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Masters of the manipulator: two new hypocrealean genera, *Niveomyces* (*Cordycipitaceae*) and *Torrubiellomyces* (*Ophiocordycipitaceae*), parasitic on the zombie ant fungus *Ophiocordyceps camponoti-floridani*

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Abstract During surveys in central Florida of the zombie-ant fungus *Ophiocordyceps camponoti-floridani*, which manipulates the behavior of the carpenter ant *Camponotus floridanus*, two distinct fungal morphotypes were discovered associated with and purportedly parasitic on *O. camponoti-floridani*. Based on a combination of unique morphology, ecology and phylogenetic placement, we discovered that these morphotypes comprise two novel lineages of fungi. Here, we propose two new genera, *Niveomyces* and *Torrubiellomyces*, each including a single species within the families *Cordycipitaceae* and *Ophiocordycipitaceae*, respectively. We generated *de novo* draft genomes for both new species and performed morphological and multi-loci phylogenetic analyses. The macromorphology and incidence of both new species, *Niveomyces coronatus* and *Torrubiellomyces zombiae*, suggest that these fungi are mycoparasites since their growth is observed exclusively on *O. camponoti-floridani* mycelium, stalks and ascomata, causing evident degradation of their fungal hosts. This work provides a starting point for more studies into fungal interactions between mycopathogens and entomopathogens, which have the potential to contribute towards efforts to battle the global rise of plant and animal mycoses.

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INTRODUCTION

Fungi occupy a wide array of ecological niches as decomposers, mutualists, and parasites of plants, animals and other fungi. Mycoparasites of other parasitic fungal lineages can impact ecosystem composition and disease dynamics by modulating their hosts' population size and transmission rate (Blackwell & Vega 2018). Despite these perceived ecosystem impacts and their biocontrol potential, mycoparasites are generally understudied, which is especially true for those that attack entomopathogenic fungi.

The order *Hypocreales* contains the widest diversity of animal parasites among the kingdom Fungi. Most hypocrealean fungi are parasites of plants and arthropods, especially insects, although some species are known to parasitize spiders, nematodes, rotifers and even immunocompromised humans, as well as other fungi (Samson et al. 1988, Kepler et al. 2013, Lombard et al. 2015, Araújo & Hughes 2016). The genus *Ophiocordyceps* comprises approximately 300 species, strictly associated with insects belonging to 13 orders (Crous et al. 2004, Araújo & Hughes 2016). Among these, one particular group stands out for its intriguing and bizarre biology, the *Ophiocordyceps unilateralis* clade sensu Araújo et al. (2018), which infect and manipulate the behavior of ants, mostly of the tribe *Camponotini*, across the globe (Andersen et al. 2009, Evans et al. 2011a, Araújo et al. 2015, 2018). Typically, *Ophiocordyceps*-infected ants, such as the Florida carpenter ant *Camponotus floridanus,* are manipulated to leave their nest and ascend vegetation, where they exhibit a fungus-adaptive 'death-grip' behaviour (Andersen et al. 2009, Araújo & Hughes 2019, Will et al. 2020). Species within the *O. unilateralis* clade are highly specialised heterotrophs that are able to form epizootics, often infecting hundreds of ants within a small area of forest (Evans 1982). After the spores encounter the host, penetrate and overcome its defences, the fungus proliferates as yeast-like cells in the haemocoel (see Araújo et al. 2020: f. 2d–f). Once established inside the host, the fungus produces secondary metabolites, proteases and other (small) secreted bioactive compounds to interact with its host and adaptively manipulate its behaviour (De Bekker et al. 2021). After the fungus kills the host, the yeast-like cells are converted into hyphae forming an endosclerotium, a compact mass of fungal mycelium that rapidly fills the host body after death (see Andersen et al. 2009: f. 3). *Ophiocordyceps* then utilizes the ant's body as a platform to grow the spore-producing structures needed for transmission to the next host (Evans et al. 2011a, Hughes et al. 2011,

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De Bekker et al. 2015, Araújo et al. 2018). However, despite being sophisticated parasites themselves, but not unlike most (if not all) life on earth, *Ophiocordyceps* species are also parasitized by other hypocrealean fungi (Evans et al. 2011a, b, Andersen & Hughes 2012, Araújo et al. 2020).

Mycoparasitism, or the parasitism of one fungus by another (Kirk et al. 2008), has independently and repeatedly appeared in a variety of fungal lineages along their evolution (Boddy 2016, Herrera et al. 2016, Blackwell & Vega 2018). Thus far, mycoparasites associated with four other *Ophiocordyceps* species from China and Thailand have been described (Wang et al. 2015b, Zhong et al. 2016, Xiao et al. 2018). However, none of these species are associated with a behaviour-manipulating *Ophiocordyceps*. In addition, mycoparasites growing on other *Ophiocordyceps*-manipulated ants, such as *Ophiocordyceps camponoti-rufipedis* in Brazil (Evans et al. 2011a, b, Andersen & Hughes 2012) and *Ophiocordyceps paltothyrei* in Ghana (Araújo et al. 2020), have been reported. These records, along with our unpublished observations of mycoparasites on *Ophiocordyceps* across North and South America, as well as in Africa

Fig. 1 Mycoparasites of *Ophiocordyceps* species pathogenic on *Camponotini* ants. a–b. Niveomyces-like growth on *Ophiocordyceps camponoti-novogranadensis* on its host, *Camponotus novogranadensis*, in Atlantic rainforest, Itacolomi, Minas Gerais, Brazil (Note yellow perithecia on the subiculum in b; c–d. on *O. camponoti-rufipedis* on *Camponotus rufipes* in Atlantic rainforest, Viçosa, Minas Gerais, Brazil; e. torrubiellomyces-like perithecia on *Ophiocordyceps camponoti-novogranadensis* on, *Camponotus novogranadensis*, habitat as a, b; f. dark perithecia produced on the mycelium of *Ophiocordyceps oecophyllae*, on *Oecophylla smaragdina* in rainforest, Licuala State Forest, Queensland, Australia.

and Australia (JPM Araújo & HC Evans pers. obs., Fig. 1), suggest that the tri-trophic interactions that we report here are not unique to Florida. Instead, they are an example of a common worldwide phenomenon. Despite this, formal species descriptions and reports have remained limited: no genomes have currently been sequenced, and little research has been done on their biology and the effects that these mycoparasitic lineages have on *Ophiocordyceps* disease dynamics and transmission. Here, we describe two new genera, *Niveomyces* (*Cordycipitaceae*) and *Torrubiellomyces* (*Ophiocordycipitaceae*) associated with and parasitic on *Ophiocordyceps camponoti-floridani* in Central Florida. Our proposal is supported by a polyphasic approach combining morphological, ecological and phylogenetic data. While we predicted the placement of both morphotypes to reside in less data-rich parts of the hypocrealean tree (complicating culture identification through GenBank alignments) and obtaining PCRs from DNA extracted directly from field specimens proved to be difficult, we produced draft genomes of both species. These draft genomes were subsequently used to obtain sequences for multi-loci phylogenetic analyses and as alignment databases to identify the correct isolates obtained from additional specimens. These genomes also add considerable data to the current low number of available mycoparasite genomes.

MATERIALS AND METHODS

Field sampling

Ophiocordyceps camponoti-floridani-manipulated ant cadavers of *C. floridanus* with visible mycoparasitic growth of both morphotypes were collected from the Black Hammock Wilderness Area (N28°42'04.7" W81°09'32.0") and Little Big Econ State Forest (N28°41'14.7" W81°09'33.4") in Central Florida. Two morphotypes were readily recognised in the field either by their characteristic cotton white hyphae that consistently covered and overgrew the host and *Ophiocordyceps* synnemata (*Niveomyces coronatus*), or by the dark perithecia (*Torrubiellomyces zombiae)* that arose directly from the fungal host. Our collection permits were provided by the Seminole County's Leisure Services Department, Greenways and Natural Lands Division and the Florida Department of Agriculture and Consumer Service's Florida Forest Service.

Fungal culturing

To isolate *N. coronatus*, sterile water droplets of ~1 µL were pipetted onto parasitised synnema. Because of the high hydrophobicity of the spore structures, droplets stayed intact and spores were released onto the water surface. Droplets with spores were streaked onto potato dextrose agar (PDA; BD Difco) and incubated at room temperature. After seven days, mycelium was transferred from single colonies to fresh PDA plates with a sterile inoculation loop for further isolation. To isolate *T. zombiae*, a sterile inoculation loop was used to pick up young, bright white, not-yet-matured fungal growth exhibited on top of *O. camponoti-floridani* and to inoculate PDA plates using the T-streak method. After incubating at room temperature for 14 d, single colonies were transferred to fresh PDA plates with a sterile inoculation loop for further isolation. Cultures of these isolates (ex-types) are deposited in the culture collection of the Westerdijk Institute (CBS 149186 = BH-Nc-1D-3 for *N. coronatus*) and (CBS 149187 = BH-Tz-4E-4 for *T. zombiae*), respectively.

To document culture characteristics of both species, the centre of fresh PDA plates was inoculated with mycelium from the two isolates using a sterile 4 mm-diam cork borer (Cole-Parmer). One half of the plates were incubated at room temperature and subject to daily light fluctuations in the lab. The other half was kept in the dark inside an incubator (Panasonic) kept at 25 °C. After 6–8 wk of radial growth on PDA, the diameter of the cultures was measured and the mycelium was examined for presence of growth differentiation.

To grow mycelium for DNA extractions, a flame-sterilised inoculation loop was used to scrape a small amount of mycelium from colonies growing on PDA plates to inoculate a sterile 250 mL Erlenmeyer flask containing 50 mL Sabouraud dextrose broth (SDB; BD Difco). Flasks were incubated at room temperature on a shaking platform (Fisher) at 120 rpm. Mycelium was harvested from the *Niveomyces* liquid culture three days after inoculation while *Torrubiellomyces* was harvested after 9 d by pouring the culture over a Buchner funnel (Fisher) with Whatman Grade 1 filter paper (Fisher) and applying suction. The mycelial-impregnated filter paper was pressed flat by hand between paper towels to remove any remaining liquid. A 1 cm^2 piece of the dried mycelium was placed in a 2 mL microcentrifuge tube (USA Scientific) containing two metal ball bearings (5/32" type 2B, grade 300, Wheels Manufacturing) and snap-frozen in liquid nitrogen for tissue disruption and DNA extraction.

Morphology

To assess the macromorphological features, images were taken using a Canon EOS 7D Mark II camera fitted with a 35 mm lens. To investigate their micromorphological features, fungal tissues were mounted on microscope slides with a drop of either lactic acid, in the case of *Torrubiellomyces*, or lacto-fuchsin stain (0.1 g acid fuchsin powder and 100 mL 85 % lactic acid) for *Niveomyces* to aid visualisation of taxonomically informative structures. The slides were visualized using a Leica DMi8 inverted microscope, mounted with a Leica MC 170 HD camera (Leica Microsystems). Type materials (holotypes and paratypes) are deposited at the New York Botanical Garden Herbarium (type numbers NY4434800 and NY4434801 for *N. coronatus* and *T. zombiae*, respectively).

DNA extraction, library preparation and whole-genome sequencing

While the morphology of the fungi described in this study appeared to be unique compared to currently described species, we predicted that the sequence submissions of potentially related species would be vastly underrepresented in GenBank. This complicated direct genetic identification of sampled specimens and derived potential isolates based on PCR amplification. As such, draft genomes were generated for both new species using DNA extracted directly from collected specimens to obtain sequences for phylogenetic analyses and serve as genetic references to confirm the identity of isolated cultures through alignments. Under a dissecting microscope, tissues of the mycoparasites were removed while taking careful consideration not to include *O. camponoti-floridani* tissue. These tissues were surface sterilized in 70 % ethanol and placed into microcentrifuge tubes (USA Scientific) along with two metal ball bearings (5/32" type 2B, grade 300, Wheels Manufacturing), and snap-frozen in liquid nitrogen using a 1600 MiniG tissue homogenizer (SPEX) at 1300 RPM for 30 s to disrupt fungal cell walls. Genomic DNA was extracted using a previously described phenol-chloroform extraction protocol (Will et al. 2020), which was quantified with a Qubit Fluorometer (Thermo Fisher) and the Qubit dsDNA High Sensitivity Assay Kit (Thermo Fisher). Subsequently, DNA libraries were prepared with the Nextera DNA Flex Library Preparation Kit (Illumina) for sequencing on an Illumina MiSeq Sequencer to generate 2 × 250 bp paired-end reads with a 50× target coverage.

Table 1 List of species, voucher and GenBank accession numbers and host associations. Species in **bold** are new taxa presented in this study.

Species	Voucher	nSSU rDNA	nLSU rDNA	*TEF*	*RPB1*	*RPB2*	Host	Reference
Aciculosporium take	MAFF 241224	AB479213	–	KP689550	–	KP689511	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	EU369070	Araneae	Kepler et al. (2017)
Akanthomyces novoguineensis	NHJ 11923	EU369095	EU369032	EU369013	EU369052	EU369072	Araneae	Kepler et al. (2017)
Akanthomyces pistillariaeformis	HUA 186131	EU369095	EU369032	EU369013	EU369052	EU369072	Lepidoptera	Kepler et al. (2017)
Albonectria rigidiuscula	CBS 315.73	–	KM231677	KM231938	KM232229	KM232378	Plant	Lombard et al. (2015)
Aphysiostroma stercorarium	ATCC 62321	AF543769	AF543792	AF543782	AY489633	EF469103	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	DQ452472	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)
	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	–	KP689514	Endophyte	Quandt et al. (2014)
Atkinsonella texensis	B6155	–	–	KP689547	–	KP689515	Endophyte	Quandt et al. (2014)
Atractium crassum	CBS 180.31	U88110	KM231919	KM232205	HQ897722	Water tap	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	EF468908	Poaceae	Quandt et al. (2014)
Balansia henningsiana	GAM 16112	AY545723	AY545727	AY489610	AY489643	DQ522413	Poaceae	Quandt et al. (2014)
Balansia obtecta	B249	–	–	KP689549	–	KC113318	Plant	Quandt et al. (2014)
Balansia pilulaeformis	AEG 94-2	AF543764	AF543788	DQ522319	DQ522365	DQ522414	Poaceae	Quandt et al. (2014)
Beauveria bassiana	ARSEF 1564	–	–	HQ880974	HQ880833	HQ880905	Insect	Quandt et al. (2014)
Beauveria blattidicola	MCA 1727	MF416593	MF416539	MF416483	MF416640	–	Blattae	Kepler et al. (2017)
Beauveria brongniartii	ARSEF 617	–	–	HQ880991	HQ880854	HQ880926	Insect	Quandt et al. (2014)
Beauveria caledonica	ARSEF 2567	AF339570	AF339520	EF469057	EF469086	–	Soil	Quandt et al. (2014)
Beauveria malawiensis	ARSEF 7760	–	–	DQ376246	HQ880897	HQ880969	Insect	Quandt et al. (2014)
Beauveria pseudobassiana	ARSEF 3405	–	–	AY531931	HQ880864	HQ880936	Insect	Quandt et al. (2014)
Bionectria aureofulva_cf	GJS 71-328	DQ862044	DQ862027	DQ862029	–	DQ862013	Plant	Quandt et al. (2014)
Bionectria ochroleuca	CBS 114056	AY489684	AY489716	AY489611	DQ842031	DQ522415	Plant	Quandt et al. (2014)
Bisifusarium delphinoides	CBS 120718	–	KM231660	EU926296	KM232210	–	Plant	Lombard et al. (2015)
Bisifusarium dimerum	CBS 108944	–	JQ434514	EU926334	KM232212	KM232363	Human	Lombard et al. (2015)
Bisifusarium domesticum	BS 116517	–	JQ434512	EU926286	KM232213	HQ897694	Saprophyte	Lombard et al. (2015)
Bisifusarium nectrioides	CBS 176.31	–	KM231659	EU926312	KM232209	HQ897721	Soil	Lombard et al. (2015)
Bisifusarium penzigii	CBS 317.34	–	KM231661	EU926324	KM232211	KM232362	Plant	Lombard et al. (2015)
Blackwellomyces aurantiacus	BCC 85060	–	MT003028	MK411598	MK411600	MT017819	Lepidoptera larva	Mongkolsamrit et al. (2020)
	BCC 85061	–	MT003029	MK411599	MK411601	MT017820	Lepidoptera larva	Mongkolsamrit et al. (2020)
Blackwellomyces calendulinus	BCC 68500	–	MT003030	MT017842	MT017802	MT017821	Coleoptera larva	Mongkolsamrit et al. (2020)
	BCC 68502	–	MT003031	MT017843	MT017803	MT017822	Coleoptera larva	Mongkolsamrit et al. (2020)
Blackwellomyces cardinalis	OSC 93610	AY184974	AY184963	EF469059	EF469088	EF469106	Lepidoptera larva	Mongkolsamrit et al. (2020)
	OSC 93609	AY184973	AY184962	DQ522325	DQ522370	DQ522422	Lepidoptera larva	Mongkolsamrit et al. (2020)
Blackwellomyces lateris	MFLU18 0663	–	MK08606	MK069471	MK084615	MK079354	Coleoptera larva	Mongkolsamrit et al. (2020)
Blackwellomyces minutus	BCC 88269	–	MT003032	MT017844	MT017804	MT017823	Coleoptera larva	Mongkolsamrit et al. (2020)
Blackwellomyces pseudomilitaris	BCC 2091	MF416589	MF416535	MF416479	–	MF416441	Lepidoptera larva	Mongkolsamrit et al. (2020)

Table 1 (cont.)

