New mycoparasitic species in the genera *Niveomyces* and *Pseudoniveomyces gen. nov*. (*Hypocreales*: *Cordycipitaceae*), with sporothrix-like asexual morphs, from Thailand

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Abstract: Four new species of the genus *Niveomyces* are described from Thailand. They were found as mycoparasites on: *Ophiocordyceps* infecting flies (*Diptera*) for *Niveomyces albus*; ants (*Hymenoptera*) for *N. formicidarum*; and leafhoppers (*Hemiptera*) for *N. hirsutellae* and *N. multisynnematus*. A new genus, *Pseudoniveomyces* with two species: *Pseudoniveo. blattae* (type species), parasitic on *Ophiocordyceps* infecting cockroaches, and *Pseudoniveo. arachnovorum*, found on a spider egg sac, are also described. These fungi share a common feature which is a sporothrix-like asexual morph. Based on our molecular data, *Sporothrix insectorum* is shown to be affiliated to the genus *Niveomyces*, and thus a new combination *N. insectorum comb. nov*. is proposed. *Niveomyces coronatus*, *N. formicidarum* and *N. insectorum* formed the *N. coronatus* species complex found on ant-pathogenic *Ophiocordyceps* from different continents. *Pseudoniveomyces* species are distinguished from *Niveomyces* spp. based on the presence of fusoid macroconidia in culture and a red pigment diffused in the medium, resembling to *Gibellula* and *Hevansia*. The molecular phylogenetic analyses also confirmed its generic status. The host/substrates associated with the genera within *Cordycipitaceae* were mapped onto the phylogeny to demonstrate that mycoparasitism also evolved independently multiple times in this family.

INTRODUCTION

Fungi constitute the most diverse kingdom of living organisms (Blackwell 2011) with highly diversified lifestyles and ecologies (Smith & Read 2008, Gibson & Hunter 2010, Pineda *et al.* 2013, Lacey *et al.* 2015). Some fungi evolved to exploit other fungi which are themselves pathogenic or parasitic on other organisms, a lifestyle called "hyperparasitism" (Boosalis 1964, Parratt & Laine 2016). Hyperparasitism by fungi on fungal pathogens of other organisms is commonly called "mycoparasitism" (Bushley *et al.* 2013, Wang *et al.* 2015, Crous *et al.* 2017, Araújo *et al.* 2020, 2022) although the initial meaning of the latter term referred to fungi parasitising other fungi whether the hosts be parasites/pathogens or not (Barnett 1963). Mycoparasitism has evolved multiple times in the Kingdom *Fungi* (Gleason *et al.* 2014). Within the *Ascomycota*, the order *Hypocreales* contains almost 3 000 species, composed of plant and animal pathogens, endophytes, insect endosymbionts, saprophytes and mycoparasites (Spatafora

et al. 2007, Sung *et al.* 2007a, Blackwell 2010, Boomsma *et al.* 2014, Matsuura *et al.* 2018). The mycoparasitic lifestyle has evolved independently multiple times in *Hypocreales* (Spatafora *et al.* 2007, Sung *et al.* 2008). For example, within *Cordycipitaceae, Lecanicillium* and *Simplicillium* are found to infect plant pathogens (*e.g.* coffee rust fungus *Hemileia vastatrix*) and entomopathogens (Vandermeer *et al.* 2009, Baiswar *et al.* 2014, Wei *et al.* 2019). *Pseudogibellula* is found to grow directly on *Ophiocordyceps*, suggesting an apparent mycoparasitism (Araújo *et al.* 2020, Mongkolsamrit *et al.* 2021), and the genus *Niveomyces* has been recently proposed as a mycoparasite of *Ophiocordyceps camponoti-floridani* (Araújo *et al*. 2022). Within *Hypocreaceae*, *Syspastospora parasitica* is found to infect a well-known insect pathogen/endophyte, *Beauveria bassiana* (Posada *et al.* 2004). Within *Ophiocordycipitaceae*, *Polycephalomyces* and *Pleurocordyceps* are found to infect a variety of cordycipitaceous entomopathogens and most recently, *Torrubiellomyces* was proposed to infect ant-pathogenic *Ophiocordyceps camponoti-*

floridani (Kaitsu *et al.* 2013, Wang *et al.* 2015, 2021, Zhong *et al.* 2016, Araújo *et al.* 2022).

The genus *Sporothrix* (*Ophiostomataceae*, *Ophiostomatales*) was established in the early 20th century by Hektoen & Perkins (1900) with *S. schenckii*, a human pathogen, as the type species of the genus. Subsequently, a link was established to the sexually reproductive genus *Ophiostoma* found essentially as pathogens of various organisms including plants and insects (Münch 1907). De Beer *et al.* (2016) established the distinction between *Ophiostoma sensu stricto* and *Sporothrix s.s.* based on molecular phylogenies. Before the monograph of de Hoog (1974), there were inconsistencies regarding what "*Sporothrix*" really was. Due to morphological plasticity, *Sporothrix* species had been linked to various ascomycetous genera such as *Graphium*, *Cephalosporium*, *Cladosporium* (Hedgcock 1906, Münch 1907), and even *Sporotrichum*, which is now recognised as being affiliated to *Basidiomycota* (von Arx 1971, Stalpers 1978). "Sporothrix-like" asexual morphs are characterised by hyaline mycelia occasionally producing holoblastic conidia on variably denticulate conidiogenous cells, forming conidia sympodially (de Hoog 1974, de Beer *et al.* 2016). This type of asexual morph is very similar to what can be found in *Beauveria* which are insect pathogens of the family *Cordycipitaceae*. *Sporothix insectorum*, a species initially found on insects (de Hoog 1974), is a good example of uncertainty regarding "*Sporothrix*". De Hoog (1993) suggested a "clavicipitalean relationship" to *Spor. insectorum* while de Beer *et al.* (2016) also suggested that this species should be compared with fungi in *Cordycipitaceae*. De Hoog (1974) described the type specimen of *Spor. insectorum* as being found on "*Paltothyreus tarsatus*" (*Hymenoptera, Formicidae)* "associated with *Gibellula* [= *Pseudogibellula*] *formicarum*", which was further supported and discussed by Araújo *et al.* (2020) and considered to be a mycoparasite of *Ophiocordyceps paltothyreum*, a commonly found ant parasite in West Africa.

Through our continuous exploration of the diversity of entomopathogenic fungi from Thailand and their associated mycoparasites, we have found new specimens with sporothrix-like asexual morphs resembling the recently proposed new genus *Niveomyces* (Araújo *et al.* 2022). They were found on dead insects infected with hypocrealean entomopathogenic fungi, strongly suggesting a mycoparasitic nutritional mode. In order to confirm whether these specimens belong to *Niveomyces* but are different from *N. coronatus* described by Araújo *et al.* (2022) and to check the taxonomic affiliation of *Spor. insectorum*, we conducted a taxonomic study including the newly found specimens and the ex-type culture of *Spor. insectorum* (CBS 756.73). We propose four new mycoparasitic species of *Niveomyces*, and the new genus, *Pseudoniveomyces*, represented so far by two species, *Pseudoniveo. arachnovorum* and *Pseudoniveo. blattae*. With these new taxa added to the *Cordycipitaceae*, we also reconstructed the ancestral host/substrate association to gain insights into the evolution of mycoparasitism in this family. Our hypothesis is that different lineages of mycoparasites have evolved multiple times independently in *Cordycipitaceae*.

MATERIALS AND METHODS

Fungal isolation, DNA extractions and PCR

The samples were found while inspecting the underside and topside of understory leaves in natural parks and community forests of Thailand (Table 1). The samples were placed in plastic boxes and transported back to the laboratory. To establish cultures, small pieces of sterile potatoes dextrose agar (PDA; potato 200 g/L, dextrose 15 g/L, agar 15 g/L) were cut with a flamed sterile needle and gently brought into contact with synnemata bearing abundant conidia, then placed on sterile PDA Petri dishes. The inoculated PDA plates were examined daily for fungal gemination and contamination. The germinating conidia without contaminations were sub-cultured on fresh PDA plates. The pure cultures were maintained at 25 °C for 2–3 wk before further examination.

The DNA of 15 samples was extracted using a procedure based on Cetyl-trimethyl-ammonium bromide (CTAB); mycelial mass grown on PDA (40–50 mg) was collected and placed into a micro centrifuge tube (1.5 mL), ground manually with a pestle in 600 μL of CTAB buffer (NaCl 1.4 M; Tris-HCl 100 mM pH 8.0; EDTA 20 mM pH 8.0, 2 % CTAB and 1 % PVP-40). The suspension was thoroughly mixed and incubated for 1 h at 65 °C. After the suspension had cooled to approximately room temperature, 600 μL of chloroform/isoamyl alcohol (24:1 v/v) was added and homogenised until an emulsion was obtained; the mixture was then centrifuged at 12 000 rpm for 15 min at 25 °C. The supernatant was transferred to a new tube (1.5 mL) and 300 μL of cold (~ 4 °C) isopropanol was added, the suspension was left in a refrigerator for 15–30 min and centrifuged at 12 000 rpm for 15 min at 4 °C. Subsequently, the supernatant was discarded, and the pellet was washed in 300 mL 70 % (v/v) ethanol then air-dried at room temperature. Finally, the DNA pellet was dissolved in 50 mL TE buffer (10 mM Tris-HCl pH 8.0, 1 mM, EDTA pH 8.0).

PCR and sequencing

Amplification of the whole internal transcribed spacer region (ITS), partial region of the large subunit of the nuclear ribosomal DNA (LSU), the translation elongation factor 1-α (*TEF1*), partial regions of genes encoding the largest (*RPB1*) and second largest (*RPB2*) subunits of RNA polymerase II were amplified. The primers used were ITS5 and ITS4 (White *et al.* 1990; ITS), LR0R and LR7 (Vilgalys & Hester 1990; LSU), EF1-983F and EF1-2218R (Rehner & Buckley 2005; *TEF1*), CRPB1 and RPB1Cr (Castlebury *et al.* 2004; *RPB1*) and RPB2-5F2 (Sung *et al.* 2007b) and fRPB2-7cR (Liu *et al.* 1999; *RPB2*). All amplification reactions were performed in 25 μL volumes consisting of 10× *Taq* Buffer with $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 1 U *Taq* DNA polymerase (Thermo Scientific), 200 μM of each of the four dNTPs (Promega, Madison, WI, USA), 0.4 M betaine and 0.2 μM of each primer, using a T100 Thermal Cycler (Bio-Rad). The amplification reactions were checked for positive amplification on 1 % agarose gel. The PCR amplicons were sent to MACROGEN (Korea) for purification and Sanger sequencing.

