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Hidden diversity of *Ophiocordyceps* parasitizing Hymenoptera: Novel evolutionary lineages and a cryptic species complex

Samuel Júlio Lima dos Santos
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Thesis submitted to the Entomology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

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Assent:

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Ao meu avô Antônio, por sempre me mostrar que, mesmo no escuro,
eu poderia ver o infinito...

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“…*This is my commitment, my modern manifesto*
I'm doing it for all of us who never got the chance…”

Get Free – Lana Del Rey

ABSTRACT

SANTOS, Samuel Júlio Lima dos, D.Sc., Universidade Federal de Viçosa, June, 2025. **Hidden diversity of *Ophiocordyceps* parasitizing Hymenoptera: Novel evolutionary lineages and a cryptic species complex**. Adviser: Simon Luke Elliot. Co-advisers: Thairine Mendes Pereira and João Paulo Machado Araújo.

Ophiocordyceps fungi are predominantly entomopathogenic, with some species capable of manipulating the behaviour of social hymenopterans. Over recent decades, taxonomic studies have revealed extensive cryptic diversity within the genus, particularly within the *O. unilateralis* species complex composed by taxa that manipulate ant behaviour. Estimations suggest that hundreds of species remain undescribed, especially among those manipulative species associated with Hymenoptera in underexplored environments such as tropical forests. This thesis aimed to investigate the cryptic diversity of entomopathogenic *Ophiocordyceps* infecting Hymenoptera, particularly ants and social wasps, in Brazilian tropical forests. For the General Introduction (Chapter I), we reviewed biological, ecological and taxonomic aspects of entomopathogenic fungi, followed by an overview of *Ophiocordyceps*-Hymenoptera interactions and the current knowledge gaps concerning bees, ants, and wasps. In Chapter II, we employed an integrative taxonomic approach to describe *O. acanthoponerae*, a new species infecting *Acanthoponera mucronata* ants in an ecotone between the Atlantic Forest and Cerrado biomes. This species represents a novel lineage of myrmecophilous *Ophiocordyceps* closely-related to *O. humbertii*, a species known to infect and manipulate social wasps. In Chapter III, we revealed that *O. humbertii* constitutes a cryptic species complex by describing seven new species associated with social wasps, uncovering at least four novel fungal lineages. This thesis significantly contributes to the understanding of cryptic fungal diversity in tropical forests and highlights the pivotal role of Hymenoptera in the evolution and diversification of behaviour-manipulating *Ophiocordyceps*.

Keywords: Atlantic Forest; Hypocreales; host manipulation; integrative taxonomy; *Ophiocordyceps acanthoponerae;* ; *Ophiocordyceps humbertii*

RESUMO

SANTOS, Samuel Júlio Lima dos, D.Sc., Universidade Federal de Viçosa, junho de 2025. **Diversidade oculta de *Ophiocordyceps* parasitas de Hymenoptera: novas linhagens evolutivas e um complexo de espécies crípticas**. Orientador: Simon Luke Elliot. Coorientadores: Thairine Mendes Pereira e João Paulo Machado Araújo.

Fungos do gênero *Ophiocordyceps* são majoritariamente entomopatogênicos, com algumas espécies capazes de manipular o comportamento de insetos sociais da ordem Hymenoptera. Nas últimas décadas, estudos taxonômicos revelaram a existência de diversas espécies crípticas para o gênero, especialmente dentro do complexo de espécies *O. unilateralis*, que manipulam o comportamento de formigas. Estimativas indicam que centenas de espécies ainda estão para serem descritas, particularmente para os grupos que infectam e manipulam o comportamento de Hymenoptera, em ambientes negligenciados como florestas tropicais. Esta tese teve como objetivo investigar a diversidade críptica de fungos entomopatogênicos do gênero *Ophiocordyceps* parasitando insetos da ordem Hymenoptera, especialmente formigas e vespas sociais em florestas tropicais do Brasil. Para a Introdução Geral (Capítulo I), inicialmente revisamos os aspectos biológicos, ecológicos e taxonômicos de fungos entomopatogênicos e, posteriormente, detalhamos os aspectos e as lacunas de conhecimento das interações entre *Ophiocordyceps* com os diferentes grupos de insetos Hymenoptera (abelhas, formigas e vespas). No Capítulo II, nós utilizamos uma abordagem de taxonomia integrativa para descrever uma nova espécie, *O. acanthoponerae*, infectando formigas *Acanthoponera mucronata* em um ecótono entre Floresta Atlântica e Cerrado. Essa nova espécie representa uma nova linhagem dentro de *Ophiocordyceps* mirmecófilos filogeneticamente relacionada a *O. humbertii*, espécie que infecta e manipula o comportamento de vespas sociais. No Capítulo III, nós revelamos através da descrição de sete novas espécies que *O. humbertii* constitui um complexo de espécies crípticas. Nossos resultados revelam a existência de pelo menos quatro novas linhagens fúngicas associadas a vespas. Essa tese contribui significativamente para o conhecimento da diversidade críptica de fungos em florestas tropicais e reforçam a importância de Hymenoptera na evolução e diversificação de *Ophiocordyceps* manipuladores de comportamento.

Palavras-chave: Floresta Atlântica; Hypocreales; manipulação comportamental; taxonomia integrativa; *Ophiocordyceps acanthoponerae*; *Ophiocordyceps humbertii*

SUMMARY

1 CHAPTER I: GENERAL INTRODUCTION 13

1.1 THE KINGDOM FUNGI 13

1.2 THE ENTOMOPATHOGENIC HYPOCREALES... 15

1.3 HYMENOPTERA-*OPHIOCORDYCEPS* INTERACTIONS 18

1.3.1 Hymenoptera 18

1.3.2 *Ophiocordyceps* 19

1.3.3 *Ophiocordyceps* and ants 20

1.3.4 *Ophiocordyceps* and wasps.. 24

1.3.5 *Ophiocordyceps* and bees 26

1.4 MAIN OBJECTIVES OF THE THESIS.. 27

1.5 REFERENCES 29

2 CHAPTER II: There is gold in the graveyard: A new lineage of zombie-ant fungi in the genus *Ophiocordyceps* (*Ophiocordycipitaceae*: *Hypocreales*) from Minas Gerais, Brazil.. 39

2.1 ABSTRACT 39

2.2 INTRODUCTION 40

2.3 MATERIALS AND METHODS 43

2.3.1 Field collections... 43

2.3.2 DNA extraction, amplification and sequencing.. 45

2.3.3 Phylogenetic analyses... 46

2.3.4 Morphological characterisation 50

2.4 RESULTS 52

2.4.1 Phylogenetic analyses... 52

2.4.2 Taxonomy 52

2.5 DISCUSSION.. 56

2.6 CONCLUSIONS 65

2.7 ACKNOWLEDGEMENTS... 65

2.8 REFERENCES 67

3 CHAPTER III: A light into a forgotten graveyard: Cryptic diversity within the wasp-parasitic *Ophiocordyceps humbertii* (*Ophiocordycipitaceae: Hypocreales*) 74

3.1 ABSTRACT 74

3.2 INTRODUCTION 75
3.3 MATERIALS AND METHODS 77
3.3.1 Specimen collections 78
3.3.2 DNA extraction, amplification and sequencing 81
3.3.3 Phylogenetic analyses 82
3.3.4 Morphological characterisation 87
3.4 RESULTS 90
3.4.1 Phylogenetic analyses 90
3.4.2 Taxonomy 92
3.5 DISCUSSION 107
3.6 CONCLUSIONS 113
3.7 ACKNOWLEDGEMENTS 114
3.8 REFERENCES 115

4 FINAL CONSIDERATIONS 122

5 SUPPLEMENTARY MATERIAL I 123

1 CHAPTER I: GENERAL INTRODUCTION

1.1 THE KINGDOM FUNGI

Global fungal diversity is estimated at 2.2 to 3.8 million species (Hawksworth & Lücking 2017, Lucking *et al.* 2021). This could be a gross underestimate, though, given figures of 11.7-13.2 million species estimated by using metabarcoding data (Wu *et al.* 2019, Phukhamsakda *et al.* 2022). Currently, less than 155,869 fungal species have been described (Bánki *et al.* 2025), which represents only 7.5% or less of the estimated total diversity. These diversity estimates help to provide insights into fungal distribution and roles across habitats, to identify knowledge gaps and to prioritise areas for further research and conservation (Hawksworth & Lücking 2017, Hyde 2022, Phukhamsakda *et al.* 2022). The main obstacles to recognize fungal diversity are the lack of research in locations considered hotspots of diversity (e.g. tropical regions), the underfunding of taxonomic studies and fieldwork on mycology, but also the existence of morphologically and ecologically cryptic species hidden under well-established names (Hawksworth & Lücking 2017, Cazabonne *et al.* 2022). It is notable that estimates of fungal diversity have become more robust over the years with the increasing number of studies in tropical environments and use of molecular techniques to discriminate species (Blackwell 2011, Hibbett *et al.* 2011, Hawksworth 2012). However, some groups of fungi are neglected from these estimates because there is still a lack of research effort and specialists to study their diversity, life-histories and ecology (Blackwell 2011, Hawksworth & Lücking 2017).

The kingdom Fungi (also known as 'true fungi' or Eumycota) is a monophyletic clade of heterotrophic organisms that do not have a well-defined synapomorphy (i.e. they lack a derived character that is shared by the descendants of a common ancestor), but are traditionally recognized for having chitinous cell walls, the α-aminoadipate pathway for the biosynthesis of lysine, and growth as yeast or mycelium (Richards *et al.* 2017, Adl 2019). These organisms can be found in a broad range of environments (terrestrial and aquatic) playing essential roles in nutrient cycling and the regulation of populations of other organisms (Naranjo-Ortiz & Gabaldón 2019, Heitman *et al.* 2020). Fungi can also exhibit a wide range of lifestyles and have a range of interactions with other organisms (e.g. lichens, ecto- or endomycorrhizae, endophytes, phytopathogens, mutualists of insects and entomopathogens (Naranjo-Ortiz & Gabaldón 2019). Currently, 12 phyla of fungi are recognized: Aphelidiomycota, Ascomycota, Basidiomycota, Blastocladiomycota, Chytridiomycota, Cryptomycota (or Rozellomycota), Entorrhizomycota, Microsporidia, Monoblepharidomycota, Mucoromycota, Neocallimastigomycota and Zoopagomycota (James *et al.* 2020, Li *et al.* 2021). Six of these phyla (Ascomycota, Basidiomycota, Chytridiomycota, Microsporidia, Mucoromycota and Zoopagomycota) include groups of insect-associated fungi that act as mutualists or pathogens for their hosts (Araújo & Hughes 2016, Biedermann & Vega 2020). These are expected to be highly diverse considering the estimated diversity of insects (approx. 5.5 million species), but are still largely understudied (Blackwell 2011, Mueller & Schmit 2007, Schmit & Mueller 2007, Stork 2017).

Ascomycota is the most diverse of the fungal phyla, with approximately 83,000 described species, representing two-thirds of the fungal species that are known (James *et al.* 2020). Their richness is expected to be higher in tropical regions of the world, but they can also be found in temperate, glacial and arid environments, or even within other organisms (Hawksworth & Lücking 2017, Wu *et al.* 2019; Maharachchikumbura *et al.* 2021). Three subphyla are recognized: Pezizomycotina, Saccharomycotina and Taphrinomycotina. The subphylum Pezizomycotina is the richest and most diverse of these, comprising 13 classes, 124 orders and 507 families of fungi that exhibit a variety of morphological traits and ecological backgrounds (Schoch *et al.* 2009, Wijayawardene *et al.* 2018). These fungi are characterized by the formation of a short-lived dikaryotic hypha and by producing a specialized sac-like structure, the ascus, where the meiotic spores (usually eight) are produced (Schoch *et al.* 2009, Naranjo-Ortiz & Gabaldón 2019). They are also recognized for being filamentous, having septate hyphae with organelles known as 'Woronin bodies'and asci surrounded by a multicellular structure named the Ascomata (or Ascocarp) (Jedd 2006, Ng *et al.* 2009, Healy *et al.* 2013, Naranjo-Ortiz & Gabaldón 2019). The subphylum Pezizomycotina includes different lineages of insect-associated fungi, especially the most important lineages of entomopathogenic fungi in the order Hypocreales (Humber *et al.* 2008, Blackwell 2010).

The delimitation of fungal species has been a major topic of discussion among mycologists over the years, especially regarding which species concept and/or species recognition criteria should be used to recognize new taxa or to apply an existing name across different groups of fungi (Cai *et al.* 2011, Jayawardena *et al.* 2021). There are more than 40 species concepts in biology and each of them have different meanings and applications according to the field and organisms studied (Crous *et al.* 2015, Samarakoon *et al.* 2016, Jayawardena *et al.* 2021). In mycology, the morphological, physiological, intersterility, host specificity and phylogenetic species recognition criteria have traditionally been used to delimit new fungal taxa, with the morphological and phylogenetic criteria being the most successful to disentangle evolutionary relationships between species (Taylor *et al*. 2000, Cai *et al*. 2011). The Morphological Species Concept relies on morphological criteria, such as similarities or divergence between morphological traits, to distinguish new species whilst the Phylogenetic Species Concept relies on nucleotide divergence as the criterion to delimit monophyletic lineages (Taylor *et al*. 2000, Giraud *et al*. 2008, Jayawardena *et al*. 2021). In recent years, the Genealogical Concordance Phylogenetic Species Recognition (GCPSR), a methodology using the phylogenetic concordance of unlinked genes to indicate a lack of genetic exchange, and thus evolutionary independence of lineages, has been widely used by mycologists to describe new species (Taylor *et al*. 2000, Crous *et al*. 2015, Jayawardena *et al*. 2021). This enables the delimitation of species even with insufficient distinguishable morphological characters or ambiguity between them. The use of a polyphasic approach (combining morphological, ecological and phylogenetic data) has also been a common practice to identify new species in fungal taxonomy (Crous *et al*. 2015).

1.2 THE ENTOMOPATHOGENIC HYPOCREALES

The order Hypocreales comprises the majority of fungal species that are considered insect pathogens (i.e. entomopathogenic fungi), distributed principally in three families: Clavicipitaceae, Cordycipitaceae and Ophiocordycipitaceae (Sung *et al*. 2007, 2008). The ancestral trait of the order is an association with plants (decomposers, phytopathogens, epiphytes and endophytes), with the insect-associated lineages arising around 160 million years ago (Sung *et al.* 2008). These analyses also suggest that the evolution of hypocrealean entomopathogens probably occurred mediated by a phenomenon known as inter- kingdom host-jumping (or host-switching), in which closely-related fungi are able to exploit nutritional resources from distantly related host species (animals, plants and other fungi) (Spatafora *et al*. 2007, Sung *et al*. 2008, Yu *et al*. 2021, Wei *et al*. 2022). This phenomenon can be considered a major driver of diversity, lifestyle differentiation and speciation for entomopathogenic fungi (Thines 2019, Wei *et al*. 2022).

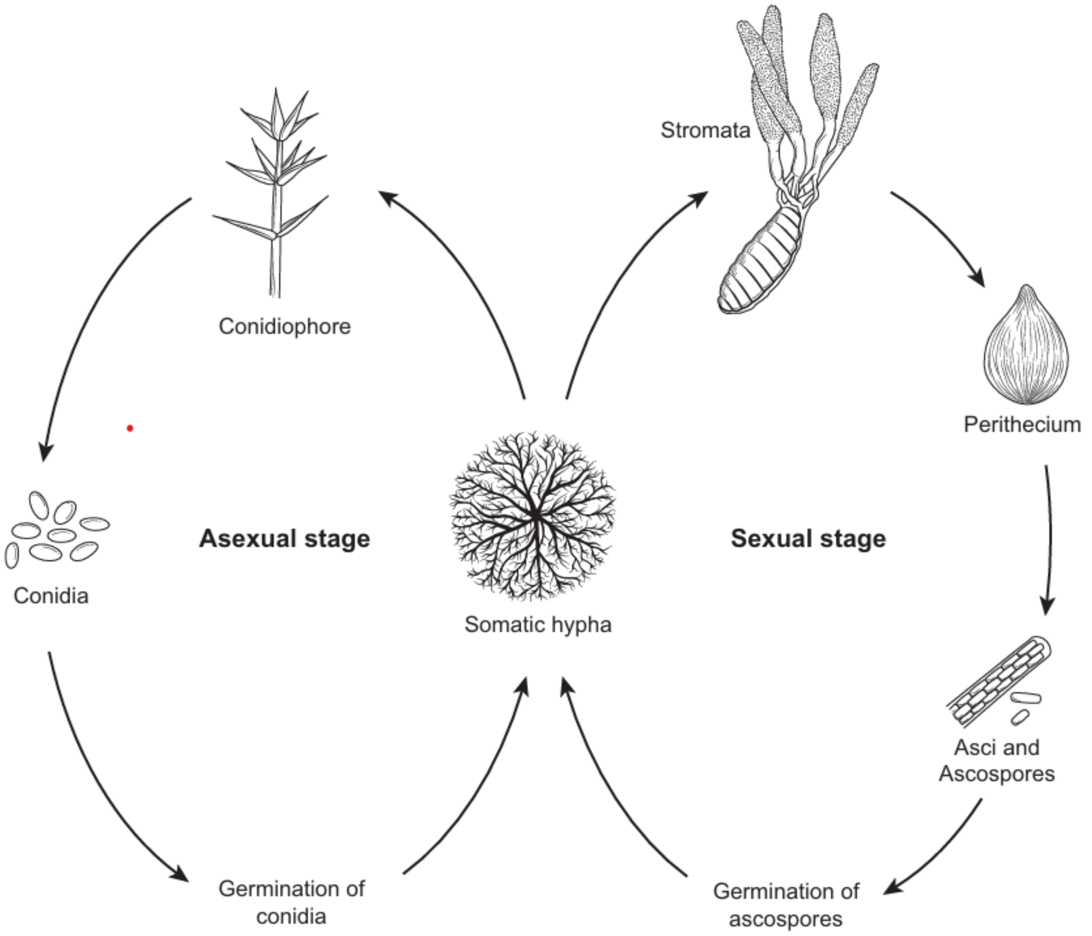

Fig. 1. Reproductive stages of Hypocreales. (Image: Vega & Kaya, 2012).

Hypocrealean fungi have an asexual state and a sexual state in their life cycles, with the latter usually produced very rarely (Vega & Kaya 2012). The asexual state usually allows fungi to reproduce more rapidly and disperse in stable environments, while sexual reproduction promotes gene recombination and genetic diversity. One curious fact is that these reproductive states have caused a lot of confusion on the taxonomy of this group in the past since fungi of the same species but in different reproductive states have been identified as different species or even different genera (Blackwell 2010, Hawksworth *et al*. 2011, Wijayawardene *et al*. 2018). To resolve this problem, mycologists established the concept called "One fungus = One name (1F1N)" where the name for the asexual state is not considered valid if the name of the sexual state is widely recognized (Hawksworth 2011, Hawksworth *et al*. 2011).

Two types of entomopathogenic fungi can be discriminated within the order Hypocreales, considering their reproductive strategies and host specificity (Vega & Kaya 2012, Kryukov, Yaroslavtseva & Glupov 2021). First, there are generalist pathogens that are eurytopic and mostly found in their asexual state (Araújo & Hughes 2016, Kryukov, Yaroslavtseva & Glupov 2021). These are represented by fungi widely used for biological control strategies, such as *Metarhizium anisopliae* (Metschn.) Sorokin, 1883 (Clavicipitaceae); *Beauveria bassiana* (Bals.-Criv.) Vuill., 1912 (Cordycipitaceae) and *Cordyceps fumosorosea* (Wize) Kepler, B. Shrestha & Spatafora, 2017 (Cordycipitaceae) (Islam *et al*. 2021). The second type are represented by specialist, obligatory and stenotopic pathogens with complex life cycles. This type is exemplified by *Ophiocordyceps* Petch, 1931 (Ophiocordycipitaceae) (Araújo & Hughes 2016, Hughes & Araújo 2016, Kryukov, Yaroslavtseva & Glupov 2021) and *Akanthomyces* Lebert, 1858 (Cordycipitaceae) (Aini *et al*. 2020, Wang *et al*. 2024). All of these fungi need to have a mechanism to infect their hosts and acquire the nutrients required to complete their life cycles (Vega & Kaya 2012, Araújo & Hughes 2016).

Generally, the infection process on insect hosts begins with spore (conidia or blastopore) attachment to the host cuticle, which is mediated by fungal proteins (Vega & Kaya 2012, Kryukov, Yaroslavtseva & Glupov 2021). Following this stage, the spore germinates and differentiates into a germ tube (with or without the subsequent formation of a structure named appresorium) (Kryukov, Yaroslavtseva & Glupov 2021). Then, the fungus penetrates the cuticle using mechanical pressure exerted by a thin penetration peg in conjunction with cuticle-degrading enzymes until it reaches the hemocoel (Ortiz-Urquiza & Keyhani 2013, Kryukov, Yaroslavtseva & Glupov 2021). Once inside the hemocoel, the fungus differentiates itself into either yeast-like blastospores or hyphal bodies and starts to consume the needed nutrients for its development (Vega & Kaya 2012). At this stage, the fungus may produce secondary metabolites that can damage the host's tissues and disrupts its physiology, eventually leading to host death (Vega & Kaya 2012, Ortiz-Urquiza & Keyhani 2013, Kryukov, Yaroslavtseva & Glupov 2021).

Some specialized entomopathogenic fungi can employ additional mechanisms to reach an environment where their growth, reproduction and dispersal are facilitated. These fungi can promote alterations in their host's regular behaviour as a consequence of infection (host reaction to disease) or by actively secreting compounds that induce behavioural changes (Hughes *et al*. 2016, de Bekker *et al*. 2015, 2017a 2017b). Since these pathogens modify their host’s phenotype to express their genotype and increase their own fitness, this kind of interaction fits the ‘parasite extended-phenotype’ as defined by Dawkins (1982). The highest diversity of behaviour-manipulating fungi is found in the order Hypocreales and the phylum Zoopagomycota (subphylum Entomophtoramycotina) (Hughes *et al*. 2016). Their origins are thought to be multiple and independent over evolutionary time (Vega & Kaya 2012, Hughes *et al*. 2016, Araújo & Hughes 2019). This subject is attracting interest and curiosity from scientists and the general population nowadays, especially regarding the manipulative fungi *Ophiocordyceps* (Ascomycota: Hypocreales) (Andersen *et al*. 2009, Evans *et al*. 2011a, Hughes *et al*. 2016; de Bekker *et al*. 2019).

1.3 HYMENOPTERA-*Ophiocordyceps* INTERACTIONS

1.3.1 Hymenoptera

Hymenoptera (ants, bees, sawflies and wasps) is one of the most diversified orders of insects, with approximately 150,000 described species (Huber *et al.* 2017). The order arose about 280 million years ago in the Permian period with the earliest hymenopterans being plant-feeding insects (Blaimer *et al.* 2023). Over evolutionary time, these insects diversified and acquired different lifestyles, including primary and secondary phytophagy, parasitoidism and predation, which reflect the ecosystem services they provide (Huber *et al.* 2017, Brock *et al*. 2021). Within the order Hymenoptera, three traditional groups can be considered in terms of their lifestyles and morphological adaptations; one is the suborder Symphyta (less derived plant-feeding hymenopterans without the wasp waist); the other two, within the suborder Apocrita (with the characteristic wasp waist that permits articulation of the abdomen and its ovipositor or stinger), are the artificial Parasitica (endoparasitoids) and the monophyletic Aculeata (stinging ectoparasitoids, pollen feeders and predators) (Branstetter *et al.* 2017, Blaimer *et al.* 2023). Note that all ants, bees and social wasps are found within the clade Aculeata (Branstetter *et al.* 2017, Blaimer *et al.* 2023).

The majority of hymenopteran families (in the taxonomic sense) are solitary, with varying degrees of parental care, but only in the clade Aculeata is it possible to find social insects, represented by all ants (Formicidae) and some groups within Vespidae (wasps) and Apidae (bees) (Huber *et al.* 2017, Blaimer *et al.* 2023). In their multigenerational colonies, these insects divide tasks related to reproduction, provision and defense to raise their offspring (O'Donnel 1998 Beshers & Fewell 2001). Regarding defense, social insect colonies face threats from competitors and parasites, with the latter being one of the major constraints to their fitness. They have therefore evolved behaviours to avoid, resist or tolerate negative interactions with these organisms (Abbot 2021). In parasitic interactions, individual and social behaviours of the hosts such as grooming, secretion of antimicrobial peptides and removal of infected nest mates can be performed to avoid infection (De Roode & Lefèvre 2012, Diez *et al.* 2014, Cremer *et al.* 2018). However, some parasites can employ strategies to overcome these defensive mechanisms, exploit their hosts and complete their life cycles. This is the case with some species of *Ophiocordyceps* associated with hymenopteran hosts (Andersen *et al*. 2009, Hughes *et al*. 2016).

1.3.2 *Ophiocordyceps*

Ophiocordyceps is one of the most diversified genera of the hypocrealean fungi, with approximately 350 species (Crous *et al*. 2004, Quandt *et al*. 2014, Mycobank 2025). It probably arose about 100 million years ago (Sung *et al*. 2008). The genus was initially proposed by Petch (1931) and included all the *Cordyceps* (Cordycipitaceae) fungi that had septate ascospores that would not turn into part-spores and that presented asci with inconspicuous apical caps (Petch 1931,1933). Later, Sung *et al*. (2007) separated and raised the family Ophiocordycipitaceae including all the *Ophiocordyceps* representatives that presented mostly firm and darkly pigmented stromata (Fig. 1A), in contrast to the fleshy and brightly pigmented stromata of *Cordyceps* (Fig. 1B). Petch's consideration of ascospores being septate or not has been put to one side, so *Ophiocordyceps* representatives with ascospores that divide into part-spores are now included within the genus (Sung *et al.* 2007).

Fig. 2. A. Firm and dark stromata of *Ophiocordyceps*. **B.** Fleshy and bright stromata *Cordyceps*. (Photos: João Araújo).

Fungi within the *Ophiocordyceps* are almost exclusively entomopathogens and are associated with nine insect orders (Hughes *et al*. 2016, Araújo & Hughes 2019). The best known species is *Ophiocordyceps sinensis* (Berk.) Sung et al. (2007), which is a pathogen of Lepidoptera. This species has been extensively researched due to medical applications of compounds these fungi produce as pharmaceuticals (Shrestha *et al.* 2013, Baral 2017, Xu *et al.*, 2016 Xue *et al.* 2024) and it is considered to be one of the most expensive fungus species in the world, selling for 31,000 USD/kg to 145,000 USD/kg (Cunningham & Long 2019, Li *et al*. 2021). As mentioned above, some *Ophiocordyceps* species are able to manipulate their hosts to achieve a suitable environment where they can grow, reproduce, disperse and infect new hosts (Hughes *et al*. 2016, Araújo & Hughes 2019). This is the case for the *Ophiocordyceps unilateralis* clade, the so-called zombie-ant fungus, which has also been a subject of interest to scientists and the general public alike because of the behavioural manipulation promoted by taxa within this clade on ant host (Andersen *et al.* 2009, Araújo & Hughes 2016, Hughes *et al.* 2016, Araújo *et al.* 2018).

All of these fungi are found in natural environments in their asexual or sexual states, which may be very different morphologically from one other (Evans 1982). In the past, these different *Ophiocordyceps* reproductive states were identified as different species (Evans 1982). Entomopathogenic genera such as *Hirsutella*, *Hymenostilbe*, *Paraisaria*, *Synglicladium* and *Sorosporella* were previously recognized as being the asexual states of *Ophiocordyceps* and their names were considered valid when two names for different states of the same fungi were accepted (Sung *et al*. 2007, Quandt *et al*., 2014). However, after the conception of "One fungus = One name (1F1N)", these genera names were considered invalid and should no longer be used in new descriptions (Hawksworth 2011, Hawksworth *et al.* 2011). Currently, the asexual characteristics of these former genera are helping to identify and separate the clades within the genus (Araújo *et al*. 2018; 2020).

1.3.3 *Ophiocordyceps* and ants

Ophiocordyceps unilateralis (Tul. & C. Tul.) Petch, 1931 is widely known for its manipulation of the behaviour of ants, especially those in the tribe Camponotini (Hymenoptera: Formicidae) (Andersen *et al*. 2009, Evans *et al*. 2011a). After infecting their hosts, these fungi promote what is known as 'summit disease' (Hughes *et al*., 2016). They manipulate hosts' behaviour and make them leave their colonies, climb to an elevated position where they will lock their mandibles (in a death-grip) and die attached to the vegetation (Fig. 2) (Andersen *et al*. 2009, Hughes *et al*. 2016). This manipulation strategy allows the fungus to develop its reproductive structures and release its spores from an elevated position (i.e. the attached host) onto a substrate where new hosts will be infected (Andersen *et al.* 2009). Aggregations of dead ants can occur at high densities in tropical forests (the so-called graveyards) and these can be permanent in the same location for many months or years (Pontoppidan *et al.* 2009, Loreto *et al.* 2014, Neto *et al.* 2019 Will *et al.* 2022). This phenomenon reveals the chronic nature of the infections promoted by *O. unilateralis* in its ant hosts (Andersen *et al.* 2012, Loreto *et al.* 2014).

Fig. 3. Diversity of morphotypes and ant hosts within *Ophiocordyceps unilateralis sensu lato*. (Photos: João Araújo).

For many years, it was suspected that *O. unilateralis* could represent a species complex to be discovered, since the collected specimens presented variations in their morphology (Fig. 2) (Petch 1931, Kobayasi 1941, Mains 1958, Samson *et al.* 1982, Evans & Samson 1984, Evans *et al.* 2011a, Araújo *et al.* 2015). However, only in the last decade, Evans *et al.* (2011a) were able to reveal a hidden diversity within this taxon, with the description of four new and distinct species of *Ophiocordyceps* infecting *Camponotus* ants. It was hypothesised by Evans *et al.* (2011a) that each ant species within the Camponotini could be infected by a specific *Ophiocordyceps* species. Since then, new species have been described worldwide that are strictly associated with their hosts (and not described for other hosts), giving more strength to this hypothesis (Luangsa-ard *et al.* 2011, Kepler *et al.* 2011, Kobmoo *et al.* 2012, 2015, Sanjuan *et al*. 2015, Araújo *et al*. 2018, 2020). A recent study, however, has shown that this interaction might not be as strict or host-specific as previously thought (Tang *et al.* 2023).

Two clades of myrmecophilous *Ophiocordyceps* are recognized according to their formerly described asexual states (or anamorph) and morphology of sexual spores (Fig. 3). Those fungi with a *Hirsutella*-like state are included in the *O. unilateralis* clade (or *sensu lato*), which is divided into the *O. unilateralis* core clade and the *O. kniphofioides* sub-clade (Evans & Samson 1984 Araújo & Hughes 2017). The former infects exclusively Camponotini ants and can be recognized by brown or black stromata that emerge laterally on the stalk (i.e. *unilateralis*). The latter can infect other ant tribes (e.g. Cephalotini, Dacetini, Dolichoderini and Paraponerini) and is usually recognized by the development of single ascostromata that grows laterally on the host's thorax, with the fertile portion covering all sides of the stalk (360º) (Evans & Samson, 1982 Araújo *et al.* 2018). The other clade, with the *Hymenostilbe*-like asexual state, is the subgenus *Neocordyceps* Kobayasi (1941), also recognized as the *O. sphecocephala* clade sensu Sung *et al.* (2007) (Araújo & Hughes 2017). This clade is associated with different insect orders, but infects mainly Hymenoptera with little evidence of host manipulation, since the majority of hosts are usually found on the ground. In ants, these fungi were found infecting Cephalotini, Dolichoderini, Ponerini and Pseudomyrmecini ants (Araújo *et al.* 2020). There are at least three morphological complexes associated with ants within this clade: *O. myrmecophila/irangiensis*, characterized by having a single slender stalk emerging from the pronotum, with a yellow to orange ovoid fertile head at the end; *O. australis*, recognized by the long thin black stipe with a fertile crimson head; and *O. lloydii* with one or two light-yellow fruiting bodies terminal on the stalk, with vertically arranged perithecia (Araújo & Hughes, 2017).

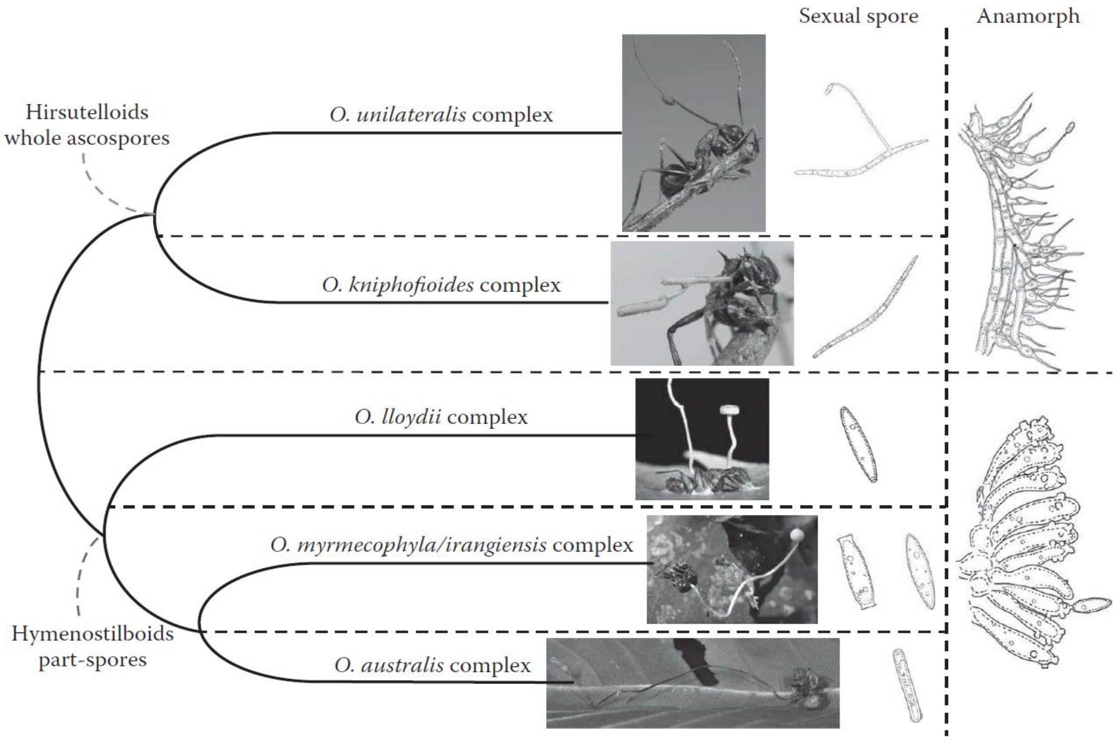

Fig. 4. Main lineages of myrmecophilous *Ophiocordyceps* with two origins related to the morphology of sexual ascospores and asexual states. (Image: Araújo & Hughes, 2017).

The manipulating fungi within *O. unilateralis s.l.* probably evolved from a non-manipulating *Ophiocordyceps* that parasitized coleopteran insects (Araújo & Hughes 2019). The diversification of hosts might have occurred through host jumps (common in the Hypocreales) from beetles to ants that shared the same ecological niche (Araújo & Hughes 2019). Additionally, the process of behavioural manipulation might have evolved as a strategy to avoid the selection pressure promoted by ants and their collective immune defense strategies (Araújo & Hughes 2019). Corroborating this hypothesis, some studies have shown that the fungus cannot develop inside its host's nest or in places with different conditions than the place where it manipulates its host to attach (i.e. plant substrates above ground) (Andersen *et al*. 2009; Loreto *et al*. 2014, Andriolli *et al*. 2019).

After the pioneering study conducted by Evans *et al*. (2011a), it has been hypothesised that hundreds of species are yet to be discovered within the genus *Ophiocordyceps* (Evans *et al*. 2011b). Tropical forests, like the Brazilian Amazon and Atlantic Forests, might be a refuge for an undiscovered diversity of *Ophiocordyceps* and other fungi (Evans *et al*. 2011b). However, the lack of systematic collection of entomopathogenic fungi in these environments is still a problem for the description of new species and their inclusion in diversity estimations

(Hawksworth & Lücking 2017, De Wint *et al.* 2024). Ecological research concerning *Ophiocordyceps*-host interactions might also be affected by the scarcity of research effort in tropical forests, given that these interactions are frequently recorded in these habitats and might be favored by the ecological conditions in these environments (Evans 1982, Evans *et al.* 2011b, Sanjuan *et al.* 2015, Hawksworth & Lücking 2017).

1.3.4 *Ophiocordyceps* and wasps

Wasps can also be hosts of parasitic *Ophiocordyceps*. These insects form a paraphyletic group within the suborder Apocrita (excluding Formicidae and Anthophila) with approximately 103,000 described species (Branstetter *et al.* 2017, Blaimer *et al.* 2023). This group is comprised of wasps that have different life-history traits, including endoparasitoids (clade Parasitica), ectoparasitoids, kleptoparasites, predators and pollen-feeders (Aguiar *et al.* 2013, Branstetter *et al.* 2017, Brock *et al.* 2021, Blaimer *et al.* 2023). The majority of wasps are solitary, but in the Vespidae (Hymenoptera: Aculeata) different levels of sociality (ranging from primitively to highly eusocial) are registered for three subfamilies (Polistinae, Stenogastrinae and Vespinae), comprising about 1,000 species of social wasps (Turillazzi 1989, Bell & Sumner 2013, Taylor *et al.* 2018, Wyatt *et al.* 2023).

To date, twelve species of *Ophiocordyceps* are recorded as being pathogens of wasps (Shrestha *et al.* 2017, Long *et al.* 2021, Chawngthu *et al.* 2021). Their asexual states are mainly recognized as *Hirsutella*-like or *Hymenostilbe*-like fungi (Sung *et al.* 2007, Montalva *et al.* 2017, Mollá *et al.* 2020). Infection records have been made in different countries, such as Austria, Brazil, China, Colombia, India, New Zealand, Thailand and Vietnam (Shrestha *et al.* 2017, Hyde *et al.* 2018, Haelewaters e*t al.* 2020, Somavilla *et al.* 2020, Long *et al.* 2021, Zohmangaiha *et al.* 2021, Barbosa & Somavilla 2022, Mai *et al.* 2022, Reason *et al.* 2022; Thuan *et al.* 2023). In Brazil, the species *Ophiocordyceps humbertii* (C.P. Robin) Sung *et al.* (2007) is frequently found in tropical forests infecting mainly social wasps (Somavilla *et al.* 2020, Sobczak *et al.* 2020, Barbosa & Somavilla 2022). In addition, Somavilla *et al.* (2019) recorded solitary wasps of the families Crabronidae and Pompilidae infected by *O. humbertii*.