Species	Voucher	nSSU rDNA	nLSU rDNA	*TEF*	*RPB1*	*RPB2*	Host	Reference
Blackwellomyces pseudomilitaris (cont.)	BCC 73634	–	–	MT017849	MT017809	MT017827	Lepidoptera larva	Mongkolsamrit et al. (2020)
	TBRC 3662	–	MT003036	MT017848	MT017808	–	Lepidoptera larva	Mongkolsamrit et al. (2020)
	BCC 1919	MF416588	MF416534	MF416478	–	MF416440	Lepidoptera larva	Mongkolsamrit et al. (2020)
Blackwellomyces roseostromatus	BCC 91360	–	MT003035	MT017847	MT017807	MT017826	Lepidoptera larva	Mongkolsamrit et al. (2020)
	BCC 91358	–	MT003033	MT017845	MT017805	MT017824	Lepidoptera larva	Mongkolsamrit et al. (2020)
	BCC 91359	–	MT003034	MT017846	MT017806	MT017825	Lepidoptera larva	Mongkolsamrit et al. (2020)
Calcarisporium arbuscula	CBS 900.68	KT945002	KX442598	KX442596	–	KX442597	Fungi	Sun et al. (2017)
Calcarisporium cordycipiticola	CGMCC 3.17905	KT944998	KX442599	KX442593	–	KX442594	Fungi	Sun et al. (2017)
Calcarisporium xilariicola	HMAS 276836	KX442602	KX442601	KX442595	–	KX442606	Fungi	Sun et al. (2017)
Calonectria brassicae	CBS 111869	–	GQ280698	FJ918567	KM232181	KM232308	Plant	Lombard et al. (2015)
Calonectria ilicicola	CBS 190.50	–	GQ280727	AY725726	KM232180	KM232307	Plant	Lombard et al. (2015)
Calonectria naviculata	CBS 101121	–	GQ280722	GQ267317	KM232182	KM232309	Plant	Lombard et al. (2015)
Campylocarpon fasciculare	CBS 112613	–	HM364313	JF735691	HM364331	KM232322	Plant	Lombard et al. (2015)
Campylocarpon pseudofasciculare	CBS 112679	–	HM364314	JF735692	HM364332	KM232323	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	–	HQ897709	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	DQ522416	Poaceae	Quandt et al. (2014)
Claviceps purpurea	cp11	EF469122	EF469075	EF469058	EF469087	EF469105	Plant	Quandt et al. (2014)
	GAM 12885	AF543765	AF543789	AF543778	AY489648	DQ522417	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	KM232350	Plant	Lombard et al. (2015)
Coccinonectria rusci	CBS 126108	–	KM231639	KM231904	KM232189	KM232349	Plant	Lombard et al. (2015)
Conoideocrella luteorostrata	NHJ 12516	EF468994	EF468849	EF468800	EF468905	EF468946	Hemiptera	Quandt et al. (2014)
Conoideocrella tenuis	NHJ 6293	EU369112	EU369044	EU369029	EU369068	EU369087	Hemiptera	Quandt et al. (2014)
Corallomycetella elegans	CBS 275.60	–	KM231710	KM231963	–	KM232393	Plant	Lombard et al. (2015)
Corallomycetella repens	CBS 358.49	–	KM231708	KM231961	KM232258	KM232391	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	EF468909	Lepidoptera	Kepler et al. (2017)
Cordyceps brongniartii	BCC 16585	JF415951	JF415967	JF416009	JN049885	JF415991	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	DQ522422	Lepidoptera	Quandt et al. (2014)
Cordyceps coccidioperitheciata	NHJ 6709	EU369110	EU369042	EU369025	EU369067	EU369086	Araneae	Quandt et al. (2014)
	DJ 29	EU369108	–	EU369027	EU369065	–	Araneae	Kepler et al. (2017)
Cordyceps confragosa	spat 08-146	MF416581	MF416528	MF416472	MF416634	MF416436	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)
	MCA 2155	MF416596	MF416542	MF416486	MF416643	–	Lepidoptera	Kepler et al. (2017)
Cordyceps kyusyuënsis	EFCC 5886	EF468960	EF468813	EF468754	EF468863	EF468917	Lepidoptera	Quandt et al. (2014)
Cordyceps locustiphila	HUA 179218	JQ895525	JQ895535	JQ958619	JX003846	JX003845	Orthoptera	Kepler et al. (2017)
Cordyceps militaris	OSC 93623	AY184977	AY184966	DQ522332	DQ522377	AY545732	Lepidoptera	Quandt et al. (2014)
Cordyceps nelumboides	BCC 2093	MF416583	MF416530	MF416473	–	MF416437	Araneae	Kepler et al. (2017)
Cordyceps neogryllotalpae	MV2498	JQ895531	JQ895539	JQ958617	JX003854	–	Orthoptera	Sanjuán et al. (2015)
Cordyceps ochraceostromata	ARSEF 5691	EF468964	EF468819	EF468759	EF468867	EF468921	Lepidoptera	Quandt et al. (2014)
Cordyceps piperis	CBS 116719	–	AY466442	DQ118749	DQ127240	EU369083	Araneae	Quandt et al. (2014)
Cordyceps pleuricapitata	NBRC 100746	KF049607	KF049625	KF049680	KF049643	KF049668	Hemiptera	Kepler et al. (2013)
	NBRC 100745	KF049606	KF049624	KF049679	KF049642	KF049667	Hemiptera	Kepler et al. (2013)
Cordyceps polyarthra	MCA 996	MF416597	MF416543	MF416487	MF416644	–	Lepidoptera	Kepler et al. (2017)
	MCA 1009	MF416598	MF416544	MF416488	MF416645	–	Lepidoptera	Kepler et al. (2017)
Cordyceps pruinosa	ARSEF 5413	AY184979	AY184968	DQ522351	DQ522397	DQ522451	Lepidoptera	Kepler et al. (2017)
Cordyceps pseudomilitaris	BCC 1919	MF416588	MF416534	MF416478	–	MF416440	Lepidoptera	Kepler et al. (2017)
	BCC 2091	MF416589	MF416535	MF416479	–	MF416441	Lepidoptera	Kepler et al. (2017)
Cordyceps rosea	spat 09-053	MF416590	MF416536	MF416480	MF416637	MF416442	Lepidoptera	Kepler et al. (2017)

Table 1 (cont.)

Species	Voucher	nSSU rDNA	nLSU rDNA	*TEF*	*RPB1*	*RPB2*	Host	Reference
Cordyceps scarabaeicola	ARSEF 5689	AF339574	AF339524	DQ522335	DQ522380	DQ522431	Coleoptera, Scarabaidae	Quandt et al. (2014)
Cordyceps sp.	EFCC 2535	EF468980	EF468835	EF468772	–	–	Coleoptera	Quandt et al. (2014)
	RCEF HP090724-04C	MF416591	MF416537	MF416481	MF416638	MF416443	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)
	BCC 12688	MF416599	MF416545	MF416489	MF416646	–	Lepidoptera	Kepler et al. (2017)
Cordyceps tuberculata	OSC 111002	DQ522553	DQ518767	DQ522338	DQ522384	DQ522435	Lepidoptera	Quandt et al. (2014)
Cosmospora arxii	CBS 748.69	–	KM231694	KM231950	KM232245	HQ897862	Fungi	Lombard et al. (2015)
Cosmospora coccinea	CBS 341.70	–	KM231692	KM231947	KM232242	HQ897777	Fungi	Kepler et al. (2012)
Cosmospora cymosa	CBS 762.69	–	KM231693	KM231948	KM232243	HQ897778	Fungi	Kepler et al. (2012)
Curvicladiella cignea	CBS 101411	–	JQ666075	KM231866	KM232141	KM232310	Plant	Kepler et al. (2012)
Cyanonectria cyanostroma	CBS 101734	–	HM626671	HM484611	GQ506017	HQ897759	Plant	Lombard et al. (2015)
Cylindrocarpon cylindroides	CBS 503.67	–	MH870763	JF735789	–	–	Plant	Vu et al. (2019)
Cylindrocarpostylus gregarius	CBS 101072	–	JQ666084	KM231870	KM232144	KM232317	Coleoptera	Lombard et al. (2015)
Cylindrocladiella camelliae	CPC 234	–	JN099249	JN099087	KM232139	KM232304	Plant	Lombard et al. (2015)
Cylindrocladiella lageniformis	CBS 340.92	–	JN099165	JN099003	JN989491	KM232303	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	KM232339	Algae	Lombard et al. (2015)
Cylindrodendrum hubeiensis	CBS 129.97	–	KM231628	KM231891	KM232164	KM232341	Plant	Lombard et al. (2015)
Dactylonectria alcacerensis	CBS 129087	–	KM231629	JF735819	KM232176	–	Plant	Lombard et al. (2015)
Dactylonectria estremocensis	CBS 129085	–	KM231630	JF735807	KM232174	KM232345	Plant	Lombard et al. (2015)
Dactylonectria macrodiyma	CBS 112615	–	HM364315	JF268750	HM364333	JF268710	Plant	Lombard et al. (2015)
Dactylonectria novozelandica	CBS 113552	–	–	JF735822	KM232175	KM232346	Plant	Lombard et al. (2015)
Dactylonectria torresensis	CBS 129086	–	KM231631	JF735870	KM232177	KM232347	Plant	Lombard et al. (2015)
Drechmeria balanoides	CBS 250.82	AF339588	AF339539	DQ522342	DQ522388	DQ522442	Nematoda	Quandt et al. (2014)
Drechmeria gunnii	OSC 76404	AF339572	AF339522	AY489616	AY489650	DQ522426	Lepidoptera	Quandt et al. (2014)
Drechmeria sinensis	CBS 567.95	AF339594	AF339545	DQ522343	DQ522389	DQ522443	Nematoda	Quandt et al. (2014)
Dussiella tuberiformis	n/a	–	–	JQ257027	JQ257015	JQ257020	Plant	Kepler et al. (2012)
Engyodontium aranearum	CBS 309.85	AF339576	AF339526	DQ522341	DQ522387	DQ522439	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)
	CBS 206.74	–	LC092912	–	–	–	Soil	Gams et al. (1984)
Epichloë gansuensis	e7080	–	–	KP689495	–	KP689494	Plant	Schardl et al. (2008)
Epichloë typhina	ATCC 56429	U32405	U17396	AF543777	AY489653	DQ522440	Poaceae	Quandt et al. (2014)
Flavocillium primulinum	JCM 18526	–	AB712264	–	–	–	Plant	Wang et al. (2020)
	JCM 18525	–	AB712263	–	–	–	Plant	Wang et al. (2020)
	JCM 18527	–	AB712265	–	–	–	Plant	Wang et al. (2020)
Fusarium lunatum	BBA 63199	–	–	–	–	HQ897766	Plant	Gräfenhan et al. (2011)
Fusarium proliferatum	CBS 189.38	–	KM231685	–	KM232238	KM232384	Plant	Lombard et al. (2015)
Fusarium sambucinum	CBS 146.95	–	KM231682	KM231941	KM232235	KM232381	Plant	Lombard et al. (2015)
Fusarium sublunatum	BBA 62431	–	–	–	–	HQ897780	Soil	Gräfenhan et al. (2011)
Fusarium venenatum	CBS 458.93	–	KM231683	KM231942	KM232236	KM232382	Plant	Lombard et al. (2015)
Fusarium verrucosa	CBS 102163	–	KM231681	KM231940	KM232234	HQ897784	Plant	Lombard et al. (2015)
Fusicolla aquaeductuum	CBS 837.85	–	KM231699	KM231955	KM232250	HQ897744	Water	Lombard et al. (2015)
Fusicolla matuoi	CBS 581.78	–	KM231698	KM231954	KM232249	HQ897720	Plant	Lombard et al. (2015)
Fusicolla violacea	CBS 634.76	–	KM231700	KM231956	KM232251	HQ897696	Hemiptera	Lombard et al. (2015)
Geejayesia desmazieri	CBS 313.34	–	U88125	–	–	HQ897703	Plant	Gräfenhan et al. (2011)
Geejayessia celtidicola	CBS 125502	–	HM626669	HM626638	KM232232	HM626685	Plant	Lombard et al. (2015)
Geejayessia cicatricum	CBS 125549	–	KM231678	HM626643	KM232231	HM626679	Plant	Lombard et al. (2015)
Geejayessia desmazierii	CBS 125507	–	HM626663	HQ728146	KM232230	HM626675	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	EU369075	Araneae	Quandt et al. (2014)
Gibellula pulchra	NHJ 10808	EU369099	EU369035	EU369018	EU369056	EU369076	Araneae	Quandt et al. (2014)

Table 1 (cont.)