Molecular phylogenies

Nucleotide sequences were assembled and edited in BioEdit v. 7.1.3 (Hall 1999). Sequences of ITS, LSU, *TEF1*, *RPB1* and *RPB2* from related cordycipitaceous species, selected from previous studies (Sung *et al.* 2001, 2007a, b, Sung & Spatafora 2004, Bischoff *et al.* 2005, Chaverri *et al.* 2005, Luangsa-Ard *et al.* 2005, Spatafora *et al.* 2007, Zare & Gams 2008, Johnson *et al.* 2009, Rehner *et al.* 2011, Kepler *et al.* 2012, 2017, Sanjuan *et al.* 2014, Tsang *et al.* 2016, Mongkolsamrit *et al.* 2018, 2020, 2021, 2022, Vu *et al.* 2019, Thanakitpipattana *et al.* 2020, Wang *et al.*

Table 1. List of taxa included in the phylogenetic analyses and their GenBank accession numbers. The accession numbers marked in **bold** font refer to sequences new in this study or have been generated by our group in Thailand. T= ex-type culture.

Species	Strain	Host/Substratum	GenBank Accession no.				
			ITS	LSU	TEF1	RPB1	RPB2
Akanthomyces aculeatus	HUA 186145[T]	*Lepidoptera*	–	MF416520[1]	MF416465[1]	–	–
	HUA 772	*Lepidoptera; Sphingidae*	KC519371[2]	KC519370[2]	KC519366[2]	–	–
Akanthomyces sulphureus	TBRC 7248[T]	*Araneae*; spider	MF140758[3]	MF140722[3]	MF140843[3]	MF140787[3]	MF140812[3]
Ascopolyporus polychrous	P.C. 546	*Hemiptera*	–	DQ118737[4]	DQ118745[4]	DQ127236[4]	–
Ascopolyporus villosus	ARSEF 6355	*Hemiptera*	AY886544[5]	AY886544[5]	DQ118750[4]	DQ127241[4]	–
Beauveria bassiana	ARSEF 1564[T]	*Lepidoptera*	HQ880761[6]	–	HQ880974[6]	HQ880833[6]	HQ880905[6]
	ARSEF 7518	*Hymenoptera*	HQ880762[6]	–	HQ880975[6]	HQ880834[6]	HQ880906[6]
Blackwellomyces cardinalis	OSC 93609	*Lepidopteran*	–	AY184962[7]	DQ522325[8]	DQ522370[8]	DQ522422[8]
	OSC 93610	*Lepidopteran*	JN049843[9]	AY184963[7]	EF469059[10]	EF469088[10]	EF469106[10]
Cordyceps militaris	OSC 93623	*Lepidoptera*	JN049825[9]	AY184966[7]	DQ522332[8]	DQ522377[8]	–
	YFCC 6587	*Lepidoptera*	–	MN576818[11]	MN576988[11]	MN576878[11]	MN576932[11]
Engyodontium parvisporum	IHEM 22910	Human bronchoscopy specimen	LC092896[12]	LC092915[12]	LC425558[13]	–	–
Engyodontium rectidentatum	CBS 206.74	Air	LC092893[12]	LC092912[12]	LC425553[13]	–	–
	CBS 641.74	Buried keratinous substance	LC092895[12]	LC092914[12]	LC425540[13]	–	–
Flavocillium bifurcatum	YFCC 6101[T]	*Lepidoptera; Noctuidae*	–	MN576781[11]	MN576951[11]	MN576841[11]	MN576897[11]
Gamszarea humicola	CGMCC 3.19303[T]	Soil	MK329092[14]	MK328997[14]	MK336027[14]	–	MK335979[14]
Gamszarea wallacei	CBS 101237[T]	*Lepidoptera*	EF641891[15]	AY184967[7]	EF469073[10]	EF469102[10]	EF469119[10]
Gibellula gamsii	BCC 28797	*Arachnida; Araneida*	MH152531[16]	MH152541[16]	MH152562[16]	MH152549[16]	MH152557[16]
	BCC 27968[T]	*Arachnida; Araneida*	MH152529[16]	MH152539[16]	MH152560[16]	MH152547[16]	–
Gibellula pulchra	BCC 47555	*Arachnida; Araneida*	MH532885[17]	OK040714[17]	MH521897[17]	MH521804[17]	–
Hevansia novoguineensis	CBS 610.80[T]	*Arachnida*	MH532831[18]	MH394646[19]	MH521885[19]	–	MH521844[19]
	BCC 42675	*Arachnida*	MZ684089[20]	MZ684004[20]	MZ707814[20]	–	MZ707835[20]
Jenniferia thomisidarum	BCC 37881[T]	*Araneae, Diaea cf. dorsata*	MZ684099[20]	MZ684010[20]	MZ707823[20]	MZ707830[20]	MZ707843[20]
	BCC 37882	*Araneae, Diaea cf. dorsata*	MZ684100[20]	MZ684011[20]	MZ707824[20]	MZ707831[20]	MZ707844[20]

Table 1. (Continued).

Species	Strain	Host/Substratum	GenBank Accession no.				
			ITS	LSU	TEF1	RPB1	RPB2
Lecanicillium antillanum	CBS 350.85	*Arachnida*	–	AF339536[21]	DQ522350[8]	DQ522396[8]	DQ522450[8]
Lecanicillium aranearum	CBS 726.73a	*Arachnida; Araneae*	–	AF339537[21]	EF468781[10]	EF468887[10]	EF468934[10]
Lecanicillium tenuipes	CBS 309.85	*Arachnida*	–	AF339526[21]	DQ522341[1]	DQ522387[1]	DQ522439[1]
Liangia sinensis	YFCC3103[T]	*Beauveria yunnanensis*	–	MN576782[11]	MN576952[11]	MN576842[11]	MN576898[11]
	YFCC3104	*Beauveria yunnanensis*	–	MN576783[11]	MN576953[11]	MN576843[11]	MN576899[11]
Neohyperdermium piperis	CBS 116719	*Hemiptera*	–	AY466442[22]	DQ118749[1]	DQ127240[4]	EU369083[23]
Neohyperdermium pulvinatum	P.C. 602	*Hemiptera*	–	DQ118738[4]	DQ118746[4]	DQ127237[4]	–
Neotorrubiella chinghridicola	BCC 39684	*Orthopterida*	–	MK632096[24]	MK632148[24]	MK632071[24]	MK632181[24]
	BCC 80733[T]	*Orthopterida*	–	MK632097[24]	MK632149[24]	MK632072[24]	MK632176[24]
Niveomyces albus	**BCC 83025[T]**	**Ophiocordyceps sp. on Diptera**	ON103032	ON103157	ON125015	ON286876	ON125027
	BCC 74477	**Ophiocordyceps sp. on Arachnida (Araneae)**	ON103033	ON103158	ON125016	ON286877	ON125028
	BCC 73628	**Ophiocordyceps sp. on Arachnida (Araneae)**	ON103034	ON103159	ON125017	–	ON125029
Niveomyces coronatus	NY 04434800[T]	*Ophiocordyceps camponoti-floridani*	–	ON493606[25]	ON513397[25]	ON513399[25]	ON513400[25]
Niveomyces formicidarum	**BCC 79346**	**Ophiocordyceps sp. on Hymenoptera**	ON103035	ON103160	ON125018	ON286878	ON125030
	BCC 83026[T]	**Ophiocordyceps sp. on Hymenoptera**	ON103036	ON103161	ON125019	ON286879	–
Niveomyces hirsutellae	**BCC 36631[T]**	**Ophiocordyceps sp. on Hemiptera**	ON103039	ON103164	ON125022	ON286882	ON125033
	BCC 36632	**Ophiocordyceps sp. on Hemiptera**	ON103040	ON103165	ON125023	ON286883	ON125034
	BCC 78482	**Ophiocordyceps sp. on Hemiptera**	ON103041	ON103166	ON125024	ON286884	ON125035
Niveomyces insectorum	CBS 756.73[T]	"*Palthothyreus tarsatus* in *Rubiaceae*, associated with *Gibellula formicarum*" *	MH860798[26]	ON103169	ON125026	ON286887	ON125038
Niveomyces multisynnematus	**BCC 90307**	**Ophiocordyceps sp. on Hemiptera**	ON103037	ON103162	ON125020	ON286880	ON125031
	BCC 90308[T]	**Ophiocordyceps sp. on Hemiptera**	ON103038	ON103163	ON125021	ON286881	ON125032
Parahevansia koratensis	NHJ 666.01	*Arachnida*	GQ250010[20]	GQ249981[20]	GQ250031[20]		
	NHJ 2662	*Lepidoptera*	GQ250008[20]	GQ249982[20]	GQ250032[20]	ON470206[20]	ON470208[20]
Parengyodontium album	CBS 368.72	A fresco	LC092891[12]	LC092910[12]	LC382183[13]	–	–
	CBS 504.83[T]	Human brain abscess	LC092880[12]	LC092899[12]	LC382177[13]	–	–

Table 1. (Continued).

