DANILO OLIVEIRA RAMOS

A HAIRY DISCOVERY: *Chaetomiaceae* ASSOCIATED WITH ROOTS OF WILD *Orchidaceae* AND THEIR POTENTIAL FOR THE BIOLOGICAL CONTROL OF ROOT DISEASES

Dissertation submitted to the Fitopatologia Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

Adviser: Olinto Liparini Pereira

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Olinto Liparini Pereira
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To my grandmother, Maria da Penha.

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“I can never read all the books I want; I can never be all the people I want and live all the lives I want. I can never train myself in all the skills I want. And why do I want? I want to live and feel all the shades, tones and variations of mental and physical experience possible in my life. And I am horribly limited.”
(Sylvia Plath)

ABSTRACT

RAMOS, Danilo Oliveira, M.Sc., Universidade Federal de Viçosa, Febuary, 2024. **A Hairy Discovery: *Chaetomiaceae* associated with roots of wild *Orchidaceae* and their potential for the biological control of root diseases**. Adviser: Olinto Liparini Pereira.

Fungal members of the *Chaetomiaceae* family can be usually found as endophytes on several plant species, including orchids. Several species of this fungal family have the potential to be used in the biological control of plant diseases. Due to the lack of information about these fungi in association with orchids, this study aimed to isolate and identify the root endophytic *Chaetomiaceae* that occur in wild *Orchidaceae* plants, based on morphological and molecular analyses. Furthermore, isolates were tested for their ability to antagonize two soil-borne pathogens, *Fusarium sacchari* and *Rhizoctonia solani*. Healthy roots from 12 orchid species were sampled in the cities of Araponga-MG, Conceição do Mato Dentro-Mg, Viçosa-MG, Brasília-DF, Goiânia-GO and Nova Friburgo-RJ. Orchid fungal endophytes were isolated using the indirect isolation and dilution-to-extinction culturing method. Fungal genomic DNA was extracted, and the partial beta-tubulin (*tub2*) and partial RNA polymerase II second large subunit (*rpb2*) gene regions were amplified. PCR products were purified and sequenced by Macrogen (South Korea). Maximum Likelihood and Bayesian Inferences were performed for phylogenetic analyses. A total of 45 isolates, from six genera belonging to *Chaetomiaceae* family were obtained, these being *Arcopilus*, *Chaetomium*, *Collariella*, *Dichotomopilus*, *Humicola* and *Pseudohumicola*. The species *Arcopilus amazonicus*, *A. aureus*, and *A. cupreus* were identified, and four new *Arcopilus* species were discovered. In the *Chaetomium* genus five species were identified, *C. coarctatum, C. cochliodes*, *C. globosum*, *C. pseudocochliodes* and *C. tenue*. A new species of *Collariella* and *Dichotomopilus* were identified, and three new species from the genus *Humicola* were discovered. In addition, *Pseudohumicola pulvericola* and a new species from this genus were isolated from orchid substrate. A dual culture test was performed to test the ability of 10 endophytic *Chaetomiaceae* strains to antagonize *F. sacchari*. Moreover, seven isolates were chosen for the greenhouse experiment with *R. solani* on common bean (*Phaseolus vulgaris*). In both biocontrol essays the strain *C. tenue* (COAD 3736) presented a potential in the control of both pathogens when compared to the other strains tested. This study unraveled the species richness of *Chaetomiaceae* associated with roots of wild orchids and presented a strain of *C. tenue* (COAD 3736) with the potential for the biological control of phytopatogenic fungi.

Keywords: Taxonomy; phylogeny; biocontrol; endophytes, orchids.

RESUMO

RAMOS, Danilo Oliveira, M.Sc., Universidade Federal de Viçosa, fevereiro, 2024. **Uma Descoberta Cabeluda: *Chaetomiaceae* associados à raízes de orquídeas selvagens e seu potencial para o controle biológico de doenças radiculares**. Orientador: Olinto Liparini Pereira.

Membros fúngicos da família *Chaetomiaceae* podem ser encontrados como endófitos em diversas espécies de plantas, inclusive orquídeas. Espécies desta família apresentam potencial para serem utilizadas no controle biológico de doenças de plantas. Devido à falta de informações sobre esses fungos em associação com orquídeas, este estudo teve como objetivo isolar e identificar espécies de *Chaetomiaceae* endofíticas radiculares que ocorrem em orquídeas silvestres, com base em análises morfológicas e moleculares. Além disso, os isolados foram testados quanto à sua capacidade de antagonizar dois patógenos do solo, *Fusarium sacchari* e *Rhizoctonia solani*. Raízes saudáveis de 12 espécies de orquídeas foram amostradas nas cidades de Araponga-MG, Conceição do Mato Dentro-Mg, Viçosa-MG, Brasília-DF, Goiânia-GO e Nova Friburgo-RJ. Fungos endofíticos de orquídea foram isolados usando o método de isolamento indireto e cultivo de diluição-à-extinção. O DNA genômico do fungo foi extraído e as regiões gênicas da segunda grande subunidade parcial da beta-tubulina (*tub2*) e da RNA polimerase II (*rpb2*) foram amplificadas. Os produtos de PCR foram purificados e sequenciados pela Macrogen (Coreia do Sul). Máxima Verossimilhança e Inferências Bayesianas foram realizadas para análises filogenéticas. Foram obtidos 45 isolados, de seis gêneros pertencentes à família *Chaetomiaceae*, sendo eles *Arcopilus*, *Chaetomium*, *Collariella*, *Dichotomopilus*, *Humicola* e *Pseudohumicola*. As espécies *Arcopilus amazonicus*, *A. aureus* e *A. cupreus* foram identificadas e quatro novas espécies de *Arcopilus* foram descobertas. No gênero *Chaetomium* foram identificadas cinco espécies, *C. coarctatum*, *C. cochliodes*, *C. globosum*, *C. pseudocochliodes* e *C. tenue*. Foram identificadas uma nova espécie de *Collariella* e *Dichotomopilus*, e três novas espécies do gênero *Humicola*. Além disso, *Pseudohumicola pulvericola* e uma nova espécie do gênero foram isoladas de substrato de orquídea. Testes de cultura pareada foram realizados para avaliar a capacidade de 10 isolados endofíticos de *Chaetomiaceae*, antogonazirem *F. sacchari*. Além disso, sete isolados foram escolhidos para o experimento em casa de vegetação com *R. solani* em feijoeiro (*Phaseolus vulgaris*). Em ambos os ensaios de biocontrole o isolado *C. tenue* (COAD 3736) apresentou potencial no controle de ambos os patógenos quando comparada às demais cepas testadas. Este estudo desvendou a riqueza de espécies de *Chaetomiaceae* associadas a raízes de orquídeas silvestres e apresentou um isolado de *C. tenue* com potencial no controle biológico de fungos fitopatogênicos.

Palavras-chave: Taxonomia; filogenia; biocontrole, endófitos, orquídeas.

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INTRODUCTION

Orchids represent a diverse group of angiosperms that have adapted to living in an extensive variety of ecosystems. It is estimated that the ancestor of orchids-initiated diversification of the group around 76–84 million years ago, in the late Cretaceous, relatively recent in the history of land plants (Ramirez *et al.*, 2007). Nevertheless, the *Orchidaceae* family is considered one of the most species-rich groups of flowering plants, with 29,524 species distributed across all continents except Antarctica (Chase *et al.*, 2015; Christenhusz and Byng, 2016; Govaerts *et al.*, 2021).

Owing to the absence of sufficient nutrients for the complete germination of seeds, symbiotic associations between *Orchidaceae* plants and fungi are mandatory during their life cycle in the wild. Orchids have a heterotrophic stage in which a mycorrhizal fungus provides a source of organic carbon (C) until the plant reaches the autotrophic stage. Even when orchids are completely mature, the mycorrhizal fungus still provides C to the plant in a nutritional strategy called mixotrophy (Selosse and Martos, 2014). Consequently, there is a possibility that changes in symbiotic associations among fungi can be related to the diversification of orchids (Wang *et al.*, 2021)

Non-mycorrhizal fungi, mainly Ascomycetes, often colonize the root tissue of orchids asymptomatically (Sarsaiya *et al.*, 2019). Although their taxonomic diversity is still poorly studied, this ecological relationship can provide physiological benefits to the host plant, such as plant growth promotion, pathogen resistance, and resistance to abiotic stress (Rodriguez *et al.*, 2009; Ma *et al.*, 2015).

The potential use of endophytes in agriculture has gained attention as an alternative method for control of diseases. The use of sustainable disease management increased research about biocontrol agents and alternatives have been discovered, such as *Clonostachys*, *Chaetomium* and *Trichoderma* species (Ghazanfar *et al.* 2018; Soytong *et al.*, 2021). However, *Chaetomium* species are little studied compared to other groups even though they have the potential to be used in biological control.

Given potential use in agriculture of *Chaetomiaceae* endophytes and the absence of studies exploring the diversity of species from this group in association with orchids, the present study aimed to isolate and identify root endophytic *Chaetomiaceae* on orchids. Therefore, the isolates obtained were tested for their potential for the biocontrol of root diseases.

CHAPTER 1: ENDOPHYTIC *Chaetomiaceae* SPECIES ASSOCIATED WITH ROOTS OF *Orchidaceae* plants.

INTRODUCTION

Among the flowering plants present in Brazil, the *Orchidaceae* family is the second most diverse group, behind *Fabaceae*, having terrestrial, rupicolous and epiphytic habits. It is estimated that around 2,692 native species occur, present in all national biomes, with around 1,490 endemic species little studied by science (BFG, 2021).

The Atlantic Forest is recognized as one of the 34 biodiversity “hotspots” around the world and is home to more than 1,400 species of orchids, many of which are mentioned on lists of endangered species. As a result, habitat reduction and exploratory collection of orchids are the main factors associated with the loss of species in nature (Stehmann *et al.*, 2009; Mittermeier *et al.*, 2004; Martinelli and Moraes, 2013; Fay, 2018).

In the state of Minas Gerais, the *Orchidaceae* family has 127 genera and 863 species, distributed between the Atlantic Forest and Cerrado biomes (Flora e Funga do Brasil, 2023). In Serra do Brigadeiro State Park, 75 species of orchids have already been recorded in Serra das Cabeças, of which more than half are epiphytes (Lana; Peluzio, 2021). Discoveries about orchids endemic to Serra do Brigadeiro have been made in the last ten years, including the species *Cattleya locatellii*, which was described in 2016. This species has rupicolous habits, occurring in the municipality of Araponga, at 1,500 meters of altitude between other Angiosperms, mainly *Vellozia* sp. (Miranda, 2016).

Among the various microorganisms capable of establishing an association with plants are endophytic fungi. These organisms are capable of colonizing plant tissues without causing apparent symptoms. In this interaction between plant and fungus, the microorganism benefits from the plant, by obtaining nutrients for its survival and the shelter provided within the host's tissues. However, the plant is also able to benefit from this interaction, since the fungus, through the production of molecules, alters the physiology of the plant and can promote its growth, increase its resistance to phytopathogens and increase its resistance to heavy metals in the soil, such as mercury (Rodriguez *et al.*, 2009; Pietro-Souza *et al.*, 2019).

The endophytic fungi most frequently associated with the roots of orchids are the mycorrhizal fungi, which form a mutualistic association with the plant. This polyphyletic group of Basidiomycota, known as rhizoctonias, forms a specific type of mycorrhiza that only occurs with orchids (Selosse and Martos, 2014). However, these plants are associated with non-mycorrhizal endophytic fungi, commonly belonging to the phylum Ascomycota. In this interaction, these fungi can play an essential role in the survival of the orchid in the natural environment (Shah *et al.*, 2019).

Endophytic fungi that are associated with *Orchidaceae* plants have great biotechnological potential, as they can produce several metabolites, which can be used in the production of drugs and bioproducts (Sarsaiya *et al.*, 2019). Endophytes from the orchid *Cremastra appendiculata*, from the species *Trichoderma tomentosum* and *Talaromyces amestolkiae*, presented action in promoting the growth of orchid and soybean plants, in addition to producing compounds capable of inhibiting the growth of phytopathogens (Wang *et al.*, 2022). Although other genera known as biological control agents, such as *Chaetomium* can be found as endophytes in orchids, most of the studies do not clarify which taxa are associated with them (Sarsaiya *et al.*, 2019).

Owing to the use of molecular tools, the taxonomy of the *Chetomiaceae* family has undergone severe changes in recent years. The application of multi-gene molecular phylogeny allowed the correct separation of genera within the family that were previously identified as *Chaetomium*. Furthermore, new genera and species have been described in closed environments, in the soil, plant remains, and endophytic associations with different plant families (Wang *et al.*, 2016a). Although the morphology is an important tool in the taxonomy of *Chaetomium* and *Chaetomium*-like fungi, phylogenetic analyses using the RNA polymerase II second largest subunit (*rpb2*), β-tubulin (*tub2*), ITS and 28S large subunit (LSU) nrDNA sequences were proved essential to correctly delimitated genera and species in the *Chaetomiaceae* family (Wang *et al.*, 2016a; Wang *et al.*, 2019; Wang *et al.*, 2022)

In a study by Wang *et al.* (2016a), five new genera were introduced into the *Chaetomiaceae* family, they are: *Amesia*, *Arcopilus*, *Collariella*, *Dichotomopilus* and *Ovotospora*. Furthermore, the genus *Humicola* was recognized in this study. Subsequently, new thermophilic genera have been introduced to the family, such as *Parvomelanocarpus*, *Thermochaetoides*, and *Xanthiomyces* (Wang *et al.*, 2022). In contrast, two new genera, *Allocanariomyces* and *Parachaetomium*, were described in endophytic association with seeds of *Triticum boeoticum* and *Aegilops triuncialis*, respectively (Mehrabi *et al.*, 2020).

Although still scarce in Brazil, studies involving the taxonomy and description of new species of the *Chaetomiaceae* family have recently been developed. A new species of the genus *Arcopilus* was described as an endophyte of *Eremanthus erythopappus*. *Arcopilus eremanthi* described by Tavares *et al*. (2021) produces metabolites with bactericidal and antioxidant activity. *Achaetomium lippiae* was described as an endophyte of *Lippia gracilis* (Viana *et al*., 2017).

Other sources of reports of *Chaetomiaceae* in Brazil are the studies on coprophilous and saprobe fungi. *Chaetomium* species are the most frequently found in animal dung, while in plant debris species of *Dichotomopilus*, *Humicola* and *Staphylotrichum* can be found besides the *Chatetomium* species (Melo *et al.*, 2012; Barbosa *et al.* 2012; Melo *et al.*, 2019).

Brazilian caves have shown to be a source of taxonomic discoveries of fungi, including *Chaetomiaceae*. Condé *et al*. (2023) described a new genus and four new species of *Chaetomiaceae* in Brazil. *Parahumicola* is a new monotypic genus of the family, with only *Parahumicola guana* found to be associated with bat guano in the Monte Cristo Cave, in the state of Minas Gerais. Furthermore, the authors described the species *Chaetomium meridionalense*, *Pseudohumicola alba* and *Pseudohumicola lutea*, in addition to the first report of *Collariella bostrychodes*, all occurring in caves in the municipality of Diamantina, MG. Moreover, Alves *et al*. (2022) described the species *Psedohumicola cecavii* in caves of Pernambuco state.

Even though reports of *Chaetomiaceae* in endophytic association with orchids are common in the literature, no studies are focusing on the taxonomy of the isolates found in these hosts. As this field is still little explored, only the genera *Chaetomium* and *Humicola* are reported to be endophytic in orchid roots (Ma *et al.*, 2015). However, there is a potential for the discovery of taxonomic and biotechnological novelties in endophytic fungi associated with *Orchidaceae*, since these plants harbor a diversity of fungi associated with their root tissue (Sarsaiya *et al*., 2019).

Due to the lack of studies focusing on the taxonomy of *Chaetomiaceae* fungi associated with *Orchidaceae* plants, especially root endophytes, this study aimed to isolate and identify these fungi using the polyphasic taxonomy approach. Therefore, wild orchids with rupiculous, terrestrial and epiphyte lifestyles had their healthy roots sampled and their endophytic fungi identified based on morphological and phylogenetic analyses.

MATERIAL AND METHODS

Sample Collection

Roots of 12 species of orchids were collected from two Brazilian biomes, the Atlantic Forest and the Cerrado, in four states: Distrito Federal, Goiás, Minas Gerais, and Rio de Janeiro (Figure 1). Samples of healthy roots of wild orchids from the species *Ancianthera teres* (Lindl.) Borba, *Catasetum hookeri* Lindl., *Cattleya jongheana* (Rchb.f.) Van den Berg, *Cattleya locatellii* (F.E.L.Miranda) Van den Berg, *Cattleya nobilior* Rchb.f., *Cattleya* sp. Lindl., *Cyclopogon congestus* (Vell.) Hoehne, *Habenaria petalodes* Lindl., *Gomesa recurva* R.Br., *Oeceoclades maculata* (Lindl.) Lindl., *Polystachya concreta* (Jacq.) Garay & Sweet, and *Zygopetalum mackayi* Hook. were collected (Fig. 1). Orchid species were identified with the help of a specialist in taxonomy of the group. Although we were not able to properly identify the second *Cattleya* species mentioned above, this rupicolous *Cattleya* was identified as belonging to the series *Parviflorae* based on morphological analysis of the vegetative parts of the plant (Van Den Berg, 2014). Samplings were made from 2019 to 2023 during different seasons during the years.

Located at the Iron Quadrangle in the central-southern part of the Minas Gerais state, in Conceição do Mato Dentro city, two rupicolous orchid species were sampled: *Acianthera teres* near Curral de Pedra Cave, and *Cattleya* sp. in Serra da Ferrugem. Both orchids grew with their roots fixed above ferruginous rocks.

In the southeastern part of Minas Gerais state, in the mesoregion known as Zona da Mata, samplings were made in the cities of Viçosa and Araponga. In the ecological reserve of Mata do Paraíso, a fragment of Atlantic Forest located in Viçosa, roots of two epiphytic species *Catasetum hookeri* and *Polystachya concreta* were sampled. Moreover, was observed that the orchid *Oeceoclades maculata* grew naturally in the ground and above leaf litter in different parts of the forest and in other fragments of Atlantic Forest of Viçosa region. Samplings were made in the summer and winter seasons, once the forest humidity is higher during the summer due to the high precipitation of rain, and drier in the winter. In addition, roots of the terrestrial species *Habenaria petalodes* were collected near the countryside of Viçosa.

Samples of roots of two endemic *Cattleya* species, *C. jongheana* and *C. locatellii*, and the species *Gomesa recurva* and *Zygopetalum mackayi*, were collected near the *Parque Estadual da Serra do Brigadeiro*, in Araponga municipality. *Cattleya jongheana* and *G. recurva* were growing epiphytically on trees in an underbrush, while *C. locatellii* was growing above rocks and *Z. mackayi* was growing on the ground.

Located at Fluminense Mountain Region of the Rio de Janeiro state, two terrestrial orchids were sampled in Nova Friburgo city, *Cyclopogon congestus* and *Oeceoclades maculata*. Both orchids were collected in an Atlantic Forest fragment, at approximately 990 m altitude during the spring season.

Samplings were made in two cities in the Cerrado region. In a gallery forest near Brasília-DF, roots of *Oeceoclades maculata* were grown above dead leaves, with their roots growing underground. Therefore, one isolate was sampled from the epiphytic species *Cattleya nobilior* in Goiânia-GO.

In addition, two non-endophytic isolates were obtained from the substrate in which an orchid was growing. Characteristic structures of *Chaetomiaceae* fungi, such as ascomata with terminal hair coiled, asci evanescent, and brown ascospores, were observed to grow saprophytically close to the roots of *Cattleya aclandiae* on the coconut shell used as substrate. Direct isolation was performed from the fungi structures.

Besides the endophytes obtained during the samples made from 2019 to 2023, two isolates identified as *Chaetomium* sp. from the roots of a *Cattleya* sp. collected in 2012 and deposited at the *Coleção de Culturas Fúngicas* of the *Laboratório de Micologia e Etiologia de Doenças Fúngicas* were included in this study.

Isolation of endophytic fungi

Endophytic fungi were isolated from healthy roots of orchids using two different methods to access the diversity present in this plant tissue: the indirect isolation method and the dilution-to-extinction cultivation method.

For indirect isolation, the methodology described by Pereira and Zambolim (2012) was used. Fragments of healthy roots were washed in running water and fragmented into small pieces of approximately 1 cm. After this procedure, they were immersed in a solution of 70% alcohol and 80% Tween for 1 min and in a sodium hypochlorite solution for 4 min, washed in sterile distilled water three times, and dried on sterilized filter paper. The entire procedure was performed in a Flow Chapel. Disinfested fragments were plated in Petri dishes containing Synthetic Nutrient Agar (SNA) medium. Four fragments were arranged per Petri dish, 12 replicates were made for each orchid species, and then plates were incubated at 25ºC for two weeks. Fungi that presented morphological characteristics of *Chaetomiaceae* were subcultured in Oatmeal Agar (OA) medium (g/L: 30 g) for future analyses.

Fig. 1: A. *Acianthera teres*. **B.** *Catasetum hookeri*. **C.** *Cattleya jongheana*. **D.** *Cattleya locatellii*. **E.** *Cattleya nobilior*. **F.** *Cattleya* sp. **G.** *Cyclopogon congestus*. **H.** *Gomesa recurva*. **I.** *Habenaria petalodes*. **J.** *Oeceoclades maculata.* **K.** *Polystachya concreta*. **L.** *Zygopetalum mackayi*.

The dilution-to-extinction cultivation method described by Collado *et al.* (2007) and modified by Leite *et al.* (2013) was used to isolate root endophytic fungi with a slow growth ratio. Healthy roots were washed under running water and then shredded. Approximately 7 g of root from each plant was used for isolation. Initially, the root fragments were superficially disinfested in a solution of 70% alcohol and 80% Tween for 1 min, in sodium hypochlorite solution for 3 min, and sterile distilled water (twice) for 1 min. The disinfested fragments were crushed in a previously sterilized blender containing 300 ml of 0.85% saline solution (NaCl). After this process, the solution was filtered through three sieves with openings of 500 µm, 212 µm and 106 µm, respectively. The material that remained on the 106 µm sieve was transferred to 50 ml Falcon tubes along with 20 ml of saline solution and centrifuged for 6 min at 7000 g. Subsequently, the supernatant was discarded, and 15 ml of Carboxymethyl Cellulose (CMC) was added and centrifuged for another 15 minutes at 4000g. After centrifugation, serial dilutions were carried out using CMC in the orders of 1:2, 1:4, 1:8, 1:16 and 1:32. Then, 40 µl of each dilution were plated in Petri dishes containing Yeast Malt Agar (YMC) medium (g/L: 2 g of yeast extract, 10 g of malt extract, 13 g of agar, 0.05 g of streptomycin, 0. 05 g tetracycline). The spread plate method was used to plate the particles, and six replicates were prepared for each dilution. Afterward, the plates were incubated at 25 °C and subcultured according to the fungal growth observed for 1 month in Petri dishes containing Oatmeal Agar (OA) medium.

All isolates obtained in this study were deposited in the Coleção de Culturas Fúngicas (CCF) of the Laboratório de Etiologia de Doenças Fúngicas de Plantas and the *Coleção Octávio Almeida Drummond* (COAD). The holotype of new species represented by metabolically inactive dried cultures, were deposited in the Herbarium VIC, all housed at Universidade Federal de Viçosa.

Identification of isolates

Morphological Analyses

The morphological characteristics of the *Chaetomiaceae* isolates were analyzed according to the recommendations of Wang *et al.* (2022). Fungal isolates were inoculated at three equidistant points on 90 mm Petri dishes. Fungal characters such as colony diameter, colour of mycelium and colour reverse, degree of sporulation, texture of aerial mycelium, presence or absence of ascomata and asexual morphs, and soluble pigment and exudates production were described in Corn Meal Agar (CMA), Malt extract Agar (MEA), Oatmeal Agar (OA) and Potato Carrot Agar (PCA) media at 25ºC for 7 days in the dark (Crous *et al.* 2009; Samson *et al.* 2010). Color names and alphanumeric codes were used according to Rayner (1970).