All social wasps in Brazil belong to the subfamily Polistinae, distributed in tree tribes (Polistini, Mischocyttarini and Epiponini) and 21 genera (Somavilla *et al.* 2021). In this subfamily, wasps can exhibit levels of eusociality from simple to highly complex (Carpenter & Marques 2001, Bell & Sumner, 2013). All of them are opportunistic and generalist foragers, able to predate on different arthropods to feed their offspring and visit a variety of plants to collect nectar, thus being involved in a wide array of ecological interactions (Detoni & Prezoto 2021, Brock *et al*. 2021). It is presumed that these social wasps have their behaviour manipulated by *O. humbertii* species in a manner similar to that in the *O. unilateralis s.l* - ant system, given that they are frequently found attached by their mandibles to plant substrates in tropical forests (Hughes *et al*. 2016, Araújo & Hughes 2019, Somavilla *et al*. 2019, 2020, Sobczak *et al*. 2020, Barbosa & Somavilla 2022).

It is salient to compare the life-history traits of Polistinae social wasps and Camponitini ants, the most studied insects in manipulative interactions with *Ophiocordyceps*, to understand how these fungi interact with their hosts. Ants are highly eusocial, with their colonies comprising thousands of individuals (Hölldobler & Wilson 1990). Workers are wingless and forage for sugar and protein sources in trails constructed on the forest floor, trees and other types of substrates (Loreto *et al*. 2014, Hansen & Akre 2019). In fact, carpenter ants are infected by *O. unilateralis s.l.* by having contact with a specialized sticky-capped spore that germinates on the surface it lands on (Evans *et al*. 2011a, Araújo *et al*. 2018). The ants generally build sheltered nests on soil or underneath stones, in dead wood or living trees (Hansen & Akre 2019, Rossi & Feldhaar 2021). In comparison, wasps display different levels of sociality and their colonies can vary from dozens to millions of individuals according to the genus (Zucci *et al*. 1995, Bell & Sumner 2013, Wyatt *et al*. 2023). They are generalist predators and forage through flight in the forest for sugar and protein sources to feed them and their offspring (Prezoto *et al*. 2019, Brock *et al*. 2021). The nesting behaviour of social wasps varies widely, since they can build exposed or enveloped hanging nests with different architectures and substrates (Wenzel 2020, Barbosa *et al*. 2021, Wyatt *et al*. 2023). The infection process by *O. humbertii* is yet to be discovered and all of these traits may impact the way social wasps are infected and also their behaviours while infected.

According to Petch (1935), *O. humbertii* was initially named as *Cordyceps humberti* by Robin based on an illustration of an infected *Vespa cincta* from Senegal presented in Saussure's *Monographie des Guêpes Sociales* (Saussure, 1853-58) (Fig. 4), without giving a formal description. In 1935, Petch conducted a revision of this fungus and published a formal description, based on the examination of a hymenopteran specimen from Sarawak, and transferred it to the genus *Ophiocordyceps* (Petch 1935). This fungus was characterized by having two clavae, each one arising from the insertion points of the wasp's wings with fertile heads, and longer, slender claves arising from the abdominal sutures (Fig. 4A) (Petch 1935).

The latter structures were then associated with *Hirsutella saussurei* (Cooke ex Sacc.) Speare 1920 (Fig. 4B) and Petch confirmed this as being the asexual state of *O. humbertii* (Petch 1935).

Fig. 5. Illustrations of *Ophiocordyceps humbertii* in Saussure's Monographie des Guêpes Sociales. **A.** *Vespa cincta* infected with the sexual state of *O. humbertii*. **B.** *Polistes americanus* infected with the asexual *H. saussurei* state.

It was hypothesised that *O. humbertii* also evolved from non-manipulating *Ophiocordyceps* of coleopteran larvae and that an intermediate host jump might have occurred from social wasps to ants in the evolution of *O. unilateralis s.l* (Araújo & Hughes 2019). However, as mentioned above, the steps of the infection process and sequence of behaviours performed by social wasps while infected by *O. humbertii* are still to be discovered. Based on morphological variation across recorded and deposited specimens in scientific collections worldwide (Somavilla *et al*. 2020, Sobczak *et al*. 2020, Barbosa & Somavilla 2022, Araújo, J., personal communication), it is also suspected that *O. humbertii* might be a new species complex to be unravelled, much like *O. unilateralis s.l.*. Multigene phylogenies have been a useful tool to solve taxonomic problems and discover new species within *Ophiocordyceps* (Wen *et al*. 2016, Fan *et al*. 2021, Long *et al*. 2021, Tang *et al*. 2023, Mongkolsamrit *et al*. 2023). Considering the importance of systematic collections of entomopathogenic fungi in biodiversity hotspots to increase the knowledge about hidden fungi diversity, Brazil's tropical forests are the perfect location to conduct a taxonomic and phylogenetic review concerning *O. humbertii* and its wasp hosts.

1.3.5 *Ophiocordyceps* and bees

There is little information in the literature concerning the diversity of *Ophiocordyceps* fungi parasitizing bees. Shrestha *et al.* (2017) reviewed *Ophiocordyceps* infecting Hymenoptera and found that the bees *Bombus equestris* Thompson, 1872 and *Apis sp.* were recorded as being infected by *Ophiocordyceps oxycephala* (Penz. & Sacc.) Sung *et al.* (2007) and *Ophiocordyceps sphecocephala* (Klotzsch ex Berk.) Sung *et al.* (2007), which also infect wasps, mostly in Asia. Somavilla *et al.* (2019) also recorded Crabonidae (Hymenoptera: Apoidea) representatives, a group that is closely-related to bees, infected by *O. humbertii* in Brazil. Besides that, there is no other information available so far. Considering the diversity of *Ophiocordyceps* parasitizing ants and wasps, it is possible that there is also hidden diversity of these fungi infecting bees. It is presently a mystery why there are so few records of bees being infected by *Ophiocordyceps*. Broadly, this could be due differences in their ecologies and life-histories traits when compared to other hymenopterans or it could be due simply to a lack of published records and research effort on this matter.

1.4 MAIN OBJECTIVES OF THE THESIS

All ants, aculeate wasps and bees descend from a common ancestor (Hymenoptera: Aculeata) (Branstetter *et al.* 2017, Blaimer *et al.* 2023). It is possible that the evolution and diversification of Hymenoptera-parasitic *Ophiocordyceps* might have been favored by the diversification of these hosts. The morphophysiological similarities between these insects and their co-existence in the same habitat might have favored the occurrence of host jumps by fungi (Araújo & Hughes 2019). This diversification process might also have been favored by the strong selective pressure that could arise from social immune behaviours (Cremer *et al.* 2018, Araújo & Hughes 2019). It is hypothesised that *Ophiocordyceps* might have evolved behavioural manipulation as a strategy to infect social insects outside of their colonies and be maintained developing within the host population (Araújo & Hughes 2019). However, there is still a lack of information concerning species diversity and host specificity of Hymenoptera-parasitic *Ophiocordyceps*, especially considering wasps and bees. Therefore, more studies on fungal collections, specimen reviews and multigene phylogenies are needed to better understand and describe the diversity of insect-associated fungi. In this thesis, we aimed to explore the diversity of *Ophiocordyceps* species associated with hymenopterans.

For Chapter II which is entitled "**There is gold in the graveyard: a new lineage of zombie-ant fungi in the genus *Ophiocordyceps* (*Ophiocordycipitaceae*: *Hypocreales*) from Minas Gerais, Brazill**", we describe a new species and novel lineage of zombie-ant fungus infecting *Acanthoponera* (Formicidae: Heteroponerini) ants at Estação Ecológica do Tripuí, Ouro Preto, Minas Gerais, Brazil. This is also the first record of Heteroponerini ants being infected by *Ophiocordyceps* fungi, expanding the host range for myrmecophilous *Ophiocordyceps*.

For Chapter III, entitled "**A light into a forgotten graveyard: Cryptic diversity within the wasp-parasitic *Ophiocordyceps humbertii* (*Ophiocordycipitaceae*: *Hypocreales*)**", we describe seven new species infecting social wasps in the Brazilian Atlantic Forest, revealing a cryptic diversity within *O. humbertii* sensu lato. One of the novel species is closely related to the hemipteran-pathogenic *H. citriformis* clade. Our results also support the existence of at least four novel lineages and expand the current knowledge of host association of wasp-parasitic *Ophiocordyceps*.

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2 CHAPTER II: There is gold in the graveyard: a new lineage of zombie-ant fungi in the genus *Ophiocordyceps* (*Ophiocordycipitaceae*: *Hypocreales*) from Minas Gerais, Brazil*

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2.1 ABSTRACT

Ophiocordyceps serves as a key model for studying cryptic fungal diversity and behavioural manipulation of hymenopterous insects. Here, we describe *Ophiocordyceps acanthoponerae*, a newly discovered species infecting *Acanthoponera mucronata* (*Heteroponerini*: *Formicidae*) in a Brazilian Atlantic rainforest-Cerrado ecotone. Morphological analyses revealed mixed traits characteristic of *Ophiocordyceps* lineages associated with ants and wasps, including leaf biting behaviour manipulation, dark brown ascostromata covering 360º of the stalk, ascospores producing capilliconidia and hirsutelloid asexual morphs. Phylogenetic analyses based on four genomic regions (SSU, LSU, *TEF* and *RPB1*) placed this species outside the traditional myrmecophilous hirsutelloid clades *O. unilateralis* and *O. kniphofioides*, and within a novel clade closely related to the wasp pathogen *O. humbertii*. This discovery represents the first record of *Ophiocordyceps* infecting *Heteroponerini* and highlights an unexplored lineage of manipulative fungi. Our findings expand the known host range for myrmecophilous *Ophiocordyceps* and underscore the importance of studying fungal diversity in under-sampled ecological niches.

KEYWORDS: *Acanthoponera*, Atlantic rainforest-Cerrado ecotone, *Heteroponerini*, host manipulation, *Ophiocordyceps acanthoponerae sp. nov.,* systematics

2.2 INTRODUCTION

The order *Hypocreales* (*Ascomycota*) comprises most of the fungal species that are known as insect pathogens, or entomopathogenic fungi distributed predominantly across three families: *Clavicipitaceae*, *Cordycipitaceae* and *Ophiocordycipitaceae* (Sung *et al.* 2007, 2008). The ancestral trait of the order is an association with plants (decomposers, phytopathogens, epiphytes and endophytes), with the insect-associated lineages arising around 160 million years ago (Sung *et al.* 2008). The evolution of hypocrealean entomopathogens probably occurred via the process of inter-kingdom host-jumping (or host-switching), in which closely-related fungi are able to exploit nutritional resources from even distantly related host taxa (e.g. animals, plants and other fungi) that share the same habitat or niche (Spatafora *et al.* 2007, Sung *et al.* 2008, Yu *et al.* 2021, Wei *et al.* 2022). This phenomenon is considered to be a major driver of diversity, lifestyle differentiation and speciation for the entomopathogenic fungi (Thines 2019, Wei *et al.* 2022).

Ophiocordyceps (*Ophiocordycipitaceae*: *Hypocreales*) is a flagship genus for the study of cryptic fungal diversity, particularly in tropical forests (Evans *et al.* 2011a). Current hypotheses suggest that *Ophiocordyceps* arose nearly 100 million years ago (Sung *et al.* 2008) and is almost exclusively comprised of pathogens of insects (nine insect orders) with approximately 350 legitimate species described (Sung *et al.* 2007, Quandt *et al.* 2014, Araújo & Hughes 2019, Mycobank 2025). The diversification process of *Ophiocordyceps* might have been favoured by successive and independent host-jumps from the ancestral host-association with *Coleoptera* larvae to other orders of insects and also between taxa within these orders (Araújo & Hughes 2019). A characteristic of the genus is firm, mostly darkly pigmented stromata, in contrast to the fleshy and brightly pigmented stromata of *Cordyceps* (Sung *et al.* 2007). The most widely known species is the lepidopteran pathogen *O. sinensis*, which has been studied extensively because of its pharmaceutical applications, especially in traditional Chinese medicine (Shrestha & Bawa 2013, Baral 2017). Within the genus, *O. unilateralis s.l.* —the so-called "zombie-ant fungus"— has also been a subject of interest to scientists and the general public alike because of its behavioural manipulation of the ant host. This has inspired successful video games and television series, as well as number of books (Andersen *et al.* 2009, Araújo & Hughes 2016, Hughes *et al.* 2016, Araújo *et al.* 2018).

Following infection by *O. unilateralis* and related taxa, the ant climbs to an elevated position in the forest understorey where it bites into the plant stem or leaf (the so-called death-grip; Hughes *et al.* 2011); dying attached to the vegetation and locked into it by its mandibles (Evans 1982, Evans & Samson 1982, Andersen *et al*. 2009). This manipulation strategy allows the fungus to optimise spore release from an elevated position onto the forest floor where foraging hosts are found (Andersen *et al*. 2009, Hughes *et al*. 2016). Dead ants are commonly found aggregated at high densities —especially, in tropical forests— forming "graveyards" or "cemeteries", which can persist in the same location for months or even years (Evans & Samson 1982, Pontoppidan *et al*. 2009, Loreto *et al*. 2014, Neto *et al*. 2019, Will *et al*. 2023). These aggregations are constantly resupplied with new infected hosts, revealing the chronic or enzootic nature of *Ophiocordyceps*-ant interactions at the colony level, rather than epizootic events (Andersen *et al.* 2012, Loreto *et al.* 2014).

Evans *et al.* (2011b) determined that the fungus previously identified as *O. unilateralis* was, in fact, a complex of species strictly associated with ants of the tribe *Camponotini* (*Formicinae*: *Formicidae*). Evans *et al.* (2011a, b) also hypothesised that species within the complex were each restricted to one species of host and, therefore, that on a global scale, tens or hundreds of species would eventually be delimited within this complex. If the one-species-one-fungus hypothesis holds, then the cosmopolitan nature of the *Camponitini* across the tropics —with almost 2,500 described species with a wide range of habitats (Feitosa & Dias 2024; Bolton 2025) — seems to make this conjecture feasible. Since 2011, new fungal species strictly associated with a particular *Camponotini* host are constantly being discovered and described worldwide, increasing the support for this hypothesis (Luangsa-ard *et al.* 2011, Kepler *et al.* 2011, Kobmoo *et al.* 2012, 2015, Sanjuan *et al.* 2015, Araújo *et al.* 2015, 2018). A recent study, however, has shown that this interaction might not be as strict or host-specific as previously thought (Tang *et al.* 2023) and more research is needed to determine the level or levels of host specificity.

These new myrmecophilous species associated with *Camponotini* are currently inserted in what is now recognized as the *O. unilateralis* clade (Luangsa-ard *et al.* 2011, Kepler *et al.* 2011, Kobmoo *et al.* 2012, 2015, Sanjuan *et al.* 2015, Araújo *et al.* 2015, 2018, Tang *et al.* 2023). This considers their phylogenetic proximity and shared morphological traits, especially ascospore morphology and their *Hirsutella*-like (or hirsutelloid) asexual states, but also host specificity (Araújo & Hughes 2017, Araújo *et al.* 2018). Among all *Ophiocordyceps* lineages that infect ants, the *O. unilateralis* core clade and the *O. kniphofioides* sub-clade are, to date, the only hirsutelloid lineages that induce 'death-grip' manipulation in their hosts (Araújo & Hughes 2017, Araújo *et al.* 2018, 2020). The *O. unilateralis* core clade exclusively infects *Camponotini* and can be recognized by the brown or black fertile region (or ascoma) that form laterally on the stalk (or stroma) (Evans & Samson 1984, Araújo & Hughes 2017, Araújo *et al.* 2018). The *O. kniphofioides* sub-clade comprises five known species and infects other ant tribes (*Cephalotini*, *Dacetini*, *Dolichoderini* and *Paraponerini*) and produces a single ascoma that grows laterally from the host's thorax, with the fertile portion covering all sides of the stalk (Evans & Samson 1982, Araújo & Hughes 2017, Araújo *et al.* 2018).

The myrmecophilous and manipulative *Ophiocordyceps* (*O. unilateralis* core clade and *O. kniphofioides* sub-clade) are closely related to another hirsutelloid lineage, composed of *O. humbertii*, which infects and induces behavioural manipulation in social wasps similar to that seen in *O. unilateralis* infections (Petch 1935, Araújo & Hughes 2019, Somavilla *et al.* 2020, Sobczak *et al.* 2020, Barbosa & Somavilla 2022). It is considered that behavioural manipulation might have occurred first in social wasps, rather than in ants, during the evolution of manipulative *Ophiocordyceps* (Araújo & Hughes 2019). All hymenopterans descend from a common ancestor, so it is possible that the morpho-physiological similarities between them follows their co-existence in the same habitat (host-habitat hypothesis) which could have facilitated host-jumps during the evolution of *Ophiocordyceps* (Branstetter *et al.* 2017, Huber *et al.* 2017, Araújo & Hughes 2019). It is also possible that the evolution of manipulative *Ophiocordyceps* was favoured by the social behaviour exhibited by the hosts, given that behavioural manipulation is considered to be a parasite strategy that helps to cope with social immunity inside colonies (Loreto *et al.* 2014, Araújo & Hughes 2019). To address these questions, it is important to expand our knowledge of *Ophiocordyceps*-infecting social hymenopterans and, in particular, those where there are features in common or in closely-related lineages with a scarcity of taxon sampling.

Here, we describe a new species of *Ophiocordyceps* infecting *Acanthoponera mucronata* (*Heteroponerini*: *Ectatomminae*: *Formicidae*) in Brazil: an ant species for which life-history and ecological data are scarce. This species has morphological traits similar to known myrmecophilous *Ophiocordyceps* species but also to the wasp-parasitic species *O. humbertii*. Our phylogenetic analyses have revealed that this new taxon also represents a new origin of myrmecophily and of host-manipulative *Ophiocordyceps* species, closely-related to the wasp pathogen, *O. humbertii*. This is also the first record of ants belonging to the *Heteroponerini* tribe being infected by *Ophiocordyceps*, expanding the host range for the genus.

2.3 MATERIALS AND METHODS

In this study, we combined morphological, ecological and molecular data for the description of the new species. Our methodologies for collection, field observations, morphological characterisation and molecular analyses are based on previous studies with other myrmecophilous *Ophiocordyceps* species (Araújo *et al*. 2015, 2018, 2020, Evans *et al*. 2011b, 2018).

2.3.1 Field collections

Collection of specimens was concentrated at the Estação Ecológica do Tripuí (EET), Ouro Preto municipality, Minas Gerais, Brazil (BR 356, km 90, R. Nossa Senhora, da Conceição, 375, 20º 23' 5'' S 43º 32' 34.8'' W) (Fig. 1). The forest reserve covers approximately 392 ha, with altitudes varying between 1,180 and 1,300 m a.s.l., and is located in the transition (or ecotone) between Atlantic Forest and Cerrado biomes, with a mixed vegetation belonging to both; including semi-deciduous mesophytic forests, Cerrado and permanent swamps (FEAM 1995, Pedralli *et al*. 1997, Werneck *et al*. 2001). The forest reserve is also hyperdiverse in arthropods, especially *Coleoptera* (Gatti & Carneiro 2019). Surveys were conducted along the "Trilha da Guarita" trail (Fig. 1) in July and October 2023, March and November 2024 and February 2025. Collections were concentrated at the same spot (20º 22' 48'' S 43º 32' 24'' W) (Fig. 1) where the infected ants formed a major graveyard. These collections consisted of careful examination of the vegetation (mainly understory shrubs), from ground level up to ca. 2 m in height. Infected ants attached to the substratum (leaves and twigs) were then transferred to sterile falcon tubes and taken to the Laboratory of Insect-Microbe Interactions at the Universidade Federal de Viçosa (LIIM-UFV) for morphological and molecular analyses. The specimens were dried in sterile falcon tubes containing filter paper and silica gel to avoid contamination by opportunistic fungi. Permits for collection and transport were provided by SISBIO (nº 86685–4) and the Instituto Estadual de Florestas (IEF; nº 037/2023). Images of the specimens were taken in situ and their positions on the vegetation were noted.

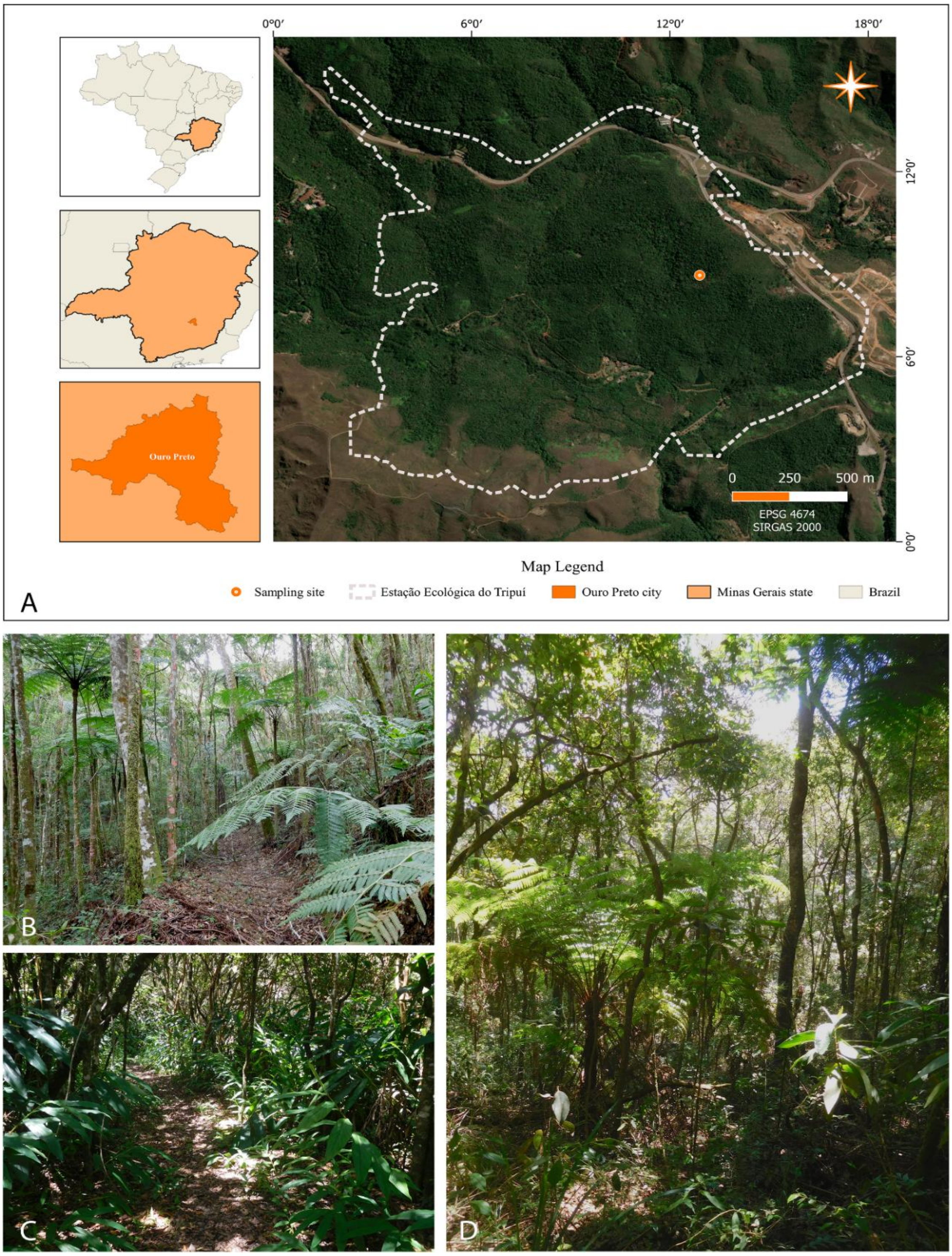

Fig. 1. Sampling site in Ouro Preto, Minas Gerais, Brazil. **A.** Map with an overview of the forest reserve, Estação Ecológica do Tripuí. **B, D.** Sampling sites at the higher and drier elevations showing the relatively open canopy and the understorey dominated by tree ferns (*Cyathea* spp.), where ant graveyards predominantly occurred. **C.** Lower, wetter parts of the trail being invaded by exotic wild ginger (*Hedychium coronarium*).

The ant hosts were identified by RMF, specialist in the taxonomy of *Heteroponerini*. Voucher specimens of *O. acanthoponerae* (Holotype and Paratypes) were deposited at the VIC Fungarium, located at UFV, at Royal Botanic Gardens Kew Fungarium (K-M: IMI) and at the Natural History Museum of Denmark, University of Copenhagen (NHMD). The specimens were transferred to Petri dishes containing filter paper and silica before being deposited in the herbaria. The remaining specimens are conserved at LIIM-UFV and at the Entomological Collection Padre Jesus Santiago Moure at the Universidade Federal do Paraná (DZUP-UFPR).

2.3.2 DNA extraction, amplification and sequencing

For molecular identification, DNA was extracted from ascostromata (ascoma + stroma) and synnemata which had been excised from the host and macerated in microtubes and then placed in liquid nitrogen. DNA extraction was performed using the Wizard™ Genomic DNA Purification Kit (Promega™, Madison, USA), following the manufacturer's recommendations. The final concentrations of the DNA extractions were determined by NanoDrop™ spectrophotometry (Thermo Scientific™, Waltham, USA).

In total, four genomic regions were amplified using polymerase chain reaction (PCR): I) the small nuclear ribosomal subunit (SSU), II) the large nuclear ribosomal subunit (LSU), III) the translation elongation factor 1-α (*TEF*), and IV) RNA polymerase II largest subunit (*RPB1*). Primers used for each region were: SSU) NS1 (5'-GTAGTCATATGCTTGTCTC-3') and NS4 (5'-CTTCCGTCAATTCCTTTAAG-3') (White *et al.* 1990), LSU) LR0R (5'-ACCCGCTGAAC-TTAAGC-3') (Hopple Jr 1994) and LR5 (5'-TCCTGAGGGAAACTTCG-3') (Vilgalys & Hester 1990), *TEF*) 983F (5'-GCYCCYGGHCAYCGTGAYTTYAT-3') and 2218R (5'- ATGACACCRACRGCRACRGTYTG-3') (Rehner & Buckley 2005), *RPB1*) RPB1-Ac: (5'-GARTGYCCDGGDCAYTTYGG-3') and RPB1_Cr (5'-CCNGCDATNTCRTTRTCCATRTA-3') (Murata *et al.* 2014).

Each 25 µL-PCR reaction contained 12.5 µL KAPA Taq ReadyMix (KAPA Biosystems™), 1 µL of each forward and reverse primers (10 ng/µL), 5 µL of DNA template, and 5.5 µL of DNAse/RNAse Free Water (ZymoBIOMICS™). The PCR reactions were placed in a Eppendorf™ thermocycler under the following conditions: for SSU and LSU, 2 min at 95 °C, 4 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 50.5 °C for 1 min, and extension at 72 °C for 2 min and final extension at 5 min at 72 °C. For *TEF*, 2 min at 94 °C, 10 cycles of denaturation at 94 °C for 30 s, annealing at 64 °C for 1 min, and extension at 72 °C for 1 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 1 min, extension at 72 °C for 1 min and final extension at 3 min at 72 °C. For *RPB1*, 2 min at 95 °C, 10 cycles of denaturation at 95 °C for 30 s, annealing at 66 °C for 1 min, and extension at 72 °C for 1 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 56.6 °C for 1 min, extension at 72 °C for 1 min and final extension at 3 min at 72 °C. After amplification, PCR products were purified and sequenced by Macrogen™ (Seoul, South Korea).

2.3.3 Phylogenetic analyses

The obtained contigs were compared to sequences of related species deposited in the NCBI (https://www.ncbi.nlm.nih.gov/) nucleotide database using BLASTn at Geneious Prime™ 2025.0.3 (Kearse *et al*. 2012) to confirm that they belonged to the genus *Ophiocordyceps*. We aligned the *O. acanthoponerae* sequences to a database with 530 OTUs from species (Supplementary material I) that broadly represent the order Hypocreales. The final database consisted of 323 SSU, 479 LSU, 360 *TEF* and 444 *RPB1* sequences.

We generated, improved (excluding gaps and removing ambiguously alignments) and combined individual gene alignments at Geneious Prime 2025.0.3. The final alignment length was 3,972 bp: with 1,242 bp for SSU, 999 bp for LSU, 1,006 bp for *TEF* and 725 bp for RBP1. We used the ModelTest-NG 0.1.7 (Darriba *et al.* 2020) on CIPRES Science Gateway 3.3 (Miller *et al.* 2012) for selecting the nucleotide substitution models, considering the lowest Akaike Information Criterion (AIC). The final dataset consisted of eight data partitions (one for SSU, one for LSU, three for *TEF* and three for *RPB1*).

Concatenated phylogenetic tree for all four genes were reconstructed performing Maximum Likelihood analysis using IQ-Tree 2.2.2.7, based on the fit-model for each region (GTR+I+G4) and generated 1,000 bootstrap replicates, (Nguyen *et al.* 2015, Chernomor *et al.* 2016, Kalyaanamoorthy *et al*. 2017, Hoang *et al*. 2018, Minh *et al*. 2020) on the CIPRES webserver. The species *Verticillium dahliae* (ATCC 16535) was used as the outgroup. The resulting consensus phylogenetic tree was visualized in FigTree 1.4.4 (Rambaut 2017) and edited in Adobe Illustrator™

Table 1. List of *Ophiocordyceps* species, vouchers, GenBank accession numbers and host associations. *Ophiocordyceps acanthoponerae* is highlighted in **bold font**.

Species	Voucher	nSSU rDNA	nLSU rDNA	*TEF*	*RPB1*	Host	Reference
Ophiocordyceps acanthoponerae	**VIMI_2023_0001**	**PV283217**	**PV283219**	**PV356076**	**PV356074**	***Acanthoponera mucronata***	**This study**
Ophiocordyceps acanthoponerae	**VIMI_2023_0002**	**PV283216**	**PV283218**	**PV356075**	–	***Acanthoponera mucronata***	**This study**
Ophiocordyceps acicularis	OSC 128580	DQ522543	DQ518757	DQ522326	DQ522371	*Coleoptera*	Quandt *et al.* (2014)
Ophiocordyceps agriotidis	ARSEF 5692	DQ522540	DQ518754	DQ522322	DQ522368	*Coleoptera*	Quandt *et al.* (2014)
Ophiocordyceps albacongiuae	RC20	KX713633	–	KX713670	–	*Hymenoptera*	Araújo *et al.* (2019)
Ophiocordyceps amazonica	HUA 186113	KJ917566	KJ917571	–	KP212903	*Orthoptera*	Sanjuan et al. (2015)
Ophiocordyceps annulata	CEM303	KJ878915	KJ878881	KJ878962	KJ878995	*Coleoptera*	Quandt *et al.* (2014)
Ophiocordyceps aphodii	ARSEF 5498	DQ522541	DQ518755	DQ522323		*Coleoptera*	Quandt *et al.* (2014)
Ophiocordyceps australis	HUA 186097	KC610786	KC610765	KC610735	KF658662	*Hymenoptera*	Sanjuan et al. (2015)
Ophiocordyceps australis	HUA 186147	KC610784	KC610764	KC610734	KF658678	*Hymenoptera*	Quandt *et al.* (2014)
Ophiocordyceps blakebarnesii	MISSOU5	KX713641	KX713610	KX713688	KX713716	*Hymenoptera*	Araújo *et al.* (2018)
Ophiocordyceps brunneipunctata	OSC 128576	DQ522542	DQ518756	DQ522324	DQ522369	*Coleoptera*	Quandt *et al.* (2014)
Ophiocordyceps buquetii	HMAS_199613	KJ878939	KJ878904	KJ878984	KJ879019	*Hymenoptera*	Araújo *et al.* (2018)
Ophiocordyceps camponoti-atricipis	A25	KX713666	–	KX713677	–	*Hymenoptera*	Araújo *et al.* (2018)
Ophiocordyceps camponoti-balzani	G104	KX713660	KX713593	KX713689	KX713703	*Hymenoptera*	Araújo *et al.* (2018)
Ophiocordyceps camponoti-bispinosi	OBIS5	KX713636	KX713616	KX713693	KX713721	*Hymenoptera*	Araújo *et al.* (2018)
Ophiocordyceps camponoti-femorati	FEMO2	KX713663	KX713590	KX713678	KX713702	*Hymenoptera*	Araújo *et al.* (2018)
Ophiocordyceps camponoti-floridani	Flx1	KX713661	–	–	–	*Hymenoptera*	Araújo *et al.* (2018)
Ophiocordyceps camponoti-hippocrepidis	HIPPOC	KX713655	KX713597	KX713673	KX713707	*Hymenoptera*	Araújo *et al.* (2018)
Ophiocordyceps camponoti-leonardi	C36	KJ201512	–	JN819013	–	*Hymenoptera*	Kobmoo *et al.* (2018)
Ophiocordyceps camponoti-nidulantis	NIDUL2	KX713640	KX713611	KX713669	KX713717	*Hymenoptera*	Araújo *et al.* (2018)
Ophiocordyceps camponoti-renggeri	RENG2	KX713632	–	KX713672	–	*Hymenoptera*	Araújo *et al.* (2018)
Ophiocordyceps camponoti-rufipedis	G108	KX713659	KX713594	KX713679	KX713704	*Hymenoptera*	Araújo *et al.* (2018)
Ophiocordyceps camponoti-saundersi	C40	KJ201519	–	JN819012	–	*Hymenoptera*	Araújo *et al.* (2018)
Ophiocordyceps citrina	TNS F18537	–	KJ878903	KJ878983	–	*Hemiptera*	Quandt *et al.* (2014)
Ophiocordyceps clavata	CEM1762	KJ878916	KJ878882	KJ878963	KJ878996	*Coleoptera*	Quandt *et al.* (2014)
Ophiocordyceps cochlidiicola	HMAS_199612	KJ878917	KJ878884	KJ878965	KJ878998	*Lepidoptera*	Quandt *et al.* (2014)
Ophiocordyceps communis	NHJ 12581	EF468973	EF468831	EF468775	–	*Coleoptera*	Quandt *et al.* (2014)
Ophiocordyceps curculionum	OSC 151910	KJ878918	KJ878885	–	KJ878999	*Coleoptera*	Quandt *et al.* (2014)
Ophiocordyceps daceti	MF01	–	KX713604	KX713667	–	*Hymenoptera*	Araújo *et al.* (2018)
Ophiocordyceps diabolica	BDS 32	MK393830	MK393322	–	–	*Hymenoptera*	Araújo *et al.* (2018)
Ophiocordyceps dipterigena	OSC 151912	KJ878920	KJ878887	KJ878967	KJ879001	*Diptera*	Quandt *et al.* (2014)
Ophiocordyceps elongata	OSC 110989	–	EF468808	EF468748	EF468856	*Lepidoptera*	Quandt *et al.* (2014)
Ophiocordyceps entomorrhiza	16250	KJ878942	–	KJ878987	KJ879021	*Coleoptera*	Quandt *et al.* (2014)
Ophiocordyceps evansii	HUA 186159	KC610796	KC610770	KC610736	KP212916	*Hymenoptera*	Sanjuan *et al.* (2015)
Ophiocordyceps formicarum	TNS F18565	KJ878921	KJ878888	KJ878968	KJ879002	*Hymenoptera*	Quandt *et al.* (2014)
Ophiocordyceps formosana	TNM F13893	KJ878908	–	KJ878956	KJ878988	*Coleoptera*	Quandt *et al.* (2014)