Species	Strain	Host/Substratum	GenBank Accession no.				
			ITS	LSU	TEF1	RPB1	RPB2
Pleurodesmospora lepidopterorum	DY 10501[T]	*Lepidoptera*	MW826576[27]	–	MW834317[27]	MW834315[27]	MW834316[27]
	DY 10502	*Lepidoptera*	MW826577[27]	–	MW834319[27]	–	MW834318[27]
Polystromomyces araneae	BCC 93301[T]	*Arachnida*	MZ684101[20]	MZ684016[20]	MZ707825[20]	MZ707832[20]	MZ707845[20]
Pseudogibellula formicarum	BCC 84257	*Ophiocordyceps flavida*	MT508782[19]	MT512653[19]	MT533480[19]	MT533473[19]	–
	CBS 433.73	*Palthothyreus tarsatus*	MH860731[26]	MH872442[26]	MT533481[19]	MT533475[19]	–
Pseudoniveomyces arachnovorum	**BCC 95818[T]**	***Arachnida*** (of spider eggs)	**OR099526**	–	**OR133172**	**OR133173**	**OR133173**
Pseudoniveomyces blattae	**BCC 53567[T]**	***Blattodea***	**ON103042**	**ON103167**	–	**ON286885**	**ON125036**
	BCC 53568	***Blattodea***	**ON103043**	**ON103168**	**ON125025**	**ON286886**	**ON125037**
Purpureocilium lilacinum	CBS 431.87	*Meloidogyn* sp. (*Nematoda*)	AY624188[28]	EF468844[10]	EF468791[10]	EF468897[10]	EF468940[10]
	CBS 284.36[T]	Soil	AY624189[28]	FR775484[29]	EF468792[10]	EF468898[10]	EF468941[10]
Samsoniella inthanonensis	TBRC 7915[T]	*Lepidoptera*	MF140761[3]	MF140725[3]	MF140849[3]	MF140790[3]	MF140815[3]
	TBRC 7916	*Lepidoptera*	MF140760[3]	MF140724[3]	MF140848[3]	MF140789[3]	MF140814[3]
Simplicillium lanosoniveum	CBS 101267	*Hemileia vastatrix*	–	AF339554[21]	DQ522357[8]	DQ522405[8]	DQ522463[8]
	CBS 704.86	*Hemileia vastatrix*	AJ292396[30]	AF339553[21]	DQ522358[8]	DQ522406[8]	DQ522464[8]

References: [1]Kepler *et al.* (2017), [2]Sanjuan *et al.* (2014), [3]Mongkolsamrit *et al.* (2018), [4]Chaverri *et al.* (2005), [5]Bischoff *et al.* (2005), [6]Rehner *et al.* (2011), [7]Sung & Spatafora (2004), [8]Spatafora *et al.* (2007), [9]Kepler *et al.* (2012), [10]Sung *et al.* (2007), [11]Wang *et al.* (2020), [12]Tsang *et al.* (2016), [13]Lee *et al.* (2018, unpublished), [14]Zhang *et al.* (2021), [15]Zare & Gams (2008), [16]Kuephadungphan *et al.* (2019), [17]Kuephadungphan *et al.* (2022), [18]Helaly *et al.* (2019), [19]Mongkolsamrit *et al.* (2021), [20]Mongkolsamrit *et al.* (2022), [21]Sung *et al.* (2001), [22]Bischoff & White (2004), [23]Johnson *et al.* (2009), [24]Thanakitpipattana *et al.* (2020), [25]Araújo *et al.* (2022), [26]Vu *et al.* (2019), [27]Chen *et al.* (2021), [28]Luangsa-ard *et al.* (2005), [29]Perdomo *et al.* (2013), [30]Zare *et al.* (2000).

* Literal description of *Sporothrix insectorum* from de Hoog (1974).

2020, Chen *et al.* 2021, Zhang *et al.* 2021) were downloaded from GenBank for phylogenetic analyses (Table 1). Sequences from the ex-type culture of *Sporothrix insectorum* (CBS 756.73) were also included, as its affiliation to *Cordycipitaceae* had been previously hypothesised (de Beer *et al.* 2016). The sequences from each marker were aligned using ClustalW (Thompson *et al.* 1994) in BioEdit (Hall 1999). A "Randomized Accelerated Maximum Likelihood" (RAxML) phylogenetic analysis was performed using RAxML-VI-HPC2 v. 8.2.12 (Stamatakis 2006, 2014) on XSEDE (http://www.phylo.org/), with the GTRGAMMA + I model and 1 000 bootstrap iterations (BS) were executed to evaluate the branch support. Bayesian inference (BI) analyses were performed by MrBayes v. 3.2 (Ronquist *et al.* 2012), with the GTR + G + I model as inferred by MrModeltest v. 2.2 (Nylander 2004). Five million generations of Markov chain Monte Carlo (MCMC) simulation were run with sampling every 1 000 generations, and discarding the first 10 % as burn-in after which the Bayesian posterior probabilities (PP) were calculated on the remaining trees. The molecular divergence based on p-distances calculated using MEGA v. 11 (Tamura *et al.* 2021) was used to aid the decision of splitting species within the *Niveomyces coronatus* complex. The sequence alignments for all datasets used in this study were submitted to Figshare https://doi.org/10.6084/m9.figshare.22716451.v4.

Morphological examination

Macro-morphological characters were described based on dry materials and photographs by using a digital Nikon D5100 camera. Micro-morphological characters, examined under a compound microscope (Olympus CX23, Olympus Corporation, Japan), were mounted with lactophenol cotton blue before measuring the sizes of the conidiogenous cells and conidia. Morphological characteristics of colonies, consisting of colour, texture, pigmentation and growth rates, were observed on two kinds of media: oatmeal agar (OA, Difco, oatmeal 60 g, agar 12.5 g, in 1 L distilled water) and PDA and incubated at 25 °C for 20 d. The colours of specimens and cultures incubated on OA and PDA were described and codified following the Sixth Royal Horticultural Society (R.H.S.) Colour Chart (2015).

Reconstruction of ancestral hosts and substrates

The hosts or substrates from which the fungal strains were isolated were classified into five categories: environment, insect, arachnid, nematode and fungus. The strains isolated from soil (*Gamzarea humicola* CGMCC3 19303, *Purpureocillium lilacinum* CBS 284.36) and from a fresco (mural painting) (*Parengyodontium album* CBS 368.72) were categorised as coming from the environment. The strains of *Simplicillium lasonovineum* and *Liangia sinensis* were reported to grow on the rust *Hemileia vastatrix* (Sung *et al.* 2001), and the entomopathogen *Beauveria bassiana* (Wang *et al.* 2020) respectively; the hosts of these two species were thus categorised as fungi. The novel taxa described in this study, *Niveomyces* spp. and *Pseudoniveomyces blattae* are mycoparasites; the hosts were also classified as fungi. Other prominent entomopathogenic fungi of the family are classified as associated with insects or arachnids.

The host/substrate information was mapped on the 5-locus phylogenetic tree and used to reconstruct the ancestral host and substrate association for the species presented in the tree, using a stochastic mapping approach (Huelsenbeck *et al.* 2003, Bollback

2006) with Markov Chain Monte Carlo to sample characters histories from posterior probability of characters distribution. This was done with the R package "phytools" (Revell 2012).

RESULTS

Phylogenetic analyses

The 5-locus phylogeny (ITS-LSU-*TEF1-RPB1-RPB2*: Fig. 1) revealed that the 15 specimens exhibiting sporothrix-like asexual morphs (*i.e. Niveomyces* spp. and *Pseudoniveomyces gen. nov.*) included in this study formed two strongly supported monophyletic clades; one included *N. insectorum* (= *Spor. insectorum*, CBS 756.73) and *N. coronatus* (NY04434800) (*i.e.* the genus *Niveomyces*, BS = 92 % / BPP = 1.00; Fig. 1), branching as a sister clade to *Pseudogibellula formicarum*, and another independent clade with full support (BS = 100 % / BPP = 1.00; *Pseudoniveomyces gen. nov.*), represented by *Pseudoniveo. blattae* and *Pseudoniveo. arachnovorum sp. nov.*, which branches as a sister taxon to a clade comprising *Gibellula*, *Hevansia* and *Jenniferia*. Four fully supported subclades could be observed within *Niveomyces,* with one subclade containing both *N. insectorum* (= *Spor. insectorum*) and the recently proposed type species for *Niveomyces*, *N. coronatus* (Araújo *et al.* 2022). This subclade is herein considered as the *N. coronatus* complex, which also includes *N. formicidarum sp. nov.* (BCC79346, BCC83026) (Fig. 1).

The single-locus phylogenetic trees from respective markers (Supplementary Figs S1–S5) consistently showed a strong grouping of BCC79346/BCC83026, separated from *N. insectorum* and *N. coronatus*. The pair BCC79346/BCC83026 is thus proposed as a new species, namely *N. formicidarum sp. nov.* Furthermore, the p-distances between the two strains of *N. formicidarum* from different markers were always lower compared to those between these strains and *Spor. insectorum* or *N. coronatus* (Fig. 2). The divergences between *N. formicidarum* and *Spor. insectorum*, and the one between *N. formicidarum* and *N. coronatus* are lower than the 3 % divergence (p-distance = 0.03) that is the required threshold for separating Operational Taxonomic Units (OTUs), but higher than the 1 % (except LSU), which has been recently used for separating species within *Cordycipitaceae* (Kuephadungphan *et al.* 2022). Based on all the markers combined, the p-distance between the two strains of *N. formicidarum* (0.0015) is also lower than those between this species and *Spor. insectorum* (mean ± sd = 0.0165 ± 0.0018), and *N. coronatus* (mean ± sd = 0.0255 ± 0.0031). The all-loci p-distance between *Spor. insectorum* and *N. coronatus* is 0.0244.