Species	Voucher	nSSU rDNA	nLSU rDNA	*TEF*	*RPB1*	*RPB2*	Host	Reference
Gibellula sp.	NHJ 10788	EU369101	EU369036	EU369019	EU369058	EU369078	Araneae	Quandt et al. (2014)
	NHJ 13158	EU369100	EU369037	EU369020	EU369057	EU369077	Araneae	Quandt et al. (2014)
	NHJ 5401	EU369102	–	–	EU369059	EU369079	Araneae	Quandt et al. (2014)
Gliocephalotrichum bulbilium	CBS 242.62	–	AY489732	KM231892	AY489664	EF469114	Soil	Lombard et al. (2015)
Gliocephalotrichum cylindrosporum	CBS 902.70	–	JQ666077	KF513408	KM232179	KM232306	Soil	Lombard et al. (2015)
Gliocephalotrichum irregularis	CBS 755.97	–	JQ666082	KF513449	KM232138	KM232302	Soil	Lombard et al. (2015)
Gliocephalotrichum longibrachium	CBS 126571	–	KM231686	KF513435	KM232178	KM232305	Leaf litter	Lombard et al. (2015)
Gliocladiopsis pseudotenuis	CBS 116074	–	JQ666080	JQ666099	KM232137	KM232301	Soil	Lombard et al. (2015)
Gliocladiopsis sagariensis	CBS 199.55	–	JQ666078	JQ666106	KM232136	KM232300	Soil	Lombard et al. (2015)
Glomerella cingulata	CBS 114054	AF543762	AF543786	AF543773	AY489659	DQ522441	Rosaceae	Quandt et al. (2014)
Haematonectria illudens	BBA 67606	–	AF178362	–	–	HQ897692	Plant	Gräfenhan et al. (2011)
Haematonectria ipomoaea	BBA 64379	–	–	–	–	HQ897753	Plant	Gräfenhan et al. (2011)
Haptocillium zeosporum	CBS 335.80	AF339589	AF339540	EF469062	EF469091	EF469109	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	EU369070	Araneae	Johnson et al. (2009)
Hevansia nelumboides	BCC 41864	JN201863	JN201873	JN201867	–	–	Araneae	Kepler et al. (2017)
Hevansia novoguineensis	NHJ 4314	EU369094	–	EU369012	EU369051	EU369071	Araneae	Kepler et al. (2017)
	NHJ 10469	EU369090	EU369031	EU369008	EU369047	–	Araneae	Kepler et al. (2017)
	NHJ 11923	EU369095	EU369032	EU369013	EU369052	EU369072	Araneae	Kepler et al. (2017)
	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	AY545731	Plant	Quandt et al. (2014)
Hydropisphaera peziza	CBS 102038	AY489698	AY489730	AY489625	AY489661	DQ522444	Plant	Quandt et al. (2014)
Hyperdermium pulvinatum	P.C. 602	–	DQ118738	DQ118746	DQ127237	–	Hemiptera	Quandt et al. (2014)
Hypocrea leucopus	CBS 122499	–	–	FJ179571	–	FJ179605	Fungi	Jaklitsch & Voglmayr (2015)
Hypocrea lutea	ATCC 208838	AF543768	AF543791	AF543781	AY489662	DQ522446	Plant	Quandt et al. (2014)
Hypocrea rufa	CBS 114374	AY489694	AY489726	AY489621	AY489656	EF692510	Fungi	Quandt et al. (2014)
Hypocrella sp.	P.C. 436.2	–	AY986922	AY986949	DQ000350	–	Hemiptera	Quandt et al. (2014)
	P.C. 603	–	AY986923	AY986950	DQ000351	–	Hemiptera	Quandt et al. (2014)
Hypomyces aurantius	GJS74-69	–	HM466684	FJ467643	–	FJ442744	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	KM232336	Plant	Lombard et al. (2015)
Ilyonectria coprosmae	CBS 119606	–	KM515910	JF735694	KM232173	KM232338	Plant	Lombard et al. (2015)
Ilyonectria destructans	CBS 264.65	–	KM515927	JF735695	KM232169	KM232334	Plant	Lombard et al. (2015)
Ilyonectria leucospermi	CBS 132809	–	KM515917	JX231129	KM232172	KM232337	Plant	Lombard et al. (2015)
Ilyonectria liriodendri	CBS 117527	–	KM515922	JF735698	KM232170	KM232335	Plant	Lombard et al. (2015)
Isaria amoenerosea	CBS 729.73	MF416604	MF416551	MF416495	MF416652	MF416446	Insect	Kepler et al. (2017)
Isaria cicadae	RCEF HP090724-31	MF416605	MF416552	MF416496	MF416653	MF416447	Cicadidae	Kepler et al. (2017)
Isaria coleopterorum	CBS 110.73	JF415965	JF415988	JF416028	JN049903	JF416006	Coleoptera	Kepler et al. (2017)
Isaria farinosa	CBS 111113	AY526474	MF416554	MF416499	MF416656	MF416450	Insect	Kepler et al. (2017)
Isaria fumosorosea	CBS 337.52	MF416607	MF416555	MF416500	MF416657	MF416451	Insect	Kepler et al. (2017)
Isaria javanica	CBS 134.22	MF416610	MF416558	MF416504	MF416661	MF416455	Insect	Kepler et al. (2017)
Isaria sp.	spat 09-050	MF416613	MF416559	MF416506	MF416663	MF416457	Lepidoptera	Kepler et al. (2017)
	spat 09-051	MF416614	MF416560	MF416507	MF416664	MF416458	Lepidoptera	Kepler et al. (2017)
Isaria tenuipes	OSC 111007	DQ522559	DQ518773	DQ522349	DQ522395	DQ522449	Lepidoptera	Quandt et al. (2014)
Lecanicillium acerosum	CBS 418.81	KM283762	KM283786	KM283810	KM283832	KM283852	–	Wang et al. (2020)
Lecanicillium antillanum	CBS 350.85	AF339585	AF339536	DQ522350	DQ522396	DQ522450	Hymenoptera	Quandt et al. (2014)
Lecanicillium aranearum	CBS 726.73a	AF339586	AF339537	EF468781	EF468887	EF468934	Araneae	Quandt et al. (2014)
Lecanicillium attenuatum	CBS 402.78	AF339614	AF339565	EF468782	EF468888	EF468935	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	DQ522466	Hemiptera	Quandt et al. (2014)

Table 1 (cont.)

Species	Voucher	nSSU rDNA	nLSU rDNA	*TEF*	*RPB1*	*RPB2*	Host	Reference
Lecanicillium sp.	CBS 639.85	KM283777	KM283801	KM283824	KM283843	KM283865	–	Quandt et al. (2014)
Leptobacillium leptobactrum	IRAN 1230	–	KU382225	–	–	–	Soil	Zare & Gams (2016)
	CBS 771.69	–	KU382224	–	–	–	Soil	Zare & Gams (2016)
Leptobacillium muralicola	CGMCC3.19014	–	MH379997	–	–	–	–	Zare & Gams (2016)
Leuconectria clusiae	ATCC 22228	AY489700	AY489732	AY489627	AY489664	EF469114	Plant	Quandt et al. (2014)
Liangia sinensis	YFCC 3104	MN576727	MN576783	MN576953	MN576843	MN576899	Fungi	Wang et al. (2020)
	YFCC 3103	MN576726	MN576782	MN576952	MN576842	MN576898	Fungi	Wang et al. (2020)
Macroconia leptosphaeria	CBS 717.74	–	KM231707	KM231959	KM232255	HQ897755	Plant/Fungi	Lombard et al. (2015)
Macroconia papilionacearum	CBS 125495	–	KM231704	KM231958	KM232254	HQ897776	Fungi	Lombard et al. (2015)
Mariannaea camptospora	CBS 209.73	–	–	KM231875	KM232147	KM232326	Soil	Lombard et al. (2015)
Mariannaea humicola	CBS 740.95	–	KM231619	KM231880	KM232153	KM232328	Soil	Lombard et al. (2015)
Mariannaea pinicola	CBS 745.88	–	AY554242	KM231879	KM232152	KM232327	Plant	Lombard et al. (2015)
Mariannaea pruinosa	ARSEF 5413	AY184979	AY184968	DQ522351	DQ522397	DQ522451	Lepidoptera	Kepler et al. (2017)
Mariannaea punicea	CBS 239.56	–	JF415981	KM231876	KM232148	JF416001	Soil	Lombard et al. (2015)
Mariannaea samuelsii	CBS 746.88;	–	KM231621	KM231882	KM232155	KM232330	Saprophyte	Lombard et al. (2015)
Metapochonia bulbillosa	CBS 145.70	AF339591	AF339542	EF468796	EF468902	EF468943	Plant	Quandt et al. (2014)
Metapochonia gonioides	891.72	AF339599	AF339550	DQ522354	DQ522401	DQ522458	Nematoda	Quandt et al. (2014)
Metapochonia rubescens	464.88	AF339615	AF339566	EF468797	EF468903	EF468944	Nematoda	Quandt et al. (2014)
Metarhizium album	ARSEF 2082	DQ522560	DQ518775	DQ522352	DQ522398	DQ522452	Hemiptera	Quandt et al. (2014)
Metarhizium anisopliae	ARSEF 3145	AF339579	AF339530	AF543774	DQ522399	DQ522453	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	EF468938	Sand dune	Quandt et al. (2014)
	CBS 399.59	EF468989	EF468842	EF468788	EF468895	EF468939	Soil	Quandt et al. (2014)
Metarhizium chlamydosporia	CBS 101244	DQ522544	DQ518758	DQ522327	DQ522372	DQ522424	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	DQ522454	Hemiptera	Quandt et al. (2014)
Metarhizium indigoticum	TNS F18553	JF415953	JF415968	JF416010	JN049886	JF415992	Coleoptera	Quandt et al. (2014)
Metarhizium kusanagiense	F18494	JF415954	JF415972	JF416014	JN049890	–	Lepidoptera	Quandt et al. (2014)
Metarhizium liangshanensis	EFCC 1523	EF468961	EF468814	EF468755	–	EF468918	Lepidoptera	Quandt et al. (2014)
	EFCC 1452	EF468962	EF468815	EF468756	–	–	Lepidoptera	Quandt et al. (2014)
Metarhizium marquandii	CBS 182.27	EF468990	EF468845	EF468793	EF468899	EF468942	Soil	Quandt et al. (2014)
Metarhizium martiale	TTZ070716-04	JF415955	JF415973	–	JN049891	–	Insect	Quandt et al. (2014)
Metarhizium owariensis	NBRC 33258	–	JF415976	JF416017	–	JF415996	Hemiptera	Kepler et al. (2014)
Metarhizium rileyi	CBS 806.71	AY624205	AY624250	EF468787	EF468893	EF468937	Lepidoptera	Quandt et al. (2014)
Metarhizium sp.	HMAS 199601	JF415957	JF415978	JF416018	JN049894	JF415998	Coleoptera	Kepler et al. (2013)
	HMAS 199603	JF415963	JF415986	JF416026	JN049901	JF416005	Coleoptera	Quandt et al. (2014)
	NHJ 12118	EF468978	EF468829	EF468768	EF468878	EF468927	Lepidoptera	Quandt et al. (2014)
	OSC 110996	EF468974	EF468832	EF468773	EF468880	EF468928	Lepidoptera	Quandt et al. (2014)
Metarhizium taii	ARSEF 5714	AF543763	AF543787	AF543775	DQ522383	DQ522434	Lepidoptera	Quandt et al. (2014)
Metarhizium yongmunense	EFCC 2135	EF468979	EF468834	EF468769	EF468877	–	Lepidoptera	Quandt et al. (2014)
	EFCC 2131	EF468977	EF468833	EF468770	EF468876	–	Lepidoptera	Quandt et al. (2014)
Microcera coccophila	CBS 310.34	–	KM231703	JF740692	JX171462	HQ897705	Hemiptera	Lombard et al. (2015)
Microcera larvarum	CBS 738.79	–	KM231701	KM231957	KM232252	KM232387	Hemiptera	Lombard et al. (2015)
Microcera rubra	CBS 638.76	–	KM231702	JF740696	KM232253	HQ897767	Hemiptera	Lombard et al. (2015)
Microhilum oncoperae	AFSEF 4358	AF339581	AF339532	EF468785	EF468891	EF468936	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)
	PC 115	–	AY986920	AY986947	DQ000348	–	Hemiptera	Quandt et al. (2014)
Moelleriella raciborskii	PC 8238	DQ372102	–	DQ384961	DQ385001	DQ452470	Hemiptera	Quandt et al. (2014)
Moelleriella reineckiana	PC 2355	DQ372092	–	DQ384970	DQ385011	DQ452474	Hemiptera	Quandt et al. (2014)
Myriogenospora atramentosa	AEG 96-32	AY489701	AY489733	AY489628	AY489665	DQ522455	Poaceae	Quandt et al. (2014)
Myrothecium inundatum	IMI158855	AY489699	AY489731	AY489626	–	–	Fungi	Chen et al. (2016)
Nalanthamala psidii	CBS 116952	–	AY864837	KM231972	KM232268	KM232401	Plant	Lombard et al. (2015)

Table 1 (cont.)

Species	Voucher	nSSU rDNA	nLSU rDNA	*TEF*	*RPB1*	*RPB2*	Host	Reference
Nalanthamala vermoesenii	CBS 230.48	–	AY554263	KM231970	KM232266	KM232399	Plant	Lombard et al. (2015)
Nectria balansae	CBS 123351	–	GQ505996	HM484525	GQ506026	KM232407	Plant	Lombard et al. (2015)
Nectria cinnabarina	CBS 114055	U32412	U00748	AF543785	AY489666	DQ522456	Betulaceae	Quandt et al. (2014)
Nectria mariae	CBS 125294	–	JF832684	JF832542	JF832789	KM232404	PLant	Lombard et al. (2015)
Nectria sp.	CBS 478.75	U47842	U17404	EF469068	EF469097	EF469115	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	KM232368	Plant	Lombard et al. (2015)
Neocosmospora phaseoli	CBS 265.50	–	KM231674	HE647964	KM232226	KM232375	Plant	Lombard et al. (2015)
Neocosmospora rubicola	CBS 320.73	–	KM231666	DQ247551	KM232218	KM232366	Soil	Lombard et al. (2015)
Neocosmospora vasinfecta	CBS 325.54	–	KM231670	KM231931	KM232222	KM232370	Soil	Lombard et al. (2015)
Neonectria candida	CBS 151.29	–	HM042436	DQ789723	KM232168	DQ789792	Plant	Lombard et al. (2015)
Neonectria lugdunensis	CBS 125485	–	KM231625	KM231887	KM232160	HQ897731	Plant	Quandt et al. (2014)
Neonectria tsugae	CBS 788.69	–	HQ232146	DQ789720	KM232161	HQ897728	Plant	Lombard et al. (2015)
Niveomyces coronatus	**FieldW**	**ON493545**	**ON493604**	**–**	**–**	**–**	***Ophiocordyceps camponoti-floridani***	**This study**
	Niveo	**ON493546**	**ON493605**	**–**	**–**	**ON513401**	***Ophiocordyceps camponoti-floridani***	**This study**
***Niveomyces coronatus* (TYPE)**	**NY04434800**	**ON493547**	**ON493606**	**ON513397**	**ON513399**	**ON513400**	***Ophiocordyceps camponoti-floridani***	**This study**
Ophiocordyceops naomipierceae	DAWKSANT	KX713664	KX713589	–	KX713701	–	Hymenoptera	Araújo et al. (2018)
Ophiocordyceps acicularis	OSC 128580	DQ522543	DQ518757	DQ522326	DQ522371	DQ522423	Coleoptera	Quandt et al. (2014)
Ophiocordyceps agriotidis	ARSEF 5692	DQ522540	DQ518754	DQ522322	DQ522368	DQ522418	Coleoptera	Quandt et al. (2014)
Ophiocordyceps albacongiuae	RC20	KX713633	–	KX713670	–	–	Hymenoptera	Araújo & Hughes (2019)
Ophiocordyceps amazonica	HUA 186113	KJ917566	KJ917571	–	KP212903	KM411980	Orthoptera	Sanjuán et al. (2015)
Ophiocordyceps annulata	CEM303	KJ878915	KJ878881	KJ878962	KJ878995	–	Coleoptera	Quandt et al. (2014)
Ophiocordyceps aphodii	ARSEF 5498	DQ522541	DQ518755	DQ522323		DQ522419	Coleoptera	Quandt et al. (2014)
Ophiocordyceps australis	HUA 186097	KC610786	KC610765	KC610735	KF658662	–	Hymenoptera	Sanjuán et al. (2015)
	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	DQ522420	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 & Hughes (2019)
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	–	KJ878954	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	–	EF468930	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	KJ878946	Hymenoptera	Quandt et al. (2014)
Ophiocordyceps formosana	TNM F13893	KJ878908	–	KJ878956	KJ878988	KJ878943	Coleoptera	Quandt et al. (2014)

Table 1 (cont.)