Ophiocordyceps forquignonii	OSC 151902	KJ878912	KJ878876	–	KJ878991	*Diptera*	Quandt *et al.* (2014)
Ophiocordyceps forquignonii	OSC 151908	KJ878922	KJ878889	–	KJ879003	*Diptera*	Quandt *et al.* (2014)
Ophiocordyceps fulgoromorphila	HUA 186139	KC610794	KC610760	KC610729	KF658676	*Hemiptera*	Sanjuan *et al.* (2015)
Ophiocordyceps fulgoromorphila	HUA 186142	KC610795	KC610761	KC610730	KF658677	*Hemiptera*	Sanjuan *et al.* (2015)
Ophiocordyceps gracilis	EFCC 8572	EF468956	EF468811	EF468751	EF468859	*Lepidoptera*	Quandt *et al.* (2014)
Ophiocordyceps gracillisima	HUA 186132	–	KC610768	KC610744	KF658666	*Coleoptera*	Sanjuan et al. (2015)
Ophiocordyceps halabalaensis	MY1308	KM655825	–	GU797109	–	*Hymenoptera*	Luangsa-ard *et al.* (2011)
Ophiocordyceps heteropoda	EFCC 10125	EF468957	EF468812	EF468752	EF468860	*Hemiptera*	Quandt *et al.* (2014)
Ophiocordyceps humbertii	MF116A	MK874747	MK875537	–	MK863828	*Hymenoptera*	Araújo *et al.* (2019)
Ophiocordyceps humbertii	MF116B	MK874748	MK875536	–	MK863829	*Hymenoptera*	Araújo *et al.* (2019)
Ophiocordyceps irangiensis	128578	DQ522556	DQ518770	DQ522345	DQ522391	*Hymenoptera*	Quandt *et al.* (2014)
Ophiocordyceps irangiensis	OSC 128577	DQ522546	DQ518760	DQ522329	DQ522374	*Hymenoptera*	Sanjuan *et al.* (2015)
Ophiocordyceps kimflemingiae	SC30	KX713629	KX713622	KX713699	KX713727	*Hymenoptera*	Araújo *et al.* (2019)
Ophiocordyceps kniphofioides	HUA 186148	KC610790	KF658679	KC610739	KF658667	*Hymenoptera*	Sanjuan et al. (2015)
Ophiocordyceps konnoana	EFCC 7315	EF468959	–	EF468753	EF468861	*Coleoptera*	Quandt *et al.* (2014)
Ophiocordyceps lloydii	OSC 151913	KJ878924	KJ878891	KJ878970	KJ879004	*Hymenoptera*	Quandt *et al.* (2014)
Ophiocordyceps longissima	HMAS_199600	KJ878926		KJ878972	KJ879006	*Hemiptera*	Quandt *et al.* (2014)
Ophiocordyceps melolonthae	OSC 110993	DQ522548	DQ518762	DQ522331	DQ522376	*Coleoptera*	Quandt *et al.* (2014)
Ophiocordyceps monacidis	MF74	KX713646	KX713606	–	–	*Hymenoptera*	Araújo *et al.* (2018)
Ophiocordyceps myrmecophila	HMAS_199620	KJ878927	KJ878893	KJ878973	KJ879007	*Hymenoptera*	Quandt *et al.* (2014)
Ophiocordyceops naomipierceae	DAWKSANT	KX713664	KX713589	–	KX713701	*Hymenoptera*	Araújo et al (2018)
Ophiocordyceps neovolkiana	OSC 151903	KJ878930	KJ878896	KJ878976	KJ879010	*Coleoptera*	Quandt *et al.* (2014)
Ophiocordyceps nigrella	EFCC 9247	EF468963	EF468818	EF468758	EF468866	*Coleoptera*	Quandt *et al.* (2014)
Ophiocordyceps nutans	OSC 110994	DQ522549	DQ518763	DQ522333	DQ522378	*Hemiptera*	Quandt *et al.* (2014)
Ophiocordyceps oecophyllae	OECO1	KX713635	–	–	–	*Hymenoptera*	Araújo *et al.* (2018)
Ophiocordyceps ootakii	J13	KX713652	KX713600	KX713681	KX713708	*Hymenoptera*	Araújo *et al.* (2018)
Ophiocordyceps palthothyreum	Palt1	MK393848	MK393345	–	–	*Hymenoptera*	Araújo *et al.* (2019)
Ophiocordyceps polyrhachis-furcata	P39	KJ201504	–	JN819003	–	*Hymenoptera*	Kobmoo *et al.* (2015)
Ophiocordyceps ponerinarum	HUA 186140	KC610789	KC610767	KC610740	KF658668	*Hymenoptera*	Sanjuan *et al.* (2015)
Ophiocordyceps pruinosa	NHJ 12994	EU369106	EU369041	EU369024	EU369063	*Lepidoptera*	Quandt *et al.* (2014)
Ophiocordyceps pulvinata	TNS-F 30044	GU904208	–	GU904209	GU904210	*Hymenoptera*	Kepler *et al.* (2011)
Ophiocordyceps purpureostromata	TNS F1843	KJ878931	KJ878897	KJ878977	KJ879011	*Coleoptera*	Quandt *et al.* (2014)
Ophiocordyceps rami	MY6736	KM655823	–	KJ201532	–	*Hymenoptera*	Kobmoo *et al.* (2015)
Ophiocordyceps ravenelii	OSC 110995	DQ522550	DQ518764	DQ522334	DQ522379	*Coleoptera*	Quandt *et al.* (2014)
Ophiocordyceps rhizoidea	NHJ 12522	EF468970	EF468825	EF468764	EF468873	*Coleoptera*	Quandt *et al.* (2014)
Ophiocordyceps satoi	J7	KX713653	KX713599	KX713683	KX713711	*Hymenoptera*	Araújo *et al.* (2018)
Ophiocordyceps sinensis	EFCC 7287	EF468971	EF468827	EF468767	EF468874	*Lepidoptera*	Quandt *et al.* (2014)
Ophiocordyceps sobolifera	KEW 78842	EF468972	EF468828	–	EF468875	*Hemiptera*	Quandt *et al.* (2014)
Ophiocordyceps sp	TNS F18495	KJ878937	KJ878901	–	KJ879017	*Coleoptera*	Quandt *et al.* (2014)
Ophiocordyceps sp.	OSC 151904	KJ878934	KJ878899	KJ878980	KJ879014	*Hemiptera*	Quandt *et al.* (2014)
Ophiocordyceps sp.	OSC 151905	KJ878935	–	KJ878981	KJ879015	*Hemiptera*	Quandt *et al.* (2014)

Ophiocordyceps sp.	CatKNZ01_OP	LC370816	LC370818	LC370821	LC370819	Hemiptera (Symbiont)	Matsuura *et al*. (2018)
Ophiocordyceps sp.	CfaKSD05_OP	LC370788	LC370790	LC370801	LC370791	Hemiptera (Symbiont)	Matsuura *et al*. (2018)
Ophiocordyceps sp.	EUTCHI_OP	LC370888	LC370890	LC370893	LC370891	Hemiptera (Symbiont)	Matsuura *et al*. (2018)
Ophiocordyceps sp.	EokKNG01_OP	LC370894	LC370896	LC370899	LC370897	Hemiptera (Symbiont)	Matsuura *et al*. (2018)
Ophiocordyceps sp.	GbiNNJ01_OP	LC370850	LC370852	LC370855	LC370853	Hemiptera (Symbiont)	Matsuura *et al*. (2018)
Ophiocordyceps sp.	GniKSD01_OP	LC370844	LC370846	LC370849	LC370847	Hemiptera (Symbiont)	Matsuura *et al*. (2018)
Ophiocordyceps sp.	HmaTKB05_OP	LC370983	LC370985	LC370988	LC370986	Hemiptera (Symbiont)	Matsuura *et al*. (2018)
Ophiocordyceps sp.	MiwITN01_OP	LC370967	LC370969	LC370972	LC370970	Hemiptera (Symbiont)	Matsuura *et al*. (2018)
Ophiocordyceps sp.	MkuYGJ01_OP	LC370960	LC370962	LC370965	LC370963	Hemiptera (Symbiont)	Matsuura *et al*. (2018)
Ophiocordyceps sp.	MopTKB06_OP	LC370921	LC370923	LC370926	LC370924	Hemiptera (Symbiont)	Matsuura *et al*. (2018)
Ophiocordyceps sp.	MosKNG01	LC370940	LC370942	LC370945	LC370943	Hemiptera (Symbiont)	Matsuura *et al*. (2018)
Ophiocordyceps sp.	MmiNNJ01_OP	LC370999	LC371001	LC371004	LC371002	Hemiptera (Symbiont)	Matsuura *et al*. (2018)
Ophiocordyceps sp.	TERVAC_OP	LC370866	LC370868	LC370871	LC370869	Hemiptera (Symbiont)	Matsuura *et al*. (2018)
Ophiocordyceps sp.	TjaTKB04_OP	LC370902	LC370904	LC370907	LC370905	Hemiptera (Symbiont)	Matsuura *et al*. (2018)
Ophiocordyceps sp.	MopTKB01_01	LC371006	LC371008	LC371011	LC371009	Hemiptera (Symbiont)	Matsuura *et al*. (2018)
Ophiocordyceps sp.	TniTKB04_OP	LC370872	LC370874	LC370877	LC370875	Hemiptera (Symbiont)	Matsuura *et al*. (2018)
Ophiocordyceps sp.	Gh41	KX713656	–	KX713668	KX713706	*Hymenoptera*	Araújo *et al.* (2019)
Ophiocordyceps sp.	OSC 151909	KJ878936	KJ878900	KJ878982	KJ879016	*Hymenoptera*	Quandt *et al.* (2014)
Ophiocordyceps sphecocephala	OSC 110998	DQ522551	DQ518765	DQ522336	DQ522381	*Hymenoptera*	Quandt *et al.* (2014)
Ophiocordyceps stylophora	OSC 111000	DQ522552	DQ518766	DQ522337	DQ522382	*Coleoptera*	Quandt *et al.* (2014)
Ophiocordyceps tiputini	QCNE 186287	KC610792	KC610773	KC610745	KF658671	*Megaloptera*	Sanjuan *et al.* (2015)
Ophiocordyceps variabilis	OSC 111003	EF468985	EF468839	EF468779	EF468885	*Coleoptera*	Quandt *et al.* (2014)
Ophiocordyceps yakusimensis	HMAS_199604	KJ878938	KJ878902	–	KJ879018	*Hemiptera*	Quandt *et al.* (2014)

2.3.4 Morphological characterisation

The samples were examined and photographed under a stereomicroscope Zeiss Discovery V20™ with an attached camera (AxioCam MRc). The photos were processed with the AxionVisionSE64™ software. Characteristics such as the host's position and attachment to the plant substrate, shape, colour and size of the sexual and asexual structures were considered for macro-morphological characterisation.

For micro-morphological characterisation, ascostromata and synnemata were removed from hosts and were hand-sectioned using a single-sided razor blade and placed on slides with lacto-glycerol (20 mL lactic acid in 40 mL glycerol and 20 mL distilled water) or lacto-fuchsin 1% (0.1 g acid fuchsin in 100 mL lactic acid). Specimens were examined under an Olympus™ BX53 light photomicroscope with an attached digital camera (Olympus Q-Color 3). The microphotographs were processed with the cellSens Dimensions™ software.

To obtain mature ascospores and to follow the germination process, specimens bearing ascostromata were taped to the lids of 9-cm-diameter Petri dish and suspended with vaseline above the dish containing a thin layer of distilled water agar (DWA) and kept in an incubator in the dark at 20 °C, to await the release of ascospores (Evans *et al.* 2011b). Specimens were examined daily under a stereomicroscope to check for spore discharge and germination. A minimum of 50 discharged (mature) ascospores were examined and measured for comparative morphology.

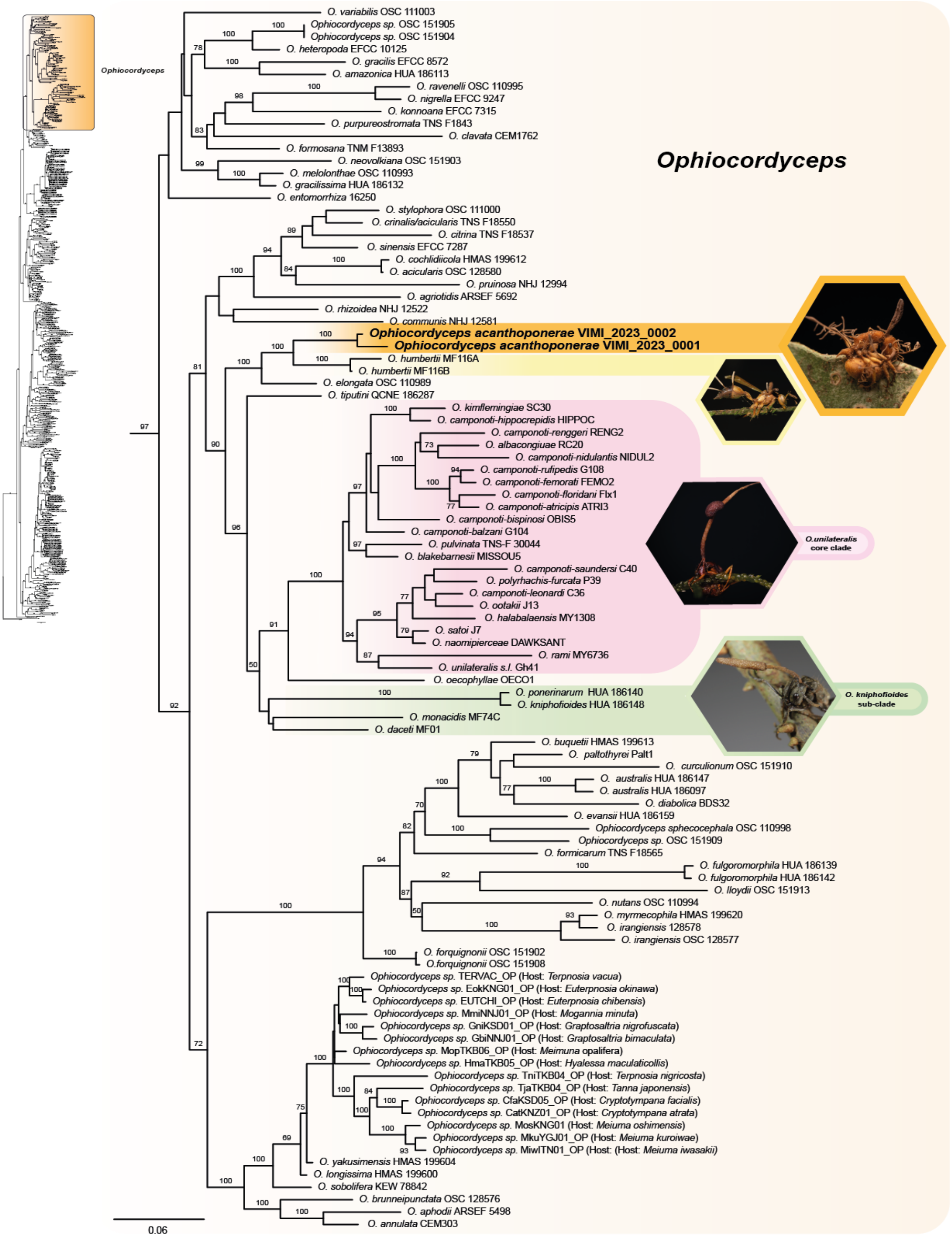

Fig. 2. Maximum likelihood tree of *Ophiocordyceps* obtained with a concatenated dataset of SSU, LSU, *TEF* and *RPB1*. Numbers on nodes represent bootstrap values > 70 %. *Ophiocordyceps acanthoponerae* sp. nov. is indicated in **bold font** and highlighted in orange. The wasp-parasitic species, *O. humbertii* (yellow), and the myrmecophilous clades, *O. unilateralis* core clade (pink) and O. *kniphofioides* sub-clade (green), are also highlighted in the tree. The whole analysis tree of the order *Hypocreales* is depicted in the top-left corner, with the position of *Ophiocordyceps* highlighted in orange.

2.4 RESULTS

2.4.1 Phylogenetic analyses

Sequences of the genomic regions SSU (= 2), LSU (= 2), *TEF* (= 2) and *RPB1* (= 1) (Table 1) from infected *Acanthoponera* samples clustered together with strong bootstrap value (BS = 100%) (Fig. 2). *Ophiocordyceps acanthoponerae* was placed in a strongly supported clade (BS = 100%) alongside the wasp pathogen *O. humbertii* and the lepidopteran pathogen *O. elongata* (Fig. 2). This clade clustered as a sister group of the clade *O. unilateralis* (BS = 90%). Building on the previous work by Araújo *et al.* (2018), *O. acanthoponerae* was positioned outside the two traditional clades of hirsutelloid *Ophiocordyceps* associated with ants, the *O. unilateralis* core clade, *O. kniphofioides* sub-clade and *O. oecophyllae*. The *O. unilateralis* core clade included 22 combined sequences of different species and was strongly supported (BS = 100%) (Fig. 2; Table 1). *Ophiocordyceps oecophyllae* clustered as a sister-group of *O. unilateralis* core clade (BS = 91%). The sub-clade *O. kniphofioides* was placed as a sister group of the *O. unilateralis* core clade together with *O. oecophyllae*, but this relationship had no support.

2.4.2 Taxonomy

Based on the morphological species concept (i.e. use of morphological traits for species description) and phylogenetic species concept (i.e. divergence of nucleotides between monophyletic lineages), a new species in the genus *Ophiocordyceps* is proposed:

Ophiocordyceps acanthoponerae S.J. Lima-Santos, H.C. Evans & J.P.M.Araújo, **sp. nov**.
MycoBank MB 859808, Figs 3–7
Etymology: Named after the host ant genus *Acanthoponera* (*Heteroponerini*: *Formicidae*).

Typus: **Brazil**, Minas Gerais state, Ouro Preto municipality, Estação Ecológica do Tripuí (20º 22' 48" S 43º 32' 24" W), on *Acanthoponera mucronata*, in forest understorey, 23 Nov. 2024, *S.J. Lima-Santos, B.L. Clemente, N.L.S. Lima & T. Mendes-Pereira* (**holotype** VIC 49609).

Paratypes: All from **Brazil**, Minas Gerais state, Ouro Preto municipality, Estação Ecológica do Tripuí (20º 22' 48" S 43º 32' 24" W), on *Acanthoponera mucronata*, in forest understorey, 12 Oct. 2023, *S.J. Lima-Santos, G.S. de Paula, M.C. Castro, M.M. Moreira & R.C. Silva,* VIC 49613; 11 Mar. 2024, *S.J. Lima-Santos, L.C. dos Reis, S.L. Elliot & H.C. Evans*, VIC 49614; 23 Nov. 2024, *B.L. Clemente, N.L.S. Lima & T. Mendes-Pereira,* VIC 49610, VIC 49611,

VIC 49612 VIC 49615, NHMD001788818, NHMD001788819, NHMD001788820; 19 Feb. 2025, *S.J. Lima-Santos*, *B.L. Clemente, G.S. de Paula & H.C. Evans* IMI 507615.

Additional specimens examined: In total 75 *Acanthoponera mucronata* ants infected by *O. acanthoponerae* were collected at different stages of infection. All of them were used to conduct fungus diagnosis. Specimens that are not deposited were located at LIIM-UFV and in the Entomological Collection Padre Jesus Santiago Moure at the Universidade Federal do Paraná (DZUP-UFPR).

Description: *External mycelium* white in early stages, becoming light brown with age, emerging from all host orifices and sutures. *Sexual morph* (Fig. 3): One or up to three stromata produced from dorsal pronotum, simple or branched, clavate to cylindrical, 2.8–4 mm in length, 0.2–0.3 mm thick, pale brown, clavate; fertile region consisting of a dark brown ascoma, covering 360º and mostly not reaching the tip of the stalk, averaging 1.6 × 0.5 mm (Fig. 3A–C). *Perithecia* immersed to partially erumpent, flask-shaped, (209–) 247 (–289) × (103–) 134 (–179) µm (Fig. 3D–E). *Asci* with 8 intertwined spores, cylindrical, hyaline, (131–) 150 (–175) × (6.0–) 7.4 (–9.0) µm; with a prominent apical cap with a narrow pore, (2.0–) 4.9 (–6.0) × (4.5–) 5.2 (–6.0) µm (Fig. 3F). *Ascospores* vermiform, hyaline, (94–) 126 (–152) × (2.0–) 2.6 (–3.0) µm, 7–septate, slightly curved, tapering towards the apices (Fig. 3G-H). *Asexual morph* (Fig. 4): *Hirsutella*-like phialides produced along the stromatal stalk and on synnemata emerging from sutures and joints. *Synnemata* pale brown or tan-coloured, single or branched, usually with secondary smaller and delicate branches produced particularly near the base; main stalks often subcapitate, produced from the dorsal pronotum, the leg joints, thorax and abdominal sutures; averaging 2.5 mm in length (Fig. 4A, B). *Phialides* lageniform, guttulate, hyaline, (10.0–) 13.7 (–16.5) × (3.0–) 4.5 (–6.5) µm, tapering to a short neck, (4.5–) 8.3 (–10.5) × (0.5–) 1.2 (–2.0) µm (Fig. 4C, D); occasionally with much longer, needle-like neck, especially on the ascostromata, up to 60 µm in length; *conidia* aseptate, tear-shaped, smooth, guttulate, hyaline, (7.5–) 8.6 (–10.0) × (2.0–) 2.2 (–2.5) µm (Fig. 4E). *Hyphal bodies* ovoid to pyriform, swollen, aseptate to septate, guttulate, hyaline, (16.0–) 29.8 (–58) × (8.0–) 11.5 (–14) µm, excised from abdomen, glistening white en masse (Fig. 4F).

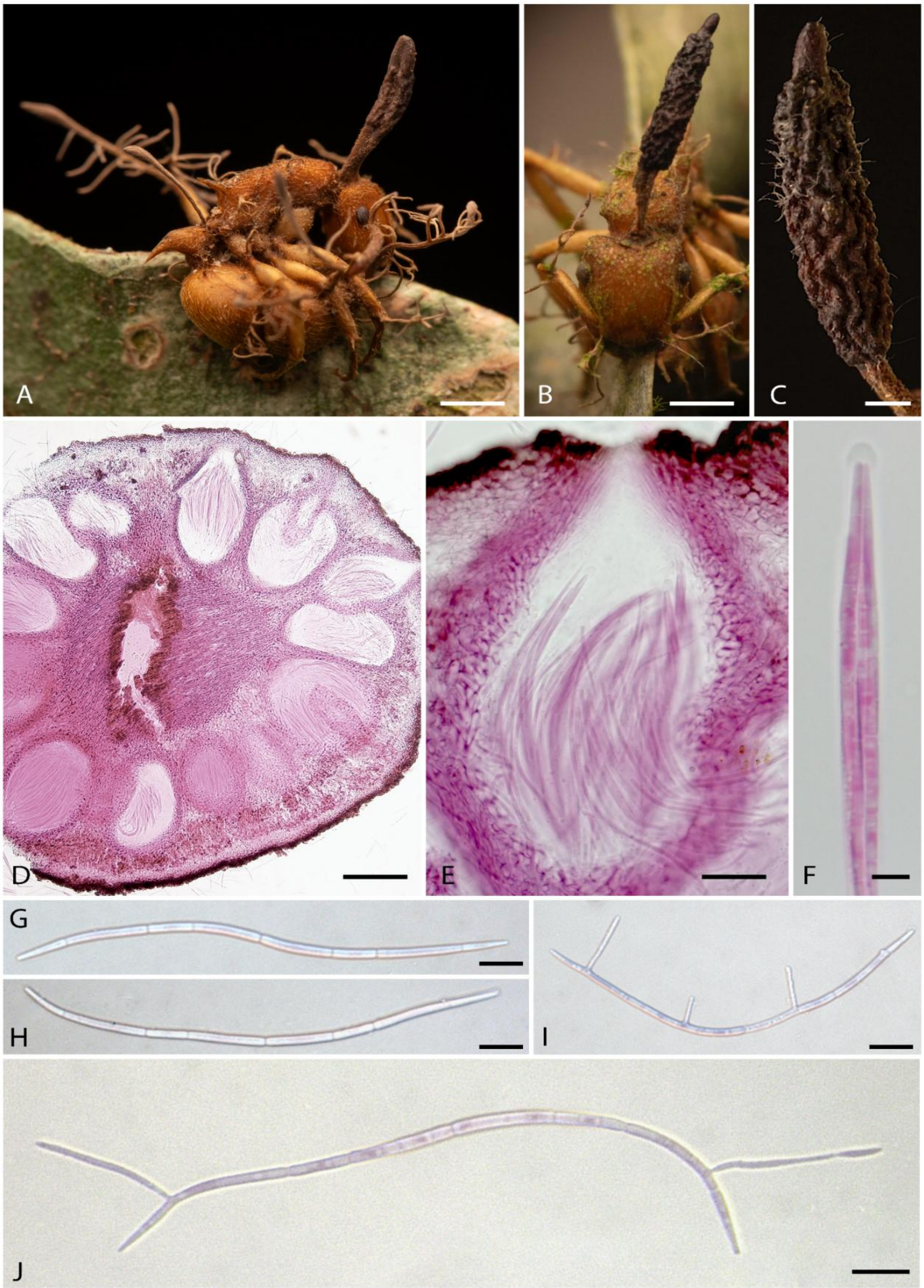

Fig. 3. *Ophiocordyceps acanthoponerae*. **A.** Holomorph with mature sexual and asexual morphs. **B-C.** Ascostromata produced from the dorsal neck region, dark brown, non-fleshy. **D.** Transversal section of the fertile part showing the buried perithecia. **E.** Perithecium with asci. **F.** Ascus, 8–spored with an apical cap. **G-H.** 7–septate ascospores. **I.** Germinated ascospore with three capilliconidiophores. **J.** Germinated ascospore bearing a capilliconidium on the right corner. Images A-C by Frederico Salles. Scale bars: A = 1 mm; B = 0.5 mm; C = 50 μm; D = 30 μm; E = 0.2 μm; F-J = 10 μm.

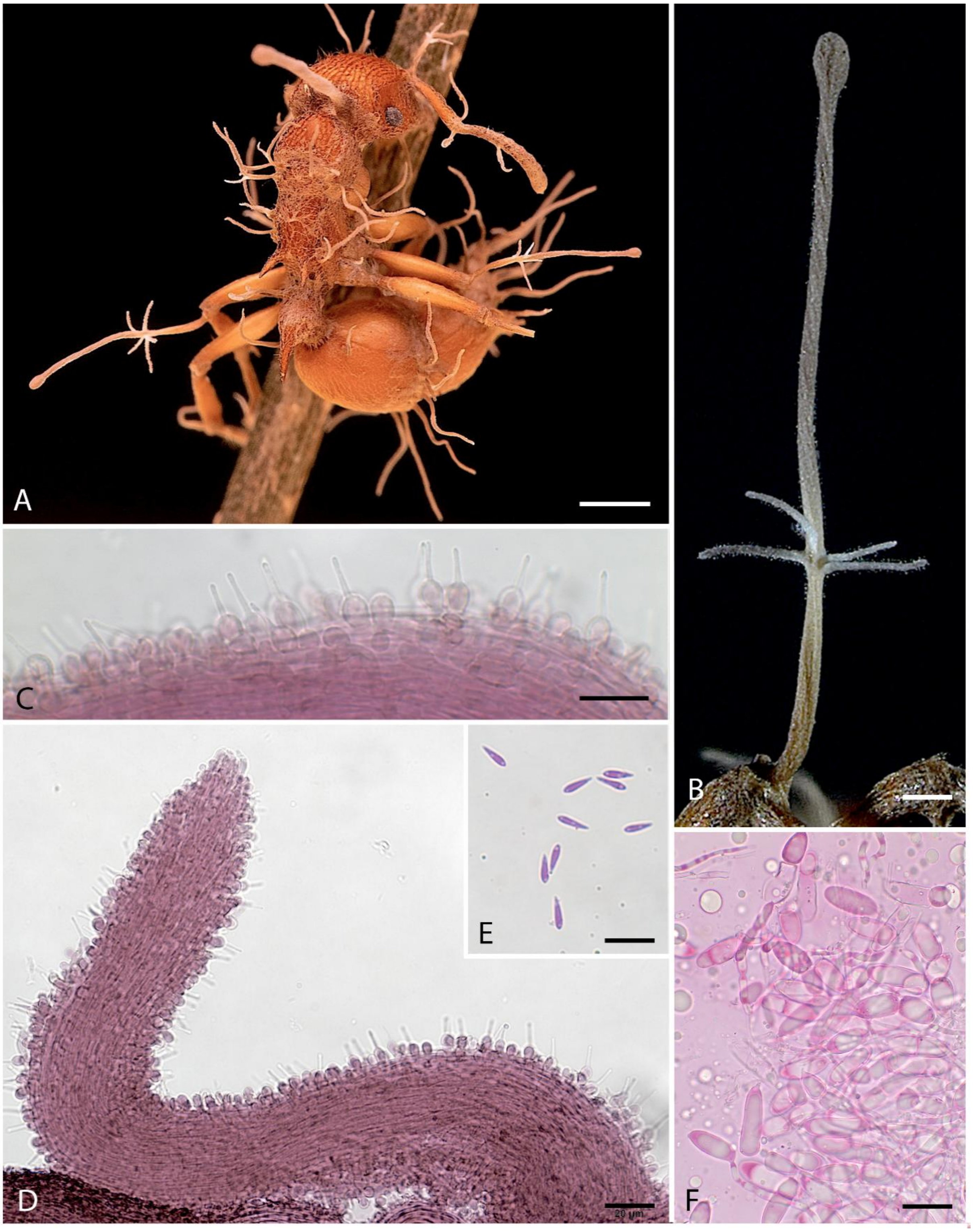

Fig. 4. *Ophiocordyceps acanthoponerae* asexual morph. **A-B.** Clavate and cylindrical branched synnemata, pale or tan-coloured arising from sutures on the body and appendages. **C-D.** Phialides along the synnemata. **E.** Tear-shaped conidia. **F.** Chains of hyphal bodies located internally in the host's abdomen. Image A by Frederico Salles. Scale bars: A = 1 mm; B = 0.2 mm; C-F = 10 µm; F = 20 µm.

Germination process: Ascospores, ejected onto DWA, germinated within 24–48 h, often accompanied by spore swelling, to produce up to four straight capilliconidiophores, averaging 17.3 × 1.3 µm (Fig. 3I); each bearing a single, terminal, fusiform capilliconidium (Fig. 3J), averaging 8.4 × 1.6 µm. Germ-tubes were not produced and no further growth was observed.

Habitat: Semi-deciduous, mesophytic forest in a transition area between Atlantic rainforest and Cerrado in the municipality of Ouro Preto, Minas Gerais, Brazil. Ouro Preto—so-named because of the dark layer of iron oxide covering the gold—was the centre of a gold rush in the 18th century; becoming famous for its many Baroque-style churches, often adorned with gold leaf: hence, the gold reference in the title of this article. Also refers to the golden colour of the workers of *A. mucronata*.

Notes: *Ophiocordyceps acanthoponerae* shares similar morphological traits with the wasp-pathogenic *O. humbertii*, such as conspicuous hirsutelloid asexual morphs, dark brown ascostromata covering 360° of the stalk and vermiform (or narrow-fusoid) ascospores. The proximity between these two species was later confirmed by our phylogenetic analysis. *O. acanthoponerae* differs from the traditional *O. unilateralis* clade by the production of acostromata covering 360° of the stalk and association with hosts from a different tribe of ants. It also differs from the *O. kniphofioides* clade by the germination of ascospores into capilliconidia. Additionally, *O. acanthoponerae* produces pale-coloured and multi-branched synnemata producing tear-shaped conidia that is not seen for other myrmecophilous *Ophiocordyceps* species described so far. Infected ants were found biting mostly on the edges of leaves—but also on the abaxial side, typically on the midrib—as well as clinging and biting onto twigs and side branches, at heights ranging from 0.5 up to 2 m above ground level. All the infected ants were found in the same location, forming one major graveyard at a specific spot along the Trilha da Guarita trail (20° 22' 48" S 43° 32' 24" W). All specimens (n = 75) of *A. mucronata* infected by *O. acanthoponerae* were found consistently attached to the vegetation with their mandibles embedded in the substratum, often with evidence of scarring; a characteristic of the so-called zombie ants, as typified by species of the *O. unilateralis* complex attacking formicine ants of the tribe *Camponotini*.

2.5 DISCUSSION

The unusual morphologies of both the asexual and sexual stages of *O. acanthoponerae* were the first indication that this could represent a new hirsutelloid species within *Ophiocordyceps* (Table 2). Myrmecophilous hirsutelloid *Ophiocordyceps* can be divided into two clades:

O. unilateralis core clade and *O. kniphofioides* sub-clade (Fig. 2). The *O. unilateralis* core clade usually produces brown or black cushion-like fertile region (single or multiple) that emerge unilaterally on the stalk, hence the name *O. unilateralis*, with ascospores that germinate via capilliconidiophores (Evans & Samson 1984, Evans *et al.* 2011b, Araújo *et al.* 2015, 2018). In comparison, the *O. kniphofioides* sub-clade typically produces a single ascostroma, covering 360º of the stalk, with ascospores that do not germinate (at least in vitro) via capilliconidiophores (Evans & Samson 1982, Sanjuan *et al.* 2015, Araújo *et al.* 2018). We have shown that *O. acanthoponerae* shares mixed traits with both clades, such as the dark brown ascostromata covering 360º of the stalk and ascospores producing capilliconidiophores and capilliconidia (Fig. 3I-J). Whilst in the asexual morph, the pale-coloured, multi-branched synnemata producing tear-shaped conidia (Fig. 4E) readily distinguish this species from other myrmecophilous hirsutelloid species described thus far.

The Maximum Likelihood phylogenetic analysis confirmed this novelty. Surprisingly, *O. acanthoponerae* clustered in a strongly supported clade (BS = 100%) outside of the traditional hirsutelloid myrmecophilous clade (*O. unilateralis* core clade + *O. oecophyllae* + *O. kniphofioides* sub-clade) (Fig. 2). This unexpected phylogenetic position corroborates the existence of a novel lineage of myrmecophilous and hirsutelloid *Ophiocordyceps*.

It is important to compare the current host association, death position and mechanisms of infection of *O. acanthoponerae*, with the two traditional clades of manipulative *Ophiocordyceps*; firstly, with the *O. unilateralis* core clade, whose species exclusively infect ants of the tribe *Camponotini* (Evans *et al.* 2011b). Their hosts are commonly found biting on leaves—the so-called death grip (Hughes *et al.* 2011)—usually at the edges or tips, but also can occur attached to palm spines, epiphytes and mosses (in tropical forests), and twigs (in temperate forests) (Table 2), with heights varying up to 2 m (Evans *et al.* 2011b, Hughes *et al.* 2016, Araújo *et al.* 2018, Loreto *et al.* 2018, van Roosmalen & de Bekker 2024). It is posited that ants are infected on or near their foraging trails when coming into contact with sticky capilliconidia, a secondary and infective spore produced on hirsutelloid conidiogenous cells (capilliconidiophores) arising from germinating ascospores that have been ejected from ascostromata on infected ants in the forest understorey (Evans *et al.* 2011a, b). This infection process has been likened, somewhat fancifully, to "a delayed-action landmine" (Evans & Boddy 2010), and the germination of the ascospores, essential for the infection process, can be simulated in vitro, following the protocol described previously in the Materials and Methods (Evans *et al.* 2011b). The morphology of the ascus, with its thickened apical cap and a narrow pore—diagnostic of the genus (Petch 1931)—is crucial for the explosive release of the ascospores and the purported infection cycle with ascospores raining on and ‘contaminating’ the ant trails. As graphically shown in Figs 5–6, the death positions and the biting strategy of *Acanthoponera* ants infected with *O. acanthoponerae* replicate the behaviours of *Camponotini* ants infected with *O. unilateralis* sensu lato. This evidence of fungal manipulation, with the ants positioned in the lower understorey above the nests and the trails emanating from them on the forest floor, justifies the classification of *O. acanthoponerae* within the zombie-ant fungi.

Table 2. Comparison of host, death position, ascospores morphology and geographic allocation of hirsutelloid species of *Ophiocordyceps*. *Ophiocordyceps acanthoponerae* sp. nov. is highlighted in **bold font**.

Species Complex	Species	Host	Death position	Stromata	Fertile region	Ascospores					Distribution	Source
						Shape	Size (µm)	Septation	Capilliconidiophore	Capilliconidia		
Unnamed lineages	***Ophiocordyceps acanthoponerae* sp. nov.**	***Acanthoponera mucronata***	**biting the edges of leaves, abaxial faces of leaves and twigs**	**1-3 stromata, sinmple or branched, produced from dorsal pronotum**	**Dark brown, covering 360º and mostly not reaching the tip of the stalk**	**vermiform**	**(94–) 126 (–152) × (1.9–) 2.6 (–3.2)**	**7**	**straight, averaging 17.3 × 1.3 µm**	**fusiform, averaging 8.4 × 1.6 µm**	**Brazilian Atlantic Forest-Cerrado ecotone**	**This study**
	O. elongata	*Apatela americana (Lepidoptera) larva and pupa*	–	1-3 stromata, arising from each host	Pale yellow, cylindric, terete, equal	cylindrical	–	–	–	–	United States of America	Petch 1937
	O. humbertii	*Hymenoptera*	biting leaf	Two stromata produced from points of insertion of the wings on each side	Dark brown, covering 360º and not reaching the tip of the stalk	narrow-fusoid	75 × 2.5	at intervals of 6 to 16 µm	–	–	Sarawak, Malaysia	Petch 1935
	O. tiputini	*Megaloptera*	leaf litter	Single, simple or branched, capitate, slender	Dark orange to deep orange, having a central core and sterile acuminate tip	elongate to fusiform	120-180 × 1.5-1.5	–	–	–	Amazon region, Ecuador and Bolivia	Sanjuan *et al.* 2015
O. unilateralis s.l.	*O. unilateralis* (Epitype)	*Camponotus sericeiventris*	biting leaf	Solitary, arising from the dorsal pronotum	Dark brown, hemisphaerical produced unilaterally	filiform	(70–) 75–85 × 2–2.5	4–5	–	–	Brazilian Atlantic Forest	Evans *et al.* 2018
	O. camponoti-atricipis	*Camponotus atriceps*	biting leaf	Single, produced from dorsal pronotum	Chocolate brown, 1–2 lateral cushions, hemispherical	vermiform	(75–) 80–85 (–100) × (2–) 3 (–3.5)	5	straight, thread-like structures, 55 µm in length	allantoid, 10–11 × 2–2.5 µm	Brazilian Central Amazon	Araújo *et al.* 2015
	O. camponoti-balzani	*Camponotus balzani*	biting leaf	Single, produced from dorsal pronotum	Brown to black, lateral cushions, disc-shaped to hemisphaerical	cylindrical	(120–) 135–175 × 4.0–5.0	14–22 (–27)	–	–	Brazilian Atlantic Forest	Evans *et al.* 2011b
	O. camponoti-bispinosi	*Camponotus bispinosus*	biting spines	Single, produced anterior to pronotum	Dark brown becoming black with age, lateral globose cushion	cylindrical	(60–) 70–75 (–80) × 4.5–5 (–6)	4–5	straight, (50–) 65 (–80) µm	slightly truncate at the base, 10–11 × 3–4 µm	Brazilian Central Amazon	Araújo *et al.* 2015
	O. camponoti-chartificis	*Camponotus chartifex*	biting leaf	Single, produced from dorsal pronotum	Chocolate brown, 1–2 lateral cushions	vermiform	75–85 × 5	9–13	long and extremely narrow hair-like, (65–)75–90(–95) µm	fusoid	Brazilian Central Amazon	Araújo *et al.* 2018
	O. camponoti-femorati	*Camponotus femoratus*	biting leaf/spines	Single, produced from dorsal pronotum	Chestnut-brown, terminal of 1–3 lateral cushions, disc-shaped to hemispherical	sinuous to curved	75–90 × 3	5	narrow, 35–40 µm long	clavate, 9 × 3 µm	Brazilian Central Amazon	Araújo *et al.* 2018
	O. camponoti-hippocrepidis	*Camponotus hippocrepidis*	biting spines	Single, produced from dorsal pronotum	Chestnut-brown, circular, produced at the middle part of stroma, laterally attached	cylindrical	75–85 × 4–5	5	straight, 45–50 µm long	truncate at base, narrowing apically, 10–11 × 4 µm	Brazilian Central Amazon	Araújo *et al.* 2018

	O. camponoti-indiani	*Camponotus indianus*	biting leaf	Multiple, arising from right and left sides of pronotum, and leg joints	Chocolate to dark brown with age, 1–4 lateral cushions, hemispherical	cylindrical	(60–) 75 (–80) × (3.5–) 4.5 (–5)	5	hair-like, 120–130 µm in length	fusoid, 13–14 µm	Brazilian Central Amazon	Araújo *et al.* 2015
	O. camponoti-melanotici	*Camponotus melanoticus*	biting leaf	Single, produced from dorsal pronotum	Dark brown to black, 1–2 lateral cushions, hemispherical	cylindrical	170–210 × 4–5	27–35	–	–	Brazilian Atlantic Forest	Evans *et al.* 2011b
	O. camponoti-nidulantis	*Camponotus nidulans*	biting saplings	Single, produced from dorsal pronotum	Light brown, 1–2 lateral cushions, disc-shaped to hemispherical	vermiform	90–105(–115) × 3–4	5	straight, extremely narrow hair-like, 50–60 µm	clavate, 9 × 2 µm	Brazilian Central Amazon	Araújo *et al.* 2018
	O. camponoti-novogranadensis	*Camponotus novogranadensis*	biting epiphytes	Single, produced from dorsal pronotum	Brown, 1–2 lateral cushions, disc-shaped to hemisphaerical	filiform	75–95 x 2.5–3.5	5–10	20–25 x 0.2–0.4 µm	reniform, (5–) 7–8 × 2.5–3.0 µm	Brazilian Atlantic Forest	Evans *et al.* 2011b
	O. camponoti-renggeri	*Camponotus renggeri*	biting leaf/moss	Single, rarely branched, produced from dorsal pronotum	Dark-brown to black, 1–2 lateral cushions, hemispherical to globose	vermiform	90–120 × 4	5–8	–	–	Brazilian Central Amazon	Araújo *et al.* 2018
	O. camponoti-rufipedis	*Camponotus rufipes*	biting leaf	Single, produced from dorsal pronotum	Pale to chestnut-brown, 1–3 lateral cushions, disc-shaped to hemisphaerical	vermiform	(75–) 80–95 (–115) × 2–3	4–7	extremely narrow hair-like structures, (45–) 60–70 (–80) µm	clavate, (7–) 9–11 × 1.5–2.5 µm	Brazilian Atlantic Forest	Evans *et al.* 2011b
	O. camponoti-sexguttati	*Camponotus sexguttatus*	biting leaf	Single, arising from the dorsal pronotum, never branching	Chestnut-brown, single lateral cushion, disc-shaped	cylindrical	120–140 × 3	7	straight, 25–30 µm	narrowing apically, 5–9 × 2 µm	Brazilian Central Amazon	Araújo *et al.* 2018
O. kniphofioides s.l.	*O. monacidis*	*Dolichoderus bispinosus*	base of trunk (moss)	Single, emerges laterally from the pronotum, rarely from the gaster	Dark orange, terminal	–	(80-) 95-110 (-120)	3-4	–	–	Brazilian Amazon	Evans *et al.* 1982; Araújo *et al.* 2018
	O. daceti	*Daceton armigerum*	leaf (not biting)	Arising from the dorsal pronotum	–	–	–	–	–	–	Brazilian Central Amazon	Araújo *et al.* 2018
	O. kniphofioides	*Cephalotes atratus*	base of trunk	Simple or branched, arising laterally from neck or upper thorax (pronotum), occasionally dorsally	Light to deep orange, terminal, clavate to conical	filiform	110-150 × 1.5-3.0	3-5	–	–	Brazilian Amazon, Colombia	Evans *et al.* 1982
	O. ponerinarum	*Paraponera clavata*	base of trunk	Simple or branched, emerging laterally from the pronotum	Acuminate	–	–	–	–	–	Brazilian Amazon, Colombia	Evans *et al.* 1982

Species of the *O. kniphofioides* sub-clade infect ant hosts belonging to the distantly related genera *Cephalotes*, *Daceton*, *Dolichoderus* and *Paraponera*. The most studied interaction involves *O. kniphofioides sensu stricto* and *Cephalotes atratus*—an arboreal myrmicine ant highly adapted to living in the forest canopy where it nests and forages on pollen and homopteran exudates with the aid of adhesive tarsal pads (Stark & Yanoviak 2018)—where infected ants aggregate on tree trunks (Table 2) (Evans & Samson 1982, Sanjuan *et al.* 2015, Araújo *et al.* 2018, Imirzian *et al.* 2020). Healthy workers scavenge on the trees attempting to remove infected cadavers and it is most probable that these become infected when coming into contact with the mucilaginous conidia produced on hirsutelloid, rhizoid-like synnemata that radiate out on the tree bark from the ant host (Evans & Samson 1982, Araújo *et al.* 2018).

