures 20, 25 and 30 °C, colonies started to grow after the first (*Escovopsis microspora*, *E. moelleri*, and *E. weberi*) or the second day (*E. aspergilloides*, *E. clavata*, *E. lentecrescens*, *E. multiformis*). Some fast-growing species (*E. microspora*, *E. moelleri*, and *E. weberi*) covered the diameter of the Petri dishes between the third and the fourth day. Therefore, we selected the fourth day as the best time to measure growth radius at 20, 25, and 30 °C.

Evaluation of colony growth on the eight agar media tested (Fig. 1) demonstrated that some media did not yield informative colony characters while some species exhibited similar morphological traits on different media. For instance, growth on all media resulted in a similar morphology for *E. microspora* and *E. weberi*. Likewise, on MA2 % colonies showed similar growth patterns to those on CYA, MEA, OA, as well as on PDA (*E. moelleri*), on CYA (*E. aspergilloides*), and on PCA and PDA (*E. clavata*, *E. lentecrescens*, and *E. multiformis*). By contrast, none of the isolates grew well on SNA (all strains exhibited inconspicuous aerial mycelia, and the morphological patterns were difficult to observe). Oatmeal Agar was also not ideal because the growth was difficult to measure due to the medium's opacity. On the other hand, except for *E. aspergilloides*, all isolates exhibited vigorous growth and notable expression of colony colour on MEA. Differences in colony growth rate were most apparent on CMD, and, on this medium, some species (*e.g.*, *E. microspora* and *E. weberi*) produced pustule-like hyphal structures on which conidiophores were often produced, similar to the sporulating tufts 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. These media are also used for the culture assessments of many other genera in the *Hypocreaceae,* which facilitates comparisons across the family.

Colonies of most *Escovopsis* species grew better (colonies develop faster and form more mycelium and conidia) at 25 °C on CMD, MEA, and PDA (Fig. 2), and the differences in macroscopic characters, especially colony colours, were most apparent on the seventh day. For instance, White (LIII73(10)) to Colonial Buff (XXX21"d) and Light Brownish Olive (XXX19"k) (*E. clavata*, *E. lentecrescens*, *E. microspora*, *E. moelleri*, *E. multiformis*, and *E. weberi*), and Light Yellow-Green (VI31d) to *Olive-Yellow (XXX23") colours (*E. aspergilloides*) were clearly distinguishable on the seventh day. Although less clear, other colours [Ecru-Olive (XXX21"i), *Vinaceous-Cinnamon (XXIX13"b), and Deep Colonial Buff (XXX21"b)] were observed on colonies of *E. weberi* and *E. microspora* on PDA and MEA. Between the seventh and tenth day, most species became Light Brownish Olive (XXX19"k) on the three media, thus, the distinction between them became less apparent (except for *E. lentecrescens*). After the 10th day, the aerial mycelium of all species started to deteriorate (collapse). Therefore, 7 d of growth at 25 °C appears optimal to evaluate the macroscopic characters of *Escovopsis* species. Supplementary Table S6 shows the conditions, adopted in this study, for macroscopic characters evaluation of the colonies.

The most informative microscopic structures for distinguishing *Escovopsis* species are: conidiophores, conidiophore branching, presence and shape of conidiophore swollen cells, vesicles, phialides, conidia, and chlamydospores. A complete micromorphological description should consider the following: (i) *Conidiophores*: The number of vesicles (mono-vesiculate, poly-vesiculate conidiophore), length, stipe (length, septum, distance of septum from the foot cell), shape (pyramidal, irregular, *etc.*), cell wall (smooth or rough), arrangement (alternate, opposite, in verticils, *etc.*); (ii) *Conidiophore branches*: length, arrangement, shape, levels of branching, stipe; (iii) *Vesicle*: length and width, shapes, presence or absence of septum, stipe; (vi) *Phialide*: where is it formed (vesicles, aerial mycelium), shape, total length and dimensions (length and width) of the base, swollen section and neck; (v) *Conidia*: formed in chains or solitary, shape, length, width, colour (individually and in mass), presence or absence of ornamentation; (vi) *Chlamydospores*: where is it formed (on aerial or submerged mycelium), arrangement (intercalary or terminal), shape, colour, length and width. Supplementary Table S7 shows the characters and parameters, selected in this study, for the evaluation of the microscopic morphology.

Escovopsis phylogeny

The analysis resulting from the combination of the five molecular markers showed that the 138 *Escovopsis* isolates distributed among 19 well supported monophyletic groups across the genus phylogeny (Fig. 3). Six out of the 19 clades correspond to the previously described species: *Escovopsis aspergilloides*, *E. clavata*, *E. lentecrescens*, *E. moelleri*, *E. multiformis*, and *E. weberi*. The ex-type strain of *E. microspora* was placed in the same clade with strains of *E. weberi* [Posterior Probability (PP) = 1; Maximum likelihood bootstrap (MLB) = 100 %, Fig. 3]. The remaining 13 clades correspond to the new species described in this study, *i.e.*, *E. breviramosa*, *E. chlamydosporosa*, *E. diminuta*, *E. elongatistipitata*, *E. gracilis*, *E. maculosa*, *E. papillata*, *E. peniculiformis*, *E. phialicopiosa*, *E. pseudocylindrica*, *E. rectangula*, *E. rosisimilis*, and *E. spicaticlavata*, (Fig. 3).

In total, five major clades can be differentiated in the multilocus phylogeny of *Escovopsis* (Fig. 3). Clade I comprises *E. breviramosa*, *E. gracilis*, *E. peniculiformis*, and *E. weberi*. Clade II comprises *E. chlamydosporosa* and *E. rectangula*. Clade III comprises by *E. elongatistipitata*, *E. moelleri*, *E. phialicopiosa*, *E. pseudocylindrica*, and *E. spicaticlavata*. Clade IV comprises *E. aspergilloides*, *E. diminuta*, *E. maculosa*, *E. lentecrescens*, and *E. rosisimilis*. Lastly, clade V comprises *E. clavata*, *E. multiformis*, and *E. papillata*. Species in Clades I, II and III, grow faster and over wider temperature ranges, and form mostly cylindrical vesicles, while species in clades IV and V grow slowly, at narrow temperature ranges, and form globose vesicles (Figs 2, 3).

In the analyses performed with the molecular markers separately, we observed that the phylogenetic placement of the 19 *Escovopsis* species may vary depending on the marker used (Supplementary Fig. S1). The *rpb*2 and *tef*1 genes, for example,

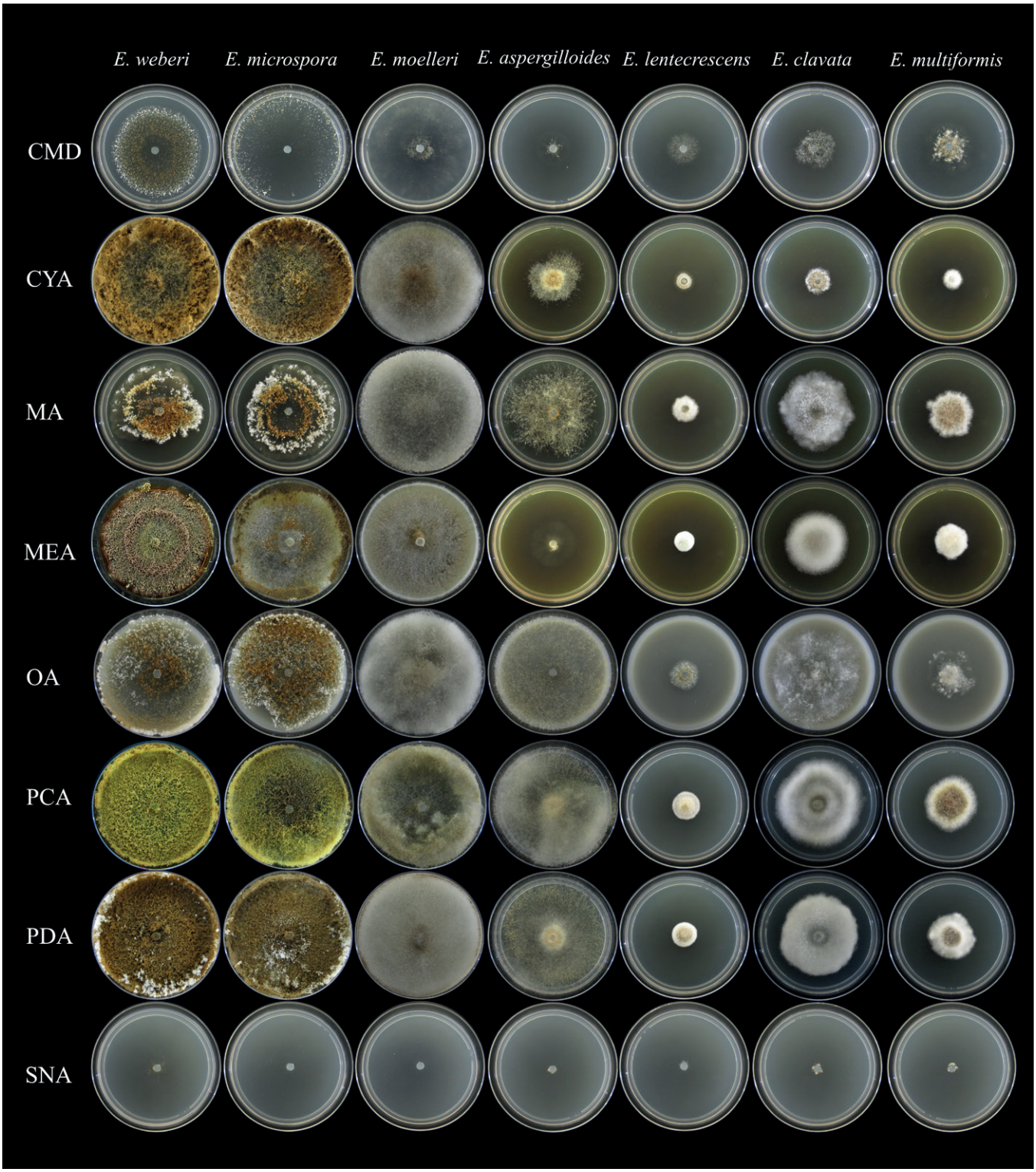

Fig. 1. Colonies of ex-type cultures of *Escovopsis aspergilloides*, *E. clavata*, *E. microspora*, *E. moelleri*, *E. multiformis*, *E. lentecrescens*, and *E. weberi* grown on eight media. The order of the species names in the figure corresponds to the phylogenetic relationships of the species shown on the tree (Fig. 3). All cultures were incubated for 7 d at 25 °C in the dark in unsealed plates.

showed each of the 19 *Escovopsis* species forming well-supported monophyletic clades (Supplementary Fig. S1A, B). The topology of these trees differs slightly from that of the tree with all the markers combined (Figs 3, S1F). In the *rpb*2 gene tree, *E. chlamydosporosa* and *E. rectangula* do not share the same common ancestor (Fig. S1A). In the case of the *tef*1 gene, *E. breviramosa* is more closely related to *E. weberi* than to *E. peniculiformis*; *E. papillata* is more closely related to clade IV, and *E. multiformis* and *E. clavata* do not share the same common ancestor, as seen in the tree based on the five markers (Fig. S1B). On the other hand, most of the species form well-supported monophyletic groups in the trees reconstructed with the ITS or *rpb*1 markers (Fig. S1C, D). However, the clades *E. breviramosa*, *E. chlamydosporosa* (ITS tree, Fig. S1C), and *E. phialicopiosa* (*rpb*1 tree, Fig. S1D) were unresolved. Likewise, in the *rpb*1 tree, *E. peniculiformis* is placed within *E. weberi* (Fig. S1D). Finally, the 28S marker was the one with the lowest resolution to separate *Escovopsis* species (Fig. S1E). While *E. breviramosa*, *E. clavata*, *E. lentecrescens*, *E. maculosa*, *E.*

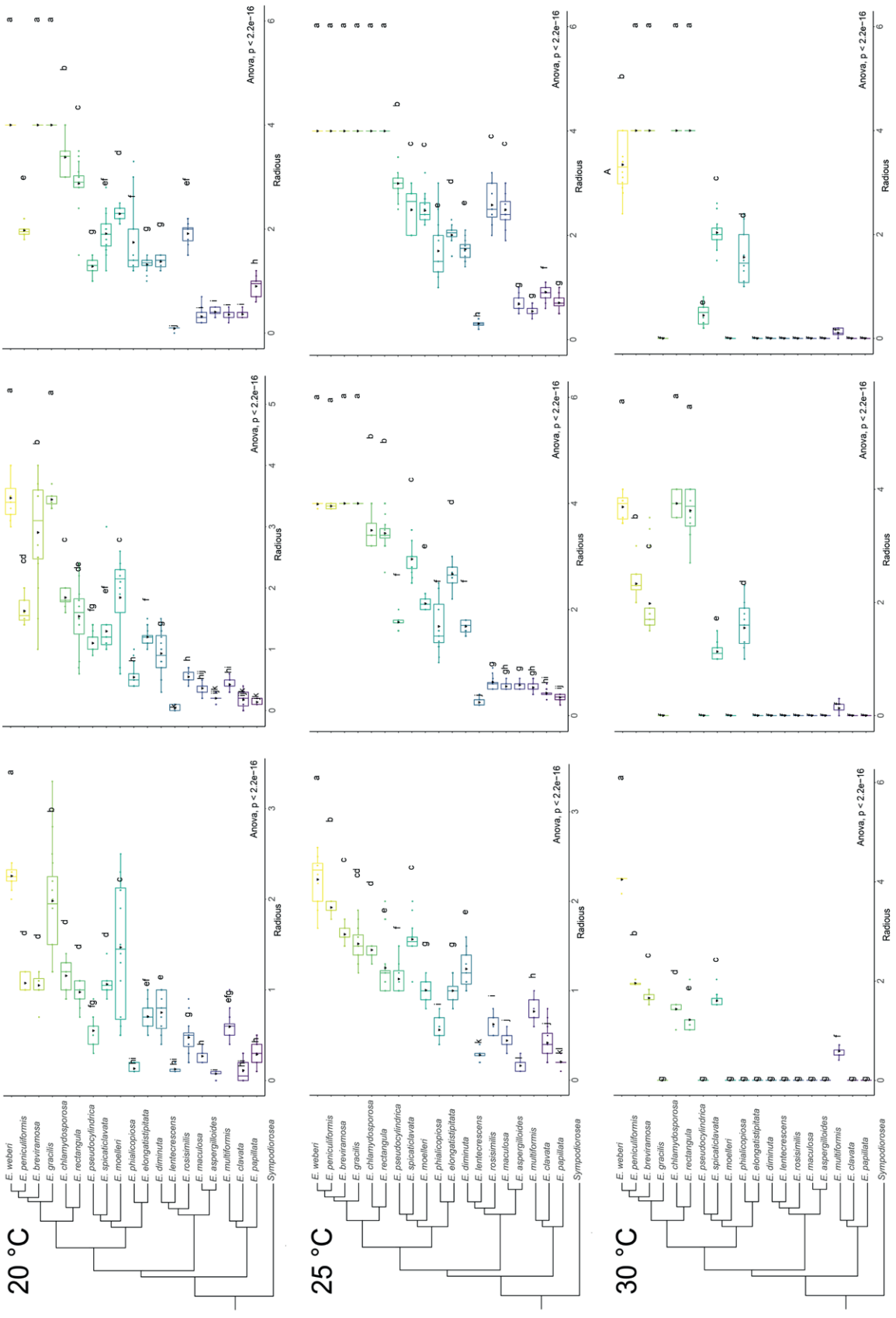

Fig. 2. Growth rate of 19 *Escovopsis* species on three culture media. Boxplots for each species are distinguished by different colours. Triangles represent the mean growth ratio, vertical lines the mid-point of the data, and upper and lower whiskers the lowest and the highest values, respectively (without considering outliers). Boxes marked with the same letter indicate species for which the mean growth rates did not differ significantly according to the Duncan's Multiple Range Test.

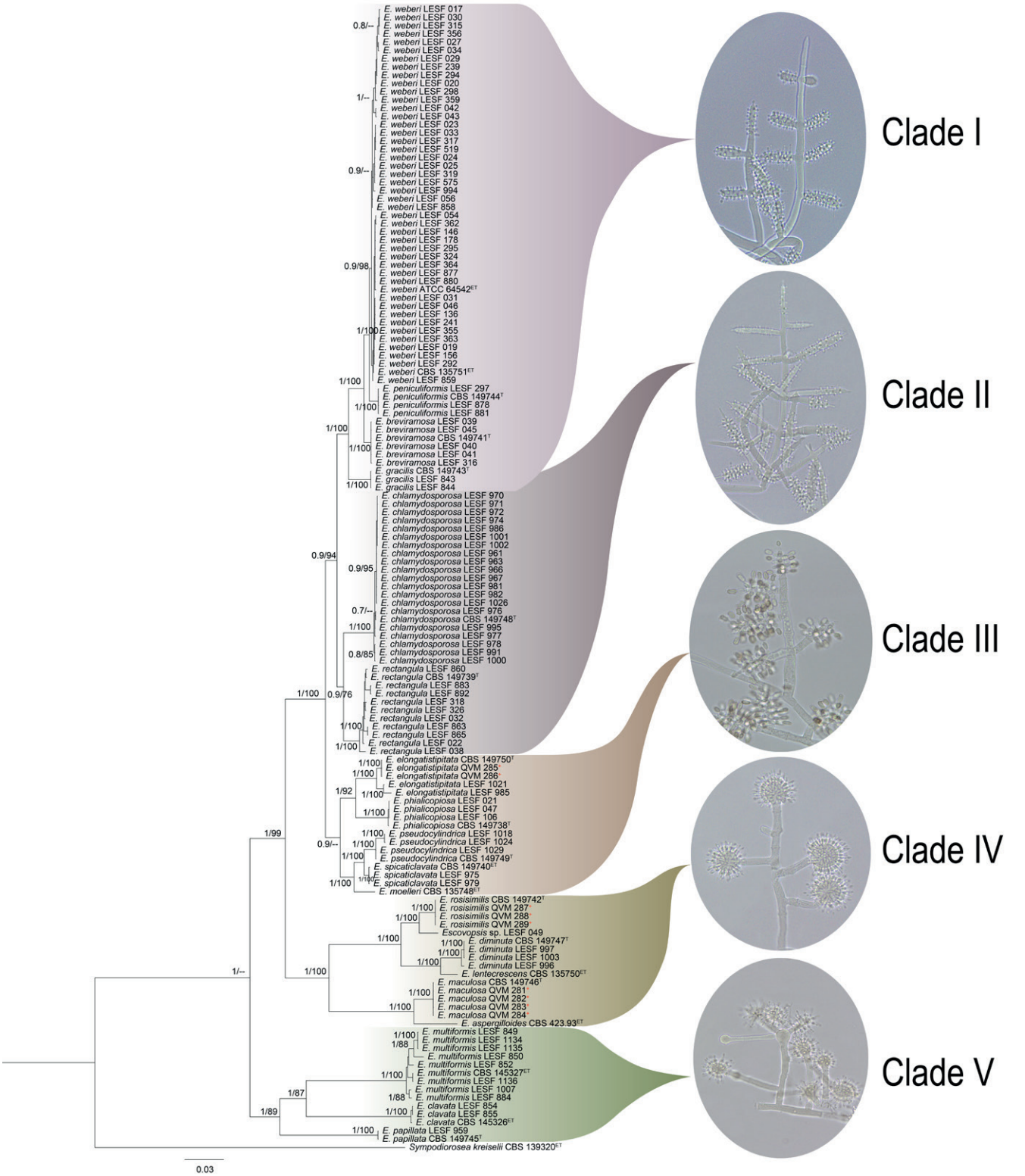

Fig. 3. Multigene phylogeny revealing relationship among 19 species of *Escovopsis* and *Sympodiorosea kreiselii* CBS 139320 as the outgroup. Highlighted boxes in different colours represent the clades I–V distinguished according to the variation of conidiophores and vesicles as depicted in the boxes. The tree was inferred using Bayesian Inference and concatenated sequences of ITS, LSU, *tef*1, *rpb*1 and *rpb*2 molecular markers. The final alignment of 3 700 characters consisted of sequences from 138 *Escovopsis* isolates. Numbers on branches indicate BI posterior probabilities > 0.69 and ML bootstrap support values > 75 %. [ET] indicates ex-type cultures and the red cross the strains that are non-viable.

multiformis, *E. papillata*, *E. rectangula*, and *E. rosisimilis* formed well-supported monophyletic groups, their phylogenetic placement, as well as the monophyly of the other species, are not well-resolved (Fig. S1E).

Morphological diversity *Escovopsis*

While species in the five main clades observed in the *Escovopsis* phylogeny share some morphological characters, each also has unique character states that differentiate them from one another (Fig. 3). Clades I and II form pyramidal conidiophores mostly producing

cylindrical vesicles and smooth-walled conidia. However, species in Clade I (*i.e*., *E. breviramosa*, *E. gracilis*, and *E. peniculiformis*) usually have septate vesicles and phialides produced both on vesicles and aerial mycelium (although the latter is less frequent). In contrast, species in Clade II have shorter and less frequently septate vesicles without phialides formed in the aerial mycelium. In addition, some species in Clade II (*e.g*., *E. chlamydosporosa*), usually form chlamydospores which are rare in species in Clade I.

In contrast, species in Clades III, IV and V form irregular-shaped conidiophores, differing among each other in the type of vesicle and conidia. Species in Clade III form clavate, cymbiform, subulate, and lanceolate vesicles, and conidia with thickened walls and ornamentation, except for *E. phialicopiosa,* in which conidia have inconspicuous ornamentation. Species in Clade IV form globose, subglobose, and capitate vesicles, and smooth-walled conidia. Species in this clade are usually slow growing, with the striking example provided by *E. lentecrescens*, the slowest growing species in the genus.

Finally, distinct from all other clades, species in Clade V have the most variable vesicle shapes, ranging from globose, subglobose, capitate, obovoid, prolate, spatulate, clavate, cymbiform, lanceolate to subulate. Also, in contrast to species in other clades, most species in Clade V can only grow between 20 and 25 °C, except for some isolates of *E. multiformis* that can also grow at 10 and 30 °C.

Taxonomy

Our taxonomic protocol is applied below to provide standardised morphological descriptions of the know species of *Escovopsis,* excluding the five species described by Marfetán *et al.* (2019) for which no cultures were available. We re-described the ex-type cultures of *E. aspergilloides*, *E. clavata*, *E. lentecrescens*, *E. moelleri*, *E. multiformis,* and *E. weberi* (Figs 4, 7, 11, 13, 14, 22). The re-description of these species provided the basis for the morphological analysis of the genus. Furthermore, based on the similarity of their sequence and morphological characters, we synonymize *E. microspora* with *E. weberi*. Finally, we describe 13 new species obtained from our field work in Argentina, Brazil, Costa Rica, Mexico, and Panama.

Escovopsis aspergilloides K.A. Seifert *et al.*, Mycologia 87: 408. 1995. MycoBank MB 413060. Fig. 4.

Diagnosis: *Escovopsis aspergilloides* forms colonies with diffuse pale-yellow to yellowish-brown colours and conidiophores with globose vesicles.

Typus: **Trinidad and Tobago**, near ASA Wright Nature Centre, isolated from a nest of *Trachymyrmex ruthae* (collected 15–20 cm below the soil surface in a wet, dense, secondary tropical rainforest by T. R. Schultz, 9 Nov. 1992), in Ithaca, New York by I.H. Chapela, no. 92110905C [**holotype** DAOM 216382 (dried agar culture), ex-type culture CBS 423.93].

Description: *Conidiophores* forming 2–15 vesicles, hyaline, irregular shape, smooth-walled, alternate or opposite, formed on aerial hyphae. Mono-vesiculate conidiophores 40–72 µm, polyvesiculate 80–300 µm long. Conidiophore stipes 24–100 × 5–6 µm, with a septum 4–6 µm from the foot cell. Conidiophore branches 32–160 µm long, formed in one to three levels, in almost right angles, alternate. Second branching level usually longer than other branching levels. Stipes on branches 10–49 µm long, with a septum 2–4 µm from conidiophore axis. *Vesicles* of various shapes, *i.e*., globose, sub-globose, capitate, obovoid, prolate and spatulate, 13–29 × 10–24 µm, aseptate, formed on the tips of conidiophore and branches. Vesicle stipe 10–40 µm long, with one or two septa. *Phialides* formed on vesicles, 6–10 µm long, ampulliform, 1–2 × 0.5–1.5 µm at the base, 3.5–4 × 2–3 µm at the swollen section and 4 × 2 µm at the neck. *Conidia* formed in chains, globose to oblong, 2.5–3 × 2–2.5 µm, Olive-Ochre (XXX21"), with smooth and slightly thickened walls. *Chlamydospores* intercalary, hyaline, 11–22 × 8–14 µm.