Beside the *N. coronatus* complex, the phylogenetic tree (Fig. 1) also revealed three other highly supported subclades within *Niveomyces*. These subclades are thus proposed respectively as novel species, namely *Niveomyces albus*, *N. multisynnematus* and *N. hirsutellae*. The monophyly of these new species was mostly recovered in the single-locus phylogenetic trees (Supplementary Figs S1–S5) except for *N. albus* for LSU. The *RPB1* phylogeny showed that the *N. coronatus* complex and *Pseudoniveo. blattae* clustered together without separation (Supplementary Fig. S4) while all the other markers consistently placed *Pseudoniveo. blattae* with *Pseudoniveo. arachnovorum*, forming the new genus close to the genera *Hevansia*, *Gibellula* and *Jenniferia* with variable levels of support. The difference of *Pseudoniveomyces* to *Niveomyces* is also supported by morphological characteristics (see Taxonomy below).
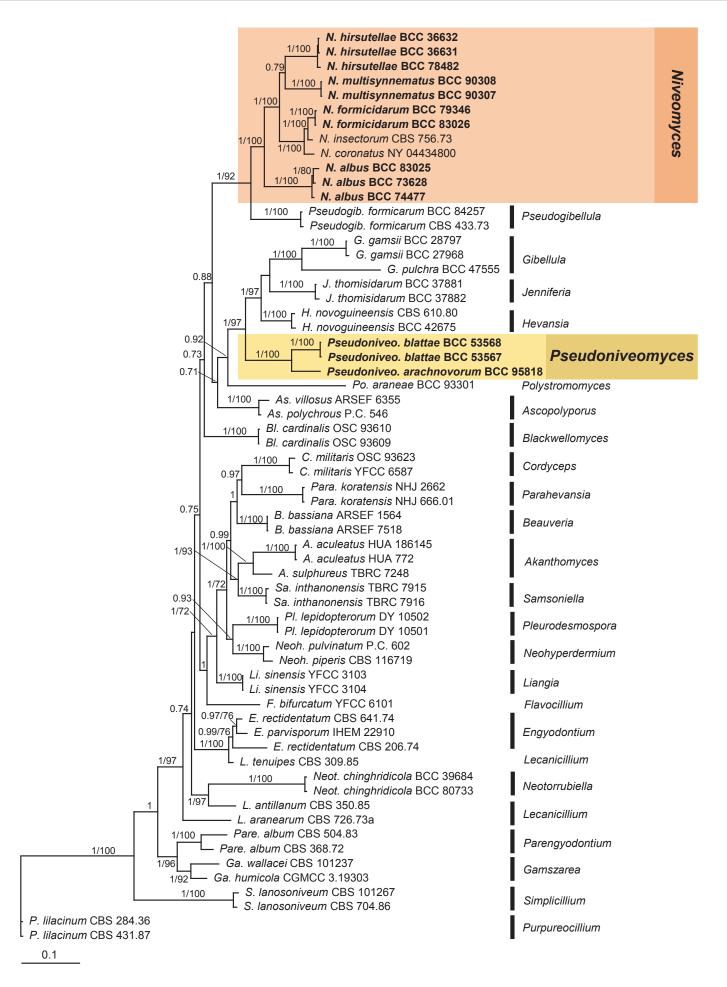
Fig. 1. The best phylogenetic tree from the Bayesian inference based on a multi-locus alignment (ITS-LSU-*TEF1-RPB1-RPB2*). The statistical support values, namely Bayesian posterior probability (PP; > 0.70) and maximum likelihood-based bootstrap (BS; > 70 %), are shown above the nodes (PP/BS).
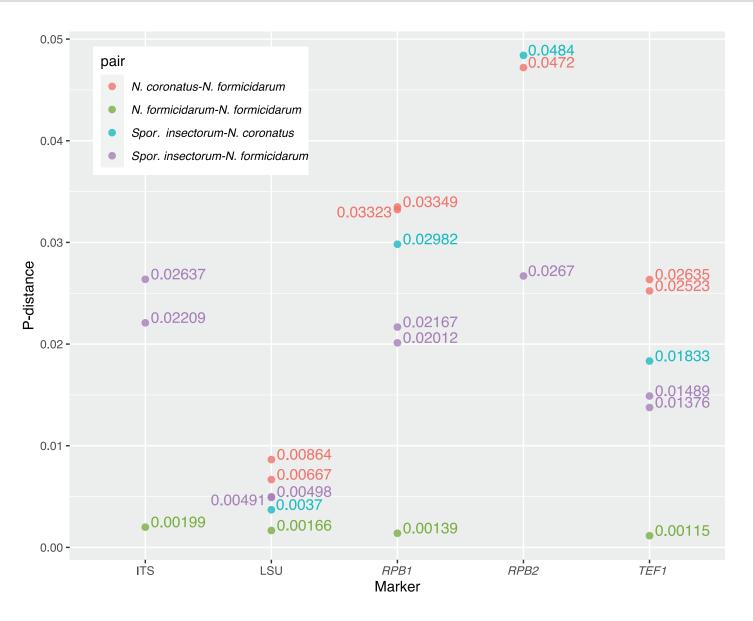
Fig. 2. A dot plot representing the p-distance values calculated between pairs of strains within the *Niveomyces coronatus* species complex for the five markers used in the molecular phylogenetic analyses.

Taxonomy

Niveomyces albus Tasanathai, Noisripoom & Kobmoo, ***sp. nov.***
MycoBank MB 846659. Fig. 3.

Etymology: The name refers to the white colour of fresh specimens in nature.

Typus: **Thailand**, Nakhon Ratchasima Province, Khao Yai National Park, on *Ophiocordyceps dipterigena s.l.* on *Diptera*, on the underside of leaves, 1 Nov. 2016, *D. Thanakitpipattana, N. Kobmoo, R. Somnuk & B. Sakolrak* (**holotype** BBH 42322, culture ex-type BCC 83025).

Sexual morph: Unknown. *Asexual morph*: *Host* covered by dense, white and cottony mycelium forming on the stromata of *Ophiocordyceps dipterigena s.l. Hyphae* septate, hyaline, smooth-walled, irregularly branched, 1–2 μm wide. *Conidiophores* mono- or synnematous, septate, cylindrical, simple, dichotomously or irregularly branched of variable length. *Conidiogenous cells* arising directly from the hyphae, cylindrical, (12–)14.5–36(–60) × 1–2 μm, bearing a rather irregularly, geniculate rachis. *Conidia*

forming singly on denticles, cylindrical with rounded ends and on apiculus, hyaline, smooth-walled, aseptate, 5–7(–10) × 1–2 μm.

Culture characteristics: Colonies on OA attaining a diam of 20–22 mm in 20 d at 25 °C, cottony, yellow white (NN155A). Colonies on PDA attaining a diam of 13–15 mm in 20 d at 25 °C, cottony, pale yellow (11D); reverse colonies with light yellow colour (12C).

Additional specimen examined: **Thailand**, Saraburi Province, Khao Yai National Park, Chet Kot Waterfall, parasitic on a fungal pathogen of spider, on the underside of leaves, 1 Jul. 2014, *K. Tasanathai, A. Khonsanit, W. Noisripoom & D. Thanakitpipattana* (BBH 38780, culture BCC 73628).

Notes: *Niveomyces albus* shows similarity to *N. formicidarum*, *N. hirsutellae* and *N. multisynnematus* in the length of the conidiogenous cells. *Niveomyces albus* differs from the three species in the size of the conidia, *N. albus* (5–10 × 1–2 μm) is larger than *N. formicidarum* (3–5 × 1–1.5 μm), *N. hirsutellae* (2–5 × 1–1.5 μm) and *N. multisynnematus* (2–5 × 1–1.5 μm).
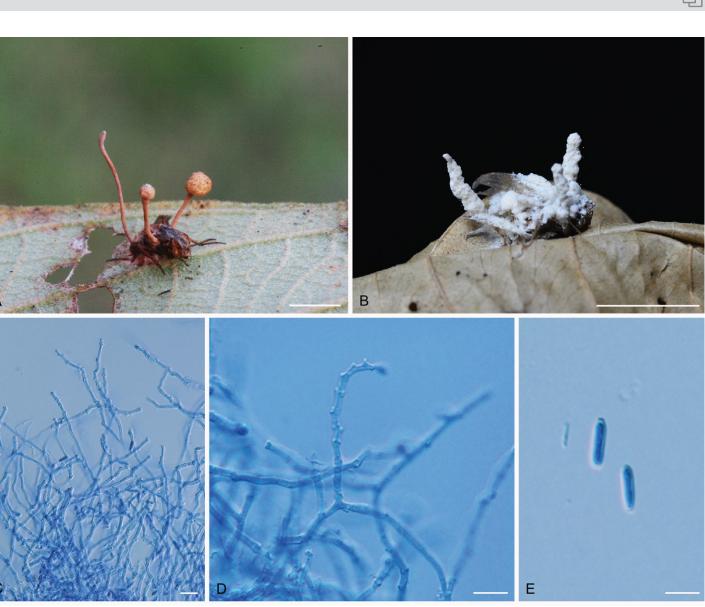
Fig. 3. *Niveomyces albus.* **A.** *Ophiocordyceps dipterigena s.l.* on a fly. **B.** *Niveomyces albus* growing on *Ophiocordyceps dipterigena s.l.* **C.** Conidiogenous cells. **D.** Close-up of conidiogenous cells with characteristic denticles. **E.** Conidia. Scale bars: A, B = 5 mm; C, D = 10 µm; E = 5 µm.

Niveomyces formicidarum Tasanathai, Noisripoom & Kobmoo, *sp. nov.* MycoBank MB 846660. Fig. 4.

Etymology: The name refers to the ant family *Formicidae.*

Typus: **Thailand**, Nakhon Ratchasima Province, Khao Yai National Park, on *Ophiocordyceps polyrhachis-furcata* (*Ophiocordycipitaceae*, *Hypocreales*, *Ascomycota*) on *Polyrhachis furcata* (*Hymenoptera*, *Formicidae*), attached to the underside of leaves, 1 Nov. 2016, *D. Thanakitpipattana, N. Kobmoo, R. Somnuk & B. Sakolrak* (**holotype** BBH 42323, culture ex-type BCC 83026).

Sexual morph: Unknown. *Asexual morph*: White and cottony mycelium forming on the stroma of *Ophiocordyceps polyrhachis-furcata*. *Hyphae* septate, hyaline, smooth-walled, irregularly branched, 1–2 µm wide. *Synnemata* > 2 mm long and 80–100 µm wide, indeterminate, simple, cylindrical, curved, occasionally dichotomously branched, consisting of longitudinal paralleled cells. *Conidiophores* mono- or synnematous, septate, cylindrical to linear, simple, dichotomously or irregularly branched of variable length. *Conidiogenous cells* arising directly from the hyphae, cylindrical, (10–)13–23.5(–33) × 1 µm, bearing a rather irregular, geniculate rachis. *Conidia* forming on denticles, ellipsoidal to cylindrical with rounded ends and an apiculus, hyaline, smooth-walled, aseptate, (3–)4–5 × 1–1.5 µm.