For the description of asexual morphs, the slide culture method (Riddell, 1950) and the inclined coverslip method (Kawato and Shinobu 1959; Nugent *et al.* 2006) were used. Slides were mounted in lactoglycerol or Shear's solution from structures grown on OA and sometimes PCA. Fungal structures were observed using an Olympus BX 53 and images were obtained using a Q-color 5 digital camera (Olympus America INC.). The morphometry of the structures, such as ascomata, ascomatal hairs, asci, ascospores, and asexual morphs was obtained using cellSens Dimension 1.9 software. At least 30 measurements were performed for each structure mentioned above.

DNA extraction, PCR, and phylogenetic analyses

Monosporic isolates were cultivated on OA with a sterile cellophane membrane from 4 to 7 days at 25 ºC in the dark. Fungal mycelium was removed from the culture medium with the aid of a sterilized wooden toothpick and transferred to 2.0 mL microtubes. To disrupt the cells physically, four steel spheres (beads) were added to the microtubes. The samples were subjected to L-BEADER 6 cell and tissue disruptor (Loccus Biotecnologia) for 30 s at 4,000 rpm. Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega) protocol, with changes made by Pinho *et al.* (2013)

Initially, the fungal DNA regions of β-tubulin (*tub2*) and RNA polymerase II (*rpb2*) of all isolates were amplified by Polymerase Chain Reaction (PCR), as these are the most informative regions for identifying *Chaetomiaceae* at the species level (Wang *et al.*, 2016b). Primers T1 (O'Donnell and Cigelnik, 1997) and Bt2b (Glass and Donaldson, 1995) or Tub4RD (Groenewald *et al.*, 2013) were used for the *tub2* region. For amplification of *rpb2*, the primers fRPB2-5F2 (Sung *et al.,* 2007) and fRPB2-7cR (Liu *et al.* 1999) were used. The isolates that represented possible new species in the analyses with the two regions mentioned above had the rDNA-Internal Transcribed Spacer (ITS) and 28S Large Subunit (rDNA-LSU) regions amplified using the primers ITS5 and LR6 (White *et al.*, 1990; Vilgalys; Hester 1990).

PCR reactions were carried out with 18 μL of Platinum® PCR SuperMix, 0.4 μL of each forward and reverse primer (10 μM) synthesized by Invitrogen (USA), 1 μL of dimethyl sulfoxide (DMSO) and 1.2 µL of genomic DNA (25 ng/µL). Nuclease-free water was used as the negative control instead of genomic DNA. The PCR conditions were set for an initial denaturation step at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C (ITS/LSU), 55 ºC (*tub2* and *rpb2*) for 30 s, elongation at 72 °C for 60 s, and a final elongation step at 72 °C for 2 min. The amplified regions were visualized by electrophoresis in a 2% agarose gel stained with GelRed™ (Biotium) in TAE buffer (1×) on the L-Pix Photodocumentator to verify purity and fragment size. The PCR products were sent to Macrogen Inc. (South Korea), where they were purified and sequenced.

The nucleotide sequences obtained were visualized and edited using FinchTV (v.1.4.0) software (Geospiza Inc.). Sequences were compared with those available in the GenBank database using the megaBLAST tool. Sequences of the type and representative isolates were obtained from GenBank to create a database. Individual alignments were performed using MAFFT v.7 (Katoh and Standley, 2013), and small adjustments were made when necessary, using the software MEGA v.7 (Kumar *et al.,* 2016). Therefore, concatenated alignments using ITS, LSU, RPB2, and TUB were performed using the software Sequence Matrix 1.8 (Vaidya *et al.,* 2011)

Bayesian Inference (BI) and maximum likelihood (ML) were performed for phylogenetic analyses. BI analyses were carried out using MrBayes (v.3.2.7) (Ronquist *et al*., 2012) within the CIPRES Science Gateway Portal (Miller *et al*., 2011). The software Mr.ModelTest 2.3 (Posada and Buckley, 2004) was used to select the nucleotide substitution model for the BI analyses. Likelihood values were calculated, and the model was selected according to the Akaike Information Criterion (AIC). Evolution models were selected for each gene region separately. Four MCMC chains were conducted simultaneously, starting the trees randomly for up to 10,000,000 generations. Trees were sampled every 1,000 generations, resulting in 10,000 trees. The first 2,500 trees were discarded from the analyses. Posterior probability values (Rannala and Yang, 1996) were determined from the consensus tree using the remaining 7,500 trees. ML analyses were carried out using RAxML-HPC2 (v 8.2.12) (Stamatakis, 2014) in the CIPRES Science Gateway Portal, using GTR+G+I as nucleotide model substitution and 1,000 bootstrap (bs) replicates, performed using rapid bootstrapping (Miller *et al*., 2011). Branches were considered significantly supported when posterior probability values were above 0.95 (BI) and bootstrap were above 75 (ML). Trees were visualized using FigTree software v. 1.4.3 (Rambaut, 2018) and exported to graphics programs.

RESULTS

Isolates

Most of the root endophytic fungi isolated from orchids could only be identified at the genus level using morphological analyses and the DNA marker ITS. Since the present study focused on the endophytic *Chaetomiaeceae*, studies involving other groups of orchid endophytes will be conducted by members of the CNPq-PROTAX project. High genus richness was observed, and the phylum *Ascomycota* represented the majority of the isolates, with only a few isolates belonging to the phyla *Basidiomycota* (1) and *Mucoromycota* (2). In addition, common endophytes of plant roots, such as *Fusarium*, *Colletotrichum* and *Trichorderma*, were identified in association with roots of almost all orchids sampled (Table 1).

The method of indirect isolation proved to be the best to isolate endophytic *Chaetomiaceae* from roots of orchids, with the majority of the isolates being from this method. The dilution-to-extinction culturing method was helpful to isolate other endophytic fungi, such as dark septate endophytes and slow growth fungi, including *Chaetomium*-like. A total of 45 strains belonging to the *Chaetomiaceae* family were obtained in this study, with 42 of them root endophytic to *Orchidaceae* plants. They represented six *Chaetomiaceae* genera, namely *Arcopilus*, *Chaetomium*, *Collariella*, *Dichotomopilus*, *Humicola* and *Pseudohumicola*. Twenty species were identified in the phylogenetic analyses, in which ten of them were taxonomical novelties.

The isolates from *Acianthera teres* (COAD 3724, COAD 3725, and COAD 3726), *Cattleya locatellii* (COAD 3723) and *Habenaria petalodes* (COAD 3727 and COAD 3728) represented four new *Arcopilus* species, with each isolate from *H. petalodes* being a new species separately. One isolate *Cattleya jongheana* (COAD 3717) and nine from *Cattleya* sp. (COAD 3710, COAD 3711, COAD 3712, COAD 3713, COAD 3714, COAD 3715, and COAD 3716) were identified belonged to *Arcopilus amazonicus*. In addition, two isolates from *Cattleya* sp. (COAD 3718 and COAD 3719) and two from *H. petalodes* (COAD 3720 and COAD 3721) were identified as *Arcopilus* *aureus*. The isolates obtained in the fungal collection were identified *Arcopilus cupreus* (COAD 3722) and *Dichotmopilus variostiolatus* (CCF 341).

In the *Chaetomium* genus, one isolate from *Cyclopogon congestus* belonged to *Chaetomium coarctatum*, while the isolates from *Oeceoclades maculata* (COAD 3732) *Polystachia concreta* (CCF 702), and *Zygopetalum mackayi* (CCF 645) were identified as *Chaetomium globosum*. Moreover, two isolates from *O. maculata* (COAD 3730 and COAD 3731) and two from *Z. mackayi* (CCF 634 and CCF 641) were identified as *Chaetomium cochliodes*. The species *Chaetomium pseudocochliodes* was identified on *P. concreta* (COAD 3733) and *Gomesa recurva* (COAD 3734 and COAD 3735), whereas *Chaetomium tenue* was identified on *O. maculata* (COAD 3736).

Five isolates identified as belonging to *Humicola* genus (COAD 3742, COAD 3743, COAD 3744, COAD 3745, and COAD 3746) represented three putative new species. A new *Collariella* species was identified among the isolates from *Cyclopogon congestus* (COAD 3737) and *O. maculata* (COAD 3738 and COAD 3739). Furthermore, one isolated from *Z. mackayi* was consired to be a new species from the *Dichotomopilus* genus (CCF 638).

In addition, three strains were isolated from the substrate of *Cattleya aclandiae,* two identified as *Pseudohumicola pulvericola* (COAD 3748 and COAD 3749) and the other one represented a new *Pseudohumicola* species. Finally, one isolate from *Cattleya nobilior* was identified as *Dichotomopilus variostiolatus* (COAD 3740).

Table 1: Root endophytic fungi identified in the roots of the orchids.

Orchid species	Family	Genus
Acianthera teres	*Chaetomiaceae*	*Arcopilus*
Acianthera teres	*Glomerellaceae*	*Colletotrichum*
Acianthera teres	*Hypocreaceae*	*Trichoderma*
Cattleya locatellii	*Bionectriaceae*	*Acremonium*
Cattleya locatellii	*Thelebolaceae*	*Antarctomyces*
Cattleya locatellii	*Chaetomiaceae*	*Arcopilus*
Cattleya locatellii	*Lophiotremataceae*	*Atrocalyx*
Cattleya locatellii	*Herpotrichiellaceae*	*Cladophialophora*
Cattleya locatellii	*Glomerellaceae*	*Colletotrichum*
Cattleya locatellii	*Conioscyphaceae*	*Conioscypha*

Orchid species	**Family**	**Genus**
Cattleya locatellii	*Incertae sedis*	*Crucellisporium*
Cattleya locatellii	*Pleosporaceae*	*Curvularia*
Cattleya locatellii	*Chaetosphaeriaceae*	*Epicoccum*
Cattleya locatellii	*Hyaloscyphaceae*	*Hyaloscypha*
Cattleya locatellii	*Mollisiaceae*	*Mollisia*
Cattleya locatellii	*Mucoraceae*	*Mucor*
Cattleya locatellii	*Aspergillaceae*	*Penicillium*
Cattleya locatellii	*Hyphodiscaceae*	*Purimyces*
Cattleya locatellii	*Trichocomaceae*	*Talaromyces*
Cattleya locatellii	*Chaetosphaeriaceae*	*Thozetella*
Cattleya locatellii	*Cladosporiaceae*	*Toxicocladosporium*
Cattleya locatellii	*Hypocreaceae*	*Trichodema*
Cattleya locatellii	*Incertae sedis*	*Tritirachium*
Cattleya locatellii	*Xylariaceae*	*Virgaria*
Catasetum hookeri	*Herpotrichiellaceae*	*Cladophialophora*
Catasetum hookeri	*Cladosporiaceae*	*Cladosporium*
Catasetum hookeri	*Chaetosphaeriaceae*	*Chloridium*
Catasetum hookeri	*Glomerellaceae*	*Colletotrichum*
Catasetum hookeri	*Nectriaceae*	*Fusarium*
Catasetum hookeri	*Chaetomiaceae*	*Humicola*
Catasetum hookeri	*Aspergillaceae*	*Penicillium*
Catasetum hookeri	*Sordariaceae*	*Sordaria*
Catasetum hookeri	*Hypocreaceae*	*Trichoderma*
Catasetum hookeri	*Xylariaceae*	*Xylaria*
Cattleya jongheana	*Chaetomiaceae*	*Arcopilus*
Cattleya nobilior	*Chaetomiaceae*	*Dichotmopilus*
Gomesa recurva	*Chaetomiaceae*	*Chaetomium*
Gomesa recurva	*Coniochaetaceae*	*Coniochaeta*
Gomesa recurva	*Nectriaceae*	*Fusarium*
Gomesa recurva	*Hypocreaceae*	*Trichoderma*
Habenaria petalodes	*Chaetomiaceae*	*Arcopilus*

Orchid species	**Family**	**Genus**
Habenaria petalodes	*Nectriaceae*	*Fusarium*
Habenaria petalodes	*Hypocreaceae*	*Trichoderma*
Oeceoclades maculata	*Chaetomiaceae*	*Collariella*
Oeceoclades maculata	–	Dark septate endophyte
Oeceoclades maculata	*Nectriaceae*	Fusarium
Oeceoclades maculata	*Chaetomiaceae*	*Humicola*
Oeceoclades maculata	*Periconiaceae*	*Periconia*
Oeceoclades maculata	*Sordariaceae*	*Sordaria*
Oeceoclades maculata	*Hypocreaceae*	*Trichoderma*
Oeceoclades maculata	*Xylariaceae*	*Xylaria*
Polystachya concreta	*Chaetomiaceae*	*Chaetomium*
Polystachya concreta	*Glomerellaceae*	*Colletotrichum*
Polystachya concreta	*Nectriaceae*	*Fusarium*
Polystachya concreta	*Botryosphaeriaceae*	*Lasiodiplodia*
Polystachya concreta	*Sporocadaceae*	*Neopestalotiopsis*
Polystachya concreta	*Sordariaceae*	*Sordaria*
Polystachya concreta	*Xylariaceae*	*Xylaria*
Cattleya sp.	*Chaetomiaceae*	*Arcopilus*
Cattleya sp.	–	Dark septate endophyte
Cattleya sp.	*Sordariaceae*	Sordaria
Cattleya sp.	*Trichocomaceae*	*Talaromyces*
Cyclopogon congestus	*Bionectriaceae*	*Acremonium*
Cyclopogon congestus	*Chaetomiaceae*	*Chaetomium*
Cyclopogon congestus	*Chaetomiaceae*	*Collariella*
Cyclopogon congestus	*Nectriaceae*	*Fusarium*
Cyclopogon congestus	*Rhizopodaceae*	*Rhizopus*
Cyclopogon congestus	*Sordariaceae*	*Sordaria*
Cyclopogon congestus	*Hypocreaceae*	*Trichoderma*
Cyclopogon congestus	*Xylariaceae*	*Xylaria*
Zygopetalum mackayi	*Chaetomiaceae*	*Chaetomium*
Zygopetalum mackayi	*Chaetomiaceae*	*Dichotomopilus*

Phylogenetic Analyses

The best model nucleotide substitution models for Bayesian Inferences chosen for each alignment are presented in Table 2. The length of each alignment and the concatenated one are indicated in the same table. Phylogenetic analyses were essential to identify correctly the isolates obtained in this study

Isolate COAD 3723, from *Cattleya locatelli*, formed a clade with high support next to *Arcopilus erermanthi*, *A. flavigenus* and *A. fusiformis*, and represents a new species. The isolate COAD 3728, from *Habenaria petalodes*, formed a basal lineage next to the species mentioned above and represents a new species (Figure 2). Besides to clustered next to each other, the isolates COAD 3723 and COAD 3728 differ from every other species from the genus by the absence of a sexual morph.

The isolates COAD 3724, COAD 3725 and COAD 3726 were found as root endophyte on *Acianthera teres*, formed a well-supported clade sister to *Arcopilus hogheensis* and *A. navicularis* and represents a new species. Furthermore, the isolate from *H. petalodes* (COAD 3727) clustered next to *Arcopilus cupreus* and represents a new species. Two isolates from *Oececlades maculata* (COAD 3738 and COAD 3739) collected in Viçosa-MG were similar to one isolate obtained from roots of *Cyclopogon congestus* (COAD 3737) in Nova Friburgo-RJ. These isolates formed a clade sister to *Collariella pachypodioides*, *C. carteri* and *C. capillicompacta* strongly supported (bs = 100 and pp =1) and represents a new *Collariella* species (Fig. 2).

In the *Humicola* genus, the isolates COAD 3745 and COAD 3746 formed a clade well supported (bs = 94 and pp =0.98) sister to *Humicola fuscoatra*. These strains were isolated as root endophytes on *O. maculata* in Viçosa-MG and constitute a new species. Moreover, other *Humicola* strain (COAD 3744) obtained from *O. maculata* in Brasília-DF formed a strongly supported clade (bs = 100 and pp =1). The isolates COAD 3742 and COAD 3743 from *Catasetum hookeri* clustered next to the isolate COAD 3744. These three isolates clustered apart from each other and represent two new species of *Humicola* (Fig. 5). The strain COAD 3747, isolated from the substrate of *Cattleya aclandiae,* clustered into the *Pseudohumicola* clade. This isolate is sister to *Pseudohumicola semispiralis* and represents a new species of the genus.

Table 2: Number of alignment positions and best nucleotide substitution models selected for Bayesian inference (BI) analyses.

	***Arcopilus* + *Collariella* phylogeny**		***Chaetomium* phylogeny**		***Dichotomopilus* phylogeny**		***Humicola* + *Pseudohumicola* phylogeny**	
Partition	Length	Best model	Length	Best model	Length	Best model	Length	Best model
ITS	641	GTR+I+G	562	GTR+I+G	565	GTR+I	547	SYM+I+G
LSU	564	GTR+I+G	702	GTR+I+G	-	-	532	GTR+I
TUB	834	HKY+I+G	770	HKY+I+G	754	HKY+G	779	GTR+I+G
RPB2	852	GTR+I+G	598	GTR+I+G	525	GTR+I+G	852	GTR+I+G
Concatenated	2891		2632		1844		2710	

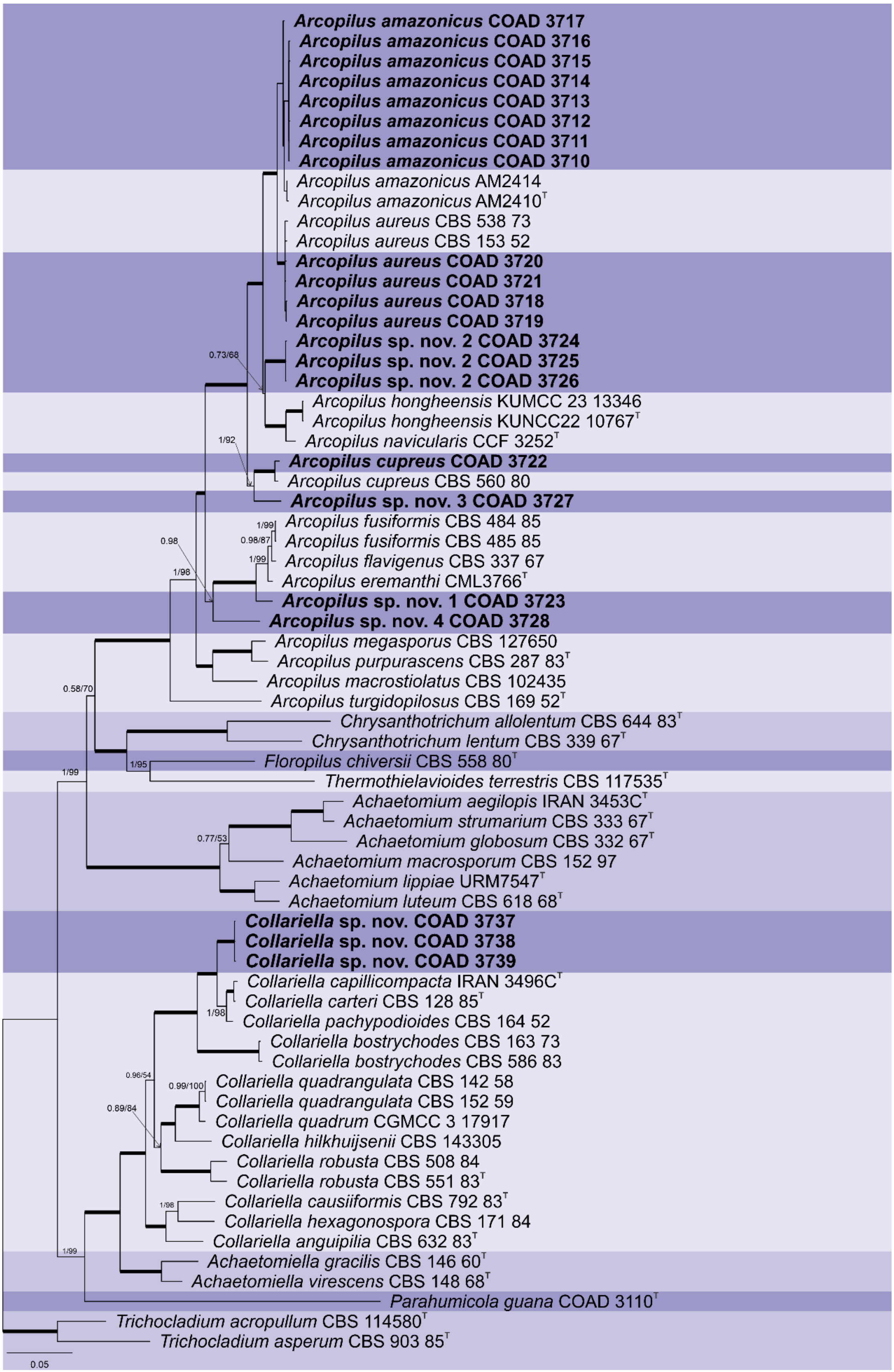


Fig. 2: Bayesian phylogenetic tree of *Arcopilus*, *Collariella* and related genera based on a concatenated dataset of ITS, LSU, *rpb2*, and *tub2* sequences. The isolates obtained in this study are shown in bold. Ex-type isolates are indicated with ”T”. The branches that presented bs= 100% and pp = 1 are thickened. The tree is rooted with *Trichocladium acropullum* CBS 114580 and *Trichocladium asperum* CBS 903 85.

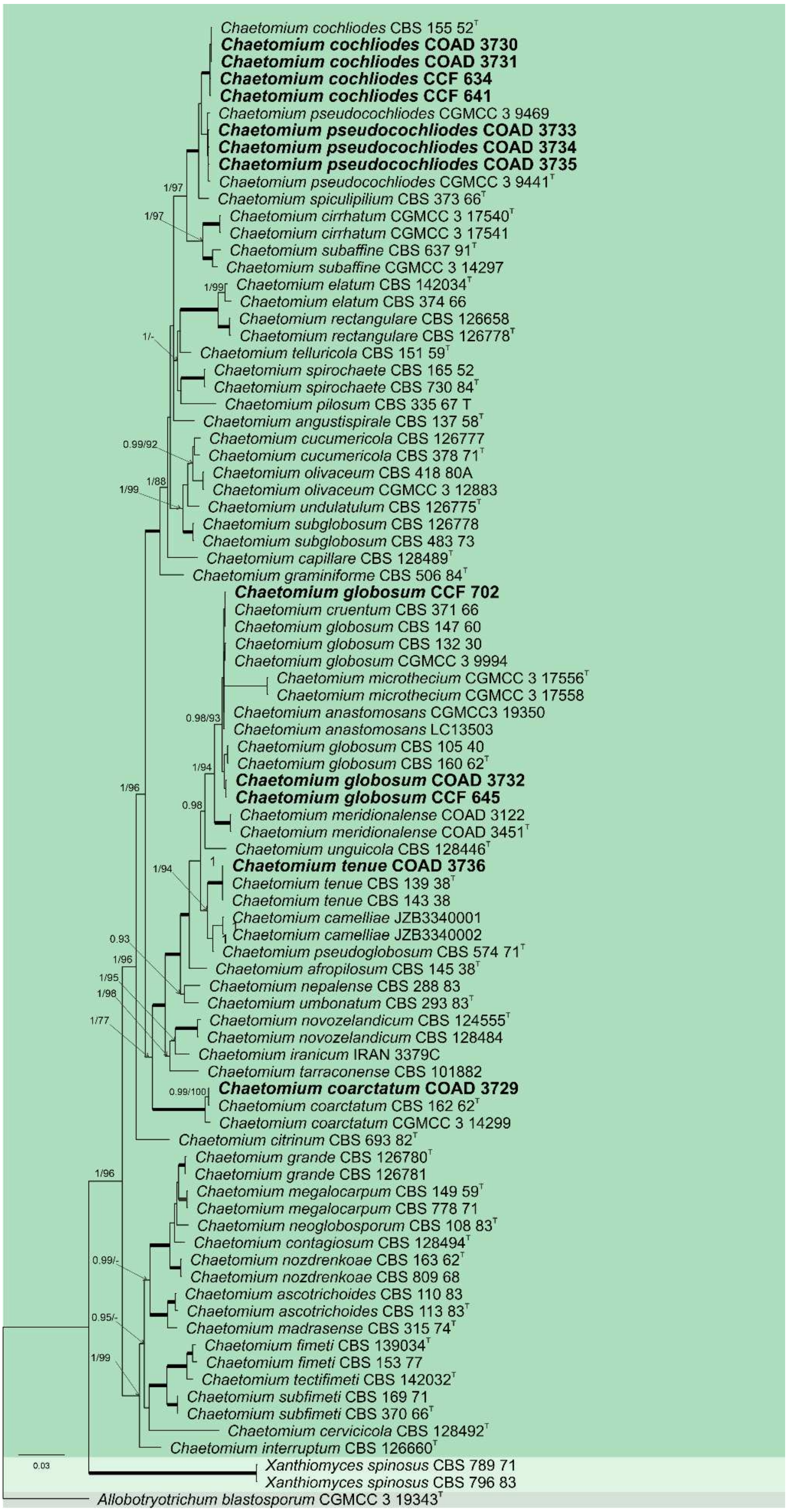


Fig. 3: Bayesian phylogenetic tree of *Chaetomium* and *Xantiomyces* based on a concatenated dataset of ITS, LSU, *rpb2*, and *tub2* sequences. The isolates obtained in this study are shown in bold. Ex-type isolates are indicated with "T". The branches that presented bs= 100% and pp = 1 are thickened. The tree is rooted with *Allobotryotrichum blastosporum* CGMCC 3.1943.