Culture characteristics: *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. At 25 °C, growth starts on second day on MEA and PDA, and on third day on CMD. *Colony* radius after 4 d at 20 °C: 0–1 mm on CMD, 2–5 mm on MEA and 5–8 mm on PDA; at 25 °C: 1–3 mm on CMD, 5–7 mm on MEA and 5–10 mm on PDA. *Colony morphology* — CMD 25 °C, 7 d: colonies with diffuse aerial mycelium, spread by stolons, Light Yellow-Green (VI31d) to Olive-Ochre (XXX21"). MEA 25 °C, 7 d: colonies with submerged mycelium forming dense circular zones, diffuse aerial mycelium forming concentric rings, White (LIII73(10)) to Picnic Yellow (IV23d) and *Olive-Yellow (XXX23") (*Olive-Yellow (XXX23") at centre and White (LIII73(10)) at margin). PDA 25 °C, 7 d: colonies with abundant aerial mycelium, spread by stolons, Picnic Yellow (IV23d) to *Olive-Yellow (XXX23") and Light Yellow-Green (VI31d) to Colonial buff (XXX21"d). Pustule-like structures and soluble pigments absent.

Ecology: Unknown, but this species has only been found in a nest of *Trachymyrmex ruthae* in a rain forest.

Distribution: Trinidad.

Notes: *Escovopsis aspergilloides* is closely related to *E. maculosa*. However, *E. aspergilloides* grows slower and forms longer and more branched conidiophores. Unlike *E. maculosa*, which has mainly globose vesicles, *E. aspergilloides* forms vesicles of various shapes, *i.e.*, globose, sub-globose, capitate, obovoid, prolate and spathulate.

Escovopsis breviramosa Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, ***sp. nov.*** Mycobank MB 847805. Fig. 5.

Etymology: "*breviramosa*" (brevi = short, ramose = branches) in reference to the short branches formed on the conidiophores of this species.

Diagnosis: *Escovopsis breviramosa* frequently has mono-vesiculate conidiophores and their polyvesiculate conidiophores have branches comprised mainly of a sessile vesicle.

Typus: **Brazil**, Bahia, Camacan, Serra Bonita, 15°23'43.0''S, 39°33'49.1''W, fungus garden of *Acromyrmex* sp., May 2015, A. Rodrigues, LESF 055 (**holotype** CBS 149741 preserved as metabolically inactive culture, ex-type culture CBS 149741).

Description: *Conidiophores* forming 2–33 vesicles, hyaline, usually pyramidal, smooth-walled, alternate or less frequent opposite, formed on aerial hyphae. Mono-vesiculate conidiophores 17–55 µm, polyvesiculate 36–430 µm long. Conidiophore stipes 3.5–130 µm × 3.5–5 µm, with a septum 1.5–28.5 µm from the foot cell.

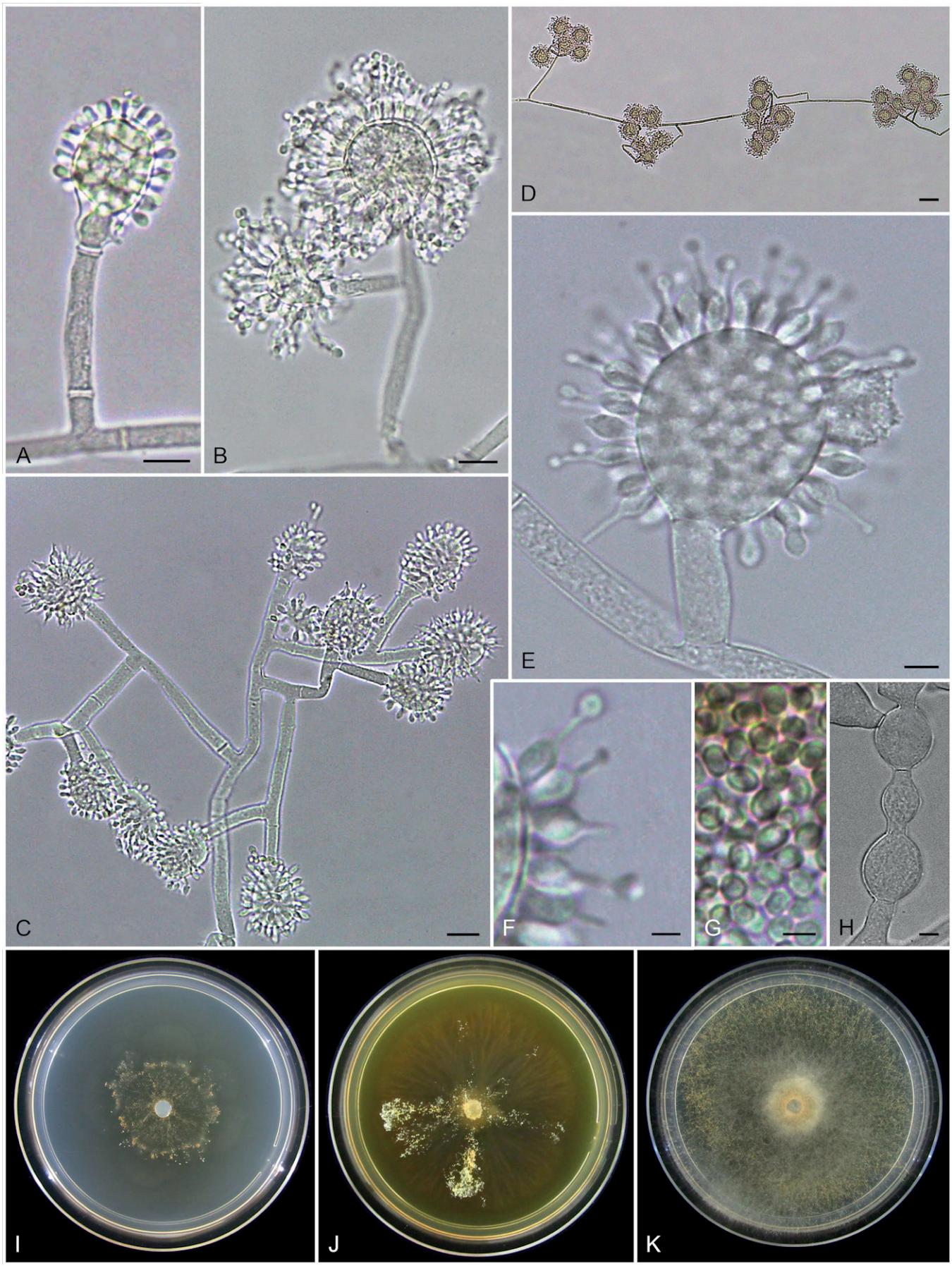

Fig. 4. Morphological characters of *Escovopsis aspergilloides* (ex-type culture CBS 423.93). **A.** Mono-vesiculate conidiophore. **B, C.** Polyvesiculate conidiophores. **D.** Conidiophore arrangement on aerial mycelium. **E.** Globose vesicle with phialides. **F.** Phialides. **G.** Conidia. **H.** Chlamydospores. **I–K.** Cultures on CMD, MEA and PDA, respectively, after 7 d of growth at 25 °C. Scale bars: A–C = 10 µm; D = 40 µm; E = 4 µm; F–H = 2 µm.

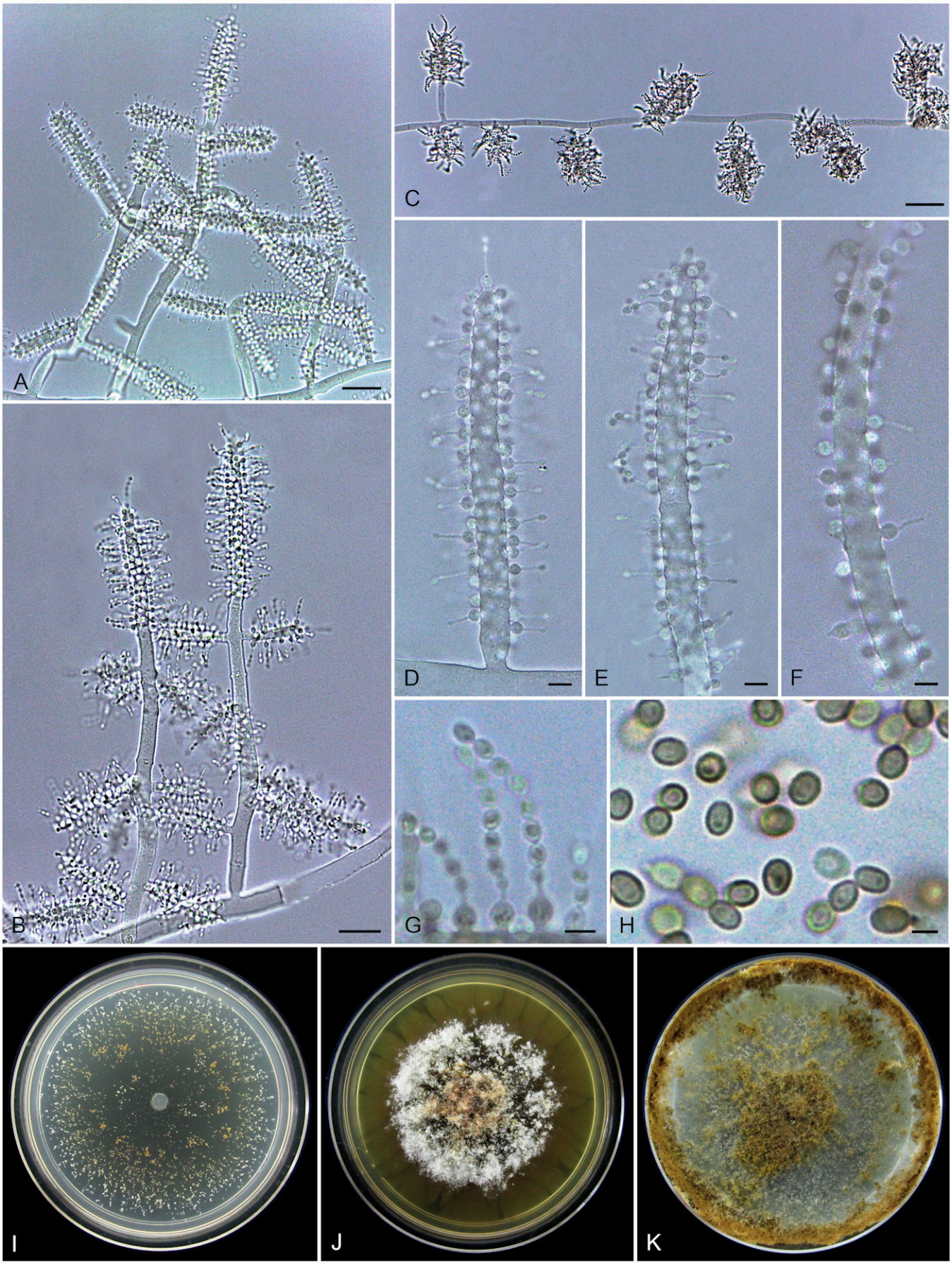

Fig. 5. Morphological characters of *Escovopsis breviramosa* (type culture CBS 149741). **A, B.** Polyvesiculate conidiophores. **C.** Arrangement of monovesiculate conidiophores on aerial mycelium. **D.** Non-septate cylindric vesicles with phialides. **E.** Septate cylindrical vesicle with phialides. **F.** Phialides on aerial mycelium. **G.** Phialides. **H.** Conidia. **I–K.** Cultures on CMD, MEA and PDA, respectively, after 7 d of growth at 25 °C. Scale bars: A–C = 20 µm; D–G = 4 µm; H = 2 µm.

Conidiophore branches 20.5–190 µm long, formed in one or three levels, at almost right angles, alternate. Stipes on branches 1–24.5 µm long, with a septum 0.5–13 µm from conidiophore axis. *Vesicles* cylindrical, 29.5–60 × 3.5–9.5 µm, predominantly aseptate, less frequently septate (1–2 septa), formed on conidiophore axis or on the axis of branches. Vesicle stipe 1–6 µm long, with one septum, rarely with two septa. *Phialides* predominantly formed on vesicles, less frequently on aerial mycelia, 4.5–12 µm long, lageniform, 0–1.5 × 0.5–2 µm at the base, 2.5–4 × 2–3 µm at the swollen section and 1–9.5 × 0.5 µm at the neck. *Conidia* formed in chains, subglobose, 1–3 × 1–2 µm, Olive-Ochre (XXX21"), with smooth and thick wall. *Chlamydospores* absent.

Culture characteristics: *Colonies* growing at 10, 20, 25, and 30 °C on CMD, PDA, and MEA. At 10 °C, growth starts between the first and second day, and at 20, 25, and 30 °C growth starts at the first day, on all media. *Colony* radius, after 4 d at 10 °C: Inconspicuous growth (the colony barely grows on the inoculum); at 20 °C: 7–12 mm on CMD, 10–40 mm on MEA and 40 mm on PDA; at 25 °C: 15–18 mm on CMD and 40 mm on MEA and PDA; at 30 °C: 15–18 mm on CMD, 15–33 mm on MEA and 40 mm on PDA. *Colony morphology* — CMD 25 °C, 7 d: Colonies with diffuse aerial mycelium, spread by stolons, abundant pustule-like formations, White (LIII73(10)) to Olive-Ochre (XXX21"). MEA 25 °C, 7 d: Colonies with aerial mycelium at centre, dense submerged mycelium at margin, pustule-like formations, White (LIII73(10)) to Olive-Ochre (XXX21"). PDA 25 °C, 7 d: Colonies with abundant aerial mycelium, spread by stolons, without pustule-like formations, White (LIII73(10)) to Olive-Ochre (XXX21") (White (LIII73(10)) at centre and Olive-Ochre (XXX21") at margin. Soluble pigments absent.

Ecology: Unknown.

Distribution: This species is found in different regions in Brazil and Panama in fungus gardens of the attine ant genera *Atta*, *Acromyrmex, Apterostigma,* and *Mycetomoellerius*.

Additional materials examined: **Brazil**, Rio Grande do Sul, Nova Petrópolis, grape orchard, 29°22'38.2"S, 50°57'18.1"W, fungus garden of *Acromyrmex ambiguus*, Jun. 2004, A. Rodrigues, LESF 039; São Paulo, Rio Claro, São Paulo State University (UNESP), 22°23'46.0"S, 47°32'43.2"W, fungus garden of *Mycetomoellerius* sp., unknown date, A. Rodrigues, LESF 316.

Notes: *Escovopsis breviramosa* is closely related to *E. gracilis* and *E. peniculiformis.* Unlike strains of *E. peniculiformis, E. breviramosa* grows at 10 °C. Conidiophores of *E. breviramosa* are usually shorter, more branched, and have shorter and broader terminal vesicles than those of *E. peniculiformis*. Unlike *E. gracilis*, strains of *E. breviramosa* grow at 10 and 30 °C. In addition, *E. breviramosa* forms wider and branched conidiophores, and wider and longer vesicles than *E. gracilis*.

Escovopsis chlamydosporosa Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, ***sp. nov.*** MycoBank MB 847806. Fig. 6.

Etymology: "*chlamydosporosa*" (*osa* = Lat *feminine* indicating abundance) in reference to the abundant chlamydospores formed by isolates of this species.

Diagnosis: *Escovopsis chlamydosporosa* forms chlamydospores (*sensu* Augustin *et al.* 2013) more frequently and abundantly than any other known *Escovopsis* species; these structures are rare or absent in most species of this genus.

Typus: **Brazil**, Amazonas, Novo Airão, Parque Nacional de Anavilhanas, 2°31'25.6"S, 60°49'32.4"W, fungus garden of *Trachymyrmex* sp. *sensu lato*, Jan. 2017, Q.V. Montoya, LESF 984 (**holotype** CBS 149748 preserved as metabolically inactive culture, ex-type culture CBS 149748).

Description: *Conidiophores* forming 2–26 vesicles, scarce, thin, hyaline, irregular shaped, smooth-walled, alternate, formed on aerial hyphae. Mono-vesiculate conidiophores 1.5–65.5 µm, polyvesiculate 45.5–380 µm long. Conidiophore stipes 4–170 µm × 3.5–5.5 µm, with a septum 0–6.5 µm from the foot cell. Conidiophore branches 16.5–97.5 µm long, formed in in one or two levels, usually at right angles, alternate. Stipes on branches 1–17.5 µm long, with a septum 1–9.5 µm from conidiophore axis. *Vesicles* cylindrical, 20.5–84.5 × 4–8 µm, predominantly aseptate, less frequently septate (one septum), formed on conidiophore axis or on the axis of branches. Vesicle stipe1–17 µm long, aseptate. *Phialides* formed on vesicles, 5.5–10 µm long, lageniform, 0.5–1.5 × 0.5–1.5 µm at the base, 2–3.5 × 1.5–2.5 µm at the swollen section, 1.5–5.5 × 0.5 µm at the neck. *Conidia* formed in chains, subglobose, 1–4 × 1–3 µm, Olive-Ochre (XXX21"), with smooth and thick wall. *Chlamydospores* very common, 10–17 × 8–16 µm, formed in chains on aerial mycelium, intercalary.

Culture characteristics: *Colonies* growing at 20, 25, and 30 °C on CMD, PDA, and MEA. Growth starts on the first day at all temperatures, on all media. *Colony* radius, after 4 d at 20 °C: 9–14 mm on CMD, 16–20 mm on MEA and 30–40 mm on PDA; at 25 °C: 13–15 mm on CMD, 32–40 mm on MEA and 40 mm on PDA; at 30 °C: 10–15 mm on CMD, 36–20 mm on MEA and 30–40 mm on PDA. *Colony morphology* — CMD 25 °C, 7 d: Colonies with scattered aerial mycelium, spread by stolons, White (LIII73(10)) to Margerite Yellow (XXX23"f). MEA 25 °C, 7 d: Colonies with cottony aerial mycelium, White (LIII73(10)) to Margerite Yellow (XXX23"f). PDA 25 °C, 7 d: Colonies with abundant cottony aerial mycelium, spread by stolons, White (LIII73(10)) to Colonial Buff (XXX21"d). White (LIII73(10)) to Colonial Buff (XXX21"d) pustule-like formations only on PDA. Soluble pigments absent.

Ecology: Unknown.

Distribution: This species is found in Novo Airão, Amazonas, Brazil in fungus gardens of the attine ant genera *Acromyrmex*, *Apterostigma*, and *Trachymyrmex*.

Additional materials examined: **Brazil**, Amazonas, Novo Airão, Parque Nacional de Anavilhanas, 2°16'15.7"S, 61°01'8.46"W, fungus garden of *Acromyrmex* sp., 24 Jan. 2017, Q.V. Montoya, LESF 961, *ibid*., LESF 963; Amazonas, Novo Airão, Parque Nacional de Anavilhanas, 2°31'26.04"S, 60°49'31.62"W, fungus garden of *Trachymyrmex* sp., 20 Jan. 2017, Q.V. Montoya, LESF 991; Amazonas, Novo Airão, Parque Nacional de Anavilhanas, 2°26'52.56"S, 59°45'53.4"W, fungus garden of *Trachymyrmex* sp., 9 Mar. 2017, Q.V. Montoya, LESF 1026.

Notes: *Escovopsis chlamydosporosa* is closely related to *E. rectangula.* Unlike strains of *E. rectangula, E. chlamydosporosa* does not grow at 10 °C. Furthermore, its conidiophores are usually longer, more branched, and irregularly shaped, compared to of *E. rectangula,* which are shorter and slightly rectangular.

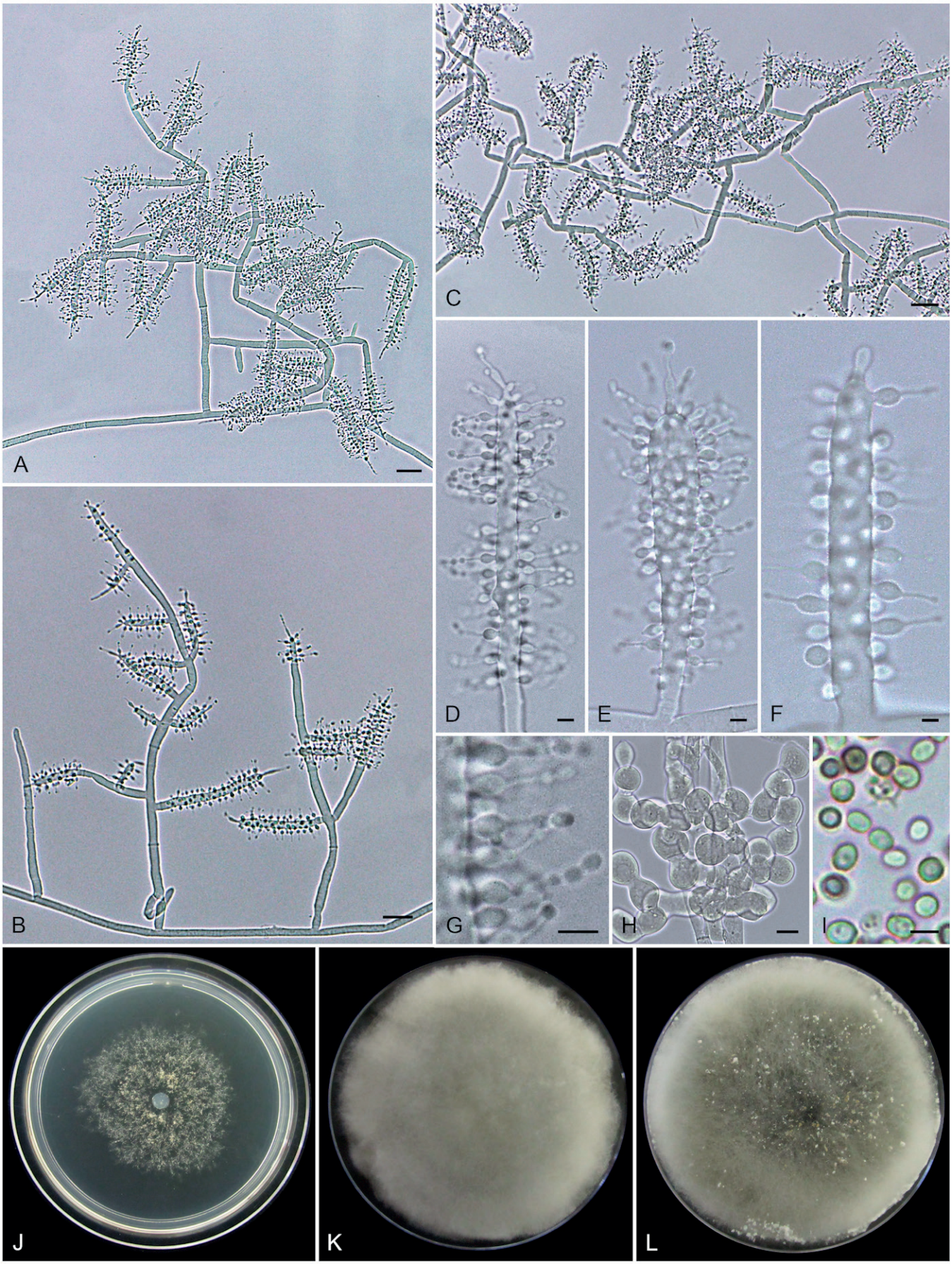

Fig. 6. Morphological characters of *Escovopsis chlamydosporosa* (type culture CBS 149748). **A, B.** Polyvesiculate conidiophores. **C.** Conidiophore arrangement on aerial mycelium. **D.** Septate cylindrical vesicle with phialides. **E.** Clavate vesicle with phialides. **F.** Non-septate cylindrical vesicle with phialides. **G.** Phialides. **H.** Chlamydospores. **I.** Conidia. **J–L.** Cultures on CMD, MEA and PDA, respectively, after 7 d of growth at 25 °C. Scale bars: A–C = 20 µm; D–H = 4 µm; I = 2 µm.

Escovopsis clavata Q.V. Montoya *et al.*, Mycokeys 46: 102. 2019. MycoBank MB 828328. Fig. 7.

Diagnosis: *Escovopsis clavata* usually forms conidiophores with swollen cells and with terminal sterile hypha (conidiophore apex does not end in a vesicle).

Typus: **Brazil**, Santa Catarina, Florianópolis, 27°44'39.6"S, 48°31'10.14"W, elev. 46 m, fungus garden of *Apterostigma* sp., Aug. 2015, A. Rodrigues, LESF 853 (**holotype** CBS H-23845 dried culture, ex-type culture CBS 145326).

Description: *Conidiophores* forming 2–8 vesicles, sometimes with swollen cells, hyaline, irregular shape, smooth-walled, alternate or opposite, formed on aerial hyphae. Mono-vesiculate conidiophores 10–50 µm, polyvesiculate up to 780 µm long. Conidiophore stipes 10–40 µm × 5–8 µm, with a septum 2–9 µm from the foot cell. Conidiophore axis usually ends in a vesicle, sometimes in an infertile hypha and less frequently in a terminal swollen cell 10–18 × 7–9 µm. Conidiophore branches 16–138 µm long (usually shorter, sometimes as long as conidiophore axis), formed in one or two levels, usually at right angles and sometimes slightly curved up or down, alternate or opposite. Swollen cells form 2–4 branches, 28–35 µm long, mostly curved upward or less frequently at right angles. Stipes on branches 9–38 µm long, with a septum 2–6 µm from conidiophore axis. *Vesicles* of various shapes, *i.e.*, globose, sub-globose, capitate, obovoid, prolate, spatulate, predominantly clavate, cymbiform, and cylindric, 9–27 × 7–20 µm, aseptate, formed on the tips of conidiophore and branches. Vesicle stipe 10–30 µm long, with two or six septa. *Phialides* formed on vesicles, 5–8 µm long, lageniform 0.5–1.5 × 0.5–1 µm at the base, 1.5–2.5 × 1–3 µm at the swollen section, 1.5–4 × 0.5 µm at the neck. *Conidia* formed in chains, ellipsoidal to oblong, 1.5–2.5 × 0.5–1.5 µm, Olive-Ochre (XXX21"), with smooth and slightly thick walls. *Chlamydospores* absent.