Additional specimen examined: **Thailand**, Phetchabun Province, Nam Nao National Park, on *Hymenoptera* (ant), on the underside of leaves, 6 Oct. 2015, *K. Tasanathai, S. Mongkolsamrit, W. Noisripoom, N. Kobmoo & R. Promharn* (BBH 44067, culture BCC 79346).

Notes: *Niveomyces formicidarum* is similar to *N. hirsutellae* and *N. multisynnematus* in the length of conidiogenous cells and conidia but differs in host association and molecular segregation. *Niveomyces formicidarum* was found on an ant (*Hymenoptera*) while *N. hirsutellae* and *N. multisynnematus* were found on leafhoppers (*Hemiptera*).

Niveomyces hirsutellae Tasanathai, Noisripoom & Kobmoo, *sp. nov.* MycoBank MB 846661. Fig. 5.

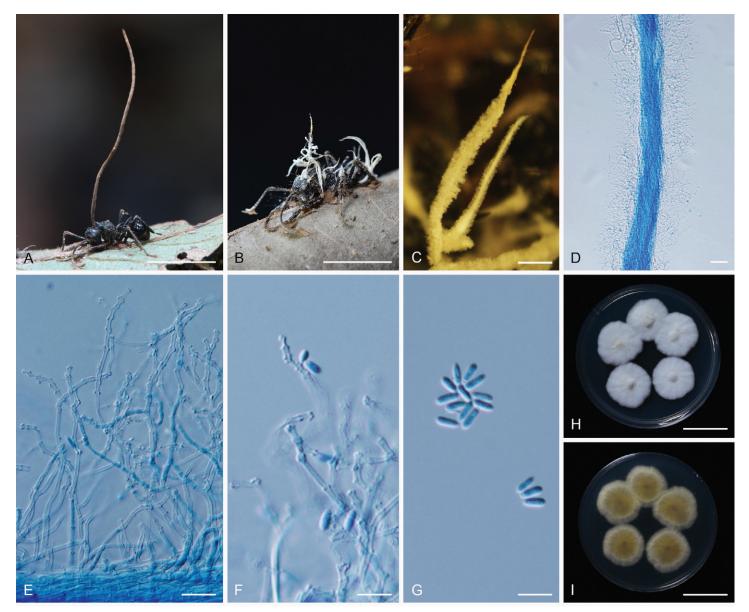*Etymology*: The name refers to the hyperparasite of *Hirsutella* species.

Fig. 4. *Niveomyces formicidarum.* **A.** *Ophiocordyceps polyrhachis-furcata* on *Polyrhachis furcata*. **B.** *Niveomyces formicidarum* growing on *Ophiocordyceps polyrhachis-furcata*. **C.** Close-up of synnemata. **D.** Close-up of synnema. **E.** Conidiogenous cells. **F.** Close-up of conidiogenous cells with characteristic of denticles with conidia. **G.** Conidia. **H, I.** Colonies on PDA: (H) obverse, (I) reverse. Scale bars: A, B = 5 mm; C = 500 µm; D = 80 µm; E–G = 10 µm; H, I = 15 mm.

Typus: **Thailand**, Phetchabun Province, Nam Nao National Park, on *Hirsutella aff*. *versicolor* on *Hemiptera* (leafhopper, *Cicadellidae*), on the underside of leaves, 29 May 2009, *K. Tasanathai, S. Mongkolsamrit & T. Chohmee* (**holotype** BBH 27064, culture ex-type BCC 36631).

Sexual morph: Unknown. *Asexual morph*: *Host* covered by dense, white to cream and cottony mycelium forming on *Hirsutella aff*. *versicolor*. *Hyphae* septate, hyaline, smooth-walled, irregularly branched, 1–2 µm wide. *Synnemata* indeterminate, simple, cylindrical, curved, occasionally dichotomously branched, > 4 mm long, 80–100 µm wide, consisting of longitudinal parallel layers of cells. *Conidiophores* mono- or synnematous, septate, cylindrical to linear, simple, dichotomously or irregularly branched of variable length. *Conidiogenous cells* arising directly from the hyphae, cylindrical, (15–)18.5–25.5(–30) × 1–1.5 µm, bearing a rather irregular, geniculate rachis. *Conidia* forming singly on denticles, ellipsoidal with an apiculus, hyaline, smooth-walled, aseptate, (2–)3–5 × 1–1.5 µm.

Additional specimens examined: **Thailand**, Phetchabun Province, Nam Nao National Park, on *Hemiptera* (leafhopper, *Cicadellidae*), on the underside of leaves, 29 May 2009, *K. Tasanathai, S. Mongkolsamrit & T. Chohmee* (BBH 26747, culture BCC36632); Samut Songkhram Province, Bang Khonthi, on *Hemiptera* (leafhopper), on the underside of leaves, 17 Aug. 2015, *K. Tasanathai, A. Khonsanit, D. Thanakitpipattana, W. Noisripoom & R. Promharn* (BBH 42358, culture BCC 78482).

Notes: *Niveomyces hirsutellae* is closely related to *N. multisynnematus*. Both species can be found on leafhoppers (insect species could not be determined) but differ in the production of multiple synnemata for *N. multisynnematus*, in the shape of conidia, and in the molecular segregation.

Niveomyces insectorum (de Hoog & H.C. Evans) Kobmoo, Tasanathai & Luangsa-ard, ***comb. nov.*** MycoBank MB 323936. *Basionym*: *Sporothrix insectorum* de Hoog & H.C. Evans, *Stud. Mycol.* **7**: 25. 1974.
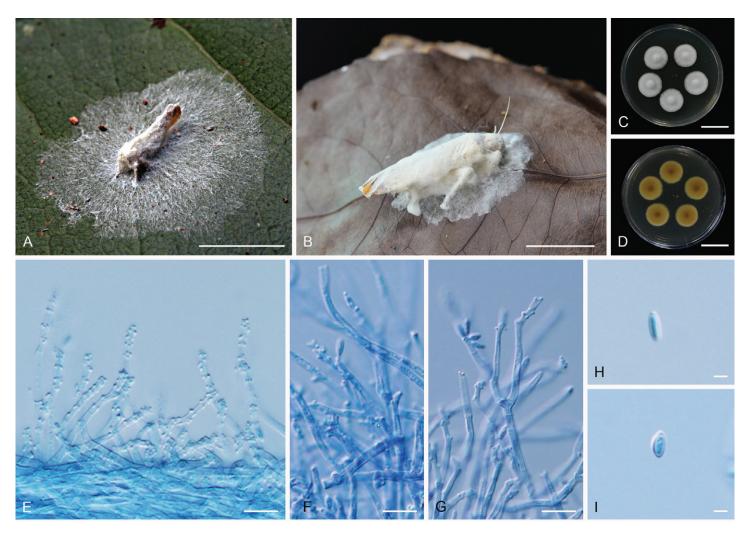
Fig. 5. *Niveomyces hirsutellae*. **A.** *Hirsutella aff. versicolor* on leafhopper. **B.** *Niveomyces hirsutellae* growing on *Hirsutella aff. versicolor*. **C, D.** Colonies on PDA: (C) obverse, (D) reverse. **E.** Conidiogenous cells. **F, G.** Close-up of conidiogenous cells with characteristic denticles with conidia attached. **H, I.** Conidium. Scale bars: A = 10 mm; B = 8 mm; C, D = 15 mm; E–G = 10 µm; H, I = 1.5 µm.

Notes: The ex-type culture of *Sporothrix insectorum* is shown here to cluster inside *Niveomyces* (Fig. 1) and therefore a new combination is proposed for this species.

Niveomyces multisynnematus Tasanathai, Noisripoom & Kobmoo, ***sp. nov.*** MycoBank MB 846662. Fig. 6.

Etymology: The name reflects the production of multiple synnemata.

Typus: **Thailand**, Samut Songkhram Province, Bang Khonthi, on *Ophiocordyceps aff. flavida* on *Hemiptera* (leafhopper, *Cicadellidae*), on the underside of leaves, 25 Mar. 2019, *K. Tasanathai, J. Luangsa-ard, S. Mongkolsamrit & R. Promharn* (**holotype** BBH 47491, culture ex-type BCC 90308).

Sexual morph: Unknown. *Asexual morph*: White and cottony mycelium forming on *Ophiocordyceps aff. flavida*. Hyphae septate, hyaline, smooth-walled, irregularly branched, 1–2 µm wide. *Synnemata* indeterminate, simple, cylindrical, curved, occasionally dichotomously branched, > 1 mm long, 50–60 µm wide, consisting of longitudinal parallel layers of cells. *Conidiophores* mono- or synnematous, septate, cylindrical, simple, dichotomously or irregularly branched of variable length. *Conidiogenous cells* arising directly from the hyphae, cylindrical, (20–)26–40(–50) × 1 µm, bearing a rather irregularly, geniculate rachis. *Conidia* singly forming on denticles, oval to ellipsoidal with an apiculus, occasionally cylindrical with rounded ends, aseptate, hyaline, smooth-walled, (2–)2.5–4(–5) × 1–1.5 µm.
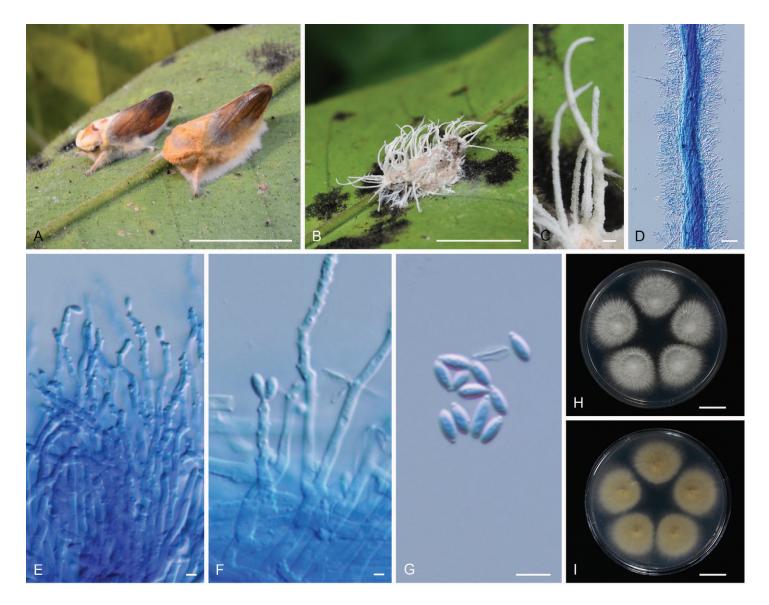
Additional specimen examined: **Thailand**, Samut Songkhram Province, Bang Khonthi, on *Hemiptera* (leafhopper), on the underside of leaves, 25 Mar. 2019, *K. Tasanathai, J. Luangsa-ard, S. Mongkolsamrit & R. Promharn* (BBH 47490, culture BCC 90307).
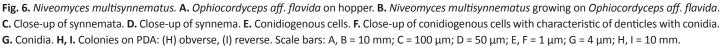
Notes: *Niveomyces multisynnematus* is closely related to *N. hirsutellae*, found on *Hemiptera* (leafhopper) on the underside of leaves. It differs from *N. hirsutellae* in the production of multiple synnemata, and in the conidial shape.