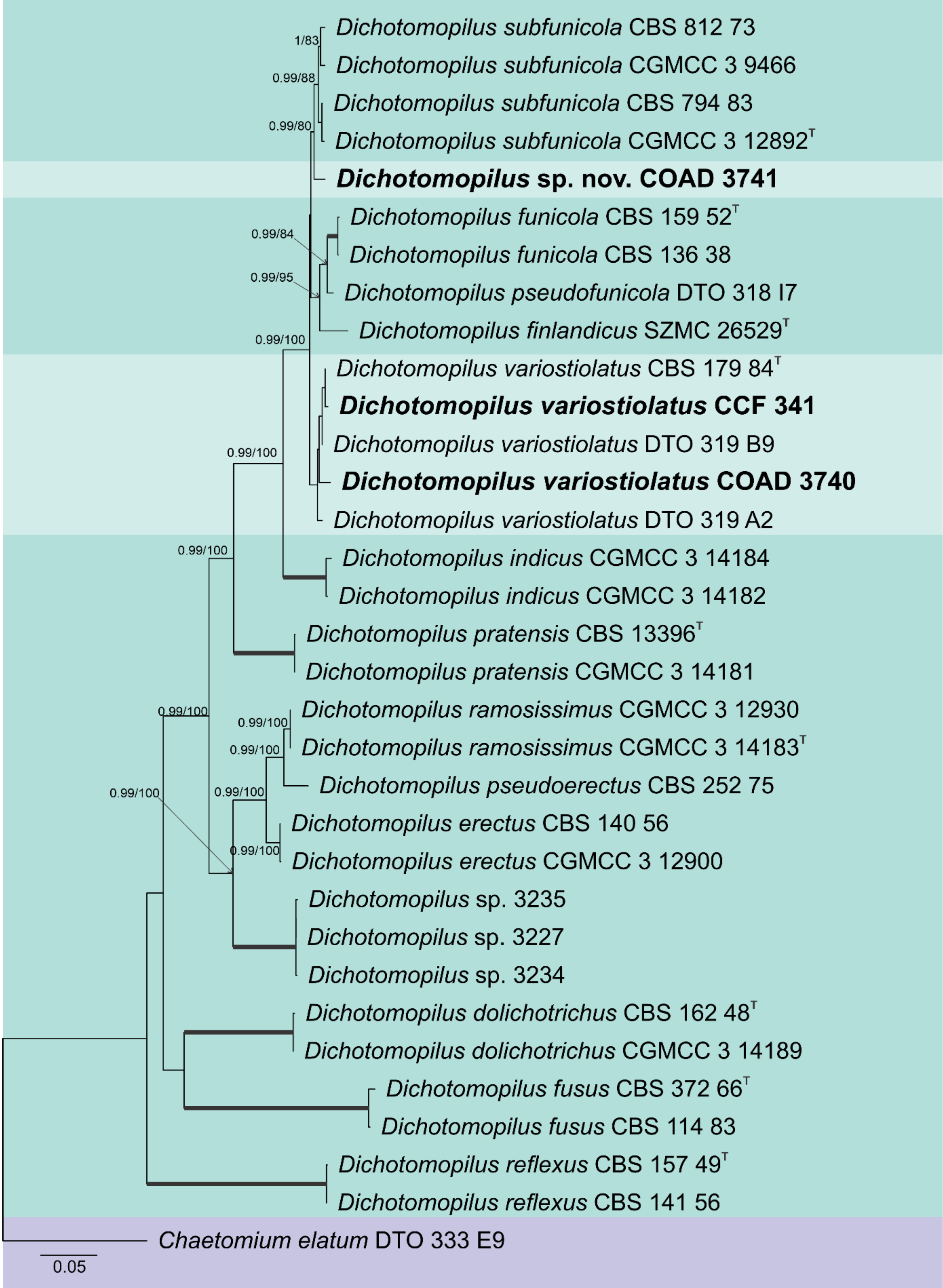


Fig. 4: Maximum-likelihood tree of *Dichotomopilus* based on a concatenated dataset of ITS, *rpb2*, and *tub2* sequences. The isolates obtained in this study are shown in bold. Ex-type isolates are indicated with ”**T**”. The branches that presented full statistical support (bs= 100% and pp = 1) are thickened. Only values above 70%, for bootstrap (bs), and above 0.95 for posterior probabilities (pp), are shown at branches. The tree is rooted with *Chaetomium elatum* DTO 333 E9.

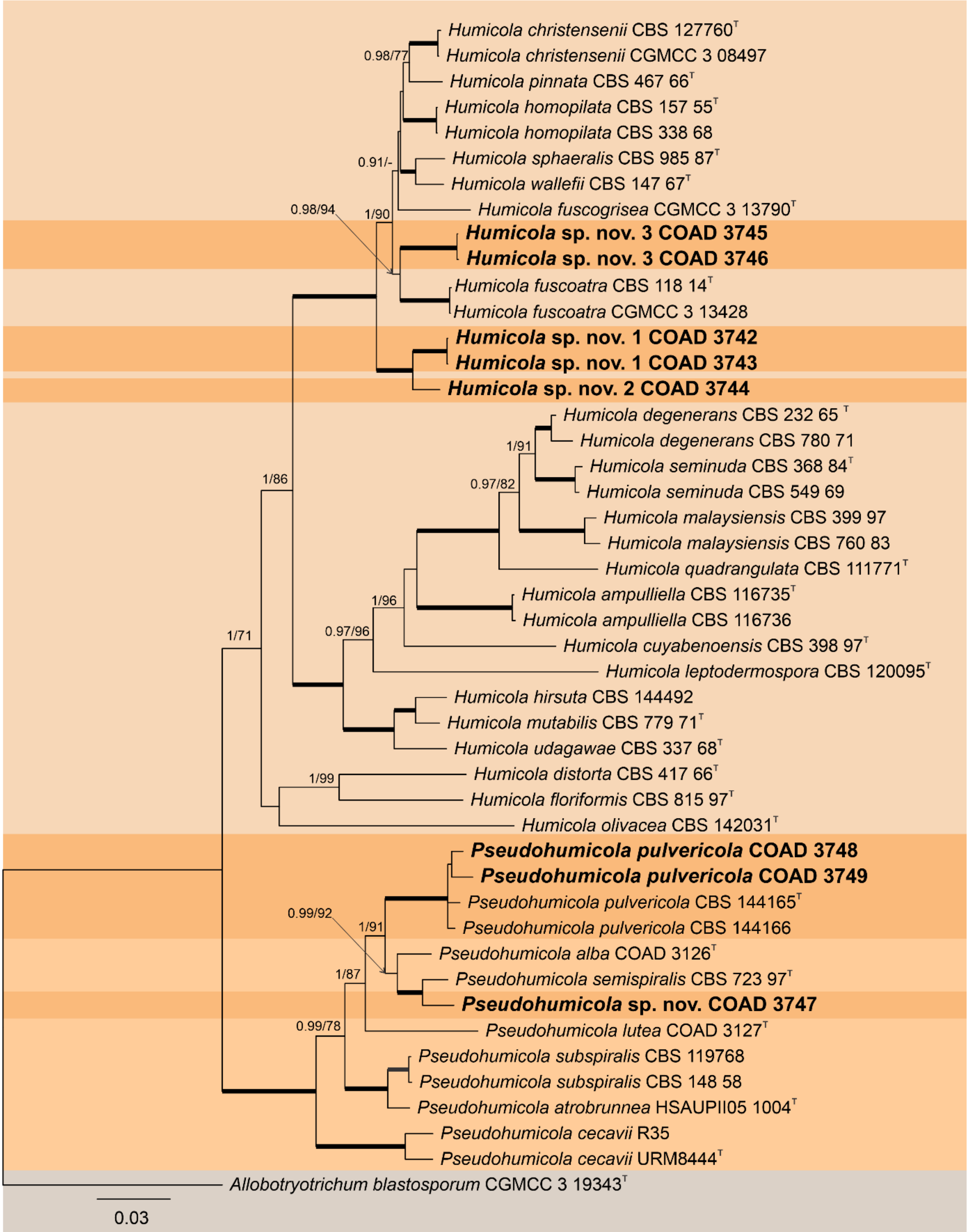


Fig. 5: Bayesian phylogenetic tree of *Pseudohumicola* and *Humicola* based on a concatenated dataset of ITS, LSU, *rpb2*, and *tub2* sequences. The isolates obtained in this study are shown in bold Ex-type isolates are indicated with ”$^{\mathbf{T}}$”. The branches that presented full statistical support (bs= 100% and pp = 1) are thickened. Only values above 70%, for bootstrap (bs), and above 0.95 for posterior probabilities (pp), are shown at branches. The tree is rooted with *Allobotryotrichum blastosporum* CGMCC 3.1943.

Taxonomy

Arcopilus X. Wei Wang & Samson, Stud. Mycol. 84:179. 2016.

Description and illustrations: Wang *et al.* (2016a).

***Arcopilus* sp. nov 1** Fig. 6.

Type: **Brazil**, Minas Gerais state, Araponga municipality, Pedra redonda, isolated from roots of *Cattleya locatellii*, Nov. 2021, collected by O.L. Pereira, isolated by D.O. Ramos (holotype VIC 49497 preserved as metabolically inactive culture, extype culture COAD 3723).

Cultures are sterile. *Arcopilus* sp.nov. 1 forms a unique lineage in the *Arcopilus* genus, forming a sister clade to *A. eremanthii*, *A. flavigenus* and *A. fusiformis*.

Culture characteristics: Colonies on OA attaining 34–37 mm diameter, edge entire, white aerial mycelium sparse, flat, circular, no ascomata production; reverse orange (7) to scarlet (5) due to the intense production of soluble pigment. Colonies on CMA attaining 48–50 mm diameter, entire margin, white aerial mycelium sparse, flat, circular, no ascomata production; reverse luteous (12) color at the center of colony due to the production of soluble pigment with the same color, and buff (45) at the margins of the colonies. Colonies on MEA attaining 46–48 mm diameter, edge entire, white aerial mycelium moderate at the center of colonies and sparse at the margins, flat, circular, no ascomata production; reverse red (2) to vinaceous (57) at the center of colony due to the production of soluble pigments, and buff at the margins. Colonies on PCA attaining 36–39 mm diameter, edge entire, white aerial mycelium moderate at the center of colonies and sparse at the margins, flat, circular, no ascomata production; reverse rosy buff (61) at the center of the colonies and buff at the margins; soluble pigments absent.

Notes: All attempts to induce sporulation in the four media cited above failed, even with the addition of sterile pine needles, sterile cellophane membrane, and filter paper (Wang *et al.*, 2022). In addition, *Arcopilus* sp. nov. 1 was grown in Synthetic Nutrient Agar (SNA) to induce sporulation since was observed that other species isolated in this medium produced ascomata, but this species didn’t produce any ascomata or conidia on SNA.

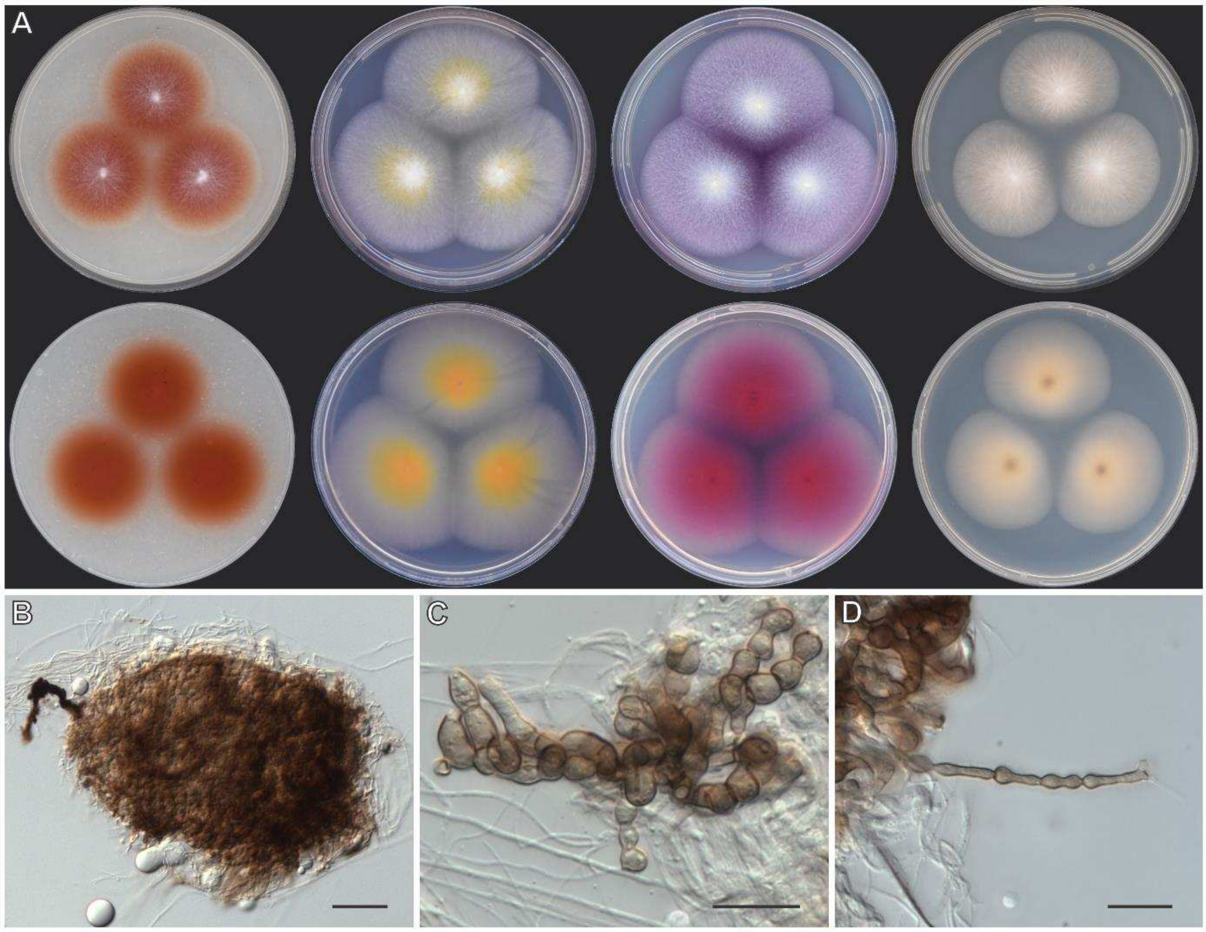


Fig. 6: *Arcopilus* sp. nov. 1 COAD 3723. **A.** Colonies from left to right (top row) OA, CMA, MEA and PCA; (bottom row) OA reverse, CMA reverse, MEA reverse and PCA reverse 7 days at 25 ºC in the dark. **B.** Tangled melanized hyphae. **C, D.** Thick walled hyphae. Scale bars: B = 50 µm, C, D = 20 µm.

***Arcopilus* sp. nov 2** Fig. 7.

Type: **Brazil**, Minas Gerais state, Conceição do Mato Dentro municipality, Curral de Pedra, isolated from roots of *Acianthera teres*, April 2022, collected by T.O. Condé, isolated by D.O. Ramos (holotype VIC 49506 preserved as metabolically inactive culture, extype culture COAD 3724).

Description: *Ascomata* superficial, ostiolate, saffron in reflected light due to ascomatal hairs, globose or subglobose, 118.4–209.3 µm high × 103.2–191.6 µm diam, homothallic. *Ascomatal wall* surface brown with textura angularis. *Terminal hairs* arcuate, apically coiled, brown, septate, verrucose, 3.1–5.6 µm wide near the base. *Lateral hairs* arcuate or inequilaterally undulated. *Asci* clavate, with eight biseriate ascospores, spore-bearing part 29.5–34.7 × 9.6–13.7 µm, evanescent. *Ascospores* dark brown, navicular in side view and fusiform in top view, smooth, (10.8–) 11.3–12.2 (–13.5) × (7.3–) 7.8–8.2 (–8.8) µm, with two germ pores. *Asexual morph* unknown.

Culture characteristics: Colonies on OA attaining 33–39 mm diameter, edge lobate, white aerial mycelium sparse to absent, flat, circular, no ascomata production; reverse buff; soluble pigment absent. Colonies on CMA attaining 33–36 mm diameter, entire margin, white aerial mycelium moderate, raised, circular, no ascomata production; reverse buff color; soluble pigments absent. Colonies on MEA attaining 33–35 mm diameter, edge entire, white aerial mycelium moderate, flat, circular, no ascomata production; reverse rosy buff; soluble pigments absent. Colonies on PCA attaining 38–40 mm diameter, edge entire, white aerial mycelium moderate at the center of colonies and sparse at the margins, raised, circular, ascomata production at the center of the colony; reverse buff; soluble pigments absent.

Other materials examined: **Brazil**, Minas Gerais state, Conceição do Mato Dentro municipality, Curral de Pedra, isolated from roots of *Acianthera teres*, April 2022, collected by T.O. Condé, isolated by D.O. Ramos (living cultures COAD 3725 and COAD 3726).

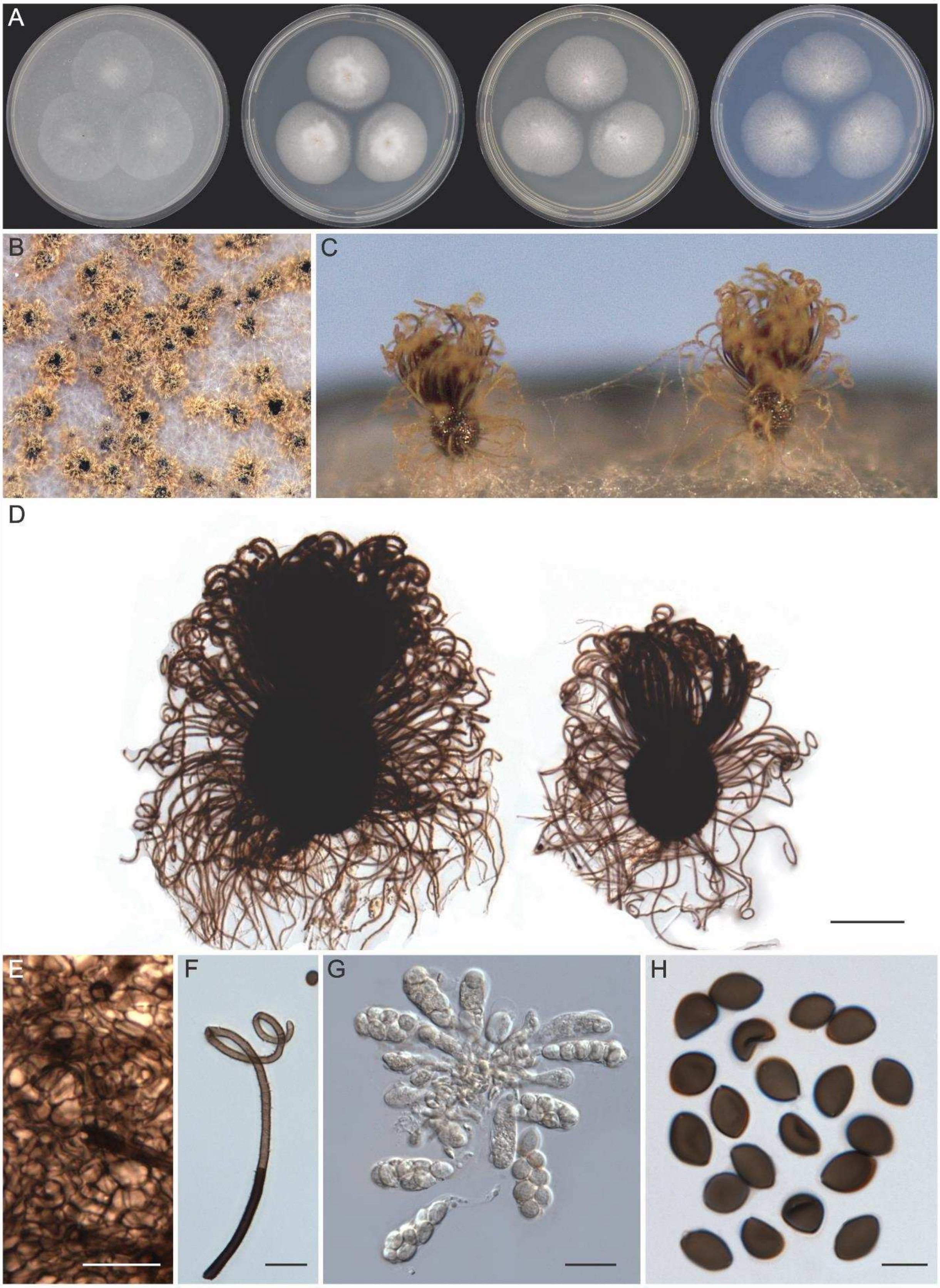


Fig. 7: *Arcopilus* sp. nov. 2 COAD 3724. **A.** Colonies on OA, CMA, MEA and PCA from left to right after 7 days at 25 ºC in the dark. **B.** Top view of ascomata on OA. **C.** Side view of mature ascomata. **D.** Ascomata. **E.** Surface of ascomatal wall **F.** Terminal ascomatal hair. **G.** Asci. **H.** Ascpospores. Scale bars: D = 100 µm; E–G = 20 µm; H = 10 µm.

***Arcopilus* sp. nov. 3** Fig. 8.

Type: **Brazil**, Minas Gerais state, Viçosa municipality, isolated from roots of *Habenaria petalodes*, April 2023, collected by O.L. Pereira, isolated by D.O. Ramos (holotype VIC 49498 preserved as metabolically inactive culture, extype culture COAD 3727).

Description: *Ascomata* superficial, ostiolate, greenish olivaceous in reflected light owing to ascomatal hairs, globose or subglobose, 110–147.6 µm high, 89–123 µm diam. *Ascomatal wall* brown, textura angularis. Terminal hairs arcuate, apically incurved, brown, septate. *Lateral hairs* recurved, sometimes straight, brown. Asci fasciculate, clavate, spore-bearing portion 24–35 × 10–15 µm, with 8 biseriate ascospores, evanescent. *Ascospores* dark brown, navicular in side view and fusiform in top view, smooth, bilaterally flattened, (12.5–) 13.5–14.1 (–15.3) × (6–) 6.8–7.2 (–8) µm, with one apical germ pores. Asexual morph unknown.

Culture characteristics: Colonies on OA attaining 40–43 mm diameter, edge entire, aerial mycelium absent, flat, circular shape, little ascomata production at the center of colony; reverse grey olivaceous (107) at the center, pale luteus (11) at the margins of the colony and with rosy vinaceous (58) soluble pigment. Colonies on CMA attaining 38–40 mm diameter, entire margin, white aerial mycelium moderate, raised, circular, no ascomata production, reverse olivaceous grey at the center of colonies and vinaceous at the edges owing to the presence of soluble pigment. Colonies on MEA attaining 40–42 mm diameter, edge entire, white aerial mycelium dense, flat, circular, no ascomata production; reverse dark brick (60) at the center and rosy buff at the margins of colonies. Colonies on PCA attaining 43–46 mm diameter, edge entire, white aerial mycelium moderate, raised, circular, little ascomata production under the mycelium at the centrer of colony; reverse greenish olivaceous; soluble pigments absent.