Species	Voucher	nSSU rDNA	nLSU rDNA	*TEF*	*RPB1*	*RPB2*	Host	Reference
Ophiocordyceps forquignonii	OSC 151908	KJ878922	KJ878889	–	KJ879003	KJ878947	Diptera	Quandt et al. (2014)
Ophiocordyceps fulgoromorphila	HUA 186139	KC610794	KC610760	KC610729	KF658676	KC610719	Hemiptera	Sanjuan et al. (2015)
	HUA 186142	KC610795	KC610761	KC610730	KF658677	–	Hemiptera	Sanjuan et al. (2015)
Ophiocordyceps gracilis	EFCC 8572	EF468956	EF468811	EF468751	EF468859	EF468912	Lepidoptera	Quandt et al. (2014)
Ophiocordyceps gracillisima	HUA 186132	–	KC610768	KC610744	KF658666	–	Coleoptera	Sanjuán et al. (2015)
Ophiocordyceps halabalaensis	MY1308	KM655825	–	GU797109	–	–	Hymenoptera	Luangsa ard et al. (2011)
Ophiocordyceps heteropoda	EFCC 10125	EF468957	EF468812	EF468752	EF468860	EF468914	Hemiptera	Quandt et al. (2014)
Ophiocordyceps humbertii	MF116b	MF116B	MK874748	MK875536	–	MK863828	Hymenoptera	Araújo & Hughes (2019)
Ophiocordyceps irangiensis	128578	DQ522556	DQ518770	DQ522345	DQ522391	DQ522445	Hymenoptera	Quandt et al. (2014)
	OSC 128577	DQ522546	DQ518760	DQ522329	DQ522374	DQ522427	Hymenoptera	Sanjuán et al. (2015)
Ophiocordyceps kimflemingiae	SC30	KX713629	KX713622	KX713699	KX713727	–	Hymenoptera	Araújo & Hughes (2019)
Ophiocordyceps kniphofioides	HUA 186148	KC610790	KF658679	KC610739	KF658667	KC610717	Hymenoptera	Sanjuán et al. (2015)
Ophiocordyceps konnoana	EFCC 7315	EF468959	–	EF468753	EF468861	EF468916	Coleoptera	Quandt et al. (2014)
Ophiocordyceps lloydii	OSC 151913	KJ878924	KJ878891	KJ878970	KJ879004	KJ878948	Hymenoptera	Quandt et al. (2014)
Ophiocordyceps longissima	HMAS_199600	KJ878926		KJ878972	KJ879006	KJ878949	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)
Ophiocordyceps neovolkiana	OSC 151903	KJ878930	KJ878896	KJ878976	KJ879010	–	Coleoptera	Quandt et al. (2014)
Ophiocordyceps nigrella	EFCC 9247	EF468963	EF468818	EF468758	EF468866	EF468920	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 & Hughes (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	EU369084	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	DQ522430	Coleoptera	Quandt et al. (2014)
Ophiocordyceps rhizoidea	NHJ 12522	EF468970	EF468825	EF468764	EF468873	EF468923	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	EF468924	Lepidoptera	Quandt et al. (2014)
Ophiocordyceps sobolifera	KEW 78842	EF468972	EF468828	–	EF468875	EF468925	Hemiptera	Quandt et al. (2014)
Ophiocordyceps sp.	TNS F18495	KJ878937	KJ878901	–	KJ879017	–	Coleoptera	Quandt et al. (2014)
	OSC 151904	KJ878934	KJ878899	KJ878980	KJ879014	–	Hemiptera	Quandt et al. (2014)
	OSC 151905	KJ878935	–	KJ878981	KJ879015	KJ878951	Hemiptera	Quandt et al. (2014)
	CatKNZ01_OP	LC370844	LC370818	LC370821	LC370819	LC370820	Hemiptera (symbiont)	Matsuura et al. (2018)
	CfaKSD05_OP	LC370995	LC370790	LC370801	LC370819	LC370800	Hemiptera (symbiont)	Matsuura et al. (2018)
	EUTCHI_OP	LC370840	LC370890	LC370893	LC370891	LC370892	Hemiptera (symbiont)	Matsuura et al. (2018)
	EokKNG01_OP	LC370992	LC370896	LC370899	LC370897	LC370898	Hemiptera (symbiont)	Matsuura et al. (2018)
	GbiNNJ01_OP	LC370850	LC370852	LC370864	LC370862	LC370854	Hemiptera (symbiont)	Matsuura et al. (2018)
	GniKSD01_OP	LC370844	LC370846	LC370849	LC370847	LC370848	Hemiptera (symbiont)	Matsuura et al. (2018)
	HmaTKB05_OP	LC370983	LC370985	LC370988	LC371009	LC370987	Hemiptera (symbiont)	Matsuura et al. (2018)
	MiwITN01_OP	LC370995	LC370969	LC370978	LC370976	LC370971	Hemiptera (symbiont)	Matsuura et al. (2018)
	MkuYGJ01_OP	LC370992	LC370962	LC370965	LC370963	LC370964	Hemiptera (symbiont)	Matsuura et al. (2018)
	MopTKB06_OP	LC370995	LC370923	LC370926	LC371009	LC370925	Hemiptera (symbiont)	Matsuura et al. (2018)
	MosKNG01	LC370857	LC370942	LC370945	LC370943	LC370944	Hemiptera (symbiont)	Matsuura et al. (2018)
	MmiNNJ01_OP	LC370857	LC371001	LC371004	LC371002	LC371003	Hemiptera (symbiont)	Matsuura et al. (2018)
	TjaTKB04_OP	LC370857	LC370904	LC370907	LC370911	LC370906	Hemiptera (symbiont)	Matsuura et al. (2018)
	MopTKB01_01	LC370857	LC371008	LC371011	LC370884	LC370885	Hemiptera (symbiont)	Matsuura et al. (2018)
	TniTKB04_OP	LC370857	LC370874	LC370877	LC370869	LC370876	Hemiptera (symbiont)	Matsuura et al. (2018)

Table 1 (cont.)

Species	Voucher	nSSU rDNA	nLSU rDNA	*TEF*	*RPB1*	*RPB2*	Host	Reference
Ophiocordyceps sp. (cont.)	Gh41	KX713656	–	KX713668	KX713706	–	Hymenoptera	Araújo & Hughes (2019)
	OSC 151909	KJ878936	KJ878900	KJ878982	KJ879016	KJ878952	Hymenoptera	Quandt et al. (2014)
Ophiocordyceps sphecocephala	OSC 110998	DQ522551	DQ518765	DQ522336	DQ522381	DQ522432	Hymenoptera	Quandt et al. (2014)
Ophiocordyceps stylophora	OSC 111000	DQ522552	DQ518766	DQ522337	DQ522382	DQ522433	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	EF468933	Coleoptera	Quandt et al. (2014)
Ophiocordyceps yakusimensis	HMAS_199604	KJ878938	KJ878902	–	KJ879018	KJ878953	Hemiptera	Quandt et al. (2014)
Ophionectria trichospora	CBS 109876	AF543766	AF543790	AF543779	AY489669	DQ522457	Plant	Quandt et al. (2014)
Orbiocrella petchii	NHJ 6209	EU369104	EU369039	EU369023	EU369061	EU369081	Hemiptera	Quandt et al. (2014)
Paecilomyces niphetodes	CBS 364.76	AY526471	JF415989	JF416029	JN049904	JF416007	Soil	Quandt et al. (2014)
Paecilomyces penicillatus	CBS 448.69	AY526493	EU553300	–	–	–	Fungi	Quandt et al. (2014)
Paracremonium contagium	CBS 110348	–	HQ232118	KM231966	KM232262	KM232396	Human	Lombard et al. (2015)
Paracremonium inflatum	CBS 485.77	–	HQ232113	KM231964	KM232260	KM232394	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)
	CBS 368.72	–	LC092910	–	–	–	Soil	Wang et al. (2020)
Penicillifer bipapillatus	CBS 420.88	–	KM231608	KM231860	KM232129	KM232295	Saprophyte	Lombard et al. (2015)
Penicillifer diparietisporus	CBS 376.59	–	KM231609	KM231861	KM232130	KM232296	Soil	Lombard et al. (2015)
Penicillifer pulcher	CBS 560.67	–	KM231610	KM231862	KM232131	KM232297	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	KF049669	Coleoptera	Quandt et al. (2014)
	NBRC 100942	JN941711	JN941430	AB972954	JN992445	AB972958	Coleoptera	Matočec et a. (2014)
Perennicordyceps prolifica	TNS-F-18547	KF049613	KF049632	KF049687	KF049649	KF049670	Hemiptera (cicada)	Kepler et al. (2017)
	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. (2012)
	NBRC 103842	JN941701	JN941440		JN992435	–	Coleoptera	Wang et al. (2021)
	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	KP276667	Fungi	Wang et al. (2021)
	YHHPA 1303						Fungi	Wang et al. (2021)
Pleurocordyceps aurantiaca	MFLUCC 17 2114	MG136905	MG136911	MG136874	MG136867	MG136871	*Ophiocordyceps*	Wang et al. (2021)
	MFLUCC 17 1394	MG136906	MG136912	MG136876	–	MG136872	*Ophiocordyceps*	Wang et al. (2021)
	MFLUCC 17 2113	MG136904	MG136910	MG136875	MG136866	MG136870	*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)
	GIMMY 9603	KF226249	KF226250	KF226252	KF226251		Lepidoptera	Wang et al. (2021)
Pleurocordyceps marginaliradians	MFLUCC 17 2276	MG136909	MG136915	MG136879		MG271930	Lepidoptera	Wang et al. (2021)
	MFLU 17 1582	MG136908	MG136914	MG136878	MG136869	MG271931	Lepidoptera	Wang et al. (2021)
Pleurocordyceps nipponica	BCC 1682	KF049620	KF049638	KF049694			Neuroptera	Wang et al. (2015a)
	BCC 18108	KF049608	KF049626	KF049681	KF049644		Neuroptera	Wang et al. (2015a)
	BCC 2325	KF049622	KF049640	KF049696	KF049655	KF049677	Neuroptera	Wang et al. (2015a)
	NHJ 4268	KF049621	KF049639	KF049695	KF049654	KF049676	Neuroptera	Wang et al. (2015a)
	BCC 1881	KF049618	KF049636	KF049692		KF049674	Neuroptera	Wang et al. (2015a)
	NHJ 7727	MF416625	MF416570	MF416518	MF416677	MF416464	–	Kepler et al. (2017)
Pleurocordyceps phaothaiensis	BCC 84557	–	MF959738	MF959741	MF959746	–	Coleoptera	Wang et al. (2021)
	BCC 84551	–	MF959735	MF959739	MF959743	–	Coleoptera	Wang et al. (2021)
	BCC 84552	–	MF959736	MF959740	MF959744	–	Coleoptera	Wang et al. (2021)
	BCC 84553	–	MF959737	MF959742	MF959745	–	Coleoptera	Wang et al. (2021)
Pleurocordyceps ramosopulvinata	SU-65	DQ118742	DQ118753	DQ127244	–	–	Hemiptera	Quandt et al. (2014)
	EFCC 5566	–	KF049627	KF049682	KF049645	–	Hemiptera	Quandt et al. (2014)
Pleurocordyceps sinensis	ARSEF 1424	KF049615	AY259544	DQ118754	DQ127245	KF049671	Coleoptera	Quandt et al. (2014)
	CN 80-2	HQ832887	HQ832886	HQ832890	HQ832888	HQ832889	*Ophiocordyceps sinensis*	Wang et al. (2021)
	GIMCC 3570	JX006097	JX006098	JX006100	JX006101	–	*Ophiocordyceps sinensis*	Wang et al. (2021)

Table 1 (cont.)

Species	Voucher	nSSU rDNA	nLSU rDNA	*TEF*	*RPB1*	*RPB2*	Host	Reference
Pleurocordyceps sinensis (cont.)	BL 4	KF049623	AY259545	KF049697	KF049656	KF049678	Myxomycete	Quandt et al. (2014)
Pleurocordyceps yunnanensis	NBRC 109984	MN586819	MN586837	MN598052	MN598043	–	*Ophiocordyceps nutans*	Wang et al. (2015a)
	NBRC 109985	MN586820	MN586838	MN598053	MN598044	–	*Ophiocordyceps nutans*	Wang et al. (2015a)
	NBRC 101760	MN586818	MN586836	MN598051	MN598042	MN598060	*Ophiocordyceps nutans*	Wang et al. (2015a)
	YHCPY 1005	KF977848	KF977848	KF977850	KF977852	KF977854	*Ophiocordyceps nutans*	Wang et al. (2015a)
	YHHPY 1006	KF977849	KF977849	KF977851	KF977853	KF977855	*Ophiocordyceps nutans*	Wang et al. (2015a)
Pochonia chlamydosporia	CBS 504.66	AF339593	AF339544	EF469069	EF469098	EF469120	Nematoda	Quandt et al. (2014)
Pochonia parasiticum	3436	EF468993	EF468848	EF468799	EF468904	EF468945	Rotifera	Quandt et al. (2014)
Polycephalomyces formosus-like	CGMCC 5.2204	MN586821	MN586839	MN598054	MN598045	MN598061	Coleoptera	Wang et al. (2021)
	CGMCC5.2205	MN586822	MN586840	MN598055	MN598046	MN598062	Coleoptera	Wang et al. (2021)
	CGMCC5.2207	MN586823	MN586841	MN598056	MN598047	MN598063	Coleoptera	Wang et al. (2021)
	CGMCC5.2206	MN586824	MN586842	MN598057	MN598048	MN598064	Coleoptera	Wang et al. (2021)
	CGMCC5.2208	MN586825	MN586843	MN598058	MN598049	MN598065	Coleoptera	Wang et al. (2021)
	CGMCC5.2203	MN586826	MN586844	MN598059	MN598050	MN598066	Coleoptera	Wang et al. (2021)
Polycephalomyces sp.	NBRC 109990	–	AB925968	–	–	–	–	Wang et al. (2021)
	NBRC 110224	–	AB925969	–	–	–	–	Wang et al. (2021)
	NBRC 109987	–	AB925983	–	–	–	–	Wang et al. (2021)
	NBRC 109988	–	AB925984	–	–	–	–	Wang et al. (2021)
Pseudogibellula formicarum	BCC 81493	–	MT512652	MT863566	MT533472	–	*Ophiocordyceps flavida*	Mongkolsamrit et al. (2021)
	BCC 84257	–	MT512653	MT533480	MT533473	–	*Ophiocordyceps flavida*	Mongkolsamrit et al. (2021)
	CBS 871.72	–	MH878295	MT863565	MT533474	–	Hemiptera; Ricania mediana	Vu et al. (2019)
	CBS 433.73	–	MH872442	MT533481	MT533475	–	Hemiptera; Ricania mediana	Vu et al. (2019)
Pseudonectria buxi	CBS 324.53	–	KM231644	KM231909	KM232194	KM232353	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	DQ522459	Buxaceae	Quandt et al. (2014)
Purpureocillium atypicolum	GNJ070713-08, Na16	KJ878907	KJ878872	KJ878955	–	–	Araneae	Quandt et al. (2014)
	CBS 744.73	EF468987	EF468841	EF468786	EF468892	–	Araneae	Quandt et al. (2014)
Purpureocillium lilacinum	CBS 284.36	AY624189	AY624227	EF468792	EF468898	EF468941	Soil	Quandt et al. (2014)
Purpureocillium takamizusanense	NHJ 3582	EU369096	EU369033	EU369014	EU369053	EU369074	Hemiptera	Quandt et al. (2014)
Rectifusarium robinianum	CBS 830.85	–	KM231656	KM231922	JX171461	JX171575	Plant	Lombard et al. (2015)
Rectifusarium ventricosum	CBS 748.79	–	KM231658	KM231924	KM232208	HQ897761	Soil	Lombard et al. (2015)
Regiocrella camerunensis	ARSEF 7682		DQ118735	DQ118743	DQ127234		Hemiptera	Quandt et al. (2014)
Rosasphaeria moravica	LMM	–	–	JF440987	–	JF440986	Saprophyte	Jaklitsch & Voglmayr (2012)
Rotiferophthora angustispora	CBS 101437	AF339584	AF339535	AF543776	DQ522402	DQ522460	Rotifera	Quandt et al. (2014)
Roumegueriella rufula	CBS 346.85	DQ522561	DQ518776	DQ522355	DQ522403	DQ522461	Nematoda	Quandt et al. (2014)
Rugonectria neobalansae	CBS 125120	–	HM364322	KM231874	KM232146	KM232321	Plant	Lombard et al. (2015)
Samsoniella alpina	YFCC 5836	MN576755	MN576811	MN576981	MN576871	MN576925	Lepidoptera	Wang et al. (2020)
Samsoniella antleroides	YFCC 6113	MN576748	MN576804	MN576974	MN576864	MN576918	Lepidoptera	Wang et al. (2020)
Samsoniella cardinalis	YFCC 5830	MN576732	MN576788	MN576958	MN576848	MN576902	Lepidoptera	Wang et al. (2020)
Samsoniella cristata	YFCC 6021	MN576735	MN576791	MN576961	MN576851	MN576905	Lepidoptera	Wang et al. (2020)
Samsoniella hepiali	YFCC 661	MN576739	MN576795	MN576965	MN576855	MN576909	Lepidoptera	Wang et al. (2020)
Samsoniella kunmingensis	YHH 16002	MN576746	MN576802	MN576972	MN576862	MN576916	Lepidoptera	Wang et al. (2020)
Samsoniella lanmaoa	YFCC 6148	MN576733	MN576789	MN576959	MN576849	MN576903	Lepidoptera	Wang et al. (2020)
Samsoniella ramosa	YFCC 6020	MN576749	MN576805	MN576975	MN576865	MN576919	Lepidoptera	Wang et al. (2020)
Samsoniella tortricida	YFCC 6013	MN576751	MN576807	MN576977	MN576867	MN576921	Lepidoptera	Wang et al. (2020)
Samsoniella yunnanensis	YFCC 1527	MN576756	MN576812	MN576982	MN576872	MN576926	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	KM232360	Soil	Lombard et al. (2015)
Sarcopodium circinosetiferum	CBS 100251	–	KM231646	KM231913	KM232197	KM232356	Soil	Lombard et al. (2015)
Sarcopodium flavolanatum	CBS 112283	–	KM231649	KM231916	KM232200	KM232358	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	EF469118	Plant	Kepler et al. (2017)
Simplicillium lamellicola	CBS 116.25	AF339601	AF339552	DQ522356	DQ522404	DQ522462	Fungi	Kepler et al. (2017)