Culture characteristics: *Colonies* growing at 20 and 25 °C on CMD, PDA, and MEA. At both temperatures, growth starts on the third day on all media. *Colony* radius, after 4 d at 20 °C: 0–3 mm on CMD, 1–4 mm on MEA and 3–5 mm on PDA; at 25 °C: 2–8 mm on CMD, 4–6 mm on MEA and 6–11 mm on PDA. *Colony morphology* — CMD 25 °C, 7 d: Colonies with diffuse aerial mycelium, Margerite Yellow (XXX23"f) to Colonial Buff (XXX21"d). MEA and PDA 25 °C, 7 d: Colonies with dense floccose aerial mycelium forming concentric rings, Margerite Yellow (XXX23"f) to Colonial Buff (XXX21"d) at centre and Margerite Yellow (XXX23"f) at margin. Rarely forming stolons. Pustule-like formations and soluble pigments absent.

Ecology: Unknown.

Distribution: This species is found in different regions of Brazil in fungus gardens of the attine ant genus *Apterostigma*.

Additional materials examined: **Brazil**, Santa Catarina, Florianópolis, 27°44'38.94"S, 48°31'9.3"W, elev. 32 m, fungus garden of *Apterostigma* sp., Aug. 2015, A. Rodrigues, LESF 854; Santa Catarina, Florianópolis, 27°44'39.49"S, 48°31'9.72"W, elev. 38 m, fungus garden of *Apterostigma* sp., Aug. 2015, A. Rodrigues, LESF 855.

Notes: *Escovopsis clavata* is closely related to *E. multiformis*. Unlike strains of *E. multiformis*, which grow at 10, 20, 25 and 30 °C, *E. clavata* only grows at 20 and 25 °C. Conidiophores of *E. clavata* are usually larger and more branched than those of *E. multiformis* and end in a sterile elongation not observed in *E. multiformis*. Compared to the latter species, swollen cells are less frequent and shorter in *E. clavata*.

Escovopsis diminuta Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, ***sp. nov.*** MycoBank MB 847814. Fig. 8.

Etymology: "*diminuta*" (*diminuta* = reduced in size) in reference to the reduced size of the conidiophores.

Diagnosis: *Escovopsis diminuta* forms short and rarely branched conidiophores with globose vesicles.

Typus: **Brazil**, Amazonas, Novo Airão, Parque Nacional de Anavilhanas, 2°31'23.4"S, 60°49'31.9"W, fungus garden of *Trachymyrmex* sp., 20 Jan. 2017, Q.V. Montoya, LESF 969 (**holotype** CBS 149747 preserved as metabolically inactive culture, ex-type culture CBS 149747).

Description: *Conidiophores* forming 2–8 vesicles, hyaline, irregularly shaped, smooth-walled, alternate, formed on aerial hyphae. Mono-vesiculate conidiophores 22–80 µm, polyvesiculate 52–130 µm long. Conidiophore stipe 8–73 × 3.5–8.5 µm, with a septum 0–5 µm from the foot cell. Conidiophore branches 21–61 µm long, formed in one level, in almost right angles, alternate or opposite. Stipes on branches 3–30 µm long, with a septum 0.5–10 µm from conidiophore axis. *Vesicles* globose 17–30 × 15–30 µm, aseptate, formed on the tips of conidiophore and branches. Vesicle stipe 0.5–10 µm long, with two septa. *Phialides* formed on vesicles, 6–9 µm long, lageniform, 0–1 × 1–2 µm at the base, 3–5 × 2–4 µm at the swollen section and 2–4 × 0.5–1 µm at the neck. *Conidia* formed in chains, oblong, 2–3 × 1.5–2.5 µm, Olive-Ochre (XXX21", smooth and thick wall. *Chlamydospores* absent.

Culture characteristics: *Colonies* growing at 20, and 25 °C on CMD, PDA, and MEA. At 20 °C growth starts on second day, and at 25 °C on the first day. *Colony* radius, after 4 d at 20 °C: 4–10 mm on CMD, 3–15 mm on MEA and 12–15 mm on PDA; at 25 °C: 10–16 mm on CMD, 15–18 mm on MEA and 14–21 mm on PDA. *Colony morphology* — CMD 25 °C, 7 d: colonies with diffuse aerial mycelia, with pustule-like formations, White (LIII73(10)) to Colonial Buff (XXX21"d) (Colonial buff (XXX21"d) at centre, White (LIII73(10)) at margin). MEA 25 °C, 7 d: colonies with dense cottony aerial mycelium, few stolons, White (LIII73(10)) to Margerite Yellow (XXX23"f), *Olive-Yellow (XXX23") to Olive-Ochre (XXX21") (*Olive-Yellow (XXX23") to Olive-Ochre (XXX21") at centre, White (LIII73(10)) to Margerite Yellow (XXX23"f) at margin). PDA 25 °C, 7 d: colonies with dense aerial mycelium, few stolons, few pustule-like formations, White (LIII73(10)), Colonial buff (XXX21"d), Olive-Ochre (XXX21") (Olive-Ochre (XXX21") to Colonial Buff (XXX21"d) at centre, White (LIII73(10)) at margin). Light Yellow-Green (VI31d) soluble pigments only on MEA.

Ecology: Unknown.

Distribution: This species was found in the Amazon regions of Brazil in fungus gardens of the attine ant genera *Apterostigma* and *Trachymyrmex*.

Additional materials examined: **Brazil**, Amazonas, Novo Airão, Parque Nacional de Anavilhanas, 2°32'02.7"S, 60°50'11.7"W, fungus garden of *Apterostigma* sp., 19 Jan. 2017, Q.V. Montoya, LESF 996; Amazonas, Novo Airão, Parque Nacional de Anavilhanas, 2°32'1.4"S, 60°50'0.4"W, fungus garden of *Trachymyrmex* sp., 21 Jan. 2017, Q.V. Montoya, LESF 1003.

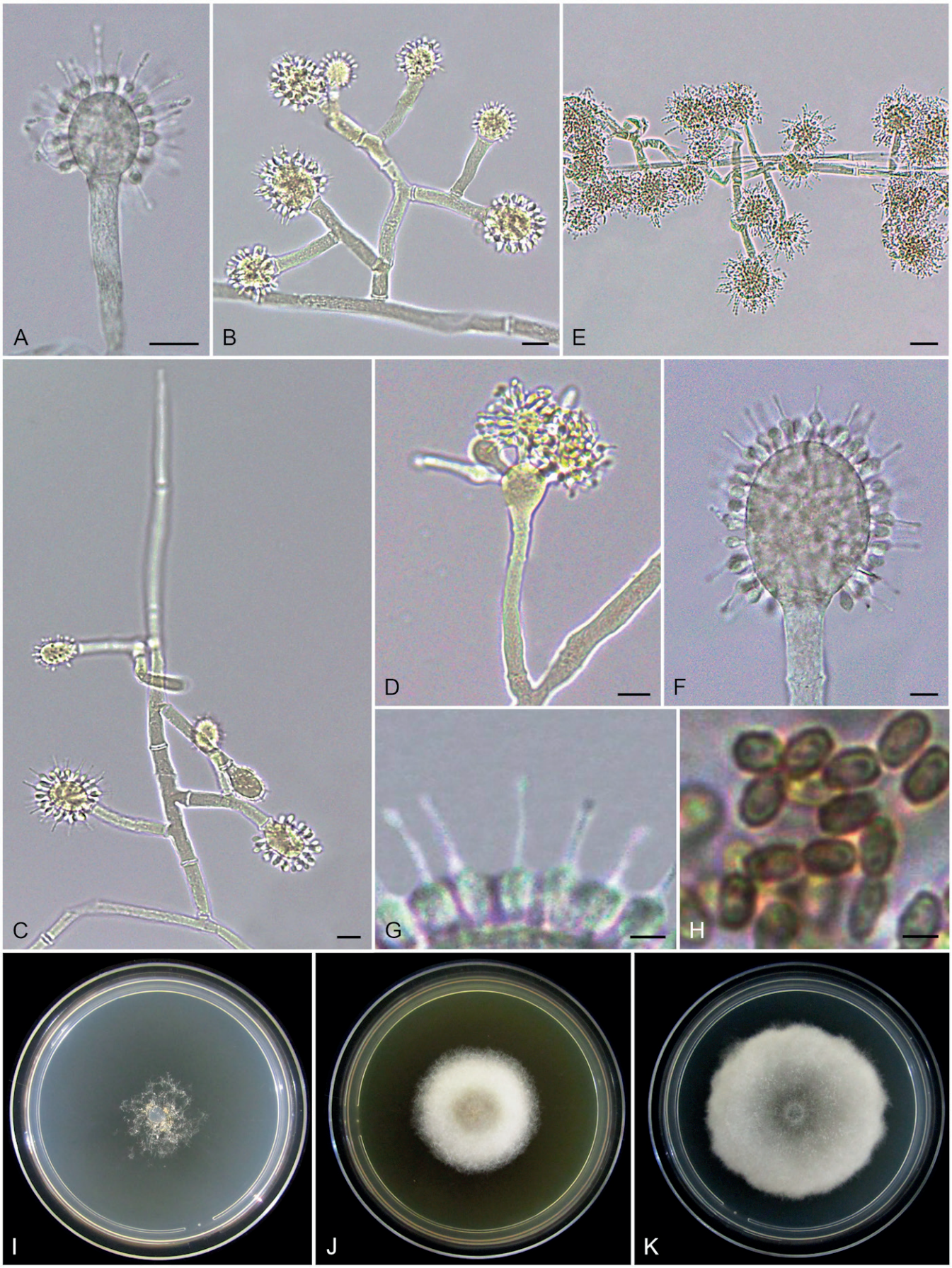

Fig. 7. Morphological characters of *Escovopsis clavata* (ex-type culture CBS 145326). **A.** Mono-vesiculate conidiophores. **B.** Polyvesiculate conidiophore. **C.** Conidiophore with infertile hypha at the apex. **D.** Conidiophore with swollen cell. **E.** Conidiophore arrangement on aerial mycelium. **F.** Clavate vesicle with phialides. **G.** Phialides. **H.** Conidia. **I–K.** Cultures on CMD, MEA and PDA, respectively, after 7 d of growth at 25 °C. Scale bars: A–D = 10 µm; E = 20 µm; F = 4 µm; G; H = 2 µm.

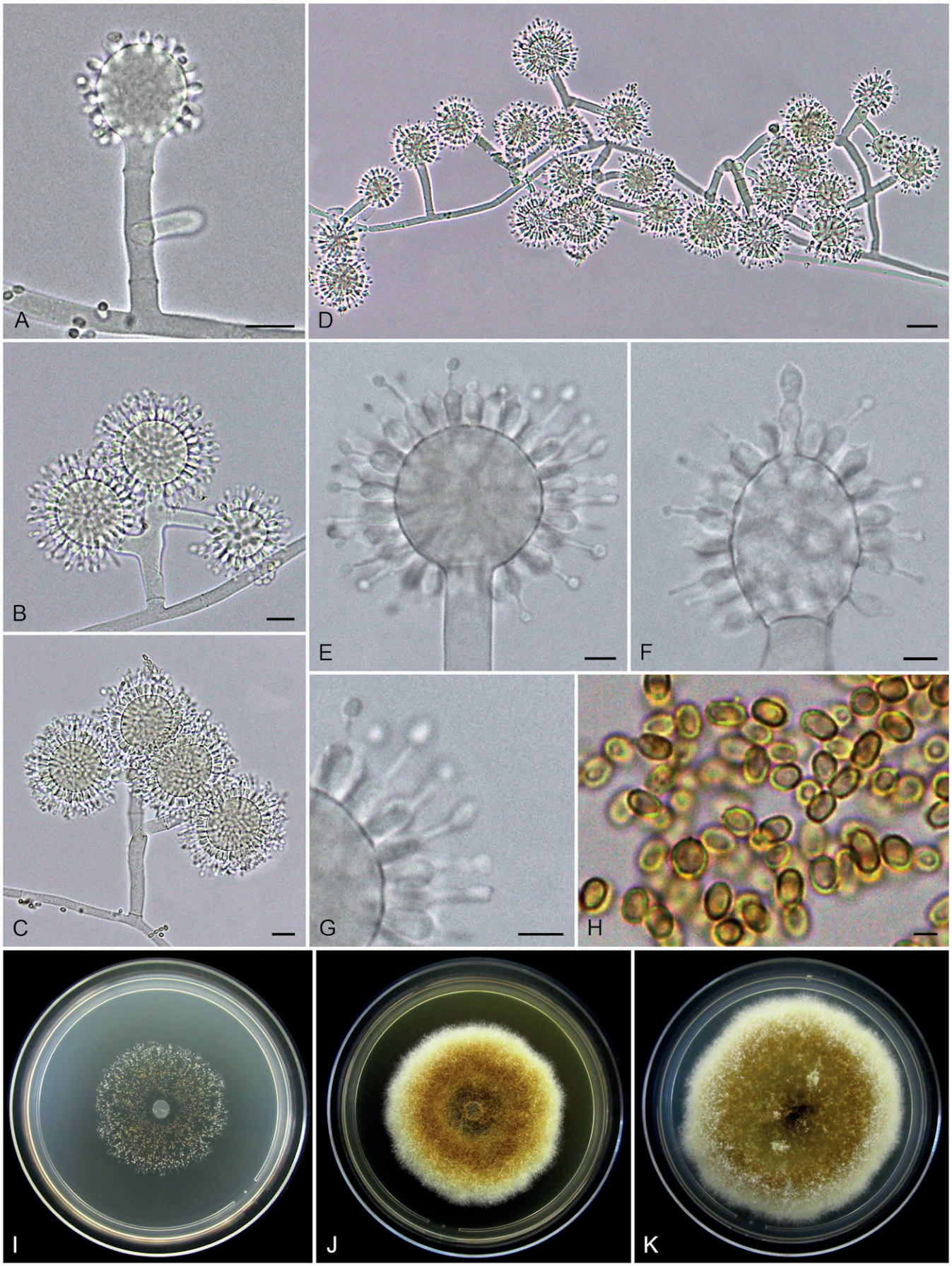

Fig. 8. Morphological characters of *Escovopsis diminuta* (type culture CBS 149747). **A.** Mono-vesiculate conidiophore. **B, C.** Polyvesiculate conidiophores. **D.** Conidiophore arrangement on aerial mycelium. **E.** Globose vesicle with phialides. **F.** Subglobose vesicle with phialides. **G.** Phialides. **H.** Conidia. **I–K.** Cultures on CMD, MEA and PDA, respectively, after 7 d of growth at 25 °C. Scale bars: A–C = 10 µm; D = 20 µm; E–G = 4 µm; H = 2 µm.

Notes: *Escovopsis diminuta* is closely related to *E. rosisimilis* and *E. lentecrescens*. *Escovopsis diminuta* grows faster than *E. rosisimilis* but slower than *E. lentecrescens*. Furthermore, *E. diminuta* has shorter conidiophores than *E. rosisimilis* and *E. lentecrescens*.

Escovopsis elongatistipitata Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, ***sp. nov.*** MycoBank MB 847810. Fig. 9.

Etymology: "*elongatistipitata*" (*elongati* = Latin feminine for elongate, *stipitata* = stipe) in reference to the elongate stipes of both the conidiophores and the conidiophore branches.

Diagnosis: *Escovopsis elongatistipitata* forms conidiophores and conidiophore branches with long stipes.

Typus: **Brazil**, Amazonas, Novo Airão, Parque Nacional de Anavilhanas, 2°31'23.4''S, 60°49'31.9''W, fungus garden of *Trachymyrmex* sp., 20 Jan. 2017, Q.V. Montoya, LESF 999, (**holotype** CBS 149750 preserved as metabolically inactive culture, ex-type culture LESF 999 = CBS 149750).

Description: *Conidiophores* forming 2–13 vesicles, hyaline, irregular shaped, smooth-walled, alternate or opposite, formed on aerial hyphae. Mono-vesiculate conidiophores rare, 54–120 µm, polyvesiculate 56.5–380 µm long. Conidiophore stipes 10.5–190 × 2–6.5 µm, with a septum 0.5–9 µm from the foot cell. Conidiophore branches 20–230 µm long, formed in one or two levels, usually at right angles, less frequently at angles less than 90°, alternate or opposite. Stipes on branches 3–220 µm long, with a septum commonly 0–2 µm and rarely 6–20 µm from conidiophore axis. *Vesicles* mainly cylindric, less frequently clavate, 14–74.5 × 2–7.5 µm, aseptate, formed on conidiophore axis or on the axis of branches. Vesicle stipe 0–76 µm long, with one or two septa. *Phialides* formed on vesicles, 4–13 µm long, lageniform, 0–1 × 1–2 µm at the base, 2–7 × 1.5–3 µm at the swollen section and 1–3.5 × 0.5–1.5 µm at the neck. *Conidia* formed in chains, oblong, 3–5 × 2–3.5 µm, Olive-Ochre (XXX21"), with ornamented and thick wall. *Chlamydospores* absent.

Culture characteristics: *Colonies* growing at 20, and 25 °C on CMD, MEA, PDA. Growth starts on second day at both temperatures, on all media. *Colony* radius, after 4 d at 20 °C: 5–9 mm on CMD, 10–15 mm on MEA and 12–15 mm on PDA; at 25 °C: 8–12 mm on CMD, 25–30 mm on MEA and 20–23 mm on PDA. *Colony morphology* — CMD 25 °C, 7 d: colonies with diffuse cottony aerial mycelium, White (LIII73(10)) to Margerite Yellow (XXX23"f). MEA 25 °C, 7 d: colonies with cottony aerial mycelium, sometimes spread by stolons, forming concentric rings, White (LIII73(10)) to Margerite Yellow (XXX23"f) and Colonial Buff (XXX21"d) (Colonial buff (XXX21"d) at centre White (LIII73(10)) to Margerite Yellow (XXX23"f) at margin). PDA 25 °C, 7 d: colonies with cottony aerial mycelium, spread by stolons, White (LIII73(10)) to Margerite Yellow (XXX23"f). Pustule-like formations and soluble pigments absent.

Ecology: Unknown.

Distribution: This species was found in the amazon regions of Brazil in fungus gardens of the attine ant *Trachymyrmex*.

Additional materials examined: **Brazil**, Amazonas, Novo Airão, Parque Naciona de Anavilhanas, 2°16'8.7''S, 59°27'32.32''W, fungus garden of *Trachymyrmex* sp., 20 Jan. 2017, Q.V. Montoya, LESF 1021; Amazonas, Novo Airão, Parque Nacional de Anavilhanas, 2°18'52.09''S, 60°27'29.41.02''W, fungus garden of *Trachymyrmex* sp., 20 Jan. 2017, Q.V. Montoya, LESF 985.

Notes: *Escovopsis elongatistipitata* is closely related to *E. phialicopiosa*. Unlike strains of the latter species, which can grow at 30 °C on MEA and PDA and do not form concentric rings on any media, *E. elongatistipitata* does not grow at 30 °C and eventually forms concentric rings on MEA and PDA.

Escovopsis gracilis Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, ***sp. nov.*** MycoBank MB 847807. Fig. 10.

Etymology: "*gracilis*" (*gracilis* = thin) in reference to the narrow conidiophores and vesicles.

Diagnosis: *Escovopsis gracilis* forms cottony colonies with disperse narrow conidiophores and vesicles.

Typus: **Brazil**, Bahia, Camacan, Serra Bonita, 14°47'56.8''S, 39°10'16.4''W, fungus garden of *Atta cephalotes*, 3 Jul. 2012, A. Rodrigues, LESF 325 (**holotype** CBS 149743 preserved as metabolically inactive culture, ex-type culture CBS 149743).

Description: *Conidiophores* forming 2–10 vesicles, scarce, thin, hyaline, irregular shaped, smooth-walled, alternate, formed on aerial hyphae. Mono-vesiculate conidiophores 27–150 µm, polyvesiculate 78–680 µm long. Conidiophore stipes 7.5–550 µm × 3–5.5 µm, with a septum 2–58.5 µm from the foot cell. Conidiophore branches 32.5–120 µm long, formed in one level, rarely in two levels, usually at angles less than 90°, less frequently at right angles, alternate. Stipes on branches 2–90 µm long, with a septum 0–22.5 µm from conidiophore axis. *Vesicles* thin, cylindrical, 19.5–81 × 2.5–5.5 µm, predominantly aseptate, less frequently septate (1–2 septa), formed on conidiophore axis or on the axis of branches. Vesicle stipe 0.5–7.5 µm long, septate (1–2 septa). *Phialides* formed on vesicles, 5–13 µm long, lageniform, 1–2 × 0.5–2 µm at the base (sometimes base absent), 3–7.5 × 2–4 µm at the swollen cell and 0.5–7 × 0.5–1 µm at the neck. *Conidia* formed in chains, subglobose, 3–7 × 2.5–4.5 µm, Olive-Ochre (XXX21"), with smooth and thick wall. *Chlamydospores* absent.

Culture characteristics: *Colonies* growing at 20, and 25 °C on CMD, PDA, and MEA. Growth starts on the first day at both temperatures, on all media. *Colony* radius, after 4 d at 20 °C: 32–33 mm on CMD, 33–37 mm on MEA and 40 mm on PDA; at 25 °C: 12–19 mm on CMD and 40 mm on MEA and PDA. *Colony morphology* — CMD 25 °C, 7 d: Colonies with scattered aerial mycelium, spread by stolons, few short White (LIII73(10)) pustule-like formations, White (LIII73(10)) to Margerite Yellow (XXX23"f). MEA 25 °C, 7 d: Colonies with abundant cottony aerial mycelium (sometimes forming concentric rings), spread by stolons, without pustule-like formations, White (LIII73(10)) to Margerite Yellow (XXX23"f). PDA 25 °C, 7 d: Colonies with abundant dense aerial mycelium (sometimes forming concentric rings), spread by stolons, abundant White (LIII73(10)) to Colonial Buff (XXX21"d) pustule-like formations, White (LIII73(10)) to Colonial buff (XXX21"d). Soluble pigments absent.

Ecology: Unknown.

Distribution: This species is found in Bahia-Brazil in fungus gardens of the attine ant *Atta cephalotes*.

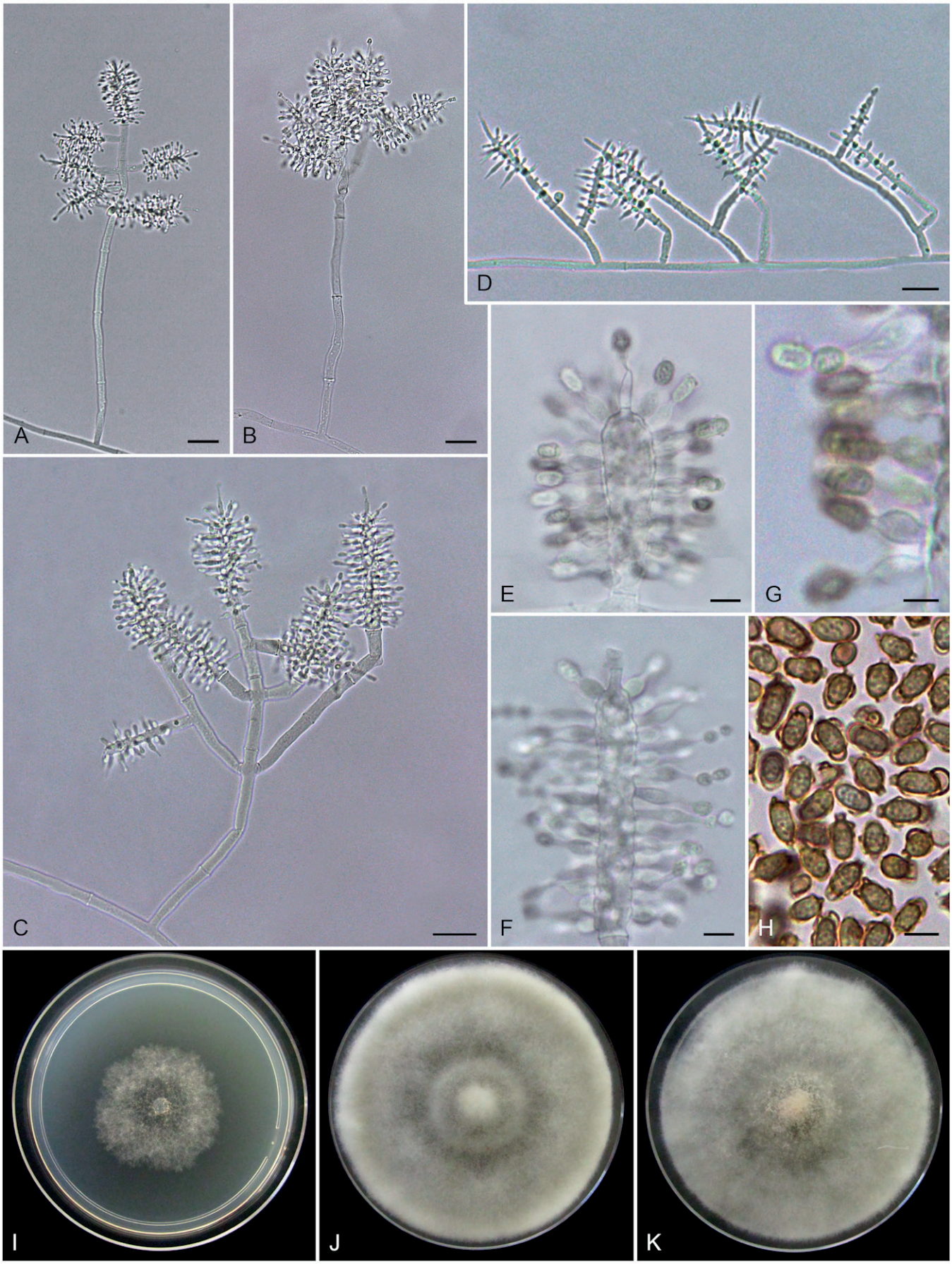

Fig. 9. Morphological characters of *Escovopsis elongatistipitata* (type culture CBS 149750). **A–C.** Polyvesiculate conidiophores. **D.** Conidiophore arrangement on aerial mycelium. **E.** Clavate vesicle with phialides. **F.** Cylindric vesicle with phialides. **G.** Phialides. **H.** Conidia. **I–K.** Cultures on CMD, MEA and PDA, respectively, after 7 d of growth at 25 °C. Scale bars: A–C = 20 µm; D = 10 µm; E–H = 4 µm.