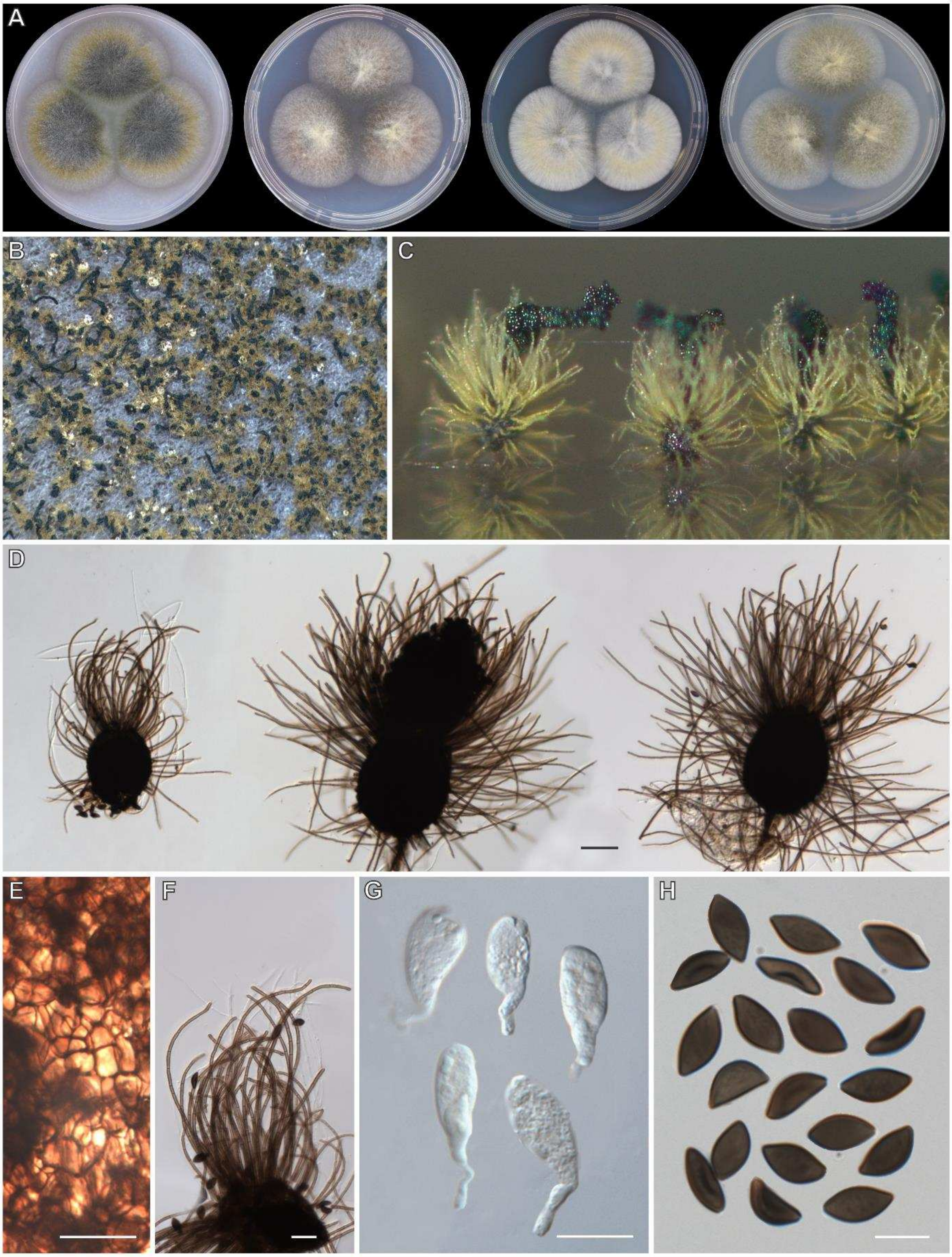


Fig. 8: *Arcopilus* sp. nov. 3 COAD 3727. **A.** Colonies on OA, CMA, MEA and PCA from left to right after 7 days at 25 ºC in the dark. **B.** Top view of ascomata on OA. **C.** Side view of mature ascomata. **D.** Ascomata. **E.** Surface of ascomatal wall **F.** Terminal ascomatal hair. Ascospores. **G.** Asci. **H.** Ascpospores. Scale bars: D = 50 µm; E–G = 20 µm; H = 10 µm.

***Arcopilus* sp. nov 4** Fig. 9.

Type: **Brazil**, Minas Gerais state, Viçosa municipality, isolated from roots of *Habenaria petalodes*, April 2023, collected by O.L. Pereira, isolated by D.O. Ramos (holotype VIC 49499 preserved as metabolically inactive culture, extype culture COAD 3728).

Cultures are sterile. Produces a melanized hyphae forming ring-shaped structure. In addition, were observed structures of tangled, melanized and campact mycelium similar to sclerotium.

Culture characteristics: Colonies on OA attaining 45–50 mm diameter, edge entire, aerial mycelium absent, flat, circular, no ascomata production; reverse olivaceous buff (89) with little soluble pigment. Colonies on CMA attaining 38–44 mm diameter, entire margin, white aerial mycelium sparse, flat, circular, no ascomata production; reverse rosy buff; soluble pigments absent. Colonies on MEA attaining 40–47 mm diameter, edge entire, white aerial mycelium moderate with exudates above, flat, circular, no ascomata production; reverse coral (38) to rosy buff at the center of colony due to the production of soluble pigments. Colonies on PCA attaining 34–42 mm diameter, edge entire or slightly undulate, white aerial mycelium moderate at the center of colonies and sparse at the edges, flat, circular, no ascomata production; reverse buff; soluble pigments absent.

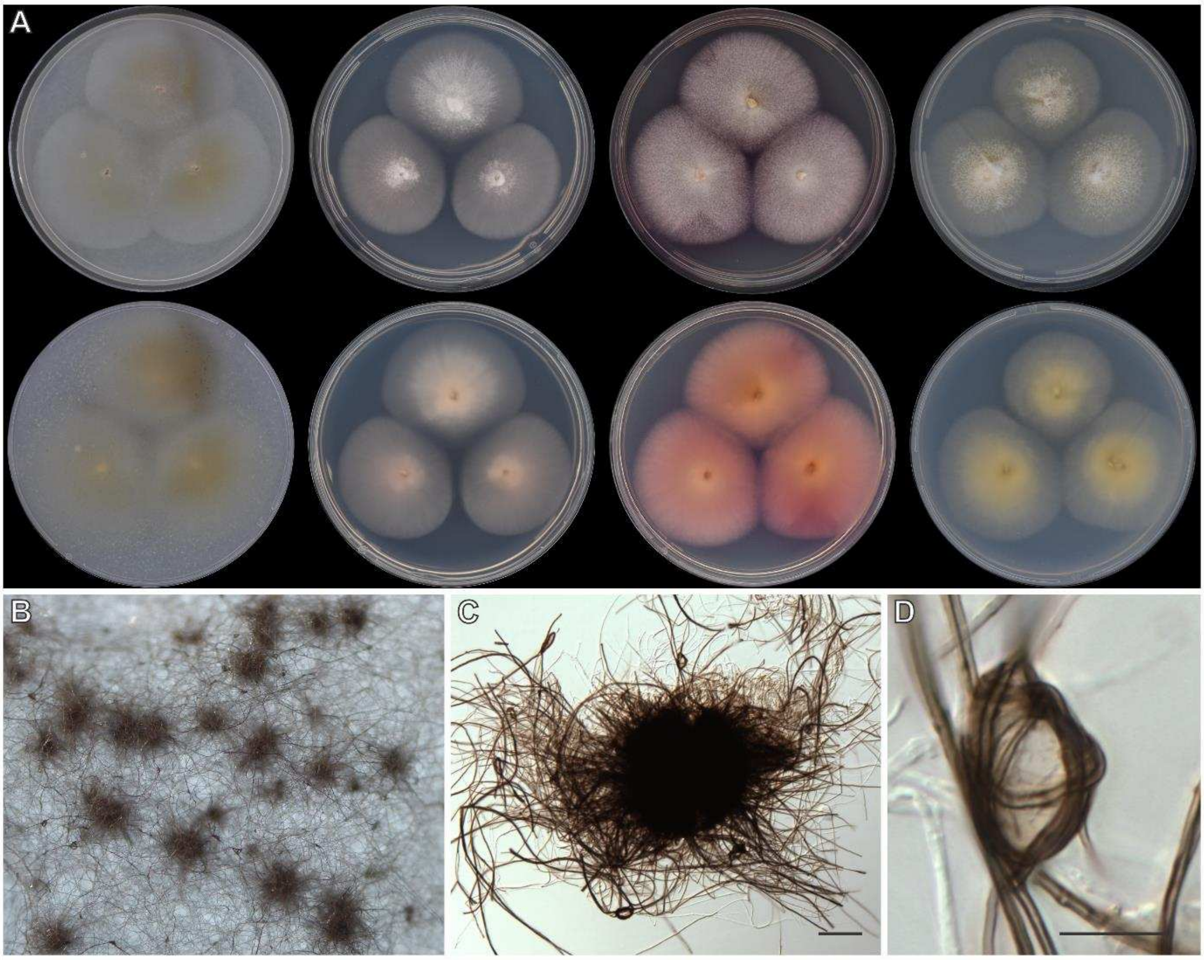


Fig. 9: *Arcopilus* sp. nov. 4 COAD 3728. **A.** Colonies from left to right (top row) OA, CMA, MEA and PCA; (bottom row) OA reverse, CMA reverse, MEA reverse and PCA reverse 7 days at 25 ºC in the dark. **B.** Top view tangled melanized hyphae on OA. **C, D.** Unique structures formed on OA. Scale bars: C = 100 µm, D = 20 µm.

Arcopilus amazonicus T.F. Sousa & G.F. Silva, Phytotaxa 456 (2): 145–156. 2020. Fig. 10.

Description: Souza *et al.* (2020)

Material eximined: **Brazil**, Minas Gerais state, Araponga municipality, isolated from roots of *Cattleya jongheana*, Nov. 2021, collected by O.L. Pereira, isolated by D.O. Ramos (living cultures COAD 3717).

Notes: *Arcopilus amazonicus* was described as endophyte on leaves of guarana plants (*Paullinia cupana*) in the Brazilian Amazon region (Souza *et al.* 2020). The species is phylogenetically close to *Arcopilus aureus*, being distinct only by the *rpb2* sequences. *Arcopilus amazonicus* differs morphologically from *A. aureus* by the ascus size, terminal hairs width and size of ascospores. To the best of our knowledge, this is the first report of this species as root endophyte of plants of the *Orchidaceae* family.

Other materials examined: **Brazil**, Minas Gerais state, Conceição do Mato Dentro municipality, Serra da Ferrugem, isolated from roots of *Cattleya* sp., April 2022, collected by T.O. Condé, isolated by D.O. Ramos (living cultures COAD 3710, COAD 3711, COAD 3712, COAD 3713, COAD 3714, COAD 3715, and COAD 3716).

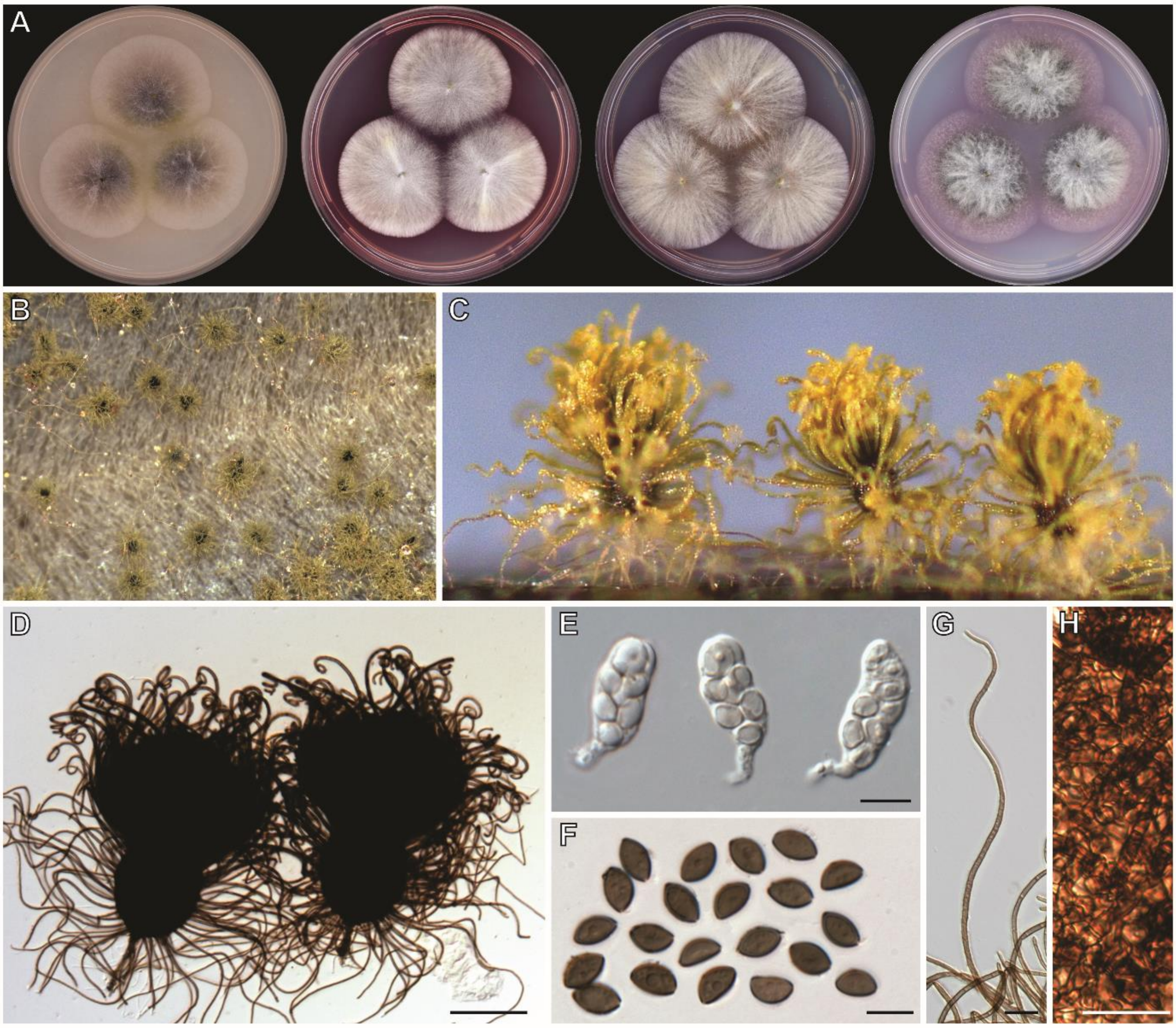


Fig. 10: *Arcopilus amazonicus* COAD 3717. **A.** Colonies on OA, CMA, MEA and PCA from left to right after 7 days at 25 ºC in the dark. **B.** Top view of ascomata on OA. **C.** Side view of mature ascomata. **D.** Ascomata. **E.** Asci. **F.** Ascospores. **G.** Terminal ascomatal hair. **H.** Surface of ascomatal wall. Scale bars: D = 100 µm; E, F = 10 µm; G, H = 20 µm.

Arcopilus aureus (Chivers) X. Wei Wang & Samson, Stud. Mycol. 84: 217. 2016. Fig. 11.

Description: Wang *et al.* (2016a)

Material eximined: **Brazil**, Minas Gerais state, Viçosa municipality, isolated from roots of *Habenaria petalodes*, April 2023, collected by O.L. Pereira, isolated by D.O. Ramos (living cultures COAD 3720 and COAD 3721).

Notes: *Arcopilus aureus* is the type species of the genus *Arcopilus* and was originally described as *Chaetomium aureum* until Wang *et al.* (2016a) established the genus *Arcopilus*. This species presents Ascomata superficial, ostiolate, subglobose with terminal hairs arcuate and brown ascospores. This is the first report of *A. aureus* as root endophyte of *Orchidaceae* plants.

Other materials examined: **Brazil**, Minas Gerais state, Conceição do Mato Dentro municipality, Serra da Ferrugem, isolated from roots of *Cattleya* sp., April 2022, collected by T.O. Condé, isolated by D.O. Ramos (living cultures COAD 3718 and COAD 3719).

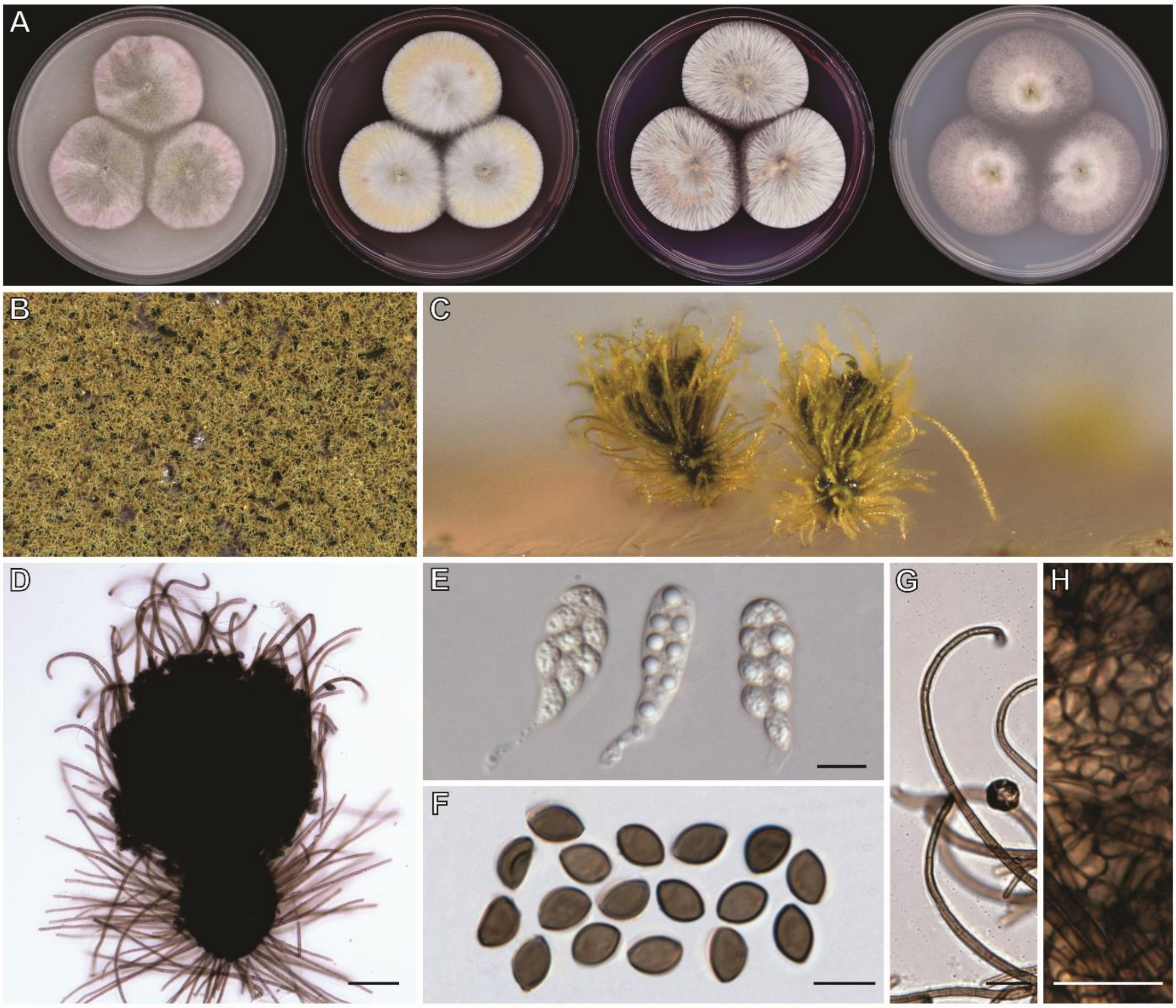


Fig. 11: *Arcopilus aureus* COAD 3721. **A.** Colonies on OA, CMA, MEA and PCA from left to right after 7 days at 25 ºC in the dark. **B.** Top view of ascomata on OA. **C.** Side view of mature ascomata. **D.** Ascomata. **E.** Asci. **F.** Ascospores. **G.** Terminal ascomatal hair. **H.** Surface of ascomatal wall. Scale bars: D = 50 µm; E, F = 10 µm; G, H = 20 µm.

Arcopilus cupreus (L.M. Ames) X. Wei Wang & Samson, Stud. Mycol. 84: 217. 2016. Fig. 12.

Description: Wang *et al.* (2016a)

Material eximined: **Brazil**, Minas Gerais state, Viçosa municipality, isolated from roots of *Cattleya* sp., September 2013, collected and isolated by O.L. Pereira (living cultures COAD 3722).

Notes: *Arcopilus cupreus* was described as *Chaetomium cupreum* but included in *Arcopilus* by Wang *et al.* (2016a). The species is characterized by ascomata superficial, globose to ovate, covered with bright red-colored hairs; asci clavate and ascospores olive-brow. This is the first report of *A. cupreus* as root endophyte of an *Orchidaceae* plant.

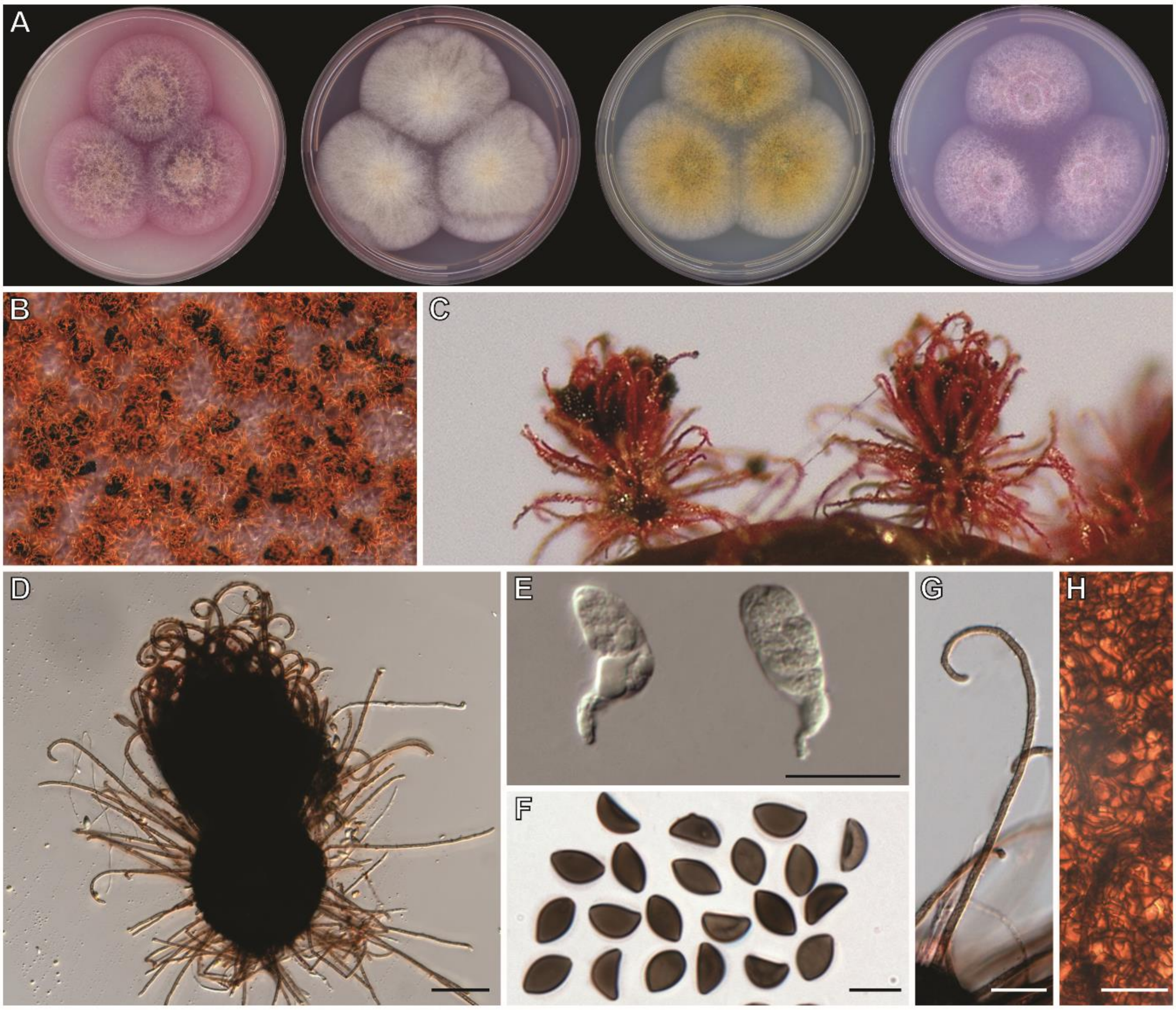


Fig. 12: *Arcopilus cupreus* COAD 3722. **A.** Colonies on OA, CMA, MEA and PCA from left to right after 7 days at 25 ºC in the dark. **B.** Top view of ascomata on OA. **C.** Side view of mature ascomata. **D.** Ascomata. **E.** Asci. **F.** Ascospores. **G.** Terminal ascomatal hair. **H.** Surface of ascomatal wall. Scale bars: D = 50 µm; F = 10 µm; E, G, H = 20 µm.