Table 1 (cont.)

Species	Voucher	nSSU rDNA	nLSU rDNA	*TEF*	*RPB1*	*RPB2*	Host	Reference
Simplicillium lanosoniveum	CBS 704.86	AF339602	AF339553	DQ522358	DQ522406	DQ522464	Fungi	Kepler et al. (2017)
Simplicillium obclavatum	CBS 311.74	AF339567	AF339517	EF468798	–	–	Fungi	Kepler et al. (2017)
Sphaerostilbella aureonitens	GJS74-87	–	HM466683	–	–	FJ442763	Fungi	Judith et al. (2015)
Sphaerostilbella berkeleyana	CBS 102308	AF543770	U00756	AF543783	AY489671	DQ522465	Hymenomycetes	Judith et al. (2015)
Stachybotrys chlorohalonata	DAOM 235557	JN939037	JN938870	–	–	–	Plant	Andersen et al. (2003)
Stachybotrys microspora	CBS 186.79	JN939037	–	DQ676604	–	DQ676580	Plant	Koster et al. (2009)
Stephanonectria keithii	GJS92-133	AY489695	AY489727	AY489622	–	–	Plant	Castlebury et al. (2004)
Striatibotrys eucylindrospora	CBS 203.61	–	KU846868	KU847078	–	KU846975	Soil	Lombard et al. (2015)
Stylonectria applanata	CBS 125489	–	KM231689	KM231944	HQ897739	HQ897739	Plant	Lombard et al. (2015)
Stylonectria wegeliana	CBS 125490	–	KM231690	KM231945	KM232240	HQ897754	Plant	Lombard et al. (2015)
Thelonectria discophora	CBS 125153	–	HM364307	KM231897	HM364339	KM232343	Plant	Lombard et al. (2015)
Thelonectria olida	CBS 215.67	–	NG 064061	–	M364334	KM232342	Plant	Vu et al. (2019)
Thelonectria trachosa	CBS 112467	–	HM364312	KM231896	HM364339	KM232413	Plant	Lombard et al. (2015)
Thyronectria lamyi	CBS 417.89	–	KM231718	JF832580	JF832830	KM232413	Plant	Lombard et al. (2015)
Thyronectria pyrrhochlora	CBS 125131	–	HM484570	HM484519	HM484584	KM232410	Plant	Lombard et al. (2015)
Thyronectria quercicola	CBS 128976	–	JF832743	JF832581	JF832831	KM232411	Plant	Lombard et al. (2015)
Thyronectria sinoptica	CBS 462.83	–	GQ506001	HM484531	GQ506031	KM232412	Plant	Lombard et al. (2015)
Tilachlidium brachiatum	CBS 505.67	–	KM231720	KM231976	KM232272	KM232415	Fungi	Lombard et al. (2015)
Tolypocladium capitatum	OSC 71233	AY489689	AY489721	AY489615	AY489649	DQ522421	Fungi	Quandt et al. (2014)
Tolypocladium cylindrosporum	NRRL 28025	AF049153	AF049173	–	–	–	Diptera	Quandt et al. (2014)
Tolypocladium fractum	OSC 110990	DQ522545	DQ518759	DQ522328	DQ522373	DQ522425	Fungi	Quandt et al. (2014)
Tolypocladium japonicum	OSC 110991	DQ522547	DQ518761	DQ522330	DQ522375	DQ522428	Fungi	Quandt et al. (2014)
Tolypocladium longisegmentum	OSC 110992	–	EF468816	–	EF468864	EF468919	Fungi	Quandt et al. (2014)
Tolypocladium ophioglossoides	CBS 100239	KJ878910	KJ878874	KJ878958	KJ878990	KJ878944	Fungi	Quandt et al. (2014)
Tolypocladium subsessilis	OSC 71235	EF469124	EF469077	EF469061	EF469090	EF469108	Coleoptera	Quandt et al. (2014)
Torrubiella ratticaudata	1915	DQ522562	DQ518777	DQ522360	DQ522408	DQ522467	Araneae	Kepler et al. (2017)
Torrubiella sp.	NHJ 7859	EU369107	–	–	EU369064	EU369085	Araneae	Kepler et al. (2017)
Torrubiella wallacei	CBS 101237	AY184978	AY184967	EF469073	EF469102	EF469119	Lepidoptera	Kepler et al. (2017)
Torrubiellomyces zombiae	**FieldB**	**ON493544**	**ON493603**	ON513395	**–**	**–**	***Ophiocordyceps camponoti-floridani***	**This study**
	Polyceph	**–**	**ON493607**	ON513394	**–**	**–**	***Ophiocordyceps camponoti-floridani***	**This study**
***Torrubiellomyces zombiae* (TYPE)**	**NY04434801**	**ON493543**	**ON493602**	ON513396	ON513398	ON513402	***Ophiocordyceps camponoti-floridani***	**This study**
Trichoderma aggressivum	CBS 100526	–	–	AF348096	–	AF545541	Fungi	Jaklitsch & Voglmayr (2012)
Trichoderma americanum	G.J.S. 92-93	–	–	–	–	DQ835455	Fungi	Jaklitsch & Voglmayr (2012)
Trichoderma deliquescens	CBS 121131	–	–	–	–	FJ179609	Fungi	Jaklitsch & Voglmayr (2012)
Trichoderma viride	GJS89-127	–	–	AY750891	–	AF545558	Fungi	Lombard et al. (2015)
Trichosphaerella ceratophora	CBS 130.82	–	KM231727	KM231983	–	KM232423	Saprophyte	Lombard et al. 2015
Tyrannicordyceps fratricida	TNS 19011	JQ257022	JQ257023	JQ257028	JQ257016	JQ257021	Fungi	Kepler et al. (2012)
Ustilaginoidea dichromenae	IB 9228	–	AF373280	JQ257025	JQ257013	JQ257018	Plant	Quandt et al. (2014)
Ustilaginoidea virens	MAFF 240421	–	JQ257011	JQ257026	–	JQ257017	Plant	Quandt et al. (2014)
Valetoniellopsis laxa	ATCC 16535	AY489705	AY489737	AY489632	AY489673	DQ522468	Saprophyte	Zhang & Blackwell (2002)
Verticillium dahliae	ATCC 16535	AY489705	AY489737	AY489632	AY489673	DQ522468	Rosaceae	Quandt et al. (2014)
Verticillium epiphytum	CBS 384.81	AF339596	AF339547	DQ522361	DQ522409	DQ522469	Urediales	Quandt et al. (2014)
Verticillium sp.	CBS 102184	AF339613	AF339564	EF468803	EF468907	EF468948	Araneae	Quandt et al. (2014)
Virgatospora echinofibrosa	CBS 110115	–	KU847270	KU847313	–	KU847293	Plant	Lombard et al. (2015)
Viridispora diparietispora	CBS 102797	AY489703	AY489735	AY489630	AY489668	DQ522471	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	HQ897715	Plant	Lombard et al. (2015)
Volutella rosea	CBS 128258	–	KM231634	KM231900	KM232185	KM232348	Soil	Lombard et al. (2015)
Xenoacremonium recifei	CBS 137.35	–	HQ232106	KM231968	KM232264	KM232397	Human	Lombard et al. (2015)
Xenocylindrocladium guianense	CBS 112179	–	JQ666073	KM231895	KM232166	KM232314	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	–	KM232313	Plant	Lombard et al. (2015)
Xenogliocladiopsis cypellocarpa	CBS 133814	–	KM231623	KM231885	KM232158	KM232332	Plant	Lombard et al. (2015)

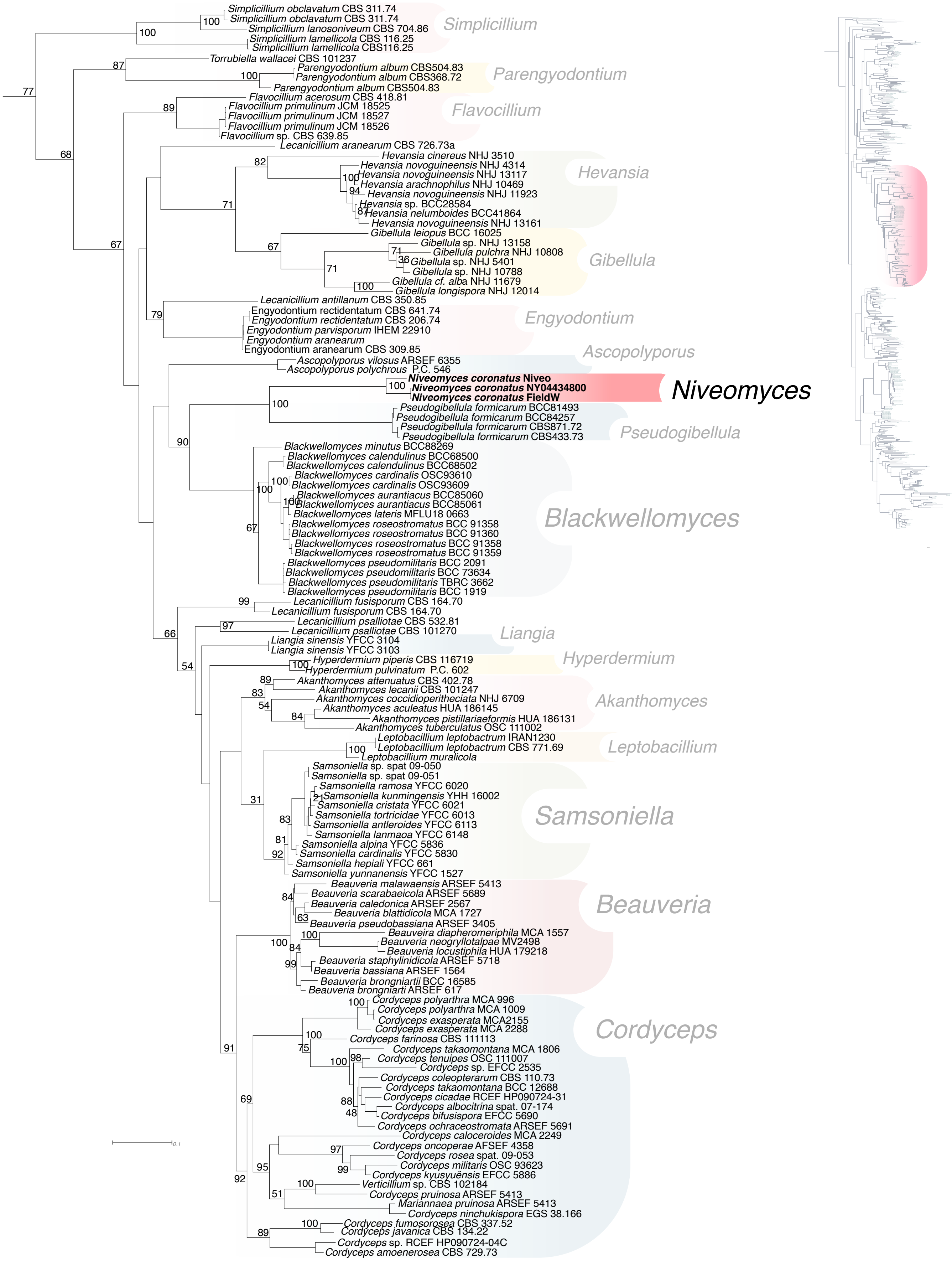

Fig. 2 Maximum likelihood tree of *Cordycipitaceae* obtained with a concatenated dataset of SSU, LSU, *TEF*, *RPB1* and *RPB2*. *Niveomyces* gen. nov. is indicated in **bold** font. The whole analysis tree of the order *Hypocreales* is depicted in the top-right corner, with the position of *Cordycipitaceae* highlighted in red.