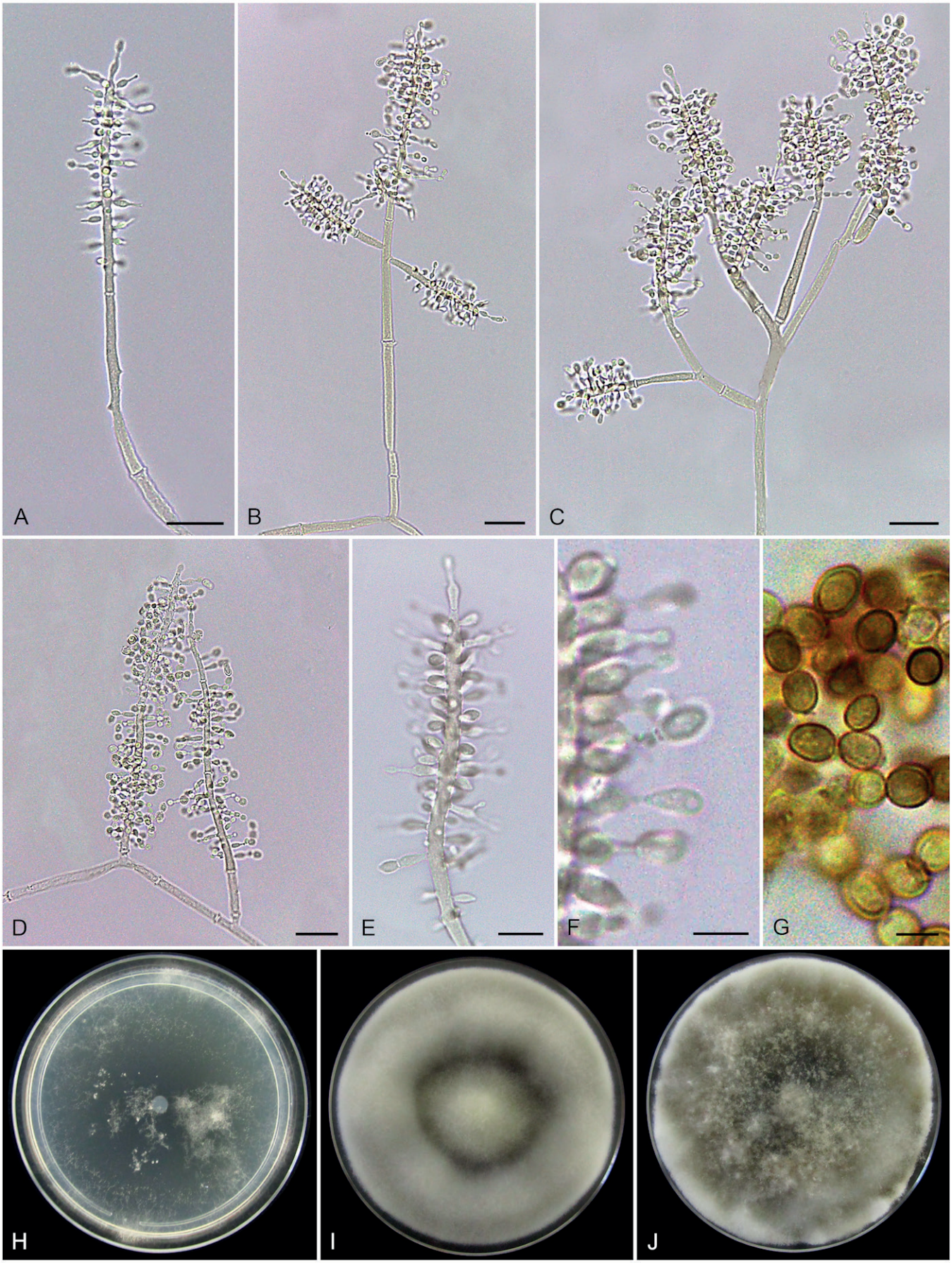

Fig. 10. Morphological characters of *Escovopsis gracilis* (type culture CBS 149743). **A.** Mono-vesiculate conidiophore. **B, C.** Polyvesiculate conidiophores. **D, E.** Cylindrical vesicle with phialides. **F.** Phialides. **G.** Conidia. **H–J.** Cultures on CMD, MEA and PDA, respectively, after 7 d of growth at 25 °C. Scale bars: A–D = 20 µm; E = 10 µm; F, G = 4 µm.

Additional materials examined: **Brazil**, Bahia, Camacan, Fazenda Paris, 14°47'51.18"S, 39°10'17.4"W, fungus garden of *Atta cephalotes*, 15 Mar. 2013, A. Rodrigues, LESF 843; Bahia, Camacan, Serra Bonita, 15°23'14.82"S, 39°33'28.38"W, fungus garden of *Atta cephalotes*, 21 Feb. 2013, A. Rodrigues, LESF 844.

Notes: *Escovopsis gracilis* is closely related to *E. breviramosa.* Unlike strains of the latter species, *E. gracilis* does not grow at 10 or 30 °C. Conidiophores of *E. gracilis* are usually thinner, longer and more branched than those of *E. breviramosa*.

Escovopsis lentecrescens H.C. Evans & J.O. Augustin, PLoS ONE 8 (12): e82265, 5. 2013. MycoBank MB 800441. Fig. 11.

Diagnosis: *Escovopsis lentecrescens* has the slowest growth rate in culture among the known species of the genus.

Typus: **Brazil**, Minas Gerais, Viçosa, Mata do Paraíso, elev. 700 m, fungal garden of *Acromyrmex subterraneus subterraneus*, Apr. 2010, J.O. Augustin & H.C. Evans, AUJ9 (**holotype** IMI 501179, **isotypes** CBS 135750, DOA628 and VIC 31755).

Description: *Conidiophores* forming 1–10 vesicles, hyaline, of irregular shape, smooth-walled, alternate or opposite, formed on aerial hyphae. Mono-vesiculate conidiophores 36–150 µm, polyvesiculate 57–200 µm long. Conidiophore stipes 28–49 µm × 5–7 µm, with a septum 2–6 µm from the foot cell. Conidiophore branches 20–80 µm long, formed in one to three levels, in almost right angles, alternate. The second branching level is usually much longer than other branching levels. Stipes on branches 7–31 µm long, with a septum up to 9 µm from conidiophore axis. *Vesicles* of various shapes, *i.e.*, predominantly globose, subglobose, spathulate, oblanceolate and cylindric, 14–27 µm × 13–27 µm, aseptate, formed on the tips of conidiophore and branches. Vesicle stipe 10–94 µm long with 1–6 septa. *Phialides* formed on vesicles, 6–8.5 µm long, ampulliform, 0.5–1 × 1–1.5 µm at the base, 4–5 × 2.5–3 µm at the swollen section and 2–3 × 0.5–0.6 µm at the neck. *Conidia* formed in chains, globose to oblong, 2–3.5 × 1.5–2 µm, Olive-Ochre (XXX21''), with smooth and slightly thick walls. Rarely *chlamydospores* intercalary, hyaline, 9.5–23 × 8.5–16 µm.

Culture characteristics: *Colonies* growing at 20, and 25 °C on CMD, PDA, and MEA. At 20 °C, growth starts on the third day, and at 25 °C, growth starts on the second day, on all media. *Colony* radius, after 4 d at 20 °C: 0–2 mm on CMD, 0–1 mm on MEA and 0–1 mm on PDA; at 25 °C: 2–3 mm on CMD, 2–5 mm on MEA and 2–3 mm on PDA. *Colony morphology* — CMD 25 °C, 7 d: Colonies with diffuse aerial mycelium, spread by stolons, *Vinaceous-Cinnamon (XXIX13"b) to Colonial Buff (XXX21"d). MEA 25 °C, 7 d: Colonies with dense cottony aerial mycelium, forming concentric rings, White (LIII73(10)) to Margerite Yellow (XXX23"f). PDA 25 °C, 7 d: Colonies with dense cottony aerial mycelium, forming concentric rings, White (LIII73(10)) to Light Brownish olive (XXX19"k) (Colonial buff (XXX21"d) to Light Brownish Olive (XXX19"k) at centre and White (LIII73(10)) at margin). Rarely forming stolons. Pustule-like formations and soluble pigments absent.

Ecology: Unknown.

Distribution: This species has been found only in one region in Brazil in fungus garden of *Acromyrmex subterraneus*.

Notes: *Escovopsis lentecrescens* is closely related to *E. diminuta*. Unlike strains of *E. diminuta*, which form yellowish-brown to brown colonies, *E. lentecrescens* usually has white to light-brown or beige colonies. In addition, conidiophores formed by *E. lentecrescens* are slightly longer and more branched than those of *E. diminuta*.

Escovopsis maculosa Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, ***sp. nov.*** MycoBank MB 847815. Fig. 12.

Etymology: "*maculosa*" (*maculosa* = mottled, full of spots) in reference to the mottled aspect of the mycelial growth displayed by strains of this species on PDA.

Diagnosis: *Escovopsis maculosa* displays a mottled aspect on the base of plates containing PDA. This is caused by a dense pattern of stolons that give the appearance of spots on the reverse of the colonies.

Typus: **Brazil**, Amazonas, Novo Airão, Parque Nacional de Anavilhanas, 2°16'15.7"S, 61°01'8.5"W, fungus garden of *Acromyrmex* sp., 24 Jan. 2017, Q.V. Montoya, LESF 962 (**holotype** CBS 149746 preserved as metabolically inactive culture, ex-type culture CBS 149746).

Description: *Conidiophores* forming 2–14 vesicles, hyaline, irregularly shaped, smooth-walled, alternate or opposite, formed on aerial hyphae. Mono-vesiculate conidiophores 44–82 µm (less frequent), polyvesiculate 57–180 µm long. Conidiophore stipes 10–83 × 4–8 µm, with a septum 0.5–15 µm from the foot cell. Conidiophore branches 26–100 µm long, formed in one level, in almost right angles, alternate or opposite. Stipes on branches 2–75 µm long, with a septum 1–8 µm from conidiophore axis. *Vesicles* globose, 13–24 × 12–22 µm, aseptate, formed on the tips of conidiophore and branches. Vesicle stipe 5–70 µm long, usually with two to three septa. *Phialides* formed on vesicles, 5–8 µm long, lageniform, 0.5–1.5 × 0.5–2 µm at the base, 2–4.5 × 2–3.5 µm at the swollen cell and 1–3.5 × 0.5–1 µm at the neck. *Conidia* formed in chains, oblong, 2–4 × 1.5–2.5 µm, Olive-Ochre (XXX21", smooth and thick walls. *Chlamydospores* absent.

Culture characteristics: *Colonies* growing at 20, and 25 °C on CMD, PDA, and MEA. At 20 °C, growth starts on third day, on all media. At 25 °C growth starts on third day, on CMA and MEA and on first day on PDA. *Colony* radius, after 4 d at 20 °C: 2–4 mm on CMD, 2–5 mm on MEA and 2–7 mm on PDA; at 25 °C: 3–5 mm on CMD, 5–7 mm on MEA and 19–30 mm on PDA. *Colony morphology* — CMD 25 °C, 7 d: colonies with diffuse aerial mycelium, usually spread by stolons, with abundant conidia, White (LIII73(10)) to Olive-Ochre (XXX21"). MEA 25 °C, 7 d: colonies with diffuse aerial mycelium, spread by stolons, Margerite Yellow (XXX23"f) to Light Yellow-Green (VI31d). PDA 25 °C, 7 d: colonies with cottony aerial mycelium, abundant stolons, Margerite Yellow (XXX23"f) to Light Yellow-Green (VI31d). colonies with a mottled aspect on the reverse of the plate on all media, but more visible on PDA. Pustule-like formations and soluble pigments absent.

Ecology: Unknown.

Distribution: This species was found in the amazon regions of Brazil in fungus garden of the attine ant genus *Acromyrmex*.

Notes: *Escovopsis maculosa* is closely related to *E. aspergilloides*. However, *E. maculosa* grows faster and forms shorter and

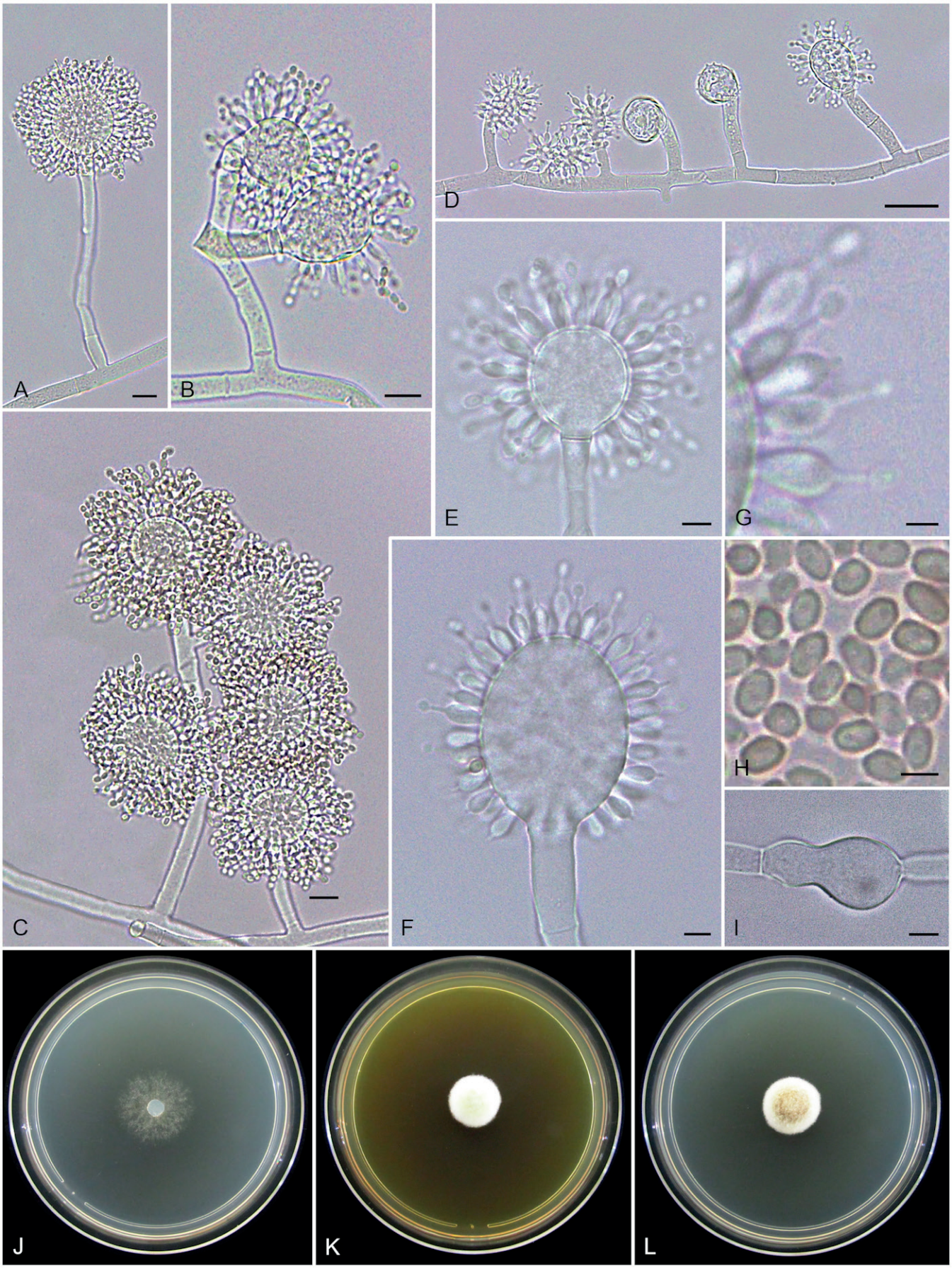

Fig. 11. Morphological characters of *Escovopsis lentecrescens* (ex-type culture CBS 135750). **A.** Mono-vesiculate conidiophores. **B, C.** Polyvesiculate conidiophore. **D.** Conidiophore arrangement on aerial mycelium. **E.** Globose vesicle with phialides. **F.** Subglobose vesicle with phialides. **G.** Phialides. **H.** Conidia. **I.** Chlamydospore. **J–L.** Cultures on CMD, MEA and PDA, respectively, after 7 d of growth at 25 °C. Scale bars: A–C = 10 µm; D = 20 µm; E, F, I = 4 µm; G, H = 2 µm.

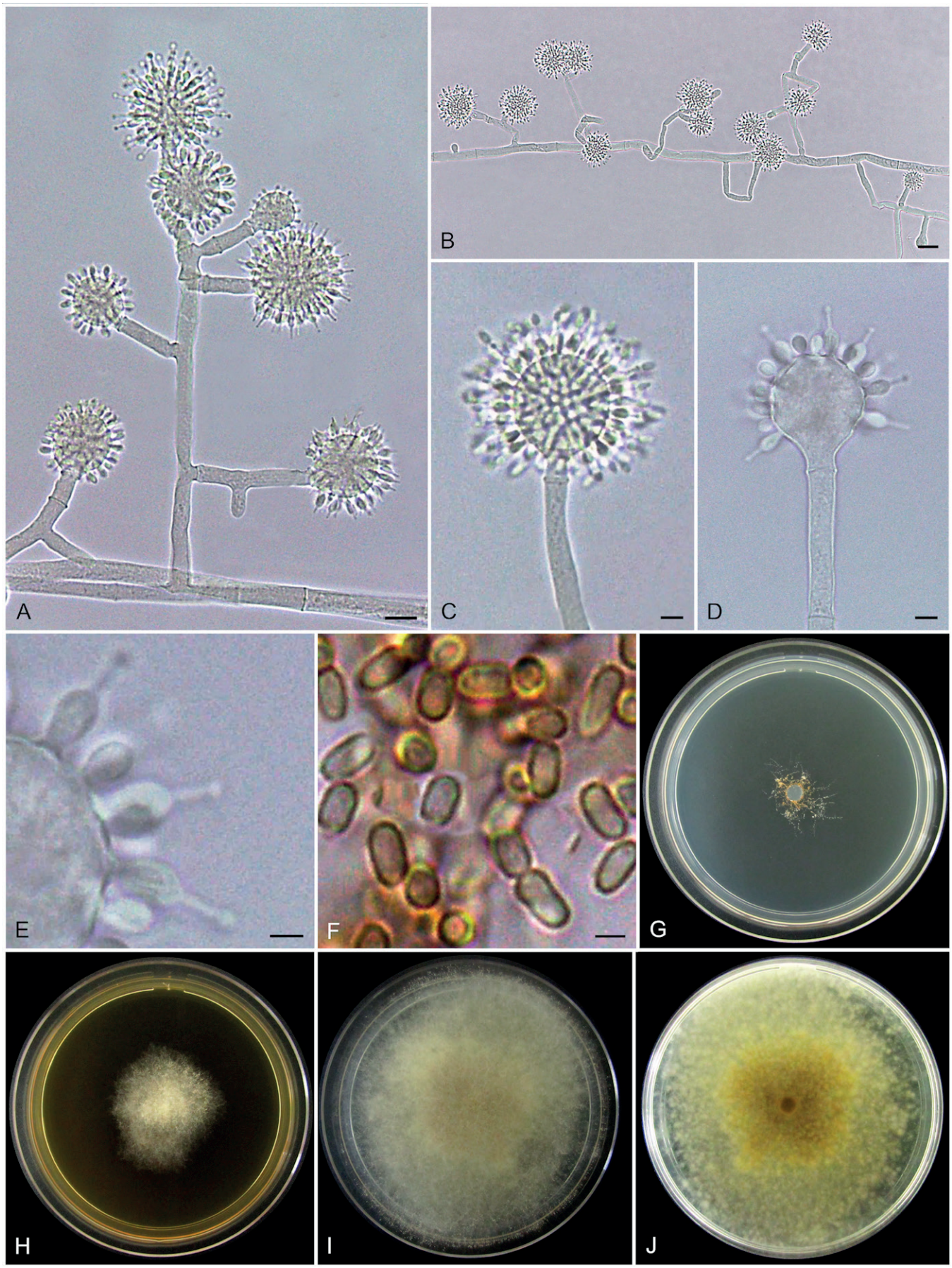

Fig. 12. Morphological characters of *Escovopsis maculosa* (type culture CBS 149746). **A.** Polyvesiculate conidiophore. **B.** Conidiophore arrangement on aerial mycelium. **C, D.** Globose vesicles with phialides. **E.** Phialides. **F.** Conidia. **G–I.** Cultures on CMD, MEA and PDA, respectively, after 7 d of growth at 25 °C. **J.** Dense areas formed by stolons visible as small dots at the bottom of the PDA medium. Scale bars: A= 20 µm, B = 20 µm; C–D = 4 µm; E–F = 2 µm.

less branched conidiophores than *E. aspergilloides*. Unlike *E. aspergilloides*, which forms vesicles of various shapes *i.e.*, globose, sub-globose, capitate, obovoid, prolate and spatulate, *E. maculosa* forms mainly globose vesicles.

Escovopsis moelleri H.C. Evans & J.O. Augustin, PLoS ONE 8 (12): e82265, 4. 2013. MycoBank MB 800440. Fig. 13.

Diagnosis: *Escovopsis moelleri* forms mostly subulate vesicles and conidia with thickened cell walls and ornamentations.

Typus: **Brazil**, Minas Gerais, Viçosa, Mata do Paraíso, elev. 700 m, fungus garden of *Acromyrmex subterraneus molestans* Forel, Mar. 2010, J.O. Augustin & H.C. Evans, AUJ5 (**holotype** IMI 501176, ex-type culture CBS 135748 = DOA626 = VIC 31753). GenBank: JQ815077 (ITS); JQ855715 (28S); MT305413 (*rpb*1); MT305538(*rpb*2); JQ855712 (*tef*1).

Description: *Conidiophores* forming 2–9 vesicles, hyaline, usually pyramidal, less frequently of irregular shape, smooth-walled, mostly alternate, less frequently opposite, formed on aerial hyphae. Mono-vesiculate conidiophores, rare, 34–54 µm long, and polyvesiculate 70–230 µm long. Conidiophores stipes 2–52 µm × 6–10 µm, with 1–5 septa, first septum 2–7 µm from the foot cell. Conidiophore branches 30–89 µm long, formed in one or two levels, in almost right angles and sometimes slightly curved upward, mostly alternated, less frequent opposite. Stipes on branches 2–20 µm long, with a septum at 2–8.5 µm from conidiophore axis. *Vesicles* of various shapes, *i.e*., subulate, oblanceolate, and clavate, 22–60 µm × 5–10 µm, predominantly aseptate and rarely with one septum (clavate-septate), formed on the tips of conidiophore and branches. Vesicle stipe 1.5–17 µm long, with one or three septa. *Phialides* formed on vesicles, 5–7 µm long, ampulliform, 1.7–3 × 0.5–1 µm at the base, 4.4–5.6 × 3.6–4.8 µm at the swollen section and 1–1.7 × 1–2 µm at the neck. *Conidia* formed on phialides, predominantly solitary, less frequent in short chains, oblong-ornamented, 6–7 × 3–3.8 µm, Olive-Ochre (XXX21"), with ornamentation and thick walls. *Chlamydospores* absent.