Chaetomium Kunze, Mykol. Hefte 1: 16. 1817.

Description and illustrations: Wang *et al.* (2016b).

Chaetomium coarctatum Sergeeva, Not. Syst. sect. Crypt. Inst. Bot. Acad. Sci. U.S.S.R. 14: 146. 1961. — Fig. 13.

Description: Wang *et al.* (2016b)

Material eximined: **Brazil**, Rio de Janeiro state, Nova Friburgo municipality, Córrego D'Antas, isolated from roots of *Cyclopogon congestus*, Jul. 2023, collected and isolated by D.O. Ramos (living cultures COAD 3729).

Notes: *Chaetomium coactatum* was first described on seeds of *Campanula medium*. After, was considered as a synonym of *Chaetomium globosum* but the phylogenetic analyses confirmed as a legitimate species (Wang *et al.*, 2016b). To the best of our knowledge, this is the first report of *C. coarctatum* in endophytic association with an *Orchidaceae* plant.

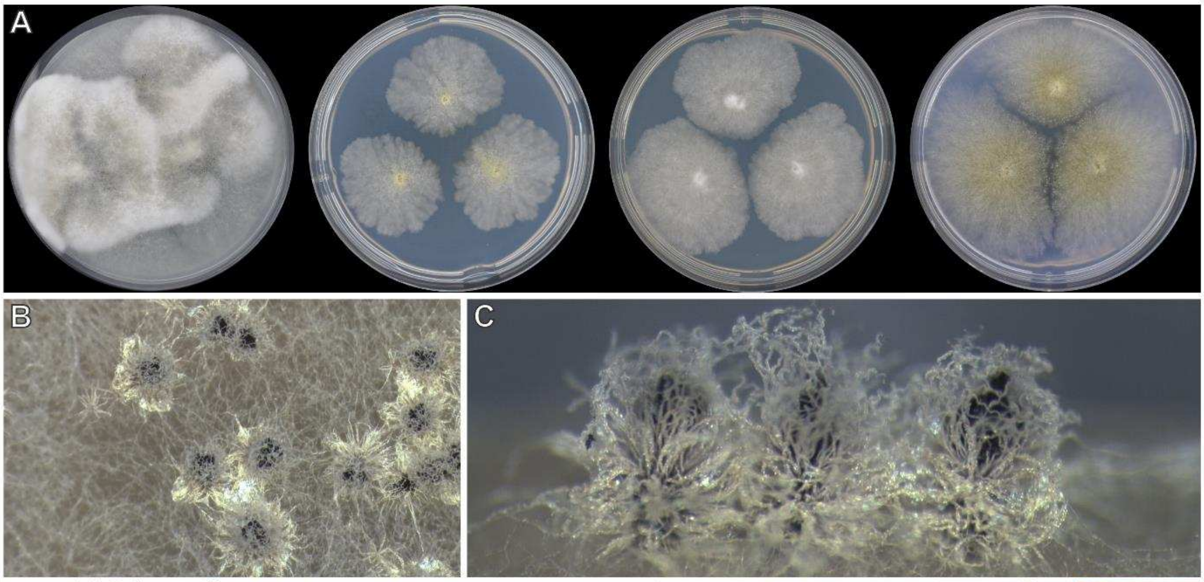


Fig. 13: *Chaetomium coarctatum* COAD 3729. **A.** Colonies on OA, CMA, MEA and PCA from left to right after 7 days at 25 ºC in the dark. **B.** Top view of ascomata on OA. **C, D.** Side view of mature ascomata. **E, F.** Ascomata. **G.** Ascospores. **H.** Terminal ascomatal hair. **I.** Surface of ascomatal wall. Scale bars: E, F = 100 µm; G = 10 µm; H, I = 20 µm.

Chaetomium cochliodes Palliser, N. Amer. Fl. 3: 61. 1910. — Fig. 14.

Description and illustration: Wang *et al.* (2016b)

Material eximined: **Brazil**, Rio de Janeiro state, Nova Friburgo municipality, Córrego D'Antas, isolated from roots of *Oeceoclades maculata*, Oct. 2022, collected and isolated by D.O. Ramos (living cultures COAD 3730 and COAD 3731).

Notes: *Chaetomium cochliodes* was introduced by Palliser (1910) but was treated as a synonym of *C. globosum* by Von Arx *et al.* (1986). *Chaetomium cochliodes* differs from other *Chaetomium* species by the distinctive coiled ascomatal hairs and was re-introduced by Wang *et al.* (2016b) based on the phylogenetic analyses of the genus.

Other materials examined: **Brazil**, Minas Gerais state, Araponga municipality, isolated from roots of *Zygopetalum mackayi*, Nov. 2021, collected and isolated by O.L. Pereira (living cultures CFF 634 and CFF 641).

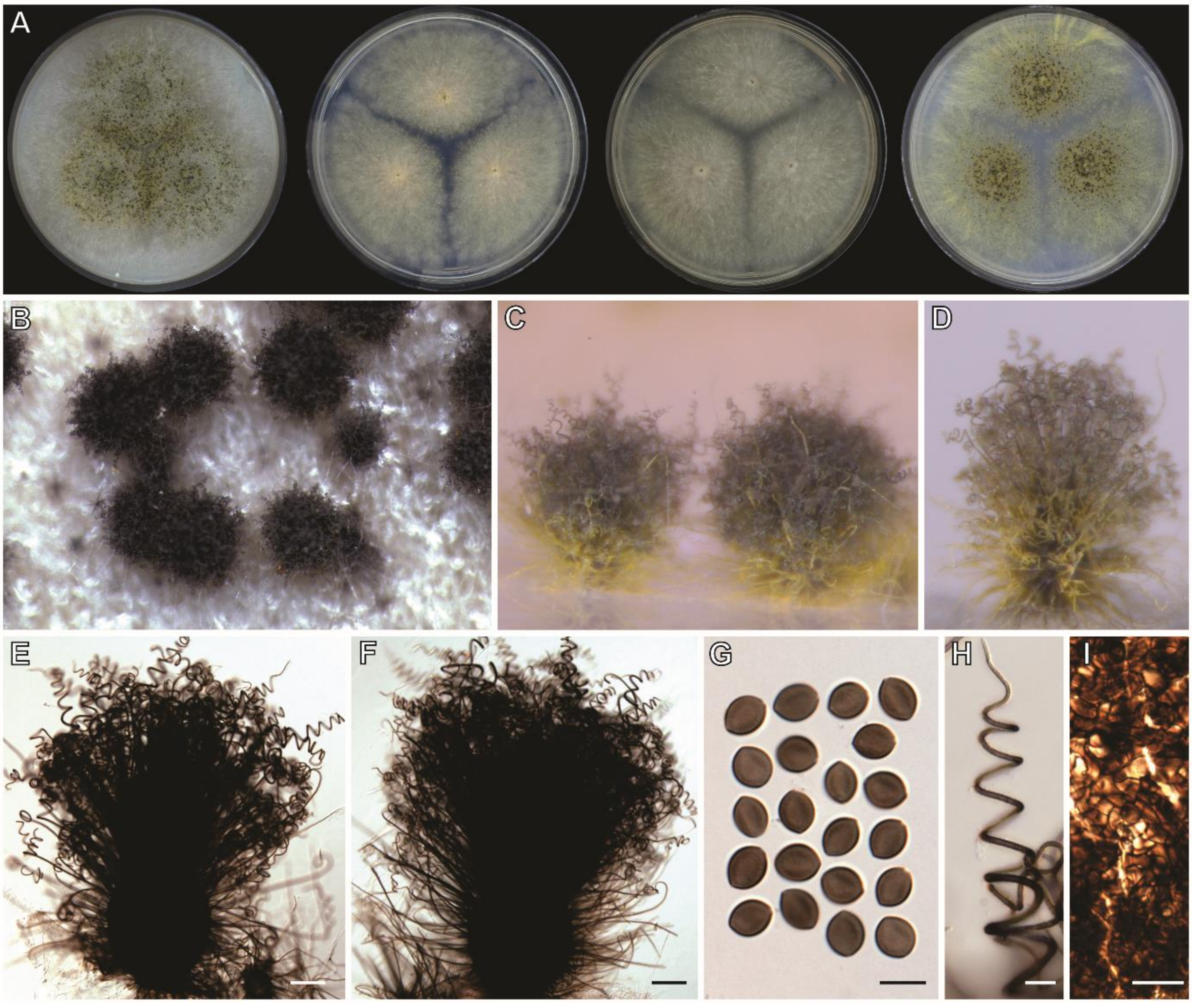


Fig. 14: *Chaetomium cochliodes* COAD 3731. **A.** Colonies on OA, CMA, MEA and PCA from left to right after 7 days at 25 ºC in the dark. **B.** Top view of ascomata on OA. **C, D.** Side view of mature ascomata. **E, F.** Ascomata. **G.** Ascospores. **H.** Terminal ascomatal hair. **I.** Surface of ascomatal wall. Scale bars: E, F = 100 µm; G = 10 µm; H, I = 20 µm.

Chaetomium globosum Kunze, Mykol. Hefte 1: 16. 1817. — Fig. 15.

Description: Wang *et al.* (2016b)

Material eximined: **Brazil**, Minas Gerais state, Viçosa municipality, Recanto das Cigarras-UFV, isolated from roots of *Oeceoclades maculata*, Sept. 2019, collected and isolated by D.O. Ramos (living cultures COAD 3732).

Notes: *Chaetomium globosum* is the type species of the genus and presented a diversity of morphological variations in exudate colours and ascomatal hairs among strains isolated from different substrates (Wang *et al.*, 2016b).

Other materials examined: **Brazil**, Minas Gerais state, Araponga municipality, isolated from roots of *Zygopetalum mackayi*, Nov. 2021, collected and isolated by O.L. Pereira (living cultures CCF 645). **Brazil**, Minas Gerais state, Viçosa municipality, Universidade Ferderal de Viçosa, isolated from roots of *Polystachia concreta*, May 2022, collected and isolated by D.O. Ramos (living cultures CCF 702).

Fig. 15: *Chaetomium globosum* COAD 3732. **A.** Colonies on OA, CMA, MEA and PCA from left to right after 7 days at 25 ºC in the dark. **B.** Top view of ascomata on OA. **C.** Side view of mature ascomata. **D.** Ascomata. **E.** Ascospores. **F.** Terminal ascomatal hair. **G.** Surface of ascomatal wall. Scale bars: D = 100 µm; E = 10 µm; F, G = 20 µm.

Chaetomium pseudocochliodes X. Wei Wang, X.Z. Liu & Crous, Persoonia 36: 113. 2016. Fig. 16.

Description: Wang *et al.* (2016b)

Material eximined: **Brazil**, Minas Gerais state, Viçosa municipality, Universidade Federal de Viçosa, isolated from roots of *Polystachia concreta*, May 2022, collected and isolated by D.O. Ramos (living cultures COAD 3733).

Notes: *Chaetomium pseudocochliodes* is phylogenetic related with *C. cochliodes* and differs from this species by the irregular ascomatal hairs and shape of ascospores with more protunding ends (Wang *et al.* 2016b).

Other materials examined: **Brazil**, Minas Gerais state, Araponga municipality, isolated from roots of *Gomesa recurva*, Aug. 2022, collected and isolated by D.O. Ramos (living cultures COAD 3734 and COAD 3735).

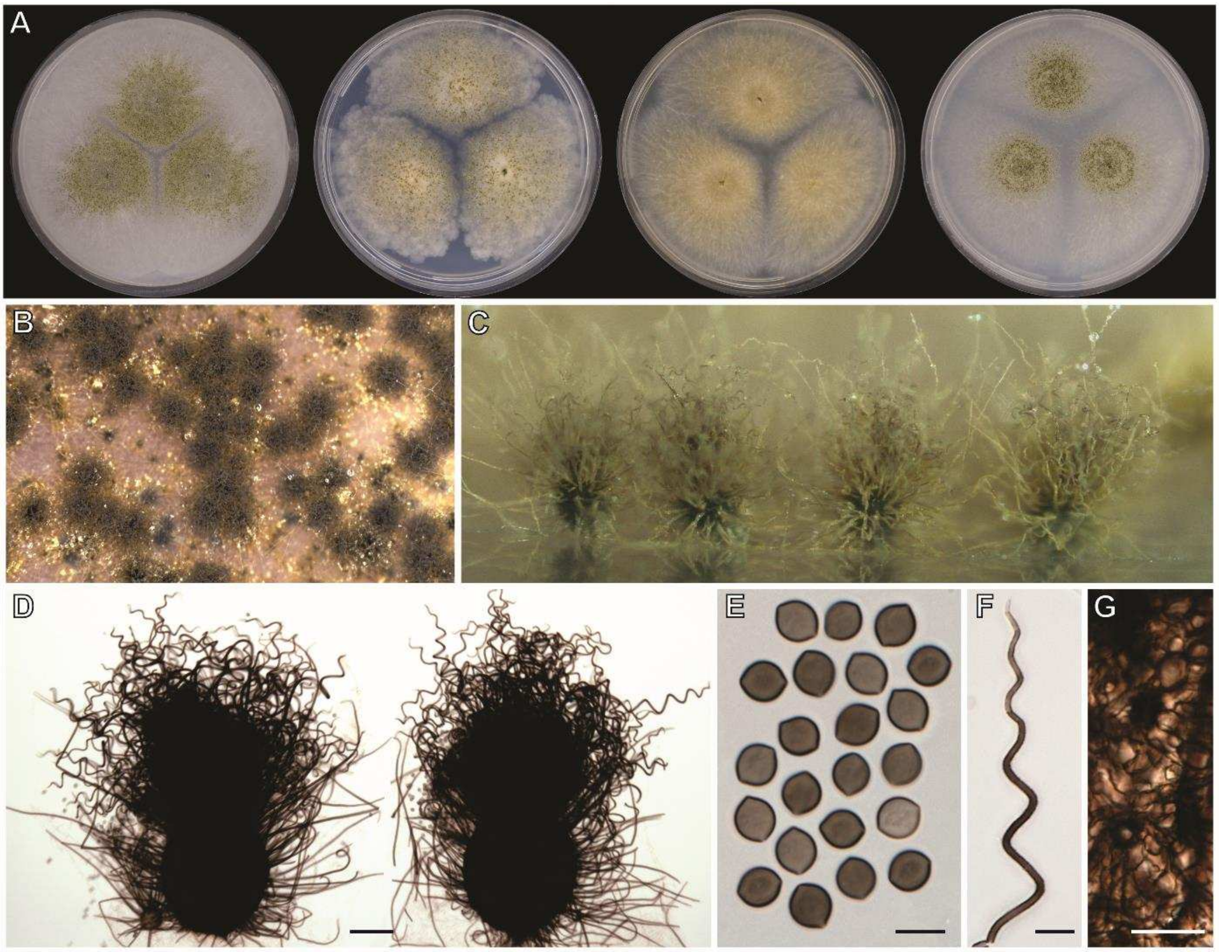


Fig. 16: *Chaetomium pseudocochliodes* COAD 3733. **A.** Colonies on OA, CMA, MEA and PCA from left to right after 7 days at 25 ºC in the dark. **B.** Top view of ascomata on OA. **C.** Side view of mature ascomata. **D.** Ascomata. **E.** Ascospores. **F.** Terminal ascomatal hair. **G.** Surface of ascomatal wall. Scale bars: D = 100 µm; E = 10 µm; F, G = 20 µm.

Chaetomium tenue Chaetomium tenue X. Wei Wang, Crous & L. Lombard, Persoonia 36: 125. 2016. Fig. 17.

Description: Wang *et al.* (2016b)

Material eximined: **Brazil**, Minas Gerais state, Viçosa municipality, Recanto das Cigarras-UFV, isolated from roots of *Oeceoclades maculata*, Sept. 2019, collected and isolated by D.O. Ramos (living cultures COAD 3736).

Notes: *Chaetomium tenue* was introduced by Wang *et al.* (2016b) and can be distinguished by its less dense and undulate ascomatal hairs, besides the elongate limoniform ascospores, when compared with *C. pseudoglobosum*, wich is phylogenetically related to *C. tenue* (Wang *et al.* 206b).

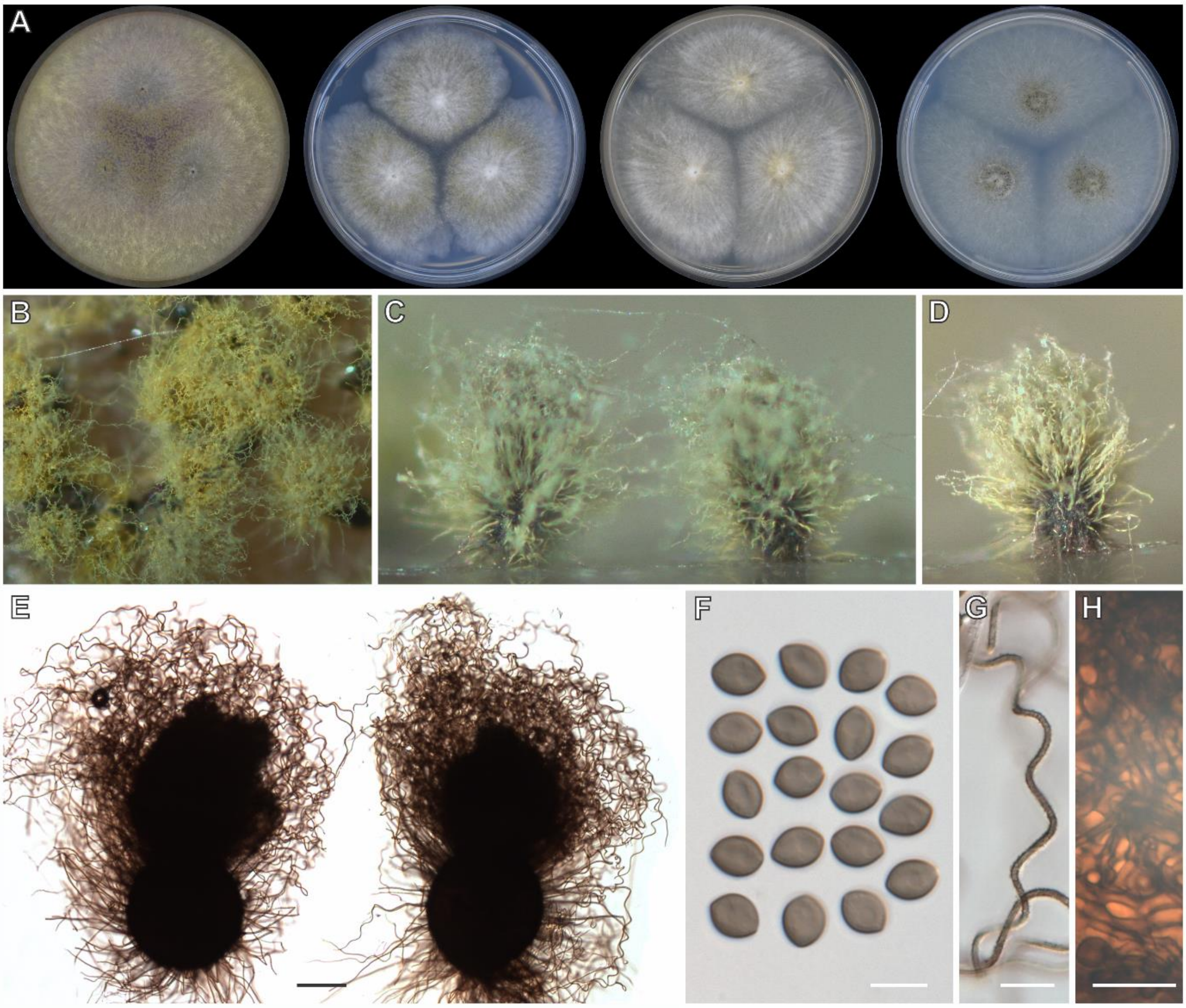


Fig. 17: *Chaetomium tenue* COAD 3736. **A.** Colonies on OA, CMA, MEA and PCA from left to right after 7 days at 25 ºC in the dark. **B.** Top view of ascomata on OA. **C, D.** Side view of mature ascomata. **E.** Ascomata under a light microscope. **F.** Ascospores. **G.** Terminal ascomatal hair. **H.** Surface of ascomatal wall. Scale bars: E = 100 µm; F = 10 µm; G, H = 20 µm.

Collariella X. Wei Wang & Samson, Stud. Mycol. 84:179. 2016.

Description and illustrations: Wang *et al.* (2016a).

***Collariella* sp. nov.** Fig. 18.

Type: **Brazil**, Rio de Janeiro state, Nova Friburgo municipality, isolated as root endophyte on *Cyclopogon congestus*, Jul. 2023, collected and isolated by D.O. Ramos (holotype VIC 49500 preserved as metabolically inactive culture, extype culture COAD 3737).

Description: *Ascomata* superficial, pale olivaceous grey owing to the ascomatal hairs in reflected light, ostiolate, 393–552 µm high, 205–252 µm diam. *Ascomatal wall* brown, textura angularis in surface view. *Terminal hairs* arising from the apical collar, conspicuously rough, dark brown, septate, erect in the lower part, spirally coiled in the upper part. *Lateral hairs* seta-like, tapering and fading towards the tips. *Asci* fasciculate, fusiform or clavate, sporebearing part 15–25 × 6–11 µm, with 8 irregularly-arranged ascospores, evanescent. *Ascospores* pale brown when mature, limoniform, bilaterally flattened, (5.7–) 6–6.4 (–6.6) × (4.4–) 5.3–5.6 (–6) µm, with one apical germ pore. Asexual morph unknown.

Culture characteristics: Colonies on OA attaining 32–36 mm diameter, edge entire, aerial mycelium absent, flat, circular shape, moderate ascomata production all over the colony; reverse luteus (12) due to the intense production of soluble pigment. Colonies on CMA attaining 23–27 mm diameter, entire margin, aerial mycelium absent, flat, circular, intense ascomata production at the center of the colony; reverse pale luteus at the center of colonies and buff at the edges. Colonies on MEA attaining 26–30 mm diameter, edge entire, aerial mycelium absent, flat, irregular shape, no ascomata production; reverse pale luteus. Colonies on PCA attaining 30–32 mm diameter, edge entire, aerial mycelium absent, flat, circular, intense ascomata production at the center of colony and little at the edges; reverse buff; soluble pigments absent.

Other materials examined: **Brazil**, Minas Gerais state, Viçosa municipality, Mata do Paraíso, isolated as root endophyte on *Oeceoclades maculata*, Aug. 2023, collected and isolated by D.O. Ramos (living cultures COAD 3738 and COAD 3739).