A closely-related species within the *O. kniphofioides* complex is *O. monacidis* on the myrmicine ant *Dolichoderus* (*Monacis*) *bispinosus* (Araújo *et al.* 2018): a strictly arboreal ant with similar foraging habits to *C. atratus* (Mackay 1993). As with diseased *C. atratus* ants, infected *D. bispinosus* ants descend from their arboreal habitats and die amongst clumps of moss on the buttress roots of upperstorey trees, where the dark-orange, poker-like ascostromata of *O. monacidis* mimick the moss sporophores (Araújo *et al.* 2018). If, as the evidence suggests (Mackay 1993), *D. bispinosus* is an arboreal ant—foraging and nesting in the canopy— with only occasional sorties to the forest floor, it is difficult to determine how the ascospores reach their target hosts and function in the infection cycle. Rather than being manipulated by the fungus, infected ants would appear to be moving away from their arboreal habitats and actively hiding within the moss substrate, which could be interpreted to be altruistic by avoiding infection of the nests and trails in the canopy. Such an interpretation has been posited regarding both *C. atratus* and *D. bispinosus* infected by another *Ophiocordyceps* species complex, *O. cucumispora sensu lato*, where both hosts when infected descend from the forest upperstorey and die hidden within the litter layer (Evans 1982, 1988, Evans & Samson 1982). The ascospores readily break into highly-distinct, cucumber-like, longitudinally-ridged and echinulate part-spores. The form and function of these unusual spores is unknown, as is how they reach their arboreal targets, although the striation and echinulation indicates the likely involvement of phoresy on a vector.

Fig. 5. Sequence of events following infection of *Acanthoponera mucronata* by *Ophiocordyceps acanthoponerae*: development of asexual morph. **A.** Recently dead ant biting into leaf midrib with no external evidence of infection. **B-D.** White silky hyphae emerging from sutures on body and appendages, including the antennae, on ants clinging to leaf margins. **E-F.** Developing synnemata, pale-coloured emerging from body and appendages. Scale bars: A-E = 1.5 mm; F = 2.5 mm.

Fig. 6. Sequence of events following infection of *Acanthoponera mucronata* by *Ophiocordyceps acanthoponerae*: development of sexual morph. **A-D.** Single immature, pale-coloured ascostroma emerging from dorsal pronotum of each ant, dying in various positions biting into leaf margins, midribs and small branches. **E-F.** Mature, dark brown ascostromata (short arrows); atypically, in **E**, it is emerging from the lateral thorax. Note the leaf scars in **A**, **C** and **E** (long arrows), caused by repetitive biting prior to death. Scale bars: A-F = 2.5 mm.

In the phylogenetic analyses, *O. acanthoponerae* clustered in a clade (BS = 100%) with sequences obtained for the wasp pathogen *O. humbertii* and the lepidopteran pathogen

O. elongata (Fig. 2). We were able to identify shared traits between the taxa associated with hymenopteran insects. Both of these taxa, *O. acanthoponerae* and *O. humbertii*, have conspicuous hirsutelloid asexual morphs, dark brown ascostromata covering 360º of the stalk and vermiform (or narrow-fusoid) ascospores (Table 2) (Petch 1935, Somavilla *et al.* 2020, Sobczak *et al.* 2020, Barbosa & Somavilla, 2022). Wasps are also manipulated by *O. humbertii* and are mostly found biting on the border of leaves (Somavilla *et al.* 2020, Sobczak *et al.* 2020, Barbosa & Somavilla 2022), in a similar way to *O. acanthoponerae*. An analysis by Araújo & Hughes (2019) placed *O. humbertii* as a sister group of *O. unilateralis* clade (BS = 100%) and it was hypothesised that an intermediate step might have occurred via social wasps, in the sense that an ancient *Ophiocordyceps* lineage jumped from wasps to ants, during the evolution of the myrmecophilous clade. Our new findings could represent such a missing link between manipulative ant- and wasp-associated *Ophiocordyceps*. Currently, studies are under way to provide a more robust phylogenetic framework for wasp-associated species, in the *O. humbertii* clade or complex, with the objective of elucidating the origins and evolution of manipulative *Ophiocordyceps* lineages.

This study is also the first record of ants belonging to the genus *Acanthoponera* (*Heteroponerini*: *Ectatomminae*: *Formicidae*) being infected by *Ophiocordyceps*. The genus *Acanthoponera* is restricted to the Neotropics and these relatively-large, golden-coloured ponerines are characterized by their prominent spines, the well-developed eyes and their long palps (Feitosa 2015, Camacho *et al.* 2022). They represent a lineage of nocturnal ants which forage exclusively on arboreal substrates, mostly in humid forests (Feitosa 2015, Feitosa & Prada-Achiardi 2019, Camacho *et al.* 2022). Workers are usually found foraging alone on the vegetation searching for small arthropods (Feitosa 2015, Feitosa & Prada-Achiardi 2019). It is thought that their colonies may harbour dozens to a few hundred individuals but data on nest structure and colony size are lacking (Feitosa 2015, and *pers. obs.*). The species *A. mucronata* has the largest distribution of the genus and is found in all Brazilian regions (Feitosa 2015, Feitosa & Prada-Achiardi 2019). This raises the questions of whether *O. acanthoponerae* is to be found across its host's range and also whether other *Ophiocordyceps* species exist that parasitise the rarer members of the genus. *Acanthoponera mucronata* shares arboreal nesting and foraging habits with other genera of ants commonly infected by manipulative *Ophiocordyceps* (i.e., *Camponotus*, *Cephalotes*, *Daceton*, *Paraponera* and *Polyrhachis*) (Feitosa & Dias 2024). This life-history trait might may have favoured infection and ultimately host-switching by *Ophiocordyceps* between hosts sharing the same ecological niche or habitat

(Sanjuan *et al.* 2015, Araújo & Hughes 2019). The fact that workers forage alone rather than in collective trails is likely to influence how *O. acanthoponerae* infects and manipulates them. Their colony size, with fewer individuals, might also indicate host-parasite dynamics different from that described for *O. unilateralis* sensu lato on *Camponotini* (Andersen *et al.* 2012, Loreto *et al.* 2014).

In their recent review of behavioural manipulation, van Roosmalen and de Bekker (2024) stressed that more studies are needed to fully elucidate the infection biology of *Ophiocordyceps*. There is accumulating evidence which indicates "that the secretion of parasite proteins and light-driven biological rhythms are likely involved in the infection and manipulation biology" (van Roosmalen & de Bekker 2024). In particular, it is posited that *Ophiocordyceps* turn their ant hosts into light seekers (Andriolli *et al*. 2019). How this would relate to a nocturnal ant species, such as *A. mucronata*, would be worthy of further investigation.

2.6 CONCLUSIONS

The morphological and phylogenetic analyses confirm *O. acanthoponerae* as a new species within the genus *Ophiocordyceps*. The fungus presents mixed morphological traits related to the myrmecophilous and manipulative clade *O. unilateralis* (*O. unilateralis* core clade and *O. kniphofioides* sub-clade) and shows similarities with the wasp-parasitic fungus, *O. humbertii*. Significantly, the phylogenetic reconstruction showed that *O. acanthoponerae* falls outside of these 'traditional' clades and indicates the existence of a new lineage of myrmecophilous and manipulative fungi within the genus *Ophiocordyceps.* This is also the first record of *Heteroponerini* ants being infected by *Ophiocordyceps*, which expands our knowledge of the host range for these fungi. More work is needed, especially on the little-researched ant-host species, to further elucidate the biological and ecological factors that may influence this host-parasite interaction.

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3 CHAPTER III: A light into a forgotten graveyard: Cryptic diversity within the wasp-parasitic *Ophiocordyceps humbertii* (*Ophiocordycipitaceae: Hypocreales*)*

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3.1 ABSTRACT

Ophiocordyceps has a pivotal role in studies of cryptic fungal diversity and behavioural manipulation of hymenopterous insects. The wasp pathogen *O. humbertii* has long been suspected to be a species complex. Here, we investigate the cryptic diversity within *O. humbertii* sensu lato by combining morphological, ecological and multilocus phylogenic data (SSU, LSU, *TEF* and *RPB1*) from 26 fungal specimens associated with wasps from the Brazilian Atlantic and Amazon forests. Our analyses support the existence of at least four distinct clades of manipulative and hirsutelloid *Ophiocordyceps* associated with wasps, leading to the description of seven new species: *O. humbertioides*, *O. prezotii*, *O. sanctaluciae*, *O. dendritica*, *O. tripuiensis*, *O. lacrimospora* and *O. pseudocitriformis*. Notably, *O. pseudocitriformis* forms a distinct lineage within the *H. citriformis* clade, expanding its known host range beyond Hemiptera. Our findings reveal that *O. humbertii* in fact constitutes a complex of cryptic species, expand the documented host association for wasp-parasitic *Ophiocordyceps* and underscore the significance of wasps in the evolutionary history of behaviour-manipulating fungi across *Hymenoptera*.

KEYWORDS: Atlantic rainforest, host manipulation, *Hymenoptera*, systematics, zombie-fungi

3.2 INTRODUCTION

The fungal genus *Ophiocordyceps* (*Ophiocordycipitaceae*: *Hypocreales*) is represented almost exclusively by species recognized as insect pathogens (or entomopathogenic fungi) (Araújo & Hughes 2016, 2019). Currently, the genus is comprised of 350 valid species (Mycobank 2025), most of which engage in parasitic interactions with hosts from at least nine insect orders (Sung *et al.* 2007, Quandt *et al.* 2014, Araújo & Hughes 2019). They probably arose almost 100 million years ago (Sung *et al.* 2008) and their diversification most likely involved successive and independent host switches (or host-jumps) between orders of insects and also between taxa within these orders, all descending from an ancestral *Coleoptera*-parasitic lineage (Araújo & Hughes 2019). According to Sung *et al.* (2007), *Ophiocordyceps* species are mostly recognized for having darkly pigmented stromata (with rare cases of brightly coloured stromata), asci with thickened ascus apexes and usually cylindrical and multiseptate ascospores that can disarticulate or not into part-spores. Their asexual states were formerly classified under the anamorphic genera *Hirsutella*, *Hymenostilbe*, *Paraisaria*, *Sorosporella and Syngliocladium*, which now help to distinguish clades within the genus (Sung *et al.* 2007, Quandt *et al.* 2014). One of the most famous species within this genus is *Ophiocordyceps unilateralis*, recognized as the “zombie-ant fungus”, regarding the behavioural manipulation it promotes in ant hosts, which has been of great interest for the general public (Andersen *et al.* 2009, Araújo & Hughes 2016, Hughes *et al.* 2016, Araújo *et al.* 2018).

In 2011, Evans *et al.* (2011a) revealed that the myrmecophilous *O. unilateralis* actually constituted a species complex strictly associated with *Camponotini* (*Formicidae*: *Hymenoptera*) hosts. Following this discovery, *Ophiocordyceps* was considered a flagship genus to study the diversity of cryptic speciation of fungi, especially in tropical forests (Evans *et al*. 2011b). Since then, a new era for taxonomic studies concerning cryptic diversity within *Ophiocordyceps* was established with the description of more than 30 cryptic species at least for the myrmecophilous *O. unilateralis* sensu lato (= s.l.) (Kepler *et al.* 2011, Luangsa-ard *et al.* 2011, Araújo *et al.* 2015, 2018, Kobmoo *et al.* 2015, Crous *et al.* 2016, Tang *et al.* 2023a, 2023b, 2023c). *Ophiocordyceps unilateralis* s.l. manipulates the behaviour of their ant hosts to achieve an elevated position from where (i.e. from the attached host) they will release their spores onto forest substrates (floor, leaves, stems) and then infect new hosts (Evans & Samson, 1984, Andersen *et al.* 2009). These manipulative fungi probably arose from non-manipulating lineages of *Ophiocordyceps* that infected burying beetle larvae after successive and independent host-jumps (Araújo & Hughes 2019); this is a common phenomenon in the evolution of Hypocreales, considering niche overlap between potential hosts for a parasitic fungus lineage. Araújo & Hughes (2019) hypothesised that this phenomenon might have occurred first in social wasps and then in ants and stated that more taxon sampling of the wasp-parasitic species should improve our comprehension of the diversification and evolution of manipulative *Ophiocordyceps* across social insects.

The species *Ophiocordyceps humbertii* is closely related to *O. unilateralis* s.l. and induces in social wasps a similar behavioural manipulation to that seen in ant hosts (Araújo & Hughes 2019, Sobczak *et al.* 2020, Somavilla *et al.* 2020, Barbosa & Somavilla 2022). This fungus was formally described by Petch (1935) infecting a wasp from Sarawak (Malaysia), and was characterized as having two clavae bearing dark brown fertile heads, each arising from the insertion points of the wasp's wings, as well as longer, slender clavae emerging from the abdominal sutures. Petch associated the latter structures with the morphology of *Hirsutella saussurei*, revised by Speare (1920), and stated them to be the asexual state of *O. humbertii*. The morphotype of *H. saussurei* is readily distinguished by its very long, branched and slender synnemata and allantoid spores embedded in gelatinous matrices (Spare 1920). After the establishment of "One fungus = One name (1F1N)", *H. saussurei* can no longer considered a valid name over *O. humbertii* (Hawksworth 2011, Hawksworth *et al.* 2011). While re-examining specimens of *H. saussurei* from different countries and based on spore measurements, Petch suspected that the fungus should be a "collective species", in the sense of the existence of cryptic speciation (Petch 1948).

Occasional fungal specimens, identified as *O. humbertii*, have been found infecting and manipulating not only social but also solitary wasps from the Brazilian Amazon and Atlantic Forests (Somavilla *et al.* 2019; Sobczak *et al.* 2020, Somavilla *et al.* 2020, Barbosa & Somavilla 2022). The morphological differences between collected and deposited specimens, the original localities and the diversity of wasp hosts provide support for Petch's assertion (1948) that *O. humbertii* should be treat as a species complex to be disentangled. Wasps themselves constitute an extremely diverse, paraphyletic grouping of hymenopteran insects (excluding *Formicidae* and *Anthophila*) (Branstetter *et al.* 2017, Blaimer *et al.* 2023) with approximately 103,000 described species (Aguiar *et al.* 2013, Brock *et al.* 2021). They exhibit a wide spectrum of social behaviours (from solitary to highly eusocial) and foraging habits (from specialist parasitoids to generalist foragers), thereby engaging in a broad array of ecological interactions (Bell & Sumner 2023, Brock *et al.* 2021, Barbosa *et al.* 2021, Detoni & Prezoto 2021). Considering the ecological and taxonomic diversity of wasps and the morphophysiological similarities shared by *Hymenoptera*, which all descend from a common ancestor (Branstetter *et al.* 2017, Blaimer *et al.* 2023), increased sampling of *O. humbertii* associated with different groups of wasps could significantly advance our current understanding of the evolution of manipulative *Ophiocordyceps* across hymenopteran hosts.

In this study, we describe seven new *Ophiocordyceps* species parasitising social wasps in the Brazilian Atlantic Forest, revealing a previously unrecognized diversity within *O. humbertii* sensu lato. One of the novel species is closely related to the hemipteran-pathogenic *Hirsutella citriformis* clade. Our findings highlight the existence of four novel lineages of wasp-parasitic *Ophiocordyceps*, comprising the newly described taxa and other morphotypes collected from the Amazon and Atlantic Forest biomes. These results confirm the hypothesis that *O. humbertii* represents a complex of cryptic species. The present study also expands the current knowledge of host associations of manipulative *Ophiocordyceps* with wasps, ranging from solitary to social host species within the Neotropical region.

3.3 MATERIALS AND METHODS

In this study, we combined morphological, ecological and molecular data for the description of the new species. Our methods for collection, field observations, morphological characterisation and molecular analyses are mainly based on previous studies with myrmecophilous *Ophiocordyceps* species (Araújo *et al*. 2015, 2018, Evans *et al*. 2011a, 2018).

3.3.1 Specimen collections

Collections were conducted in conservation units in the Brazilian Atlantic Forests: Estação Ecológica do Tripuí (Ouro Preto municipality, Minas Gerais), Estação de Pesquisas, Treinamento e Educação Ambiental (EPTEA) Mata do Paraíso (Viçosa municipality, Minas Gerais), Reserva Betary (Iporanga municipality, São Paulo), Reserva Biológica Augusto Ruschi and Estação Biológica de Santa Lúcia (both in Santa Teresa municipality, Espírito Santo) (Fig. 1, Table 1). Permits for collection and transport were provided by SISBIO (nº 86685-4) and Instituto Estadual de Florestas (IEF; nº 037/2023). We also received samples from the Central Amazonia, Reserva Ducke (Manaus, Amazonas), and meridional Amazonian region, Alta Floresta, Mato Grosso) (Fig. 1, Table 1). Additionally, we included sequences for *O. humbertii s.l.* specimens (W1, W10, W13, W14 and W15) in our phylogenetic analysis, which were collected by JPMA in Reserva Ducke and are now located in New York Botanical Garden (NYBG). Unfortunately, these latter specimens were not morphologically characterized.

Estação Ecológica do Tripuí (EET; 20º 23' 45" S, 43º 34' 33" W) is a conservation unit of approximately 392 ha, with altitudes varying between 1,180 and 1,300 m a.s.l. The forest reserve is located in the transition (or ecotone) between Atlantic Forest and Cerrado biomes, with a mixed vegetation belonging to both; including semi-deciduous mesophytic forests, Cerrado and permanent swamps (FEAM 1995, Pedralli et al. 1997, Werneck et al. 2001). The collections were conducted on the “Trilha da Guarita” trail in October 2023 and November 2024.

Estação de Pesquisas, Treinamento e Educação Ambiental Mata do Paraíso (MP; 20°48' 00"S 42° 52' 1.19" W) is a reserve of approximately 400 ha, with altitudes varying between 690 and 870 m a.s.l. The forest is classified as Altimontane Semideciduous Forest (Oliveira-Filho & Fontes 2000) or Montane Seasonal Semideciduous Forest (Veloso et al. 1991). We searched for wasps on the “Trilha dos Gigantes” trail in March 2023.

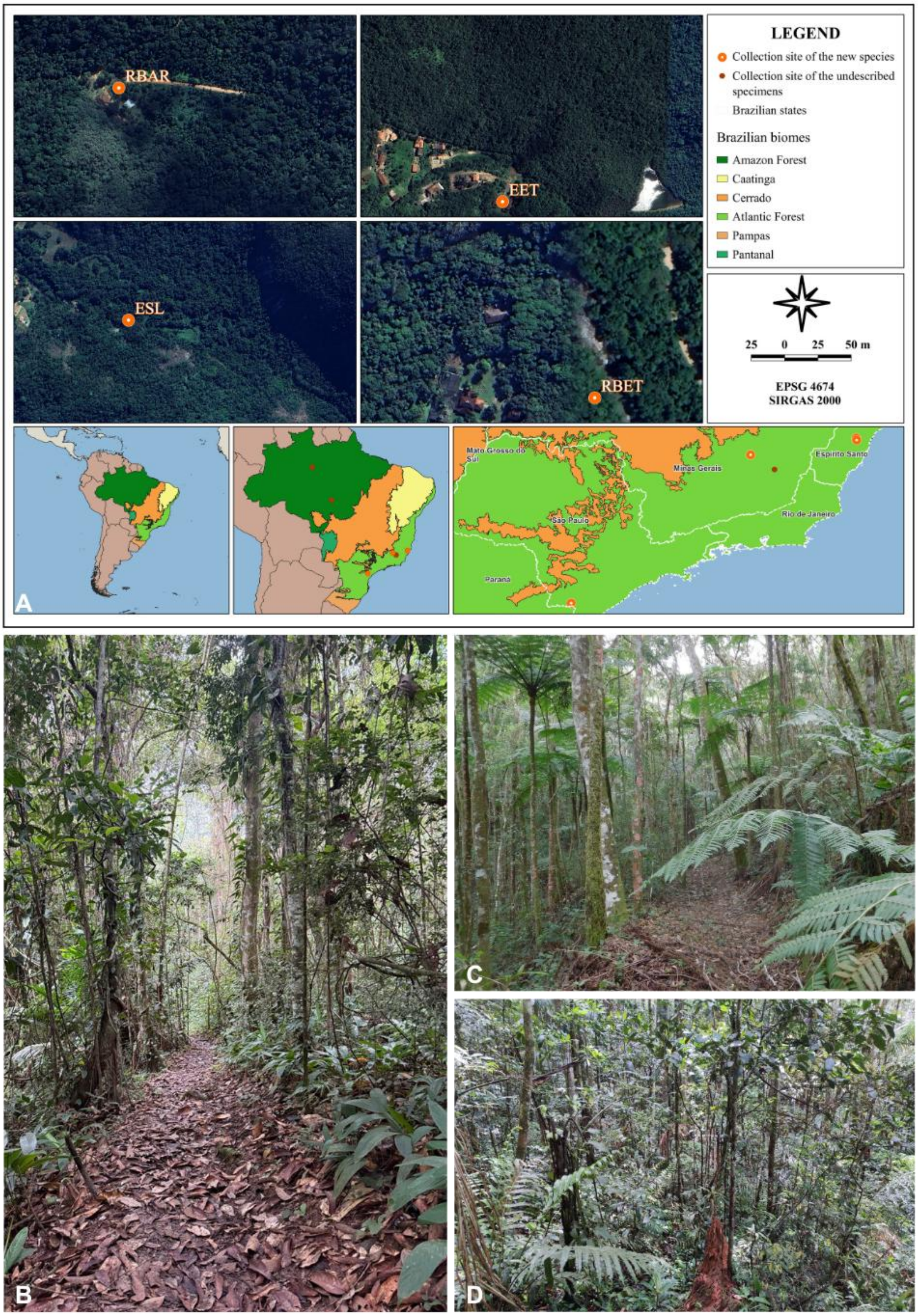

Fig. 1. Collection sites of *O. humbertii* sensu lato. **A.** Map with an overview of the Brazilian biomes highlighting the collection sites from where new species were collected. **B.** Understory vegetation from Reserva Betary (RBET). **D.** Understory vegetation from Estação Ecológica do Tripuí (EET) **C.** Understory vegetation from Reserva Biológica Augusto Ruschi (RBAR).

Table 1. Samples, host, deposit and collection information of *O. humbertii* sensu lato from this study. The new species are highlighted in **bold font**.

Sample/Isolation number	Host	Deposit Information	Voucher	Collection site	Biome
Ophiocordyceps humbertii MF116	–	New York Botanical Garden	–	Reserva Ducke, Amazonas state, Brazil	Central Amazon
O. humbertii s.l. 92 AM	*Agelaia* sp.	Herbarium VIC - UFV	49640	Alta Floresta, Mato Grosso state, Brazil	Meridional Amazon
O. humbertii s.l. 31 EET	*Polybia fastidiosuscula*	Herbarium VIC - UFV	49641	Estação Ecológica do Tripuí, Minas Gerais state, Brazil	Ecotone Atlantic Forest - Cerrado
O. humbertii s.l. 32 EET	*Agelaia angulata*	Herbarium VIC - UFV	49642	Estação Ecológica do Tripuí, Minas Gerais state, Brazil	Ecotone Atlantic Forest - Cerrado
O. humbertii s.l. 119 EET	*Agelaia multipicta*	Herbarium VIC - UFV	49643	Estação Ecológica do Tripuí, Minas Gerais state, Brazil	Ecotone Atlantic Forest - Cerrado
O. humbertii s.l. 121 EET	*Polybia* sp.	Herbarium VIC - UFV	49644	Estação Ecológica do Tripuí, Minas Gerais state, Brazil	Ecotone Atlantic Forest - Cerrado
O. humbertii s.l. 120 EET	*Polybia* sp.	Herbarium VIC - UFV	49645	Estação Ecológica do Tripuí, Minas Gerais state, Brazil	Ecotone Atlantic Forest - Cerrado
O. humbertii s.l. 122 EET	*Agelaia vicina*	Herbarium VIC - UFV	49646	Estação Ecológica do Tripuí, Minas Gerais state, Brazil	Ecotone Atlantic Forest - Cerrado
O. humbertii s.l. 29 MP	*Agelaia multipicta*	Herbarium VIC - UFV	49647	EPTEA Mata do Paraíso, Minas Gerais state, Brazil	Atlantic Forest
O. humbertii s.l. 18 RBAR	*Apoica* sp.	Herbarium VIC - UFV	49648	Reserva Biológica Augusto Ruschi, Espírito Santo state, Brazil	Atlantic Forest
O. humbertii s.l. 80 RBET	*Agelaia angulata*	Herbarium VIC - UFV	49649	Reserva Betary, São Paulo state, Brazil	Atlantic Forest
O. humbertii s.l. W1	*Agelaia fulvofasciata*	New York Botanical Garden	–	Reserva Ducke, Amazonas state, Brazil	Central Amazon
O. humbertii s.l. W10	*Agelaia* sp.	New York Botanical Garden	–	Reserva Ducke, Amazonas state, Brazil	Central Amazon
O. humbertii s.l. W13	*Polistes claripenis*	New York Botanical Garden	–	Reserva Ducke, Amazonas state, Brazil	Central Amazon
O. humbertii s.l. W14	*Polybia dimidiata*	New York Botanical Garden	–	Reserva Ducke, Amazonas state, Brazil	Central Amazon
O. humbertii s.l. W15	*Agelaia constructor*	New York Botanical Garden	–	Reserva Ducke, Amazonas state, Brazil	Central Amazon
***O. humbertioides* 12 ESL**	***Agelaia vicina***	**Herbarium VIC - UFV**	**49651**	**Estação Biológica de Santa Lúcia, Espírito Santo state, Brazil**	**Atlantic Forest**
***O. humbertioides* 2 RBAR**	***Agelaia vicina***	**Herbarium VIC - UFV**	**49650**	**Reserva Biológica Augusto Ruschi, Espírito Santo state, Brazil**	**Atlantic Forest**
***O. humbertioides* 3 RBAR**	***Agelaia vicina***	**Herbarium VIC - UFV**	**49652**	**Reserva Biológica Augusto Ruschi, Espírito Santo state, Brazil**	**Atlantic Forest**
***O. humbertioides* 116 RBAR**	***Agelaia vicina***	**Herbarium VIC - UFV**	**49653**	**Reserva Biológica Augusto Ruschi, Espírito Santo state, Brazil**	**Atlantic Forest**
***O. humbertioides* 117 RBAR**	***Agelaia vicina***	**Herbarium VIC - UFV**	**49654**	**Reserva Biológica Augusto Ruschi, Espírito Santo state, Brazil**	**Atlantic Forest**
***O. humbertioides* 118 RBAR**	***Agelaia vicina***	**Herbarium VIC - UFV**	**49655**	**Reserva Biológica Augusto Ruschi, Espírito Santo state, Brazil**	**Atlantic Forest**
***O. dendritica* 114 RBET**	***Mischocyttarus* sp.**	**Herbarium VIC - UFV**	**49659**	**Reserva Betary, São Paulo state, Brazil**	**Atlantic Forest**
***O. lacrimospora* 123 EET**	***Polybia* sp.**	**Herbarium VIC - UFV**	**49661**	**Estação Ecológica do Tripuí, Minas Gerais state, Brazil**	**Ecotone Atlantic Forest - Cerrado**
***O. prezotii* 83 RBET**	***Agelaia angulata***	**Herbarium VIC - UFV**	**49656**	**Reserva Betary, São Paulo state, Brazil**	**Atlantic Forest**
***O. prezotii* 130 RBET**	***Agelaia angulata***	**Herbarium VIC - UFV**	**49657**	**Reserva Betary, São Paulo state, Brazil**	**Atlantic Forest**
***O. pseudocitriformis* 113 RBET**	***Angiopolybia* sp.**	**Herbarium VIC - UFV**	**49662**	**Reserva Betary, São Paulo state, Brazil**	**Atlantic Forest**
***O. pseudocitriformis* 131 RBET**	***Pompillidae***	**Herbarium VIC - UFV**	**49663**	**Reserva Betary, São Paulo state, Brazil**	**Atlantic Forest**
***O. sanctaluciae* 11 ESL**	***Agelaia vicina***	**Herbarium VIC - UFV**	**49658**	**Estação Biológica de Santa Lúcia, Espírito Santo state, Brazil**	**Atlantic Forest**
***O. tripuiensis* 124 EET**	***Agelaia angulata***	**Herbarium VIC - UFV**	**49660**	**Estação Ecológica do Tripuí, Minas Gerais state, Brazil**	**Ecotone Atlantic Forest - Cerrado**

Reserva Biológica Augusto Ruschi (RBAR; 3,573 ha; 19° 54' 26" S, 40° 33' 11" W) and Estação Biológica de Santa Lúcia (ESL; 440 ha; 19° 57' 54"S, 40° 32' 24" W) are two areas classified as dense ombrophilous mountain forests and the climate classified as humid subtropical (Veloso & Goes-Filho 1982). At Estação Biológica de Santa Lúcia, we searched for wasps along the banks of the Timbuí River and on the river trail of the research station. At Reserva Biológica Augusto Ruschi, collection was conducted along the waterfall trail. The collections were conducted in January 2023 and October 2024.

Reserva Betary (RBET; 24° 35' 16.80" S, 48° 37' 40.80" W) covers 60 ha of Atlantic Forest, 100 m a.s.l., and is situated within the protective zone of the Parque Estadual Turístico do Alto Ribeira (PETAR). The area is classified as dense ombrophilous forest in an advanced regeneration status (Falaschi *et al*. 2019, Rebouças *et al*. 2021). The collections were conducted mostly on the 'Betary' trail continually from September 2023 to February 2025.

Our sampling methodology consisted of the careful inspection of the vegetation (mainly understory shrubs) close to the ground up to ca. 2 m height. Infected wasps attached to the substratum (leaves and twigs) were transferred to sterile tubes and taken to the Laboratory of Insect-Microbe Interactions at the Universidade Federal de Viçosa (LIIM-UFV) for morphological and molecular analyses. The wasp hosts were identified by comparing morphological characters while parasitic fungi were identified by comparing morphological and molecular characters. Voucher specimens (Holotype and Paratypes) were deposited at the VIC Fungarium, Universidade Federal de Viçosa, Brazil (VIC-UFV).

3.3.2 DNA extraction, amplification and sequencing

For molecular identification, DNA was directly extracted from ascostromata (ascoma or fertile region + stromata) and synnemata. The structures were directly removed from infected wasps and macerated in microtubes placed in liquid nitrogen to freeze. DNA extraction was performed using the Wizard™ Genomic DNA Purification Kit (Promega™, Madison, USA), following the manufacturer's recommendations. The concentration and final quality of the DNA extraction were determined using NanoDrop™ spectrophotometer (Thermo Scientific™, Waltham, USA).

In total, four genomic regions were amplified using polymerase chain reaction (PCR): I) the small nuclear ribosomal subunit (SSU); II) the large nuclear ribosomal subunit (LSU); III) the translation elongation factor 1-α (*TEF*); and IV) RNA polymerase II largest subunit (*RPB1*). The primers used for each region were: SSU: NS1 (5'-GTAGTCATATGCTTGTCTC-3') and NS4 (5'-CTTCCGTCAATTCC-TTTAAG-3') (White *et al.* 1990); LSU: LR0R (5'-

ACCCGCTGAAC-TTAAGC-3’) (Hopple Jr 1994) and LR5 (5’-TCCTGAGGGAAACTTCG-3’) (Vilgalys & Hester 1990); *TEF*: 983F (5’-GCYCCYGGHCAYCGTGAYTTYAT-3’) and 2218R (5’-ATGACACCRACRG-CRACRGTYTG-3’) (Rehner & Buckley 2005); RPB1: (5’-CCWGGYTTYATCAAGAARGT-3’) and RPB1Cr_oph (5’-CTGVCCMGCRATGTCGTTGTCCAT -3’) (Araújo *et al.* 2018).

Each 25 µL-PCR reaction contained 12.5 µL KAPA Taq ReadyMix (KAPA Biosystems™), 1 µL of each forward and reverse primer (10 ng/µL), 5 µL of DNA template, and 5.5 µL of DNAse/RNAse Free Water (ZymoBIOMICS™). The PCR reactions were placed in an Eppendorf™ thermocycler under the following conditions. For SSU and LSU (1) 2 min at 95 °C, (2) 4 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 2 min, followed by (3) 35 cycles of denaturation at 94 °C for 30 s, annealing at 50.5 °C for 1 min, and extension at 72 °C for 1 min and (4) 5 min at 72 °C. For *TEF* (1) 2 min at 94 °C, (2) 10 cycles of denaturation at 94 °C for 30 s, annealing at 64 °C for 1 min, and extension at 72 °C for 1 min, followed by (3) 35 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 1 min, and extension at 72 °C for 1 min and (4) 3 min at 72 °C. For *RPB1*(1) 2 min at 95 °C, (2) 10 cycles of denaturation at 95 °C for 30 s, annealing at 66 °C for 1 min, and extension at 72 °C for 1 min, followed by (3) 35 cycles of denaturation at 95 °C for 30 s, annealing at 56.6 °C for 1 min, and extension at 72 °C for 1 min and (4) 3 min at 72 °C. After amplification, PCR products were purified and sequenced by Macrogen™ (Seoul, South Korea). The generated bidirectional sequences (forward and reverse) were assembled into contigs in Geneious Prime™ 2025.0.3 (Geneious Prime 2024).

3.3.3 Phylogenetic analyses

The obtained contigs were compared to sequences of relates species deposited in the NCBI (https://www.ncbi.nlm.nih.gov/) nucleotide database using BLASTn at Geneious Prime™ 2025.0.3 (Kearse *et al.* 2012) to confirm that they belonged to the genus *Ophiocordyceps*. We aligned the obtained sequences of wasp-parasitic *Ophiocordyceps* to a database with 537 OTUs from species (Table 2; Supplementary material I) that broadly represent the order *Hypocreales*. The final database consisted of 323 SSU, 479 LSU, 395 *TEF* and 476 RBP1 sequences.

We generated, improved (excluding gaps and removing ambiguous alignments) and combined individual gene alignments at Geneious Prime 2025.0.3. The final alignment length was 3,954 bp: with 1,116 bp for SSU, 1,031 bp for LSU, 1,072 bp for *TEF* and 939 bp for RBP1. We used the Modeltest-NG 0.1.7 (Darriba *et al.* 2020) on CIPRES Science Gateway v.3.3 (Miller *et al.* 2010) for selecting the nucleotide substitution models and considering the lowest Akaike Information Criterion (AIC). The final dataset consisted of eight data partitions (one for SSU, one for LSU, three for *TEF* and three for RBP1).

A concatenated phylogenetic tree for all four genes was reconstructed performing a Maximum Likelihood analysis, based on the fit-model for each region (GTR+I+G4) and generating 1,000 bootstrap replicates, using IQ-Tree 2.2.2.7 (Nguyen *et al.* 2015, Chernomor *et al*. 2016, Kalyaanamoorthy *et al*. 2017, Hoang *et al*. 2018, Minh *et al*. 2020) on CIPRES webserver. The species *Verticillium dahliae* (*Glomerellales*) ATCC 16535 was used as the outgroup. The resulting phylogenetic tree was visualized on FigTree v.1.4.4 (Rambaut 2017) and edited on Adobe Illustrator™.