Culture characteristics: *Colonies* growing at 10, 20, and 25 °C on CMD, PDA and MEA. At 10 °C, growth starts between second and third day. At 20 °C, growth starts between first and second day and at 25 °C, growth starts on the first day, on all media. *Colony* radius, after 4 d at 10 °C: Inconspicuous growth (the colony barely grows on the inoculum); at 20 °C: 23–38 mm on CMD, 35–40 mm on MEA and 31–40 mm on PDA; at 25 °C: >40 mm on CMD, MEA and PDA (colonies reach the plate edge between the third and fourth day). *Colony morphology* — CMD 25 °C, 7 d: Colonies with thin aerial mycelium, spread by stolons and submerged mycelium, Margerite Yellow (XXX23"f) to Colonial Buff (XXX21"d). MEA 25 °C, 7 d: colonies with short aerial mycelium, mostly spread by submerged mycelium, White (LIII73(10)) to Margerite Yellow (XXX23"f). PDA 25 °C, 7 d: Colonies with cottony aerial mycelium, spread predominantly by submerged mycelium and less by stolons, Margerite Yellow (XXX23"f) to Olive-Ochre (XXX21") (Olive-Ochre (XXX21") at centre and Margerite Yellow (XXX23"f) at margin). Pustule-like formations and soluble pigments absent.

Ecology: Unknown.

Distribution: This species has been found only in one region in Brazil in fungus garden of *Acromyrmex subterraneus molestans*.

Notes: *Escovopsis moelleri* is closely related to *E. spicaticlavata*. Unlike species of its sister clade, which form clavate vesicles, those of *E. moelleri* are mostly subulate. In addition, *E. moelleri* does not grow at 30 °C and at 25 °C its colonies grow faster than those of *E. spicaticlavata*.

Escovopsis multiformis Q.V. Montoya *et al.*, Mycokeys 46: 106. 2019. MycoBank MB 828329. Fig. 14.

Diagnosis: *Escovopsis multiformis* is characterized by forming vesicles of multiple shapes. Swollen cells are commonly present on the conidiophores.

Typus: **Brazil**, Santa Catarina, Florianópolis, 27°28'11.28''S, 48°22'39.48''W, elev. 119 m, fungus garden of *Apterostigma* sp., Aug. 2015, A. Rodrigues, LESF 847 (**holotype** CBS H-23846, ex-type culture CBS 145327). GenBank: MH715091 (ITS); MH715105 (28S); MT305420 (*rpb*1); MT305545 (*rpb*2); MH724265 (*tef*1).

Description: *Conidiophores* forming 2–9 vesicles, sometimes with swollen cells, hyaline, usually of irregular shape, smooth-walled, alternate or opposite, formed on aerial hyphae. Mono-vesiculate conidiophores 66–130 µm, and polyvesiculate 82–290 µm long. Conidiophore stipes 16–56 µm × 7–9 µm, with 1–3 septa, first septum 1–2 µm from the foot cell. Conidiophore axis usually ends in a vesicle, and sometimes in a swollen cell 16–34 µm × 9–20 µm. Conidiophore branches 32–84 µm long (usually short, sometimes as long as the conidiophore axis), formed in one or three branching levels, usually at right angles and sometimes slightly curved upward, alternate. Swollen cells form 2–6 branches, 28–35 µm long, mostly curved upward, less frequently at right angles. Swollen-cell branch usually ends in a vesicle but sometimes forms an additional swollen cell with 2–4 new branches. Stipes on branches 22–70 µm long, with a septum at 1–2 µm from conidiophore axis. *Vesicles* of various shapes, *i.e*., globose, predominantly subglobose, capitate, obovoid, prolate, spatulate, cymbiform, and cylindric, 12–27 × 9–17 µm, aseptate, formed on the tips of conidiophore and branches. Vesicle stipe 22–70 µm long, with one or four septa. *Phialides* formed on vesicles, 6–10 µm long, lageniform, 1–2.5 × 0.5–1µm at the base, 2.5–4.5 × 2–3.5 µm at the swollen section, 1–4.5 × 0.5–1 µm at the neck. *Conidia* formed in chains, globose to oblong, 2.5–3.5 µm × 1.5–2.5 µm, Olive-Ochre (XXX21"), with smooth and slightly thick wall. *Chlamydospores* absent.

Culture characteristics: *Colonies* growing at 10 °C on PDA and MEA, and at 20, 25 and 30 °C on CMD, PDA and MEA. At 10 °C growth starts between the second and third day on PDA and MEA and after fourth day on CMD. At 20 °C and 30 °C, growth starts on second day on CMD and on third day on MEA and PDA. At 25 °C, growth starts on second day on all media. *Colony* radius, after 4 d at 10 °C: Inconspicuous growth (the colony barely grows on the inoculum); at 20 °C: 4–10 mm on CMD, 3–6 mm on MEA and 2–5 mm on PDA; at 25 °C: 6–10 mm on CMD, 4–7 mm on MEA and 4–7 mm on PDA; at 30 °C: 4–7 mm on CMD, mm on 0–3 MEA and 0–2 mm on PDA. *Colony morphology* — CMD 25 °C, 7 d: Colonies with diffuse aerial mycelium, spread by stolons, submerged mycelium forming dense circular zones, pustule-like formations, Margerite Yellow (XXX23"f) to Colonial buff (XXX21"d). MEA 25 °C, 7 d: Colonies with dense cottony mycelium, White (LIII73(10)) to Margerite Yellow (XXX23"f), forming Margerite Yellow (XXX23"f) exudates. PDA 25 °C, 7 d: Colonies with raised cottony mycelium, White (LIII73(10)) to Olive-Ochre (XXX21") colours (Olive-Ochre (XXX21") at centre and White (LIII73(10)) at margin). Rarely forming stolons on MEA and PDA. Soluble pigments absent.

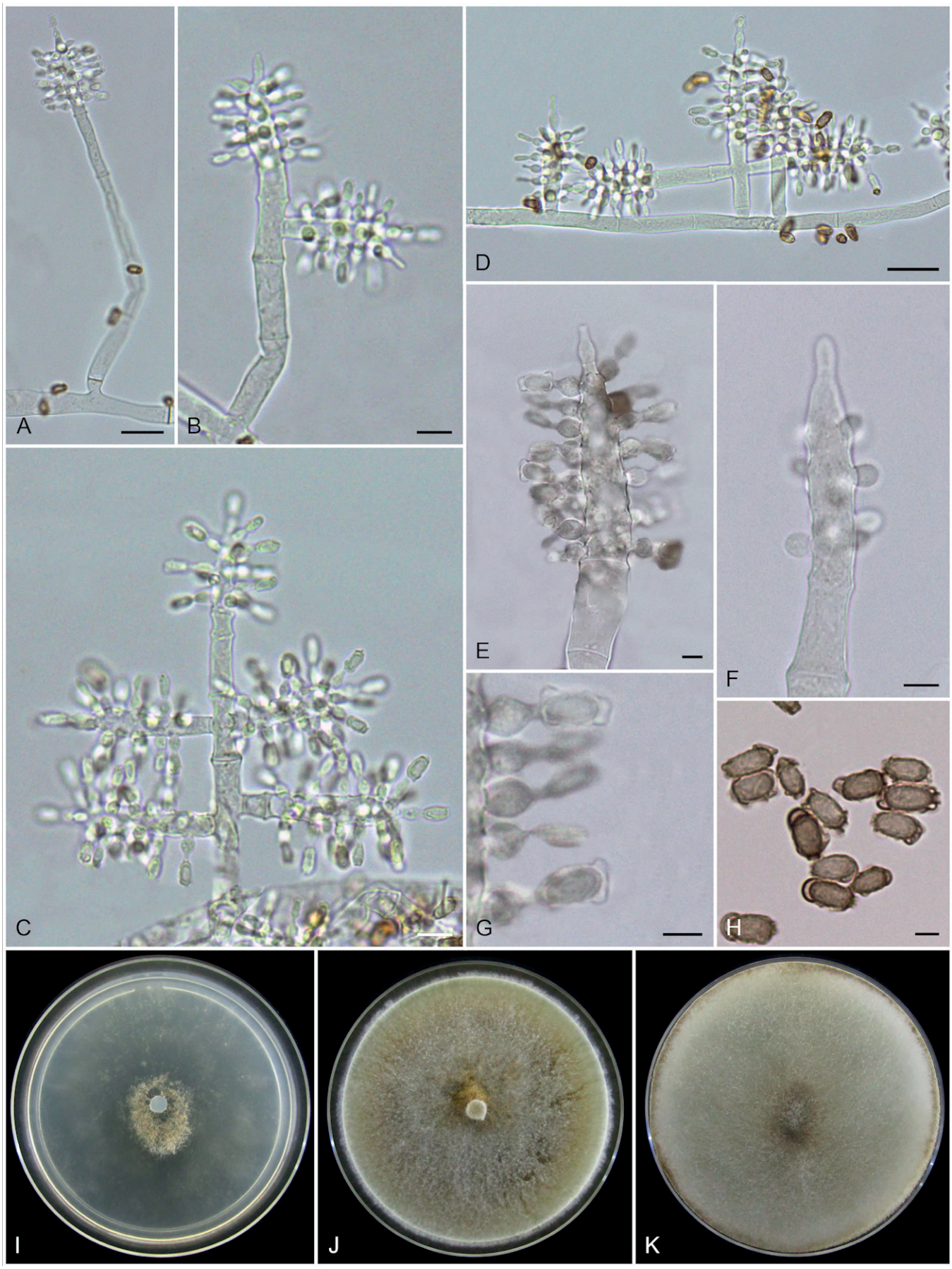

Fig. 13. Morphological characters of *Escovopsis moelleri* (ex-type culture CBS 135748). **A.** Mono-vesiculate conidiophore. **B, C.** Polyvesiculate conidiophore. **D.** Conidiophore arrangement on aerial mycelium. **E.** Subulate vesicle. **F.** Lanceolate vesicle. **G.** Phialides with conidia. **H.** Ornamented conidia. **I–K.** Cultures on CMD, MEA and PDA, respectively, after 7 d of growth at 25 °C. Scale bars: D, G = 20 µm; E, F = 10 µm; H–K = 4 µm.

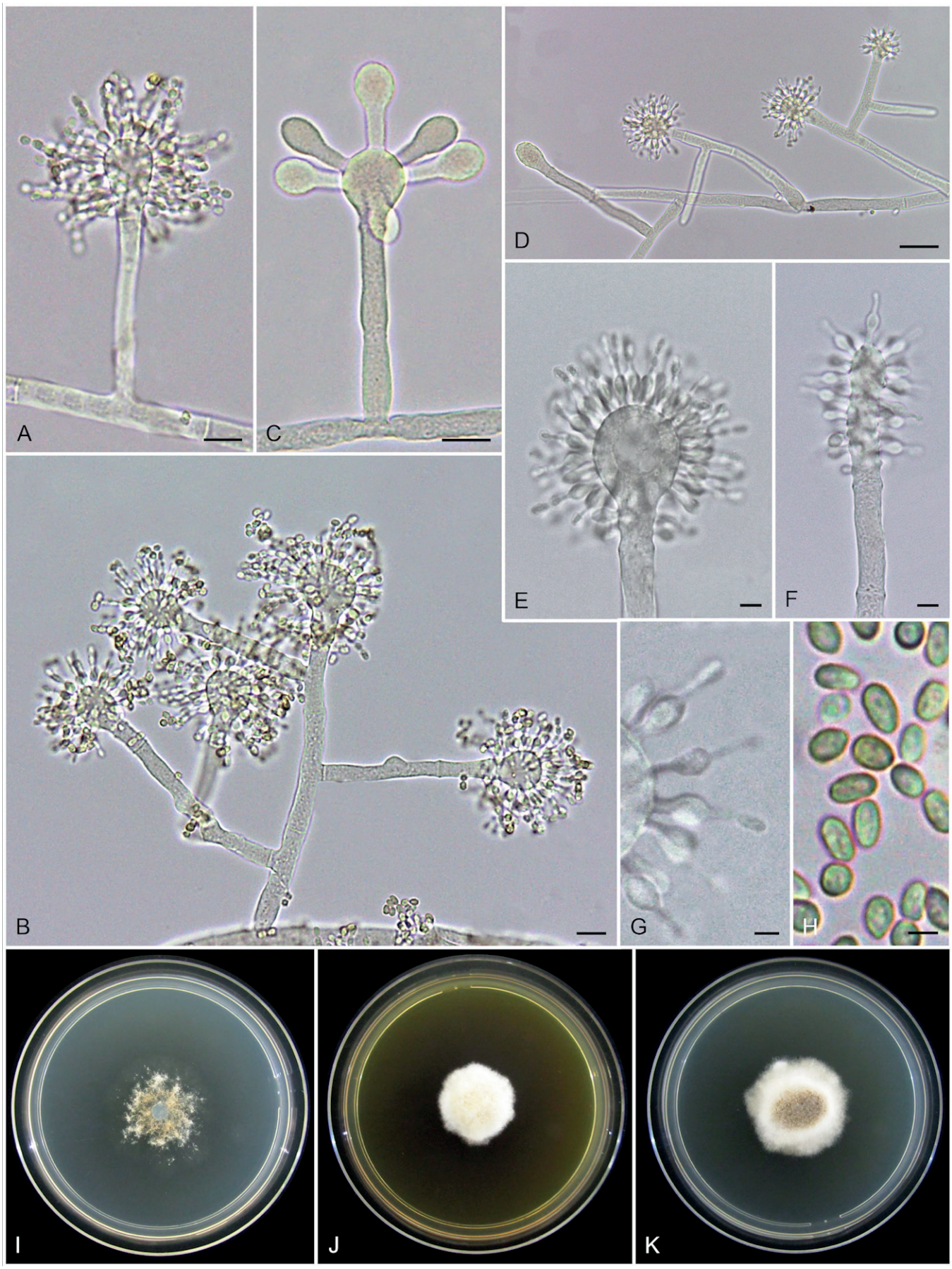

Fig. 14. Morphological characters of *Escovopsis multiformis* (ex-type culture CBS 145327). **A.** Mono-vesiculate conidiophore. **B.** Polyvesiculate conidiophores. **C.** Conidiophore with swollen cell. **D.** Conidiophore arrangement on aerial mycelium. **E.** Capitate vesicle with phialides. **F.** Cylindrical vesicles with phialides. **G.** Phialides. **H.** Conidia. **I–K.** Cultures on CMD, MEA and PDA, respectively, after 7 d of growth at 25 °C. Scale bars: A–C = 10 µm; D = 20 µm; E, F = 4 µm; G, H = 2 µm.

Ecology: Unknown.

Distribution: This species is found in different regions in Brazil and Panama in fungus gardens of the attine genus *Apterostigma*.

Additional material examined: **Brazil**, Mato Grosso, Cotriguaçu, 09°49'22.74"S, 58°15'32.04"W, elev. 252 m, fungus garden of *Apterostigma* sp., Oct. 2017. Q.V. Montoya, LESF 1136.

Notes: *Escovopsis multiformis* is closely related to *E. clavata*. Unlike strains of *E. clavata,* which grow only at 20 and 25 °C, *E. multiformis* also grows at 10 and 30 °C. Conidiophores of *E. multiformis* are usually shorter and less branched than those of *E. clavata*, and frequently with swollen cells more frequently, which are larger than those of *E. clavata*.

Escovopsis papillata Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, ***sp. nov.*** MycoBank MB 847816. Fig. 15.

Etymology: "*papillata*" (*papillata* = shaped like a nipple) in reference to the nippled aspect of some immature vesicles before they form phialides.

Diagnosis: *Escovopsis papillata* usually has some vesicles that have a papilla at the terminal part (nipple-shaped). This is more common on immature vesicles when these start to form the phialides.

Typus: **Brazil**, Amazonas, Novo Airão, Parque Nacional de Anavilhanas, 2°31'25.8"S, 60°49'28.62"W, fungus garden of *Apterostigma* sp., 23 Jan. 2017, Q.V. Montoya, LESF 960 (**holotype** CBS 149745 preserved as metabolically inactive culture, ex-type culture CBS 149745). GenBank: OQ589840 (ITS); OQ589790 (28S); OQ596413 (*rpb*1); OQ603883 (*rpb*2); OQ603933 (*tef*1).

Description: *Conidiophores* forming 2–7 vesicles, hyaline, irregularly shaped, smooth-walled, mostly alternate and less opposite, formed on aerial hyphae. Mono-vesiculate conidiophores 6.5–116 µm, polyvesiculate 45–170 µm long. Conidiophore stipes 7–76 × 3–7 µm, with a septum 0.5–13 µm from the foot cell. Conidiophore branches 25–72 µm long, formed in one level, at right and less than 90° angles, commonly opposite and less frequently alternate. Stipes on branches 9–84 µm long, with a septum 0–4 µm from conidiophore axis. *Vesicles* mostly obovoid, 18–59 × 15–31 µm, aseptate, formed on the tips of conidiophore and branches. Vesicle stipe 6.5–115 µm long with two to three septa. *Phialides* formed on vesicles, 5–8 µm long, lageniform, 0.5–2 × 1–2 µm at the base, 2.5–5 × 1.5–3 µm at the swollen cell and 2–3 × 0.5–1 µm at the neck. *Conidia* formed in chains, oblong, 2–5 × 1.5–2.5 µm, Olive-Ochre (XXX21", smooth and thick wall. *Chlamydospores* absent.

Culture characteristics: *Colonies* growing at 20, and 25 °C on CMD, PDA, and MEA. At 20 °C, growth starts on the third day, and between the second and third day at 25 °C. *Colony* radius, after 4 d at 20 °C: 2–5 mm on CMD, 1–2 mm on MEA and 5–10 mm on PDA; at 25 °C: 1–4 mm on CMD, 2–4 mm on MEA and 5–10 mm on PDA. *Colony morphology* — CMD 25 °C, 7 d: colonies with diffuse aerial mycelium, White (LIII73(10)) to Margerite Yellow (XXX23"f). MEA 25 °C, 7 d: colonies with dense aerial mycelium, few stolons, Margerite Yellow (XXX23"f) to Light Yellow-Green (VI31d). PDA 25 °C, 7 d: colonies with diffuse fluffy aerial mycelium, few stolons, White (LIII73(10)) and Margerite Yellow (XXX23"f) to Colonial Buff (XXX21"d) (Colonial buff (XXX21"d) at centre, White (LIII73(10)) and Margerite Yellow (XXX23"f) at margin). Pustule-like formations and pigments absent.

Ecology: Unknown.

Distribution: This species was found in the Amazon regions of Brazil in fungus gardens of the attine ant genus *Apterostigma*.

Additional material examined: **Brazil**, Amazonas, Novo Airão, Parque Nacional de Anavilhanas, 2°31'25.8"S, 60°49'28.62"W, fungus garden of *Apterostigma* sp., 20 Jan. 2017, Q.V. Montoya, LESF 959.

Notes: *Escovopsis papillata* is closely related to *E. clavata* and *E. multiformis*. *Escovopsis papillata* grows slower than *E. clavata* and *E. multiformis* and does not grow at 30 °C, as is also the case for some strains of *E. multiformis*. In addition, unlike strains of *E. clavata* and *E. multiformis*, which form conidiophores with swollen cells, *E. papillata* lacks such structures on its conidiophores.

Escovopsis peniculiformis Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, ***sp. nov.*** MycoBank MB 847804. Fig. 16.

Etymology: "*peniculiformis*" (*peniculus* = duster, *formis* = shape) in reference to the duster shape of the conidiophores.

Diagnosis: *Escovopsis peniculiformis* is characterized by forming conidiophores with short branches and very long and thin vesicles at the apex of their conidiophores.

Typus: **Panama**, Gamboa, fungus garden of *Atta colombica*, 19 Jan. 2001, N.M. Gerardo, LESF 876 (**holotype** CBS 149744 preserved as metabolically inactive culture, ex-type culture CBS 149744). GenBank: KM817101 (ITS); OQ589724 (28S); OQ596347 (*rpb*1); OQ603817 (*rpb*2); KM817162 (*tef*1).

Description: *Conidiophores* forming 2–25 vesicles, hyaline, usually pyramidal, smooth-walled, alternate or less frequent opposite, formed on aerial hyphae. Mono-vesiculate conidiophores 9–77 µm, polyvesiculate 40–1 380 µm long. Conidiophore stipes 12–570 × 3.5–7 µm, with a septum 1.5–29.5 µm from the foot cell. Conidiophore branches 11–86 µm long, mostly formed by long vesicles, rarely in one or two levels, in almost right angles, alternate and opposite. Stipes on branches 1–24 µm long, with a septum 1–9 µm from conidiophore axis. *Vesicles* cylindrical, 12–280 × 4.5–7 µm, predominantly aseptate and less frequently septate, predominantly formed on conidiophore axis, less frequently on the axis of branches. Vesicle stipe 0.5–49 µm long, with one or two septa. The terminal vesicle is usually the longest and thinnest and appear to be an extension of the conidiophore apex rather than a vesicle. *Phialides* formed mainly on vesicles and less frequently on the aerial mycelium, 4.5–10 µm long, lageniform, 0.5–1 × 0.5–1.5 µm at the base, 2–4 × 1.5–3 µm at the swollen section and 1–6 × 0.5–1 µm at the neck. *Conidia* formed in chains, ellipsoidal, 1.5–3.5 × 1–2.5 µm, Olive-Ochre (XXX21"), with smooth and thick wall. *Chlamydospores* absent.

Culture characteristics: *Colonies* growing at 20, 25, and 30 °C on CMD, PDA, and MEA. Growth starts between the first and second day at all temperatures and on all media. *Colony* radius, after 4 d at 20 °C: 10–12 mm on CMD, 14–20 mm on MEA and 18–22 mm on PDA; at 25 °C: 18–30 mm on CMD, 39–40 mm on MEA and > 40 mm on PDA (colonies reach plate edge on third day); at 30 °C: 19–20 mm on CMD, 20–25 mm on MEA and 40 mm

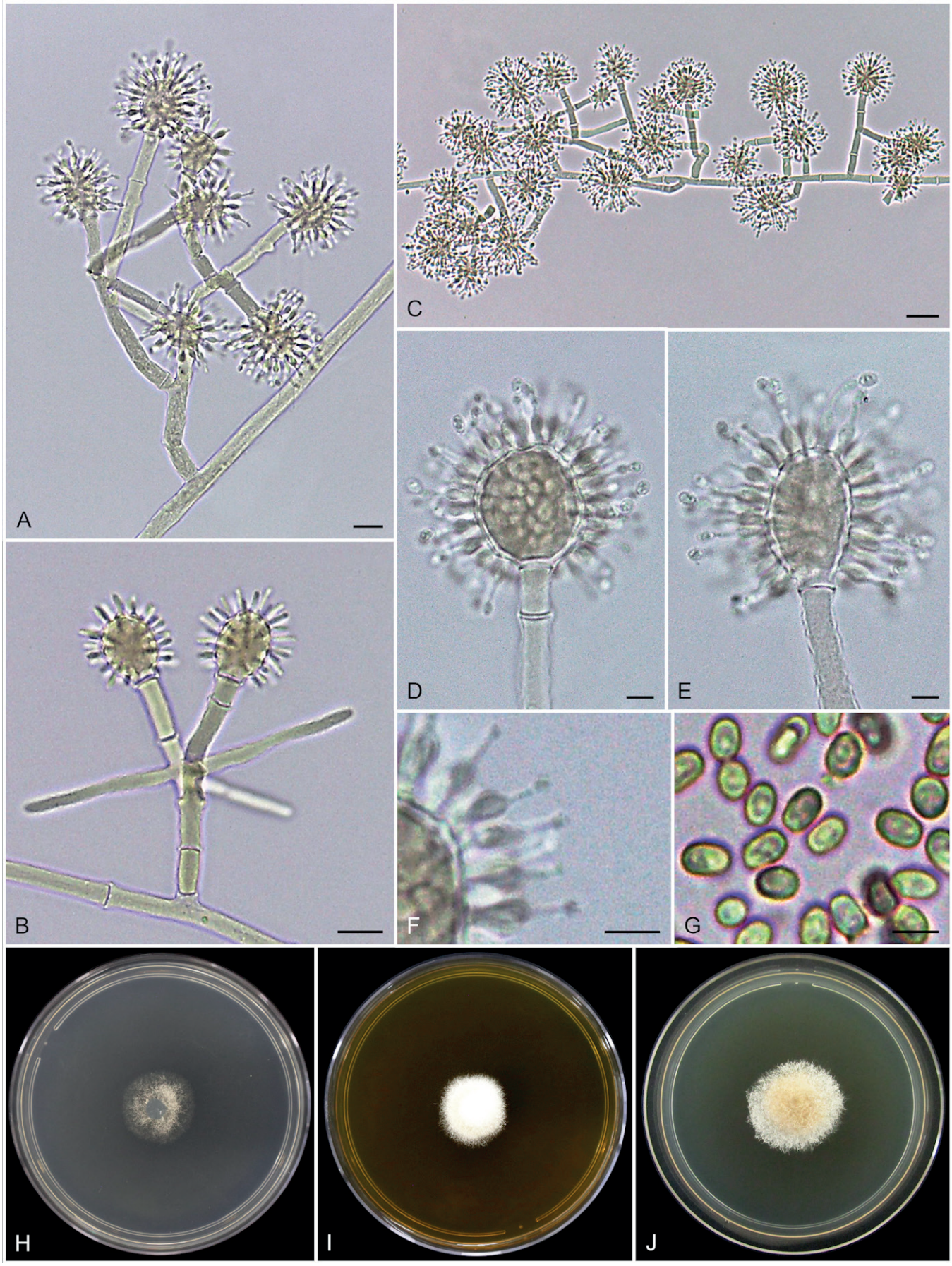

Fig. 15. Morphological characters of *Escovopsis papillata* (type culture CBS 149745). **A, B.** Polyvesiculate conidiophores. **C.** Conidiophore arrangement on aerial mycelium. **D.** Cylindrical vesicle with phialides. **E.** Clavate vesicle with phialides. **F.** Phialides. **G.** Conidia. **H–J.** Cultures on CMD, MEA and PDA, respectively, after 7 d of growth at 25 °C. Scale bars: A, B = 10 µm; C = 20 µm; D–G = 4 µm.