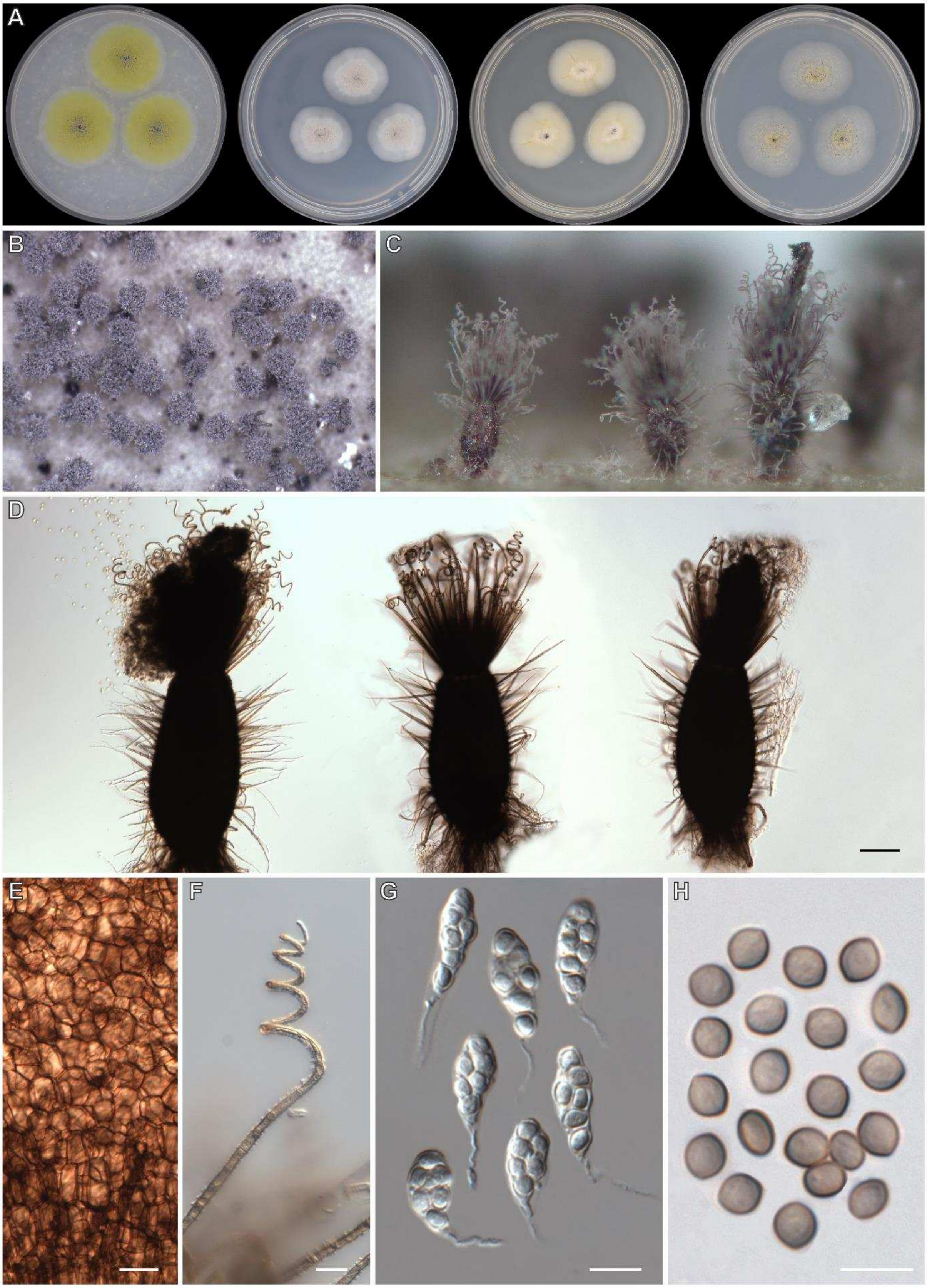


Fig. 18: *Collariella* sp. nov. COAD 3737. **A.** Colonies on OA, CMA, MEA and PCA from left to right after 7 days at 25 ºC in the dark. **B.** Top view of ascomata on OA. **C.** Side view of mature ascomata. **D.** Ascomata under a light microscope. **E.** Surface of ascomatal wall. **F.** Terminal ascomatal hair. **G.** Asci. **H.** Ascospores. Scale bars: D = 100 µm; E, F = 20 µm; G, H = 10 µm.

Dichotomopilus X. Wei Wang & Samson, Stud. Mycol. 84:179. 2016
Description and illustrations: Wang *et al.* (2016a).

Dichotomopilus **sp. nov.** Fig. 19.

Type: **Brazil**, Minas Gerais state, Araponga municipality, isolated from roots of *Zygopetalum mackayi*, Nov. 2021, collected and isolated by O.L. Pereira (holotype VIC 49501 preserved as metabolically inactive culture, extype culture COAD 3741).

Description: *Ascomata* superficial, ostiolate, dark gray in reflected light, subglobose to ovate, 160–219.7 µm high × 130.8–201 µm diam. *Ascomatal wall* brown, textura intricata or angularis. *Terminal hairs* dark brown, septate, unbranched or dichotomously branched 1–3 times at the top. *Lateral hairs* seta-like, tapering and fading towards tips. *Asci* fasciculate, clavate, with 8 ascospores irregularly arranged, spore-bearing portion 13–19 × 7–12 µm, evanescent. *Ascospores* brown, ovate to slightly elongate ovate, bilaterally flattened, (4.8–) 5–5.2 (–5.6) × (4–) 4.3–4.6 (–5.5) µm, with an apical germ. Asexual morph unknown.

Culture characteristics: Colonies on OA attaining 35–37 mm diameter, edge entire to undulate, white aerial mycelium sparse, flat, circular, intense ascomata production all over the colony; reverse pale luteus owing to the presence of soluble pigment. Colonies on CMA attaining 47–60 mm diameter, edge entire to undulate, white aerial mycelium dense, flat, circular, ascomata production at the center of colony; reverse buff; soluble pigment absent. Colonies on MEA reaching over 75 mm diameter, edge entire, white aerial mycelium intense, flat, circular, no ascomata production; reverse pale luteus due to the presence of soluble pigments. Colonies on PCA attaining 59–75 mm diameter, edge entire, white aerial mycelium sparse, flat, circular, intense ascomata production; reverse buff; soluble pigment absent.

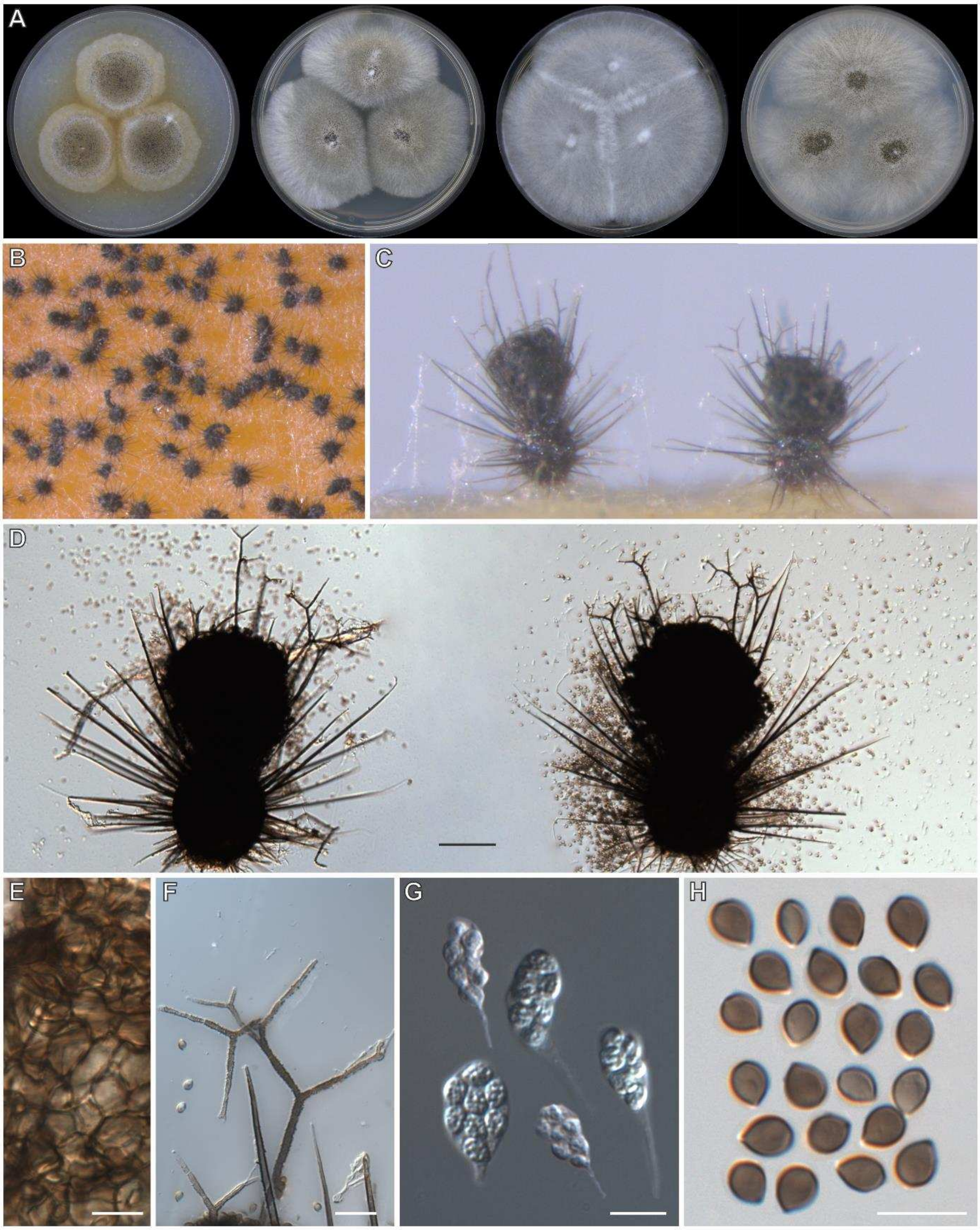


Fig. 19: *Dichotomopilus* sp. nov. COAD 3741. **A.** Colonies on OA, CMA, MEA and PCA from left to right after 7 days at 25 ºC in the dark. **B.** Top view of ascomata on OA. **C, D.** Side view of mature ascomata. **E, F.** Ascomata under a light microscope. **G.** Ascospores. **H.** Terminal ascomatal hair. **I.** Surface of ascomatal wall. Scale bars: D = 100 µm; E, G, H = 10 µm; F = 20 µm.

Dichotomopilus variostiolatus (A. Carter) X. Wei Wang & Samson, Studies in Mycology 84: 203. 2016. Fig. 20.

Description: Wang *et al.* (2016a)

Material eximined: **Brazil**, Goiás state, Goiânia municipality, isolated as root endophyte on *Cattleya nobilior*, Jan. 2023, collected and isolated by P.T.S. Nogueira (living cultures COAD 3740).

Notes: Fist introduced as *Chaetomium variostiolatum* by Carter (1983) and combined to *Dichotomopilus variostiolatus* by Wang *et al.* (2016a) when the genus was established. This species can produce terminal hairs unbranched seta-like or dichotomously branched terminal hairs. Both isolates of this study produced the two types of terminal hairs, similar to that was observed by Wang *et al.* (2016a).

Other materials examined: **Brazil**, Minas Gerais state, Viçosa municipality, isolated from roots of *Cattleya* sp., 2012, collected and isolated by O.L. Pereira (living cultures CCF 341).

Fig. 20: *Dichotomopilus variostiolatus* COAD 3740. **A.** Colonies on OA, CMA, MEA and PCA from left to right after 7 days at 25 ºC in the dark. **B.** Top view of ascomata on OA. **C, D.** Side view of mature ascomata. **E.** Ascomata under a light microscope. **F.** Asci. **G.** Ascospores. **H.** Terminal ascomatal hair. **I.** Surface of ascomatal wall. Scale bars: E = 100 µm; F, G = 10 µm; H, I = 20 µm.

Humicola Traaen, Nytt Mag. Naturvidensk. 52: 31. 1914.

Description and illustration: Wang *et al.* (2019)

***Humicola* sp. nov. 1** Fig. 21.

Type: **Brazil**, Minas Gerais state, Viçosa municipality, Mata do Paraíso, isolated as root endophyte on *Catasetum hookeri*, Jun. 2023, collected and isolated by D.O. Ramos (holotype VIC 49502 preserved as metabolically inactive culture, extype culture COAD 3743).

Description: *Ascomata* superficial, ostiolate, hairs in reflected light, elongate obpyriform, obclavate or ampulliform below, apically attenuated to an elongate conical or short cylindrical neck, 135.6–290.2 µm high, 63–159 µm diam at the widest part. *Ascomatal wall* brown, composed of textura angularis. *Terminal hairs* arising from the extension of the adjacent ostiolar cells, seta-like and delicate, smooth, flexuous at the top. *Lateral hairs* are similar to terminal ones, tapering and fading towards the tips. *Asci* clavate to cylindrical, with spore-bearing part 17.8–26.8 × 6–9.7 µm, with 8 biseriate ascospores, evanescent. *Ascospores* brown, limoniform, umbonate at both ends, bilaterally flattened, (6.3–) 7.3–7.9 (–8.9) × 6.3–6.7(–7.8) µm, with one apical germ pore.

Culture characteristics: Colonies on OA attaining 25–28 mm diameter, edge entire, aerial mycelium absent, flat, circular, intense production of ascomata, conidia production was not observed; reverse pale vinaceous (85) owing to the presence of pigments. Colonies on CMA attaining 25–28 mm diameter, edge entire to fimbriate, white aerial mycelium sparse with little exudate at the center of the colony, flat, circular shape, moderate ascomata production all over the colony; reverse buff; soluble pigment absent. Colonies on MEA attaining 27–30 mm diameter, entire margin, white aerial mycelium thick, texture floccose, flat, circular, ascomata and conidia were not observed; reverse buff; soluble pigments absent. Colonies on PCA attaining 25–28 mm diameter, edge entire, aerial mycelium absent, flat, circular, moderate ascomata production concentrated in the center of the colony, conidia were not observed; reverse buff; soluble pigments absent.

Other materials examined: **Brazil**, Minas Gerais state, Viçosa municipality, isolated as root endophyte of *Catasetum hookeri*, Jun. 2023, collected and isolated by D.O. Ramos (living culture COAD 3742).

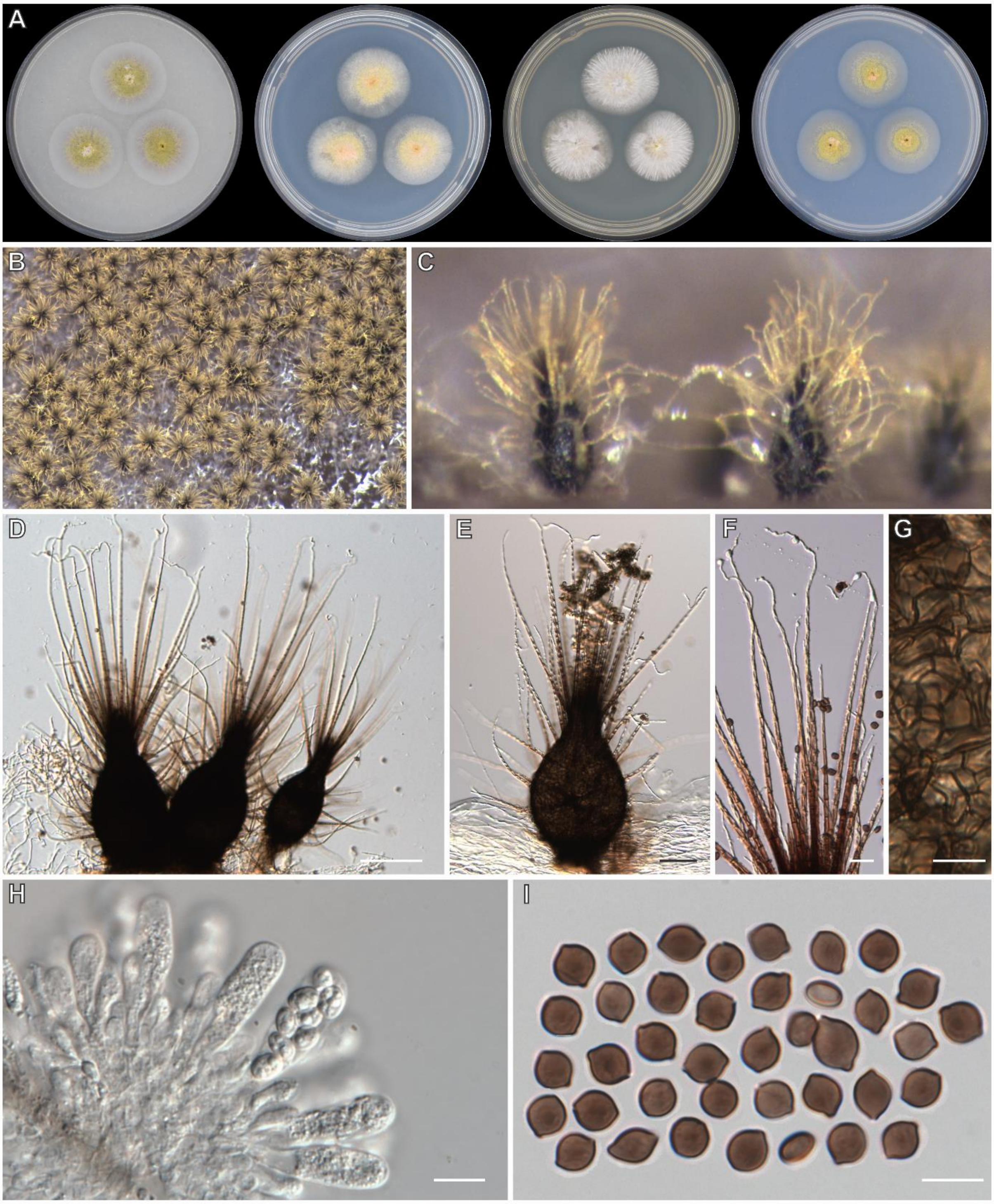


Fig. 21: *Humicola* sp. nov. 1 COAD 3743. **A.** Colonies on OA, CMA, MEA and PCA from left to right after 7 days at 25 ºC in the dark. **B.** Top view of ascomata on OA. **C.** Side view of mature ascomata. **D, E.** Ascomata. **F.** Terminal ascomatal hair Ascospores. **G.** Surface of ascomatal wall. **H.** Asci. **I.** Ascospores. Scale bars: D = 100 µm; E = 50 µm; F = 20 µm; G–I = 10 µm.

***Humicola* sp. nov. 2** Fig. 22.

Type: **Brazil**, Distrito Federal, Brasília municipality, isolated as root endophyte on *Oeceoclades maculata*, Aug. 2023, collected and isolated by D.O. Ramos (holotype VIC 49507 preserved as metabolically inactive culture, extype culture COAD 3744).

Description: *Ascomata* superficial, ostiolate, hairs in reflected light, elongate obpyriform, obclavate or ampulliform below, apically attenuated to an elongate conical or short cylindrical neck, 272.7–339.6 µm high, 131.3–173 µm diam at the widest part. *Ascomatal wall* brown, composed of textura angularis. *Terminal hairs* arising from the extension of the adjacent ostiolar cells, seta-like and delicate, smooth, flexuous at the top. *Lateral hairs* are similar to terminal ones, tapering and fading towards the tips. *Asci* clavate to cylindrical, with spore-bearing part 18.3–26 × 6.1–10.2 µm, with 8 biseriate ascospores, evanescent. *Ascospores* brown, limoniform, umbonate at both ends, bilaterally flattened, (7.3–) 7.7–8.3(–9.7) × (5.6–) 6.1–6.5 (–7.2) µm, with one apical germ pore.

Somatic hyphae hyaline. *Aleuroconidia-like* conidia produced laterally on hyphae on branches, solitary or in clusters of two or more, subglobose or occasionally pyriform, single-celled, with a pale brown, thick wall when mature, (7–) 7.3–7.7 (–7.9) × (6.6–) 7.1–7.7 (–8.6) µm.

Culture characteristics (7 Days at 25ºC in the dark): Colonies on OA attaining 32–34 mm diameter, edge entire, aerial mycelium absent, flat, circular, intense production of ascomata at the center of the colony, asexual morph was not observed; reverse pale vinaceous due to pigment production. Colonies on CMA attaining 28–32 mm diameter, entire margin, white aerial mycelium sparse, flat, irregular shape, moderate ascomata production all over the colony; reverse buff; soluble pigment absent. Colonies on MEA attaining 33–37 mm diameter, edge entire, white aerial mycelium with hypha tuffs, flat, circular, sexual and asexual morphs were not observed; reverse rosy buff; soluble pigments absent. Colonies on PCA attaining 30–33 mm diameter, edge entire, white aerial mycelium with mycelial strands, flat, circular, moderate ascomata production, conidia were not observed; reverse buff; soluble pigments absent.

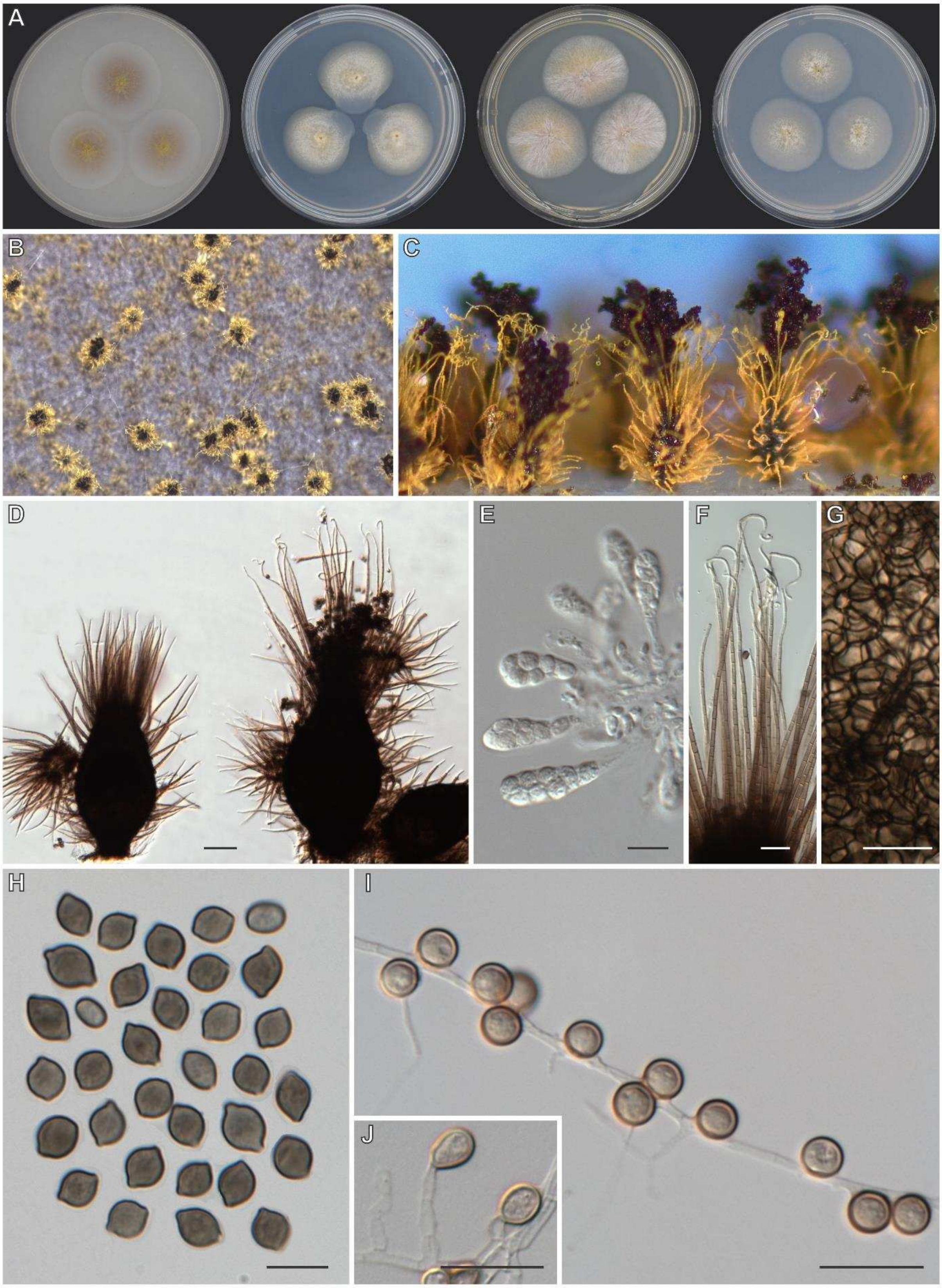


Fig. 22: *Humicola* sp. nov. 2 COAD 3744. **A.** Colonies on OA, CMA, MEA and PCA from left to right after 7 days at 25 ºC in the dark. **B.** Top view of ascomata on OA. **C.** Side view of mature ascomata. **D.** Ascomata. **E.** Asci. **F.** Terminal ascomatal hair Ascospores. **G.** Surface of ascomatal wall. **H.** Ascospores. **I, J.** Aleuroconidia-like and hyphae. Scale bars: D = 50 µm; E, H = 10 µm; F, G, I, J = 20 µm.