Pseudoniveomyces Tasanathai, Noisripoom & Kobmoo, ***gen. nov.*** MycoBank MB 846491.

Etymology: Referring to the phenotypic similarity of the asexual morph to *Niveomyces*.

Type species: *Pseudoniveomyces blattae* Tasanathai, Noisripoom & Kobmoo

Fig. 6. *Niveomyces multisynnematus.* **A.** *Ophiocordyceps aff. flavida* on hopper. **B.** *Niveomyces multisynnematus* growing on *Ophiocordyceps aff. flavida.* **C.** Close-up of synnemata. **D.** Close-up of synnema. **E.** Conidiogenous cells. **F.** Close-up of conidiogenous cells with characteristic of denticles with conidia. **G.** Conidia. **H, I.** Colonies on PDA: (H) obverse, (I) reverse. Scale bars: A, B = 10 mm; C = 100 µm; D = 50 µm; E, F = 1 µm; G = 4 µm; H, I = 10 mm.

Sexual morph: Unknown. *Asexual morph*: *Mycelium* white to cream, covering the host. *Vegetative hyphae* septate and hyaline. *Conidiogenous cells* arising from undifferentiated hyphae, consisting of elongate or cylindrical cells, with characteristic denticles that are crowded at the apex and less frequent towards the base. *Type I conidia (microconidia)*, hyaline, aseptate, smooth- and thin-walled, ovoid to ellipsoid, formed singly on the denticles, produced on specimen and solid media. *Type II conidia (macroconidia)* hyaline, aseptate, smooth, thin-walled, fusoid, produced on solid media. Colonies on PDA, OA produce a pale red diffusate in solid medium.

Pseudoniveomyces blattae Tasanathai, Noisripoom & Kobmoo, **sp. nov.** MycoBank MB 846762. Fig. 7.

Etymology: The name refers to the host – a cockroach.

Typus: **Thailand**, Nakhon Nayok Province, Khao Yai National Park, *Ophiocordyceps* sp. on cockroach, on the underside of leaves, 7 Jun. 2012, *K. Tasanathai, S. Mongkolsamrit, A. Khonsanit, W. Noisripoom & P. Srikitikulchai* (**holotype** BBH 32477, culture ex-type BCC 53567).

Sexual morph: Unknown. *Asexual morph*: White to cream and cottony mycelium forming on the stromata of *Ophiocordyceps* sp. on cockroach, flattened, scattered. *Conidiophores* mono- or synnematous, *conidiogenous cells* arising from undifferentiated cylindrical to linear cells, (12–)15–71(–90) × (1.5–)2–2.5(–3) µm bearing a rather irregular, geniculate rachis, scattered. Two types of *conidia*: *Type I (microconidia)*, produced on specimen and on OA, forming singly on denticles, hyaline, smooth-walled, one-celled, ovoid to ellipsoid, 5–7(–8) × 2–3 µm; *Type II (macroconidia)*, produced on PDA, hyaline, fusoid, occasionally septate, 5–9(–12) × 1–2 µm.

Culture characteristics: Colonies on OA attaining a diam of 15–18 mm in 20 d at 25 °C, cottony with high mycelium density, flattened, white, reverse deep pink (180D) produce pale red pigment diffusing in the medium. Sporulation observed after 14 d. Conidiogenous cells cylindrical arising from aerial hyphae, producing microconidia, hyaline, oval to ellipsoidal, 5–7(–8) × 2–3 µm. Colonies on PDA attaining a diam of 12–15 mm in 20 d at 25 °C, cottony with high mycelium density, white, moderate purplish red to dark purplish pink pigment diffusing in the
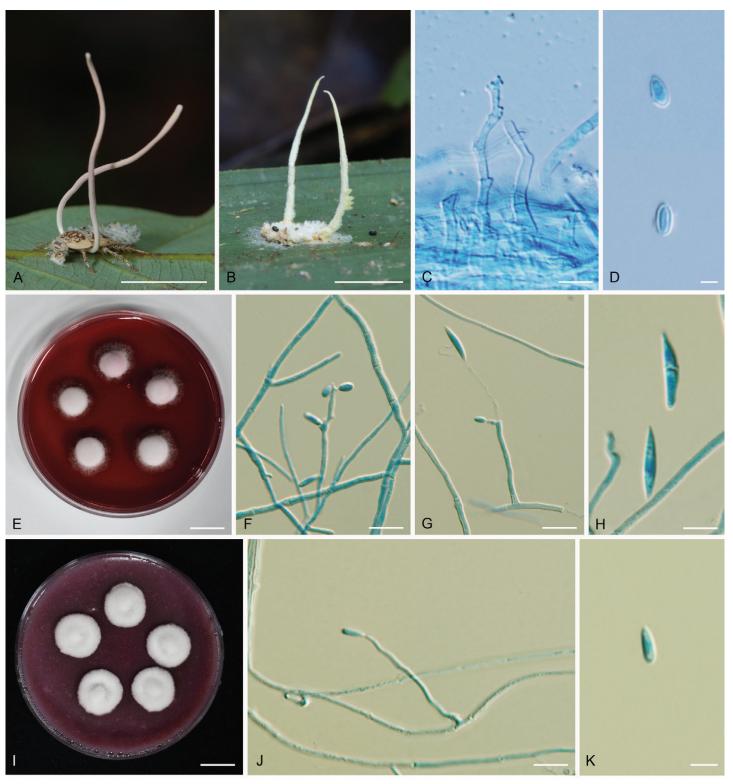
Fig. 7. *Pseudoniveomyces blattae.* **A.** *Ophiocordyceps* sp. on cockroach. **B.** *Pseudoniveomyces blattae* growing on *Ophiocordyceps* sp. **C.** Conidiogenous cells observed directly on the specimen. **D.** Conidia. **E.** Colonies on PDA. **F.** Conidiogenous cells and Type I conidia on PDA. **G.** Conidiogenous cells with a Type I and a Type II conidium on PDA. **H.** Type II conidia on PDA. **I.** Colonies on OA. **J.** Conidiogenous cells and Type I conidia on OA. **K.** Type I conidium on OA. Scale bars: A, B = 5 mm; C, F, G, J = 10 μm; D = 3 μm; E, I = 10 mm; H = 5 μm; K = 4 μm.

medium, reverse moderate red. Sporulation observed after 14 d. Conidiogenous cells arising from aerial hyphae, solitary, producing both microconidia and macroconidia. Macroconidia hyaline, fusiform, 5–9(–12) × 1–2 μm, occasionally septate.

Notes: *Pseudoniveomyces blattae* shows similarity to *Pseudoniveo. arachnovorum* in the production of a pale red pigment diffused in OA and PDA. Morphological comparison

between *Pseudoniveo. blattae* and *Pseudoniveo. arachnovorum* shows similarity in the conidial shape but *Pseudoniveo. blattae* has shorter conidia than *Pseudoniveo. arachnovorum*.

Pseudoniveomyces arachnovorum Tasanathai, Noisripoom & Kobmoo, ***sp. nov.*** MycoBank MB 849232. Fig. 8.

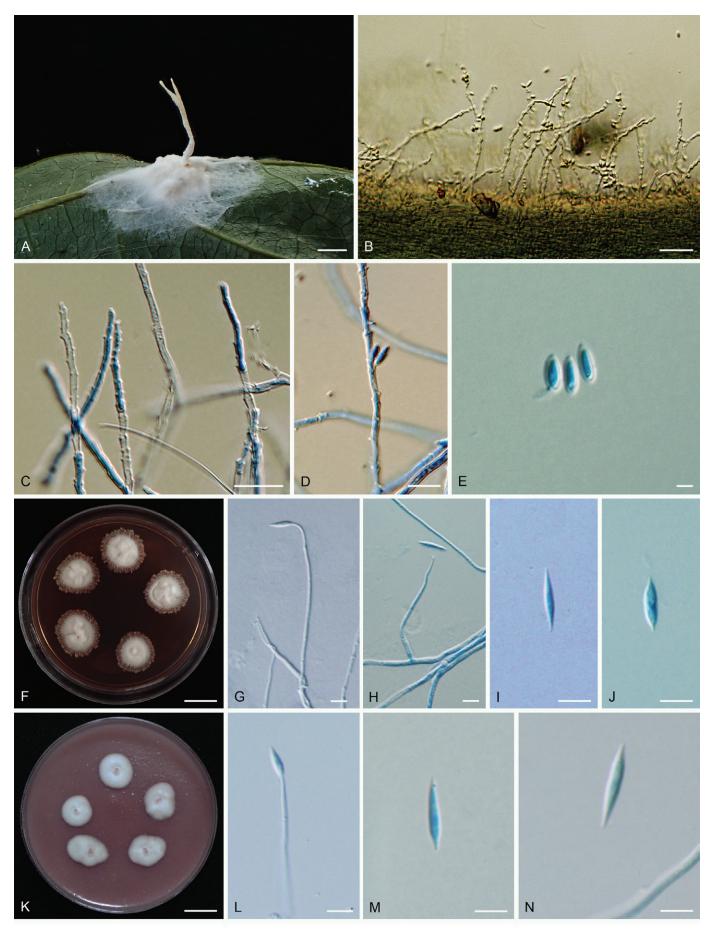*Etymology*: The name refers to the host - spider egg sac.