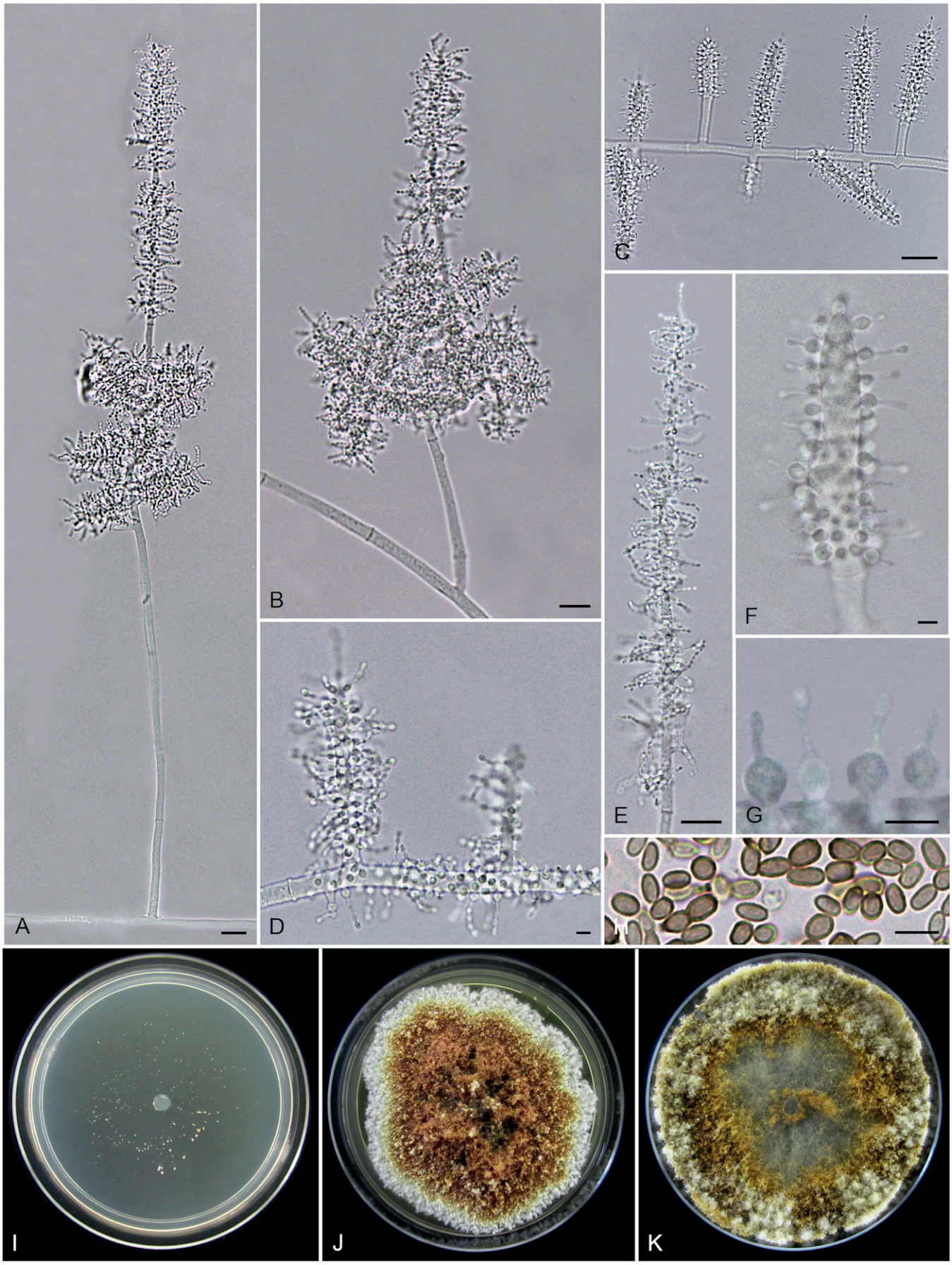

Fig. 16. Morphological characters of *Escovopsis peniculiformis* (type culture CBS 149744). **A, B.** Polyvesiculate conidiophores. **C.** Arrangement of monovesiculate conidiophores on aerial mycelium. **D.** Phialides on vesicles and on aerial mycelia. **E, F.** Cylindrical vesicle with phialides. **G.** Phialides. **H.** Conidia. **I–K.** Cultures on CMD, MEA and PDA, respectively, after 7 d of growth at 25 °C. Scale bars: A–C, E = 20 µm; D, F–H = 4 µm.

on PDA. *Colony morphology* — CMD 25 °C, 7 d: Colonies with submerged and diffuse aerial mycelium, spread by stolons, White (LIII73(10)) to Olive-Ochre (XXX21"). MEA 25 °C, 7 d: Colonies with dense aerial mycelium, spread by stolons, Colonial buff (XXX21"d) to Light Brownish olive (XXX19"k) at centre and Light Yellow-Green (VI31d) to White (LIII73(10)) at margin. On this medium, colonies sometimes Deep Colonial Buff (XXX21"b) and *Vinaceous-Cinnamon (XXIX13"b), with submerged mycelium forming dense circular zones. PDA 25 °C, 7 d: Colonies forming abundant aerial mycelium, spread by stolons, White (LIII73(10)) and *Olive-Yellow (XXX23") to Ecru-Olive (XXX21"i) at the centre and White (LIII73(10)) to Margerite Yellow (XXX23"f) at margin. Commonly forming pustule-like formations. Rarely forming soluble pigments.

Ecology: Unknown.

Distribution: This species is found in Panama and Austin (Texas, USA) in fungus gardens of the attine ant genera *Atta*, *Acromyrmex,* and *Apterostigma*.

Additional materials examined: **Panama**, fungus garden of *Apterostigma* sp., 6 Jan. 2003, U.G. Mueller, LESF878. **USA**, Texas, Austin, 30°22'9.9"N; 97°47'49.8"W, elev. 157.8 m, fungus garden of fungus-growing ant, 19 Nov. 2005, U.G. Mueller, LESF 297.

Notes: *Escovopsis peniculiformis* is closely related to *E. weberi.* However, unlike strains of *E. weberi, E. peniculiformis* does not grow at 10 °C. Conidiophores of *E. peniculiformis* are usually longer and less branched than those of *E. weberi* and unlike strains of *E. weberi* (which form phialides only on vesicles)*, E. peniculiformis* forms phialides on both the vesicles and aerial mycelium (less frequently).

Escovopsis phialicopiosa Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, ***sp. nov.*** MycoBank MB 847809. Fig. 17.

Etymology: "*phialicopiosa*" (*phiali* = phialide, *copiosa* = abundant) in reference to the abundant number of phialides formed by strains of this species on their vesicles.

Diagnosis: *Escovopsis phialicopiosa* forms conidiophores with vesicles covered with phialides to such an extent that they are difficult to observe individually.

Typus: **Brazil**, Minas Gerais, Uberlândia, Panga Ecological Station, 19°17'17.5"S, 48°39'40.2"W, fungus garden of *Trachymyrmex* sp., 22 Sep. 2008, A. Rodrigues, LESF 048 (**holotype** CBS 149738 preserved as metabolically inactive culture, ex-type culture CBS 149738). GenBank: KM817088 (ITS); OQ589739 (28S); OQ596362 (*rpb*1); OQ603832 (*rpb*2); KF240731 (*tef*1).

Description: *Conidiophores* forming 2–11 vesicles, hyaline, pyramidal, smooth-walled, alternate, formed on aerial hyphae. Mono-vesiculate conidiophores 14–41 µm, polyvesiculate 12–150 µm long. Conidiophore stipes 0–79 µm × 3–7.5 µm, with a septum 0–14 µm from the foot cell. Conidiophore branches 11–62 µm long, formed mostly by a vesicle, rarely with two levels, usually at right angles, alternate or opposite. Stipes on branches 0.5–6 µm long, with a septum 0–1.5 µm from conidiophore axis. *Vesicles* mostly prolate, 13–42 × 6–12.5 µm, aseptate, formed on conidiophore axis or on the axis of branches. Vesicle stipe 0–1 µm long, aseptate. *Phialides* formed on vesicles, 4.5–8 µm long, lageniform, 0.5–1 × 0.5–1.5 µm at the base, 2–3 × 1.5–2.5 µm at the swollen section and 2–4 × 0.5–1 µm at the neck. *Conidia* formed in chains, ellipsoidal, 1.5–4 × 1–2.5 µm, Olive-Ochre (XXX21"), with smooth and thick wall. *Chlamydospores* absent.

Culture characteristics: *Colonies* growing at 20, 25 °C, on CMD, PDA, and MEA and only on PDA and MEA at 30 °C. Growth starts on the third day at 20, and 25 °C on CMD, and between the first and second day at all temperatures, on PDA, and MEA. *Colony* radius, after 4 d at 20 °C: 1–2 mm on CMD, 4–10 mm on MEA and 12–33 mm on PDA; at 25 °C: 4–7 mm on CMD, 11–25 mm on MEA and 10–25 mm on PDA; at 30 °C: 10–23 mm on MEA and 10–24 mm on PDA. *Colony morphology* — CMD 25 °C, 7 d: colonies with scatter aerial mycelium, few conidia, without pustule-like formation, White (LIII73(10)) to Margerite Yellow (XXX23"f). MEA 25 °C, 7 d: colonies with dense cottony aerial mycelium, spread by stolons, few pustule-like formations, White (LIII73(10)) to Margerite Yellow (XXX23"f) PDA 25 °C, 7 d: colonies with wispy cottony aerial mycelium, spread by stolons, pustule-like formations, White (LIII73(10)) to Olive-Ochre (XXX21"). Soluble pigments absent.

Ecology: Unknown.

Distribution: This species is distributed in different regions in Brazil in fungus gardens of the attine ants *Atta sexdens, Mycetomoellerius dichrous,* and *Trachymyrmex* sp. *sensu lato*.

Additional materials examined: **Brazil**, Goiás, Fazenda Pau, fungus garden of *Trachymyrmex* sp., 8 Apr. 2008, A. Rodrigues, LESF 047; Minas Gerais, Uberlândia, Panga Ecological Station, 19°17'17.5"S, 48°39'40.2"W, fungus garden of *Mycetomoellerius dichrous*, 22 Sep. 2008, A. Rodrigues, LESF 106; São Paulo, Rio Claro, São Paulo State University (UNESP), fungus garden of *Atta sexdens*, unknown date, A. Rodrigues, LESF 021.

Notes: *Escovopsis phialicopiosa* is closely related to *E. elongatistipitata.* Unlike strains of the latter species, which do not grow at 30 °C and eventually form concentric rings on MEA and PDA, colonies of *E. phialicopiosa* grow at 30 °C on MEA and PDA and does not produce concentric rings on any media. *Escovopsis phialicopiosa* forms conidiophores with shorter stipes than those of *E. elongatistipitata*.

Escovopsis pseudocylindrica Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, ***sp. nov.*** MycoBank MB 847811. Fig. 18.

Etymology: "*pseudocylindrica*" (*pseudo* = false, *cylindrica* = Latin feminine of cylindrical) in reference to the "cylindrical" collapsed vesicles observed in old colonies of this species.

Diagnosis: *Escovopsis pseudocylindrica* forms conidiophores with oblong vesicles that start collapsing after 7 d, as they form phialides and conidia.

Typus: **Brazil**, Amazonas, Novo Airão, Parque Nacional de Anavilhanas, 2°31'29.64"S, 60°49'28.92"W, fungus garden of *Trachymyrmex* sp., 20 Jan. 2017, Q.V. Montoya, LESF 993 (**holotype** CBS 149749 preserved as metabolically inactive culture, ex-type culture CBS 149749). GenBank: OQ589819 (ITS); OQ589769 (28S); OQ596392 (*rpb*1); OQ603862 (*rpb*2); OQ603912 (*tef*1).

Description: *Conidiophores* forming 2–14 vesicles, hyaline, irregularly shaped, smooth-walled, alternate, less frequent opposite, formed on aerial hyphae. Mono-vesiculate conidiophores 20–36

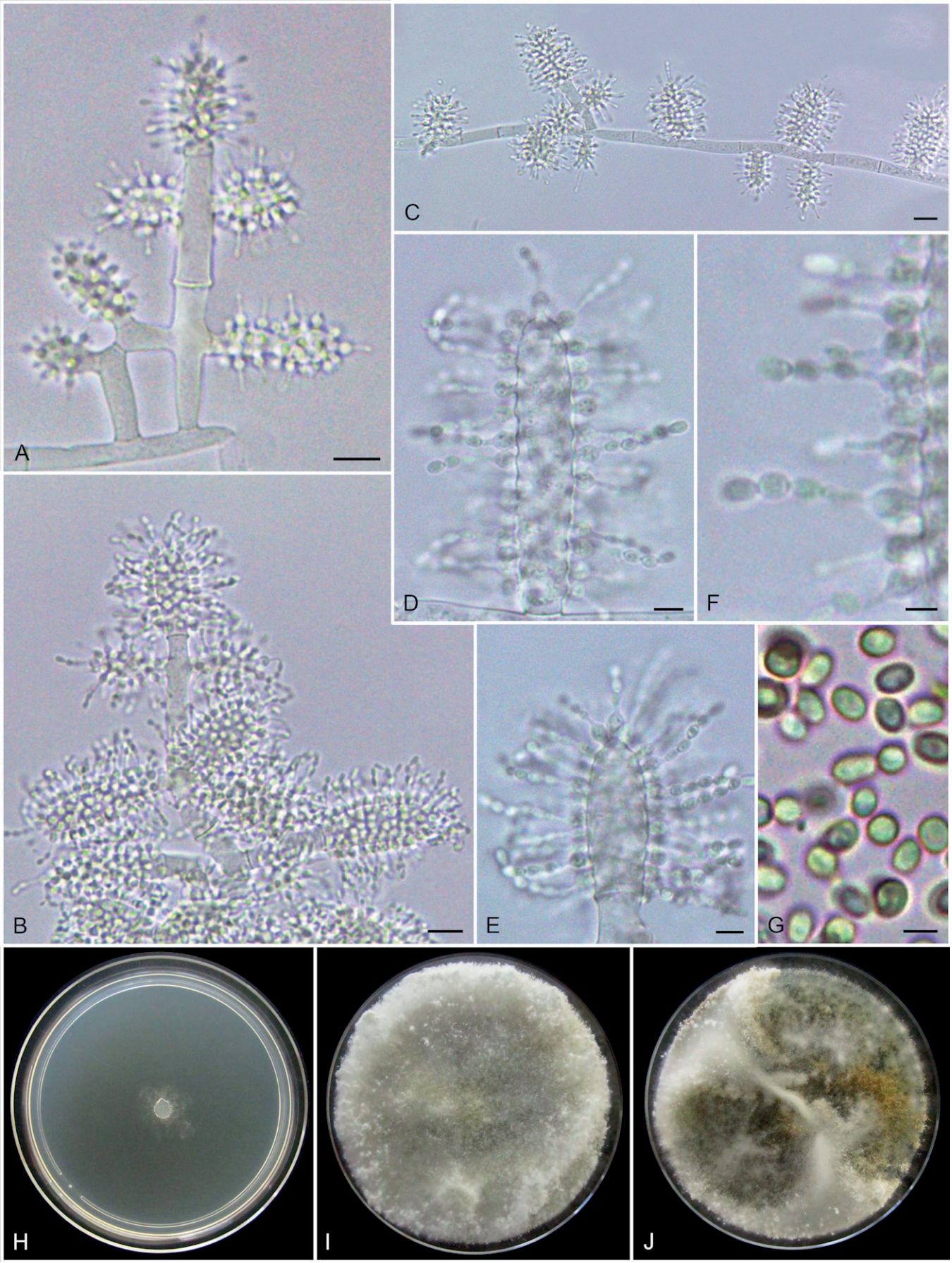

Fig. 17. Morphological characters of *Escovopsis phialicopiosa* (type culture CBS 149738). **A, B.** Polyvesiculate conidiophores. **C.** Conidiophore arrangement on aerial mycelium. **D.** Cylindric vesicle with phialides. **E.** Ellipsoidal vesicle with phialides. **F.** Phialides. **G.** Conidia. **H–J.** Cultures on CMD, MEA and PDA, respectively, after 7 d of growth at 25 °C. Scale bars: A–C = 10 µm; D, E = 4 µm; F, G = 2 µm.

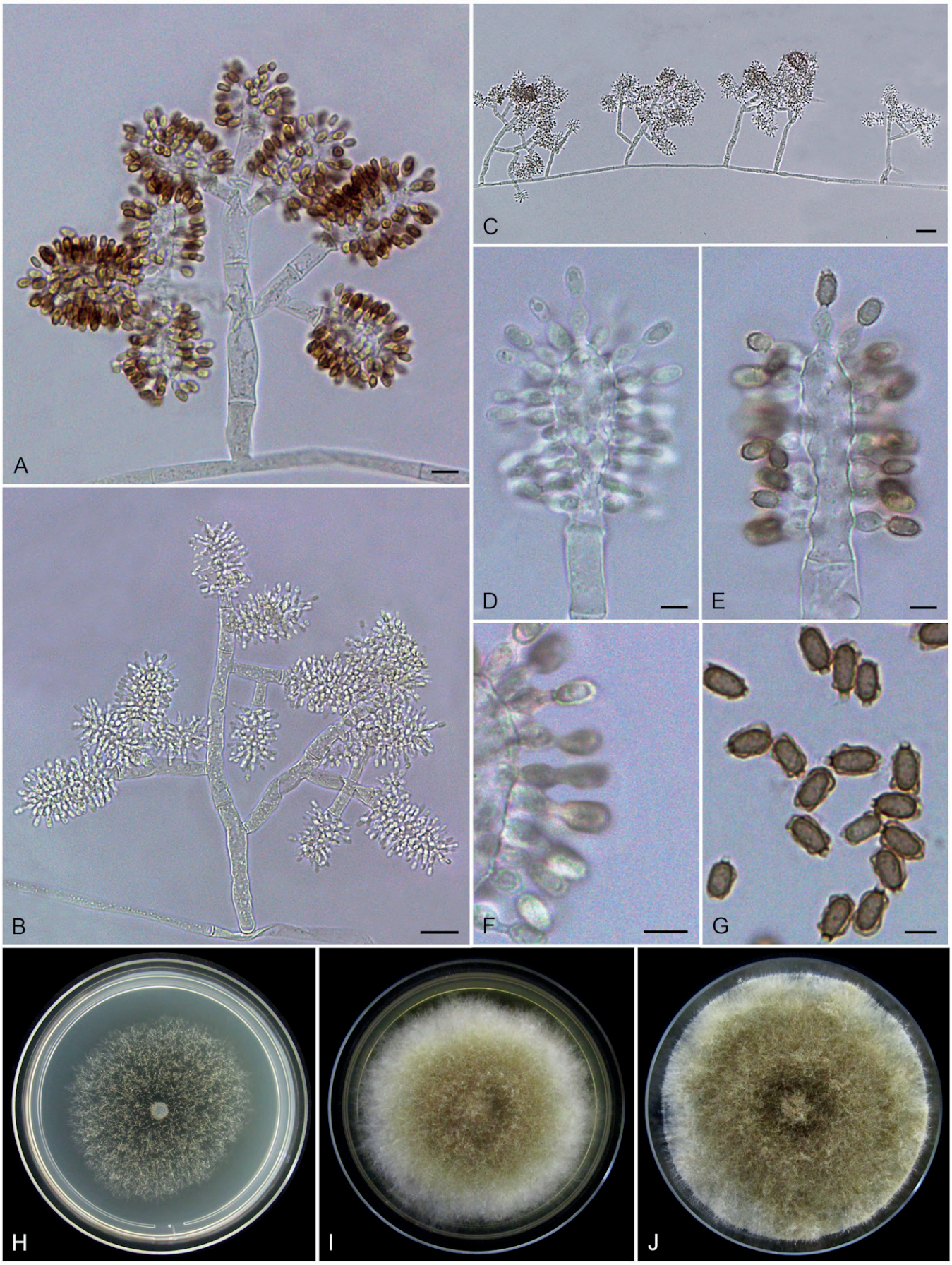

Fig. 18. Morphological characters of *Escovopsis pseudocylindrica* (type culture CBS 149749). **A, B.** Polyvesiculate conidiophores. **C.** Conidiophore arrangement on aerial mycelium. **D.** Young clavate vesicle with phialides. **E.** Old withered vesicle with phialides. **F.** Phialides. **G.** Conidia. **H–J.** Cultures on CMD, MEA and PDA, respectively, after 7 d of growth at 25 °C. Scale bars: A = 10 µm; B = 20 µm; C = 40 µm; D–G = 4 µm.

µm, polyvesiculate 46–270 µm long. Conidiophore stipes 2–112 × 4–8 µm, with a septum 2–14 µm from the foot cell. Conidiophore branches 21–148 µm long, formed in one or two levels, at angles less than 90°, alternate or opposite. Stipes on branches 1.5–38.5 µm long, with a septum 0.5–4 µm from conidiophore axis. *Vesicles* mainly prolate, 9–45 × 4–12 µm, aseptate, formed on the tips of conidiophore and branches. Vesicle stipe 1–26 µm long, with one or three septa. *Phialides* formed on vesicles, 4–7 µm long, lageniform, 0–1.5 × 1–2 µm at the base, 2–4 × 2–3 µm at the swollen section and 1–2.5 × 0.5–1 µm at the neck. *Conidia* formed in chains, oblong, 2–6 × 3–4 µm, Olive-Ochre (XXX21"), with ornamented and thick wall. *Chlamydospores* absent.

Culture characteristics: *Colonies* growing at 20, 25 °C on CMD, PDA, and MEA, and only on PDA at 30 °C. At 20 °C growth starts on the second day, at 25 °C on the first day, and at 30 °C on third day. *Colony* radius, after 4 d at 20 °C: 3–9 mm on CMD, 9–14 mm on MEA and 10–15 mm on PDA; at 25 °C: 10–15 mm on CMD, 16–20 mm on MEA and 26–35 mm on PDA; at 30 °C: 2–6 mm on PDA. *Colony morphology* — CMD 25 °C, 7 d: colonies with diffuse aerial mycelium, Margerite Yellow (XXX23"f) and White (LIII73(10)) to Colonial Buff (XXX21"d). MEA and PDA 25 °C, 7 d: colonies with cottony aerial mycelium, spread by stolons; White (LIII73(10)), Colonial buff (XXX21"d), Olive-Ochre (XXX21") and Ecru-Olive (XXX21"i) (Ecru-Olive (XXX21"i) at centre, White (LIII73(10)) at margin); sometimes Light Yellow-Green (VI31d), *Olive-Yellow (XXX23"). Pustule-like formations and soluble pigments absent.

Ecology: Unknown.

Distribution: This species was found in the amazon regions of Brazil in fungus garden of the attine ant *Trachymyrmex*.

Additional material examined: **Brazil**, Amazonas, Novo Airão, Parque Nacional de Anavilhanas, S2°16'9.3"S, 59° 27'32.54"W, fungus garden of *Trachymyrmex* sp., 24 Jan. 2017, Q.V. Montoya, QVM157, LESF 1029.

Notes: *Escovopsis pseudocylindrica* is closely related to *E. spicaticlavata*. Unlike strains of the latter species, which grow only on PDA at 30 °C, *E. pseudocylindrica* can grow on all media at this temperature. In addition, conidiophores of *E. pseudocylindrica* are more branched than those of *E. spicaticlavata*.

Escovopsis rectangula Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, ***sp. nov.*** MycoBank MB 847808. Fig. 19.

Etymology: "*rectangula*" (*rectangula* = right angled) in reference to the slightly rectangular shape of the conidiophores formed by strains of this species.

Diagnosis: *Escovopsis rectangula* forms slightly rectangular conidiophores, usually with branches formed by a long cylindrical vesicle.

Typus: **Brazil**, Rondônia, Fazenda São Sebastião, fungus garden of *Acromyrmex* sp., 7 Oct. 2018, A. Rodrigues, LESF 050 (**holotype** CBS 149739 preserved as metabolically inactive culture, ex-type culture CBS 149739). GenBank: KM817091 (ITS); OQ589729 (28S); OQ596352 (*rpb*1); OQ603822 (*rpb*2); KM817152 (*tef*1).

Description: *Conidiophores* forming 2–34 vesicles, hyaline, slightly rectangular shape, smooth-walled, alternately, formed on aerial hypha. Mono-vesiculate conidiophores 24–56 µm, polyvesiculate 47–250 µm long. Conidiophore stipe 2.5–72.5 µm × 4–6.5 µm, with a septum 0–12.5 µm from the foot cell. Conidiophore branches 16.5–220 µm long, formed in one or two levels, usually at right angles, alternate. Stipes on branches 1–87 µm long, with a septum 1–28 µm from conidiophore axis. *Vesicles* cylindrical, 20–58 × 4–9 µm, predominantly non-septate, less frequently septate (1 septum), formed on conidiophore axis or on the axis of branches. Vesicle stipe 1–12 µm long, septate (1 septum). *Phialides* formed on vesicles, 5–8 µm long, lageniform, 0.5–1 × 0.5–2 µm at the base, 2–3.5 × 1–3 µm at the swollen section and 1–5 × 0.5 µm at the neck. *Conidia* formed in chains, subglobose, 2–4 × 1.5–3 µm, Olive-Ochre (XXX21"), with smooth and thick wall. *Chlamydospores* absent.