***Humicola* sp. nov. 3** Fig. 23.

Type: **Brazil**, Minas gerais state, Viçosa municipality, Mata do Paraíso, isolated as root endophyte on *Oeceoclades maculata*, Jun. 2023, collected and isolated by D.O. Ramos (holotype VIC 49503 preserved as metabolically inactive culture, extype culture COAD 3745).

Description: *Ascomata* superficial, ostiolate, hairs in reflected light, elongate obpyriform, obclavate or ampulliform below, apically attenuated to an elongate conical or short cylindrical neck, 208–280 μm high, 90.3–136.2 μm diam at the widest part. *Ascomatal wall* brown, composed of textura angularis. *Terminal hairs* arising from the extension of the adjacent ostiolar cells, seta-like and delicate, smooth, flexuous at the top. *Lateral hairs* are similar to terminal ones, tapering and fading towards the tips. *Asci* clavate to cylindrical, with spore-bearing part 21.6–28.8 × 7.5–12 μm, with 8 biseriate ascospores, evanescent. *Ascospores* brown, limoniform, umbonate at both ends, bilaterally flattened, (7–) 7.7–8.3 (–9.4) × (5.4–) 6.3–6.7 (–7.4) μm, with one apical germ pore.

Somatic hyphae hyaline. *Aleuroconidia-like* conidia produced laterally on hyphae, solitary or in clusters of two, subglobose to ovate, single-celled, with a pale brown, thick wall when mature, (8–) 8.6–9.6 (–11) × (7–) 7.5–8.7(–9.5) μm.

Culture characteristics (7 Days at 25ºC in the dark): Colonies on OA attaining 28–30 mm diameter, edge entire, aerial mycelium absent, flat, circular, moderate production of ascomata at the center of the colony, conidia production was not observed; reverse dark vinaceous (82) owing to soluble pigment. Colonies on CMA attaining 30–33 mm diameter, entire margin, white aerial mycelium sparse, flat, circular, moderate ascomata production at the center of the colony; reverse buff; soluble pigment absent. Colonies on MEA attaining 30–34 mm diameter, edge entire, white aerial mycelium sparse, flat, circular, ascomata and conidia were not observed; reverse dark vinaceous at the center and pale vinaceous at the margins of colonies due to the presence of pigments. Colonies on PCA attaining 28–32 mm diameter, edge entire, white aerial mycelium sparse at the center of the colony and absent at the margins, flat, circular, little ascomata production, aleuroconidia-like at the center of the colony; reverse buff; soluble pigments absent.

Other materials examined: **Brazil**, Minas Gerais state, Viçosa municipality, isolated as root endophyte of *Oeceoclades maculata*, Jul. 2023, collected and isolated by D.O. Ramos (living culture COAD 3746).

Fig. 23: *Humicola* sp. nov. 3 COAD 3745. **A.** Colonies on OA, CMA, MEA and PCA from left to right after 7 days at 25 ºC in the dark. **B.** Top view of ascomata on OA. **C.** Side view of mature ascomata. **D.** Ascomata. **E.** Asci. **F.** Terminal ascomatal hair. **G.** Surface of ascomatal wall. **H.** Ascospores. **J.** Aleuroconidia-like and hyphae. Scale bars: D = 100 µm; E, H, K, L = 10 µm; F, G, H = 20 µm.

Pseudohumicola X. Wei Wang P.J. Han, F.Y. Bai Houbraken, Stud. Mycol. 101: 96. 2022.

Description and illustration: Wang *et al.* (2022)

***Pseudohumicola* sp. nov.** Fig. 24.

Type: **Brazil**, Minas Gerais state, Viçosa municipality, isolated from coconut shell used as substrate of *Cattleya aclandiae*, Jun. 2022, collected and isolated by P.T.S Nogueira (holotype VIC 49504 preserved as metabolically inactive culture, extype culture COAD 3747).

Description: *Somatic hyphae* hyaline. *Aleuroconidia-like* conidia produced laterally on hyphae, but more commonly continuous with the hyphae, solitary or in clusters of three or more, subglobose or occasionally cylindrical or pyriform, single-celled, with a pale brown, thick wall when mature, (7.2–) 9–10 (–14.8) × (6.9–) 7.9–8.5 (–9.6) µm. *Acremonium-like conidiophores* arising laterally from hyphae, branched, sometimes unbranched, aseptate, or sometimes septate, 23.4– 41.8 µm long. *Conidiogenous cells* phialidic, 5.2–21 µm long, 1.5–3.6 µm wide near the base of phialides. *Conidia* hyaline, born in basipetal chains or held in false heads, aseptate, clavate, sometimes cylindrical, (3.2–3.8–4.2 (–5.1) × (1.2–) 1.4–1.8 (–2) µm. Sexual morph not observed.

Culture characteristics (7 Days at 25ºC in the dark): Colonies on OA attaining 42–46 mm diameter, edge entire, aerial mycelium sparse, raised, circular, moderate production of conidia; reverse buff with little production of pale luteus pigment. Colonies on CMA attaining 38–43 mm diameter, entire margin, white aerial mycelium sparse, flat, intense production of conidia at the center of colonies; reverse olivaceous at the center and buff at the margins of colonies; soluble pigment absent. Colonies on MEA attaining 45–48 mm diameter, edge little undulate, white aerial mycelium abundant, raised, circular, conidia production was not observed; reverse pale luteus; soluble pigments absent. Colonies on PCA attaining 34–37 mm diameter, edge erose or dentate, white aerial mycelium moderate at the center and sparse at the edges, concave with raised margins, circular, intense production of conidia; reverse olivaceous buff; soluble pigments absent.

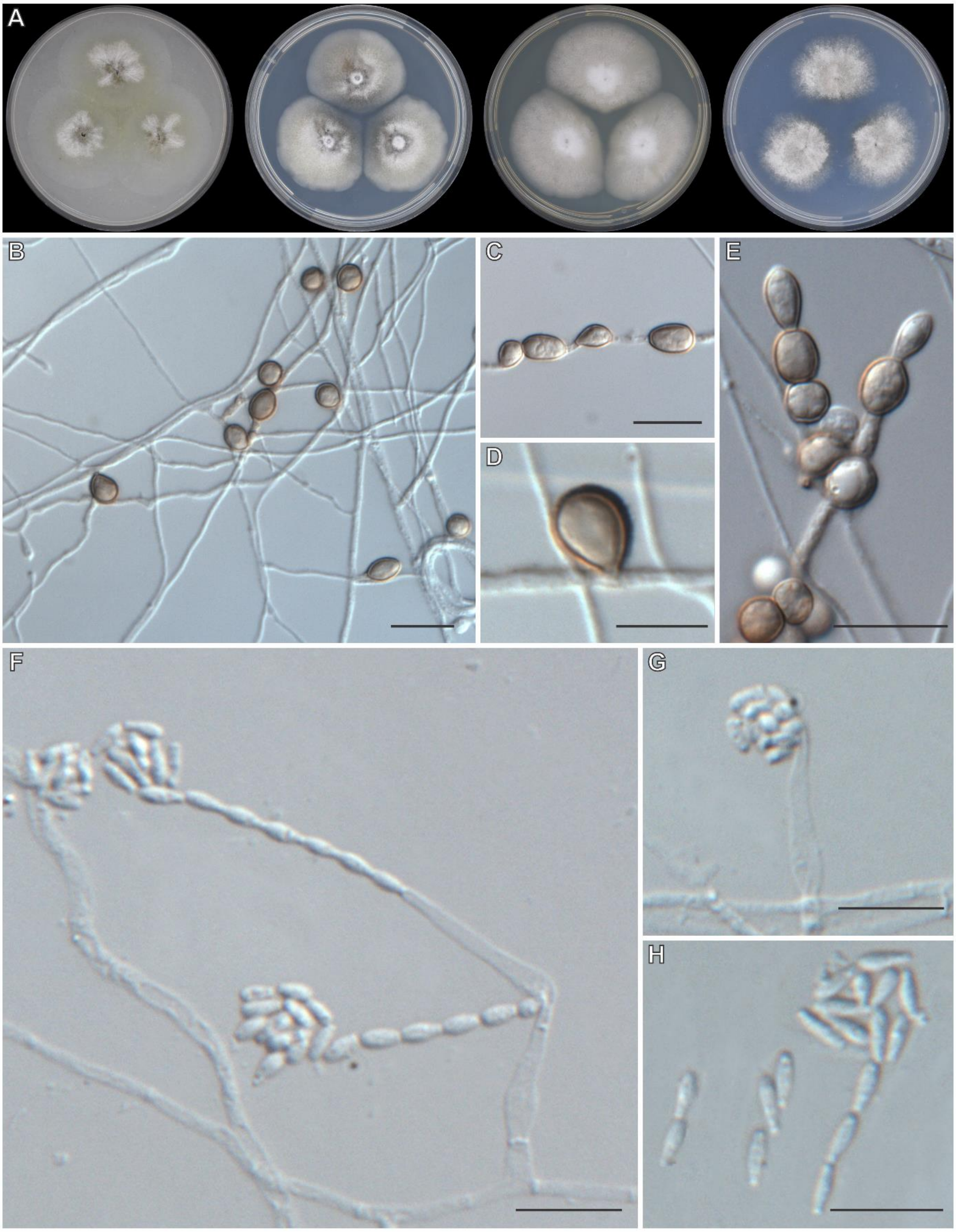


Fig. 24: *Pseudohumivola* sp. nov. COAD 3747. **A.** Colonies on OA, CMA, MEA and PCA from left to right after 7 days at 25 ºC in the dark. **B–E.** Aleuroconidia-like and hyphae. **F, G.** Acremonium-like condiophores. **H.** Hyaline conidia. Scale bars: B, C, E = 20 µm; D, F–H = 10 µm.

Pseudohumicola pulvericola (X. Wei Wang, Houbraken & Seifert) X. Wei Wang, P.J. Han, F.Y. Bai & Houbraken, Studies in Mycology 101: 196. 2022. Fig. 25.

Description: Wang *et al.* (2019)

Material eximined: **Brazil**, Minas Gerais state, Viçosa municipality, isolated from the substrate of *Cattleya aclandiae*, Jun. 2022, collected and isolated by P.T.S. Nogueira (living cultures COAD 3748 and COAD 3749).

Notes: *Pseudohumicola pulvericola* was known only by its asexual morph, with Aleuroconia-like and Acremonium-like conidia. Here, the sexual morph was observed and described. To induce the production of reproductive structures, isolate COAD 3748 was cultivated on OA with sterile paper filter. The production of ascomata begins after 3 weeks under the paper filter.

Ascomata superficial, ostiolate, ellipsoidal to ovoid, 260–408 µm high, 210–308 µm diam, growing under the aerial mycelium. *Ascomatal wall* brown, texture angular. *Terminal hairs* flexuous, septate, verrucose near the base. *Lateral hairs* straight or flexuous. *Asci* clavate, with spore-bearing portion 20–24.7 × 6–8.3 µm, with 8 biseriate ascospores, evanescent. *Ascospores* pale brown, limoniform, bilaterally flattened, (8.8–) 10-10.6 (–11.3) × (6.5–) 8–8.6 (–9.3), with one apical germ pore.

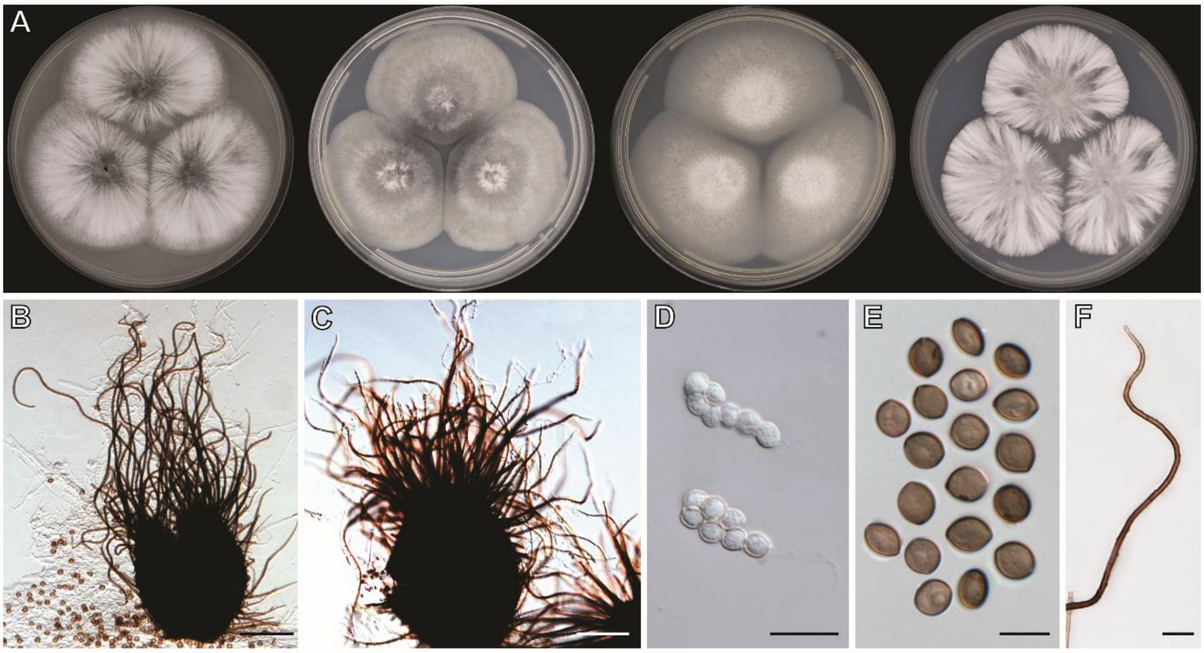


Fig. 25: *Pseudohumivola pulvericola* COAD 3748. **A.** Colonies on OA, CMA, MEA and PCA from left to right after 7 days at 25 ºC in the dark. **B, C.** Ascomata. **D.** Asci. **E.** Ascospores **F.** Terminal ascomatal hair. Scale bars: B, C = 100 µm; E = 10 µm; D, F = 20 µm.

DISCUSSION

In this study, five *Chaetomiaceae* genera, *Arcopilus*, *Chaetomium*, *Collariella*, *Dichotomopilus* and *Humicola*, were isolated as root endophyte of orchids, and one genus, *Pseudohumicola*, associated with the substrate of an orchid. Using the polyphasic approach, 10 putative new species were identified on the analyses. In addition, seven *Chaetomiaceae* species were reported for the first time as endophytes on roots of wild orchids.

Because it was found in almost all the locations where the collections were made, the orchid *Oeceoclades maculata* was the most frequently sampled species. A wide ecological amplitude, vegetative reproduction, and autogamous flowers make this species capable of spreading throughout South America, and it is considered a naturalized species (Wetterer and Wetterer, 2022). Three genera and six *Chaetomiaceae* species were identified in endophytic association with the roots of *O. maculata*, including three new species, two belonging to the *Humicola* genus, and one from *Collariela*. In addition, *Chaetomium cochliodes* and *Chaetomium tenue* were identified as root endophytes, with *C. tenue* found only in this orchid.

The genus *Arcoplius* showed the greatest species richness compared to the other *Chaetomiaceae* genera in this study, with seven species identified in seven different orchid species. However, four of these *Arcopilus* species have been identified as new to science. *Arcopilus* sp. nov. 1 and *Arcopilus* sp. nov. 4 lack sexual or asexual reproduction and are phylogenetically related. Moreover, it was observed that both species produced tangled melanized hyphae forming an amorphous structure. In the *Chaetomiaceae* family, there are species such as *Chaetomium capillare*, *C. cervicicola*, and *C. cucumericola*, which are considered to be sterile because of the absence of a reproductive structure, even when the majority of *Chaetomium* species produce these structures (Wang *et al*., 2016b). Although all *Arcopilus* species known to date have their sexual morphs, we speculate that these two species could have lost their ability to sporulate because of their association with the plant host.

Four new species of *Arcopilus* were identified in native orchids, and two of these, *Arcopilus* sp. nov. 1 and *Arcopilus* sp. nov. 2, were isolated from endemic rupicolous orchids, *Cattleya locatellii* and *Acianthera teres*, respectively (Flora e Funga do Brasil, 2024).

Recent studies have revealed taxonomic novelties of the genus *Arcopilus* in endophytic association with different groups of plants, with two of these discoveries in Brazil (Souza *et al*., 2020, Tavares *et al*., 2022). The species *Arcopilus amazonicus*, which was described in the Brazilian Amazon Forest as an endophyte on the leaves of *Paullinia cupana*, was identified in the same association but with the roots of two orchids from the Atlantic Forest, *Cattleya jongheana*, and *Cattleya* sp. This may indicate that *A. amazonicus* is widely distributed in Brazil. Although we were not able to properly identify the second *Cattleya* species mentioned above, this rupicolous *Cattleya* was identified as belonging to the series *Parviflorae* based on morphological analysis of the vegetative parts of the plant (Van Den Berg, 2014).

In Brazil, *Arcopilus cupreus* was reported associated with the plant tissue of *Syagrus coronata*, a palm native to the Brazilian biome Caatinga (Fortes; Vitória, 2022). We identified this species in association with the roots of *Cattleya* sp.; however, because it was an isolate recovered from the fungal collection from the *Laboratório de Micologia e Etiologia de Doenças Fúngicas de Plantas,* there was no information about the location or orchid from which the fungus was isolated.

As mentioned by Wang *et al.* (2016b), phylogenetic analyses of *tub2* and *rpb2* sequences were sufficient to distinguish *Chaetomium* species. Although no new *Chaetomium* species were discovered, the genus showed high species richness, with five species identified in five orchids. *Chaetomium* is the most reported genus from the *Chaetomiaceae* family in endophytic associations with orchids (Ma *et al*., 2015; Lee *et al*., 2017). *Chaetomium globosum* was the only species found in this study as a root endophyte on three different orchids: *Oeceoclades maculata*, *Polystachia concreta,* and *Zygopetalum mackayi*. This species is frequently reported as an endophyte in plants from different groups, including orchids (Adit *et al*., 2022). In Brazil, *C. globosum* was isolated from the leaf litter of *Syagrus coronata* (Fortes; Vitória, 2022) and as a root endophyte of *Musa* sp. (Santana, 2023).

In addition to being found in *Oeceoclades maculata*, *Chaetomium cochliodes* was isolated as a root endophyte from another terrestrial orchid, *Zygopetalum mackayi*. This *Chaetomium* species was capable of colonizing endophytically the root tissue of barley, soybean, wheat, and other plants used in agriculture (Kopylov *et al*., 2021), but has never been reported in association with roots of *Orchidaceae* plants. Nonetheless, *C. cochliodes* has already been reported in goat dung in Brazil (Melo *et al*., 2019).

Chaetomium coarctatum was found to be a root endophyte in *Avena fatua* and showed the ability to improve the growth of barley under salt stress (Bouzouina *et al*., 2021). To the best of our knowledge, this is the first report of *C. coarctatum* as an endophyte of an *Orchidaceae* plant. In addition, the species *Chaetomium pseudocochliodes* and *Chaetomium tenue*, which have never been reported before in endophytic association with any plant, were isolated from *Gomesa recurva* and *Polystachia concreta* and *Oeceoclades maculata*, respectively. This is the first report of *C. pseudocochlioides* and *C. tenue* as endophytes.

So far, the only *Collariella* species reported in Brazil are *Collariella botrychodes*, on animal dung, and in the leaf litter from a cave (Melo *et al*., 2012; Condé *et al*., 2023). However, the genus *Collariella* was founded in endophytic association with *Melocactus ernestii*, a cactus present in the Brazilian Caatinga biome (Ferreira-Silva *et al*. 2021). In this study, we reported for the first time a *Collariella* species as an endophyte on *Orchidaceae* plants. *Collariella* sp. nov. was isolated from the roots of two orchids, *Cyclopogon congestus* and *Oeceoclades maculata*, in two Brazilian states, indicating that this species may be distributed in the southeastern region of Brazil.

Dichotomopilus species have been identified to be associated with plants in Brazil. Fortes and Vitória (2022) identified the species *Dichotomopilus funicola* and *D. indicus* on the leaf litter of *Syagrus coronata*, and Barbosa *et al*. (2012) isolated *D. funicola* from plant debris. Furthermore, Santana (2022) identified a new species of *Dichotomopilus* and *D. variostiolatus* as root endophytes of *Musa* sp. In this study, *D. variostiolatus* was also isolated as a root endophyte, but from *Cattleya nobilior* and *Cattleya* sp., suggesting that this species may be a common endophyte in the roots of Brazilian plants.

A new *Dichotomopilus* species is presented in this study as a root endophyte of *Zigopetalum mackayi*. This species is closely related to *D. subfunicola* but grouped separately from *D. subfunicola* in both *tub2* and *rpb2* phylogeny, even though the rDNA-ITS sequences showed no differences between these two species.

Although two of the new species from *Humicola* were described from *O. maculata*, they are not phylogenetically related. *Humicola* sp. nov. 2., from Brasília-DF, clustered next to *Humicola* sp. nov. 1, that was isolated as endophyte of *Catasetum hookeri* from Viçosa municipiality, forming a basal clade inside the genus. However, the *Humicola* sp. nov. 3, isolated *from O. maculata* in Viçosa clustered next to *Humicola fuscoatra*.

Recently, three species of the genus *Pseudohumicola* have been described in Brazilian caves (Condé *et al*., 2023; Alves *et al.,* 2022). Here, a new species of *Pseudohumicola* was identified growing on the substrate close to the roots of *Cattleya aclandiae*. This species is phylogenetically close to *P. alba,* described in Brazil, and *P. subspiralis*. The phylogeny of *rpb2* and *tub2* loci clustered this new species as sister to *P. subspiralis*; however, the phylogeny of rDNA-ITS grouped *Pseudohumicola* sp. nov. with *Humicola fuscogrisea*. Although ascomata were observed on the substrate from which the fungus was isolated, all attempts to induce the production of these structures *in vitro* failed.

Furthermore, *P. pulvericola* was isolated from the same substrate as *Pseudohumicola* sp. nov.; this is the first report of this species in Brazil. This species was described from house dust by Wang *et al.* (2019) and was isolated from coconut shell used as a substrate for orchids. Although *P. pulvericola* was characterized only by the production of their asexual morph by Wang *et al.* (2019), the isolate *P. pulvericola* COAD 3748 produced the sexual morph, that has never been observed before. *Pseudohumicola* species identified here were not found in endophytic association, but as decomposers on an organic substrate, which coincides with the species from this genus, that were found in soil, dust, paper, or leaf litter (Wang *et al.* 2019; Wang *et al.* 2022).

CONCLUSION

This study unraveled the species richness of *Chaetomiaceae* fungi associated with the roots of wild *Orchidaceae* in Brazil. Twenty species from six genera belonging to the *Chaetomiaceae* family were identified among the 12 orchid species collected.

Half of the total species identified in the morphological and phylogenetic analyses were new to science and will be proposed in accordance with the International Code of Nomenclature for Algae, Fungi, and Plants. Orchids appear to harbor a great diversity of *Chaetomiaceae*, even though they were not the most abundant fungi during the isolations.