Table 2. List of *Ophiocordyceps/Hirsutella* species, vouchers, GenBank accession numbers and host associations. Species from this study are highlighted in **bold font**. * = yet to be deposited in Genbank.

Species	Voucher	SSU	LSU	*TEF*	*RPB1*	Host	Reference
Hirsutella citriformis	ARSEF 1035	KM652064	KM652105	KM651989	KM652030	*Hemiptera*	Simmons *et al*. (2015)
Hirsutella citriformis	ARSEF 1446	KM652065	KM652106	KM651990	KM652031	*Hemiptera*	Simmons *et al*. (2015)
Hirsutella citriformis	CHE-CNRCB 335	KY587216	–	KY587203	KY587213	*Hemiptera*	Berlanga-Padilha *et al*. (2018)
Hirsutella citriformis	CHE-CNRC 339	KY587217	–	KY587204	KY587214	*Hemiptera*	Berlanga-Padilha *et al*. (2018)
Hirsutella citriformis	CHE-CNRC 375	KY587218	–	KY587205	KY587215	*Hemiptera*	Berlanga-Padilha *et al*. (2018)
Ophiocordyceps acanthoponerae	VIMI_2023_0001	PV283217	PV283219	PV356076	PV356074	*Hymenoptera*	Lima-Santos *et al*. (2025)
Ophiocordyceps acanthoponerae	VIMI_2023_0002	PV283216	PV283218	PV356075	–	*Hymenoptera*	Lima-Santos *et al*. (2025)
Ophiocordyceps albacongiuae	RC20	KX713633	–	KX713670	–	*Hymenoptera*	Araújo *et al*. (2019)
Ophiocordyceps blakebarnesii	MISSOU5	KX713641	KX713610	KX713688	KX713716	*Hymenoptera*	Araújo *et al*. (2018)
Ophiocordyceps camponoti-atricipis	ATRI3	KX713666	–	KX713677	–	*Hymenoptera*	Araújo *et al*. (2018)
Ophiocordyceps camponoti-balzani	G104	KX713660	KX713593	KX713689	KX713703	*Hymenoptera*	Araújo *et al*. (2018)
Ophiocordyceps camponoti-bispinosi	OBIS5	KX713636	KX713616	KX713693	KX713721	*Hymenoptera*	Araújo *et al*. (2018)
Ophiocordyceps camponoti-femorati	FEMO2	KX713663	KX713590	KX713678	KX713702	*Hymenoptera*	Araújo *et al*. (2018)
Ophiocordyceps camponoti-floridani	Flx2	–	KX713592	KX713674	–	*Hymenoptera*	Araújo *et al*. (2018)
Ophiocordyceps camponoti-floridani	Flo4	KX713662	KX713591	–	–	*Hymenoptera*	Araújo *et al*. (2018)
Ophiocordyceps camponoti-hippocrepidis	HIPPOC	KX713655	KX713597	KX713673	KX713707	*Hymenoptera*	Araújo *et al*. (2018)
Ophiocordyceps camponoti-leonardi	C36	KJ201512	–	JN819013	–	*Hymenoptera*	Kobmoo *et al*. (2018)
Ophiocordyceps camponoti-nidulantis	NIDUL2	KX713640	KX713611	KX713669	KX713717	*Hymenoptera*	Araújo *et al*. (2018)
Ophiocordyceps camponoti-renggeri	RENG2	KX713632	–	KX713672	–	*Hymenoptera*	Araújo *et al*. (2018)
Ophiocordyceps camponoti-rufipedis	G108	KX713659	KX713594	KX713679	KX713704	*Hymenoptera*	Araújo *et al*. (2018)
Ophiocordyceps camponoti-saundersi	C40	KJ201519	–	JN819012	–	*Hymenoptera*	Araújo *et al*. (2018)
Ophiocordyceps daceti	MF01	–	KX713604	KX713667	–	*Hymenoptera*	Araújo *et al*. (2018)
O. dendritica	**114RBET**	**PV645090**	**PV645082**	**PV641701**	**PV641768**	***Mischocyttarus* sp. (*Vespidae*)**	**This study**
Ophiocordyceps elongata	OSC 110989	–	EF468808	EF468748	EF468856	*Lepidoptera*	Quandt *et al*. (2014)
Ophiocordyceps halabalaensis	MY1308	KM655825	–	GU797109	–	*Hymenoptera*	Luangsa-ard *et al*. (2011)
Ophiocordyceps humbertii	MF116A	MK874747	MK875537	–	MK863828	*Hymenoptera*	Araújo *et al*. (2019)
Ophiocordyceps humbertii	MF116B	MK874748	MK875536	–	MK863829	*Hymenoptera*	Araújo *et al*. (2019)
***O. humbertii* s.l.**	**92AM**	**PV642464**	**PV642454**	**PV641695**	**PV641763**	***Agelaia* sp. (*Vespidae*)**	**This study**
***O. humbertii* s.l.**	**31EET**	**PV642465**	**PV642449**	**PV641691**	**–**	***Polybia fastidiosuscula* (*Vespidae*)**	**This study**
***O. humbertii* s.l.**	**32EET**	**PV642467**	**PV642456**	**PV641696**	**PV641762**	***Agelaia angulata* (*Vespidae*)**	**This study**
***O. humbertii* s.l.**	**119EET**	**PV642463**	**PV642451**	**PV641694**	**–**	***Agelaia multipicta* (*Vespidae*)**	**This study**
***O. humbertii* s.l.**	**121EET**	**PV642461**	**PV642452**	**PV641699**	**PV641757**	***Polybia* sp. (*Vespidae*)**	**This study**
***O. humbertii* s.l.**	**120EET**	**PV642462**	**PV642455**	**PV641693**	**PV641756**	***Polybia* sp. (*Vespidae*)**	**This study**
***O. humbertii* s.l.**	**122EET**	**PV642468**	**PV642457**	**PV641697**	**PV641758**	***Agelaia vicina* (*Vespidae*)**	**This study**
***O. humbertii* s.l.**	**29MP**	**PV645091**	**PV645085**	**–**	**PV641765**	***Agelaia multipicta* (*Vespidae*)**	**This study**
***O. humbertii* s.l.**	**18RBAR**	**PV645087**	**PV645084**	**–**	**PV641767**	***Apoica* sp. (*Vespidae*)**	**This study**

O. humbertii s.l.	**80RBET**	**PV645089**	**PV645083**	**–**	**PV641766**	**_Agelaia angulata_ (_Vespidae_)**	**This study**
O. humbertii s.l.	**W1**	**–**	**Sequenced***	**Sequenced***	**Sequenced***	**_Agelaia fulvofasciata_ (_Vespidae_)**	**This study**
O. humbertii s.l.	**W10**	**Sequenced***	**Sequenced***	**–**	**Sequenced***	**_Agelaia_ sp. (_Vespidae_)**	**This study**
O. humbertii s.l.	**W13**	**–**	**Sequenced***	**–**	**Sequenced***	**_Polistes claripenis_ (_Vespidae_)**	**This study**
O. humbertii s.l.	**W14**	**Sequenced***	**Sequenced***	**Sequenced***	**Sequenced***	**_Polybia dimidiata_ (_Vespidae_)**	**This study**
O. humbertii s.l.	**W15**	**Sequenced***	**Sequenced***	**Sequenced***	**Sequenced***	**_Agelaia constructor_ (_Vespidae_)**	**This study**
O. humbertioides	**2RBAR**	**PV642466**	**PV642458**	**PV641700**	**PV641760**	**_Agelaia vicina_ (_Vespidae_)**	**This study**
O. humbertioides	**3RBAR**	**PV642460**	**PV642453**	**PV641692**	**PV641761**	**_Agelaia vicina_ (_Vespidae_)**	**This study**
O. humbertioides	**12ESL**	**PV642469**	**PV642450**	**PV641698**	**PV641759**	**_Agelaia vicina_ (_Vespidae_)**	**This study**
Ophiocordyceps kimflemingiae	SC30	KX713629	KX713622	KX713699	KX713727	*Hymenoptera*	Araújo *et al*. (2019)
Ophiocordyceps kniphofioides	HUA 186148	KC610790	KF658679	KC610739	KF658667	*Hymenoptera*	Sanjuán *et al*. (2015)
O. lacrimospora	**123EET**	**–**	**PV645081**	**PV641702**	**PV641764**	**_Polybia_ sp. (_Vespidae_)**	**This study**
Ophiocordyceps monacidis	MF74	KX713646	KX713606	–	–	*Hymenoptera*	Araújo *et al*. (2018)
Ophiocordyceops naomipierceae	DAWKSANT	KX713664	KX713589	–	KX713701	*Hymenoptera*	Araújo *et al*. (2018)
Ophiocordyceps oecophyllae	OECO1	KX713635	–	–	–	*Hymenoptera*	Araújo *et al*. (2018)
Ophiocordyceps ootakii	J13	KX713652	KX713600	KX713681	KX713708	*Hymenoptera*	Araújo *et al*. (2018)
Ophiocordyceps polyrhachis-furcata	P39	KJ201504	–	JN819003	–	*Hymenoptera*	Kobmoo *et al*. (2015)
Ophiocordyceps ponerinarum	HUA 186140	KC610789	KC610767	KC610740	KF658668	*Hymenoptera*	Sanjuan *et al*. (2015)
O. prezotii	**83RBET**	**PV643881**	**PV643883**	**PV641690**	**PV641754**	**_Agelaia angulata_ (_Vespidae_)**	**This study**
O. prezotii	**130RBET**	**PV643882**	**PV643884**	**–**	**PV641753**	**_Agelaia angulata_ (_Vespidae_)**	**This study**
O. pseudocitriformis	**113RBET**	**PV645070**	**PV645071**	**PV647905**	**PV647907**	**_Angiopolybia_ sp. (_Vespidae_)**	**This study**
O. pseudocitriformis	**131RBET**	**PV645069**	**PV645072**	**–**	**PV647906**	**_Pompillidae_ (_Vespidae_)**	**This study**
Ophiocordyceps pulvinata	TNS-F 30044	GU904208	–	GU904209	GU904210	*Hymenoptera*	Kepler *et al*. (2011)
Ophiocordyceps rami	MY6736	KM655823	–	KJ201532	–	*Hymenoptera*	Kobmoo *et al*. (2015)
O. sanctaluciae	**11ESL**	**PV642448**	**PV642459**	**PV641704**	**PV641755**	**_Agelaia vicina_ (_Vespidae_)**	**This study**
Ophiocordyceps satoi	J7	KX713653	KX713599	KX713683	KX713711	*Hymenoptera*	Araújo *et al*. (2018)
Ophiocordyceps tiputini	QCNE 186287	KC610792	KC610773	KC610745	KF658671	*Megaloptera*	Sanjuan *et al*. (2015)
O. tripuiensis	**124EET**	**PV645088**	**PV645086**	**PV641703**	**PV641769**	**_Agelaia angulata_ (_Vespidae_)**	**This study**
Ophiocordyceps unilateralis s.l.	Gh41	KX713656	–	KX713668	KX713706	*Hymenoptera*	Araújo *et al*. (2019)
Ophiocordyceps unilateralis	SERI1	KX713628	KX713626	KX713675	KX713730	*Hymenoptera*	Araújo *et al*. (2020)
Ophiocordyceps unilateralis	SERI2	KX713627	–	KX713676	KX713731	*Hymenoptera*	Araújo *et al*. (2020)

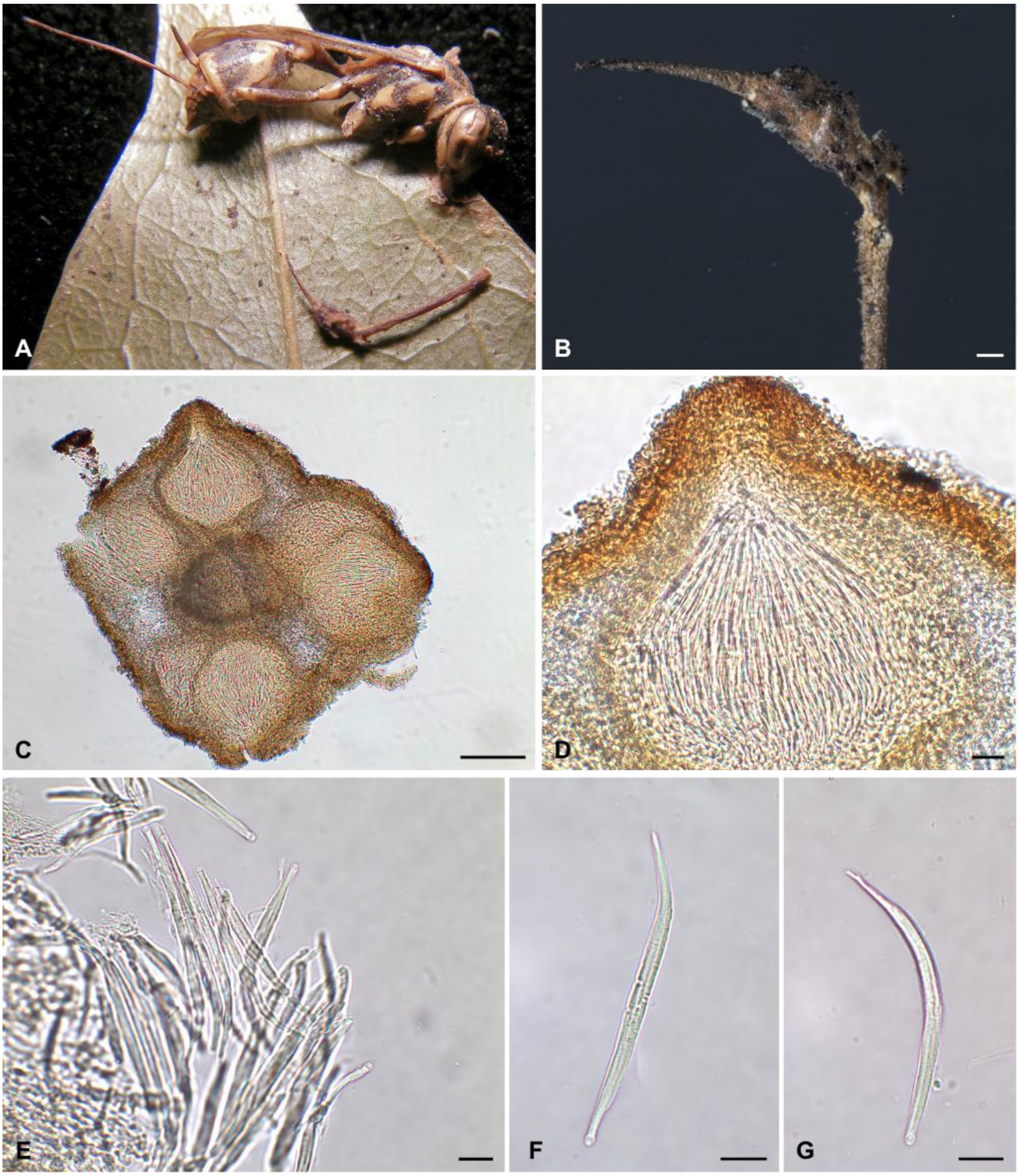

Fig. 2. Original specimen described by Petch as *O. humbertii*. **A.** *Ropalidini* wasp biting onto leaf with a broken ascostromata clavae placed in the bottom-right corner. **B.** Broken ascostromata clavae. **C.** Transversal section of ascostromata showing the perithecial arrangement. **D.** Perithecium. **E.** Cluster of asci erupting from the perithecium. **F-G.** Asci. Scales bars: B = 0.2 mm; C = 100 µm; D-G = 20 µm.

3.3.4 Morphological characterisation

To properly describe the new species herein proposed, we had access to the original *O. humbertii* specimen (Fig. 2) described by Petch (1935). This specimen was collected by P. Synge, a botanist listed as one of collectors of The Oxford University Expedition to Sarawak (Malaysia) in 1932 (Harrison 1933). This is the same expedition and country listed in Petch's description. The specimen is located in the Fungi (Overseas) Ascomycotina collection, under the code 1.1.19.3, at Royal Botanic Gardens, Kew. In his description, Petch stated that the specimen bore two clavae emerging from the thorax, from the points of insertion of the wings on each side, with the upper part (or apex) being hair-like. The fertile region (or ascoma) was dark brown, not terminal and not lateral as in *O. unilateralis*. The spores were narrow-fusoid, measuring 75 × 2.5 µm, and septate.

Petch also stated the existence of three dark brown hair-like clavae from the abdomen, which he described as the asexual *H. saussurei*, without giving more morphological details or measurements of these latter structures. Host identification was not provided, but the host is an exemplar of the tribe Ropalidini (*Polistinae*), that does not occur in the Neotropical region (recognized by AS). The specimen was also not treated as a holotype, even though it is the only exemplar used in a formal description for this species.

Samples were examined and photographed under a stereomicroscope ZEISS SteREO Discovery.V20™ with an attached camera (AxioCam MRc). Characteristics such as the host's position and attachment to the plant substrate, shape, color, and size of the macroscopic sexual and asexual structures were considered for macro-morphological characterisation.

For micro-morphological characterisation, stromata and synnemata were removed from hosts and were sectioned by hand and placed on slides with lactoglycerol (20 mL lactic acid in 40 mL glycerol and 20 mL distilled water). They were examined under a Nikon Eclipse E200™ photomicroscope with an attached digital camera (Moticam 3+™ with 3.0 megapixels). Microscopic fungal structures such as ascocarps, perithecia, asci, ascospores, secondary ascospores (i.e. capilliconidiophore and capilliconidia), phialides and asexual spores (i.e. conidia) were characterized in terms of arrangement, color, shape and size (Table 3). Only specimens that were evidently morphologically distinguished from the original description of *O. humbertii* and with sufficient characters to be measured and compared were selected for species description.

Table 3. Comparison of host, death position, stromata, ascospores and synnemata morphology, and geographic distribution of hirsutelloid species of *Ophiocordyceps*. The new species are highlighted in **bold font**.

			Stromata			Ascospores			Synnemata		Phialides		Conidia				
Species	**Host**	**Death position**	**Production**	**Fertile heads**	**Shape**	**Mean Size (µm)**	**Septation**	**Capilliconidia Mean Size (µm)**	**Ramification**	**Production**	**Shape**	**Mean Size (µm)**	**Shape**	**Mean Size (µm)**	**Mucous Matrix**	**Distribution**	**Reference**
Ophiocordyceps humbertii	*Ropalidini*	biting leaf	Insertion of the wings	Dark brown	Narrow-fusoid	75 × 2.5	6 to 16µm apart	–	–	Abdomen	–	–	–	–	–	Sarawak, Malaysia	Petch 1935
Hirsutella saussurei	*Polistes* spp.	–	–	–	–	–	–	–	Branched	Host's body	Lageniform	–	Allantoid	9-11 x 1-1.5	yes	North Carolina, Hawaii, California, British West Indies	Spare 1920
Ophiocordyceps humbertioides	***Agelaia vicina***	**biting leaf**	**Insertion of the wings**	**Brown**	**Vermiform**	**91 × 2.5**	**7-septate**	**7.7 × 2**	**Few and simple**	**Abdomen, legs and antennae**	**Lageniform**	**14.2 × 3.3**	**Fusiform to allantoid**	**14.2 × 3.3**	**no**	**Espiríto Santo state, Brazil**	**This study**
Ophiocordyceps prezotii	***Agelaia angulata***	**biting leaf**	**Insertion of the wings**	**Light brown**	**Vermiform**	**104 × 2.8**	**7-septate**	**–**	**Few and simple**	**Legs and antennae**	**Lageniform**	**13.9 × 3.5**	**Allantoid**	**7 × 2**	**no**	**São Paulo state, Brazil**	**This study**
Ophiocordyceps sanctaluciae	***Agelaia vicina***	**biting leaf**	**Insertion of the wings**	**Brown**	**–**	**–**	**–**	**–**	**Multiple and simple**	**All host's orifices and sutures**	**Lageniform**	**31.5 × 4.2**	**Allantoid**	**6.4 × 2.2**	**yes**	**Espiríto Santo state, Brazil**	**This study**
Ophiocordyceps dendritica	***Mischocyttarus* sp.**	**on leaf**	**–**	**–**	**–**	**–**	**–**	**–**	**Multiple and branched**	**All host's orifices and sutures**	**Lageniform**	**28 × 4**	**Fusoid**	**7.4 × 2**	**yes**	**São Paulo state, Brazil**	**This study**
Ophiocordyceps tripuiensis	***Agelaia angulata***	**biting leaf**	**Insertion of the wings**	**–**	**–**	**–**	**–**	**–**	**Multiple and simple**	**All host's orifices and sutures**	**Lageniform**	**32.3 × 4.2**	**Fusoid to allantoid**	**7.3 × 2**	**yes**	**Minas Gerais state, Brazil**	**This study**
Ophiocordyceps lacrimospora	***Polybia* sp.**	**biting leaf**	**Insertion of the wings**	**–**	**–**	**–**	**–**	**–**	**Multiple and simple**	**All host's orifices and sutures**	**Lageniform**	**28.2 × 3.5**	**Tear-shaped**	**7.8 × 2.**	–	**Minas Gerais state, Brazil**	**This study**
Hirsutella citriformis	Hemiptera	–	–	–	–	–	–	–	Multiple, simple or with short and stumpy branches	Host's body	Simple, sessile or subsessile	–	Fusoid	5.5 - 8.5 × 1.5 - 1.8	yes	New Zealand, Porto Rico and Hawaii	Spare 1920
Ophiocordyceps pseudocitriformis	***Angiopolybia* sp.**	**biting leaf**	**–**	**–**	**–**	**–**	**–**	**–**	**Multiple, with short and stumpy branches**	**All host's orifices and sutures**	**Lageniform**	**25.7 × 3.4**	**Fusoid to allantoid**	**6.5 × 1.8**	**yes**	**São Paulo state, Brazil**	**This study**
Ophiocordyceps acanthoponerae	*Acanthoponera mucronata (Formicidae)*	biting leaf	Dorsal pronotum	Dark brown	Vermiform	126 × 2.6	7	126 × 2.6	Single or branched, with secondary smaller and delicate branches	All host's orifices and sutures	Lageniform	13.7 × 4.5	Tear-shaped	8.6 × 2.2	–	Minas Gerais state, Brazil	Lima-Santos *et al.* 2025
Ophiocordyceps elongata	*Apatela americana (Lepidoptera)*	–	One to three arising from each host	Pale yellow	Cylindric, equal	2 in diameter	4-12µm apart	–	–	–	–	–	–	–	–	Tennessee, North Carolina, Maine	Petch 1937

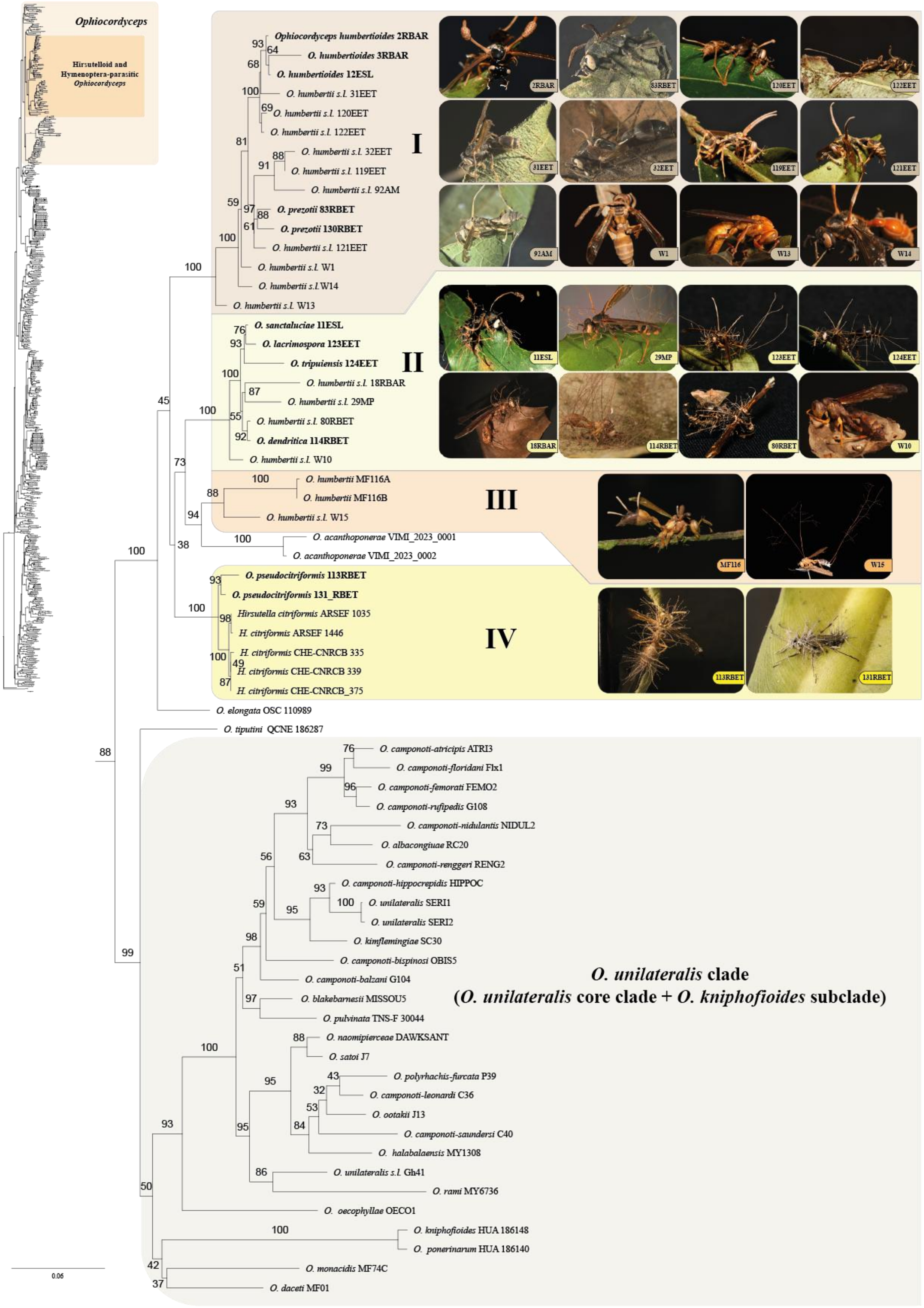

Fig. 3. Maximum likelihood tree of *Ophiocordyceps* obtained with a concatenated dataset of SSU, LSU, TEF and *RPB1*. Numbers on nodes represent bootstrap values. New species are indicated in **bold font**. The Clade I (pale brown), Clade II (pale yellow), Clade III (pale orange), Clade IV (strong yellow) and also the *O. unilateralis* clade (grey) are highlighted in the tree. The whole analysis tree of the order *Hypocreales* is depicted in the top-left corner, with the position of the highlighted *Ophiocordyceps* clades indicated in orange.

3.4 RESULTS

3.4.1 Phylogenetic analyses

We obtained sequences for four genomic regions from 26 specimens of *O. humbertii* sensu lato (SSU=23; LSU=26; TEF=19; *RPB1*=24) (Table 2). Our Maximum Likelihood reconstruction with the obtained sequences revealed the existence of at least four clades composed of manipulative wasp-parasitic fungi (Fig. 3). These four clades were grouped together in a major clade that was strongly supported (BS=100%) alongside the lepidopteran pathogen *O. elongata*. This major clade is also closely-related to the manipulative and myrmecophilous *O. unilateralis* clade (*O. unilateralis* core clade + *O. kniphofioides* sub-clade) with 88% of bootstrap support.

The first clade, here referred to as Clade I, is strongly supported (BS=100%) and is formed by the two newly proposed species, *O. humbertioides* (BS=93%) and *O. prezotii* (BS=88%), but also by the undescribed specimens: *O. humbertii* s.l. 120 EET, *O. humbertii* s.l. 122 EET, *O. humbertii* s.l. 31 EET, *O. humbertii* s.l. 32 EET, *O. humbertii* s.l. 119 EET, *O. humbertii* s.l. 121 EET, *O. humbertii* s.l. 92 AM, *O. humbertii* s.l. W1, *O. humbertii* s.l. W13 and *O. humbertii* s.l. W14. The second clade, referred to as Clade II, is also strongly supported (BS=100%) and is represented by the newly described species *Ophiocordyceps sanctaluciae*, *Ophiocordyceps dendritica*, *Ophiocordyceps tripuiensis* and *O. lacrimospora*, but also by the undescribed specimens: *O. humbertii* s.l. 29 MP, *O. humbertii* s.l. 18 RBAR, *O. humbertii* s.l. 80 RBET and *O. humbertii* s.l. W10.

The third clade (BS=88%), Clade III, is composed of *O. humbertii* MF116 and the undescribed *O. humbertii* s.l. W15. This clade is a sister group of the newly myrmecophilous *O. acanthoponerae* (BS=94%) (Lima-Santos *et al*. 2025, *under review*). The fourth clade, Clade IV, is represented by *H. citriformis* (Spare 1920), widely recognized as a hemipteran pathogen. This clade is strongly supported (BS=100%). We propose a new species within this clade, *O. pseudocitriformis*, infecting wasps, that is strongly supported (BS=93%) and is placed outside of the Hemiptera-associated specimens.

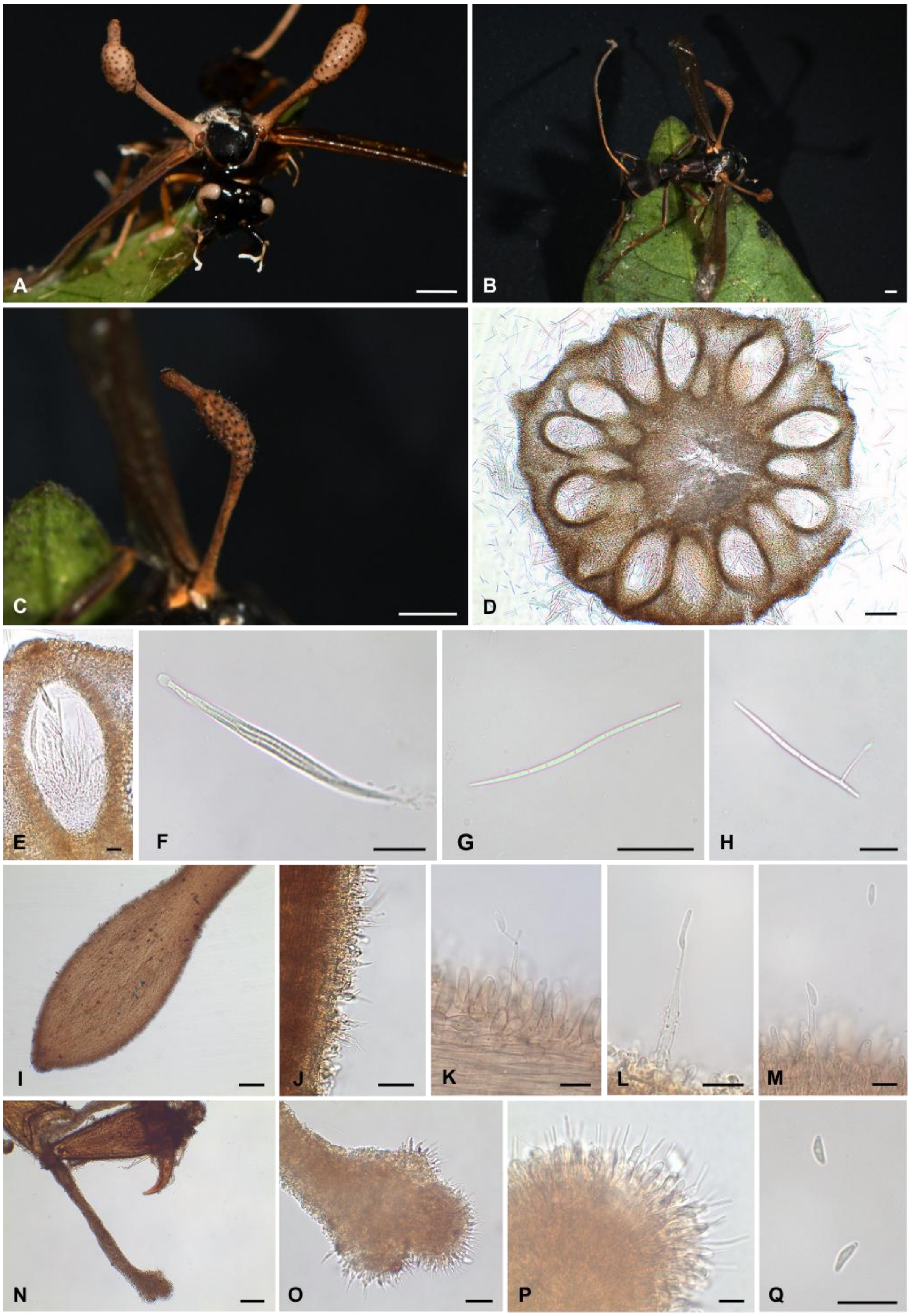

Fig 4. *Ophiocordyceps humbertioides*. **A-B.** Infected *Agelaia vicina* biting onto leaf. **C.** Ascostromata. **D.** Transversal section of ascostromata showing the perithecial arrangement. **E.** Perithecium. **F.** Asci. **G.** Ascospores. **H.** Germinated ascospores with capilliconidium. **I.** Tip of the synnema emerging from the host's abdomen. **J.** Phialides from synnema of the abdomen. **K-M.** Phialides producing conidia in the synnema of the abdomen. **N.** Synnema emerging from the host's leg. **O-P.** Phialides from the synnema of the leg. Q. Conidia from the synnema of the leg. Scale bars: A-C = 1 mm; D = 100 µm; E-H = 20 µm; I = 100 µm; J = 20 µm; K-M = 10 µm; N= 100 µm; O = 20 µm; P-Q = 10 µm.

3.4.2 Taxonomy

Based on the morphological species concept (i.e. relying on overlapping morphological traits for species delimitation) and phylogenetic species concept (i.e. relying on divergence of nucleotides between monophyletic lineages), new species in the genus *Ophiocordyceps* are proposed:

Ophiocordyceps humbertioides **sp. nov.** S.J. Lima-Santos, T. Mendes-Pereira & T.G. Kloss

MycoBank MB: Not deposited yet, Fig. 4

Etymology: The name refers to the similarity between the proposed species with *O. humbertii*.

Typus. **Brazil**, Espírito Santo state, Santa Teresa municipality, Reserva Biológica Augusto Ruschi (19° 54' 26" S, 40° 33' 11" W), infecting workers of *A. vicina* in forest understorey, 17 Jan. 2023, S.J. Lima-Santos, T. Mendes-Pereira & T.G. Kloss (**holotype** VIC 49652; Isolate number: 3RBAR; Genbank: SSU = PV642460; LSU = PV642453; TEF = PV641692; *RPB1* = PV641761).

Paratypes: **Brazil**, Espírito Santo state, Santa Teresa municipality, Reserva Biológica Augusto Ruschi (19° 54' 26" S, 40° 33' 11" W), infecting workers of *A. vicina* in forest understorey, 17 Jan. 2023, S.J. Lima-Santos, T. Mendes-Pereira & T.G. Kloss, VIC 49650 (Isolate number: 2RBAR; Genbank: SSU = PV642466; LSU = PV642458; TEF = PV641700; *RPB1* = PV641760); 15 Oct. 2024, T. Mendes-Pereira & T.G. Kloss, VIC 49653 (Isolate number: 116RBAR), VIC 49654 (Isolate number: 117RBAR), VIC 49655 (Isolate number: 118RBAR). **Brazil**, Espírito Santo state, Santa Teresa municipality, Estação Biológica de Santa Lúcia (19° 56′ 10″ S, 40° 36′ 06″ W), infecting a worker of *A. vicina* in forest understorey, 19 Jan. 2023, T. Mendes-Pereira & T.G. Kloss, VIC 49651 (Isolate number: 12ESL; Genbank: SSU = PV642469; LSU = PV642450; TEF = PV641698; *RPB1* = PV641759).

Description: *External mycelium* produced from all host orifices and sutures, white in the early stages becoming light brown at maturity. *Stromata* produced from the insertion of the wing on each side, averaging (4.6–) 5.5 (–7.1) × (0.2–) 0.4 (–0.9) mm [(min.–) mean length (–max.) × (min.–) mean width (–max.)] , clavate, brown, with a rounded apex (Fig. 4A-C); *Fertile region* consisting of a brown ascoma, covering all the sides of the stroma and not reaching the tip of the stroma, averaging (1.5–) 2.2 (–3.3) × (0.2–) 0.7 (–1) mm; *Perithecia* immersed to partially erumpent, flask shaped, (231.5–) 291.6 (–361) × (88–) 160.4 (–225) µm (Fig. 4D-E); *Asci* with 8 intertwined ascospores, hyaline, (86.7–)134.2 (–159.5) × (5.5–) 160.4 (–10.4) µm (Fig. 4F), with a prominent apical cap, averaging (4.6–) 5.8 (–8.4) × (4–) 5.7 (–7.1) µm; *Ascospores* hyaline, thin-walled, vermiform, 7-septate, (72–) 91 (–108) × (1.5–) 2.5 (–3.3) µm (Fig. 4G-H). *Asexual morph*: *Synnemata* from host's abdomen clavate, simple, brown, with a rounded apex, growing up to 20 mm (Fig. 4I); *Phialides* lageniform to cylindrical, simple to verrucose on the base, hyaline, (14.6–) 24.4 (–31.3) × (2–) 3.6 (–4.9) µm, tapering into a neck 6.7–22 µm in length (Fig. 4J-N); *Conidia* oblong to cylindrical, (5.1–) 7.8 (–9.9) × (1.4–) 2.3 (–3.3) µm (Fig. 4L-N). *Smaller synnemata* emerging from host's antennae and legs, clavate, simple or branched, brown, with a lobed apex, (0.5–) 0.8 (–1.1) × (0.04–) 0.07 (–0.1) mm (Fig. O-P); *Phialides* lageniform, (8.7–) 14.2 (–17.4) × (2.6–) 3.3 (–3.7) µm, tapering into a neck 3.9–9.4 µm in length (Fig. 4P-Q); *Conidia* fusiform to allantoid, guttulate, (8.7–) 14.2 (–17.4) × (2.6–) 3.3 (–3.7) µm (Fig. 4R).

Germination process: Ascospore germinating into a straight capilliconidiophore, averaging 20 × 2 µm, bearing a single capilliconidium, averaging 7.7 × 2 µm (Fig. 4H).