Fig. 8. *Pseudoniveomyces arachnovorum*. **A.** *Pseudoniveomyces arachnovorum* on spider eggs in the sac. **B.** Conidiogenous cells with Type I conidia observed directly on the specimen. **C, D.** Close-up of conidiogenous cells with characteristic denticles and Type I conidia. **E.** Type I conidia extracted from the specimen. **F.** Colonies on PDA. **G, H.** Conidiogenous cells with Type II conidia on PDA. **I, J.** Type II Conidia on PDA. **K.** Colonies on OA. **L.** Conidiogenous cells with a conidium on OA. **M, N.** Type II Conidia on OA. Scale bars: A = 1 mm; B = 150 µm; C, D = 20 µm; E = 3 µm; F, K = 10 mm; G–J, L = 10 µm; M, N = 5 µm.

Typus: **Thailand**, Songkhla Province, Khao Nam Khang National Park, on spider eggs in the sac on the underside of leaves, 15 Dec. 2022, *B. Sakolrak, W. Himaman & P. Jangsantear* (**holotype** BBH49635, culture ex-type BCC 95818).

Sexual morph: Unknown. *Asexual morph*: White to cream and cottony mycelium forming on spider egg sacs. *Conidiophores* mono- or synnematous. *Conidiogenous cells* directly from the hyphae, cylindrical, > 420 μm long, 15–20 μm wide, bearing a rather irregularly, geniculate rachis. Two types of *conidia*: Type I (*microconidia*), produced on specimen, forming singly on denticles, ovoid to ellipsoidal with an apiculus, occasionally cylindrical with rounded ends, aseptate, hyaline, smooth-walled, (4–)5–6(–7) × 2–3 μm; Type II (*macroconidia*) produced on solid media (OA and PDA), fusiform, smooth-walled, 10–19 × 1.5–2 μm.

Culture characteristics: Colonies on OA attaining 10–15 mm diam in 20 d at 25 °C, cottony with high mycelium density, white, reverse deep pink (180D) produce pale red pigment diffusing in the medium. Conidiogenous cells cylindrical arising from aerial hyphae. Conidia forming on denticles, fusiform, smooth-walled, aseptate, (10–11)–16 × 1.5–2 μm. Colonies on PDA attaining a diam of 10–12 mm in 20 d at 25 °C, cottony with high mycelium density, white, moderate purplish red to dark purplish pink pigment diffusing in the medium, reverse moderate red. Conidiogenous cells arising from aerial hyphae, solitary. Conidia hyaline, fusiform, (10–)12–16(–19) × 1.5–2 μm.

Notes: *Pseudoniveomyces arachnovorum* shows similarity to *Pseudoniveo. blattae* in the production of a pale red pigment diffused in OA and PDA but differ in the conidial length of *Pseudoniveo. arachnovorum* in being longer than that of *Pseudoniveo. blattae*.

Reconstruction of ancestral hosts/substrates

The reconstruction of ancestral hosts/substrates from the 5-locus phylogenetic tree (Fig. 9 and Table 2) showed that the most recent common ancestor (MRCA) of *Cordycipitaceae* (MRCA 1) was versatile with the highest probability of being an environmental fungus (0.430). The genus *Simplicillium* constitutes a deep basal lineage departing from the MRCA 1 along with the other genera which have the MRCA (MRCA 2) also the most probably being from the environment. The genera *Parengyodontium* and *Gamszarea* appeared also as a deep lineage of *Cordycipitaceae* with the MRCA (MRCA 3) being the most probably from the environment. The remaining taxa formed a conspicuous lineage including in majority pathogens of insects and spiders, with the MRCA (MRCA 4) inferred to be
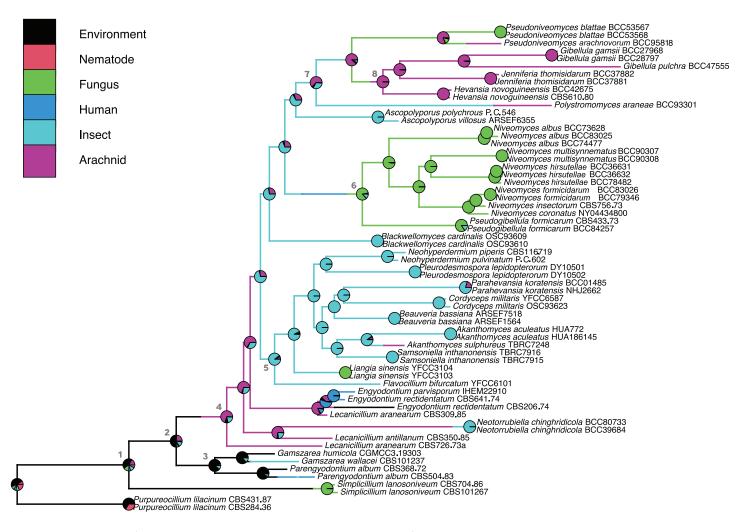

Fig. 9. Evolution of hosts/substrates association as inferred by mapping the hosts/substrates, from which the fungal strains were isolated, onto the 5-locus phylogenetic tree. The pie charts on the internal nodes showed the posterior probabilities of association to different hosts/substrates of the most recent common ancestors (MRCAs) at those nodes. The numbers denote major MRCAs of the evolutionary history of the *Cordycipitaceae*.

Table 2. Posterior probabilities (PP) of ancestral hosts/substrates at major nodes (MRCAs: most recent common ancestors) inferred from the 5-locus Bayesian phylogenetic tree. The MRCA numbers correspond to those appearing in Fig. 9. The PP corresponding to different hosts/substrates are written in the following order: environment/human/nematode/insect/arachnid/fungus. The highest PP value per MRCA is highlighted with a **bold** font.

MRCA	Posterior Probability
1	**0.430** / 0.051 / 0.078 / 0.123 / 0.167 / 0.151
2	**0.522** / 0.031 / 0.026 / 0.134 / 0.235 / 0.052
3	**0.815** / 0.030 / 0.006 / 0.098 / 0.042 / 0.009
4	0.068 / 0.010 / 0.003 / 0.208 / **0.701** / 0.010
5	0.003 / 0.005 / 0.001 / **0.897** / 0.073 / 0.021
6	0.009 / 0.006 / 0.005 / 0.121 / 0.019 / **0.840**
7	0.010 / 0.008 / 0.013 / 0.316 / **0.622** / 0.031
8	0.000 / 0.000 / 0.001 / 0.005 / **0.992** / 0.002

most probably associated to spiders. In this lineage, we can notice a lineage of mostly insect fungi (MRCA 5). There is an almost exclusive lineage of mycoparasites (MRCA 6), including the new *Niveomyces* species discovered in this study with the highest probability as a pathogen of fungi (0. 849). The new genus *Pseudoniveomyces* did not depart from the MRCA 7 but branched as the sister clade to the group of *Gibellula*, *Hevansia* and *Jenniferia* whose most recent common ancestor (MRCA 8) was found to be most probably a spider pathogen. In effect, *Pseudoniveomyces* branched within an almost exclusive clade of spider pathogens whose most recent common ancestor (MRCA 7) was thus inferred to be versatile but with the predominance for arachnids. *Liangia sinensis*, purported mycoparasite of *Beauveria yunnanensis*, appeared among taxa of which the MRCA is most probably an entomopathogen (MRCA 5).

DISCUSSION

New species and a new genus of mycoparasites in *Cordycipitaceae*

In this study, we describe four new species within *Niveomyces* (*N. albus*, *N. formicidarum*, *N. multisynnematus* and *N. hirsutellae*) and a new genus, *Pseudoniveomyces*, with two species (*Pseudoniveo. arachnovorum* and *Pseudoniveo. blattae*). *Niveomyces* and *Pseudoniveomyces* species are characterised by the presence of a sporothrix-like asexual morph and their mostly mycoparasitic nutritional mode.

The species within *Niveomyces* share common morphological features which are scattered, denticulate, conidiogenous cells arising either from undifferentiated hyphae (*N. albus*), or from indeterminate synnemata (*N. formicidarum*, *N. hirsutellae* and *N. multisynnematus*) forming terminally or laterally. The conidia are hyaline, smooth-walled, aseptate and formed singly on denticles, having an ovoid to cylindrical shape with rounded ends. The new genus, *Pseudoniveomyces*, is characterised by the presence of two types of conidia, Type I (microconidia), which is homologous to the conidia in *Niveomyces*, and Type II