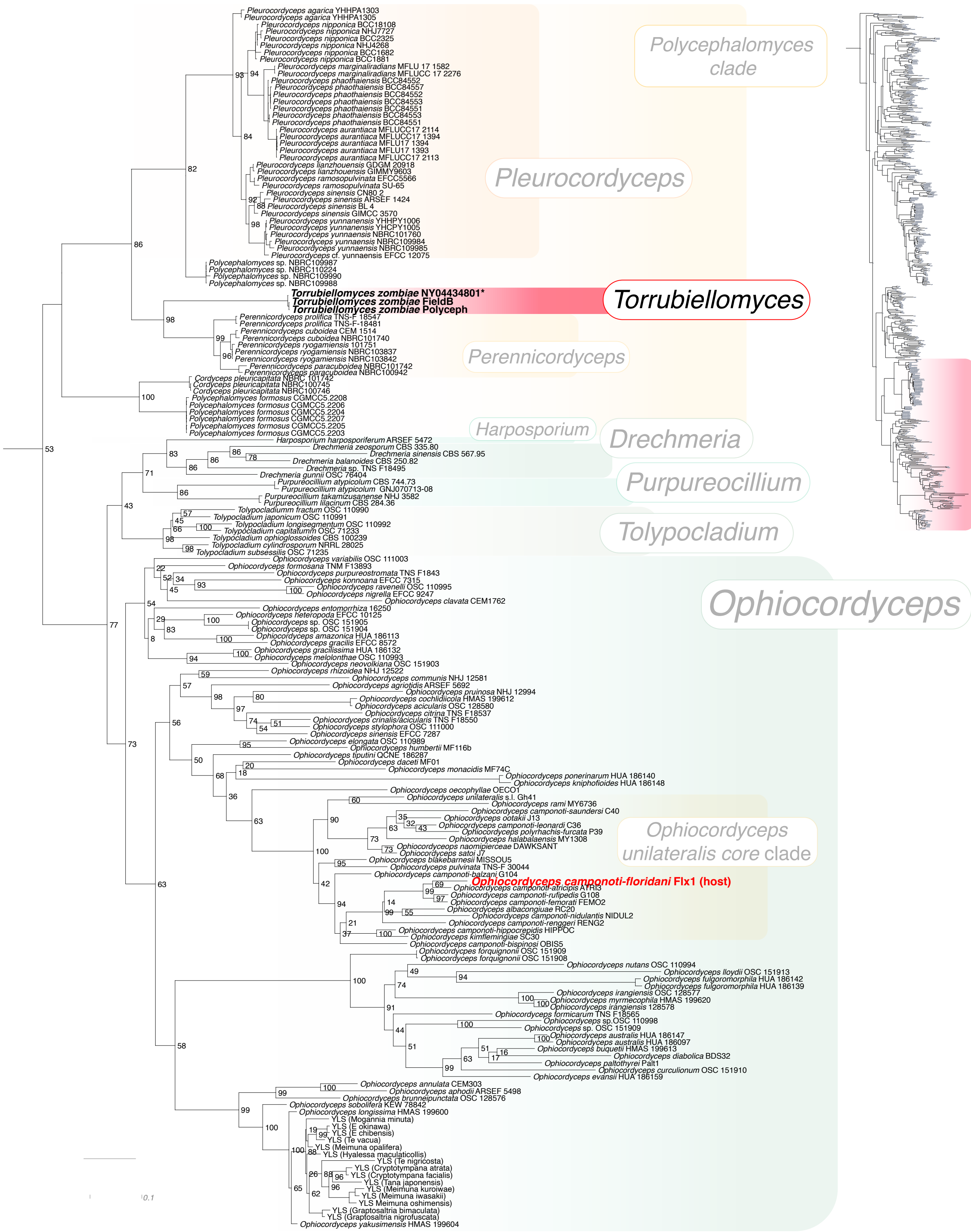

Fig. 3 Maximum likelihood tree of *Ophiocordycipitaceae* obtained with a concatenated dataset of SSU, LSU, *TEF*, *RPB1* and *RPB2*. *Torrubiellomyces* gen. nov. is indicated in **bold** font. The host of *Niveomyces* and *Torrubiellomyces*, *O. camponoti-floridani*, is indicated in red. The whole analysis tree of the order *Hypocreales* is depicted in the top-right corner, with the position of *Ophiocordycipitaceae* highlighted in red.

Genome assembly and gene prediction

Prior to assembling the two mycoparasite genomes, the raw sequence data were filtered and trimmed using the BBDuk plugin in Geneious Prime v. 20.2.3 with default parameters. Subsequently, to confirm the quality of these trimmed reads, fastQC was used (Andrews 2010). The genomes were then assembled *de novo* using the SPAdes assembly algorithm (Bankevich et al. 2012) and the quality of the assemblies was confirmed through QUAST (Gurevich et al. 2013). As expected from samples taken directly from the field, the QUAST outputs showed bacterial contamination in the genomic data, indicated by the presence of two distinct mean G-C % peaks; one large peak comprising fungal reads (30–80 %) and a second, much smaller peak comprising bacterial contaminants (0–30 %). The bacterial contaminants were removed from both genomes by manually removing sequences with a mean G-C % that fell within the contaminant peak (i.e. < 30 %). The effectiveness of this bacterial filtering was confirmed using MG-RAST (Keegan et al. 2016). We determined the completeness of the genomes after bacterial filtering with BUSCO (Seppey et al. 2019), using the *Hypocreales* lineage (fungi_odb9), and CEGMA (Parra et al. 2007). Scaffolds shorter than 1 000 bp were discarded. Gene predictions were performed with Augustus v. 3.3 (Stanke & Morgenstern 2005) using the previously generated parameters for *O. camponoti-floridani* (Will et al. 2020) and the software parameters for *Fusarium graminearum*. The draft genomes are available through GenBank under the accession numbers: JADHZA000000000 (*Torrubiellomyces zombiae*) and JAFEME000000000 (*Niveomyces coronatus*). The genome assembly, gene predictions and functional annotations can also be interactively analysed and downloaded through https://fungalgenomics.science.uu.nl.

Functional annotation

The predicted proteins in our draft genomes were functionally annotated using PFAM (El-Gebali et al. 2019) and mapped to their corresponding gene ontology (GO) terms. Transmembrane domains were annotated using TMHMM v. 2.0 (Krogh et al. 2001) and signal peptides using SignalP-5.0 (Nielsen et al. 1997). Proteins with a secretory signal were considered small secreted proteins if they were shorter than 300 amino acids and did not contain a transmembrane domain (except in the first 40 amino acids). We used a SMURF-based pipeline to predict secondary metabolite clusters (Khaldi et al. 2010, De Bekker et al. 2015). BlastP, with an E-value cutoff of 1e-10, was used to search the MEROPS database for proteases (Rawlings et al. 2018).

DNA extraction, PCR and phylogenetics

Using our *de novo* assembled and annotated mycoparasite genomes as protein Blast databases, we obtained and verified sequences for ribosomal 18S (SSU), ribosomal 28S (LSU), translation elongation factor 1-alpha (*TEF*), and RNA Polymerase II Subunits (*RPB1* and *RPB2*) for phylogenetic placement. We also extracted DNA from additional specimens and liquid cultures of the isolates for PCR amplification of these genes. We extracted DNA as previously described (Will et al. 2020). We amplified genes with Phusion polymerase (New England Biolabs) using the primers and PCR programs published in Araújo et al. (2018), with cycle lengths and temperatures adjusted as per the recommendations provided in the Phusion polymerase protocol. We aligned the obtained sequences in Geneious to a database comprised of 531 species (Table 1) that broadly represented the order *Hypocreales*. Each locus was individually aligned with MAFFT (Katoh & Standley 2013) and concatenated into a single combined dataset using Geneious v. 11.1.5. The concatenated files, along with a position (POS) file for each gene, were imported into CIPRES (Miller et al. 2012). The final alignment length was 4 770 bp: 1 244 bp for SSU, 939 bp for LSU, 963 bp for *TEF*, 639 bp for *RPB1* and 985 bp for *RPB2*. We performed maximum likelihood analysis with RAxML v. 8.2.4 (Stamatakis 2014) on a concatenated dataset containing all five genes. The dataset consisted of 11 partitions, two for SSU and LSU and nine for each codon position of the three protein coding genes: *TEF*, *RPB1* and *RPB2*. We employed the GTRGAMMA model of nucleotide substitution during the generation of 1 000 bootstrap replicates. Visualization and graphic adjustments were made in Dendroscope (Huson & Scornavaca 2012) and further edited in Adobe Illustrator.

RESULTS

Phylogenetics

The phylogenetic results recovered the overall topology presented in previous studies (Quandt et al. 2015, Kepler et al. 2017, Araújo et al. 2018, Araújo & Hughes 2019). To determine the phylogenetic placement of the two mycoparasitic species, a comprehensive phylogenetic tree of the order *Hypocreales* was generated, adapted from Araújo & Hughes (2019). Based on the phylogenetic results, both species, *N. coronatus* and *T. zombiae*, formed distinct and well-supported monophyletic clades, BS = 100 for *Niveomyces* and BS = 98 for *Torrubiellomyces* (Fig. 2, 3).

According to the data, *Niveomyces* occupies a basal branch within the family *Cordycipitaceae* (Fig. 2), while *Torrubiellomyces* sits in a basal clade within *Ophiocordycipitaceae* (Fig. 3). *Niveomyces* formed a unique, distinctive and relatively long-branched clade, while *Torrubiellomyces* fell within the *Polycephalomyces* clade. In order to investigate the relationships of *Torrubiellomyces*, we sampled *Polycephalomyces* s.lat. (*Polycephalomyces* insertae sedis, *Pleurocordyceps* and *Perennicordyceps*); including a range of species representing distinct ecologies, such as animal and fungal parasites (Kepler et al. 2013, Matočec et al. 2014, Xiao et al. 2018, Wang et al. 2021) (Fig. 3). Our phylogeny suggests the *Polycephalomyces* clade as the most basal lineage within *Ophiocordycipitaceae* (BS = 53) and strongly supports *Torrubiellomyces* as a distinct genus (BS = 98), closely related to a clade strictly associated with insects: *Perennicordyceps cuboidea*, *Pe. Paracuboidea*, *Pe. ryogamiensis* (all on Coleoptera), and *Pe. prolificus* (on Hemiptera) (Fig. 3) (Kepler et al. 2013).

Taxonomy

Based on a combination of morphological, ecological and phylogenetic data, we introduce two new genera and two new species of mycoparasites within the *Hypocreales*. *Torrubiellomyces zombiae* and *Niveomyces coronatus* were both collected parasitizing *Ophiocordyceps camponoti-floridani*, a ubiquitous entomopathogen of the ant *Camponotus floridanus* in Florida, USA.

Niveomyces J.P.M. Araújo & C. de Bekker, *gen. nov*. – MycoBank MB 839229

Etymology. Name reflects the ‘snowy’ (Lat.: *niveus*) appearance of this fungus.

Type species. *Niveomyces coronatus* J.P.M. Araújo & C. de Bekker

Diagnosis: *Niveomyces* is diagnosed by its mycoparasitic nature, the production of spiky, white, slender, velvety synnemata and unique characters of the conidiogenous cells, which exhibit multiple denticles along the phialides with a crown-like apex, producing conidia singly.

Mycelium white to pale yellow, often covering the host entirely. *Vegetative hyphae* septate and hyaline. *Synnemata* multiple,

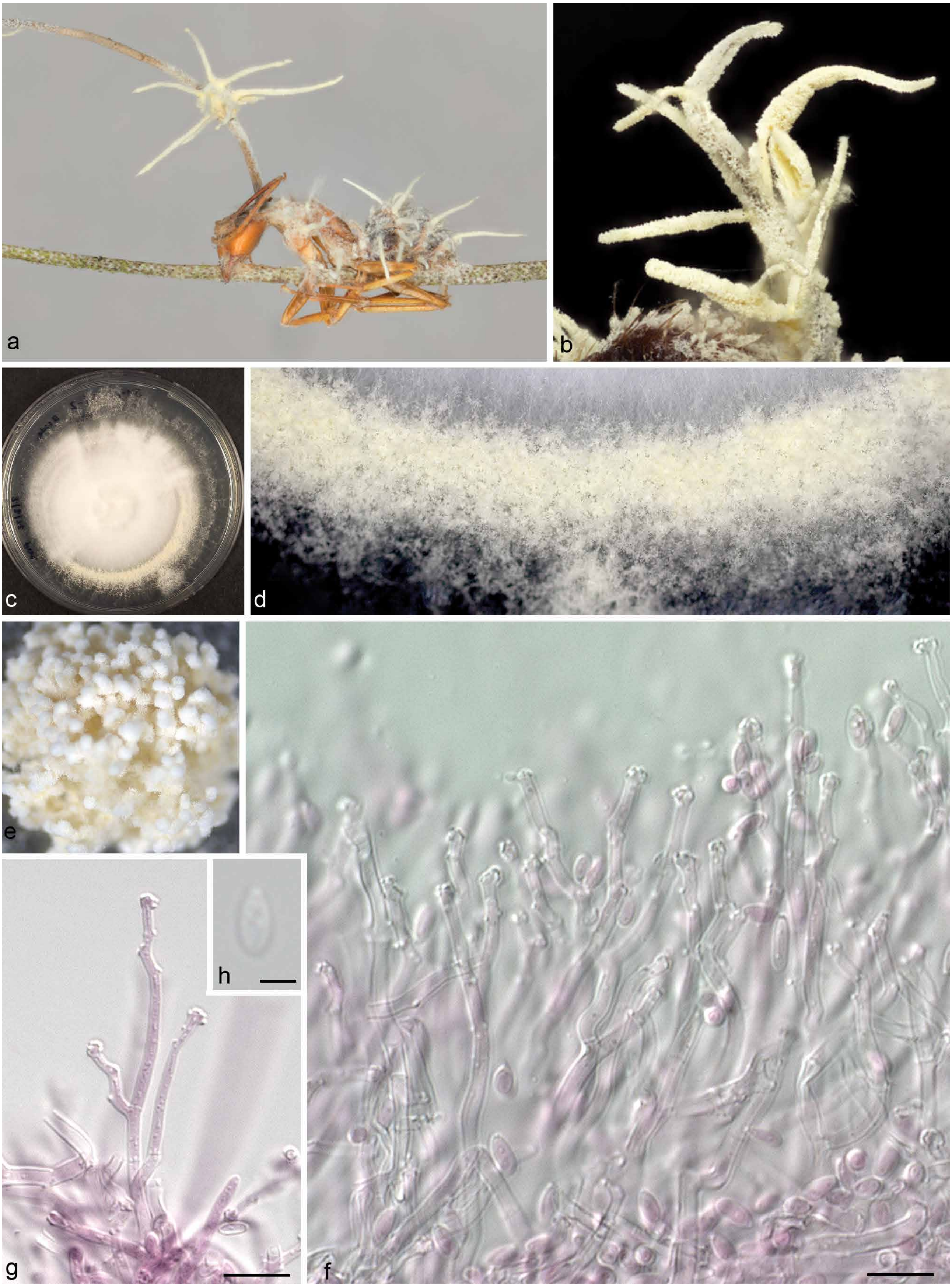

Fig. 4 *Niveomyces coronatus* growing on *Ophiocordyceps camponoti-floridani*, a pathogen of the ant *Camponotus floridanus*. a. View of the tri-trophic system ant-entomopathogenic fungi-mycoparasite; b. close-up of *N. coronatus* synnemata; c. PDA culture after 60 d; d. close-up of culture edge; e. close-up of sporodochia formed in culture; f. layer of phialides (hymenium); g. close-up of apical and lateral conidiogenous cells; h. conidium. — Scale bars: f–g = 10 μm, h = 5 μm.

spiky, erect, slightly sinuous to straight, not branched, tapering towards the apex, covered by hymenium-like layer of conidiogenous cells. *Conidiogenous cells* polyblastic, elongated, irregular, hyaline, cylindrical, with characteristic denticles that are crowded on the apical part and less frequent towards the base. *Conidia* globose to ovoid formed singly on the denticles. Produces micromorphological features, such as conidiogenous cells and conidia, identical on both specimen and in culture. *Sexual morph* unknown.