Habitat: Dense ombrophilous mountain forests belonging to the Atlantic Forest in the municipality of Santa Teresa, Espírito Santo state, Brazil.

Notes: *O. humbertioides* differs from the original *O. humbertii* mostly regarding the shape of the ascostromata, with brown fertile regions and rounded apexes, whilst *O. humbertii* presents ascostromata with hair-like apexes. They are also different considering host association, with *O. humbertii* being associated with a *Ropalidini* wasp species that does not occur in the neotropics whilst *O. humbertioides* infects the neotropical *A. vicina*. Additionally, we observed that this species is ubiquitous along the trails of both Reserva Biológica Augusto Ruschi and Estação Biológica de Santa Lúcia reserves. We have constantly collected infected *A. vicina* at different stages of infection at different months of the year.

***Ophiocordyceps prezotii* sp. nov.**, S.J. Lima-dos-Santos, O.H.P. Della-Torre & J.P.M. Araújo

MycoBank MB: Not deposited yet, Fig. 5

Etymology: Named after Professor Fabio Prezoto, a renowned Brazilian entomologist and professor at the Federal University of Juiz de Fora, Brazil (UFJF). His career has been marked by the mentorship and training of numerous researchers in the field of behaviour and biology of social wasps, including SJLS and BCB. He is also one of the pioneers in highlighting the economic importance of social wasps, emphasizing their role in the biological control of agricultural pests.

Typus: **Brazil**, São Paulo state, Iporanga municipality, Reserva Betary (24° 35' 09" S, 48° 35' 34' W), infecting worker of *A. angulata* in forest understorey, 13 Nov. 2023, B. Sanderman & O.H.P. Della-Torre (**holotype** VIC 49656; Isolate number: 83RBET; Genbank: SSU = PV643881; LSU = PV643883; TEF = PV641690; *RPB1* = PV641754).

Paratypes: **Brazil**, São Paulo state, Iporanga municipality, Reserva Betary (24° 35' 09" S, 48° 35' 34' W), infecting worker of *A. angulata* in forest understorey, 12 Sep. 2024, O.H.P. Della-Torre, VIC 49657 (Isolate number: 130RBET; Genbank: SSU = PV643882; LSU = PV643884; *RPB1* = PV641753).

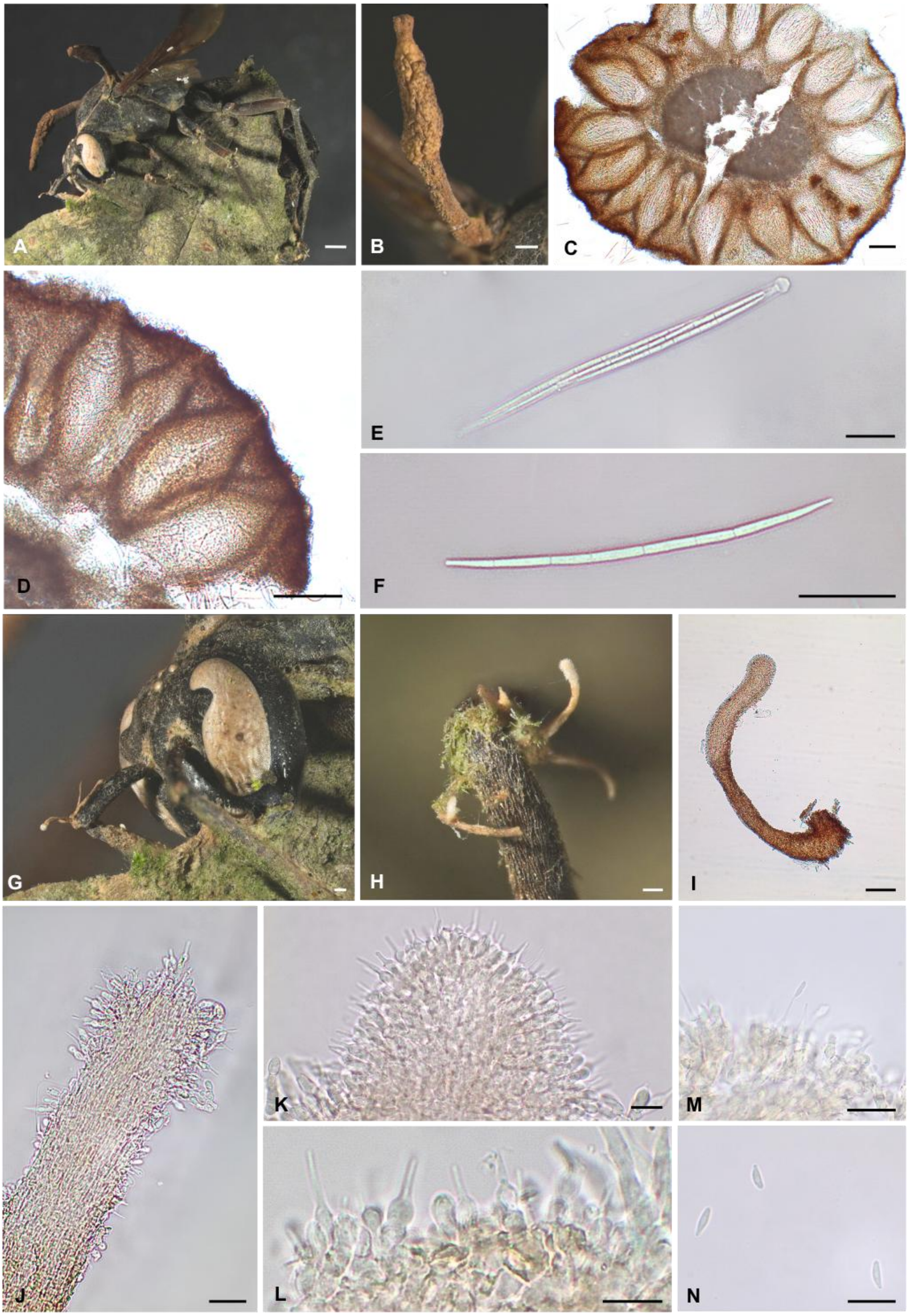

Fig 5. *Ophiocordyceps prezotii*. **A.** Infected *Agelaia angulata* biting onto a leaf. **B.** Ascostromata. **C.** Transversal section of ascostromata showing the perithecial arrangement. **D.** Perithecium. **E.** Asci. **G.** Ascospores. **G-H.** Synnemata emerging from the host's joints. **I-J.** Synnemata. **K-L.** Phialides. **M-N.** Conidia. Scale bars: A =1 mm; B = 0.5 mm; C = 100 µm; D-F = 20 µm; G = 0.2 mm; H = 0.1 mm; I = 100 µm; J-K = 20 µm; L-N = 10 µm.

Description: *External mycelium* produced from all orifices and sutures, white in the early stages becoming light brown at maturity. *Stromata* produced in pairs, each emerging from the insertion of the wing and the thorax on each side, averaging 5.5 × 0.5 mm, clavate, light brown, tapering into a truncate apex (Fig. 5A-B); *Fertile region* consisting of a light brown ascoma produced around the stroma, covering approximately half of its length, sub-terminal, averaging 2.75 × 0.96 mm (Fig. 5B); *Perithecia* immersed to partially erumpent, flask shaped, (267–) 348 (–446) × (120–) 180 (–238) µm (Fig. 5C-D); *Asci* with 8 intertwined ascospores, hyaline, (106–)139 (–189) × (6.4–) 8.7 (–11.5) µm (Fig. 5E), with prominent apical caps, averaging 3–6 × 6–9 µm; *Ascospores* hyaline, thin-walled, vermiform, 7-septate, (75–)104 (–136) × (2–) 2.8 (–3.5) µm (Fig. 5F). *Asexual morph*: *Synnemata* clavate, simple or branched, light brown, produced from the leg joints and antennae, with apical light-cream fertile regions, averaging 0.5 × 0.05 mm (Fig. 5G-I); *Phialides* lageniform, hyaline, (9–) 13.9 (–19.8) × (2.5–) 3.5 (–4.9), tapering into a neck 3.2–10.7 µm in length (Fig. 5J-L); *Conidia* allantoid, guttulate, (5.4–) 7 (–8.6) × (1.4–) 2 (–2.7) µm (Fig. 5M-N).

Germination process: Not observed, only dried material was studied.

Habitat: Dense ombrophilous forest in an advanced regeneration status belonging to a fragment of Atlantic Forest in the Iporanga municipality, São Paulo state, Brazil.

Notes: This species is readily distinguished from *O. humbertii* and *O. humbertioides* mainly regarding the light brown colour and truncate apexes of the ascostromata.

Ophiocordyceps sanctaluciae **sp. nov**., S.J. Lima-Santos, T. Mendes-Pereira & T.G. Kloss

MycoBank MB: Not deposited yet, Fig. 6

Etymology: Named after the conservation unit Estação Biológica de Santa Lúcia, located at Santa Teresa municipality, Espírito Santo state, from where the species was collected.

Typus: **Brazil**, Espírito Santo state, Santa Teresa municipality, Estação Biológica de Santa Lúcia (19° 56′ 10″ S, 40° 36′ 06″ W), infecting worker of *A. vicina* in forest understorey, 18 Jan. 2023, S.J. Lima-Santos, T. Mendes-Pereira & T.G. Kloss (**holotype** VIC 49658; Isolate number: 11ESL; Genbank: SSU = PV642448; LSU = PV642459; TEF = PV641704; *RPB1* = PV641755).

Description: *External mycelium* light brown emerging from the host's sutures. *Sexual morph:* Two immature, dark brown stromata, with acute apex, arising from the insertion of the wing and the thorax on each side (Fig. 6A-B). *Asexual morph*: *Synnemata* clavate with a lobed apex, light brown to white towards the apex, not branched, produced from all host's orifices and sutures, 0.78 – 20.2 × 0.04 – 0.11 mm (Fig. 6C-D); *Phialides*, lageniform, hyaline, (16.5–) 31.5 (–44.5) × (2.7–) 4.2 (–5.1) µm (Fig. 6E-I), tapering into a long neck 0.99 – 34.8 µm in length; *Conidia* fusoid to allantoid, hyaline, guttulate, 1–2 conidia embedded in mucous matrices when mature, (3.7–) 6.4 (–8.5) × (1.2–) 2.2 (–3.2) µm (Fig. 6G-J).

Habitat: Dense ombrophilous mountain forests belonging to the Atlantic Forest in the municipality of Santa Teresa, Espírito Santo state, Brazil.

Notes: This species is readily distinguished from the other species in Clade II mainly regarding the production of simple and clavate synnemata with lobed apexes. These lobed apexes were not observed for the other species within Clade II.

Fig 6. *Ophiocordyceps sanctaluciae*. **A.** Infected *Agelaia vicina* biting onto a leaf. **B.** Immature Ascostromata. **C-E.** Synnema. **F-I.** Phialides and conidia imbedded in mucous matrices. **J.** Conidium without mucous matrix. Scale bars: A = 2 mm; B = 0.5 mm; C = 100 µm; D-G = 20 µm; H-J = 10 µm.

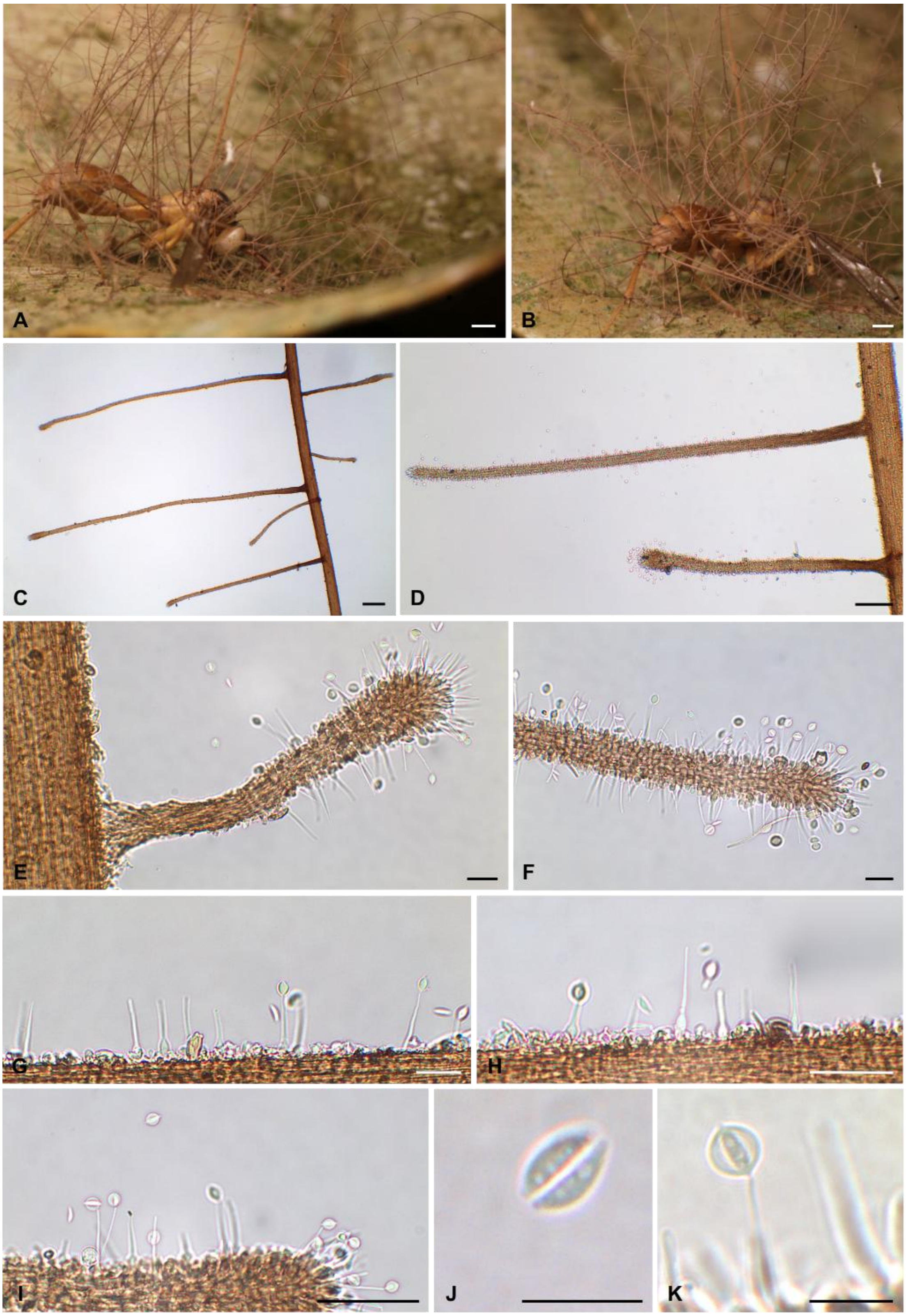

Fig. 7. *Ophiocordyceps dendritica*. **A-B.** Infected *Mischocyttarus sp.* attached to a leaf. **C-D.** Synnema with short and long branches. **E-F.** Close-up on the synnema branches. G-I) Phialides and conidia; J. Conidia. K. Conidium imbedded in a mucous matrix. Scale bars: A-B =1 mm; C = 200 µm; D = 100 µm; E-I = 20 µm; J-K = 10 µm.

Ophiocordyceps dendritica **sp. nov.** S.J. Lima-Santos & O.H.P. Della-Torre

MycoBank MB: Not deposited yet, Fig. 7

Etymology: Name refers to the branched aspect of the synnemata similar to neural dendrites or tree branches.

Typus: **Brazil**, São Paulo state, Iporanga municipality, Reserva Betary (24° 35' 09" S, 48° 35' 34' W), infecting worker of *Mischocyttarus* sp. in forest understorey, 02 May 2024, O.H.P. Della-Torre (**holotype** VIC 49659; Isolate number: 114RBET; Genbank: SSU = PV645090; LSU = PV645082; TEF = PV641701; *RPB1* = PV641768).

Description: *External mycelium* light brown emerging from the host's sutures. *Sexual morph:* not observed. *Asexual morph: Synnemata* very long, growing more than 14 mm, light brown, branched, produced from all host's orifices and sutures (Fig. 7A-B); *Long and short branches*, clavate, light brown, 0.25–2.5 × 0.02–0.04 mm Fig. 7C-F); *Phialides*, lageniform, hyaline, (17–) 28 (–35) × (2.9–) 4 (–5.3) µm tapering into a long neck 11–26.5 µm in length (Fig. 7G-I); *Conidia* fusoid, hyaline, guttulate, embedded in mucous matrices when mature, (5.6–) 7.4 (–8.8) × (1.1–) 2 (–2.9) µm Fig. 7I-K).

Habitat: Dense ombrophilous forest in an advanced regeneration status belonging to a fragment of Atlantic Forest in the Iporanga municipality, São Paulo state, Brazil.

Notes: This species is readily distinguished from the other species in Clade II mainly regarding the production of several branches emerging from the synnemata. All the other species described for Clade II (*O. sanctaluciae*, *O. tripuiensis* and *O. lacrimospora*) were observed producing simple or rarely branched synnemata.

Ophiocordyceps tripuiensis **sp. nov.** S.J. Lima-dos-Santos

MycoBank MB: Not deposited yet, Fig. 8

Etymology: Named after the conservation unit Estação Ecológica do Tripuí, located in the Ouro Preto municipality, Minas Gerais state, from where the species was collected.

Typus: **Brazil**, Minas Gerais state, Ouro Preto municipality, Estação Ecológica do Tripuí (43º 34' 33" W, 20º 23' 45" S), infecting *A. angulata*, in forest understorey, 24 Nov. 2024, S.J. Lima-Santos, B.L. Clemente, N.L.S. Lima & T. Mendes-Pereira (**holotype** VIC 49660; Isolate number: 124EET; Genbank: SSU = PV645088; LSU = PV645086; TEF = PV641703; *RPB1* = PV641769).

Description: *External mycelium* light brown emerging from the host's sutures. *Sexual morph:* Up to six immature stromata, light brown, with acute to clavate apex, arising from the insertion of the wing and the thorax on each side (Fig. 8A-B). *Asexual morph: Synnemata* acute to clavate, straight, light brown to white towards the apex, rarely branched, produced from all host's orifices and sutures, 0.7 – 12.8 × 0.03 – 0.2 mm (Fig. 8A-D); *Phialides*, lageniform, hyaline, (23–) 32.3 (–67.8) × (2.7–) 4.2 (–5.5) µm tapering into a long neck ranging from 14.9 to up 54.6 µm in length (Fig. 8E-H); *Conidia* fusoid to allantoid, guttulate, hyaline, (5–) 7.3 (–8.6) × (1.5–) 2.13 (–2.6) µm (Fig. 8F-J). Only one conidium was observed embedded with a gelatinous matrix (Fig. 8H).

Habitat: Semi-deciduous, mesophytic forest; forming a transition area between Atlantic rainforest and Cerrado in the municipality of Ouro Preto, Minas Gerais, Brazil.

Notes: This species is very similar to *O. lacrimospora*, however *O. tripuiensis* is readily distinguished for the production of fusoid to allantoid conidia whilst *O. lacrimospora* produces tear-shaped conidia.

***Ophiocordyceps lacrimospora* sp. nov.,** S.J. Lima-dos-Santos

MycoBank MB: Not deposited yet, Fig. 9

Etymology: The name refers to the lachrymose or tear-shaped aspect of the conidia.

Typus: **Brazil**, Minas Gerais state, Ouro Preto municipality, Estação Ecológica do Tripuí (43º 34' 33" W, 20º 23' 45" S), infecting *Polybia* sp., in forest understorey, 24 Nov. 2024, S.J. Lima-Santos, B.L. Clemente, N.L.S. Lima & T. Mendes-Pereira (**holotype** VIC 49661; Isolate number: 123EET; Genbank: LSU = PV645081; TEF = PV641702; *RPB1* = PV641764).

Description: *External mycelium* light brown emerging from the host's sutures. *Sexual morph:* Two immature, light brown stromata, with clavate apex, arising from the insertion of the wing and the thorax on each side (Fig. 9A-B). *Asexual morph: Synnemata* clavate, straight, light brown to white towards the apex, never branched, produced from all host's orifices and sutures, 0.7 – 14.1 × 0.04 – 0.3 mm (Fig. 9A-D); *Phialides* lageniform, hyaline, (16–) 28.2 (–35.9) × (2.7–) 3.5 (–4.4) µm, tapering into a long neck ranging from 8.8 to up 27.3 µm in length (Fig.

9D-G); *Conidia* tear-shaped or lachrymose, guttulate, hyaline, (6.3–) 7.8 (–9.5) × (1.7–) 2.08 (–2.4) µm. Conidia were not observed embedded with a gelatinous matrix (Fig. 9H-I).

Habitat: Semi-deciduous, mesophytic forest; forming a transition area between Atlantic rainforest and Cerrado in the municipality of Ouro Preto, Minas Gerais, Brazil.

Notes: This species is mainly characterised by producing tear-shaped conidia that were not observed for the other species in Clade II. We also did not observed conidia being embedded by mucous matrices for this species in contrast to other species in this clade.

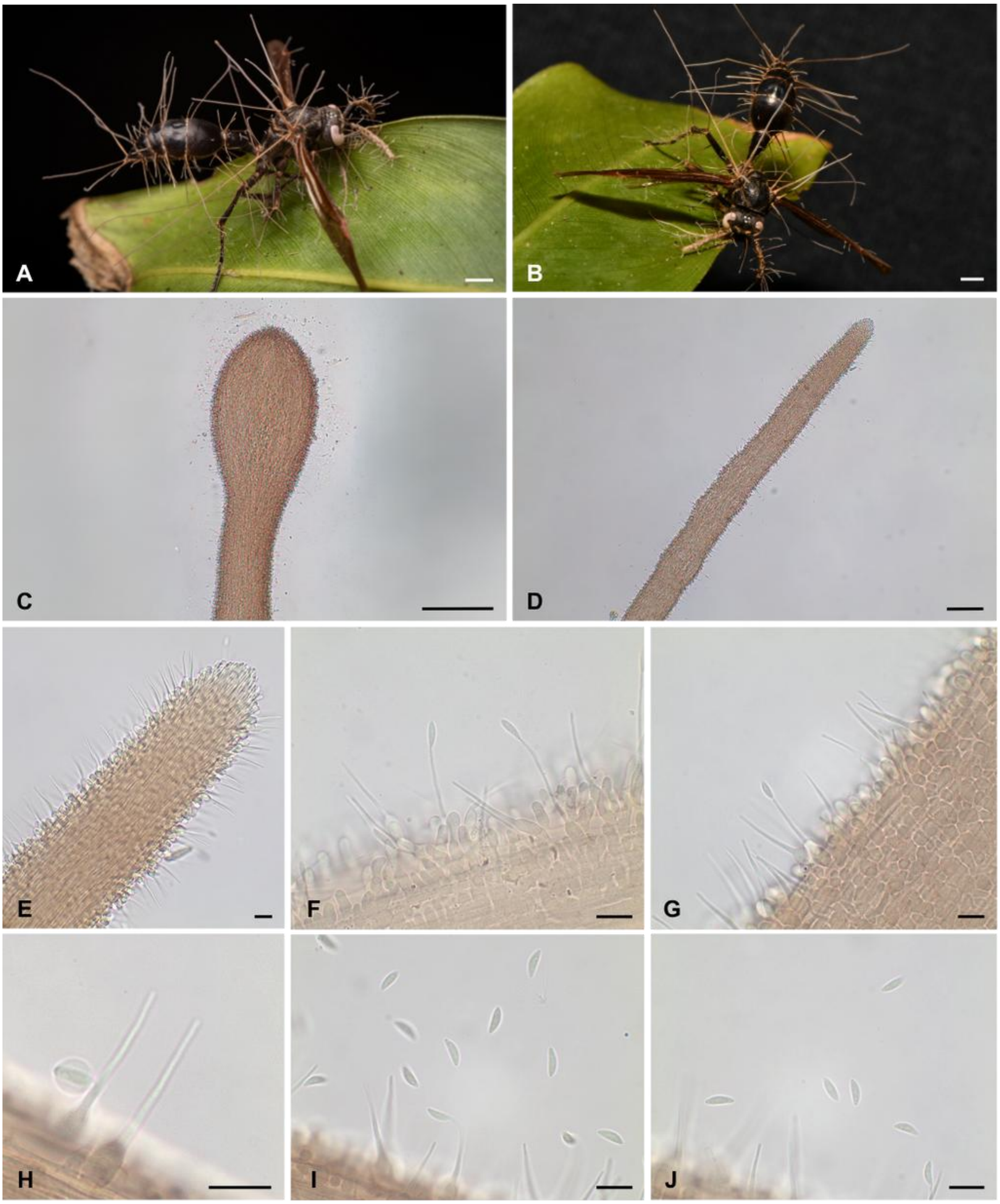

Fig. 8. *Ophiocordyceps tripuiensis*. **A-B.** Infected *Agelaia angulata* biting onto a leaf. **C-D.** Tip of the synnemata. **E.** Close-up on the synnema tip. **F-G.** Phialides producing conidia. **H.** Conidium with mucous matrix. **I-J.** Conidia. Scale bars: A-B = 2 mm; C-D = 100 µm; E = 20 µm; F-J = 10 µm.

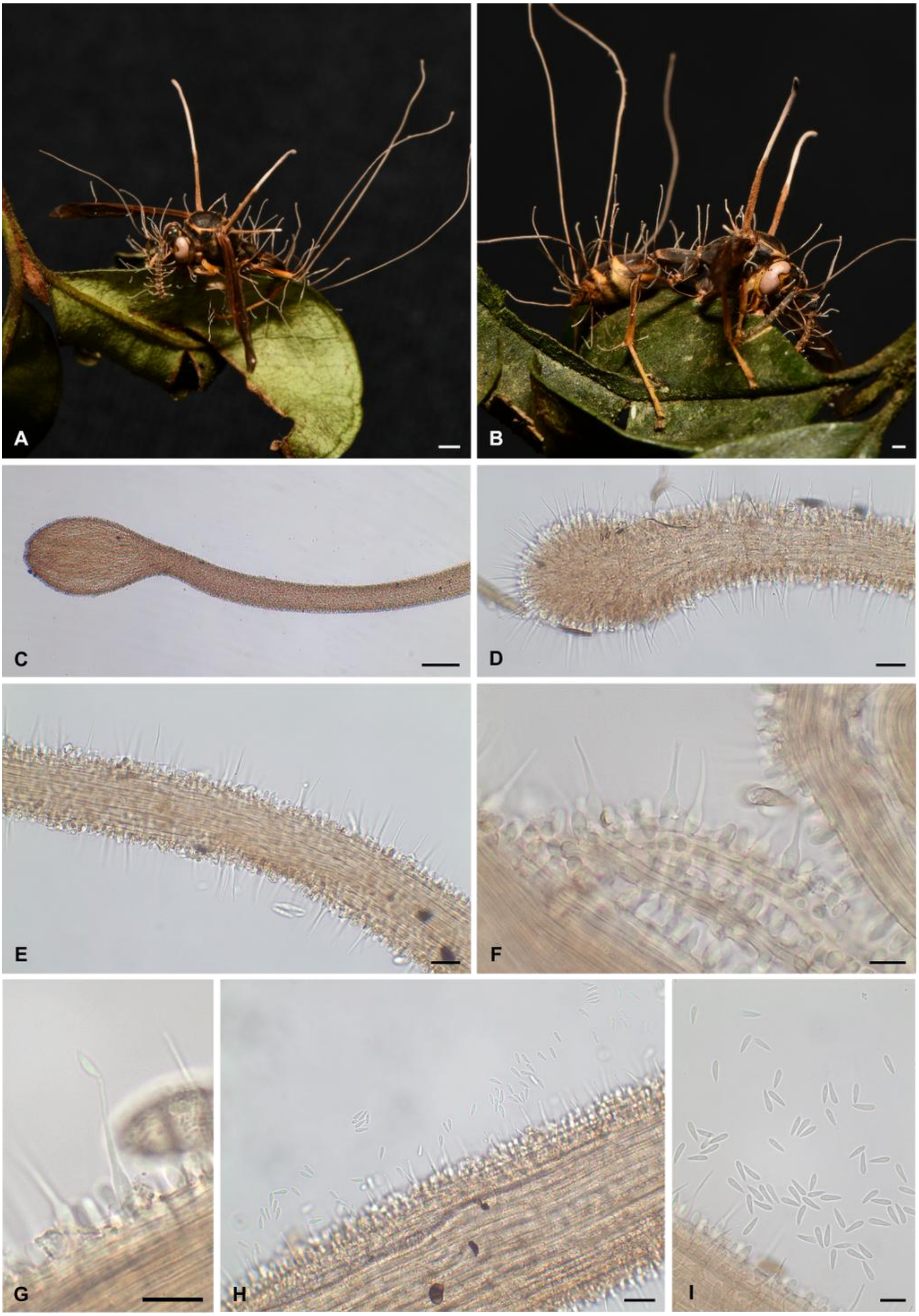

Fig. 9. *Ophiocordyceps lacrimospora*. **A-B.** Infected *Polybia sp.* biting onto a leaf. **C-E.** Synnemata. **F-H.** Phialides producing conidia. **I.** Conidia. Scale bars: A = 2 mm; B = 1 mm; C = 100 µm; D-E = 20 µm; F-G = 10 µm; H = 20; I = 10 µm.

***Ophiocordyceps pseudocitriformis* sp. nov.** S.J. Lima-dos-Santos & O.H.P. Della-Torre

MycoBank MB: Not deposited yet, Fig. 10

Etymology: The name refers to the similarity between the proposed species with *H. citriformis*.

Typus: **Brazil**, São Paulo state, Iporanga municipality, Reserva Betary (24° 35' 09" S, 48° 35' 34' W), infecting a male *Angiopolybia* sp. in forest understorey, 18 Apr. 2024, O.H.P. Della-Torre (**holotype** VIC 49662; Isolate number: 113RBET; Genbank: SSU = PV645070; LSU = PV645071; TEF = PV647905; *RPB1* = PV647907).

Paratypes: **Brazil**, São Paulo state, Iporanga municipality, Reserva Betary (24° 35' 09" S, 48° 35' 34' W), infecting *Pompilidae* in forest understorey, 11 Apr. 2024, O.H.P. Della-Torre, VIC 49663 (Isolate number: 131RBET; Genbank: SSU = PV645069; LSU = PV645072; *RPB1* = PV647906).

Description: *External mycelium* light brown emerging from the host's sutures. *Sexual morph:* not observed. *Asexual morph*: *Synnemata* clavate, brown, branched, produced from all host's orifices and sutures, (0.4–) 2.9 (–4.9) × (0.02–) 0.04 (–0.07) mm (Fig. 10A-C); *Branches short and stumpy*, light brown, (49.8–) 152 (–330) × (28.4 –) 43 (–69.2) µm (Fig. 10D-F); *Phialides*, lageniform, hyaline, (13.5–) 25.7 (–33.6) × (1–) 3.4 (–5.2) µm, tapering into a long neck 7.8 – 26.8 µm in length (Fig. 10E-I); *Conidia* fusoid, hyaline, guttulate, embedded in mucous matrices when mature, (4.4–) 6.5 (– 8.4) × (1.1–) 1.8 (–2.9) µm (Fig. 10G-J).

Habitat: Dense ombrophilous forest in an advanced regeneration status belonging to a fragment of Atlantic Forest in the Iporanga municipality, São Paulo state, Brazil.

Notes: *O. pseudocitriformis* is morphologically similar to *Hirsutella citriformis*, especially regarding the production of several synnemata, with short and stumpy branches, and fusoid conidia embedded in mucous matrices. *H. citriformis* is described and widely recognized as a specific pathogen of hemipteran insects (Spare 1920). Considering the original location of its description, host-association and our phylogenetic analysis, it seems clear to propose a new species similar to *H. citriformis*, but infecting a different host –wasps.

Fig. 10. *Ophiocordyceps pseudocitriformis*. **A-B.** Infected *Angiopolybia sp*. biting onto a leaf. **C.** Synnema with short and stumpy branches. **D-E.** Close-up on synnemata branches. **F.** Tip of the synnema; **G-I.** Phialides producing conidia. J). Close-up on the conidia imbedded with a mucous matrix. Scale bars: A-B =1 mm; C= 200 µm; D =100 µm; E-F = 20 µm; G-J = 10 µm.

3.5 DISCUSSION

Prior to this study, our knowledge of manipulative and hirsutelloid (i.e. with *Hirsutella*-like asexual state) *Ophiocordyceps* in wasps could be summarized as original descriptions of *O. humbertii* (or *C. humbertii*) and of *H. saussurei* (Speare 1920), with a number of subsequent reports or collections of specimens of one or other of these infecting mostly social wasps. This is not dissimilar to the situation with *O. unilateralis* in ants or another hypocrealean fungus associated with ants, *Escovopsis*, up until ca. 2010 (see Evans *et al*. 2011a, 2011b, Augustin *et al*. 2013, Elliot *et al*. 2025). Here we have used morphological observations and comparison with the original *O. humbertii* to separate specimens from Brazil into four clades and to describe seven new species, these supported by our phylogenetic analysis (Fig. 3).

Each of the four clades has novel species and/or strong indications of novel species that will hopefully be described in future. There are also interesting (we believe) questions of the fungus' morphology in each clade that likely give clues about the interactions of these fungi with their given hosts in the field, such as the amount of produced synnemata across different clades and the production of mucous matrices embedding conidia for certain species. Here we discuss the four clades in detail before turning to more general questions about the situation of this group within the *Ophiocordycipitaceae*, followed by considerations on the behavioural manipulation of wasps, the high frequency of samples in the asexual state, collection effort and host association and how all of these topics can help to elucidate the host-parasite interactions documented in this study.

To begin with Clade I, we were able to describe two novel species, *O. humbertioides* and *O. prezotii*, and also demonstrate the existence of various cryptic species yet to be described. Specimens within this clade present strong morphological similarities with Petch's *O. humbertii* (1935), especially regarding the position (e.g. emerging from the insertion of wings) and format of the ascostromata and arrangement of the synnemata (e.g. few emerging from the abdomen). Even though our phylogeny indicates the existence of more cryptic wasp-parasitic species for this clade, morphological features (i.e. synnemata and conidia) were insufficient to take measurements and conduct comparisons given that most of the samples were immature (i.e. with few conidia being produced) or in the asexual state, with the latter being very difficult to distinguish between samples from this clade.

Clade II is represented by the newly described *O. dendritica*, *O. lacrimospora*, *O. sanctaluciae*, *O. tripuiensis* and other undescribed specimens readily distinguished by the production of several slender synnemata emerging from all host's orifices and sutures, resembling those of Speare's *H. saussurei* (1920). Regarding these observations, there is the slightly possibility that *H. saussurei* was originally wrongly associated with *O. humbertii*. Petch's specimen presented strong morphological similarities with Clade I (Fig. 2), while Speare's specimen is more similar to Clade II, particularly in the production of several synnemata and mucoid conidia (Fig. 11A-B). Additionally, Petch (1935) did not give any details of the synnemata, phialides or conidia of the specimen he examined, which would have allowed proper comparison with Speare's observations, and this leaves room for taxonomic ambiguity. We cannot go further in this discussion as we only had access to the ascostromata of Petch's specimen and we also have no information concerning the deposition of Speare's specimens in collections. Mains (1951) stated that most of specimens studied by Speare have been presumably lost and are not located in reference mycological collections. The resolution of these taxonomic uncertainties regarding the status of *O. humbertii* would require access to both Petch's and Spare's collections and the conduction of comprehensive morphological and molecular analyses.

Clade III is comprised of *O. humbertii* MF116 and the undescribed *O. humbertii* s.l. W15. Both specimens lack proper morphological characterisation and a formal description must be provided. This clade is closely related to the recently described *O. acanthoponerae* (Lima-Santos *et al*. 2025, *under review*), that infects the ant *Acanthoponera mucronata*, and it shares with that species conspicuous *Hirsutella*-like asexual morphs and dark brown ascostromata covering all the sides of the stalk (360º).

In Clade IV, we describe the novel species *O. pseudocitriformis*. Surprisingly, this species is clustered within the *Hemiptera*-parasitic *H. citriformis* clade, sharing strong morphological traits (i.e. synnemata emerging from all host's orifices and sutures with short and stumpy branches). *Hirsutella citriformis* is widely recognized as a pathogen of *Hemiptera* (Spare 1920) (Fig. 11A-B) with potential use in biological control (Hall *et al*. 2012, Casique-Valdes *et al*. 2011, Pérez-Gonzales *et al*. 2015, 2016, 2022, 2023). However, taxonomy and phylogenetic relationships of this taxon are not well described (Pérez-Gonzales *et al*. 2015, Simmons *et al*. 2015). Pérez-Gonzales *et al*. (2015) stated that collected specimens of *H. citriformis* could be very diverse regarding morphological structures, such as phialides and conidia. This could indicate the existence of a cryptic species complex within *H. citrifomis* to be unraveled. Unfortunately, as mentioned before, most of the specimens studied by Speare appear to have been lost and a new type is deposited in the Farlow Herbarium at Harvard

University (Fig. 11C) (Mains 1951). Considering all the information aligned with the distribution of the specimens, host association and our morphological and phylogenetic results, we propose the new species *O. pseudocitriformis*. This expands the host range for the *H. citriformis* clade, traditionally associated with *Hemiptera*, and highlights the existence of a new lineage of hirsutelloid wasp-parasitic *Ophiocordyceps*.

All of the four lineages from this study were clustered in a major clade (BS = 100%) with the lepidopteran pathogen *O. elongata*. This major clade is closely related (BS = 88%) to the famous *O. unilateralis* clade (*O. unilateralis* core clade + *O. kniphofioides* subclade). The *O. unilateralis* core clade infects exclusively ants from the tribe *Camponotini*, with high levels of host specificity, while the *O. kniphofioides* subclade infects distantly related ant genera *Cephalotes*, *Daceton*, *Dolichoderus* and *Paraponera*. The *O. unilateralis* core clade is also known for manipulating the behaviour of ants, causing them to climb and die while biting onto the vegetation – the so-called death grip (Evans & Samson, 1984, Andersen *et al*. 2009, Araújo & Hughes, 2016). This manipulation strategy allows the fungus to develop its reproductive structures and release its ascospores from an elevated position on or near the foraging trails of the ants (Andersen *et al*. 2009, Araújo & Hughes, 2016). When the ascospores land in the substrate they germinate into a sticky capilliconidia that will have contact and infect new hosts (Araújo & Hughes 2016, Evans *et al*. 2011). This whole process was compared to a "a delayed-action landmine" by Evans & Boddy (2010) and is also considered to be a parasite strategy to cope with social immunity inside colonies (Loreto *et al*. 2014, Araújo & Hughes 2019). For *O. kniphofioides* sensu stricto, the infection process also might involve the production of mucilaginous conidia produced on hirsutelloid and rhizoid-like synnemata that emerges from the host (Evans & Samson 1982, Araújo *et al*. 2018). It is salient to ask to what degree behavioural manipulation and transmission between host follow a similar pattern in wasp-parasitic *Ophiocordyceps*.