(macroconidia), fusoid and occasionally septate, was observed only on solid media. The phylogenetic analyses suggest that it is more related to *Hevansia*, *Gibellula* and *Jenniferia*. *Pseudoniveomyces* shares similar morphological features to *Niveomyces* but differs in the production of the two types of conidia (Fig. 7D, H) and the colour of axenic culture on solid media resembling *Hevansia* and *Gibellula*. The phylogenetic evidence with the distinctive macroconidia of *Pseudoniveo. blattae* and *Pseudoniveo. arachnovorum* supports the status of a distinct genus to *Niveomyces* although both genera produce sporothrix-like asexual morphs. The size of conidiophores and conidia are largely overlapping between the species within *Niveomyces* and *Pseudoniveomyces*. Our finding reinforces the idea that the species diversity of hypocrealean entomopathogenic and mycoparasitic fungi is still largely underexploited due to the existence of cryptic species. There are many closely related species with overlapped morphological characters as shown by molecular phylogenies (Tasanathai *et al.* 2019, 2022, Khonsanit *et al.* 2020, Mongkolsamrit *et al.* 2020) and genomics data (Kobmoo *et al.* 2019, 2021). Most of the novel species described here were found on entomopathogenic fungi which are *Ophiocordyceps* (or *Hirsutella*) growing on different insects: *Niveomyces albus* on *Diptera*-associated *Ophiocordyceps*, *N. multisynnematus* and *N. hirsutellae* on *Hemiptera*-associated *Ophiocordyceps* (= *Hirsutella*), *N. formicidarum* (as well as *N. coronatus* complex) on ant-associated *Ophiocordyceps*. *Pseudoniveomyces blattae* was found on an undescribed *Ophiocordyceps* infecting a cockroach. *Pseudoniveomyces arachnovorum* was found on a spider egg sac. *Pseudoniveomyces arachnovorum* is thus not a strict mycoparasite. Due to the limited number of specimens per species, we do not recommend the insect host identity as an absolute criterion for identification. However, our findings suggest the association of *Niveomyces* species with *Ophiocordyceps* parasitising specific host groups. For example, the *N. coronatus* complex is associated with *Ophiocordyceps* infecting ants (*O. camponoti-floridani* infecting *Camponotus floridanus* for *N. coronatus*, *O. polyrhachis-furcata* infecting *Polyrhachis furcata* for *N. formicidarum* and *N. insectorum* for *O. paltothyreum* infecting *Paltothyreus tarsatus*) (de Hoog 1974, Araújo *et al.* 2020, 2022). Other species are formed by an association with *Ophiocordyceps* infecting *Hemiptera* (*N. hirsutellae* and *N. multisynnematus* on *Hirsutella*) and *Diptera* (*N. albus* in *O. dipterigena s.l.*). The fact that *Niveomyces* species appeared to only parasitise *Ophiocorydyceps* (*Hirsutella*) which are specific pathogens to various insects, warrants further investigation as to why this ecological niche produces such a host-specific diversification. Furthermore, no sexual morph is yet known for *Niveomyces* and *Pseudoniveomyces*. The potential lack of sexual reproduction could contribute to a reduced gene flow between host-specific species, exacerbating the specialisation to different *Ophiocordyceps* species. Future studies further exploring the diversity of *Niveomyces* that infect entomopathogens associated with other hosts should contribute to confirm or refute this pattern.

The affiliation of *Sporothrix insectorum* to *Cordycipitaceae*

De Hoog (1993) proposed a "clavicipitalean" relationship for *Sporothrix insectorum*. De Beer *et al.* (2016) even suggested that its sequences should be compared to *Cordycipitaceae*. Our study presents strong molecular evidence that *Sporothrix*

insectorum is affiliated to the *N. coronatus* species complex in the *Cordycipitaceae*. Considering that the *Spor. insectorum* ex-type strain (CBS 756.73) was isolated in Ghana while *N. coronatus* has been described from North America (Araújo *et al.* 2022), and the two specimens of *N. formicidarum* are from Thailand (BCC79346 and BCC83026); they were thus discovered from different biogeographic regions. The divergence between these taxa is also higher than 1 %, further supporting the status of *N. formicidarum* as a new species from Thailand, and that *Spor. insectorum* becomes a synonym of *N. insectorum*.

The evolution of mycoparasitism in *Cordycipitaceae* and *Hypocreales*

It was established that the fungi of the order *Hypocreales* were derived from a plant pathogenic ancestor (Spatafora *et al.* 2007, Zhang *et al.* 2018). This order contains the most conspicuous group of fungal pathogens of plants and animals (Berbee 2001). The evolution of host specificity of fungal pathogens has received much attention during the last decade, particularly through genomic studies to elucidate underlying molecular mechanisms (Baroncelli *et al.* 2016, Zhang *et al.* 2018, St. Leger & Wang 2020). The existence of mycoparasites within *Hypocreales* has been increasingly documented (Wang *et al.* 2014, Zhong *et al.* 2016, Crous *et al.* 2017, Mongkolsamrit *et al.* 2021). Our study has added supplementary taxa to the list. Mycoparasitism appears overall relatively minor within this order and has evolved independently multiple times. *Niveomyces* formed with *Pseudogibellula* a unique lineage of mycoparasites while *Pseudoniveomyces* might have evolved independently from the common ancestor shared with *Gibellula*, *Hevansia* and *Jenniferia*. *Pseudoniveomyces arachnovorum* was found on a spider egg sac and thus cannot be described as a mycoparasite. It is possible that mycoparasites from *Cordycipitaceae* can maintain a potent entomopathogenicity and be occasionally found on insects and spiders. The exoskeleton of insects and arachnids as well as fungal cell wall are composed of chitins. Entomopathogenic fungi are specialised to secrete enzymes such as chitinases allowing the penetration into the insect body (Da Silva *et al.* 2005, Staats *et al.* 2013). They are therefore evolutionarily weaponised to also exploit fungi. Other mycoparasites exist also in other *Hypocrealean* families, *e.g.*, *Polycephalomyces* and *Torrubiellomyces* in *Ophiocordycipitaceae* (Wang *et al.* 2015, Crous *et al.* 2017, Araújo *et al.* 2022) and *Syspastospora* in *Hypocreaceae* (Posada *et al.* 2004). Overall, our finding confirms the fact that mycoparasitism has evolved multiple times in the evolution of *Hypocreales*.

Previous studies of *Cordycipitaceae* show that *Simplicillium* and *Lecanicillium* are basal to other genera in *Cordycipitaceae* (Sung *et al.* 2007a, Mongkolsamrit *et al.* 2018, 2020). However, these did not include *Gamszarea* and *Parengyodontium*. Our study includes a comprehensive list of genera of *Cordycipitaceae*, showing that *Gamszarea* and *Parengyodontium* constitute basal lineages in *Cordycipitaceae*. *Simplicillium* and *Lecanicillium* have a broad spectrum of hosts and substrates including fungal pathogens of plants (Vandermeer *et al.* 2009, Baiswar *et al.* 2014) and insects (Wei *et al.* 2019) and are also known for their entomopathogenic potentials (Zhou *et al.* 2020, Sujithra *et al.* 2021). *Parengyodontium* has been isolated as a human pathogen and from environmental samples (soil, air, material clean surface) (Tsang *et al.* 2016, Zhang *et al.* 2021). *Gamzarea* also appeared to be an ecologically versatile genus with species found from soil and insects (Zhang *et al.* 2021). Both *Parengyodontium* and *Gamszarea* form a deep lineage close to *Simplicillium* and *Lecanicillium*. Otherwise, *Cordycipitaceae* contains prominent entomopathogenic genera such as *Beauveria* (Imoulan *et al.* 2017, Khonsanit *et al.* 2020), *Blackwellomyces* (Mongkolsamrit *et al.* 2020), *Cordyceps* (Mongkolsamrit *et al.* 2018), *Gibellula* and *Hevansia* (Kuephadungphan *et al.* 2020, 2022). These genera are known only as entomopathogens, except *Beauveria* which was reported to cause an infection in an immune-suppressed human individual (Henke *et al.* 2002), and occasionally as endophytes (Brownbridge *et al.* 2012).

Overall, it seems that *Cordycipitaceae* might have originated from an ecologically versatile ancestor with the capacity to exploit various substrates and hosts. It then evolved to become specialised pathogens of insects and spiders while some lineages, as evidenced by *Niveomyces* and *Pseudoniveo. blattae*, have evolved as specialised mycoparasites. *Sporothrix insectorum* was described as growing on an ant "associated to *Gibellula formicarum*" following de Hoog (1974). It has been thus unclear whether it is an entomopathogen co-occurring with *G. formicarum* which is now reclassified as *Pseudogibellula formicarum* (Samson & Evans 1973, Mongkolsamrit *et al.* 2021), or a mycoparasite exploiting *Pseudogib. formicarum*. As *Spor. insectorum* is clearly affiliated to *Niveomyces* which has been shown to be mycoparasites (Araújo *et al.* 2022), it is highly probable that this species is also parasitic on entomopathogens. *Pseudogibellula formicarum* was originally described as an ant pathogen (Samson & Evans 1973, Samson *et al.* 1989) and later documented as pathogenic to the glassy-winged sharpshooter (*Homalodisca coagulata*, *Hemiptera*) (Kanga *et al.* 2004, Boucias *et al.* 2007) whereas Mongkolsamrit *et al.* (2021) has found it growing on *Ophiocordyceps flavida* occurring on leafhoppers in Thailand, which suggests a mycoparasitism. The ecology of *Pseudogib. formicarum* is thus ambiguous. It is possible that *Pseudogib. formicarum* is a mycoparasite which still has a potent entomopathogenicity. The genus *Liangia* with a lecanicillium-like asexual morph has been shown to be a mycoparasite on *Beauveria yunnanensis* (Wang *et al.* 2020). Therefore, it appears that mycoparasitism has also evolved multiple times in the *Cordycipitaceae*. It would be interesting in the future to sequence the genome of these fungal mycoparasites and compare them to other species with different substrate utilisation from the same family as well as to mycoparasites from other families of *Hypocreales*. This will contribute to a better understanding of the genetic and genomic mechanisms behind the evolutionary trajectory towards mycoparasitism.

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Supplementary information

Fig. S1. The best phylogenetic tree from Bayesian inference based on ITS. The statistical support values, namely Bayesian posterior probability (PP; > 0.70) and maximum likelihood-based bootstrap (BS; > 70 %), are shown above the nodes (PP/BS).

Fig. S2. The best phylogenetic tree from Bayesian inference based on LSU. The statistical support values, namely Bayesian posterior probability (PP; > 0.70) and maximum likelihood-based bootstrap (BS; > 70 %), are shown above the nodes (PP/BS).

Fig. S3. The best phylogenetic tree from Bayesian inference based on *TEF1*. The statistical support values, namely Bayesian posterior probability (PP; > 0.70) and maximum likelihood-based bootstrap (BS; > 70 %), are shown above the nodes (PP/BS).

Fig. S4. The best phylogenetic tree from Bayesian inference based on *RPB1*. The statistical support values, namely Bayesian posterior probability (PP; > 0.70) and maximum likelihood-based bootstrap (BS; > 70 %), are shown above the nodes (PP/BS).

Fig. S5. The best phylogenetic tree from Bayesian inference based on *RPB2*. The statistical support values, namely Bayesian posterior probability (PP; > 0.70) and maximum likelihood-based bootstrap (BS; > 70 %), are shown above the nodes (PP/BS).