Hosts — Entomopathogenic fungi.

Distribution — USA, but probably worldwide.

Niveomyces coronatus J.P.M. Araújo & C. de Bekker, *sp. nov.* — MycoBank MB 844049; Fig. 4

Etymology. Name reflects the characteristic crown of denticles on top of the conidiogenous cells.

Typus. USA, Florida, Seminole County, Oviedo, Little Big Econ State Forest, N28°41'14.7" W81°09'33.4", over-growing *Ophiocordyceps camponoti-floridani*, a fungal pathogen of *Camponotus floridanus*, 10 June 2017, *de Bekker* (holotype NY04434800).

Diagnosis: White mycelium covering the host almost entirely, producing multiple spike-like synnemata; exhibiting abundant characteristic conidiogenous cells bearing multiple denticles, especially at the apical part producing globose to ovoid conidia.

Mycelium white to light yellow, growing abundantly on the host. *Synnemata* multiple, not branching, white, slender, erect, arising from the subiculum that covers the host almost entirely, narrowing towards the end, averaging 311.9 × 65.2 μm; covered by a hymenium-like layer of dense conidiogenous cells. *Conidiogenous cells* (12–)17(–25) × 1.5–2 μm, polyblastic, cylindrical, hyaline, irregular, sometimes capitate, bearing crowded hyaline denticles on the apical part, often descending sparsely along almost the entire cell. *Conidia* (3.7–)4.5(–5.5) × 1.5–2(–2.5) μm formed singly on the denticles, solitary, ovoid to globose, one-celled, hyaline and smooth-walled.

Culture characteristics — Colonies on PDA reach a diameter of 65–70 mm after 6 wk incubation at room temperature. Mycelium white during early stages becoming light yellow with age; remaining thin without spore formation when grown at 25 °C in total darkness; at room temperature, under regular day-night light fluctuations, aerial hyphae and conidia similar to those observed on field specimens formed at the periphery of the colony after 8 wk. Synnemata were produced after 10 wk of incubation.

Host — *Ophiocordyceps camponoti-floridani*.

Distribution — Florida, USA.

Ecology — Parasitic on *Ophiocordyceps camponoti-floridani*, an entomopathogen of the Florida carpenter ant, *Camponotus floridanus*, a ground-dwelling ant, commonly nesting in dead wood or soil. *Ophiocordyceps*-manipulated *C. floridanus* are predominantly found in elevated position, ranging from 0.1 m up to at least 2.5 m in height, clinging on and biting on epiphytic plants of the family *Bromeliaceae* (specifically, *Tillandsia recurvata* and *T. usneoides*) in mesic hammock habitats with evergreen canopy.

A similar species has been found parasitising both *Ophiocordyceps camponoti-novogranadensis* on its host, *Camponotus novogranadensis*, and *O. camponoti-rufipedis* on *Camponotus rufipes* in remnant Atlantic rainforest in Minas Gerais, Brazil (H.C. Evans pers. obs.). Here, they are common mycoparasites – especially in the wet season – and may be exerting some control of *Ophiocordyceps* infections (see Fig. 1). It remains to be determined if these are *N. coronatus* or another member of the genus.

Torrubiellomyces J.P.M. Araújo & C. de Bekker, *gen. nov*. – MycoBank MB 844048

Etymology. Name reflects the resemblance to torrubiella-like fungi with the production of superficial perithecia on a subiculum directly on the host, without a stipe.

Type species. *Torrubiellomyces zombiae* J.P.M. Araújo & C. de Bekker

Diagnosis: *Torrubiellomyces* is diagnosed by its mycoparasitic habit, its initially white, then brown to black perithecia produced directly on the fungal host tissue. In culture, it exhibits a characteristic production of viscous conidial masses, similar to those produced by *Pleurocordyceps*, but without a stipe (synnema) on the host or in culture (Fig. 5, 6).

Mycelium initially white, then brown growing on the insect sutures where the fungal host emerges; producing solitary or clusters of superficial perithecia that turn black and powdery with age; no synnemata or stalk produced on the host or in culture. *Ascospores* filiform, disarticulating into part-spores upon maturity. In culture, producing light cream mycelium with pools or masses of viscous conidia in sporodochia (Fig. 6c), never forming synnemata. Only one type of conidiogenous cell observed.

Hosts — Entomopathogenic fungi.

Distribution — USA, but probably worldwide.

Torrubiellomyces zombiae J.P.M. Araújo & C. de Bekker, *sp. nov*. — MycoBank MB844050; Fig. 5, 6

Etymology. Name reflects its pathogenic association with the zombie-ant fungus *O. camponoti-floridani,* which manipulates its host's behaviour during infection.

Typus. USA, Florida, Seminole County, Oviedo, Little Big Econ State Forest, N28°41'14.7" W81°09'33.4", on *Ophiocordyceps camponoti-floridani* pathogenic on *Camponotus floridanus*, 12 June 2017, *de Bekker* (holotype NY04434801).

Diagnosis: Multiple perithecia produced solitarily or forming aggregations on multiple parts of the host mycelium, turning black and powdery with age. Asexual morph only observed in culture, forming pools of viscous conidial masses in sporodochia, lacking a stipe, one type of conidiogenous cell observed; producing ovoid, hyaline, smooth conidia.

Mycelium scarce, pale to brown; growing sparsely, producing brown perithecia becoming black and powdery with age, produced directly on the host synnemata or on host mycelium that emerges from the ant joints and sutures. *Perithecia* superficial, ovoid, solitary or forming dense aggregations, (420–)480(–525) × (205–)280(–310) μm, rugose when dry. *Asci* hyaline, capitate, cylindrical, 225 × 6.5–7 μm. *Ascospores* arranged in a spiral within the ascus, readily breaking into part-spores often still within the ascus. Part-spores hyaline, cuboid to globose, and often varying in size within a single ascus when mature, exhibiting a corn cob-like aspect (Fig. 5f), (1.8–)2.4(–3.2) × (1.5–)2(–2.6) μm. Asexual morph not observed on any of the field-collected specimens.

Culture characteristics — Colonies on PDA reaching a diameter of c. 40–48 mm after 6 wk incubation at room temperature. Mycelium white during early stages; becoming light cream with age, dense and reverse brown. Synnemata never observed, but conidial masses produced after 6–8 wk directly on the subiculum; pale cream, usually surrounded by sterile perithecium-like structures. Phialides producing large number of conidia, forming viscous masses (Fig. 6c); cylindrical to subulate, usually slightly curved, (5.5–)8(–12) × (1.5–)1.7(–2.2) μm, tapering gradually towards the apex. Conidia solitary, single celled, smooth-walled, hyaline, ovoid, 2.5–3 × 1.5–1.8 μm.

Host — *Ophiocordyceps camponoti-floridani.*

Distribution — Florida, USA.

Ecology — As above.

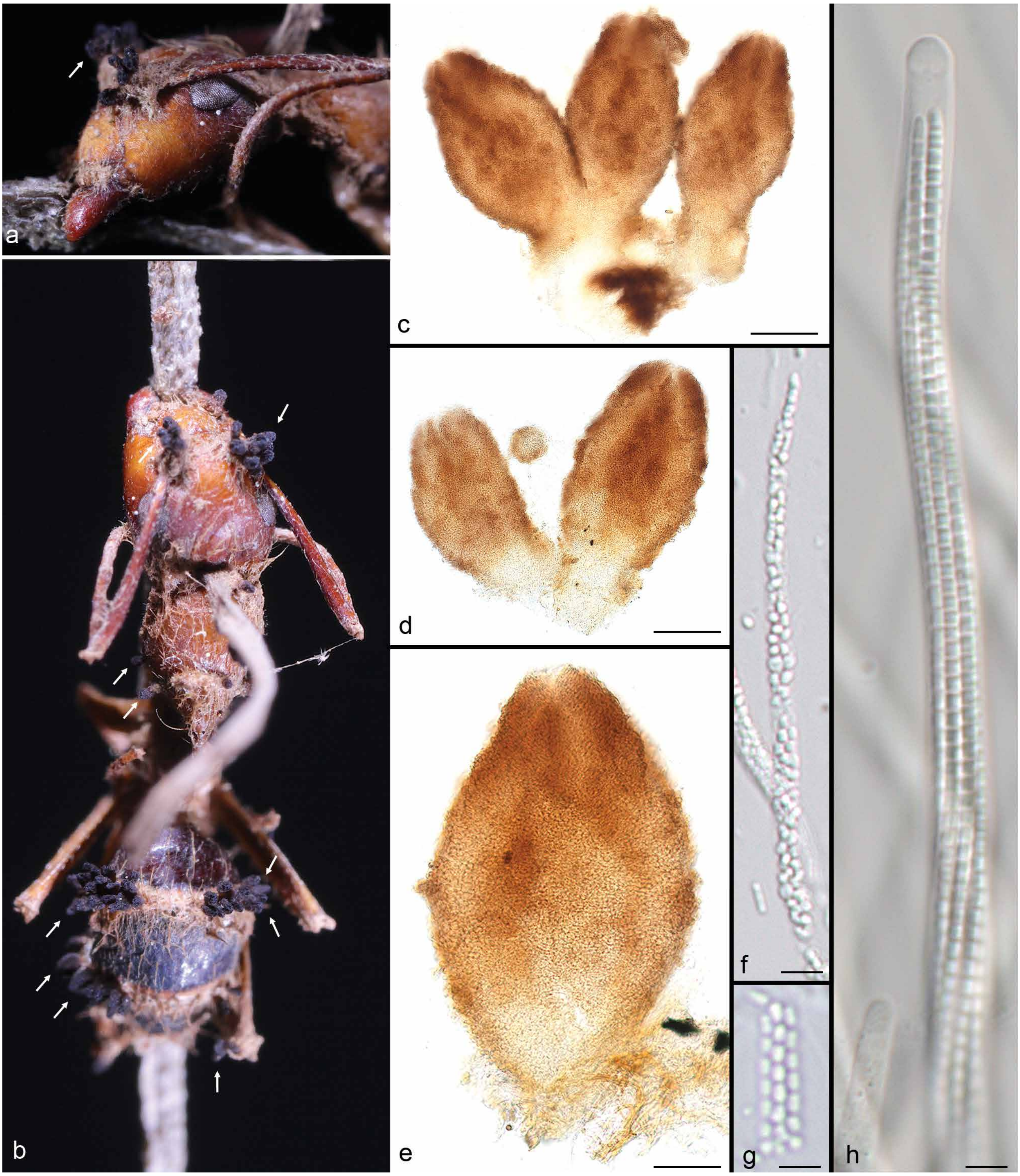

Fig. 5 *Torrubiellomyces zombiae* growing on *Ophiocordyceps camponoti-floridani*, a pathogen of the ant *Camponotus floridanus*. a. Close-up showing perithecia emerging from the antennal plate of the ant; b. general overview of a typical perithecial arrangement in clusters or less often singly produced; c–e. perithecia; f. mature ascospores disarticulated prior to release, forming corn cob-like asci; g. cluster of part-spores; h. ascus showing the ascospores already sub-divided into part-spores. — Scale bars: c–d = 100 µm, e = 50 µm, f = 10 µm, g–h = 5 µm.

A similar species has been found parasitising *O. camponoti-novogranadensis* on its host, *Camponotus novogranadensis* in Brazil and *O. oecophyllae* infecting *Oecophylla smaragdina* in Australis (see above; Fig. 1e and 1f, respectively). Similar perithecia have also been found on the mycelium of *Ophiocordyceps oecophyllae* (Araújo et al. 2018), a pathogen of the weaver ant *Oecophylla smaragdina* in the rainforest of tropical Queensland, Australia. It remains to be determined if these records are *T. zombiae* or a related species of the genus.

Draft genomes

As part of the species descriptions, the genomes of the two novel fungal species, *N. coronatus* and *T. zombiae*, were sequenced and assembled *de novo*. Our assembly of the *N. coronatus* genome resulted in a total genome size of 31.95 Mbp, made up by 1 357 contigs, with an N50 of 49 324 bp and a G+C content of 51.11 %. The genome contains 8 930 protein encoding sequences of which 6 433 sequences (72.04 %) were functionally annotated with PFAM domains and 4 159 (46.57 %) received GO annotations. In addition, this genome is predicted to contain 766 genes with secretion signals, 1 714

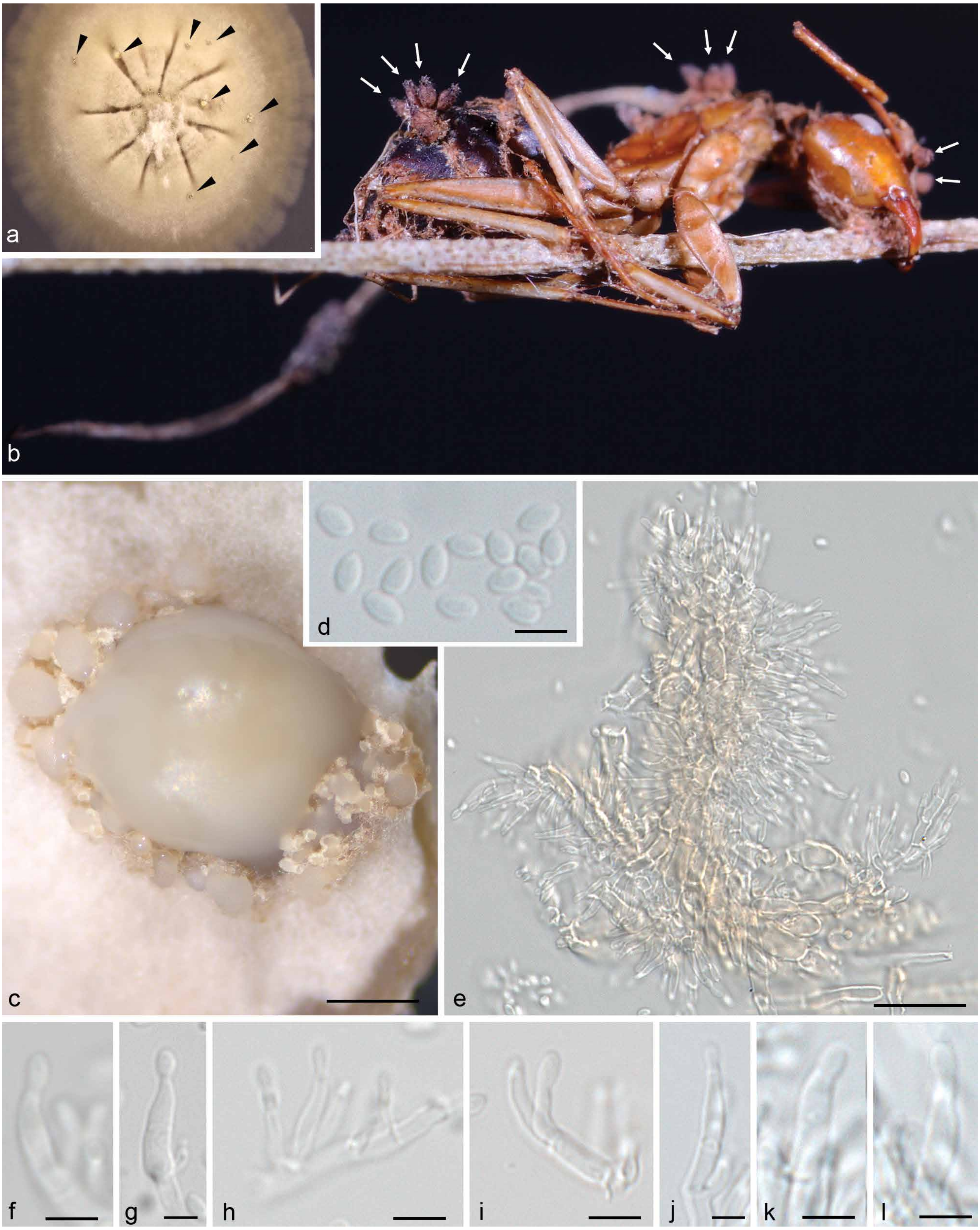

Fig. 6 *Torrubiellomyces zombiae* in culture. a. PDA plate after 60 d, arrows indicate the pools of viscous conidia produced in sporodochia scattered over the plate; b. overview of the specimen, white arrows indicate *T. zombiae* perithecia emerging from the fungal host tissue; c. close-up of sporodochium with pools of viscous conidia; d. conidia; e. cluster of phialides; f–l. phialides. — Scale bars: c = 1000 µm, d = 3 µm, e = 20 µm, f–l = 5 µm.

genes with transmembrane domains, 304 transcription factors, 336 proteases and 53 secondary metabolite clusters. The set of gene predictions was determined to be 93.1 % complete (i.e., BUSCO completeness, CEGMA was 97.16 %). The assembled *T. zombiae* genome was calculated to be 27.11 Mbp in size, consisting of 1 725 contigs with an N50 of 41 529 bp and a G+C content of 51.49 %. Our annotation predicted the genome to contain 8 422 protein encoding sequences with 5 985 (71.06 %) of those sequences containing known PFAM domains and 3 812 (45.26 %) receiving GO annotations. Additionally, this genome was functionally annotated to have 675 genes with secretion signals, 1 496 genes with transmembrane domains, 242 transcription factors, 274 proteases and 32 secondary metabolite clusters. The set of gene predictions was found to be 94.48 % complete (i.e., BUSCO completeness, CEGMA was 98.03 %) (Table 2).