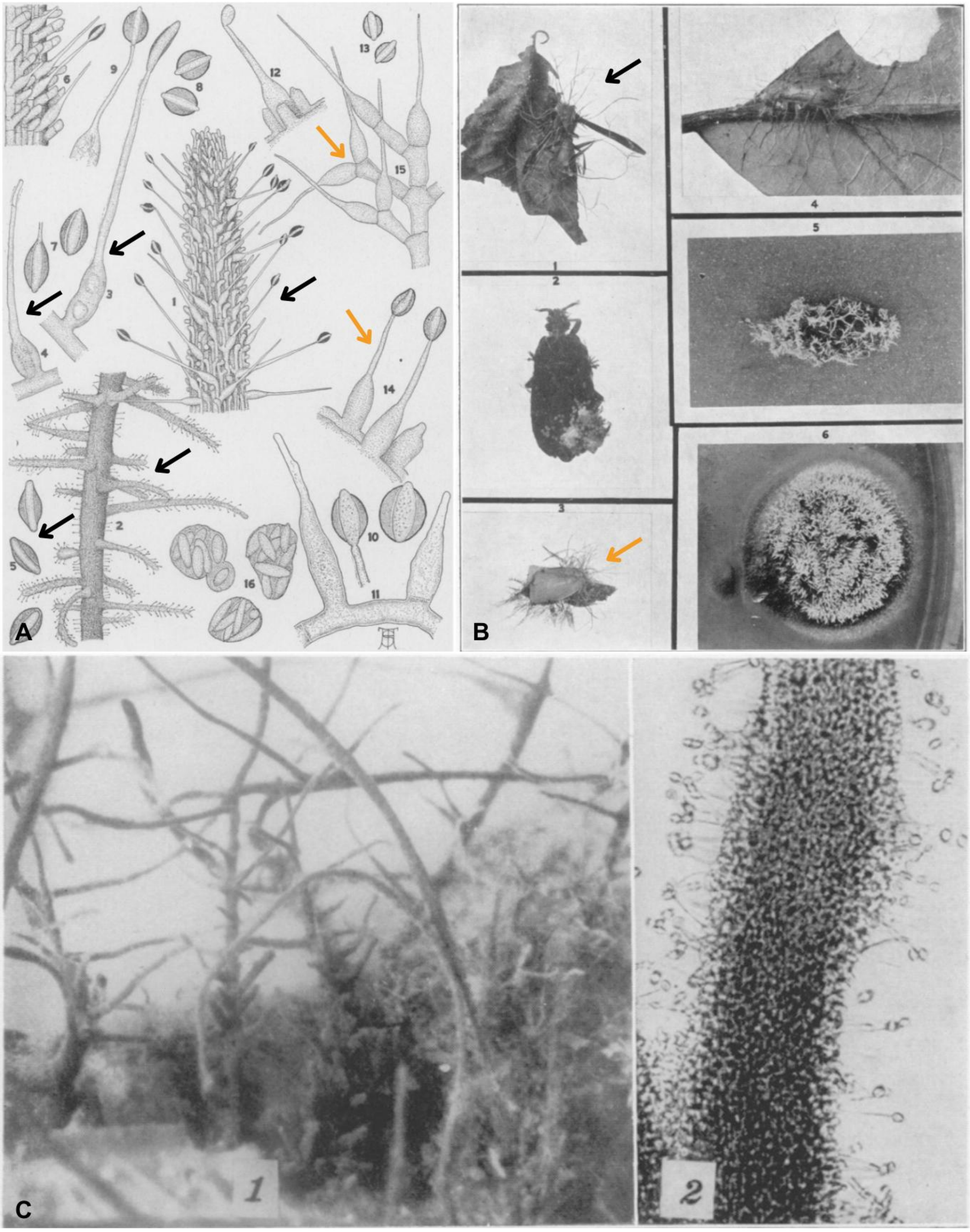

Fig. 6. Specimens of hirsutelloid *Ophiocordyceps* examined by Spare (1920) and Mains (1951). **A.** Spare's illustrations of the asexual structures of *H. saussurei* (black arrows) and *H. citriformis* (orange arrows). **B.** Original specimens and hosts examined to revise *H. saussurei* (black arrow) and describe *H. citriformis* (orange arrow). **C.** *Hirsutella citriformis* type deposited in Farlow Herbarium (FH) at Harvard University.

In our study, infected wasps were found biting vegetation, mostly leaves, indicating that they are manipulated in a similar way to ants infected by *O. unilateralis* sensu lato. This behaviour is also documented for wasps in previous literature (Sobczak *et al.* 2020; Somavilla *et al*. 2020, Barbosa & Somavilla 2022). Araújo & Hughes (2019) hypothesised that social wasp hosts might have been an intermediate step in the evolution of the myrmecophilous *O. unilateralis* clade. The phylogenetic proximity of Clade III with *O. acanthoponerae* could represent a missing link between lineages of manipulative ant- and wasp-associated *Ophiocordyceps* in this regard. However, the mechanisms and steps of the infection process promoted by *O. humbertii* sensu lato in wasp hosts are yet to be completed elucidated. In this study, we observed the germination of ascospores into capilliconidia only for the new *O. humbertioides* (Fig. 4H), placed in Clade I. This observation may indicate that, at least for Clade I, the infection process occurs similarly to that "delayed-action landmine" observed for *O. unilateralis* core clade (Evans & Boddy 2010). On the other hand, it should be considered that social wasps do not forage in collective trails as ants do, instead they forage individually by flying through the forest in search of sugar and protein sources to feed their colonies and offspring (Detoni & Prezoto 2021). Therefore, the infection process likely follows a different pattern for these hosts.

It is salient to note that a common observation in our collection of *Ophiocordyceps*-infected wasp cadavers is their extremely low density when compared with *Ophiocordyceps*-infected ants and other hypocrealean-killed arthropods, notably spiders killed by *Gibellula*. The collector could easily walk past ten or twenty of each of those before finding a single wasp cadaver. There may be several explanations for this, notably of course that there may be wasp cadavers higher up in the forest or even in the canopy, unnoticed by the collector. It could, though, indicate that the density of wasp-parasitising *Ophiocordyceps* is indeed far lower than the other examples mentioned above. Investigating this question would require a comprehensive study of *Ophiocordyceps* in wasps in a given area, akin to that conducted by Loreto and collaborators (2014) for *Ophiocordyceps* in ants and this undertaking promises to be far from trivial. We do suspect, however, that the great mobility of wasps in comparison to other potential arthropod hosts may allow for a considerably lower density of the pathogen within a host population, a hypothesis that it would be interesting to investigate with epidemiological modelling.

We also observed that the majority of the collected specimens were in their asexual state. This state allows fungi to reproduce faster and disperse in stable environments while the sexual state promotes recombination and genetic diversity. For the *O. unilateralis* clade, the role of *Ophiocordyceps* asexual morphotypes is yet to be fully understood (Evans & Samson, 1984, Araújo *et al*. 2018), but considering that the two types of conidia — mucoid and non-mucoid (without a mucous matrix) — are observed for this group it is possible that the mucoid conidia are likely to be contact spores or splash-dispersed while non-mucoid conidia are possibly dispersed by air. Evans & Samson (1982) discuss that these mucous matrices in *O. kniphofioides* subclade might contribute to the dispersal process by helping the conidia to adhere to new hosts or, indirectly, by forming a protective barrier against desiccation and irradiation during dry seasons, maintaining a seasonal carry-over. We did not observed conidia with mucoid sheaths for specimens in Clade I. We speculate that these are most-likely to be dispersed aerially, as has been posited for a type of non-mucoid conidia observed in the *O. kniphofioides* subclade (Evans & Samson 1982). We have recorded specimens with conidia embedded in mucous matrices only for Clades II and IV, just as observed by Speare (1920) for the asexual *H. saussurei* and *H. citriformis*. They are probably contact- or splash-dispersed just as the mucoid conidia are also observed for the *O. kniphofioides* subclade (Evans & Samson 1982).

Most of the collected hosts from Clades I and II belong to the genus *Agelaia* (Table 1), a representative of the swarm-founding social *Epiponini* (*Polistinae*) wasps. Their geographic distribution ranges from Mexico to northern Argentina (Richards 1978, Silveira & Carpenter 1995, Cooper 2000, 2001, Andena *et al*. 2024), and they are a conspicuous part of the social wasp fauna in much of tropical America (Jeanne, 1991). The genus is composed of 32 valid species (Carpenter & Grimaldi, 1997, Andena *et al*. 2024). These can exhibit considerable variation in body size, morphological traits, nest architecture, and colony population, with some species reaching colony sizes of millions of adults (Zucchi *et al*. 1995, Andena *et al*. 2024). They usually build their nests in cavities, subterranean or arboreal substrates (Wenzel 2020, Barbosa *et al*. 2021). In this study, we recorded the species *A. angulata* and *A. vicina* being hosts of more than one *Ophiocordyceps* species (Table 1). Information about natural history traits of *A. angulata* is scarce and we are limited to conjecture how these traits might influence host-parasite dynamic. The species *A. vicina* represents the largest colony and population size among all social wasps, with millions of individuals (Zuchi *et al*. 1995). It is possible that, for this species, the host-parasite dynamic might be similar to that *O. unilateralis*, considered as a chronic disease for the populous colonies of *Camponotini* (Andersen *et al*. 2012, Loreto *et al*. 2014), but more work is needed to completely elucidate how *Ophiocordyceps* species can affect wasp colonies.

Besides *Agelaia*, we also recorded infected wasps from the genera *Polistes (Polistini)*, *Apoica (Epiponini)*, *Angiopolybia (Epiponini)*, and *Polybia (Epiponini)*. Spare (1920) recorded *Polistes spp*. infected in the *H. saussurei* asexual state as well. These genera exhibit marked differences in key social traits, ranging from primitively eusocial species, such as *Polistini*, to highly eusocial ones, like *Epiponini*. These differences include colony size — from a few dozen individuals in *Polistes* to several hundred in *Polybia* — as well as colony founding strategies, hierarchical interactions, caste differentiation, worker lifespan and colony survival rates (Jandt *et al*. 2014, Jeanne 2021, Bell & Sumner 2023). Such variation in social structure likely influences how *Ophiocordyceps* infects and manipulates individuals from each genus. Additionally, we collected a solitary *Pompilidae* infected by the newly proposed *O. pseudocitriformis* (Table 1). Somavilla *et al*. (2019) also recorded solitary wasps from *Crabronidae* and *Pompilidae* infected by a fungus, identified as *O. humbertii*, in an epizootic event in the Brazilian Amazon region. The fungus morphotype observed in that study is not the same collected by the present one. It is feasible to speculate that the morphophysiological similarities shared by all hymenopterans, from solitary to social species that descend from a common ancestor, might have facilitated the interactions and diversification of *Ophiocordyceps* across the whole order (Branstetter *et al*. 2017, Araújo & Hughes 2019, Blaimer *et al*. 2023).

3.6 CONCLUSIONS

Considering all of our observations, it is clear the existence of a deep and complex scenario for the interactions of *Ophiocordyceps* with wasps, from solitary to eusocial, yet to be completely unraveled. Our results confirm the existence of a cryptic species complex under *O. humbertii*, with four novel lineages associated with solitary and social wasps from Amazon and Atlantic Forest. We propose seven new species, distinguished by unique morphological traits and supported by molecular data. One of these lineages is phylogenetically related to *H. citriformis*, traditionally recognized as hemipteran pathogens. These novel lineages are closely-related with manipulative and myrmecophilous *Ophiocordyceps*, such as *O. unilateralis* clade and *O. acanthoponerae*, and also present strong evidence of behavioural manipulation (i.e. leaf biting). This highlights the ecological and evolutionary importance of wasp-parasitic species and suggest that social hymenopterans had a pivotal role in the evolution of behaviour manipulation within *Ophiocordyceps*. Considering the vast taxonomic, behavioural and ecological diversity of wasps worldwide, future studies regarding cryptic speciation of wasp-parasitic

Ophiocordyceps across different regions of the world are essential to better understand the global diversity and evolutionary patterns of these fungal parasites.

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4 FINAL CONSIDERATIONS

This thesis contributes to the current knowledge of cryptic diversification of *Ophiocordyceps* associated with Hymenoptera in tropical ecosystems and suggest the existence of a deep and complex scenario for these interactions yet to be fully unveiled. The employment of an integrative taxonomic approach that combined morphological characterisation, multigene phylogenies and ecological data, allows us to reveal significant findings in both chapters of the thesis. In Chapter II, we discovered a novel myrmecophilous lineage represented by the description of *O. acanthoponerae* infecting *A. mucronata*, placed outside of the traditional hirsutelloid and myrmecophilous clades, *O. unilateralis* and *O. kniphofioides*, and closely-related to the wasp-parasitic *O. humbertii*. In Chapter III, we confirmed that *O. humbertii* truly constitutes a cryptic species complex, demonstrated by the description of four novel lineages and seven new species infecting wasps from Brazilian Atlantic Forest.

Our discoveries not only expand the diversity and host range of *Ophiocordyceps*, but also support the hypothesis that social hymenopterans might have played a crucial role in the evolution and diversification of behaviour-manipulating fungal species within the genus. Moreover, the observation that several distinct fungal lineages were hidden within a single species name (i.e. *O. humbertii*) reinforce the necessity of systematic sampling and taxonomic reviews of *Ophiocordyceps* species, especially in tropical ecosystems where most fungal diversity remains undescribed. This thought can also be expanded regarding host association with Hymenoptera, which is highly diverse and presents a wide spectrum of behaviours and ecological interactions. Our results strongly suggest that studying cryptic fungal diversity in tropical environments is not only important for taxonomy, but also to a better understanding of ecological and evolutionary process in which these fungi may be inserted. Future studies should consider a broader geographic sampling, collection of a wide range of hymenopteran hosts—especially solitary and primitively eusocial species—and the combination of morphological, genomic, ecological and behavioural data to enhance the current knowledge of the global diversity and evolutionary patterns of Hymenoptera-associated *Ophiocordyceps*.

5 SUPPLEMENTARY MATERIAL I

Table S1. List of species from the order *Hypocreales*, vouchers, GenBank accession numbers and host associations, with exception of *Ophiocordyceps* which is depicted in Chapter II and III.

Species	Voucher	nSSU rDNA	nLSU rDNA	*TEF*	*RPB1*	Host	Reference
Aciculosporium take	MAFF 241224	AB479213	–	KP689550	–	Plant	Quandt *et al.* (2014)
Akanthomyces aculeatus	HUA 186145	MF416572	MF416520	MF416465	–	*Lepidoptera*	Kepler *et al.* (2017)
Akanthomyces arachnophilus	NHJ 10469	EU369090	EU369031	EU369008	EU369047	*Araneae*	Kepler *et al.* (2017)
Akanthomyces cinereus	NHJ 3510	EU369091	–	EU369009	EU369048	*Araneae*	Kepler et al. (2017)
Akanthomyces novoguineensis	NHJ 11923	EU369095	EU369032	EU369013	EU369052	*Araneae*	Kepler *et al.* (2017)
Akanthomyces pistillariaeformis	HUA 186131	EU369095	EU369032	EU369013	EU369052	*Lepidoptera*	Kepler *et al.* (2017)
Albonectria rigidiuscula	CBS 315.73	–	KM231677	KM231938	KM232229	Plant	Lombard *et al.* (2015)
Aphysiostroma stercorarium	ATCC 62321	AF543769	AF543792	AF543782	AY489633	Dung	Quandt *et al.* (2014)
Aschersonia aleyrodis	P.C. 445	–	AY986900	AY986925	DQ000326	*Hemiptera*	Chaverri *et al.* (2008)
Aschersonia andropogonis	P.C. 535	AY986901	AY986926	DQ000327	–	*Hemiptera*	Chaverri *et al.* (2008)
Aschersonia basicystis	P.C. 457	–	AY986904	AY986929	DQ000330	*Hemiptera*	Chaverri *et al.* (2008)
Aschersonia blumenaviensis	P.C. 597	–	AY986905	AY986930	DQ000331	*Hemiptera*	Chaverri *et al.* (2008)
Aschersonia cubensis	P.C. 440	–	AY986907	AY986932	DQ000333	*Hemiptera*	Chaverri *et al.* (2008)
Aschersonia incrassata	P.C. 595	–	AY986909	AY986934	DQ000335	*Hemiptera*	Chaverri *et al.* (2008)
Aschersonia marginata	BCC 1765	DQ372093	–	DQ384958	DQ385010	*Hemiptera*	Chaverri *et al.* (2008)
Aschersonia napoleonae	P.C. 737	–	AY986910	AY986936	DQ000337	*Hemiptera*	Chaverri *et al.* (2008)
Aschersonia rhombispora	P.C. 467	–	AY986908	AY986933	DQ000334	*Hemiptera*	Quandt *et al.* (2014)
Aschersonia sp.	P.C. 627	–	AY986916	AY986942	DQ000343	*Hemiptera*	Quandt *et al.* (2014)
Aschersonia sp.	P.C. 569	–	AY986915	AY986941	DQ000342	*Hemiptera*	Quandt *et al.* (2014)
Aschersonia turbinata	M.C.A. 2432	–	AY986912	AY986938	DQ000339	*Hemiptera*	Quandt *et al.* (2014)
Aschersonia viridans	M.L. 202i	–	AY986913	AY986939	DQ000340	*Hemiptera*	Quandt *et al.* (2014)
Ascopolyporus polychrous	P.C. 546	–	DQ118737	DQ118745	DQ127236	*Hemiptera*	Kepler *et al.* (2017)
Ascopolyporus villosus	ARSEF 6355	–	AY886544	DQ118750	DQ127241	*Hemiptera*	Quandt *et al.* 2014
Atkinsonella hypoxylon	B4728	–	–	KP689546	–	Endophyte	Quandt *et al.* 2014
Atkinsonella texensis	B6155	–	–	KP689547	–	Endophyte	Quandt *et al.* 2014
Atractium crassum	CBS 180.31	U88110	KM231919	KM232205	HQ897722	Water	Lombard *et al.* (2015)
Atractium stilbaster	CBS 410.67	–	KM231654	KM231920	KM232206	*Plant*	Lombard *et al.* (2015)
Balansia epichloë	AEG 96-15a	EF468949		EF468743	EF468851	*Poaceae*	Quandt *et al.* (2014)
Balansia henningsiana	GAM 16112	AY545723	AY545727	AY489610	AY489643	*Poaceae*	Quandt *et al.* (2014)
Balansia obtecta	B249	–	–	KP689549	–	*Plant*	Quandt *et al.* (2014)
Balansia pilulaeformis	AEG 94-2	AF543764	AF543788	DQ522319	DQ522365	*Poaceae*	Quandt *et al.* (2014)
Beauveria bassiana	ARSEF 1564	–	–	HQ880974	HQ880833	Insect	Quandt *et al.* (2014)
Beauveria blattidicola	MCA 1727	MF416593	MF416539	MF416483	MF416640	*Blattae*	Kepler *et al.* (2017)
Beauveria brongniartii	ARSEF 617	–	–	HQ880991	HQ880854	Insect	Quandt *et al.* (2014)
Beauveria caledonica	ARSEF 2567	AF339570	AF339520	EF469057	EF469086	*Soil*	Quandt *et al.* (2014)

Beauveria malawiensis	ARSEF 7760	–	–	DQ376246	HQ880897	Insect	Quandt *et al.* (2014)
Beauveria pseudobassiana	ARSEF 3405	–	–	AY531931	HQ880864	Insect	Quandt *et al.* (2014)
Bionectria aureofulva_cf	GJS 71-328	DQ862044	DQ862027	DQ862029	–	Plant	Quandt *et al.* (2014)
Bionectria ochroleuca	CBS 114056	AY489684	AY489716	AY489611	DQ842031	Plant	Quandt *et al.* (2014)
Bisifusarium delphinoides	CBS 120718	–	KM231660	EU926296	KM232210	Plant	Lombard *et al.* (2015)
Bisifusarium dimerum	CBS 108944	–	JQ434514	EU926334	KM232212	Human	Lombard *et al.* (2015)
Bisifusarium domesticum	BS 116517	–	JQ434512	EU926286	KM232213	Saprophyte	Lombard *et al.* (2015)
Bisifusarium nectrioides	CBS 176.31	–	KM231659	EU926312	KM232209	Soil	Lombard *et al.* (2015)
Bisifusarium penzigii	CBS 317.34	–	KM231661	EU926324	KM232211	Plant	Lombard *et al.* (2015)
Blackwellomyces aurantiacus	BCC 85060	–	MT003028	MK411598	MK411600	*Lepidoptera* larvae	Mongkolsamrit *et al.* (2020)
Blackwellomyces aurantiacus	BCC 85061	–	MT003029	MK411599	MK411601	*Lepidoptera* larvae	Mongkolsamrit *et al.* (2020)
Blackwellomyces calendulinus	BCC 68500	–	MT003030	MT017842	MT017802	*Coleoptera* larvae	Mongkolsamrit *et al.* (2020)
Blackwellomyces calendulinus	BCC 68502	–	MT003031	MT017843	MT017803	*Coleoptera* larvae	Mongkolsamrit *et al.* (2020)
Blackwellomyces cardinalis	OSC 93610	AY184974	AY184963	EF469059	EF469088	*Lepidoptera larvae*	Mongkolsamrit *et al.* (2020)
Blackwellomyces cardinalis	OSC 93609	AY184973	AY184962	DQ522325	DQ522370	*Lepidoptera larvae*	Mongkolsamrit *et al.* (2020)
Blackwellomyces lateris	MFLU18 0663	–	MK08606	MK069471	MK084615	*Coleoptera larvae*	Mongkolsamrit *et al.* (2020)
Blackwellomyces minutus	BCC 88269	–	MT003032	MT017844	MT017804	*Coleoptera larvae*	Mongkolsamrit *et al.* (2020)
Blackwellomyces pseudomilitaris	BCC 2091	MF416589	MF416535	MF416479	–	*Lepidoptera larvae*	Mongkolsamrit *et al.* (2020)
Blackwellomyces pseudomilitaris	BCC 73634	–	–	MT017849	MT017809	*Lepidoptera larvae*	Mongkolsamrit *et al.* (2020)
Blackwellomyces pseudomilitaris	TBRC 3662	–	MT003036	MT017848	MT017808	*Lepidoptera larvae*	Mongkolsamrit *et al.* (2020)
Blackwellomyces pseudomilitaris	BCC 1919	MF416588	MF416534	MF416478	–	*Lepidoptera larvae*	Mongkolsamrit *et al.* (2020)
Blackwellomyces roseostromatus	BCC 91360	–	MT003035	MT017847	MT017807	*Lepidoptera larvae*	Mongkolsamrit *et al.* (2020)
Blackwellomyces roseostromatus	BCC 91358	–	MT003033	MT017845	MT017805	*Lepidoptera larvae*	Mongkolsamrit *et al.* (2020)
Blackwellomyces roseostromatus	BCC 91359	–	MT003034	MT017846	MT017806	*Lepidoptera larvae*	Mongkolsamrit *et al.* (2020)
Calcarisporium arbuscula	CBS 900.68	KT945002	KX442598	KX442596	–	Fungi	Sun *et al.* (2017)
Calcarisporium cordycipiticola	CGMCC 3.17905	KT944998	KX442599	KX442593	–	Fungi	Sun *et al.* (2017)
Calcarisporium xilariicola	HMAS 276836	KX442602	KX442601	KX442595	–	Fungi	Sun *et al.* (2017)
Calonectria brassicae	CBS 111869	–	GQ280698	FJ918567	KM232181	Plant	Lombard *et al.* (2015)
Calonectria ilicicola	CBS 190.50	–	GQ280727	AY725726	KM232180	Plant	Lombard *et al.* (2015)
Calonectria naviculata	CBS 101121	–	GQ280722	GQ267317	KM232182	Plant	Lombard *et al.* (2015)
Campylocarpon fasciculare	CBS 112613	–	HM364313	JF735691	HM364331	Plant	Lombard *et al.* (2015)
Campylocarpon pseudofasciculare	CBS 112679	–	HM364314	JF735692	HM364332	Plant	Lombard *et al.* (2015)
Chaetopsina acutispora	CBS 667.92	–	KM231636	KM231901	KM232187	Litter	Lombard *et al.* (2015)
Chaetopsina fulva	CBS 142.56	–	KM231637	KM231902	KM232188	Plant	Lombard *et al.* (2015)
Chaetopsina penicillata	CBS 608.92	–	KM231638	KM231903	–	Plant	Lombard *et al.* (2015)
Claviceps fusiformis	ATCC 26019	DQ522539	U17402	DQ522320	DQ522366	*Poaceae*	Quandt *et al.* (2014)
Claviceps paspali	ATCC 13892	U32401	U47826	DQ522321	DQ522367	*Poaceae*	Quandt *et al.* (2014)
Claviceps purpurea	cp11	EF469122	EF469075	EF469058	EF469087	Plant	Quandt *et al.* (2014)
Claviceps purpurea	GAM 12885	AF543765	AF543789	AF543778	AY489648	*Poaceae*	Quandt *et al.* (2014)
Clonostachys rosea	GJS90-227	Y489684	AY489716	AY489611	–	Plant	Castlebury *et al.* (2004)

Coccinonectria pachysandricola	CBS 501.63	–	KM231640	KM231905	KM232190	Plant	Lombard *et al.* (2015)
Coccinonectria rusci	CBS 126108	–	KM231639	KM231904	KM232189	Plant	Lombard *et al.* (2015)
Conoideocrella luteorostrata	NHJ 12516	EF468994	EF468849	EF468800	EF468905	*Hemiptera*	Quandt *et al.* (2014)
Conoideocrella tenuis	NHJ 6293	EU369112	EU369044	EU369029	EU369068	*Hemiptera*	Quandt *et al.* (2014)
Corallomycetella elegans	CBS 275.60	–	KM231710	KM231963	–	Plant	Lombard *et al.* (2015)
Corallomycetella repens	CBS 358.49	–	KM231708	KM231961	KM232258	Plant/Soil	Lombard *et al.* (2015)
Cordyceps albocitrina	spat 07-174	MF416575	–	MF416467	MF416629	*Lepidoptera*	Kepler *et al.* (2017)
Cordyceps bifusispora	EFCC 5690	EF468952	EF468806	EF468746	EF468854	*Lepidoptera*	Kepler *et al.* (2017)
Cordyceps brongniartii	BCC 16585	JF415951	JF415967	JF416009	JN049885	*Coleoptera*	Quandt *et al.* (2014)
Cordyceps caloceroides	MCA 2249	MF416578	MF416525	MF416470	MF416632	*Araneae*	Kepler *et al.* (2017)
Cordyceps cardinalis	OSC 93609	AY184973	AY184962	DQ522325	DQ522370	*Lepidoptera*	Quandt *et al.* (2014)
Cordyceps coccidioperitheciata	NHJ 6709	EU369110	EU369042	EU369025	EU369067	*Araneae*	Quandt *et al.* (2014)
Cordyceps coccidioperitheciata	DJ 29	EU369108	–	EU369027	EU369065	*Araneae*	Kepler *et al.* (2017)
Cordyceps confragosa	spat 08-146	MF416581	MF416528	MF416472	MF416634	*Hemiptera*	Kepler *et al.* (2017)
Cordyceps diapheromeriphila	MCA 1557	MF416582	MF416529	–	–	*Phasmida*	Quandt *et al.* (2014)
Cordyceps exasperata	MCA 2288	MF416592	MF416538	MF416482	MF416639	*Lepidoptera*	Kepler *et al.* (2017)
Cordyceps exasperata	MCA 2155	MF416596	MF416542	MF416486	MF416643	*Lepidoptera*	Kepler *et al.* (2017)
Cordyceps kyusyuënsis	EFCC 5886	EF468960	EF468813	EF468754	EF468863	*Lepidoptera*	Quandt *et al.* (2014)
Cordyceps locustiphila	HUA 179218	JQ895525	JQ895535	JQ958619	JX003846	*Orthoptera*	Kepler *et al.* (2017)
Cordyceps militaris	OSC 93623	AY184977	AY184966	DQ522332	DQ522377	*Lepidoptera*	Quandt *et al.* (2014)
Cordyceps nelumboides	BCC 2093	MF416583	MF416530	MF416473	–	*Araneae*	Kepler *et al.* (2017)
Cordyceps neogryllotalpae	MV2498	JQ895531	JQ895539	JQ958617	JX003854	*Orthoptera*	Sanjuan et al. (2014)
Cordyceps ochraceostromata	ARSEF 5691	EF468964	EF468819	EF468759	EF468867	*Lepidoptera*	Quandt *et al.* (2014)
Cordyceps piperis	CBS 116719	–	AY466442	DQ118749	DQ127240	*Araneae*	Quandt *et al.* (2014)
Cordyceps pleuricapitata	NBRC 100746	KF049607	KF049625	KF049680	KF049643	*Hemiptera*	Kepler *et al.* (2013)
Cordyceps pleuricapitata	NBRC 100745	KF049606	KF049624	KF049679	KF049642	*Hemiptera*	Kepler *et al.* (2013)
Cordyceps polyarthra	MCA 996	MF416597	MF416543	MF416487	MF416644	*Lepidoptera*	Kepler *et al.* (2017)
Cordyceps polyarthra	MCA 1009	MF416598	MF416544	MF416488	MF416645	*Lepidoptera*	Kepler *et al.* (2017)
Cordyceps pruinosa	ARSEF 5413	AY184979	AY184968	DQ522351	DQ522397	*Lepidoptera*	Kepler *et al.* (2017)
Cordyceps pseudomilitaris	BCC 1919	MF416588	MF416534	MF416478	–	*Lepidoptera*	Kepler *et al.* (2017)
Cordyceps pseudomilitaris	BCC 2091	MF416589	MF416535	MF416479	–	*Lepidoptera*	Kepler *et al.* (2017)
Cordyceps rosea	spat 09-053	MF416590	MF416536	MF416480	MF416637	*Lepidoptera*	Kepler *et al.* (2017)
Cordyceps scarabaeicola	ARSEF 5689	AF339574	AF339524	DQ522335	DQ522380	*Coleoptera, Scarabaidae*	Quandt *et al.* (2014)
Cordyceps sp.	EFCC 2535	EF468980	EF468835	EF468772	–	*Coleoptera*	Quandt *et al.* (2014)
Cordyceps sp.	RCEF HP090724-04C	MF416591	MF416537	MF416481	MF416638	*Lepidoptera*	Kepler *et al.* (2017)
Cordyceps staphylinidicola	ARSEF 5718	EF468981	EF468836	EF468776	EF468881	*Coleoptera*	Kepler *et al.* (2017)
Cordyceps takaomontana	MCA 1806	MF416595	MF416541	MF416485	MF416642	*Lepidoptera*	Kepler *et al.* (2017)
Cordyceps takaomontana	BCC 12688	MF416599	MF416545	MF416489	MF416646	*Lepidoptera*	Kepler *et al.* (2017)
Cordyceps tuberculata	OSC 111002	DQ522553	DQ518767	DQ522338	DQ522384	*Lepidoptera*	Quandt *et al.* (2014)

Cosmospora arxii	CBS 748.69	–	KM231694	KM231950	KM232245	Fungi	Lombard *et al.* (2015)
Cosmospora coccinea	CBS 341.70	–	KM231692	KM231947	KM232242	Fungi	Kepler et al. (2012)
Cosmospora cymosa	CBS 762.69	–	KM231693	KM231948	KM232243	Fungi	Kepler et al. (2012)
Curvicladiella cignea	CBS 101411	–	JQ666075	KM231866	KM232141	Plant	Kepler et al. (2012)
Cyanonectria cyanostroma	CBS 101734	–	HM626671	HM484611	GQ506017	Plant	Lombard *et al.* (2015)
Cylindrocarpon cylindroides	CBS 503.67	–	MH870763	JF735789	–	Plant	Vu *et al*. (2019)
Cylindrocarpostylus gregarius	CBS 101072	–	JQ666084	KM231870	KM232144	*Coleoptera*	Lombard *et al.* (2015)
Cylindrocladiella camelliae	CPC 234	–	JN099249	JN099087	KM232139	Plant	Lombard *et al.* (2015)
Cylindrocladiella lageniformis	CBS 340.92	–	JN099165	JN099003	JN989491	Plant	Lombard *et al.* (2015)
Cylindrocladiella parva	CBS 114524	–	JN099171	JN099009	KM232140	Plant	Lombard *et al.* (2015)
Cylindrodendrum album	CBS 301.83	–	KM231626	KM231889	KM232162	*Algae*	Lombard *et al.* (2015)
Cylindrodendrum hubeiensis	CBS 129.97	–	KM231628	KM231891	KM232164	Plant	Lombard *et al.* (2015)
Dactylonectria alcacerensis	CBS 129087	–	KM231629	JF735819	KM232176	Plant	Lombard *et al.* (2015)
Dactylonectria estremocensis	CBS 129085	–	KM231630	JF735807	KM232174	Plant	Lombard *et al.* (2015)
Dactylonectria macrodiyma	CBS 112615	–	HM364315	JF268750	HM364333	Plant	Lombard *et al.* (2015)
Dactylonectria novozelandica	CBS 113552	–	–	JF735822	KM232175	Plant	Lombard *et al.* (2015)
Dactylonectria torresensis	CBS 129086	–	KM231631	JF735870	KM232177	Plant	Lombard *et al.* (2015)
Drechmeria balanoides	CBS 250.82	AF339588	AF339539	DQ522342	DQ522388	*Nematoda*	Quandt *et al.* (2014)
Drechmeria gunnii	OSC 76404	AF339572	AF339522	AY489616	AY489650	*Lepidoptera*	Quandt *et al.* (2014)
Drechmeria sinensis	CBS 567.95	AF339594	AF339545	DQ522343	DQ522389	*Nematoda*	Quandt *et al.* (2014)
Dussiella tuberiformis	n/a	–	–	JQ257027	JQ257015	Plant	Kepler *et al.* (2012)
Engyodontium aranearum	CBS 309.85	AF339576	AF339526	DQ522341	DQ522387	*Araneae*	Kepler *et al.* (2017)
Engyodontium parvisporum	IHEM 22910	–	LC092915	–	–	*Hemiptera*	Gams *et al.* (1984)
Engyodontium rectidentatum	CBS 641.74	–	LC092914	–	–	Soil	Gams *et al.* (1984)
Engyodontium rectidentatum	CBS 206.74	–	LC092912	–	–	Soil	Gams *et al.* (1984)
Epichloë gansuensis	e7080	–	–	KP689495	–	Plant	Schardl *et al.* (2009)
Epichloë typhina	ATCC 56429	U32405	U17396	AF543777	AY489653	*Poaceae*	Quandt *et al.* (2014)
Flavocillium primulinum	JCM 18526	–	AB712264	–	–	Plant	Wang *et al.* (2020)
Flavocillium primulinum	JCM 18525	–	AB712263	–	–	Plant	Wang *et al.* (2020)
Flavocillium primulinum	JCM 18527	–	AB712265	–	–	Plant	Wang *et al.* (2020)
Fusarium lunatum	BBA 63199	–	–	–	–	Plant	Gräfenhan *et al.* (2011)
Fusarium proliferatum	CBS 189.38	–	KM231685	–	KM232238	Plant	Lombard *et al.* (2015)
Fusarium sambucinum	CBS 146.95	–	KM231682	KM231941	KM232235	Plant	Lombard *et al.* (2015)
Fusarium sublunatum	BBA 62431	–	–	–	–	Soil	Gräfenhan *et al.* (2011)
Fusarium venenatum	CBS 458.93	–	KM231683	KM231942	KM232236	Plant	Lombard *et al.* (2015)
Fusarium verrucosa	CBS 102163	–	KM231681	KM231940	KM232234	Plant	Lombard *et al.* (2015)
Fusicolla matuoi	CBS 581.78	–	KM231698	KM231954	KM232249	Plant	Lombard *et al.* (2015)
Fusicolla violacea	CBS 634.76	–	KM231700	KM231956	KM232251	*Hemiptera*	Lombard *et al.* (2015)
Fusicolla aquaeductuum	CBS 837.85	–	KM231699	KM231955	KM232250	Water	Lombard *et al.* (2015)
Geejayesia desmazieri	CBS 313.34	–	U88125	–	–	Plant	Gräfenhan *et al.* (2011)