The ecological role of the association between *Chaetomiaceae* species and orchids is still unclear, however knowing the diversity of species may help future studies to explore this field of study. Endemic and endangered orchid species presented taxonomic novelties among the endophytic fungi associated with their root, although new species were identified in widely distributed orchids, such as *Oeceoclades maculata*.

CHAPTER 2: POTENTIAL OF ENDOPHYTIC *Chaetomiaceae* ON BIOLOGIAL CONTROL OF ROOT DISEASES

INTRODUCTION

Root endophytic fungi from orchids are a source of metabolites and compounds that have the potential to be used in the pharmaceutical industry and agriculture for the biological control of plant pathogens (Ma *et al*., 2015). Some species of orchid mycorrhizal fungi can be used in the control of soil-borne pathogens, such as *Rhizoctonia solani*, with results similar to well-studied biocontrol agents, such as *Trichoderma harzianum* (Espinosa *et al*., 2013; Pereira *et al.,* 2021). In addition to being used in biocontrol, root endophytes from orchids can also be used as plant growth promoters (Shah *et al*., 2019).

Species of *Chaetomiaceae* are among endophytic fungi from orchids that have potential use in biological control. These fungi produce metabolites with antiviral, antibacterial and antifungal activities (Ibrahim *et al*., 2021). *Chaetomium globosum* is one of the most studied species in the family, with potential use in agriculture as a biofertilizer and biological control agent (Zhai *et al*. 2018). Studies showed that *C. globosum* was capable of suppressing and reducing the primary inoculum of *Diaporthe phaseolorum* in soybean (Pereira; Dhingra, 1997; Dhingra *et al*., 2002). Moreover, metabolites from other strains of this species presented nematicidal activity against *Meloidogyne incognita* and *M. javanica* (Yang et *al*., 2013; Khan *et al*., 2019).

A product based on *Chaetomium globosum* and *Arcopilus cupreus*, namely Ketomium®, is registered as a mycofungicide for the biological control of plant pathogens in China, Cambodia, Laos, Thailand, and Vietnam. This product has been used to control several diseases caused by phytopathogenic fungi and oomycetes (Soytong *et al*., 2021).

In Brazil, despite the growing market demand for the use and development of products for biological control, species of *Chaetomiaceae* are still little explored for this purpose. Therefore, this study aimed to test the potential of endophytic *Chaetomiaceae* species isolated from the roots of orchids for the biocontrol of soil-borne pathogens. The pathogen *Fusarium sacchari* was chosen for the antagonism test *in vitro*. This pathogen is kwon to cause root diseases in several commercial crops, including sugar cane and corn, and was recently isolated as a pathogen of orchids in Brazil. In addition, a pathogen with historical relevance in agriculture, *Rhizoctonia solani*, isolated from common bean was used in the greenhouse assays.

MATERIAL AND METHODS

In vitro antagonism test against *Fusarium sacchari*

The isolates obtained in the previous chapter, COAD 3710 (*Arcopilus amazonicus*), COAD 3723 (*Arcopilus* sp. nov. 1), COAD 3725 (*Arcopilus* sp. nov. 2), COAD 3731 (*Chaetomium cochliodes*), COAD 3732 (*Chaetomium globosum*), CCF 702 (*Chaetomium globosum*), COAD 3733 (*Chaetomium pseudocochliodes*), COAD 3734 (*Chaetomium pseudocochliodes*), COAD 3736 (*Chaetomium tenue*), and COAD 3741 (*Dichotomopilus* sp. nov.) were tested for their antagonistic activity against *Fusarium sacharii* using the methodology of Dang *et al.* (2023). A strain of *F. sacharii* (CCF 873) isolated from *Vanda* sp. in São Paulo state causing root rot was obtained from the fungal collection of the *Laboratório de Micologia e Etiologia de Doenças Fúngicas de Plantas*.

Endophytic and phytopathogenic fungi were cultivated at 25 °C for 7 days in the dark on Potato Dextrose Agar (PDA) medium. Discs approximately 5 mm in diameter, containing the pathogen mycelium, were placed 1.5 cm away from the edges of Petri dishes measuring 8.5 cm in diameter. At the opposite end to the phytopathogens, a 5 mm diameter disc containing mycelium of the endophytic strains was deposited, 1.5 cm away from the edge of the plate. As a control, the phytopathogen was grown alone. Four replicates were used for each combination of endophytic and phytopathogenic fungi, and the entire experiment was conducted in a randomized experimental design. Each experiment was conducted until the pathogen colonies cover the entire plate of control treatments.

The *in vitro* antagonism tests were evaluated by the percentage of inhibition of the growth of phytopathogens by the endophytic fungi. The percentage of inhibition was calculated following the equation described by Rahayu *et al.* (2021):

$$PICM = R1 - R2/R1 \times 100\%$$

Where:

PICM = Percentage of inhibition of mycelial growth

R1 = Radius of the phytopathogen colony on the control plate

R2 = Radius of the phytopathogen colony on the plate with the endophytic fungus

Each plate used in the experiments was evaluated as an experimental unit. Each treatment was averaged across the four replicates. Afterwards, the data was subjected to analysis of variance and the means of the treatments were compared using the Tukey test ($p<0.05$), using the MiniTab software.

Greenhouse experiments

Seven root-endophytic *Chaetomiaceae* strains obtained in this study were selected as antagonists of the soil-borne pathogen *Rhizoctonia solani*. This pathogen was chosen for this assay because of its high aggressiveness in common beans, which makes it easier to evaluate the results. The endophytes were chosen based on the literature on *Chaetomiaceae* used for biological control and ascospore production over a period of 14 days; those that had little spore production or grew slowly were not used for the tests. The isolates selected were COAD 3717 (*Arcopilus amazonicus*), COAD 3721 (*Arcopilus aureus*), COAD 3722 (*Arcopilus cupreus*), *Chaetomium cochliodes*, COAD 3732 (*Chaetomium globosum*), COAD 37333 (*Chaetomium pseudochochliodes*), and COAD 3736 (*Chaetomium tenue*). A strain *of Rhizoctonia solani* phytopathogenic to common bean (*Phaseolus vulgaris*) was given by *Unidade de Controle Biológico* at Universidade Federal de Viçosa.

Chaetomiaceae isolates were cultivated in oatmeal agar (OA) medium for 14 days at 25 °C in the dark. A spore suspension was prepared using sterile water and Tween 80%. The concentration was adjusted to 5×10^6 ascospores mL $^{-1}$ by dilution and counting using a Neubauer chamber for each isolate. The pathogen was cultivated in sterile rice grains for seven days at 25 °C in the dark.

Seeds of BRSMG Marte, a cultivar of common, were sown in plastic pots of 950 mL (20 cm long and 7 cm deep). Each pot received approximately 400 g of red sandy loam soil that was previously solarized, and six seeds were sown at depths of 3 cm and 1 cm from each other. A rice grain colonized with *R. solani* was placed 1 cm apart at the same depth as the first seed of common bean (Fig. 26). Four pots were used per treatment, and each one was considered a repetition. As a negative control, 15 mL sterile water and Tween 80% were used to drench the soil. As a positive control, only seeds of common bean were sown without the pathogen, and 15 mL of sterile water was used to drench the soil. Each of the seven treatments received 15 mL of ascospore suspension to drench as soon as the seeds and pathogens were sown.

The pots were kept in a greenhouse for 14 d at 28 ± 3 °C, with photoperiod of 12 h. Observations were recorded daily for interactions between antagonists and pathogen. A final evaluation was performed 14 d after the seeds were drenched with the spore suspension. The evaluation was based on disease incidence. Plants that presented injuries in the root collar region above 1.5 cm were considered severely affected by the pathogen. Moreover, the germination of the seeds and the presence or absence of symptoms of damping-off were used to determine with the pathogen was affecting the seedlings.

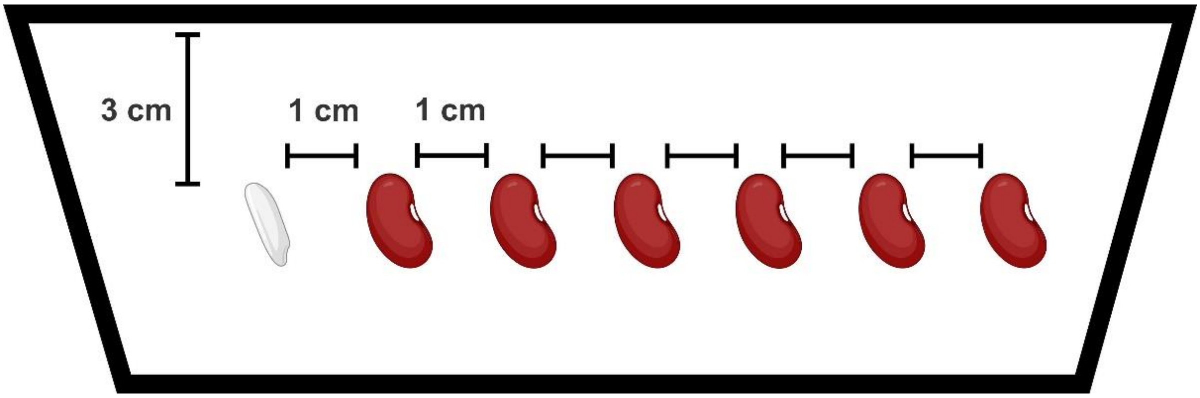


Fig. 26: Representative scheme of the arrangement of the common bean seeds and the rice grain colonized with *R. solani*.

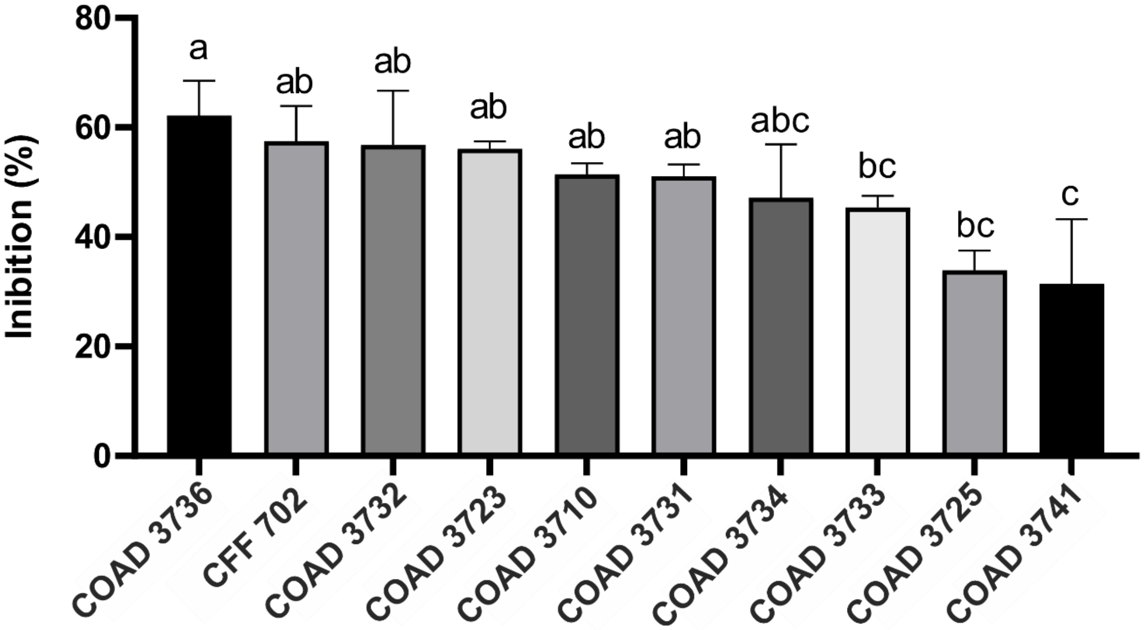
RESULTS

Inhibition of growth of *Fusarium sacchari* in a dual culture test

The result of inhibition percentage obtained with each isolate are shown in Graphic 1. Among the root endophytic strains tested, the isolate COAD 3736 (*C. tenue*) was statistically the best antagonist activity against *Fusarium sacchari* with an average of 62% inhibition of the pathogen *in vitro*. All the isolates presented antagonism by competition, other types of antagonism, such as inhibition and parasitism, were not observed in the tests.

The isolates CCF 702 (*C. globosum*), COAD 3732 (*C. globosum*), COAD 3723 (*Arcopilus* sp. nov. 1), COAD 3710 (*A. amazonicus*), and COAD 3731 (*C. cochliodes*) presented an average of growth inhibition above 50% on the pathogen growth, with 58%, 57%, 56%, 51% and 51%, respectively.

Although the isolates COAD 3731, COAD 3733 (*C. pseudocochliodes*), and COAD 3734 (*C. pseudocochliodes*) were able to inhibit the growth of *F. sachari*, at the end of the experiment, we observed that the pathogen was starting to grow above these isolates, which could indicate possible parasitism; however, this was not analyzed in this study. The same was observed for COAD 3725 (*Arcopilus* sp. nov. 2) and COAD 3741 (*Dichotomopilus* sp. nov.).



Graphic 1: The inhibition percentage of orchid root endophytic strains. Letters above the columns shows the significantly statistical differences of means based on Tukey Test at 5% significant level.

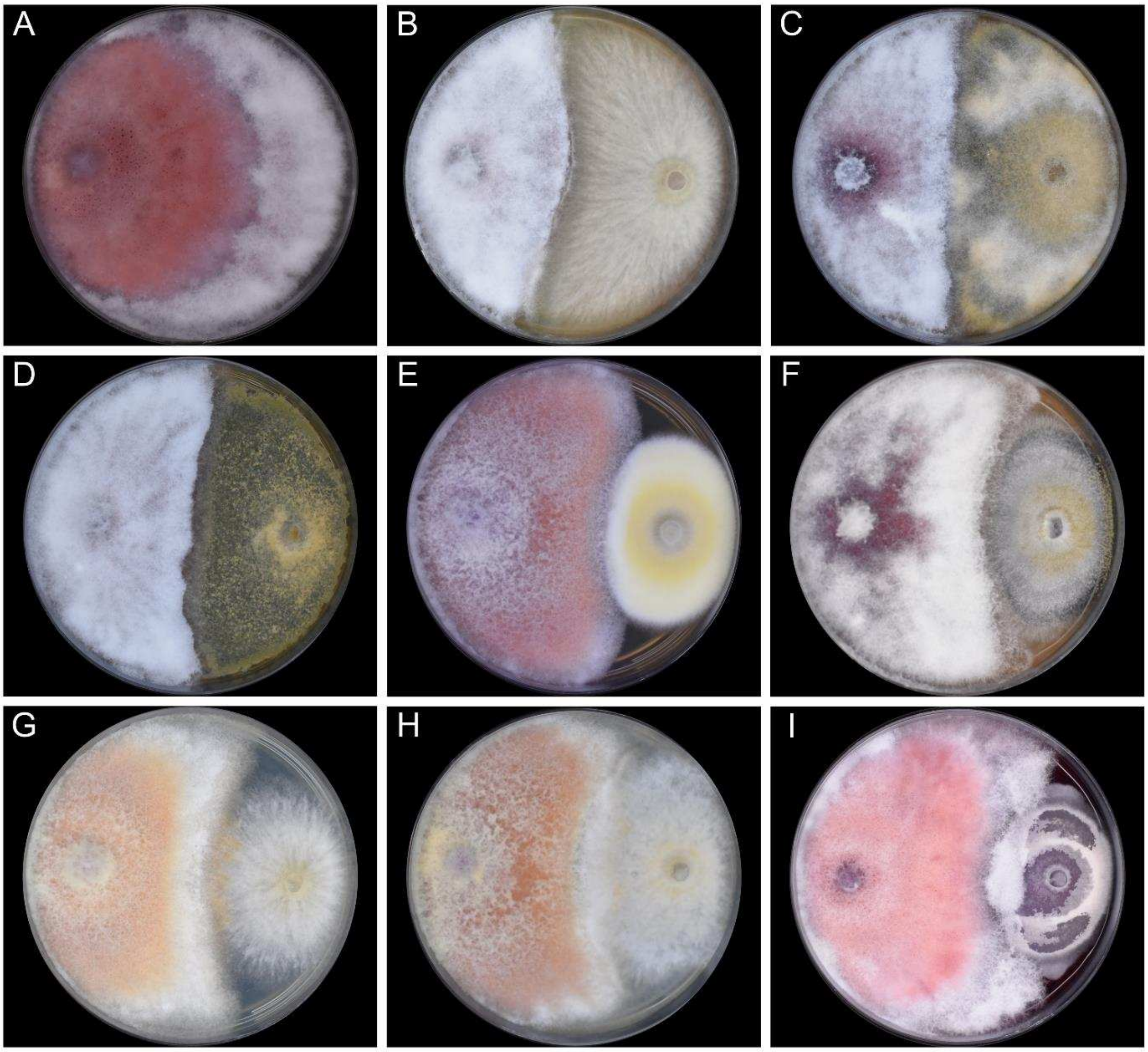


Fig. 27: Inhibition of *Fusarium sacchari* by root endophytic *Chaetomiaceae* isolates after 14 days at 25 °C on PDA. **A.** Control (*F. sacchari*). **B.** COAD 3736 (*C. tenue*)**. C.** CCF 702 (*C. globosum*). **D.** COAD 3732 (*C. globosum*). **E.** COAD 3723 (*Arcopilus* sp. nov. 1). **F.** COAD 3710 (*A. amazonicus*). **G.** COAD 3731 (*C. cochliodes*). **H***.* COAD 3734 (*C. pseudocochliodes*). **I.** COAD 3725 (*Arcopilus* sp. nov. 2).

Greenhouse experiments with *Rhizoctonia solani*

In the positive control all the seeds germinated, showing that the soil and the seeds were appropriated to the essay and didn't interfere in the results (Fig.1 A). Negative control was used to compare with the treatments that received the spore suspension of the endophytic isolates. Three out of four repetitions of the negative control presented the five first seedlings, from the rice with *R. solani*, severely affected by the pathogen, showing dumping off and root collar rot symptoms (injuries > 2cm). While the sixth seedlings were not affected by the pathogen after 14 days. In the fourth repetition, two seeds near to the pathogen didn't germinate and all the seedling presented symptoms, but the sixth plant was little affected (Fig.1 B). Moreover, we consider that the pathogen was not able to completely colonize the region where the last seed of common bean was placed.

The isolate COAD 3736 (*C. tenue*) presented the best results in this essay. In the four repetitions, all the seeds germinated and in the first seven days no apparent symptoms were observed. After the nineth day damping off symptoms were observed in the first two seedlings near to where the pathogen was placed. In the final evaluation these seedlings were affected, with injuries of approximately 1.5 cm in the collar region. The third seedling was not affected by the pathogen in two repetitions, whereas in the other two were little affected (lesions < 1 cm). In all the repetitions, the fifth and sixth seedlings presented no symptoms (Fig.1 I).

The results of the treatment with COAD 3717 (*A. amazonicus*) were not consistent, since in one repetition the last three seedlings presented no symptoms of the disease, while in other two repetitions the last two plants were not affected (Fig. 1 C). However, in one repetition one seed didn't geminate and all the five plants that grown were severely affected by the pathogen. Therefore, we were not able to affirm that this isolate has the potential to antagonize *R. solani.*

The treatment with COAD 3721 (*A. aureus*) showed inconsistent results similar to what was observed with COAD 3717. Nevertheless, in one repetition of this treatment none of the plants presented any symptoms of the pathogen, while other repetitions only the three seedlings near to where the pathogen was placed were showing symptoms. In the other two repetitions only the sixth plant was not affected.

The treatments with COAD 3722 (*A. cupreus*), COAD 3730 (*C. cochliodes*), COAD 3732 (*C. globosum*), and COAD 3733 (*C. pseudochochliodes*) didn't present the ability to control the pathogen. The seedlings of these treatments were more affected by *R. solani* than the negative control. In the treatment with COAD 3722 all the plants were severely affected, with injuries above 2 cm, and the first seed not germinated or died during the experiment. since in all the repetitions the first seed didn't geminate, and all the plants were presented injuries in root collar region. Treatments with COAD 3730, COAD 3732, and COAD 3733 presented similar results, and all the plants were showing dumping off after 14 days of the inoculation.

Fig. 28: Seedling of common bean after 14 days of inoculation with *Rhizoctonia solani* **A.** Positive control. **B.** Negative control. **C.** COAD 3717 (*A. amazonicus*). D. COAD 3721 (*A. aureus*). **E.** COAD 3722 (*A. cupreus*). **F.** COAD 3730 (*C. cochliodes*). G. COAD 3732 (*C. globosum*). **H.** COAD 3733 (*C. pseudochochliodes*). **I.** COAD 3736 (*C. tenue*).

DISCUSSION

The strain COAD 3736 identified as *Chaetomium tenue*, showed the potential to inhibit the growth of *Fusarium sacchari in vitro* by competition. Due to the limitations during this study, this pathogen was not tested on greenhouses. However, the same strain, COAD 3736, presented disease suppression on common bean seedlings infected with *Rhizoctonia solani* in the greenhouse assays. Although symptoms of the disease caused by *R. solani* were observed on plants treated with the spore suspension of *C. tenue*, more plants survived without injuries caused by the pathogen when compared with the negative control and the other treatments. Even the plants that were affected by the fungus were visually healthier than the those treated with other endophytic strains. Since the seedlings of common bean were not showing apparent symptoms after seven days of this treatment, a second application of spore suspension could be applied after this period as an alternative to avoid the progression of the disease. Another alternative could be increasing the concentration of the spore suspension, to favor the antagonist in the competition with the pathogen in the soil. Therefore, no studies applying *Chaetomium tenue* for the biological control of plant pathogens were founded on literature and is the first attempt to use a strain of this species as a potential biocontrol agent.

The strain COAD 3732 (*C. globosum*) that presented an inhibition percentage of 58% on *F. sacchari* in the dual culture tests, however in the greenhouse essay against *R. solani*, the same strain showed results worse than the negative control. *Chaetomium globosum* strains are known for their potential on the biological control of phytopathogenic fungi, Oomycetes and nematodes, including *R. solani* and *Fusarium* species (Soytong *et al.*, 2021; Bairwa *et al.*, 2023). However, this strain was only capable of inhibiting *Fusarium sacchari*, as well as CCF 702, on the tests performed in this study. As observed by Wang *et al.* (2016b), different populations of *C. globosum* can present high diversity of substrates and life styles. These variations influence the ability of these strains on the production of metabolites and other compounds that make them capable of being used in biological control.

Along with *C. globosum*, *C. cochliodes* was one of the first *Chaetomiaceae* species applied to control *Fusarium* spp. and *Helminthosporium victoriae* (Soytong *et al.*, 2021). Even though the strains tested were not capable of antagonizing with the pathogens chosen for the essays, these fungi can be beneficial to plants. Kopylov *et al.* (2021) observed that strains of *C. cochliodes* increased the activity of succinate dehydrogenase in the roots of different plants, such as soybean and wheat. This enzyme activity is used to identify mycorrhizal associations, due to their influence on the metabolism of root cells.

The species *Arcopilus cupreus*, previously named *Chaetomium cupreum*, has been reported as a biocontrol agent against several plant pathogens (Soytong *et al.*, 2021). In both assays, none of the endophytic *Arcopilus* strains was able to antagonize the pathogens. However, strain COAD 3722 of *A. cupreus* has not been tested against *F. sacchari*. Among the *Arcopilus* species tested against this pathogen, strain COAD 3723 (*Arcopilus* sp. nov. 1) showed the best result, with 56% growth inhibition.

Strains belonging to other genera obtained as root endophytes of orchids were not tested against *F. sacchari* and *R. solani*, because of their slow growth or slow production of spores. However, other assays could be performed with these strains, and they produced secondary metabolites that could be tested for their potential for the biocontrol of plant pathogens.

CONCLUSION

Biological control assays revealed that a strain of *Chaetomium tenue* stood out among the strains tested and proved to be a potential biocontrol agent for soil-borne pathogens such as *Fusarium sacchari* and *Rhizoctonia solani*. The other strains tested had inconsistent results in the experiments, and future analyses are necessary to evaluate their potential.

Studies on the application of orchid endophytes have been focusing mainly on metabolites with pharmaceutical or industrial potential, however applications of these fungi in agriculture are in the spotlight nowadays. The present study contributes to knowledge about these fungi and their potential application in the control of diseases caused by phytopathogenic fungi.

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