Table 2 Draft genome details and statistics on genome assembly, gene predictions, annotations and completeness.

Property	*Torrubiellomyces zombiae*	*Niveomyces coronatus*
Sequences in assembly	1725	1357
Total assembly length (Mbp)	27,11	31,95
Assembly GC content (%)	51,49	51,11
Assembly gaps (%)	0	0
L50 number (#)	189	200
N50 length (bp)	41529	49324
Genes	8422	8930
Gene length (median)	1382	1452
Transcript length (median)	1233	1302
Exon length (median)	294	323
CDS length (median)	1230	1299
Protein length (median)	410	433
Spliced genes (total, %)	6294 (74.73%)	6450 (72.23%)
Exons per gene (median)	2	2
Intron length (median)	64	67
Introns per spliced gene (median)	2	2
Gene density (genes / Mbp)	310,68	279,49
Coding content of assembly (bp, %)	12740001 (47.0%)	13987488 (43.78%)
Proteins with internal stops (total, %)	0 (0.0%)	0 (0.0%)
Unique PFAM domains	3803	3786
Genes with PFAM (total, %)	5985 (71.06%)	6433 (72.04%)
Genes with GO (total, %)	3812 (45.26%)	4159 (46.57%)
Genes with signalP (total, %)	675 (8.01%)	766 (8.58%)
Genes with TMHMM (total, %)	1496 (17.76%)	1714 (19.19%)
Genes annotated as TF (total, %)	242 (2.87%)	304 (3.4%)
Genes annotated as MEROPS protease (total, %)	266 (3.16%)	319 (3.57%)
Genes annotated as CAZyme (total, %)	224 (2.66%)	296 (3.31%)
Secondary metabolite clusters	32	38
CEGMA completeness (%)	98,03	97,16
BUSCO2 completeness (fungi_odb9)	Complete: 94.48 % (Single-copy: 93.79 %, Duplicated: 0.69 %), Fragmented: 3.79 %, Missing: 1.72 %	Complete: 93.1 % (Single-copy: 92.07 %, Duplicated: 1.03 %), Fragmented: 2.41 %, Missing: 4.48 %

DISCUSSION

Few mycoparasitic species of entomopathogenic fungi have been formally described, thus far, including recent records of *Polycephalomyces* on *Ophiocordyceps* species from Asia (Wang et al. 2015b, Zhong et al. 2016, Xiao et al. 2018). However, mycoparasites associated with behaviour-manipulating *Ophiocordyceps* have only been noted in the field as an ecological aspect of those interactions (Andersen & Hughes 2012, Araújo et al. 2020, Mongkolsamrit et al. 2021). In this study, we present two new genera *Niveomyces* and *Torrubiellomyces*, which were recorded consistently infecting the zombie-ant fungus *O. camponoti-floridani*. We also provide their annotated draft genomes, which we used as a means to genetically identify the correctly cultured isolates that were obtained from field specimens and to extract sequences for phylogenetic analysis. Only a limited number of hypocrealean mycoparasites has been sequenced so far, including *Tolypocladium ophioglossoides* (Quandt et al. 2015), several *Trichoderma* species such as *Trichoderma virens* (Kubicek et al. 2011), *Trichoderma atroviride* (Kubicek et al. 2011) and *Trichoderma reesei* (Martinez et al. 2008), *Escovopsis weberi* (De Man et al. 2016), and *Clonostachys rosea* (Karlsson et al. 2015). The draft genomes that we generated for *N. coronatus* and *T. zombiae*, therefore, represent a significant contribution to the still scarce, existing mycoparasite genomics data. Currently, these data are too scattered across the *Hypocreales* to conduct meaningful comparative genomics analyses into mycoparasite signatures. However, we hope that this study will stimulate more research into mycoparasitism and generate additional draft genomes in order to make such analyses more worthwhile.

Both mycoparasites are well-supported by the comprehensive phylogeny as novel and unique lineages in the families *Cordycipitaceae* and *Ophiocordycipitaceae* of the order *Hypocreales*. *Torrubiellomyces zombiae* is placed as a new lineage within the *Polycephalomyces* clade, as sister to *Perennicordyceps*, a genus composed exclusively of entomopathogenic species. This suggests that its origins are from an insect-associated ancestor shared with *Perennicordyceps*. However, other species within the *Polycephalomyces* clade are also parasitic on entomopathogenic fungi. For example: *Pleurocordyceps yunnaensis* on *Ophiocordyceps nutans*, a pathogen of stink bugs; *Pl. aurantiaca* on *O. barnesii*, a pathogen of melolonthid larvae and *Pl. agarica* on an unidentified *Ophiocordyceps* species also pathogenic on melolonthid larvae; demonstrating the affinity of this group to exploit entomopathogens (Wang et al. 2015b, Xiao et al. 2018). Furthermore, *Niveomyces coronatus* resides within a part of the *Hypocreales* tree that, thus far, largely contains entomopathogens. This suggests that its mycoparasitism may have evolved from a previous animal parasitic relationship.

Regarding morphological features, *Niveomyces coronatus* exhibits snow-white mycelium that often completely covers the host, producing multiple synnemata on a subiculum or directly on the host tissue; while its sister genus – *Pseudogibellula* – has gibellula-like conidiophores but which, unlike the phialidic heads of *Gibellula*, has heads of conidiogenous cells producing conidia sympodially on minute denticles, leaving protuberant scars (Samson & Evans 1973, Araújo et al. 2020, Mongkolsamrit et al. 2021). *Pseudogibellula* is a monotypic genus and the type species, *P. formicarum* has been described as a 'strongly competitive fungus on insect substrates and frequently exploits ant cadavers killed by other fungal pathogens' (Samson & Evans 1973). However, it was also reported to cause local epizootics on at least six ant species in evergreen forest in Ghana, as well as being a pathogen of several Homopteran hosts in cocoa farms (Samson & Evans 1973). Thus, *P. for-*

micarum has been considered – seemingly, ambiguously – as both an opportunistic mycoparasite and as an entomopathogen. Both Araújo et al. (2020) and Mongkolsamrit et al. (2021) have recorded *P. formicarum* in a purported mycoparasitic association with *Ophiocordyceps* pathogens of ants and leafhoppers, respectively. However, these authors reasoned that the fungus may also be an entomopathogen, based on evidence of primary infection of insect hosts (Homoptera: Cicadellidae) in both Brazil and the USA. In the latter, this involved both *in vivo* and *in vitro* studies of the interaction and from the results of pathogenicity experiments, it was concluded that *P. formicarum* is a primary entomopathogen and responsible for field epizootics of the glassy-winged sharpshooter, *Homalodisca coagulata*: a major agricultural pest in Florida (Kanga et al. 2004, Boucias et al. 2006). In fact, Boucias et al. (2006) noted differences in conidiophore morphology in the Florida isolate and considered that this could be a novel species of *Pseudogibellula*. A more detailed molecular analysis of the various geographical and host isolates of *P. formicarum* seems warranted, especially to compare the purported mycoparasitic and entomopathogenic strains.

This apparent inter-kingdom jump – with mycoparasitic species evolving from entomopathogens to parasitise related entomopathogenic fungi – is analogous to mycoparasitism in the *Urediniomycetes*, where rust relatives of the genus *Tubercularia* are parasitic on rust fungi; having evolved from a plant parasitic lineage (Lutz et al. 2004a–c). The plant parasitic *Helicobasidium* sexual morph has a wide host range, whereas the *Tubercularia* mycoparasitic species show a high degree of specificity within their rust hosts. It remains to be confirmed if the mycoparasites described here, as well as *P. formicarum* s.lat., have similar levels of specificity within their entomopathogenic fungal hosts.

Torrubiellomyces is only known from its sexual morph and it is easily recognized in the field by the formation of single or clusters of brown to black superficial perithecia that are produced directly on the mycelium of the *Ophiocordyceps* host fungus. In culture, *Torrubiellomyces* forms viscous conidia that are characteristic of species belonging to the *Polycephalomyces* clade. However, it differs from other closely related genera (*Perennicordyceps*, *Pleurocordyceps* and *Polycephalomyces* s.str.) by the absence of a stipe (synnema) supporting the formation of viscous conidia, which in *Torrubiellomyces* are produced in sporodochia *in vitro* (see Fig. 6c).

Field observations

The fungi that we consistently find growing on *Ophiocordyceps* have always been considered as their associated mycoparasites. However, one could perhaps argue that they could also be growing saprophytically on dead insect or fungal tissue or act as entomopathogens that co-infect the insect host of *Ophiocordyceps*. The latter is especially enticing considering the phylogenetic placement of both species among fungal groups that broadly include entomopathogenic species. However, based on their unique morphology, which indicates that both *N. coronatus* and *T. zombiae* only grow on top of *Ophiocordyceps* tissue, it is more likely that they are indeed mycoparasites as posited in other studies dealing with interactions between entomopathogenic fungi and other antagonistic fungi (Wang et al. 2015b). Our field observations supports this conclusion. Although in some cases *N. coronatus* and *T. zombiae* growth was observed less than a week after a new *O. camponoti-floridani*-infected ant cadaver was found, they also appeared up to nine months after initial *Ophiocordyceps* infection, suggesting that these mycoparasites are able to infect *Ophiocordyceps* species at any stage of its development. Moreover, *O. camponoti-floridani* mycelium begins to emerge from a fresh ant cadaver within one to two days after its death, providing already sufficient tissue for the mycoparasite to inhabit. This seemingly rules out the possibility of either species being an entomopathogen that coinfects the ant. Moreover, already within the first hours after an ant's manipulation and following death, *Ophiocordyceps* completely colonizes the ant's body, consuming all ant tissue, besides the cuticle, to gain the energy needed to grow the fruiting body (De Bekker et al. 2015). This makes it unlikely that either of the two species that we describe here would be able to saprophytically consume the insect cadaver. Moreover, the appearance of both mycoparasitic species in the first few weeks after death of the ant host, when *O. camponoti-floridani* is fresh and actively growing, seems to rule out the possibility of either species being an opportunistic saprophyte that merely feeds on dead *O. camponoti-floridani* tissue. In fact, even months after ant manipulation and death have taken place, *Ophiocordyceps* is often found to be alive and is still able to produce stalks with ascoma and consequently release spores. Taken together, our morphological and field observations confirm previous assumptions that the fungi we found in association with *Ophiocordyceps* species are indeed mycoparasites.

Conclusions

Here, we describe two new genera of fungi – *Niveomyces* and *Torrubiellomyces* – parasitic on the zombie-ant fungus, *Ophiocordyceps camponoti-floridani*, from a small sample area in central Florida. Collections of similar fungi have been made from other *Ophiocordyceps* species, especially those attacking ants, in South America, Africa, Asia and Australia. It is likely, therefore, that such mycoparasites are pantropical and that these tri-trophic interactions are important contributors to the 'natural balance' in their respective ecosystems. The diversity and host specificity within these new genera, as well as in related genera, such as *Pseudogibellula*, remains to be determined. However, it is possible that such mycoparasitic genera harbour a potentially large and untapped reservoir of undocumented fungal diversity, especially when we consider the diversity of entomopathogenic fungi worldwide (Araújo & Hughes 2016). This is part of the 'hidden fungal biodiversity' described by Blackwell & Vega (2018) and evidence suggests that there was a diverse range of mycoparasites in existence over 400 million years ago (Berbee et al. 2017, Krings et al. 2017). Mycoparasites of plant pathogens also constitute this 'cryptic' biodiversity and are now being more intensively studied because of their potential for biological control of plant diseases. The classical biological control approach, involving surveys in the centres of origin or diversity of the target plant pathogen, has yielded a surprising diversity from relatively small sample sizes. Mycoparasites associated with frosty pod of cacao (*Moniliophthora roreri*) on its wild *Theobroma* host in the forests of western Ecuador (Evans et al. 2003) and those associated with coffee leaf rusts (*Hemileia* spp.) on their wild *Coffea* hosts in Africa (Colmán et al. 2021, Rodríguez et al. 2021) provide evident examples. Recent estimates of extant fungal species, based on a fungal census of soils and a fungal/plant ratio of 17/1, has put the number near six million species (Taylor et al. 2014). These authors concluded that: "98 % of fungi remain undescribed and that many of these species occupy unique niches". Clearly, entomopathogenic fungi and their mycoparasites would fall into the unique-niche category. Currently, arthropods are considered to be the most diverse and species-abundant group of organisms on the planet with estimates of 5–10 million species (Ødegaard 2000). If each arthropod species hosts at least one unique fungal pathogen – as postulated for the beetle-infecting *Laboulbeniales* (Bass & Richards 2011) – then entomopathogenic fungi and their mycoparasites would constitute an immensely richer group than even the most recent data suggest. Certainly, preliminary evidence from studies of the zombie-ant fungi – in which species complexes have been identified that may be composed of hundreds of taxa – supports this assumption (Araújo et al. 2018, 2020).

Availability of data and material

The annotated genomes are deposited in GenBank: accession numbers JADHZA000000000 (*Torrubiellomyces zombiae*) and JAFEME000000000 (*Niveomyces coronatus*). The genome assembly, gene predictions and functional annotations can also be analysed interactively at https://fungalgenomics.science.uu.nl. Sequences generated in this study for phylogenetic analysis have also been deposited in GenBank (see Table 1 for accession numbers). Holotypes are deposited at the New York Botanical Garden Herbarium (type numbers NY4434800 and NY4434801 for *N. coronatus* and *T. zombiae*, respectively). Cultures are deposited in the culture collection of the Westerdijk Fungal Biodiversity Institute (CBS 149186 and CBS 149187 for *N. coronatus* and *T. zombiae*, respectively).

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Declaration on conflict of interest The authors declare that there is no conflict of interest.

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