Culture characteristics: *Colonies* growing at 10, 20, 25, and 30 °C on CMD, PDA, and MEA. At 10 °C growth starts between second and third day, and at 20, 25, and 30 °C growth starts on the first day, on all media. *Colony* radius, after 4 d at 10 °C: Inconspicuous growth (the colony barely grows on the inoculum) but with few conidia production; at 20 °C: 8–11 mm on CMD, 6–24 mm on MEA and 13–35 mm on PDA; at 25 and 30 °C: 10–20 mm on CMD, 27–40 mm on MEA and 40 mm on PDA. *Colony morphology* — CMD 25 °C, 7 d: colonies with scattered aerial mycelium, abundant short pustule-like formations, White (LIII73(10)) to Olive-Ochre (XXX21") (Olive-Ochre (XXX21") at centre, White (LIII73(10)) at margin). MEA 25 °C, 7 d: colonies with dense cottony aerial mycelium, without pustule-like formations, White (LIII73(10)) to Margerite Yellow (XXX23"f). PDA 25 °C, 7 d: colonies with dense cottony aerial mycelium, spread by stolons, abundant pustule-like formations, White (LIII73(10)) to Light Brownish olive (XXX19"k) (White (LIII73(10)) at centre, Light Brownish olive (XXX19"k) at margin forming a ring). Soluble pigments absent.

Ecology: Unknown.

Distribution: This species is found in Brazil, Mexico and Panama in fungus gardens of the attine ant genera *Acromyrmex*, *Apterostigma*, and *Trachymyrmex*.

Additional materials examined: **Brazil**, Pernambuco, Frei Caneca, fungus garden of *Atta cephalotes*, 21 Jan. 2004, A. Rodrigues, LESF 022; Bahia, Camacan, Santa Cruz State University (UESC), 14°47'56.8"S, 39°10'16.4"W, fungus garden of *Atta cephalotes*, 15 Mar. 2013, A. Rodrigues, LESF 326. **Mexico**, Guadeloupe island, fungus garden of *Acromyrmex octospinosus*, 24 Dec. 2003, N.M. Gerardo, LESF 865. **Panama**, fungus garden of *Apterostigma dentigerum*, 9 Jul. 2002, N.M. Gerardo, LESF 863.

Notes: *Escovopsis rectangula* is closely related to *E. chlamydosporosa.* Unlike strains of the latter species, which do not grow at 10 °C and usually form chlamydospores, *E. rectangula* grows at 10 °C and rarely forms these structures. Conidiophores of *E. rectangula* are short, less branched and have a slightly rectangular shape, while conidiophores of *E. chlamydosporosa* are longer, more branched, and irregularly shaped.

Escovopsis rosisimilis Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, ***sp. nov.*** MycoBank MB 847813. Fig. 20.

Etymology: "*rosisimilis*" (*rosi* = roses, *similis* = like) in reference to the shape of blooming roses displayed by the conidiophore aggregations on the aerial mycelium.

Diagnosis: *Escovopsis rosisimilis* forms clusters of short, tangled conidiophores on the aerial mycelium that resemble blooming

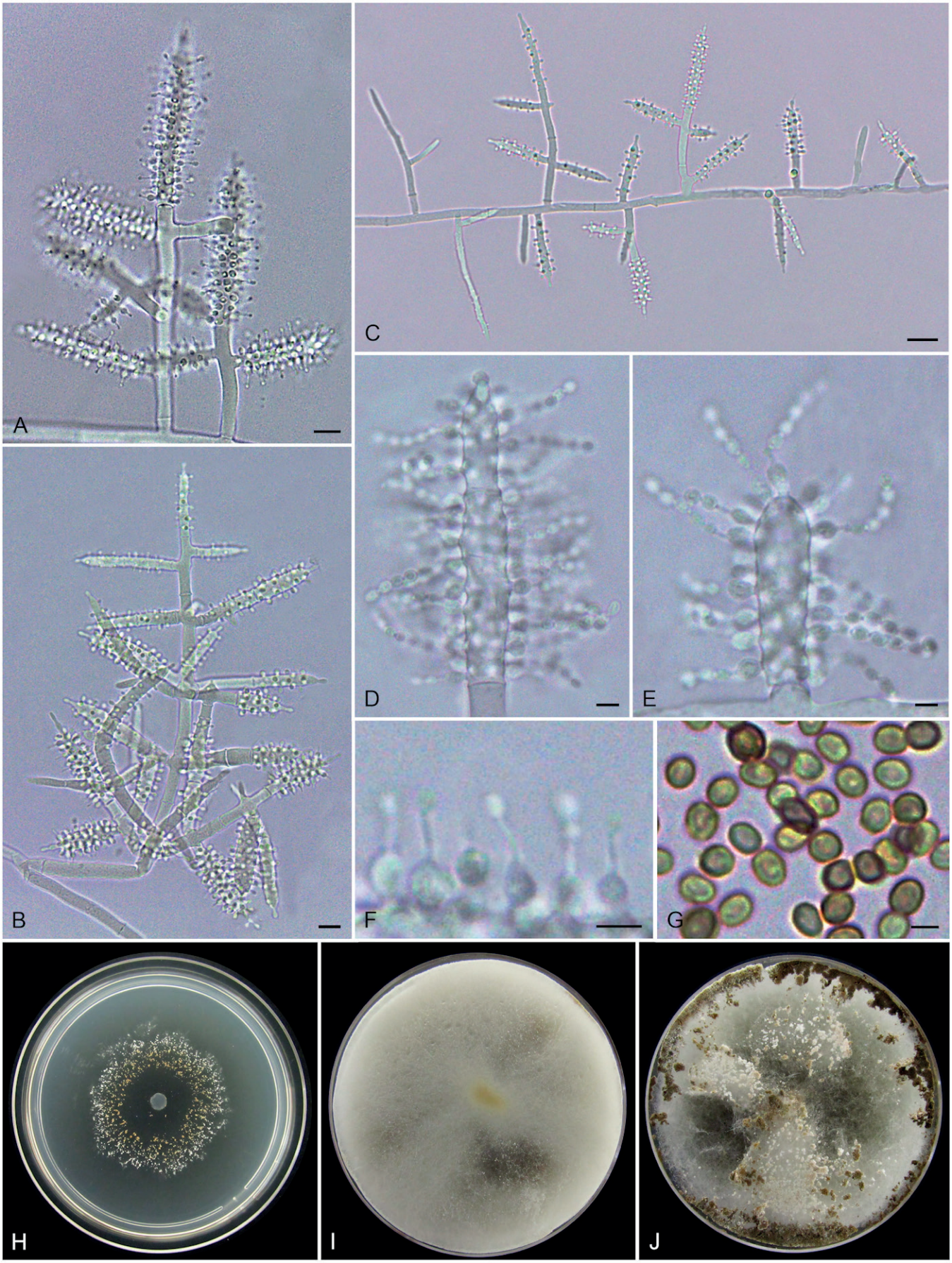

Fig. 19. Morphological characters of *Escovopsis rectangula* (type culture CBS 149739). **A, B.** Polyvesiculate conidiophores. **C.** Conidiophore arrangement on aerial mycelium. **D.** Septate cylindrical vesicle with phialides. **E.** Non-septate cylindrical vesicle with phialides. **F.** Phialides. **G.** Conidia. **H–J.** Cultures on CMD, MEA and PDA, respectively, after 7 d of growth at 25 °C. Scale bars: A, B = 10 µm; C = 20 µm; D–F = 4 µm; G = 2 µm.

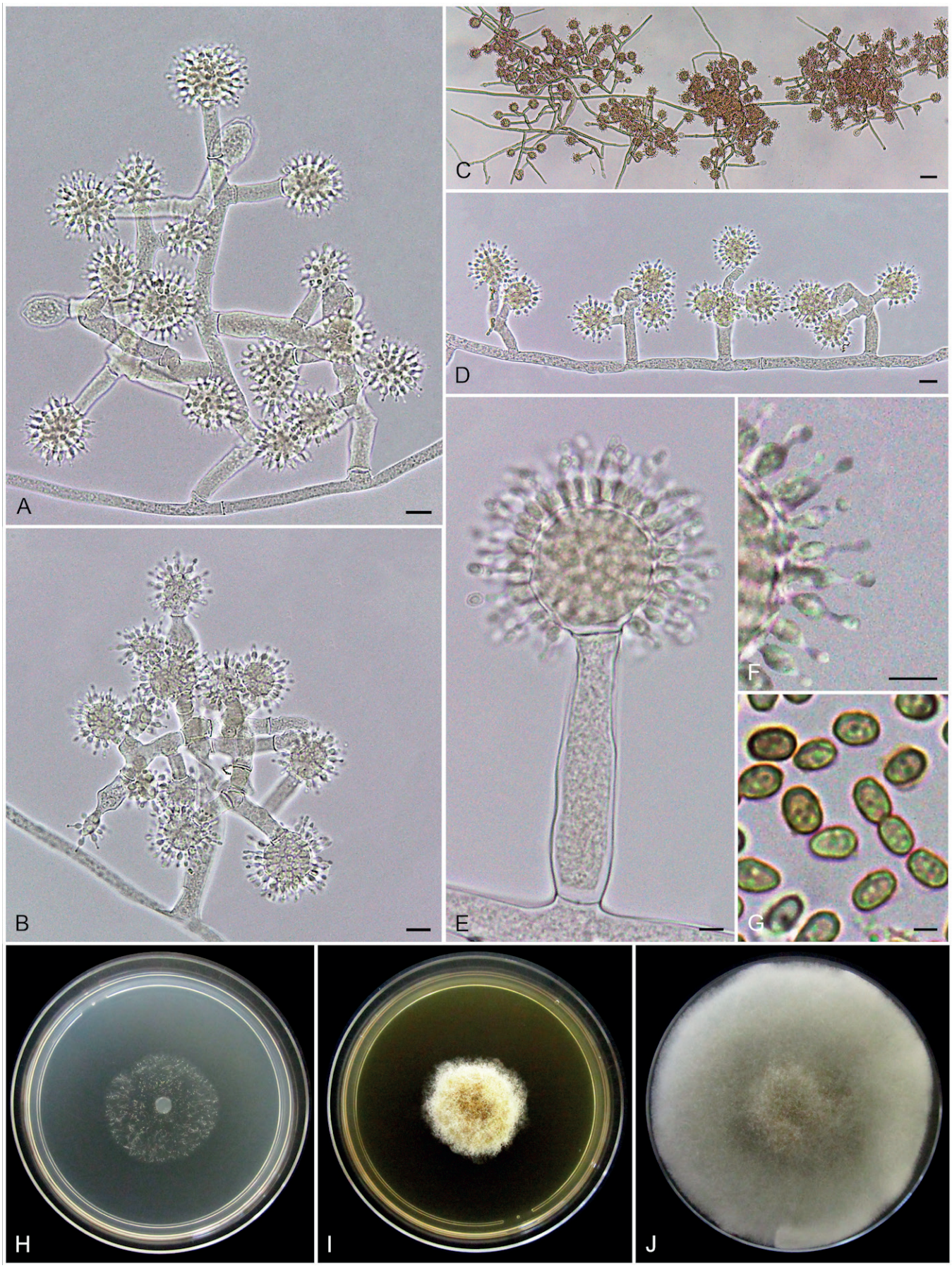

Fig. 20. Morphological characters of *Escovopsis rosisimilis* (type culture CBS 149742). **A, B.** Polyvesiculate conidiophores. **C.** Clusters of conidiophores on aerial mycelium. **D.** Conidiophore arrangement on aerial mycelium. **E.** Globose vesicle with phialides. **F.** Phialides. **G.** Conidia. **H–J.** Cultures on CMD, MEA and PDA, respectively, after 7 d of growth at 25 °C. Scale bars: A, B, D = 10 µm; C = 40 µm; E, F = 4 µm; G = 2 µm.

roses.
Typus: **Brazil**, Minas Gerais, Uberlândia, Panga Ecological Station, 19°17'17.5"S, 48°39'40.2"W, fungus garden of *Trachymyrmex* sp., 20 Aug. 2008, A. Rodrigues, LESF 135 (**holotype** CBS 149742 preserved as metabolically inactive culture, ex-type culture CBS 149742). GenBank: KM817086 (ITS); OQ589740 (28S); OQ596363 (*rpb*1); OQ603833 (*rpb*2); KM817148 (*tef*1).

Description: *Conidiophores* forming 2–16 vesicles, hyaline, irregularly shaped, smooth-walled, alternate or opposite, formed on aerial hyphae. Mono-vesiculate conidiophores 21–80 µm, polyvesiculate 47–210 µm long. Conidiophore stipes 5–65 × 5–15 µm, with a septum 1–7 µm from the foot cell. Conidiophore branches 21–110 µm long, formed in up to two levels, in almost right angles, alternate or opposite. Stipes on branches 5–38 µm long, with a septum 1–9 µm from conidiophore axis. *Vesicles* globose, 13–62 × 14–58 µm, aseptate, formed on the tips of conidiophore and branches. Vesicle stipe 2–34 µm long, with two septa. *Phialides* formed on vesicles, 5–8 µm long, lageniform, 0.5–1 × 0.5–2 µm at the base, 2–3 × 1–3 µm at the swollen section and 1.5–3 × 0.5–1 µm at the neck. *Conidia* formed in chains, oblong, 1.5–3 × 1–2 µm, Olive-Ochre (XXX21"), smooth and thick wall. *Chlamydospores* absent.

Culture characteristics: *Colonies* growing at 20, and 25 °C on CMD, PDA, and MEA. At 20 and 25 °C growth starts on the third day on CMD and MEA, and on the second day on PDA, on all media. *Colony* radius, after 4 d at 20 °C: 2–18 mm on CMD, 4–7 mm on MEA and 15–22 mm on PDA; at 25 °C: 5–7 mm on CMD, 5–10 mm on MEA and 20–32 mm on PDA. *Colony morphology* — CMD 25 °C, 7 d: colonies with diffuse aerial mycelium, Margerite Yellow (XXX23"f) to Light Yellow-Green (VI31d). MEA 25 °C, 7 d: colonies with dense aerial mycelium, Margerite Yellow (XXX23"f) to Picnic Yellow (IV23d) and *Olive-Yellow (XXX23"). PDA 25 °C, 7 d: colonies with dense cottony aerial mycelium, spread by stolons, mostly White (LIII73(10)) and less frequently Light Yellow-Green (VI31d) (Light Yellow-Green (VI31d) at centre, White (LIII73(10)) at margin). Pustule-like formations and soluble pigments absent.

Ecology: Unknown.

Distribution: This species was found in Minas Gerais and in the Amazon regions of Brazil in fungus gardens of the attine ant *Trachymyrmex*.

Notes: *Escovopsis rosisimilis* is closely related to *E. diminuta* and *E. lentecrescens*. *Escovopsis rosisimilis* grows faster than *E. lentecrescens*, but slower than *E. diminuta*. Furthermore, *E. rosisimilis* forms slightly longer and more entangled conidiophores on the aerial mycelium than *E. lentecrescens* and *E. diminuta*.

Escovopsis spicaticlavata Q.V. Montoya, M.J.S. Martiarena & A. Rodrigues, ***sp. nov.*** MycoBank MB 847812. Fig. 21.

Etymology: "*spicaticlavata*" (*spicati* = spikes, *clava* = club) in reference to the spiked club shape of the vesicles formed by this species.

Diagnosis: *Escovopsis spicaticlavata* forms irregularly shaped conidiophores with mostly prolate vesicles that resemble a spiked club because of the phialides jutting out from it.

Typus: **Brazil**, Amazonas, Manaus, Biological Dynamics of Forest Fragments Project (PDBFF–Camp 41), 2°26'54.84"S, 59°46'10.02"W, fungus garden of *Paratrachymyrmex diversus*, 9 Jan. 2009, A. Rodrigues, LESF 052 (**holotype** CBS 149740 preserved as metabolically inactive culture, ex-type culture CBS 149740). GenBank: KM817093 (ITS); MH715124 (28S); MT305437 (*rpb*1); MT305562 (*rpb*2); KM817154 (*tef*1).

Description: *Conidiophores* forming 2–15 vesicles, sometimes with swollen cells, hyaline, irregular shaped, smooth-walled, alternate or opposite, formed on aerial hyphae. Mono-vesiculate conidiophores rarely, 22–110 µm long, polyvesiculate 63.5–400 µm long. Conidiophore stipe 18.5–150 × 3–10 µm, with a septum mostly 0–5 µm and rarely 8–15 µm from the foot cell. Conidiophore axis usually ends in a vesicle, less frequently in a terminal swollen cell. Conidiophore branches 28–120 µm long, formed on conidiophore axis or on swollen cells, in one level, almost at right angles, sometimes curved upward or down, alternate or opposite. Conidiophore branches sometimes ends in a swollen cell. Swollen cells 11–25 × 9–17 µm, form up to three branches. Stipes on branches 5–38 µm long, with a septum 0–7 µm from conidiophore axis. *Vesicles* mainly prolate, 14.5–39 × 7–15 µm, aseptate, formed on conidiophore axis or on swollen cells. Vesicle stipe 2–69 µm long, with one or four septa. *Phialides* formed on vesicles, 5–9.5 µm long, lageniform, 0–2.5 × 1–2 µm at the base, 2–5.5 × 2–3 µm at the swollen section and 1–3 × 0.5–1.5 µm at the neck. *Conidia* formed in chains, oblong, 2–5 × 2–3.5 µm, Olive-Ochre (XXX21"), with ornamented thick wall. *Chlamydospores* absent.

Culture characteristics: *Colonies* growing at 20, 25, and 30 °C on CMD, PDA, and MEA. At 20 °C, growth starts on the second day and at 25 and 30 °C on the first day. *Colony* radius, after 4 d at 20 °C: 9–14 mm on CMD, 10–30 mm on MEA and 15–28 mm on PDA; at 25 °C: 15–20 mm on CMD, 25–35 mm on MEA and 20–30 mm on PDA; at 30 °C: 15–20 mm on CMD, 25–35 mm on MEA and 20–28 mm on PDA. *Colony morphology* — CMD 25 °C, 7 d: colonies with diffuse aerial mycelium, White (LIII73(10)) or Margerite Yellow (XXX23"f) to Colonial buff (XXX21"d). MEA 25 °C, 7 d: colonies with diffuse cottony aerial mycelium, spread by stolons, White (LIII73(10)) or Margerite Yellow (XXX23"f) to *Olive-Yellow (XXX23") (*Olive-Yellow (XXX23") at centre, White (LIII73(10)) to Margerite Yellow (XXX23"f) at margin). PDA 25 °C, 7 d: Colonies with cottony aerial mycelium, spread by stolons, White (LIII73(10)) and Colonial Buff (XXX21"d) (Colonial Buff (XXX21"d) at centre, White (LIII73(10)) at margin). Pustule-like formations and soluble pigments absent.

Ecology: Unknown.

Distribution: This species was found in the Amazon regions of Brazil in fungus garden of the attine ant *Trachymyrmex*.

Additional materials examined: **Brazil**, Amazonas, Novo Airão, Parque Nacional de Anavilhanas, 2°31'25.3"S, 60°49'33.1"W, fungus garden of *Trachymyrmex* sp., 20 Jan. 2017, Q.V. Montoya, LESF 975; Amazonas, Novo Airão, Parque Nacional de Anavilhanas, fungus garden of *Trachymyrmex* sp., 24 Jan. 2017, Q.V. Montoya, LESF 979.

Notes: *Escovopsis spicaticlavata* is closely related to *E. pseudocylindrica*. Unlike strains of the latter species, which grow at 30 °C on all media, *E. spicaticlavata* grows only on PDA at this temperature. Conidiophores of *E. spicaticlavata* are less branched than those of *E. pseudocylindrica*.

Escovopsis weberi J.J. Muchovej & Della Lucia, Mycotaxon 37: 192. 1990. MycoBank MB 127786. Fig. 22.
Synonym: *Escovopsis microspora* H.C. Evans & J.O. Augustin,

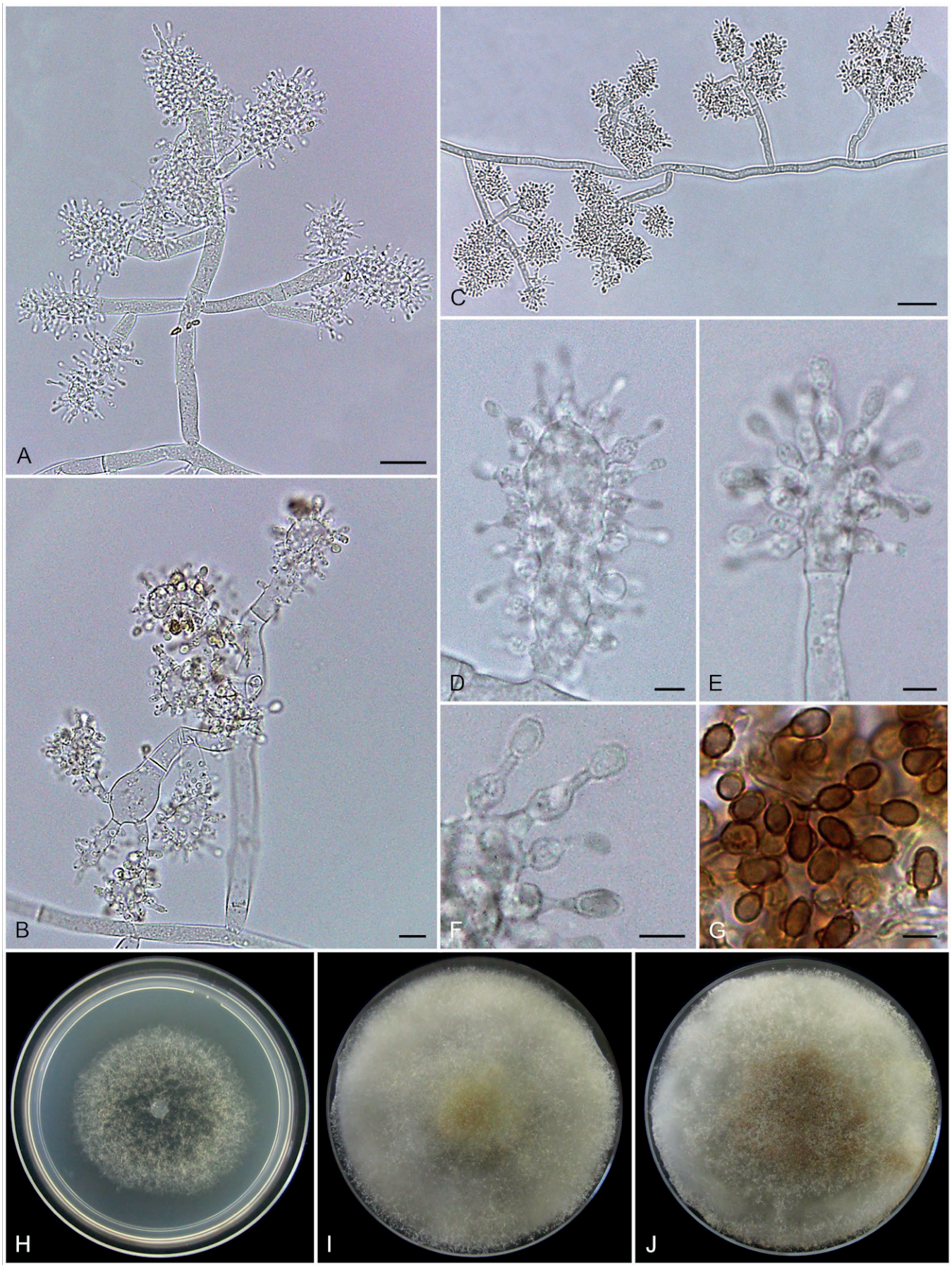

Fig. 21. Morphological characters of *Escovopsis spicaticlavata* (type culture CBS 149740). **A.** Polyvesiculate conidiophore. **B.** Conidiophore with swollen cell on the branch. **C.** Conidiophore arrangement on aerial mycelium. **D, E.** Clavate vesicles with phialides. **F.** Phialides. **G.** Conidia. **H–J.** Cultures on CMD, MEA and PDA, respectively, after 7 d of growth at 25 °C. Scale bars: A, C = 20 µm; B = 10 µm; D–G = 4 µm.