Geejayessia celtidicola	CBS 125502	–	HM626669	HM626638	KM232232	Plant	Lombard *et al.* (2015)
Geejayessia cicatricum	CBS 125549	–	KM231678	HM626643	KM232231	Plant	Lombard *et al.* (2015)
Geejayessia desmazierii	CBS 125507	–	HM626663	HQ728146	KM232230	Plant	Lombard *et al.* (2015)
Gibellula cf. alba	NHJ 11679			EU369016	EU369054	*Araneae*	Wang *et al.* (2020)
Gibellula leiopus	BCC 16025	MF416602	MF416548	MF416492	MF416649	*Araneae*	Kepler *et al.* (2017)
Gibellula longispora	NHJ 12014	EU369098	–	EU369017	EU369055	*Araneae*	Quandt *et al.* (2014)
Gibellula pulchra	NHJ 10808	EU369099	EU369035	EU369018	EU369056	*Araneae*	Quandt *et al.* (2014)
Gibellula sp.	NHJ 10788	EU369101	EU369036	EU369019	EU369058	*Araneae*	Quandt *et al.* (2014)
Gibellula sp.	NHJ 13158	EU369100	EU369037	EU369020	EU369057	*Araneae*	Quandt *et al.* (2014)
Gibellula sp.	NHJ 5401	EU369102	–	–	EU369059	*Araneae*	Quandt *et al.* (2014)
Gliocephalotrichum bulbilium	CBS 242.62	–	AY489732	KM231892	AY489664	Soil	Lombard *et al.* (2015)
Gliocephalotrichum cylindrosporum	CBS 902.70	–	JQ666077	KF513408	KM232179	Soil	Lombard *et al.* (2015)
Gliocephalotrichum irregularis	CBS 755.97	–	JQ666082	KF513449	KM232138	Soil	Lombard *et al.* (2015)
Gliocephalotrichum longibrachium	CBS 126571	–	KM231686	KF513435	KM232178	Leaf Litter	Lombard *et al.* (2015)
Gliocladiopsis pseudotenuis	CBS 116074	–	JQ666080	JQ666099	KM232137	Soil	Lombard *et al.* (2015)
Gliocladiopsis sagariensis	CBS 199.55	–	JQ666078	JQ666106	KM232136	Soil	Lombard *et al.* (2015)
Glomerella cingulata	CBS 114054	AF543762	AF543786	AF543773	AY489659	*Rosaceae*	Quandt *et al.* (2014)
Haematonectria illudens	BBA 67606	–	AF178362	–	–	Plant	Gräfenhan *et al.* (2011)
Haematonectria ipomoaea	BBA 64379	–	–	–	–	Plant	Gräfenhan *et al.* (2011)
Haptocillium zeosporum	CBS 335.80	AF339589	AF339540	EF469062	EF469091	*Nematoda*	Quandt *et al.* (2014)
Harposporium harposporiferum	ARSEF 5472	AF339569	AF339519	DQ118747	DQ127238	*Nematoda*	Quandt *et al.* (2014)
Hevansia arachnophilus	NHJ 10469	EU369090	EU369031	EU369008	EU369047	*Araneae*	Kepler *et al.* (2017)
Hevansia cinereus	NHJ 3510	EU369091	–	EU369009	EU369048	*Araneae*	Johnson *et al.* (2009)
Hevansia nelumboides	BCC 41864	JN201863	JN201873	JN201867	–	*Araneae*	Kepler *et al.* (2017)
Hevansia novoguineensis	NHJ 4314	EU369094	–	EU369012	EU369051	*Araneae*	Kepler *et al.* (2017)
Hevansia novoguineensis	NHJ 10469	EU369090	EU369031	EU369008	EU369047	*Araneae*	Kepler *et al.* (2017)
Hevansia novoguineensis	NHJ 11923	EU369095	EU369032	EU369013	EU369052	*Araneae*	Kepler *et al.* (2017)
Hevansia novoguineensis	NHJ 13161	EU369093	–	EU369011	EU369050	*Araneae*	Kepler *et al.* (2017)
Hevansia sp.	BCC28584	GQ249965	GQ249989	GQ250040	–	*Araneae*	Johnson *et al.* (2009)
Hirsutella crinalis/acicularis	TNS F18550	KJ878911	KJ878875	KJ878959	–	*Coleoptera*	Quandt *et al.* (2014)
Hydropisphaera erubescens	ATCC 36093	AY545722	AY545726	DQ522344	DQ522390	Plant	Quandt *et al.* (2014)
Hydropisphaera peziza	CBS 102038	AY489698	AY489730	AY489625	AY489661	Plant	Quandt *et al.* (2014)
Hyperdermium pulvinatum	P.C. 602	–	DQ118738	DQ118746	DQ127237	*Hemiptera*	Quandt *et al.* (2014)
Hypocrea leucopus	CBS 122499	–	–	FJ179571	–	Fungi	Jaklitsch & Voglmayr 2015
Hypocrea lutea	ATCC 208838	AF543768	AF543791	AF543781	AY489662	Plant	Quandt *et al.* (2014)
Hypocrea rufa	CBS 114374	AY489694	AY489726	AY489621	AY489656	Fungi	Quandt *et al.* (2014)
Hypocrella sp.	P.C. 436.2	–	AY986922	AY986949	DQ000350	*Hemiptera*	Quandt *et al.* (2014)
Hypocrella sp.	P.C. 603	–	AY986923	AY986950	DQ000351	*Hemiptera*	Quandt *et al.* (2014)
Hypomyces aurantius	GJS74-69	–	HM466684	FJ467643	–	Fungi	Sun *et al.* (2017)
Hypomyces polyporinus	ATCC 76479	AF543771	AF543793	AF543784	AY489663	*Hymenomycetes*	Quandt *et al.* (2014)

Ilyonectria capensis	CBS 132815	–	KM515908	JX231119	KM232171	Plant	Lombard *et al.* (2015)
Ilyonectria coprosmae	CBS 119606	–	KM515910	JF735694	KM232173	Plant	Lombard *et al.* (2015)
Ilyonectria destructans	CBS 264.65	–	KM515927	JF735695	KM232169	Plant	Lombard *et al.* (2015)
Ilyonectria leucospermi	CBS 132809	–	KM515917	JX231129	KM232172	Plant	Lombard *et al.* (2015)
Ilyonectria liriodendri	CBS 117527	–	KM515922	JF735698	KM232170	Plant	Lombard *et al.* (2015)
Isaria amoenerosea	CBS 729.73	MF416604	MF416551	MF416495	MF416652	Insect	Kepler *et al.* (2017)
Isaria cicadae	RCEF HP090724-31	MF416605	MF416552	MF416496	MF416653	*Cicadidae*	Kepler *et al.* (2017)
Isaria coleopterorum	CBS 110.73	JF415965	JF415988	JF416028	JN049903	*Coleoptera*	Kepler *et al.* (2017)
Isaria farinosa	CBS 111113	AY526474	MF416554	MF416499	MF416656	Insect	Kepler *et al.* (2017)
Isaria fumosorosea	CBS 337.52	MF416607	MF416555	MF416500	MF416657	Insect	Kepler *et al.* (2017)
Isaria javanica	CBS 134.22	MF416610	MF416558	MF416504	MF416661	Insect	Kepler *et al.* (2017)
Isaria sp.	spat 09-050	MF416613	MF416559	MF416506	MF416663	*Lepidoptera*	Kepler *et al.* (2017)
Isaria sp.	spat 09-051	MF416614	MF416560	MF416507	MF416664	*Lepidoptera*	Kepler *et al.* (2017)
Isaria tenuipes	OSC 111007	DQ522559	DQ518773	DQ522349	DQ522395	*Lepidoptera*	Quandt *et al.* (2014)
Lecanicillium acerosum	CBS 418.81	KM283762	KM283786	KM283810	KM283832	–	Wang *et al.* (2020)
Lecanicillium antillanum	CBS 350.85	AF339585	AF339536	DQ522350	DQ522396	*Hymenoptera*	Quandt *et al.* (2014)
Lecanicillium aranearum	CBS 726.73a	AF339586	AF339537	EF468781	EF468887	*Araneae*	Quandt *et al.* (2014)
Lecanicillium attenuatum	CBS 402.78	AF339614	AF339565	EF468782	EF468888	*Araneae*	Quandt *et al.* (2014)
Lecanicillium fusisporum	CBS 164.70	AF339598	AF339549	EF468783	EF468889	*Hymenomycetes*	Quandt *et al.* (2014)
Lecanicillium lecanii	CBS 101247	AF339604	AF339555	DQ522359	DQ522407	*Hemiptera*	Quandt *et al.* (2014)
Lecanicillium sp.	CBS 639.85	KM283777	KM283801	KM283824	KM283843	–	Quandt *et al.* (2014)
Leptobacillium leptobactrum	IRAN 1230	–	KU382225	–	–	Soil	Zare & Gams (2016)
Leptobacillium leptobactrum	CBS 771.69	–	KU382224	–	–	Soil	Zare & Gams (2016)
Leptobacillium muralicola	CGMCC3.19014	–	MH379997	–	–	–	Zare & Gams (2016)
Leuconectria clusiae	ATCC 22228	AY489700	AY489732	AY489627	AY489664	Plant	Quandt *et al.* (2014)
Liangia sinensis	YFCC 3104	MN576727	MN576783	MN576953	MN576843	Fungi	Wang *et al.* (2020)
Liangia sinensis	YFCC 3103	MN576726	MN576782	MN576952	MN576842	Fungi	Wang *et al.* (2020)
Macroconia leptosphaeria	CBS 717.74	–	KM231707	KM231959	KM232255	Plant/Fungi	Lombard *et al.* (2015)
Macroconia papilionacearum	CBS 125495	–	KM231704	KM231958	KM232254	Fungi	Lombard *et al.* (2015)
Mariannaea camptospora	CBS 209.73	–	–	KM231875	KM232147	Soil	Lombard *et al.* (2015)
Mariannaea humicola	CBS 740.95	–	KM231619	KM231880	KM232153	Soil	Lombard *et al.* (2015)
Mariannaea pinicola	CBS 745.88	–	AY554242	KM231879	KM232152	Plant	Lombard *et al.* (2015)
Mariannaea pruinosa	ARSEF 5413	AY184979	AY184968	DQ522351	DQ522397	*Lepidoptera*	Kepler *et al.* (2017)
Mariannaea punicea	CBS 239.56	–	JF415981	KM231876	KM232148	Soil	Lombard *et al.* (2015)
Mariannaea samuelsii	CBS 746.88;	–	KM231621	KM231882	KM232155	*Saprophyte*	Lombard *et al.* (2015)
Metapochonia bulbillosa	CBS 145.70	AF339591	AF339542	EF468796	EF468902	Plant	Quandt *et al.* (2014)
Metapochonia gonioides	891.72	AF339599	AF339550	DQ522354	DQ522401	*Nematoda*	Quandt *et al.* (2014)
Metapochonia rubescens	464.88	AF339615	AF339566	EF468797	EF468903	*Nematoda*	Quandt *et al.* (2014)
Metarhizium album	ARSEF 2082	DQ522560	DQ518775	DQ522352	DQ522398	*Hemiptera*	Quandt *et al.* (2014)
Metarhizium anisopliae	ARSEF 3145	AF339579	AF339530	AF543774	DQ522399	*Coleoptera*	Quandt *et al.* (2014)

Metarhizium atrovirens	TNM 1732	JF415950	JF415966	–	JN049884	*Coleoptera*	Quandt *et al.* (2014)
Metarhizium carneum	CBS 239.32	EF468988	EF468843	EF468789	EF468894	sand dune	Quandt *et al.* (2014)
Metarhizium carneum	CBS 399.59	EF468989	EF468842	EF468788	EF468895	Soil	Quandt *et al.* (2014)
Metarhizium chlamydosporia	CBS 101244	DQ522544	DQ518758	DQ522327	DQ522372	*Diplopoda*	Quandt *et al.* (2014)
Metarhizium cylindrosporum	TNS 16371	JF415964	JF415987	JF416027	JN049902	*Hemiptera*	Quandt *et al.* (2014)
Metarhizium flavoviride	ARSEF 2037	AF339580	AF339531	DQ522353	DQ522400	*Hemiptera*	Quandt *et al.* (2014)
Metarhizium indigoticum	TNS F18553	JF415953	JF415968	JF416010	JN049886	*Coleoptera*	Quandt *et al.* (2014)
Metarhizium kusanagiense	F18494	JF415954	JF415972	JF416014	JN049890	*Lepidoptera*	Quandt *et al.* (2014)
Metarhizium liangshanensis	EFCC 1523	EF468961	EF468814	EF468755	–	*Lepidoptera*	Quandt *et al.* (2014)
Metarhizium liangshanensis	EFCC 1452	EF468962	EF468815	EF468756	–	*Lepidoptera*	Quandt *et al.* (2014)
Metarhizium marquandii	CBS 182.27	EF468990	EF468845	EF468793	EF468899	Soil	Quandt *et al.* (2014)
Metarhizium martiale	TTZ070716-04	JF415955	JF415973	–	JN049891	Insect	Quandt *et al.* (2014)
Metarhizium owariensis	NBRC 33258	–	JF415976	JF416017	–	*Hemiptera*	Kepler *et al.* (2014)
Metarhizium rileyi	CBS 806.71	AY624205	AY624250	EF468787	EF468893	*Lepidoptera*	Quandt *et al.* (2014)
Metarhizium sp.	HMAS 199601	JF415957	JF415978	JF416018	JN049894	*Coleoptera*	Kepler *et al.* (2013)
Metarhizium sp.	HMAS 199603	JF415963	JF415986	JF416026	JN049901	*Coleoptera*	Quandt *et al.* (2014)
Metarhizium sp.	NHJ 12118	EF468978	EF468829	EF468768	EF468878	*Lepidoptera*	Quandt *et al.* (2014)
Metarhizium sp.	OSC 110996	EF468974	EF468832	EF468773	EF468880	*Lepidoptera*	Quandt *et al.* (2014)
Metarhizium taii	ARSEF 5714	AF543763	AF543787	AF543775	DQ522383	*Lepidoptera*	Quandt *et al.* (2014)
Metarhizium yongmunense	EFCC 2135	EF468979	EF468834	EF468769	EF468877	*Lepidoptera*	Quandt *et al.* (2014)
Metarhizium yongmunensis	EFCC 2131	EF468977	EF468833	EF468770	EF468876	*Lepidoptera*	Quandt *et al.* (2014)
Microcera coccophila	CBS 310.34	–	KM231703	JF740692	JX171462	*Hemiptera*	Lombard *et al.* (2015)
Microcera larvarum	CBS 738.79	–	KM231701	KM231957	KM232252	*Hemiptera*	Lombard *et al.* (2015)
Microcera rubra	CBS 638.76	–	KM231702	JF740696	KM232253	*Hemiptera*	Lombard *et al.* (2015)
Microhilum oncoperae	AFSEF 4358	AF339581	AF339532	EF468785	EF468891	*Lepidoptera*	Quandt *et al.* (2014)
Moelleriella africana	PC 736	–	AY986917	AY986943	DQ000344	*Hemiptera*	Quandt *et al.* (2014)
Moelleriella macrostroma	PC 605	–	AY986919	AY986946	DQ000347	*Hemiptera*	Quandt *et al.* (2014)
Moelleriella macrostroma	PC 115	–	AY986920	AY986947	DQ000348	*Hemiptera*	Quandt *et al.* (2014)
Moelleriella raciborskii	PC 8238	DQ372102	–	DQ384961	DQ385001	*Hemiptera*	Quandt *et al.* (2014)
Moelleriella reineckiana	PC 2355	DQ372092	–	DQ384970	DQ385011	*Hemiptera*	Quandt *et al.* (2014)
Myriogenospora atramentosa	AEG 96-32	AY489701	AY489733	AY489628	AY489665	*Poaceae*	Quandt *et al.* (2014)
Myrothecium inundatum	IMI158855	AY489699	AY489731	AY489626	–	Fungi	Chen *et al.* (2016)
Nalanthamala psidii	CBS 116952	–	AY864837	KM231972	KM232268	Plant	Lombard *et al.* (2015)
Nalanthamala vermoesenii	CBS 230.48	–	AY554263	KM231970	KM232266	Plant	Lombard *et al.* (2015)
Nectria balansae	CBS 123351	–	GQ505996	HM484525	GQ506026	Plant	Lombard *et al.* (2015)
Nectria cinnabarina	CBS 114055	U32412	U00748	AF543785	AY489666	*Betulaceae*	Quandt *et al.* (2014)
Nectria mariae	CBS 125294	–	JF832684	JF832542	JF832789	Plant	Lombard *et al.* (2015)
Nectria_cf sp.	CBS 478.75	U47842	U17404	EF469068	EF469097	Plant	Quandt *et al.* (2014)
Nectriopsis violacea	CBS 424.64	AY489687	AY489719	–	AY489646	Plant	Castlebury *et al.* (2004)
Neoclaviceps monostipa	INBio 6-141		AF245293	AY986983	DQ000353	Plant	Chaverri *et al.* (2005)

Neocosmospora ambrosia	CBS 571.94	–	KM231668	KM231929	KM232220	Plant	Lombard *et al.* (2015)
Neocosmospora phaseoli	CBS 265.50	–	KM231674	HE647964	KM232226	Plant	Lombard *et al.* (2015)
Neocosmospora rubicola	CBS 320.73	–	KM231666	DQ247551	KM232218	Soil	Lombard *et al.* (2015)
Neocosmospora vasinfecta	CBS 325.54	–	KM231670	KM231931	KM232222	Soil	Lombard *et al.* (2015)
Neonectria tsugae	CBS 788.69	–	HQ232146	DQ789720	KM232161	Plant	Lombard *et al.* (2015)
Neonectria candida	CBS 151.29	–	HM042436	DQ789723	KM232168	Plant	Lombard *et al.* (2015)
Neonectria lugdunensis	CBS 125485	–	KM231625	KM231887	KM232160	Plant	Quandt *et al.* (2014)
Niveomyces coronatus	FieldW	ON493545	ON493604	–	–	*Ophiocordyceps camponoti-floridani*	Araújo *et al.* (2022)
Niveomyces coronatus	Niveo	ON493546	ON493605	–	–	*Ophiocordyceps camponoti-floridani*	Araújo *et al.* (2022)
Niveomyces coronatus	NY04434800	ON493547	ON493606	ON513397	ON513399	*Ophiocordyceps camponoti-floridani*	Araújo *et al.* (2022)
Ophionectria trichospora	CBS 109876	AF543766	AF543790	AF543779	AY489669	Plant	Quandt *et al.* (2014)
Orbiocrella petchii	NHJ 6209	EU369104	EU369039	EU369023	EU369061	*Hemiptera*	Quandt *et al.* (2014)
Paecilomyces niphetodes	CBS 364.76	AY526471	JF415989	JF416029	JN049904	Soil	Quandt *et al.* (2014)
Paecilomyces penicillatus	CBS 448.69	AY526493	EU553300	–	–	Fungi	Quandt *et al.* (2014)
Paracremonium contagium	CBS 110348	–	HQ232118	KM231966	KM232262	Human	Lombard *et al.* (2015)
Paracremonium inflatum	CBS 485.77	–	HQ232113	KM231964	KM232260	Human	Lombard *et al.* (2015)
Paramyrothecium roridum	ATCC 16297	–	AY489676	AY489708	AY489603	–	Castlebury *et al.* (2004)
Parengyodontium album	CBS 504.83	–	LC092899	–	–	Soil	Wang *et al.* (2020)
Parengyodontium album	CBS 368.72	–	LC092910	–	–	Soil	Wang *et al.* (2020)
Penicillifer bipapillatus	CBS 420.88	–	KM231608	KM231860	KM232129	Saprophyte	Lombard *et al.* (2015)
Penicillifer diparietisporus	CBS 376.59	–	KM231609	KM231861	KM232130	Soil	Lombard *et al.* (2015)
Penicillifer pulcher	CBS 560.67	–	KM231610	KM231862	KM232131	Soil	Lombard *et al.* (2015)
Perennicordyceps cuboideus	CEM 1514	KF049609	KF049628	KF049683	–	*Hypocreales*	Quandt *et al.* (2014)
Perennicordyceps paracuboidea	NBRC 101742	KF049611	KF049630	KF049685	KF049647	*Coleoptera*	Quandt *et al.* (2014)
Perennicordyceps paracuboidea	NBRC 100942	JN941711	JN941430	AB972954	JN992445	*Coleoptera*	Matočec *et al.* (2014)
Perennicordyceps prolifica	TNS-F-18547	KF049613	KF049632	KF049687	KF049649	*Hemiptera (cicada)*	Kepler *et al.* (2017)
Perennicordyceps prolifica	TNS-F-18481	KF049612	KF049631	KF049686	KF049648	*Hemiptera (cicada)*	Kepler *et al.* (2017)
Perennicordyceps ryogamiensis	NBRC 101751	KF049614	KF049633	KF049688	KF049650	*Coleoptera*	Kepler *et al.* (2017)
Perennicordyceps ryogamiensis	NBRC 103842	JN941701	JN941440		JN992435	*Coleoptera*	Wang *et al.* (2021)
Perennicordyceps ryogamiensis	NBRC 103837	JN941702	JN941439	–	JN992436	*Coleoptera*	Wang *et al.* (2021)
Phytocordyceps ninchukispora	EGS 38.166	EF468992	EF468847	EF468794	EF468901	Plant	Kepler *et al.* (2017)
Pleurocordyceps agarica	YHHPA 1305	KP276655	KP276651	KP276659	KP276663	Fungi	Wang *et al.* (2021)
Pleurocordyceps agarica	YHHPA 1303					Fungi	Wang *et al.* (2021)
Pleurocordyceps aurantiaca	MFLUCC 17 2114	MG136905	MG136911	MG136874	MG136867	*Ophiocordyceps*	Wang *et al.* (2021)
Pleurocordyceps aurantiaca	MFLUCC 17 1394	MG136906	MG136912	MG136876	–	*Ophiocordyceps*	Wang *et al.* (2021)
Pleurocordyceps aurantiaca	MFLUCC 17 2113	MG136904	MG136910	MG136875	MG136866	*Ophiocordyceps*	Wang *et al.* (2021)
Pleurocordyceps cf. yunnaensis	EFCC 12075	KJ878909	KJ878873	KJ878957	KJ878989	–	Kepler *et al.* (2013)
Pleurocordyceps lianzhouensis	GDGM 20918	KF226245	KF226246	KF226248	KF226247	*Lepidoptera*	Wang *et al.* (2021)
Pleurocordyceps lianzhouensis	GIMMY 9603	KF226249	KF226250	KF226252	KF226251	*Lepidoptera*	Wang *et al.* (2021)
Pleurocordyceps marginaliradians	MFLUCC 17 2276	MG136909	MG136915	MG136879		*Lepidoptera*	Wang *et al.* (2021)

Pleurocordyceps marginaliradians	MFLU 17 1582	MG136908	MG136914	MG136878	MG136869	*Lepidoptera*	Wang *et al.* (2021)
Pleurocordyceps nipponica	BCC 1682	KF049620	KF049638	KF049694		*Neuroptera*	Wang *et al.* (2021)
Pleurocordyceps nipponica	BCC 18108	KF049608	KF049626	KF049681	KF049644	*Neuroptera*	Wang *et al.* (2021)
Pleurocordyceps nipponica	BCC 2325	KF049622	KF049640	KF049696	KF049655	*Neuroptera*	Wang *et al.* (2021)
Pleurocordyceps nipponica	NHJ 4268	KF049621	KF049639	KF049695	KF049654	*Neuroptera*	Wang *et al.* (2021)
Pleurocordyceps nipponica	BCC 1881	KF049618	KF049636	KF049692		*Neuroptera*	Wang *et al.* (2021)
Pleurocordyceps nipponica	NHJ 7727	MF416625	MF416570	MF416518	MF416677	–	Kepler *et al.* (2017)
Pleurocordyceps phaothaiensis	BCC 84557	–	MF959738	MF959741	MF959746	*Coleoptera*	Wang *et al.* (2021)
Pleurocordyceps phaothaiensis	BCC 84551	–	MF959735	MF959739	MF959743	*Coleoptera*	Wang *et al.* (2021)
Pleurocordyceps phaothaiensis	BCC 84552	–	MF959736	MF959740	MF959744	*Coleoptera*	Wang *et al.* (2021)
Pleurocordyceps phaothaiensis	BCC 84553	–	MF959737	MF959742	MF959745	*Coleoptera*	Wang *et al.* (2021)
Pleurocordyceps ramosopulvinata	SU-65	DQ118742	DQ118753	DQ127244	–	*Hemiptera*	Quandt *et al.* (2014)
Pleurocordyceps ramosopulvinata	EFCC 5566	–	KF049627	KF049682	KF049645	*Hemiptera*	Quandt *et al.* (2014)
Pleurocordyceps sinensis	ARSEF 1424	KF049615	AY259544	DQ118754	DQ127245	*Coleoptera*	Quandt *et al.* (2014)
Pleurocordyceps sinensis	CN 80-2	HQ832887	HQ832886	HQ832890	HQ832888	*Ophiocordyceps sinensis*	Wang *et al.* (2021)
Pleurocordyceps sinensis	GIMCC 3570	JX006097	JX006098	JX006100	JX006101	*Ophiocordyceps sinensis*	Wang *et al.* (2021)
Pleurocordyceps sinensis	BL 4	KF049623	AY259545	KF049697	KF049656	*Myxomycete*	Quandt *et al.* (2014)
Pleurocordyceps yunnanensis	NBRC 109984	MN586819	MN586837	MN598052	MN598043	*Ophiocordyceps nutans*	Wang *et al.* (2015)
Pleurocordyceps yunnanensis	NBRC 109985	MN586820	MN586838	MN598053	MN598044	*Ophiocordyceps nutans*	Wang *et al.* (2015)
Pleurocordyceps yunnanensis	NBRC 101760	MN586818	MN586836	MN598051	MN598042	*Ophiocordyceps nutans*	Wang *et al.* (2015)
Pleurocordyceps yunnanensis	YHCPY 1005	KF977848	KF977848	KF977850	KF977852	*Ophiocordyceps nutans*	Wang *et al.* (2015)
Pleurocordyceps yunnanensis	YHHPY 1006	KF977849	KF977849	KF977851	KF977853	*Ophiocordyceps nutans*	Wang *et al.* (2015)
Pochonia chlamydosporia	CBS 504.66	AF339593	AF339544	EF469069	EF469098	*Nematoda*	Quandt *et al.* (2014)
Pochonia parasiticum	3436	EF468993	EF468848	EF468799	EF468904	*Rotifera*	Quandt *et al.* (2014)
Polycephalomyces formosus-like	CGMCC 5.2204	MN586821	MN586839	MN598054	MN598045	*Coleoptera*	Wang *et al.* (2021)
Polycephalomyces formosus-like	CGMCC5.2205	MN586822	MN586840	MN598055	MN598046	*Coleoptera*	Wang *et al.* (2021)
Polycephalomyces formosus-like	CGMCC5.2207	MN586823	MN586841	MN598056	MN598047	*Coleoptera*	Wang *et al.* (2021)
Polycephalomyces formosus-like	CGMCC5.2206	MN586824	MN586842	MN598057	MN598048	*Coleoptera*	Wang *et al.* (2021)
Polycephalomyces formosus-like	CGMCC5.2208	MN586825	MN586843	MN598058	MN598049	*Coleoptera*	Wang *et al.* (2021)
Polycephalomyces formosus-like	CGMCC5.2203	MN586826	MN586844	MN598059	MN598050	*Coleoptera*	Wang *et al.* (2021)
Polycephalomyces sp.	NBRC 109990	–	AB925968	–	–	–	Wang *et al.* (2021)
Polycephalomyces sp.	NBRC 110224	–	AB925969	–	–	–	Wang *et al.* (2021)
Polycephalomyces sp.	NBRC 109987	–	AB925983	–	–	–	Wang *et al.* (2021)
Polycephalomyces sp.	NBRC 109988	–	AB925984	–	–	–	Wang *et al.* (2021)
Pseudogibellula formicarum	BCC 81493	–	MT512652	MT863566	MT533472	*Ophiocordyceps flavida*	Mongkolsamrit *et al.* (2021)
Pseudogibellula formicarum	BCC 84257	–	MT512653	MT533480	MT533473	*Ophiocordyceps flavida*	Mongkolsamrit *et al.* (2021)
Pseudogibellula formicarum	CBS 871.72	–	MH878295	MT863565	MT533474	*Hemiptera; Ricania mediana*	Vu *et al.* (2019)
Pseudogibellula formicarum	CBS 433.73	–	MH872442	MT533481	MT533475	*Hemiptera; Ricania mediana*	Vu *et al.* (2019)
Pseudonectria buxi	CBS 324.53	–	KM231644	KM231909	KM232194	Plant	Lombard *et al.* (2015)
Pseudonectria foliicola	CBS 122566	–	KM231643	KM231908	KM232193	Plant	Lombard *et al.* (2015)

Pseudonectria rousseliana	CBS 114049	AF543767	U17416	AF543780	AY489670	*Buxaceae*	Quandt *et al.* (2014)
Purpureocillium atypicolum	GNJ070713-08, Na16	KJ878907	KJ878872	KJ878955	–	*Araneae*	Quandt *et al.* (2014)
Purpureocillium atypicolum	CBS 744.73	EF468987	EF468841	EF468786	EF468892	*Araneae*	Quandt *et al.* (2014)
Purpureocillium lilacinum	CBS 284.36	AY624189	AY624227	EF468792	EF468898	Soil	Quandt *et al.* (2014)
Purpureocillium takamizusanense	NHJ 3582	EU369096	EU369033	EU369014	EU369053	*Hemiptera*	Quandt *et al.* (2014)
Rectifusarium robinianum	CBS 830.85	–	KM231656	KM231922	JX171461	Plant	Lombard *et al.* (2015)
Rectifusarium ventricosum	CBS 748.79	–	KM231658	KM231924	KM232208	Soil	Lombard *et al.* (2015)
Regiocrella camerunensis	ARSEF 7682		DQ118735	DQ118743	DQ127234	*Hemiptera*	Quandt *et al.* (2014)
Rosasphaeria moravica	LMM	–	–	JF440987	–	Saprophyte	Jaklitsch & Voglmayr (2012)
Rotiferophthora angustispora	CBS 101437	AF339584	AF339535	AF543776	DQ522402	*Rotifera*	Quandt *et al.* (2014)
Roumegueriella rufula	CBS 346.85	DQ522561	DQ518776	DQ522355	DQ522403	*Nematoda*	Quandt *et al.* (2014)
Rugonectria neobalansae	CBS 125120	–	HM364322	KM231874	KM232146	Plant	Lombard *et al.* (2015)
Samsoniella alpina	YFCC 5836	MN576755	MN576811	MN576981	MN576871	*Lepidoptera*	Wang *et al.* (2020)
Samsoniella antleroides	YFCC 6113	MN576748	MN576804	MN576974	MN576864	*Lepidoptera*	Wang *et al.* (2020)
Samsoniella cardinalis	YFCC 5830	MN576732	MN576788	MN576958	MN576848	*Lepidoptera*	Wang *et al.* (2020)
Samsoniella cristata	YFCC 6021	MN576735	MN576791	MN576961	MN576851	*Lepidoptera*	Wang *et al.* (2020)
Samsoniella hepiali	YFCC 661	MN576739	MN576795	MN576965	MN576855	*Lepidoptera*	Wang *et al.* (2020)
Samsoniella kunmingensis	YHH 16002	MN576746	MN576802	MN576972	MN576862	*Lepidoptera*	Wang *et al.* (2020)
Samsoniella lanmaoa	YFCC 6148	MN576733	MN576789	MN576959	MN576849	*Lepidoptera*	Wang *et al.* (2020)
Samsoniella ramosa	YFCC 6020	MN576749	MN576805	MN576975	MN576865	*Lepidoptera*	Wang *et al.* (2020)
Samsoniella tortricida	YFCC 6013	MN576751	MN576807	MN576977	MN576867	*Lepidoptera*	Wang *et al.* (2020)
Samsoniella yunnanensis	YFCC 1527	MN576756	MN576812	MN576982	MN576872	*Lepidoptera*	Wang *et al.* (2020)
Samulesia rufobrunnea	PC 613	–	AY986918	AY986944	DQ000345	*Hemiptera*	Quandt *et al.* (2014)
Sarcopodium circinatum	CBS 587.92	–	KM231651	JF832545	KM232202	Soil	Lombard *et al.* (2015)
Sarcopodium circinosetiferum	CBS 100251	–	KM231646	KM231913	KM232197	Soil	Lombard *et al.* (2015)
Sarcopodium flavolanatum	CBS 112283	–	KM231649	KM231916	KM232200	Plant	Lombard *et al.* (2015)
Sarcopodium macalpinei	CBS 115296	–	KM231647	KM231914	KM232198	Plant	Lombard *et al.* (2015)
Sarcopodium vanillae	CBS 100582	–	HQ232174	KM231911	KM232195	Plant	Lombard *et al.* (2015)
Shimizuomyces paradoxus	EFCC 6564	EF469130	EF469083	EF469072	EF469101	Plant	Kepler *et al.* (2017)
Simplicillium lamellicola	CBS 116.25	AF339601	AF339552	DQ522356	DQ522404	Fungi	Kepler *et al.* (2017)
Simplicillium lanosoniveum	CBS 704.86	AF339602	AF339553	DQ522358	DQ522406	Fungi	Kepler *et al.* (2017)
Simplicillium obclavatum	CBS 311.74	AF339567	AF339517	EF468798	–	Fungi	Kepler *et al.* (2017)
Sphaerostilbella aureonitens	GJS74-87	–	HM466683	–	–	Fungi	Judith *et al.* (2015)
Sphaerostilbella berkeleyana	CBS 102308	AF543770	U00756	AF543783	AY489671	*Hymenomycetes*	Judith *et al.* (2015)
Stachybotrys chlorohalonata	DAOM 235557	JN939037	JN938870	–	–	Plant	Schoch *et al.* (2012)
Stachybotrys microspora	CBS 186.79	JN939037	–	DQ676604	–	Plant	Koster *et al.* (2009)
Stephanonectria keithii	GJS92-133	AY489695	AY489727	AY489622	–	Plant	Castlebury *et al.* (2004)
Striatibotrys eucylindrospora	CBS 203.61	–	KU846868	KU847078	–	Soil	Lombard *et al.* (2015)
Stylonectria applanata	CBS 125489	–	KM231689	KM231944	HQ897739	Plant	Lombard *et al.* (2015)

Stylonectria wegeliana	CBS 125490	–	KM231690	KM231945	KM232240	Plant	Lombard *et al.* (2015)
Thelonectria discophora	CBS 125153	–	HM364307	KM231897	HM364339	Plant	Lombard *et al.* (2015)
Thelonectria olida	CBS 215.67	–	NG 064061	–	M364334	Plant	Vu *et al.* (2019)
Thelonectria trachosa	CBS 112467	–	HM364312	KM231896	HM364339	Plant	Lombard *et al.* (2015)
Thyronectria lamyi	CBS 417.89	–	KM231718	JF832580	JF832830	Plant	Lombard *et al.* (2015)
Thyronectria pyrrhochlora	CBS 125131	–	HM484570	HM484519	HM484584	Plant	Lombard *et al.* (2015)
Thyronectria quercicola	CBS 128976	–	JF832743	JF832581	JF832831	Plant	Lombard *et al.* (2015)
Thyronectria sinoptica	CBS 462.83	–	GQ506001	HM484531	GQ506031	Plant	Lombard *et al.* (2015)
Tilachlidium brachiatum	CBS 505.67	–	KM231720	KM231976	KM232272	Fungi	Lombard *et al.* (2015)
Tolypocladium capitatum	OSC 71233	AY489689	AY489721	AY489615	AY489649	Fungi	Quandt *et al.* (2014)
Tolypocladium cylindrosporum	NRRL 28025	AF049153	AF049173	–	–	*Diptera*	Quandt *et al.* (2014)
Tolypocladium fractum	OSC 110990	DQ522545	DQ518759	DQ522328	DQ522373	Fungi	Quandt *et al.* (2014)
Tolypocladium japonicum	OSC 110991	DQ522547	DQ518761	DQ522330	DQ522375	Fungi	Quandt *et al.* (2014)
Tolypocladium longisegmentum	OSC 110992	–	EF468816	–	EF468864	Fungi	Quandt *et al.* (2014)
Tolypocladium ophioglossoides	CBS 100239	KJ878910	KJ878874	KJ878958	KJ878990	Fungi	Quandt *et al.* (2014)
Tolypocladium subsessilis	OSC 71235	EF469124	EF469077	EF469061	EF469090	*Coleoptera*	Quandt *et al.* (2014)
Torrubiella ratticaudata	1915	DQ522562	DQ518777	DQ522360	DQ522408	*Araneae*	Kepler *et al.* (2017)
Torrubiella sp.	NHJ 7859	EU369107	–	–	EU369064	*Araneae*	Kepler *et al.* (2017)
Torrubiella wallacei	CBS 101237	AY184978	AY184967	EF469073	EF469102	*Lepidoptera*	Kepler *et al.* (2017)
Torrubiellomyces zombiae	FieldB	ON493544	ON493603	ON513395	–	*Ophiocordyceps camponoti-floridani*	Araújo *et al.* (2022)
Torrubiellomyces zombiae	Polyceph	–	ON493607	ON513394	–	*Ophiocordyceps camponoti-floridani*	Araújo *et al.* (2022)
Torrubiellomyces zombiae	NY04434801	ON493543	ON493602	ON513396	ON513398	*Ophiocordyceps camponoti-floridani*	Araújo *et al.* (2022)
Trichoderma aggressivum	CBS 100526	–	–	AF348096	–	Fungi	Jaklitsch & Voglmayr (2012)
Trichoderma americanum	G.J.S. 92-93	–	–	–	–	Fungi	Jaklitsch & Voglmayr (2012)
Trichoderma deliquescens	CBS 121131	–	–	–	–	Fungi	Jaklitsch & Voglmayr (2012)
Trichoderma viride	GJS89-127	–	–	AY750891	–	Fungi	Lombard *et al.* (2015)
Trichosphaerella ceratophora	CBS 130.82	–	KM231727	KM231983	–	*Saprophyte*	Lombard *et al.* (2015)
Tyrannicordyceps fratricida	TNS 19011	JQ257022	JQ257023	JQ257028	JQ257016	Fungi	Kepler *et al.* (2012)
Ustilaginoidea dichromenae	IB 9228	–	AF373280	JQ257025	JQ257013	Plant	Quandt *et al.* (2014)
Ustilaginoidea virens	MAFF 240421	–	JQ257011	JQ257026	–	Plant	Quandt *et al.* (2014)
Valetoniellopsis laxa	ATCC 16535	AY489705	AY489737	AY489632	AY489673	Saprophyte	Zhang & Blackwell (2002)
Verticillium dahliae	ATCC 16535	AY489705	AY489737	AY489632	AY489673	*Rosaceae*	Quandt *et al.* (2014)
Verticillium epiphytum	CBS 384.81	AF339596	AF339547	DQ522361	DQ522409	*Urediales*	Quandt *et al.* (2014)
Verticillium sp.	CBS 102184	AF339613	AF339564	EF468803	EF468907	*Araneae*	Quandt *et al.* (2014)
Virgatospora echinofibrosa	CBS 110115	–	KU847270	KU847313	–	Plant	Lombard *et al.* (2015)
Viridispora diparietispora	CBS 102797	AY489703	AY489735	AY489630	AY489668	Soil	Quandt *et al.* (2014)
Volutella ciliata	CBS 483.61	–	KM231635	HM364356	KM232186	Soil	Lombard *et al.* (2015)
Volutella consors	CBS 139.79	–	KM231633	KM231899	KM232184	Plant	Lombard *et al.* (2015)
Volutella rosea	CBS 128258	–	KM231634	KM231900	KM232185	Soil	Lombard *et al.* (2015)
Xenoacremonium recifei	CBS 137.35	–	HQ232106	KM231968	KM232264	Human	Lombard *et al.* (2015)

Xenocylindrocladium guianense	CBS 112179	–	JQ666073	KM231895	KM232166	Soil/Plant litter	Lombard *et al.* (2015)
Xenocylindrocladium serpens	CBS 128439	–	KM231688	KM231894	KM232165	Plant	Lombard *et al.* (2015)
Xenocylindrocladium subverticillatum	CBS 113660T	–	KM231687	KM231893	–	Plant	Lombard *et al.* (2015)
Xenogliocladiopsis cypellocarpa	CBS 133814	–	KM231623	KM231885	KM232158	Plant	Lombard *et al.* (2015)