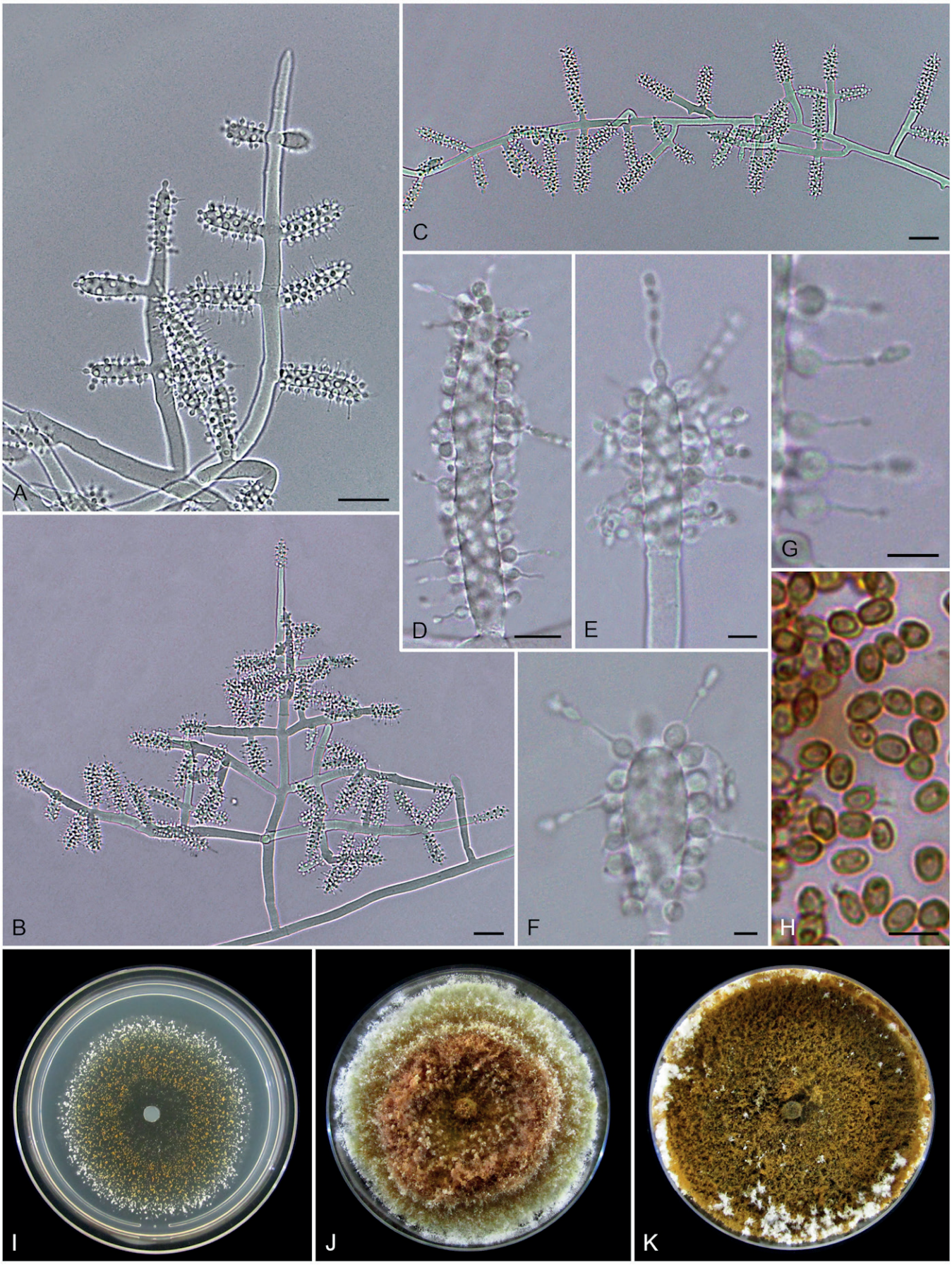

Fig. 22. Morphological characters of *Escovopsis weberi* (ex-type culture ATCC 64542). **A–B.** Polyvesiculate conidiophore. **C.** Arrangement of mono- and polyvesiculate conidiophores on aerial mycelium. **D.** Septate cylindrical vesicle. **E.** Cylindrical vesicle. **F.** Clavate vesicle. **G.** Phialides. **H.** Conidia. **I–K.** Cultures on CMD, MEA and PDA, respectively, after 7 d of growth at 25 °C**.** Scale bars: A–C = 20 µm; D–H = 4 µm.

PLoS ONE 8: e82265, 4. 2013. MycoBank MB 800442.
Diagnostic characters: *Escovopsis weberi* grows faster than other known *Escovopsis* species and exhibits the most variable colony colours in the genus.

Typus: **Brazil**, Minas Gerais, Viçosa, ant colony, 25 Mar. 1987, Della Lucia (Herbário, UFV–Universidade Federal de Viçosa) (**holotype** ATCC 64542 preserved as metabolically inactive culture). GenBank: KF293285 (ITS); KF293281 (28S); MT305412 (*rpb*1); MT305537 (*rpb*2); MZ170961 (*tef*1).

Description: *Conidiophores* forming 1–48 vesicles, hyaline, usually pyramidal, less frequently of irregular shape, smooth-walled, alternate or less frequent opposite, formed on aerial hyphae. Mono-vesiculate conidiophores 20–60 µm, polyvesiculate 30–570 µm long. Conidiophore stipes 45–90 × 5–7 µm, with a septum 2–5 µm from the foot cell. Conidiophore branches 34–120 µm long, formed in one or two levels, in almost right angles and sometimes slightly curved upward, mostly alternate and occasionally opposite. Stipes on branches 10–36 µm long, with a septum 1.5–3 µm from conidiophore axis. *Vesicles* of various shapes, *i.e.*, cylindrical, clavate, or filiform, 21–63 × 6–12 µm, predominantly aseptate, less frequently septate (1–2 septa), formed directly on conidiophore axis or on the axis of branches. Vesicle stipe 1–12 µm long, without septa. *Phialides* formed on vesicles, 7–9 µm long, lageniform, 0.5–1 × 1–1.5 µm at the base, 2.5–3 × 2.5–3 µm at the swollen section and 3–4 × 0.5–0.8 µm at the neck. *Conidia* formed in chains, ellipsoidal to oblong, 2.5–3 × 1.5–2.5 µm, Olive-Ochre (XXX21"), with smooth and thick wall. *Chlamydospores* absent.

Culture characteristics: *Colony* growing at 10, 20, 25, and 30 °C on CMD, PDA, and MEA. At 10 °C, growth starts on the third day, and at 20, 25, and 30 °C, growth starts on the first day on all media. *Colony* radius, after 4 d at 10 °C: Inconspicuous growth (the colony barely grows on the inoculum); at 20 °C: 20–24 mm on CMD, 30–40 mm on MEA and 40 mm on PDA; at 25 °C: 17–36 mm on CMD, 24–40 mm on MEA and > 40 mm on PDA (in this case colonies reach plate edge on third day); at 30 °C: 37–40 mm on CMD, 12–15 mm on MEA and 37–40 mm on PDA. *Colony morphology* — CMD 25 °C, 7 d: Colonies with diffuse aerial mycelium, spread by stolons, forming small pustule-like structures, White (LIII73(10)) to Olive-Ochre (XXX21"). MEA 25 °C, 7 d: colonies with abundant aerial mycelium; varying in colour, usually Colonial buff (XXX21"d) to Olive-Ochre (XXX21") at centre (often Picnic Yellow (IV23d) and Deep Colonial Buff (XXX21"b) are also observed), surrounded by *Vinaceous-Cinnamon (XXIX13"b) to Colonial buff (XXX21"d) and Margerite Yellow (XXX23"f) to Ecru-Olive (XXX21"i) regions, White (LIII73(10)) to Light Yellow-Green (VI31d) at margin; submerged of *E. weberi* examined here. mycelium forming dense circular zones, with White (LIII73(10)) to Olive-Ochre (XXX21") pustules. PDA 25 °C, 7 d: colonies with abundant aerial mycelium spread by stolons; White (LIII73(10)) to Olive-Ochre (XXX21"), sometimes Picnic Yellow (IV23d) to Ecru-Olive (XXX21"i) and *Vinaceous-Cinnamon (XXIX13"b) to Deep Colonial Buff (XXX21"b); eventually with pustule-like formations. Occasionally forming soluble pigments.

Ecology: Some representatives of this species are opportunists in the fungus gardens of leaf-cutting ants and a few strains were reported as mycoparasites of *Leucoagaricus gongylophorus*, the fungal cultivar of *Atta*.

Distribution: Across Brazil.

Additional materials examined: **Brazil**, Bahia, Ilhéus, Santa Cruz State University (UESC), 14°47'56.8"S, 39°10'16.4"W, fungus garden of *Acromyrmex balzanii*, unknown collection date, A. Rodrigues, LESF 054; Mato Grosso, Alta Floresta, fungus garden of *Atta cephalotes*, unknown collection date, A. Rodrigues, LESF 023; Rio Grande do Sul, Chuvisca, grassland, 30°50'10.2"S, 51°55'10.4"W, fungus garden of *Acromyrmex lundii*, unknown collection date, A. Rodrigues, LESF 042; Rio Grande do Sul, Chuvisca, grassland, 30°50'10.2"S, 51°55'10.4"W, fungus garden of *Acromyrmex heyeri*, unknown collection date, A. Rodrigues, LESF 043; São Paulo, Botucatu, Fazenda Santana, 22°50'45.8"S, 48°26'09.4"W, fungus garden of *Atta sexdens rubropilosa*, unknown collection date, A. Rodrigues, LESF 019; São Paulo, Botucatu, Fazenda Santana, 22°50'46.4"S, 48°26'09.6"W, fungus garden of *Atta capiguara*, unknown collection date, A. Rodrigues, LESF 292; São Paulo, Corumbataí, Fazenda Corumbataí, 22°17'22"S, 47°39'23"W, fungus garden of *Atta sexdens*, unknown collection date, A. Rodrigues, LESF 031; São Paulo, Corumbataí, Fazenda Corumbataí, 22°17'21.7"S, 47°39'22.8"W, fungus garden of *Atta sexdens rubropilosa*, unknown collection date, A. Rodrigues, LESF 156; São Paulo, Thermas de Santa Bárbara, 22°49'10.6"S, 49°16'06.2"W, fungus garden of *Atta laevigata*, unknown collection date, A. Rodrigues, LESF 324.

Notes: *Escovopsis weberi* is closely related to *E. peniculiformis*. The conidiophores of *E. weberi* are usually shorter but more branched than those of *E. peniculiformis*. Furthermore, the vesicles of *E. weberi* are shorter and wider than those of *E. peniculiformis*. The morphological characters of *E. weberi* do not differentiate it from *E. microspora*. In addition, the ex-type strains have only one nucleotide difference in the ITS, *rpb*1 and *rpb*2 sequences, no difference in the 28S and *tef*1, and they form together with other isolates of *E. weberi* a well-supported clade. Although *E. microspora* was described based on the supposition that the conidial sizes differ from *E. weberi*, the measurements of Augustin *et al.* (2013) are within the range observed in the broader sampling

Dichotomous key to known *Escovopsis* species

1a. Colony growth < 20 mm at 25 °C on PDA 2
1b. Colony growth > 20 mm at 25 °C on PDA 8

2a. Aerial mycelium Margerite Yellow (XXX23"f), Light Yellow-Green (VI31d) to Picnic Yellow (IV23d) on CMD 3
2b. Aerial mycelium White (LIII73(10)) on CMD 6

3a. Colonies growing at 10 °C on CMD, PDA, and MEA ..... *E. multiformis*
3b. Colonies not growing at 10 °C 4

4a. Aerial mycelium Light Yellow-Green (VI31d) to Picnic Yellow (IV23d) on CMD ..... *E. aspergilloides*
4b. Aerial mycelium Light Brownish olive (XXX19"k) on CMD 5

5a. Aerial mycelium Margerite Yellow (XXX23"f) on CMD ..... *E. clavata*
5b. Aerial mycelium Light Yellow-Green (VI31d) on CMD ..... *E. lentecrescens*

6a. Aerial mycelium Margerite Yellow (XXX23"f) on CMD 7
6b. Aerial mycelium *Olive-Yellow (XXX23") on CMD ..... *E. diminuta*

7a. Aerial mycelium White (LIII73(10)) on MEA ..... *E. phialicopiosa*
7b. Aerial mycelium Light Yellow-Green (VI31d) on MEA ..... *E. papillata*

8a. Aerial mycelium Margerite Yellow (XXX23"f) on MEA 9
8b. Aerial mycelium Colonial buff (XXX21"d) on MEA 16

9a. Colonies with clusters of short, tangled conidiophores on the aerial mycelium ..... *E. rosisimilis*
9b. Colonies without clusters of short, tangled conidiophores on the aerial mycelium 10

10a. Colonies not growing at 10 °C 11
10b. Colonies growing at 10 °C on CMD, PDA, and MEA 14

11a. Colonies growing at 30 °C on CMD, PDA, and MEA 12
11b. Colonies do not grow at 30 °C on CMD, PDA, and MEA 13

12a. Colonies with a mottled aspect on the reverse of the plate ..... *E. maculosa*
12b. Colonies with a uniform aspect on the reverse of the plate ..... *E. gracilis*

13a. Colonies forming abundant chlamydospores ..... *E. chamydosporosa*
13b. Colonies rarely forming chlamydospores ..... *E. spicaticlavata*

14a. Aerial mycelium forming slightly rectangular conidiophores ..... *E. rectangula*
14b. Aerial mycelium forming pyramidal or irregularly shaped conidiophores 15

15a. Colonies growing at 30 °C on CMD, PDA, and MEA, conidia ornamented ..... *E. moelleri*
15b. Colonies not growing at 30 °C on any media, conidia without ornamentations ..... *E. weberi*

16a. Colonies growing at 10 °C on CMD, PDA, and MEA ..... *E. breviramosa*
16b. Colonies not growing at 10 °C on CMD, PDA, and MEA 17

17a. Aerial mycelium white on CMD, Light Yellow-Green (VI31d) on PDA and *Olive-Yellow (XXX23") and Ecru-Olive (XXX21"i) on MEA ..... *E. pseudocylindrica*
17b. Aerial mycelium, Colonial buff (XXX21"d) or Margerite Yellow (XXX23"f) on CMD, Margerite Yellow (XXX23"f) on PDA and without *Olive-Yellow (XXX23") and Ecru-Olive (XXX21"i) colours on MEA 18

18a. Colonies growing at 30 °C ..... *E. peniculiformis*
18b. Colonies not growing at 30 °C ..... *E. elongatistipitata*

DISCUSSION

Here we provide a new taxonomic framework comprising a set of laboratory conditions (media, temperatures and time of evaluation), morphological characters and phylogenetic markers to evaluate the morphology and species concepts in the genus *Escovopsis*. Following this framework, we redescribed the ex-type cultures of six *Escovopsis* species (Figs 4, 7, 11, 13, 14, 22), including the type of the genus, *E. weberi*, synonymised *E. microspora* with *E. weberi*, and introduced thirteen new species. Our standardised approach provides a solid basis for future phylogenetic and morphological studies of the diversity and speciation of these *Hypocreaceae* fungi.

The description of new fungal species is not a simple task (Raja *et al.* 2017, 2021), as an integrated molecular and morphological analysis is needed for accurate species delimitation (Taylor *et al.* 2000, Lücking *et al.* 2020, 2021). This forms a basis for a taxonomic framework that enables to meet the challenges of describing of new fungal species (Raja *et al.* 2017, 2021, Senanayake *et al.* 2020, Koukol & Delgado 2021). Since the discovery of *Escovopsis* by Möller (1893), only twelve species of this genus have been described (Seifert *et al.* 1995, Augustin *et al.* 2013, Masiulionis *et al.* 2015, Meirelles *et al.* 2015a). This is mainly because: (i) for a long time, fungi of different genera were treated as *Escovopsis* without any taxonomic support, (ii) only the ITS region or *tef*1 genes were sequenced for most of the isolates (which prevented a multilocus analysis), and (iii) the taxonomic uncertainties have long hampered interspecific morphological comparisons and assessment of the morphological diversity of described and new species in the genus (Montoya *et al.* 2019, 2021).

Although the disagreements between alternative phylogenetic hypotheses of *Escovopsis* were recently solved (Montoya *et al.* 2021), this is the first time that a multilocus analysis following the GCPSR species concept is used in combination with a standardised

morphological evaluation to assess the diversity of these attine colony inhabitants. Our results show that the combined analysis of the five molecular markers, sequenced in this study, provides a strong support to distinguish the species of *Escovopsis* and to clarify their phylogenetic relationships. However, separate analyses performed with each of the molecular markers shows that the 28S region does not resolve species in this genus. The limitation of the 28S region to distinguish phylogenetic relationships between other *Hypocreaceae* genera, *i.e.*, *Protocrea*, *Sphaerostilbella*, *Hypomyces* and *Trichoderma*, has been reported in several studies (Põldmaa *et al.* 1999, Põldmaa 2000, Druzhinina & Kubicek 2005, Jaklitsch & Samuels 2011). Therefore, future studies may consider to exclude this molecular marker from the sequencing. On the other hand, while the ITS region and the *rpb*1 gene provide adequate resolution for some species, these are unable to distinguish others (mainly those that form clade I, Fig. S1C, D). Although the trees reconstructed with ITS, *rpb*1 and 28S have different topologies, the species *E. clavata*, *E. diminuta*, *E. maculosa*, *E. multiformis*, and *E. rectangula* remain as well-supported and separate clades in the three trees (Fig. S1). On the other hand, *rpb*2 and *tef*1 were the most suitable genes to delimitate well-supported *Escovopsis* species and larger monophyletic groups. The tree of *rpb*2 and that of the five markers combined share the same topology and differ slightly from that of *tef*1 (Fig. S1). Interestingly, *rpb*2 and *tef*1 genes are considered molecular barcodes for genus *Trichoderma* (Chaverri *et al.* 2015), a sister clade of *Escovopsis*. Future studies should analyse the application of these genes as barcodes to assess the diversity and identify new species in *Escovopsis*.

Based on the high genetic diversity, as shown here and in ecological studies (Gerardo *et al.* 2006a, Meirelles *et al.* 2015b), a comparable morphological diversity was also expected in *Escovopsis*. While our results show abundant morphological and physiological differentiation among *Escovopsis* species; growth rates at different temperatures, colony colours and vesicle shapes appear to be the most diagnostic characters. Briefly, species in clades I, II, and III (in this order) are fast-growing, grow over wider temperature ranges and have cylindrical vesicles, while species in clades IV and V, grow slowly and at narrow temperature ranges, and form globose vesicles (Figs 2, 3). Future studies are needed to evaluate, from an evolutionary perspective, whether these characters are related to the diversification or the ecology of species in this genus.

On the other hand, many species in the family *Hypocreaceae* are ecologically associated with plants as versatile symbionts (Jaklitsch 2009, Jaklitsch & Samuels 2011, Bailey & Melnick 2013, Guzmán-Guzmán *et al.* 2019). However, in the genera *Hypomyces* and *Trichoderma* that are closely related to *Escovopsis*, many species also act as mycoparasites (Põldmaa 2000, Druzhinina *et al.* 2011, Atanasova *et al.* 2013, Chaverri & Samuels 2013, Kubicek *et al.* 2019, Mukherjee *et al.* 2022). Species of *Trichoderma*, that are mycoparasites, can form specialized structures, such as hooks, papilla-like, and coiling hyphae, and produce enzymes that help the fungus penetrate and degrade host cell walls (Brotman *et al.* 2010, Druzhinina *et al.* 2011). In *Escovopsis,* only few strains of *E. weberi* have been reported to be specialized, and virulent mycoparasites of the attine cultivars, *Leucoagaricus* sp. (Currie *et al.* 1999, Currie 2001, Currie *et al.* 2003), or to form specialized structures, apparently to parasitise it (Marfetán *et al.* 2015). Isolates of this species are also able to produce metabolites that are harmful to the fungal cultivars, the ants, and the ants' associated bacteria *Pseudonocardia* (Boya *et al.* 2017, Heine *et al.* 2018, Batey *et al.* 2020). Despite this, recent studies using many *Escovopsis* species (including many isolates of *E. weberi*) have proven that most of the species of this genus have low virulence (Mendonça *et al.* 2021) and an opportunistic nature modulated by the susceptibility of the ant cultivars (Jiménez-Gómez *et al.* 2021). In this study, we carried out a detailed analysis of the microscopic structures of *Escovopsis*. Notwithstanding, we did not observe any structure that would resemble a specialized structure, *i.e.*, hooks, papilla-like, and coiling hyphae (Druzhinina *et al.* 2011), related to parasitizing other fungi. However, we evaluated the isolates in axenic culture conditions. Considering the variability of synapomorphic characters of the close relatives of *Escovopsis*, it is expected that species of this genus present not only characters of mycoparasites but also related to other lifestyles. Future studies are needed to evaluate all *Escovopsis* species in co-cultures with the ant cultivars or other microorganisms and to check whether these species (other than *E. weberi*) are capable of producing specific structures or secondary metabolites to parasitize other fungi or damage the fungal cultivars, the ants, or their associated bacteria.

Finally, in contrast to their closest relatives (*e.g.*, *Trichoderma* and *Hypomyces sensu lato,* among others), many of which are ubiquitous (Jaklitsch 2009), and can infect various host fungi cultivated by humans (Tamm & Põldmaa 2013), *Escovopsis* has only been found in association with attine ant colonies. Cocladogenesis and genomic analyses suggested a coevolutionary history between *Escovopsis* (as the ant fungus garden parasites), the attine ants, and their mutualistic *Basidiomycota* fungi (Currie *et al.* 2003, Gotting *et al.* 2022). Although these studies did not consider the taxonomic analyses that revealed that the group of fungi they named as *Escovopsis* correspond to different genera, *i.e.*, *Escovopsis*, *Sympodiorosea* and *Luteomyces* (Montoya *et al.* 2021), the data suggests that *Escovopsis* maintains an ancient symbiotic relationship with the ants. Considering that close ecological relationships maintained for millions of years, such as mutualism or parasitism, influence the adaptation of the species by multiple selection pressures (Kessler & Halitschke 2009, Hutchinson *et al.* 2018), it is expected that morphological variations, observed in this study, throughout the *Escovopsis* phylogeny are related to its symbiotic relationships with the attine ants and/or their fungal cultivars. Studies on the taxonomy of *Escovopsis* are, like those on the ecological (Currie *et al*. 2003, Gerardo *et al.* 2004, 2006b, Taerum *et al.* 2007, Folgarait *et al.* 2011, Diego *et al.* 2014, Marfetán *et al.* 2015, Birnbaum & Gerardo 2016), biochemical (Boya *et al.* 2017, Heine *et al.* 2018), and genomic (De Man *et al.* 2016) aspects, important to answer this question. Future studies should combine all these approaches to shed light on the environmental and ecological pressures that affect morphological diversity in *Escovopsis* and their influence on the evolution of attine ants.

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DECLARATION ON CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

QVM, MJSM and AR designed the study. QVM carried out the morphological and phylogenetic analyses. QVM and MJSM carried out *in vitro* growth experiments and statistical analysis. QVM wrote the manuscript. QVM, MJSM and AR reviewed and proofread the manuscript. All authors read and approved the final manuscript.

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Table S1. Culture media used to standardize the parameters to assess morphological characters of *Escovopsis* species.
Table S2. Measurements of the colony radius of the *Escovopsis* ex-type cultures, and the new described species.
Table S3. Molecular markers, primers and Polymerase Chain Reaction (PCR) conditions.
Table S4. Strains and their associated metadata used to reveal the phylogenetic relationships of *Escovopsis* species described by Marfetán *et al.* (2019) (Fig. S2).
Table S5. Morphological features used to construct the dichotomous key of *Escovopsis* species.
Table S6. Data recoding sheet to evaluate the macroscopic characters of the colonies of *Escovopsi*s species.
Table S7. Data recoding sheet to evaluate the microscopic characters of *Escovopsis* species.
Fig. S1. Phylogeny revealing relationship among 19 species of *Escovopsis*, based on each molecular marker: (A) *rpb*2, (B) *tef*1, (C) ITS, (D) *rpb*1, (E) LSU, and (E) the combination of all of them (concatenated). Phylogenies shown were inferred using Bayesian Inference (BI) and *Sympodiorosea kreiselii* CBS 139320 was used as the outgroup. Numbers on branches indicate BI posterior probabilities (PP) and Maximum Likelihood bootstrap support values (MLB), respectively. Hyphens (--) indicate MLB < 70 %. ET indicates ex-type cultures and red crosses the non-viable strains. See Table 1 for all strains and their associated metadata used to infer these phylogenetic trees.
Fig. S2. Phylogeny revealing the relationship between *Escovopsis* species described by Marfetán *et al.* (2018). The tree was reconstructed to include the LSU sequences (in the green box) generated by Marfetán *et al.* (2018). The phylogeny was reconstructed using Bayesian Inference (BI) and Maximum Likelihood (ML) and and *Sympodiorosea kreiselii* CBS 139320 was used as the outgroup. Numbers on branches indicate BI posterior probabilities (PP) and Maximum Likelihood bootstrap support values (MLB), respectively. Hyphens (--) indicate MLB < 70 %. ET indicates ex-type cultures and red crosses the non-viable strains. See Table S4 for all strains and their associated metadata.
Fig. S3. Dichotomous key, in a cladogram format, revealing the relationship among *Escovopsis* species. The cladogram was reconstructed using 68 morphological features from species of *Escovopsis* in "rpart" library (Therneau & Atkinson 2019) in R v. 3.6.3. The final cladogram was manually edited using Adobe Illustrator CC v. 17.1. Information on branches was used to construct the taxonomic key and the leaves correspond to each *Escovopsis* species. See Table S5 for all associated data used to infer this cladogram.